Synthesis of some new *N*-(alkyl/aralkyl)-*N*-(4-methoxyphenethyl) benzenesulfonamides as antibacterial agents against *Escherichia coli*

Muhammad Athar Abbasi[1*], Zareen Fatima[1], Aziz-ur-Rehman[1], Sabahat Zahra Siddiqui[1], Syed Adnan Ali Shah[2,3], Muhammad Shahid[4] and Hina Fatima[4]

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

Abstract: The present study comprises the synthesis of a new series of benzenesulfonamides derived from *N*-sulfonation of 2-(4-methoxyphenyl)-1-ethanamine (1). The synthesis was initiated by the reaction of 2-(4-methoxyphenyl)-1-ethanamine (1) with benzenesulfonyl chloride (2), to yield *N*-(4-methoxyphenethyl)benzenesulfonamide (3). This parent molecule 3 was subsequently treated with various alkyl/aralkyl halides (4a-j) in *N,N*-dimethylformamide (DMF) and in the presence of a weak base lithium hydride (LiH) to obtain various *N*-(alkyl/aralkyl)-*N*-(4-methoxyphenethyl) benzenesulfonamides (5a-j). The characterization of these derivatives was carried out by spectroscopic techniques like IR, ^{1}H-NMR, and ^{13}C-NMR. Elemental analysis also supported this data. The biofilm inhibitory action of all the synthesized compounds was carried out on *Escherichia coli* and some of the compounds were identified to be very suitable inhibitors of this bacterial strain. Furthermore, the molecules were also tested for their cytotoxicity behavior to assess their utility as less cytotoxic therapeutic agents.

Keywords: 2-(4-Methoxyphenyl)-1-ethanamine, benzenesulfonyl chloride, *Escherichia coli*, biofilm inhibition, toxicity.

INTRODUCTION

Sulfonamides have an important role in the field of medicine such as antimicrobial, antiviral, anticancer and anti-diabetic agents. These agents have wide history to control infection of diseases but with the routine use of these microbial agents, these may cause various diseases like hypersensitivity, drugs resistivity, anxiety, supra-infection and nutritional deficiencies (Alsughayer *et al*., 2011; Baskin & Wang, 2002; Supuran *et al*., 2003).

Biofilms are aggregates of microbes formed by the adhering microbial cells in polymeric substances. Mostly, biofilms are highly resistant to antibiotics and are therefore being keenly studied in quite a lot of scientific disciplines, together with evolutionary biology, drinking water treatment and biomedicines. The growth of a microbial biofilm is exercised by attachment of free-floating microorganisms to a surface. Later, these dialectical materialist microbes are entrenched frequently within a self-generated medium of extracellular polymeric chemicals (EPS) usually composed of extracellular DNA, proteins, and polysaccharides. These bacterial biofilms can cause several infection causing diseases and develop much resistance (Shahid *et al*., 2015).

The molecular structures of compounds are the main reason behind the varied toxicity of compounds. Similarly, by the structural modification, the antibacterial activity is also varied in different molecules (Abbasi *et al*. 2015). Polyfunctional compounds have gained much importance in drugs discovery program (Backes *et al*, 1996). Due to increasing resistance of infectious microbes day by day against the available antibacterial agents, the exploration of new drug candidates is necessity of the time. Therefore, in continuation of our work on sulfonamides (Abbasi *et al*, 2016a, Abbasi *et al*, 2016b), hereby we report the biofilm inhibition study of a new series of sulfonamides against *Escherichia coli* along with the evaluation of their cytotoxicity profile.

MATERIALS AND METHODS

General

Chemicals of Sigma Aldrich & Alfa Aesar (Germany) were utilized and solvents of analytical grades were supplied by local suppliers. Melting points were recorded by using open capillary tube method on Griffin and George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate and *n*-hexane (30:70) as mobile phase), preliminary purity of compounds was noted at 254nm. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. ^{1}H-NMR signals were noted at 600 MHz and ^{13}C-NMR at 150MHz in $CDCl_3$ using Bruker spectrometers.

Synthesis of N-(4-methoxyphenethyl) benzenesulfonamide (3)

2-(4-Methoxyphenyl)-1-ethanamine (1, 2g) was added in

**Corresponding author:* e-mail: atrabbasi@yahoo.com; abbasi@gcu.edu.pk

distilled water (5mL) at room temp. contained in a 25mL round bottom flask and stirred for 15min. The pH of the solution was maintained by the addition of aq. Na_2CO_3 soln. Then, equimolar benzenesulfonyl chloride (2) was addition into the reaction mixture and it was further stirred for 8-9hrs. The completion of the reaction was observed by TLC and after completion; the reaction mixture was quenched with ice cold water (200mL). The obtained solid was filtered, washed with distilled water, and dried to yield corresponding *N*-(4-methoxyphenethyl) benzenesulfonamide (3). ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.81 (dd, J =1.1, 8.2 Hz,, 2H, H-2' & H-6'), 7.57 (tt, J = 1.2, 7.4 Hz, 1H, H-4'), 7.49 (br.t, J = 8.5 Hz, 2H, H-3' & H-5'), 6.98 (d, J = 8.5 Hz, 2H, H-2 & H-6), 6.80 (d, J =8.6, 2H, H-3 & H-5), 3.78 (s, 3H, CH_3-9), 3.20 (br.t, J = 6.8 Hz, 2H, CH_2-8), 2.70 (br.t, J = 6.8 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): δ 158.54 (C-4), 140.00 (C-1'), 132.58 (C-4'), 129.68 (C-3' & C-5'), 129.45 (C-1), 129.08 (C-2 & C-6), 127.02 (C-2' & C-6'), 114.23 (C-3 & C-5), 55.27 (C-9), 44.37 (C-8), 34.88 (C-7). Anal. Calc. for $C_{15}H_{17}NO_3S$ (291.09): Calculated: C, 61.83; H, 5.88; N, 4.81. Found: C, 61.95; H, 5.97; N, 4.89.

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

A calculated amount (0.1mmol) of 3 was taken in a round bottomed flask (50mL), and it was dissolved in dimethyl formamide DMF (10.0mL). To this, lithium hydride (0.1mmol) was added as a base. This mixture was stirred for almost 30 minutes at room temperature for activation. At last, equimolar quantity of particular alkyl/aryl halide (4a-j) was added and this solution was kept stirring for three hours. The reaction's progress was checked with intervals until a single spot was evident on TLC. At completion of reaction, distilled water was added to carry out precipitation of the corresponding product in 5a-j. The product was filtered, washed with distilled water and utilized for further study.

Biological studies

Assessment of Biofilm Inhibition

The inhibition of bacterial (*Escherichia coli)* biofilm formation was assessed by the micro titer-plate method as described by Stepanovic *et al.* (2000). The wells of a sterile 24-well flat bottomed plastic tissue culture plate were filled with 100µL of nutrient broth (Oxoid, UK). Two concentrations, that is, 2.5 and 5.0 µg of testing samples (dissolved in 1mL of DMSO), were added in different wells. Finally, 20µL of bacterial suspension containing 1×10^9 CFU/mL was inoculated. Positive control well contained Ciprofloxacin and nutrient broth (Oxoid, UK) while negative control well contained nutrient broth and microbial strain. Afterwards, plates were covered and then incubated aerobically for 24 hours at 37^{o}C. Thereafter, the contents of each well were behed thrice with 220µL of sterile phosphate buffer (pH: 7.2). To remove all non-adherent bacteria, plates were vigorously shaken. Then, attached leftover bacteria were fixed with 220mL of 99% methanol per well. Next, after 15min, plates were emptied and left to dry. Then, plates were stained for 5min with 220mL of 50% crystal violet per well. Surplus stain was rinsed of using distilled water. Then plates were air-dried and the bound dye was resolubilized with 220µL of 33% (v/v) glacial acetic acid per well. The optical density (OD) of each well was measured at 630nm using microplate reader (Biotek, USA). All the tests were carried thrice against selected bacterial strains and the results were averaged. The bacterial growth inhibition (Inhibition %) was calculated using the following formula.

$$\text{Inhibition \%} = \frac{[(OD)]_{630\ \text{sample}}}{OD_{630\ \text{control}}} \times 100$$

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 30min 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:

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

Spectral characterization of synthesized compounds

N-(4-Methoxyphenethyl)-N-(2-propyl)benzenesulfonamide (5a)

White amorphous solid; yield: 81%; m.p.: 191-192^{o}C; Mol. formula: $C_{18}H_{23}NO_3S$; Mol. mass: 333 g/mol: IR (KBr, cm^{-1})υ_{max}: 3080 (C-H str. of aromatic ring), 2878 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1164 (C-O-C stretching ether), 1120 (C-N); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.80 (dd, J =1.5, 8.6 Hz,, 2H, H-2' & H-6'), 7.56 (tt, J = 1.7, 8.6 Hz, 1H, H-4'), 7.48 (br.t, J = 8.6 Hz, 2H, H-3' & H-5'), 6.98 (d, J = 8.5 Hz, 2H, H-2 & H-6), 6.80 (d, J =8.5, 2H, H-3 & H-5), 4.30 (m, 1H, H-2"), 3.78 (s, 3H, CH_3-9), 3.20 (br.t, J = 6.6 Hz, 2H, CH_2-8), 2.70 (br.t, J = 6.8 Hz, 2H, CH_2-7), 1.03 (d, J = 6.7 Hz, 6H, CH_3-1" & CH_3-3"); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): δ 158.57 (C-4), 140.02 (C-1'), 132.60 (C-4'), 129.70 (C-3' & C-5'), 129.44 (C-1), 129.10 (C-2 & C-6), 127.04 (C-2' & C-6'), 114.26 (C-3 & C-5), 55.30 (C-9), 53.20 (C-2"), 44.38 (C-8), 34.90 (C-7), 21.52 (C-1" & C-3"). Anal. Calc. for $C_{18}H_{23}NO_3S$ (333.14): Calculated: C, 64.84; H, 6.95; N, 4.20. Found: C, 64.91; H, 7.11; N, 4.27.

N-(1-Butyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5b)

White powder solid; yield: 83%; m.p.: 213-214^{o}C ; Mol. formula: $C_{19}H_{25}NO_3S$; Mol. mass: 347 g/mol: IR (KBr, cm^{-1})υ_{max}: 3070 (C-H str. of aromatic ring), 2855 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1164(C-O-C stretching of ether), 1125 (C-N); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.80 (dd, *J* =1.1, 8.5 Hz, 2H, H-2' & H-6'), 7.49 (tt, *J* = 1.8, 7.9 Hz, 1H, H-4'), 7.47 (br.t, *J* = 8.1 Hz, 2H, H-3' & H-5'), 7.04 (d, *J* = 8.5 Hz, 2H, H-2 & H-6), 6.81 (d, *J* = 8.5, 2H, H-3 & H-5), 3.77 (s, 3H, CH_3-9), 3.29 (br.t, *J* = 8.0 Hz, 2H, CH_2-8), 3.14 (t, *J* = 7.5 Hz, 2H, CH_2-1"), 2.78 (br.t, *J* = 8.2 Hz, 2H, CH_2-7), 1.48 (quint, *J* = 7.6 Hz, 2H, CH_2-2"), 1.28 (sext, *J* = 7.4 Hz, 2H, CH_2-3"), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3-4") ; ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.31 (C-4), 140.06 (C-1'), 132.28 (C-4'), 130.52 (C-1), 129.68 (C-3' & C-5'), 129.04 (C-2 & C-6), 127.04 (C-2' & C-6'), 114.01 (C-3 & C-5), 55.25 (C-9), 49.96 (C-8), 48.31 (C-1"), 34.86 (C-7), 30.64 (C-2"), 19.87 (C-3"), 13.69 (C-4"). Anal. Calc. for $C_{19}H_{25}NO_3S$ (347.16): Calculated: C, 65.68; H, 7.25; N, 4.03. Found: C, 65.86; H, 7.34; N, 4.17.

N-(1-Hexyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5c)

Dull white solid; yield: 87%; m.p.: 195-196 ^{o}C , Mol. formula: $C_{21}H_{29}NO_3S$; Mol. mass: 375 g/mol: IR (KBr, cm^{-1})υ_{max}: 3084 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1591 (C=C aromatic str.), 1165 (C-O-C stretching of ether), 1130 (C-N); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.80 (dd, *J* = 2.0,7.8 Hz,, 2H, H-2' & H-6'), 7.49 (tt, *J* = 1.2, 7.4 Hz, 1H, H-4'), 6.89 (br.t, *J* = 7.1 Hz, 2H, H-3' & H-5'), 7.08 (d, *J* =8.2 Hz, 2H, H-2 & H-6), 6.98 (d, *J* = 8.2, 2H, H-3 & H-5), 3.78 (s, 3H, CH_3-9), 3.29 (br.t, *J* = 7.8 Hz, 2H, CH_2-8), 3.14 (t, *J* = 7.6Hz, 2H, CH_2-1"), 2.78 (br.t, *J* = 8.0 Hz, 2H, CH_2-7), 1.49 (quint, *J* = 7.1 Hz, 2H, CH_2-2"), 1.29-1.20 (m, 6H, CH_2-3"- CH_2-5"), 0.87 (t, *J* = 7.0 Hz, 3H, CH_3-6"); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.50 (C-4), 140.07 (C-1'), 132.27 (C-4'), 130.53 (C-1), 129.70 (C-3' & C-5'), 129.07 (C-2 & C-6), 127.05 (C-2' & C-6'), 113.99 (C-3 & C-5), 55.27 (C-9), 49.96 (C-8), 48.59 (C-1"), 34.88 (C-2), 31.69 (C-4"), 28.88 (C-3"), 28.54 (C-2"), 22.54 (C-5"), 14.04 (C-6"). Anal. Calc. for $C_{21}H_{29}NO_3S$ (375.19): Calculated: C, 67.17; H, 7.78; N, 3.73. Found: C, 67.26; H, 7.84; N, 3.82.

N-(4-Methoxyphenethyl)-N-(1-octyl) benzenesulfonamide (5d)

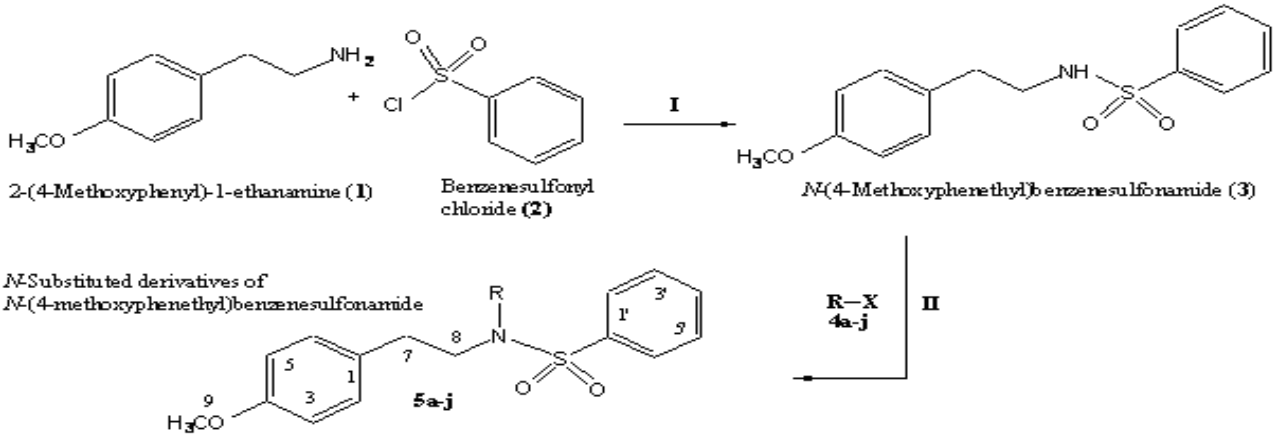
Light yellow solid; yield: 79%; m.p.: 157-158 ^{o}C , Mol. formula: $C_{23}H_{33}NO_3S$; Mol. mass: 403 g/mol: IR (KBr, cm^{-1})υ_{max}: 3080 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1592 (C=C aromatic str.), 1150 (C-O-C stretching of ether), 1125 (C-N); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.80 (dd, *J* = 1.1, 8.2 Hz, 2H, H-2' & H-6'), 7.54 (tt, *J* = 2.0, 8.1 Hz, 1H, H-4'), 7.47 (br.t, *J* = 8.5 Hz, 2H, H-3' & H-5'), 7.06 (d, *J* = 8.5, 2H, H-3 & H-5), 6.81 (d, *J* = 8.6 Hz, 2H, H-2 & H-6), 3.78 (s, 3H, CH_3-9), 3.30 (br.t, *J* = 8.0 Hz, 2H, CH_2-8), 3.13 (t, *J* = 7.6 Hz, 2H, CH_2-1"), 2.78 (br.t, *J* = 8.2 Hz, 2H, CH_2-7), 1.49 (quint., *J* = 7.2 Hz, 2H, CH_2-2"), 1.28 (br.s, 8H, CH_2-3" - CH_2-6"), 1.28 (sext., *J* = 6.8 Hz, 2H, CH_2-7"), 0.88 (t, *J* = 7.2 Hz, 3H, CH_3-8"); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.33 (C-4), 140.13 (C-1'), 132.27 (C-4'), 130.56 (C-1), 129.70 (C-3' & C-5'), 128.99 (C-2 & C-6), 127.07 (C-2' & C-6'), 114.02 (C-3 & C-5), 55.26 (C-9), 49.96 (C-8), 48.60 (C-1"), 34.90 (C-7), 31.76 (C-6"), 29.19 (C-4"), 29.16 (C-5"), 28.55 (C-3"), 26.70 (C-2"), 22.63 (C-7"), 14.07(C-8"). Anal. Calc. for $C_{22}H_{23}NO_3S$ (381.4): Calculated: C, 68.45; H, 8.24; N, 3.47. Found: C, 68.57; H, 8.31; N, 3.52.

N-(4-Methoxyphenethyl)-N-(3-phenylpropyl) benzenesulfonamide (5e)

White crystalline solid; yield: 77%; m.p.: 181-182 ^{o}C ; Mol. formula: $C_{24}H_{27}NO_3S$; Mol. mass: 409 g/mol: IR (KBr, cm^{-1})υ_{max}: 3079 (C-H str. of aromatic ring), 2890 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1170 (C-O-C stretching of ether), 1117 (C-N); ^{1}H-NMR (600 MHz $CDCl_3$, δ in ppm): δ 7.76 (dd, *J* =1.0, 8.1 Hz, 2H, H-2' & H-6'), 7.54 (tt, *J* = 1.2, 8.1 Hz, 1H, H-4'), 7.47 (br.t, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.27-7.26 (m, 2H, H-2" & H-6"), 7.21-7.19 (m, 3H, H-3", H-4" & H- 5"), 7.20 (d, *J* = 8.5 Hz, 2H, H-2 & H-6), 6.80 (d, *J* = 8.5 Hz, 2H, H-3 & H-5), 3.74 (s, 3H, CH_3-9), 3.38 (t, *J* = 6.5 Hz, 2H, CH_2-9"), 3.16 (br.t, *J* = 8.4 Hz, 2H, CH_2-8), 2.75 (t, *J* = 6.7 Hz, 2H, CH_2-7"), 2.58 (br.t, *J* = 7.9 Hz, 2H, CH_2-7), 2.16 (quint., *J* = 6.6 Hz, 2H, CH_2-8"); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.34 (C-4), 141.13 (C-1"), 139.92 (C-1'), 132.36 (C-4') 130.40 (C-1), 129.70 (C-3' & C-5'), 129.04 (C-2 & C-6), 128.45 (C-3" & C-5"), 128.33 (C-2" & C-6"), 127.08 (C-2' & C-6'), 126.04 (C-4"), 114.04 (C-3 & C-5), 55.26 (C-9), 50.19 (C-8), 48.14 (C-9"), 34.83 (C-7), 32.90 (C-7"), 30.11 (C-8"). Anal. Calc. for $C_{24}H_{27}NO_3S$ (409.17): Calculated: C, 70.39; H, 6.65; N, 3.42. Found: C, 70.47; H, 6.73; N, 3.55.

N-Benzyl-N-(4-methoxyphenethyl) benzenesulfonamide (5f)

Light brown solid; yield: 81%; m.p.: 187-188 ^{o}C; Mol. formula: $C_{22}H_{23}NO_3S$; Mol mass: 381 g/mol: IR (KBr, cm^{-1})υ_{max}: 3080 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1165(C-O-C stretching of ether), 1122 (C-N); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.84 (dd, *J* = 0.8, 8.5, 2H, H-2' & H-6'), 7.56 (tt, *J* = 0.9, 8.5 Hz, 1H, H-4'), 7.50 (br.t, *J* = 8.5 Hz, 2H, H-3' & H-5'), 7.32-7.28 (m, 5H, H-2"- H-6"), 6.84 (d, *J* = 8.6, 2H, H-2 & H-6), 6.74 (d, *J* = 8.6 Hz, 2H, H-3 & H-5), 4.34 (s, 2H, CH_2-7"), 3.74 (s, 3H, CH_3-9), 3.26 (br.t, *J* = 7.92 Hz, 2H, CH_2-8), 2.56 (br.t, *J* = 7.9 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.24 (C-4), 140.15 (C-1'), 136.16 (C-1"), 132.50 (C-4'), 130.43 (C-1), 129.60 (C-3' & C-5'), 129.14 (C-2 & C-6), 128.63 (C-3" & C-5"), 128.46 (C-2" & C-6"), 127.91 (C-4"),



Scheme 1: Outline for the synthesis of different *N*-Substituted derivatives (5a-j) of *N*-(4-methoxyphenethyl) benzenesulfonamide. Reagents & Conditions: (I) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 2-3 hrs. (II) DMF/LiH/stirring at RT for 0.5 hrs for activation/addition of R-X (4a-j) and then stirring for 4-5 hrs.

Table 1: Different alkyl/aralkyl substituents in 4a-j and 5a-j

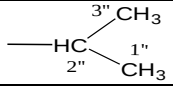
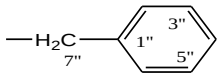
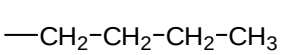
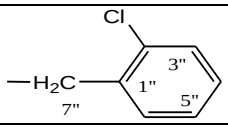
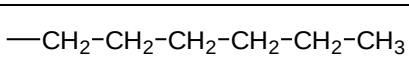
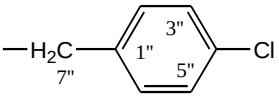
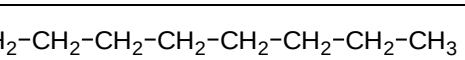
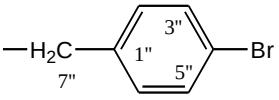
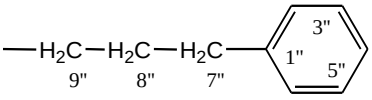
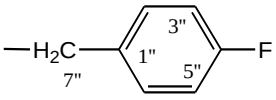
Compd.	-R	Compd.	-R
4a, 5a		4f, 5f	
4b, 5b		4g, 5f	
4c, 5c		4h, 5h	
4d, 5d		4i, 5i	
4e, 5e		4j, 5j	

Table 2: *Escherichia coli* biofilm inhibition and cytotoxicity of 5a-j

Sample	Sample Absorbance	Control Absorbance	% Inhibition	Cytotoxicity (%)
5a	0.626	0.821	23.75	1.54
5b	0.673	0.821	18.02	46.09
5c	0.652	0.821	20.58	2.92
5d	0.485	0.821	40.92	4.54
5e	0.739	0.821	9.987	1.64
5f	0.767	0.821	6.57	7.54
5g	0.81	0.821	1.33	4.75
5h	0.726	0.821	11.57	43.15
5i	0.712	0.821	13.27	6.32
5j	0.432	0.821	47.38	5.76
Ampicillin	0.219	0.821	73.32	-
Triton				97.82
PBS				1.08

Note: Ampicillicin was used as a positive control (antibacterial). Negative control (% Inhibition) = 73.32. PBS (% Hemolysis) = 1.08.

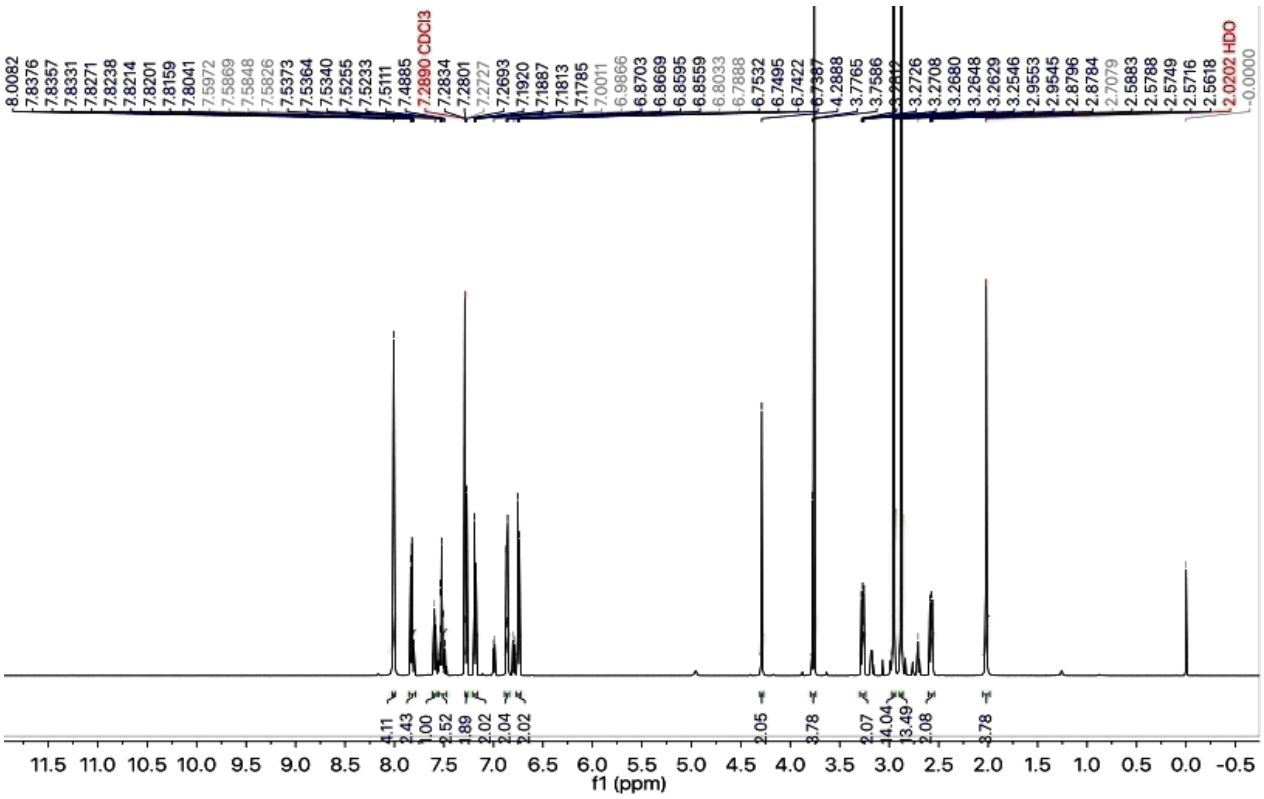


Fig. 1: ^{1}H-NMR spectrum of 5h.

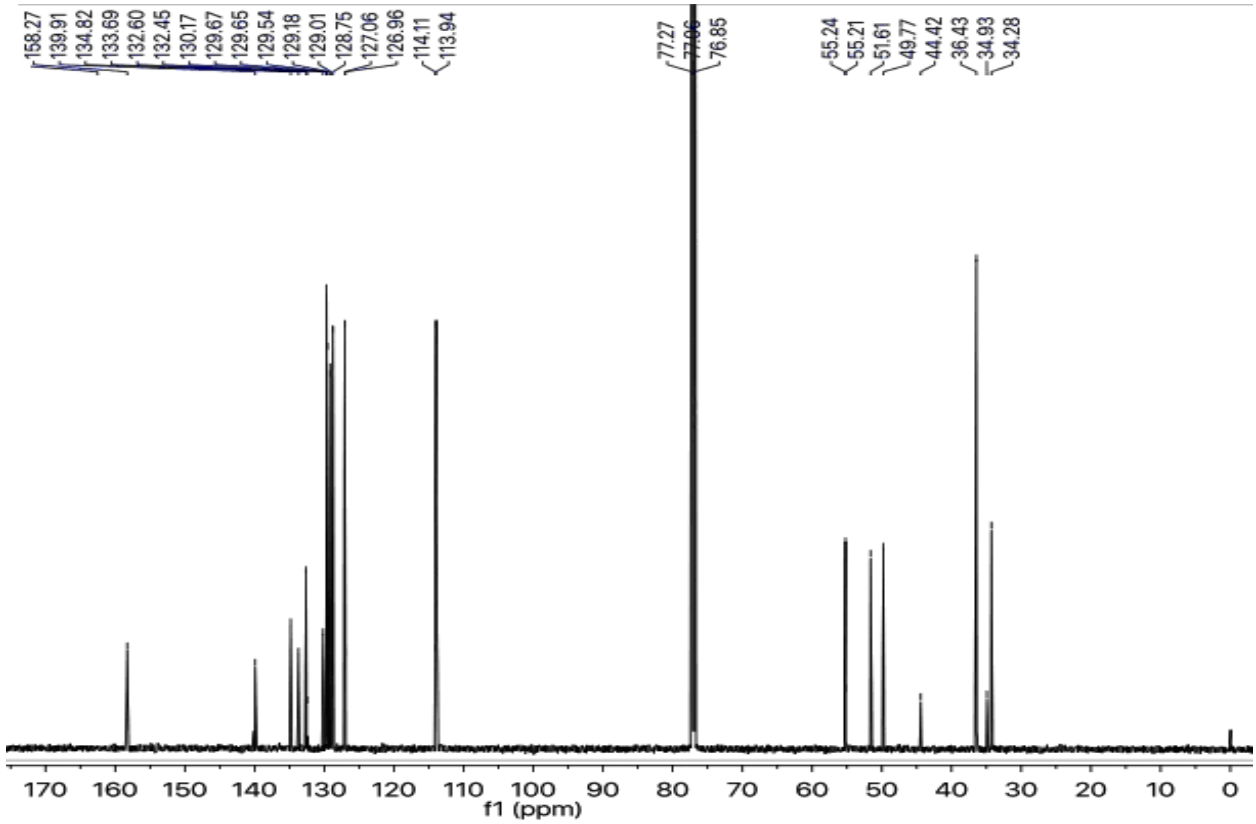


Fig. 2: ^{13}C-NMR spectrum of 5h.

127.11 (C-2' & C- 6'), 113.94 (C-3 & C-5), 55.23 (C-9), 52.23 (C-7"), 49.65 (C-8), 43.33 (C-7). Anal. Calc. for $C_{22}H_{23}NO_3S$ (381.14): Calculated: C, 69.26; H, 6.08; N, 3.67. Found: C, 69.39; H, 6.15; N, 3.74.

N-(2-Chlrobenzyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5g)

White solid; yield: 77%; m.p.: 163-164^{o}C; Mol. formula: $C_{22}H_{22}ClNO_3S$; Mol. mass: 415 g/mol): IR (KBr, cm^{-1})υ_{max}: 3090 (C-H str. of aromatic ring), 2868 (C-H str. of aliphatic), 1591 (C=C aromatic str.), 1166 (C-O-C stretching of ether), 1140 (C-N), 695 (C-Cl stretching); ^{1}H-NMR (600 MHz; $CDCl_3$, δ in ppm): δ 7.84 (dd, J = 1.6, 8.6 Hz, 2H, H-2' & H-6'), 7.58 (tt, J = 1.3, 7.4 Hz, 1H, H-4'), 7.51 (br.t, J = 7.3 Hz, 2H, H3' & H-5'), 7.34 (dd, J = 1.6, 7.7 Hz, 1H, H-3"), 7.26-7.23 (m, 2H, H-4" & H-5", merged in the signal of $CDCl_3$), 7.20 (dd, J = 1.9,7.4 Hz, 1H, H-6"), 6.91 (d, J = 8.6 Hz, 2H, H-2 & H-6), 6.74 (d, J = 8.6 Hz, 2H, H-3 & H-5), 4.52 (s, 2H, CH_2-7"), 3.74 (s, 3H, CH_3-9), 3.33 (br.t, J = 8.3 Hz, 2H, CH_2-8), 2.59 (br.t, J = 8.2 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.27 (C-4), 139.90 (C-1'), 134.11 (C-1"), 133.19 (C-2"), 132.61 (C-4'), 130.40 (C-6"), 130.14 (C-1), 129.63 (C-5'), 129.45 (C-3"), 129.19 (C-2' & C-6'), 129.01 (C-5"), 127.19 (C-4"), 127.13 (C-6'), 113.97 (C-3 & C-5), 55.24 (C-9), 50.66 (C-7"), 49.24 (C-8), 34.31 (C-7). Anal. Calc. for $C_{22}H_{22}ClNO_3S$ (415.10): Calculated: C, 63.53; H, 5.33; N, 3.37. Found: C, 63.61; H, 5.48; N, 3.45.

N-(4-Chlrobenzyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5h)

Dull white solid; yield: 84%; m.p: 151-152 ^{o}C; Mol. formula: $C_{22}H_{22}ClNO_3S$; Mol. mass: 415 g/mol: IR (KBr, cm^{-1})υ_{max}: 3085 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1165 (C-O-C stretching of ether), 1140 (C-N), 690 (C-Cl stretching); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): δ 7.83 (dd, J =1.9, 7.1 Hz, 2H, H-2' & H-6'), 7.58 (tt, J = 1.9, 7.4 Hz, 1H, H-4'), 7.53 (br.t, J = 7.1 Hz, 2H, H-3' & H-5'), 7.27 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.18 (d, J = 8.1 Hz, 2H, H-3" & H-5"), 6.86 (d, J = 8.6 Hz, 2H, H-2 & H-6), 6.74 (d, J =8.7 Hz, 2H, H-3 & H-5), 4.28 (s, 2H, CH_2-7"), 3.75 (s, 3H, CH_3-9), 3.27 (br.t, J = 7.9 Hz, 2H, CH_2-8), 2.57 (br.t, J = 8.0 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.27 (C-4), 139.91 (C-1'), 134.82 (C-1"), 133.69 (C-4") 132.60 (C-4'), 130.17 (C-1), 129.65 (C-3" & C-5"), 129.54 (C-3' & C-5'), 129.18 (C-2 & C-6), 128.75 (C-2" & C-6"), 127.06 (C-2' & C-6'), 113.94 (C-3 & C-5), 55.21 (C-9), 51.61 (C-7"), 49.77 (C-8), 34.28 (C-7). Anal. Calc. for $C_{22}H_{22}ClNO_3S$ (415.10): Calculated: C, 63.53; H, 5.33; N, 3.37. Found: C, 63.65; H, 5.42; N, 3.44.

N-(4-Bromobenzyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5i)

White creamy powder; yield: 89%; m.p.: 213-214^{o}C; Mol. Formula: $C_{22}H_{22}BrNO_3S$; Mol. mass: 459 g/mol: IR (KBr, cm^{-1})υ_{max}: 3080 (C-H str. of aromatic ring), 2882 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1160(C-O-C stretching of ether), 1122 (C-N), 540 (C-Br stretching); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ in ppm): δ 7.83 (dd, J =1.1, 7.2 Hz, 2H, H-2' & H-6'), 7.58 (tt, J = 1.2, 7.5 Hz, 1H, H-4'), 7.52 (br.t, J = 7.9 Hz, 2H, H-3' & H-5'), 7.24 (d, J = 9.0, 2H, H-3" & H-5"), 7.12 (d, J = 8.3 Hz, 2H, H-2" & H-6"), 6.86 (d, J = 8.5, 2H, H-2 & H-6), 6.74 (d, J = 8.5 Hz, 2H, H-3 & H-5), 4.26 (s, 2H, CH_2-7"), 3.76 (s, 3H, CH_3-9), 3.26 (br.t, J = 8.0 Hz, 2H, CH_2-8), 2.57 (br.t, J = 7.9 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 158.29 (C-4), 139.91 (C-1'), 135.34 (C-1"), 132.62 (C-4'), 131.75 (C-3" & C-5"), 130.18 (C-1), 130.00 (C-2" & C-6"), 129.58 (C-3' & C-5'), 129.20 (C-2 & C-6), 127.11 (C-2' & C-6'), 121.85 (C-4"), 55.25 (C-9), 51.70 (C-7"), 49.81 (C-8), 34.22(C-7). Anal. Calc. for $C_{22}H_{22}BrNO_3S$ (459.05): Calculated: C, 57.39; H, 4. 28; N, 3.04. Found: C, 57.45; H, 4.37; N, 3.15.

N-(4-Fluorobenzyl)-N-(4-methoxyphenethyl) benzenesulfonamide (5j)

White solid; yield: 83%; m.p.: 188-189 ^{o}C; Mol. formula: $C_{22}H_{22}FNO_3S$; Mol. mass: 399 g/mol: IR (KBr, cm^{-1})υ_{max}: 3085 (C-H str. of aromatic ring), 2875 (C-H str. of aliphatic), 1590 (C=C aromatic str.), 1164 (C-O-C stretching of ether), 1120 (C-N), 1195 (C-F stretching); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ in ppm): δ 7.83 (dd, J = 1.5, 8.5, 2H, H-2' & H-6'), 7.58 (tt, J = 1.2, 7.3 Hz, 1H, H-4'), 7.51 (br.t, J = 7.3 Hz, 2H, H-3' & H-5'), 7.22 (dd, J = 5.3, 8.5 Hz, 2H, H-2" & H-6", multiplicity due to coupling with F_{19}), 6.99 (br.t, J = 8.6, 2H, H-3" & H-5", due to coupling of F_{19}), 6.85 (d, J = 8.6 Hz, 2H, H-2 & H-6), 6.74 (d, J = 8.6 Hz, 2H, H-3 & H-5), 4.29 (s, 2H, CH_2-7"), 3.75 (s, 3H, CH_3-9), 3.25 (br.t, J = 7.9 Hz, 2H, CH_2-8), 2.56 (br.t, J = 8.1 Hz, 2H, CH_2-7); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ in ppm): 162.51 (C-4"), 158.27 (C-4), 140.00 (C-1'), 132.56 (C-4'), 131.98 (C-1") 130.26 (C-1), 130.09 & 130.04 (C-2" & C-6", due to effect of F_{19}),129.55 (C-3' & C-5'), 129.16 (C-2 & C-6), 127.07 (C-2' & C-6'), 115.57 & 115.43 (C-3" & C-5", due to effect of F_{19}), 113.95 (C-3 & C-5), 55.21 (C-9), 51.56 (C-7"), 49.67 (C-8), 34.33 (C-7). Anal. Calc. for $C_{22}H_{22}FNO_3S$ (399.13): Calculated: C, 66.14; H, 5.55; N, 3.51. Found: C, 66.25; H, 5.63; N, 3.65.

RESULTS

In the presented work, ten derivatives, 5a-j, were synthesized starting with 2-(4-methoxyphenyl)-1-ethanamine (1) according to the outline illustrated in Scheme 1 and table 1. The synthesis was carried out by reacting 2-(4-methoxyphenyl)-1-ethanamine (1) with benzenesulfonyl chlorides (2) in distilled water and in the presence of sodium carbonate to obtain *N*-(4-methoxyphenethyl) benzene sulfonamide (3). Then, in the second step, this parent 3 was treated with different alkyl/aryl (4a-j) in the presence of DMF and LiH as

activator to achieve the targeted sulfonamides, 5a-j. The structures of the synthesized molecules were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectral techniques. The corresponding 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 biofilm inhibition study against one bacterial strain i.e. *Escherichia coli* and these results are tabulated in table 2. Their cytoxicity profile was also studied through hemolytic activity and these results are also shown in table 2.

DISCUSSION

The synthesis of targeted *N*-sulfonated derivatives (5a-j) was accomplished by a facile strategy (Abbasi *et al*., 2016a) and all the compounds were obtained in good yields. The synthesized compounds were structurally confirmed through spectral data of IR, ^{1}H-NMR, ^{13}C-NMR and by elemental analysis. One of the compounds is discussed hereby in detail for the expediency of the readers. The molecule 5h, was obtained as a dull white solid. The molecular formula of this compound was established 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, $C_{22}H_{22}ClNO_3S$. Various functionalities in this molecule were affirmed by its IR spectrum. The absorption band at 1368 cm^{-1} in its IR spectrum was characteristic for -SO_2 stretching in this molecule while other functional groups were assigned on the basis of observed peaks at 3042 cm^{-1} (C-H Ar), 1648 cm^{-1} (C=C Ar), 1137 cm^{-1} (C-O-C stretching of ether), and 690 (C-Cl stretching). The ^{1}H-NMR spectrum of this molecule demonstrated overall eleven resonances. The benzenesulfonyl moiety was characterized by three typical signals in aromatic region at δ 7.83 (dd, J =1.9, 7.1 Hz, 2H, H-2' & H-6'), 7.58 (tt, J = 1.9, 7.4 Hz, 1H, H-4'), and 7.53 (br.t, J = 7.1 Hz, 2H, H-3' & H-5'). The substitution of 4-chlorobenzyl moiety was also identified by three signals, two *ortho*-coupled doublets at δ 7.27 (d, J =8.4 Hz, 2H, H-2"&H-4") and 7.18 (d, J =8.1, 2H, H-1"& H-5"), along with a benzylic methylene singlet at δ 4.28 (s, 2H, CH_2-7"). The 4-methoxyphenethyl unit in this molecule was ascribed by an A_2B_2 spin system represented by two doublets at δ 6.86 (d, J = 8.6 Hz, 2H, H-2 & H-6), 6.74 (d, J = 8.7 Hz, 2H, H-3 & H-5), a singlet of methoxyl group at δ 3.75 and two triplets of adjacent methylene groups at δ 3.27 (br.t, J = 7.9 Hz, 2H, CH_2-8) and 2.57 (br.t, J = 8.0 Hz, 2H, CH_2-7). The ^{13}C-NMR spectrum demonstrated overall sixteen 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, $C_{22}H_{22}ClNO_3S$, of the compound. The benzensulfonyl moiety was signified by four signals δ 139.91(C-1'), 132.60 (C-4'), 129.54 (C-3'& C-5'), and 127.06 (C-2' & C-6'). The substituted 4-chlorobenzyl moiety was represented by five resonances at δ 134.82 (C-1"), 133.69 (C-4"), 129.65 (C-3" & C-5"), 128.75(C-2"& C-6"), and 51.61 (C-7"). The 4-methoxyphenethyl unit, integral part of the starting amine (**1**), was clearly demonstrated by seven resonances in the spectrum at δ 158.27 (C-4), 130.17 (C-1), 129.18 (C-2 & C-6), 113.94 (C-3 & C-5), 55.21 (C-9), 49.77 (C-8), and 34.28 (C-7). The ^{1}H-NMR and ^{13}C-NMR spectra of this molecule are shown in fig. 1 and fig. 2, respectively. So, on the basis of afore mentioned collective evidences, the structure of 5h was confirmed *N*-(4-Chlrobenzyl)-*N*-(4-methoxy phenethyl) benzenesulfonamide. A similar spectral analysis was exercised for the structural characterization of other derivatives in the synthesized series.

Biofilm inhibition and structure-activity relationship

The antibacterial activity of derivatives, 5a-j, was checked by biofilm inhibition method using a gram negative bacterial pathogenic strain i.e. *Escherichia coli*. Ampicillin was used as a standard drug in this assay to compare the antibacterial potential of the synthesized molecules. From the results (table 2), it was apparent that the presence of different groups at nitrogen atom in 5a-j molecules, was rendering an increase or decrease in the antibacterial potential of the respective compound. Here, it was observed that among the series, maximum inhibition (47.38%) was given by 5j against *Escherichia coli* and it can be attributed to the presence of an inductively electron withdrawing fluoro group at C-4" position in this molecule. The percentage inhibition of the standard Ampicillicin was noted as 73.32%. The other compounds 5a, 5c and 5d also showed moderate biofilm inhibition against this strain with percentage of 40.92%, 23.75% and 20.58%, respectively.

Hemolytic activity

All the synthesized compounds, 5a-j, were also subjected to hemolytic assay to find out their cytotoxicity profile. Results of percentage hemolysis are shown in table 2. Our results showed that all compounds of this series have moderate toxicity towards red blood cell membrane. High membrane toxicity was shown by the compound 5b (46.09%) and 5h (43.15%) while very low toxicity was recorded in case of 5a (1.54%) and 5e (1.64%). A moderate toxicity was observed for molecule 5c (2.92%), 5d (4.54%), 5f (7.54%), 5g (4.75%), 5i (6.32%), 5j (5.76%) relative to Triton-X having % hemolysis of 97.82%.

CONCLUSION

On the basis of biofilm inhibition study, it was concluded that out of the synthesized molecules, 5j exhibited relatively good antibacterial potential against *Escherichia* coli and it also possessed a moderate toxicity, so this

molecule can be utilized further as a safe therapeutic agent in drug designing.

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