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Article

Synthesis and *in vitro* Antibacterial Activity of Quaternization 10-Methoxycanthin-6-one Derivatives

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Abstract: Natural products are an important source of antibacterial agents. Canthin-6-one alkaloids have displayed potential antibacterial activity based on our previous work. In order to improve the activity, twenty-two new 3-N-benzylated 10-methoxy canthin-6-ones were designed and synthesized through quaternization reaction. The *in vitro* antibacterial activity against three bacteria was evaluated by double dilution method. Four compounds (**6f**, **6i**, **6p** and **6t**) displayed 2-fold superiority (minimum inhibitory concentration (MIC) = 3.91 μg/mL) against agricultural pathogenic bacteria *R. solanacearum* and *P. syringae* than agrochemical propineb. Moreover, the structure–activity relationships (SARs) were also carefully summarized in order to guide the development of antibacterial canthin-6-one agents.

Keywords: 10-methoxycanthin-6-one; quaternization; antibacterial; SARs

1. Introduction

Healthcare systems, farming, and the food production industry are the main sectors driving antibiotic consumption [1]. To maintain animal welfare and food security, there are many circumstances where antibiotics are required in farming and food production, including for livestock, poultry, aquaculture, and crops [2–5]. *Bacillus cereus* could cause food poisoning [6]. *Ralstonia solanacearum* and *Pseudomonas syringae* are major components of plant pathogens [7]. We could see that all of these diseases caused by bacteria constitute a major threat to humans' life and property. Antibiotics have a massive impact on both health and society. Yet we are in increasing danger of losing them because the efforts of pharmaceutical industry are channeled towards some

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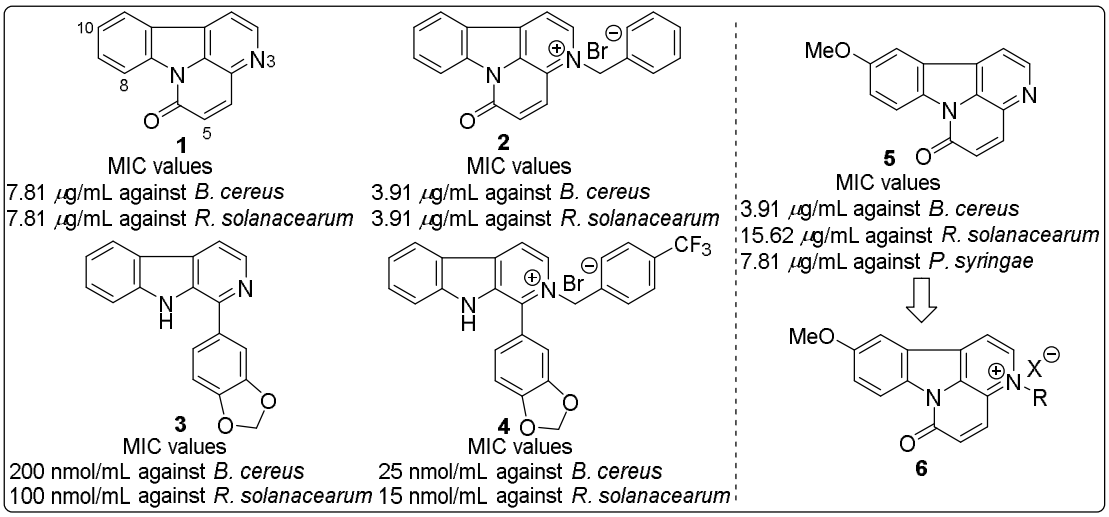


Figure 1. Design of the quaternization 10-methoxycanthin-6-one analogues.

diseases that have more favorable prospects for a return on investment [8]. Therefore, we need to develop new candidates to enrich the types of antibacterial agents and provided more options for solving the issue of "lack of antibiotics".

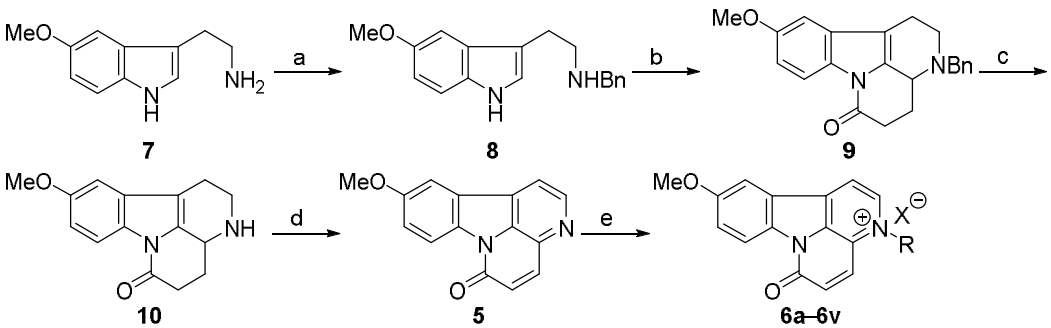
Natural products, millions of years of evolution, are fashioned by natural selection to interact with cellular targets with high efficiency and selectivity and to avoid resistance. Specially, they are privileged in the sphere of antibiotic development [8,9]. About 200 natural products were directly used as antibiotic drugs and improvements by semi-synthesis on these scaffolds generated another 200–300 drugs [8,9].

Canthin-6-one **1**, mainly from the Rutaceae and Simaroubaceae families, are a subclass of the tryptophan-derived *β*-carboline alkaloids [10,11]. In our previous work, we found that quaternization modification could significantly improve the antibacterial activity (**Figure 1**) [12,13]. For example, the antibacterial activity of compounds **2** and **4** against *B. cereus* enhanced 2-fold and 8-fold, respectively. Interestingly, 10-methoxycanthin-6-one **5**, *per se* has good antibacterial activity [14,15]. With the aim of finding more promising antibacterial canthin-6-one analogues, we report here a simple idea and design to generate the bioactive compounds by quaternization reaction of 10-methoxycanthin-6-one.

2. Results and Discussion

2.1. Chemistry

The synthetic route of target compounds **6a–6v** was outlined in **Scheme 1**. The natural product 10-methoxycanthin-6-one **5** could be easily obtained by sequentially Borch reduction, Pictet-Spengler reaction, catalytic hydrogen transfer reaction and oxidation reaction [15]. With this



No.	R	X	No.	R	X	No.	R	X

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6a	-CH_2Ph(*o*-F)	Br	**6i**	-CH_2Ph(*m*-Me)	Br	**6q**	-CH_2Ph(*o*-Br)	Br
6b	-CH_2Ph(*p*-F)	Br	**6j**	-CH_2Ph(*m*-OMe)	Cl	**6r**	-CH_2Ph(*p*-Br)	Br
6c	-CH_2Ph(*m*-F)	Br	**6k**	-CH_2Ph(*o*-CF_3)	Br	**6s**	-CH_2Ph(*m*-Br)	Br
6d	-CH_2Ph(*m*-CN)	Cl	**6l**	-CH_2Ph(*p*-CF_3)	Br	**6t**	-CH_2Ph(*m*-I)	Br
6e	-CH_2Ph(*o*-Cl)	Cl	**6m**	-CH_2Ph(*m*-CF_3)	Br	**6u**	-CH_2Ph	Br
6f	-CH_2Ph(*m*-Cl)	Cl	**6n**	-CH_2Ph(2,5-difluoro)	Br	**6v**	-CH_2Ph	Cl
6g	-CH_2Ph(*o*-Me)	Br	**6o**	-CH_2Ph(2,4-difluoro)	Br			
6h	-CH_2Ph(*p*-Me)	Br	**6p**	-CH_2Ph(3,4-dichloro)	Br			

Scheme1. Synthetic route and chemical structures of compounds **5** and **6a–6v**. Reagents and conditions: (a) i: benzaldehyde, MeOH, r.t.; ii: $NaBH_4$, r.t., 98%; (b) α-ketoglutaric acid, *p*-TSA, dry toluene:dioxane = 3:2, DST, reflux, 83%; (c) $HCOONH_4$, 5% Pd/C, MeOH:toluene = 1:1, heated, 75%; (d) 5% Pd/C, xylene, reflux, 90%;(e) CH_3CN, reflux, r.t., 42–72%.

compound **5** in hand, attention was focused on modifying the 3-N position. Benzyl group in this position significantly increased the activity (**Figure 1**). So we synthesized a series of quaternization 10-methoxycanthin-6-one derivatives **6a–6v** (42–72% yields) with diverse substituted benzyl group which varied in electron-inducing ability and substitution position.

All the structures of the target compounds (**5** and **6a–6v**) were confirmed by ^1H NMR, ^{13}C NMR and HRMS spectra. In the NMR spectra of compound **6a**, the signal of the methylene group was detected around δ = 6.39 ppm and δ = 56.4 ppm, respectively, which indicated that the quaternization 10-methoxycanthin-6-one derivatives were successfully synthesized. Moreover, the signal of $[M–Br]^+$ could be found at 359.1189 Da in HRMS spectra of compound **6a** (error = 0.28 ppm), which conformed to the theoretical value 359.1190 Da within the allowable error range (error < 5 ppm).

2.2. Antibacterial Activity

The *in vitro* antibacterial activity of compounds **5** and **6a–6v** were evaluated against three kinds of bacteria (*B. cereus, R. solanacearum and P. syringae*) using the double dilution method giving the MIC values [16]. Fosfomycin sodium and agrochemical propineb were used as the positive controls [14,17]. The antibacterial results (**Table 1**) revealed that most of the quaternization 10-methoxycanthin-6-one derivatives displayed good *in vitro* biological activity against *R. solanacearum* and *P. syringae* compared with agrochemical propineb. Similar result was also proposed by Bazina *et al.* [18]. Eight compounds (**6e**, **6f**, **6i**, **6k**, **6l**, **6m**, **6p** and **6t**) displayed 4-fold superiority (MIC = 3.91 μg/mL) against *R. solanacearum* than lead compound **5** and 2-fold superiority than propineb. Five compounds (**6f**, **6h**, **6i**, **6p** and **6t**) displayed 2-fold superiority (MIC = 3.91 μg/mL) against *P. syringae* than lead compound **5** and propineb. Unfortunately, quaternization modification did not improve the activity for *B. cereus*. It is worth mentioning that four compounds (**6p**, **6q**, **6r** and **6t**) still have potential antibacterial activity against *B. cereus* compared with natural canthin-6-one **1**. Overall, compounds **6p** and **6t** were considered to be the highly active derivatives against these three bacteria.

Table 1. Antibacterial activity of compounds **6a–6v** against three bacteria (MIC, μg/mL).

No.	*B. cereus*	*R. solanacearum*	*P. syringae*	No.	*B. cereus*	*R. solanacearum*	*P. syringae*
6a	15.63	15.63	15.63	**6n**	31.25	7.81	15.63
6b	15.63	15.63	15.63	**6o**	15.63	15.63	7.81
6c	31.25	7.81	15.63	**6p**	7.81	3.91	3.91

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6d	62.50	15.63	15.63	**6q**	7.81	7.81	15.63
6e	15.63	3.91	7.81	**6r**	7.81	7.81	7.81
6f	15.63	3.91	3.91	**6s**	15.63	7.81	7.81
6g	15.63	7.81	7.81	**6t**	7.81	3.91	3.91
6h	15.63	7.81	3.91	**6u**	15.63	15.63	15.63
6i	15.63	3.91	3.91	**6v**	15.63	15.63	15.63
6j	15.63	15.63	7.81	**5**	3.91	15.63	7.81
6k	15.63	3.91	7.81	F.S.[a]	3.91	-	-
6l	15.63	3.91	7.81	P.[a]	3.91	7.81	7.81
6m	15.63	3.91	15.63				

[a] Fosfomycin sodium; Propineb.

2.3. Structure–activity relationships

Based on the antibacterial activity data, the structure–activity relationships were carefully investigated for *R. solanacearum* and *P. syringae*. Halogen anion did not affect the activity. For example, the antibacterial activity of compounds **6u** and **6v** are equal. The substituted benzyl groups are favorable for most of the quaternization derivatives. The detailed SARs of diverse substituted benzyl group which varied in electron-inducing ability and substitution position were summarized in **Table 2**. In terms of halogen atom substitution, fluorine substituted benzyl groups are disadvantageous for improving the activity. Chlorine and iodine substituents may be better. In most instances, large sterically hindered groups such as methyl and trifluoromethyl are beneficial for activity enhancement. However, cyano and methoxy groups were exceptional and complex which need further study. Multiple substitutions showed different activity trends. Compound **6p** exhibited excellent antibacterial activity compared with agrochemical propineb.

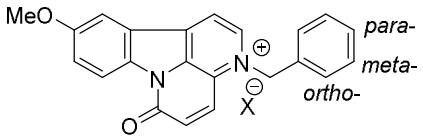

Table 2. Structure–activity relationships against *R. solanacearum* and *P. syringae*.

Location	Bacteria strains	SARs
Ortho-position	*R. solanacearum*	F < Br = Me < Cl = CF3
	P. syringae	F = Br < Cl = Me = CF3
Meta-position	*R. solanacearum*	CN = OMe < F = Br < Cl = I = Me = CF3
	P. syringae	F = CN = CF3 < OMe = Br < Cl = I = Me
Para-position	*R. solanacearum*	F < Br = Me < CF3
	P. syringae	F < Br = CF3 < Me

3. Materials and Methods

3.1. General Details

All the reagents and solvents were obtained locally or purified according to standard methods. Melting points were determined using a digital melting-point apparatus and were uncorrected. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using a Bruker Avance III 500 MHz instrument (Bruker, Madison, WI, USA) with TMS as the internal standard and dimethylsulfoxide (DMSO-d_6) as the solvent. High-resolution mass spectroscopy (HRMS) was undertaken using an AB SCIEX Triple TOF 5600+ spectrometer. The silica gel and GF254 silica gel of analytical thin-layer chromatography (TLC) were produced by the Qingdao Haiyang Chemical Co., Ltd..

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3.2. Synthesis of Target Compounds **6a–6v**

The 10-methoxy canthin-6-one **5** could be easily obtained according to our previous work [15]. Compound **5** was dissolved in CH_3CN (50 mL) and the diverse substituted benzyl bromide or chloride (5 eq.) was added. The solution was then stirred at 80 °C until the reaction is completed. The reaction solution was concentrated under reduced pressure, and purified by flash column chromatography using chloroform/methanol (30:1, *v*/*v*) as the eluent.

3-(2-Fluorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6a**: yield 60%; yellow-green solid powder; m.p. 224.2–224.7 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.29 (d, J = 6.4 Hz, 1H), 8.96 (d, J = 6.4 Hz, 1H), 8.59 (d, J = 10.2 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.25 (d, J = 2.5 Hz, 1H), 7.57 (m, 1H), 7.51–7.46 (m, 1H), 7.40 (d, J = 10.2 Hz, 1H), 7.34 (m, 2H), 7.24 (d, J = 7.6 Hz, 1H), 6.39 (s, 2H), 3.96 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.5, 142.6, 136.7, 136.3, 134.6, 134.5, 132.0, 131.9, 130.6, 130.2, 130.0, 125.7, 124.1, 122.8, 120.1, 117.9, 116.5, 116.3, 109.3, 58.6, 56.4. HRMS (ESI) m/z calcd for $C_{22}H_{16}BrFN_2O_2$ $[M–Br]^+$ 359.1190, found 359.1189.

3-(4-Fluorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6b**: yield 61%; yellow-green solid powder; m.p. 233.2–235.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.32 (d, J = 6.4 Hz, 1H), 8.96 (d, J = 6.4 Hz, 1H), 8.60 (d, J = 10.2 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.27 (d, J = 2.6 Hz, 1H), 7.68 (m, 1H), 7.65–7.45 (m, 3H), 7.35 (m, 2H), 6.35 (s, 2H), 3.95 (s, 3H). HRMS (ESI) m/z calcd for $C_{22}H_{16}BrFN_2O_2$ $[M–Br]^+$ 359.1190, found 359.1188.

3-(3-Fluorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6c**: yield 65%; brick yellow solid powder; m.p. 241.6–241.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (d, J = 6.4 Hz, 1H), 9.00 (d, J = 6.4 Hz, 1H), 8.64 (d, J = 10.2 Hz, 1H), 8.40 (d, J = 9.0 Hz, 1H), 8.27 (d, J = 2.6 Hz, 1H), 7.65–7.45 (m, 3H), 7.38 (d, J = 10.2 Hz, 1H), 7.32–7.10 (m, 2H), 6.31 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.5, 142.4, 136.5, 136.2, 134.6, 134.6, 131.3, 131.3, 130.6, 130.5, 130.3, 130.1, 124.1, 122.6, 120.2, 117.8, 116.5, 116.4, 109.3, 58.5, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{16}BrFN_2O_2$ $[M–Br]^+$ 359.1190, found 359.1190.

3-(3-Cyanobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium chloride **6d**: yield 45%; brick red solid powder; m.p. 225.8–227.7 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (d, J = 6.2 Hz, 1H), 8.99 (d, J = 6.2 Hz, 1H), 8.50 (d, J = 10.2 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.24 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 10.2 Hz, 1H), 6.42 (s, 2H), 3.96 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.7, 142.4, 136.7, 136.3, 136.1, 134.7, 134.5, 133.8, 130.9, 130.8, 130.3, 128.9, 127.0, 126.1, 124.2, 122.7, 120.3, 118.0, 117.8, 109.4, 57.8, 56.6. HRMS (ESI) m/z calcd for $C_{23}H_{16}ClN_3O_2$ $[M–Cl]^+$ 366.1237, found 366.1235.

3-(2-Chlorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium chloride **6e**: yield 44%; yellow solid powder; m.p. 231.2–232.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.28 (d, J = 6.4 Hz, 1H), 9.02 (d, J = 6.4 Hz, 1H), 8.56 (d, J =10.2 Hz, 1H), 8.40 (d, J = 9.0 Hz, 1H), 8.26 (d, J = 2.6 Hz, 1H), 7.97–7.65 (m, 1H), 7.59 (m, 1H), 7.42–7.28 (m, 3H), 6.88–6.78 (m, 1H), 6.34 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.8, 157.6, 142.6, 139.9, 136.4, 136.3, 135.1, 134.6, 134.5, 130.3, 130.1, 129.5, 129.4, 127.9, 125.0, 124.1, 122.6, 120.2, 117.8, 109.5, 59.7, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{16}Cl_2N_2O_2$ $[M–Cl]^+$ 375.0894, found 375.0892.

3-(3-Chlorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium chloride **6f**: yield 47%; brick yellow solid powder; m.p. 229.5–231.2 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (d, J = 6.4 Hz, 1H), 8.99 (d, J = 6.4 Hz, 1H), 8.57 (d, J = 10.2 Hz, 1H), 8.42 (d, J = 9.0 Hz, 1H), 8.26 (d, J = 2.6 Hz, 1H), 7.96–7.68 (m, 2H), 7.54 (m, 1H), 7.37 (d, J = 10.2 Hz, 1H), 7.22 (d, J = 8.4 Hz, 2H), 6.32 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ158.7, 157.5, 142.6, 136.7, 136.3, 134.6, 134.5, 132.0, 131.9, 130.9, 130.2, 130.0, 125.7, 124.1, 122.8, 120.1, 117.9, 116.5, 116.3, 109.3, 59.6, 56.4. HRMS (ESI) m/z calcd for $C_{22}H_{16}Cl_2N_2O_2$ $[M–Cl]^+$ 375.0894, found 375.0893.

10-Methoxy-3-(2-methylbenzyl)-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6g**: yield 68%; brick yellow solid powder; m.p. > 280 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.18 (d, J = 6.4 Hz, 1H), 9.00 (d, J = 6.4 Hz, 1H), 8.44 (d, J = 8.9 Hz, 2H), 8.31 (d, J = 2.4 Hz, 1H), 7.57 (m, 1H), 7.38–7.33 (m, 2H), 7.28 (t, J = 7.4 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H), 6.55 (d, J = 7.7 Hz, 1H), 6.33 (s, 2H), 3.96 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.7, 142.4, 136.7, 136.3, 136.0, 134.7, 134.5, 133.8, 130.9,

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130.8, 130.3, 128.9, 127.0, 126.1, 124.2, 122.7, 120.3, 117.8, 109.4, 57.8, 56.6, 19.3. HRMS (ESI) m/z calcd for $C_{23}H_{19}BrN_2O_2$ $[M–Br]^+$ 355.1441, found 355.1439.

10-Methoxy-3-(4-methylbenzyl)-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6h**: yield 71%; yellow solid powder; m.p. 228.6–228.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (d, J = 6.4 Hz, 1H), 8.98 (d, J = 6.4 Hz, 1H), 8.57 (d, J = 10.2 Hz, 1H), 8.35 (d, J = 9.0 Hz, 1H), 8.25 (d, J = 2.6 Hz, 1H), 7.49 (m, 1H), 7.34 (d, J = 10.2 Hz, 1H), 7.28 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 9.2 Hz, 3H), 6.27 (s, 2H), 3.92 (s, 3H), 2.25 (s, 3H). HRMS (ESI) m/z calcd for $C_{23}H_{19}BrN_2O_2$ $[M–Br]^+$ 355.1441, found 355.1439.

10-Methoxy-3-(3-methylbenzyl)-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6i**: yield 70%; yellow solid powder; m.p. 222.4–222.7 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (d, J = 6.3 Hz, 1H), 9.03 (d, J = 6.3 Hz, 1H), 8.47 (d, J = 8.9 Hz, 2H), 8.32 (d, J = 2.4 Hz, 1H), 7.61 (m, 1H), 7.36–7.31 (m, 2H), 7.29 (t, J = 7.4 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 6.55 (d, J = 7.7 Hz, 1H), 6.35 (s, 2H), 3.98 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.7, 142.6, 136.7, 136.3, 136.0, 134.7, 134.5, 133.8, 130.9, 130.8, 130.4, 128.9, 127.0, 126.1, 124.2, 122.7, 120.3, 117.9, 109.4, 59.8, 56.6, 21.4. HRMS (ESI) m/z calcd for $C_{23}H_{19}BrN_2O_2$ $[M–Br]^+$ 355.1441, found 355.1443.

10-Methoxy-3-(3-methoxybenzyl)-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium chloride **6j**: yield 43%; brick yellow solid powder; m.p. 225.3–226.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.41 (d, J = 6.3 Hz, 1H), 8.97 (d, J = 6.3 Hz, 1H), 8.57 (d, J = 10.2 Hz, 1H), 8.39 (d, J = 9.0 Hz, 1H), 8.25 (d, J = 2.6 Hz, 1H), 7.46 (m, 1H), 7.34 (d, J = 10.2 Hz, 1H), 7.28 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 9.2 Hz, 3H), 6.37 (s, 2H), 3.92 (s, 3H), 3.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.8, 157.6, 142.7, 136.6, 136.3, 136.2, 134.7, 134.7, 132.0, 130.7, 130.5, 130.3, 130.0, 126.1, 125.4, 124.1, 122.7, 120.3, 117.8, 109.4, 58.5, 56.5, 56.5. HRMS (ESI) m/z calcd for $C_{23}H_{19}ClN_2O_3$ $[M–Cl]^+$ 406.1078, found 355.1443.

10-Methoxy-6-oxo-3-(2-(trifluoromethyl)benzyl)-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6k**: yield 70%; yellow solid powder; m.p. 243.6–244.1 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.22 (d, J = 6.4 Hz, 1H), 9.00 (d, J = 6.3 Hz, 1H), 8.47 (d, J = 9.0 Hz, 1H), 8.29 (t, J = 6.7 Hz, 2H), 7.96 (d, J = 7.6 Hz, 1H), 7.69–7.55 (m, 3H), 7.36 (d, J = 10.2 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 6.51 (s, 2H), 3.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.6, 142.6, 139.6, 136.7, 136.3, 134.7, 134.6, 130.5, 130.0, 129.8, 129.5, 128.6, 126.4, 125.5, 124.1, 123.3, 122.7, 120.3, 117.9, 109.4, 58.5, 56.6. HRMS (ESI) m/z calcd for $C_{23}H_{16}BrF_3N_2O_2$ $[M–Br]^+$ 409.1158, found 409.1155.

10-Methoxy-6-oxo-3-(4-(trifluoromethyl)benzyl)-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6l**: yield 68%; yellow solid powder; m.p. 238.5–238.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.43 (d, J = 6.2 Hz, 1H), 9.01 (d, J = 6.2 Hz, 1H), 8.63 (d, J = 10.1 Hz, 1H), 8.41 (d, J = 8.9 Hz, 1H), 8.28 (d, J = 2.6 Hz, 1H), 7.92 (s, 1H), 7.77 (d, J = 5.2 Hz, 1H), 7.64 (s, 2H), 7.59–7.46 (m, 1H), 7.39 (d, J = 10.1 Hz, 1H), 6.43 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.6, 142.6, 136.6, 136.3, 136.2, 134.8, 134.7, 132.0, 130.5, 130.0, 126.1, 125.4, 125.0, 124.1, 122.7, 120.3, 117.8, 109.4, 58.5, 56.6. HRMS (ESI) m/z calcd for $C_{23}H_{16}BrF_3N_2O_2$ $[M–Br]^+$ 409.1158, found 409.1159.

10-Methoxy-6-oxo-3-(3-(trifluoromethyl)benzyl)-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6m**: yield 72%; brick red solid powder; m.p. 223.3–223.7 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (d, J = 6.4 Hz, 1H), 8.99 (d, J = 6.4 Hz, 1H), 8.53 (d, J = 10.2 Hz, 1H), 8.40 (d, J = 9.0 Hz, 1H), 8.26 (d, J = 2.6 Hz, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.64–7.48 (m, 3H), 7.36 (d, J = 10.2 Hz, 1H), 6.42 (s, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.6, 142.7, 139.7, 136.7, 136.2, 134.8, 134.73, 130.5, 130.0, 129.8, 129.5, 128.6, 126.4, 125.5, 124.1, 123.3, 122.7, 120.3, 117.9, 109.3, 58.6, 56.6. HRMS (ESI) m/z calcd for $C_{23}H_{16}BrF_3N_2O_2$ $[M–Br]^+$ 409.1158, found 409.1157.

3-(2,5-Difluorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6n**: yield 61%; yellow solid powder; m.p. 245.1–245.8 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.32 (d, J = 6.4 Hz, 1H), 8.99 (d, J = 6.3 Hz, 1H), 8.58 (d, J = 10.2 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.29 (d, J = 2.6 Hz, 1H), 7.57 (m, 1H), 7.50–7.29 (m, 3H), 7.20 (m, 1H), 6.39 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.6, 142.8, 136.8, 136.3, 134.7, 134.6, 130.7, 130.1, 124.2, 124.2, 124.1, 122.8, 120.2, 118.1, 117.9, 117.9, 116.6, 116.4, 109.4, 56.6, 53.9. HRMS (ESI) m/z calcd for $C_{22}H_{15}BrF_2N_2O_2$ $[M–Br]^+$ 377.1096, found 377.1093.

3-(2,4-Difluorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6o**: yield 58%; yellow solid powder; m.p. 230.6–231.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (d, J = 6.4 Hz, 1H), 8.98 (d, J = 6.4 Hz, 1H), 8.68 (d, J = 10.2 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.27 (d, J = 2.6 Hz, 1H),

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7.58 (m, 1H), 7.64–7.45 (m, 3H), 7.38 (d, *J* = 10.2 Hz, 1H), 6.39 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 157.6, 142.8, 136.7, 136.6, 134.6, 134.5, 130.6, 130.5, 124.2, 124.2, 124.1, 122.6, 120.2, 118.1, 117.9, 117.8, 116.6, 116.4, 109.3, 56.7, 53.9. HRMS (ESI) m/z calcd for $C_{22}H_{15}BrF_2N_2O_2$ $[M–Br]^+$ 377.1096, found 377.1094.

3-(3,4-Dichlorobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6p**: yield 46%; orange solid powder; m.p. 223.7–224.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (d, *J* = 6.4 Hz, 1H), 9.00 (d, *J* = 6.4 Hz, 1H), 8.60 (d, *J* = 10.2 Hz, 1H), 8.41 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 2.6 Hz, 1H), 7.95–7.67 (m, 2H), 7.37 (d, *J* = 10.2 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.38 (s, 2H), 3.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.5, 142.4, 136.5, 136.2, 134.6, 134.6, 131.3, 131.3, 130.6, 130.5, 130.3, 130.1, 124.1, 122.6, 120.2, 117.8, 116.5, 116.4, 109.3, 58.5, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{15}BrF_2N_2O_2$ $[M–Br]^+$ 377.1096, found 377.1094.

3-(2-Bromobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6q**: yield 62%; brick yellow solid powder; m.p. 235.5–236.0 °C,;^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (d, *J* = 6.4 Hz, 1H), 8.97 (d, *J* = 6.4 Hz, 1H), 8.46 (m, 2H), 8.28 (d, *J* = 2.6 Hz, 1H), 7.82 (m, 1H), 7.59 (m 1H), 7.42–7.28 (m, 3H), 6.88–6.78 (m, 1H), 6.34 (s, 2H), 3.96 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.6, 142.6, 136.9, 136.4, 134.7, 134.6, 134.2, 133.6, 131.2, 130.9, 130.2, 129.1, 129.0, 124.1, 122.8, 122.4, 120.2, 117.9, 109.4, 59.6, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{16}Br_2N_2O_2$ $[M–Br]^+$ 419.0389, found 419.0390.

3-(4-Bromobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6r**: yield 65%; yellow solid powder; m.p. 234.5–235.2 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.21 (d, *J* = 6.4 Hz, 1H), 8.92 (d, *J* = 6.4 Hz, 1H), 8.65 (m, 2H), 8.28 (d, *J* = 2.6 Hz, 1H), 7.86 (m, 1H), 7.79 (m, 1H), 7.39–7.24 (m, 3H), 7.13 (d, *J* = 10.2 Hz, 1H), 6.36 (s, 2H), 3.96 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 142.6, 136.8, 136.4, 134.7, 134.2, 133.8, 132.2, 130.5, 130.2, 129.4, 129.5, 124.1, 122.8, 122.4, 120.2, 117.9, 109.5, 59.6, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{16}Br_2N_2O_2$ $[M–Br]^+$ 419.0389, found 419.0387.

3-(3-Bromobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6s**: yield 64%; brick red solid powder; m.p. 238.7–239.1 °C;^{1}H NMR (500 MHz, DMSO-d_6) δ 9.30 (d, *J* = 6.4 Hz, 1H), 9.01 (d, *J* = 6.4 Hz, 1H), 8.47 (d, *J* = 9.0 Hz, 1H), 8.31 (t, *J* = 6.7 Hz, 2H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.59–7.45 (m, 3H), 7.16 (d, *J* = 10.2 Hz, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 6.51 (s, 2H), 3.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7, 157.5, 142.6, 136.7, 136.3, 134.6, 134.5, 132.0, 131.9, 130.9, 130.2, 130.0, 125.7, 124.1, 122.8, 120.1, 117.9, 116.5, 116.3, 109.3, 59.6, 56.4. HRMS (ESI) m/z calcd for $C_{22}H_{16}Br_2N_2O_2$ $[M–Br]^+$ 419.0389, found 419.0386.

3-(3-Iodobenzyl)-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6t**: yield 42%; red solid powder; m.p. 245.6–246.3 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.36 (d, *J* = 6.4 Hz, 1H), 8.99 (d, *J* = 6.4 Hz, 1H), 8.57 (d, *J* = 10.2 Hz, 1H), 8.40 (d, *J* = 9.0 Hz, 1H), 8.26 (d, *J* = 2.6 Hz, 1H), 7.96–7.68 (m, 2H), 7.54 (m, 1H), 7.37 (d, *J* = 10.2 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.28 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ13C NMR (126 MHz, DMSO-d_6) δ 158.6, 157.5, 142.5, 138.3, 136.6, 136.2, 134.8, 134.6, 134.6, 130.3, 130.2, 130.1, 124.1, 122.7, 120.2, 117.8, 116.5, 116.4, 109.3, 96.1, 58.7, 56.6. HRMS (ESI) m/z calcd for $C_{22}H_{16}BrIN_2O_2$ $[M–Br]^+$ 467.0251, found 467.0251.

3-Benzyl-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium bromide **6u**: yield 65%; yellow solid powder; m.p. 224.1–224.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (d, *J* = 6.4 Hz, 1H), 8.99 (d, *J* = 6.2 Hz, 1H), 8.60 (d, *J* = 10.2 Hz, 1H), 8.40 (d, *J* = 9.0 Hz, 1H), 8.26 (d, *J* = 2.3 Hz, 1H), 7.54 (m, 1H), 7.51–7.29 (m, 6H), 6.32 (s, 2H), 3.94 (s, 3H). HRMS (ESI) m/z calcd for $C_{22}H_{17}BrN_2O_2$ $[M–Br]^+$ 341.1284, found 341.1282.

3-Benzyl-10-methoxy-6-oxo-6H-indolo[3,2,1-de][1,5]naphthyridin-3-ium chloride **6v**: yield 56%; yellow solid powder; m.p. 222.4–222.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.38 (d, *J* = 6.4 Hz, 1H), 9.01 (d, *J* = 6.4 Hz, 1H), 8.58 (d, *J* = 10.2 Hz, 1H), 8.40 (d, *J* = 9.0 Hz, 1H), 8.28(d, *J* = 2.3 Hz, 1H), 7.56 (m, 1H), 7.50–7.27 (m, 6H), 6.33 (s, 2H), 3.96 (s, 3H). HRMS (ESI) m/z calcd for $C_{22}H_{17}ClN_2O_2$ $[M–Br]^+$ 341.1284, found 341.1280.

3.3. Antibacterial assay

MICs were determined as described by the National Committee for Clinical Laboratory Standards [19]. *B. cereus* (CGMCC 1.1846) was purchased from the China General Microbiological Culture Collection Center. *R. solanacearum* and *P. syringae* were provided by the College of Plant

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Protection, Northwest A&F University [20]. The MIC was defined as the minimum inhibitory concentration, each compound resulting in visible inhibition on bacteria growth (incubation at 37 °C for 12–14 h). Each bacterial suspension was adjusted to a concentration of 1 × 10^5 CFU/mL. All compounds were thoroughly dried before weighing. Initially, the compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solutions. The tested compounds (**5** and **6a–6v**) and reference drugs were then prepared in liquid Luria–Bertani media. The required concentrations were 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 μg/mL, respectively (DMSO < 0.5%).

4. Conclusions

In this study, twenty-two new quaternarization 10-methoxycanthin-6-one derivatives were designed and synthesized based on our previous work. Their antibacterial activity was evaluated against three bacterial strains including two kinds of agricultural pathogenic bacteria. Four compounds (**6f**, **6i**, **6p** and **6t**) displayed 2-fold superiority (MIC = 3.91 μg/mL) against *R. solanacearum* and *P. syringae* than agrochemical propineb. Simultaneously, the structure–activity relationships were summarized which provided some important guidance for the development of antibacterial agents. Overall, this work further demonstrated here the antibacterial potential of canthin-6-one scaffold, enriched the types of candidate antibiotics and provided more options for solving the issue of "lack of antibiotics".

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Sample Availability: Samples of all the compounds in this paper are available from the authors.