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

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Abstract: In the planned research work, the nucleophilic substitution reaction of 1-[(*E*)-3-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. *Bacillus subtilis* and *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-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 *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 *et al*., 2014). They also exhibited a good inhibitory potential against acetylcholinesterase, butyrlcholimesterase, chymotrypsin (Abbasi *et al*., 2014), α-glucosidase and lipoxygenase enzymes (Abbasi *et al*., 2015). Some of the sulfonamide derivatives showed a large antimicrobial activity towards *Pseudomonas aeroginosa* and *Escherichia coli* and inhibition of proliferation of breast carcinoma cell line MCF7 (Poreba *et al*., 2015) and also exhibited high antitumor activity and low toxicity (Huang *et al.*, 2001). Some sulfonamide derivatives are used as carbonic anhydrase inhibitors of commercial importance (Vullo *et al*., 2013). They are also effective for the treatment of urinary, intestine and ophthalmic infections, scalds, as well as ulcerative colitis (Wilson *et al*., 2004).

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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 *et al*., 2005). Some piprazine-sulfonamide derivatives act as potent and selective antagonists at postsynaptic 5-HT_{1A} receptors (Peglion *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-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 (ν, cm^{-1}) were recorded by KBr pellet method in the Jasco-320-A spectrophotometer. ^{1}H-NMR spectra (δ, 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. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system.

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 of aq. Na_2CO_3 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 1×10^9 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 mL of 99% methanol per well. After every 15 min, the plates were emptied and left to dry. Then, by using 220 mL of 50% crystal violet per well, the plates were stained for 5 min. Surplus stain was rinsed by using distilled water. Then plates were re-solubilized 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 carried thrice and the results were averaged. The bacterial growth inhibition (Inhibition %) was calculated using the following formula.

$$\text{Inhibition } \% = 100 - \frac{(OD_{630 \text{of sample}} \times 100)}{OD_{630 \text{control}}}$$

Hemolytic activity

Bovine blood samples was collected in EDTA that was diluted with saline (0.9% NaCl), and centrifuge at 1000xg for 10min. The erythrocytes separated diluted in phosphate buffer saline of pH 7.4 and a suspension was 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:

$$\text{(\%) of Hemolysis} = \frac{\text{Absorbance of Sample} - \text{Absorbance of Negative Control}}{\text{Absorbance of Positive Control}} \times 100$$

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 $C_{20}H_{24}N_2O_2S$; Mol. mass 356 $gmol^{-1}$; yield: 81%; melting point 137°C; IR (KBr, υ/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.); ^{1}H-NMR (600 MHz, $CDCl_3$, 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, CH_2-1'), 3.03 (br.s, 4H, CH_2-2 & CH_2-6), 2.57-2.56 (m, 4H, CH_2-3 & CH_2-5), 2.42 (s, 3H, CH_3-4'''); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm: 143.66 (C-1'''), 136.60 (C-1''), 133.66 (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 $C_{20}H_{24}N_2O_2S$ (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: $C_{23}H_{30}N_2O_2S$; Mol. mass: 398 $gmol^{-1}$; yield: 87%; melting point 149 °C; IR (KBr, υ/cm^{-1}): 3088 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), d 1594 (C=C aromatic str.), 1112 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, 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, CH_2-1'), 3.05 (br.s, 4H, CH_2-2 & CH_2-6), 2.59-2.58 (m, 4H, CH_2-3 & CH_2-5), 1.34 (s, 9H, 4-C$(CH_3)_3$); ^{13}C-NMR (150 MHz, $CDCl_3$, 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-

6"), 125.99 (C-3'" & C-5'"), 125.44 (C-3'), 60.59 (C-1'), 52.16 (C-3 & C-5), 46.07 (C-2 & C-6), 35.16 [4-$C(CH_3)_3$], 31.10 [4-$C(CH_3)_3$]; Anal. Calc. for $C_{23}H_{30}N_2O_2S$ (398.54): C, 69.31; H, 7.59, N, 7.03. Found: C, 69.17; H, 7.68, N, 6.89.

1-[(4-Fluorophenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl]piperazine (3c)

White crystalline solid; Mol. formula: $C_{19}H_{21}FN_2O_2S$; Mol. mass: 360 gmol^{-1}; yield: 79%; melting point: 154 ^oC; 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.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.77 (dd, *J* = 4.9, 8.2 Hz, due to coupling with F_{19}, 2H, H-2'" & H-6'"), 7.33 (br.d, *J* = 7.7 Hz, 2H, H-2" & H-6"), 7.29 (br.t, *J* = 7.4 Hz, 2H, H-3'" & H-5'"), 7.23-7.19 (m, 3H, 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 (br.d, *J* = 6.9 Hz, 2H, CH_2-1'), 3.05 (br.s, 4H, CH_2-2 & CH_2-6), 2.58-2.57 (m, 4H, CH_2-3 & CH_2-5); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 166.11 & 164.42 (due to coupling of $F_{19,}$ C-4'"), 136.56 (C-1"), 133.72 (C-2'), 131.5 & 131.48 (due to effect of F_{19} isotope, C-1'"), 130.55 & 130.49 (C-2'" & C-6'"), 128.60 (C-5"), 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-5), 46.07 (C-2 & C-6); Anal. Calc. for $C_{19}H_{21}FN_2O_2S$ (360.44): C, 63.31; H, 5.87, N, 7.77. Found: C, 63.13; H, 5.73, N, 7.89.

1-[(4-Bromophenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl]piperazine (3d)

Off white crystalline solid; Mol. formula: $C_{19}H_{21}BrN_2O_2S$; Mol. mass: 421 gmol^{-1}; yield: 81%; melting point: 139 ^oC; 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.); ^{1}H-NMR (600 MHz, $CDCl_3$, 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 (br.d, J = 7.1 Hz, 2H, H-2" & H-6"), 7.29 (br.t, *J* = 7.7 Hz, 2H, H-3" & H-5"), 7.23 (br.t, *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 (br.d, *J* = 6.8 Hz, 2H, CH_2-1'), 3.07 (br.s, 4H, CH_2-2 & CH_2-6), 2.61 (br.s, 4H, CH_2-3 & CH_2-5); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 136.46 (C-1"), 134.40 (C-2'), 132.36 (C-5'"), 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"), 60.46 (C-1'), 52.00 (C-3 & C-5), 45.94 (C-2 & C-6); Anal. Calc. for $C_{19}H_{21}BrN_2O_2S$ (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-[(E)-3-phenyl-2-propenyl]piperazine* (*3e*)**

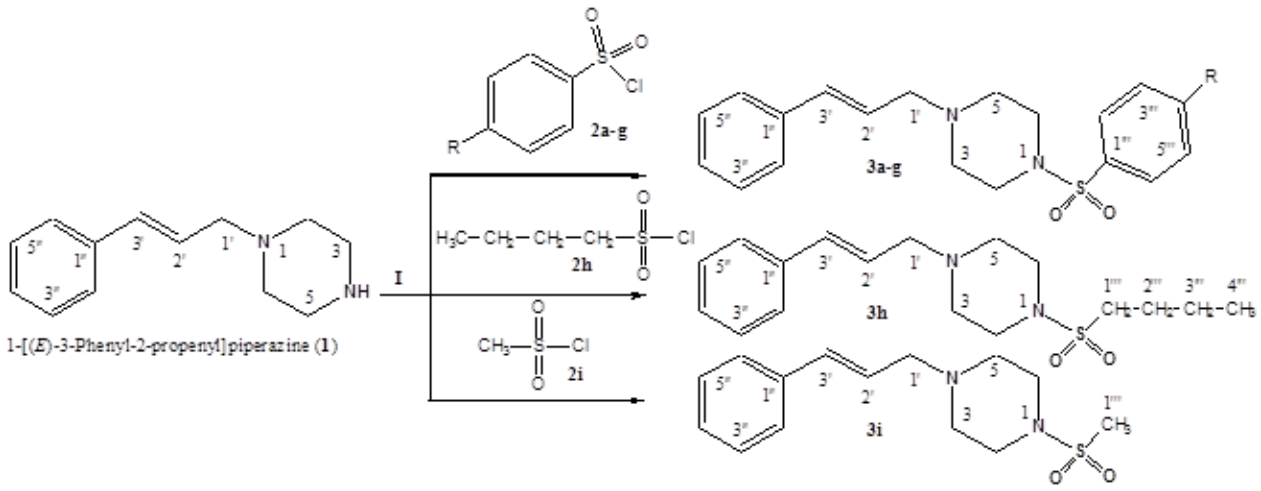
White crystalline solid; Mol. Formula: $C_{19}H_{21}N_3O_4S$; Mol. Mass: 387 gmol^{-1}; yield: 83%; melting point: 146 ^oC; IR (KBr, υ/cm^{-1}): 3080 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1111 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 8.37 (d, *J* = 8.6 Hz, 2H, H-3'" & H-5'"), 7.94 (d, *J* = 8.7 Hz, 2H, H-2'" & H-6'"), 7.33 (br.d, *J* = 8.6 Hz, 2H, H2" & H-6"), 7.29 (br.t, *J* = 8.4 Hz, 2H, H-3" & H-5"), 7.22 (br.t, *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 (br.d, *J* = 6.7 Hz, 2H, CH_2-1'), 3.11 (br.s, 4H, CH_2-2 & CH_2-6), 2.60-2.58 (m, 4H, CH_2-3 & CH_2-5); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 150.22 (C-4'"), 141.71 (C-1'"), 136.49 (C-1"), 133.80 (C-2'), 128.95 (C-2'" & C-6'"), 128.61 (C-3" & C-5"), 127.77 (C-4"), 126.30 (C-2" & C-6"), 125.34 (C-3'), 124.28 (C-3'" & C-5'"), 60.45 (C-1'), 52.01 (C-3 & C-5), 46.06 (C-2 & C-6); Anal. Calc. for $C_{19}H_{21}N_3O_4S$ (387.45): C, 58.90; H, 5.46, N, 10.85. Found: C, 58.81; H, 5.39, N, 10.33.

4-[(E)-3-Phenyl-2-propenyl]-1-(phenylsulfonyl)piperazine (3f)

White crystalline solid, Mol. Formula: $C_{19}H_{22}N_2O_2S$; Mol. Mass: 342 gmol^{-1}; yield: 89%; melting point: 153 ^oC; IR (KBr, υ/cm^{-1}): 3080 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1109 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.73 (distorted dd, *J* = 1.0, 7.5 Hz, 2H, H-2'" & H-6'"), 7.55 (br.t, *J* = 7.7 Hz, 2H, H-3'" & H-5'"), 7.64 (br.t, *J* = 7.4 Hz, 1H, H-4'"), 7.40 (br.t, *J* = 7.4 Hz, 1H, H-4"), 7.34 (br.d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.31 (br.t, *J* = 8.2 Hz, 2H, H-3" & H-5"), 6.73 (d, *J* = 15.8 Hz, 1H, H-3'), 6.32 (td, *J* = 7.4, 15.8 Hz, 1H, H-2'), 3.82 (br.d, *J* = 7.4 Hz, 2H, CH_2-1'), 3.17-3.13 (m, 4H, CH_2-3 & CH_2-5), 3.01 (br.s, 4H, CH_2-2 & CH_2-6); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 144.71 (C-1'"), 135.44 (C-1"), 134.50 (C-2'), 133.83 (C-4'"), 129.63 (C-3'" & C-5'"), 128.80 (C-3" & C-5"), 128.34 (C-2'" & C-6'"), 127.44 (C-2" & C-6"), 127.12 (C-4"), 125.76 (C-3'), 59.54 (C-1'), 50.75 (C-2 & C-6), 43.09 (C-3 & C-5); Anal. Calc. for $C_{19}H_{22}N_2O_2S$ (342.45): C, 66.64; H, 6.48, N, 8.18. Found: C, 66.69; H, 6.50, N, 8.13.

1-[(4-Acetamidophenyl)sulfonyl]-4-[(E)-3-phenyl-2-propenyl]piperazine (3g)

White crystalline solid; Mol. Formula: $C_{21}H_{25}N_3O_3S$, Mol. Mass: 399 gmol^{-1}; yield: 92%; melting point: 159^oC; IR (KBr, υ/cm^{-1}): 3084 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1660 (C=O str.), 1592 (C=C aromatic str.), 1110 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 8.33 (d, *J* = 8.7 Hz, 1H, NH), 7.72 (d, *J* =8.7 Hz, 2H, H-2'" & H-6'"), 7.65 (d, *J* = 8.7 Hz, 2H, H-3'" & H-5'"), 7.33 (br.d, *J* = 7.0 Hz, 2H, H-2" & H-6"), 7.27 (br.t, *J* = 7.7 Hz, 2H, H-3" & H-5"), 7.20 (br.t, *J* = 7.3 Hz, 1H, H-4"), 6.49 (d, *J* =15.9 Hz, 1H, H-3'), 6.12 (td, *J* = 6.8, 15.8 Hz, 1H, H-2'), 3.13 (br.d, *J* = 6.8 Hz, 2H, CH_2-1'), 3.04 (br.s, 4H, CH_2-2 & CH_2-6), 2.57-2.55 (m, 4H, CH_2-3 & CH_2-5), 2.20 (s, 3H, 4-NH-CO-CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 169.25 (4-NH-CO-CH_3), 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'"),



Scheme 1: Protocol for the synthesis of various *N*-sulfonated derivatives of 1-[(*E*)-3-phenyl-2-propenyl]piperazine. Reagents & Conditions: (I) H_2O/Na_2CO_3/pH 9-10/stirring at RT for 8-9 hrs.

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

Compd.	3a	3b	3c	3d	3e	3f	3g
-R	4-CH_3	4-$C(CH_3)_3$	4-F	4-Br	4-NO_2	-H	4-NH-CO-CH_3

Table 2: *Bacillus subtilis* biofilm inhibition.

Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i	Positive control
Absorbance	0.233	0.741	0.278	0.832	0.258	0.543	0.732	0.609	0.301	0.195
% Inhibition	77.17	27.42	72.77	28.51	74.73	46.81	28.33	40.35	70.51	80.9

Table 3: *Escherichia coli* biofilm inhibition.

Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i	Positive control
Absorbance	0.324	0.526	0.453	0.317	0.546	0.428	0.701	0.646	0.621	0.131
% Inhibition	66.93	46.32	53.77	67.65	44.28	56.32	28.46	34.08	36.63	86.63

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

Table 4: Cytotoxic potential through hemolytic activity.

Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i	Triton-X
% Hemolysis	34.75	43.45	23.35	34.15	35.65	42.15	25.35	49.25	15.45	87.67

Note: PBS (% Hemolysis) = 1.03.

52.02 (C-3 & C-5), 46.05 (C-2 & C-6), 24.64 (4-NH-CO-CH_3); Anal. Calc. for $C_{21}H_{25}N_3O_3S$ (399.50): C, 63.13; H, 6.31, N, 10.52. Found: C, 63.19; H, 6.43, N, 10.45.

1-(Butylsulfonyl)-4-[(E)-3-phenyl-2-propenyl]piperazine (3h)

White crystalline solid; Mol. formula $C_{17}H_{26}N_2O_2S$; 322 $gmol^{-1}$; yield: 79%; melting point 129 ^oC; IR (KBr, υ/cm^{-1}): 3080 (C-H str. of aromatic ring), 2878 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1112 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.38 (br.d, *J* = 7.7 Hz, 2H, H-2" & H-6"), 7.31 (br.t, *J* = 7.5 Hz, 2H, H-3" & H-5"), 7.23 (br.t, *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, CH_2-3 & CH_2-5), 3.22 (d, *J* = 6.8 Hz, 2H, CH_2-1'), 2.91 (dist.t, *J* = 7.9 Hz, 2H, CH_2-1'"), 2.62-2.61 (m, 4H, CH_2-2 & CH_2-6), 1.80 (m, 2H, CH_2-2'"), 1.44 (sext., *J* = 7.5 Hz, 2H, CH_2-3'"), 0.94 (t, *J* = 7.7 Hz, 3H, CH_3-4'"); ^{13}C-NMR (150 MHz, $CDCl_3$, 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'"). Anal. Calc. for $C_{17}H_{26}N_2O_2S$ (322.46): C, 63.32; H, 8.13, N, 8.69. Found: C 63.19; H, 8.21, N, 8.81.

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

White crystalline solid; Mol. formula $C_{14}H_{20}N_2O_2S$; 280.38 $gmol^{-1}$; % yield 79%; melting point 132°C; IR (KBr, υ/cm^{-1}): 3078 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1594 (C=C aromatic str.), 1109 (C-N-C 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_2-3 & CH_2-5), 3.20 (br.d, *J* = 6.9 Hz, 2H, CH_2-1'), 2.78 (s, 3H, CH_3-1'"), 2.62-2.61 (m, 4H, CH_2-2, CH_2-6); ^{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 $C_{14}H_{20}N_2O_2S$ (280.38): C, 59.97; H, 7.19; N, 9.99. Found: C 59.81; H, 7.28; N, 9.84.

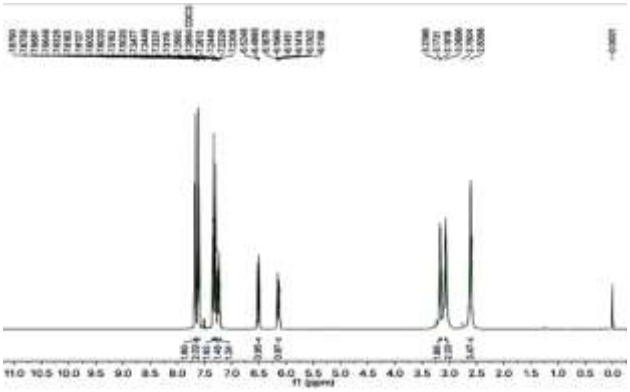


Fig. 1: ^{1}H-NMR spectrum of 3d.

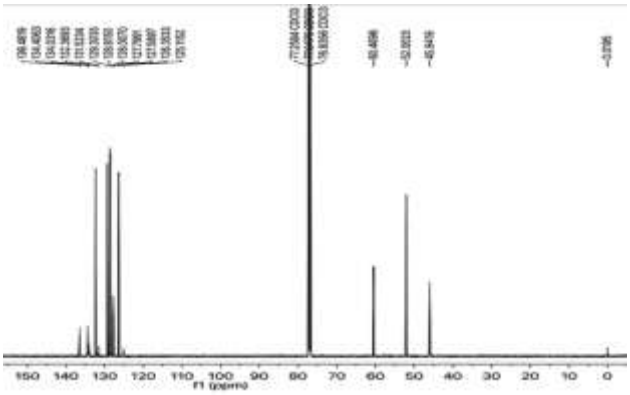


Fig. 2: ^{13}C-NMR spectrum of 3d molecule.

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.

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 formal, $C_{19}H_{21}BrN_2O_2S$. Various functionalities in this molecule were confirmed by its IR spectrum. The characteristic peaks appeared at n 3082 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1112 (C-N-C bond str) cm^{-1}. A typical A_2B_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_2-1'). The central 1,4-disubstitued piperazine core of this molecule was represented by two peaks at δ 3.07 (br.s, 4H, CH_2-2 & CH_2-6), and 2.61 (br.s, 4H, CH_2-3 & CH_2-5). 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 ($C_{19}H_{21}BrN_2O_2S$) 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

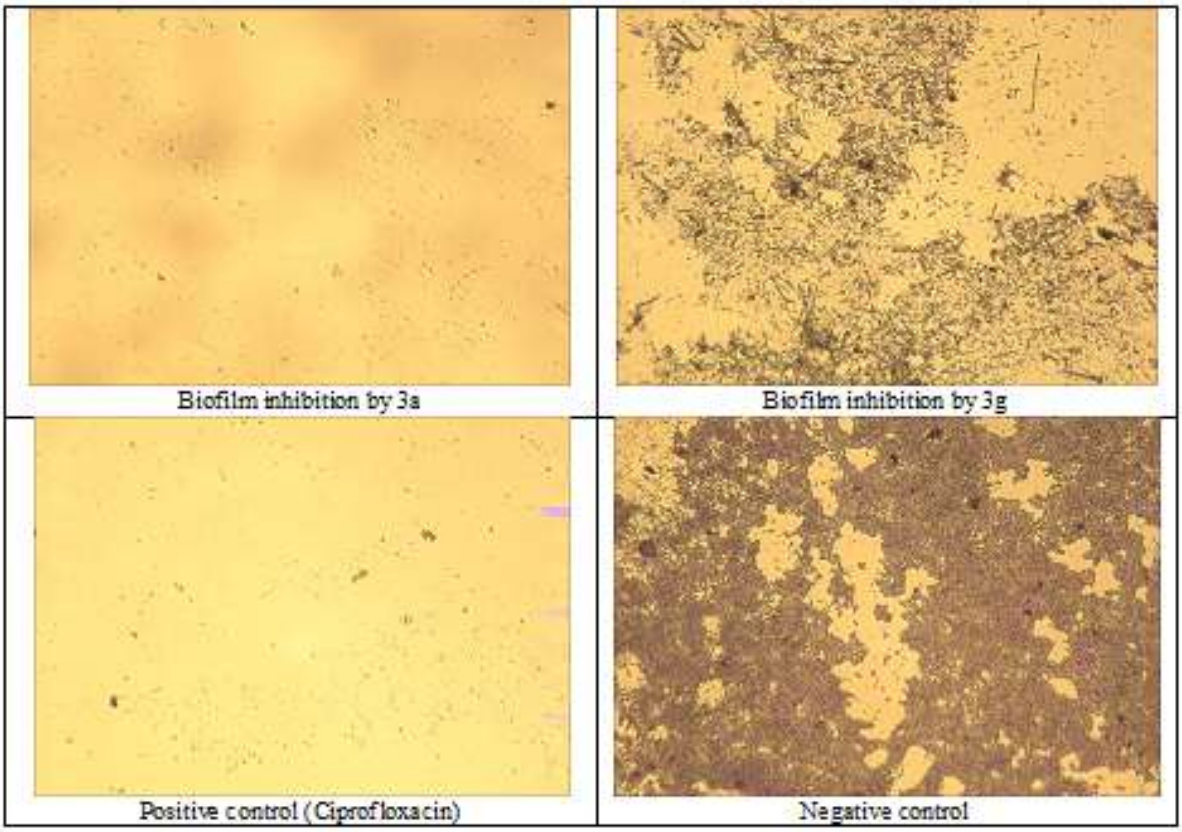


Fig. 3: Phase contrast microscopic view of inhibition of *Bacillus subtilis* biofilm.

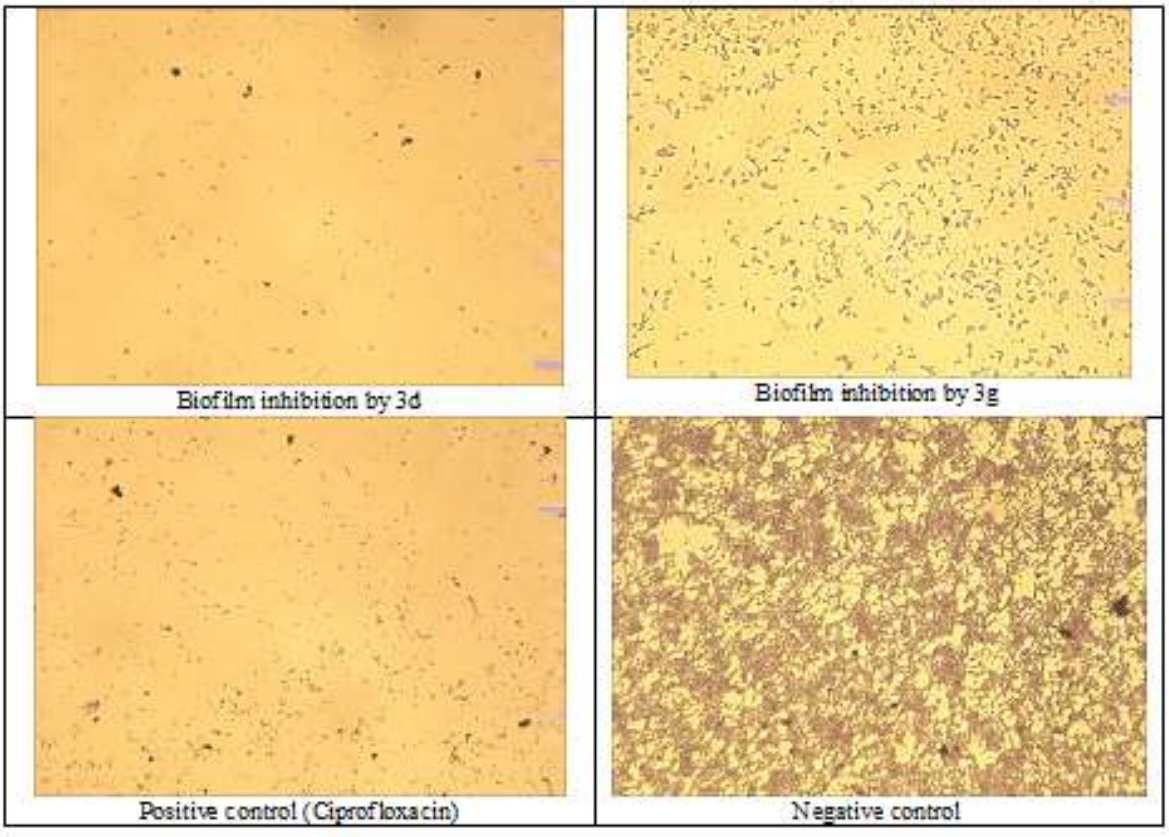


Fig. 4: Phase contrast microscopic view of inhibition of *Escherichia coli* biofilm.

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-substitued 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 ^{1}H-NMR and ^{13}C-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.

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