Synthesis and Antibacterial Activity Evaluation of Novel (*E*)-4-(4-((arylidene)amino)phenoxy)coumarin Derivatives

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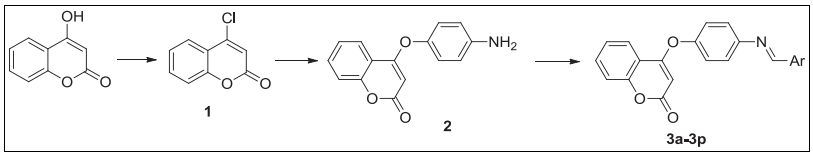


A series of novel (***E***)-4-(4-((arylidene)amino)phenoxy)coumarin derivatives were synthesized from 4-hydroxycoumarin in three step reactions, and their antibacterial activities against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas citri* subsp. *Citri* (*Xcc*) *in vitro* were evaluated. Result found that most of the target compounds exhibited pronounced antibacterial activities. Among the target compounds, **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** exhibited excellent antibacterial activities against *Xoo*, with EC_{50} values of 143.9, 127.4, 133.8, 145.8, 138.4, 116.9, 134.6, and 121.8 μg/mL, respectively, which were better than that of thiadiazole copper (203.6 μg/mL). Moreover, compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** showed good antibacterial activities against *Xcc*, with EC_{50} values of 118.4, 126.3, 117.2, 105.3, 102.3, 95.2, 96.0, and 88.2 μg/mL, respectively, which were similar to that of thiadiazole copper (138.3 μg/mL).

INTRODUCTION

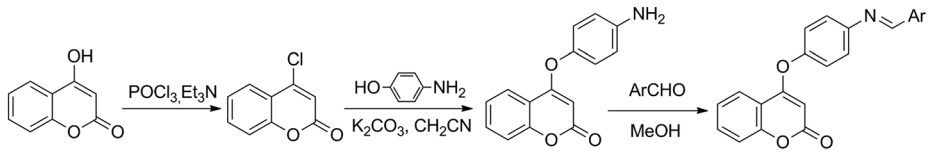
The bacterial genus *Xanthomonas* comprises a number of gram-negative plant pathogenic bacteria that cause a variety of severe plant diseases [1]; *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas citri* subsp. *Citri* (*Xcc*) are two kinds of important *Xanthomonas* species, which lead to defoliation, dieback and fruit drop, reducing yields, and causing billions of dollars of economic losses worldwide [2]. The pathogen can also be spread by people directly and potentially on various surfaces including plant material, clothing, and various equipment or implements [3]. To date, some of the traditional bactericides (thiadiazole copper, kocide, and streptomycin) and control methods are insufficient to manage the disease [4,5]. Therefore, development of novel antibacterial agents remains a daunting task in pesticide science.

Coumarin, the most important classes of benzopyrones, belongs to natural as well as synthetic origin that exhibits diverse biological activities, including anticoagulants [6], anticancer [7–11], antioxidant [12], anti-HIV [13], antimalarial [14,15], anti-depressant-like [16], and antimicrobial [17] activities. Previous research on coumarin-6-sulfonamides with a free C4-azidomethyl group as antimicrobials was reported [18]. During recent years, some of researches demonstrated that novel iodinated-4-aryloxymethylcoumarins derivatives exhibited potent anticancer and antimycobacterial activities [19], and coumarin containing isoxazoles, pyrimidinthiones, and pyrimidin-2-ones exhibited pronounced antimycobacterial and antimicrobial activities [20]. Moreover, imines derivatives have gained attention because of showing extensive biological activities [21–27]. Some of the imine derivatives containing the 4(3*H*)-quinazolinone moiety can effectively control tobacco bacterial wilt, tomato bacterial wilt, and *Xoo* [28]. Our research group is interested in imine derivatives and synthesized a serial of novel Schiff base derivatives containing quinazolin-4(3*H*)-one moiety with antibacterial properties against tobacco and tomato bacterial wilt (Chen, M.-H.; Wang, X.-B.; Tang, B.-C.; Zhang, X. Synthesis and antibacterial evaluation of novel Schiff base derivatives containing 4(3H)-quinazolinone moiety. Submitted.). For further study of antibacterial activity of novel imine derivatives we synthesized a series of *(E)*-4-(4-((arylidene)amino)phenoxy)coumarin derivatives and evaluated their antibacterial activities against *Xoo* and *Xcc in vitro* and the structure-activity relationship (SAR) analyses of antibacterial activity were also discussed.

RESULTS AND DISCUSSION

Chemistry. The synthetic route of novel (*E*)-4-(4-((arylidene)amino)phenoxy)coumarin derivatives was shown in Scheme 1. The title compounds were synthesized from 4-hydroxycoumarin in three steps including chlorination, etherification, and condensation reactions. The structures of

Scheme 1. Synthetic route of the title compounds **3a–3p**.



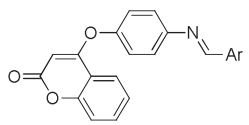
3a: R = Ph; **3b**: R = 4-MePh; **3c**: R = 4-OMePh; **3d**: R = 2-OMePh;
3e: R = 3,4-diOMePh; **3f**: R = 2-FPh; **3g**: R = 4-FPh; **3h**: R = 4-ClPh;
3i: R = 2-ClPh; **3j**: R = 2-F-6-ClPh; **3k**: R = 4-BrPh; **3l**: R = 3-BrPh;
3m: R = 2-BrPh; **3n**: R = 2-NO_2Ph; **3o**: R = 3-NO_2Ph; **3p**: R = 4-NO_2Ph.

the synthesized compounds were confirmed on the basis of IR, NMR spectra, and elemental analysis. The IR spectral data of compounds **3a–3p** showed characteristic absorption bands at 1723–1709 cm^{-1}, which were assigned to C=O of coumarin. The characteristic −CH=N− stretching bands were observed at about 1630−1621 cm^{-1}. In the ^1H NMR spectra, depending on the structure, the characteristic H_3 of coumarin cycle were observed as a signal at about 5.30−5.25 ppm. A singlet ranging from 8.95−8.58 ppm belonged to –N=CH− proton. The chemical shifts at 162.59−161.63 and 161.70−154.04 ppm confirmed the existence of C=O, and −N=CH− groups in ^{13}C NMR, respectively.

Antibacterial activity. The synthesized compounds (**3a–3p**) were evaluated for antibacterial activity against *Xoo* and *Xcc in vitro*. Biology results were listed in Table 2 and indicated that most of the synthesized compounds exhibited appreciable antibacterial activities against *Xoo* and *Xcc*. Among the title compounds, **3f**, **3 g**, **3 h**, **3i**, **3j**, **3n**, **3o**, and **3p** showed excellent antibacterial activities against *Xoo* at 200 μg/mL, with the inhibition rates of 83.5%, 86.2%, 81.8%, 80.3%, 87.8%, 97.3%, 94.2% and 96.5%, respectively, which were better than that of the commercial bactericide thiadiazole copper (68.7%). The antibacterial activities of compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3o**, and **3p** against *Xoo* at 100 μg/mL are 40.5%, 43.6%, 44.1%, 42.5%, 46.6%, 43.3%, 43.9%, 43.1%, 54.2%, 48.7%, and 50.4%, respectively, which were better than that of thiadiazole copper (33.5%). Additionally, compounds **3f**, **3g**, **3j**, **3n**, **3o**, and **3p** demonstrated good antibacterial activities against *Xcc*, with the inhibition rates of 80.7%, 85.4%, 87.4%, 96.1%, 95.3%, and 93.6%, respectively, which were superior to that of thiadiazole copper (76.2%) at 200 μg/mL.

Table 1

Antibacterial activities of the target compounds **3a–3p** against *Xoo* and *Xcc in vitro*.



		Xoo		*Xcc*	
Compd.	Ar	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL
3a	Ph	56.8 ± 1.7	35.3 ± 2.4	60.1 ± 2.1	32.8 ± 1.5
3b	4-MePh	48.2 ± 2.6	27.1 ± 2.6	43.4 ± 2.5	24.5 ± 2.4
3c	4-OMePh	46.7 ± 1.9	25.0 ± 1.0	40.3 ± 1.5	27.3 ± 3.3
3d	2-OMePh	47.2 ± 1.7	25.5 ± 2.8	41.8 ± 2.3	29.2 ± 2.7
3e	3,4-diOMePh	39.1 ± 2.8	26.1 ± 1.2	40.4 ± 2.0	20.4 ± 2.5
3f	2-FPh	83.5 ± 2.4	40.5 ± 2.5	80.7 ± 2.6	52.8 ± 2.4
3 g	4-FPh	86.2 ± 2.5	43.6 ± 1.2	85.4 ± 2.3	57.1 ± 2.5
3 h	4-ClPh	81.8 ± 1.2	44.1 ± 1.2	72.7 ± 2.3	55.6 ± 2.5
3i	2-ClPh	80.3 ± 3.2	42.5 ± 2.7	74.8 ± 2.7	53.0 ± 2.6
3j	2-F-6-ClPh	87.8 ± 1.4	46.6 ± 1.8	87.4 ± 1.6	57.9 ± 1.7
3 k	4-BrPh	78.2 ± 2.5	43.3 ± 1.6	78.7 ± 2.7	46.1 ± 3.5
3 l	3-BrPh	75.2 ± 1.5	43.9 ± 2.5	73.8 ± 2.1	42.1 ± 3.4
3 m	2-BrPh	77.0 ± 3.2	43.1 ± 2.7	70.3 ± 1.8	43.2 ± 4.1
3n	4-NO_2Ph	97.3 ± 2.7	54.2 ± 3.5	96.1 ± 1.5	58.1 ± 2.2
3o	3-NO_2Ph	94.2 ± 2.0	48.7 ± 2.0	95.3 ± 1.4	62.6 ± 3.2
3p	2-NO_2Ph	96.5 ± 1.1	50.4 ± 1.8	93.6 ± 2.5	58.3 ± 2.6
Thiadiazole copper		68.7 ± 2.1	33.5 ± 1.7	76.2 ± 1.3	51.2 ± 1.2

Table 2

EC_{50} values of the target compounds **3a–3p** against *Xoo* and *Xcc*.

Compd.	EC_{50} (μg/mL)	
	Xoo	*Xcc*
3a	273.2 ± 1.3	235.1 ± 2.7
3b	260.5 ± 2.0	274.1 ± 1.1
3c	290.9 ± 1.6	303.4 ± 1.6
3d	287.5 ± 2.0	294.3 ± 2.3
3e	308.3 ± 1.7	336.1 ± 3.5
3f	143.9 ± 2.6	118.4 ± 2.0
3 g	127.4 ± 2.0	126.3 ± 1.4
3 h	133.8 ± 1.6	117.2 ± 2.6
3i	145.8 ± 2.1	105.3 ± 1.7
3j	138.4 ± 2.7	102.3 ± 2.1
3 k	180.4 ± 3.2	145.6 ± 1.3
3 l	186.8 ± 1.9	153.1 ± 2.5
3 m	172.1 ± 2.5	149.0 ± 1.8
3n	116.9 ± 1.7	95.2 ± 1.2
3o	134.6 ± 2.5	96.0 ± 2.2
3p	121.8 ± 1.6	88.2 ± 3.0
Thiadiazole copper	203.6 ± 3.0	138.3 ± 1.3

Compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** showed good antibacterial activities against *Xcc* (52.8%, 57.1%, 55.6%, 53.0%, 57.9%, 58.1%, 62.6%, and 58.3%, respectively) at 100 μg/mL compared with thiadiazole copper (51.2%).

Based on the preliminary bioassays, the EC_{50} values of the test compounds as well as thiadiazole copper were summarized in Table 2. Notably, compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** exhibited excellent antibacterial activities against *Xoo*, with EC_{50} values of 143.9, 127.4, 133.8, 145.8, 138.4, 116.9, 134.6, and 121.8 μg/mL, respectively, which were better than that of thiadiazole-copper (203.6 μg/mL). Meanwhile, compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** showed good antibacterial activities against *Xcc in vitro*, with EC_{50} values of 118.4, 126.3, 117.2, 105.3, 102.3, 95.2, 96.0, and 88.2 μg/mL, respectively, which were similar to that of thiadiazole copper (138.3 μg/mL). Moreover, compounds **3a**, **3b**, **3c**, **3d**, and **3e** showed poor antibacterial activities against *Xoo* and *Xcc*.

As an extension of this approach, the SAR were deduced on the basis of the activity values in Tables 1 and 2. It was found that the type of Ar substituted groups has significant effect on antibacterial activities of the target compounds. When Ar is 2-FC_6H_4, 4-FC_6H_4, 2-ClC_6H_4, 4-ClC_6H_4, 2-BrC_6H_4, 4-BrC_6H_4, 3-BrC_6H_4, 2-F-6-ClC_6H_3, 2-$NO_2C_6H_4$, 3-$NO_2C_6H_4$, and 4-$NO_2C_6H_4$, the corresponding compounds exhibited excellent antibacterial activities against *Xoo*. On the other hand, when Ar is 4-MeC_6H_4, 4-$OMeC_6H_4$, and 2-$OMeC_6H_4$, the corresponding title compounds exhibited poor antibacterial activities against *Xoo*. Meanwhile, it was interesting to note that when Ar is 2-FC_6H_4, 4-FC_6H_4, 2-ClC_6H_4, 4-ClC_6H_4, 2-F-6-ClC_6H_3, 2-$NO_2C_6H_4$, 3-$NO_2C_6H_4$, and 4-$NO_2C_6H_4$, the corresponding compounds exhibited shown excellent antibacterial activities against *Xcc*. Compounds with 4-MeC_6H_4, 4-$OMeC_6H_4$, and 2-$OMeC_6H_4$ also showed unsatisfactory antibacterial activities against *Xcc*. Therefore, SAR results indicated that compounds with electron-withdrawing groups (Cl, F, and NO_2) exhibited excellent antibacterial activities against *Xoo* and *Xcc*, and compounds with electron-donating groups (Me and OMe) showed poor antibacterial activities against *Xoo* and *Xcc*.

CONCLUSIONS

In summary, a series of novel (*E*)-4-(4-((arylidene) amino)phenoxy)coumarin derivatives have been synthesized and evaluated for antibacterial activities against *Xoo* and *Xcc in vitro*. Result found that the target compounds exhibited pronounced antibacterial activities. Among the target compounds, **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** exhibited excellent antibacterial activities against *Xoo*, with EC_{50} values of 143.9, 127.4, 133.8, 145.8, 138.4, 116.9, 134.6, and 121.8 μg/mL, respectively, which were better than that of thiadiazole copper (203.6 μg/mL). Compounds **3f**, **3g**, **3h**, **3i**, **3j**, **3n**, **3o**, and **3p** showed good antibacterial activities against *Xcc*, with EC_{50} values of 118.4, 126.3, 117.2, 105.3, 102.3, 95.2, 96.0, and 88.2 μg/mL, respectively, which were similar to that of thiadiazole copper (138.3 μg/mL). SAR results indicated that compounds with electron-withdrawing groups (Cl, F, and NO_2) exhibited excellent antibacterial activities against *Xoo* and *Xcc*, and compounds with electron-donating groups (Me and OMe) showed poor antibacterial activities against *Xoo* and *Xcc*. To the best of our knowledge, it is the first report on the synthesis and antibacterial activities of novel (*E*)-4-(4-((arylidene)amino)phenoxy)coumarin derivatives.

EXPERIMENTAL

General procedures. The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China). ^1H NMR and ^{13}C NMR (solvent DMSO-d_6) spectral analyses were performed on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. The IR spectra were recorded on a Bruker Vector 22 spectrometer in a KBr disk (Bruker Corporation, Switzerland). Elemental analysis was performed on an Elementar Vario-III CHN analyzer. All solvents were dried and redistilled before used.

General procedure for synthesis of 4-chlorocoumarin (1). This compound was synthesized by following literature known methods [29].

General procedure for synthesis of 4-(4-aminophenoxy) coumarin (2). A mixture of compound **1** (1.0 mmol), 4-aminophenol (1.0 mmol), and K_2CO_3 (1.2 mmol) was dissolved in CH_3CN (30 mL) and refluxed. After refluxing for 4 h, the reaction solution was poured into ice water to produce solid precipitates. The resulting precipitates were then filtered and washed with water. The purified compound **2** were recrystallized from ethanol.

4-(4-Aminophenoxy)coumarin (2). Brown solid; mp 163–165°C; yield 84.8%; ^{1}H NMR (DMSO-d_6, 500 MHz, i>:/i>: 8.17 (d, 1H, J = 7.0 Hz, Coumarin-5-H), 7.60 (t, 1H, J = 5.0 Hz, coumarin-7-H), 7.34–7.30 (m, 2H, Coumarin-6,8-H); 6.95 (d, 2H, J = 8.5 Hz, Ar-H), 6.62 (d, 2H, J = 8.5 Hz, Ar-H), 5.22 (s, 2H, NH_2), 4.95 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.37, 161.83, 153.64, 151.52, 133.86, 131.13, 125.04, 123.49, 117.14, 112.72, 107.86, 106.08, 93.06; MS (ESI) *m/z*: 254.2 ([M + H]$^+$).

General procedure for synthesis of title compounds (3a–3p). Aromatic aldehyde (1.2 mmol) was added to a solution of 4-(4-aminophenoxy)-2H-chromen-2-one (**2**) (1.0 mmol) in anhydrous ethanol (15 mL). The resulting mixture was refluxed for 1–2 h. Upon completion of reaction, the solvent was removed under depressurization, and the residue was recrystallized from ethanol. The product was then filtered, washed, and dried to obtain (*E*)-4-(4-((arylidene)amino)phenoxy)-2H-chromen-2-one derivatives. The physical characteristics, IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis data for all the target compounds were shown below.

(E)-4-(4-((benzylidene)amino)phenoxy)coumarin (3a). White solid; mp 192–194°C; yield, 70.1%; IR (KBr): 1713, 1630, 1608, 1186 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.70 (s, 1H, CH = N), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.98(d, 2H, J = 7.5 Hz, Ar-H), 7.78 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.56–7.42 (m, 9H, coumarin-6,8-H, Ar-H), 5.26 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.51, 162.19, 161.70, 153.64, 150.60, 150.43, 136.39, 133.97, 132.23, 129.41, 129.33, 125.08, 123.60 123.54, 122.66, 117.16, 115.34; MS (ESI) *m/z*: 342.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{15}NO_3$: C, 77.41; H, 4.43; N, 4.10. Found: C, 77.16; H, 4.48; N, 4.31.

(E)-4-(4-((4-methylbenzylidene)amino)phenoxy)coumarin (3b). White solid; mp 197–199°C; yield, 78.0%; IR (KBr): 1722, 1623, 1606, 1186 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.65 (s, 1H, CH = N), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.86 (d, 2H, J = 7.5 Hz, Ar-H), 7.77 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.51–7.34 (m, 8H, coumarin-6,8-H, Ar-H), 5.25 (s, 1H, coumarin-3-H), 2.39 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.52, 161.93, 161.70, 153.63, 150.56, 150.45, 142.29, 133.97, 133.88, 130.01, 129.35, 125.07, 123.59, 123.49, 122.62, 117.16, 115.34, 93.39, 21.74; MS (ESI) *m/z*: 356.2 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 77.73; H, 4.82; N, 3.94. Found: C, 77.91; H, 4.47; N, 4.03.

(E)-4-(4-((4-methoxybenzylidene)amino)phenoxy)coumarin (3c). Brown solid; mp 203–205°C; yield, 69.6%; IR (KBr): 1717, 1623, 1602, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.61 (s, 1H, CH = N), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.92 (d, 2H, J = 7.5 Hz, Ar-H), 7.78 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.51–7.40 (m, 6H, coumarin-6,8-H, Ar-H), 7.10 (d, 2H, J = 8.5 Hz, Ar-H), 5.25 (s, 1H, coumarin-3-H), 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.55, 162.59, 161.71, 161.33, 153.63, 150.77, 150.24, 133.96, 131.15, 129.31, 125.07, 123.58, 123.41, 122.58, 117.16, 115.34, 114.84, 93.36, 55.91; MS (ESI) *m/z*: 372.2 ([M + H]$^+$); *Anal*. Calcd. for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.55; H, 4.43; N, 3.62.

(E)-4-(4-((2-methoxybenzylidene)amino)phenoxy)coumarin (3d). Brown solid; mp 207–210 °C; yield, 78.6%; IR (KBr): 1723, 1625, 1608, 1173 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.90 (s, 1H, CH = N), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 8.04 (d, 1H, J = 7.5 Hz, Ar-H), 7.78 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.57–7.38 (m, 7H, coumarin-6,8-H, Ar-H), 7.19-7.02 (m, 2H, Ar-H), 5.27 (s, 1H, coumarin-3-H), 3.91 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.50, 161.71, 159.92, 156.97, 153.64, 151.10, 150.46, 133.97, 127.42, 125.08, 124.21, 123.60, 122.66, 121.24, 117.16, 15.35, 112.62, 93.43, 56.33; MS (ESI) *m/z*: 372.2 ([M + H]$^+$); *Anal*. Calcd. for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.46; H, 4.39; N, 3.62.

(E)-4-(4-((3,4-dimethoxybenzylidene)amino)phenoxy) coumarin (3e). Brown solid; mp 214–216°C; yield, 73.1%; IR (KBr): 1709, 1624, 1606, 1185 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.58 (s, 1H, CH = N), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.78 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.57–7.38 (m, 7H, coumarin-6,8-H, Ar-H), 7.12 (d, 1H, J = 8.0 Hz, Ar-H), 5.27 (s, 1H, coumarin-3-H), 3.85 (s, 2H, 2OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.53, 161.71, 161.58, 153.63, 152.51, 150.75, 150.23, 149.54, 133.95, 129.40, 125.06, 124.87, 123.57, 122.59, 117.15, 115.34, 111.80, 109.81, 93.35, 56.18, 55.97; MS (ESI) *m/z*: 402.2 ([M + H]$^+$); *Anal*. Calcd. for $C_{24}H_{19}NO_5$: C, 71.81; H, 4.77; N, 3.49. Found: C, 71.66; H, 4.88; N, 3.37.

(E)-4-(4-((2-fluorobenzylidene)amino)phenoxy)coumarin (3f). Gray solid; mp 210–213°C; yield, 80.2%; IR (KBr): 1723, 1622, 1606, 1172 cm^{-1}; ^{1}H NMR

(DMSO-d_6, 500 MHz, ppm) δ: 8.84 (s, 1H, CH = N), 8.13 (t, 1H, J = 6.5 Hz, Ar-H), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.77 (t, 1H, J = 7.0 Hz, coumarin-7-H), 7.64-7.61 (m, 1H, Ar-H), 7.51–7.37 (m, 8H, coumarin-6,8-H, Ar-H), 5.28 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.44, 161.70, 154.84, 153.64, 150.91, 150.24, 134.36, 133.97, 128.44, 125.50, 125.08, 123.67, 123.70, 122.70, 117.16, 116.87, 116.71, 115.34, 93.49; MS (ESI) *m/z*: 360.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 73.53; H, 3.93; N, 3.90. Found: C, 73.68; H, 3.84; N, 3.73.

(E)-4-(4-((4-fluorobenzylidene)amino)phenoxy)coumarin (3g). Gray solid; mp 209–211°C; yield, 64.1%; IR (KBr): 1718, 1623, 1608, 1186 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.63 (s, 1H, CH = N), 8.08 (d, 1H, J = 7.0 Hz, coumarin-5-H), 7.97 (d, 2H, J = 8.5 Hz, Ar-H), 7.78 (t, 1H, J = 8.0 Hz, coumarin-7-H), 7.65 (d, 2H, J = 8.5 Hz, Ar-H), 7.54–7.39 (m, 6H, coumarin-6,8-H, Ar-H), 5.27 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.41, 161.63, 160.91, 153.60, 151.76, 150.22, 136.43, 135.29, 133.97,130.92, 128.56, 125.15, 123.62, 123.44, 122.68, 117.19, 115.45; MS (ESI) *m/z*: 360.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 73.53; H, 3.93; N, 3.90. Found: C, 73.59; H, 3.79; N, 3.88.

(E)-4-(4-((4-chlorobenzylidene)amino)phenoxy)coumarin (3h). Gray solid; mp 214–216°C; yield, 81.3%; IR (KBr): 1717, 1621, 1608, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.72 (s, 1H, CH = N), 8.07 (d, 1H, J = 7.0 Hz, coumarin-5-H), 7.99 (d, 2H, J = 8.5 Hz, Ar-H), 7.78 (t, 1H, J = 8.0 Hz, coumarin-7-H), 7.62 (d, 2H, J = 8.5 Hz, Ar-H), 7.51–7.37 (m, 6H, coumarin-6,8-H, Ar-H), 5.26 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.47, 161.69, 160.94, 153.63, 150.76, 150.07, 136.77, 135.25, 133.97,130.92, 129.56, 125.07, 123.62, 123.57 122.68, 117.15, 115.32; MS (ESI) *m/z*: 376.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 70.31; H, 3.75; N, 3.73. Found: C, 70.15; H, 3.83; N, 3.90.

(E)-4-(4-((2-chlorobenzylidene)amino)phenoxy)coumarin (3i). Gray solid; mp 208–210°C; yield, 68.7%; IR (KBr): 1715, 1621, 1606, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.93 (s, 1H, CH = N), 8.20 (d, 2H, J = 7.5 Hz, Ar-H), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.78 (t, 1H, J = 8.0 Hz, coumarin-7-H), 7.62–7.43 (m, 9H, coumarin-6,8-H, Ar-H), 5.30 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.40, 161.68, 157.77, 153.64, 151.03, 150.08, 135.71, 133.96, 133.70, 133.02, 130.67, 128.99, 128.25, 125.07, 123.69, 123.59, 122.77, 117.15, 115.33, 93.53; MS (ESI) *m/z*: 376.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 70.31; H, 3.75; N, 3.73. Found: C, 70.15; H, 3.83; N, 3.90.

(E)-4-(4-((2-chloro-6-fluorobenzylidene)amino)phenoxy) coumarin (3j). Brown solid; mp 219–221°C; yield, 70.1%; IR (KBr): 1714, 1623, 1608, 1176 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.80 (s, 1H, CH = N), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.77 (t, 1H, J = 8.0 Hz, coumarin-7-H), 7.61–7.58 (m, 1H, Ar-H), 7.51–7.39 (m, 8H, coumarin-6,8-H, Ar-H), 5.30 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.38, 161.68, 155.57, 151.19, 150.23, 135.36, 133.97, 133.68, 133.61, 126.83, 125.08, 123.60, 123.49, 122.82, 117.15, 116.43, 116.26, 115.33, 93.56; MS (ESI) *m/z*: 394.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 67.10; H, 3.33; N, 3.56. Found: C, 67.19; H, 3.67; N, 3.70.

(E)-4-(4-((4-bromobenzylidene)amino)phenoxy)coumarin (3k). Gray solid; mp 229–231°C; yield, 72.8%; IR (KBr): 1716, 1622, 1608, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.71 (s, 1H, CH = N), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.91 (d, 1H, J = 8.0 Hz, Ar-H), 7.78–7.75 (m, 3H, coumarin-7-H, Ar-H), 7.51–7.42 (m, 6H, coumarin-6,8-H, Ar-H), 5.25 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.47, 161.69, 161.10, 153.64, 150.78, 150.06, 135.57, 133.98, 132.49, 131.11, 125.79, 125.08, 123.63, 122.69, 117.16, 115.33, 93.45; MS (ESI) *m/z*: 420.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 62.87; H, 3.36; N, 3.33. Found: C, 62.69; H, 3.48; N, 3.47.

(E)-4-(4-((2-bromobenzylidene)amino)phenoxy)coumarin (3l). Gray solid; mp 235–237°C; yield, 70.9%; IR (KBr): 1722, 1624, 1608, 1175 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.85 (s, 1H, CH = N), 8.17 (d, 1H, J = 7.5 Hz, Ar-H), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.79–7.75 (m, 2H, coumarin-7-H, Ar-H), 7.57–7.46 (m, 8H, coumarin-6,8-H, Ar-H), 5.30 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.39, 161.68, 160.09, 153.64, 151.04, 149.99, 134.36, 133.91, 129.42, 128.7, 126.01, 125.07, 123.66, 123.59, 122.81, 117.15, 115.33, 93.55; MS (ESI) *m/z*: 420.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 62.87; H, 3.36; N, 3.33. Found: C, 62.72; H, 3.56; N, 3.41.

(E)-4-(4-((3-bromobenzylidene)amino)phenoxy)coumarin (3m). Gray solid; mp 231–233°C; yield, 65.1%; IR (KBr): 1717, 1622, 1608, 1184 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.70 (s, 1H, CH = N), 8.13 (s, 1H, Ar-H), 8.07 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.96(d, 1H, J = 8.0 Hz, Ar-H), 7.78–7.75 (m, 2H, coumarin-7-H, Ar-H), 7.50–7.45 (m, 7H, coumarin-6,8-H, Ar-H), 5.26 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ: 166.44, 161.69, 160.71, 153.64, 150.91, 149.85, 138.68, 134.72, 133.98, 131.64, 131.52, 128.29, 125.09, 123.70, 123.59, 122.71, 117.16,

115.33; MS (ESI) *m/z*: 420.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 62.87; H, 3.36; N, 3.33. Found: C, 62.72; H, 3.43; N, 3.54.

(E)-4-(4-((4-nitrobenzylidene)amino)phenoxy)coumarin (3n). Yellow solid; mp >250°C; yield, 79.1%; IR (KBr): 1713, 1623, 1608, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ 8.95 (s, 1H, CH = N), 8.21 (d, 1H, J = 7.0 Hz, Ar-H), 8.15 (d, 1H, J = 8.5 Hz, Ar-H), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.92 (t, 1H, J = 8.5 Hz, Ar-H), 7.82–7.75 (M, 2H, Ar-H), 7.51–7.46 (m, 6H, coumarin-6,8-H, Ar-H), 5.30 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ 166.34, 161.66, 158.23, 153.63, 151.21, 149.85, 149.62, 134.36, 133.97, 132.60, 130.43, 130.11, 125.11, 125.07, 123.77, 123.58, 122.83, 117.15, 115.31; MS (ESI) *m/z*: 387.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 68.39; H, 3.56; N,7.25. Found: C, 68.14; H, 3.42; N, 7.13.

(E)-4-(4-((3-nitrobenzylidene)amino)phenoxy)coumarin (3o). Yellow solid; mp >250°C; yield, 71.5%; IR (KBr): 1718, 1625, 1608, 1185 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ 8.90 (s, 1H, CH = N), 8.76 (s, 1H, Ar-H), 8.40 (d, 2H, J = 9.0 Hz, Ar-H), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.86 (t, 1H, J = 8.5 Hz, Ar-H), 7.79 (t, 1H, J = 6.5 Hz, Ar-H), 7.54–7.46 (m, 6H, coumarin-6,8-H, Ar-H), 5.27 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ 166.41, 161.68, 160.28, 153.63, 151.14, 149.50, 148.74, 137.94 135.23, 133.98, 131.13, 126.39, 125.09, 123.84, 123.58, 123.39, 122.77, 117.16, 115.32, 93.52; MS (ESI) *m/z*: 387.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 68.39; H, 3.56; N,7.25. Found: C, 68.11; H, 3.82; N, 7.46.

(E)-4-(4-((4-nitrobenzylidene)amino)phenoxy)coumarin (3p). Yellow solid; mp >250°C; yield, 68.2%; IR (KBr): 1717, 1627, 1608, 1183 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ 8.90 (s, 1H, CH = N), 8.40 (d, 1H, J = 9.0 Hz, Ar-H), 8.23 (s, 1H, J = 9.0 Hz, Ar-H), 8.08 (d, 1H, J = 8.0 Hz, coumarin-5-H), 7.79 (t, 1H, J = 7.5 Hz, Ar-H), 7.56–7.47 (m, 6H, coumarin-6,8-H, Ar-H), 5.27 (s, 1H, coumarin-3-H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm) δ 167.33, 161.69, 160.42, 153.65, 151.32, 149.54, 148.75, 130.31, 125.11, 124.64, 123.93, 123.60, 122.80, 117.67, 115.67; MS (ESI) *m/z*: 387.1 ([M + H]$^+$); *Anal*. Calcd. for $C_{22}H_{18}N_2O_2$: C, 68.39; H, 3.56; N,7.25. Found: C, 68.01; H, 3.78; N, 7.39.

Antibacterial biological assay. The synthesized compouds (**3a–3p**) were evaluated for antibacterial activity against *Xoo* and *Xcc in vitro* by the turbidimeter test [30]. Commercial agricultural antibacterial thiadiazole copper was used as control. The test compounds were dissolved in 150 μL of dimethylformamide and diluted with Tween-20 (0.1%) to prepare different concentrations of 100 and 200 μg/mL. 1 mL of sample liquid was added to the nontoxic nutrient broth (NB, 1.5 g beef extract, 2.5 g peptone, 0.5 g yeast powder, 5.0 g glucose, and 500 mL distilled water, pH 7.0 − 7.2) liquid medium in 4 mL tubes. Then, 40 μL of NB medium containing tobacco bacterial wilt was added to 5 mL of solvent NB medium containing the test compounds or thiadiazole-copper. The inoculated test tubes were incubated at 30 ± 1°C with continuous shaking at 180 rpm for 48 h. Culture growth was monitored with a spectrophotometer by measuring the optical density at 600 nm (OD_{600}) given by corrected turbidity values [28]. The relative inhibitory rate I of the circle mycelium compared with a blank assay was calculated as follows:

$$I(\%) = (C_{tur} - T_{tur})/C_{tur} \times 100.$$

C_{tur} is the corrected turbidity value of bacterial growth on untreated NB

T_{tur} is the corrected turbidity value of bacterial growth on treated NB.

Similarly, the solvent for tomato bacterial wilt was SM (10.0 g peptone, 5.0 g glucose, 1.0 g casein acid hydrolysate, 1000 mL distilled water, pH 7.0 − 7.2), and thiadiazole copper served as positive control.

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