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N-Sulfonated derivatives of (2-furoyl)piperazine: Promising antibacterial agents with mild cytotoxicity

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Abstract: 2-Furoyl-1-piperazine (1) was treated with a series of alkyl/aryl sulfonyl chlorides (2a-i) under benignant conditions to obtain its *N*-sulfonated derivatives (3a-i). These compounds were screened for their antibacterial potential against pathogenic bacteria. The low Minimum Inhibitory Concentration (MIC) values of these molecules, in comparison of ciprofloxacin, demonstrated their high antibacterial potential. Cytotoxic activities were ascertained through their hemolytic potential and mild hemolytic profiles of these compounds proved them to be promising compounds for drug designing and development.

Keywords: Piperazine, furan, sulfonamides, antibacterial, hemolytic activity.

INTRODUCTION

The discovery of new drug molecules through natural or synthetic routes is ongoing research from a number of years. Similarly, some biologically active compounds have been modified to synthesize new compounds consisting of active pharmacophores (Mohs and Greig, 2017). Organic chemists have employed piperazine (1,4-diazinane) to synthesize different derivatives with more bioactive potential. The applications of piperazine in medicinal chemistry include the synthesis of antimicrobial (Jalager *et al.,* 2019; Tang *et al.,* 2020) and anti-viral agents (Dou *et al.,* 2012). The piperazine derivatives also have vast applications in the fields of engineering (Bruder *et al.,* 2011) and polymer (Yamaguchi *et al.,* 2010).

Sulfonamides bear sulfonyl group (-$SO_2N<$) (Ordonez *et al.*, 2011). Sulfonamides are good antibacterial agents because the sulfonyl functionality perturbs folic acid synthesis and thus reduces the bacterial growth (El-Sayed *et al.*, 2011; Ozbek *et al.,* 2019; Saeedi *et al.,* 2014). Sulfonamide compounds have been used as preventive agents in chemotherapy against various diseases. These are also reported to possess antifungal, anti-inflammatory, antioxidant and enzyme inhibitory activities (Thach *et al*., 2021; Abbasi *et al*., 2020[a]; El-Sayed *et al*., 2011; Hassan *et al*., 2021; Zoumpoulakis *et al*., 2012). Moreover, these are broadly used as anticancer (Mun *et al.,* 2012), antiviral agents as well as effective inhibitor against HIV protease (Clercq *et al.,* 2001). Sulfonamides have been found as biologically active as they have shown significant cytotoxic effects against breast cancer cells (Mirian *et al.,* 2011). A list of infections including livestock herbs, urinary tract and gastrointestinal ones was also treated by sulfonamides (Root *et al.*, 2021; Apaydin *el al.,* 2019). So, the immense therapeutic potential of sulfonamides prompted us to synthesize some new molecules bearing piperazine functionality and to explore their antibacterial potentials and hemolytic behaviors.

MATERIALS AND METHODS

All chemicals and reagents required for experimental work were bought from Alfa Aesar and sigma Aldrich. Analytical grade solvents were provided by local suppliers. Griffin and George apparatus were used to collect melting point data by using open capillary method. Thin layer chromatography plates were utilized for determination of initial purity of compounds at 254 nm. Gradient solvent system used was comprised of ethyl acetate and n-hexane in 30:70 and played a role as mobile phase. IR spectra were recorded by KBr pellet method on a Jasco-320-A spectrometer. Bruker spectrometers were used for recording ^{1}H-NMR signals at 400 and 600 MHz. $CDCl_3$ was solvent used for this purpose. For the purpose of recording ^{13}C-NMR signals at 150 MHz in $CDCl_3$ Broker spectrometer was used.

Synthesis of 4-(alkyl/aryl sulfonyl)-1-(2-furoyl)piperazine (3a-i)

Suspension of 2-furoyl-I-piperazine (22.0 mmol; 1) was prepared by dispersing it in 20.0 mL of distilled water, taken in a 100 ml round bottom flask. 10% Na_2CO_3 solution was used to adjust the pH of suspension towards alkalinity in range 9-10. Then different alkyl/aryl sulfonyl chlorides (22.0 mmol; 2a-i) were gradually added in small patches, one in each reaction. Reaction mixture was

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stirred for 3 to 4 hours. Progress of the reaction was monitored by TLC. Mixture was allowed to stand for 60 minutes after adding ice cold distilled water in excess, thereby, precipitates were formed in good yield. After filtration, the obtained precipitates were washed with distilled water and then air dried to acquire title compounds in purified form.

Physical and Spectral Characterization

4-(Methylsulfonyl)-1-(2-furoyl)piperazine (3a)

White amorphous solid; Yield: 87%; m.p.: 94-96°C; Mol. For.: $C_{10}H_{14}N_2O_4S$; Mol. Mass.: 258 g/mol; IR (KBr, ν, cm^{-1}): 3027 (Ar C-H), 2836 (R C-H), 1603 (C=O), 1584, 1453 (C=C), 1323 (S=O), 1156 (C-O-C), 1078 (C-N-C), 655 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.43 (dist. d, *J* = 0.9 Hz, 1H, H-5), 7.02 (d, *J* = 2.6 Hz, 1H, H-3), 6.45 (dd, *J* = 1.8, 3.4 Hz, 1H, H-4), 3.87 (br.s, 4H, CH_2-3' & CH_2-5'), 3.24 (br.t, *J* = 5.1 Hz, 4H, CH_2-2' & CH_2-6'), 2.73 (s, 3H, CH_3-1"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.1 (C-6), 147.4 (C-2), 144.0 (C-5), 117.5 (C-4), 111.6 (C-3), 45.9 (C-2', C-3', C-5', C-6'), 34.7 (C-1", merged owing to ring flipping).

4-(2-Naphthylsulfonyl)-1-(2-furoyl)piperazine (3b)

White amorphous solid; Yield: 90%; m.p.: 91-93°C; Mol. For.: $C_{19}H_{18}N_2O_4S$; Mol. Mass.: 370 g/mol; IR (KBr, ν, cm^{-1}): 3038 (Ar C-H), 2835 (R C-H), 1612 (C=O), 1587 (C=C), 1333 (S=O), 1198 (C-O-C), 1161 (C-N-C), 657 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 8.35 (br.s, 1H, H-1"), 8.01-7.98 (m, 1H, H-8"), 7.95 (br.d, *J* = 8.1 Hz, 1H, H-5"), 7.70-7.64 (m, 4H, H-3", H-4", H-6", H-7"), 7.44 (dist. d, *J* = 1.6 Hz, 1H, H-5), 6.99 (d, *J* = 3.4 Hz, 1H, H-3), 6.46 (dd, *J* = 1.8, 3.4 Hz, 1H, H-4), 3.92 (br.s, 4H, CH_2-3' & CH_2-5'), 3.18 (dist. t, *J* = 5.1 Hz, 4H, CH_2-2' & CH_2-6'); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.8 (C-6), 147.4 (C-2), 143.8 (C-5), 135.0 (C-6"), 132.4 (C-1", C-9", merged), 132.2 (C-4"), 129.4 (C-5"), 129.2 (C-3", C-7"), 129.1 (C-10"), 127.9 (C-8"), 127.7 (C-2"), 117.3 (C-4), 111.4 (C-3), 46.2 (C-2', C-3', C-5', C-6', merged owing to ring flipping).

4-(Phenylsulfonyl)-1-(2-furoyl)piperazine (3c)

White amorphous solid; Yield: 85%; m.p.: 96-98°C; Mol. For.: $C_{15}H_{16}N_2O_4S$; Mol. Mass.: 320 g/mol; IR (KBr, *ν*, cm^{-1}): 3039 (Ar C-H), 2841 (R C-H), 1618 (C=O), 1588 (C=C), 1337 (S=O), 1159 (C-O-C), 1088 (C-N-C), 658 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.78 (d, *J* = 7.8 Hz, 2H, H-2", H-6"), 7.66 (t, *J* = 7.5 Hz, 1H, H-4"), 7.59 (t, *J* = 7.6 Hz, 2H, H-3", H-5"), 7.47 (br.s, 1H, H-5), 7.03 (d, *J* = 3.4 Hz, 1H, H-4), 6.49 (dd, *J* = 3.7, 2.1 Hz, 1H, H-3), 3.92 (br.s, 4H, CH_2-3', CH_2-5'), 3.12 (t, *J* = 5.0 Hz, 4H, CH_2-2', CH_2-6'); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.9 (C-6), 147.4 (C-2), 143.9 (C-5), 135.3 (C-1"), 133.2 (C-4"), 129.2 (C-3", C-5"), 127.7 (C-2", C-6"), 117.7 (C-4), 111.5 (C-3), 46.2 (C-2', C-3', C-5', C-6', merged owing to ring flipping).

4-(4-Acetamidophenylsulfonyl)-1-(2-furoyl)piperazine (3d)

White amorphous solid; Yield: 82%; m.p.: 92-94°C; Mol. For.: $C_{17}H_{19}N_3O_5S$; Mol. Mass.: 377 g/mol; IR (KBr, ν, cm^{-1}): 3038 (Ar C-H), 2839 (R C-H), 1652, 1606 (C=O), 1590 (C=C), 1389 (S=O), 1163 (C-O-C), 1175 (C-N-C), 656 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.93 (br.s, 1H, N-H), 7.70 (br.d, *J*=8.6 Hz, 2H, H-3", H-5"), 7.66 (t, *J*=6.5 Hz, 2H, H-2", H-6"), 7.45 (br. d, *J*=0.9 Hz, 1H, H-5), 6.99 (br. d, *J*=2.5 Hz, 1H, H-3), 6.46 (dd, *J*= 3.4, 1.7 Hz, 1H, H-4), 3.88 (br.s, 4H, CH_2-3', CH_2-5'), 3.06 (t, *J*=5.0 Hz, 4H, CH_2-2', CH_2-6'), 2.19 (s, 3H, CH_3-8"); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 168.8 (C-7"), 158.9 (C-6), 147.2 (C-2), 144.0 (C-4"), 142.6 (C-5), 129.6 (C-1"), 128.9 (C-2", C-6"), 119.3 (C-3", C-5"), 117.4 (C-4), 111.5 (C-3), 46.2 (C-2', C-3', C-5', C-6', merged owing to ring flipping), 24.6 (C-8").

4-(4-Methylphenylsulfonyl)-1-(2-furoyl)piperazine (3e)

White amorphous solid; Yield: 80%; m.p.: 90-92°C; Mol. For.: $C_{16}H_{18}N_2O_4S$; Mol. Mass.: 334 g/mol; IR (KBr, *ν*, cm^{-1}): 3046 (Ar C-H), 2838 (R C-H), 1658 (C=O), 1581 (C=C), 1335 (S=O), 1157 (C-O-C), 1092 (C-N-C), 661 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.62 (d, *J*= 6.4 Hz, 2H, H-2", H-6"), 7.44 (br. d, *J* = 0.9 Hz, 1H, H-5), 7.33 (d, *J* = 8.0 Hz, 2H, H-3", H-5"), 6.99 (dist.d, *J* = 2.6 Hz, 1H, H-3), 6.45 (dd, *J* = 3.4, 1.8 Hz, 1H, H-4), 3.88 (br.s, 4H, CH_2-3', CH_2-5'), 3.06 (t, *J* = 5.1 Hz, 4H, CH_2-2', CH_2-6'), 2.42 (s, 3H, CH_3-7"); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.8 (C-6), 147.4 (C-2), 144.1 (C-5), 143.9 (C-1"), 132.2 (C-4"), 129.8 (C-3", C-5"), 127.7 (C-2", C-6"), 117.3 (C-4), 111.5 (C-3), 46.2 (C-2', C-3', C-5', C-6', merged owing to ring flipping), 21.5 (C-7").

4-(4-Bromophenylsulfonyl)-1-(2-furoyl)piperazine (3f)

White amorphous solid; Yield: 88%; m.p.: 97-99°C; Mol. For.: $C_{15}H_{15}BrN_2O_4S$; Mol. Mass.: 398 g/mol; IR (KBr, *ν*, cm^{-1}): 3038 (Ar C-H), 2838 (R C-H), 1614 (C=O), 1587 (C=C), 1395 (S=O), 1162 (C-O-C), 1084 (C-N-C), 653 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.72 (d, *J*= 6.6 Hz, 2H, H-2", H-6"), 7.64 (d, *J*=6.6 Hz, 2H, H-3", H-5"), 7.48 (dist.d, *J*=0.9 Hz, 1H, H-5), 7.05 (dist.d, *J* = 2.5 Hz, 1H, H-3), 6.50 (dd, *J*=3.4, 1.7 Hz, 1H, H-4), 3.93 (br. s, 4H, CH_2-3', CH_2-5'), 3.11 (t, *J* 5.1 Hz, 4H, CH_2-2', CH_2-6'); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.8 (C-6), 147.3 (C-2), 143.9 (C-5), 134.4 (C-1"), 132.4 (C-3", C-5"), 129.1 (C-2", C-6"), 128.4 (C-4"), 117.5 (C-4), 111.5 (C-3), 46.1 (C-2', C-3', C-5', C-6', merged owing to ring flipping).

4-(4-Acetylphenylsulfonyl)-1-(2-furoyl)piperazine (3g)

White amorphous solid; Yield: 86%; m.p.: 88-90 °C; Mol. For.: $C_{17}H_{18}N_2O_5S$; Mol. Mass: 362 g/mol; IR (KBr, *ν*, cm^{-1}): 3035 (Ar C-H), 2839 (R C-H), 1709, 1618 (C=O), 1587 (C=C), 1331 (S=O), 1159 (C-O-C), 1095 (C-N-C), 658 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.88 (d, *J* = 7.1 Hz, 2H, H-2", H-6"), 7.82 (d, *J* = 6.6 Hz, 2H, H-

3", H-5"), 7.49-7.47 (m, 1H, H-5), 7.05-7.03 (m, 1H, H-3), 6.50-6.48 (m, 1H, H-4), 3.94 (br. s, 4H, CH_2-3', CH_2-5'), 3.14 (t, J = 5.0 Hz, 4H, CH_2-2', CH_2-6'), 2.65 (s, 3H, CH_3-8"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 189.7 (C-7"), 158.9 (C-6), 147.3 (C-2), 144.0 (C-5), 142.5 (C-1"), 138.9 (C-4"), 135.8 (C-3", C-5"), 128.9 (C-2", C-6"), 117.5 (C-4), 111.6 (C-3), 46.2 (C-2', C-3', C-5', C-6', merged owing to ring flipping), 19.0 (C-8").

4-(2,4,6-Trimethylphenylsulfonyl)-1-(2-furoyl) piperazine (3h)

White amorphous solid; Yield: 84%; m.p.: 94-96^{o}C; Mol. For.: $C_{18}H_{22}N_2O_4S$; Mol. Mass: 362 g/mol; IR (KBr, v, cm^{-1}): 3088 (Ar C-H), 2846 (R C-H), 1619 (C=O), 1589 (C=C), 1329 (S=O), 1159 (C-O-C), 1081 (C-N-C), 657 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.49 (dist.d, J=0.9 Hz, 1H, H-5), 7.06 (dist.d, J=3.4 Hz, 1H, H-3), 6.99 (s, 2H, H-3" & H-5"), 6.51 (dd, J=1.8, 3.4 Hz, 1H, H-4), 3.86 (br.s, 4H, CH_2-3' & CH_2-5'), 3.27 (br.t, J 5.2 Hz, 4H, CH_2-2' & CH_2-6'), 2.65 (s, 6H, CH_3-7" & CH_3-9"), 2.65 (s, 3H, CH_3-8"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.0 (C-6), 147.5 (C-2), 143.9 (C-1"), 143.0 (C-5), 140.5 (C-2" & C-6"), 132.1 (C-4"), 130.9 (C-3" & C-5"), 117.2 (C-4), 111.5 (C-3), 44.5 (C-2', C-3', C-5', C-6', merged owing to ring flipping), 22.9 (C-7", C-8", C-9", merged).

4-(4-Methoxyphenylsulfonyl)-1-(2-furoyl)piperazine (3i)

White amorphous solid; Yield: 88%; m.p.: 90-92^{o}C; Mol. For.: $C_{16}H_{18}N_2O_5S$; Mol. Mass: 350 g/mol; IR (KBr, v, cm^{-1}): 3029 (Ar C-H), 2838 (R C-H), 1617 (C=O), 1581 (C=C), 1328 (S=O), 1159 (C-O-C), 1098 (C-N-C), 663 (C-S); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.71-7.69 (m, 4H, H-2", H-3", H-5", H-6"), 7.47 (dist.d, J=0.9 Hz, 1H, H-5), 7.03-7.01 (m, 1H, H-3), 6.49 (dd, J=3.4, 1.8 Hz, 1H, H-4), 3.91 (br.s, 4H, CH_2-3', CH_2-5'), 3.89 (m, 3H, CH_3-7"), 3.08 (t, J=5.0 Hz, 4H, CH_2-2', CH_2-6'); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 163.3 (C-4"), 158.9 (C-6), 147.4 (C-2), 143.9 (C-5), 129.8 (C-1"), 126.7 (C-2", C-6"), 117.3 (C-4), 114.4 (C-3", C-5"), 111.5 (C-3), 46.2 (C-2', C-3', C-5', C-6' merged owing to ring flipping), 55.6 (C-7").

Antibacterial activity assay

Monitoring of synthesized compounds 3a-i was done for checking antibacterial activity against pathogenic bacteria. 96-Wells sterilized microplates were utilized for evaluating antimicrobial activity assay (Eloff et al., 1998). Test samples were dissolved in suitable solvent and taken in wells. Different samples of Tryptic Soy Broth (TSB) were taken in order to determine Minimum Inhibitory Concentration (MIC) values. The basic principle of determination corresponds to increase or decrease in total number of cells in life cycle of microbial growth. Optical density of samples was determined using 630 nm microplate reader Results were evaluated in terms of increase or decrease of absorption capacity of the tested medium (Kaspady et al., 2009; Yang et al., 2006).

Minimum inhibitory concentrations (MIC)

100µL of nutrient broth was poured in all wells of 96 well plates (micro dilution plates). Subsequently added 100µL of samples in the first well and used its two-fold diluted form by making the use of dilution method. Later on all the wells were cultured with 20µL of bacterial strains and incubation at 37^{o}C for 24 hours were carried out. Resazurine was used as bacterial growth indicator and color changes from blue to pink scored as bacterial growth initiation (Abbasi *et al.,* 2020[b]).

STATISTICAL ANALYSIS

After three-folds performance and analysis of statistical data by using Microsoft Excel 2010, results are mentioned in terms of ± SEM. EZ Fit Perrella Scientific Inc. Amherst USA software was used to find out minimum inhibitory concentration (MIC) value under different dilutions values (ranging 5-30μg/well).

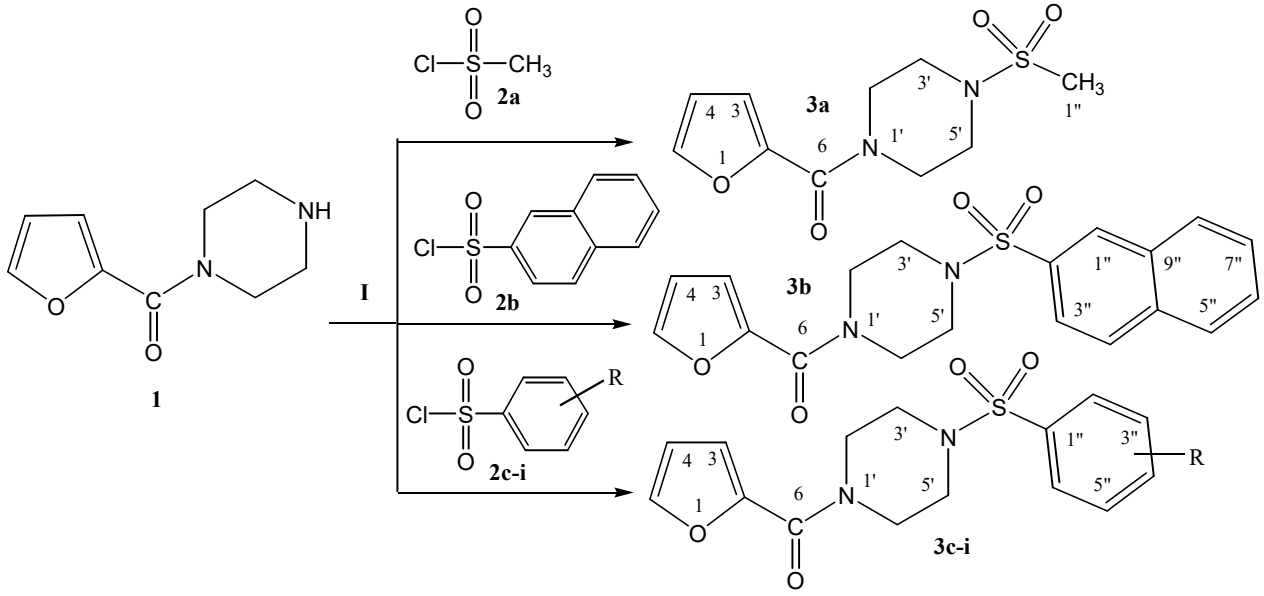
Hemolytic activity assay

The compounds were subjected to hemolytic activity analysis using reported method (Powell *et al*., 2000; Sharma *et al.*, 2001; Rodríguez *et al*., 2017). Collection of fresh heparinized bovine blood was done from volunteers after approval and recommendation from Department of Clinical, Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Samples in polystyrene tube were centrifuged at l000xg for 10 min followed by removal of supernatant. Erythrocytes thus separated were washed and diluted with phosphate buffer to adjust the pH at 7.4. To this prepared suspension, synthetic compounds were added and cell count was determined by haemacytometer. Synthetic compounds (20µL of solution; one in each experiment) were added (made by 10mg/mL) in 180µL of Red Blood Cells (RBCs) suspension and incubated for 30 min at room temperature. Phosphate Buffered Saline (PBS) and Triton 100-X were used as negative and positive controls respectively (Shahid *et al.,* 2013). The %age of hemolysis was taken as by using formula:

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

RESULTS

The synthesis of targeted sulfonamides, 3a-i, is outlined in scheme 1. The method of synthesis is explicated in the experimental section. After structural corroboration, the compounds were subjected to antibacterial and hemolytic activities. The results of antibacterial activity of synthesized compounds are depicted as % inhibition values and MIC Minimum Inhibitory Concentration (table 1 & table 2). The hemolytic activity is given as % lysis in table 3.



R **3c** = H, **3d** = 4-CH_3CONH, **3e** = 4-CH_3, **3f** = 4-Br, **3g** = 4-CH_3CO, **3h** = 2,4,6-trimethyl, **3i** = 4-CH_3O

Scheme 1: Outline for the synthesis of different sulfonamides (3a-i). Reagents & Conditions: (I) Aq. Na_2CO_3 soln./pH 9-10/stirring at room temperature (RT) for 3-4 hours.

Table 1: Antibacterial activity (inhibition %) of the synthesized compounds.

Compound	Inhibition (%)				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
3a	76.40±0.9	89.20±0.20	73.50±0.20	75.50±0.81	68.00±0.53
3b	68.75±0.95	72.35±1.14	60.57±0.43	70.55±0.95	67.15±0.95
3c	77.80±1.20	79.25±0.25	75.00±0.25	79.25±1.15	73.43±0.98
3d	75.85±0.35	74.80±0.20	68.21±0.86	78.70±1.20	64.86±0.05
3e	75.85±1.45	71.50±1.20	77.79±1.07	77.400±1.00	64.56±1.04
3f	74.05±1.55	74.30±0.50	65.36±0.35	75.85±0.53	64.64±1.00
3g	63.50±0.8	58.60±1.27	50.21±0.30	65.80±0.60	52.64±1.00
3h	78.45±0.65	77.00±1.27	65.93±0.60	76.55±1.25	67.29±0.44
3i	84.75±0.85	75.75±0.14	76.21±1.00	74.30±0.20	66.07±0.95
Ciprofloxacin	92.87±0.91	92.27±0.64	92.34±0.35	91.63±0.05	90.57±0.35

Table 2: Antibacterial activity (MIC, μM) of the synthesized compounds.

Compound	MIC (μM)				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
3a	9.26±0.05	7.89±0.79	9.02±0.20	8.01±0.33	10.37±0.51
3b	9.11±0.91	9.14±0.28	13.87±0.58	10.38±0.19	10.35±0.69
3c	9.24±0.90	8.27±0.73	9.26±0.37	8.05±0.10	10.38±0.38
3d	9.98±0.95	9.02±0.36	9.87±0.86	8.10±0.47	12.64±0.75
3e	9.93±0.05	10.48±0.58	8.11±0.47	8.39±0.21	12.58±0.83
3f	9.36±0.55	9.47±0.79	10.24±0.16	9.12±0.22	12.67±0.79
3g	11.57±0.90	14.36±0.47	17.63±0.85	12.06±0.58	17.46±0.38
3h	8.98±0.61	8.19±0.37	10.47±0.70	8.09±0.42	10.38±0.57
3i	8.10±0.85	9.46±0.43	9.74±0.36	10.21±0.57	10.97±0.63
Ciprofloxacin	7.28±0.11	7.31±0.14	7.22±0.76	7.29±0.05	7.86±0.42

Table 3: Hemolytic activity of the synthesized compounds.

Compound	Hemolytic activity
	% Hemolysis
3a	83.83
3b	70.88
3c	27.87
3d	56.27
3e	81.20
3f	86.32
3g	29.13
3h	82.85
3i	88.57
PBS	0.43
Triton	100

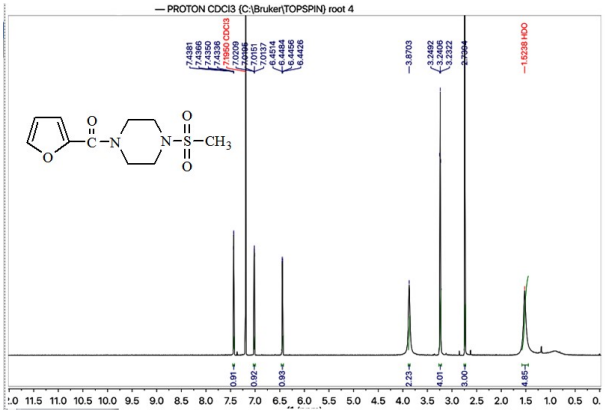


Fig. 1a: ^{1}H-NMR spectrum of 4-(methylsulfonyl)-1-(2-furoyl)piperazine (3a).

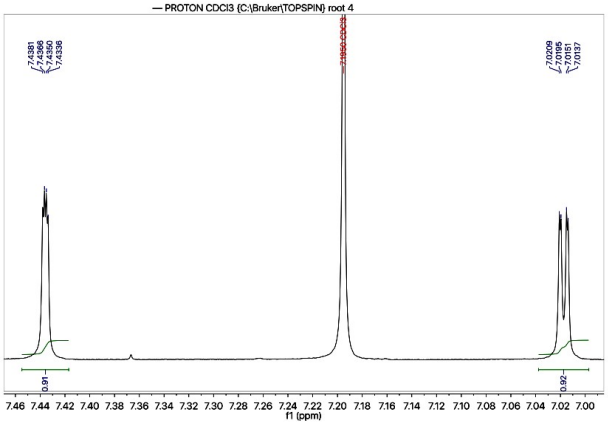


Fig. 1b: Expanded downfield region of ^{1}H-NMR spectrum of 3a.

DISCUSSION

Chemistry

4-(Methylsulfonyl)-1-(2-furoyl)piperazine (3a) was purified as white amorphous solid and its molecular formula $C_{10}H_{14}N_2O_4S$ was ascertained by counting the number of protons in its ^{1}H-NMR spectrum (fig. 1a-d) and number of carbon resonances in its ^{13}C-NMR spectrum (fig. 2). The presence of prominent functionalities was also confirmed by IR absorption bands at 3027 (Ar C-H), 2836 (R C-H), 1603 (C=O), 1584, 1453 (C=C), 1323 (S=O), 1156 (C-O-C), 1078 (C-N-C), and 655 (C-S).

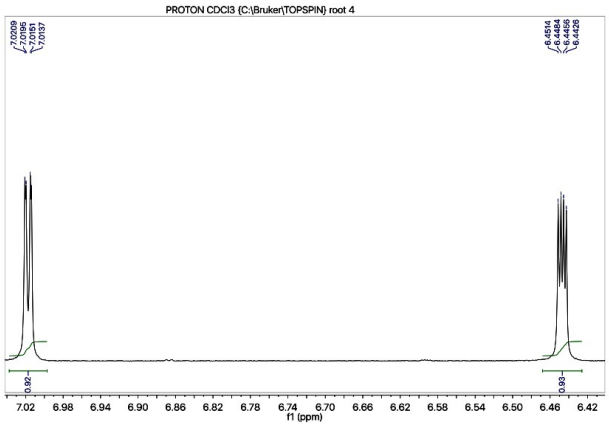


Fig. 1c: Expanded part of aromatic region of ^{1}H-NMR spectrum of 3a.

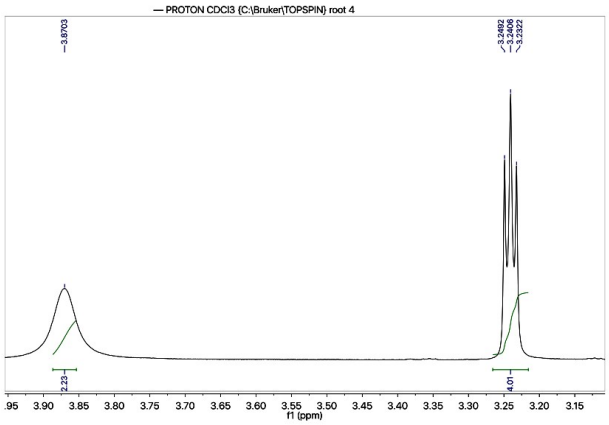


Fig. 1d: Expanded part of aliphatic region of ^{1}H-NMR spectrum of 3a.

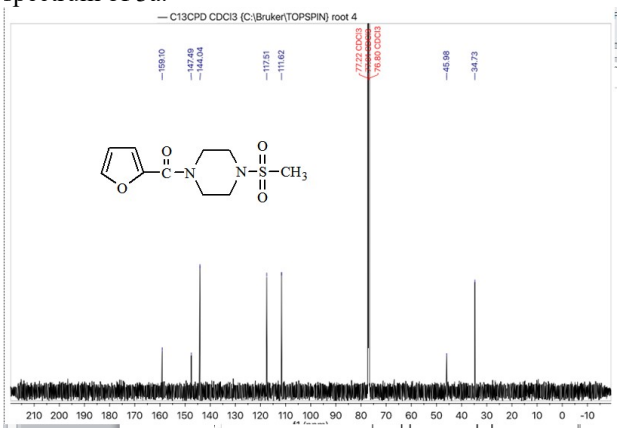


Fig. 2: ^{13}C-NMR spectrum of 3a.

The singlet at δ 2.73 (s, 3H, CH_3-1") was allotted to methyl group directly attached to sulfonyl group. The furan moiety showed three singles, with one-proton

integration each, at δ 7.43 (dist.d, J=0.9 Hz, 1H, H-5), 7.02 (d, J=2.6 Hz, 1H, H-3) and 6.45 (dd, J=1.8, 3.4 Hz, 1H, H-4). The piperazine moiety presented one broad singlet and one triplet, for the overall eight-portons, resonating at δ 3.87 (br.s, 4H, CH_2-3' & CH_2-5') and 3.24 (br.t, J=5.1 Hz, 4H, CH_2-2' & CH_2-6'). The ^{13}C-NMR spectrum (fig. 2) further augmented the structural interpretation. The carbon core of furoyl moiety was fully confirmed by five signals of 159.1 (C-6), 147.4 (C-2), 144.0 (C-5), 117.5 (C-4) and 111.6 (C-3). The four carbons of piperidine moiety were merged into one signal at δ 45.9 (C-2', C-3', C-5', C-6'), owing to usual ring flipping. Similarly, the appearance of a singlet at δ 34.7 (C-1") was rational for a methyl group attached to sulfonyl functionality. So, on the basis of cumulative spectral evidences the structure of 3a was affirmed as 4-(methylsulfonyl)-1-(2-furoyl)piperazine. Same protocol was followed for the structural characterization of other synthesized compounds.

Antibacterial activity

The synthesized compounds remained efficient inhibitors of all the considered bacterial strains. The lower MIC values, which are comparable to the reference, ciprofloxacin, supported better activity of these compounds. Against *S. typhi*, 4-(4-methoxy phenylsulfonyl)-1-(2-furoyl)piperazine (3i) remained the most active compound with MIC value of 8.10±0.85µM in comparison of that of reference, 7.28±0.11µM. In broader interpretation, only 3g was the less active against this strain. 4-(Methylsulfonyl)-1-(2-furoyl)piperazine (3a) was the most active compound against *E. coli*, with MIC value of 7.89±0.79µM, relative to MIC of reference, 7.31 ±0.14µM. Against *P. aeruginosa*, 4-(4-methylphenyl sulfonyl)-1-(2-furoyl)piperazine (3e) was the most active one with MIC value of 8.11±0.47µM. The reference was having MIC 7.22±0.76µM in this case. Against *B. subtilis,* 4-(methylsulfonyl)-1-(2-furoyl)piperazine (3a) was the most superb one with MIC value of 8.01±0.33 µM in comparison of the reference having value of 7.29± 0.50 µM. Against *S. aureus,* 4-(2-naphthylsulfonyl)-1-(2-furoyl)piperazine (3b) was identified as best active molecule bearing MIC value of 10.35±0.69µM, relative to the reference exhibiting MIC value of 7.86±0.82µM. In general, all the synthesized molecules exhibited convincing antibacterial inhibition potential, except 3g.

Hemolytic activity

Two compounds, 3c bearing phenyl and 3g bearing 4-acetylphenyl groups, exhibited very mild hemolytic activity with % lysis of 27.87 and 29.13%, respectively, relative to the Triton-X-100 (% lysis 100). Highest % lysis was shown by 3i (% lysis 88.57) yet it is much lower than positive control Triton-X-100. So, in general it is pertinent to say that the studied molecules are not toxic and might be utilized as safe therapeutic agents.

CONCLUSION

It was concluded from the investigated study that targeted sulfonamides were successfully synthesized in good yields by a benign and cost effective method. Most of the synthesized compounds exhibited remarkable antibacterial potentials with mild cytotoxicity. So, such molecules might serve as valued lead compounds in drug discovery and development program.

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