Bioorganic & Medicinal Chemistry Letters 18 (2008) 6462–6467

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vitro evaluation of the antifungal activities of dihydropyrimidinones

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ARTICLE INFO

Article history:
Received 16 August 2008
Accepted 15 October 2008
Available online 18 October 2008

Keywords:
Dihydropyrimidine
Solvent-free
Antifungal activity

ABSTRACT

Copper (II) chloride in the absence of any solvent, efficiently catalyses the synthesis of dihydropyrimidinones (80–96% yields) by the Biginelli reaction. Six compounds were selected and examined their antifungal activities against the radial growth of three fungal species viz., *Trichoderma hammatum*, *Trichoderma koningii* and *Aspergillus niger*.

Owing to their remarkable pharmacological properties such as calcium channel blockers, antitumor and antiinflammatory activities, dihydropyrimidinones and their derivatives have increasingly attracted the attention of synthetic chemists.[1–5] Moreover, the dihydropyrimidine-5-carboxylate core has been found in several marine natural products which are potent HIVgp-120-CD4 inhibitors.[6,7]

However, despite the potential utility of dihydropyrimidines as bioactive compounds, their antifungal activities are very rarely studied.[8] Thus we have aimed at further investigation of these synthesized dihydropyridine derivatives as antifungal potential using *Trichoderma hammatum*, *Trichoderma koningii* and *Aspergillus niger* as model pathogens.

Polyfunctionalized dihydropyrimidines are prepared by a multicomponent reaction (MCR) that was first reported by Biginelli in 1893, involving a one-pot condensation of an aldehyde, β-ketoester and urea under strongly acidic conditions.[9] Recently, a variety of methods for promoting the Biginelli reaction including solid phase reactions,[10] microwave irradiation,[11] and catalytic reactions [12] have been developed.

In continuation of our studies on the cupric chloride promoted intramolecular ring cyclization[13] and C–C bond forming reactions,[14] we are reporting herewith a simple and practical method for the synthesis of dihydropyrimidinones by an improved Biginelli protocol using catalytic amount of $CuCl_2{\cdot}2H_2O$ chloride under solvent-free conditions. The antifungal activities of six of the selected compounds are also reported.

The reaction of benzaldehyde **1** (10 mmol), ethylacetoacetate **2** (10 mmol) and urea **3** (12 mmol) was investigated previously using 20 mol% $CuCl_2{\cdot}2H_2O$ at 100 °C for 1 h. The heterogeneous reaction mixture containing the solid cupric chloride was stirred rapidly. Cupric chloride and urea dissolves gradually as the reaction proceeds and after 20 min the mixture turns to be clear oil. After 30 min of stirring, the oil starts solidifying and the solid mass was heated under the same temperature for 1 h (monitored by TLC). The resulting solid was crushed, washed with cold water (200 mL) and filtered. The solid was dried and recrystallized from hot ethanol to afford the analytically pure product **4a** with 96% yield (Scheme 1).

The same process was successfully extended to a wide range of structurally varied aldehydes **1**, urea/thiourea **3** and β-dicarbonyl compounds **2**, to yield the corresponding 3,4-dihydropyrimidin-2(1*H*)-ones **4b–n** (Table 1) in excellent yields (80–96%). Many of the pharmacological relevant substitution patterns on the aromatic ring could be introduced with high efficiency. Aromatic aldehydes carrying electron-donating substituent afforded high yields of products in high purity. The products obtained under solvent-free conditions are of high purity and do not require any chromatographic separation.[15]

The antifungal activity of compounds **4a–n** on *T. hammatum*, *T. koningii* and *A. niger* growth and was evaluated. The results of in vitro antifungal activities showed that the title compounds were active against nearly all fungi tested to some extent. Most deriva-

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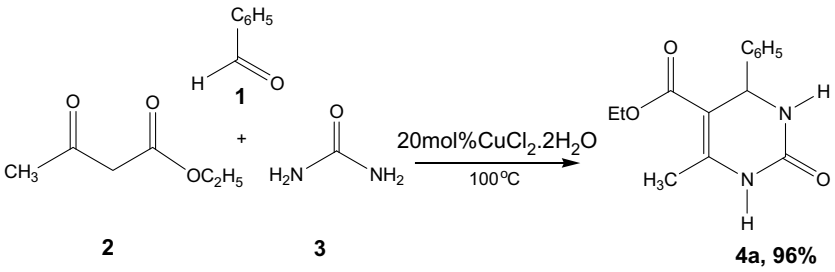
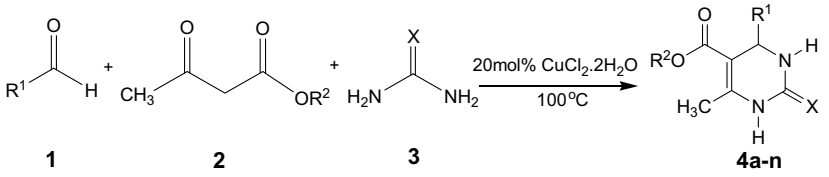
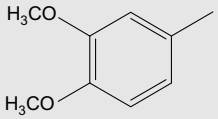
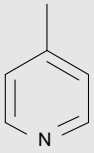
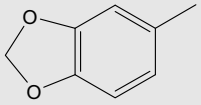
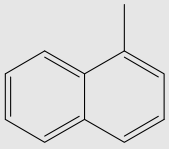
0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.10.063



Scheme 1.

Table 1
$CuCl_2{\cdot}2H_2O$-catalyzed synthesis of dihydropyrimidines under solvent-free conditions.



Dihydropyridines	R_1	R_2	X	Yield (%)	Mp (°C) found	Mp (°C) reported
4a	C_6H_5	C_2H_5	O	96	208–209	209–210[12g]
4b	C_6H_5	C_2H_5	S	90	208–209	207–208[12e]
4c	2-HOC_6H_4	C_2H_5	O	95	199–200	200–202[12h]
4d	2-HOC_6H_4	C_2H_5	S	86	811–182	183–185[12b]
4e	4-$Me_2NC_6H_4$	C_2H_5	S	85	209–210	209–210[12e]
4f	4-$Me_2NC_6H_4$	C_2H_5	O	85	253–254	256–258[12j]
4g	4-$CH_3OC_6H_4$	C_2H_5	O	96	200–202	199–201[12k]
4h	4-HOC_6H_4	C_2H_5	O	90	237–238	236–238[12g]
4i	4-ClC_6H_4	C_2H_5	O	92	215–216	216–217[12g]
4j	4-$CH_3C_6H_4$	C_2H_5	O	90	205–206	205–206[12e]
4k		C_2H_5	O	90	174–175	–
4l		C_2H_5	O	80	250–252	–
4m		C_2H_5	O	92	188–190	–
4n		C_2H_5	O	89	247–248	–

tives showed significant in vitro antifungal activities against tested fungi. However, we have selected the best six compounds (**4a–f**) exhibiting higher growth inhibition of the tested fungi with low minimum inhibitory concentration (MIC)[16] values included in the range of 0.025–0.35 μg/mL and a detailed report of the evaluation of the six selected compounds are given below.

Poison food technique of Falck[17] was used for in vitro study of the antifungal activities. The effect of six different chemical compounds **4a–f** on the radial growth of three fungal species viz., *T. hammatum*, *T. koningii* and *A. niger* was assessed by amending the compounds in potato dextrose agar (PDA) medium.[18] Stock solutions of tested compounds were prepared in acetone. Each compound from the stock solution was added to the molten sterilized PDA medium in conical flasks separately to get a final concentration of 0.5%, 1%, 3%, 5% and 7%, respectively. Each PDA medium (20 mL) containing the compounds of the five different concentrations was mixed thoroughly and poured into each 90 mm petriplate.

Petriplates without any chemical compounds serve as control. In each case, three replicates were taken. The petriplates after solidification were inoculated separately with mycelial disc (9 mm) of test fungi taken from 4 days old pure cultures aseptically

and incubated at 25 ± 1 °C in B.O.D. incubator till the mycelial growth in the control reached a maximum growth. The radial growth of the test fungi were recorded at 24 h intervals by measuring the colony diameter, compared with control, were taken as a measure of fungitoxicity. Growth inhibition (%) of test fungus was determined by using the formula given by Vincent.[19]

$$\text{Growth inhibition } (\%) = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

The effect of six compounds **4a-f** on radial growth (colony diameter) of the three fungal species are presented in Table 2–7 **4a**–**f** and the percent inhibition on radial growth of the three test fungi by these six compounds in relation to untreated plates are also provided (Table 2.1–7.1). Regarding *T. hammatum*, the highest growth inhibition, that is, 100% growth inhibition was shown by compound **4a** at 7% concentration (0.35 μg/mL) followed by compound **4d** and **4e**, respectively. After 24 and 48 h, 50% growth inhibition was shown at 3% concentration of all the compounds and after 72 h except compounds **4b** and **4e**, the remaining compounds **4a**, **4c**, **4d** and **4f** show 50% growth inhibition.

The increase growth of *T. koningii* and *A. niger* over control were also recorded from 0.5% to 1% of some compounds. In case of *T. koningii*, highest growth inhibition was shown by the compound **4c**, followed by **4a** and **4e**, respectively, at 7% concentration.

Table 2
Evaluation of different concentrations of dihydropyrimidine **4a** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	14.66	48.25	81.00	11.66	31.50	78.25	2.16	4.41	9.66
0.5	13.00	32.33	79.00	11.00	25.75	77.00	2.58	7.25	12.33
1	11.00	37.66	71.00	10.25	25.00	69.75	1.50	5.00	10.41
3	2.62	13.50	29.66	—	11.87	23.33	0.66	2.08	4.41
5	—	1.50	5.50	—	—	6.62	0.33	0.83	2.58
7	—	—	—	—	—	3.50	—	0.91	2.25

—, no growth.

Table 2.1
Inhibition rates of radial growth (%) of test fungi by different concentrations of dihydropyrimidine **4a** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	11.32	32.99	2.46	5.66	18.25	1.59	0.00 (19.44)	0.00 (64.39)	0.00 (27.63)
1	24.96	21.94	12.34	12.09	20.63	10.86	30.55	0.00 (13.37)	0.00 (7.76)
3	82.12	72.02	63.38	100	62.31	70.18	69.44	52.83	54.34
5	100	96.89	93.20	100	100	91.53	84.72	81.17	73.29
7	100	100	100	100	100	95.52	100	79.36	76.70

The values in parentheses denoted percentage of increase growth over control.

Table 3
Evaluation of different concentrations of dihydropyrimidine **4b** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	18.50	64.33	81.00	17.33	55.33	78.66	3.00	5.50	8.25
0.5	15.33	58.66	81.00	23.58	64.33	81.00	7.00	10.83	17.00
1	9.66	52.33	81.00	22.00	57.50	81.00	3.00	8.25	14.50
3	6.08	20.83	50.50	7.62	17.66	29.33	2.00	2.87	5.25
5	2.00	4.50	4.08	1.87	7.00	15.87	2.00	2.50	3.66
7	—	—	3.25	1.50	5.00	12.25	—	—	—

Table 3.1
Inhibition rates of radial growth (%) of test fungi by different concentrations of dihydropyrimidine **4b** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	17.13	8.81	0.00	0.00 (36.06)	0.00 (16.26)	0.00 (2.97)	0.00 (133.00)	0.00 (96.90)	0.00 (106.06)
1	47.78	18.65	0.00	0.00 (26.94)	0.00 (3.92)	0.00 (2.97)	0.00	0.00 (50.00)	0.00 (75.75)
3	67.13	67.62	37.65	56.03	68.08	62.71	33.33	47.81	36.36
5	89.18	93.00	94.96	89.20	87.34	79.82	33.33	54.54	55.63
7	100.00	100.00	95.98	91.34	90.96	84.42	100.00	100.00	100.00

The values in parentheses denoted percentage of increase growth over control.

Table 4
Evaluation of different concentrations of dihydropyrimidine **4c** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	19.00	52.00	77.83	17.25	48.00	74.50	5.00	12.00	14.00
0.5	20.08	62.16	81.00	18.37	53.75	79.25	5.16	7.83	9.33
1	15.00	40.33	65.50	18.25	45.50	74.00	5.37	9.87	11.75
3	6.87	16.25	31.83	8.50	29.37	55.75	3.83	4.25	5.50
5	2.00	3.00	3.83	3.50	14.00	33.00	3.00	4.12	4.75
7	—	—	2.50	—	—	2.00	—	—	1.00

—, no growth.

Table 4.1
Inhibition rates of radial growth (%) of test fungi by different concentrations of dihydropyrimidine **4c** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	0.00 (5.68)	0.00 (19.53)	0.00 (4.07)	0.00 (6.49)	0.00 (11.97)	0.00 (6.37)	0.00 (3.20)	34.75	33.35
1	21.05	22.44	15.84	0.00 (5.79)	5.20	0.67	0.00 (7.40)	17.75	16.07
3	63.84	68.75	59.10	50.72	38.81	25.16	37.60	64.58	60.71
5	89.47	94.23	95.07	79.71	70.83	55.70	40.00	65.66	66.07
7	100.00	100.00	96.78	100.00	100.00	97.31	100.00	100.00	92.85

The values in parentheses denoted percentage of increase growth over control.

Table 5
Evaluation of different concentrations of dihydropyrimidine **4d** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	13.25	51.16	80.66	11.41	32.00	80.00	3.33	5.66	7.83
0.5	9.75	48.00	81.00	13.66	49.00	81.00	6.33	13.00	15.16
1	6.66	24.83	49.83	10.00	36.33	68.50	2.66	11.00	16.00
3	2.50	9.75	19.00	5.66	30.66	53.83	1.50	3.50	6.00
5	—	—	2.00	—	2.75	9.33	1.25	2.83	5.33
7	—	—	1.33	—	2.00	7.16	1.25	2.16	5.00

—, no growth.

Table 5.1
Inhibition rates of radial growth (%) of test fungi by different concentrations dihydropyrimidine **4d** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	26.41	61.76	0.00 (0.42)	0.00 (19.71)	0.00 (53.12)	0.00 (1.25)	0.00 (90.09)	0.00 (129.68)	0.00 (93.61)
1	49.73	51.46	38.22	12.35	0.00 (113.53)	14.37	20.12	0.00 (94.34)	0.00 (104.34)
3	81.13	80.94	76.44	50.39	4.18	32.71	54.95	38.16	23.37
5	100.00	100.00	97.52	100.00	91.40	88.33	62.46	50.00	31.92
7	100.00	100.00	98.35	100.00	93.75	91.05	62.46	61.83	36.14

The values in parentheses denoted percentage of increase growth over control.

After 24, 48 and 72 h, 50% growth inhibition was shown at 3% concentration of the compounds **4a**, **4b** and **4f**.

Similarly, 100% growth inhibition of *A. niger* were recorded from the compounds **4b**, **4e** and **4f**, respectively. At 3% concentration, the compounds **4a** and **4f** show 50% growth inhibition of *A. niger*.

Inhibitory effects of the six compounds on each test fungus were different. Thus compound **4a** was the most potent against *T. hammatum* resulting in 100% growth inhibition with MIC value of 0.35 μg/mL. The radial growth of *T. koningii* after 24 and 48 h were found to be inhibited completely (100%) by compound **4c** and compounds **4b**, **4e** and **4f** were the most potent against *A. niger*, with MIC value of 0.35 μg/mL each.

In conclusion, a broad range of structurally diverse 1,3-dicarbonyl compounds, aldehydes and urea/thiourea are subjected under the one-pot Biginelli cyclocondensation catalyzed by cupric chloride under solvent-free conditions to produce the corresponding dihydropyrimidinones. In view of the simplicity, environmentally friendly nature and high yields, the present procedure of the synthesis of dihydropyrimidin-2(1*H*)-ones provides a simple, efficient, cost-effective and green modification of the Biginelli's reac-

Table 6
Evaluation of different concentrations of dihydropyrimidine **4e** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	14.00	44.33	75.50	13.00	32.00	74.00	2.50	5.00	8.33
0.5	12.75	42.00	69.16	15.00	38.00	76.00	4.00	10.16	16.66
1	11.00	38.83	65.00	12.50	37.83	75.00	2.16	9.33	16.00
3	3.50	11.33	45.00	7.58	26.00	62.33	1.75	3.00	4.00
5	—	—	2.50	1.00	4.41	8.66	—	—	1.80
7	—	—	1.83	—	1.50	6.58	—	—	—

—, no growth.

Table 6.1
Inhibition rates of radial growth (%) of test fungi by different concentrations of dihydropyrimidine **4e** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	8.92	5.25	8.39	0.00 (15.38)	0.00 (18.75)	0.00 (2.70)	0.00 (60.00)	0.00 (103.20)	0.00 (100.00)
1	21.42	12.40	13.90	3.84	0.00 (18.21)	0.00 (1.35)	13.60	0.00 (86.60)	0.00 (92.07)
3	75.00	74.44	40.39	41.69	18.75	15.77	30.00	40.00	51.98
5	100.00	100.00	96.68	92.30	86.21	88.29	100.00	100.00	78.39
7	100.00	100.00	97.57	100.00	95.31	91.10	100.00	100.00	100.00

The values in parentheses denoted percentage of increase growth over control.

Table 7
Evaluation of different concentrations of dihydropyrimidine **4f** on the growth of the test fungi (average of three replicates).

Concentration (%)	Radial growth (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0	15.83	55.33	81.00	12.50	31.00	78.33	3.50	12.00	18.66
0.5	17.25	57.16	81.00	12.00	28.00	42.66	2.16	7.00	12.00
1	16.33	56.16	81.00	11.33	16.00	40.50	2.00	6.33	10.00
3	3.58	13.66	21.83	2.00	11.00	31.66	—	3.00	4.83
5	2.50	3.66	10.66	—	4.00	16.33	—	1.50	2.16
7	—	2.00	5.00	—	4.00	15.66	—	—	—

—, no growth.

Table 7.1
Inhibition rates of radial growth (%) of test fungi by different concentrations of dihydropyrimidine **4f** (average of three replicates).

Concentration (%)	Percentage inhibition of radial growth over control (mm)								
	Trichoderma hammatum			*Trichoderma koningii*			*Aspergillus niger*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
0.5	0.00 (8.97)	0.00 (3.30)	0.00	4.00	9.67	45.53	38.28	41.66	35.69
1	0.00 (3.15)	0.00 (1.50)	0.00	9.36	48.38	48.29	42.85	47.25	46.40
3	77.38	75.31	73.04	84.00	64.51	59.58	100.00	75.00	74.11
5	84.20	93.38	86.83	100.00	87.09	79.15	100.00	87.50	88.42
7	100.00	96.38	93.82	100.00	87.09	80.00	100.00	100.00	100.00

The values in parentheses denoted percentage of increase growth over control.
—, no growth.

tion. Evaluation of the antifungal activities revealed that 4-aryl-1,4-dihydropyridines **4a–f** showed significant in vitro antifungal activities against tested fungi with low MIC values in the range 0.025–0.35 μg/mL.

Acknowledgments

O.M.S. is thankful to CSIR project (No. 01(2135)/07/EMR-II) for financial assistance. We also thank SAIF, CDRI, Lucknow & SAIF, NEHU, Shillong for the spectral recordings.

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15. To a mixture of aldehyde **1** (10 mmol), β-dicarbonyl compound **2** (10 mmol) and urea **3** (12 mmol), $CuCl_2{\cdot}2H_2O$ (2 mmol, 20 mol%) was added at room temperature. After it was stirred for 5 min, the resulting mixture was heated at 100 °C in a preheated oil bath for 20 min–1 h (monitored by TLC). The reaction mixture was brought to room temperature, crushed and 100 mL of cold water was added and stirred for 5–10 min. The solid was filtered, washed with ice-cold water (100 mL) and then recrystallized from hot ethanol to afford pure product.
5-(Ethoxycarbonyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one ***4a****:* ^{1}H NMR (DMSO-d_6): δ 1.17 (t, J = 7.1 Hz, 3H), 2.30 (s, 3H), 4.05 (q, J = 7.1 Hz, 2H), 5.25 (s, 1H), 7.19–7.35 (m, 6H), 8.98 (br s, 1H, NH); IR (KBr): 3242, 1721, 1637 cm^{-1}. EIMS: m/z (%) 260 (M^+), 232, 184, 156, 138, 43. Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.64; H, 6.25; N, 10.92.
Compound ***4k****:* ^{1}H NMR (DMSO-d_6): δ 1.15 (t, J = 7.1 Hz, 3H), 2.30 (s, 3H), 3.85 (s, 6H), 4.05 (q, J = 7.1 Hz, 2H), 5.25 (s, 1H), 6.80–6.85 (m, 3H), 7.25 (br s, 1H, NH), 8.90 (br s, NH); IR (KBr): 3253, 1721, 1687 cm^{-1}. EIMS: m/z (%) 320 (M^+), 292, 273, 247, 138, 97.
Compound ***4l****:* ^{1}H NMR (DMSO-d_6): δ 1.15 (t, J = 7.1 Hz, 3H), 2.30 (s, 3H), 4.00 (q, J = 7.1 Hz, 2H), 5.35(s, 1H), 7.20–7.25 (m, 2H), 8.40–8.58 (m, 2H), 9.00 (br s, 1H, NH), 9.40 (br s, NH); ^{13}C NMR: δ 13.7, 17.7, 49.6, 59.9, 106.4, 124.7, 124.8, 139.3, 146.6, 150.8, 156.8, 165.2; IR (KBr): 3290, 1712, 1679, 1589 cm^{-1}. EIMS: m/z (%) 261 (M^+), 233, 216. Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.70; H, 5.63; N, 16.28.
Compound ***4m****:* ^{1}H NMR (DMSO-d_6): δ 1.15 (t, J = 7.1 Hz, 3H), 2.35 (s, 3H), 4.10 (q, J = 7.1 Hz, 2H), 5.95 (s, 2H), 6.75–6.85 (m, 3H), 8.25 (br s, 1H, NH), 8.90 (br s, NH); IR (KBr): 3353, 1701, 1647 cm^{-1}. EIMS: m/z (%) 304 (M^+), 275, 258, 231, 183, 69.
Compound ***4n****:* ^{1}H NMR (DMSO-d_6): δ 0.95 (t, J = 7.1 Hz, 3H), 2.45 (s, 3H), 3.95 (q, J = 7.1 Hz, 2H), 5.25 (s, 1H), 6.91–7.20 (m, 2H), 7.45–7.60 (m, 2H), 7.85 (t, J = 8.2 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 8.35 (d, J = 8.2 Hz, 1H), 9.15 (br s, NH); IR (KBr): 3253, 1699, 1647, 1435 cm^{-1}. EIMS: m/z (%) 310 (M^+), 217, 176, 231, 133, 69.
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18. Two hundred and fifty grams of peeled and sliced potatoes were washed in tap water and boiled in 500 mL distilled water for 30 min or till it could be crushed by a glass rod and the extract was filtered into a beaker (1000 mL) through a muslin cloth. Twenty grams of dextrose was added to the extract and its volume was made up to 1000 mL by adding additional distilled water to make potato dextrose broth. Agar was dissolved to the potato dextrose broth. The medium thus prepared was dispensed into conical flasks and sterilized in an autoclave at 15l bs psi pressure for 20 min after wrapping the cotton plugs with paper. This sterilized PDA medium was stored for further experiment.
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