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Short communication

Design, synthesis and *in vitro* antibacterial/antifungal evaluation of novel 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl)quinoline-3-carboxylic acid derivatives

Zhiyi Yu [a], Guanying Shi [b], Qiu Sun [a], Hong Jin [c], Yun Teng [b], Ke Tao [b], Guoping Zhou [a], Wei Liu [a], Fang Wen [c], Taiping Hou [b,*]

[a] West China School of Pharmacy, Sichuan University, Chengdu 610064, PR China
[b] Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu, Sichuan 610064, PR China
[c] Key Laboratory of Green Chemistry and Technology of Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan 610064, PR China

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ABSTRACT

A series of 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl)quinoline-3-carboxylic acid (norfloxacin) derivatives were prepared according to the principle of combinating bioactive substructures and tested for their activities against five plant pathogenic bacteria and three fungi *in vitro*. The preliminary bioassays indicated that almost all synthesized target compounds retained the antibacterial activities of norfloxacin and had some antifungal activities as carboxylic acid amide compounds. The activities of compounds **1** and **22** against *Xanthomonas oryzae* were better than norfloxacin and all tested compounds had better antibacterial activities as compared to the agricultural streptomycin sulfate (a commercial bactericide) against *X. oryzae*, *Xanthomonas axonopodis* and *Erwinia aroideae*. Additionally, compounds **2** and **20** displayed good antifungal activities against *Rhizoctonia solani* and their inhibition of growth reached 83% and 94% respectively at the concentration of 200 mg/L.

1. Introduction

Ever since the discovery of the first marketed quinolone, nalidixic acid (a naphthyridine) in 1962 by Lesher et al. [1,2], numerous structural modifications have been made in the quinoline nucleus in order to produce agents with a broader spectrum of activity and applicability [3]. The real breakthrough didn't come until the 1980s with the introduction of a fluorine molecule into this basic nucleus at position R6 renaming the compound fluoroquinolone [3,4]. Since then, fluoroquinolones have been widely and rapidly adopted in the treatment of ocular infections, with topical, intravitreal and systemic routes of administration being used, because they have been shown in most situations to be equivalent to combination therapies and they are also effective against polyresistant organisms such as *Pseudomonas aeruginosa* and Enterobacteriaceae [5–7]. Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazineyl)quinoline-3-carboxylic acid], a 6-fluorinated compound with a piperazine ring at position R7, is one of the commonly used fluoroquinolones, and it has activities against a broad spectrum of gram-negative and certain gram-positive bacteria [1,8]. Although norfloxacin has already been widely used and studied in veterinary and human medicine [9], its application to pesticide science has not been reported to our knowledge.

In the agricultural chemistry field, carboxylic acid amide fungicides have played an important role in pesticide science. Since the first carboxylic acid amide fungicide carboxin was discovered by von Schmeling and Kulla [10], a series of carboxylic acid amide fungicides with novel structures have successively emerged [11]. These carboxylic acid amide fungicides can inhibit the growth of pathogens and cause their eventual death by interfering with the pathogens' respiration [12,13]. Nowadays, they have been mainly used to control diseases caused by plant pathogenic fungi and some of them can also control diseases caused by plant pathogenic bacteria [12].

In this context, the principle of combinating bioactive substructures was used in this study in order to integrate norfloxacin with carboxylic acid amide fungicides to search for novel lead compounds with a broader antimicrobial spectrum and better biological activities (Fig. 1 shown as an example in which furcarbanil is a marketed fungicide). Herein, different kinds of carboxylic

* Corresponding author. Tel.: +86 28 85410992.
E-mail address: houtp@scu.edu.cn (T. Hou).

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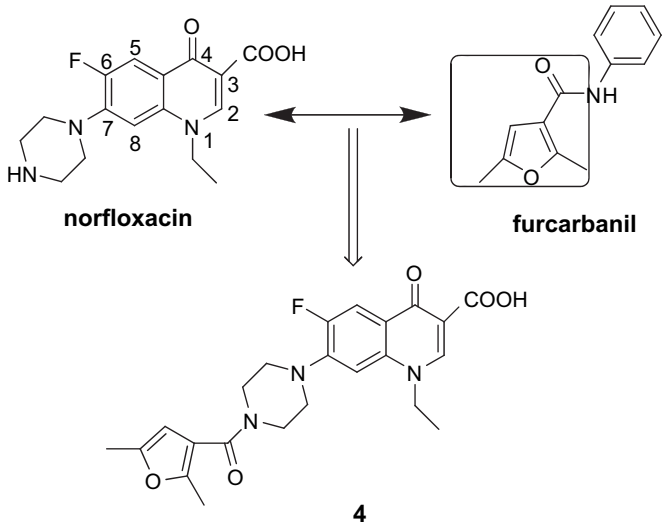


Fig. 1. Design of compound **4**.

acids regarded as pharmacophores among carboxylic acid amide fungicides have been connected to the amino group in C7-piperazinyl-[norfloxacin] to give a series of novel compounds (Table 1) and a detailed discussion of the methodology used to prepare the aforementioned compounds is given. Their bioactivities against five plant pathogenic bacteria (*Xanthomonas oryzae*, *Xanthomonas axonopodis*, *Erwinia aroideae*, *Bacillus subtilis* and *Ralstonia solanacearum*) and three plant pathogenic fungi (*Rhizoctonia solani*, *Gibberella zeae* and *Valsa mali*) were also evaluated, and the results are discussed subsequently.

2. Results and discussion

2.1. Chemistry

The intermediates **1a**, **2a**, **3a**–**3e** and **4a**–**4i** used in this study were synthesized by Method 1 [14], 2 [15], 3 [16] or 4 [17,18] depicted in Scheme 1 according to the literatures and they were used in the subsequent reactions without further purification. 3-Methylbenzofuran-2-carboxylic acid and 2,4,5-trimethylfuran-3-carboxylic acid were synthesized in other studies by our laboratory, and the other carboxylic acids were purchased from commercial sources.

The synthesis pathways of all target compounds are shown in Schemes 2 and 3. Their ^{1}H NMR data and melting points are listed in the synthesis procedures of the title compounds. Compounds **1**–**8**, **10**–**28** were prepared by the method shown in Scheme 2 successfully. Nevertheless, compound **9** couldn't be synthesized by the same method since the nicotinoyl chloride synthesized in Scheme 2 existed in the form of salt and was not suitable for the next reaction condition. However, compound **9** could be synthesized using the method expressed in Scheme 3 and the yield was relatively good [19]. Before the procedure shown in Scheme 2 was discovered, we had tried many other ways to synthesize these compounds such as by reacting in a sodium hydroxide solution or tetrahydrofuran and triethylamine solution with carboxylic acid chloride and norfloxacin and so on. Unfortunately, almost all of them ended in failure because of too many by-products and poor yields of the target compounds.

2.2. Biological activity

Compounds **1**–**28** and norfloxacin were evaluated *in vitro* to explore their antibacterial activities using the agar well-diffusion method [20,21] and their antifungal activities were also studied using the plate growth rate method [22,23].

2.2.1. Antibacterial activity

The antibacterial activities of all tested compounds against *X. oryzae*, *X. axonopodis*, *E. aroideae*, *B. subtilis* and *R. solanacearum* were screened at a concentration of 50 mg/L using the agar well-diffusion method. The results were calculated and expressed as diameters of inhibition to bacteria (Table 2).

As shown in Table 2, all the title compounds (**1**–**28**) retained the antibacterial activities of norfloxacin (the parent compound) *in vitro*. In particular, compounds **1** and **22** had better antibacterial activities than norfloxacin against *X. oryzae*. In addition, the antibacterial activities of all compounds, including norfloxacin, against *X. oryzae*, *X. axonopodis* and *E. aroideae* were better than the agricultural streptomycin sulfate, a commercial bactericide.

The diameters of inhibition of compounds **1**–**28** against *X. oryzae* ranged from 4.44 to 22.94 mm at a concentration of 50 mg/L, while that of norfloxacin was 22.19 mm. The preliminary bioassay results indicated that compounds **2**–**21** and **23**–**28** had lower activities than norfloxacin, but compounds **1** and **22** were more active than the parent compound. Furthermore, all compounds tested had better activities than the agricultural streptomycin sulfate *in vitro*.

When it comes to *X. axonopodis* and *E. aroideae*, the preliminary bioassay results indicated a lower antibacterial activity by tested compounds **1**–**28** than norfloxacin at a concentration of 50 mg/L. However, all of them were more active than the agricultural streptomycin sulfate *in vitro*. The diameters of inhibition of compound **22** against *X. axonopodis* and *E. aroideae* were 15.83 and 17.85 mm respectively, and the values were close to norfloxacin whose inhibition zones were 17.74 and 21.52 mm respectively.

Compounds **1**, **22**, **24** and **25**, for which the diameters of inhibition against *B. subtilis* were 22.49, 20.66, 20.08 and 22.74 mm respectively, displayed similar activities as compared with the agricultural streptomycin sulfate (the diameter of inhibition for it was 24.99 mm). Nevertheless, all the other compounds tested had much lower activities than the agricultural streptomycin sulfate. In addition, the preliminary test results also showed that all synthesized compounds, including the agricultural streptomycin sulfate, were less active than norfloxacin.

The activities of all synthesized compounds were poor against *R. solanacearum* and much lower than the agricultural streptomycin sulfate. At the same time, norfloxacin had a better activity than both the title compounds and the agricultural streptomycin sulfate.

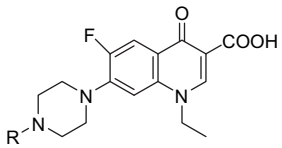
2.2.2. Antifungal activity

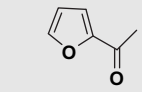
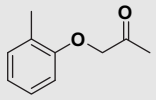
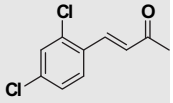
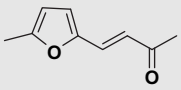
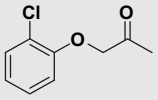
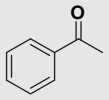
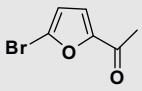
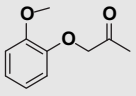
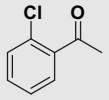
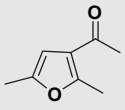
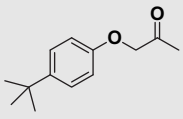
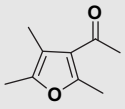
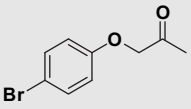
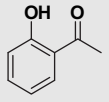
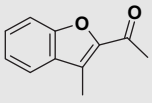
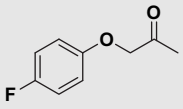
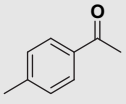
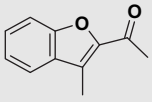
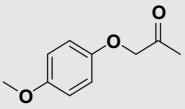
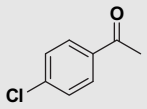
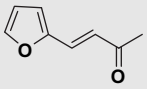
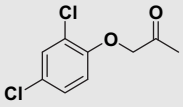
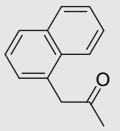
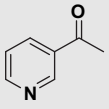
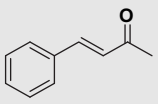
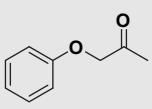
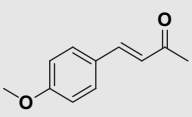
Synthesized target compounds **1**–**28** and norfloxacin were screened for their anti-plant pathogenic fungi activities *in vitro* and the inhibition of growth of the antifungal agents at 200 mg/L is summarized in Table 3.

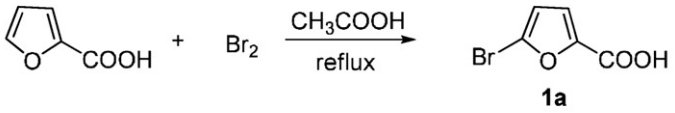
The result of the target compounds' activities against *R. solani in vitro* showed that almost all the target compounds except compounds **13**, **21** and **25** were more active than norfloxacin, and the inhibition of growth by compounds **2** and **20** was up to 83% and 94% respectively which indicated that their activities were relatively close to carbendazim (its inhibition of growth was 100%), a commercial fungicide.

Against *G. zeae* and *V. mali*, the preliminary bioassay results indicated that the activities of all tested compounds were not very good as compared to carbendazim, a commercial fungicide.

Table 1
Synthesized norfloxacin derivatives.

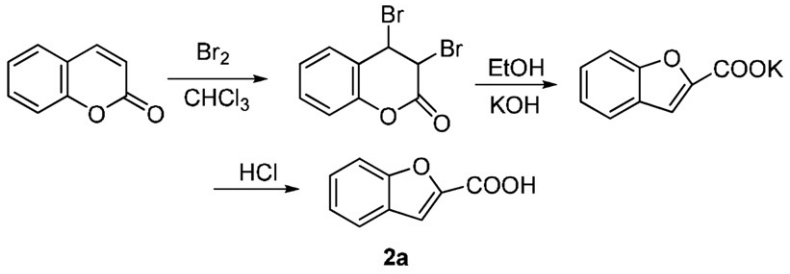


Compound	R	Compound	R	Compound	R
1		**11**		**21**	
2		**12**		**22**	
3		**13**		**23**	
4		**14**		**24**	
5		**15**		**25**	
6		**16**		**26**	
7		**17**		**27**	
8		**18**		**28**	
9		**19**			
10		**20**			



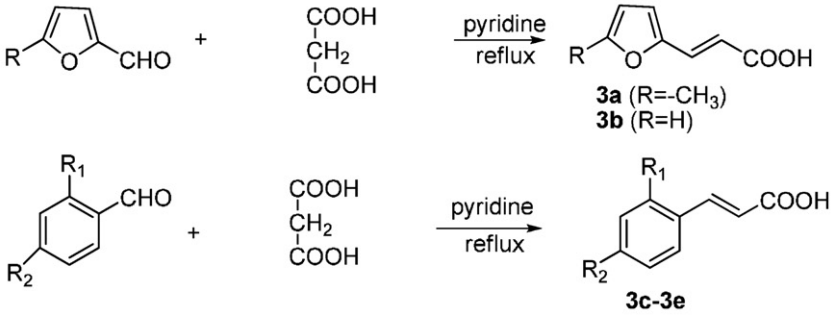
1a

Method 1. Preparation of 5-bromo-2-furoic acid



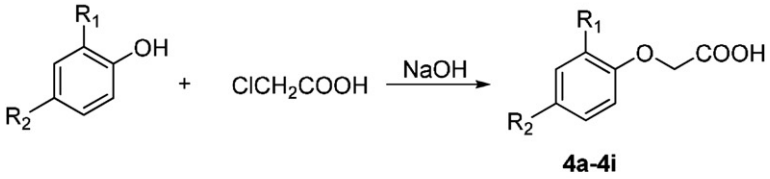
2a

Method 2. Preparation of Benzofuran-2-carboxylic acid



3c:R_1=R_2=H **3d**:R_1=H, R_2=-OCH_3 **3e**:R_1=R_2=Cl

Method 3. Preparation of substituted acrylic acids

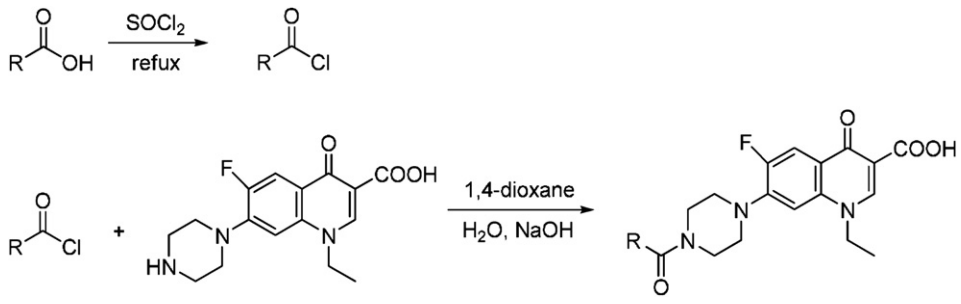


4a-4i

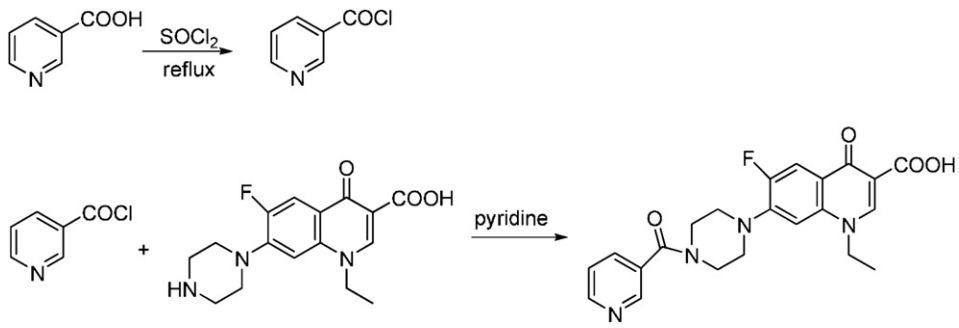
4a:R_1=R_2=H **4b**:R_1=-CH_3,R_2=H **4c**:R_1=Cl,R_2=H **4d**:R_1=-OCH_3,R_2=H
4e:R_1=H,R_2=-$C(CH_3)_3$ **4f**:R_1=H,R_2=Br **4g**:R_1=H,R_2=F **4h**:R_1=H,R_2=-OCH_3
4i:R_1=R_2=Cl

Method 4. Preparation of substituted phenoxyacetic acid

Scheme 1. Preparation of intermediates.



Scheme 2. Synthesis pathway of compounds **1**–**8**, **10**–**28**.



Scheme 3. Synthesis pathway of compound **9**.

Table 2
Antibacterial activities of compounds **1**–**28** and norfloxacin at 50 mg/L.

Compound	Inhibition zone (diameter in mm)				
	X. oryzae	*X. axonopodis*	*E. aroideae*	*B. subtilis*	*R. solanacearum*
1	22.94	15.49	13.85	22.49	9.81
2	18.10	10.24	11.19	16.99	6.97
3	13.02	12.08	13.69	18.91	6.72
4	18.10	11.66	11.74	15.74	8.39
5	12.69	11.08	12.44	13.91	5.31
6	8.35	8.66	11.02	11.16	8.56
7	5.44	5.24	5.94	6.99	3.56
8	15.27	12.91	14.94	19.43	5.72
9	7.94	6.58	9.19	8.74	1.06
10	15.35	14.99	13.77	17.33	4.89
11	14.19	10.74	13.44	15.33	6.22
12	17.19	10.41	11.77	14.16	4.67
13	11.94	10.99	12.94	17.74	4.97
14	5.81	6.41	8.52	10.79	10.89
15	8.02	10.74	8.94	12.66	7.47
16	11.02	11.08	13.27	13.91	3.31
17	14.52	12.49	15.19	17.58	6.31
18	10.02	8.16	8.52	9.91	0.50
19	11.69	12.08	10.85	15.16	4.64
20	5.35	6.83	8.02	8.91	0
21	4.44	5.91	2.92	2.08	0
22	22.69	15.83	17.85	20.66	5.92
23	16.85	14.24	16.00	18.99	8.97
24	16.52	14.58	16.19	20.08	8.14
25	16.69	14.41	16.52	22.74	2.06
26	19.69	12.99	14.60	17.08	3.22
27	17.69	12.91	16.60	18.49	1.47
28	9.35	9.99	10.44	13.16	0
Norfloxacin	22.19	17.74	21.52	30.49	19.31
Streptomycin	0.81	0.20	0.50	24.99	17.27
DMSO	0	0	0	0	0

"Streptomycin" represents the agricultural streptomycin sulfate.

Nevertheless, the data in Table 3 showed that almost half of the tested compounds (such as **7** and **24**) had better antifungal activities than norfloxacin against *G. zeae*, and the inhibition of growth by compounds **4**, **5**, **7**, **22**–**24** and **26** was also higher than norfloxacin against *V. mali*.

3. Conclusion

In summary, a series of novel 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl) quinoline-3-carboxylic acid (norfloxacin) derivatives have been synthesized by employing a convenient and practical process and tested for their antibacterial activities against *X. oryzae*, *X. axonopodis*, *E. aroideae*, *B. subtilis* and *R. solanacearum* and antifungal activities against *R. solani*, *G. zeae* and *V. mali*. The above biological test results showed that all the synthesized target compounds had kept the antibacterial activities of norfloxacin (the synthetic precursor), and the activities of compounds **1** and **22** were even better than norfloxacin against *X. oryzae* at 50 mg/L. Furthermore, most of the title compounds had better antibacterial activities as compared with the agricultural streptomycin sulfate (a commercial bactericide) and better antifungal activities than norfloxacin. Meanwhile, the inhibition of growth by compounds **2** and **20** against *R. solani* reached 83% and 94% respectively at a concentration of 200 mg/L. In addition, although the synthesized compounds don't possess both good antibacterial and antifungal activities, these preliminary results are promising and beneficial for further studies in developing a new sort of widely used antimicrobial agents in the agricultural chemistry field. Moreover, further design and biological evaluation of these compounds are ongoing in our laboratory.

4. Experimental protocols

4.1. Chemistry

4.1.1. Materials

All chemicals and solvents were purchased from commercial sources unless otherwise specified. All bacteria and fungi were obtained from the Institute of Pesticide and Crop Protection, Sichuan University. Melting points were determined using an X-4 micro-melting point apparatus (Beijing second optical instrument factory, PR China) and are uncorrected. ^{1}H NMR spectra were recorded in deuterochloroform solution on a Bruker 400 MHz spectrometer, using tetramethylsilane (TMS) as an internal standard. HRMS data were obtained on an ESI-TOFMS instrument (Agilent 6210).

4.1.2. Synthesis of target compounds

4.1.2.1. Preparation of carboxylic acid chlorides. Thionyl chloride (15 mL) was added into the corresponding acid (5 mmol), and then the mixture was heated under reflux for 2 h with the help of a drying tube filled with anhydrous calcium chloride. When the reaction was completed, the excess thionyl chloride was removed under reduced pressure. The crude products were used in the subsequent reactions without further purification.

*4.1.2.2. General procedures for compounds **1**–**8** and **10**–**28**.* A mixture of sodium hydroxide (0.7 g, 17.5 mmol), 1,4-dioxane (15 mL), water (15 mL), and norfloxacin (1.6 g, 5 mmol) was dissolved and cooled to 0 °C in an ice bath. Under stirring, carboxylic acid chloride synthesized in "Section 4.1.2.1" was added dropwise to the mixture with the temperature maintained below 3 °C. Afterwards, the resulting reaction mixture was stirred for another 5 h at room temperature. Then the mixture was poured into ice-water and acidified with hydrochloric acid to pH 4. Finally, the generated precipitates were collected by filtration, washed

Table 3
Antifungal activities of compounds **1**–**28** and norfloxacin at 200 mg/L.

Compound	Inhibition of growth (%)			Compound	Inhibition of growth (%)		
	R. solani	*G. zeae*	*V. mali*		*R. solani*	*G. zeae*	*V. mali*
1	14	7	8	**16**	52	15	12
2	83	25	25	**17**	60	17	10
3	47	26	27	**18**	72	5	17
4	16	25	29	**19**	29	5	9
5	65	24	40	**20**	94	1	5
6	17	7	9	**21**	0	2	6
7	65	45	33	**22**	25	23	42
8	20	8	7	**23**	5	27	41
9	12	11	17	**24**	15	34	44
10	30	17	8	**25**	0	15	17
11	34	20	19	**26**	36	28	37
12	3	10	12	**27**	21	27	16
13	0	14	7	**28**	5	12	0
14	53	28	14	Norfloxacin	0	18	27
15	49	6	9	Carbendazim	100	100	95

successively with water and dried. The pure products **1**–**8** and **10**–**28** were obtained by recrystallization in DMF.

Compound **1**: white crystals, yield: 55%, m.p. 295–296 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.98 (s, 1H), 8.70 (s, 1H), 8.13 (d, J = 12.8 Hz, 1H), 7.51–7.54 (m, 1H), 7.11 (d, J = 3.4 Hz, 1H), 6.87 (d, J = 6.8 Hz, 1H), 6.53 (dd, J = 1.7 Hz, 1H), 4.33 (q, J = 7.3 Hz, 2H), 3.99–4.16 (m, 4H), 3.39 (t, J = 5.0 Hz, 4H), 1.60 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{21}H_{20}FN_3O_5$ ($[M+H]^+$): found 414.1464, calcd. 414.1460.

Compound **2**: brown crystals, yield: 93%, m.p. 211–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 15.01 (s, 1H), 8.69 (s, 1H), 8.11 (d, J = 12.8 Hz, 1H), 7.46 (d, J = 15.0 Hz, 1H), 6.86 (d, J = 6.8 Hz, 1H), 6.73 (d, J = 15.0 Hz, 1H), 6.49 (d, J = 3.3 Hz, 1H), 6.09 (d, J = 3.3 Hz, 1H), 4.33 (q, J = 7.3 Hz, 2H), 3.78–4.03 (m, 4H), 3.28–3.43 (m, 4H), 2.36 (s, 3H), 1.60 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{24}H_{24}FN_3O_5$ ($[M+H]^+$): found 454.1780, calcd. 454.1773.

Compound **3**: white crystals, yield: 56%, m.p. 229–230 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 15.00 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.09 (d, J = 3.5 Hz, 1H), 6.87 (d, J = 6.9 Hz, 1H), 6.48 (d, J = 3.5 Hz, 1H), 4.33 (q, J = 7.3 Hz, 2H), 3.99–4.14 (m, 4H), 3.40 (t, J = 5.1 Hz, 4H), 1.59 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{21}H_{19}BrFN_3O_5$ ($[M+H]^+$): found 492.0565, calcd. 492.0565.

Compound **4**: brown crystals, yield: 71%, m.p. 224–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.98 (s, 1H), 8.69 (s, 1H), 8.11 (d, J = 12.8 Hz, 1H), 6.85 (d, J = 6.8 Hz, 1H), 5.95 (s, 1H), 4.32 (q, J = 7.2 Hz, 2H), 3.79–4.01 (m, 4H), 3.23–3.37 (m, 4H), 2.37 (s, 3H), 2.26 (s, 3H), 1.61 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{23}H_{24}FN_3O_5$ ($[M+H]^+$): found 442.1776, calcd. 442.1773.

Compound **5**: yellow crystals, yield: 68%, m.p. 266–268 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.99 (s, 1H), 8.68 (s, 1H), 8.09 (d, J = 12.8 Hz, 1H), 6.86 (d, J = 6.8 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.12–4.12 (m, 8H), 2.27 (s, 3H), 1.92 (s, 3H), 1.65 (s, 3H), 1.60 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{24}H_{26}FN_3O_5$ ($[M+H]^+$): found 456.1932, calcd. 456.1929.

Compound **6**: white crystals, yield: 65%, m.p. 278–279 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.98 (s, 1H), 8.70 (s, 1H), 8.14 (d, J = 12.8 Hz, 1H), 7.67–7.71 (m, 1H), 7.52–7.57 (m, 1H), 7.43–7.47 (m, 1H), 7.42 (s, 1H), 7.30–7.35 (m, 1H), 6.89 (d, J = 6.8 Hz, 1H), 4.34 (q, J = 7.2 Hz, 2H), 4.04–4.23 (m, 4H), 3.43 (t, J = 5.0 Hz, 4H), 1.61 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{25}H_{22}FN_3O_5$ ($[M+H]^+$): found 464.1616, calcd. 464.1616.

Compound **7**: light yellow crystals, yield: 88%, m.p. 140–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 15.00 (s, 1H), 8.70 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.61–7.65 (m, 1H), 7.47–7.50 (m, 1H), 7.40–7.46 (m, 1H), 7.30–7.36 (m, 1H), 6.89 (d, J = 6.8 Hz, 1H), 4.34 (q, J = 7.3 Hz, 2H), 4.04 (t, J = 5.0 Hz, 4H), 3.42 (t, J = 5.0 Hz, 4H), 2.51 (s, 3H), 1.61 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{26}H_{24}FN_3O_5$ ($[M+H]^+$): found 478.1773, calcd. 478.1773.

Compound **8**: white crystals, yield: 86%, m.p.198–199 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 15.00 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.52 (d, J = 15.0 Hz, 1H), 7.47 (d, J = 1.7 Hz, 1H), 6.86 (d, J = 6.8 Hz, 1H), 6.83 (d, J = 15.0 Hz, 1H), 6.60 (d, J = 3.4 Hz, 1H), 6.49 (dd, J = 1.7 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.83–4.06 (m, 4H), 3.28–3.44 (m, 4H), 1.60 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{23}H_{22}FN_3O_5$ ($[M+H]^+$): found 440.1618, calcd. 440.1616.

Compound **10**: yellow crystals, yield: 55%, m.p. 236–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.69 (s, 1H), 8.11 (d, J = 12.8 Hz, 1H), 7.27–7.36 (m, 2H), 6.94–7.06 (m, 3H), 6.82 (d, J = 6.8 Hz, 1H), 4.76 (s, 2H), 4.31 (q, J = 7.2 Hz, 2H), 3.83–3.92 (m, 4H), 3.21–3.36 (m, 4H),1.60 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{24}H_{24}FN_3O_5$ ($[M+H]^+$): found 454.1774, calcd. 454.1773.

Compound **11**: green crystals, yield: 27%, m.p. 232–233 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.95 (s, 1H), 8.68 (s, 1H), 8.10 (d, J = 12.8 Hz, 1H), 7.11–7.22 (m, 2H), 6.86–6.96 (m, 2H), 6.82 (d, J = 6.8 Hz, 1H), 4.77 (s, 2H), 4.32 (q, J = 7.2 Hz, 2H), 3.89 (t, J = 4.9 Hz, 4H), 3.20–3.37 (m, 4H), 2.26 (s, 3H), 1.57 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{25}H_{26}FN_3O_5$ ($[M+H]^+$): found 468.1930, calcd. 468.1929.

Compound **12**: light yellow crystals, yield: 21%, m.p. 249–250 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.95 (s, 1H), 8.70 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.28–7.43 (m, 2H), 6.93–7.08 (m, 2H), 6.83 (d, J = 6.9 Hz, 1H), 4.84 (s, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.83–3.97 (m, 4H), 3.21–3.38 (m, 4H), 1.59 (t, J = 7.1 Hz, 3H); HRMS (ESI) for $C_{24}H_{23}ClFN_3O_5$ ($[M+H]^+$): found 488.1384, calcd. 488.1383.

Compound **13**: yellowish green crystals, yield: 58%, m.p. 241–242 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.97 (s, 1H), 8.69 (s, 1H), 8.11 (d, J = 12.8 Hz, 1H), 6.88–7.04 (m, 4H), 6.82 (d, J = 6.8 Hz, 1H), 4.80 (s, 2H), 4.32 (q, J = 7.2 Hz, 2H), 3.88–3.97 (m, 4H), 3.87 (s, 3H), 3.25–3.36 (m, 4H), 1.59 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{25}H_{26}FN_3O_6$ ($[M+H]^+$): found 484.1884, calcd. 484.1878.

Compound **14**: light yellow crystals, yield: 20%, m.p. 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.69 (s, 1H), 8.10 (d, J = 12.8 Hz, 1H), 7.32 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 6.8 Hz, 1H), 4.73 (s, 2H), 4.32 (q, J = 7.2 Hz, 2H), 3.83–3.93 (m, 4H), 3.26–3.37 (m, 4H), 1.59 (t, J = 7.2 Hz, 3H), 1.29 (s, 9H); HRMS (ESI) for $C_{28}H_{32}FN_3O_5$ ($[M+H]^+$): found 510.2402, calcd. 510.2399.

Compound **15**: light green crystals, yield: 24%, m.p. 248–249 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.94 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.40 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 6.8 Hz, 1H), 4.74 (s, 2H), 4.32 (q, J = 7.3 Hz, 2H), 3.82–3.90 (m, 4H), 3.22–3.35 (m, 4H), 1.59 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{24}H_{23}BrFN_3O_5$ ($[M+H]^+$): found 532.0876, calcd. 532.0878.

Compound **16**: green crystals, yield: 19%, m.p. 232–234 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.94 (s, 1H), 8.70 (s, 1H), 8.12 (d, J = 12.7 Hz, 1H), 6.73–7.04 (m, 5H), 4.73 (s, 2H), 4.32 (q, J = 7.2 Hz, 2H), 3.81–3.92 (m, 4H), 3.19–3.42 (m, 4H), 1.61 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{24}H_{23}F_2N_3O_5$ ($[M+H]^+$): found 472.1679, calcd. 472.1679.

Compound **17**: brown crystals, yield: 35%, m.p. 217–218 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.68 (s, 1H), 8.09 (d, J = 12.8 Hz, 1H), 6.79–6.95 (m, 5H), 4.71 (s, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.87 (t, J = 4.9 Hz, 4H), 3.77 (s, 3H), 3.24–3.36 (m, 4H), 1.59 (t, J = 7.1 Hz, 3H); HRMS (ESI) for $C_{25}H_{26}FN_3O_6$ ($[M+H]^+$): found 484.1878, calcd. 484.1878.

Compound **18**: light green crystals, yield: 26%, m.p. 243–244 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.94 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.7 Hz, 1H), 7.39 (s, 1H), 7.21 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 6.3 Hz, 1H), 4.82 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.82–3.95 (m, 4H), 3.21–3.39 (m, 4H), 1.60 (t, J = 7.1 Hz, 3H); HRMS (ESI) for $C_{24}H_{22}Cl_2FN_3O_5$ ($[M+H]^+$): found 522.0994, calcd. 522.0993.

Compound **19**: yellow crystals, yield: 80%, m.p. 252–254 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.99 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.8 Hz, 1H), 7.74 (d, J = 15.4 Hz, 1H), 7.50–7.59 (m, 2H), 7.35–7.44 (m, 3H), 6.91 (d, J = 15.4 Hz, 1H), 6.86 (d, J = 6.7 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.83–4.09 (m, 4H), 3.26–3.46 (m, 4H), 1.58 (t, J = 7.1 Hz, 3H); HRMS (ESI) for $C_{25}H_{24}FN_3O_4$ ($[M+H]^+$): found 450.1829, calcd. 450.1824.

Compound **20**: brown crystals, yield: 62%, m.p. 248–249 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 15.00 (s, 1H), 8.69 (s, 1H), 8.12 (d, J = 12.7 Hz, 1H), 7.71 (d, J = 15.2 Hz, 1H), 7.50 (d, J = 8.3 Hz, 2H), 6.82–7.02 (m, 3H), 6.78 (d, J = 15.2 Hz, 1H), 4.32 (q, J = 7.2 Hz, 2H), 3.87–4.06 (m, 4H), 3.85 (s, 3H), 3.23–3.46 (m, 4H), 1.60 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{26}H_{26}FN_3O_5$ ($[M+H]^+$): found 480.1931, calcd. 480.1929.

Compound **21**: light green crystals, yield: 65%, m.p. 265–266 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.69 (s, 1H), 8.13 (d, J = 12.8 Hz, 1H), 7.99 (d, J = 15.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.46 (s, 1H), 7.27–7.30 (m, 1H), 6.84–6.92 (m, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.77–4.05 (m, 4H), 3.25–3.46 (m, 4H), 1.58 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{25}H_{22}Cl_2FN_3O_4$ ($[M+H]^+$): found 518.1049, calcd. 518.1044.

Compound **22**: light green crystals, yield: 68%, m.p. 263–264 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.97 (s, 1H), 8.67 (s, 1H), 8.07 (d, J = 12.8 Hz, 1H), 7.42–7.53 (m, 5H), 6.86 (d, J = 6.8 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.11–4.20 (m, 8H), 1.59 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{23}H_{22}FN_3O_4$ ($[M+H]^+$): found 424.1680, calcd. 424.1667.

Compound **23**: light yellow crystals, yield: 70%, m.p. 262–263 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.68 (s, 1H), 8.09 (d, J = 12.8 Hz, 1H), 7.31–7.48 (m, 4H), 6.86 (d, J = 6.8 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.23–4.01 (m, 8H), 1.59 (t, J = 7.1 Hz, 3H); HRMS (ESI) for $C_{23}H_{21}ClFN_3O_4$ ($[M+H]^+$): found 458.1277, calcd. 458.1277.

Compound **24**: light green crystals, yield: 59%, m.p. 269–270 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.96 (s, 1H), 8.68 (s, 1H), 8.09 (d, J = 12.8 Hz, 1H), 7.59–7.65 (m, 1H), 7.37–7.46 (m, 1H), 7.27–7.34 (m, 2H), 6.86 (d, J = 6.8 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.23–4.23 (m, 8H), 1.59 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{23}H_{21}BrFN_3O_4$ ($[M+H]^+$): found 502.0772, calcd. 502.0772.

Compound **25**: green crystals, yield: 32%, m.p. 286–287 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.94 (s, 1H), 9.57 (s, 1H), 8.70 (s, 1H), 8.14 (d, J = 12.8 Hz, 1H), 7.36–7.42 (m, 1H), 7.28–7.33 (m, 1H), 7.03–7.08 (m, 1H), 6.88–6.94 (m, 1H), 6.87 (d, J = 6.9 Hz, 1H), 4.33 (q, J = 7.3 Hz, 2H), 4.01 (t, J = 5.0 Hz, 4H), 3.37 (t, J = 5.0 Hz, 4H), 1.60 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{23}H_{22}FN_3O_5$ ($[M+H]^+$): found 440.1616, calcd. 440.1616.

Compound **26**: brownish yellow crystals, yield: 91%, m.p. 243–244 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.98 (s, 1H), 8.66 (s, 1H), 8.06 (d, J = 12.8 Hz, 1H), 7.36 (d, J = 7.9 Hz, 2H), 7.24 (d, J = 7.9 Hz, 2H), 6.85 (d, J = 6.8 Hz, 1H), 4.32 (q, J = 7.0 Hz, 2H), 3.18–4.18 (m, 8H), 2.39 (s, 3H), 1.58 (t, J = 7.0 Hz, 3H); HRMS (ESI) for $C_{24}H_{24}FN_3O_4$ ($[M+H]^+$): found 438.1826, calcd. 438.1824.

Compound **27**: green crystals, yield: 41%, m.p. 242–243 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.95 (s, 1H), 8.69 (s, 1H), 8.11 (d, J = 12.8 Hz, 1H), 7.38–7.46 (m, 4H), 6.86 (d, J = 6.7 Hz, 1H), 4.32 (q, J = 7.2 Hz, 2H), 3.59–4.12 (m, 4H), 3.24–3.46 (m, 4H), 1.60 (t, J = 7.2 Hz, 3H); HRMS (ESI) for $C_{23}H_{21}ClFN_3O_4$ ($[M+H]^+$): found 458.1278, calcd. 458.1277.

Compound **28**: light yellow crystals, yield: 63%, m.p. 288–289 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.95 (s, 1H), 8.68 (s, 1H), 8.09 (d, J = 12.8 Hz, 1H), 7.99–8.04 (m, 1H), 7.87–7.92 (m, 1H), 7.78–7.83 (m, 1H), 7.50–7.60 (m, 2H), 7.41–7.47 (m, 1H), 7.35–7.40 (m, 1H), 6.77 (d, J = 6.8 Hz, 1H), 4.29 (q, J = 7.3 Hz, 2H), 4.23 (s, 2H), 3.96 (t, J = 4.9 Hz, 2H), 3.69 (t, J = 4.9 Hz, 2H), 3.29 (t, J = 4.9 Hz, 2H), 3.12 (t, J = 4.9 Hz, 2H), 1.58 (t, J = 7.3 Hz, 3H); HRMS (ESI) for $C_{28}H_{26}FN_3O_4$ ($[M+H]^+$): found 488.1983, calcd. 488.1980.

4.1.2.3. Synthesis of compound ***9***. Nicotinoyl chloride prepared under procedure "Section 4.1.2.1" was a light yellow solid. After cooling to room temperature, norfloxacin (1.6 g, 5 mmol) and pyridine (20 mL) were added. Then the reaction mixture was vigorously stirred for 10 h at ambient temperature. When completed, the final mixture was concentrated under reduced pressure and then poured into ice-water and neutralized with hydrochloric acid. The precipitates were found, collected by filtration, washed with water, dried and recrystallized from DMF to give the title compound **9**.

Compound **9**: light green crystals, yield: 52%, m.p. 198–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.95 (s, 1H), 8.67–8.77 (m, 3H), 8.01–8.22 (m, 2H), 7.38–7.46 (m, 1H), 6.75–7.02 (m, 1H), 3.17–4.46 (m, 10H), 1.62–1.68 (m, 3H); HRMS (ESI) for $C_{22}H_{21}FN_4O_4$ ($[M+H]^+$): found 425.1619, calcd. 425.1620.

4.2. Biological assay

4.2.1. The assay of antibacterial activity

The antibacterial activities of the tested compounds against *X. oryzae*, *X. axonopodis*, *E. aroideae*, *B. subtilis* and *R. solanacearum* were screened using the agar well-diffusion method [20,21].

Using a sterile pipette, 0.8 mL of twenty-four-hour-old bacteria inoculums was added to 60 mL of the sterile molten agar which had been cooled to 45 °C, mixed well and poured into three sterile Petri dishes (each dish given 20 mL). After the agar solidified, the wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. The recommended concentration of the tested sample (50 mg/L using DMSO as the solvent) was added into respective wells. Other wells were supplemented with DMSO and the agricultural streptomycin sulfate serving as negative and positive controls, respectively. Each sample was repeated three times. The plates were incubated immediately at 37 °C for 24 h. The diameters (mm) of inhibition were measured and used to express the antibacterial activities of the tested compounds.

4.2.2. The assay of antifungal activity

Compounds **1**–**28** and norfloxacin were screened for their anti-plant pathogenic fungi activities against *R. solani*, *G. zeae* and *V. mali in vitro* using the plate growth rate method [22,23].

Each tested compound was dissolved in DMSO, added to the sterile culture medium (PDA) at 45 °C, mixed to homogeneity and transferred to sterile Petri dishes to solidify. For primary screenings, compounds were used at a concentration of 200 mg/L. At the same time, carbendazim (a commercial fungicide) and one equivalent of DMSO were used as positive and negative controls, respectively.

Afterwards, a mycelium agar disc (5 mm diameter) of the target fungi was placed in the center of PDA plates, and then the plates were incubated at 28 °C in the dark until the target fungi used as the negative control covered the plate's surface. Then the diameters of all fungi in the cultures were measured and the results were reported as the inhibition of growth following Abbott's formula: [(% living in control − % living in treatment)/% living in control × 100] [24]. Each compound was tested three times.

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