JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Novel Ferulic Amide Ac6c Derivatives: Design, Synthesis, and Their Antipest Activity

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Cite This: https://doi.org/10.1021/acs.jafc.1c03892 Read Online

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ABSTRACT: Thirty-eight novel ferulic amide 1-aminocyclohexane carboxylic acid (Ac6c) derivatives **D1**−**D19** and **E1**−**E19** were designed and synthesized, and their antibacterial, antifungal, and insecticidal activities were tested. Most of the synthesized compounds displayed excellent activity against*Xanthomonas oryzae* pv. *oryzae* (*Xoo*), with EC_{50} values ranging from 11.6 to 83.1 μg/mL better than that of commercial bismerthiazol (BMT, EC_{50} = 84.3 μg/mL), as well as much better performance compared to that of thiediazole copper (TDC, EC_{50} = 137.8 μg/mL). **D6** (EC_{50} = 17.3 μg/mL), **D19** (EC_{50} = 29.4 μg/mL), **E3** (EC_{50} = 29.7 μg/mL), **E9** (EC_{50} = 27.0 μg/mL), **E10** (EC_{50} = 18.6 μg/mL), and **E18** (EC_{50} = 20.8 μg/mL) showed much higher activity on *Xanthomonas oryzae* pv. *oryzicola* compared with BMT (EC_{50} = 80.1 μg/mL) and TDC (EC_{50} = 124.7 μg/mL). In relation to controlling the fungus, *Rhizoctonia solani*, **E1**, **E10**, and **E13** had much lower EC_{50} values of 0.005, 0.140, and 0.159 μg/mL compared to hymexazol at 74.8 μg/mL. Further *in vivo* experiments demonstrated that **E6** and **E12** controlled rice bacterial leaf blight disease better than BMT and TDC did. Scanning electron microscopy (SEM) studies revealed that **E12** induced the *Xoo* cell membrane collapse. Moreover, **D13** (73.7%), **E5** (80.6%), and **E10** (73.4%) also showed moderate activity against *Plutella xylostella*. These results indicated that the synthesized ferulic amide Ac6c derivatives showed promise as candidates for treating crop diseases.

KEYWORDS: *ferulic amide, Ac6c derivatives, synthesis, antibacterial activity, antifungal activity, insecticidal activity*

INTRODUCTION

Crop pests and pathogens often result in quality and yield losses in agricultural production.[1−3] About 16−20% of the major food crop production is lost worldwide each year due to preharvest diseases.[4] *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*) are good examples, as they can cause devastating leaf blight and leaf stripe in rice crops and often lead to serious disease outbreaks, which reduce crop yield by 20%, rising to 50% under severe infection.[5−8] In addition, as one of the most aggressive pathogens, *Xanthomonas axonopodis* pv *citri* (*Xac*) in citrus can cause serious canker and cut down citrus production worldwide; also, as it is difficult to control once plants are infected, it has a serious impact on the entire citrus industry.[9−11] Currently, the use of chemical pesticides is still the main measurement taken to control crop diseases and pests. However, there is a severe shortage of available control agents and a limited number of actions to take against these diseases. This is especially true given the continuous increase in resistance or cross-resistance by the pests and pathogens to the existing pesticides;[12,13] therefore, the prevention and control of crop pests are becoming more and more difficult. Hence, exploration of new active molecules with novel modes of action that exhibit high activity yet possess low-risk profiles for plant pests and pathogens is urgently needed.

Natural products (NPs) play a significant role in exploring novel pesticides. So far, more than 50% of the available commercial pesticides have been derived from NPs, these include conventional neonicotinoids, strobilurins, pyrethroids, ethylicin, fenpiclonil, and fludioxonil, all of which mimic NPs.[1] Nowadays, NP-based products are still hot topics for researching and developing novel agrochemicals due to their unique properties including biocompatibility with the environment and their novel modes of action.[14−16] As an important natural product, the ferulic acid skeleton is the most widely used today as it is the lead compound in NP-based drug design.[1] Many molecules derived from ferulic acid have shown various physiological functions such as antioxidant,[17] antibacterial,[18] anticancer,[19] anticoagulant,[20] and anti-inflammatory actions.[21] In particular, ferulic acid based compounds have also been developed for inhibiting plant diseases in recent years. Some novel ferulic acid skeleton-based compounds, such as amide derivatives,[22] chalcone derivatives,[23] α-amino phosphonates derivatives,[24] acylhydrazone derivatives,[25] and others, have reportedly shown promising activity in relation to plant disease control. In particular, combining an amino group with ferulic acid could lead to ferulic amides, which can potentially be used as fungicides in agriculture.[24−28]

1-Aminocyclohexane carboxylic acid (Ac6c) is also an important non-proteinogenic quaternary α-amino acid, which plays an important role in synthetic antimicrobial peptide and drug discovery.[29] The compounds or peptide analogues containing Ac6c have shown excellent bioactivities including anticonvulsant,[30] antiproliferative,[31] antitumor,[32] antibacteri-

Received: June 29, 2021
Revised: August 3, 2021
Accepted: August 11, 2021

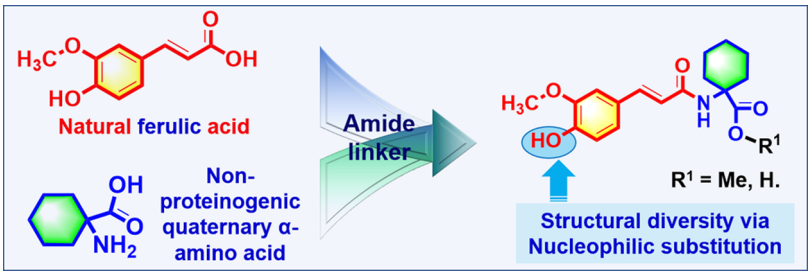
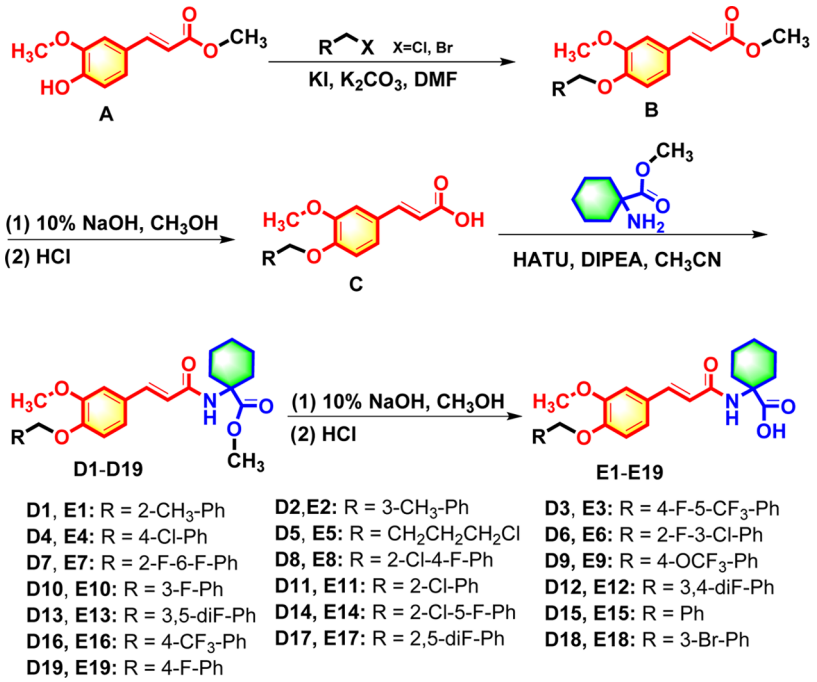


Figure 1. Design strategy for the target molecules.

Scheme 1. Synthetic Route of the Title Compounds D1−D19 and E1−E19



al,[33] insecticidal,[34] herbicidal,[35] antifungal,[36] and plant growth regulator activities toward improving drought resistance in plants.[27,28] Some Ac6c derivatives also display excellent activity levels against *Phytophthora infestans* in tomato plants.[37] So far, about ∼150 Ac6c pharmacophore-containing compounds have been discovered.[38] Moreover, the Ac6c structure displays two superiorities: (1) The cyclic ring and quaternary α-carbon atom in Ac6c could restrict its ability to become an intrinsically stable conformation. This feature often results in a more efficient and selective ligand than a nonrestricted one when they act upon the target.[39,40] (2) Ac6c possesses a saturated cyclohexane core, ensuring that Ac6c derivatives are more lipophilic.[41] These factors have ensured that interest in Ac6c derivatives has grown.

The method of "substructure combination" is often used in pesticide design.[42] The rationale of this method is integrated with different substructures in some active compounds *via* a functional linker (such as amide, ether, etc);[23,43] based on this method, a potentially active compound might be obtained.[23,24,42,43] Hence, encouraged by the qualities described above, we sought to combine the ferulic acid core with Ac6c *via* a functional amide, then achieve structural diversity through the reaction site at the hydroxyl on ferulic acid to produce novel ferulic amide Ac6c derivatives (Figure 1). Antibacterial activities of these derivatives were then tested on *Xoo*, *Xoc*, and *Xac*, and antifungal activities were evaluated against *Cytospora mandshurica*, *Colletotrichum gloeosporioides*, *Gibberella zeae*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, and *Rhizoctonia solani*. Insecticidal activity against diamondback moth (*Plutella. xylostella*) was also investigated. Successful results showed that most of the designed compounds showed outstanding antibacterial activity. *In vivo* experiments and the morphological influence on *Xoo* were also considered.

MATERIALS AND METHODS

Instruments and Chemicals. ^{1}H, ^{13}C, and ^{19}F NMR spectra were detected on a JEOL ECX 500 NMR (JEOL Ltd., Tokyo, Japan) or an AVANCE III HD 400M NMR (Bruker Corporation, Fallanden, Switzerland) spectrometer using dimethyl sulfoxide (DMSO) as the solvent. The melting points of the ferulic amide Ac6c derivatives **D1−D19** and **E1−E19** were measured by a binocular microscope melting point apparatus (XT-4, Beijing Tech Instrument Co., Beijing, China). High-resolution mass spectrometry (HR-MS) was given by an Orbitrap liquid chromatography−mass spectrometry (LC-MS) (Q-Exative, Thermo Scientific). Scanning electron microscopy (SEM) images were obtained and visualized using Nova NanoSEM 450 (Thermo Fisher Scientific, Massachusetts). Ferulic acid methyl ester was bought from TCI (Tokyo, Japan) and the substituted halogenated hydrocarbons and 1-aminocyclohexane carboxylic acid were supplied by Accela (Shanghai, China). All of the reactions were monitored by TLC.

General Procedure for Preparing D1−D19 and E1−E19. Scheme 1 depicts the synthetic route for the title compounds **D1−D19** and **E1−E19**. They were provided *via* several steps that started from

https://doi.org/10.1021/acs.jafc.1c03892
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. *In Vitro* Antibacterial Activity[a] of the Target Compounds against *Xoo*, *Xac*, and *Xoc*

compd.	*Xoo* inhibition rate (%)		*Xoc* inhibition rate (%)		*Xac* inhibition rate (%)	
	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
D1	71.4 ± 1.4	56.2 ± 0.6	33.2 ± 4.6	32.3 ± 2.3	64.2 ± 0.2	27.5 ± 2.9
D2	56.0 ± 2.9	36.9 ± 2.6	40.9 ± 1.2	38.1 ± 4.7	47.3 ± 1.2	45.2 ± 4.8
D3	87.9 ± 1.7	62.6 ± 2.3	41.8 ± 1.4	39.3 ± 0.2	66.4 ± 4.0	30.5 ± 0.7
D4	40.4 ± 3.8	30.5 ± 3.0	45.1 ± 2.1	40.2 ± 2.4	30.2 ± 4.5	24.9 ± 1.3
D5	90.3 ± 3.6	82.4 ± 2.6	41.3 ± 3.3	29.1 ± 1.3	75.2 ± 3.3	42.2 ± 0.2
D6	70.5 ± 1.4	63.0 ± 0.7	64.8 ± 2.2	55.9 ± 1.1	79.7 ± 1.1	45.0 ± 2.7
D7	67.7 ± 2.7	62.1 ± 2.1	45.0 ± 4.7	36.3 ± 3.0	47.8 ± 2.8	48.7 ± 4.6
D8	57.8 ± 2.8	42.7 ± 4.1	56.3 ± 4.5	41.5 ± 0.2	74.8 ± 4.5	39.3 ± 4.5
D9	95.9 ± 1.0	90.0 ± 0.2	59.6 ± 1.7	50.5 ± 5.0	53.5 ± 4.2	12.5 ± 2.4
D10	41.1 ± 0.6	35.0 ± 0.2	35.1 ± 4.3	28.0 ± 4.9	22.8 ± 1.4	8.5 ± 1.0
D11	46.5 ± 0.0	23.3 ± 3.4	43.9 ± 3.7	40.6 ± 0.6	50.8 ± 1.8	33.1 ± 4.8
D12	71.8 ± 1.6	57.2 ± 2.0	68.5 ± 4.0	60.6 ± 2.9	89.1 ± 3.2	77.3 ± 0.7
D13	68.0 ± 3.7	61.6 ± 0.1	70.2 ± 3.6	68.7 ± 4.7	45.4 ± 4.8	25.3 ± 1.6
D14	28.9 ± 2.4	26.2 ± 2.5	39.7 ± 2.9	36.9 ± 1.6	34.8 ± 2.8	24.9 ± 1.8
D15	69.3 ± 1.6	73.7 ± 3.1	40.8 ± 1.3	38.1 ± 3.6	36.7 ± 3.6	23.4 ± 0.6
D16	65.1 ± 1.9	52.3 ± 1.1	34.5 ± 2.2	21.9 ± 1.2	20.4 ± 4.7	17.1± 1.1
D17	38.2 ± 1.7	21.6 ± 1.9	32.6 ± 0.2	19.1 ± 3.7	35.8 ± 4.1	10.0 ± 0.4
D18	21.1 ± 5.0	19.5±3.8	58.5 ± 0.7	48.6 ± 1.1	8.9 ± 1.9	2.7 ± 1.5
D19	74.1 ± 4.9	63.1 ± 4.3	70.7 ± 2.6	53.2 ± 1.0	59.2 ± 4.8	42.8 ± 4.1
E1	61.4 ± 1.4	44.2 ± 0.6	55.0 ± 4.9	43.8 ± 1.3	54.6 ± 4.9	47.8 ± 3.3
E2	67.4 ± 3.7	59.1 ± 0.3	46.2 ± 3.7	28.4 ± 1.3	50.7 ± 2.1	14.3 ± 0.5
E3	55.9 ± 3.2	47.0 ± 0.4	76.4 ± 2.4	61.1 ± 4.1	71.1 ± 3.9	54.2 ± 4.5
E4	86.9 ± 0.5	55.2 ± 0.9	75.2 ± 3.9	54.4 ± 1.5	20.0 ± 2.5	7.6 ± 2.6
E5	72.9 ± 3.5	58.2 ± 0.2	42.8 ± 2.0	40.1 ± 0.7	56.8 ± 0.6	33.6 ± 2.1
E6	97.8 ± 0.8	87.0 ± 4.8	55.9 ± 2.1	39.3 ± 3.5	58.1 ± 4.3	41.1 ± 4.3
E7	96.9 ± 0.4	95.1 ± 1.3	37.7 ± 2.7	34.4 ± 4.7	74.4 ± 3.3	51.5 ± 3.8
E8	93.5 ± 0.1	66.8 ± 1.3	53.9 ± 3.7	41.3 ± 4.0	53.9 ± 3.0	37.2 ± 0.5
E9	97.4 ± 0.1	91.8 ± 0.1	74.1 ± 0.7	63.3 ± 1.2	69.9 ± 0.1	46.4 ± 2.0
E10	67.9 ± 1.7	63.6 ± 3.2	80.8 ± 0.7	76.4 ± 2.3	54.7 ± 0.6	35.9 ± 1.8
E11	50.3 ± 3.1	30.7 ± 2.7	56.2 ± 2.9	19.2 ± 0.9	53.1 ± 4.2	41.1 ± 1.6
E12	90.1 ± 0.6	88.7 ± 3.7	54.5 ± 4.4	43.1 ± 1.1	47.6 ± 4.6	40.4 ± 1.0
E13	94.3 ± 1.2	81.2 ± 3.1	54.6 ± 1.6	47.4 ± 4.4	81.8 ± 1.9	67.5 ± 3.7
E14	88.3 ± 3.5	69.5 ± 1.3	37.0 ± 2.5	28.8 ± 2.4	28.5 ± 4.3	72.6 ± 2.1
E15	97.9 ± 1.0	76.1 ± 1.8	32.9 ± 1.8	25.9 ± 2.4	27.2 ± 4.7	7.1 ± 3.2
E16	97.2 ± 1.7	79.7 ± 4.6	65.2 ± 3.1	54.0 ± 0.6	72.1 ± 4.7	50.5 ± 3.4
E17	88.7 ± 4.1	84.7 ± 3.1	62.3 ± 2.9	58.2 ± 1.3	70.5 ± 1.7	38.1 ± 1.4
E18	68.5 ± 1.4	50.4 ± 3.4	69.6 ± 0.4	60.6 ± 3.4	65.2 ± 1.1	51.5 ± 4.5
E19	74.9 ± 4.9	63.0 ± 4.3	67.4 ± 4.1	43.5 ± 0.7	65.9 ± 1.3	39.4 ± 3.1
BMT[b]	67.8 ± 2.6	47.3 ± 2.7	59.0 ± 0.7	50.8 ± 2.8	62.8 ± 1.7	44.8 ± 5.0
TDC[b]	65.8 ± 0.5	34.6 ± 1.9	60.8 ± 3.4	42.0 ± 2.2	51.0 ± 1.2	22.0 ± 2.4

[a]Average of three replicates. [b]Commercialized bactericides bismerthiazol (BMT) and thiediazole copper (TDC).

ferulic acid methyl ester as the starting materials, involving two intermediates **B** and **C**.[43−45] First, the intermediates **B** and **C** were prepared using a classical literature method with ferulic acid methyl ester used as the starting material.[43,44] Then, the target compounds **D1−D19** were synthesized in accordance with previous reports.[45] Finally, due to the conjugate effect of a double bond in α,β-unsaturated amide, the target compounds **D1−D19** were selectively hydrolyzed to prepare the target compounds **E1−E19**.[44] The details of this synthesis process are shown in the Supporting Information (SI).

***In Vitro* Antibacterial Activity Test.** Using the turbidimetric method in refs 46−48, *in vitro* antibacterial activities of **D1−D19** and **E1−E19** against *Xoo*, *Xac*, and *Xoc* were tested. BMT and TDC were used as positive controls. The test method utilized is given in the Supporting Information.

***In Vivo* Antibacterial Activity Test.** The curative and protective activities of compounds **E6** and **E12** for controlling rice leaf blight were tested *in vivo* by employing a previously reported method with little modification.[49] This test method is provided in the Supporting Information (SI).

Scanning Electron Microscopy (SEM) Characterization. The influence of the most active compound **E12** on *Xoo* was observed using SEM in accordance with methods previously published,[48,50] as also shown in the SI.

***In Vitro* Antifungal Bioassay.** The antifungal activities of **D1−D19** and **E1−E19** against *F. oxysporum* (*F. o.*), *C. mandshurica* (*C. m.*), *C. gloeosporioides* (*C. g.*), *G. zeae* (*G. z.*), *S. sclerotiorum* (*S. s.*), and *R. solani* (*R. s.*) were evaluated using a previously reported method,[51] in which fungicides hymexazol (**HM**) and carbendazim were put to positive control use. The test particulars can be found in the SI.

Insecticidal Activity Test on Plutella Xylostella (P. xylostella). The insecticidal activities of **D1−D19** and **E1−E19** were obtained using previously published methods.[52,53] The insecticide chlorpyrifos was employed as a positive control, and distilled water containing 1% DMSO was used as a blank control.

https://doi.org/10.1021/acs.jafc.1c03892
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 2. EC_{50} Values of Target Compounds against *Xoo*[a]

compd.	toxic regression equation	r	EC_{50} (μg/mL)	compd.	toxic regression equation	r	EC_{50}(μg/mL)
D1	$y = 1.23x + 3.26$	0.92	25.6 ± 1.1	**E1**			
D2				**E2**	$y = 0.41x + 4.10$	0.92	77.7 ± 10.0
D3	$y = 0.63x + 4.11$	0.92	26.8 ± 8.9	**E3**	$y = 0.31x + 4.41$	0.95	83.1 ± 8.9
D4				**E4**	$y = 1.42x + 2.60$	0.92	49.3 ± 5.2
D5	$y = 1.81x + 2.12$	0.9	39.1 ± 2.1	**E5**	$y = 1.13x + 2.96$	0.99	62.9 ± 9.8
D6	$y = 0.71x + 3.77$	0.91	53.5 ± 1.0	**E6**	$y = 1.71x + 2.83$	0.98	14.6 ± 1.5
D7	$y = 0.42x + 4.20$	0.97	78.2 ± 6.1	**E7**	$y = 1.34x + 2.73$	0.93	49.5 ± 8.8
D8				**E8**	$y = 2.02x + 2.09$	0.97	27.2 ± 0.5
D9	$y = 1.57x + 2.58$	0.91	34.6 ± 8.1	**E9**	$y = 1.65x + 2.42$	0.93	24.0 ± 0.3
D10				**E10**	$y = 2.42x + 1.37$	0.97	31.1 ± 1.4
D11				**E11**	$y = 0.46x + 4.19$	0.99	55.3 ± 6.5
D12	$y = 0.88x + 3.38$	0.93	67.8 ± 5.7	**E12**	$y = 1.00x + 3.93$	0.98	11.6 ± 3.6
D13	$y = 2.07x + 1.47$	0.97	49.6 ± 1.1	**E13**	$y = 1.53x + 2.95$	0.92	14.8 ± 1.5
D14				**E14**	$y = 1.25x + 2.97$	0.94	18.4 ± 5.2
D15	$y = 0.81x + 3.94$	0.92	19.9 ± 1.9	**E15**	$y = 2.04x + 2.04$	0.93	28.1 + 1.1
D16	$y = 0.60x + 3.94$	0.94	59.6 ± 4.3	**E16**	$y = 1.59x + 2.96$	0.91	19.1 ± 5.0
D17				**E17**	$y = 1.21x + 3.26$	0.96	27.3 ± 4.3
D18				**E18**	$y = 0.53x + 3.89$	0.97	128.2 ± 5.2
D19	$y = 1.14x + 3.02$	0.95	54.4 ± 6.4	**E19**			
BMT[b]	$y = 0.83x + 3.39$	0.91	84.3 ± 9.1	**TDC**[b]	$y = 1.30x + 2.21$	0.94	137.8 ± 4.3

[a]Average of three replicates. [b]Commercialized bactericides bismerthiazol (BMT) and thiediazole copper (TDC).

Table 3. EC_{50} Values of Target Compounds against *Xoc*[a]

compd.	toxic regression equation	r	EC_{50} (μg/mL)	compd.	toxic regression equation	r	EC_{50}(μg/mL)
D1				**E1**			
D2				**E2**			
D3				**E3**	$y = 0.75x + 3.92$	0.93	29.7 ± 2.3
D4				**E4**	$y = 0.84x + 3.58$	0.91	47.7 ± 4.7
D5				**E5**			
D6	$y = 0.21x + 4.74$	1.00	17.3 ± 8.2	**E6**			
D7				**E7**			
D8				**E8**			
D9	$y = 0.72x + 3.59$	1.00	91.5 ± 5.2	**E9**	$y = 0.68x + 4.02$	0.96	27.0 ± 3.0
D10				**E10**	$y = 0.82x + 3.96$	0.94	18.6 ± 1.9
D11				**E11**			
D12	$y = 0.59x + 3.93$	0.95	63.4 ± 9.6	**E12**			
D13	$y = 0.85x + 3.72$	0.91	31.9 ± 4.1	**E13**	$y = 0.29x + 4.40$	0.92	119.0 ± 2.8
D14				**E14**			
D15				**E15**			
D16				**E16**	$y = 0.32x + 4.50$	0.95	38.7 ± 4.9
D17				**E17**	$y = 0.35x + 4.46$	0.96	32.7 ± 2.3
D18	$y = 0.84x + 3.24$	0.93	122.8 ± 3.2	**E18**	$y = 0.49x + 4.35$	0.92	20.8 ± 6.1
D19	$y = 0.59x + 4.14$	0.96	29.4 ± 7.4	**E19**	$y = 1.22x + 2.50$	0.96	109.9 ± 6.5
BMT[b]	$y = 0.47x + 4.10$	0.98	80.1 ± 4.3	**TDC**[b]	$y = 0.87x + 3.17$	0.97	124.7 ± 5.0

[a]Average of three replicates. [b]Commercialized bactericides bismerthiazol (BMT) and thiediazole copper (TDC).

RESULTS AND DISCUSSION

Chemistry. As exhibited in the synthetic route (Scheme 1). The intermediate **B** was easily prepared *via* a reaction of starting material **A** with benzyl chloride or benzyl bromine in the presence of KI and K_2CO_3 using *N*,*N*-dimethylformamide as a solvent. Intermediate **B** was hydrolyzed to obtain intermediate **C**, which underwent a reaction with methyl 1-aminocyclohexane-1-carboxylate to obtain the title compounds **D1**−**D19**.[43−45] After the hydrolysis of the title compounds **D1**−**D19**, the title compounds **E1**−**E19** were prepared.[44] The compounds **D1**−**D19** and **E1**−**E19** were characterized by ^{1}H, ^{13}C, and ^{19}F NMR, and high-resolution liquid chromatography−mass spectroscopy (HRMS), and the spectrum information is listed in the Supporting Information.

Analysis of *In Vitro* Activity toward *Xoo*, *Xoc*, and *Xac*. *In Vitro Activity Analysis on Xoo.* The preliminary activity *in vitro* against bacterium *Xoo* is listed in Table 1, and the concentration for 50% of maximal effect (EC_{50}) is listed in Table 2. As shown in Table 1, most of the title compounds exhibited good activity at both 100 and 50 μg/mL. Particularly, the activities of compounds **D9**, **E6**, **E7**, **E8**, **E9**, **E13**, **E15**, and **E16** were close to 100% at 100 μg/mL, and compounds **D3**, **D5**, **E4**, **E12**, **E4**, **E12**, **E14**, and **E17** recorded more than 88% activities at 100 μg/mL, which were much higher than that of BMT (67.8%) and TDC (65.8%). While the concentration was set to

https://doi.org/10.1021/acs.jafc.1c03892
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 4. Protection and Curative Activities of Compound E6 and E12 against Rice Bacterial Leaf Blight under Greenhouse Conditions at 200 μg/mL *In Vivo*

	protection activity (14 days after spraying)			curative activity (14 days after spraying)		
treatment	morbidity (%)	disease index (%)	control efficiency (%)[a]	morbidity (%)	disease index (%)	control efficiency (%)[a]
E6	100	44.6	49.4	100	51.5	38.6
E12	100	46.7	47.0	100	46.7	44.4
BMT	100	56.9	35.3	100	56.0	33.2
TDC	100	60.0	31.9	100	60.0	28.5
CK[b]	100	88.1		100	83.9	

[a]Statistical analysis was conducted by ANOVA method under the condition of assumed equal variances ($P > 0.05$) and not assumed equal variances ($P < 0.05$). Different uppercase letters indicate the values of protection activity with significant difference among different treatment groups at $P < 0.05$. [b]Blank control.

Figure 2. Protective activities of compounds **E6** and **E12** against rice leaf blight at 200 μg/mL.

50 μg/mL, the activities of compounds **D5**, **D9**, **E7**, **E9**, **E12**, and **E13** were also higher than 80%. As shown in Table 2, the EC_{50} values of the active title compounds ranged from 11.6 to 128.2 μg/mL. Most of their EC_{50} values were much lower than these of BMT (84.3 μg/mL) and TDC (137.8 μg/mL).

In Vitro Activity Analysis on Xoc. The preliminary activity *in vitro* against bacterium *Xoc* is listed in Table 1, and the EC_{50} is listed in Table 3. Compared with the activity toward *Xoo*, the activity toward *Xoc* was relatively lower, but there were also 15 compounds that had lower EC_{50} values ranging from 17.3 to 122.8 μg/mL, compared to that of TDC (124.7 μg/mL), and eleven compounds that had lower EC_{50} values than that of BMT (80.1 μg/mL).

In Vitro Activity Analysis on Xac. With regards to controlling *Xac* bacterium, most of the title compounds did not exhibit good activity, but compounds **D12** and **E13** showed higher activities, beyond 80% higher, than both BMT (62.8%) and TDC (51.0%) (Table 1).

Structure−Activity Relationship Analysis of Compounds D1−D19 and E1−E19. Based on the results of Tables 1−3, the structure−activity relationship (SAR) was deduced, which concluded the following points: (i) With the same R group, the antibacterial activities of compounds **D1−D19** and their hydrolysates **E1−E19** are quite different. Generally, most of the hydrolysate **E** with group −COOH had higher activity *in vitro* than that of compound **D**. For example, the active compounds **E6** (R = 2-F-3-Cl-Ph; EC_{50} 14.6 μg/mL),

https://doi.org/10.1021/acs.jafc.1c03892
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Figure 3. Curative activities of compounds **E6** and **E12** against rice leaf blight at 200 μg/mL.

E12 (R = 3,4-diF-Ph; EC_{50} 11.6 μg/mL), and **E13** (R = 3,5-diF-Ph; EC_{50} 14.8) had much lower EC_{50} values against *Xoo* than compounds **D6** (R = 2-F-3-Cl-Ph; EC_{50} 53.5 μg/mL), **D12** (R = 3,4-diF−Ph; EC_{50} 67.8 μg/mL), and **D13** (R = 3,5-diF-Ph; EC_{50} 49.6 μg/mL). The carboxyl strengthening the hydrophobic reaction could be a potential reason for these increases in activity.[36] (ii) The R group including a weak electron-donating group $-CH_3$, strong electron-withdrawing groups $-CF_3$, $-F$, and $-OCF_3$, and moderate electron-withdrawing groups $-Cl$ and $-Br$ could lead to significant differences in antibacterial activity at different positions of the phenyl group. In terms of *Xoo*, the location effect is listed as follows: **D1** (R = 2-CH_3-Ph) > **D2** (R = 3-CH_3-Ph); **D4** (R = 4-Cl-Ph) > **D11** (R = 2-Cl-Ph); **D10** (R = 3-F-Ph) > **D19** (R = 4-F-Ph); **D12** (R = 3,4-diF-Ph) > **D13** (R = 3,5-diF-Ph) > **D17** (R = 2,5-diF-Ph); **E2** (R = 3-CH_3-Ph) > **E1** (R = 2-CH_3-Ph); **E4** (R = 4-Cl-Ph) > **E11** (R = 2-Cl-Ph); **E19** (R = 4-F-Ph) > **E10** (R = 3-F-Ph); and **E13** (R = 3,5-diF-Ph) > **E12** (R = 3,4-diF-Ph) > **E17** (R = 2,5-diF-Ph). The location effect also existed when controlling *Xoc* and *Xac*. (iii) Halogen atoms substituted in phenyl significantly affected the antibacterial activity. When one or two halogen atoms are substituted in phenyl, the title compounds could have a higher antibacterial activity against both *Xoc* and *Xac* to some degree when compared with **D15** (R = Ph) and **E15** (R = Ph). Moreover **D18**, in which the phenyl is substituted by the Br atom, or in other compounds where the phenyl is substituted by Cl atom, showed relatively lower activity compared with the compounds where phenyl was substituted by an F atom including **D3** and **E3** (R = 4-F-5-CF_3-Ph); **D6** and **E6** (R = 2-F-3-Cl-Ph); **D7** and **E7** (R = 2-F-6-F-Ph); **D8** and **E8** (R = 2-Cl-4-F-Ph); **D9** and **E9** (R = 4-OCF_3-Ph); **D10** and **E10** (R = 3-F-Ph); **D13** and **E13** (R = 3,5-diF-Ph); and **D19** and **E19** (R = 4-F-Ph). The potential factor is that the introduction of fluorine atoms could improve the lipophilicity and bioavailability of these compounds.[54,55]

***In Vivo* Antibacterial Activity Analysis.** According to the results in Tables 1−3, it was clear that the title compounds of hydrolysates **E6** and **E12** showed the lowest EC_{50} values against *Xoo*, reaching 14.6 and 11.6 μg/mL. Therefore, **E6** and **E12** were selected to verify their applications within rice crops on rice blight at 200 μg/mL in a greenhouse. As shown in Table 4 and Figures 2 and 3, the protective activities of both **E6** and **E12** were 49.4 and 47.0%, which were both higher than BMT (35.3%) and TDC (31.9%). In addition, the curative activity of both **E6** and **E12** were measured at 38.6 and 44.4%, which were also higher than those of BMT (33.2%) and TDC (28.5%). These results showed that compounds **E6** and **E12** had an effective efficiency on rice bacterial blight in rice crops and could therefore be considered as antibacterial agent candidates.

https://doi.org/10.1021/acs.jafc.1c03892
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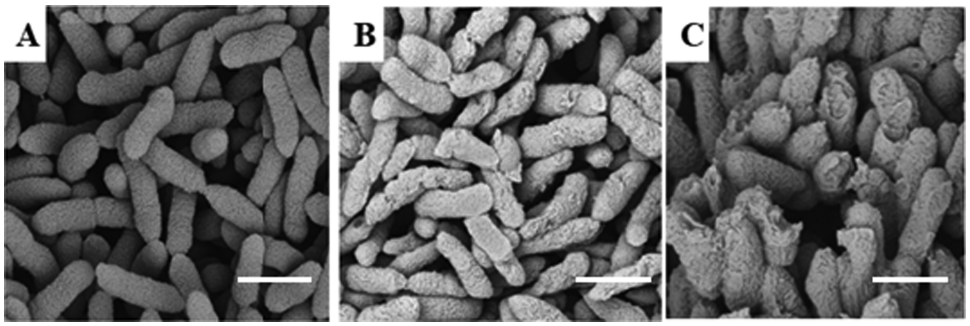

Figure 4. SEM images for the influence of **E12** on *Xoo* at (A) 0 μg/mL, (B) 25.0 μg/mL, and (C) 50.0 μg/mL.

Table 5. Antifungal Activities of the Target Compounds D1−D19 and E1−E19 at 50 μg/mL *In Vitro*[a]

	inhibition rate (%)					
compd.	*F. o.*[b]	*C. m.*[c]	*C. g.*[d]	*G. z.*[e]	*S. s.*[f]	*R.s.*[g]
D1	7.8 ± 2.6	9.1 ± 1.0	20.9 ± 2.4	10.7 ± 0.4	28.1 ± 1.2	54.0 ± 1.3
D2	0	7.0 ± 0.8	22.1 ± 3.7	11.5 ± 1.0	28.1 ± 3.9.	54.9 ± 2.2
D3	5.7 ± 1.0	8.2 ± 1.3	24.1 ± 3.2	24.2 ± 2.5	23.9 ± 1.6	53.6 ± 2.7
D4	6.3 ± 2.0	10.5 ± 0.9	32.1 ± 1.0	12.5 ± 5.0	16.3 ± 1.2	99.4 ± 1.0
D5	15.0 ± 3.3	9.9 ± 2.5	21.5 ± 3.7	44.2 ± 0.5	36.0 ± 0.9	12.8 ± 3.4
D6	12.1 ± 1.7	17.3 ± 3.5	22.4 ± 1.4	4.3 ± 1.7	23.4 ± 0.9	98.2 ± 0.0
D7	16.4 ± 4.0	14.3 ± 4.0	19.1 ± 0.9	6.8 ± 0.9	29.1 ± 0.0	57.6 ± 2.2
D8	11.8 ± 3.6	11.4 ± 0.9	24.2 ± 0.5	32.5 ± 3.6	17.1 ± 1.2	44.6 ± 0.0
D9	8.9 ± 2.2	7.0 ± 1.8	21.8 ± 0.0	20.6 ± 3.9	20.7 ± 0.5	49.5 ± 0.4
D10	11.2 ± 2.6	8.5 ± 0.5	20.6 ± 2.3	10.0 ± 2.6	19.7 ± 0.0	15.2 ± 4.5
D11	12.9 ± 1.7	14.3 ± 0.5	22.4 ± 3.7	23.9 ± 3.7	29.1 ± 1.6	45.1 ± 0.4
D12	13.5 ± 3.0	13.7 ± 3.3	13.6 ± 1.8	19.2 ± 2.1	23.6 ± 1.6	32.1 ± 2.7
D13	10.9 ± 3.9	14.6 ± 1.0	28.6 ± 3.2	20.1 ± 1.3	13.9 ± 0.6	41.1 ± 3.6
D14	8.6 ± 1.7	13.5 ± 4.8	29.8 ± 2.9	22.2 ± 3.1	25.7 ± 2.5	48.6 ± 1.3
D15	5.7 ± 2.2	8.5 ± 1.0	10.6 ± 0.5	0	20.7 ± 1.6	30.4 ± 0.9
D16	12.5 ± 1.3	9.4 ± 1.3	19.7 ± 5.0	49.6 ± 0.9	35.8 ± 2.8	47.7 ± 3.1
D17	14.4 ± 3.0	12.9 ± 4.0	20.3 ± 1.0	16.2 ± 0.9	24.4 ± 2.8	38.1 ± 4.1
D18	26.4 ± 1.8	15.8 ± 1.8	13.6 ± 4.5	38.7 ± 2.5	34.1 ± 3.0	79.9 ± 2.4
D19	13.5 ± 0.5	14.6 ± 1.8	7.3 ± 1.6	0	33.4 ± 0.4	50.6 ± 2.9
E1	12.0 ± 0.9	8.9 ± 0.3	7.4 ± 0.4	15.9 ± 2.3	71.5 ± 2.8	100.0 ± 1.0
E2	3.4 ± 2.6	5.9 ± 2.5	12.3 ± 0.0	27.7 ± 3.2	26.9 ± 2.1	36.2 ± 3.5
E3	50.1 ± 2.2	13.0 ± 2.8	12.7 ± 0.4	22.7 ± 0.9	31.1 ± 1.8	56.4 ± 4.8
E4	33.7 ± 3.9	32.6 ± 3.7	18.0 ± 3.1	39.9 ± 5.0	47.3 ± 0.5	41.3 ± 4.8
E5	0	0	0	0	0	0
E6	33.0 ± 0.3	23.4 ± 3.3	26.3 ± 0.9	8.6 ± 1.4	34.2 ± 2.3	4.2 ± 2.0
E7	21.4 ± 0.9	5.7 ± 1.5	31.9 ± 1.0	12.1 ± 4.2	24.8 ± 3.0	83.6 ± 2.9
E8	43.4 ± 1.1	8.1 ± 2.4	43.9 ± 0.9	3.6 ± 0.9	24.8 ± 0.0	41.3 ± 1.0
E9	41.4 ± 0.4	30.7 ± 4.1	37.1 ± 4.1	49.7 ± 2.3	67.1 ± 0.4	84.0 ± 1.6
E10	36.4 ± 4.6	5.5 + 1.9	19.3 ± 3.5	12.4 ± 3.0	1.1 ± 1.7	95.6 ± 1.4
E11	42.0 ± 1.5	23.6 ± 4.0	30.1 ± 3.7	50.9 ± 3.6	33.6 ± 3.5	22.3 ± 4.0
E12	24.3 ± 1.2	0	23.7 ± 2.3	0	24.2 ± 3.0	4.8 ± 1.0
E13	22.3 ± 1.8	13.2 ± 2.2	12.0 ± 0.5	26.4 ± 4.8	25.9 ± 2.7	81.2 ± 0.5
E14	22.6 ± 1.7	0	29.5 ± 1.0	0	17.7±1.3	82.2 ± 2.4
E15	16.9 ± 2.0	5.9 ± 1.1	34.7 ± 3.9	0	20.8 ± 1.3	82.2 ± 2.4
E16	36.0 ± 0.9	12.2 ± 0.3	38.3 ± 3.7	50.9 ± 3.6	42.1 ± 4.9	56.9 ± 4.0
E17	31.7 ± 3.8	38.2 ± 2.6	32.5 ± 1.8	0.6 ± 4.2	31.6 ± 1.7	42.3 ± 1.9
E18	27.4 ± 4.4	29.8 ± 1.6	39.2 ± 3.1	29.7 ± 4.2	38.5 ± 2.6	28.1 ± 3.1
E19	18.0 ± 3.7	10.0 ± 1.7	21.3 ± 3.3	0	30.5 ± 2.0	57.7 ± 0.0
HM[h]	37.2 ± 3.7	43.0 ± 3.1	61.2 ± 2.3	58.5 ± 4.3	48.6 ± 1.9	65.7 ± 1.4
carbendazim	98.3 ± 0.1	100.0 ± 0.0	99.4 ± 1.0	98.3 ± 0.0	97.0 ± 2.1	100.0 ± 0.0

[a]The average of three trials. [b]*F. o.* = *FF. oxysporum*. [c]*C. m.* = *C. mandshurica*. [d]*C. g.* = *C. gloeosporioides*. [e]*G. z.* = *G. zeae*. [f]*S. s.* = *S. sclerotiorum*. [g]*R. s.* = *R. solani*. [h]Commercialized agrofungicide hymexazol (HM).

Scanning Electron Microscopy (SEM) Characterization Analysis. With the help of SEM, morphological observation of bacterium *Xoo* was carried out and morphological differences following treatment with the most active compound **E12** were

https://doi.org/10.1021/acs.jafc.1c03892
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Table 6. EC_{50} values of Active Compounds against Fungi *S. s.*, and *R. s. In Vitro*[a]

comp.	pathogens	regression equation	correlation coefficient (r)	EC_{50}(μg/ mL)
E1	*S. s.*	$y = 0.41x + 4.27$	0.94	60.6 ± 4.6
E9	*S. s.*	$y = 1.12x + 3.16$	0.94	43.8 ± 4.5
HM[b]	*S. s.*	$y = 0.99x + 3.03$	0.94	96.3 ± 3.6
carbendazim	*S. s.*	$y = 1.31x + 4.86$	0.92	1.3 ± 0.7
D4	*R. s.*	$y = 1.89x + 3.58$	0.95	5.6 ± 1.1
D6	*R. s.*	$y = 2.27x + 2.57$	0.90	11.7 ± 0.7
D18	*R. s.*	$y = 0.66x + 4.33$	0.98	10.1 ± 1.3
E1	*R. s.*	$y = 0.41x + 5.93$	0.94	0.005 ± 0.004
E7	*R. s.*	$y = 0..51x + 4.78$	0.96	2.6 ± 1.0
E9	*R. s.*	$y = 0..47x + 4.91$	0.99	1.6 ± 1.0
E10	*R. s.*	$y = 0.64x + 5.28$	0.91	0.370 ± 0.117
E13	*R. s.*	$y = 0.29x + 5.23$	0.95	0.159 ± 0.002
E14	*R. s.*	$y = 0.53x + 4.74$	0.91	3.1 ± 0.3
E15	*R. s.*	$y = 0.44x + 4.85$	0.96	2.1 ± 0.4
HM[b]	*R. s.*	$y = 0.76x + 3.57$	0.92	74.8 ± 0.7
carbendazim	*R. s.*	$y = 1.21x + 5.09$	0.90	0.8 ± 0.5

[a]Average of three replicates. [b]The agricultural fungicide hymexazol (HM) was used to compare the antifungal activities of the title compounds.

analyzed in more depth. As shown in Figure 4 A, the bacterium *Xoo* grown without the addition of the compound solution treatment had slender cells with a hard surface, thus classified as a good appearance. After the treatment of **E12**, the cell membranes of *Xoo* collapsed to a varying degree; as the **E12** concentrations increased, cell membrane damage severity also increased (Figure 4B,C). From the SEM characterization of *Xoo*, it was clear that **E12** resulted in cell membrane deformation or rupture, which could be the potential mechanism to inhibit bacterial growth.

***In Vitro* Antifungal Activity Analysis.** The preliminary antifungal activities of **D1**−**D19** and **E1**−**E19** against six fungi, including *F. o.*, *C. m.*, *C. g.*, G. z., S. s., and *R. s.* are concluded in Table 5, and the EC_{50} values of the active compounds are shown in Table 6. The data revealed that the compounds displayed good to excellent antifungal activity against *F. o., S. s., and R. s.* at 50 μg/mL, particularly **E3** and **E8**, which had good activities at 50.1 and 43.4% against *F. o.*, **E9**, which had good activities at 41.4, 67.1, and 84.0% against *F. o.*, *S. s.*, and *R. s.*, respectively, **E11**, which had good activity at 42.0% against *F. o.*, **E1**, which had excellent activity at 71.5% and 100.0% against *S. s.* and *R. s.*, respectively, **D4**, which showed significantly high activity at 99.4% against *S. s.*, **D6**, which showed significantly high activity at 98.2% against *S. s.*, **D18**, which showed excellent activity at 79.9% against *S. s.*, **E7**, which showed excellent activity at 83.6% against *S. s.*, **E10**, which showed significantly high activity at 95.6% against *S. s.*, **E13**, which showed excellent activity at 81.2% against *S. s.*, **E14**, which showed excellent activity 82.2% against *S. s.*, and **E15**, which showed excellent activity at 82.2% against *S. s.* All of the antifungal activity levels above were higher than those of **HM** (37.2, 48.6, and 65.7% against *F. o., S. s.*, and *R. s.*, respectively), and antifungal activity against *R. s.* of compounds **D4**, **D6**, **E1**, and **E10** were also similar to that of carbendazim 100%. As shown in Table 6, the active compounds **E1** and **E9** provided impressive actions toward *S. s.*, with the EC_{50} values of 60.4 and 43.8 μg/mL, respectively, which were evidently higher than that of HM (96.3 μg/mL). Moreover, the active compounds **D4**, **D6**, **D18**, **E1**, **E7**, **E9**, **E10**, **E13**, **E14**, and **E15** had the EC_{50} values of 5.6, 11.7, 10.1, 0.005, 2.6, 1.6, 0.37, 0.159, 3.1, and 2.1 μg/mL, respectively, much better than the HM levels (74.8 μg/mL). The EC_{50} values of compounds **E1**, **E10**, and **E13** were also lower than that of carbendazim (0.8 μg/mL). Among them, the most active compound **E1** (R = 2-CH_3−Ph) had the lowest EC_{50} value, which reached 0.005 μg/mL and effectively controlled the growth of the fungus *R. s.* at a range of concentrations (Figure S1). From Tables 5 and 6, it can be observed that the synthesized compounds had the highest activity against *R. s.* but no activity against *C. m.*, *C. g.*, or *G. z.* It should be noted that most of the hydrolysates **E** obtained *via* hydrolysis of **D** also exhibited a higher activity than the title compound **D** itself. These results indicated that synthesized novel ferulic amide Ac6c derivatives could also be considered as antifungal agents for further structural modification.

Insecticidal Activity Analysis. Table 7 lists the results of the insecticidal activities for **D1**−**D19** and **E1**−**E19** against *P.*

Table 7. Insecticidal Activities of the Title Compounds against *P. xylostella*

compd.	*P. xylostella* 500 μg/mL mortality rate (%)[a]	compd.	*P. xylostella* 500 μg/mL mortality rate (%)[a]
D1	50.3 ± 1.0	**E1**	33.3 ± 1.5
D2	40.2 ± 1.0	**E2**	37.1 ± 3.0
D3	40.6 ± 2.5	**E3**	47.4 ± 3.8
D4	55.1 ± 0.5	**E4**	57.6 ± 1.7
D5	53.9 ± 1.6	**E5**	80.6 ± 0.6
D6	37.3 ± 1.2	**E6**	23.8 ± 1.2
D7	45.4 ± 0.5	**E7**	63.0 ± 0.6
D8	6.7 ± 1.0	**E8**	33.4 ± 1.0
D9	67.9 ± 0.5	**E9**	20.7 ± 2.1
D10	57.8 ± 0.5	**E10**	73.4 ± 1.5
D11	37.0 ± 0.5	**E11**	50.7 ± 4.2
D12	60.3 ± 0.5	**E12**	20.9 ± 0.6
D13	73.7 ± 2.0	**E13**	47.3 ± 1.0
D14	57.3 ± 2.0	**E14**	5.4 ± 3.2
D15	63.1 ± 0.5	**E15**	33.7 ± 1.7
D16	67.5 ± 1.0	**E16**	50.9 ± 2.0
D17	40.9 ± 1.0	**E17**	50.2 ± 2.0
D18	47.4 ± 0.5	**E18**	30.8 ± 1.5
D19	50.6 ± 2.3	**E19**	30.1 ± 4.3
blank control	0	**chlorpyrifos**	100.0 ± 0.0

[a]average of three replicates.

https://doi.org/10.1021/acs.jafc.1c03892
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xylostella at 500 μg/mL. Some compounds showed moderate insecticidal activity, such as compounds **D13** (R = 3.5-diF−Ph), **E5** (R = $CH_2CH_2CH_2Cl$), and **E10** (R = 3-F−Ph) showed activities of 73.7, 80.6, and 73.4%, respectively. Obviously, the synthesized compounds did not exhibit good insecticidal activity toward *P. xylostella*, but the structural modification of these structures for exploring the insecticidal molecules is ongoing in our laboratory.

In conclusion, novel ferulic amide Ac6c derivatives were synthesized in the present work, and bioassays targeting three kinds of bacteria (*Xoo*, *Xoc*, and *Xac*), six kinds of fungi (*F. oxysporum*, *C. mandshurica*, *C. gloeosporioides*, *G. zeae*, *S. sclerotiorum*, and *R. solani*), and the pest *P. xylostella* were carried out. Some more active synthesized compounds were compared against commercial pesticides, and screening indicated that they should be considered as agrochemical candidates, such as **E6** and **E12**, which showed significantly higher activity against *Xoo* and also performed well in the pot experiment and SEM of *in vivo* studies. **E1** whose EC_{50} value reached 0.005 toward the fungus *R. solani* was an example. Through the structure−activity relationship, it was shown that in most cases, the hydrolysis of the title compound **D** resulted in the more active title compound **E**, which could be an inspiration for the discovery of agrochemical agents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c03892.

Mycelium growth and extension of *R. solani* after treatment with different concentrations of **E1** on agar medium (Figure S1). All of the general procedures of the experiments, the characterizations, physical, analytical data, and NMR copies of the synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to express our thanks for the financial support from NSFC (Natural Science Foundation of China) (Nos. 21762012, 32072445, and 21562012), the Program of Introducing Talents to Chinese Universities (111 Program, D20023), and the S & T Planning Project of Guizhou Province (Nos. [2017] 1402 and [2017] 5788).

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