A Convenient Synthesis of Bioactive 5-Arylidenebarbiturates

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Summary: A convenient one pot green chemistry route has been developed for the synthesis of 5-arylidenebarbiturates by way of an acetic acid catalyzed Knoevenagel condensation of a variety of aromatic aldehydes with barbituric acid. The target compounds were obtained in 50-90 % yield and characterized by elemental analysis and different spectral techniques such as IR, NMR and EIMS. All of these were screened for antimicrobial activity.

Key words: 5-Arylidenebarbiturates, Synthesis, Knoevenagel Condensation, Antimicrobial Activity.

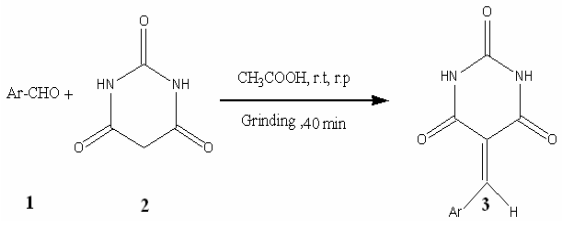
Introduction

Barbiturates are 2,4,6-trioxypyrimidine derivatives of barbituric acid. Many of these are widely used as sedative hypnotic [1-2], antispasmodic, anticonvulsant and local anesthetic drugs [3-5]. The Knoevenagel condensation of aldehydes with active methylene compounds is widely employed in organic synthesis and are catalyzed by bases [6,7] , Lewis acids [8], surfactants [9], zeolites [10] and heterogeneous catalysts[11,12] in aqueous medium or solvent free conditions [13]. Some of these reactions are also performed on solid supports, promoted under infrared [14], ultrasound or microwave [15, 16] radiations [17-19].

In our previous work [1], we reported a facile synthesis of 5- arylidenethiobarbituric acids by Knoevenagel condensation of thiobarbituric acid with different aromatic aldehydes. As a further extension of this strategy, we now wish to report a convenient synthesis of 5-arylidene -barbiturates **3a-3s** by way of a one pot Knoevenagel condensation between a variety of aromatic aldehydes **1a - 1s** with barbituric acid **2** under solvent free conditions in presence of a catalytic amount of acetic acid (Scheme-1).

Results and Discussions

Mechanochemical approach has several advantages such as solvent free environment, cleaner process, reduced reaction time, better yield and no expensive catalysts. By using this convenient synthetic route, a series of 5-arylidenebarbiturates were synthesized (Table-1). All the synthesized compounds were obtained in good (>50%) to excellent (90 %) yield. The products were collected as powdered material and their structures elucidated on the basis of IR, mass, ^{1}H-and ^{13}C-NMR spectroscopic data. From the results it can be inferred that the aldehydes having electron donating groups give higher yields compared to the aldehydes with electron withdrawing groups (Scheme-1)



1 2

Scheme-1: Synthesis of 5-Arylidenebarbiturates.

Antibacterial and antifungal activities of the newly synthesized compounds were determined using the standard methods (Tables-2-4). Antibacterial activity was determined against two gram positive and four gram negative bacteria. Bacterial growth inhibition is expressed in terms of MIC_{50} (µg/mL) which is defined as the minimum amount of substance to inhibit 50% bacterial growth. Table-2 showed that compounds **3g**, **3h**, **3m**, **3r** were inhibitory against the gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. MIC_{50} values were comparable to the standard ampicillin. Compound **3f** inhibited growth of *Escherichia coli* and *Pseudomonas aeruginosa* as effectively as the standards gentamycin and ciprofloxacin. Compounds

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3l and **3q** inhibited growth of *Shigella sonnei*. Growth of *P. aeruginosa* was inhibited by **3a**, **3b**, **3d**, **3f**, **3n**, **3o**, **3p**, **3s**. Some of these compounds were more effective to kill this bacterium than the standards, as is indicated by their low MIC_{50} values. None of the compound exhibited inhibition of *Salmonella typhi*.

Table-1: Results of Synthesis of 5-Arylidenebarbituric Acids.

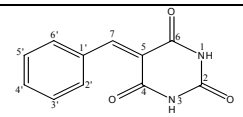
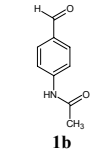
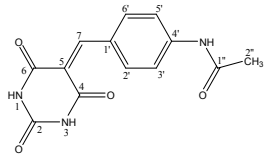
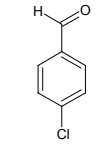
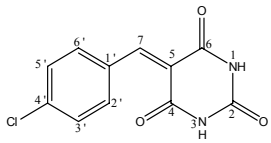
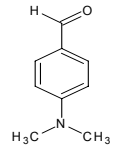
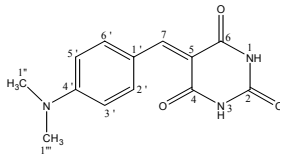
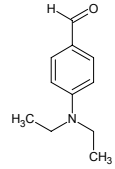
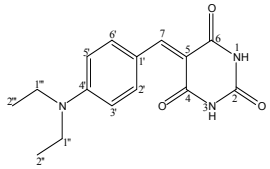
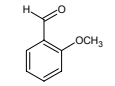
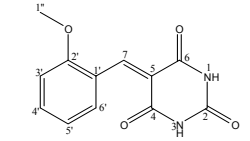
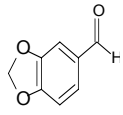
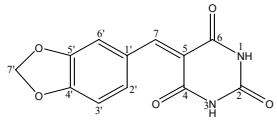
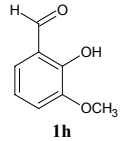
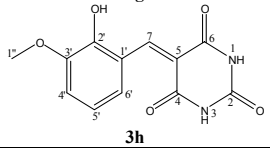
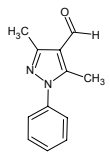
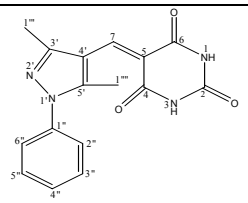
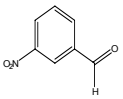
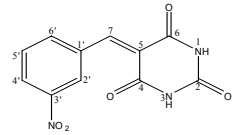
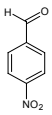
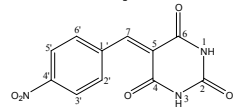
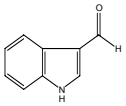
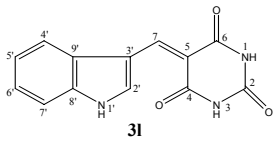
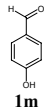
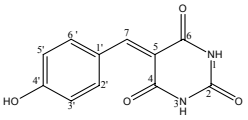
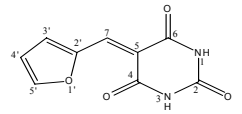
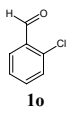
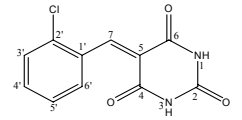
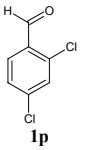
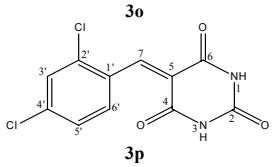
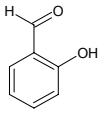
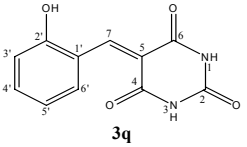
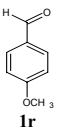
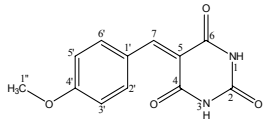
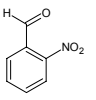
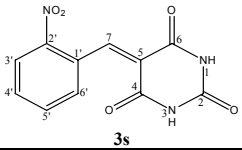
Entry	Ar (Aldehydes)	Time (min)	Product
1	 **1a**	30	 **3a**
2	 **1b**	45	 **3b**
3	 **1c**	30	 **3c**
4	 **1d**	30	 **3d**
5	 **1e**	30	 **3e**
6	 **1f**	30	 **3f**
7	 **1g**	30	 **3g**
8	 **1h**	30	 **3h**
9	 **1i**	45	 **3i**
10	 **1j**	45	 **3j**
11	 **1k**	45	 **3k**
12	 **1l**	45	 **3l**
13	 **1m**	30	 **3m**
14	 **1n**	30	 **3n**
15	 **1o**	30	 **3o**
16	 **1p**	30	 **3p**
17	 **1q**	30	 **3q**
18	 **1r**	30	 **3r**
19	 **1s**	45	 **3s**

Table-2: Antibacterial assay by broth dilution method in 96-wells plates. MIC values were determined by suitable dilution of test compounds. Results are mean of three independent experiments (mean ± sem, n=3). MIC_{50} is in µg/ mL.

Compound	Basillus subtilis (+)	Staphylococus aureus (+)	E. coli(-)	Shigella sonnei (-)	Salmonella typhi (-)	P. aureginosa (-)
	MIC50	MIC50	MIC50	MIC50	MIC50	MIC50
3a	17.75±0.42	13.74±0.66	11.68±0.31	11.33±0.60	10.02±0.91	8.49 ±0.93
3b	13.19±0.27	13.69±0.15	13.77±0.21	12.19±0.31	19.19±0.14	10.07±0.43
3d	13.27±0.33	18.66±0.21	14.81 ±1.3	13.21±0.38	13.79±0.25	9.16 ±0.23
3e	14.26±0.32	13.19±0.31	13.16±0.21	16.11±0.22	14.69±0.33	13.12±0.12
3f	15.55±0.53	12.20 ±1.6	8.24 ±0.75	17.22±0.31	16.08±0.23	8.69 ±0.31
3g	14.28±0.14	11.17±0.61	13.91±0.33	13.00±0.43	15.13±0.93	-
3h	13.37±0.45	11.09±0.75	12.33±0.21	14.21±0.21	14.74±0.25	14.62±0.17
3i	16.94±0.27	12.04±0.33	13.96±0.23	15.15±0.24	12.89±0.22	11.32±0.31
3j	13.11±0.32	14.86±0.23	13.27±0.24	18.29±0.12	15.62±0.20	15.52±0.18
3l	14.29±0.41	12.33±0.22	17.99±0.51	10.09±0.43	-	16.39±0.43
3m	11.23±0.32	-	14.65±0.23	13.19 ±1.6	19.00±0.00	12.62±0.26
3n	12.11±0.70	-	19.22±0.24	12.16±0.11	14.63±0.18	11.39±0.36
3o	14.51±0.42	14.66±0.28	11.63±0.61	14.19±0.42	14.79±0.21	10.63±0.14
3p	15.29±0.41	12.72±0.41	10.06±0.24	15.00±0.27	12.62±0.12	9.31 ±0.00
3q	16.11±0.23	17.69±0.43	15.13±0.08	11.36±0.37	12.12±0.00	13.66±0.29
3r	11.43±0.74	14.33±0.44	15.96±0.32	12.09±0.24	13.46±0.31	14.47±0.31
3s	-	-	-	-	12.93±0.74	8.92 ±0.32
Ampicilin	11.66±0.14	10.69±0.06	11.32±0.13	11.98±0.13	10.85±0.16	12.33±0.15
Gentamycin	10.36±0.13	8.42 ± 0.12	8.21 ± 0.02	9.31 ± 0.18	11.21±0.31	10.89±0.11
Ciprofloxacin	8.36 ± 0.12	9.42 ± 0.11	8.21 ± 0.02	7.31 ± 0.08	7.59 ± 0.11	11.03±0.10

Antifungal activity was determined against two fungi, *Fusarium solani* and *Aspergillus niger* as given in Table-3. All the compounds showed low to high degree of fungal growth inhibition on 7th day of measurement of growth. However, on 14th day of measurement, compounds **3p** and **3q** showed maximum antifungal activity compared with the standard clotrimazole. When compounds were tested against *Candida albicans*, none of the compound showed good inhibitory activity except **3a** and **3b** compared to the control clotrimazole (Table-4).

Table-3: Antifungal activity by slant method. Results are percent inhibition of three independent methods and expressed as µg/ mL. Error is <5%.

Compound	Inhibition (%)			
	Fusarium solani		Aspergillus niger	
	7-days	14-days	7-days	14-days
3a	53.10	4.90	43.20	2.50
3b	80.20	64.20	80.20	72.80
3d	72.80	54.30	61.70	33.30
3e	58.00	16.00	58.00	3.70
3f	56.80	37.00	72.80	0.00
3g	40.70	0.00	82.70	56.80
3h	69.10	4.90	69.10	42.00
3i	64.20	21.00	43.20	-1.20
3j	42.00	2.50	48.10	3.70
3k	48.10	18.50	44.40	0.00
3l	43.20	3.70	54.30	2.50
3m	51.90	1.20	60.50	16.00
3n	46.90	2.50	69.10	3.70
3o	51.90	-2.50	76.50	60.50
3p	76.50	75.30	85.20	64.20
3q	77.80	67.90	77.80	69.10
3r	74.10	55.60	44.40	4.90
3s	76.50	61.70	69.10	42.00
Clotrimazole	97.50	97.50	96.30	96.30

Experimental

All chemicals used were of analytical grade. Melting points were taken on the GallenKamp melting point apparatus. The IR spectra as nujol mulls were recorded on Perkin-Elmer FTIR (Spectrum RXI) spectrometer. Mass spectra were recorded on low resolution mass data Finnigan EIMS mass spectrometer MAT 312 having a data system MASPEC. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker/ XWIN-NMR instruments having frequency 300, 400 and 500 MHz using DMSO-d_6 as a solvent and TMS as an internal reference. The elemental analysis was carried out on CARLO ERBA elemental analyzer.

Table-4: Antifungal activity against *Candida albicans* by dilution broth method. MIC_{50} is in µg/ mL (mean±sem, n=3).

Comounds	*Candida albicans*
	MIC 50
3a	9.61±0.19
3b	8.64±0.22
3d	15.78 ±0.08
3e	14.59 ±0.23
3f	14.00 ±0.32
3g	-
3h	11.09 ±0.45
3i	12.45±0.37
3j	-
3k	-
3l	-
3m	-
3n	-
3o	-
3p	-
3q	-
3r	-
3s	-
Clotrimazole	9.36 ±0.11

General Procedure

General procedure is same as mentioned previously [1]. A mixture of barbituric acid (0.01 mol) and the relevant aldehyde (0.01 mol) were

ground for 30 min using pestle and mortar, followed by drop-wise addition of 1 mL acetic acid. The product was treated with boiling water (2-3 mL), dried and washed with n-hexane (3-5 mL) to give 5-arylidenebarbiturate **3a-3s** (Table-1).

5-benzylidene-2,4,6(1H,3H,5H)-pyrimidinetrione **(3a)**

Amorphous solid; yield 75 %. M.p 259 °C. IR (nujol) ν_{max} cm^{-1}: 3200 (NH), 1755 (C=O), 1673 (Ar C=O str), 1582 (Ar C=C), 1564 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.31 (s, 1H, NH), 11.22 (s, 1H, NH), 8.20 (s, 1H, C = CH), 7.45-8.01 (m, 5H, Ar); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.3 (C-4), 161.5 (C-6), 119.2 (C-5), 154.6 (C-7), 150.1 (C-2), 133.0 (C-4′), 132.6 (C-1′), 132.1(C-6′ and C-2′), 128.0 (C-3′ and C-5′). EIMS *m/z* (rel. int. %): [M$^+$] 215 (100), 216 (65), 172 (59), 102 (48), 76 (20), 63 (19).

N-(4-{[2,4,6-trioxotetrahydro-5(2H)-pyrimidinylidene]methyl}phenyl)acetamide **(3b)**

Amorphous solid; yield 73 %. M.p > 250 °C. IR (nujol) ν_{max} cm^{-1}: 3304 (NH), 1738 (C=O), 1664 (C=C), 1563 (NH def), 1499 (C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.25 (s, 1H, NH), 11.11 (s, 1H, NH), 6.40 (d, J = 8.82 Hz, 2H, H3′ and H-5′), 8.20 (d, J = 8.10 (s, 1H, C=CH, H-7), 7.65 (d, J = 8.81 Hz, 2H, H-2′ and H-6′); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 169.0 (NH-CO, C-1″), 165.8 (C-4), 163.7 (C-6), 161.9 (C-2), 154.1 (C-7), 150.1 (C-4′), 143.6 (C-1′), 136.0 (C-5′ and C-3′), 126.0 (C-5), 117.7 (C-2′ and C-6′), 24.1 (CO-CH$_3$, C-2″). EIMS *m/z* (rel. int. %): [M$^+$] 231 (100), 187 (47), 144 (13), 131 (15), 117 (24), 82 (80), 63 (22).

5-(4-chlorobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3c)**

Amorphous solid; yield 88 %. M.p 260 °C. IR (nujol) ν_{max} cm^{-1}: 3213 (NH), 1755 (C=O), 1669 (Ar C=O str), 1550 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.35 (s, 1H, NH), 11.20 (s, 1H, NH), 8.04 (d, J = 8.8 Hz, 2H, H-3′ and H-5′), 8.22 (s, 1H, H-7), 7.54 (d, J = 8.6 Hz, 2H, H-2′ and H-6′); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.1 (C-4 and C-6), 161.5 (C-2), 150.1 (C-7), 136.6 (C-4′), 134.6 (C-6′ and C-2′), 131.5 (C-1′), 128.0 (C-3′ and C-5′), 119.6 (C-5). EIMS *m/z* (rel. int. %): [M$^+$] 251 (7), 250 (14), 249 (20), 205 (16), 172 (11), 163 (8), 136 (13), 82 (100), 50 (29).

5-[4-(dimethylamino)benzylidene]-2,4,6(1H,3H,5H)-pyrimidinetrione **(3d)**

Amorphous solid; yield 86 %. M.p 274 °C. IR (nujol) ν_{max} cm^{-1}: 3191 (NH), 1729 (C=O), 1680 (C=O), 1606 (Ar C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.21 (s, 1H, NH), 8.40 (s, 1H, H-7), 8.20 (dd, J = 1.5, 6.7 Hz, 2H, H-3′ and H-5′), 7.22 (dd, J = 1.2, 6.7 Hz, 2H, H-2′ and H-6′), 3.81 (s, 6H, H-1″ and H-1‴); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.6 (C-4), 162.6 (C-6), 155.3 (C-2), 154.1 (C-7), 150.2 (C-4′), 138.9 (C-2′ and C-6′), 119.9 (C-1′), 111.1 (C-3′ and C-5′), 109.5 (C-5), 40.0 (C-1″ and C-2‴). EIMS *m/z* (rel. int. %): [M$^+$] 259 (100), 215 (13), 144 (14), 172 (10), 101 (6), 72 (11).

5-[4-(diethylamino)benzylidene]-2,4,6(1H,3H,5H)-pyrimidinetrione **(3e)**

Amorphous solid; yield 84 %. M.p 268 °C. IR (nujol) ν_{max} cm^{-1}: 3375 (NH), 3068 (CH), 1739 (C=O), 1668 (Ar C=O str), 1550 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.21 (s, 1H, NH), 8.40 (s, 1H, H-7), 8.22 (dd, J = 1.5, 6.7 Hz, 2H, H-3′ and H-5′), 7.20 (dd, J = 1.2, 6.7 Hz, 2H, H-2′ and H-6′), 3.80 (q, J = 6.0 Hz, 4H, CH$_2$, H-1″ and H-1‴), 1.22 (t, J = 6.8 Hz, 6H, CH$_3$, H- H-2″ and H-2‴); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.6 (C-4), 162.6 (C-6), 155.1 (C-2), 152.1 (C-7), 150.2 (C-4′),139.4 (C-2′ and C-6′), 119.6 (C-1′), 108.9 (C-5), 110.8 (C-3′ and C-5′), 44.7 (C-1″ and C-1‴), 12.0 (C-2″ and C-2‴). EIMS *m/z* (rel. int. %): [M$^+$] 272 (100), 287 (40), 244 (15), 130 (6), 100 (7), 56 (8).

5-(2-methoxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3f)**

Amorphous solid; yield 79 %. M.p 267 °C. IR (nujol) ν_{max} cm^{-1}: 3198 (NH), 1733 (C=O), 1705 (C=O), 1677 (Ar C=O str), 1555 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.19 (s, 1H, NH), 8.40 (s, 1H, H-7), 7.92 (dd, J = 6.7 Hz, 1H, H-5′), 7.50 (dt, J = 8.46, 8.3 Hz, 1H, H-3′), 7.41 (dd, J = 3.1, 2.9 Hz, 1H, H-2′), 6.91 (dt, J = 15.0, 14.0 Hz, 1H, H-4′), 3.80 (s, 3H, OCH$_3$); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.4 (C-4), 161.4 (C-6), 159.0 (C-2), 150.2 (C-7), 149.8 (C-6′),134.1 (C-4′), 132.4 (C-2′), 121.5 (C-1′), 119.4 (C-5), 118.6 (C-3′), 110.9 (C-5′), 55.9 (OCH$_3$). EIMS *m/z* (rel. int. %): [M$^+$] 215 (100), 172 (61), 131 (13), 102 (12), 89 (41), 63 (31).

5-(1,3-benzodioxol-5-ylmethylene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3g)**

Amorphous solid; yield 68 %. M.p 255 °C. IR (nujol) ν_{max} cm^{-1}: 3209 (NH), 3094 (CH), 1732 (C=O), 1691 (C=O), 1667 (Ar C=O), 1551 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.40 (s, 1H, NH), 11.61 (s, 1H, NH), 8.40 (s, 1H, H-7), 7.32 (dd, J = 1.0, 6.5 Hz, 1H, H-5′), 5.85 (s, 2H, H-7′), 7.24 (d, J = 1.5 Hz, 1H, H-1′), 7.11 (d, J = 6.5 Hz, 1H, H-4′);

^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.9 (C-4), 162.2 (C-6), 160.1 (C-2), 154.9 (C-7), 148.2 (C-3′),147.0 (C-4′), 133.6 (C-1′ and C-6′), 112.3 (C-2′), 108.3 (C-5′), 120.6 (C-5), 102.3 (C-7′). EIMS *m/z* (rel. int. %): [M$^+$] 260 (100), 216 (29), 173 (15), 145 (12), 62 (27).

5-(2-hydroxy-3-methoxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3h)**

Amorphous solid; yield 81 %. M.p 261 °C. IR (nujol) ν_{max} cm^{-1}: 3338 (NH), 1744 (C=O), 1696 (C=O), 1667 (Ar C=O str), 1553 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.31 (s, 1H, NH), 11.20 (s, 1H, NH), 8.40 (s, 1H, H-7), 7.80 (dt, J = 6.0, 1.0 Hz, 1H, H-5′), 7.20 (dt, J = 1.0, 7.0 Hz, 1H, H-3′), 6.51 (dt, J = 6.0, 7.0 Hz, 1H, H-4′), 3.81 (s, 3H, H-1″); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.9 (C-4), 162.5 (C-6), 161.0 (C-2), 150.0 (C-7), 148.6 (C-2′), 147.3 (C-3′), 123.9 (C-1′), 120.9 (C-5′), 119.6 (C-5), 117.7 (C-6′), 115.6 (C-4′). EIMS *m/z* (rel. int. %): [M$^+$] 262 (100), 245 (82), 202 (32), 105 (29), 133 (15), 51 (67), 77 (42).

5-[(3,5-dimethyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2,4,6(1H,3H,5H)-pyrimidinetrione **(3i)**

Amorphous solid; yield 79 %. M.p > 250 °C. IR (nujol) ν_{max} cm^{-1}: 1759 (C=O), 1705 (C=O), 1652 (Ar C=O str), 1568 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.31 (s, 1H, NH), 11.20 (s, 1H, NH), 8.51 (s, 1H, H-7), 7.11-7.60 (m, 5H, Ar H), 2.40 (s, 1H, H-1‴), 2.32 (s, 1H, H-1⁗). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.3 (C-4), 163.3 (C-6), 161.3 (C-2), 150.3 (C-5′), 145.1 (C-7), 143.5 (C-3′), 138.4 (C-1″), 129.3 (C-3″ and C-5″), 128.1 (C-4″), 124.5 (C-2″ and C-6″), 116.2 (C-5), 115.9 (C-4′), 13.2 (C-3‴,$\underline{C}H_3$), 13.5(C-5⁗,$\underline{C}H_3$). EIMS *m/z* (rel. int. %): [M$^+$] 310 (86), 293 (81), 250 (13), 118 (20), 77 (100), 52 (21).

5-(3-nitrobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3j)**

Amorphous solid; yield 55 %. M.p 245 °C. IR (nujol) ν_{max} cm^{-1}: 3233 (NH), 3069, 1740 (C=O), 1685 (Ar C=O str), 1598 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 8.35 (s, 1H, H-7), 8.52 (dd, J = 1.3, 1.2 Hz, 1H, H-5′), 8.32 (dt, J = 5.16, 4.9 Hz, 1H, H-1′), 7.81 (dt, J = 7.8 Hz, 1H, H-3′), 7.73 (dt, J = 8.0, 7.9 Hz, 1H, H-2′), 3.84 (s, 3H, O$\underline{C}H_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 162.8 (C-4), 161.1 (C-6), 151.2 (C-2), 150.2 (C-7), 147.1 (C-5′), 138.4 (C-1′), 134.5 (C-4′), 134.9 (C-6′), 130.9 (C-3′), 129.4 (C-2′), 121.6 (C-5); EIMS *m/z* (rel. int. %): [M$^+$] 244 (19), 214 (15), 101 (52), 75 (87), 51 (100).

5-(4-nitrobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3k)**

Amorphous solid; yield 63 %. M.p 275 °C. IR (nujol) ν_{max} cm^{-1}: 3234 (NH), 1741 (C=O), 1712 (C=O), 1515 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.41 (s, 1H, NH), 8.40 (s, 1H, H-7), 8.39 (d, J = 8.39 Hz, 2H, H-3′ and H-5′), 8.02 (d, J = 1.8 Hz, 2H, H-2′ and H-6′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 168.6 (C-4), 162.6 (C-6), 161.1 (C-2), 151.0 (C-7), 147.9 (C-4′), 139.9 (C-1′), 132.2 (C-2′ and C-6′), 128.0(C-5), 122.6 (C-3′ and C-5′). EIMS *m/z* (rel. int. %): [M$^+$] 261 (51), 244 (100), 214 (55), 101 (46), 89 (86), 51 (70).

5-(1H-indol-3-ylmethylene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3l)**

Amorphous solid; yield 77 %. M.p > 300 °C. IR (nujol) ν_{max} cm^{-1}: 3274 (NH), 3155 (C=O), 3022 (CH), 1726 (C=O), 1687 (C=O), 1639 (Ar C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 9.40 (s, 1H, NH, H-1′), 8.70 (s, 1H, H-7), 7.31-7.83 (m, 5H, Ar-H). ^{13}C-NMR (DMSO-d_6, 100MHz) δ: 164.5 (C-4 and C-6), 163.2 (C-2), 143.7 (C-7), 139.7 (C-8′), 138.4 (C-9′), 129.1 (C-2′), 122.6 (C-4′ and C-7′), 120.8 (C-5), 117.6 (C-5′ and C-6′), 108.6 (C-3′). EIMS *m/z* (rel. int. %): [M$^+$] 255 (100), 169 (29), 140 (31), 114 (48), 89 (19), 63 (43).

5-(4-hydroxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3m)**

Amorphous solid; yield 87 %. M.p > 300 °C. IR (nujol) ν_{max} cm^{-1}: 3159 (NH), 1728 (C=O), 1533 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 8.40 (s, 1H, H-7), 7.72 (dd, J = 1.2, 7.1 Hz, 2H, H-2′ and H-6′), 7.42 (dd, J = 1.5, 7.1 Hz, 2H, H-3′ and H-5′); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.0 (C-4), 162.9 (C-6 and C-2), 155.4 (C-7), 150.1 (C-4′), 138.2 (C-2′ and C-6′), 123.4 (C-1′), 115.4 (C-3′ and C-5′), 114.1 (C-5). EIMS *m/z* (rel. int. %): [M$^+$] 232 (100), 188 (53), 145 (22), 89 (27).

5-(2-furylmethylene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3n)**

Amorphous solid; yield 71 %. M.p 250 °C. IR (nujol) ν_{max} cm^{-1}: 3144 (NH), 3038 (NH), 1744 (C=O), 1704 (C=O), 1652 (Ar H), 1565 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH),

11.21 (s, 1H, NH), 8.01 (s, 1H, H-7), 7.40 (d, J = 2.1 Hz, 1H, H-5′), 6.40 (d, J = 5.1 Hz, 1H, H-3′), 6.21 (d, J = 3.7 Hz, 1H, H-4′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.2 (C-4 and C-6), 162.0 (C-2), 151.1 (C-2′), 150.2 (C-7), 150.1 (C-5′), 126.4 (C-3′), 115.1 (C-4), 112.8 (C-5). EIMS *m/z* (rel. int. %): [M$^+$] 206 (82), 178 (62), 135 (40), 119 (32), 92 (42), 79 (55), 52 (100).

5-(2-chlorobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3o)**

Amorphous solid; yield 81 %. M.p 264 °C. IR (nujol) ν_{max} cm^{-1}: 3209 (NH), 3081 (NH), 1751 (C=O), 1693 (C=O), 1597 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 8.20 (s, 1H, H-7), 7.32-7.74 (m, 4H, Ar); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.6 (C-4), 162.6 (C-6), 161.1 (C-2), 151.0 (C-2′), 150.2 (C-1′), 145.2 (C-7), 132.2 (C-3′), 128.0 (C-6′), 122.8 (C-4′), 122.6 (C-5), 122.5 (C-5′). EIMS *m/z* (rel. int. %): [M$^+$] 215 (100), 172 (83), 128 (18), 83 (59), 50 (20).

5-(2,4-dichlorobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3p)**

Amorphous solid; yield 82 %. M.p 269 °C. IR (nujol) ν_{max} cm^{-1}: 3197 (NH), 3060 (NH), 1760 (C=O), 1687 (C=O), 1577 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 8.40 (s, 1H, H-7), 7.91 (d, J = 1.5 Hz, 1H, H-2′), 7.80 (dd, J = 1.5, 6.5 Hz, 1H, H-4′), 7.72 (d, J = 6.5 Hz, 1H, H-5′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 162.4 (C-4 and C-6), 160.9 (C-2), 150.1 (C-2′), 148.3 (C-4′), 135.5 (C-7), 134.1 (C-1′), 128.4 (C-3′), 128.2 (C-6′), 126.6 (C-5′), 122.3 (C-5). EIMS *m/z* (rel. int. %): [M$^+$] 249 (75), 206 (100), 99 (19), 74 (14).

5-(2-hydroxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3q)**

Amorphous solid; yield 81 %. M.p 280 °C. IR (nujol) ν_{max} cm^{-1}: 3159 (NH), 1728 (C=O), 1658 (C=O), 1603 (Ar C=C), 1533 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.30 (s, 1H, NH), 11.11 (s, 1H, NH), 8.40 (s, 1H, H-7), 4.7 (s, 1H, OH), 6.81-8.15 (m, 4H, Ar). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.7 (C-5), 161.7 (C-3), 158.9 (C-1), 150.0 (C-7), 134.6 (C-2′), 132.8 (C-1′), 132.2 (C-6′), 119.9 (C-4′), 118.2 (C-5′), 117.1 (C-3′), 115.4 (C-4). EIMS *m/z* (rel. int. %): [M$^+$] 173 (38), 143 (50), 89 (57), 63 (100).

5-(4-methoxybenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3r)**

Amorphous solid; yield 80 %. M.p 297 °C. IR (nujol) ν_{max} cm^{-1}: 3187 (NH), 1726 (C=O), 1649 (Ar C=O str), 1510 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.26 (1H, s, NH), 11.25 (1H, s, NH), 8.14 (s, 1H, H-7), 7.13-8.44 (m, 4H, Ar), 3.90 (s, 3H, H-1″). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.9 (C-6), 163.4 (C-4), 162.2 (C-2), 154.9 (C-7), 150.4 (C-4′), 137.5 (C-6′ and C-2′), 125.1 (C-1′), 115.5 (C-5), 113.9 (C-3′ and C-5′), 55.7 (C-1″). EIMS *m/z* (rel. int. %): [M$^+$] 246 (100), 202 (40), 89 (29), 63 (20).

5-(2-nitrobenzylidene)-2,4,6(1H,3H,5H)-pyrimidinetrione **(3s)**

Amorphous solid; yield 61 %. M.p 248 °C. IR (nujol) ν_{max} cm^{-1}: 3202 (NH), 1758 (C=O), 1684 (Ar C=O str), 1531 (NH def). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.40 (s, 1H, NH), 11.21 (s, 1H, NH), 8.22 (s, 1H, H-7), 8.50 (dt, J = 8.1, 3.2 Hz, 1H, H-3′), 7.68 (m, 1H, H-5′), 7.55 (dt, J = 7.4, 2.9 Hz, 1H, H-6′). 7.50 (dt, J = 7.5, 6.9 Hz, 1H, H-4′); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 162.3 (C-4 and C-6), 161.1 (C-2), 152.2 (C-7), 150.2 (C-2′), 146.2 (C-1′), 133.7 (C-6′), 131.6 (C-3′), 130.3 (C-4′), 130.1 (C-5′), 120.5 (C-5). EIMS *m/z* (rel. int. %): [M$^+$] 215 (100), 172 (76), 89 (55), 63 (30).

Determination of Antibacterial and Antifungal Activities

Antimicrobial activity was performed by broth dilution method which is based on the measurement of bacterial or fungal cell numbers as a function of absorbance as the growth proceeds [20-22]. Fresh bacterial cultures of four gram-negative and two gram-positive bacteria after suitable dilutions were poured into wells (180 µL) containing 20 µg/well of test compound to total volume of 200 µL. The initial absorbance of the culture was maintained at 0.12-0.19 at 540 nm [20-21]. After incubation at 37^{o}C for 16-24 hours in 96-well microplates, the absorbance was measured at 540 nm using Synergy HT BioTek® USA microplate reader. Data was interpreted as: Inhibition (%) = 100 (X – Y) / X where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Ciprofloxacin, gentamycin and ampicillin were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions in the range 5-30 µg/well. MIC_{50} value was calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software. Antifungal activity was performed as mentioned above**.** *Candida albicans* was maintained on stock Saboraud dextrose agar culture medium. The test samples, 20 µg/well, were added to wells containing 180 µL fungal growth showing absorbance between 0.54-0.56 at 405 nm. All the assay was performed accordingly [22]. Clotrimazole

was taken as standard. Antifungal activity was also determined by standard slant method for mycelia fungi *Fusarium solani* and *Aspergillus niger* as mentioned [1].

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