Synthesis of 2-Furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives as suitable antibacterial agents with mild cytotoxicity

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Abstract: The aim of the present research work was synthesis of some 2-furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives and to ascertain their antibacterial potential. The cytotoxicity of these molecules was also checked to find out their utility as possible therapeutic agents. The synthesis was initiated by reacting furyl(-1-piperazinyl)methanone (1) in *N,N*-dimethylformamide (DMF) and lithium hydride with different aralkyl halides (2a-j) to afford 2-furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives (3a–j). The structural confirmation of all the synthesized compounds was done by IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR spectral techniques and through elemental analysis. The results of *in vitro* antibacterial activity of all the synthesized compounds were screened against Gram-negative (*S. typhi*, *E. coli, P. aeruginosa*) and Gram-positive (*B. subtilis, S. aureus*) bacteria and were found to be decent inhibitors. Amongst the synthesized molecules, 3e showed lowest minimum inhibitory concentration MIC = 7.52±0.μg/mL against *S. Typhi*, credibly due to the presence of 2-bromobenzyl group, relative to the reference standard, ciprofloxacin, having MIC = 7.45±0.58μg/mL.

Keywords: Furyl(-1-piperazinyl)methanone, aralkyl halides, antibacterial activity, cytotoxicity studies.

INTRODUCTION

Piperazine is a medicinally heterocyclic nucleus which consists a six membered ring containing two nitrogen atoms at 1 and 4 positions in the ring. The piperazine has been classified as a privileged structure and is frequently found in biologically active compounds across a number of different therapeutic areas (Faist *et al.,* 2012), which encompass anti-microbial, anti-tubercular, anti-psychotic, anti-convulsant, anti-depressant, anti-inflammatory, cytotoxic, anti-malarial, anti-arrhythmic, anti-oxidant and anti-viral activities (Kulig *et al.,* 2007).

The development of bacterial resistance against available antibiotics is a need of hour for the search of new antibacterial agents (Bhattacharjee *et al.,* 2005). Numerous researchers worked to eliminate pathogenic microorganisms from biologically active components isolated from plants (Tepe *et al.,* 2005). The initiation of antimicrobial investigations is resistance, which microorganisms have gained against antibiotics that increases the applications of essentials oils or plants against a wide range of bacteria (Gram-negative and Gram-positive) including antibiotic, fungal and yeast resistant species (Jimenez-Arellanes *et al.,* 2003, Hammer *et al.,* 1999, Hammer *et al.,* 1998 and Nelson *et al.,* 1997).

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The discovery of the therapeutic potential of nitrofuran derivatives by Dodd and Stillman in 1944 during their research for antibacterial agents (Kedderis *et al.,* 1988), paved the way to design and synthesis various 5-nitrofuran analogs with a broad-spectrum activity against Gram-negative & positive bacteria and even some protozoa (Jorge *et al.,* 2009). Although the mechanism of action of nitrofurans is not completely understood, previous studies accounted that under anaerobic conditions, the nitro group of the nitrofurans is reduced with formation of toxic free radical (Viodé *et al.,* 1999). So, the incorporation of a nitro group at position 5 of furan ring resulted in a marked increase in antibacterial activity (Kedderis *et al.,* 1988).

Depression is a chronic, persistent and life-threatening disease that is present in 20 % of the population across the globe (Srinath *et al.,* 2010). The etiology of the disease is suboptimal concentrations of the monoamine neurotransmitters serotonin (5-HT) and norepinephrine (NE) in the central nervous system (CNS). It is also due to consequence of dysfunctional endocrine, immune and neurotransmitter systems (Lucas *et al.,* 2010). Selective serotonin reuptake inhibitors (SSRIs) have great success in treating depression and have fewer and less severe side-effects than first-generation drugs (Zhou *et al.,* 2008), reported a series of analogues of arylpiperazine-4-yl-cyclohexylindole as shown in (fig. 1) evaluated as 5-HT transporter inhibitor and 5-HT$_{1A}$ receptor antagonist.

In continuation of our previous efforts (Hussain *et al.,* 2016) and in search of new therapeutic agents, hereby we report a facile and benign synthesis of different *N*-aralkylated derivatives of furyl(-1-piperazinyl)methanone, which might be utilized as possible antibacterial agents.

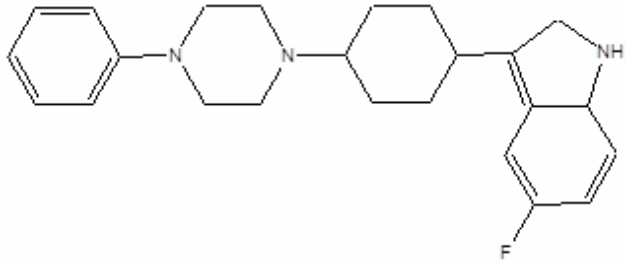


Fig. 1: Structure of 5-fluoro-3-[4-(4-phenylpiperazin-1-yl)cyclohexyl]-2,7-dihydro-*1H*-indole.

MATERIALS AND METHODS

General

Chemicals were purchased from Sigma Aldrich & Alfa Aesar (Germany) & solvents of analytical grade were supplied by local suppliers. By using open capillary tube method, melting points were taken on Griffin and George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate & *n*-hexane (30:70) as mobile phase), initial purity of compounds was detected at 254 nm. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. ^{1}H-NMR signals were recorded at 500 MHz and ^{13}C-NMR at 150 MHz in $CDCl_3$ using Bruker spectrometers. EI-MS signals were recorded by utilizing a JMS-HX-110 spectrometer.

Synthesis of 2-furyl[(4-aralkyl)-1-piperazinyl] methanone derivatives (3a-j)

2-Furyl(-1-piperazinyl)methanone (1.66mmol; 1) was added in *N,N*-dimethylformamide (5mL) & lithium hydride (0.42mmol) which is added in 25mL round bottom flask at room temperature and stirred for 15 min. The corresponding aralkyl halides (1.66mmol, 2a-j) were added into the reaction mixture & further stirred for 4-5 hrs. The reaction mixture was then monitored by TLC. After completion, the reaction mixture was quenched with ice cold water (100mL). The obtained solid was filtered, washed with distilled water followed by drying to yield the corresponding 2-furyl[(4-aralkyl)-1-piperazinyl] methanone derivatives (3a–j). In some cases, compounds isolated via solvent extraction using chloroform / ethyl acetate.

2-Furyl(4-benzyl-1-piperazinyl)methanone (3a)

Light brown crystalline solid; Yield: 90%; m.p: 102-104^{o}C; Mol. F.: $C_{16}H_{18}N_2O_2$; Mol. Mass: 270 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3080 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1664 (C=O str.), 1592 (C=C aromatic str.), 1188 (C-O-C bond str.), 1109 (C-N-C bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.61 (d, *J*= 7.5 Hz, 2H, H-2" & H-6"), 7.49 (d, *J*=1.6 Hz, 1H, H-5), 6.98 (d, *J*=3.4 Hz, 1H, H-3), 7.46-7.36 (m, 3H, H-3" to H-5"), 6.45 (dd, *J*=1.7, 3.4 Hz, 1H, H-4), 5.15 (s, 2H, CH_2-7"), 4.05 (br.s, 4H, CH_2-2' & CH_2-6'), 2.43 (br.t, *J*= 4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.07 (C-6), 148.08 (C-2), 143.55 (C-5), 137.61 (C-1"), 129.15 (C-3" & C-5"), 128.34 (C-2" & C-6"), 127.29 (C-4"), 116.20 (C-3), 111.20 (C-4), 62.91 (C-7"), 53.13 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 270 $[M]^+$, 175 $[C_{11}H_{15}N_2]^+$, 161 $[C_{11}H_{15}N]^{\bullet+}$, 146 $[C_{10}H_{12}N]^+$, 134 $[C_9H_{12}N]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$, 93 $[C_7H_7]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{18}N_2O_2$: C 71.09, H 6.71, N 10.36; found C 70.85, H 6.57, N 10.19.

2-Furyl[4-(2-chlorobenzyl)-1-piperazinyl]methanone (3b)

Light brown crystalline solid; Yield: 93%; m.p: 97-99^{o}C; Mol. F.: $C_{16}H_{17}ClN_2O_2$; Mol. Mass: 304 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3084 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1651 (C=O str.), 1583 (C=C aromatic str.), 1190 (C-O-C bond str.), 1110 (C-N-C bond str.), 661 (C-Cl bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.50-7.47 (m, 1H, H-6"), 7.46 (d, *J*=1.7 Hz, 1H, H-5), 7.35 (dd, *J*=1.4, 7.8 Hz, 1H, H-3"), 7.23 (dt, *J*=1.4, 7.8 Hz, 1H, H-5"), 7.21 (dt, *J*=1.7, 7.6 Hz, 1H, H-4"), 6.98 (d, *J*=2.8 Hz, 1H, H-3), 6.47 (dd, *J*=1.7, 3.7 Hz, 1H, H-4), 3.81 (br.s, 4H, CH_2-2' & CH_2-6'), 3.66 (s, 2H, H-7"), 2.57 (br.t, *J*=4.9 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.01 (C-6), 147.77 (C-2), 143.64 (C-5), 136.28 (C-1"), 132.78 (C-2"), 130.87 (C-6"), 128.66 (C-4"), 127.25 (C-5"), 124.69 (C-3"), 116.20 (C-3), 111.17 (C-4), 61.54 (C-7"), 53.00 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 306 $[M + 2]^+$, 304 $[M]^+$, 269 $[C_{16}H_{17}N_2O_2]^+$, 209 $[C_{11}H_{14}ClN_2]^+$, 195 $[C_{11}H_{14}ClN]^{\bullet+}$, 180 $[C_{10}H_{11}ClN]^+$, 168 $[C_9H_{11}ClN]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 125 $[C_7H_6Cl]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{17}ClN_2O_2$: C 63.05, H 5.62, N 9.19; found C 62.91, H 5.53, N 9.10.

2-Furyl[4-(3-chlorobenzyl)-1-piperazinyl]methanone (3c)

Off white crystalline solid; Yield: 90%; m.p: 102-104^{o}C; Mol. F.: $C_{16}H_{17}ClN_2O_2$; Mol. Mass: 304 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3077 (C-H str. of aromatic ring), 2878 (C-H str. of aliphatic), 1658 (C=O str.), 1589 (C=C aromatic str.), 1194 (C-O-C bond str.), 1119 (C-N-C bond str.), 665 (C-Cl bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.47 (d, *J*=1.6 Hz, 1H, H-5), 7.36 (d, *J*=2.6 Hz, 1H, H-2"), 7.26 (dd, *J*=3.0, 8.7 Hz, 1H, H-6"), 7.19-7.12 (m, 2H, H-4" & H-5"), 6.97 (d, *J*=3.4 Hz, 1H, H-3), 6.48 (dd, *J* 1.7, 3.4 Hz, 1H, H-4), 5.15 (s, 2H, CH_2-7"), 4.05 (br.s, 4H, CH_2-2' & CH_2-6'), 2.45 (br.t, *J*=4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.07 (C-6), 147.98 (C-2), 143.59 (C-5), 139.93 (C-1"), 134.30 (C-3"), 129.59 (C-5"), 129.01 (C-2"), 127.47 (C-6"), 127.12 (C-4"), 116.31 (C-3), 111.24 (C-4), 62.25 (C-7"), 53.14 (C-2', C-3', C-5', C-6'); EI-MS (*m/z*): 306 $[M + 2]^+$,

304 $[M]^+$,269 $[C_{16}H_{17}N_2O_2]^+$, 209 $[C_{11}H_{14}ClN_2]^+$, 195 $[C_{11}H_{14}ClN]^{\bullet+}$, 180 $[C_{10}H_{11}ClN]^+$, 168 $[C_9H_{11}ClN]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 125 $[C_7H_6Cl]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{17}ClN_2O_2$: C 63.05, H 5.62, N 9.19; found C 62.91, H 5.52, N 9.07.

2-Furyl[4-(4-chlorobenzyl)-1-piperazinyl]methanone (3d)

White amorphous solid; Yield: 87%; m.p: 105-107°C; Mol. F.: $C_{16}H_{17}ClN_2O_2$; Mol. Mass: 304 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3086 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1659 (C=O str.), 1584 (C=C aromatic str.), 119 (C-O-C bond str.), 1112 (C-N-C bond str.), 667 (C-Cl bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.51 (d, *J*=8.1 Hz, 2H, H-3" & H-5"), 7.45 (d, *J*=1.6 Hz, 1H, H-5), 7.01 (d, *J*=8.1 Hz, 2H, H-2" & H-6"), 6.99 (d, *J* =3.5 Hz, 1H, H-3), 6.47 (dd, *J*=1.7, 3.4 Hz, 1H, H-4), 5.21 (s, 2H, CH_2-7"), 4.08 (br.s, 4H, CH_2-2' & CH_2-6'), 2.47 (br.t, *J*=4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.06 (C-6), 147.98 (C-2), 143.58 (C-5), 136.25 (C-1"), 130.34 (C-3" & C-6"), 128.49 (C-2" & C-6"), 133.00 (C-4"), 116.29 (C-3), 111.23 (C-4), 62.10 (C-7"), 53.09 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 306 $[M + 2]^+$, 304 $[M]^+$,269 $[C_{16}H_{17}N_2O_2]^+$, 209 $[C_{11}H_{14}ClN_2]^+$, 195 $[C_{11}H_{14}ClN]^{\bullet+}$, 180 $[C_{10}H_{11}ClN]^+$, 168 $[C_9H_{11}ClN]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 125 $[C_7H_6Cl]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{17}ClN_2O_2$: C 63.05, H 5.62, N 9.19; found C 62.95, H 5.53, N 9.08.

2-Furyl[4-(2-bromobenzyl)-1-piperazinyl]methanone (3e)

Light brown liquid; Yield: 78%; Mol. F.: $C_{16}H_{17}BrN_2O_2$; Mol. Mass: 348g/mol; IR (KBr, cm^{-1}) υ_{max}: 3082 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1660 (C=O str.), 1592 (C=C aromatic str.), 1170 (C-O-C bond str.), 1118 (C-N-C bond str.), 623 (C-Br bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.47 (d, *J*=1.6 Hz, 1H, H-5), 7.34 (dd, *J*=2.5, 8.1 Hz, 1H, H-3"), 7.19 (dd, *J*=3.0, 8.4 Hz, 1H, H-6"), 7.13-7.06 (m, 2H, H-4" & H-5"), 6.98 (d, *J*=3.4 Hz, 1H, H-3), 6.46 (dd, *J*=1.7, 3.4 Hz, 1H, H-4), 5.17 (s, 2H, CH_2-7"), 4.06 (br.s, 4H, CH_2-2' & CH_2-6'), 2.45 (br.t, *J*=4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.01(C-6), 147.54(C-2), 143.64(C-5), 136.51(C-1"), 132.78(C-3"), 130.87(C-6"), 128.66(C-4"), 127.27(C-5"), 124.69(C-2"), 116.24(C-3), 111.17(C-4), 61.54(C-7"), 53.23(C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 348$[M]^+$, 269$[C_{16}H_{17}N_2O_2]^+$, 253 $[C_{11}H_{14}BrN_2]^+$, 239$[C_{11}H_{14}BrN]^{\bullet+}$, 224$[C_{10}H_{11}BrN]^+$, 212 $[C_9H_{11}BrN]^+$, 152$[C_8H_{10}NO_2]^+$, 138$[C_7H_8NO_2]^+$, 169 $[C_7H_6Br]^+$, 95$[C_5H_3O_2]^+$, 44$[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{17}BrN_2O_2$: C 55.03, H 4.91, N 8.02; found C 54.91, H 4.80, N 7.94.

2-Furyl[4-(4-bromobenzyl)-1-piperazinyl]methanone (3f)

White amorphous solid; Yield: 80%; m.p: 105-107°C; Mol. F.: $C_{16}H_{17}BrN_2O_2$; Mol. Mass: 348g/mol; IR (KBr, cm^{-1}) υ_{max}: 3087 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1658 (C=O str.), 1580 (C=C aromatic str.), 1194 (C-O-C bond str.), 1116 (C-N-C bond str.), 621 (C-Br bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.57 (d, *J*=6.5 Hz, 2H, H-3" & H-5"), 7.49 (d, *J*=6.9 Hz, 2H, H-2" & H-6"), 7.47 (d, *J*=1.6 Hz, 1H, H-5), 6.94 (d, *J* =3.5 Hz, 1H, H-3), 6.47 (dd, *J*=1.7, 3.4 Hz, 1H, H-4), 5.04 (s, 2H, CH_2-7"), 4.05 (br.s, 4H, CH_2-2' & CH_2-6'), 2.53 (br.t, *J* = 4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.12 (C-6), 147.01 (C-2), 142.58 (C-5), 136.20 (C-1"), 130.39 (C-3" & C-6"), 128.49 (C-2" & C-6"), 133.00 (C-4"), 116.27 (C-3), 111.21 (C-4), 62.10 (C-7"), 53.03 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 348 $[M]^+$, 269 $[C_{16}H_{17}N_2O_2]^+$, 253 $[C_{11}H_{14}BrN_2]^+$, 239 $[C_{11}H_{14}BrN]^{\bullet+}$, 224 $[C_{10}H_{11}BrN]^+$, 212 $[C_9H_{11}BrN]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 169 $[C_7H_6Br]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{16}H_{17}BrN_2O_2$: C 55.03, H 4.91, N 8.02; found C 54.90, H 4.82, N 7.90.

2-Furyl[4-(4-fluorobenzyl)-1-piperazinyl]methanone (3g)

White amorphous solid; Yield: 86%; m.p: 107-109°C; Mol. F.: $C_{16}H_{17}FN_2O_2$; Mol. Mass: 288 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3089 (C-H str. of aromatic ring), 2884 (C-H str. of aliphatic), 1650 (C=O str.), 1589 (C=C aromatic str.), 1194 (C-O-C bond str.), 1102 (C-F bond str.), 1110 (C-N-C bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.48 (distorted d, *J*=0.8 Hz, 1H, H-5), 7.31 (dd, *J*=8.3, 5.6 Hz, 2H, H-2" & H-6"), 7.03 (br.t, *J*=8.6 Hz, 2H, H-3" & H-5"), 6.99 (d, *J*=3.4 Hz, 1H, H-3), 6.48 (dd, *J*=1.5, 3.0 Hz, 1H, H-4), 3.82 (br.s, 4H, CH_2-2' & CH_2-6'), 3.52 (s, 2H, H-7"), 2.50 (dd, *J*=4.6 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.32 (C-6), 147.90 (C-2), 143.39 (C-5), 136.20 (C-1"), 130.12 (C-3" & C-6"), 128.67 (C-2" & C-6"), 133.03 (C-4"), 116.98 (C-3), 111.21 (C-4), 62.19 (C-7"), 53.42 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 288 $[M]^+$, 269 $[C_{16}H_{17}N_2O_2]^+$, 193 $[C_{11}H_{14}FN_2]^+$, 179 $[C_{11}H_{14}FN]^{\bullet+}$, 164 $[C_{10}H_{11}FN]^+$, 152 $[C_9H_{11}FN]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 109 $[C_7H_6F]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd for $C_{16}H_{17}FN_2O_2$: C 66.65, H 5.94, N 9.72; found C 66.56, H 5.84, N 9.61.

2-Furyl[4-(2-methylbenzyl)-1-piperazinyl]methanone (3h)

White amorphous solid; Yield: 88%; m.p: 94-96°C; Mol. F.: $C_{17}H_{20}N_2O_2$; Mol. Mass: 284 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3085 (C-H str. of aromatic ring), 2884 (C-H str. of aliphatic), 1650 (C=O str.), 1589 (C=C aromatic str.), 1193 (C-O-C bond str.), 1114 (C-N-C bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.46 (d, *J*=1.6 Hz, 1H, H-5), 7.23 (d, *J*=8.2 Hz, 1H, H-6"), 7.18-7.12 (m, 3H, H-3" to H-5"), 6.97 (d, *J*=3.4 Hz, 1H, H-3), 6.46 (dd, *J*= 1.7, 3.4 Hz, 1H, H-4), 4.03 (br.s, 4H, CH_2-2' & CH_2-6'), 3.49 (s, 2H, CH_2-7"), 2.49 (br.t, *J*=4.4 Hz, 4H, CH_2-3' & CH_2-5'), 2.38 (s, 3H, CH_3-1'"); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.82 (C-6), 147.59 (C-2), 143.42 (C-5),

136.18 (C-1"), 135.86 (C-2"), 130.55 (C-3"), 128.61 (C-6"), 127.67 (C-4"), 124.99 (C-5"), 116.37 (C-3), 111.81 (C-4), 61.09 (C-7"), 53.02 (C-2', C-3', C-5' & C-6'); EI-MS (*m/z*): 284 $[M]^+$, 269 $[C_{16}H_{17}N_2O_2]^+$, 189 $[C_{12}H_{17}N_2]^+$, 175 $[C_{12}H_{17}N]^{\bullet+}$, 160 $[C_{11}H_{14}N]^+$, 148 $[C_{10}H_{14}N]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 105 $[C_8H_9]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{17}H_{20}N_2O_2$: C 71.81, H 7.09, N 9.85; found C 71.69, H 6.88, N 9.72.

2-Furyl[(4(2-phenethyl)-1-piperazinyl)]methanone (3i)

Off white liquid; Yield: 80 %; Mol. F.: $C_{17}H_{20}N_2O_2$; Mol. Mass: 284 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3082 (C-H str. of aromatic ring), 2887 (C-H str. of aliphatic), 1654 (C=O str.), 1583 (C=C aromatic str.), 1192 (C-O-C bond str.), 1113 (C-N-C bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.45 (d, *J*=1.6 Hz, 1H, H-5), 7.35-7.17 (m, 5H, H-2" to H-6"), 6.93 (d, *J*=3.4 Hz, 1H, H-3), 6.49 (dd, *J*= 1.7, 3.4 Hz, 1H, H-4), 4.37 (t, *J*=6.7 Hz, 2H, CH_2-7"), 4.05 (br.s, 4H, CH_2-2' & CH_2-6'), 3.19 (t, *J* = 6.6 Hz, 2H, CH_2-8"), 2.41 (br.t, *J*=4.4 Hz, 4H, CH_2-3' & CH_2-5'); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.03 (C-6), 147.86 (C-2), 143.68 (C-5), 139.67 (C-1"), 128.64 (C-3" & C-5"), 128.48 (C-2" & C-6"), 126.25 (C-4"), 116.47 (C-3), 111.29 (C-4), 60.13 (C-8"), 53.50 (C-2', C-3', C-5' & C-6'), 33.26 (C-7"); EI-MS (*m/z*): 284 $[M]^+$, 189 $[C_{12}H_{17}N_2]^+$, 175 $[C_{13}H_{17}N]^{\bullet+}$, 160 $[C_{11}H_{14}N]^+$, 148 $[C_{10}H_{14}N]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 105 $[C_8H_9]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd. for $C_{17}H_{20}N_2O_2$: C 71.81, H 7.09, N 9.85; found C 71.71, H 6.92, N 9.70.

2-Furyl[(4-(3-phenylpropyl)-1-piperazinyl]methanone (3j)

Light brown liquid; Yield: 75%; Mol. F.: $C_{18}H_{22}N_2O_2$; Mol. Mass: 298 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3083 (C-H str. of aromatic ring), 2881 (C-H str. of aliphatic), 1659 (C=O str.), 1586 (C=C aromatic str.), 1195 (C-O-C bond str.), 1119 (C-N-C bond str.); ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 7.49 (d, *J*=1.5 Hz, 1H, H-5), 7.18-6.96 (m, 5H, H-2" to H-6"), 6.94 (d, *J* = 3.4 Hz, 1H, H-3), 6.45 (dd, *J* = 1.5, 3.4 Hz, 1H, H-4), 4.06 (br.s, 4H, CH_2-2' & CH_2-6'), 3.57 (t, *J* = 7.7 Hz, 2H, CH_2-9"), 2.45 (br.t, *J*= 4.4 Hz, 4H, CH_2-3' & CH_2-5'), 2.39 (t, *J*=7.7 Hz, 2H, CH_2-7"), 1.81 (m, 2H, CH_2-8"); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 159.03 (C-6), 147.87 (C-2), 143.66 (C-5), 141.82 (C-1"), 128.38 (C-3" & C-5"), 128.36 (C-2" & C-6"), 125.87 (C-4"), 116.34 (C-3), 111.26 (C-4), 57.88 (C-9"), 53.00 (C-2', C-3' , C-5' and C-6'), 33.51 (C-7"), 28.23 (C-8"); EI-MS (*m/z*): 298 $[M]^+$, 203 $[C_{13}H_{19}N_2]^+$, 189 $[C_{13}H_{19}N]^{\bullet+}$, 174 $[C_{12}H_{16}N]^+$, 162 $[C_{11}H_{16}N]^+$, 152 $[C_8H_{10}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 119 $[C_9H_{11}]^+$, 95 $[C_5H_3O_2]^+$, 44 $[C_2H_4O]^+$; Anal. Calcd for $C_{18}H_{22}N_2O_2$: C 72.46, H 7.43, N 9.39; found C 72.27, H 7.31, N 9.25.

Antibacterial activity

The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments**.** The method is rooted in the principle that microbial cell number increases as the microbial growth keep on in a log phase of growth which outcome in increased absorbance of broth medium (Kaspady *et al.,* 2009 and Yang *et al.,* 2006). Three gram-negative (*S. typhi, E. coli* & *P. aeruginosa*) & two gram-positive bacteria (*B. subtilis, S. aureus*) bacteria were included in the study. The organisms were maintained on stock culture agar medium. The test samples with suitable solvents and dilutions were pipette into wells (20μg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180μL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540nm. The total volume in each well was kept to 200μL. The incubation was done at 37^{o}C for 16-24 hours with lid on the microplate. The absorbance was measured, before and after incubation and the difference was noted as an index of bacterial growth at 540 nm by using micro plate reader. The % inhibition was calculated by using the formula:

$$\text{Inhibition}(\%) = \frac{X - Y}{X} \times 100$$

Where, X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n=3, ± SEM), Ciprofloxacin was taken as standard.

STATISTICAL ANALYSIS

The results are written as mean ± SEM after performance in three-folds and statistical analysis by Microsoft Excel 2010. Minimum inhibitory concentration (MIC) was calculated by using different dilutions (ranging 5-30 μg/well) and EZFit Perrella Scientific Inc. Amherst USA software.

Hemolytic activity

Hemolytic activity was done by the reported method (Sharma *et al.* 2001 and Powell *et al.,* 2000). Bovine blood was obtained from the Department of Clinical Medicine & Surgery, University of Agriculture, Faisalabad, Pakistan. After centrifugation, separation & washing, the % RBCs lysis was computed by noting the absorbance.

RESULTS

In the present research, various 2-Furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives were synthesized according to the outline illustrated in (Scheme 1; Table 1). The synthesis was carried out by reacting furyl(-1-piperazinyl)methanone (1) in *N,N*-dimethylformamide (DMF) and lithium hydride with different aralkyl halides (2a-j) to obtain various 2-furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives (3a–j). The structures of the targeted molecules were confirmed by EI-MS, IR, ^{1}H-NMR and ^{13}C-NMR spectral techniques and the spectral data is given in the experimental section. These

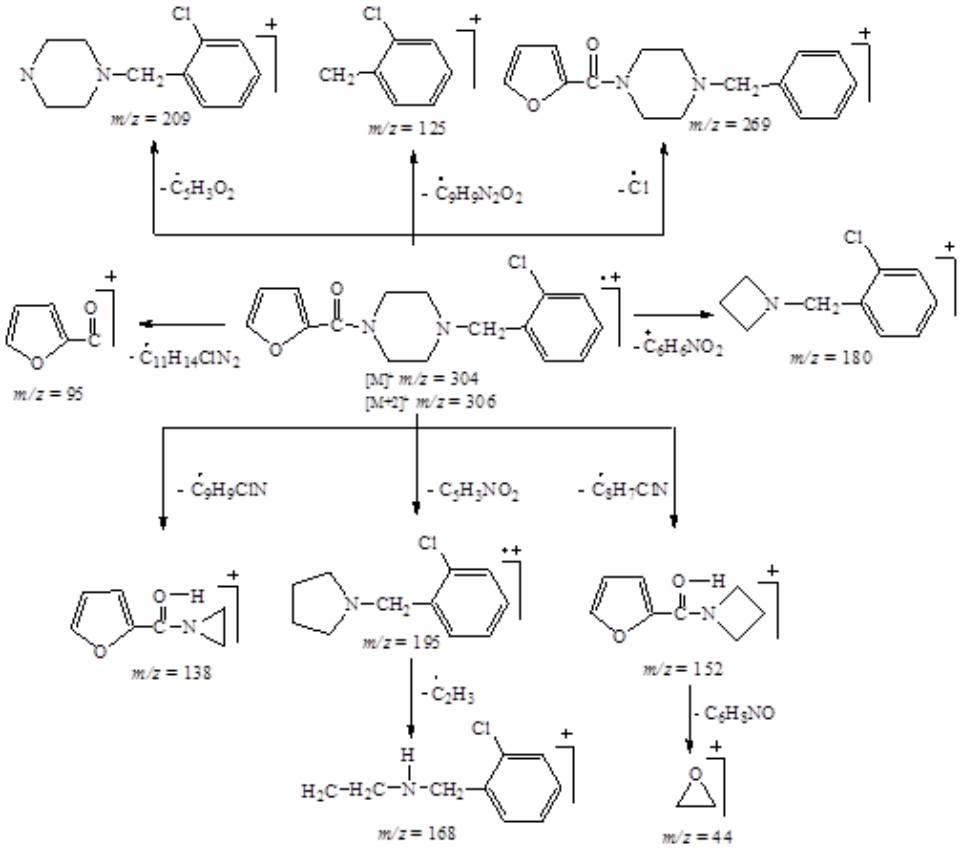


Fig. 2: Suggested mass fragmentation pattern of 2-furyl[4-(2-chlorobenzyl)-1-piperazinyl]methanone (3b).

Table 1: Different substituents (-R) of 3a-h in scheme 1.

Compd.	3a	3b	3c	3d	3e	3f	3g	3h
-R	-H	2-Cl	3-Cl	4-Cl	2-Br	4-Br	4-F	2-CH_3

molecules were then screened for their antibacterial potential against three gram negative and two gram positive bacterial strains and the results are tabulated in table 2. Moreover, their cytotoxicity was also profiled as hemolytic activity on bovine blood and results are columned in table 2.

DISCUSSION

The synthesis of targeted 2-furyl[(4-aralkyl)-1-piperazinyl] methanone derivatives (3a-j) was accomplished by a facile strategy (Nayak *et al*., 2014) and all the compounds were obtained in very good yields. The synthesized compounds were structurally corroborated through spectral data of IR, EI-MS, ^{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 3b was synthesized as light brown crystalline solid having melting point 97-99^{o}C and molecular formula $C_{16}H_{17}ClN_2O_2$, which was confirmed by EI-MS showing $[M]^+$ peak at *m/z* 304 g/mol and by counting the number of protons in its ^{1}H-NMR spectrum. In IR spectrum, characteristic peaks appeared at 3084 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1651 (C=O str.), 1583 (C=C aromatic str.), 1190 (C-O-C bond str.), 1110 (C-N-C bond str.) and 661 (C-Cl bond str.) confirmed the presence of 2-chlorobenzyl group and 2-furyl(-1-piperazinyl)methanone ring. The presence of 2-chlorobenzyl group in this molecule was evident by a distinct fragment ion peak at *m/z* 125 and this assignment was also supported by an ion peak at *m/z* 180 for 1-(2-chlorobenzyl)azetidine part. The suggested mass fragmentation pattern of this molecule is given in Figure 2. In ^{1}H-NMR spectrum, a 2-substituted aromatic ring was evident from the signals δ 7.50-7.47 (m, 1H, H-6"), 7.35 (dd, *J* =1.4, 7.8 Hz, 1H, H-3"), 7.23 (dt, *J*=1.4, 7.8 Hz, 1H, H-5"), and 7.21 (dt, *J*=1.7, 7.6 Hz, 1H, H-4"). Three other peaks in aromatic region at δ 7.46 (d, *J*=1.7 Hz, 1H, H-5), 6.98 (d, *J*=2.8 Hz, 1H, H-3) and 6.47 (dd, *J*=1.7, 3.7 Hz, 1H, H-4) confirmed the presence of furoyl moiety. In the aliphatic region of its ^{1}H-NMR spectrum, a singlet at δ 3.66 represented a benzylic methylene while the piperazine moiety was ascertained by a broad singlet at δ 3.81 having integration of four-protons for CH_2-2' & CH_2-6' and another broad singlet at δ 2.57 for two other methylene groups (CH_2-3' & CH_2-5'). The ^{13}C-NMR

Table 2: Antibacterial activity (% age inhibition / MIC) and cytotoxicity (hemolytic activity) of the 2-furyl[(4-aralkyl)-1-piperazinyl]methanones (3a-j).

Antibacterial Activity %age Inhibition / MIC (μg/mL)						Hemolytic Activity
Compd. No.	*S. typhi (-)*	*E. coli (-)*	*P.aeruginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*	%
3a	71.45±0.3/ 9.63±0.71	90.55±0.8/ 7.97±0.98	70.23±0.1/ 10.94±0.44	59.43±0.7/ 13.87±0.10	49.85±0.7/ -	5.10
3b	72.91±1.0/ 9.43±0.67	82.60±0.7/ 8.68±0.42	67.99±0.6/ 10.48±0.70	76.54±0.2/ 9.01±0.19	53.19±0.3/ 19.22±0.57	14.64
3c	73.33±0.4/ 9.56±0.44	77.70±0.3/ 8.79±0.16	58.62±0.9/ 15.89±0.23	72.60±0.8/ 9.92±0.45	48.95±0.9/ -	5.85
3d	72.47±0.2/ 9.39±0.61	82.75±0.8/ 8.86±0.24	57.78±0.1/ 15.64±0.79	82.30±0.2/ 8.75±0.71	51.76±0.6/ 18.97±0.68	11.29
3e	72.52±0.3/ 7.52±0.3	58.25±0.9/ 16.83±0.54	53.20±0.9/ 18.65±0.49	60.00±0.5/ 15.59±0.13	46.00±0.4/ -	10.04
3f	77.82±0.4/ 8.79±0.22	65.15±0.6/ 10.67±0.4	42.71±0.0/ -	65.63±0.5/ 10.83±0.4	31.47±0.1/ -	15.89
3g	70.21±0.6/ 9.80±0.96	72.95±0.3/ 9.77±0.92	53.29±0.4/ 18.54±0.53	70.83±0.9/ 9.87±0.48	37.67±0.3/ -	16.31
3h	69.74±0.8/ 9.93±0.74	62.15±0.4/ 12.59±0.5	53.52±0.8/ 1796±0.43	64.53±0.7/ 10.56±0.4	37.95±0.2/ -	13.38
3i	76.23±0.6/ 8.98±0.49	71.40±0.5/ 9.90±0.46	71.29±0.3/ 9.89±0.41	81.43±0.0/ 9.11±0.45	48.23±0.8/ -	12.97
3j	60.43±0.5/ 14.52±0.7	61.80±0.9/ 13.58±0.1	42.39±0.5/ -	63.40±0.7/ 11.76±0.0	57.14±0.2/ 15.42±0.5	17.49
Ciprofl-oxacin	91.05±0.6/ 7.45±0.58	92.32±0.4/ 7.16±0.58	92.50±0.3/ 7.14±0.18	92.02±05/ 7.29±0.90	91.44±0.6/ 7.80±0.19	
PBS						0.09
Triton						100

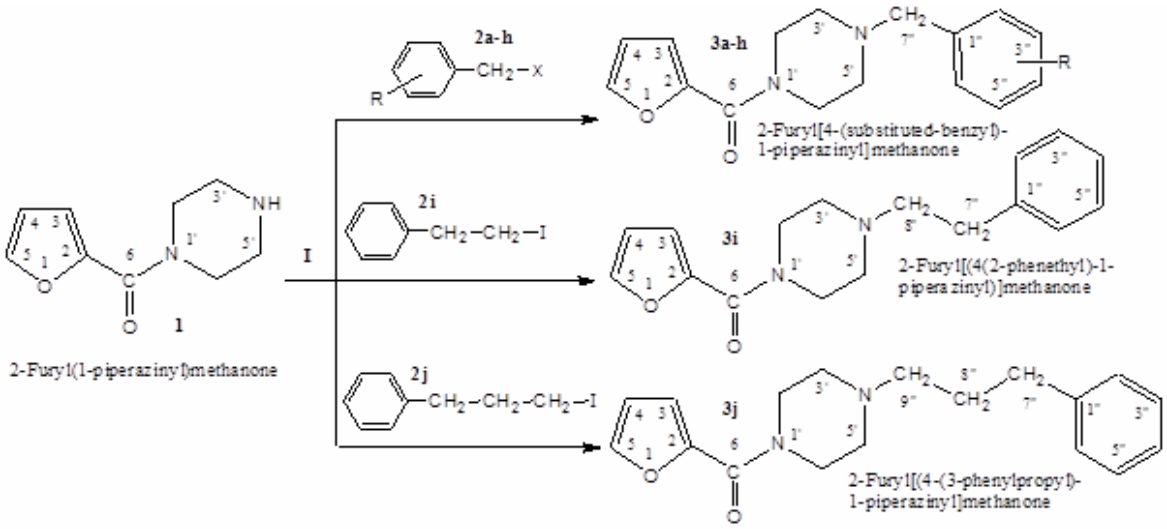
spectrum demonstrated total thirteen carbon resonances, out of which four were quaternary carbons, seven were methine carbons while two carbon resonances were overall assigned to five methylene carbons in the molecule. The carbons of furoyl moiety resonated at δ 159.01 (C-6), 147.77 (C-2), 143.64 (C-5), 116.20 (C-3), and 111.17 (C-4) while 2-chlorobenzyl group was depicted by the carbon resonances at δ 136.28 (C-1"), 132.78 (C-3"), 130.87 (C-6"), 128.66 (C-4"), 127.25 (C-5"), 124.69 (C-2"), and 61.54 (C-7"). The discrete carbon resonances for piperazine ring were not observed, most probably due to ring flipping, however one broad signal at δ 53.00 (C-2', C-3', C-5' and C-6') represented this moiety. The suggested mass fragmentation pattern of this molecule is sketched in fig. 3. The ^{1}H-NMR spectrum of this molecule is shown in fig. 4. On the basis of aforementioned spectral evidences, the structure of 3b was confirmed as 2-furyl[4-(2-chlorobenzyl)-1-piperazinyl] methanone. In a similar way, all the synthesized molecules, 3a-j, were characterized by elemental analysis and by their spectral data of IR, EI-MS, ^{1}H-NMR, and ^{13}C-NMR.

Antibacterial activity

The results of *in vitro* antibacterial activity of all the synthesized molecules were screened against three Gram-negative and two Gram-positive bacteria and displayed decent inhibition. The results are tabulated as % age inhibition / MIC values in Table 2. The synthesized molecule 3e showed lowest MIC=7.52±0.3μg/mL against *S. Typhi*, credibly because of the presence of 2-bromobenzyl group. In the case of *E. Coli,* molecule 3a showed lowest MIC (7.97±0.9858μg/mL) due to the substitution of benzyl group while against *P. Aeruginosa,* 3i exhibited lowest MIC value (9.89±0.41μg/mL), which might be due to the presence of 2-phenylethyl group in this molecule. Against *B. Subtilis,* the lowest MIC value (8.75±0.7μg/mL) of 3d might be attributed to the substitution of 4-chlorobenzyl group on piperazine unit. Against *S. Aureus,* the compound 3j possessed lowest MIC value (15.42±0.5μg/mL) which might be an outcome of the induction of 3-phenylpropyl group in this molecule. In general we can say the most of the compounds exhibited very decent antibacterial potential against the studied bacterial strains.

Hemolytic activity

It was ascribed from the results of hemolytic study (table 2) that most of the molecules displayed very mild cytotoxicity values except 3k (73.55%), yet it was lower than the positive control (Triton-X-100). The lowest activity was shown by molecule 3a (5.10%), although it was little higher than the negative controls (PBS). So, these molecules might be further tested for their



Scheme 1: Outline for the synthesis of 2-furyl[(4-aralkyl)-1-piperazinyl]methanone derivatives (3a-j). Reagents & Conditions: (I) DMF/LiH/stirring for 4-5 hrs.

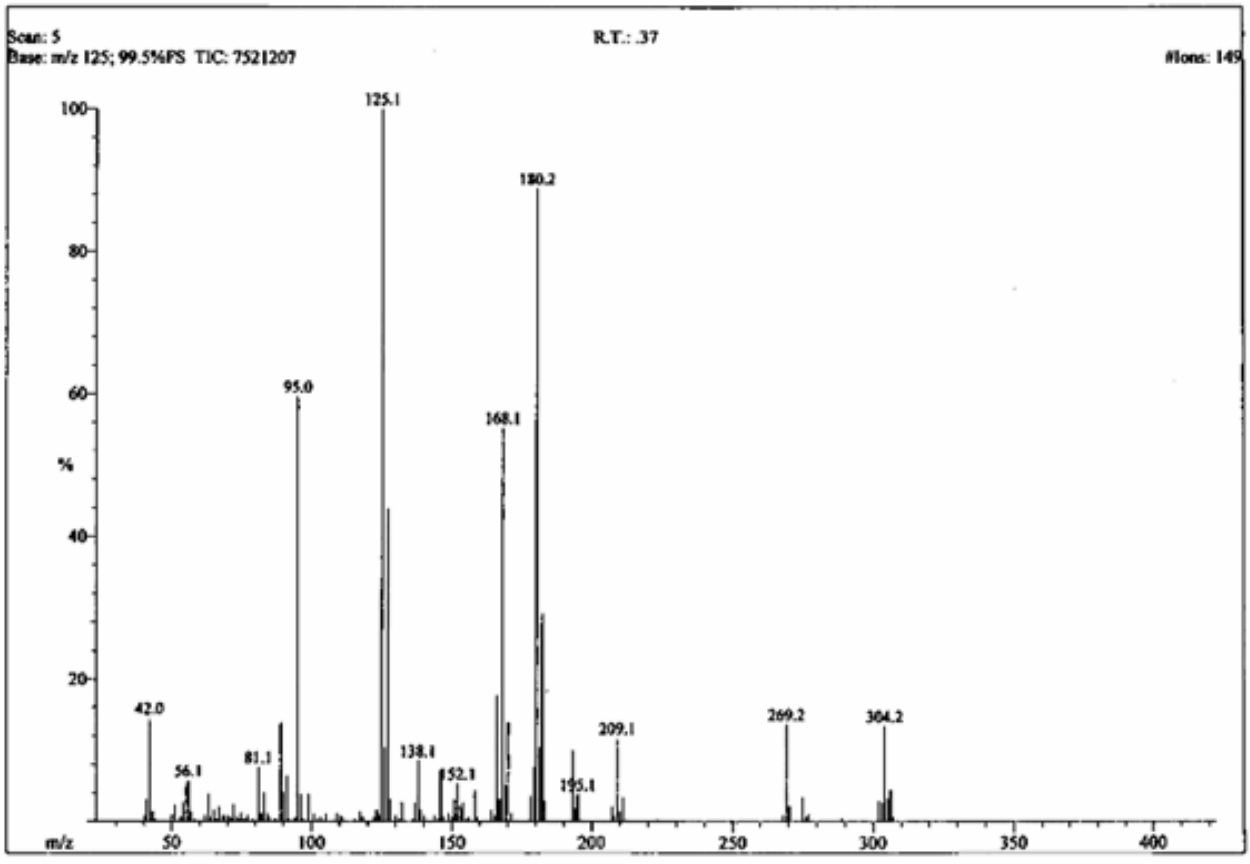


Fig. 3: EI-MS spectrum of 2-furyl[4-(2-chlorobenzyl)-1-piperazinyl]methanone (3b).

therapeutic applications in the drug designing program because of their mild cytotoxicity.

CONCLUSION

Indeed, it is the need of hour to introduce new pharmacologically active drugs to help pharmacy against the increasing resistance of microorganisms. From the present study, it can be concluded that most of the synthesized piperazine derivatives exhibited very mild cytotoxicity and displayed very decent antibacterial potential; therefore, these molecules can be utilized as suitable therapeutic entrants for bacteria related ailments.

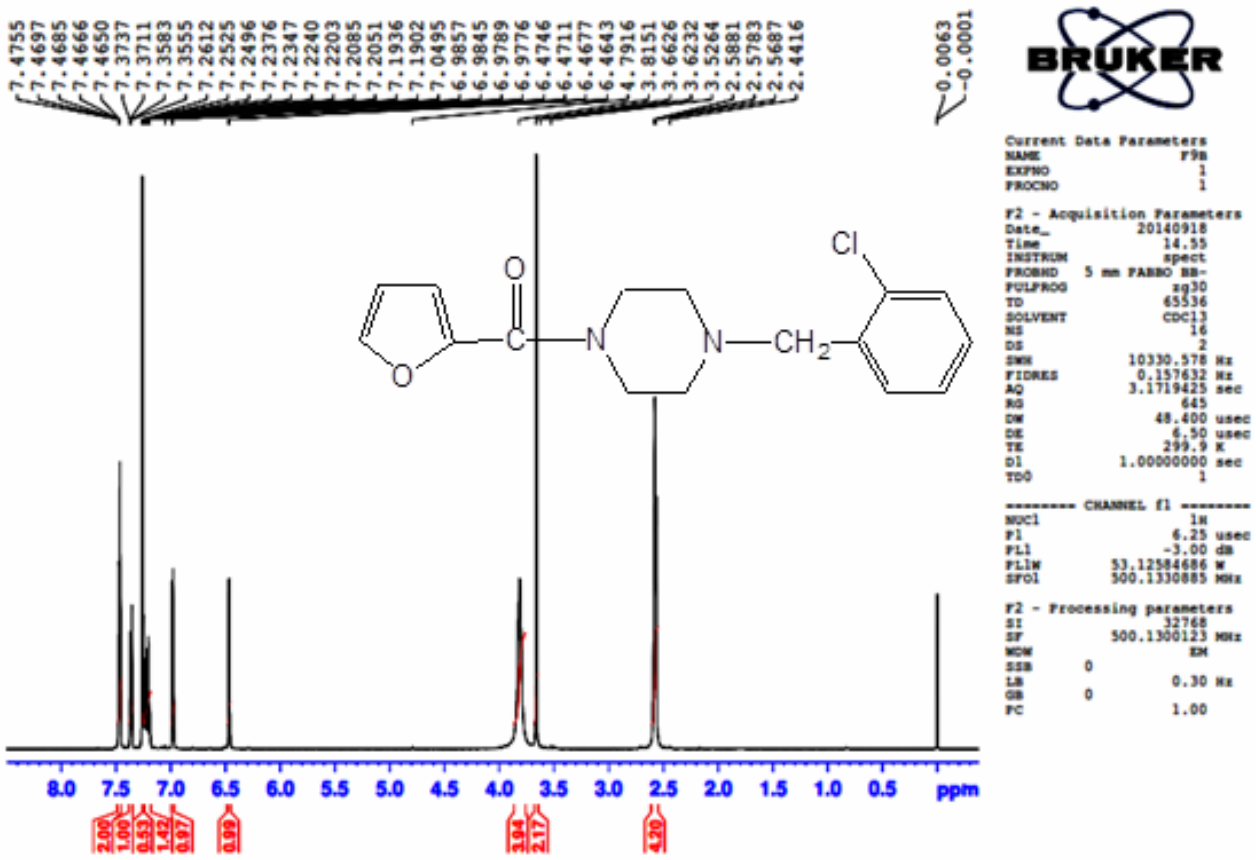


Fig. 4: [1]H-NMR spectrum of 2-furyl[4-(2-chlorobenzyl)-1-piperazinyl]methanone (3b).

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