Tropical Journal of Pharmaceutical Research July 2018; 17 (7): 1397-1406
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i7.25

Original Research Article

Synthesis of 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(substituted)propanamides as promising antibacterial agents with mild cytotoxicity

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Sent for review: 31 October 2016 *Revised accepted: 15 June 2018*

Abstract

Purpose: *To evaluate the antibacterial activity and cytotoxicity of a series of molecules with amalgamation of furoyl, piperazine and amide moieties.*
Methods: *New derivatives, namely 3-[4-(2-furoyl)-1-piperazinyl]-N-(substituted) propanamides, were synthesized and evaluated for their antibacterial activity and toxicity to mammalian cells. The synthesis was initiated by treating different aryl/aralkyl amines (**1a-u**) with 3-bromopropionyl chloride (**2**) to obtain the solid electrophiles **3a-u**, which were collected by filtration. Thereafter, the different N-aryl/aralkyl-3-bromopropionamides (**3a-u**) and 2-furoyl-1-piperazine (**4**) at equimolar ratios were allowed to react in acetonitrile and in the presence of a base, K_2CO_3, to form the target compounds, **5a-u**. Structural elucidation was carried out using EI-MS (electron impact mass spectrometry), IR (infrared) and 1H-NMR (proton nuclear magnetic resonance). The antibacterial activity of the synthesized compounds was evaluated against various bacterial strains. Furthermore, hemolysis was determined to assess cytotoxicity using bovine red blood cells.*
Results: *Molecules **5g**, **5a**, **5p**, **5g** and **5i** were found to be potent agents against S. aureus, S. typhi, P. aeruginosa, E. coli and B. subtilis with respective minimum inhibitory concentration (MIC) values of 8.34 ± 0.55, 8.37 ± 0.12, 8.65 ± 0.57, 8.97 ± 0.12 and 9.24 ± 0.50 µM, compared to 7.80 ± 0.19, 7.45 ± 0.58, 7.14 ± 0.58, 7.16 ± 0.58 and 7.29 ± 0.90 µM for the reference standard, ciprofloxacin. The most active compounds, **5a**, **5g**, **5i** and **5p**, showed a hemolysis of 15.48, 8.03, 5.52 and 4.35 %, respectively.*
Conclusion: *The synthesized compounds exhibit good antibacterial activity. The hemolysis data indicate that these compounds have a low toxicity level. However, in vivo studies are required to ascertain their potentials as new drug candidates.*

Keywords: *4-(2-Furoyl)-1-piperazine, ^{1}H-NMR, EI-MS, Antimicrobial activity, Hemolytic activity*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

There are wide possibilities for the utilization of furan derivatives as biologically active compounds. Piperazine derivatives are known to possess the properties of triple reuptake inhibitors [1], norovirus inhibitors [2], cannabinoid CB1 receptor agonists [3], antagonists for the melanocortin-4 receptor [4], antimicrobials [5], enzyme inhibitors [6], etc. This moiety has also found applications in the field of engineering [7] and polymers [8]. The amides are known to possess valuable biological activities [9-11].

These valuable biological activities of furan and amide moieties prompted us to introduce some new molecules with the amalgamation of these moieties and to evaluate their potential as antibacterial agents.

EXPERIMENTAL

General

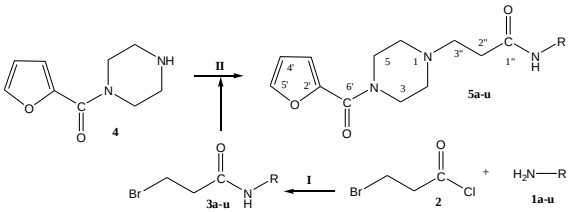
Chemicals and solvents were from Sigma Aldrich and Alfa Aesar and purchased through a local supplier. All solvents were further used without purification. A Griffin and George melting point apparatus was utilized to read the melting points of synthesized compounds, and melting points were uncorrected. Thin layer chromatography (TLC) run on silica-coated aluminum plates helped to confirm product formation. The mobile phase was a mixture of ethyl acetate and *n*-hexane and spots were visualized under a UV lamp at 254 nm. A Jasco-320-A spectrometer was used to record IR peaks by the KBr pellet method. Bruker spectrometers were used to record ^{1}H-NMR signals at 500 MHz in $CHCl_3$-d_1, and a JMS-HX-110 spectrometer recorded EIMS signals.

General procedure for synthesis of *N*-aryl/aralkyl-3-bromopropanamides (3a-u)

A calculated amount of substituted aryl/aralkyl/alkyl amines (**1a-u**; 15.0 mmol) was placed in a 250-mL iodine flask containing distilled water (15.0 mL) and 10 % Na_2CO_3. The pH was adjusted to 9-10. 3-Bromopropionyl chloride (**2**; 15.0 mmol) was then added dropwise to the reaction mixture for 2-5 min. A solid precipitate appeared on vigorous shaking. TLC was used to monitor the reaction progress. The products, *N*-(aryl/aralkyl/alkyl)-3-bromopropanamides (**3a-u**), were collected by filtration and washed and dried.

General procedure for synthesis of 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(aryl/aralkyl/alkyl) propanamides (5a-u)

4-(2-Furoyl)-1-piperazine (**4**; 0.1 g, 4.5 mmol) and solid K_2CO_3 (13.5 mmol) were dissolved in acetonitrile (18 mL) in a round-bottom flask (100 mL). After refluxing for 0.5 h, the electrophiles *N*-aryl/aralkyl/alkyl-3-bromopropanamides (**3a-u**; 4.5 mmol) were added and refluxing was continued for 3-4 h. The reaction was monitored by TLC. The final compounds were acquired after the addition of excess distilled water, and the precipitate was filtered out, washed and dried.



Scheme 1: Outline for the synthesis of 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(substituted) propanamides (5a-u). For –R group, see structures of derivatives in Figure 1.

Reagents and conditions

(I) 10 % sodium carbonate, pH = 9 - 10, stir for 3 - 4 h, room temperature; (II) acetonitrile, potassium carbonate, reflux for 0.5 h; and (III) reflux for 3 - 4 h.

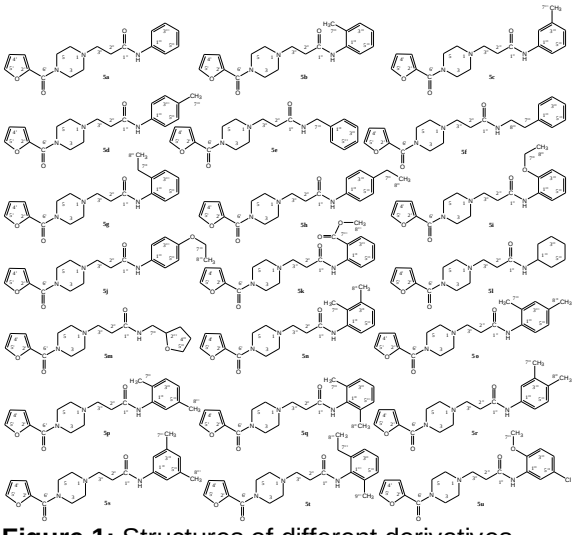


Figure 1: Structures of different derivatives

Antibacterial activity assay

The broth microdilution method was employed to evaluate the antibacterial activity of the synthesized compounds. The synthesized compounds at different concentrations were

mixed with selected bacterial strains, and the change in absorbance before and after incubation was noted [12,13].

Evaluation of hemolytic activity

Hemolytic activity was studied using a previously reported method [14,15]. A blood sample was collected from a cow, and red blood cells (RBCs) were isolated for use. Triton X-100 and phosphate buffer saline (PBS) were employed as the positive and negative controls, respectively.

Statistical analysis

Microsoft Excel 2010 was utilized for statistical analysis of results which were expressed as mean ± SEM (n = 3).

RESULTS

The target molecules, 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(aryl/aralkyl/alkyl)propanamides (**5a-u**), were synthesized as outlined in Scheme-1 and structures of different derivatives are also shown in Figure 1.

Spectral characteristics of synthesized molecules (5a-u)

3-[4-(2-Furoyl)-1-piperazinyl]-N-phenylpropanamide (5a)

Gray-brown amorphous solid; yield: 90 %; m.p.: 105-107 ^{o}C; Mol. F.: $C_{18}H_{21}N_3O_3$; Mol. Mass.: 327; IR (KBr, cm^{-1}) *v*: 3406 (N-H), 3086 (Ar C-H), 2882 (R C-H), 1657 (C=O), 1582 (Ar C=C), 1197 (C-O-C), 1110 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.48 (distorted d, *J* = 1.5 Hz, 1H, H-5'), 7.33-7.27 (m, 5H, H-2''' to H-6'''), 7.04 (d, *J* = 3.5 Hz, 1H, H-3'), 6.49 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.94 (br.s, 4H, CH_2-3, CH_2-5), 2.77 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.65 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.63 (m, 4H, CH_2-2, CH_2-4); EIMS (*m/z*): 327 $[M]^+$, 216 $[C_{13}H_{16}N_2O]^{\bullet+}$, 203 $[C_{12}H_{15}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 147 $[C_9H_9NO]^+$, 138 $[C_7H_8NO_2]^+$, 119 $[C_7H_5NO]^{\bullet+}$, 95 $[C_5H_3O_2]^+$, 93 $[C_6H_7N]^{\bullet+}$.

3-[4-(2-Furoyl)-1-piperazinyl]-N- (2-methylphenyl) propanamide (5b)

Light brown-pink amorphous solid; yield: 91 %; m.p.: 120-122 ^{o}C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, cm^{-1}) *v*: 3411 (N-H), 3069 (Ar C-H), 2877 (R C-H), 1657 (C=O), 1576 (Ar C=C), 1204 (C-O-C), 1114 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.39 (d, *J* = 7.6 Hz, 1H, H-6'''), 7.24 (d, *J* = 7.6 Hz, 1H, H-3'''), 7.14 (br.t, *J* = 7.6 Hz, 1H, H-5'''), 7.11 (dt, *J* = 6.6, 1.0 Hz, 1H, H-4'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3'') 2.25 (s, 3H, CH_3-7'''); EIMS (*m/z*): 341 $[M]^+$, 230 $[C_{14}H_{18}N_2O]^{\bullet+}$, 217 $[C_{13}H_{17}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 161 $[C_{10}H_{11}NO]^+$, 138 $[C_7H_8NO_2]^+$, 133 $[C_8H_7NO]^{\bullet+}$, 107 $[C_7H_9N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(3-methylphenyl) propanamide (5c)

Grey-white crystalline solid; yield: 92 %; m.p: 116-118 ^{o}C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, cm^{-1}) *v*: 3405 (N-H), 3083 (Ar C-H), 2882 (R C-H), 1656 (C=O), 1575 (Ar C=C), 1209 (C-O-C), 1118 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.36 (br.s, 1H, H-2'''), 7.31 (br.d, *J* = 8.0 Hz, 1H, H-6'''), 7.17 (br.t, *J* = 8.0 Hz, 1H, H-5'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.89 (br.d, *J* = 7.2 Hz, 1H, H-4'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.31 (3H, s, CH_3-7'''); EIMS (*m/z*): 341 $[M]^+$, 230 $[C_{14}H_{18}N_2O]^{\bullet+}$, 217 $[C_{13}H_{17}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 161 $[C_{10}H_{11}NO]^+$, 138 $[C_7H_8NO_2]^+$, 133 $[C_8H_7NO]^{\bullet+}$, 107 $[C_7H_9N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

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

White crystalline solid; yield: 94 %; m.p: 122-124 ^{o}C; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, cm^{-1}) *v*: 3415 (N-H), 3072 (Ar C-H), 2874 (R C-H), 1651 (C=O), 1586 (Ar C=C), 1209 (C-O-C), 1108 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.40 (d, *J* = 8.3 Hz, 2H, H-2''', H-6'''), 7.12 (d, *J* = 8.2 Hz, 2H, H-3''', H-5'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.31 (s, 3H, CH_3-7'''); EIMS (*m/z*): 341 $[M]^+$, 230 $[C_{14}H_{18}N_2O]^{\bullet+}$, 217 $[C_{13}H_{17}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 161 $[C_{10}H_{11}NO]^+$, 138 $[C_7H_8NO_2]^+$, 133 $[C_8H_7NO]^{\bullet+}$, 107 $[C_7H_9N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-benzylpropanamide (5e)

Light-brown, sticky amorphous solid; yield: 90 %; Mol. F.: $C_{19}H_{23}N_3O_3$; Mol. Mass: 341; IR (KBr, cm^{-1}) *v*: 3410 (N-H), 3092 (Ar C-H), 2891 (R C-H), 1661 (C=O), 1588 (Ar C=C), 1195 (C-O-C), 1112 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'),

7.27-7.19 (m, 5H, H-2''' to H-6'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 4.41 (s, 2H, CH_2-7'''); 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''); EIMS (*m/z*): 341 $[M]^+$, 230 $[C_{14}H_{18}N_2O]^{\bullet+}$, 217 $[C_{13}H_{17}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 161 $[C_{10}H_{11}NO]^+$, 138 $[C_7H_8NO_2]^+$, 133 $[C_8H_7NO]^{\bullet+}$, 107 $[C_7H_9N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2-phenylethyl) propanamide (5f)

Brown amorphous solid; yield: 90 %; M.P: 107-109 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3410 (N-H), 3090 (Ar C-H), 2880 (R C-H), 1659 (C=O), 1581 (Ar C=C), 1197 (C-O-C), 1115 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.12-7.10 (m, 5H, H-2''' to H-6'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 3.37 (t, *J* = 6.2 Hz, 2H, CH_2-8'''), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.13 (t, *J* = 6.2 Hz, 2H, CH_2-7'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethylphenyl) propanamide (5g)

Light-brown amorphous solid; yield: 90 %; m.p: 95-97 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3407 (N-H), 3087 (Ar C-H), 2883 (R C-H), 1651 (C=O), 1580 (Ar C=C), 1200 (C-O-C), 1115 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.18 (dd, *J* = 9.0, 3.0 Hz, 1H, H-6'''), 7.14 (dt, *J* = 8.0, 2.0 Hz, 1H, H-5'''), 7.06 (dt, *J* = 8.5, 3.0 Hz, 1H, H-4'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.95 (dd, *J* = 8.0, 3.0 Hz, 1H, H-3'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2-7'''), 1.01 (t, *J* = 7.5 Hz, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(4-ethylphenyl) propanamide (5h)

Brown amorphous solid; yield: 90 %; m.p: 106-108 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3409 (N-H), 3090 (Ar C-H), 2881 (R C-H), 1658 (C=O), 1581 (Ar C=C), 1197 (C-O-C), 1118 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.06 (d, *J* = 8.0 Hz, 2H, H-2''', H-6'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.96 (d, *J* = 8.0 Hz, 2H, H-3''', H-5'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.54 (q, *J* = 7.5 Hz, 2H, CH_2-7'''), 1.15 (t, *J*= 7.5 Hz, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethoxyphenyl) propanamide (5i)

Light-brown amorphous solid; yield: 90%; M.P: 137-139 ^oC; Mol. F.: $C_{20}H_{25}N_3O_4$; Mol. Mass: 371; IR (KBr, cm^{-1}) *v*: 3409 (N-H), 3086 (Ar C-H), 2881 (R C-H), 1658 (C=O), 1583 (Ar C=C), 1197 (C-O-C), 1116 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 8.63 (br.d, *J* = 8.4 Hz, 1H, H-6'''), 8.00 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3'''), 7.55 (dt, *J* = 8.8, 1.2 Hz, 1H, H-5'''), 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.12 (dt, *J* = 8.6, 1.2 Hz, 1H, H-4'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 3.73 (q, *J* = 7.5 Hz, 2H, CH_2-7'''), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 1.14 (t, *J* = 7.5 Hz, 3H, CH_3-8'''); EIMS (*m/z*): 371 $[M]^+$, 260 $[C_{15}H_{20}N_2O_2]^{\bullet+}$, 247 $[C_{14}H_{19}N_2O_2]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 191 $[C_{11}H_{13}NO_2]^+$, 163 $[C_9H_9NO_2]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 137 $[C_8H_{11}NO]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(4-ethoxyphenyl) propanamide (5j)

Tea-pink amorphous solid; yield: 90 %; M.P: 142-144 ^oC; Mol. F.: $C_{20}H_{25}N_3O_4$; Mol. Mass: 371; IR (KBr, cm^{-1}) *v*: 3412 (N-H), 3084 (Ar C-H), 2887 (R C-H), 1659 (C=O), 1583 (Ar C=C), 1195 (C-O-C), 1112 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.93 (d, *J* = 8.4 Hz, 1H, H-2''', H-6'''), 6.75 (d, *J* = 8.0 Hz, 1H, H-3''', H-5'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.94 (q, *J* = 7.5, 2H, CH_2-7'''), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 1.32 (t, *J* = 7.5, 3H, CH_3-8'''); EIMS (*m/z*): 371 $[M]^+$, 260 $[C_{15}H_{20}N_2O_2]^{\bullet+}$, 247 $[C_{14}H_{19}N_2O_2]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 191 $[C_{11}H_{13}NO_2]^+$, 163 $[C_9H_9NO_2]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 137 $[C_8H_{11}NO]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2-methoxycarbonylphenyl) propanamide (5k)

Light-brown amorphous solid; yield: 90%; m.p: 107-109 ^{o}C; Mol. F.: $C_{20}H_{23}N_3O_5$; Mol. Mass: 385; IR (KBr, cm^{-1}) *v*: 3408 (N-H), 3088 (Ar C-H), 2884 (R C-H), 1655 (C=O), 1584 (Ar C=C), 1199 (C-O-C), 1114 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 8.76 (br.d, *J* = 8.4 Hz, 1H, H-6'''), 8.12 (br.d, *J* = 7.7 Hz, 1H, H-3'''), 7.50 (br.t, *J* = 7.7 Hz, 1H, H-5'''), 7.49 (distorted d, *J* = 1.5 Hz, 1H, H-5'), 7.12 (br.t, *J* = 7.7 Hz, 1H, H-4'''), 7.02 (d, *J* = 3.5 Hz, 1H, H-3'), 6.50 (dd, *J* = 1.6, 3.4 Hz, 1H, H-4'), 3.92 (br.s, 4H, H-3, H-5), 3.90 (s, 3H, CH_3-8''') 2.76 (t, *J* = 6.1 Hz, 2H, H-2''), 2.61 (t, *J* = 6.2 Hz, 2H, H-3''), 2.60 (m, 4H, H-2, H-4); EIMS (*m/z*): 385 $[M]^+$, 284 $[C_{15}H_{18}N_2O_3]^{\bullet+}$, 261 $[C_{14}H_{17}N_2O_3]^+$, 205 $[C_{11}H_{11}NO_3]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 177 $[C_9H_7NO_3]^{\bullet+}$, 151 $[C_8H_9NO_2]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-cyclohexylpropanamide (5l)

Dark-brown amorphous solid; yield: 94 %; m.p: 109-111 ^{o}C; Mol. F.: $C_{18}H_{27}N_3O_3$; Mol. Mass: 333; IR (KBr, cm^{-1}) *v*: 3407 (N-H), 3079 (Ar C-H), 2884 (R C-H), 1652 (C=O), 1584 (Ar C=C), 1198 (C-O-C), 1107 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 3.85-3.83 (m, 1H, H-1'''), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 1.86-1.14 (m, 10H, CH_2-2''' to CH_2-6'''); EIMS (*m/z*): 333 $[M]^+$, 222 $[C_{17}H_{18}N_2O]^{\bullet+}$, 209 $[C_{16}H_{17}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 152 $[C_{13}H_{11}NO]^+$, 138 $[C_7H_8NO_2]^+$, 125 $[C_7H_{11}NO]^{\bullet+}$, 99 $[C_6H_{13}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-tetrahydrofuran-2-ylmethylpropanamide (5m)

Light-brown amorphous solid; yield: 90 %; m.p: 107-109 ^{o}C; Mol. F.: $C_{17}H_{25}N_3O_4$; Mol. Mass: 335; IR (KBr, cm^{-1}) *v*: 3406 (N-H), 3085 (Ar C-H), 2884 (R C-H), 1655 (C=O), 1584 (Ar C=C), 1196 (C-O-C), 1112 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.48 (distorted d, *J* = 1.4 Hz, 1H, H-5'), 7.05 (d, *J* = 3.4 Hz, 1H, H-3'), 6.48 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 4.05-3.98 (m, 1H, H-2'''), 3.92 (br.s, 4H, H-3, H-5), 3.84-3.82 (m, 1H, H_{eq}-5'''), 3.71-3.67 (m, 1H, H_{ax}-5'''), 3.21 (dd, *J* = 14.0, 6.8 Hz, 1H, H_a-6'''), 3.11 (dd, *J* = 14.8, 7.2 Hz, 1H, H_b-6'''), 2.76 (t, *J* = 6.2 Hz, 2H, H-2''), 2.65 (t, *J* = 6.2 Hz, 2H, H-3''), 2.62 (m, 4H, CH_2-2, CH_2-4), 2.03-1.95 (m, 1H, H_{eq}-4'''), 1.90-1.84 (m, 1H, H_{eq}-3'''), 1.69-1.59 (m, 2H, H_{ax}-3''', H_{ax}-4'''); EIMS (*m/z*): EIMS (*m/z*): 335 $[M]^+$, 224 $[C_{12}H_{20}N_2O_2]^{\bullet+}$, 211 $[C_{11}H_{19}N_2O_2]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 155 $[C_8H_{13}NO_2]^+$, 138 $[C_7H_8NO_2]^+$, 127 $[C_6H_9NO_2]^{\bullet+}$, 99 $[C_5H_9NO]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2,3-dimethylphenyl) propanamide (5n)

Light-brown amorphous solid; yield: 90 %; m.p: 121-123 ^{o}C; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3410 (N-H), 3075 (Ar C-H), 2880 (R C-H), 1649 (C=O), 1580 (Ar C=C), 1205 (C-O-C), 1110 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.57 (br.d, *J* = 7.6 Hz, 1H, H-6'''), 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.11 (br.t, *J* = 8.0 Hz, 1H, H-5'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 7.03 (br.d, *J* = 7.6 Hz, 1H, H-4'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.32 (s, 3H, CH_3-7'''), 2.14 (s, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2,4-dimethylphenyl) propanamide (5o)

Pink-brown amorphous solid; yield: 92 %; m.p: 128-130 ^{o}C; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3405 (N-H), 3075 (Ar C-H), 2882 (R C-H), 1646 (C=O), 1578 (Ar C=C), 1201 (C-O-C), 1109 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.68 (br.d, *J* = 7.6 Hz, 1H, H-6'''), 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.97 (br.d, *J* = 7.6 Hz, 1H, H-5'''), 6.96 (br.s, 1H, H-3'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.25 (s, 3H, CH_3-7'''), 2.20 (s, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

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

Off-white amorphous solid; yield: 94 %; m.p: 125-127 ^{o}C; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3412 (N-H), 3073 (Ar C-H), 2881 (R C-H), 1650 (C=O), 1584 (Ar C=C), 1200 (C-O-C), 1109 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.78 (br.s, 1H, H-6'''), 7.48 (distorted d, *J* = 1.5 Hz, 1H, H-5'), 7.05 (d, *J* = 7.7 Hz, 1H, H-3'''), 7.04 (d, *J* = 3.5 Hz, 1H, H-3'), 6.87 (br.d, *J* = 7.5 Hz, 1H, H-4'''), 6.49 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.94 (br.s, 4H, CH_2-3, CH_2-5), 2.77 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.65 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.63 (m, 4H, CH_2-2, CH_2-4), 2.32 (s, 3H, CH_3-7'''), 2.26 (s, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193

$[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2,6-dimethylphenyl)propanamide (5q)

Off-white amorphous solid; yield: 90 %; m.p.: 126-128 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass.: 355; IR (KBr, cm^{-1}) *v*: 3412 (N-H), 3079 (Ar C-H), 2885 (R C-H), 1655 (C=O), 1579 (Ar C=C), 1201 (C-O-C), 1114 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.06-7.01 (m, 3H, H-3''' to H-5'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.16 (s, 6H, CH_3-7''', CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(3,4-dimethylphenyl) propanamide (5r)

White crystalline solid; yield: 89 %; m.p: 130-132 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3407 (N-H), 3076 (Ar C-H), 2879 (R C-H), 1649 (C=O), 1580 (Ar C=C), 1207 (C-O-C), 1105 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.29 (br.d, *J* = 8.0 Hz, 1H, H-6'''), 7.26 (br.s, 1H, H-2'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 7.03 (br.d, *J* = 8.0 Hz, 1H, H-5'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.21 (s, 3H, CH_3-7'''), 2.18 (s, 3H, CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(3,5-dimethylphenyl) propanamide (5s)

Light-brown amorphous solid; yield: 93 %; m.p.: 128-130 ^oC; Mol. F.: $C_{20}H_{25}N_3O_3$; Mol. Mass: 355; IR (KBr, cm^{-1}) *v*: 3409 (N-H), 3070 (Ar C-H), 2886 (R C-H), 1646 (C=O), 1575 (Ar C=C), 1208 (C-O-C), 1117 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.15 (br.s, 2H, H-2''', H-6'''), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.73 (br.s, 1H, H-4'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.26 (s, 6H, CH_3-7''', CH_3-8'''); EIMS (*m/z*): 355 $[M]^+$, 244 $[C_{15}H_{20}N_2O]^{\bullet+}$, 231 $[C_{14}H_{19}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 175 $[C_{11}H_{13}NO]^+$, 147 $[C_9H_9NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 121 $[C_8H_{11}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(2-ethyl-6-methylphenyl) propanamide (5t)

Red-brown, sticky amorphous solid; yield: 90 %; m.p: 131-133 ^oC; Mol. F.: $C_{21}H_{27}N_3O_3$; Mol. Mass: 369; IR (KBr, cm^{-1}) *v*: 3406 (N-H), 3089 (Ar C-H), 2887 (R C-H), 1655 (C=O), 1588 (Ar C=C), 1198 (C-O-C), 1110 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 6.98-7.14 (m, 3H, H-3''' to H-5'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, H-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''), 2.46 (q, *J* = 7.5 Hz, 2H, CH_2-7'''), 1.97 (s, 3H, CH_3-9'''), 1.02 (t, *J* = 7.5, 3H, CH_3-8'''); EIMS (*m/z*): 369 $[M]^+$, $[C_{11}H_{15}N_2O_2]^+$, 258 $[C_{16}H_{22}N_2O]^{\bullet+}$, 245 $[C_{15}H_{21}N_2O]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 189 $[C_{12}H_{15}NO]^+$, 161 $[C_{10}H_{11}NO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 135 $[C_9H_{13}N]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

3-[4-(2-Furoyl)-1-piperazinyl]-N-(5-chloro-2-methoxyphenyl) propanamide (5u)

Light-brown amorphous solid; yield: 90 %; m.p.: 112-114 ^oC; Mol. F..: $C_{19}H_{22}ClN_3O_4$; Mol. Mass: 391; IR (KBr, cm^{-1}) *v*: 3407 (N-H), 3089 (Ar C-H), 2884 (R C-H), 1655 (C=O), 1584 (Ar C=C), 1197 (C-O-C), 1109 (C-N-C); ^{1}H-NMR (500 MHz, $CDCl_3$, *δ* in ppm): 8.52 (d, *J* = 2.1Hz, 1H, H-6'''), 7.49 (distorted d, *J* = 1.0 Hz, 1H, H-5'), 7.05 (d, *J* = 3.5 Hz, 1H, H-3'), 7.01 (dd, *J* = 8.5, 2.1 Hz, H-4'''), 6.79 (d, *J* = 8.5 Hz, H-3'''), 6.05 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'), 3.92 (s, 3H, H_3C-8'''), 3.91 (br.s, 4H, CH_2-3, CH_2-5), 2.78 (t, *J* = 6.2 Hz, 2H, CH_2-2''), 2.67 (m, 4H, CH_2-2, CH_2-4), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-3''); EIMS (*m/z*): 393 $[M + 2]^+$, 391 $[M]^+$, 280 $[C_{14}H_{17}ClN_2O_2]^{\bullet+}$, 267 $[C_{13}H_{16}ClN_2O_2]^+$, 211 $[C_{10}H_{10}ClNO_2]^+$, 193 $[C_{10}H_{13}N_2O_2]^+$, 183 $[C_8H_6ClNO_2]^{\bullet+}$, 157 $[C_7H_6ClNO]^{\bullet+}$, 138 $[C_7H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$.

Antibacterial and hemolytic activities

All synthesized molecules were screened against Gram-positive and Gram-negative bacteria and were found to be good to excellent inhibitors. The results are given as % inhibition and MIC values in Tables 1 and 2. The % hemolysis of the synthesized compounds is also given in Table 2. The results for compounds were compared with that for Triton X-100 and phosphate-buffered saline (PBS).

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

Compound	Inhibition (%)				
	S. typhi (-)	***E. coli (-)***	***P. aeruginosa (-)***	***B. subtilis (+)***	***S. aureus (+)***
5a	81.75±0.40	60.65±0.05	65.70±0.20	68.15±0.35	75.53±0.60
5b	75.87±0.73	64.80±0.70	75.39±0.04	66.60±0.50	60.85±0.15
5c	73.00±0.60	65.05±0.75	77.21±0.68	64.05±0.45	64.00±0.48
5d	73.20±0.80	61.45±0.65	71.79±0.71	68.65±0.65	64.00±0.30
5e	81.37±0.77	69.70±0.50	68.46±0.35	66.85±0.15	86.30±0.50
5f	83.20±0.60	61.15±0.25	59.36±0.89	72.85±0.85	68.80±0.65
5g	84.83±0.82	80.15±0.05	69.86±0.07	61.15±0.85	86.35±0.65
5h	78.23±0.37	72.75±1.00	67.93±0.36	71.20±0.10	84.55±0.15
5i	82.67±0.07	65.25±0.95	43.57±0.50	70.55±0.25	79.80±0.30
5j	85.73±0.40	64.40±0.45	47.00±0.34	70.65±0.85	77.50±0.90
5k	80.00±0.33	62.90±0.60	61.21±0.36	63.30±0.60	70.30±0.80
5l	79.07±0.17	74.71±0.01	70.90±0.12	60.15±0.25	78.90±0.15
5m	79.63±0.83	56.80±0.56	57.39±0.82	67.80±0.58	66.75±0.45
5n	75.93±1.00	62.60±0.50	68.46±0.75	69.65±0.95	66.00±0.43
5o	71.70±0.37	58.65±0.05	63.18±0.96	64.65±0.35	56.25±0.55
5p	73.70±0.57	60.20±0.20	82.71±0.21	67.25±0.85	62.15±0.05
5q	75.87±0.60	64.70±0.14	51.32±0.18	66.90±0.60	62.60±0.25
5r	82.80±078	67.95±0.85	70.29±0.50	60.20±0.80	78.85±0.05
5s	82.00±0.34	71.05±0.55	72.93±0.21	65.50±0.60	72.05±0.75
5t	78.83±0.57	52.85±0.85	35.00±0.71	57.00±0.50	83.85±0.35
5u	81.40±0.60	60.00±0.30	38.36±0.07	64.60±0.30	73.30±0.34
Ciprofloxacin	***91.05±0.68***	***92.32±0.42***	***92.50±0.34***	***92.02±053***	***91.44±0.64***

Table 2: Antibacterial activity (MIC) and hemolytic activity of the synthesized compounds

Compound	MIC (µM)					Hemolytic activity
	S. typhi (-)	***E. coli (-)***	***P. aeruginosa (-)***	***B. subtilis (+)***	***S. aureus (+)***	%
5a	8.37±0.12	12.92±0.11	10.29±0.30	9.97±0.77	9.22±0.76	15.48
5b	9.65±0.17	12.87±0.51	9.19±0.52	10.21±0.44	14.28±0.75	8.41
5c	9.87±0.45	12.76±0.90	8.97±0.10	10.13±0.89	12.46±0.50	61.93
5d	9.74±0.13	15.31±0.78	9.46±0.13	10.22±0.48	11.97±0.73	3.98
5e	9.28±0.63	10.49±0.64	9.96±0.49	10.32±0.24	8.65±0.50	3.12
5f	9.41±0.23	14.72±0.10	15.98±0.10	9.76±0.15	10.53±0.30	35.56
5g	8.45±0.31	8.97±0.12	9.89±0.30	13.52±0.12	8.34±0.55	8.03
5h	9.12±0.23	9.52±0.75	10.43±0.74	9.47±0.73	8.73±0.90	64.18
5i	8.41±0.11	10.87±0.19	-	9.24±0.50	8.93±0.64	5.52
5j	8.68±0.17	14.71±0.31	9.85±0.28	10.61±0.10	10.15±0.30	4.78
5k	8.97±0.27	14.73±0.75	13.57±0.59	12.63±0.97	9.76±0.12	8.41
5l	9.10±0.43	16.58±0.80	8.67±0.94	9.29±0.89	13.12±0.37	3.93
5m	9.11±0.24	15.75±0.39	15.97±0.44	12.75±0.27	10.46±0.51	7.61
5n	9.56±0.38	14.75±0.19	9.85±0.56	9.87±0.50	10.53±0.53	15.88
5o	10.23±0.87	16.79±0.45	12.42±0.40	12.87±0.14	16.95±0.45	2.97
5p	10.01±0.49	15.89±0.79	8.65±0.57	10.24±0.87	14.75±0.70	4.35
5q	9.44±0.39	12.65±0.33	19.85±0.10	10.67±0.50	13.74±0.60	4.30
5r	8.98±0.54	9.80±0.41	9.34±0.56	11.54±0.23	9.65±0.34	10.87
5s	8.68±0.64	10.89±0.23	9.78±0.42	14.32±0.61	8.98±0.23	15.89
5t	9.34±0.50	17.42±0.15	-	15.98±0.28	8.85±0.12	20.08
5u	8.70±0.98	16.32±0.71	-	11.85±0.52	9.53±0.89	12.89
Ciprofloxacin	***7.45±0.58***	***7.16±0.58***	***7.14±0.18***	***7.29±0.90***	***7.80±0.19***	
PBS						***0.09***
Triton X-100						***100***

Note: Minimum inhibitory concentration (MIC) was calculated using different concentrations (ranging 5 - 30 µg/well) and EZ-Fit software (Perrella Scientific Inc. Amherst, NH, USA)

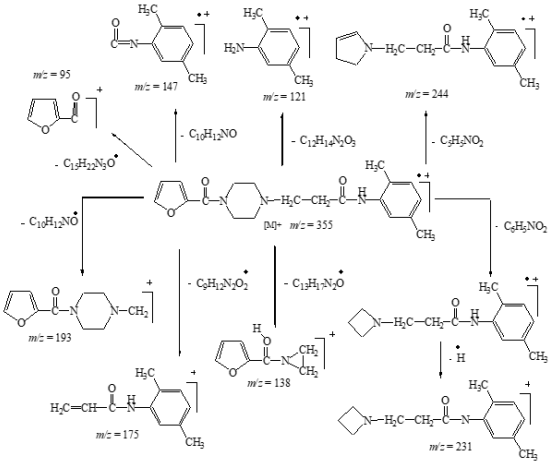


Figure 2: Mass fragmentation pattern of **5p**

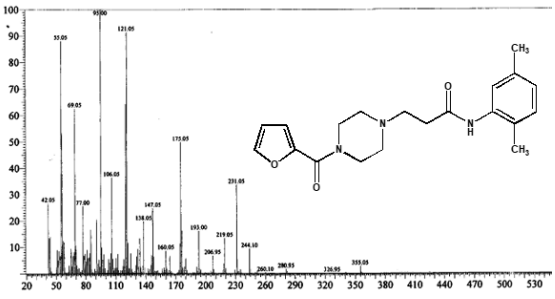


Figure 3: EIMS spectrum of **5p**

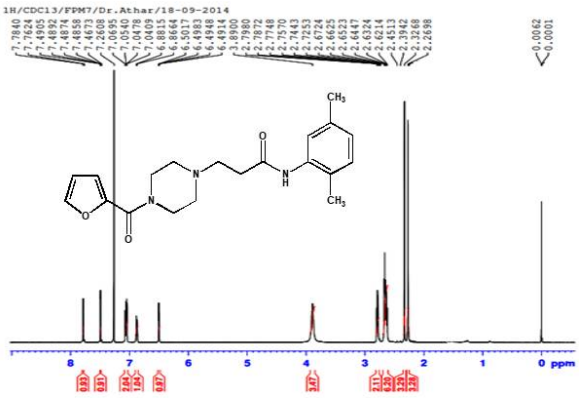


Figure 4: ^{1}H-NMR spectrum of **5p**

DISCUSSION

Some 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(substituted) propanamides (**5a-u**) were synthesized to introduce new promising antibacterial agents with mild cytotoxicity. The synthesis has been outlined in scheme 1 and structures of these targeted molecules are shown in Figure 1. The procedures of synthesis and conditions of reactions are described in the experimental section. The structural assignment of one of the compounds is described hereby for clear understanding of interpretational decorum of the synthesized compounds.

Compound **5p** was obtained as an amorphous solid having an off-white color and melting point of 125 - 127 ^oC with Mol. F. of $C_{20}H_{25}N_3O_3$, corroborated through EI-MS with $[M]^+$ peak of 355. The fragment of *N*-furoyl group appeared at *m/z* 95 and that of 2,5-dimethylaniline moiety at *m/z* 121.

The IR spectrum displayed a list of peaks for dominant functionalities of the amidic carbonyl group and furoylpiperazine at 3412 (N-H), 3073 (Ar C-H), 2881 (R C-H), 1650 (C=O), 1584 (Ar C=C), 1200 (C-O-C) and 1109 (C-N-C). In the aromatic region of ^{1}H-NMR spectrum, three signals resonating at *δ* 7.78 (br.s, 1H, H-6'''), 7.05 (d, *J* = 7.7 Hz, 1H, H-3''') and 6.87 (br.d, *J* = 7.5 Hz, 1H, H-4''') were typical for the 2,5-dimethylphenyl ring. Three protons of the furan ring resonated at *δ* 7.48 (distorted d, *J* = 1.5 Hz, 1H, H-5'), 7.04 (d, *J* = 3.5 Hz, 1H, H-3') and 6.49 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4'). In the aliphatic region of the ^{1}H-NMR spectrum, two signals resonated at *δ* 3.94 (br.s, 4H, CH_2-3, CH_2-5) and 2.64-2.62 (m, 4H, CH_2-2, CH_2-4) for the piperazine ring; two signals at *δ* 2.77 (t, *J* = 6.2 Hz, 2H, CH_2-2'') and 2.65 (t, *J* = 6.2 Hz, 2H, CH_2-3'') for two methylene groups of propanamide; and two signals at *δ* 2.32 (s, 3H, CH_3-7''') and 2.26 (s, 3H, CH_3-8''') for two methyl groups attached to the aromatic ring at positions 2 and 5. The ^{1}H-NMR spectrum of **5p** is shown in Figure 4. The synthesized molecule was designated 3-(4-(furan-2-carbonyl) piperazin-1-yl)-*N*-(2,5-dimethylphenyl) propanamide, and other compounds were also structurally elucidated.

The different synthesized 3-[4-(2-furoyl)-1-piperazinyl]-*N*-(substituted) propanamides showed notable activity against both Gram-positive and Gram-negative bacterial strains compared to ciprofloxacin. A comparison of the two compounds **5a** bearing an unsubstituted phenyl group and **5l** bearing an aliphatic cyclohexyl group showed that **5a** was more active against *S. typhi*, *E. coli* and *S. aureus,* but **5l**, more against *P. aeruginosa* and *B. subtilis*. The presence of an aromatic system at the nitrogen of the propanamoyl group had a positive effect on the bioactivity of the synthesized molecule. The hemolysis results for compound **5a** showed that it is more toxic than **5l**. The increase in the distance of the unsubstituted phenyl ring from the nitrogen of the amidic group resulted in an increase in bioactivity potential but this effect was found to be the reverse for a too long aliphatic chain. Therefore, compound **5e** bearing a benzyl group was found to be more

active than **5a** bearing a phenyl group, but **5f** bearing a phenylethyl group was the least active.

Furthermore, **5e** was the least toxic compound among these three. No considerable difference in bioactivity potential was found between the three compounds **5b-d**, bearing a methyl-substituted phenyl group at the *ortho*, *meta* and *para* positions. When a 2-methylphenyl group (compound **5b**) was replaced by a 2-ethylphenyl group (compound **5g**), the bioactivity potential was found to be increased and also with low toxicity. The presence of an ethoxy group at the *ortho* position of the phenyl ring (**5i**) did not favor higher bioactivity.

A similar order of bioactivity was found for 4-methylphenyl (compound **5d**) and 4-ethylphenyl (compound **5h**), but **5h** was more toxic. 4-Ethoxyphenyl (compound **5j**) had a little less antibacterial activity compared to **5h** but it possessed much less toxicity. This shows that the substitution of ethyl at the *ortho* position of the phenyl ring (compound **5g**) resulted in more activity with low toxicity as compared to that at the *para* position (compound **5h**). A similar pattern of bioactivity was observed for the ethoxy group at the *ortho* and *para* positions. Among those with dimethylphenyl groups, compounds **5n-q**, bearing one methyl at the *ortho* position and a second at other positions turned out to be less effective compared to compounds **5r** and **5s**, bearing one methyl at the *meta* position and a second at other positions. Among compounds **5n-q**, **5n** was the most active, but it showed high toxicity. The other three showed comparable potential, with no considerable effect of variation in position of substitution. Both compounds **5r** and **5s** also showed a similar order of activity.

Overall, all synthesized molecules showed impressive antibacterial activity against *S. typhi.* The aromatic rings were responsible for more interaction, and the alkyl groups in better positions accounted for higher antibacterial activity. The compounds exhibiting low toxicity could be further subjected to *in vivo* study to assess their application in a drug design program.

CONCLUSION

The findings of this study show that almost all the derivatives are active against all the strains of Gram-positive and Gram**-**negative bacteria tested. Only a few of the synthesized molecules possess moderate cytotoxicity. Thus, these molecules have some potential as therapeutic agents, but further studies are required in this regard.

DECLARATIONS

Acknowledgement

The authors acknowledge the Higher Education Commission of Pakistan for financial support. Dr. A. Leyva (USA) provided English editing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest with regard to this study.

Contribution of authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ghulam Hussain synthesized and characterized all the compounds presented in this research paper. Muhammad Athar Abbasi supervised the project. Aziz-ur-Rehman and Sabahat Zahra Siddiqui are co-workers in this project. Rabia Malik evaluated the antibacterial activity of the synthesized molecules, under the supervision of Irshad Ahmad. Zahid Mushtaq determined the hemolytic activity of the synthesized molecules, under the supervision of Muhammad Shahid. Syed Adnan Ali Shah evaluated the spectral data of the synthesized compounds.

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