## ORIGINAL RESEARCH ARTICLES

# SYNTHESIS, CHARACTERIZATION, *IN VITRO* ANTIMICROBIAL AND ANTI-INFLAMMATORY EVALUATIONS OF {5'-(SUBSTITUTED ARYL)-2-FURANYL}-3,4-DIHYDRO-*1H*-PYRIMIDINE-2-ONE DERIVATIVES

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**ABSTRACT**

In the present study, a series of 5'-(substituted aryl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones has been designed and synthesized with Beginelli reaction derivatives carried out from the reaction of ethyl acetoacetate 1 with substituted ureas 2 and substituted aryl furfuraldehydes 3 in presence of acetic acid to yield {5'-(substituted aryl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones [4a-k (X=O), 4l-v (X=S)]. The target candidates were confirmed by FTIR, NMR, Mass spectrometry and by elemental analysis. The present exploration also required for *in vitro* antibacterial activity against Gram-positive strains of bacteria, namely *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 11633), *Staphylococcus epidermidis* (ATCC 155), *Enterococccus faecalis* (ATCC 14506) and Gram negative bacterial strains, namely *Shigella dysenteriae* (ATCC 13313), *Escherichia coli* (ATCC11303), *Klebsiella pneumoniae* (ATCC 10031) and *Salmonella typhi* (MTCC 733). Tested antimicrobial activity revealed that compounds 4k and 4v showed very promising antibacterial activity and were found to be most effective when tested against strains of *K. pneumonia, S. aureus, S. typhi, S. epidermidis* and *E. faecalis,* although rest synthesized derivatives also showed significant antibacterial activity. The novel synthesized compounds also exerted remarkable *in vitro* anti-inflammatory biological activity as reflected by utilization of the percent inhibition of protein denaturation method.

**Keywords**: Thiourea, Biginelli reaction, antimicrobial activity, antiinflammatory activity

## INTRODUCTION

Bacterial infection is tremendous public health concern worldwide. Organizational Research that Group-International Medical Statistics outlines revealed that most of the pharmaceutical market is established on the antibacterial agent worldwide[1], on the other hand, inflammation becomes the most common disease due to several reasons globally. Therapeutically, NSAIDs are extensively used in inflammation, pain and arthritis as well[2]. Because they prevent the enzyme cyclooxygenase (COX) from producing prostaglandins, NSAIDs have anti-inflammatory and analgesic effects[3]. The inducible COX-2 is accompanied with various clinical conditions associated with inflammations while the cytoprotective properties of prostaglandins are caused by the constitutively expressed COX-1[4]. According to comprehensive literature study, Biginelli reaction derivatives are associated with antibacterial activity, 1-adrenergic receptor antagonist, anticancer, calcium channel inhibitors, antiinflammatory, anti-viral, antifungal and antioxidant activities[5]. The Biginelli reaction, which produces 3,4-dihydro-1*H*-pyrimidine-2-ones via condensation of aromatic aldehyde, urea, and ethyl acetoacetate in the presence of ethanol as solvent and different acids as catalysts, is one of the most well-known examples of multicomponent reactions[6-7], however importance of [5-(substituted aryl)-2-furfuraldehyde] moiety in the previously synthesized imines derivatives is well documented as significant antibacterial activity[8], therefore [5-(substituted aryl)-2-furfuraldehydes] were used as aromatic aldehydes. The molecular manipulation is required for new drugs of favorable lead derivatives with enhanced biological activity.

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In the present work, we report 22 such analogues [4a-k (X=O), 4l-v (X=S)] by using major building blocks such as ethyl acetoacetate 1, aromatic aldehyde [5-(substituted aryl)-2-furfuraldehydes] 2, and urea/thiourea 3 in addition of glacial acetic acid The novel derivatives were further characterized and investigated for antimicrobial and anti-inflammatory biological potencies.

## MATERIALS AND METHODS

A series of {5'-(substituted aryl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones [4a-k (X=O), 4l-v (X=S)] have been synthesized from the condensation of 5-(substituted aryl)-2-furfuraldehyde ($R_1$a-k), ethyl acetoacetate and urea/thiourea with ethanol as solvent and glacial acetic acid as catalyst as shown in Scheme 1. All synthesized derivatives were confirmed by spectral characterization and analyzed by using FT-IR, mass spectroscopy $^1$H and $^{13}$C NMR, and CHN analysis. IR spectra were carried out on a Bruker, ALPHA EATR FT-IR spectrometer while, NMR spectra were taken as a DPX-300NMR spectrometer by using $CDCl_3$ solution. $^1$H NMR and $^{13}$C NMR were measured at 300 MHz and 75 MHz, respectively. Chemical shifts are described in $\delta$ deshielded relative to TMS ($Me_4Si$). Mass spectra (DART-MS) were recorded on Bruker Daltonics' micrOTOF-Q II™ ESI-Qq-TOF mass spectrometer by electron spray ionization. Elemental analyses were carried out in Heraeus CHN rapid elemental analyzer and recorded inherent values ±0.05 % (Theoretical values). Progress of reactions was checked by TLC. All the compounds were subjected to determination of melting points by capillary method.

## BIOLOGICAL EVALUATIONS

### *In vitro* antimicrobial activity

The antimicrobial activity was evaluated against pathogens, *E. faecalis, S. aureus, S. epidermidis* and *B. cereus* representing Gram positive bacterial strains and *S. dysenteriae, E. coli*, *K. pneumonia* and *S. typhi*, representing Gram negative bacterial strains, using agar well diffusion methodlogy[9]. The broth culture of the various bacterial strains was put overnight and was converted to about $10^5$ colony forming units (CFU mL$^{-1}$) using sterile aqueous media, and 100 L of diluted inoculum was spread across petri plates containing 25 mL of nutritional agar medium. The wells of about 6 mm in diameter were constructed by using sterilized filter paper (Whatman No. 1). Test samples were arranged in three disc sets and placed on three portions with one disc. Ampicillin (20 µg disc$^{-1}$) was Ampicillin used as reference drug. There was no significant inhibition in the negative control. The discs were sterile and previously soaked in a DMF solution (25 µg mL$^{-1}$) of the newly synthesized derivatives [4a-k (X=O), 4l-v (X=S)] and accommodated in nutrient agar culture broth and incubated for 24 h at 38 °C. Zone of growth inhibition is expressed in mm, surrounding discs were setup in triplicate sets and determination was accomplished by differentiation with that produced by standard drug.

### Evaluation of *in vitro* anti-inflammatory activity (Albumin denaturation assay)

The anti-inflammatory activity of target derivatives [4a-k (X=O), 4l-v (X=S)] was determined by using *in vitro* inhibition of albumin denaturation technique study[10]. The final concentrations were prepared to be 25, 50, 100, and 200 g mL$^{-1}$ after the reaction mixture (5 mL) was made up of 0.2 mL of egg albumin, 2.9 mL of phosphate buffered saline (pH: 6.4), and 2.0 mL of test compounds of different concentrations. Double-distilled water in a comparable amount was utilized as the control. The mixtures were incubated for 5 minutes at 70 °C after being incubated at 38 °C for around 15 minutes. A vehicle was used as a blank for the subsequent absorbance measurement at 660 nm. Then, percentage of inhibition of denaturation was calculated from control without the added drug. Albumin denaturation assay was done in triplicate set and the average value determined. The absorbance was calculated using indomethacin at final concentrations of 50 and 100 g mL$^{-1}$ as a reference medication. The following formula was used to compute the protein denaturation inhibition percentage:

$$\% \text{ Inhibition} = \frac{\text{Abs(Control)} - \text{Abs(Sample)}}{\text{Abs(Control)}} \times 100$$

### Statistical evaluation

Statistical evaluation was accomplished by *t*-test and analysis of variance (ANOVA) statistics. the values of mean ± SEM, n=3, zone of inhibition was determined for antibacterial activity and % of inhibition for albumin denaturation assay were carried *in vitro* anti-inflammatory activity of compounds [4a-k (X=O), 4l-v (X=S)].

## CHEMISTRY

### Synthetic approach for {5'-(substituted aryl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones [4a-k (X=O), 4l-v (X=S)]

A mixture of ethyl acetoacetate (14 mmoles) 1, 5-(phenyl substituted)-2-furfuraldehyde (10 mmoles) 2, urea/thiourea (22 mmoles) 3 in 50 mL of 95 % of ethanol was refluxed until the completion of reaction with addition

of 1 mL of acetic acid. Thin layer chromatography was used for monitoring the progress of the reaction. After the reaction was finished, the reaction mixture was filtered, washed, and then the final crystals were separated. The desired target compounds [4a-k (X=O), 4l-v (X=S)] thus obtained were re-crystallized with ethanol.

**Characterization of compounds [4a-k (X=O), 4l-v (X=S)]**

**{5'-(4-Nitrophenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one (4a):** mp 201-203 °C; yield, 53 %;IR ($\nu_{max,}$ $cm^{-1}$): 1168 (C-O-C), 546, 1464, 1263, 1167, 1108, 861, 741 (C=C, C-H, aryl ring),1708 (C=O, ester str) $^1$H NMR (300 MHz, DMSO-$d_6$): δ, 6.70-8.32 (m, Ar-H), 10.12 (1H,s, N-H), 4.67 (2H,s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR:(75 MHz, DMSO) δ 5.43 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 135.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 357.31($M^{+1}$); Anal: Calculated for $C_{17}H_{15}N_3O_6$(%): C, 57.14; H, 4.23; N, 11.76; O, 26.87. Found: C, 58.16; H, 4.76; N, 12.30; O, 25.86.

**{5'-(4-Chlorophenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4b**): mp 126-128 °C; yield, 55 %;IR; $\nu_{max,}$ ($cm^{-1}$): 1163 (C-O-C), 546, 1460, 1261, 1167, 1107, 861, 741 (C=C, C-H, aryl ring),1700 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.65-8.32 (m, Ar-H), 10.13 (1H, s, N-H), 4.69 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.35 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.40 (1H,s, -NH), 5.37 (1H, d, -NH), 26.09 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.3 (C-2'', C-6''), 121.2 (C-3'', C-5''), 110.1 (C-2'), 115.1 (C-3'), 132.12 (Ar, C-1), 134.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 346.16 ($M^{+1}$); Anal: Calculated for $C_{17}H_{15}ClN_2O_4$(%): C, 58.88; H, 4.36;N, 8.08; O, 18.46; Cl, 10.22 Found: C, 58.50; H, 4.87; N, 8.13; O, 18.56; Cl, 10.43.

**{5'-(4-Bromohenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4c**): mp 125-128 °C; yield, 61 %;IR; $\nu_{max}$, ($cm^{-1}$): 1158 (C-O-C), 548, 1464, 1261, 1169, 1107, 862, 745 (C=C and C-H of aryl ring),1703 (C=O, ester str) $^1$H NMR: (300 MHz, DMSO-d6) δ 6.65-8.32 (m, Ar-H), 10.13 (1H, s, N-H), 4.63 (2H, s, $OCH_3$), 2.32 (3H, s, $CH_3$), 7.45 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.41 (1H,s, -NH), 5.32 (1H, d, -NH), 26.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 121.3 (C-2'', C-6''), 121.2 (C-3'', C-5''), 110.4 (C-2'), 114.1 (C-3'), 132.12 (Ar, C-1), 134.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 391.21 ($M^{+1}$); Anal: Calculated for $C_{17}H_{15}BrN_2O_4$ (%): C, 52.19; H, 3.86; N, 7.16; Br, 20.42; N, 16.36 Found: C, 52.45; H, 3.80; N, 7.22; Br, 20.32; N, 16.11.

**{5'-(4-Methylphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4d**): mp 130-133 °C; yield, 54 %;IR; $\nu_{max}$, ($cm^{-1}$): 1164 (C-O-C), 542, 1461, 1261, 1169, 1103, 862, 742 (C=C and C-H of aryl ring),1704 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.65-8.32 (m, Ar-H), 10.16 (1H, s, N-H), 4.60 (2H, s, $OCH_3$), 2.30 (3H, s, $CH_3$), 7.45 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.40 (1H,s, -NH), 5.32 (1H, d, -NH), 26.11 ($CH_3$), 135.2 (C-1''), 121.6 (C-4''), 121.3 (C-2'', C-6''), 120.2 (C-3'', C-5''), 110.4 (C-2'), 114.3 (C-3'), 132.12 (Ar, C-1), 134.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 326.34 ($M^{+1}$); Anal: Calculated for $C_{18}H_{18}N_2O_4$(%): C, 66.24; H, 5.56;N, 8.58; O, 19.61 Found: C, 66.20; H, 5.50; N, 8.34; O, 19.42.

**{5'-(4-Methoxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4e**): mp 140-144 °C; yield, 59 %; IR; $\nu_{max}$, ($cm^{-1}$): 1166 (C-O-C), 1518, 1461, 1263, 1166, 1098, 851, 740 (C=C and C-H of aryl ring), $^1$H NMR: (300 MHz, DMSO-d6) δ 6.65-8.39 (m, Ar-H), 11.16 (1H, s, N-H), 4.65 (2H, s, $OCH_3$), 2.32 (3H, s, $CH_3$), 7.49 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.41 (1H,s, -NH), 5.32 (1H, d, -NH), 26.12 ($CH_3$), 135.3 (C-1''), 121.6 (C-4''), 121.7 (C-2'', C-6''), 120.6 (C-3'', C-5''), 110.8 (C-2'), 114.3 (C-3'), 131.12 (Ar, C-1), 134.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 342.34 ($M^{+1}$); Anal: Calculated for $C_{18}H_{18}N_2O_5$(%): C, 64.40; H, 5.12; N, 7.91; O, 22.58 Found: C, 64.23; H, 5.10; N, 7.86; O, 22.48.

**{5'-(2,4-Dinitrophenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4f**): mp 124-127 °C; yield, 63 %;IR; $\nu_{max}$, ($cm^{-1}$): 1167 (C-O-C), 548, 1466, 1263, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.70-8.32 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.43 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 402.34 ($M^{+1}$); Anal: Calculated for $C_{17}H_{14}N_4O_8$ (%): C, 52.36; H, 3.30; N, 12.21. Found: C, 52.40; H, 3.33; N, 12.26.

**{5'-(4-Sulfoxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4g**): mp130-134 °C; yield, 69 %; IR; $\nu_{max}$, ($cm^{-1}$): 1169 (C-O-C), 547, 1466, 1263, 1167, 1106, 861, 812 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.71-8.33 (m, Ar-H), 10.17 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.30 (3H, s, $CH_3$), 7.33 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.43 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 126.1 (C-2'', C-6''), 122.9 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.3 (Ar, C-2 , C-6); MS (ESI): *m/z*, 392.38 (M+1); Anal: Calculated for

$C_{17}H_{16}N_2O_7S$ (%): C, 52.04; H, 4.11; N, 7.14; O, 28.54; S, 8.17. Found: C, 51.78; H, 4.09; N, 7.11; O, 28.48; S, 8.12.

**{5'-(2-Carboxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4h**): mp 120-124 °C; yield, 62 %; IR; $\nu_{max}$, ($cm^{-1}$): 1170 (C-O-C), 548, 1466, 1264, 1167, 1105, 861, 812 (C=C and C-H of aryl ring),1706 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.73-8.32 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.13 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.2 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.2 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 356.32 ($M^{+1}$); Anal: Calculated for $C_{18}H_{16}N_2O_6$ (%): C, 60.67; H, 4.16; N, 7.86; O, 26.94. Found: C, 60.11; H, 4.09; N, 7.80; O, 26.23.

**{5'-(3-Chlorophenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4i**): mp 141-143 °C; yield, 63 %; IR; $\nu_{max}$, ($cm^{-1}$): 1161 (C-O-C), 546, 1460, 1268, 1167, 1107, 861, 741 (C=C and C-H of aryl ring),1700 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.65-8.33 (m, Ar-H), 10.13 (1H, s, N-H), 4.69 (2H, s, $OCH_3$), 2.22 (3H, s, $CH_3$), 7.35 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.40 (1H,s, -NH), 5.37 (1H, d, -NH), 26.09 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.3 (C-2'', C-6''), 121.2 (C-3'', C-5''), 110.1 (C-2'), 115.1 (C-3'), 132.12 (Ar, C-1), 134.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 346.16 ($M^{+1}$). Anal; Calcd for $C_{17}H_{15}ClN_2O_4$(%): C, 58.88; H, 4.36; N, 8.08; O, 18.46; Cl, 10.22 Found: C, 58.51; H, 4.87; N, 8.13; O, 18.56; Cl, 10.43.

**{5'-(4-Carboxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4j**): mp 135-137 °C; yield, 61 %; IR; $\nu_{max}$, ($cm^{-1}$): 1169 (C-O-C), 542, 1466, 1262, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1700 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.77-8.32 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.32 (3H, s, $CH_3$), 7.37 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 120.5 (C-4''), 127.1 (C-2'', C-6''), 123.5 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.1 (Ar, C-2 , C-6); MS (ESI): *m/z*, 356.32 ($M^{+1}$). Anal: Calculated for $C_{18}H_{16}N_2O_6$ (%): C, 60.67; H, 4.16; N, 7.86; O, 26.94. Found: C, 60.11; H, 4.09; N, 7.80; O, 26.23.

**{5'-(4-Sulfacetamidohenyl)-furan-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4k**): mp 139-142 °C; yield, 61 %; IR; $\nu_{max}$, ($cm^{-1}$): 1175 (C-O-C), 548, 1468, 1263, 1168, 1108, 861, 812 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.87-8.85 (m, Ar-H), 10.19 (1H, s, N-H), 4.69 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.33 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.43 (1H,s, -NH), 5.36 (1H, d, -NH), 25.13 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.5 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 433.43 ($M^{+1}$); Anal: Calculated for $C_{19}H_{19}N_3O_7S$ (%): C, 52.65; H, 4.42; N, 9.69.; O, 25.84; S,7.40. Found: C, 52.40; H, 4.40; N,9.45; O,25.11; S,7.16.

**{5'-(4-Nitrophenyl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4l**): mp 187-190 °C; yield, 59 %; IR; $\nu_{max}$, ($cm^{-1}$): 1167 (C-O-C), 547, 1466, 1263, 1167, 1108, 863, 812 (C=C and C-H of aryl ring),1707 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.71-8.38 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.8 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.2 (Ar, C-2 , C-6); MS (ESI): *m/z*, 373.28 ($M^{+1}$); Anal: Calculated for $C_{17}H_{15}N_3O_5S$(%): C, 54.68; H, 4.05; N, 11.25; O, 21.42; S, 8.59. Found: C, 54.70; H, 4.07; N, 11.22; O, 21.44; S, 8.58.

**{5'-(4-Chlorophenyl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4m**): mp 124-127 °C; yield, 58 %; IR; $\nu_{max}$, ($cm^{-1}$): 1163 (C-O-C), 547, 1466, 1263, 1167, 1108, 861, 813 (C=C and C-H of aryl ring),1701 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.75-8.40 (m, Ar-H), 10.15 (1H, s, N-H), 4.64 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.32 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.42 (1H,s, -NH), 5.36 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.5 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.1 (Ar, C-2 , C-6); MS (ESI): *m/z*, 362.86 (M+1); Anal: Calculated for $C_{17}H_{15}ClN_2O_3S$(%): C, 56.27; H, 4.17; N, 7.72; Cl, 9.77; O, 13.23; S, 8.84. Found: C, 56.29; H, 4.16; N, 7.70; Cl, 9.79; O, 13.20; S, 8.87.

**{5'-(4-Bromohenyl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4n**): mp 125-128 °C; yield, 63 %; IR; $\nu_{max}$, ($cm^{-1}$): 1166 (C-O-C), 546, 1466, 1262, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) δ 6.69-8.32 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) δ 5.43 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 407.28($M^{+1}$); Anal: Calculated for $C_{17}H_{15}BrN_2O_3S$ (%): C, 50.13; H, 3.71; N, 6.88; Br, 19.62; O, 11.79;S, 7.87. Found: C, 50.12; H, 3.71; N, 6.90; Br, 19.65; O, 11.68; S, 7.83.

**{5'-(4-Methylphenyl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4o**): mp 132-135 °C; yield, 62 %; IR;

$\nu_{max}$, ($cm^{-1}$): 1171 (C-O-C), 552, 1469, 1263, 1167, 1107, 861, 816 (C=C and C-H of aryl ring),1710 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.68-8.40 (m, Ar-H), 10.19 (1H, s, N-H), 4.69 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.35 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.44 (1H,s, -NH), 5.39 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.2 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.3 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.5 (Ar, C-2 , C-6); MS (ESI): *m/z*, 342.14 ($M^{+1}$); Anal: Calculated for $C_{18}H_{18}N_2O_3S$(%): C, 63.14; H, 5.30; N, 8.18; O, 14.02; S, 9.36. Found: C, 63.17; H, 5.31; N, 8.13; O, 14.01; S, 9.32.

**{5'-(4-Methoxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4p**): mp 125-128 °C; yield, 59 %; IR; $\nu_{max}$, ($cm^{-1}$): 1168 (C-O-C), 551, 1467, 1263, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1703 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.81-8.42 (m, Ar-H), 10.15 (1H, s, N-H), 4.68 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.31 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.43 (1H,s, -NH), 5.33 (1H, d, -NH), 25.15 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 133.0 (Ar, C-1), 133.4 (Ar, C-2 , C-6); MS (ESI): *m/z*, 358.41 ($M^{+1}$); Anal: Calculated for $C_{18}H_{18}N_2O_4S$(%): C, 60.32; H, 5.06; N, 7.82; O, 17.86; S, 8.95.Found: C, 62.32; H, 5.04; N, 7.80; O, 17.86; S, 8.92.

**{5'-(2,4-Dinitrophenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4q**): mp 122-125 °C; yield, 62 %; IR; $\nu_{max}$, ($cm^{-1}$): 1167 (C-O-C), 547, 1465, 1263, 1168, 1108, 861, 811 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.68-8.32 (m, Ar-H), 10.14 (1H, s, N-H), 4.62 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.33 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.47 (1H,s, -NH), 5.37 (1H, d, -NH), 25.11 ($CH_3$), 135.5 (C-1''), 120.4 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.2 (Ar, C-2 , C-6); MS (ESI): *m/z*, 418.34 ($M^{+1}$); Anal: Calcd for $C_{17}H_{14}N_4O_7S$(%): C, 48.80; H, 3.37; N, 13.39; O, 26.77; S, 7.66. Found: C, 48.65; H, 3.37; N, 13.40; O, 26.65; S, 7.68.

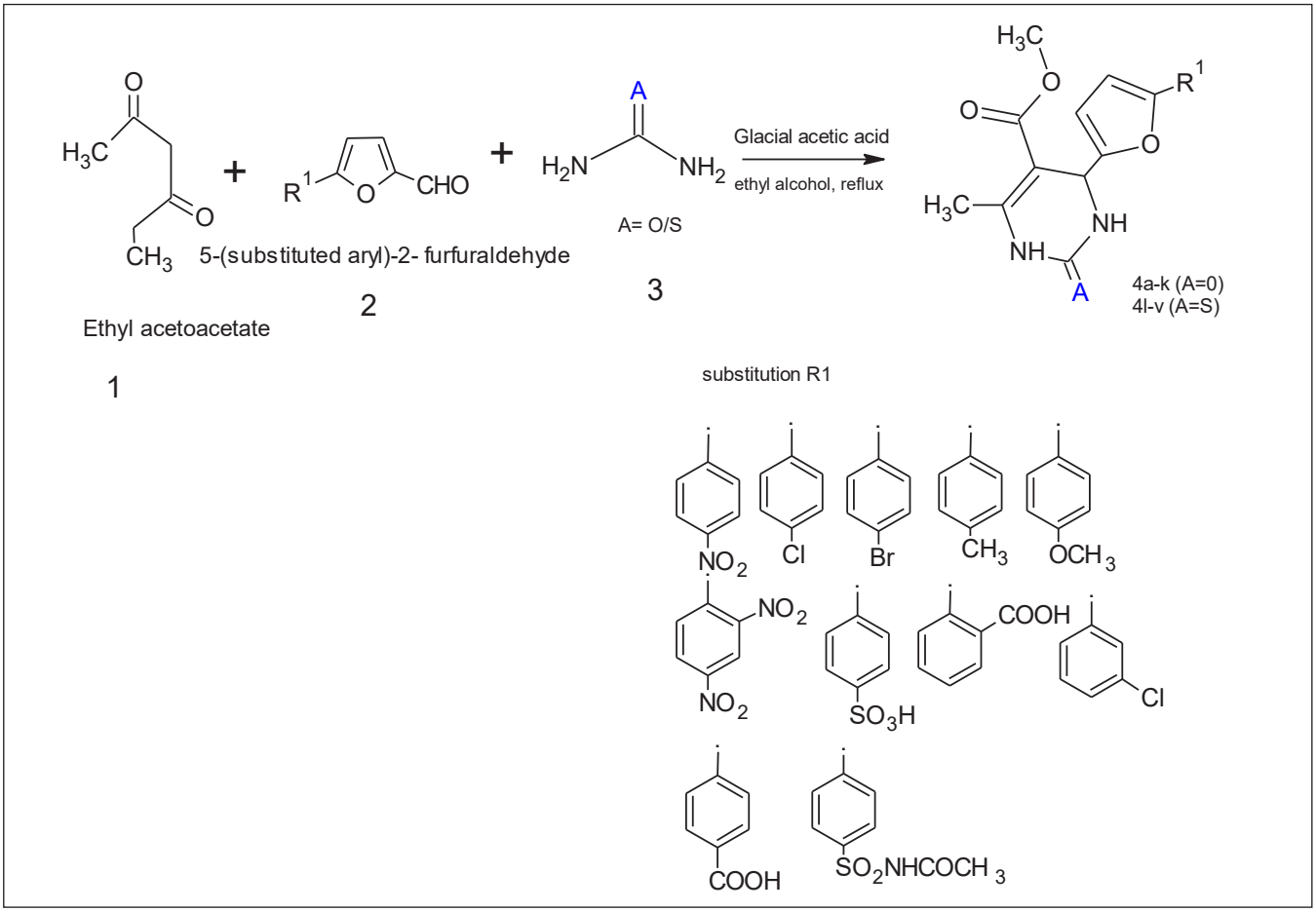
**{5'-(4-Sulfoxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4r**): mp 134-136°C; yield, 68 %; IR; $\nu_{max}$, ($cm^{-1}$): 1167 (C-O-C), 548, 1466, 1263, 1167, 1108, 861, 816 (C=C and C-H of aryl ring),1700 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.92-8.65 (m, Ar-H), 10.13 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.33 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.44 (1H,s, -NH), 5.38 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.5 (C-3'', C-5''), 110.6 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.7 (Ar, C-2 , C-6); MS (ESI): *m/z*, 408.44 ($M^{+1}$); Anal: Calculated for $C_{17}H_{16}N_2O_6S$ (%): C, 49.99; H, 3.95; N, 6.86; O, 23.50; S, 15.70. Found: C, 49.99; H, 3.95; N, 6.86; O, 23.50; S, 15.70

**{5'-(2-Carboxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4s**): mp 127-130 °C; yield, 61 %; IR; $\nu_{max}$, ($cm^{-1}$): 1176 (C-O-C), 549, 1466, 1263, 1167, 1109, 861, 812 (C=C and C-H of aryl ring),1705 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.69-8.32 (m, Ar-H), 10.18 (1H, s, N-H), 4.68 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.35 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 120.5 (C-4''), 127.1 (C-2'', C-6''), 123.3 (C-3'', C-5''), 110.2 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.3 (Ar, C-2 , C-6); MS (ESI): *m/z*, 372.29(M+1); Anal: Calcd for $C_{18}H_{16}N_2O_5S$(%): C, 58.03; H, 4.32; N, 7.52; O, 21.48; S, 8.61. Found: C, 57.15; H, 4.11; N, 7.28; O, 21.40; S, 8.65.

**{5'-(3-Chlorophenyl)- 2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4t**): mp 135-139 °C; yield, 63 %; IR; $\nu_{max}$, ($cm^{-1}$): 1169 (C-O-C), 549, 1466, 1263, 1168, 1108, 861, 813 (C=C and C-H of aryl ring),1701 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.69-8.32 (m, Ar-H), 10.15 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.36 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 120.5 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.1 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.6 (Ar, C-2 , C-6); MS (ESI): *m/z*, 362.83 ($M^{+1}$); Anal: Calculated for $C_{17}H_{15}ClN_2O_3S$(%): C, 56.27; H, 4.17; N, 7.72; Cl, 9.77; N, 7.72; O, 13.23; S, 8.84. Found: C, 56.19; H, 4.11; N, 7.68; Cl, 9.70; N, 7.56; O, 13.14; S, 8.65.

**{5'-(4-Carboxyphenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4u**): mp 130-134 °C; yield, 59 %; IR; $\nu_{max}$, ($cm^{-1}$): 1170 (C-O-C), 547, 1468, 1263, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1698 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.69-8.48 (m, Ar-H), 10.19 (1H, s, N-H), 4.68 (2H, s, $OCH_3$), 2.29 (3H, s, $CH_3$), 7.36 (2H, s, furfural), $^{13}$C NMR: (75 MHz, DMSO) $\delta$ 5.44 (1H,s, -NH), 5.37 (1H, d, -NH), 25.12 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.3 (C-2'', C-6''), 123.4 (C-3'', C-5''), 110.3 (C-2'), 116.1 (C-3'), 132.0 (Ar, C-1), 133.2 (Ar, C-2 , C-6); MS (ESI): *m/z*, 372.29 ($M^{+1}$); Anal: Calculated for $C_{18}H_{16}N_2O_5S$(%): C, 58.05; H, 4.33; N, 7.52; O, 21.48; S, 8.61. Found: C, 57.15; H, 4.11; N, 7.28; O, 21.40; S, 8.65.

**{5'-(4-Sulfacetamidohenyl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-one** (**4v**): mp 142-145 °C; yield, 60 %; IR; $\nu_{max}$, ($cm^{-1}$): 1166 (C-O-C), 544, 1466, 1263, 1167, 1108, 861, 812 (C=C and C-H of aryl ring),1704 (C=O, ester str)$^1$H NMR: (300 MHz, DMSO-d6) $\delta$ 6.73-8.38 (m, Ar-H), 10.16 (1H, s, N-H), 4.67 (2H, s, $OCH_3$), 2.24 (3H, s, $CH_3$), 7.33 (2H, s, furfural), $^{13}$C NMR: (75




**Scheme 1: Synthetic protocol of compounds [4a-k (X=O), 4l-v (X=S)]**

MHz, DMSO) δ 5.44 (1H,s, -NH), 5.36 (1H, d, -NH), 25.11 ($CH_3$), 135.7 (C-1''), 122.6 (C-4''), 127.1 (C-2'', C-6''), 123.2 (C-3'', C-5''), 110.3 (C-2'), 116.1 (C-3'), 132.2 (Ar, C-1), 133.6 (Ar, C-2 , C-6); MS (ESI): *m/z*, 449.50 (M+1); Anal: Calculated for $C_{19}H_{19}N_3O_6S_2$ (%): C, 50.77; H, 4.26; N, 9.35; O, 21.36; S, 14.27 Found: C, 50.27; H, 4.20; N, 9.12; O, 21.01; S, 14.25.

### Molecular docking studies

The Molegro Virtual Docker (MVD) version 7.0 was used to carry out the molecular docking studies. Using the protein data bank (PDB), the proteins 3FRA, 6Y3C, and 5KIR were retrieved. ChemDraw professional 16.0 software was used to create the ligands' 2D structures, and Chem3D was used to transform them to 3D. Utilizing the MM2 force field, the energy of ligands was reduced to the absolute minimum. The proteins were prepared and water molecules were removed from the proteins. With the aid of MVD, the grid generation (0.30 Å), MolDock score, and conformers assessment were completed. The PLP (Piecewise Linear Potential) scoring function is the source of the MolDock scoring formulas. The co-crystallographic ligand and protein amino acid residues were bound in a spherical area within 10 Å, which was selected as the active site. The computations were performed using the default values. With a maximum of 1500 iterations, 10 separate runs for each compound were carried out. The active side was supposed to be a rigid molecule, while the ligands were considered to be flexible.

## RESULTS AND DISCUSSION

### Chemistry

The Biginelli reaction derivatives [4a-k (X=O), 4l-v (X=S)] were synthesized by using major building blocks such as ethyl acetoacetate 1, aromatic aldehyde (5-(substituted aryl)-2-furfuraldehyde)2, and urea/thiourea **3** in glacial acetic acid. The purity of synthesized derivatives was confirmed by spectroscopic techniques such as FT-IR, $^1H$ and $^{13}C$ NMR and mass spectroscopy as well as by CHN analysis. The IR spectra of compounds [4a-k (X=O), 4l-v (X=S)] showed for C-O-C at 1167-1169; C=O of esters at 1708 $cm^{-1}$. Synthesized compounds exhibited the typical protons signals in $^1H$-NMR spectra

accounting for $CH_3$, $OCH_3$, N-H and aromatic groups at 2.28-2.29, 4.62-4.69, 10.12-10.19 and 6.70-8.30 ppm δ range. In $^{13}C$-NMR spectra of synthesized compounds, the signals at 5.43-5.47 and 25.11-25.13ppm were found to correspond to NH and $CH_3$.

## Biological studies

### *In vitro* antibacterial activity

The target candidates [4a-k (X=O), 4l-v (X=S)] were screened for their antibacterial activity. Ampicillin was used as standard drug. The bioassay was performed against *B. cereus, S. aureus*, *S. epidermidis* and *E. faecalis* represented as Gram positive bacterial strains, *S. dysenteriae*, *S. typhi, E. coli* and *K. pneumonia* represented as Gram-negative bacterial strains. The target candidates [4a-k (X=O), 4l-v (X=S)] showed significant activity against bacterial strains. The results of antibacterial studies are presented in Table I [4a-k (X=O)] and Table II [4l-v (X=S)] and comparison of compounds noted in Fig. 1 and Fig. 2. Disk diffusion method was used as a tool for antimicrobial activities. It was noted that among tested derivatives [4a-k (X=O), 4l-v (X=S)], compounds (4c, 4j, 4f,4g and 4i), containing *p*-bromo, *p*-carboxylic, *o, p*-dinitro, *p*-sulfoxy and *m*-chloro as electron withdrawing groups on aryl substitution, were found to be moderately active against *B. cereus*, *E. faecalis, S. dysenteriae*, *K. pneumonia* and *S. epidermidis,* whereas the compounds (4q, 4r, 4t, 4n) containing electron withdrawing groups (*o,p*-dinitro, *p*-sulfoxy, *m*-chloro, *p*-bromo) aryl substitution were found to moderately active against *S. dysenteriae, K. pneumonia S aureus*, *B. cereus* and *S. dysenteriae,* when compared with ampicillin as standard drug. Particularly, compounds 4k and 4v containing (*p*-sulfacetamido) phenyl substituent showed most activity then ampicillin when tested against *S aureus*, *K. pneumoniae, S. epidermidis* and *S. typhi.* While, other molecules, against both Gram-positive and Gram-negative bacterial strains, had strong antibacterial action.

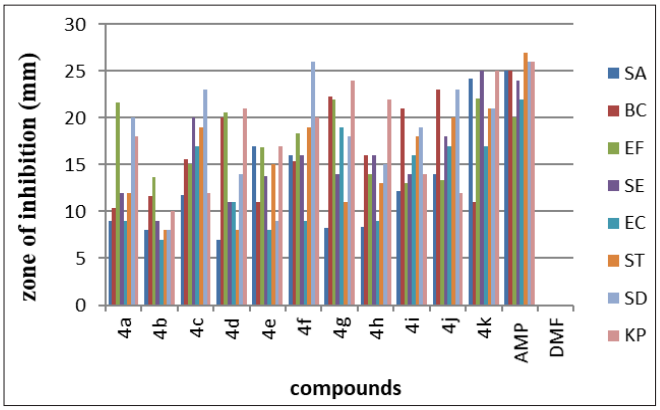



**Fig. 1: Comparison of antibacterial activity of compounds 4a-k**

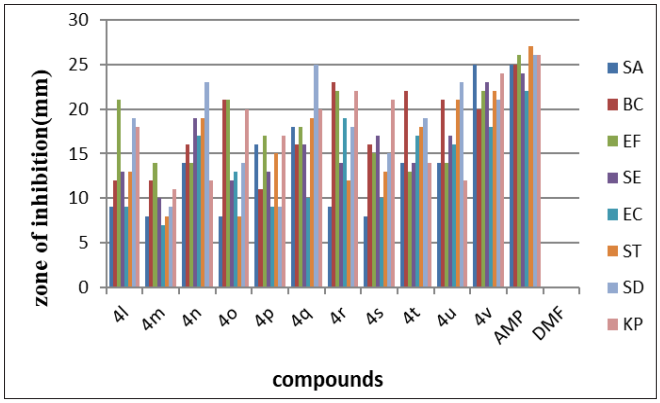



**Fig. 2: Comparison of antibacterial activity of compounds 4l-v**

### *In vitro* anti-inflammatory activity

Inhibition of albumin denaturation technique was used for *in vitro* anti-inflammatory activity. All the compounds [4a-k (X=O), 4l-v (X=S)] showed significant albumin denaturation inhibition absorbance. All compounds exhibited inhibition in the increasing order of concentration. Compounds 4c and 4q showed inhibition of 64.23% and 63.98%, respectively. Comparison of compounds is given in Fig. 3, while inhibition of albumin denaturation of all compounds is given in Table III. All the other tested compounds revealed moderate activity when compared to the standard indomethacin.

Antimicrobial and *in vitro* anti-inflammatory activities postulated that by phenyl ring substitution at 5$^{th}$ position of

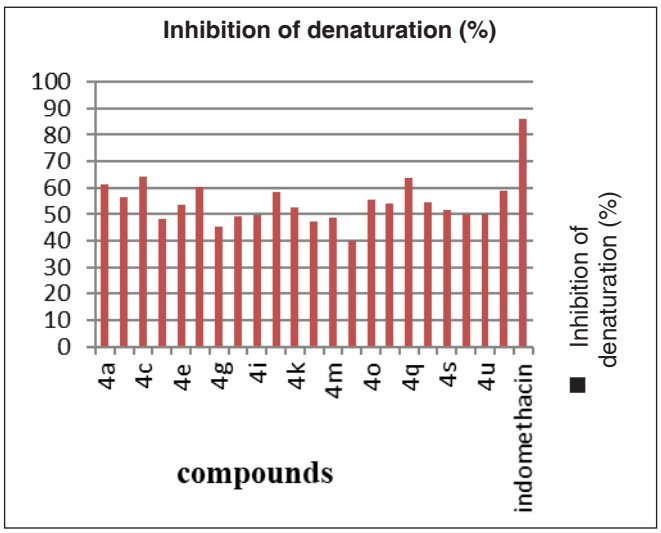



**Fig. 3: Comparison of inhibition of denaturation (%) of compounds 4a-v**

**Table I: Antibacterial-sensitivity testing of compounds 4a-k (X=O)**

| Com-pound | Antibacterial activity Zone of inhibition (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram-positive | | | | Gram-negative | | | |
| S. No | SA | BC | EF | SE | EC | ST | SD | KP |
| 4a | 9±0.00 | 10.4±0.12 | 21.6±0.13 | 12±0.00 | 9±0.00 | 12.4±0.13 | 20±0.00 | 18±0.00 |
| 4b | 8±0.00 | 11.7±0.15 | 13.7±0.17 | 9±0.00 | 7±0.00 | 7.5±0.08 | 8±0.00 | 10±0.00 |
| 4c | 11.8±0.18 | 15.6±0.15 | 15±0.00 | 20±0.00 | 17.6±0.29 | 19±0.00 | 22.7±0.28 | 12.0±0.00 |
| 4d | 7±0.00 | 20±0.00 | 20.6±0.34 | 11±0.00 | 11.7±0.44 | 8±0.00 | 13.8±0.25 | 21±0.00 |
| 4e | 17±0.00 | 11±0.24 | 16.9±0.17 | 13.8±0.24 | 8±0.00 | 14.8±0.14 | 9±0.00 | 16.3±0.29 |
| 4f | 16±0.00 | 15.4±0.17 | 18.4±0.28 | 16±0.00 | 9±0.00 | 19.1±0.23 | 25.3±0.24 | 20±0.00 |
| 4g | 8.2±0.31 | 22.3±0.48 | 22±0.00 | 14±0.30 | 19±0.00 | 11±0.38 | 17.15±0.12 | 24±0.30 |
| 4h | 8.3±0.04 | 16±0.12 | 14±0.00 | 16.4±0.20 | 9.2±0.34 | 13±0.00 | 15±0.00 | 22±0.00 |
| 4i | 12.26±0.32 | 21±0.00 | 13±0.00 | 14±0.00 | 16±0.00 | 17.8±0.25 | 19±0.00 | 14±0.00 |
| 4j | 14±0.00 | 23±0.00 | 13.4±0.14 | 18.2±0.17 | 17±0.00 | 20±0.00 | 23.2±0.45 | 11.8±0.33 |
| 4k | 24.2±0.18 | 11±0.00 | 22.1±0.20 | 25±0.00 | 17±0.00 | 21.4±0.53 | 21±0.00 | 25±0.00 |
| AMP | 25±0.00 | 25±0.00 | 26±0.00 | 24±0.00 | 22±0.00 | 27±0.00 | 26±0.00 | 26±0.00 |
| DMF | ……. | …….. | …… | ……. | …… | …… | …… | …… |

*All the values are expressed as mean ± SEM of triplicate*

- AMP =Ampicillin
- DMF = Dimethyl formamide
- SA = *Staphylococcus aureus* (ATCC 11633)
- ST = *Salmonella typhi* (MTCC 733)
- SE = *Staphylococcus epidermidis* (ATCC 155)
- SD = *Shigella dysenteriae* (ATCC 13313)
- EC = *Escherichia coli* (ATCC 10536)
- BC = *Bacillus cereus* (ATCC 11778)
- EF = *Enterococccus faecalis* (ATCC 14506)
- KP = *Klebsiella pneumoniae* (ATCC 10031)

**Table II: Antibacterial-sensitivity testing of compounds 4l-v (X=S)**

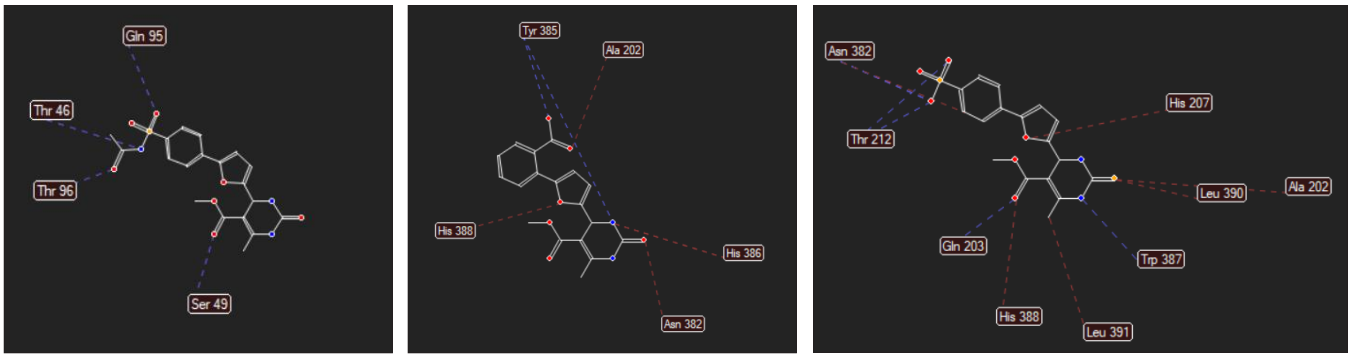
| Com-pound | Antibacterial activity Zone of inhibition (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram-positive | | | | Gram-negative | | | |
| S. No. | SA | BC | EF | SE | EC | ST | SD | KP |
| 4l | 9±0.00 | 11.4±0.12 | 20.6±0.23 | 13±0.00 | 9±0.00 | 12.4±0.16 | 19±0.00 | 18±0.00 |
| 4m | 8±0.00 | 11.9±0.15 | 14.01±0.17 | 10±0.00 | 7±0.00 | 8±0.08 | 9±0.00 | 11±0.00 |
| 4n | 13.8±0.46 | 15.9±0.19 | 14±0.00 | 19±0.00 | 16.6±0.22 | 19±0.00 | 22.6±0.27 | 12.0±0.00 |
| 4o | 8±0.00 | 21±0.00 | 20.9±0.34 | 12±0.00 | 12.7±0.43 | 8±0.00 | 14±0.25 | 20±0.00 |

| Com-pound | Antibacterial activity Zone of inhibition (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram-positive | | | | Gram-negative | | | |
| S. No. | SA | BC | EF | SE | EC | ST | SD | KP |
| 4p | 16±0.00 | 11±0.33 | 17.00±0.00 | 12.98±0.22 | 9±0.00 | 14.9±0.14 | 9±0.00 | 16.4±0.32 |
| 4q | 18±0.00 | 16±0.15 | 18.4±0.29 | 16±0.11 | 10±0.00 | 19.1±0.23 | 25±0.24 | 20±0.00 |
| 4r | 8.8±0.42 | 22.6±0.48 | 22±0.12 | 14±0.31 | 19±0.11 | 12±0.38 | 17.15±0.12 | 22±0.30 |
| 4s | 8.3±0.07 | 16±0.12 | 15±0.31 | 16.2±0.21 | 9.6±0.35 | 13±0.00 | 15±0.00 | 21±0.00 |
| 4t | 13.26±0.33 | 22±0.00 | 13±0.00 | 14±0.00 | 17±0.00 | 17.8±0.28 | 19±0.00 | 14±0.00 |
| 4u | 14±0.00 | 21±0.00 | 13.76±0.15 | 16.56±0.17 | 16±0.00 | 21±0.00 | 22.2±0.45 | 11.9±0.33 |
| 4v | 25.2±0.32 | 20±0.00 | 22.1±0.23 | 23±0.00 | 18±0.00 | 21.4±0.32 | 20.43±0.16 | 24±0.00 |
| AMP | 25±0.00 | 25±0.00 | 26±0.00 | 24±0.00 | 22±0.00 | 27±0.00 | 26±0.00 | 26±0.00 |
| DMF | ……. | …….. | …… | ……. | …… | …… | …… | …… |

*All the values are expressed as mean ± SEM of triplicate*

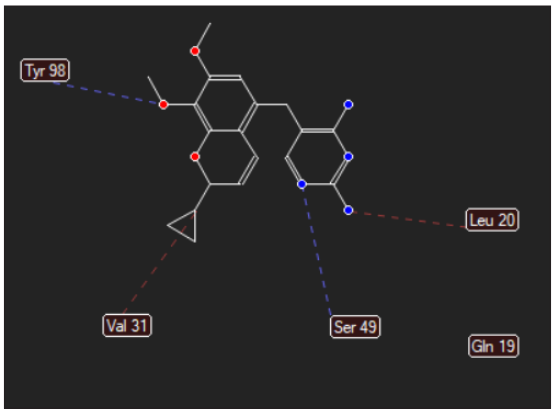
- AMP =Ampicillin
- DMF = Dimethyl formamide
- SA = *Staphylococcus aureus* (ATCC 11633)
- ST = *Salmonella typhi* (MTCC 733)
- SE = *Staphylococcus epidermidis* (ATCC 155)
- SD = *Shigella dysenteriae* (ATCC 13313)
- EC = *Escherichia coli* (ATCC 10536)
- BC = *Bacillus cereus* (ATCC 11778)
- EF = *Enterococccus faecalis* (ATCC 14506)
- KP = *Klebsiella pneumoniae* (ATCC 10031)

furfural ring in bioactive compounds {5'-(substituted aryl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones [4a-k (X=O), 4l-v (X=S)] the targeted novel synthesized derivatives showed promising antibacterial and anti-inflammatory activities. It is concluded that i substituted aryl ring presented at 5$^{th}$ position of furfural ring which is attached on 3,4-dihydro-*1H*-pyrimidine-2-ones, would be beneficial for intensifying the antimicrobial and anti-inflammatory activities. Preparation of the bioactive compounds with broad spectrum for clinically useful activities, was highly encouraging and opens a way for the futuristic scaffolds based on medicinal chemistry work.

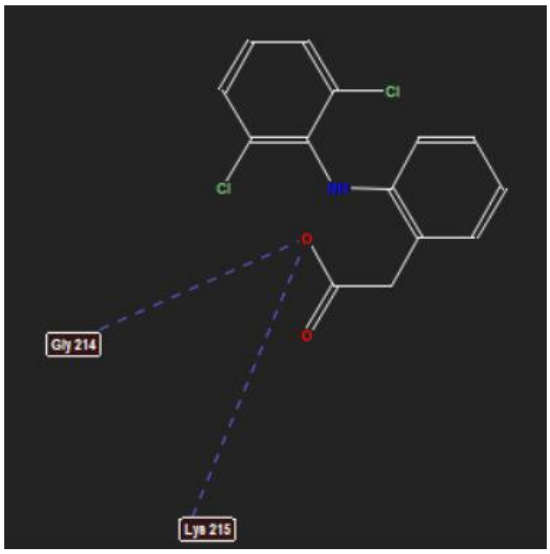



**(4k)** **(4h)** **(4r)**

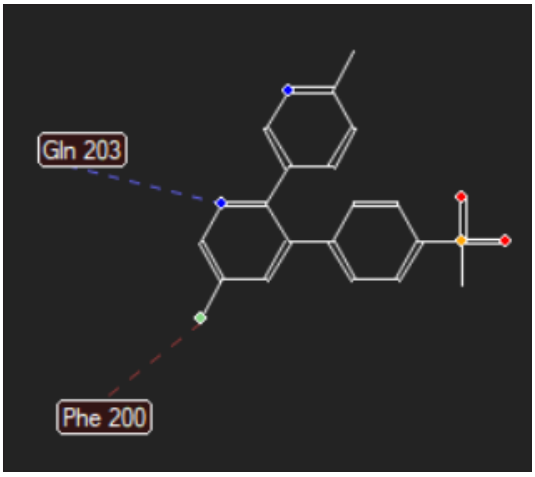
**Fig. 4: The 2D poses of compounds 4k, 4h and 4r against DHFR, COX-1 and COX-2 proteins, respectively, that showed hydrogen bonding interactions and steric interactions with various amino acids**




(A)




(B)




(C)

**Fig. 5: 2D poses of reference compounds of DHFR, COX-1 and COX-2 proteins: (A) Iclaprim, (B) Diclofenac sodium and (C) Etoricoxib**

**Table III: *In vitro* anti-inflammatory activity of compounds 4a-k (X=O), 4l-v (X=S)**

| Compound | Absorbance Value* | Inhibition of denaturation (%) |
|---|---|---|
| 4a | 0.0404 | 61.25 |
| 4b | 0.0341 | 56.45 |
| 4c | 0.0406 | 64.23 |
| 4d | 0.0234 | 48.30 |
| 4e | 0.0202 | 53.36 |
| 4f | 0.0150 | 60.16 |
| 4g | 0.0321 | 45.34 |
| 4h | 0.0128 | 48.97 |
| 4i | 0.0412 | 49.46 |
| 4j | 0.0136 | 58.34 |
| 4k | 0.0210 | 52.43 |
| 4l | 0.0302 | 47.12 |
| 4m | 0.0210 | 48.65 |
| 4n | 0.0401 | 39.87 |
| 4o | 0.0203 | 55.49 |
| 4p | 0.0267 | 53.87 |
| 4q | 0.0289 | 63.98 |
| 4r | 0.0324 | 54.76 |
| 4s | 0.0348 | 51.69 |
| 4t | 0.0319 | 49.98 |
| 4u | 0.0178 | 50.23 |
| 4v | 0.0184 | 58.98 |
| indomethacin | 0.0150 | 86.13 |

**(Mean+SD)*

## Molecular docking studies

To determine the potential binding mechanisms of all synthetic molecules (4a-4v), molecular docking experiments were conducted utilising the high-resolution crystal structures of *S. aureus* dihydrofolate reductase (DHFR), COX-1, and COX-2 proteins (PDB ID: 3FRA, 6Y3C, 5KIR respectively). These synthetic molecules were docked using the Molegro Virtual Docker (MVD) programme. Table IV lists probable hydrogen bond interactions with different amino acid residues based on MolDock scores. Most of compounds showed

**Table IV: Molecular docking score and interactions of compounds**

| S. No. | Compound | MolDock Scores | | |
|---|---|---|---|---|
| | | 3FRA | 6Y3C | 5KIR |
| 1 | 4a | -123.607 | -120.953 | -105.978 |
| 2 | 4b | -103.198 | -108.621 | -99.196 |
| 3 | 4c | -102.714 | -70.543 | -100.911 |
| 4 | 4d | -103.596 | -105.594 | -113.228 |
| 5 | 4e | -100.630 | -114.627 | -109.823 |
| 6 | 4f | -119.595 | -84.412 | -108.232 |
| 7 | 4g | -102.461 | -102.279 | -97.012 |
| 8 | 4h | -108.401 | -142.132 | -121.084 |
| 9 | 4i | -101.834 | -103.874 | -104.415 |
| 10 | 4j | -108.435 | -114.584 | -104.694 |
| 11 | 4k | -132.695 | -127.012 | -68.512 |
| 12 | 4l | -118.424 | -115.923 | -102.206 |
| 13 | 4m | -101.292 | -95.143 | -98.310 |
| 14 | 4n | -101.904 | -91.776 | -97.530 |
| 15 | 4o | -98.505 | -97.159 | -51.320 |
| 16 | 4p | -102.367 | -114.991 | -79.635 |
| 17 | 4q | -96.753 | -88.617 | -98.252 |
| 18 | 4r | -113.128 | -109.351 | -140.128 |
| 19 | 4s | -102.931 | -98.985 | -136.67 |
| 20 | 4t | -96.510 | -98.224 | -104.451 |
| 21 | 4u | -106.467 | -91.719 | -103.008 |
| 22 | 4v | -108.306 | -96.227 | -134.750 |
| 23 | Iclaprim | -109.593 | - | - |
| 24 | Diclofenac sodium | - | -59.617 | - |
| 25 | Etoricoxib | - | - | -63.260 |
| **S. No.** | **Compound** | **H-Bonding interaction with amino acids** | | |
| | | **3FRA** | **6Y3C** | **5KIR** |
| 1 | 4a | Thr121, Asn18, Leu5, Tyr98 | His388, Tyr385, Asn382, Thr212, Phe210 | Asn222, Thr212, His214, Asn382 |
| 2 | 4b | Ala7, Thr46, Phe92 | Asn382, Thr212 | Thr212, His386 |
| 3 | 4c | Thr121, Thr46, Phe92 | Thr212, Phe210, Asn382 | Thr212, His386 |

| S. No. | Compound | H-Bonding interaction with amino acids | | |
|---|---|---|---|---|
| | | 3FRA | 6Y3C | 5KIR |
| 4 | 4d | Ala7, Thr46, Phe92 | Phe210, Gln289 | Tyr148, Thr212, Phe210 |
| 5 | 4e | Ser49, Ala7, Thr46, Phe92 | Trp387, Thr212, Asn382 | Thr212, His386 |
| 6 | 4f | Thr121, Asn18, Ser49, Phe92, Leu28 | Thr206, Phe210, Gln289 | Asn222, Thr212, His214, Asn382 |
| 7 | 4g | Ser49, Ala7, Thr46 | His274, Gln203, Thr206 | Lys215, Phe210, Tyr148, Thr212, Asn382 |
| 8 | 4h | Ser49, Ala7, Thr46, Phe92 | Tyr385 | Thr206 |
| 9 | 4i | Ala7, Thr46, Phe92 | - | - |
| 10 | 4j | Leu28, Ala7, Thr46, Phe92 | Ala199, Thr212, Phe210, Asn382 | Glu290, Thr212, His214, Asn382 |
| 11 | 4k | Ser49, Thr46, Thr96, Gln95 | Thr212, Gln203, Thr206, His388 | Thr212, His386 |
| 12 | 4l | Asn18, Thr121, Phe92 | Thr212, Gln203, Thr206, His388 | Asn222, Thr212, His214, Asn382 |
| 13 | 4m | Ala7 | His207 | Hiss388 |
| 14 | 4n | Ala7, Ile14 | - | - |
| 15 | 4o | Ala7, Ile14 | His207 | - |
| 16 | 4p | Ala7, Ile14 | Thr212, Gln203, Thr206, His388 | Gln203 |
| 17 | 4q | Thr121, Asn18, Phe92 | Thr212, Gln289, Thr206 | Thr206, Trp387 |
| 18 | 4r | Ala7, Ile14, Leu28, Tyr98 | Thr212, Thr206 | Thr212, Asn382, Gln203, Trp387 |
| 19 | 4s | Gln19, Leu20, Ile14, Thr46, Tyr98 | Thr212, Thr206, His207, Asn382 | Thr206, Tyr385 |
| 20 | 4t | Tyr98, Ala7, Ile14 | - | - |
| 21 | 4u | Leu28, Ala7 | Gln289 | Glu290, Thr212, Asn382, His214 |
| 22 | 4v | Gln19, Ala7, Ile14 | Trp387 | Thr212, Gln203, Trp387 |
| 23 | Iclaprim | Tyr98, Ser49 | - | - |
| 24 | Diclofenac sodium | - | Gly214, Lys215 | - |
| 25 | Etoricoxib | - | - | Gln203 |

higher MolDock scores than reference compounds. The compound 4k exhibited excellent binding energy of -132.695 kcal $mol^{-1}$ against DHFR receptor than reference compound iclaprim. This compound showed four hydrogen bond interactions with various amino acids of 3FRA protein such as Thr46, Ser49, Gln95 and Thr96. Compounds 4h and 4r showed good binding energy (-142.132 and -140.128 respectively) against COX-1 and COX-2, respectively. Compound 4h made two hydrogen bond interaction with Tyr385 amino acid of COX-1. Compound 4r formed five hydrogen bonds with Asn382, Thr212, Gln203 and Trp387 and steric interaction with with Asn382, His388, Leu391, His207, Leu390 and Ala202 amino acids of COX-2. The 2D poses of compounds 4k, 4h and 4r against 3FRA, 6Y3C and 5KIR, respectively, and their respective reference compounds such as iclaprim, diclofenac sodium and etoricoxib are shown in Fig. 4 and Fig. 5 respectively.

## CONCLUSION

In the class of nitrogen-containing aromatic heterocyclic scaffolds, pyrimidine always grabs interest of the medicinal chemists. In this study a series of 5'-(substituted aryl)-2-furanyl}-3,4-dihydro-*1H*-pyrimidine-2-ones have been designed and synthesized with Beginelli reaction. The structures of the target candidates were confirmed by FTIR, NMR, Mass spectrometry and by elemental analysis. The *in vitro* antibacterial activity was performed against Gram-positive strains of bacteria *B. cereus*, *S. aureus*, *S. epidermidis* and *E. faecalis* and Gram-negative bacterial strains *S. dysenteriae*, *E. coli*, *K. pneumoniae* and *S. typhi*. Among the synthesized compounds, 4k and 4v found to be most effective when tested against strains of *K. pneumonia, S. aureus, S. typhi, S. epidermidis* and *E. faecalis* in anti-bacterial assay. The remaining synthesized derivatives showed significant antibacterial activity along with remarkable *in vitro* anti-inflammatory activity.

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