Regular Article

Synthesis and Antibacterial Activities of Yanglingmycin Analogues

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The synthesis of Yanglingmycin and its enantiomer, along with eighteen Yanglingmycin analogues is reported. The structures were confirmed mainly by analyses of NMR spectral data. Antibacterial activity assays showed that Yanglingmycin and some of its analogues exhibited significant antibacterial activities against two important agricultural pathogenic bacteria, *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae*, with minimum inhibitory concentration (MIC) values ranging from 3.91 to 15.62 μg/mL. The antibacterial activities exhibited by Yanglingmycin and its analogues are promising, suggesting potential in the development of compounds for novel bactericides.

Key words Yanglingmycin; structural analogue; substituted cyclic azole; antibacterial activity

Bacterial diseases pose a major threat to agricultural production worldwide. For example, *Ralstonia solanacearum*, which causes plant bacterial wilt, is one of the most destructive plant pathogens, and affects many regions of the world.[1] It is estimated that *R. solanacearum* is responsible for US$ 1 billion in losses each year.[2] *Pseudomonas syringae* pv. *actinidiae*, which causes kiwifruit canker, is another devastating plant pathogen and has received significant attention in recent years.[3–6] Thus, there has been considerable interest in development of bactericides for *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae*. Unfortunately, there have been few reports of effective bactericides against these pathogens. In the process of screening new agricultural antibiotics, we report the synthesis a new broad spectrum antibiotic, Yanglingmycin (Fig. 1), which was found to be effective against the previously mentioned pathogens.[7] Although Yanglingmycin can be isolated from the fermentation broth of *Streptomyces djakartensis*, the yield is too low to conduct experiments to determine the mechanism of bactericidal action. In order to investigate the bactericidal activities of Yanglingmycin and its analogues, Yanglingmycin and its analogues were designed and synthesized. In this paper, Yanglingmycin and its enantiomer and eighteen Yanglingmycin analogues were synthesized and their antibacterial activities against *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae* are reported.

Results

Chemistry Chart 1 describes the synthesis of all target compounds. Benzonitrile, 2-hydroxybenzonitrile, and 4-hydroxybenzonitrile were used as starting material for the Pinner reaction to obtain the corresponding methyl benzimidate hydrochlorides,[8] which were then reacted with each the following compounds: L-serine methyl ester hydrochloride, D-serine methyl ester hydrochloride, L-cysteine methyl ester hydrochloride, D-cysteine methyl ester hydrochloride, L-2,3-diaminopropionic acid methyl ester hydrochloride, and D-2,3-diaminopropionic acid methyl ester hydrochloride.[9,10] Amino acid methyl ester hydrochlorides were obtained by reacting the corresponding amino acids with AcCl in MeOH.[11] The products were reduced by $LiAlH_4$ to obtain Yanglingmycin and its analogues.

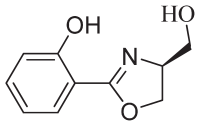

Fig. 1. Structure of Yanglingmycin

Antimicrobial Assay for Antimicrobial Activity of Yanglingmycin and Its Analogues All synthesized compounds were evaluated for their *in vitro* antibacterial activities against *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae*. The corresponding inhibition zone diameters and minimum inhibitory concentrations (MICs) were determined using the filter paper method and the micro-broth dilution method, respectively, and are presented in Tables 1 and 2. Yanglingmycin, its enantiomer, and eighteen Yanglingmycin analogues synthesized by systematically varying the heteroatom and substituent configuration on the heterocycle and substituents on the phenyl group have been characterized. Some of the synthesized compounds showed more potent antibacterial activities against *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae* than that of Ampicillin. In particular, compounds **4.2a** and **4.2a′** exhibited MIC values of 3.91 μg/mL for *Ralstonia solanacearum* and 7.81 μg/mL for *Pseudomonas syringae* pv. *actinidiae*. Compounds **4.1a** and **4.1a′** exhibited MIC values of 15.62 μg/mL for *Ralstonia solanacearum* and 7.81 μg/mL for *Pseudomonas syringae* pv. *actinidiae*. In comparison, compound **3.1a** exhibited MIC values greater than 125 μg/mL for both pathogen species. None of the other compounds characterized exhibited significant antibacterial activity.

Discussion

A comparison among the compounds screened shows that

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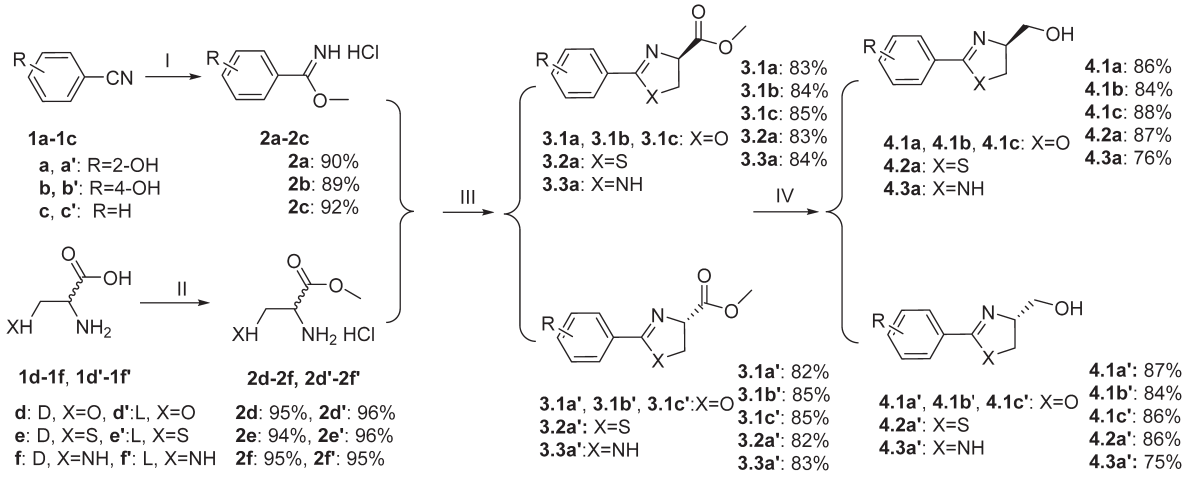

Reagents and conditions: (I) AcCl, MeOH, 16 h; (II) AcCl, MeOH, reflux, 2 h; (III) NEt_3, CH_2Cl_2, reflux, 24 h; (IV) $LiAlH_4$, Et_2O, 1 h.

Chart 1. Synthesis of Yanglingmycin and Its Analogues

Table 1. Diameter of Inhibition Zone of Yanglingmycin Analogues against the Tested Bacteria

Compounds	Diameter of inhibition zone (mm) in 1 μL/disk (mean±S.D.)	
	Ralstonia solanacearum	*Pseudomonas syringae* pv. *actinidiae*
3.1a	9.2±0.77 (++)	—
3.1a′	—	—
3.1b	—	—
3.1b′	—	—
3.1c	—	—
3.1c′	—	—
3.2a	—	—
3.2a′	—	—
3.3a	—	—
3.3a′	—	—
4.1a	17.2±0.34 (+++)	11.7±0.58 (++)
4.1a′	17.3±0.63 (+++)	12.6±0.58 (++)
4.1b	—	—
4.1b′	—	—
4.1c	—	—
4.1c′	—	—
4.2a	14.5±0.71 (+++)	8.7±0.46 (+)
4.2a′	13.5±0.71 (+++)	8.0±0.62 (+)
Ampicillin	13.6±0.21 (+++)	14.6±0.58 (++)

All values were means of three replicates, "+++" means transparent; "++" means clear; "+" means visible; "—" means no inhibitory effect.

holding all other structural components constant, 2-hydroxy substitution at the phenyl group produced the most active compounds. Yanglingmycin analogues with a 4-hydroxy substituted phenyl group and or an unsubstituted phenyl group did not exhibit significant bactericidal activities. The data suggest that the 2-substitution on the phenyl group elicits a geometric effect, since the same functional group at a different position on the phenyl group has dramatically lower activity. Similarly, if we consider the effect of the functional group at the chiral center on the heterocycle of Yanglingmycin analogues, we find that the hydroxymethyl substitution yields considerably improved bactericidal activities compared to methoxycarbonyl substition. Enantiomeric configuration of the substituents on the heterocycle was not observed to elicit a significant change in antibacterial activity. Focusing on the heteroatom substitution, compounds with oxygen and sulfur heteroatoms on the heterocycle presented better antibacterial activities than those with a nitrogen heteroatom on the heterocycle. Since the oxazole and thiazole are isoelectronic, they can be expected to share similar chemical properties and activities. On the other hand, the imidazole has an extra proton on the nitrogen heteroatom. Proton transfer between the two nitrogen atoms in the imidazole could cause enough of a structural change to disrupt antibacterial activity, which is consistent with lower bactericidal activity of Yanglingmycin analogues with nitrogen heteroatom substitution on the heterocycle. According to the MIC data, compounds **4.2a** and **4.2a′** were approximately 4 times more active than **4.1a** and **4.1a′** although the inhibition zone diameters of **4.2a** and **4.2a′** ranged from 16 to 37% lower than the inhibition diameters of **4.1a** and **4.1a′**. Since the MICs of compounds **4.2a** and **4.2a′** suggest significantly higher activity than those of **4.1a** and **4.1a′**, but the inhibition zone diameters of these (**4.1a**, **4.1a′**, **4.2a**, **4.2a′**) compounds are somewhat similar, it is likely that the diffusivity of the compounds in the inoculated culture medium during the antimicrobial activity screening process, carried out with the filter paper method, is the cause for this discrepancy. Further variation of substituents on the phenyl group in Yanglingmycin analogues may further improve upon the selectivity of antibacterial activity.

Conclusion

Yanglingmycin and its structural analogues have been synthesized. A number of the compounds displayed higher bactericidal activity than ampicillin towards *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *actinidiae*. Different substitutions for the heteroatom, on the phenyl group, and on the heterocycle suggest that 2-substitution of the phenyl group, a more polar functional group on the heterocycle yield, and oxygen or sulfur heteroatom substitution improved bactericidal activity.

Experimental

General Solvents were of analytical reagent (AR) grade unless otherwise mentioned. TLC was performed on E. Merck $60F_{254}$ silica gel plates. Column chromatography was car-

Table 2. MIC of Yanglingmycin Analogues against the Tested Bacteria

Tested bacteria	Minimum inhibitory concentration (μg/mL)					
	4.1a	**4.1a′**	**4.2a**	**4.2a′**	**3.1a**	Ampicillin
Ralstonia solanacearum	15.62	15.62	3.91	3.91	>125	>125
Pseudomonas syringae pv. *actinidiae*	7.81	7.81	7.81	7.81	>125	>125

ried out with silica gel (Qingdaohaiyang Co., Ltd., Qingdao, Shandong, China); compounds were eluted with petroleum ether and ethyl acetate in sequence. Melting point (mp) was measured using Yanagimoto apparatus (uncorrected). ^{1}H- and ^{13}C-NMR were obtained on a Bruker-Avance-500 spectrometer with DMSO-d_6 or $CDCl_3$ as solvent and $SiMe_4$ as internal standard. MS were recorded under electrospray ionization (ESI) conditions using a Thermo LCQ Fleet instrument.

General Procedure for the Synthesis of 2a–c Acetyl chloride (11.4 mL, 160 mmol) was added dropwise to a stirred solution of corresponding substituted benzonitrile (**1a**–**c**) (20 mmol) in anhydrous methanol (9.7 mL, 240 mmol) at room temperature. TLC analysis showed complete consumption of the substituted benzonitrile starting material. The product was observed to precipitate out from the mixture as a white solid. Then the mixture was filtered and washed by ethyl acetate, and the product was dried under low pressure to give methyl benzimidate hydrochloride (**2a**–**c**) as a white powder. The yields of **2a**, **b** and **c** were 90%, 89%, 92%, respectively.

General Procedure for the Synthesis of 2d–f and 2d′–f′ Acetyl chloride (1.6 mL, 22 mmol) was added to MeOH (100 mL), and the solution was cooled to 0°C. The solution was stirred for 5 min. Amino acid (L-serine, D-serine, L-cysteine, D-cysteine, L-2,3-diaminopropionic acid, and D-2,3-diaminopropionic acid) (20 mmol) was then added to the acetyl chloride solution in methanol (MeOH), and the solution was heated to reflux for 2 h, then cooled to room temperature. The reaction was evaporated under reduced pressure and gave a colorless solid. The solid was washed with CH_2Cl_2 (20 mL) to give amino acid methyl ester hydrochloride (**2d**–**f**, **2d′**–**f′**) as a white solid. The yields of **2d**, **d′**, **e**, **e′**, **f** and **f′** were 95%, 96%, 94%, 96%, 95%, 95%, respectively.

General Procedure for the Synthesis of 3.1a–3.3a′ To a mixture of the hydrochloride salt of each substituted methyl benzimidate (**2a**–**c**) (1 mmol) and corresponding amino acid methyl ester hydrochloride (1 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (0.14 mL, 1 mmol). The reaction solution was stirred 24 h at room temperature, and the reaction was checked for completion by TLC. The insoluble salts were filtered off and the filtrate was washed with an $NaHCO_3$ saturated aqueous solution, dried over Na_2SO_4. The organic layer was concentrated under vacuum, and the crude product was chromatographed on silica gel to obtain the products (**3.1a–3.3a′**). The yields of **3.1a**, **a′**, **b**, **b′**, **c**, **c′**, **3.2a**, **a′**, **3.3a**, **a′** were 83%, 82%, 84%, 85%, 85%, 85%, 83%, 82%, 84%, 83%, respectively.

General Procedure for the Synthesis of 4.1a–4.2a′ $LiAlH_4$ (52.8 mg, 1.1 mmol) was added slowly in parts to a stirred solution of **3.1a–3.2a′** (1 mmol) in Et_2O (10 mL). After 1 h, the reaction was completed (checked by TLC). Ethyl acetate was added, and then the mixture was washed with saturated aq. $NaHCO_3$ (2×5 mL), H_2O (2×5 mL), saturated aq. NaCl (2×5 mL), and dried with anhydrous Na_2SO_4. The mixture was concentrated under vacuum, and the crude product was chromatographed on silica gel to obtain the products (**4.1a–4.3a′**). The yields of **4.1a**, **a′**, **b**, **b′**, **c**, **c′**, **4.2a**, **a′**, **4.3a**, **a′** were 86%, 87%, 84%, 84%, 88%, 86%, 87%, 86%, 76%, 75%, respectively.

(*R*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1a**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 11.65 (1H, br s), 7.67 (1H, dd, J=1.5, 7.0 Hz), 7.40 (1H, m), 7.02 (1H, dd, J=0.5, 8.5 Hz), 6.89 (1H, m), 4.99 (1H, dd, J=7.5, 10.5 Hz), 4.68 (1H, dd, J=7.5, 9.0 Hz), 4.58 (1H, dd, J=9.0, 10.5 Hz), 3.81 (3H, s). ESI-MS m/z: 222 $[M+H]^+$. $[\alpha]_D^{20}$ −35.0 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1a′**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 11.65 (1H, br s), 7.66 (1H, dd, J=1.5, 7.0 Hz), 7.40 (1H, m), 7.02 (1H, dd, J=0.5, 8.5 Hz), 6.88 (1H, m), 4.99 (1H, dd, J=7.5, 10.5 Hz), 4.68 (1H, dd, J=7.5, 9.0 Hz), 4.58 (1H, dd, J=9.0, 10.5 Hz), 3.81 (3 H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 170.98, 167.58, 159.94, 134.05, 128.38, 118.83, 116.95, 110.05, 68.92, 67.19, 52.83. ESI-MS m/z: 222 $[M+H]^+$. $[\alpha]_D^{20}$ +32.1 (c=0.1, MeOH).

(*R*)-2-(4-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1b**) White solid. mp: 128–130°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.76 (2H, d, J=8.5 Hz), 6.77 (2H, d, J=8.5 Hz), 4.94 (1H, dd, J=7.5, 10.5 Hz), 4.71 (1H, dd, J=7.5, 8.5 Hz), 4.61 (1H, dd, J=8.5, 10.5 Hz), 3.73 (3H, s). ESI-MS m/z: 222 $[M+H]^+$. $[\alpha]_D^{20}$ −139.8 (c=0.1, MeOH).

(*S*)-2-(4-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1b′**) White solid. mp: 128–130°C, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.76 (2H, d, J=8.5 Hz), 6.77 (2H, d, J=8.5 Hz), 4.94 (1H, dd, J=7.5, 10.5 Hz), 4.71 (1H, dd, J=7.5, 8.5 Hz), 4.61 (1H, dd, J=8.5, 10.5 Hz), 3.74 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 171.56, 167.57, 160.77, 130.71, 117.37, 115.67, 69.72, 67.53, 52.76. ESI-MS m/z: 222 $[M+H]^+$. $[\alpha]_D^{20}$ +138.8 (c=0.1, MeOH).

(*R*)-2-Phenyl-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1c**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.98 (2H, m), 7.50 (1H, m), 7.41 (2H, m), 4.96 (1H, dd, J=8.0, 10.5 Hz), 4.70 (1H, dd, J=8.0, 8.5 Hz), 4.60 (1H, dd, J=8.5, 10.5 Hz), 3.82 (3H, s). ESI-MS m/z: 206 $[M+H]^+$. $[\alpha]_D^{20}$ −130.4 (c=0.1, MeOH).

(*S*)-2-Phenyl-4-methoxycarbonyl-4,5-dihydrooxazole (**3.1c′**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.98 (2H, m), 7.50 (1H, m), 7.41 (2H, m), 4.96 (1H, dd, J=8.0, 10.5 Hz), 4.70 (1H, dd, J=8.0, 8.5 Hz), 4.60 (1H, dd, J=8.5, 10.5 Hz), 3.82 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 171.66, 166.35, 131.90, 128.62, 128.38, 126.94, 69.57, 68.63, 52.74. ESI-MS m/z: 206 $[M+H]^+$. $[\alpha]_D^{20}$ +128.2 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydrothiazole (**3.2a**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 12.30 (1H, br s), 7.39 (2H, m), 7.02 (1H, dd, J=1.0, 8.0 Hz), 6.88 (1H, m), 5.35 (1H, dd, J=8.0, 9.5 Hz), 3.82 (3H, s), 3.68 (1H, dd, J=8.0, 11.0 Hz), 3.59 (1H, dd, J=9.5, 11.0 Hz). ESI-MS m/z: 238 $[M+H]^+$. $[\alpha]_D^{20}$ +13.7 (c=0.1, MeOH).

(*R*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydro-

thiazole (**3.2a′**) Colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 12.30 (1H, brs), 7.39 (2H, m), 7.01 (1H, dd, J=1.0, 8.0 Hz), 6.88 (1H, m), 5.35 (1H, dd, J=8.0, 9.5 Hz), 3.82 (3H, s), 3.68 (1H, dd, J=8.0, 11.0 Hz), 3.59 (1H, dd, J=9.5, 11.0 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.33, 170.61, 159.16, 133.59, 130.80, 118.97, 117.28, 115.95, 76.68, 52.91, 33.67. ESI-MS m/z: 238 $[M+H]^+$. $[\alpha]_D^{20}$ −14.1 (c=0.1, MeOH).

(*R*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydroimidazole (**3.3a**) White solid. mp: 154–156°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.35 (2H, m), 7.01 (1H, dd, J=1.0, 8.0 Hz), 6.84 (1H, m), 4.54 (1H, t, J=8.0 Hz), 4.15 (2H, d, J=8.0 Hz), 3.80 (3H, s). ESI-MS m/z: 221 $[M+H]^+$. $[\alpha]_D^{20}$ −11.7 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-methoxycarbonyl-4,5-dihydroimidazole (**3.3a′**) White solid. mp: 152–154°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.34 (2H, m), 7.01 (1H, dd, J=1.0, 8.0 Hz), 6.84 (1H, m), 4.54 (1H, t, J=8.0 Hz), 4.15 (2H, d, J=8.0 Hz), 3.80 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.02, 165.67, 160.64, 133.03, 126.41, 118.24, 117.63, 111.42, 52.75. ESI-MS m/z: 221 $[M+H]^+$. $[\alpha]_D^{20}$ +13.7 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole (Yanglingmycin) (**4.1a**) Colorless needle-shaped crystal. mp: 78–80°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.66 (1H, dd, J=1.5, 7.5 Hz), 7.39 (1H, m), 7.01 (1H, d, J=8.0 Hz), 6.89 (1H, m), 4.50 (2H, m), 4.36 (1H, t, J=6.0 Hz), 3.89 (1H, dd, J=3.5, 11.5 Hz), 3.71 (1H, dd, J=3.5, 11.5 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 166.93, 159.78, 133.68, 128.22, 118.81, 116.75, 110.40, 68.58, 66.87, 63.95. ESI-MS m/z: 194 $[M+H]^+$. $[\alpha]_D^{20}$ −16.4 (c=0.1, MeOH).

(*R*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole (**4.1a′**) White solid. mp: 76–78°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.66 (1H, dd, J=1.5, 7.5 Hz), 7.38 (1H, m), 7.00 (1H, d, J=8.0 Hz), 6.88 (1H, m), 4.52 (2H, m), 4.38 (1H, m), 3.90 (1H, dd, J=3.5, 11.5 Hz), 3.71 (1H, dd, J=3.5, 11.5 Hz). ESI-MS m/z: 194 $[M+H]^+$. $[\alpha]_D^{20}$ +17.3 (c=0.1, MeOH).

(*S*)-2-(4-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole (**4.1b**) White solid. mp: 192–194°C. ^{1}H-NMR ($(CD_3)_2SO$, 500 MHz) δ: 10.04 (1H, s), 7.69 (2H, d, J=9.0 Hz), 6.81 (2H, d, J=9.0 Hz), 4.79 (1H, t, J=5.5 Hz), 4.39 (1H, m), 4.24 (2H, m), 3.59 (1H, m), 3.42 (1H, m). ^{13}C-NMR ($(CD_3)_2SO$, 125 MHz) δ: 163.25, 160.66, 130.17, 118.83, 115.65, 69.95, 68.43, 63.74. ESI-MS m/z: 194 $[M+H]^+$. $[\alpha]_D^{20}$ −51.6 (c=0.1, MeOH).

(*R*)-2-(4-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole (**4.1b′**) White solid. mp: 192–194°C. ^{1}H-NMR ($(CD_3)_2SO$, 500 MHz) δ: 10.04 (1H, s), 7.69 (2H, d, J=9.0 Hz), 6.81 (2H, d, J=9.0 Hz), 4.78 (1H, t, J=5.5 Hz), 4.38 (1H, m), 4.24 (2H, m), 3.58 (1H, m), 3.42(1H, m). ^{13}C-NMR ($(CD_3)_2SO$, 125 MHz) δ: 163.27, 160.67, 130.18, 118.80, 115.65, 69.96, 68.41, 63.72. ESI-MS m/z: 194 $[M+H]^+$. $[\alpha]_D^{20}$ +50.2 (c=0.1, MeOH).

(*S*)-2-Phenyl-4-hydroxymethyl-4,5-dihydrooxazole (**4.1c**) White solid. mp: 81–83°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.88 (2H, m), 7.45 (1H, m), 7.37 (2H, m), 4.48 (1H, m), 4.42 (1H, m), 4.34 (1H, m), 3.97 (1H, m), 3.67 (1H, m). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 165.58, 131.54, 128.35, 128.30, 69.27, 68.08, 63.94. ESI-MS m/z: 178 $[M+H]^+$. $[\alpha]_D^{20}$ −51.1 (c=0.1, MeOH).

(*R*)-2-Phenyl-4-hydroxymethyl-4,5-dihydrooxazole (**4.1c′**) White solid. mp: 82–84°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.88 (2H, m), 7.46 (1H, m), 7.37 (2H, m), 4.48 (1H, m), 4.43 (1H, m), 4.34 (1H, m), 3.97 (1H, m), 3.67 (1H, m). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 165.59, 131.54, 128.35, 128.30, 69.27, 68.06, 63.92. ESI-MS m/z: 178 $[M+H]^+$. $[\alpha]_D^{20}$ +47.6 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrothiazole (**4.2a**) White solid. mp: 43–45°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.41 (1H, dd, J=1.5, 7.5 Hz), 7.35 (1H, m), 6.99 (1H, dd, J=1.0, 8.0 Hz), 6.88 (1H, td, J=1.0, 7.5 Hz), 4.87 (1H, m), 3.97 (1H, dd, J=5.0, 11.0 Hz), 3.83 (1H, dd, J=5.0, 11.0 Hz), 3.42 (1H, dd, J=7.5, 11.0 Hz), 3.34 (1H, dd, J=7.5, 11.0 Hz). ESI-MS m/z: 210 $[M+H]^+$. $[\alpha]_D^{20}$ +15.8 (c=0.1, MeOH).

(*R*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrothiazole (**4.2a′**) White solid. mp: 44–46°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.41 (1H, dd, J=1.5, 7.5 Hz), 7.35 (1H, m), 6.99 (1H, dd, J=1.0, 8.0 Hz), 6.88 (1H, td, J=1.0, 7.5 Hz), 4.87 (1H, m), 3.97 (1H, dd, J=5.0, 11.5 Hz), 3.83 (1H, dd, J=5.0, 11.5 Hz), 3.42 (1H, dd, J=7.5, 11.0 Hz), 3.34 (1H, dd, J=7.5, 11.0 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.48, 158.96, 133.23, 130.66, 118.99, 117.06, 116.18, 77.69, 64.08, 32.69. ESI-MS m/z: 210 $[M+H]^+$. $[\alpha]_D^{20}$ −16.3 (c=0.1, MeOH).

(*S*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydroimidazole (**4.3a**) Yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.80(1H, brs), 7.53 (1H, dd, J=1.5, 8.0 Hz), 7.21 (1H, m), 6.71 (1H, dd, J=0.5, 8.5 Hz), 6.62 (1H, m), 4.03 (1H, m), 3.73 (1H, m), 3.49 (1H, m), 3.44 (1H, m), 3.39 (1H, m). ESI-MS m/z: 193 $[M+H]^+$. $[\alpha]_D^{20}$ +25.7 (c=0.1, MeOH).

(*R*)-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydroimidazole (**4.3a′**) Yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.80 (1H, brs), 7.53 (1H, dd, J=1.5, 8.0 Hz), 7.21 (1H, m), 6.71 (1H, dd, J=0.5, 8.5 Hz), 6.62 (1H, m), 4.03 (1H, m), 3.73 (1H, m), 3.49 (1H, m), 3.44 (1H, m), 3.39 (1H, m). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.50, 170.62, 137.91, 132.76, 123.51, 120.77, 115.53, 68.93, 65.22, 46.76. ESI-MS m/z: 193 $[M+H]^+$. $[\alpha]_D^{20}$ −25.3 (c=0.1, MeOH).

Filter Paper Assay The antibacterial activities of compounds against two strains of bacteria were evaluated by the filter paper assay. The standard bacterial strains were obtained from College of Plant Protection, Northwest A&F University. Ampicillin (Sigma, Shanghai, China) was used as a positive control. Mueller–Hinton (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China) agar was used as an assay medium. The medium at 45°C was mixed with a suspension containing the bacterial pathogen at approximately 108 colony forming units (CFU)·mL^{-1}. Next, the mixture was poured on 9 cm Petri dishes. The tested compounds were dissolved in acetone at the concentration of 1000 ppm, The filter papers (6 mm in diameter) were impregnated with 1 μL/disc of each compound, then were completely dried and placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism. Experiments were run in triplicate.

Minimum Inhibitory Concentration (MIC) Antibacterial activities were also measured by the micro-broth dilution method in 96-well culture plates using the Mueller–Hinton broth, according to the National Committee for Clinical Laboratory Standards.[12] The tested bacteria were incubated in the Mueller–Hinton broth for 12 h at 30°C at 190 rpm, and the spore concentration was diluted to approximately 1×10^5–1×10^6 CFU/mL with Mueller–Hinton broth. After incubation for 24 h at 30°C, the MICs were examined.

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Conflict of Interest The authors declare no conflict of interest.

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