# Synthesis of some new 2-[4-(2-furoyl)-1-piperazinyl]-*N*-aryl/aralkyl acetamides as potent antibacterial agents

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**Abstract**: In this work, a new series of 2-[4-(2-furoyl)-1-piperazinyl]-*N*-aryl/aralkyl acetamides has been synthesized and evaluated for their antibacterial potential. The synthesis was initiated by the reaction of different aryl/aralkyl amines (1a-u) with 2-bromoacetylbromide (2) to obtain *N*-aryl/aralkyl-2-bromoacetamides (3a-u). Equimolar quantities of different *N*-aryl/aralkyl-2-bromoacetamides (3a-u) and 2-furoyl-1-piperazine (4) was allowed to react in acetonitrile and in the presence of $K_2CO_3$, to form 2-[4-(2-furoyl)-1-piperazinyl]-*N*-aryl/aralkyl acetamides (5a-u). The structural elucidation was done by EI-MS, IR and $^1$H-NMR techniques of all the synthesized compounds. All of the synthesized molecules were active against various Gram positive and Gram negative bacterial strains. Among them 5o and 5c showed very excellent MIC values. The cytotoxicity of the molecules was also checked to find their utility as possible therapeutic agents, where 5c (0.51%) and 5g (1.32%) are found to be least toxic in the series.

**Keywords**: 2-Furoyl-1-piperazine; $^1$H-NMR; antibacterial activity; hemolytic activity.

## INTRODUCTION

Piperazines and their derivatives are important pharmacophores and are accompanied in many marketed drugs, e.g. the Merck HIV protease inhibitor, Crixivan (Rossen *et al.,* 1995), and drugs under development (Cliffe *et al.,* 1993). The polar and stable amide functionality is the key unit amongst organic molecules and also in naturally occurring materials e.g. peptides and proteins. It has a wide range of applications where it is used as intermediates or as an active pharmaceutical products or prodrugs (Albericio*,* 2004). The stable and polar amide functionality is an important unit among the organic molecules present in natural occurring materials (e.g., peptides and proteins). It is also found in many synthetic substances as intermediates or as active pharmaceutical products or pro drugs (Brown *et al.,* 1999). Differentially substituted polyamine moieties are found as either key pharmacophoric element or an important structural scaffold in a large number of biochemical targets across all the therapeutic areas (Rynbrandt *et al.,* 1971). In the absence of inherent steric or electronic influences, conditions that are generally required for monoacetylation are either the use of excess diamine to avoid a statistical distribution of products (starting diamine, mono- and diacetylated products), or the reaction of one amine moiety by the use of structurally sophisticated selective acetylating agents which are not in accordance with the concept of atom economy (Murakami *et al.,* 1997).

Several species of bacteria are pathogenic and cause infectious diseases (Bahiru *et al.,* 2013 and Lowy*,* 1998). The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people per year, mostly in sub-Saharan Africa (Adjuik *et al.,* 2006).

## MATERIALS AND METHODS

### *General*

The chemicals employed in this study were of analytical grade and purchased from Merck and Alfa Aesar. By using open capillary tube method, melting points were taken on Griffin & George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate and *n*-hexane (30:70) as mobile phase). Spots were visualized at 254 nm on UV lamp. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. $^1$H-NMR signals were recorded at 500 MHz in $CDCl_3$ by using Bruker spectrometer. EIMS signals were recorded by utilizing a JMS-HX-110 spectrometer.

### *Synthesis of N-aryl/aralkyl-2-bromoacetamides (3a-u)*

Aryl/aralkyl amines (15.0mmol, 1a-u) were taken in an iodine flask in 15mL of distilled water and 10% $Na_2CO_3$

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solution was added to adjust the pH at 9 and 2-bromoacetyl bromide (15.0 mmol, 2) was then added drop wise in the reaction mixture in 2-5 min. duration. After complete addition, the iodine flask was manually shaken (vigorously) & stirred at room temperature for 3-4 hours till the formation of solid precipitates. The reaction progress was monitored by thin layer chromatography (TLC). The obtained solids were filtered, washed with distilled water & dried to yield the corresponding, *N*-(aryl/aralkyl)-2-bromoacetamides (3a-u).

***Synthesis of 2-[4-(2-furoyl)-1-piperazinyl]-N-aryl/aralkyl acetamide (5a-u)***

2-Furoyl-1-piperazine (4.5 mmol, 4) solubilized in 20 mL acetonitrile was taken in a 100 mL round bottom flask, followed by addition of $K_2CO_3$ (13.5 mmol). The contents were refluxed for half an hour after which the synthesized electrophiles, *N*-aryl/aralkyl-2-bromoacetamides (4.5 mmol, 3a-u), were added & reaction mixture was refluxed for 4-5 hours. The reaction progress was monitored by thin layer chromatography (TLC). Distilled water was added in the reaction mixture to acquire the precipitates. Precipitates were filtered, washed and dried to obtain pure products 2-[4-(2-furoyl)-1-piperazinyl]-*N*-aryl/aralkyl acetamide (5a-u).

***2-[4-(2-Furoyl)-1-piperazinyl]-N-phenylacetamide (5a)***

Off white amorphous solid; Yield: 92%; m.p: 120-122°C; Mol. F.: $C_{17}H_{19}N_3O_3$; Mol. Mass: 313; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3085 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1658 (C=O str.), 1581 (C=C aromatic str.), 1196 (C-O-C bond str.), 1110 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.49 (distorted d, *J*=1.5 Hz, 1H, H-5'), 7.35-7.29 (m, 5H, H-2''' to H-6'''), 7.06 (d, *J*=3.6 Hz, 1H, H-3'), 6.49 (dd, *J*=3.6, 1.7 Hz, 1H, H-4'), 3.99 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.18 (s, 2H, $CH_2$ -2''), 2.65 (m, 4H, $CH_2$-2 & $CH_2$-6); EIMS (*m/z*): 313 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-benzylacetamide (5b)***

Light brown amorphous solid; Yield: 92 %; m.p: 150-152 °C; Mol. F.: $C_{18}H_{21}N_3O_3$; Mol. Mass: 327; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3412 (N-H stretching), 3095 (C-H str. of aromatic ring), 2892 (C-H str. of aliphatic), 1661 (C=O str.), 1589 (C=C aromatic str.), 1190 (C-O-C bond str.), 1114 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.49 (distorted d, *J*=1.3 Hz, 1H, H-5'), 7.29-7.11 (m, 5H, H-2''' to H-6'''), 7.05 (d, *J*=3.6 Hz, 1H, H-3'), 6.04 (dd, *J*=3.5, 1.7 Hz, 1H, H-4'), 4.44 (s, 2H, $CH_2$-1''''), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.22 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6); EIMS (*m/z*): 327 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 106 $[C_7H_8N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-methylphenyl)acetamide (5c)***

Brown yellow amorphous solid; Yield: 90%; m.p: 192-194°C; Mol. F.: $C_{18}H_{21}N_3O_3$; Mol. Mass: 327; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3410 (N-H stretching), 3068 (C-H str. of aromatic ring), 2870 (C-H str. of aliphatic), 1655 (C=O str.), 1580 (C=C aromatic str.), 1207 (C-O-C bond str.), 1110 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.50 (distorted d, *J*=1.2 Hz, 1H, H-5'), 7.42 (d, *J*= 7.7 Hz, 1H, H-6'''), 7.28 (d, *J*=7.7 Hz, 1H, H-3'''), 7.15 (br.t, *J*=7.6, 1.3 Hz, 1H, H-5'''), 7.10 (dd, *J*=6.6, 1.1 Hz, 1H, H-4'''), 7.04 (d, *J*=3.4 Hz, 1H, H-3'), 6.05 (dd, *J*= 3.4, 1.7 Hz, 1H, H-4'), 3.94 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.30 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.25 (s, 3H, $CH_3$-1''''); EIMS (*m/z*): 327 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 106 $[C_7H_8N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(3-methylphenyl) acetamide (5d)***

Grey brown amorphous solid; Yield: 92%; m.p: 189-191°C; Mol. F.: $C_{18}H_{21}N_3O_3$; Mol. Mass: 327; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3407 (N-H stretching), 3081 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1660 (C=O str.), 1579 (C=C aromatic str.), 1205 (C-O-C bond str.), 1115 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.48 (distorted d, *J*=1.2 Hz, 1H, H-5'), 7.39 (s, 1H, H-2'''), 7.37 (d, *J*=8.1 Hz, 1H, H-6'''), 7.19 (t, *J*=8.1 Hz, 1H, H-5'''), 7.06 (d, *J*=3.5 Hz, 1H, H-3'), 6.95 (d, *J*=7.3 Hz, 1H, H-4'''), 6.07 (dd, *J*=3.5, 1.7 Hz, 1H, H-4'), 3.92 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.26 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.31 (3H, s, $CH_3$-1''''); EIMS (*m/z*): 327 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 106 $[C_7H_8N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(4-methylphenyl)acetamide (5e)***

Off white amorphous solid; Yield: 95%; m.p: 196-198°C; Mol. F.: $C_{18}H_{21}N_3O_3$; Mol. Mass: 327; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3414 (N-H stretching), 3075 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1652 (C=O str.), 1580 (C=C aromatic str.), 1208 (C-O-C bond str.), 1110 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.40 (d, *J*=8.3 Hz, 2H, H-3'' & H-5''), 7.12 (d, *J*=8.2 Hz, 2H, H-2'' & H-6''), 7.05 (d, *J*=3.5 Hz, 1H, H-3'), 6.05 (dd, *J*=3.4, 1.7 Hz, 1H, H-4'), 3.91 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.20 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.31 (s, 3H, $CH_3$-1''''); EIMS (*m/z*): 327 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 106 $[C_7H_8N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(4-hydroxyphenyl)-N-methylacetamide (5f)***

Grey amorphous solid; Yield: 92%; m.p: 150-152°C; Mol. F.: $C_{18}H_{21}N_3O_4$; Mol. Mass: 343; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3412 (N-H stretching), 3356 (O-H stretching), 3090 (C-H str. of aromatic ring), 2895 (C-H str. of aliphatic), 1664 (C=O str.), 1589 (C=C aromatic str.), 1199 (C-O-C bond str.), 1112 (C-N-C bond str.); $^1$H-NMR (500 MHz,

$CDCl_3$, $\delta$ in ppm); 7.49 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.05 (d, *J*=3.5 Hz, 1H, H-3'), 6.85 (d, *J*=8.7 Hz, 2H, H-2''', H-6'''), 6.69 (d, *J*=8.7 Hz, 2H, H-3''' & H-5'''), 6.05 (dd, *J*=3.4, 1.7 Hz, 1H, H-4'), 3.91 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.26 (s, 2H, $CH_2$-2''), 3.14 (s, 3H, $CH_3$-1''''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6); EIMS (*m/z*): 343 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 122 $[C_7H_8NO]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethylphenyl)acetamide (5g)***

Pink brown amorphous solid; Yield: 95%; m.p: 104-106 $^oC$; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3083 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1655 (C=O str.), 1577 (C=C aromatic str.), 1198 (C-O-C bond str.), 1118 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.49 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.19 (dd, *J*=8.8, 3.1 Hz, 1H, H-6'''), 7.15 (dt, *J*=8.8, 2.1 Hz, 1H, H-5'''), 7.05 (dt, *J*=8.5, 3.1 Hz, 1H, H-4'''), 7.06 (d, *J*=3.6 Hz, 1H, H-3'), 6.96 (dd, *J*=8.8, 3.1 Hz, 1H, H-3'''), 6.05 (dd, *J*=3.4, 1.6 Hz, 1H, H-4'), 3.93 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.25 (s, 2H, $CH_2$-2''), 2.66 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.48 (q, *J*= 7.5 Hz, 2H, $CH_2$-1''''), 1.01 (t, *J*=7.5 Hz, 3H, $CH_3$-2''''); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(4-ethylphenyl)acetamide (5h)***

Off white amorphous solid; Yield: 90 %; m.p: 112-114 $^oC$; Mol. F.: $C_{29}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3093 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1656 (C=O str.), 1580 (C=C aromatic str.), 1199 (C-O-C bond str.), 1115 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.48 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 8.1 Hz, 2H, H-2''' & H-6'''), 7.03 (d, *J*=3.5 Hz, 1H, H-3'), 6.98 (d, *J*= 8.1 Hz, 2H, H-3''' & H-5'''), 6.08 (dd, *J*=3.5, 1.6 Hz, 1H, H-4'), 3.92 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.28 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.58 (q, *J*=7.6 Hz, 2H, $CH_2$-1''''), 1.18 (t, *J*=7.6 Hz, 3H, $CH_3$-2''''); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethoxyphenyl)acetamide (5i)***

Light pink amorphous solid; Yield: 88%; m.p: 165-167$^oC$; Mol. F.: $C_{29}H_{23}N_3O_4$; Mol. Mass: 357; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3088 (C-H str. of aromatic ring), 2881 (C-H str. of aliphatic), 1650 (C=O str.), 1580 (C=C aromatic str.), 1198 (C-O-C bond str.), 1115 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 8.66 (d, *J*=8.5 Hz, 1H, H-6'''), 8.05 (dd, *J*=8.1, 1.2 Hz, 1H, H-3'''), 7.59 (dt, *J*=8.9, 1.2 Hz, 1H, H-5'''), 7.49 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.19 (dt, *J*=7.6 Hz, 1H, H-4'''), 7.06 (d, *J*=3.5 Hz, 1H, H-3'), 6.05 (dd, *J*=3.5, 1.7 Hz, 1H, H-4'), 3.92 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.75 (q, *J*=7.5 Hz, 2H, $CH_2$-1''''), 3.22 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 1.16 (t, *J*=7.4 Hz, 3H, $CH_3$-2''''); EIMS (*m/z*): 357 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 136 $[C_8H_{10}NO]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(4-ethoxyphenyl)acetamide (5j)***

Light purple amorphous solid; Yield: 93 %; m.p: 170-172 $^oC$; Mol. F.: $C_{19}H_{23}N_3O_4$; Mol. Mass: 357; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3412 (N-H stretching), 3083 (C-H str. of aromatic ring), 2889 (C-H str. of aliphatic), 1662 (C=O str.), 1577 (C=C aromatic str.), 1199 (C-O-C bond str.), 1108 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 8.68 (d, *J*=8.5 Hz, 1H, H-6'''), 8.09 (dd, *J* = 8.2, 1.3 Hz, 1H, H-3'''), 7.50 (dt, *J*=8.8, 1.2 Hz, 1H, H-5'''), 7.49 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.15 (d, *J*=7.6 Hz, 1H, H-2'''), 7.06 (d, *J* = 3.5 Hz, 1H, H-3'), 6.07 (dd, *J*=3.5, 1.6 Hz, 1H, H-4'), 3.95 (q, *J*=7.6, 2H, $CH_2$-1''''), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.24 (s, 2H, $CH_2$-2''), 2.69 (m, 4H, $CH_2$-2 & $CH_2$-6), 1.36 (t, *J*=7.5, 3H, $CH_3$-2''''); EIMS (*m/z*): 357 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 136 $[C_8H_{10}NO]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-methoxycarbonylphenyl)acetamide (5k)***

Off white amorphous solid; Yield: 90 %; m.p: 134-136$^oC$; Mol. F.: $C_{19}H_{21}N_3O_5$; Mol. Mass: 371; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3412 (N-H stretching), 3080 (C-H str. of aromatic ring), 2884 (C-H str. of aliphatic), 1652 (C=O str.), 1588 (C=C aromatic str.), 1199 (C-O-C bond str.), 1110 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ / ppm); 8.81 (br.d, *J* = 8.4 Hz, 1H, H-3'''), 8.07 (dd, *J* = 8.0, 1.5 Hz, 1H, H-6'''), 7.57 (br.t, *J* = 8.6 Hz, 1H, H-5'''), 7.50 (d, *J* = 1.4 Hz, 1H, H-5'), 7.13 (br.t, *J* = 7.6 Hz, 1H, H-4'''), 7.04 (d, *J* = 3.4 Hz, 1H, H-3'), 6.51 (dd, *J* = 3.4, 1.8 Hz, 1H, H-4'), 4.04 (m, 4H, $CH_2$-3 & $CH_2$-5), 3.94 (s, 3H, $CH_3$-2''''), 3.20 (s, 2H, $CH_2$-2''), 2.75 (br.s, 4H, $CH_2$-2 & $CH_2$-6); EIMS (*m/z*): 371 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 150 $[C_8H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2,3-dimethylphenyl)acetamide (5l)***

Off white amorphous solid; Yield: 80%; m.p: 133-135$^oC$; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3415 (N-H stretching), 3070 (C-H str. of aromatic ring), 2888 (C-H str. of aliphatic), 1647 (C=O str.), 1582 (C=C aromatic str.), 1204 (C-O-C bond str.), 1116 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.60 (d, *J*=7.6 Hz, 1H, H-6'''), 7.49 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.10 (t, *J*=8.0 Hz, 1H, H-5'''), 7.06 (d, *J*=3.4 Hz, 1H, H-3'), 7.04 (d, *J*=7.6 Hz, 1H, H-4'''), 6.07 (dd, *J*=3.4, 1.7 Hz, 1H, H-4'), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.27 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.35 (s,

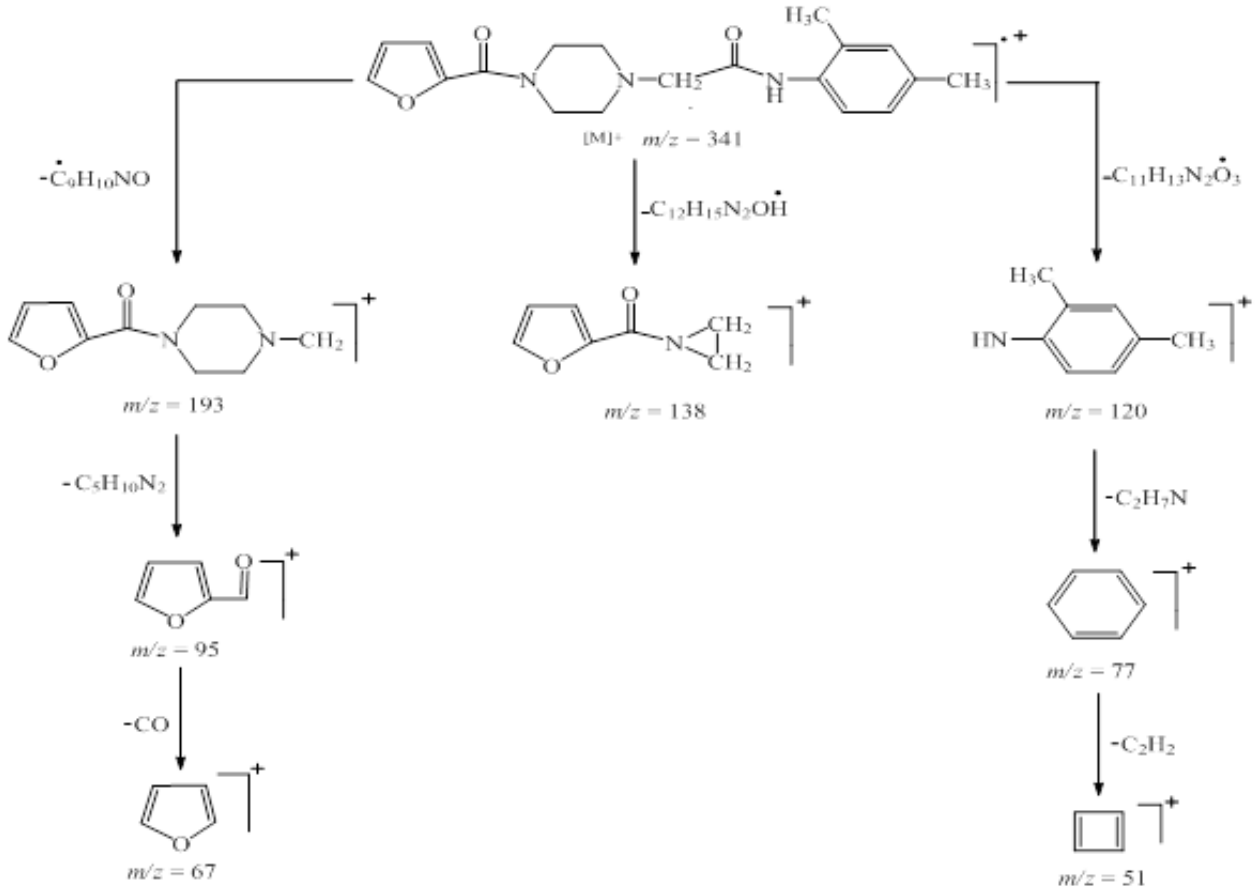



**Fig. 1**: Proposed Mass fragmentation pattern of 5m.

3H, $CH_3$-1''''), 2.15 (s, 3H, $CH_3$-2''''); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2,4-dimethylphenyl)acetamide (5m)***

Light pink amorphous solid; Yield: 85%; m.p: 147-149°C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3070 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1649 (C=O str.), 1576 (C=C aromatic str.), 1200 (C-O-C bond str.), 1114 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.73 (d, *J*=7.5 Hz, 1H, H-6'''), 7.47 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.05 (d, *J*=3.6 Hz, 1H, H-3'), 6.99 (d, *J*=7.6 Hz, 1H, H-5'''), 6.98 (s, 1H, H-3'''), 6.05 (dd, *J*=3.4, 1.6 Hz, 1H, H-4'), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.25 (s, 2H, $CH_2$-2''), 2.68 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.25 (s, 3H, $CH_3$-1''''), 2.20 (s, 3H, $CH_3$-2''''); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2,5-dimethylphenyl)acetamide (5n)***

Light brown amorphous solid; Yield: 84%; m.p: 140-142°C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3410 (N-H stretching), 3071 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1653 (C=O str.), 1581 (C=C aromatic str.), 1204 (C-O-C bond str.), 1100 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.79 (br.s, 1H, H-6'''), 7.49 (distorted d, *J*=1.5 Hz, 1H, H-5'), 7.05 (d, *J*=7.8 Hz, 1H, H-3'''), 7.04 (d, *J*=3.4 Hz, 1H, H-3'), 6.88 (br.d, *J*=7.6 Hz, 1H, H-4'''), 6.49 (dd, *J* = 3.4, 1.6 Hz, 1H, H-4'), 3.97 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.20 (s, 2H, $CH_2$-2''), 2.65 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.32 (s, 3H, $CH_3$-1''''), 2.28 (s, 3H, $CH_3$-2''''); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2,6-dimethylphenyl)acetamide (5o)***

Off white amorphous solid; Yield: 88%; m.p: 146-148°C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3411 (N-H stretching), 3078 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1655 (C=O str.), 1579 (C=C aromatic str.), 1205 (C-O-C bond str.), 1118 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.47 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.09-7.01 (m, 3H, H-3''' to H-5'''), 7.01 (d, *J*=3.5 Hz, 1H, H-3'), 6.00 (dd, *J*= 3.4, 1.7 Hz, 1H, H-4'), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.27 (s, 2H, $CH_2$-2''), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.15

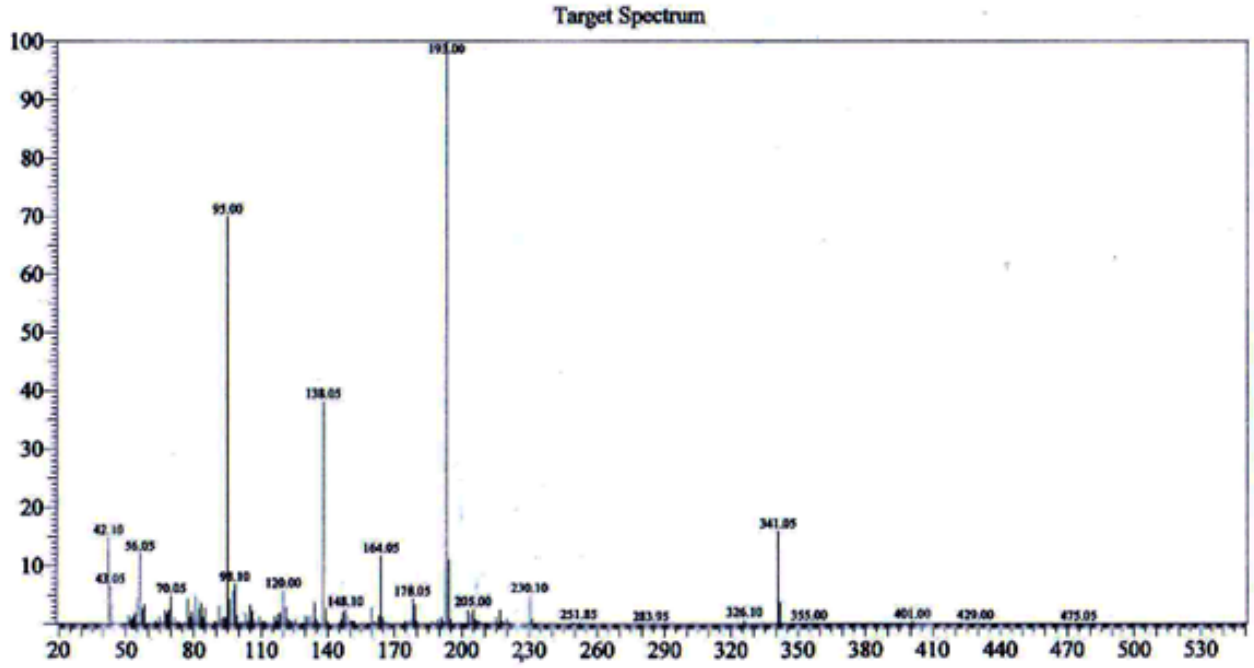



**Fig. 2**: EIMS spectrum of compound 5m.

(s, 6H, $CH_3$-1"", $CH_3$-2""); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(3,4-dimethylphenyl)acetamide (5p)***

Light brown amorphous solid; Yield: 89%; m.p: 145-147°C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3406 (N-H stretching), 3070 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1652 (C=O str.), 1587 (C=C aromatic str.), 1209 (C-O-C bond str.), 1108 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.49 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.35 (d, *J*= 8.0 Hz, 1H, H-6'"), 7.20 (s, 1H, $CH_2$-2'"), 7.05 (d, *J*=3.5 Hz, 1H, H-3'), 7.01 (d, *J*=8.0 Hz, 1H, H-5'"), 6.03 (dd, *J*= 3.4, 1.5 Hz, 1H, H-4'), 3.97 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.25 (s, 2H, H-2"), 2.66 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.24 (s, 3H, $CH_3$-1""), 2.10 (s, 3H, $CH_3$-2""); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(3,5-dimethylphenyl)acetamide (5q)***

Yellow brown amorphous solid; Yield: 90%; m.p: 128-130°C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3413 (N-H stretching), 3078 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1648 (C=O str.), 1571 (C=C aromatic str.), 1204 (C-O-C bond str.), 1117 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm): 7.49 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.14 (s, 2H, H-2'" & H-6'"), 7.06 (d, *J*=3.4 Hz, 1H, H-3'), 6.70 (s, 1H, H-4'"), 6.05 (dd, *J*=3.4, 1.6 Hz, 1H, H-4'), 3.92 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.27 (s, 2H, $CH_2$-2"), 2.68 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.20 (s, 6H, $CH_3$-1"", $CH_3$-2""); EIMS (*m/z*): 341 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 120 $[C_8H_{10}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethyl-6-methylphenyl)acetamide (5r)***

Light brown sticky amorphous solid; Yield: 88%; m.p: 150-152°C; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3086 (C-H str. of aromatic ring), 2887 (C-H str. of aliphatic), 1654 (C=O str.), 1587 (C=C aromatic str.), 1190 (C-O-C bond str.), 1112 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.50 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.07 (d, *J*= 3.5 Hz, 1H, H-3'), 7.14-6.95 (m, 3H, H-3'" to H-5'"), 6.01 (dd, *J*=3.4, 1.6 Hz, 1H, H-4'), 3.92 (br.s, 4H, H-3 & H-5), 3.25 (s, 2H, $CH_2$-2"), 2.65 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.48 (q, *J*=7.4 Hz, 2H, $CH_2$-1""), 1.98 (s, 3H, $CH_3$-3""), 1.02 (t, *J*=7.4, 3H, $CH_3$-2""); EIMS (*m/z*): 355 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 134 $[C_9H_{12}N]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(5-chloro-2-methoxyphenyl)acetamide (5s)***

Brown amorphous solid; Yield: 86%; m.p: 132-134°C; Mol. F.: $C_{18}H_{20}ClN_3O_4$; Mol. Mass: 377; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3087 (C-H str. of aromatic ring), 2886 (C-H str. of aliphatic), 1650 (C=O str.), 1581 (C=C aromatic str.), 1193 (C-O-C bond str.), 1118 (C-N-C bond str.); $^1$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 8.54 (d, *J*=2.1Hz, 1H, H-6'"), 7.49 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.06 (d, *J*=3.6 Hz, 1H, H-3'), 7.04 (dd, *J*=8.5, 2.1 Hz, H-4'"), 6.82 (d, *J*=8.5 Hz, H-3'"), 6.05 (dd, *J*=3.4, 1.7 Hz, 1H, H-4'), 3.95 (s, 3H, $H_3C$-1""), 3.90 (br.s, 4H, $CH_2$-

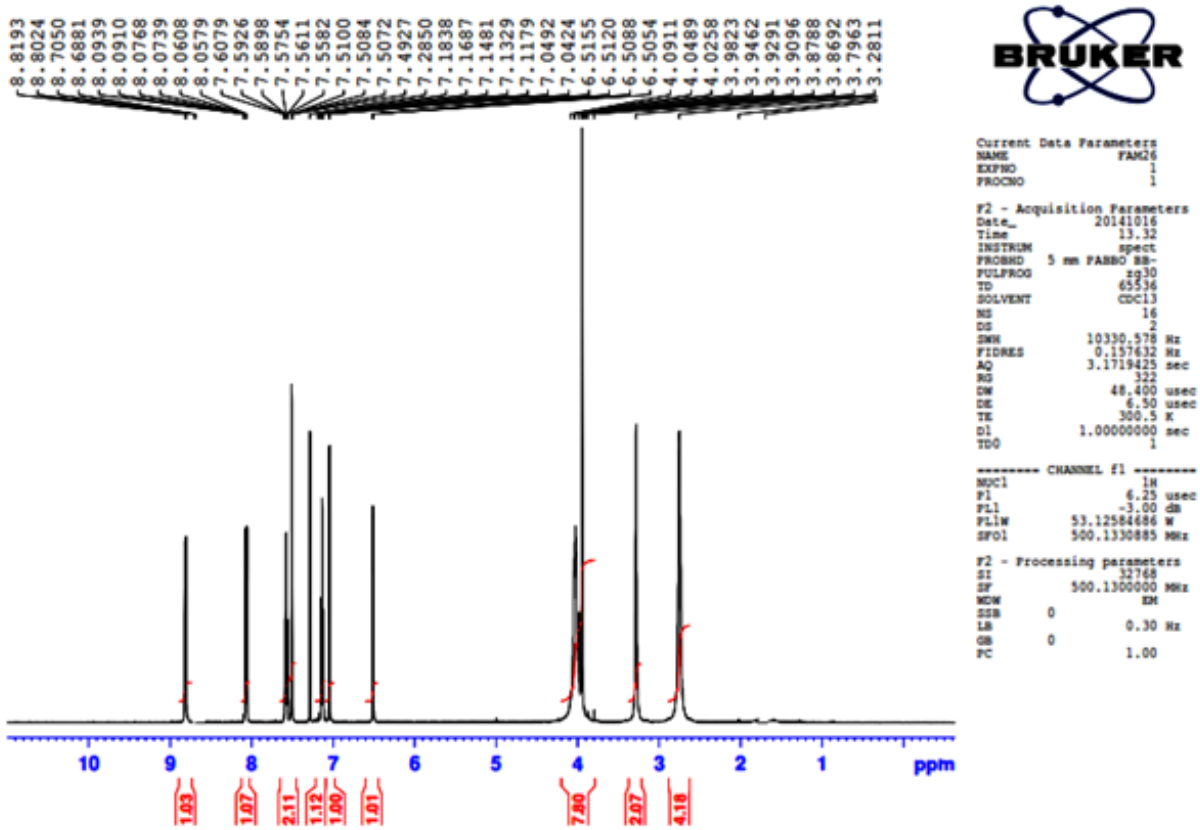



**Fig. 3**: $^{1}$H-NMR spectrum of compound **5k**.

3 & $CH_2$-5), 3.20 (s, 2H, $CH_2$-2"), 2.60 (m, 4H, $CH_2$-2 & $CH_2$-6); EIMS (*m/z*): 379 $[M + 2]^+$, 377 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 156 $[C_7H_7NOCl]^+$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-(2-methyl-6-nitrophenyl)acetamide (5t)***

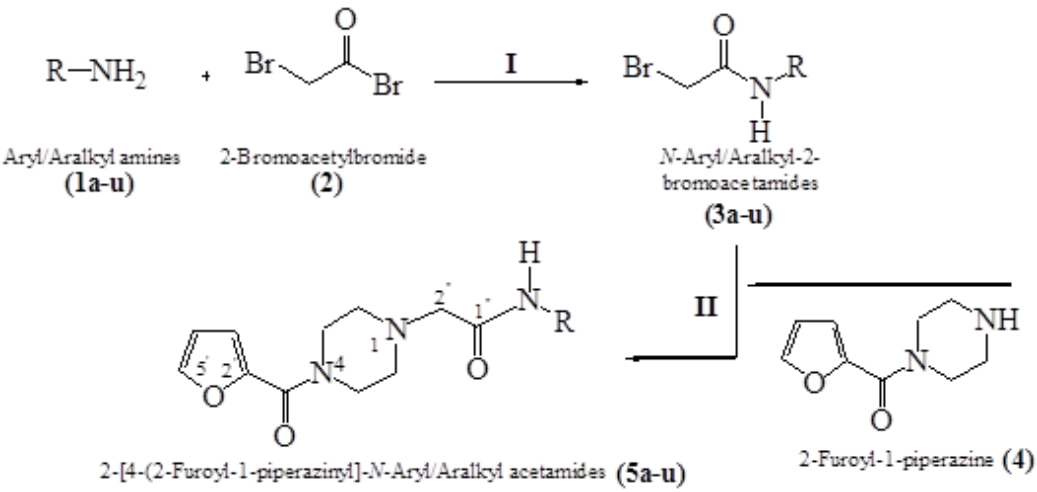
Yellow amorphous solid; Yield: 81%; m.p: 145-147$^o$C; Mol. F.: $C_{18}H_{20}N_4O_5$; Mol. Mass: 372; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3087 (C-H str. of aromatic ring), 2887 (C-H str. of aliphatic), 1650 (C=O str.), 1583 (C=C aromatic str.), 1196 (C-O-C bond str.), 1110 (C-N-C bond str.); $^{1}$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.95 (d, *J*=8.5 Hz, 1H, H-5'"), 7.49 (distorted d, *J*=1.0 Hz, 1H, H-5'), 7.29 (d, *J*=9.0 Hz, 1H, H-3'"), 7.05 (d, *J*=3.5 Hz, 1H, H-3'), 6.57 (t, *J*=9.0 Hz, 1H, H-4'"), 6.05 (dd, *J*=3.4, 1.7 Hz, 1H, H-4'), 3.91 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.25 (s, 2H, $CH_2$-2"), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 2.23 (s, 3H, $CH_3$-1'"); EIMS (*m/z*): 372 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 151 $[C_7H_7N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$, 77 $[C_6H_5]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3O]^+$.

***2-[4-(2-Furoyl)-1-piperazinyl]-N-cyclohexylacetamide (5u)***

Grey brown amorphous solid; Yield: 94%; m.p: 126-128 $^o$C; Mol. F.: $C_{17}H_{25}N_3O_3$; Mol. Mass: 319; IR (KBr, $cm^{-1}$) $\nu_{max}$: 3408 (N-H stretching), 3072 (C-H str. of aromatic ring), 2887 (C-H str. of aliphatic), 1655 (C=O str.), 1580 (C=C aromatic str.), 1195 (C-O-C bond str.), 1103 (C-N-C bond str.); $^{1}$H-NMR (500 MHz, $CDCl_3$, $\delta$ in ppm); 7.49 (distorted d, *J*=1.1 Hz, 1H, H-5'), 7.04 (d, *J*=3.5 Hz, 1H, H-3'), 6.05 (dd, *J*=3.4, 1.5 Hz, 1H, H-4'), 3.90 (br.s, 4H, $CH_2$-3 & $CH_2$-5), 3.89-3.84 (m, 1H, CH-1'"), 3.28 (s, 2H, $CH_2$-2"), 2.67 (m, 4H, $CH_2$-2 & $CH_2$-6), 1.86-1.14 (m, 10H, $CH_2$-2'" to $CH_2$-6'"); EIMS (*m/z*): 319 $[M]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 138 $[C_7H_8NO_2]^+$, 98 $[C_6H_{12}N]^+$, 95 $[C_5H_3O_2]^+$, 67 $[C_4H_3O]^+$.

***Antibacterial activity***

The antibacterial activity was done in sterile 96-wells micro plates 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*) 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 (20 µg / well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between




| Comp. | -R | Comp. | -R | Comp. | -R |
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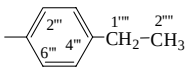
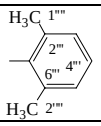
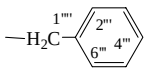
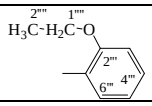
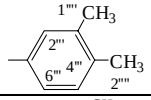
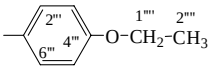
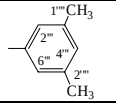
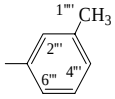
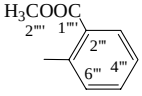
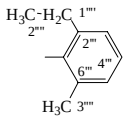
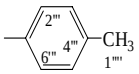
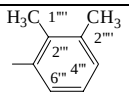
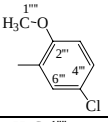
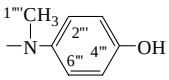
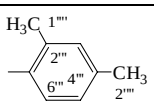
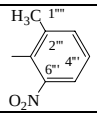
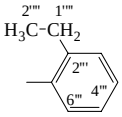
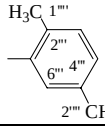
| 5a |  | 5h |  | 5o |  |
| 5b |  | 5i |  | 5p |  |
| 5c |  | 5j |  | 5q |  |
| 5d |  | 5k |  | 5r |  |
| 5e |  | 5l |  | 5s |  |
| 5f |  | 5m |  | 5t |  |
| 5g |  | 5n |  | 5u |  |

**Scheme 1**: Outline for the synthesis of 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(aryl/aralkyl) acetamides (5a-u). Reagents & conditions: (I) 10% $Na_2CO_3$, pH 9-10, Stirring at room temperature for 3-4 hours; (II) Acetonitrile, $K_2CO_3$, reflux for 4-5 hours

0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37°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 microplate reader. The % inhibition was calculated by using the formula:

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

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). Human blood was obtained from voluntaries after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. After centrifugation, separation & washing, the % RBCs lysis was computed by noting the absorbance.

## RESULTS

The purpose of this research work was to synthesize new molecules to evaluate their biological activities against different enzymes and antimicrobial agents. Further the cytotoxicity of molecules was checked. The need of hour is to introduce pharmacologically active drugs to help pharmacy because of increasing resistance of microorganisms for existing drugs. In the demoed research work, different 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(substituted)acetamides (5a-u) were synthesized in a row of steps. The synthesis was initiated by the treatment of different aryl/aralkyl amines (1a-u) with 2-bromoacetyl bromide (2) to obtain solid *N*-aryl/aralkyl-2-bromoacetamide (3a-u), which were collected through filtration. Then, equimolar ratios of different *N*-aryl/aralkyl-2-bromoacetamide (3a-u) and 2-furoyl-1-piperazine (4) were allowed to react in acetonitrile and in the presence of base, $K_2CO_3$, to form the target compounds, (5a-u; Scheme 1) where all were screened for antimicrobial & hemolytic activities.

## DISCUSSION

The compound 5m was synthesized as light pink amorphous solid with melting point of 147-149$^o$C and Mol. F.: of $C_{19}H_{23}N_3O_3$, that was confirmed by EI-MS with $[M]^+$ peak of 341. The distinct peak at *m/z* 193 was related to 2-Furoyl-1-piperazine-*N*-methylene group and that at *m/z* 95 to the furan-2-carbaldehyde part of the molecule. The fragmentation pattern and mass spectrum of this molecule is given in (fig. 1 & 2). In IR spectrum, characteristic peaks appeared at 3408 (N-H stretching), 3070 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1649 (C=O str.), 1576 (C=C aromatic str.), 1200 (C-O-C bond str.), 1114 (C-N-C bond str.) confirming the presence of acetamide and 2-furoyl-1-piperazine ring. . The compound 5k was synthesized as off white amorphous solid with melting point of 134-136$^o$C and Mol. F.: of $C_{19}H_{21}N_3O_5$, In $^1$H-NMR spectrum four signals of aromatic proton appeared at $\delta$ 8.81 (br.d, $J$ = 8.4 Hz, 1H, H-3'''), 8.07 (dd, $J$ = 8.0, 1.5 Hz, 1H, H-6'''), 7.57 (br.t, $J$=8.6 Hz, 1H, H-5'''), 7.13 (br.t, $J$=7.6 Hz, 1H, H-4'''), were typical for 1,2-disubstituted aromatic ring. Furan ring showed three peaks in aromatic region at $\delta$ 7.50 (d, $J$=1.4 Hz, 1H, H-5'), 7.04 (d, $J$ = 3.4 Hz, 1H, H-3'), 6.51 (dd, $J$=1.8, 3.4 Hz, 1H, H-4'), In the aliphatic region of the $^1$H-NMR spectrum, signals appeared at $\delta$ 4.04 (m, 4H, $CH_2$-2 & $CH_2$-6) and 2.75 (br.s, $CH_2$-3 & $CH_2$-5) for piperazine ring 3.94 (s, 3H, $CH_3$-2''''), for methoxycarbonyl group, and 3.20 (s, 2H, $CH_2$-1'') for methylene group of acetamide. The $^1$H-NMR spectrum of this molecule is given in (fig. 3). On the basis of these spectral evidences, the structure for compound 5k was appointed as 2-[4-(2-Furoyl)-1-piperazinyl]-*N*-(2-methoxycarbonylphenyl)acetamide. All the synthesized molecules, (5a-u), were characterized by IR, $^1$H-NMR and EI-MS spectral analysis.

***Antibacterial activity***

All the synthesized molecules were screened against Gram bacteria and were found to be excellent to good inhibitors. The results are tabulated as MIC values in (table 1). All of the synthesized compounds showed activities against the both Gram-positive and Gram-negative bacterial strains. Against *S. typhi* the lowest MIC value is shown by 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2-methylphenyl)acetamide (5c) 7.98±0.33 respectively most probably due to the presence of 2-methylphenyl groups. In the case of *E. coli* molecules 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2-methylphenyl)acetamide (5c) showed lowest MIC value 9.21±0.75 credibly because of the presence of 2-methylphenyl group. In the case of *P. aeruginosa* molecules 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2-ethoxyphenyl)acetamide (5i) showed lowest MIC value 8.15±0.70 may be due to presence of 2-ethoxyphenyl group. In the case of *B. subtilis* molecule 2-[4-(2-furoyl)-1-piperazinyl]-*N*-cyclohexylacetamide (5u) showed lowest MIC value 8.16±0.54 respectively predominantly because of the presence of cyclohexyl group. Against *S. aureus* molecule 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2,5-dimethylphenyl)acetamide (5n) showed lowest MIC value 7.36±0.70 respectively it may be due to induction of 2,5-dimethylphenyl group. Overall 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2,6-dimethylphenyl) acetamide (5o) and 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(2-methylphenyl)acetamide (5c) respectively showed excellent MIC value regarding to following order in all bacterial strains *S. typhi >B. subtilis >P. aeruginosa> S. aureus >E. coli* and *S. typhi >S. aureus >P. aeroginosa> E. coli >B. subtilis* due to the presence of 2,6-dimethylphenyl and 2-methylphenyl groups which are responsible for more interaction and may be an important discovery for the antibacterial inhibition potential.

***Hemolytic activity***

The highest hemolytic activity was shown by 5u (83.97 %) but lesser than the positive control (Triton-X-100). The lowest activity was shown by 5c (0.51%) but higher than the negative controls (PBS). Couple of molecules is

**Table 1**: Antibacterial activity (MIC) and hemolytic activity of 2-[4-(2-furoyl)-1-piperazinyl]-*N*-(aryl/aralkyl) acetamides (5a-u).

| Compound | MIC (µM) | | | | | Hemolytic activity |
|---|---|---|---|---|---|---|
| | S. typhi (-) | E. coli (-) | P. aeruginosa (-) | B. subtilis (+) | S. aureus (+) | % age |
| 5a | 9.54±0.78 | 12.78±0.66 | 12.50±0.15 | 11.78±0.58 | 10.23±0.49 | 23.74 |
| 5b | 9.09±0.65 | 10.64±0.33 | 9.12±0.59 | 8.57±0.50 | 9.10±0.15 | 9.62 |
| 5c | 7.98±0.33 | 9.21±0.75 | 9.13±0.46 | 9.77±0.10 | 8.98±0.13 | 0.51 |
| 5d | 8.90±0.67 | 14.78±0.53 | 11.78±0.20 | 8.22±0.90 | 8.57±0.46 | 54.76 |
| 5e | 8.76±0.80 | 9.34±0.35 | 10.19±0.56 | 8.96±0.50 | 9.41±0.54 | 34.94 |
| 5f | 8.98±0.24 | 10.99±0.13 | 10.43±0.68 | 9.12±0.53 | 8.95±1.92 | 10.74 |
| 5g | 8.45±0.72 | 10.13±0.56 | 8.87±0.15 | 9.53±0.34 | 8.68±0.20 | 1.32 |
| 5h | 8.98±0.32 | 10.20±0.43 | 9.76±0.32 | 8.79±0.65 | 8.63±0.40 | 21.28 |
| 5i | 8.87±0.31 | 10.89±0.19 | 8.15±0.70 | 8.91±0.12 | 8.00±0.30 | 22.43 |
| 5j | 9.43±0.59 | 10.65±0.90 | 10.00±0.45 | 9.80±0.45 | 8.45±0.15 | 76.65 |
| 5k | 8.79±0.26 | 10.77±0.29 | 11.20±0.54 | 8.67±0.52 | 8.45±1.02 | 2.27 |
| 5l | 8.11±0.78 | 10.32±0.23 | 8.86±0.50 | 8.43±0.65 | 7.89±0.45 | 1.94 |
| 5m | 8.45±0.90 | 10.56±0.12 | 8.52±0.16 | 9.22±0.50 | 7.65±0.50 | 30.89 |
| 5n | 8.90±0.65 | 9.34±0.56 | 8.97±0.90 | 8.72±0.48 | 7.36±0.70 | 1.45 |
| 5o | 8.01±0.24 | 9.79±0.23 | 8.46±0.56 | 8.46±0.52 | 8.80±0.57 | 21.05 |
| 5p | 8.23±0.69 | 12.45±0.37 | 10.54±0.54 | 8.90±0.46 | 8.96±0.41 | 7.06 |
| 5q | 8.13±0.67 | 9.33±0.45 | 8.61±0.40 | 9.49±0.20 | 8.14±0.57 | 21.80 |
| 5r | 9.12±0.61 | 10.21±0.50 | 9.01±0.56 | 9.59±0.38 | 8.86±0.10 | 38.76 |
| 5s | 9.23±0.65 | 9.78±0.67 | 8.94±0.50 | 8.67±0.14 | 8.23±0.69 | 9.04 |
| 5t | 8.60±0.34 | 10.39±0.90 | 10.00±0.50 | 8.50±0.23 | 8.57±0.34 | 6.29 |
| 5u | 8.11±0.24 | 9.42±0.10 | 10.63±0.78 | 8.16±0.54 | 8.43±0.41 | 83.97 |
| Ciprofloxacin | 7.83±0.78 | 8.01±0.12 | 7.98±0.89 | 7.22±0.67 | 7.00±1.54 | |
| PBS | | | | | | 0.05 |
| Triton | | | | | | 98.76 |

highly toxic while all other molecules might be further tested for their application in drug designing program because of moderate toxicity (table 1).

## CONCLUSION

The synthesized molecules were confirmed by spectral data. All of the synthesized molecules were active against Gram-positive and Gram-negative bacterial strains, while (5o) and (5c) respectively showed excellent MIC value. The cytotoxic results were also processed to evaluate the cytotoxicity of the synthesized molecules and were found that 5c (0.51%) and 5g (1.32%) are least toxic. Hence, these molecules are recommended for the drug designing program for the pharmaceutical industries of developing countries.

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## REFERENCES

Adjuik M, Smith T, Clark S, Todd J, Garrib A, Kinfu Y, Kahn K, Mola M, Ashraf A, Masanja H, Adazu U, Sacarlal J, Alam N, Marra A, Gbangou A, Mwageni E and Binka F (2006). Cause-specific mortality rates in sub-Saharan Africa and Bangladesh. *Bull. World Health Organ*., **84**: 181-188.

Albericio F (2004). Developments in peptide and amide synthesis. *Curr. Opin. Chem. Biol*., **8**: 211-221.

Bahiru AA, Emire SA and Ayele AK (2013). The prevalence of antibiotic resistant Escherichia coli isolates from fecal and water sources. *Acad. J. Microbiol. Res.,* **1**: 01-10.

Brown W (1999). Taltirelin: Tanabe Seiyaku. *Idrugs*., **2**: 1059-1068.

Cliffe IA, Brightswell CI, Fletcher A, Forster EA, Mansell HL, Reilly Y, Routledge C and White AC (1993). (S)-*N*-tert-Butyl-3-(4-(2-methoxyphenyl) piperazin-1-yl)-2-phenylpropanamide [(S)-WAY-100135]: a selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. *J. Med. Chem*., **36**: 1509-1510.

Kaspady M, Narayanaswamy VK, Raju M and Rao GK (2009). Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioesters. *Letters in Drug Design & Discovery*., **6**: 21-28.

Lowy FD (1998). Is Staphylococcus aureus an intracellular pathogen. *Trends Microbiol*., **8**: 341-344.

Murakami Y, Kondo K, Miki K, Aldyama Y, Watanabe T and Yokoyama Y (1997). The ortho-substituted *N*,*N*-diacetylaniline as a selective acylating reagent. *Tetrahedron Lett*., **38**: 3751-3754.

Powell WA, Catranis CM and Maynard CA (2000). Design of self-processing antimicrobial peptide for plant protection. *Lett. Appl. Microbiol*., **31**: 163-168.

Rossen K, Weissman SA, Sagar J, Reamer RA, Askin DA, Volante RP and Reider PJ (1995). Asymmetric hydrogenation of tetrahydropyrazines: Synthesis of (S)-piperazine-2-tert-butylcarboxamide, an intermediate in the preparation of the HIV protease inhibitor indinavir. *Tetrahedron Lett*., **36**: 6419-6422.

Rynbrandt R and Schmidt F (1971). *Cis*-1-(2-(*p*-anisidinomethyl)cyclohexyl)piperidine and related compounds, Oral hypoglycemic agents. *J. Med. Chem*., **14**: 54-56.

Sharma P, Sharma JD (2001). In vitro hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *J. Ethnopharmacol*., **74**: 239-243.

WHO mortality data (2002), WHO Mortality Database: www.who.int/healthinfo/cod/en/index2.html

Yang CR, Zang Y, Jacob MR, Khan SI, Zhang YJ and Li XC (2006). Antifungal activity of C-27 steroidal saponins. *Antimicrobial Agents and Chemotherapy*, **50**: 1710-1714.