Synthesis of some \(N\)-sulfonated derivatives of \(1-[(E)-3\text{-phenyl-2-propenyl}]\)piperazine as suitable antibacterial agents

Muhammad Athar Abbasi\(^1\)*, Mubashar Ijaz\(^1\), Aziz-ur-Rehman\(^1\), Sabahat Zahra Siddiqui\(^1\), Syed Adnan Ali Shah\(^2\), Muhammad Shahid\(^2\) and Hina Fatima\(^3\)
\(^1\)Department of Chemistry, Government College University, Lahore, Pakistan
\(^2\)Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
\(^3\)Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Abstract: In the planned research work, the nucleophilic substitution reaction of \(1-[(E)-3\text{-phenyl-2-propenyl}]\)piperazine (1) was carried out with different sulfonyl chlorides (2a-g) at pH 9-10 to synthesize its different \(N\)-sulfonated derivatives (3a-g). The structures of the synthesized compounds were characterized by their proton nuclear magnetic resonance (\(^1\)H-NMR), carbon-nuclear magnetic resonance (\(^13\)C-NMR) and Infra Red (IR) spectral data, along with CHN analysis. The inhibition potential of the synthesized molecules was ascertained against two bacterial pathogenic strains i.e. \textit{Bacillus subtilis} and \textit{Escherichia coli}. It was inferred from the results that some of the compounds were very suitable inhibitors of these bacterial strains. Moreover, their cytotoxicity was also profiled and it was outcome that most of these molecules possessed moderate cytotoxicity.

Keywords: \(1-[(E)-3\text{-phenyl-2-propenyl}]\)piperazine, sulfonamide derivatives, biofilm inhibition, antibacterial, cytotoxicity.

INTRODUCTION

The development of microbial resistance against many antibiotics has ringed the alarm bell for researchers to prepare the new drug molecules. Various bacterial species, being pathogenic, cause infectious diseases (Bahiru \textit{et al.}, 2013; Lowy, 1998). The most familiar fatal diseases caused by bacteria are tuberculosis and respiratory infections which are responsible for the death of about 2 million people per year in sub-Saharan Africa (WHO mortality data, 2002). Sulfonamides have great importance in pharmaceutical sciences because they effectively act against different pathogens. Sulfonamides are commonly used as antibacterial, anti-convulsant, analgesic, anti-inflammatory, antiplatelet and anti-tumoral, hypoglycaemic, anti-thyroid, anti-carbonic anhydrase, diuretic and COX-inhibitors (Irshad \textit{et al.}, 2014). They also exhibited a good inhibitory potential against acetylcholinesterase, butyrylcholinesterase, chymotrypsin (Abbasi \textit{et al.}, 2014), \(\alpha\)-glucosidase and lipoxygenase enzymes (Abbasi \textit{et al.}, 2015). Some of the sulfonamide derivatives showed a large antimicrobial activity towards \textit{Pseudomonas aeruginosa} and \textit{Escherichia coli} and inhibition of proliferation of breast carcinoma cell line MCF7 (Poreba \textit{et al.}, 2015) and also exhibited high antitumor activity and low toxicity (Huang \textit{et al.}, 2001). Some sulfonamide derivatives are used as carbonic anhydride inhibitors of commercial importance (Vullo \textit{et al.}, 2013). They are also effective for the treatment of urinary, intestine and ophthalmic infections, scalds, as well as ulcerative colitis (Wilson \textit{et al.}, 2004).

Piperazine molecules, having nitrogen atoms in heterocyclic ring, have a vast importance in pharmaceutical sciences. Piperazine derivatives act as good anti-malarial, anti-bacterial and anti-fungal agents (Todorovic \textit{et al.}, 2005). Some pipazine-sulfonamide derivatives act as potent and selective antagonists at postsynaptic 5-HT\(_{1A}\) receptors (Peglion \textit{et al.}, 1995).

The purpose of our research was to prepare such type of compounds bearing both sulfonamide and piperazine moieties together, which might overcome the overwhelming resistance of the some microbes. So, under the cover of this project, some new \(N\)-sulfonated derivatives of \(1-[(E)-3\text{-phenyl-2-propenyl}]\)piperazine were synthesized as possible antibacterial agents.

MATERIALS AND METHODS

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and \(n\)-hexane as solvent system. Spots were detected by UV\(_{254}\). Gallonkamp apparatus was used to detect melting points in capillary tubes. IR spectra (\(\nu\), cm\(^{-1}\)) were recorded by KBr pellet method in the Jasco 360IR spectrophoto meter. \(^1\)H-NMR spectra (\(\delta\), ppm) were recorded at 600 MHz (\(^13\)C-NMR spectra, at 150 MHz) in DMSO-\(d_6\) using the Bruker Advance III 600 As- cend spectrometer using BBO probe. El-MS spectra were measured on a JEOL JMS-600H instrument with data processing system.

\*Corresponding author: e-mail: abbas@gcu.edu.pk

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Synthesis

Synthesis of 1-[(E)-3-phenyl-2-propenyl]piperazine derivatives (3a-g)
1-[(E)-3-Phenyl-2-propenyl]piperazine (1; 0.2g) was added in distilled water (5 mL) contained in a 25 mL round bottom flask and stirred for 15 min at 25°C. The pH of the solution was maintained by the addition ofaq. Na₂CO₃ soln. After that, respective sulfonyl chlorides (2a-g, 0.15g-0.20g; one in each reaction) were added into the reacting reagent, which was further stirred for 8-9 hrs. The reaction was monitored by TLC till single spot and at completion; the reaction mixture was quenched with chilled water (100 mL). Precipitates of the product were filtered out and washed followed by drying to yield the subsequent N-sulfonated derivative of 1-[(E)-3-phenyl-2-propenyl]piperazine (3a-g).

Biological studies

Assessment of bacterial biofilm inhibition
Microtiter-plate method was used for the assessment of the inhibition of bacterial (Bacillus subtilis/Escherichia coli) biofilm formation as described by Stepanovic et al. (2000). 24-Well flat bottomed plastic tissue culture plate wells of a sterile were filled with 100 µL of nutrient broth (Oxoid, UK). Concentration, which was 1.0 µg of the testing sample (dissolved in 1 mL of DMSO), was added in different wells. At last, 20 µL of the bacterial suspension containing 1x10⁸ CFU/mL was inoculated. The well of positive control was contained with Ciprofloxacin and nutrient broth (Oxoid, UK) whereas the well of negative control contained nutrient broth and microbial strain. After that, plates were covered and aerobically incubated for 24 hours at 37°C. Subsequently, using sterile phosphate buffer (pH: 7.2) of 220 µL the contents of each well were beheld thrice. Plates were vigorously shaken to remove all non-adherent bacteria. Then, the bacteria which attached on plates were fixed with 220 µL of 33% (v/v) glacial acetic acid per well after air-dried and the bound dye. By using 630 nm microplate reader (Biotek, USA) the optical density (OD) of each well was measured. Against selected bacterial strains, all the tests were made. Add 20µL of synthetic compounds solution (10 mg/mL) in 180µL of RBCs suspension and incubate for 30 min at room temperature. PBS was used as negative control and Triton 100-X was taken as positive control (Sharma et al. 2001; Powell et al., 2000). The %age of hemolysis was taken as by using formula:

\[
\text{% Hemolysis} = \frac{\text{Absorbance of Sample} - \text{Absorbance of Negative Control} \times 100}{\text{Absorbance of Positive Control}}
\]

STATISTICAL ANALYSIS

All the measurements were carried out in triplicate and statistical analysis was performed by Microsoft Excel 2010. The results are presented as mean ± SEM with 96 % CL.

Spectral characterization of synthesized compounds
1-[(4-Methylphenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl]piperazine (3a)
White crystalline solid; Mol. formula C₂₀H₂₂N₂O₂S; Mol. mass 356 gmol⁻¹; yield: 81%; melting point 137°C; IR (KBr, µ/cm⁻¹): 3080 (C-H str. of aromatic ring), 2878 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1109 (C-N=C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ ppm): 7.63 (d, J = 8.2 Hz, 2H, H-2'' & H-6''), 7.33 (br.d, J = 8.5 Hz, 2H, H-2'' & H-6''), 7.30 (d, J=8.5 Hz, 2H, H-3'' & H-5''), 7.27 (br.t, J = 7.9 Hz, 2H, H-3'' & H-5''), 7.21 (br.t, J = 8.2 Hz, 1H, H-4''), 6.49 (d, J=15.8 Hz, 1H, H-3'), 6.13 (td, J = 6.8,15.7 Hz, 1H, H-2'), 3.13 (br.d, J = 6.8 Hz, 2H, CH₂-1'), 3.03 (br.s, 4H, CH₂-2 & CH₂-6), 2.57-2.56 (m, 4H, CH₂-3 & CH₂-5), 2.42 (s, 3H, CH₃-4'''); ¹³C-NMR (150 MHz, CDCl₃, δ ppm): 143.66 (C-1''), 136.60 (C-1'), 136.66 (C-2'), 132.25 (C-4''), 129.62 (C-3'' & C-5''), 128.58 (C-3'' & C-5''), 127.91 (C-2'' & C-6''), 127.68 (C-4''), 126.31 (C-2'' & C-6''), 125.59 (C-3'), 60.53 (C-1'), 52.14 (C-3 & C-5), 46.07 (C-2 & C-6); Anal. Calc. for C₂₀H₂₂N₂O₂S (356.48): C, 67.38; H, 6.79; N, 7.86. Found: C, 67.67; H, 6.67, N, 7.89.

1-[(4-tert-Butyl)phenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl]piperazine (3b)
White crystalline solid; Mol. formula: C₂₃H₂₅N₂O₂S; Mol. mass: 398 gmol⁻¹; yield: 87%; melting point 149 °C; IR (KBr, µ/cm⁻¹): 3088 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1594 (C=C aromatic str.), 1112 (C-N=C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ ppm): 7.66 (d, J=8.6 Hz, 2H, H-2'' & H-6''), 7.52 (d, J=8.6 Hz, 2H, H-3'' & H-5''), 7.33 (br.d, J = 8.1 Hz, 2H, H-2'' & H-6''), 7.28 (br.t, J = 9.0 Hz, 2H, H-3'' & H-5''), 7.22 (br.t, J = 9.0 Hz, 1H, H-4''), 6.49 (d, J=15.8 Hz, 1H, H-3'), 6.13 (td, J = 6.8, 15.8 Hz, 1H, H-2'), 3.14 (dd, J = 1.4, 6.8 Hz, 2H, CH₂-1''), 3.05 (br.s, 4H, CH₂-2 & CH₂-6), 2.59-2.58 (m, 4H, CH₂-3 & CH₂-5), 1.34 (s, 9H, 4(CH₃)); ¹³C-NMR (150 MHz, CDCl₃, δ ppm): 156.66 (C-4''), 136.55 (C-1''), 133.82 (C-2'), 131.99 (C-1''), 128.58 (C-3'' & C-5''), 127.80 (C-2'' & C-6''), 127.71 (C-4''), 126.32 (C-2'' & C-
1-[4-(Fluorophenyl)sulfonyl]-4-[4-(E)-3-phenyl-2-propenyl]piperazine (3c)
White crystalline solid; Mol. formula: C_{19}H_{21}F_{2}N_{2}O_{4}S; Mol. mass: 360 gmol; yield: 79%; melting point: 154°C; IR (KBr, υ/cm⁻¹): 3082 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1594 (C=C aromatic str.), 1107 (C-N-C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ, ppm): 7.77 (dd, J = 4.9, 8.2 Hz, due to coupling with F₁₀, 2H, H-2'' & H-6''), 7.33 (brd, J = 7.7 Hz, 2H, H-2'' & H-6''), 7.29 (brt, J = 7.4 Hz, 2H, H-3'' & H-5''), 7.23-7.19 (m, 13H, H-4', H-3' & H-5''), 6.50 (d, J = 15.8 Hz, 1H, H-3'), 6.13 (td, J = 6.7, 15.9 Hz, 1H, H-2'), 3.14 (brd, J = 6.9 Hz, 2H, CH₂-1'), 3.05 (brs, 4H, CH₂-2 & CH₂-6), 2.58-2.57 (m, 4H, CH₂-3 & CH₂-5); ¹³C-NMR (150 MHz, CDCl₃, δ ppm): 166.11 & 164.42 (due to coupling of F₁₀, C-4''), 136.56 (C-1''), 137.32 (C-2''), 131.5 & 131.48 (due to effect of F₁₀ isotope, C-1''), 130.55 & 130.49 (C-2'' & C-6''), 128.60 (C-3''), 127.72 (C-4''), 126.32 (C-6''), 125.51 (C-3), 116.36 & 116.22 (C-3'' & C-5''), 60.51 (C-1'), 52.06 (C-3 & C-4''), 46.07 (C-2 & C-6); Anal. Calc. for C_{19}H_{21}F_{2}N_{2}O_{4}S (360.44): C, 53.46; H, 5.87, N, 7.77. Found: C, 53.43; H, 5.73; N, 7.89.

1-[4-(Bromophenyl)sulfonyl]-4-[4-(E)-3-phenyl-2-propenyl]piperazine (3d)
Off white crystalline solid; Mol. formula: C_{19}H_{21}BrN_{2}O_{4}S; Mol. mass: 421 gmol; yield: 81%; melting point: 139°C; IR (KBr, υ/cm⁻¹): 3082 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1590 (C-C aromatic str.), 1112 (C-N-C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ, ppm): 7.66 (d, J = 8.5 Hz, 2H, H-2'' & H-6''), 7.60 (d, J = 8.5 Hz, 2H, H-3'' & H-5''), 7.34 (brd, J = 7.1 Hz, 2H, H-2'' & H-6''), 7.29 (brt, J = 7.0 Hz, 2H, H-3'' & H-5''), 7.23 (brt, J = 7.3 Hz, 1H, H-4''), 6.50 (d, J = 15.8 Hz, 1H, H-3'), 6.14 (td, J = 6.8, 15.8 Hz, 1H, H-2'), 3.17 (brd, J = 6.8 Hz, 2H, CH₂-1'), 3.07 (brs, 4H, CH₂-2 & CH₂-6), 2.61 (brs, 4H, CH₂-3 & CH₂-5); ¹³C-NMR (150 MHz, CDCl₃, δ, ppm): 136.46 (C-1''), 134.40 (C-2''), 132.36 (C-3''), 131.51 (C-1''), 129.30 (C-6''), 128.61 (C-3''), 128.00 (C-3''), 127.79 (C-4''), 127.58 (C-4''), 126.35 (C-2''), 126.46 (C-1'), 52.00 (C-3 & C-5'), 45.94 (C-2 & C-6); Anal. Calc. for C_{19}H_{21}BrN_{2}O_{4}S (421.35): C, 54.16; H, 5.02, N, 6.65. Found: C, 54.10; H, 5.19, N, 6.71.

1-[4-(Nitrophenyl)sulfonyl]-4-[4-(E)-3-phenyl-2-propenyl]piperazine (3e)
White crystalline solid; Mol. Formula: C_{19}H_{21}N_{2}O_{4}S; Mol. Mass: 387 gmol; yield: 83%; melting point: 146°C; IR (KBr, υ/cm⁻¹): 3080 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1111 (C-N-C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ, ppm): 8.37 (d, J = 8.6 Hz, 2H, H-3'' & H-5''), 7.94 (d, J = 8.7 Hz, 2H, H-2'' & H-6''), 7.33 (brd, J = 8.6 Hz, 2H, H-2'' & H-6''), 7.29 (brt, J = 8.4 Hz, 4H, H-3'' & H-5''), 7.22 (brt, J = 7.9 Hz, 1H, H-4''), 6.50 (d, J = 15.8 Hz, 1H, H-3'), 6.12 (td, J = 6.7, 15.8 Hz, 1H, H-2'), 3.15 (brd, J = 6.7 Hz, 2H, CH₂-1'), 3.11 (brs, 4H, CH₂-2 & CH₂-6), 2.60-2.58 (m, 4H, CH₂-3 & CH₂-5); ¹³C-NMR (150 MHz, CDCl₃, δ ppm): 169.25 (4-NH-OC-CH₃), 142.54 (C-4''), 136.5 (C-1''), 133.76 (C-2''), 129.57 (C-1''), 128.98 (C-2'' & C-6''), 128.59 (C-5''), 127.71 (C-4''), 126.33 (C-6'), 125.38 (C-3''), 119.37 (C-3'' & C-5'').
Synthesis of some N-sulfonated derivatives of 1-[(E)-3-phenyl-2-propenyl]piperazine as suitable antibacterial agents

Table 1: Different substituents (-R) in scheme 1.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>3g</th>
</tr>
</thead>
<tbody>
<tr>
<td>-R</td>
<td>3</td>
<td>4-CH₃</td>
<td>4-CH(CH₃)₂</td>
<td>4-F</td>
<td>4-BR</td>
<td>4-NO₂</td>
<td>-H</td>
</tr>
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</table>

Table 2: *Bacillus subtilis* biofilm inhibition.

<table>
<thead>
<tr>
<th>Compound</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>3g</th>
<th>3h</th>
<th>3i</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.233</td>
<td>0.741</td>
<td>0.278</td>
<td>0.832</td>
<td>0.258</td>
<td>0.543</td>
<td>0.732</td>
<td>0.609</td>
<td>0.301</td>
<td>0.195</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>77.17</td>
<td>72.42</td>
<td>72.77</td>
<td>28.51</td>
<td>74.73</td>
<td>46.81</td>
<td>28.33</td>
<td>40.35</td>
<td>70.51</td>
<td>80.9</td>
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</tbody>
</table>

Table 3: *Escherichia coli* biofilm inhibition.

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<tr>
<th>Compound</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>3g</th>
<th>3h</th>
<th>3i</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.324</td>
<td>0.526</td>
<td>0.453</td>
<td>0.517</td>
<td>0.546</td>
<td>0.428</td>
<td>0.701</td>
<td>0.646</td>
<td>0.621</td>
<td>0.131</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>66.93</td>
<td>46.32</td>
<td>53.77</td>
<td>67.65</td>
<td>44.28</td>
<td>56.32</td>
<td>28.46</td>
<td>34.08</td>
<td>36.63</td>
<td>86.63</td>
</tr>
</tbody>
</table>

Note: Ciprofloxacin was used as a positive control. Negative control (% Inhibition) = 1.021.

Table 4: Cytotoxic potential through hemolytic activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>3g</th>
<th>3h</th>
<th>3i</th>
<th>Triton-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Hemolysis</td>
<td>34.75</td>
<td>43.45</td>
<td>23.35</td>
<td>34.15</td>
<td>35.65</td>
<td>42.15</td>
<td>25.35</td>
<td>49.25</td>
<td>15.45</td>
<td>87.67</td>
</tr>
</tbody>
</table>

Note: PBS (% Hemolysis) = 1.03.

1-(Butylsulfonyl)-4-[(E)-3-phenyl-2-propenyl]piperazine (3h)
White crystalline solid; Mol. formula C₂₁H₂₆N₄O₃S; 322 g/mol; yield: 79%; melting point 129 °C; IR (KBr, μm⁻¹): 3080 (νC-H str. of aromatic ring), 2878 (νC-H str. of aliphatic), 1590 (νC=C aromatic str.), 1112 (νC-N,C bond str.); ¹H-NMR (600 MHz, CDCl₃, δ ppm): 7.38 (brd, J = 7.7 Hz, 2H, H-2" & H-6"), 7.31 (brt, J = 7.5 Hz, 2H, H-3" & H-5"), 7.23 (brt, J = 7.3 Hz, 1H, H-4"), 6.55 (d, J = 15.8 Hz, 1H, H-3'), 6.22 (td, J = 6.8, 15.8 Hz, 1H, H-2'), 3.35-3.34 (m, 4H, CH₂-3 & CH₂-5), 3.22 (d, J = 6.8 Hz, 2H, CH₂-1'), 2.91 (dist.t, J = 7.9 Hz, 2H, CH₂-1"'), 2.62-2.61 (m, 4H, CH₂-2 & CH₂-6), 1.80 (m, 2H, CH₂-2"'), 1.44 (sext., J = 7.5 Hz, 2H, CH₂-3"), 0.94 (t, J = 7.7 Hz, 3H, CH₃-4"'); ¹³C-NMR (150 MHz, CDCl₃, δ ppm): 136.53 (C-1'), 134.01 (C-2'), 128.62 (C-3' & C-5'), 127.78 (C-4'), 126.38 (C-2" & C-6'), 125.23 (C-3'), 60.68 (C-1'), 52.67 (C-2' & C-6), 48.91 (C-1"'), 45.64 (C-3 & C-5), 25.02 (C-2"'), 21.75 (C-3"'), 13.57 (C-4"').

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1-(Methylsulfonyl)-4-[(E)-3-phenyl-2-propenyl]piperazine (3i)

White crystalline solid; Mol. formula C14H15N2O2S; 280.38 g mol\(^{-1}\); % yield 79%; melting point 132°C; IR (KBr, u/cm\(^{-1}\)): 3078 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1594 (C=C aromatic str.), 1109 (C-N bond str.); \(^1\)H-NMR (600 MHz, CDCl\(_3\), δ, ppm): 7.38 (br.d, J = 7.1 Hz, 2H, H-2'' & H-3''), 7.32 (br.t, J = 7.4 Hz, 2H, H-3'' & H-5''), 7.24 (br.t, J = 7.4 Hz, 1H, H-4''); 6.54 (d, J = 15.8 Hz, 1H, H-3'), 6.22 (td, J = 6.8, 15.8 Hz, 1H, H-2'), 3.28-3.26 (m, 4H, CH-2'' & CH-3''), 3.20 (br.d, J = 6.9 Hz, 2H, CH-2'); 2.78 (s, 3H, CH-1''), 2.62-2.61 (m, 4H, CH-2', CH-3'); \(^13\)C-NMR (150 MHz, CDCl\(_3\), δ, ppm): 136.59 (C-1''), 133.77 (C-2''), 128.62 (C-3'' & C-5''); 127.75 (C-4''), 126.35 (C-2'' & C-6''), 125.55 (C-3''), 60.63 (C-1''), 52.33 (C-2 & C-6), 45.88 (C3 & C-5), 34.12 (C-1''). Anal. Calc. for C\(_{14}\)H\(_{15}\)N\(_2\)O\(_2\)S: C, 59.97; H, 7.19; N, 9.99. Found: C 59.81; H, 7.28; N, 9.84.

DISCUSSION

The synthesis of targeted N-sulfonated derivatives (3a–i) was accomplished by a facile strategy and all the compounds were obtained in very good yields. The synthesized compounds were structurally corroborated through spectral data of IR, \(^13\)C-NMR, \(^1\)H-NMR, and by elemental analysis. One of the synthesized molecules is discussed hereby in detail for the expediency of the readers. The molecule, 3d, was obtained as a light brown crystalline solid. The molecular formula of this compound was recognized by counting the number of protons in its \(^1\)H-NMR spectrum and number of carbon resonances in its \(^13\)C-NMR spectrum. The CHN analysis data was also in agreement with its molecular formula, C\(_{14}\)H\(_{21}\)BrN\(_2\)O\(_2\)S. Various functionalities in this molecule were confirmed by its IR spectrum. The characteristic peaks appeared at n(C=H) 3082 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1112 (C-N bond str) cm\(^{-1}\). A typical AB\(_2\) spin system for a (4-bromophenyl)sulfonyl moiety was observed in aromatic region of its \(^1\)H-NMR spectrum, by virtue of two ortho-coupled doublets at δ 7.66 (d, J = 8.5 Hz, 2H, H-2'' & H-6''), and 7.60 (d, J = 8.5 Hz, 2H, H-3'' & H-5''). The (E)-3-phenyl-2-propenyl (trans-cinnamyl) moiety in the molecule was deduced by three signals of a phenyl ring at δ 7.34 (br.d, J = 7.1 Hz, 2H, H-2'' & H-6''), 7.29 (br.t, J = 7.7 Hz, 2H, H-3'' & H-5''), and 7.23 (br.t, J = 7.3 Hz, 1H, H-4'') along with other three characteristic signals of (E)-2-propenyl unit at δ 6.50 (d, J = 15.8 Hz, 1H, H-3'), 6.14 (td, J = 6.8, 15.8 Hz, 1H, H-2') and 3.17 (br.d, J = 6.8 Hz, 2H, CH-2'). The central 1,4-disubstituted piperazine core of this molecule was represented by two peaks at δ 3.07 (br.s, 4H, CH-2 & CH-3), and 2.61 (br.s, 4H, CH-2 & CH-3). The \(^13\)C-NMR spectrum demonstrated overall thirteen carbon resonances because various sets of duplet symmetrical carbons were present in the molecule and surely each duplet resonated at same position, thus reducing the total number of carbon signals in the spectrum relative to the molecular formula (C\(_{14}\)H\(_{21}\)BrN\(_2\)O\(_2\)S) of the compound. The six carbons of aromatic ring in the (4-bromophenyl)sulfonyl moiety were represented by four signals at δ 131.51 (C-1''), 129.30 (C-2'' & C-6''), 128.4 (C-3'' & C-5'), and 127.58 (C-4''). Similarly, the six carbons of phenyl ring in trans-cinnamyl moiety were also represented by four signals at δ 159.42 (C-1), 133.77 (C-2), 127.75 (C-4), 126.35 (C-2 & C-6), 125.55 (C-3), 60.63 (C-1''), 52.33 (C-2 & C-6), 45.88 (C3 & C-5), 34.12 (C-1'').

RESULTS

In our present research work, different derivatives of 1-[(E)-3-phenyl-2-propenyl]piperazine (1) were synthesized according to the outline illustrated in (Scheme 1; table 1). The synthesis was carried out by reacting 1-[(E)-3-phenyl-2-propenyl]piperazine (1) with different sulfonyl chlorides (2a–i) in distilled water and in the presence of sodium carbonate to obtain various N-sulfonated derivatives (3a–i). The structures of targeted compounds were confirmed by IR, \(^13\)C-NMR and \(^1\)H-NMR spectral techniques and the spectral data is given in the experimental section. The CHN analysis data also supported their structural assignments. Their antibacterial potential was then ascertained by the bacterial biofilm inhibition study against two strains i.e. Bacillus subtilis and Escherichia coli and these results are tabulated in table 2 and table 3, respectively. Their cytotoxicity profile was also studied through hemolytic study and the results are shown in table 4.

Fig. 1: \(^1\)H-NMR spectrum of 3d.

Fig. 2: \(^13\)C-NMR spectrum of 3d molecule.
Synthesis of some N-sulfonated derivatives of 1-[(E)-3-phenyl-2-propenyl]piperazine as suitable antibacterial agents

Fig. 3: Phase contrast microscopic view of inhibition of *Bacillus subtilis* biofilm.

Fig. 4: Phase contrast microscopic view of inhibition of *Escherichia coli* biofilm.
δ 136.46 (C-1’’), 128.61 (C-3’’ & C-5’’), 127.79 (C-4’’ & C-6’’), and 126.35 (C-2’’). In a similar way, the four carbons in 1,4-di-substituted piperazine ring were represented by two resonances at δ 52.00 (C-3 & C-5), 45.94 (C-2 & C-6). However, three carbons of (E)-2-propenyl unit in the molecule, were attributed by three discrete resonances at δ 134.40 (C-2’’), 128.00 (C-3’’) and 60.46 (C-1’’). The 1H-NMR and 13C-NMR spectra of this molecule are shown in fig. 1 and fig. 2, respectively. So, on account of aforementioned evidences, the structure of molecule 3d was confirmed and it was named as 1-[(4-bromophenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl] piperazine. A similar pattern was adopted for the structural characterization of other derivatives in the synthesized series.

**Bacterial biofilm inhibition and structure-activity relationship**

The antibacterial activity of derivatives, 3a-i, was checked by bacterial biofilm inhibition method using two bacterial pathogenic strains, one of which was gram positive (*Bacillus subtilis*) and other was a gram negative (*Escherichia coli*) strain. Ciprofloxacin was used as a standard drug in both assays to compare the antibacterial potential of the synthesized molecules, because this standard molecule also possesses a piperazine ring in its structure. From the results (table 2 & table 3), it was evident that the presence of different groups at C-4’’ position in 3a–g molecules, resulted in an increase or decrease in the antibacterial potential of respective compound. Here, it was noted that among the series, maximum inhibition (77.7%) was given by 3a against *B. subtilis* and it can be attributed to the existence of an electron donating methyl group at C-4’’ position in this molecule. The percentage inhibition of this molecule was very close to the standard Ciprofloxacin having value of 80.9%. The other compounds 3c, 3e and 3i also showed good biofilm inhibition against this strain with percentage of 72.77%, 74.73% and 70.51%, respectively. The phase contrast microscopic views of inhibition of *Bacillus subtilis* biofilm by 3a and 3g are shown in fig. 3. Against *Escherichia coli*, the molecule 3d was found to be more suitable antibacterial agent with percentage inhibition of 67.65%. In this molecule, the enhanced potential might be an outcome of the presence of bromo group at C-4’’ position. The compound 3a also showed a reasonable inhibition (66.93%) against this strain, relative to Ciprofloxacin (86.63%). The phase contrast microscopic views of inhibition of *Escherichia coli* biofilm by 3d and 3g are shown in fig. 4.

**Hemolytic activity**

All the synthesized compounds, 3a-i, were also subjected to hemolytic assay to find out their cytotoxicity profile. Results of percentage hemolysis are shown in table 4. Our results showed that all compounds of this series exhibited moderate toxicity towards red blood cell membrane. Maximum membrane toxicity was shown by the compound 3h (49.15%), but it was less that positive control, Triton-X having % hemolysis of 87.67%. Minimum toxicity in the series was recorded for 3i (15.45%) and moderate toxicity was observed for 3a (34.75%) and 3d (34.15%). In general approximation, it can be inferred that most of the compounds possess mild cytotoxicity, so these can be considered as possible therapeutic agents.

**CONCLUSION**

On the basis of biofilm inhibition study, it was concluded that the molecule 3a exhibited good antibacterial potential against *Bacillus subtilis* while against *Escherichia coli*, 3d, was identified as more suitable antibacterial agent. It is noteworthy to mention here that both these molecules bear moderate cytotoxicity and hence can be regarded as safe therapeutic entrants.

**ACKNOWLEDGEMENT**

Special thanks are paid to Higher Education Commission of Pakistan for the financial support for this study.

**REFERENCES**


Synthesis of some N-sulfonated derivatives of 1-[(E)-3-phenyl-2-propenyl]piperazine as suitable antibacterial agents