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Synthesis of 2-furyl-4-arylidene-5(4H)-oxazolones as new potent antibacterial agents against phyto-pathogenic and nitrifying bacteria

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Crop losses due to bacterial pathogens are a major global concern. Most of the available pesticides for these pathogens suffer from various drawbacks such as complicated synthesis, high cost, high toxicity, pesticide resistance and environmental hazards. To overcome these drawbacks, the present study was undertaken to find a potent bactericide. Therefore, a series of compounds comprising bioactive furyl and oxazolone rings was synthesized under microwave irradiation and screened for *in vitro* antibacterial activity. The reactions were completed in fewer than 2 minutes with minimal use of solvents and resulted in high yields. These compounds were screened for antibacterial activity against plant pathogens, *Xanthomonas oryzae*, *Ralstonia solanacearum* and nitrifying bacteria, *Nitrosomonas* species under laboratory conditions. Five compounds were active as antibacterial agents against *Xanthomonas oryzae* and *Ralstonia solanacearum*. However, all compounds were effective against the *Nitrosomonas* species and the best one was 2-furyl-4-(3-methoxy-4-hydroxybenzylidene)-5(4H)-oxazolone. The study revealed the fast and environmentally friendly synthesis of bioactive title compounds, which also hold promise to be used as prototypes for the discovery of potent analogues.

Keywords: Oxazolone, azlactones, *Xanthomonas oryzae*, *Ralstonia solanacearum*, *Nitrosomonas.*

Introduction

Xanthomonas oryzae and *Ralstonia solanacearum* are responsible for severe losses as high as 50–75 % to many important crops, mainly rice and Solanaceous plants.[1–3] The nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* species, are responsible for the low nitrogen use efficiency of nitrogenous fertilizers amounting to US $17 billion in annual nitrogen losses[4] worldwide. Several pesticides[5,6] like carboxin + thiram, Streptomycin + Tetracycline, Nitrapyrin are in use against these pathogens. Most of these are still not fully satisfactory due to one or more of the following disadvantages like complicated synthesis, high volatility, high toxicity or ecotoxicity, low stability, high application rate, the addition of costly formulants, high cost, and pesticide resistance.[7–9] Therefore, an ideal bactericide is still elusive. It needs to be simple, safe, efficient, persistent, specific and economic in use.

Since their first synthesis,[10] 5(4H)-oxazolones have emerged as a dependable class of synthetic intermediates and several biologically active molecules.[11–22] These have been extensively used in the synthesis of organic compounds.[18–20] Oxazolone derivatives have been recently claimed to exhibit antifungal, antifeedant and appreciable antibacterial activity against *Escherichia coli* and *Xanthomonas citri*.[21,22]

In order to exploit the biopotential of 5(4H)-oxazolones and bactericidal property of furyl group especially against the *Nitrosomonas*,[23,24] the present study was undertaken to synthesize a series of 2-furyl-4-substituted-5(4H)-oxazolones as potential bactericidal agents.

Materials and methods

Chemicals and instruments

All of the chemicals used were purchased from Sigma-Aldrich and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on precoated Merck silica gel $60F_{254}$, and the spots were visualized either under UV or by iodine vapor. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer model 2000 FT-IR spectrophotometer as KBr pellet, and values are expressed as ν max cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were

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Table 1. Elemental-analytical data of 2-furyl-4-arylidene-5(4H)-oxazolones.

			% C		% H		% N	
S. no.	*Molecular formula*	*FW*	*Cal*	*Obs*	*Cal*	*Obs*	*Cal*	*Obs*
1	$C_{14}H_8FNO_3$	257.21	65.37	65.29	3.13	3.14	5.45	5.47
2	$C_{14}H_8ClNO_3$	273.67	61.44	61.44	2.95	2.92	5.12	5.11
3	$C_{14}H_8ClNO_3$	273.67	61.44	61.52	2.95	3.00	5.12	5.13
4	$C_{14}H_8BrNO_3$	318.12	52.86	52.74	2.53	2.51	4.40	4.42
5	$C_{14}H_8N_2O_5$	284.22	59.16	60.03	2.84	2.74	9.86	9.86
6	$C_{14}H_9NO_4$	255.22	65.88	65.37	3.55	3.65	5.49	5.47
7	$C_{15}H_{11}NO_4$	269.25	66.91	66.86	4.12	4.11	5.20	5.18
8	$C_{15}H_{11}NO_3$	253.25	71.14	71.15	4.38	4.39	5.53	5.54
9	$C_{15}H_{11}NO_3$	253.25	71.14	71.16	4.38	4.37	5.53	5.48
10	$C_{17}H_{15}NO_3$	281.10	72.58	72.61	5.37	5.31	4.98	4.89
11	$C_{14}H_7Cl_2NO_3$	308.11	54.57	54.48	2.29	2.33	4.55	4.58
12	$C_{14}H_7Cl_2NO_3$	308.11	54.57	54.25	2.29	2.28	4.55	4.59
13	$C_{14}H_9NO_5$	271.22	62.00	62.14	3.34	3.31	5.16	5.18
14	$C_{16}H_{13}NO_5$	299.27	64.21	64.26	4.38	4.39	4.68	4.65
15	$C_{15}H_{11}NO_5$	285.25	63.16	63.14	3.89	3.88	4.91	4.90
16	$C_{16}H_{13}NO_5$	299.27	64.21	64.32	4.38	4.45	4.68	4.64
17	$C_{17}H_{15}NO_6$	329.30	62.00	62.03	4.59	4.52	4.25	4.25
18	$C_{16}H_{14}N_2O_3$	282.29	68.07	68.06	5.00	5.05	9.92	10.01
19	$C_{13}H_8N_2O_3$	240.21	65.00	65.03	3.36	3.35	11.66	11.59
20	$C_{18}H_{11}NO_3$	289.28	74.73	74.98	3.83	3.82	4.84	5.82
21	$C_{22}H_{13}NO_3$	339.34	77.87	77.18	3.86	3.88	4.13	4.13
22	$C_{13}H_{13}NO_3$	231.24	67.52	67.27	5.67	5.65	6.06	6.02

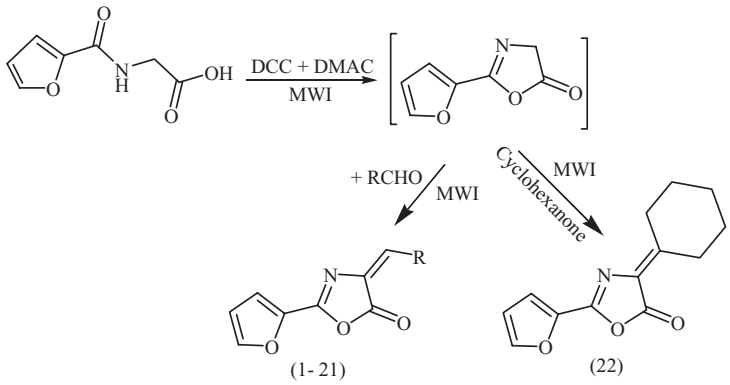
recorded on a Bruker Spectrospin spectrometer (400 and 75.5 MHz), using tetramethylsilane as an internal standard. The chemical shift values were recorded on δ scale, and the coupling constants (J) are in Hertz. Elemental analysis for all compounds was performed on a Carlo Erba model EA-1108 elemental analyzer and data of C, H, and N were within ±0.4 % of calculated values as reported in Table 1.

Synthesis of 2-furyl-4-arylidene-5(4H)-oxazolones

Various solid and solution-phase methodologies were attempted (Table 2) and the optimized protocol for the synthesis is as follows. A mixture of furoyl glycine (5 mmol), N,N-dicyclohexylcarbodiimide (DCC) (6 mmol) and N,N-dimethyl acetamide (DMAC) (3 mL) contained in a conical flask (150 mL) was irradiated under microwaves for 30 seconds.[22] Then aldehyde (5 mmol) was added to the mixture and further irradiated for 30–90 seconds. The mixture was cooled and 50 mL water was added after completion of the reaction as depicted in Figure 1. The aqueous layer was decanted and gummy residue was treated with 15 % aq. acetic acid (100 mL). The mixture was stirred for 30 min and extracted with diethyl ether (2 × 50 mL). Dicyclohexylurea, separated at the interface of two layers, was removed by gravity filtration. The organic layer was washed with 5 % sodium hydrogen carbonate (50 mL) and sodium hydrogen sulphite (50 mL) solution. Finally the ethereal layer was washed with water, dried over anhydrous sodium sulphate and evaporated to afford the product which was recrystallized from benzene. The completion of reaction was monitored by TLC using 20 % ethyl acetate: hexane as eluting solvent system and verified by analytical and spectral techniques. The twenty two 2-furyl-4-arylidene-5(4H)-oxazolones were synthesized using this model.

Table 2. Method development (for 5 milli mole of Furoyl glycine (FG) and aldehyde each).

S. no.	*Reaction conditions*	*Result*
	SOLID PHASE	
1	Anhydrous alumina (acidic) 5g + FG + aldehyde	No reaction
2	Anhydrous alumina (basic) 5g + FG + aldehyde	No reaction
3	Anhydrous alumina (neutral) 5g + FG + aldehyde	No reaction
4	Anhydrous potassium carbonate 5g + FG + aldehyde	No reaction
5	Anhydrous nano kaolinite 5g + FG + aldehyde	No reaction
6	Anhydrous nano bentonite 5g + FG + aldehyde	No reaction
	SOLUTION PHASE	
7	Acetic anhydride (7.5 mL) + FG + aldehyde	70 % yield
8a	DCC (6 milli mole) + DMAC (5 mL) + FG + aldehyde	70–80 % yield
8b	DCC (6 milli mole) + DMAC (3 mL) + FG + aldehyde	70–80 % yield



Sr. No.	R	Sr. No.	R
1	4-F-C_6H_4	12	2,6-$(Cl)_2$-C_6H_3
2	2-Cl-C_6H_4	13	2,4-$(OH)_2$-C_6H_3
3	4-Cl-C_6H_4	14	2,4-$(OCH_3)_2$-C_6H_3
4	3-Br-C_6H_4	15	3-(OCH_3)-4-(OH)-C_6H_3
5	3-NO_2-C_6H_4	16	3-(OC_2H_5)-4-(OH)-C_6H_3
6	4-OH-C_6H_4	17	3,4,5-$(OCH_3)_3$-C_6H_2
7	3-OCH_3-C_6H_4	18	4-$(N(CH_3)_2)$-C_6H_4
8	2-CH_3-C_6H_4	19	2-pyridyl
9	3-CH_3-C_6H_4	20	1-naphthyl
10	4-$CH(CH_3)_2$-C_6H_4	21	9-anthryl
11	2,4-$(Cl)_2$-C_6H_3		

Fig. 1. Synthesis of 2-Furyl-4-(arylidene)-5(4H)-oxazolones.

2-Furyl-4-(4-flurobenzylidene)-5(4H)-oxazolones (1). MWI 80 sec.; Yield 80 %; mp 121°C; ir: 1823, 1685; ^{1}H NMR: δ 6.76–6.88 (dd, J = 4.8, 1.6 Hz, 1H, H-4-furyl), 7.11–7.21 (m, 1H, H-3-furyl), 7.28 (d, J = 10.4 Hz, 2H, H-3, H-5-phenyl), 7.36 (d, J = 7.6 Hz, 2H, H-2, H-6-phenyl), 7.44–7.53 (m, 1H, H-5-furyl), 7.78 (s, 1H, CH = C).

2-Furyl-4-(2-chlorobenzylidene)-5(4H)-oxazolones (2). MWI 60 sec.; Yield 75 %; mp 127°C; ir: 1811, 1689; ^{1}H NMR: δ 6.76–6.88 (dd, J = 4.8, 1.6 Hz, 1H, H-4-furyl), 7.11–7.21 (m, 1H, H-3-furyl), 7.28 (d, J = 10.4 Hz, 2H, H-3, H-5-phenyl), 7.36 (d, J = 7.6 Hz, 2H, H-2, H-6-phenyl), 7.44–7.53 (m, 1H, H-5-furyl), 7.78 (s, 1H, CH = C).

2-Furyl-4-(4-chlorobenzylidene)-5(4H)-oxazolones (3). MWI 60 sec.; Yield 72 %; mp 201°C; ir: 1799, 1664; ^{1}H NMR: δ 6.68 (s, 1H, H-4-furyl), 7.190 (s, 1H, H-3-furyl), 7.36 (s, 1H, CH = C), 7.44 (d, J = 7.8 Hz, 2H, H-2, H-6-phenyl), 7.75 (s, 1H, H-5-furyl), 8.11 (d, J = 7.8 Hz, 2H, H-3, H-5-phenyl).

2-Furyl-4-(3-bromobenzylidene)-5(4H)-oxazolones (4). MWI 60 sec.; Yield 76 %; mp 180°C; ir: 1812, 1684; ^{1}H NMR: δ 6.68–6.69 (dd, J = 5.2, 1.6 Hz, 1H, H-4-furyl), 7.33–7.38 (m, 1H, H-3-furyl), 7.43–7.54 (m, 1H, H-5-phenyl), 7.56–7.57 (m, 1H, H-6-phenyl), 7.58–7.59 (m, 1H, H-4-phenyl), 7.74 (s, 1H, CH = C), 7.79–7.83 (m, 1H, H-5-furyl), 8.03–8.22 (m, 1H, H-2-phenyl).

2-Furyl-4-(3-nitrobenzylidene)-5(4H)-oxazolones (5). MWI 120 sec.; Yield 68 %; mp 202°C; ir: 1817, 1670; ^{1}H NMR: δ 6.70 (dd, J = 5.6, 1.6 Hz, 1H, H-4-furyl,), 7.43 (d, J = 3.6 Hz, 1H, H-3-furyl), 7.67 (dd, J = 10.8, 2.8 Hz, 1H, H-4-phenyl), 7.82 (d, J = 0.8 Hz, 1H, H-5-furyl), 8.27 (d, J = 2.8 Hz, 1H, H-6-phenyl), 8.29 (dd, J = 3.2, 1.2 Hz, 1H,

H-5-phenyl), 8.50 (d, J = 8.0 Hz, 1H, H-2-phenyl), 9.02 (s, 1H, CH = C).

2-Furyl-4-(4-hydroxybenzylidene)-5(4H)-oxazolones (6). MWI 80 sec.; Yield 75 %; mp 137°C; ir: 1804, 1653; ^{1}H NMR: δ 5.5 (1H, OH), 6.66–6.67 (dd, J = 5.2, 1.6 Hz, 1H, H-4-furyl), 7.20–7.21 (d, J = 3.2 Hz, 2H, H-3, H-5-phenyl), 7.27–7.30 (m, 1H, H-3-furyl), 7.34–7.35 (m, 1H, H-5-furyl), 7.54–7.56 (d, J = 8.8 Hz, 2H, H-2, H-6-phenyl), 7.77 (s, 1H, CH = C).

2-Furyl-4-(3-methoxybenzylidene)-5(4H)-oxazolones (7). MWI 80 sec.; Yield 79 %; mp 165°C; ir: 1789, 1653; ^{1}H NMR: δ 3.82 (s, 3H, OCH_3), 6.61–6.62 (dd, J = 5.2, 2.0 Hz, 1H, H-4-furyl), 6.95–6.96 (dd, J = 3.6, 0.4 Hz, 1H, H-3-furyl), 6.97–6.99 (m, 1H, H-4-phenyl), 7.22–7.29 (m, 1H, H-2-phenyl), 7.32–7.36 (m, 1H, H-6-phenyl), 7.66–7.76 (m, 2H, H-5-furyl, H-5-phenyl), 7.77 (s, 1H, CH = C).

2-Furyl-4-(2-methylbenzylidene)-5(4H)-oxazolones (8). MWI 100 sec.; Yield 72 %; mp 145°C; ir: 1776, 1650; ^{1}H NMR: δ 2.10 (s, 3H, H-methyl), 6.51–6.52 (m, 1H, H-4-furyl), 6.54–6.55 (dd, J = 5.6, 2.0 Hz, 1H, H-3-furyl), 7.19–7.20 (dd, J = 4.0, 0.4 Hz, 1H, H-3- phenyl), 7.29–7.30 (m, 2H, H-4, H-5-phenyl), 7.48–7.49 (m, 1H, H-5-furyl), 7.63–7.64 (dd, J = 2.8, 0.8 Hz, 1H, H-6-phenyl), 7.74 (s, 1H, CH = C).

2-Furyl-4-(3-methylbenzylidene)-5(4H)-oxazolones (9). MWI 100 sec.; Yield 73 %; mp 140°C; ir: 1769, 1658; ^{1}H NMR: δ 2.10 (s, 3H, CH_3), 6.54–6.55 (dd, J = 5.2, 1.6 Hz, 1H, H-4-furyl), 7.19–7.27 (m, 2H, H-3-furyl, H-4 phenyl), 7.30–7.42 (m, 2H, H-5-furyl, H-3-phenyl), 7.44–7.63 (m, 1H, H-5-phenyl), 7.63–7.67 (m, 1H, H-6-phenyl), 7.69 (s, 1H, CH = C).

2-Furyl-4-(4-isopropylbenzylidene)-5(4H)-oxazolones (10). MWI 90 sec.; Yield 81 %; mp 161°C; ir: 1795, 1666; ^{1}H NMR: δ 1.26 (d, 6H, 2 CH_3), 3.02 (heptet, 1H, CH), 7.26–7.30 (m, 1H, H-4-furyl), 7.31–7.35 (m, 1H, H-3-furyl), 7.40 (d, J = 8.4 Hz, 2H, H-3, H-5-phenyl), 7.64 (s, 1H, CH = C), 7.83–8.01 (m, 1H, H-5-furyl), 8.02 (d, J = 8.0 Hz, 2H, H-2, H-6-phenyl).

2-Furyl-4-(2,4-dichlorobenzylidene)-5(4H)-oxazolones (11). MWI 90 sec.; Yield 80 %; mp 152°C; ir: 1823, 1673; ^{1}H NMR: δ 6.49–6.58 (m, 1H, H-4-furyl), 6.60–6.66 (m, 1H, H-3-furyl), 7.20–7.26 (m, 1H, H-4-phenyl), 6.92–6.95 (m, 1H, H-5-phenyl), 7.25–7.47 (m, 2H, H-5-furyl, H-6-phenyl), 7.63 (s, 1H, H-3-phenyl), 7.70 (s, 1H, CH = C).

2-Furyl-4-(2,6-dichlorobenzylidene)-5(4H)-oxazolones (12). MWI 120 sec.; Yield 82 %; mp 125°C; ir: 1805, 1654; ^{1}H NMR: δ 6.48–6.50 (dd, J = 4.8, 1.6 Hz, 1H, H-4-furyl), 7.09–7.15 (m, 1H, H-3-furyl), 7.20–7.26 (m, 1H, H-4-phenyl), 7.27–7.37 (m, 2H, H-3, H-5-phenyl), 7.40–7.53 (m, 1H, H-5-furyl), 8.25 (s, 1H, CH = C).

2-Furyl-4-(2,4-dihydroxybenzylidene)-5(4H)-oxazolones (13). MWI 100 sec.; Yield 76 %; mp 149°C; ir: 1758, 1623; ^{1}H NMR: δ 4.28 (s, 2H, OH), 6.44 (s, 1H, H-3-phenyl), 6.76–6.79 (dd, J = 5.6, 2.0 Hz, 1H, H-4-furyl), 6.80–6.81 (m, 2H, H-5-phenyl, H-3-furyl), 7.06 (s, 1H, H-3-phenyl), 7.28 (d, J = 8.4 Hz, 1H, H-6-phenyl), 7.58 (d, J = 8.4 Hz, 1H, H-5-furyl), 9.69 (s, 1H, CH = C).

2-Furyl-4-(2,4-dimethoxybenzylidene)-5(4H)-oxazolones (14). MWI 80 sec.; Yield 76 %; mp 134°C; ir: 1785, 1669; ^{1}H NMR: δ 3.90 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.44 (s, 1H, H-3-phenyl), 6.50–6.54 (m, 1H, H-4-furyl), 6.56 (d, J = 2.4 Hz, 1H, H-3-furyl), 6.63 (d, J = 2.0 Hz, 1H, H-5-phenyl), 7.28 (d, J = 3.2 Hz, 1H, H-6-phenyl), 7.62 (d, J = 0.8 Hz, 1H, H-5-furyl), 7.82 (s, 1H, CH = C).

2-Furyl-4-(3-methoxy-4-hydroxybenzylidene)-5(4H)-oxazolones (15). MWI 90 sec.; Yield 80 %; mp 172°C; ir: 1763, 1685; ^{1}H NMR: δ 3.88 (s, 3H, OCH_3), 5.78 (s, 1H, OH), 7.04–7.11 (m, 1H, H-4-furyl), 7.17–7.24 (m, 1H, H-3-furyl), 7.26 (s, 1H, H-2-phenyl), 7.74 (d, J = 1.6 Hz, 1H, H-5-phenyl), 7.46 (d, J = 1.6 Hz, 1H, H-6-phenyl), 7.47–7.48 (m, 1H, H-5-furyl), 7.63 (s, 1H, CH = C).

2-Furyl-4-(3-ethoxy-4-hydroxybenzylidene)-5(4H)-oxazolones (16). MWI 90 sec.; Yield 79 %; mp 164°C; ir: 1752, 1678; ^{1}H NMR: δ 1.45 (t, 3H, CH_3), 4.13 (q, 2H, OCH_2), 5.75 (s, 1H, OH), 6.88–6.89 (dd, J = 5.2, 2.0 Hz, 1H, H-4-furyl), 7.25 (s, 1H, H-2-phenyl), 7.19 (d, J = 7.6 Hz, 1H, H-3-furyl), 7.43 (d, J = 1.6 Hz, 1H, H-5-phenyl), 7.45 (d, J = 1.6 Hz, 1H, H-6-phenyl), 7.46 (d, J = 1.6 Hz, 1H, H-5-furyl), 7.56 (s, 1H, CH = C).

2-Furyl-4-(3,4,5-trimethoxybenzylidene)-5(4H)-oxazolones (17). MWI 100 sec.; Yield 81 %; mp 168°C; ir: 1788, 1664; ^{1}H NMR: δ 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.65–6.56 (dd, J = 5.2, 2.0 Hz, 1H, H-4-furyl), 7.15 (d, J = 9.6 Hz, 2H, H-2, H-6-phenyl), 7.30–7.31 (dd, J = 4.4, 0.8 Hz, 1H, H-3-furyl), 7.56 (s, 1H, CH = C), 7.74–7.75 (dd, J = 2.4, 0.8 Hz, 1H, H-5-furyl).

2-Furyl-4-(4-N,N-dimethylaminobenzylidene)-5(4H)-oxazolones (18). MWI 80 sec.; Yield 82 %; mp 157°C; ir: 1774, 1654; ^{1}H NMR: δ 6.62–6.63 (dd, J = 4.8, 1.6 Hz, 1H, H-4-furyl), 6.72–6.84 (m, 1H, H-3-furyl), 7.21 (d, J = 4.8 Hz, 1H, H-3, H-5-phenyl), 7.23–7.50 (m, 1H, H-5-furyl), 7.71 (s, 1H, CH = C), 7.72–7.75 (d, J = 8.8 Hz, 2H, H-2, H-6-phenyl).

2-Furyl-4-(2-pyridylbenzylidene)-5(4H)-oxazolones (19). MWI 60 sec.; Yield 72 %; mp 163°C; ir: 1815, 1686; ^{1}H NMR: δ 6.53–6.54 (dd, J = 4.8, 1.6 Hz, 1H, H-4-furyl), 6.58–6.59 (dd, J =

5.2, 1.6 Hz, 1H, H-3-furyl), 7.25–7.32 (m, 1H, H-4-pyridyl), 7.33–7.37 (m, 1H, H-5-pyridyl), 7.44–7.50 (m, 1H, H-3-pyridyl), 7.55–7.61 (m, 1H, H-6-pyridyl), 7.71 (s, 1H, CH = C), 7.76–7.96 (m, 1H, H-5-furyl).

2-Furyl-4-(2-naphthylbenzylidene)-5(4H)-oxazolones (20). MWI 100 sec.; Yield 74 %; mp 155°C; ir: 1823, 1680; ^{1}H NMR: δ 6.54–6.56 (dd, J = 5.6, 1.6 Hz, 1H, H-4-furyl), 7.25–7.30 (m, 2H, H-3-furyl, H-3-naphthyl), 7.60–7.62 (m, 2H, H-6, H-7-naphthyl), 7.63–7.64 (m, 1H, H-2-naphthyl), 7.66–7.68 (m, 2H, H-5-furyl, H-4-naphthyl), 7.70 (s, 1H, CH = C), 7.92–7.99 (m, 2H, H-5, H-8-naphthyl).

2-Furyl-4-(9-anthrylbenzylidene)-5(4H)-oxazolones (21). MWI 120 sec.; Yield 80 %; mp 108°C; ir: 1795, 1672; ^{1}H NMR: δ 7.50–7.54 (dd, J = 5.4, 0.4 Hz, 1H, H-4-furyl), 7.56–7.51 (m, 1H, H-3-furyl), 7.67–7.69 (m, 1H, H-5-furyl), 7.70–8.02 (m, 4H, H-2, H-3, H-6, H-7-anthryl), 8.08 (s, 1H, CH = C), 8.53–8.72 (m, 4H, H-1, H-4, H-5, H-8-anthryl), 8.98 (s, 1H, H-10-anthryl).

2-Furyl-4-(cyclohexylidene)-5(4H)-oxazolones (22). MWI 90 sec.; Yield 76 %; mp 147°C; ir: 1764, 1652; ^{1}H NMR: δ 1.62–1.74 (m, 6H, 3 CH_2), 2.40–2.54 (m, 4H, 2 CH_2), 6.48–6.52 (dd, J = 2.8, 1.6 Hz, 1H, H-4-furyl), 6.58–6.60 (dd, 1H, H-3-furyl, J = 1.6 Hz), 7.46–7.56 (m, 1H, H-5-furyl).

Antimicrobial activity against Xanthomonas oryzae *and* Ralstonia solanacearum

Agar disc diffusion method. The test compounds were screened for their antibacterial activity against the gram negative *Xanthomonas oryzae* and *Ralstonia solanacearum.* Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth (NB) that were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 2×10^6 colony forming units (CFUs/mL) for bacteria. *In vitro* antibacterial activity was screened by using Nutrient Agar (NA) obtained from Himedia (Delhi). The NA plates were prepared by pouring 25 mL of molten media into sterile petri plates. The plates were allowed to solidify for 3–4 h. On the surface of the media, microbial suspension was spread with the help of sterilized L-shaped loop. All the synthesized compounds (100 μg/mL) were loaded on 6 mm sterile disc. Standard anti-bacterial drug streptomycin (10 μg/mL) was also tested under similar conditions. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were then kept for incubation at 37°C for 24 h. DMSO was used as a solvent for all the compounds and as a control. After 24 h, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Table 3. Activity of oxazolones (1–22) against plant pathogenic bacteria.

	**Zone of inhibition (diameter) in mm*		
Compounds	X. oryzae	R. solanacearum	*Mean*
7	9	8	8.5
13	1.7	2.3	2
17	0	1.4	0.7
19	21	17	19
20	9	12	10.5
21	20	21	20.5
22	10	13	11.5
Streptomycin	20	13	16.5

*Compounds 1–6, 8–12, 14–16 and 18 showed no zone of inhibition.

The studies were performed in triplicate. Zone of inhibition in mm of synthesized compounds and standard drugs are shown in Table 3.

Antibacterial activity against nitrifying bacteria. Test chemicals along with the reference bactericide for nitrifying bacteria, nitrapyrin and the starting material, furoyl glycine were evaluated for their effect on nitrifying bacteria. These chemicals were tested at 1, 5 and 10 % dose of applied urea-N according to the following method.

The soil of the following properties Sand 60.5 %; Silt 18.0 %; Clay 21.5 %; Water holding capacity 35.5 %; Bulk density 1.51 mg/kg; Organic C 0.5 %; Available N 58.72 mg/kg; Ammonium-N 4.2 mg/kg; Nitrite-N traces; Nitrate-N 8.54 mg/kg; pH (Soil : water :: 1 : 2.5) 7.9; EC at 25°C 0.35 dSm^{-1} was collected for experiment from the farm of the Institute. The experiments were laid following completely randomized design (CRD) with three replicates. Fertilizer-N was applied @ 200 mg kg^{-1} urea-N in each sample. For each set of treatments, a control (treated with only 200 mg kg^{-1} urea-N without the test chemicals) was taken.

Procedure

The experiments were laid following completely randomized design (CRD). The experiment was conducted in triplicate with concomitant controls. Fifty gram air-dried soil was taken per plastic beaker (100 mL). Calculated amount of acetonic solution of test chemical (0.1, 0.5 and 1.0 mg for 1, 5 and 10 % dose of applied urea-N, respectively) was added to each beaker and mixed thoroughly. Same volume of acetone was added in all the treatments including control followed by thorough mixing. An amount measuring 10 mg urea-N (200 mg urea-N per kg of soil) in aqueous solution was added to all beakers. Balance volume of distilled water was added to each beaker to bring the moisture to one half of water holding capacity of the soil and mixed thoroughly. For nitrapyrin, the soil was prepared in a similar way.

All the beakers were accurately weighed, labeled and incubated in a BOD incubator at 28 ± 1°C, and 98 % relative humidity. Soil moisture was maintained by adding distilled water every day after taking the difference of weight if necessary.

Samples were drawn on 7th, 14th, 21st, and 28th day of incubation. Five grams of soil was withdrawn from the beaker and extracted with aqueous sodium sulfate (50 mL, 1M) by shaking on a reciprocal shaker for one hr. The soil samples were filtered and estimated for ammonium, nitrite and nitrate-N by indophenol blue, sulfanilic acid and phenol-disulfonic acid methods,[25] respectively. The contents of ammonium, nitrite and nitrate-N were obtained from the standard curves and expressed in milligrams per kilogram. The nitrification rate (NR) and percent nitrification inhibition (NI) were calculated using Sahrawat's formulas[26] for assessing the effectiveness of test chemicals on *Nitrosomonas* and *Nitrobacter* species. After sampling, incubation was continued in the same beaker. Results obtained from the *in vitro* soil incubation study are reported in Table 4.

Results and discussion

Synthesis

For the synthesis of title compounds, 2-furoyl-4-arylidene-5(4H)-oxazolones, solid as well as solution phase reactions were attempted (Table 2). The 4-fluorobenzaldehyde and furoyl glycine was used as a model system for studying the reaction under different conditions. Solid phase approaches using silica, alumina (acidic, basic and neutral), anhydrous potassium carbonate, nano-clays like hydrophilic kaolinite and hydrophilic bentonite did not result in a fruitful manner. On the other hand, the solution phase reactions tried that were based on our earlier reported microwaves assisted syntheses of 2-phenyl-4-arylidene-5(4H)-oxazolones.[27,28] These methods were also successful to yield the title compounds and the methods were further compared for their greenness and efficiency.

In view of the eco-friendliness and energy efficiency of the microwave synthesis with DCC and DMAC,[28] this method was adopted for the synthesis of twenty two 2-furyl-4-arylidene-5(4H)-oxazolones including 16 novel compounds. The method was standardized and optimized for the title compounds in terms of reaction time, amount of energy transfer solvent and power level of microwave. The reactions were completed in fewer than 2 minutes with 68–82 % yields as compared to a 28–51 % yield in conventional procedure.[29] The minimal use of solvents confirmed the greenness[30] of the present synthesis of these compounds.

The spectral analysis confirmed the formation of oxazolone derivatives. As a representative example, IR spectra of 2-furyl-4-(2′-chlorobenzylidene)-5(4H)-oxazolone showed aromatic C-H stretching at 2925 cm^{-1}. The peaks at 1689 and 1811 cm^{-1} were assigned to C = N and C = O groups in the oxazolone. Eight signals were observed in its ^{1}H NMR spectrum. Aromatic protons appeared as multiplets at δ 7.43–7.45, 7.49–7.51, 7.52–7.53 and 7.61–7.63. A double doublet at δ 7.41–7.42 and two multiplets at δ 6.88–6.89, 7.76–7.781 were due to protons for furyl ring. The proton attached to olefinic carbon appeared at δ 7.78 as a singlet.

Fourteen signals were viewed in the ^{13}C NMR spectrum of the same compound establishing the presence of 14 carbons. Aromatic carbons were observed at δ 126.7, 127.69, 128.30, 130.02, 131.09 and 131.25. Furyl carbons appeared at δ 114.14, 120.71, 132.88 and 133.05. The peak at δ 131.90 and 132.02 were assigned to carbon-4 in the oxazolone ring and olefinic carbon, respectively. The carbon attached to nitrogen appeared at δ 150.23 whereas peak for carbonyl group in the oxazolone observed at δ 167.16.

Bioassay

Antibacterial activity against Xanthomonas oryzae *and* Ralstonia solanacearum. Substituted phenyl compounds (like 3-methoxy phenyl, 2,4-dihydroxy phenyl, 3,4,5-trimethoxy phenyl) were very mildly active as antibacterial agents having mean zone of inhibition ranging from 0 to 8.5 mm against both type of bacteria (Table 3). Among the phenyl substituted compounds, 2-furyl-4-(3-methoxybenzylidene)-5(4H)-oxazolones **(7)** has shown maximum antibacterial activity against both the test fungi. The activity profile of compounds drastically enhanced against both the bacterial species on increasing the number of aromatic rings (compounds 20 and 21) in the molecule. This observation was just opposite to the behavior of these compounds for their effect on nitrification inhibition. Also, the activity increased considerably by 2 to 3 folds on replacing one carbon by nitrogen in the phenyl ring (compound 19). With the substitution of saturated, cyclohexyl ring in place of phenyl ring, better activity was observed. (Fig. 2)

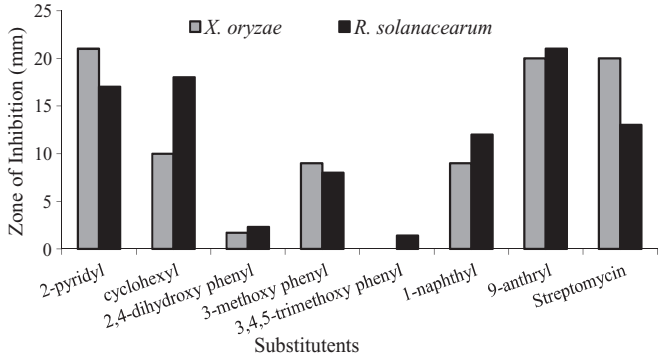


Fig. 2. Effect of various substituents on antibacterial activity against *X. oryzae* and *R solanacearum.*

Table 4. Effect of oxazolones (1–22) and furoyl glycine on rate of nitrification.

Compounds	*R- group*	*Dose (% of applied urea-N)*	*Nitrification inhibition (%)***			
			7th Day	*14th Day*	*21st Day*	*28th Day*
1	4-F-C_6H_4	1	79	36	39	23
		5	85	43	42	27
		10	93	54	49	38
2	2-Cl-C_6H_4	1	71	43	34	13
		5	80	60	37	19
		10	83	65	40	24
3	4-Cl-C_6H_4	1	56	30	18	13
		5	69	37	22	19
		10	88	50	27	23
4	3-Br-C_6H_4	1	58	55	24	22
		5	66	60	26	24
		10	87	69	30	29
5	3-NO_2-C_6H_4	1	64	49	36	24
		5	73	58	44	31
		10	78	61	51	37
6	4-OH-C_6H_4	1	54	49	33	25
		5	63	54	35	30
		10	87	63	41	33
7	3-OCH_3-C_6H_4	1	54	47	31	24
		5	59	50	33	27
		10	63	55	38	33
8	2-CH_3-C_6H_4	1	77	34	33	13
		5	85	41	39	19
		10	87	47	42	23
9	3-CH_3-C_6H_4	1	86	65	64	44
		5	91	71	64	49
		10	96	72	72	64
10	4-$CH(CH_3)_2$-C_6H_4	1	79	66	59	48
		5	86	69	64	54
		10	92	72	67	59
11	2,4-$(Cl)_2$-C_6H_3	1	81	61	55	41
		5	86	64	60	46
		10	94	71	62	55
12	2,6-$(Cl)_2$-C_6H_3	1	80	68	42	34
		5	84	72	42	35
		10	87	78	44	42
13	2,4-$(OH)_2$-C_6H_3	1	75	65	59	51
		5	82	69	64	58
		10	89	72	67	62
14	2,4-$(OCH_3)_2$-C_6H_3	1	73	52	36	29
		5	82	65	40	34
		10	89	75	41	35
15	3-(OCH_3)-4-(OH)-C_6H_3	1	79	66	66	62
		5	87	73	70	68
		10	92	81	75	70
16	3-(OC_2H_5)-4-(OH)-C_6H_3	1	74	56	50	32
		5	82	66	54	37
		10	88	72	61	45
17	3,4,5-$(OCH_3)_3$-C_6H_2	1	81	60	49	33
		5	87	66	53	38
		10	89	62	61	43
18	4-$(N(CH_3)_2)$-C_6H_4	1	78	35	39	19
		5	81	43	42	24
		10	88	53	49	37

(Continued on next page)

Table 4. Effect of oxazolones (1–22) and furoyl glycine on rate of nitrification. *(Continued)*

Compounds	*R- group*	*Dose (% of applied urea-N)*	*Nitrification inhibition (%)***			
			7th Day	*14th Day*	*21st Day*	*28th Day*
19	2-pyridyl	1	78	58	44	31
		5	81	61	51	38
		10	89	67	56	43
20	1-naphthyl	1	53	31	23	15
		5	66	42	28	20
		10	92	53	33	25
21	9-anthryl	1	79	61	50	34
		5	86	66	53	38
		10	92	63	59	43
22	2-furyl-4-(cyclohexylidene)-5(4H)-oxazolones	1	81	66	61	42
		5	87	71	61	47
		10	92	69	69	59
23	Furoyl glycine	1	22	20	18	11
		5	37	23	22	11
		10	49	40	33	15
24	Nitrapyrin	1	89	85	80	74
		5	94	90	88	85
		10	83	78	72	71
LSD (5 %)		–	4.6	3.9	3.7	2.4

** Figures are rounded to the nearest whole number.

Antibacterial activity against nitrifying bacteria. The synthesized oxazolones exhibited considerably higher ammonium-N content (Table 4) as compared to urea alone during the entire incubation period. The ammonium-N content for all the test chemicals were 146–198, 101–186, 56–171 and 21–142 mg/kg on 7th, 14th, 21st and 28th days, respectively showing the bactericidal effect against *Nitrosomonas* species. The ammonium-N content were 82, 75, 26 and 4 mg/kg on respective 7th, 14th, 21st and 28th days of incubation in urea alone. The reference, nitrapyrin, showed 174–195, 161–190, 153–182 and 147–176 mg/kg ammonium-N content on respective 7th, 14th, 21st and 28th days. The respective ammonium-N content for furoyl glycine, starting material, was in the range of 19–142 mg/kg on all sampling days. The compound 2-Furyl-4-(3-methoxy-4-hydroxybenzylidene)-5(4H)-oxazolone was best among the test compounds in inhibiting the growth of *Nitrosomonas* species throughout the incubation period.

The nitrite-N content remained insignificant in all the samples on all the sampling days. This observation established the target specific action of these compounds against *Nitrosomonas sp*. Significantly lower nitrate-N content was observed in all the test chemicals, 4–165 mg/kg as compared to urea, 103–167 mg/kg. All the test chemicals performed significantly superior to urea alone. The corresponding data for nitrapyrin and furoyl glycine were 4–58 and 58–157 mg/kg nitrate-N, respectively.

These synthesized oxazolones were effective inhibitors of nitrifying bacterial growth showing 53–96, 30–81, 18–75 and 13–70 % nitrification inhibition (NI) at 7th, 14th, 21st and 28th days, respectively. Nitrapyrin, the reference inhibitor, at 1–10 % doses showed 83–94, 78–90, 72–88 and 71–85 % nitrification inhibition on 7th, 14th, 21st and 28th days, respectively. The NI for furoyl glycine were 22–49, 20–40, 18–33 and 11–15 % at 1–10 % doses on 7th, 14th, 21st and 28th days, respectively. All the compounds showed an increase in NI with the increase in dose.

The best overall performance was observed with compound 15 with 62–92 % NI followed by compound 9 with 44–96 % NI during the entire incubation period at all doses. Other potent molecules were compound 22, 11, 10 and 13. Among the series, 2-furyl-4-(3-methoxy-4-hydroxy benzylidene)-5(4H)-oxazolone and 2-furyl-4-(3-methylbenzylidene)-5(4H)-oxazolone were emerged as the promising ones.

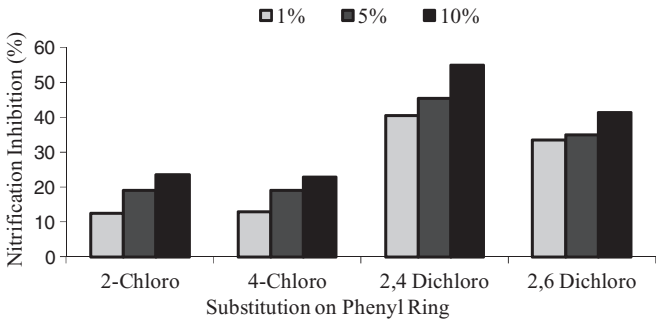


Fig. 3. Influence of chlorine atoms of 4-benzylidene substitution on nitrification inhibition.

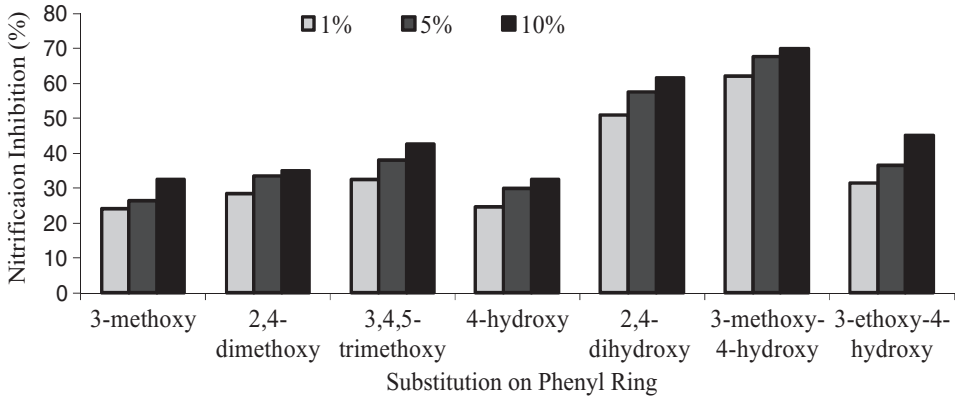


Fig. 4. Effect of number and position of hydroxy and alkoxy groups on nitrification inhibition.

Structure activity relationship studies

An increase in the number of chlorine atoms in the phenyl ring in the 5(4H)-oxazolone resulted in the drastic improvement in the activity. About 2 to 3-folds increase in the nitrification inhibitory activity was observed with dichloro phenyl substituted oxazolones (34–55 % NI on 28th day) as compared to monochloro phenyl substituted oxazolones (13–24 % NI on 28th day). (Fig. 3)

Study of effect of number, position of hydroxy and alkoxy groups in the phenyl ring revealed that introduction of hydroxy group at 2 and 4-position respectively in the 4-hydroxy derivative and 3-methoxy derivative of oxazolone demonstrated the enormous increase, about two-fold, in the nitrification inhibitory activity. (Fig. 4)

The saturated ring type i.e. cyclohexyl substituted oxazolones was more active with 42–59 % NI at all doses on the 28th day of incubation indicating the better performance of saturated ring type as compared to unsaturated rings. (Fig. 5)

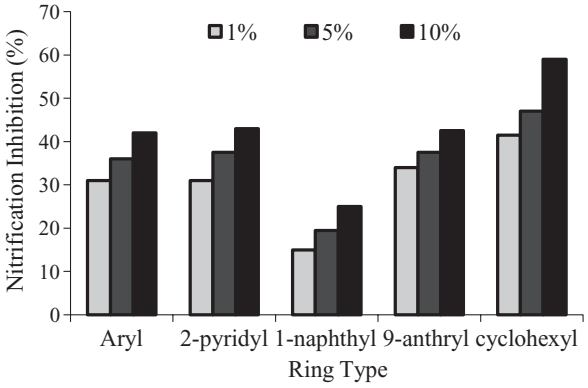


Fig. 5. Effect of ring type at 4-position of Oxazolones on nitrification inhibition.

Conclusion

The synthesized compounds were moderately effective against *Xanthomonas oryzae* and *Ralstonia solanacearum*. The compound 2-Furyl-4-(9-anthrylbenzylidene)-5(4H)-oxazolones (21) having the bulkiest substitution has shown the highest antibacterial activity. On the other hand, these oxazolones were effective inhibitors of nitrifying bacteria. The presence of insignificant nitrite-N in all test chemicals suggested their target specific and unique action against *Nitrosomonas* species exhibiting the safety of these molecules in the environment. The best performers, 2-furyl-4-(3-methoxy-4-hydroxy benzylidene)-5(4H)-oxazolone with NI 70–92 % and 2-furyl-4-(3-methyl benzylidene)-5(4H)-oxazolone with NI 64–96 % at 10 % dose during 7–28 days emerged as potent nitrification inhibitors. These potent 2-furyl-4-arylidene-5(4H)-oxazolones hold promise to be further exploited as nitrification inhibitors and antibacterial agents.

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