

## Synthesis of new bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one derivatives from dehydroacetic acid and its analogues as antibacterial agents

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Dedicated to Prof. Rosa Maria Claramunt on the occasion of her 65th anniversary

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**Abstract.** Synthesis of some new bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3** was accomplished by the reaction of 3- and/or 4-substituted 5-aminopyrazoles **2** with 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (dehydroacetic acid, DHAA) **1a** and its analogues **1b-c** in refluxing ethanol. A tentative mechanism for the formation of **3** is proposed. These compounds were evaluated for their antibacterial activity against two Gram positive bacteria i.e., *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8505) and two Gram negative bacteria i.e. *Escherichia coli* (MTCC 119) and *Pseudomonas aeruginosa* (MTCC 741) using agar well diffusion assay technique and minimum inhibitory concentration (MIC) method. One of the compound **3d** displayed significant antibacterial activity.

**Keywords:** Dehydroacetic acid, bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones, 5-aminopyrazoles antibacterial agents

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### Introduction

3-Acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (dehydroacetic acid, DHAA) **1a**, having several electrophilic sites, is susceptible to attack by nucleophilic reagents at carbonyl of the acetyl side chain at position-3, carbon terminating the conjugating carbon chain at 6-position, lactone carbonyl at 2-position and carbonyl at 4-position.<sup>1-2</sup> Binucleophiles such as mono-substituted hydrazines and hydroxylamine are known to react with DHAA to give a wide variety of products, e.g. pyrazoles, bipyrazoles, bis-isoxazole etc., under different reaction conditions.<sup>3-6</sup> 5-Amino-1*H*-pyrazoles like mono-substituted hydrazines have been utilized as active synthons for synthesis of many heterocyclic compounds, e.g., pyrazolo[1,5-*a*]pyrimidines, pyrazolo[3,4-*b*]pyridines, etc.<sup>7-11</sup> Large differences in the nucleophilicity of the two nitrogens in 5-amino-1*H*-

pyrazoles, one as *endo* nitrogen of pyrazole ring and the other as amino group at 5-position, makes it an ideal binucleophile. Thus, the reaction of 5-aminopyrazoles with  $\beta$ -diketones may take place with the participation of a primary amino group in the first step and subsequent cyclization by the attack of ring nitrogen or *vice versa* to give regioisomeric pyrazolo[1,5-*a*]pyrimidines.<sup>7,10-14</sup>

A literature survey reveals that the pyrazolo[1,5-*a*]pyrimidine skeleton has been applied in various therapeutic areas such as antitumor, antidiabetic, benzodiazepine receptor activities, etc.<sup>15-16</sup> Also, the pyrazolopyrimidines<sup>17</sup> are selective inhibitor of cyclic 3',5'-adenosine monophosphate (cAMP) phosphodiesterases *in vitro*, and some of them possess anxiolytic properties comparable to those of benzodiazepines.<sup>18</sup>

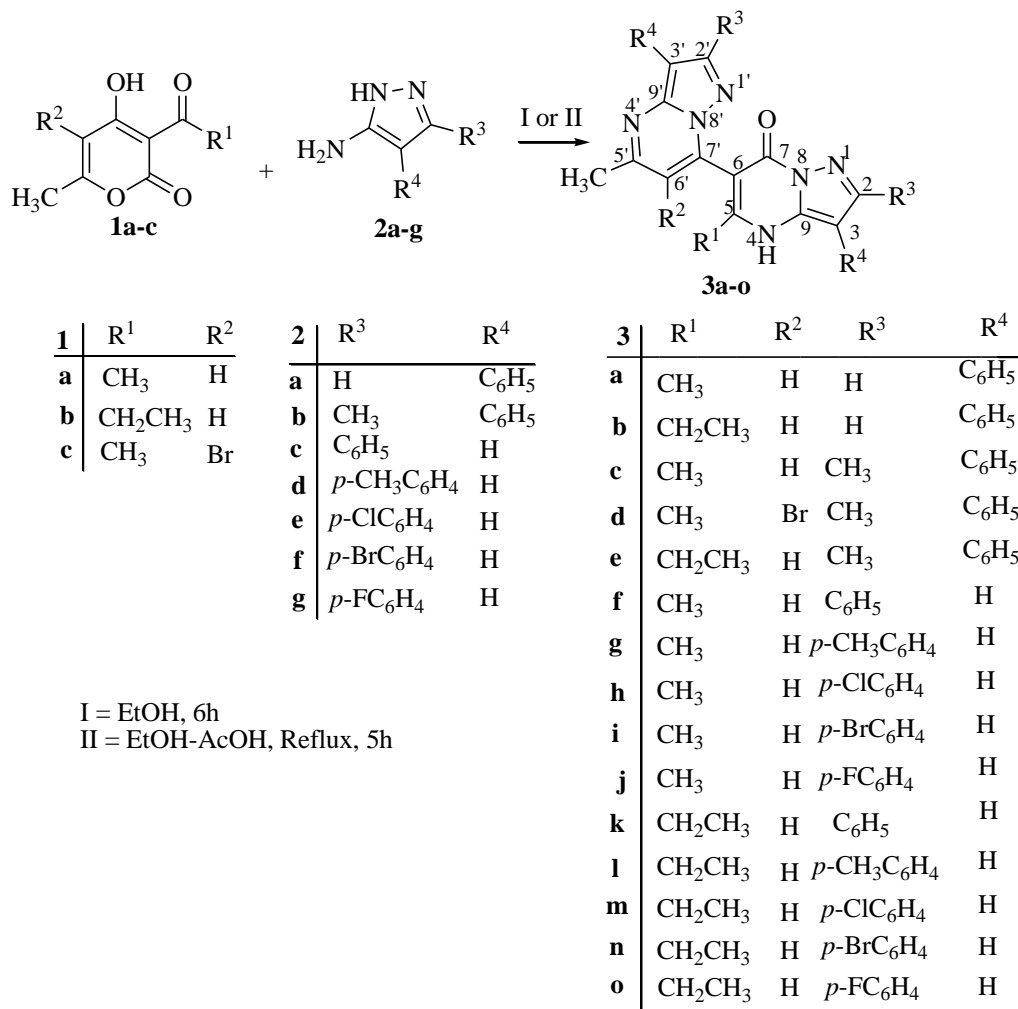
We have a constant need for novel compounds or principles to combat bacteria that have become resistant to conventional antibacterials. Therefore, in continuation of our work on the synthesis of pyrazole moiety<sup>19-20</sup> and fused heterocycles,<sup>7,11, 21-24</sup> we now have aim to determine antibacterial activity and to describe the regioselective synthesis of new fused bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones, by the reaction of DHAA and its analogues with 5-aminopyrazoles.

## Results and Discussion

The reaction of 5-amino-4-phenyl-1*H*-pyrazole **2a**, obtained by the formylation of benzylcyanide followed by the reaction with hydrazine hydrate, was initially carried out with an equimolar amount of DHAA **1a** in refluxing ethanol for 6 h which afforded 5,5'-dimethyl-3,3'-diphenyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one **3a** (**Scheme 1**). The yield of the reaction was very low as some DHAA was left unreacted, however, on using two equivalents of 5-aminopyrazole **2a** there was a tremendous improvement in the yield of the reaction from 35% to 79% and the tlc showed disappearance of DHAA. Also, using a catalytic amount of acetic acid in ethanol resulted in the reduction of reaction time to 5 h. The structure of the compound **3a** was identified on the basis of the IR, <sup>1</sup>H, <sup>13</sup>C NMR and mass spectra. The IR spectrum of **3a** showed absorption bands at 3472 and 1637 cm<sup>-1</sup> due to N-H and C=O stretch, respectively. The <sup>1</sup>H NMR spectrum of **3a** shows the presence of two singlets at  $\delta$  2.28 and 2.72 ppm due to the presence of two methyl protons at positions 5 and 5' respectively. Appearance of three sharp singlets each of one proton intensity at  $\delta$  6.89, 7.99 and 8.35 ppm corresponding to bi(pyrazolopyrimidine) 6', 2 and 2'-H shows the formation of bi(pyrazolo[1,5-*a*]pyrimidine) nucleus.<sup>25-26</sup> The <sup>1</sup>H NMR spectrum of **3a** also showed one exchangeable proton at  $\delta$  13.05 ppm due to NH present at position-4.

To generalize the reaction, different substituted bi(pyrazolo[1,5-*a*]pyrimidines) **3b-3e** were prepared by reacting differently substituted 5-amino-4-phenyl-1*H*-pyrazoles **2a-b** with DHAA **1a** and its analogues **1b-c** in a ratio of 2:1 (**Scheme 1**). All the compounds were identified on the basis of the IR, <sup>1</sup>H, <sup>13</sup>C NMR and mass spectral data. In the <sup>1</sup>H NMR spectrum of **3b**, one of the methyl of DHAA is replaced by ethyl group and a typical pattern of triplet at  $\delta$  1.14 ppm and quartet at  $\delta$  3.06 ppm appeared along with the other signals as were obtained in the spectrum of

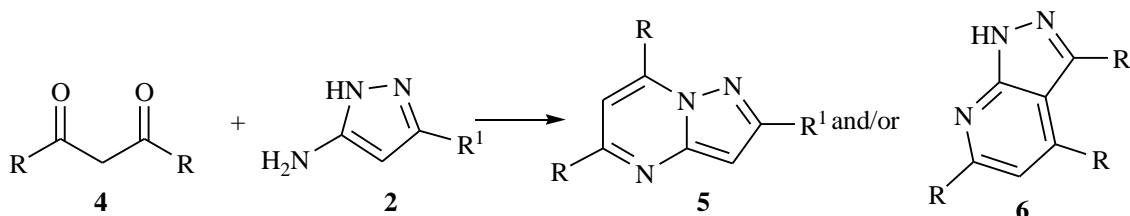
**3a**. Proton present at 6'-H disappears in **3d** as this proton is replaced by bromine atom. Also, two singlets at about  $\delta$  7.99 and 8.35 ppm, which appeared for two pyrazole ring 2 and 2'-H in **3a** and **3b**, were replaced by that of two singlets of methyl protons between  $\delta$  2.39-2.51 ppm and 2.55-2.65 ppm in **3c-3d** for 2 and 2'-CH<sub>3</sub>, respectively. The <sup>13</sup>C NMR spectra of **3a-e** also showed 12 signals for the basic skeleton along with other indispensable signals for the substituents, thus confirming the condensation of two moles of pyrazole moiety with one mole of DHAA, and its analogues.



**Scheme 1.** Synthesis of bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3a-o** from 5-aminopyrazoles **2a-g** and DHAA and its analogues **1a-c**.

An extensive literature survey of the chemical behavior of *N*-unsubstituted 5-amino-1*H*-pyrazoles **2** ring having vacant 4-position revealed that it may react with electrophiles such as  $\beta$ -diketones **4** to yield a mixture of structural isomers pyrazolo[1,5-*a*]pyrimidines **5** and pyrazolo[3,4-*b*]pyridines **6** (**Scheme 2**) depending upon the nature of substituent on  $\beta$ -

diketones.<sup>27</sup> Solvent also plays a crucial role in controlling the structure of the product as acetic acid leads to the formation of a mixture of pyrazolo[3,4-*b*]pyridine **6** and pyrazolo[1,5-*a*]pyrimidine **5**. Pyrazolopyrimidine **5** was formed as the only reaction product when DMSO was used as solvent whereas on boiling the reactants in ethanol in the presence of TEA yielded pyrazolopyridine **6** predominantly.<sup>28</sup>



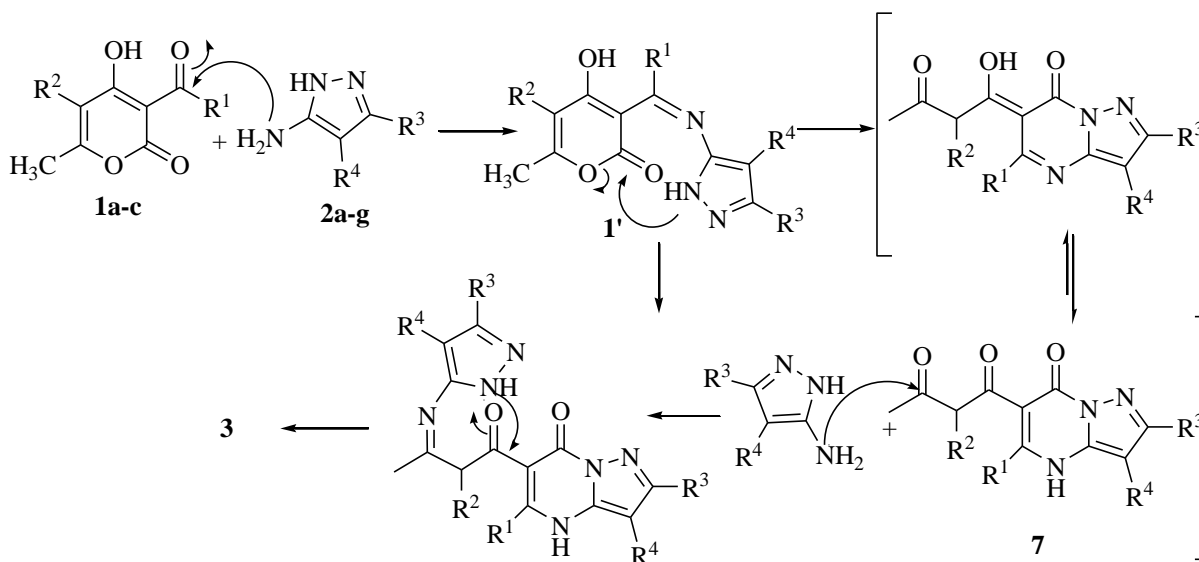
**Scheme 2.** Expected products from the reaction between *N*-unsubstituted 5-amino-1*H*-pyrazoles **2** having vacant 4-position and β-diketones **4**.

After having performed reactions with **2a** and **2b**, it was thought of investigating the structure of products on treating 5-amino-3-aryl-1*H*-pyrazoles **2c-g** (unsubstituted at position 4) with DHAA and its analogues **1a-b** (**Scheme 1**). 5-Amino-3-aryl-1*H*-pyrazoles **2c-g**, obtained from the condensation of α-cyanoacetophenones and hydrazine hydrate in ethanol, on refluxing with DHAA and its analogues **1a-b** resulted in the formation of single products which were characterized on the basis of their IR, <sup>1</sup>H, <sup>13</sup>C NMR and mass spectra as bi(pyrazolopyrimidinyl)-7-ones **3f-o**. No trace of pyrazolo[3,4-*b*]pyridine was detected in the tlc and the NMR of the crude reaction mixture.

The <sup>1</sup>H NMR spectra of bi(pyrazolopyrimidines) **3f-o** were almost identical with those of bi(pyrazolopyrimidines) **3a-e**. One important difference is that the spectra showed three singlets of one proton intensity each between δ 6.71-6.82 ppm, 7.02-7.09 ppm and 7.08-7.19 ppm corresponding to 6', 3 and 3'-H, respectively, showing the presence of two pyrazolopyrimidine nuclei having a vacant 3-position. The <sup>1</sup>H NMR spectra of **3f-o** displayed only one exchangeable proton at about δ 13.05 ppm due to NH present at position-4. Had the structure been pyrazolopyridine then the signals for 3 and 3'-H would not have appeared rather exchangeable signals for three NH protons would have been observed in the NMR and IR spectra.

A plausible mechanism leading to the formation of bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3a-o** is depicted in **Scheme 3**. An amino group of 5-aminopyrazole **2** attacks on carbonyl of acetyl side chain of **1** to generate a Schiff's base intermediate **1'**, followed by the subsequent nucleophilic attack of the *endo* nitrogen on the lactone carbonyl of DHAA to provide a pyrazolo[1,5-*a*]pyrimidinyl-β-diketone **7** through an intramolecular pyran ring opening (**Scheme 3**). This mechanism is supported by the fact that 5-aminopyrazoles react with β-ketoesters to produce pyrazolo[1,5-*a*]pyrimidinyl-7-ones as the single product involving the first attack of primary amino group on the keto group and subsequent attack of *endo* N on ester group.<sup>17,29-30</sup> Efforts were made to isolate pyrazolo[1,5-*a*]pyrimidinyl-β-diketone **7** by performing the reaction at room temperature or in the cold or by adding the solution of 5-aminopyrazole **2b** drop wise to

the solution of DHAA **1** or by using two equivalent of DHAA **1** and one equivalent of 5-aminopyrazole **2b** but the reaction did not stop at this level as the resulting  $\beta$ -diketones **7** has greater reactivity towards nucleophiles and the second mole of 5-aminopyrazole **2** reacted further with the  $\beta$ -diketone **7** to yield the bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3**.



**Scheme 3.** Plausible mechanism leading to the formation of bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3a-o**.

### *In vitro* antibacterial assay

**Primary screening.** The antibacterial activities of these compounds, 5'-methyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3a-o** were evaluated against two Gram-positive bacteria i.e. *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8505) and two Gram-negative bacteria i.e. *Escherichia coli* (MTCC 119) and *Pseudomonas aeruginosa* (MTCC 741) at three different concentrations i.e. 10, 50 and 100  $\mu\text{g/mL}$  (**Table 1**) using agar well diffusion assay technique<sup>31-32</sup> and minimum inhibitory concentration (MIC) method (**Table 2**).

The antimicrobial activities of the compounds were compared against the standard drugs, ampicillin, chloramphenicol (25  $\mu\text{g/mL}$ ) (+ve control) and DMSO (-ve control). These tests were performed in triplicate and the mean of diameter of zone of growth inhibition was taken.

**Table 1.** *In vitro* antibacterial activity of compounds **3a-o** by using well diffusion method

Compound	Concentration ( $\mu\text{g/mL}$ )	Diameter of zone of growth inhibition (mm)			
		<i>B. subtilis</i>	<i>B. stearo-thermophilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<b>3a</b>	10	-	-	-	-
	50	-	-	-	-
	100	-	-	13	12
<b>3b</b>	10	09	-	-	-
	50	21	19	13	16
	100	49	36	29	31
<b>3c</b>	10	-	10	11	09
	50	12	23	24	25
	100	31	38	46	43
<b>3d</b>	10	16	13	13	17
	50	31	29	28	30
	100	65	57	53	59
<b>3e</b>	10	-	-	-	-
	50	09	-	16	17
	100	25	23	34	39
<b>3f</b>	10	-	-	-	-
	50	-	-	-	-
	100	10	11	13	-
<b>3g</b>	10	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
<b>3h</b>	10	-	-	-	-
	50	-	-	-	-
	100	11	14	12	-
<b>3i</b>	10	-	-	-	-
	50	-	-	-	-
	100	-	13	11	10
<b>3j</b>	10	-	-	-	-
	50	-	-	-	-
	100	10	12	13	12
<b>3k</b>	10	-	-	-	-
	50	-	11	10	-
	100	10	16	14	09
<b>3l</b>	10	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-

**Table 1 (continued)**

<b>3m</b>	10	-	-	-	-
	50	-	-	-	-
	100	-	14	13	-
<b>3n</b>	10	-	-	-	-
	50	-	-	-	-
	100	10	12	11	-
<b>3o</b>	10	-	-	-	-
	50	-	-	-	-
	100	11	13	10	10
Chloramphenicol <sup>b</sup>	25	17	16	26	24
Ampicillin <sup>b</sup>	25	34	35	39	41

<sup>a</sup>“-” means not determined or no activity whatever applies. <sup>b</sup>Chloramphenicol and Ampicillin has been used as a standard drugs.

**Table 2.** Minimum inhibitory concentration (MIC\*) (μg/mL) of test compounds

Compounds	<i>B. subtilis</i>	<i>B. stearothermophilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<b>3a</b>	>200	>200	100	100
<b>3b</b>	6.25	12.25	25	12.25
<b>3c</b>	25	6.25	6.25	6.25
<b>3d</b>	3.12	6.25	6.25	3.12
<b>3e</b>	50	100	12.25	12.25
<b>3f</b>	100	100	100	>200
<b>3g</b>	>200	>200	>200	>200
<b>3h</b>	100	100	100	>200
<b>3i</b>	200	100	100	100
<b>3j</b>	100	100	100	100
<b>3k</b>	100	50	50	100
<b>3l</b>	>200	>200	>200	>200
<b>3m</b>	>200	100	100	>200
<b>3n</b>	100	100	100	>200
<b>3o</b>	100	100	100	100
<b>Chloramphenicol</b>	0.19	<0.19	<0.19	<0.19
<b>Ampicillin</b>	<0.19	<0.19	<0.19	<0.19

\*Means of triplicates.

From the **Table 1**, it may be concluded that the compounds bearing phenyl substituent at position-3 of pyrazolopyrimidine ring i.e. 3,3'-diphenyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **3a-d** showed good result as compared to compounds having vacant 3-position **3f-o**. More

importantly, compound **3d** bearing bromine at position-6' exhibited the maximum zone of inhibition followed by that for compounds **3c** and **3b** at concentration of 100, 50 and 10  $\mu\text{g/mL}$  against both Gram-positive and Gram-negative bacteria as compared to other compounds (**3a**, **3e-o**). Compounds **3g** and **3l** having p-methylphenyl at 2 and 2'-position showed no zone of inhibition at concentrations 10, 50, 100  $\mu\text{g/mL}$ . The antibacterial activity of these compounds was also compared with two commercially available antibiotics namely Chloramphenicol and Ampicillin. It is clear from the **Table 1** that the compound **3d** has the zone of inhibition compared to the standard antibiotic Chloramphenicol when used at the concentration of 25  $\mu\text{g/mL}$  whereas no compound is as effective as the Ampicillin when used at the concentration of 25  $\mu\text{g/mL}$ .

In the whole series, the MIC value of various synthesized compounds was evaluated between 200-0.19  $\mu\text{g/mL}$  against Gram-positive and Gram-negative bacteria (**Table 2**). The compound **3d** was found to be the most effective against all Gram-positive and Gram-negative bacteria having the lowest MIC value of 3.12  $\mu\text{g/mL}$  against *B. subtilis* and *P. aeruginosa*, 6.25  $\mu\text{g/mL}$  against *B. stearothermophilus* and *E. coli*. Compound **3c** showed MIC value of 6.25  $\mu\text{g/mL}$  against *B. stearothermophilus*, *P. aeruginosa* and *E. coli* while 25  $\mu\text{g/mL}$  against *B. subtilis* comparable with commercial antibiotics Chloramphenicol and Ampicillin which showed MIC value of <0.19 and 0.19  $\mu\text{g/mL}$  against all the bacteria. Whereas the compounds **3g** and **3l** are having the MIC value greater than 200  $\mu\text{g/mL}$ .

## Conclusions

A series of 5'-methyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7ones **3a-o** were synthesized from the reaction of substituted 5-amino-1*H*-pyrazoles with DHAA and its analogues and were evaluated for their antibacterial activity against both Gram-positive and Gram-negative bacteria. Treatment of 5-aminopyrazoles with or without a substituent at position-4 with DHAA and its analogues afforded single compound having two prazolopyrimidine nuclei. Compounds bearing phenyl substituent at position-3 of pyrazolopyrimidine ring **3a-d** showed good result as compared to compounds having vacant 3-position **3f-o**. It is interesting to note that compound **3d** displayed significant antibacterial activity compared to the standard antibiotic Chloramphenicol.

## Experimental Section

**General.** The IR spectra of the compounds were recorded on Buck Scientific IR M-500 spectrophotometer using KBr pellets ( $\nu_{\text{max}}$  in  $\text{cm}^{-1}$ ),  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained on a Bruker instrument at 300 and 75 MHz, respectively, chemical shifts are expressed in  $\delta$ -scale downfield from TMS as an internal standard. Elemental analysis was performed at RSIC, Lucknow, India. High resolution mass spectra (HRMS) were measured in EI mode on a Kratos



MS-50 spectrometer, Mass Spectrometry Facility, University of California, San Francisco, USA. The <sup>1</sup>H NMR signals of the aromatic protons of substituents R<sup>3</sup> (at positions 2 and 2' - compounds **3g-o**), are marked with the sign ".

**5,5'-Dimethyl-3,3'-diphenyl-4H-[6,7']bi(pyrazolo[1,5-a]pyrimidinyl)-7-one (3a).** To an ethanolic solution of 5-amino-4-phenyl-1H-pyrazole **2a** (0.318g, 2 mmol) was added 3-acetyl-4-hydroxy-6-methyl-2H-pyran-2-one **1a** (0.168g, 1 mmol) and refluxed for 6 hr. The reaction was monitored by TLC. After completion of the reaction, solvent was distilled under reduced pressure. Solid thus obtained was washed with little cold ethanol and recrystallized from ethanol to give **3a**: yield 79%, mp 213-15 °C; IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3463, 1631. <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{H}}$ : 2.28 (s, 3H, 5-CH<sub>3</sub>), 2.72 (s, 3H, 5'-CH<sub>3</sub>), 6.89 (s, 1H, 6'-H), 7.23-7.26 (m, 1H, Ph), 7.34-7.38 (m, 1H, Ph), 7.42-7.46 (m, 2H, Ph), 7.46-7.50 (m, 2H, Ph), 7.55-7.56 (m, 2H, Ph), 7.99 (s, 1H, 2-H), 8.08-8.10 (m, 2H, Ph), 8.35 (s, 1H, 2'-H), 13.05 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{C}}$ : 18.01, 24.95, 94.62, 99.21, 109.64, 112.82, 125.91, 126.10, 127.06, 128.19, 128.56, 128.85, 129.98, 132.19, 134.38, 136.58, 140.11, 141.95, 145.13, 151.45, 154.54, 158.77. MS ( $m/z$ ): Calcd 433.17, found 433.17 ([M+H]<sup>+</sup>); Anal. Calcd for C<sub>26</sub>H<sub>20</sub>N<sub>6</sub>O: C, 72.21; H, 4.66; N, 19.43%. Found: C, 72.19; H, 4.62; N, 19.25%.

This reaction procedure without modification was used for the synthesis of all other compounds **3b-o**.

**5-Ethyl-5'-methyl-3,3'-diphenyl-4H-[6,7']bi(pyrazolo[1,5-a]pyrimidinyl)-7-one (3b).** Yield 87%, mp 195-98 °C; IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3462, 1629; <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{H}}$ : 1.14 (t, *J* 7.56Hz, 3H, CH<sub>3</sub>), 2.72 (s, 3H, 5'-CH<sub>3</sub>), 3.08 (q, *J* 7.56Hz, 2H, CH<sub>2</sub>), 6.86 (s, 1H, 6'-H), 7.23-7.27 (m, 1H, Ph), 7.34-7.38 (m, 1H, Ph), 7.42-7.46 (m, 2H, Ph), 7.47-7.51 (m, 2H, Ph), 7.54-7.56 (m, 2H, Ph), 7.99 (s, 1H, 2-H), 8.08-8.10 (m, 2H, Ph), 8.34 (s, 1H, 2'-H), 13.08 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{C}}$ : 13.69, 24.88, 25.32, 94.53, 99.12, 109.48, 112.67, 125.83, 125.98, 126.97, 128.10, 128.49, 128.77, 129.87, 132.09, 140.14, 141.89, 143.21, 145.03, 151.31, 155.27, 156.15, 158.74. MS ( $m/z$ ): Calcd 447.18, found 447.19 ([M+H]<sup>+</sup>); Anal. Calcd for C<sub>27</sub>H<sub>22</sub>N<sub>6</sub>O: C, 72.63; H, 4.97; N, 18.82%. Found: C, 72.51; H, 4.86; N, 18.54%.

**2,5,2',5'-Tetramethyl-3,3'-diphenyl-4H-[6,7']bi(pyrazolo[1,5-a]pyrimidinyl)-7-one (3c).** Yield 75%, mp 228-30 °C; IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3458, 1632; <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{H}}$ : 2.22 (s, 3H, 5-CH<sub>3</sub>), 2.40 (s, 3H, 2-CH<sub>3</sub>), 2.55 (s, 3H, 2'-CH<sub>3</sub>), 2.64 (s, 3H, 5'-CH<sub>3</sub>), 6.78 (s, 1H, 6'-H), 7.26-7.31 (m, 1H, Ph), 7.37-7.41 (m, 1H, Ph), 7.44-7.51 (m, 6H, Ph), 7.72-7.74 (m, 2H, Ph), 13.07 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{C}}$ : 12.98, 14.17, 17.92, 24.68, 100.05, 104.51, 108.26, 112.16, 125.91, 127.13, 128.24, 128.66, 128.76, 129.76, 130.60, 132.40, 138.12, 139.58, 146.13, 150.88, 151.51, 153.58, 154.67, 158.26. MS ( $m/z$ ): Calcd 461.20, found 461.53 ([M+H]<sup>+</sup>); Anal. Calcd for C<sub>28</sub>H<sub>24</sub>N<sub>6</sub>O: C, 73.03; H, 5.25; N, 18.25%. Found: C, 73.21; H, 5.66; N, 18.16%.

**2,5,2',5'-Tetramethyl-3,3'-diphenyl-6'-bromo-4H-[6,7']bi(pyrazolo[1,5-a]pyrimidinyl)-7-one (3d).** Yield 80%, mp 240 °C (d); IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3471, 1640; <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO)  $\delta_{\text{H}}$ : 2.36

(s, 3H, 5-CH<sub>3</sub>), 2.51 (s, 3H, 2-CH<sub>3</sub>), 2.65 (s, 3H, 2'-CH<sub>3</sub>), 2.83 (s, 3H, 5'-CH<sub>3</sub>), 7.28-7.38 (m, 4H, Ph), 7.42-7.49 (m, 4H, Ph), 7.71-7.73 (m, 2H, Ph), 13.05 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO) δ<sub>C</sub>: 10.50, 14.38, 14.61, 21.91, 100.11, 106.20, 108.52, 116.34, 126.62, 126.80, 128.38, 128.61, 128.83, 128.92, 131.20, 132.99, 138.32, 139.44, 147.36, 150.81, 151.51, 154.41, 154.77, 157.57. MS (*m/z*): Calcd 541.11, 539.11 (1:1), found 539.11, 541.11 (1:1) ([M+H]<sup>+</sup>/[M+H+2]<sup>+</sup>); Anal. Calcd for C<sub>28</sub>H<sub>23</sub>BrN<sub>6</sub>O: C, 62.34; H, 4.30; N, 15.58%. Found: C, 62.22; H, 4.22; N, 15.21%.

**5-Ethyl-2,2',5'-trimethyl-3,3'-diphenyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3e).** Yield 81%, mp 230 °C; IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3465, 1635; <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO) δ<sub>H</sub>: 1.14 (t, *J* 7.16Hz, 3H, CH<sub>3</sub>), 2.39 (s, 3H, 2-CH<sub>3</sub>), 2.53 (s, 3H, 2'-CH<sub>3</sub>), 2.63 (s, 3H, 5'-CH<sub>3</sub>), 2.89 (q, *J* 7.16Hz, 2H, CH<sub>2</sub>), 6.75 (s, 1H, 6'-H), 7.26-7.30 (m, 1H, Ph), 7.36-7.40 (m, 1H, Ph), 7.44-7.48 (m, 4H, Ph), 7.51-7.53 (m, 2H, Ph), 7.72-7.75 (m, 2H, Ph), 13.06 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO) δ<sub>C</sub>: 12.95, 13.67, 14.14, 24.69, 25.31, 99.48, 104.49, 108.29, 112.11, 125.89, 127.09, 128.24, 128.63, 128.74, 129.77, 130.64, 132.42, 138.13, 139.58, 146.13, 151.52, 151.57, 154.81, 155.66, 158.22. MS (*m/z*): Calcd 475.22, found 475.23 ([M+H]<sup>+</sup>); Anal. Calcd for C<sub>29</sub>H<sub>26</sub>N<sub>6</sub>O: C, 73.40; H, 5.52; N, 17.71%. Found: C, 73.23; H, 5.62; N, 17.53%.

**5,5'-Dimethyl-2,2'-diphenyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3f).** Yield 82%, mp >330 °C; IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3063, 1744, 1705, 1620, 1551, 1520, 1458; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 2.22 (s, 3H, 5-CH<sub>3</sub>), 2.60 (s, 3H, 5'-CH<sub>3</sub>), 6.79 (s, 1H, 6'-H), 7.06 (s, 1H, 3-H), 7.15 (s, 1H, 3'-H), 7.34-7.53 (m, 6H, 2Ph-3", 4", 5"-H), 7.92 (d, 2 H, *J* 6.9 Hz, Ph-2", 6"-H), 8.04 (d, 2H, *J* 6.9 Hz, Ph-2", 6"-H), 13.03 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 18.20, 24.56, 87.06, 92.84, 99.97, 113.14, 126.61, 126.71, 129.28, 129.36, 129.77, 132.31, 132.89, 140.46, 149.91, 154.47, 154.98, 159.40. MS (*m/z*): Calcd 433.17, found 433.20 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>26</sub>H<sub>20</sub>N<sub>6</sub>O: C, 72.21; H, 4.66; N, 19.43%. Found: C, 72.18; H, 4.70; N, 19.40%.

**5,5'-Dimethyl-2,2'-di(*p*-methylphenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3g).** Yield 85%, mp 280 °C (d); IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3479, 3240, 3132, 3063, 2916, 1659, 1620, 1558, 1497, 1466; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 2.20 (s, 3H, 5-CH<sub>3</sub>), 2.32 (s, 3H, CH<sub>3</sub>-Ph), 2.37 (s, 3H, CH<sub>3</sub>-Ph), 2.59 (s, 3H, 5'-CH<sub>3</sub>), 6.73 (s, 1H, 6'-H), 7.02 (s, 1H, 3-H), 7.08 (s, 1H, 3'-H), 7.23 (d, 2 H, *J* 7.8 Hz, Ph-2", 6"-H), 7.30 (d, 2H, *J* 7.8 Hz, Ph-2", 6"-H), 7.81 (d, 2H, *J* 7.8 Hz, Ph-3", 5"-H), 7.92 (d, 2H, *J* 7.8 Hz, Ph-3", 5"-H), 13.02 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 18.27, 21.33, 24.69, 86.76, 92.58, 100.09, 112.89, 126.56, 126.69, 129.77, 129.84, 130.34, 138.76, 139.18, 140.45, 142.41, 150.08, 150.53, 154.39, 154.63, 154.96, 158.91; MS (*m/z*): Calcd 461.20, found 461.28 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>28</sub>H<sub>24</sub>N<sub>6</sub>O: C, 73.02; H, 5.25; N, 18.25%. Found: C, 73.07; H, 5.28; N, 18.21%.

**5,5'-Dimethyl-2,2'-di(*p*-chlorophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3h).** Yield 81%, mp >330 °C; IR (ν<sub>max</sub>, cm<sup>-1</sup>): 2962, 1612, 1582, 1435; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 2.22 (s, 3H, 5-CH<sub>3</sub>), 2.60 (s, 3H, 5'-CH<sub>3</sub>), 6.82 (s, 1H, 6'-H), 7.08 (s, 1H, 3-H), 7.19 (s, 1H, 3'-H), 7.48 (d, 2 H, *J* 8.7 Hz, Ph-2", 6"-H), 7.56 (d, 2H, *J* 8.7Hz, Ph-2", 6"-H), 7.94 (d, 2H, *J* 8.7 Hz, Ph-3", 5"-H), 8.07 (d, 2H, *J* 8.7 Hz, Ph-3", 5"-H), 13.05 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR

(DMSO, d<sup>6</sup>)  $\delta_C$ : 18.35, 24.75, 79.59, 87.20, 93.13, 100.10, 113.29, 128.35, 128.53, 129.27, 129.33, 131.47, 132.02, 133.85, 134.24, 140.48, 142.62, 150.12, 150.86, 153.18, 153.63, 154.52, 159.25; MS (*m/z*): calcd 501.09, 503.09, 505.18 found 501.19, 503.18, 505.18 (9:6:1) ([M+H]<sup>+</sup>/[M+H+2]<sup>+</sup>/[M+H+4]<sup>+</sup>); Anal. Calcd. for C<sub>26</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>6</sub>O: C, 62.29; H, 3.62; N, 16.76%. Found: C, 62.24; H, 3.67; N, 16.70%.

**5,5'-Dimethyl-2,2'-di(*p*-bromophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3i).**

Yield 88%, mp 290 °C(d); IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3001, 1612, 1582, 1435; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>)  $\delta_H$ : 2.22 (s, 3H, 5-CH<sub>3</sub>), 2.60 (s, 3H, 5'-CH<sub>3</sub>), 6.80 (s, 1H, 6'-H), 7.07 (s, 1H, 3-H), 7.17 (s, 1H, 3'-H), 7.61 (d, 2 H, *J* 8.4 Hz, Ph-2", 6"-H), 7.70 (d, 2H, *J* 8.4 Hz, Ph-2", 6"-H), 7.88 (d, 2H, *J* 8.4 Hz, Ph-3", 5"-H), 8.00 (d, 2H, *J* 8.4 Hz, Ph-3", 5"-H), 13.05 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>)  $\delta_C$ : 18.35, 24.78, 79.64, 87.18, 93.15, 100.13, 113.30, 122.50, 127.70, 128.62, 128.81, 131.85, 132.17, 132.23, 132.40, 132.51, 140.47, 142.59, 150.15, 150.81, 153.18, 159.20; MS (*m/z*): Calcd 588.99, 590.99, 592.99 (1:2:1), found 588.97, 590.97, 592.97 (1:2:1) ([M+H]<sup>+</sup>/[M+H+2]<sup>+</sup>/[M+H+4]<sup>+</sup>); Anal. Calcd. for C<sub>26</sub>H<sub>18</sub>Br<sub>2</sub>N<sub>6</sub>O: C, 52.90; H, 3.07; N, 14.24%. Found: C, 52.94; H, 3.11; N, 14.22%.

**5,5'-Dimethyl-2,2'-di(*p*-fluorophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3j).**

Yield 84%, mp 310 °C (d); IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3124, 3063, 1659, 1620, 1574; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>)  $\delta_H$ : 2.18 (s, 3H, 5-CH<sub>3</sub>), 2.58 (s, 3H, 5'-CH<sub>3</sub>), 6.71 (s, 1H, 6'-H), 7.03 (s, 1H, 3-H), 7.09 (s, 1H, 3'-H), 7.21- 7.33 (m, 4H, Ph-2", 6"-H), 7.92-7.96 (m, 2H, Ph-3", 5"-H), 7.99-8.08 (m, 2H, Ph-3", 5"-H), 13.04 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR(DMSO, d<sup>6</sup>)  $\delta_C$ : 18.32, 24.78, 79.55, 87.23, 93.16, 100.10, 113.30, 122.62, 128.54, 128.96, 129.37, 131.47, 132.49, 133.56, 134.12, 140.43, 142.60, 150.16, 150.88, 153.16, 153.56, 154.45, 159.23; MS (*m/z*): Calcd 468.15, found 469.53 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>26</sub>H<sub>18</sub>F<sub>2</sub>N<sub>6</sub>O: C, 66.66; H, 3.87; N, 17.94%. Found: C, 66.69; H, 3.89; N, 17.98%.

**5-Ethyl-5'-methyl-2,2'-diphenyl-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3k).**

Yield 88%, mp >330 °C; IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3742, 3618, 3163, 3124, 3070, 2986, 2885, 2824, 2361, 1705, 1659, 1620, 1574, 1520, 1443; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>)  $\delta_H$ : 1.13 (t, 3H, *J* 7.5 Hz, CH<sub>3</sub>), 2.34 (q, 2H, *J* 7.5 Hz, CH<sub>2</sub>), 2.60 (s, 3H, 5'-CH<sub>3</sub>), 6.78 (s, 1H, 6'-H), 7.07 (s, 1H, 3-H), 7.15 (s, 1H, 3'-H), 7.40-7.53 (m, 6H, 2Ph-3", 4", 5"-H), 7.92 (d, 2 H, *J* 6.6 Hz, Ph-2", 6"-H), 8.03 (d, 2H, *J* 6.6 Hz, Ph-2", 6"-H), 13.05 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>)  $\delta_C$ : 13.36, 24.72, 25.56, 87.14, 92.97, 99.57, 113.04, 126.63, 126.79, 129.23, 129.29, 129.66, 132.55, 133.08, 140.46, 142.71, 150.07, 154.42, 154.89, 155.09, 159.22; MS (*m/z*): Calcd 447.19, found 447.27 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>27</sub>H<sub>22</sub>N<sub>6</sub>O: C, 72.63; H, 4.97; N, 18.82%. Found: C, 72.68; H, 4.94; N, 18.78%.

**5-Ethyl-5'-methyl-2,2'-di(*p*-methylphenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one (3l).**

Yield 88%, mp >330 °C; IR ( $\nu_{\max}$ , cm<sup>-1</sup>): 3132, 3055, 3024, 2970, 2924, 2854, 1744, 1659, 1620, 1566, 1497, 1443; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>)  $\delta_H$ : 1.13 (t, 3H, *J* 7.5 Hz, CH<sub>3</sub>), 2.31 (s, 3H, CH<sub>3</sub>), 2.36 (q, 2H, *J* 7.5 Hz, CH<sub>2</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 2.59 (s, 3H, 5'-CH<sub>3</sub>), 6.73 (s, 1H, 6'-H), 7.03 (s, 1H, 3-H), 7.10 (s, 1H, 3'-H), 7.22 (d, 2 H, *J* 7.8 Hz, Ph-2", 6"-H), 7.31 (d, 2H, *J* 7.8 Hz, Ph-2", 6"-H), 7.79 (d, 2H, *J* 7.8 Hz, Ph-3", 5"-H), 7.92 (d, 2H, *J* 7.8 Hz, Ph-3", 5"-H), 13.03 (s, 1H, NH,

exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 13.39, 21.35, 24.73, 25.54, 86.89, 92.64, 99.58, 112.85, 126.56, 126.73, 129.78, 138.75, 139.17, 140.43, 154.43, 154.94, 159.03; MS (*m/z*): Calcd 475.22, found 475.29 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>29</sub>H<sub>26</sub>N<sub>6</sub>O: C, 73.40; H, 5.52; N, 17.71%. Found: C, 73.46; H, 5.48; N, 17.75%.

**5-Ethyl-5'-methyl-2,2'-di(*p*-chlorophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one**

**(3m).** Yield 88%, mp > 320 °C; IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3742, 3649, 3618, 3124, 3055, 2978, 2885, 2831, 2361, 1774, 1651, 1620, 1558, 1520, 1443; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 1.15 (t, 3H, *J* 7.2 Hz, CH<sub>3</sub>), 2.36 (q, 2H, *J* 7.2 Hz, CH<sub>2</sub>), 2.61 (s, 3H, 5'-CH<sub>3</sub>), 6.81 (s, 1H, 6'-H), 7.09 (s, 1H, 3-H), 7.19 (s, 1H, 3'-H), 7.48 (d, 2H, *J* 8.4 Hz, Ph-2", 6"-H), 7.57 (d, 2H, *J* 8.4 Hz, Ph-2", 6"-H), 7.93 (d, 2H, *J* 8.4 Hz, Ph-3", 5"-H), 8.07 (d, 2H, *J* 8.4 Hz, Ph-3", 5"-H), 13.12 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 13.38, 24.76, 25.56, 87.31, 93.21, 99.63, 113.24, 128.34, 128.56, 129.26, 129.32, 131.52, 132.04, 133.85, 134.26, 140.44, 142.83, 150.15, 153.67, 154.73, 155.19, 159.36; MS (*m/z*): calcd 515.11, 517.11, 519.11 found 515.20, 517.19, 519.19 (9:6:1) ([M+H]<sup>+</sup>/[M+H+2]<sup>+</sup>/[M+H+4]<sup>+</sup>); Anal. Calcd. for C<sub>27</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>6</sub>O: C, 62.92; H, 3.91; N, 16.31%. Found: C, 62.88; H, 3.92; N, 16.33%.

**5-Ethyl-5'-methyl-2,2'-di(*p*-bromophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one**

**(3n).** Yield 84%, mp 300 °C (d); IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3186, 3132, 3078, 2924, 2854, 1674, 1620, 1558, 1520, 1435; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 1.12 (t, 3H, *J* 7.2 Hz, CH<sub>3</sub>), 2.35 (q, 2H, *J* 7.2 Hz, CH<sub>2</sub>), 2.61(s, 3H, 5'-CH<sub>3</sub>), 6.80 (s, 1H, 6'-H), 7.08 (s, 1H, 3-H), 7.18 (s, 1H, 3'-H), 7.61 (d, 2H, *J* 8.1 Hz, Ph-2", 6"-H), 7.69 (d, 2H, *J* 8.1 Hz, Ph-2", 6"-H), 7.86 (d, 2H, *J* 8.1 Hz, Ph-3", 5"-H), 8.00 (d, 2H, *J* 8.1 Hz, Ph-3", 5"-H), 13.07 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 13.38, 24.76, 25.58, 87.30, 99.62, 113.27, 122.92, 128.61, 128.82, 132.22, 140.44, 142.82, 150.16, 153.31, 154.75, 155.20, 159.32, 159.37. MS (*m/z*): Calcd 603.00, 605.00, 607.00 (1:2:1), found 603.14, 605.14, 607.13 (1:2:1) ([M+H]<sup>+</sup>/[M+H+2]<sup>+</sup>/[M+H+4]<sup>+</sup>); Anal. Calcd. for C<sub>27</sub>H<sub>20</sub>Br<sub>2</sub>N<sub>6</sub>O: C, 53.66; H, 3.34; N, 13.91%. Found: C, 53.62; H, 3.42; N, 13.94%.

**5-Ethyl-5'-methyl-2,2'-di(*p*-fluorophenyl)-4*H*-[6,7']bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-one**

**(3o).** Yield 87%, mp 290 °C (d); IR (ν<sub>max</sub>, cm<sup>-1</sup>): 3124, 3055, 2978, 1612, 1528, 1443; <sup>1</sup>H NMR (DMSO, d<sup>6</sup>) δ<sub>H</sub>: 1.12 (t, 3H, *J* 7.2 Hz, CH<sub>3</sub>), 2.37 (q, 2H, *J* 7.2 Hz, CH<sub>2</sub>), 2.60 (s, 3H, 5'-CH<sub>3</sub>), 6.77 (s, 1H, 6'-H), 7.07 (s, 1H, 3-H), 7.14 (s, 1H, 3'-H), 7.22-7.27 (m, 2H, Ph-2", 6"-H), 7.730-7.36 (m, 2H, Ph-2", 6"-H), 7.93-7.97 (m, 2H, Ph-3", 5"-H), 8.06-8.11 (m, 2H, Ph-3", 5"-H), 13.04 (s, 1H, NH, exchangeable with D<sub>2</sub>O). <sup>13</sup>C NMR (DMSO, d<sup>6</sup>) δ<sub>C</sub>: 13.37, 24.76, 25.55, 87.30, 92.92, 113.22, 116.04, 116.27, 128.82, 128.94, 140.45, 142.79, 150.16, 153.49, 153.94, 154.76, 155.07, 159.24; MS (*m/z*): Calcd 483.17, found 483.56 ([M+H]<sup>+</sup>); Anal. Calcd. for C<sub>27</sub>H<sub>20</sub>F<sub>2</sub>N<sub>6</sub>O: C, 67.21; H, 4.18; N, 17.42%. Found: C, 67.25; H, 4.15; N, 17.40%.

**Evaluation of antibacterial activity.** For the evaluation of antibacterial activity of the compounds, the size of inoculum was adjusted to approximately 10<sup>8</sup> colony-forming units cfu/mL by suspending the culture in sterile distilled water. Petri dishes containing 25 mL of nutrient agar medium were swabbed with 100 μL culture of the respective bacterial strains and kept for 15 min for the absorption of culture. Using a sterile cork borer, ~8mm diameter well was

bored in the seeded agar plates and a 100  $\mu$ L volume of each test compound of concentration 10, 50, 100  $\mu$ g/mL, reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. All the plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the zone of inhibition around the well.

**Determination of minimum inhibitory concentration (MIC).** MIC of all the compounds was determined by the agar well diffusion method. Petri dishes containing 25 mL nutrient agar medium was swabbed with the 100  $\mu$ L culture of inoculum size of approximately  $10^8$  colony-forming units cfu/ml. Twofold serial dilution ranging from 200 – 0.19 of chemically synthesised compound were made. Dilutions were made in DMSO. Using a sterile cork borer, ~8mm diameter well was bored in the seeded agar plates and a 100  $\mu$ L volume of different dilutions of each test compound was added into the wells. These plates were incubated at 37 °C for 24 h.

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