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FULL PAPER

CHEMISTRY & BIODIVERSITY

Design, Synthesis and Antibacterial Activity of Novel Pyrimidine-Containing 4*H*-Chromen-4-One Derivatives**

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A series of pyrimidine-containing 4*H*-chromen-4-one derivatives were designed and synthesized by combining bioactive substructures. Preliminary biological activity results showed that most of the compounds displayed significant inhibitory activities *in vitro* against *Xanthomonas axonopodis pv. Citri* (*X. axonopodis*), *Xanthomonas oryzae pv. oryzae* (*X. oryzae*) and *Ralstonia solanacearum* (*R. solanacearum*). In particular, compound 2-[(3-{[5,7-dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-3-yl]oxy}propyl)sulfanyl]-4-(4-methylphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**4c**) demonstrated a good inhibitory effect against *X. axonopodis* and *X. oryzae*, with the half-maximal effective concentration (EC_{50}) values of 15.5 and 14.9 μg/mL, respectively, and compound 2-[(3-{[5,7-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-3-yl]oxy}propyl)sulfanyl]-4-(3-fluorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**4h**) showed the best antibacterial activity against *R. solanacearum* with an EC_{50} value of 14.7 μg/mL. These results were better than commercial reagents bismerthiazol (BT, 51.7, 70.1 and 52.7 μg/mL, respectively) and thiodiazole copper (TC, 77.9, 95.8 and 72.1 μg/mL, respectively). *In vivo* antibacterial activity results indicated that compound **4c** displayed better curative (42.4%) and protective (49.2%) activities for rice bacterial leaf blight than BT (35.2, 39.1%) and TC (30.8, 27.3%). The mechanism of compound **4c** against *X. oryzae* was analyzed through scanning electron microscopy (SEM). These results indicated that pyrimidine-containing 4*H*-chromen-4-one derivatives have important value in the research of new agrochemicals.

Keywords: 4*H*-chromen-4-one derivatives, pyrimidine, antibacterial activity, scanning electron microscopy.

Introduction

Plant diseases can cause huge losses in the yield of economic crops every year, which one of the main reasons is bacterial infection. Such as, the citrus canker, tobacco bacterial wilt and rice bacterial leaf blight are caused by *Xanthomonas axonopodis pv. citri*, *Ralstonia solanacearum and Xanthomonas oryzae pv. oryzae*, respectively.[1–3] These bacteria are very serious and hard to manage effectively in agricultural production. Therefore, these agrochemicals (bismerthiazol, thiodiazole-copper and azoxy strobin) are often used to prevent bacterial infection.[4,5] But prolonged and abuse of traditional chemical pesticides will enhance the resistance of the bacterial, and are harmful to the environment and human health.[6–8] Thus, to explore novel antibacterial agents with highly-efficient, broad-spectrum, and environmentally is still an arduous task in pesticide science.

3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphen-yl)-4*H*-chromen-4-one (called "myricetin") belongs to a class of natural products which are widely found in fruits, vegetables, and herbs. It has attracted the attention of more and more researchers, due to

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the unique chemical structure and diverse biological activities.[9] Pharmacological research have shown that myricetin and its derivatives present antiviral,[10,11] antibacterial,[12–14] antioxidation,[15,16] anticancer,[17–19] anti-inflammatory[20] and other biological properties, but most of them are limited to the field of medicine and have few applications in agriculture. In previous work, myricetin was took as the lead compound and active fragments were introduced, a mass of myricetin derivatives with significant biological activity were discovered. In 2017, Zhong described myricetin derivatives containing 1,3,4-thiadiazole structure, which displayed good inhibitory effect on phytopathogenic bacteria.[21] Recently, Jiang reported a series of dithiocarbamate-containing 4*H*-chromen-4-one derivatives, which have good in vitro antibacterial activity against *X. axonopodis* and *X. oryzae*, with an EC_{50} values were 0.11 and 1.58 μg/mL, respectively, which were far better than commercial agents bismerthiazol and thiodiazole copper.[22]

Pyrimidine is an important class of heterocyclic compounds, which has a wide range of applications in medicine and pesticides research, due to its remarkable biological and pharmacological properties, such as antibacterial,[23–25] antiviral,[26,27] insecticide,[28,29] anticancer,[30,31] antimalarial,[32] herbicida[33] and other biological activities.[34] In 2019, Zhang reported a series of derivatives containing pyrimidine ethers were synthesized, which have a good control effect on cucumber downy mildew.[35] Recently, Bai described that some pyrimidine derivatives have significantly inhibitory efficiencies against Gram-positive bacteria, Gram-negative bacteria and the fungus *Candida albicans*.[36] In order to develop novel, highly efficient and environment friendly bactericides, we introduced the pyrimidine moiety into 4*H*-chromen-4-one, and a variety of pyrimidine-containing 4*H*-chromen-4-one derivatives were synthesized and their antibacterial activity were evaluated (Figure 1).

Results and Discussion

Chemistry

The structures of the title compounds **4a**–**4x** were characterized by ^{1}H-NMR, ^{13}C-NMR, ^{19}F NMR and HRMS. In ^{1}H-NMR spectra, multiplet signals at δ 8.00–6.50 ppm shown the presence of protons in aromatic nucleuses, and a singlet at δ 4.00–3.70 ppm revealed the presence of -OCH_3 group, the -O-CH_2- and -S-CH_2- characteristic groups between the 4*H*-chromen-4-one skeleton and the substituted pyrimidine were observed at 4.10–3.37 ppm. The chemical shifts of ^{13}C-NMR spectra were 172.57–160.77 ppm, 109.12–106.14 ppm, 27.80–32.12 ppm in which confirmed the existence of C=O, -CN and -CH_2- groups, respectively. The HRMS spectra of the title compounds shown absorption signals of $[M+H]^+$ ions that was consistent with their molecular weight.

To further confirm the structures of the title compounds, compound **4g** was successfully cultured a single crystal and studied by single-crystal X-ray analysis as a representative example. The tested single crystal was crystallized from DMSO and acetone mixture solution containing compound **4g**. As shown in *Figure 2B*, C00T…O007 and O00A…C01A were two important intramolecular hydrogen bonds, which combine with 4*H*-chromen-4-one and pyrimidine heterocycle fragments to construct the main molecular scaffold of target compound **4g**. Besides, as presented in *Figure 2C*, four intermolecular hydrogen bonds (O008…H01B, C00J…C00L, C00J…C00L, H01B…O008)

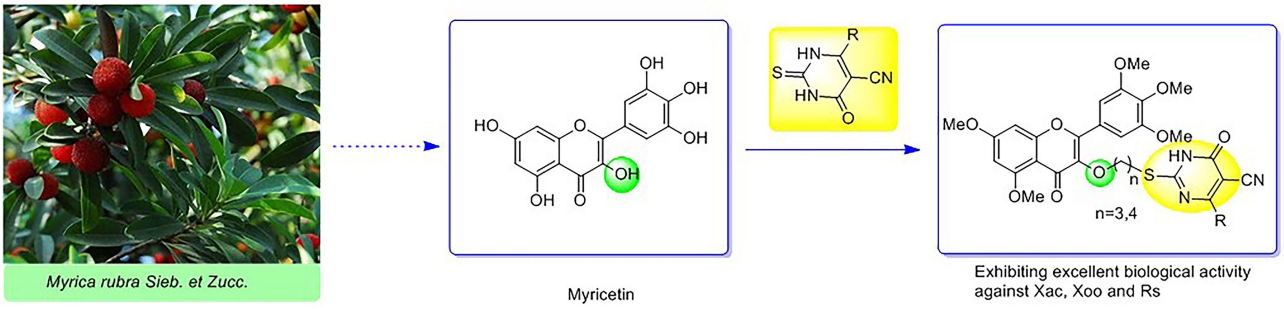


Figure 1. Design strategy for target molecules.

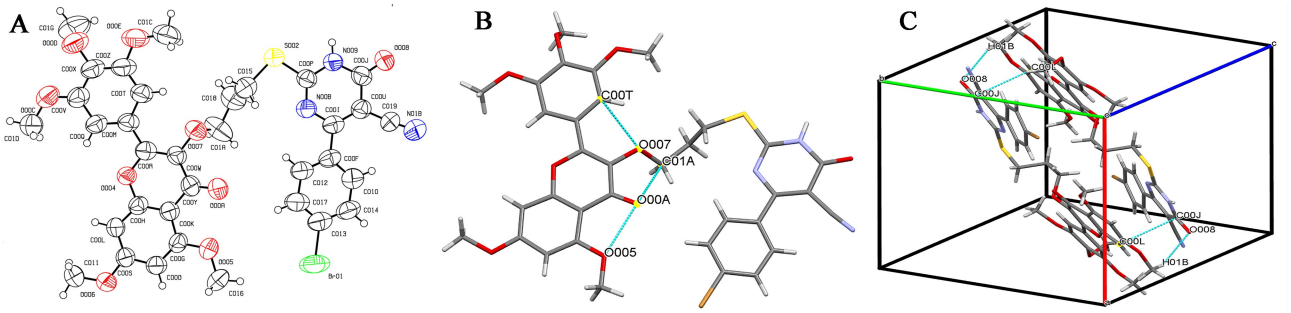


Figure 2. Crystal structural (A, B) and crystal packing (C) diagram of title compound **4g**.

between neighboring molecules were primary forces for establishing the three-dimensional structure of target compound **4g**. crystallographic data listed in *Table 1*. The deposition number is CCDC 2022970.

Antibacterial Activity of the Title Compounds against X. axonopodis, X. oryzae and R. solanacearum in Vitro

The *in vitro* antibacterial activities of the title compounds **4a**–**4x** against *X. axonopodis*, *X. oryzae* and *R. solanacearum* were determined using turbidimeter tests, and the bismerthiazol, thiadiazole copper, and myricetin were tested as the controls. Preliminary bioassays results in *Table 2* indicated that some compounds with good antibacterial activities against *X. axonopodis*, *X. oryzae and R. solanacearum*. The inhibition rates of the compounds **4b**, **4c**, **4h** and **4o** against *X. axonopodis* at 100 and 50 μg/mL were 80.4 and 51.4%, 91.3 and 69.0%, 85.3 and 51.4%, 83.2 and 50.5%, respectively, which were better than BT (66.4 and 42.3%), TC(56.9 and 38.3%) and myricetin(44.9 and 27.5%). The inhibition rates of compounds **4c**, **4h** and **4p** against *X. oryzae* at 100 μg/mL were 89.7, 81.3 and 80.8%, respectively, which were superior to BT (50.5%), TC (47.5%) and myricetin (44.0%). Note that the inhibition rates of **4c**, **4h**, **4n**, **4o** and **4p** against *X. oryzae* at 50 μg/mL were 72.1, 56.0, 54.7, 49.5 and 56.1%, respectively, which were superior to BT (31.0%), TC (29.5%) and myricetin (33.3%). Furthermore, **4c**, **4d**, **4h**, **4k**, **4l** and **4o** have fine antibacterial activities against *R. solanacearum* at 100 μg/mL with the inhibition rates of 90.9, 83.9, 92.8, 88.7, 83.2 and 90.4%, respectively, which were better than BT (70.3%), TC (57.4%) and myricetin (50.7%). Similarly, the percentage inhibition of **4c**, **4h**, **4k** and **4o** against *R. solanacearum* at 50 μg/mL were 72.6, 76.3, 66.4 and 72.0%, respectively, which exceeded BT (44.6%), TC (36.7%) and myricetin (29.6%).

Based on the preliminary bioassays results in *Table 2*, the EC_{50} values of some target compounds against *X. axonopodis*, *X. oryzae* and *R. solanacearum* were further evaluated as shown in *Table 3*. Compounds **4c**, **4h** and **4o** exhibited fine antibacterial activities against *X. axonopodis*, with the EC_{50} values of 15.5, 28.3, and 29.4 μg/mL, respectively, which exceeded BT (50.3 μg/mL), and TC (83.2 μg/mL). Compounds **4c**, **4h**, **4n** and **4p** demonstrated good inhibitory effects against *X. oryzae* with the EC_{50} values of 14.9, 23.8, 28.6 and 24.8 μg/mL, which were better than BT (72.0 μg/mL) and TC (99.2 μg/mL). Compounds **4c**, **4d**, **4h**, **4k**, **4l** and **4o** have better antibacterial activity against *R. solanacearum* with the

Table 1. Crystal data of title compound **4g**.

Crystal Data	
C_{34} H_{30} BrN_3O_9 S	Colorless/block
Mr = 736.57	ρ = 1.430
triclinic/P-1	Z = 2
a = 12.025(3) [Å]	α = 103.276(15) [°]
b = 12.509(4) [Å]	β = 112.727(15) [°]
c = 14.791(4) [Å]	γ = 102.837(17) [°]
F(000) = 832	V = 1873.79(9) [$Å^3$]
Data Collection	
T_{min} = 0.310, T_{max} = 0.752	Absorption coefficient: 2.537 [mm^{-1}]
3580 Independent reflections	θ_{min} = 3.870°, θ_{max} = 64.332°
18889 Reflections collected	−13 < = h < = 13, −14 < = k < = 14, −17 < = l < = 16
Refinement	
Goodness-of-fit: 1.090	R indices (all data) R1 = 0.1186, wR2 = 2964
$\Delta\rho_{max}$ = 0.426 $eÅ^{-3}$, $\Delta\rho_{min}$ = −0.750 $eÅ^{-3}$	R indices [I > 2r(I)] R1 = 0.0835, wR2 = 2591
438 parameter	

Table 2. Antibacterial activities of the title compounds (**4a**–**4x**) against *X. axonopodis*, *X. oryzae* and *R. solanacearum in vitro*.[a]

Compd.	*X. axonopodis* (%)		*X. oryzae* (%)		*R. solanacearum* (%)	
	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
4a	37.9 ± 2.8	25.6 ± 1.8	26.3 ± 1.6	13.6 ± 2.0	50.5 ± 0.6	39.2 ± 2.2
4b	80.4 ± 0.3	51.4 ± 0.9	20.7 ± 2.4	13.1 ± 4.2	46.9 ± 1.6	32.1 ± 2.1
4c	91.3 ± 0.6	69.0 ± 1.4	89.7 ± 0.9	72.1 ± 1.8	90.9 ± 1.7	72.6 ± 2.2
4d	76.7 ± 3.5	40.9 ± 1.6	75.1 ± 0.8	41.70 ± 2.4	83.9 ± 1.4	51.2 ± 0.3
4e	28.7 ± 4.2	20.4 ± 2.6	50.3 ± 3.7	24.7 ± 3.0	34.3 ± 4.2	24.5 ± 1.6
4f	39.6 ± 2.9	28.9 ± 2.0	29.8 ± 1.8	18.1 ± 3.7	35.2 ± 3.6	27.9 ± 1.2
4g	25.3 ± 3.3	16.7 ± 3.1	31.2 ± 3.9	22.1 ± 5.4	67.0 ± 0.9	40.8 ± 1.2
4h	85.3 ± 0.7	51.4 ± 2.0	81.3 ± 0.6	56.0 ± 2.2	92.8 ± 0.6	76.3 ± 1.2
4i	26.4 ± 2.0	25.6 ± 1.0	26.6 ± 2.1	12.5 ± 0.7	37.4 ± 5.6	19.7 ± 4.0
4j	73.5 ± 0.8	41.9 ± 1.6	45.2 ± 2.1	21.8 ± 5.6	51.2 ± 1.1	30.2 ± 1.3
4k	31.5 ± 2.7	24.2 ± 1.7	32.8 ± 1.9	21.5 ± 1.6	88.7 ± 1.4	66.4 ± 0.31
4 l	32.2 ± 1.7	21.96 ± 5.1	58.9 ± 1.7	32.5 ± 3.1	83.2 ± 1.6	50.9 ± 2.3
4m	47.1 ± 3.2	26.4 ± 2.2	47.7 ± 1.1	28.7 ± 1.9	51.8 ± 2.7	25.1 ± 1.3
4n	77.4 ± 1.5	42.9 ± 3.2	78.4 ± 0.6	54.7 ± 2.1	28.5 ± 3.4	17.7 ± 1.6
4o	83.2 ± 0.8	50.5 ± 2.3	74.9 ± 0.3	49.6 ± 2.1	90.4 ± 0.4	72.0 ± 0.9
4p	27.1 ± 1.9	20.3 ± 1.4	80.8 ± 1.7	56.1 ± 2.2	25.9 ± 3.5	14. ± 5.8
4q	23.7 ± 2.8	19.9 ± 2.8	47.7 ± 1.3	34.4 ± 1.5	48.4 ± 1.2	35.4 ± 1.5
4r	24.5 ± 1.3	16.8 ± 1.5	26.7 ± 2.4	12.8 ± 1.9	62.6 ± 0.7	39.2 ± 2.9
4s	21.9 ± 1.5	14.7 ± 1.4	45.2 ± 1.5	21.1 ± 3.9	22.2 ± 2.5	12.5 ± 3.4
4t	20.2 ± 2.5	17.7 ± 1.2	26.3 ± 4.5	21.6 ± 2.4	44.5 ± 3.8	33.0 ± 1.9
4u	45.5 ± 3.4	29.2 ± 6.3	23.8 ± 5.4	18.5 ± 1.0	53.4 ± 1.7	39.4 ± 2.2
4v	27.8 ± 1.5	19.7 ± 4.8	32.6 ± 1.9	27.9 ± 3.6	57.5 ± 1.3	37.5 ± 2.7
4w	29.1 ± 0.8	20.7 ± 1.3	44.4 ± 0.7	28.8 ± 3.0	80.7 ± 1.8	59.1 ± 1.5
4x	41.8 ± 3.7	26.2 ± 2.6	68.6 ± 3.5	45.6 ± 2.5	61.9 ± 1.2	33.2 ± 4.4
Myr[b]	44.9 ± 1.4	27.5 ± 3.01	44.0 ± 1.2	33.3 ± 0.9	50.7 ± 2.3	29.6 ± 1.1
BT[c]	56.9 ± 0.7	38.3 ± 0.9	47.5 ± 0.3	29.5 ± 1.0	70.3 ± 2.1	44.6 ± 2.5
TC[c]	66.4 ± 1.0	42.3 ± 1.4	50.5 ± 1.0	31.0 ± 0.7	57.4 ± 2.0	36.7 ± 1.8

[a] Average of three replicates. [b] The lead compound of myricetin. [c] The commercial bactericides bismerthiazol (BT) and thiediazole copper (TC).

EC_{50} values of 16.1, 23.1, 14.7, 17.5, 24.8 and 16.0 μg/mL, respectively, which were superior to BT (43.5 μg/mL) and TC (64.3 μg/mL).

Structure-Activity Relationships of Antibacterial Activities

As indicated in *Tables 2* and *Table 3*, the R groups had significant impact on the antibacterial activity, for example, **4c** ($n=3$, R = 4-CH_3-Ph), **4h** ($n=3$, R = 3-F-Ph), **4o** ($n=4$, R = 4-CH_3-Ph) demonstrated a fine inhibitory effect against *X. axonopodis* with the EC_{50} values of 15.5, 28.3 and 29.4 μg/mL,. Compounds **4c** ($n=3$, R = 4-CH_3-Ph), **4d** ($n=3$, R = 4-OCH_3-Ph) and **4p** ($n=4$, R = 4-OCH_3-Ph) demonstrated good inhibitory effects against *X. oryzae* with the EC_{50} value of 14.9, 23.8 and 24.8 μg/mL, which were better than other substituents. The presence of donor electron groups and heterocycles effectively enhanced the antibacterial activities against *R. solanacearum*, such as compounds **4c** ($n=3$, R = 4-CH_3-Ph), **4d** ($n=3$, R = 4-OCH_3-Ph), **4h** ($n=3$, 3-F-Ph), **4k** ($n=3$, R = furan), **4l** ($n=3$, R = pyridyl) and **4o** ($n=4$, R = 4-CH_3-Ph) had better antibacterial activity against *R. solanacearum* with the EC_{50} values of 16.1, 23.1, 14.7, 17.5, 24.8 and 16.0 μg/mL, respectively, which were superior to that of BT (43.5 μg/mL) and TC (64.3 μg/mL) and other substituents. From this analysis, when R were substituted by 4-CH_3-Ph, 4-CH_3O-Ph, 3-F-Ph, the corresponding compound exhibited remarkable antibacterial activities against *X. axonopodis*, *X. oryzae* and *R. solanacearum*.

In Vivo Bioactivity of **4c** *Against Rice Bacterial Leaf Blight*

The antibacterial test shown that compound **4c** has significant anti-*X. oryzae* biological activity *in vitro*. To further verify the antibacterial activity of **4c**, a pot experiment was carried out *in vivo*, and the experiments of **4c** against rice bacterial leaf blight were determined using a leaf-cutting method. As shown in *Table 4* and *Figure 3*, when the concentration of

Table 3. The EC_{50} values of the title compounds against *X. axonopodis*, *X. oryzae* and *R. solanacearum in vitro*.[a]

Tested bacterial	Compds.	Toxic regression equation	r^2	EC_{50}/(μg/mL)
X. axonopodis	**4b**	y = 1.5705x + 2.6602	0.9826	30.9 ± 2.0
	4c	y = 1.9107x + 2.7256	0.9912	15.5 ± 1.7
	4d	y = 1.5736x + 2.5280	0.9825	37.2 ± 2.1
	4h	y = 1.7100x + 2.5176	0.9809	28.3 ± 1.1
	4j	y = 1.4978x + 2.6114	0.9873	39.3 ± 0.9
	4n	y = 1.6002x + 2.5541	0.9947	33.7 ± 2.2
	4o	y = 1.7781x + 2.3894	0.9811	29.4 ± 2.4
	BT[c]	y = 1.3743x + 2.6450	0.9854	50.3 ± 2.0
	TC[c]	y = 1.3153x + 2.5117	0.9822	83.2 ± 1.7
X. oryzae	**4c**	y = 1.4639x + 3.2826	0.9906	14.9 ± 0.9
	4d	y = 1.2237x + 3.1804	0.9693	30.7 ± 1.6
	4h	y = 1.4072x + 3.0639	0.9961	23.8 ± 1.8
	4n	y = 1.2358x + 3.1996	0.9773	28.6 ± 2.6
	4o	y = 1.2425x + 3.1428	0.9892	31.2 ± 0.9
	4p	y = 1.3657x + 3.0943	0.9978	24.8 ± 1.1
	BT[c]	y = 1.3199x + 2.5633	0.9848	72.0 ± 1.9
	TC[c]	y = 1.0632x + 2.8928	0.9796	99.2 ± 2.7
R. solanacearum	**4c**	y = 1.6321x + 3.0312	0.9929	16.1 ± 2.3
	4d	y = 1.4199x + 3.0625	0.9780	23.1 ± 1.3
	4h	y = 1.6908x + 3.0236	0.9969	14.7 ± 1.2
	4k	y = 1.4931x + 3.1422	0.9857	17.5 ± 2.0
	4l	y = 1.4276x + 3.0175	0.9801	24.8 ± 1.0
	4o	y = 1.5422x + 3.1423	0.9926	16.0 ± 1.3
	4w	y = 1.4597x + 2.8546	0.9833	29.5 ± 2.5
	BT[c]	y = 1.4267x + 2.5425	0.9543	43.5 ± 3.3
	TC[c]	y = 1.2906x + 2.5962	0.9821	64.3 ± 2.1

[a] Average of three replicates. [c] The commercial agricultural antibacterial agents bismerthiazol (BT) and thiodiazole-copper (TC) were used as control agents.

Table 4. Curative and protection activity of compound **4c** against *X. oryzae* under greenhouse conditions at 200 μg/mL (14 days after spraying).[a]

Treatment	Curative activity (%)		Protection activity (%)	
	Disease index	Control efficiency	Disease index	Control efficiency
4c	48.6	42.4	45.7	49.2
BT[c]	54.8	35.2	51.4	39.1
TC[c]	59.0	30.8	61.9	27.3
Negative control	84.4	–	84.4	–

[a] The experiments were repeated three times. [c] The commercial agricultural antibacterial agents bismerthiazol (BT) and thiodiazole-copper (TC) were used as control agents.

compound **4c** was 200 μg/mL, the curative activity against rice bacterial blight was 42.4%, the result better than BT (35.2%) and TC (30.8%). Meanwhile, compound **4c** demonstrated excellent protection activity against bacterial blight of rice with the percentage inhibition of 49.2%, which was superior to BT (39.1%) and TC (27.3%). These results suggested that compound **4c** can effectively prevent the rice bacterial blight.

Scanning Electron Microscopy (SEM) Study

The mechanism of compound against *X. oryzae* was analyzed by SEM. As shown in *Figure 4*, the surface morphology of *X. oryzae* were smooth and remains intact without compound to treatment (*Figure 4A*). When the bacteria were treated with compound **4c** at concentration of 50 μg/mL, bacteria cell membrane began to appear different degrees wrinkled (*Figure 4B*).

Figure 3. Curative and protective activity of compound **4c** on the bacterial leaf blight of rice.

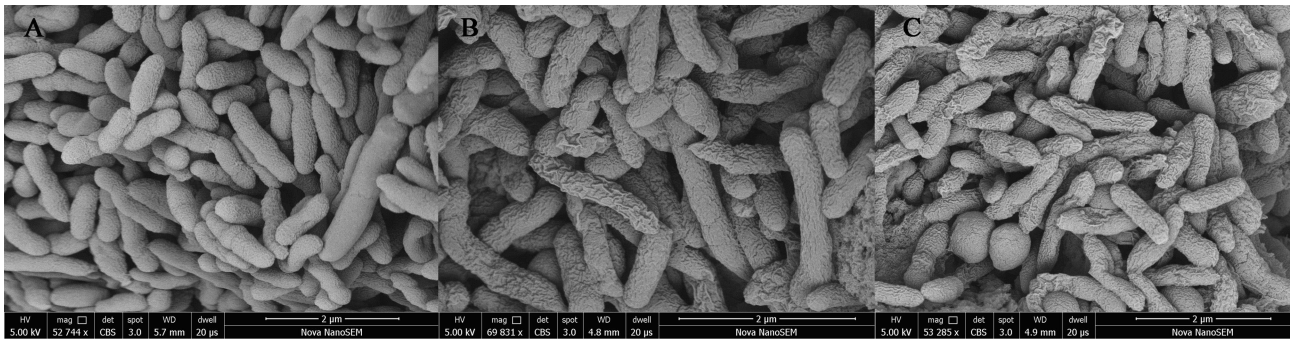


Figure 4. SEM images for *X. oryzae* after incubated in different concentration of compound **4c**. (A) 0 μg/mL, (B) 50 μg/ mL, and (C) 100 μg/mL. Scale bar for (A, B, and C) are 2 μm.

When the concentration of compound increased to 100 μg/mL, the bacterial cell membrane showed invagination and rupture (*Figure 4C*). Therefore, the compound **4c** destroyed the cell membrane structure, to achieve the purpose of antibacterial.

Conclusions

In summary, aiming to develop novel and high-efficient agents with better biological activities, a series of pyrimidine-containing 4*H*-chromen-4-one derivatives were designed and synthesized. *In vitro*

antibacterial bioassays showed that most of compounds exhibited significant antibacterial activities against *X. axonopodis*, *X. oryzae* and *R. solanacearum*. In particular, compound **4c** exhibited the best antibacterial activities against *X. axonopodis*, *X. Oryzae*, and compound **4h** exhibited the better antibacterial activity against *R. solanacearum* than TC and BT. *In vivo* antibacterial bioassays shown that compound **4c** was more effective than BT and TC in reducing bacterial blight of rice. Furthermore, the Scanning electron microscopy of **4c** against *X. oryzae* showed that compound destroyed the cell membrane structure of the bacteria, and the damage of the cell membrane was more serious as the concentration increased. These results indicated that the pyrimidine-containing 4*H*-chromen-4-one derivatives have better antibacterial activity and could be further study as potential replacement templates in the search for novel antibacterial agents.

Experimental Section

General

All solvents and reagents were purchased from Shanghai Titan Scientific Co., Ltd, and were analytical grade or chemically pure. The melting point of all compounds (**4a**–**4x**) were determined by the *X*-4B melting point apparatus (Beijing Tech Instrument Co, Beijing. China). ^{1}H-NMR, ^{13}C-NMR and ^{19}F NMR were obtained on Bruker Ascend-400 spectrometer (Bruker Optics, Germany) Tetramethylsilane (TMS) as internal standard and DMSO was used as solvent; Mass spectral studies were performed on a quadrupole electrostatic field orbitrap mass spectrograph (Thermo Scientific, USA). The synthetic route of pyrimidine-containing 4*H*-Chromen-4-one derivatives was shown in *Scheme 1*.

Chemistry

General Procedure for the Synthesis of Intermediate **1**

Based on the previous reported method,[26,37] the ethyl cyanoacetate (5 mmol), substituted aromatic aldehyde (5 mmol), thiourea (5 mmol) and anhydrous K_2CO_3 (7.5 mmol) were added in ethanol (50 mL) for refluxed for about 4–6 h. After the reaction was completed (the reaction was monitored by TLC), mixture was cooled to room temperature and diluted with ice water, a large amount of solids was precipitated as the pH adjusted with glacial acetic acid to weak acidity, the residue was filtered and dried with suction, recrystallized with absolute ethanol to obtain Intermediate **1**

General Procedure for the Synthesis of Intermediate **2**

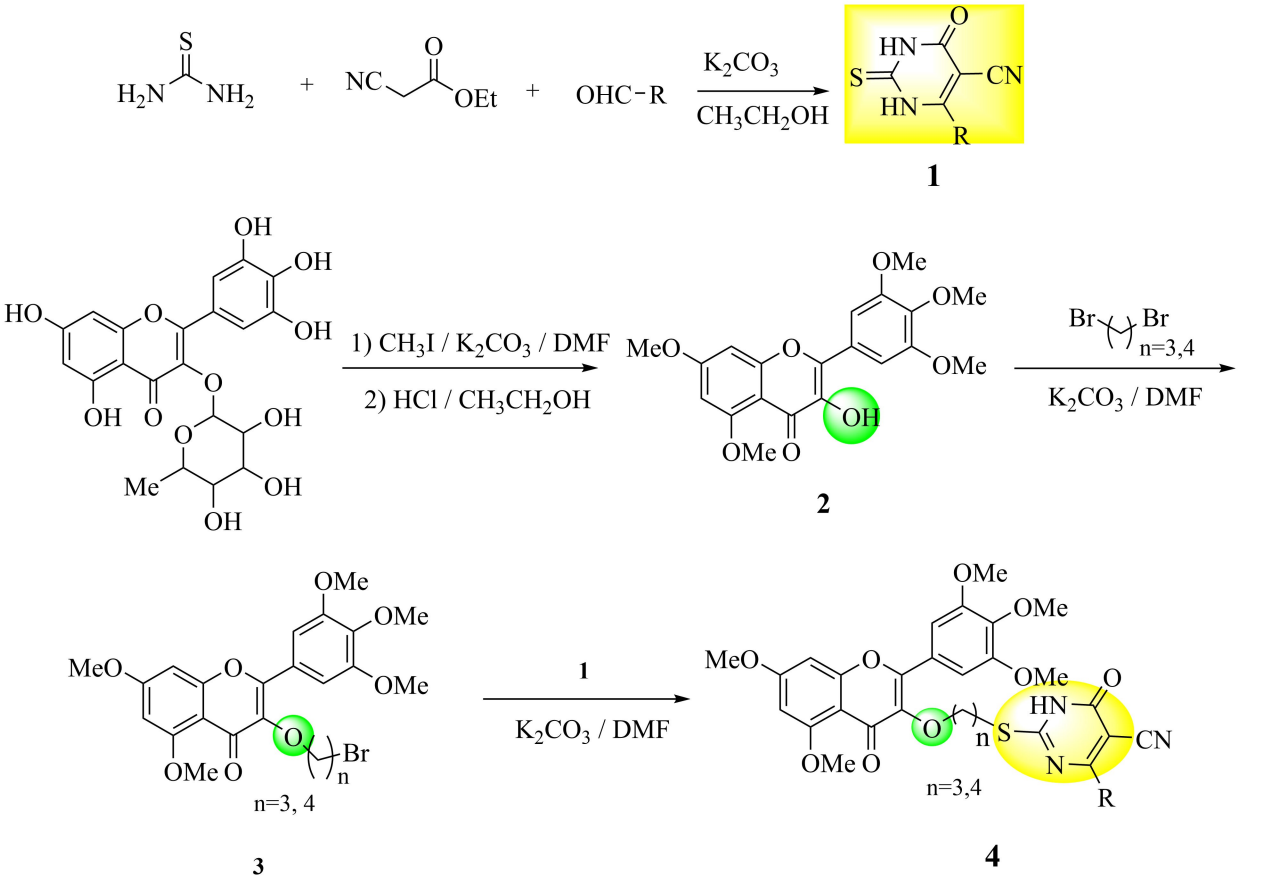
Myricetin iodomethane and anhydrous K_2CO_3 were stirred in N, N-dimethylformamide (DMF) for 48 h, and then, it was extracted with methylene chloride and concentrated. Then deglycosylation with concentrated hydrochloric acid under reflux in ethanol to prepare 3-hydroxy-3′,4′,5′,5,7-pentamethoxymyricetin (Intermediate **2**).[22]

General Procedure for the Synthesis of Intermediate **3**

Intermediate **2** (2 mmol) and K_2CO_3 (3 mmol) were dissolved in certain amount of *N*, *N*-dimethylformamide (DMF), stirred at room temperature for 0.5–1 h, the different dibromoalkane (2 mmol) was added and the reaction system was stirred at room temperature for 10 h. Mixture was dispersed in 50 mL ice water when the reaction completed(the reaction was monitored by TLC), a white solid separated out, the liquid was removed by filtration, and the solid was continuously stirred in a mixed solution of 30 mL petroleum ether and ethyl acetate for 3–4 h, then, filtered and dried under reduced pressure to obtain Intermediate **3**.[38]

General Procedure for the Synthesis of Title Compounds **4a**–**4x**

A solution of intermediates **1** (3 mmol), intermediates **3** (3 mmol) and K_2CO_3 (4.5 mmol) in 50 mL DMF was stirred under reflux for 4–5 h, after reaction was completed (the reaction was monitored by TLC). The mixture was cooled to room temperature, then, poured into 80 mL ice water and extracted with ethyl acetate (3×30 mL), organic layer was washed with 5% hydrochloric acid solution, saturated $NaHCO_3$ aqueous solution, saturated NaCl solution successively and dried with anhydrous Na_2SO_4. Solvent removed under vacuum and crude product was separated by column chromatography with petroleum ether/ethyl acetate ($V:V=1:1$) to obtain the title compound **4a**. Based on the similar method, the title compounds **4b**–**4x** were prepared. All the Spectroscopic data and Spectrographic of compound **4a**–**4x** were provided in Supporting Material.



4a: n=3 R= C_6H_5 | **4g**: n=3 R=4-Br-C_6H_4 | **4m**: n=4 R= C_6H_5 | **4s**: n=4 R=3-Br-C_6H_4
4b: n=3 R=3-CH_3-C_6H_4 | **4h**: n=3 R=3-F-C_6H_4 | **4n**: n=4 R=3-CH_3-C_6H_4 | **4t**: n=4 R=4-Br-C_6H_4
4c: n=3 R=4-CH_3-C_6H_4 | **4i**: n=3 R=4-F-C_6H_4 | **4o**: n=4 R=4-CH_3-C_6H_4 | **4u**: n=4 R=3-F-C_6H_4
4d: n=3 R=4-OCH_3-C_6H_4 | **4j**: n=3 R=Thiophen-2-yl | **4p**: n=4 R=4-OCH_3-C_6H_4 | **4v**: n=4 R=4-F-C_6H_4
4e: n=3 R=3-Cl-C_6H_4 | **4k**: n=3 R=Furan-2-yl | **4q**: n=4 R=3-Cl-C_6H_4 | **4w**: n=4 R=Furan-2-yl
4f: n=3 R=4-Cl-C_6H_4 | **4l**: n=3 R=Pyridin-4-yl | **4r**: n=4 R=4-Cl-C_6H_4 | **4x**: n=4 R=Pyridin-4-yl

Scheme 1. The synthesis of target compounds.

Antibacterial Bioassays

Bacteriostatic Activity Test in Vitro

The *in vitro* antibacterial activities of all the target compounds against *X. axonopodis*, *X. oryzae* and *R. solanacearum* were determined by turbidimetric method.[39] The test method was provided in *Supporting Information.*

In Vivo Antibacterial Activity

The protection and curative activities of compound **4c** against bacterial blight of rice were tested through potted plants using complete randomized block design according to a previous method with slight modifications.[38] Commercial agents BT and TC were used as positive control. The test method was provided in *Supporting Information.*

Scanning Electron Microscopy

To explore the mechanism of compounds **4c** against *X. oryzae*, scanning electron microscopy (SEM) analysis was taken according to previous method.[40] This method is provided in *Supporting Information.*

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Author Contribution Statement

This study is an outcome of constructive discussion with Wei Xue and Shijun Su, Mei Chen and Shijun Su carry out their synthesis and characterization experiments and carried out the ^{1}H-NMR, ^{13}C-NMR, ^{19}F NMR and HRMS spectral analyses, XueMei Tang, Feng Peng and Tingting Liu performed the antibacterial activity; Qing Zhou, Wenliang Zhan and Ming He assists in scanning electron microscope; Shijun Su and Chengwei Xie were involved in the drafting of the manuscript and revising the manuscript. All authors read and approved the final manuscript.

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