Design and Enantioselective Synthesis of Chiral Pyranone Fused Indole Derivatives with Antibacterial Activities against *Xanthomonas oryzae* pv. *oryzae* for Protection of Rice

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ABSTRACT：

A new class of chiral pyranone fused indole derivatives were prepared by means of N-heterocyclic carbene (NHC) organocatalysis and demonstrated notable antibacterial activity against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Bio-assays showed that compounds (3*S,*4*R*)-**5b**, (3*S,*4*R*)-**5d** and (3*S,*4*R*)-**5l** exhibited promising *in vitro* efficacy against *Xoo*, with EC_{50} values of 9.05, 9.71 and 5.84 mg/L, respectively, which were superior to that of the positive controls with commercial antibacterial agents, bismerthiazol (BT, EC_{50} = 27.8 mg/L) and thiodiazole copper (TC, EC_{50} = 70.1 mg/L). Furthermore, single enantiomer (3*S,*4*R*)-**5l** was identified as an optimal structure to display 55.3% and 52.0% curative and protective activities against *Xoo in vivo* tests at a concentration of 200 mg/L, which slightly surpassed the positive control with TC (curative and protective activities of 47.2% and 48.8%, respectively). Mechanistic studies through molecular docking analysis revealed preliminary insights into the distinct anti-*Xoo* activity of the two single enantiomers (3*S,*4*R*)-**5l** and (3*R,*4*S*)-**5l**, wherein the (3*S,*4*R*)-configured stereoisomer could form a more stable interaction with *Xoo*DHPS (dihydropteroate synthase). These findings underscore the significant anti-*Xoo* potential of these chiral pyranone fused indole derivatives, and shall inspire further exploration as promising lead structures for a novel class of bactericides to combat bacterial infections and other plant diseases.

KEYWORDS: Chiral indole derivatives, Bacterial activity, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), Structure-activity relationship, Enantioselective synthesis

INTRODUCTION

Rice stands as the world's most vital crop, serving as the primary calorie source for over half of the global population.[1-4] Rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a rod-shaped, Gram-negative bacterium, poses a significant threat to rice production,[5-7] that has led to crop yield decreases ranging from 10% to 50% and enormous economic losses in agriculture every year.[8-10] The transmission of rice bacterial leaf blight primarily occurs through lesions on leaf tips, margins, and wounds, giving rise to symptoms such as leaf wilt, necrosis, and other abnormal growth patterns.[11,12] Among various control measures, chemical pesticides are pivotal for the management of the rice bacterial leaf blight disease in crop protection,[13-16] such as bismerthiazol (BT) and thiodiazole copper (TC) which are widely adopted as bactericides in China to control this disease. Somewhat unfortunately, the efficacies of these agrochemicals are largely short of expectation. For instance, the control efficiency of bismerthiazol is only modest, standing at 25.4% when used at a high dosage of 200 mg/L.[17] Moreover, the frequent use of these chemical agents has led to the development of notable resistance among plant pathogens.[18,19] Therefore, the search for new class, highly effective and environment friendly anti-bacterial agents to prevent or cure *Xoo* infection continues to be of fundamental importance and a highly urgent task for crop protection.

The structural derivatization and optimization studies on natural occurring biologically active molecules as potential leads have emerged as an appealing strategy for the discovery and development of novel green pesticides.[20-25] Research reveals

that certain natural products or their derivatives featuring indole structures demonstrate commendable biological activity while with low toxicity towards non-target organisms and environment.[26] Noteworthy examples include indole-3-butyric acid, ethychlozate, and Indometacin.[27-29] Similarly, natural molecules with pyran moieties, such as indole-3-butyric acid, ethychlozate, indometacin, viridepyronone, coumarins, chromone, and osthole, show significant biological activity that could be applied in combating pathogenic microorganisms.[30-33]

On the other hand, the development and application of chiral pesticides are of increasing significance in modern crop protection.[34-36] Generally, only one single enantiomer is notably active against the target while the other paired isomer is less or inactive, that thus offering an ideal approach to reduce the excess use of chemical agents by elimination of the less effective stereoisomer in the ingredients.[37,38] For instance, the herbicidal activity of metolachlor as developed by Syngenta is mainly attributed from the *S*-configured single enantiomer, while its opposite stereoisomer, *R*-metolachlor is basically inactive and displays notable toxicity (10 times higher than *S*-metolachlor).[39,40] Dong *et al*. found that 1*S,*4*R*-(–)-benzovindiflupyr, a widely used commercial fungicide, exhibited 1.7–54.5 times greater activity against six specific phytopathogens compared to the other enantiomer 1*R,*4*S*-(+)-benzovindiflupyr (Figure 1A).[41] Up to now, approximately 44% of the registered agrochemicals feature at least one stereogenic element as revealed by recent statistical data, and the number of chiral pesticides is still in dramatic increase every year.[42] However, most of these agents are sold in their racemic form in the market despite of their chiral structures

and remarkable difference on their biological activities. One of the major problems that hinder the study and development of chiral pesticides rely on the notable shortage of simple practical methods for preparation of highly optically enriched chiral agrochemicals.

Inspired from the naturally occurring pyranone and indole frameworks that show promising biological activity, we envisioned to design a new class of chiral heterocycles by incorporation of these two elegant scaffolds for development of novel antibacterial agents (Figure 1B). Here, we disclosed the enantioselective preparation of a diverse set of chiral fused indoles and exploration of their antibacterial activities against *Xoo*. While most of the studied compounds exhibited significant activities against *Xoo*, the stereochemical configuration (3*R,*4*S*- or 3*S,*4*R*-single isomers) for these chiral fused indole derivatives showed clear impact on the inhibitory activities against *Xoo*. Preliminary insights into the distinct antibacterial properties of the two single enantiomers were revealed by molecular docking studies of (3*R,*4*S*)- and (3*S,*4*R*)-**5l** with *Xoo* dihydropteroate synthase, wherein the (3*S,*4*R*)-configured stereoisomer could form a more stable interaction with dihydropteroate synthase. The findings in this study underscores the fundamental impact of different absolute configurations of chiral compounds on their biological activities, and shall inspire further explorations of chiral pyranone fused indole heterocyclic molecules as potential drug candidates to control the plant pathogen diseases.

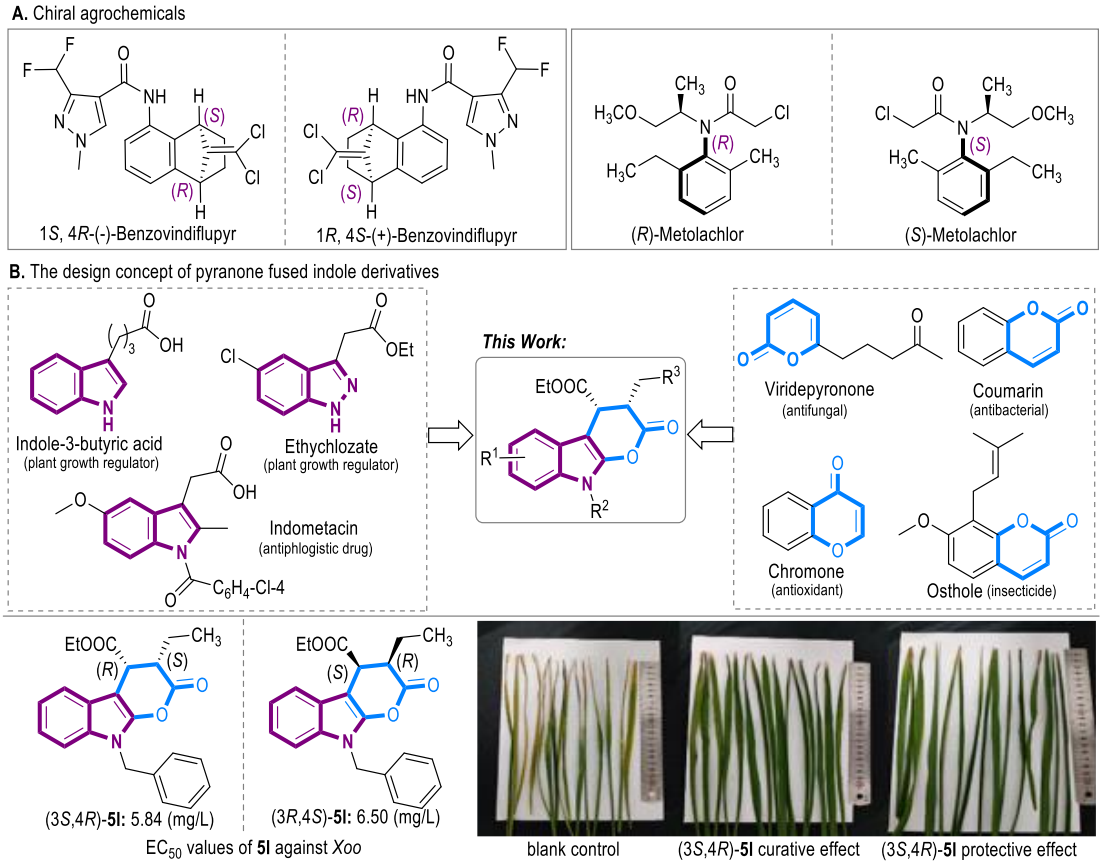

Figure 1. Design of chiral pyranone fused indole derivatives for potential treatment of rice plant *Xoo* infections.

MATERIALS AND METHODS

Chemicals and Instruments. All reagents and dry solvents were purchased from Energy Chemical, Aladdin, and Bide. NMR spectra were recorded using a Bruker ASCEND (AVANCE III HD 400 or 300 MHz) spectrometer, with deuterated chloroform ($CDCl_3$) employed as the solvent. Chiral HPLC analyses were conducted on a Shimadzu LC-20AT instrument, employing Daicel Chiracel columns at 25 °C. Chiral columns from Daicel Chemical Industries, models IB, IC, and ID, were utilized in the 4.6 × 250 mm^2 size configuration.

General procedure for the preparation of precursor 3.

A previous synthetic method with slight modifications was employed for the preparation of compound **3**.[43] In a stirred solution of isatin **1** (10.8 mmol) in dichloromethane (30.0 mL), the appropriate Wittig reagent (13.0 mmol) was added at 0 °C. The resulting mixture was stirred at 0 °C for 12 h. After removing the volatiles, the residue was subjected to purification by column chromatography (petroleum ether/ethyl acetate = 20/1) to yield the desired compound **2**. Subsequently, the title compound **2** (2.47 mmol) was combined with di-*tert*-butyl-dicarbonate (2.96 mmol, 646 mg) and 4-(dimethylamino)pyridine (0.25 mmol, 30.2 mg) in dichloromethane (15.0 mL) and kept to react at room temperature for approximately 2 h. The solvent was subsequently removed under reduced pressure, and the resulting mixture was purified through column chromatography (petroleum ether/ethyl acetate = 30/1) to afford the desired compound **3**.

Synthesis and *In Vitro* Anti-*Xoo* Activity of Target Compound 5.

The title indole derivatives **5** were prepared according to a previous synthetic

protocol.[43] In a stirred solution of aliphatic aldehyde compound **4** (1.0 mmol) in dichloromethane (5.0 mL), DL-proline (0.2 mmol, 23.0 mg) was introduced. After cooling to 0 °C, N-chlorosuccinimide (NCS, 0.9 mmol, 120 mg) was gradually added to the reaction system over a period of over 10 min. Subsequently, the reaction mixture was stirred at 0 ℃ for 1 h. Then about 4/5 of the solvent was removed under *vacuuo*, followed by filtration to give a clear filtrate, which was added to a 4 mL oven-dried vial containing a solution of compound **3** (0.10 mmol), NHC **A** (0.02 mmol, 8.4 mg), (Note: Racemates of **5** were prepared by using a racemic mixture of the NHC catalyst **A**, while (3*R*,4*S*)-**5** and (3*S*,4*R*)-**5** were prepared by using (+)-NHC **A** and (−)-NHC **A** respectively.) *N*, *N*-diisopropylethylamine (DIEA, 0.12 mmol, 35.0 mg) in 2.0 mL of 1,4-dioxane as the solvent. The reaction mixture was then stirred at 30 °C (oil bath) until TLC analysis confirmed the complete conversion of the starting material. Following this, the solvent was evaporated under reduced pressure, and the mixture was directly purified by column chromatography on silica gel (using a 20/1 petroleum ether/ethyl acetate ratio) to yield the desired pure products **5**, with isolated yields ranging from 21 to 98%.

***In Vivo* Antibacterial Activity against Rice Bacterial Leaf Blight.**

For more detailed information regarding the *in vitro* and *in vivo* experimental methods and additional specifics concerning *Xoo*, please refer to the **Supporting Information**.

HPLC analysis condition. The racemic products, employed to determine the er values were synthesized with a racemic catalyst. Details of the HPLC separation

conditions for each compound are provided in the **Supporting Information**.

Molecular Docking. Initially, the primary gene sequence of *Xoo*DHPS (dihydropteroate synthase) was acquired from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). Homology modeling was employed to derive the crystal structure of the GenBank LOC4343918. Subsequently, the resulting protein crystal underwent processing using the Protein Preparation Wizard module of the Schrödinger software. This entailed protein preprocessing, native ligand state restoration, optimization of H-bond assignments, protein energy minimization, and the removal of water molecules. At the same time, the 2D sdf structure files of compounds (3*S,*4*R*)-**5l** and (3*R,*4*S*)-**5l** were subjected to processing using Schrödinger's LigPrep module, generating their respective 3D chiral conformations. Utilizing the SiteMap module within Schrödinger, the best binding sites were predicted. Subsequently, the Receptor Grid Generation module was employed, configuring the optimal enclosing box to comprehensively encompass the predicted binding sites. This facilitated the extraction of the active site of the dihydropteroate synthase protein. Lastly, the processed compounds (3*S,*4*R*)-**5l** and (3*R,*4*S*)-**5l** were individually subjected to molecular docking with the active site of the dihydropteroate synthase protein, utilizing the highest accuracy XP docking approach. A lower docking score indicated reduced binding free energy and increased binding stability between the compound and the protein.

RESULTS AND DISCUSSION

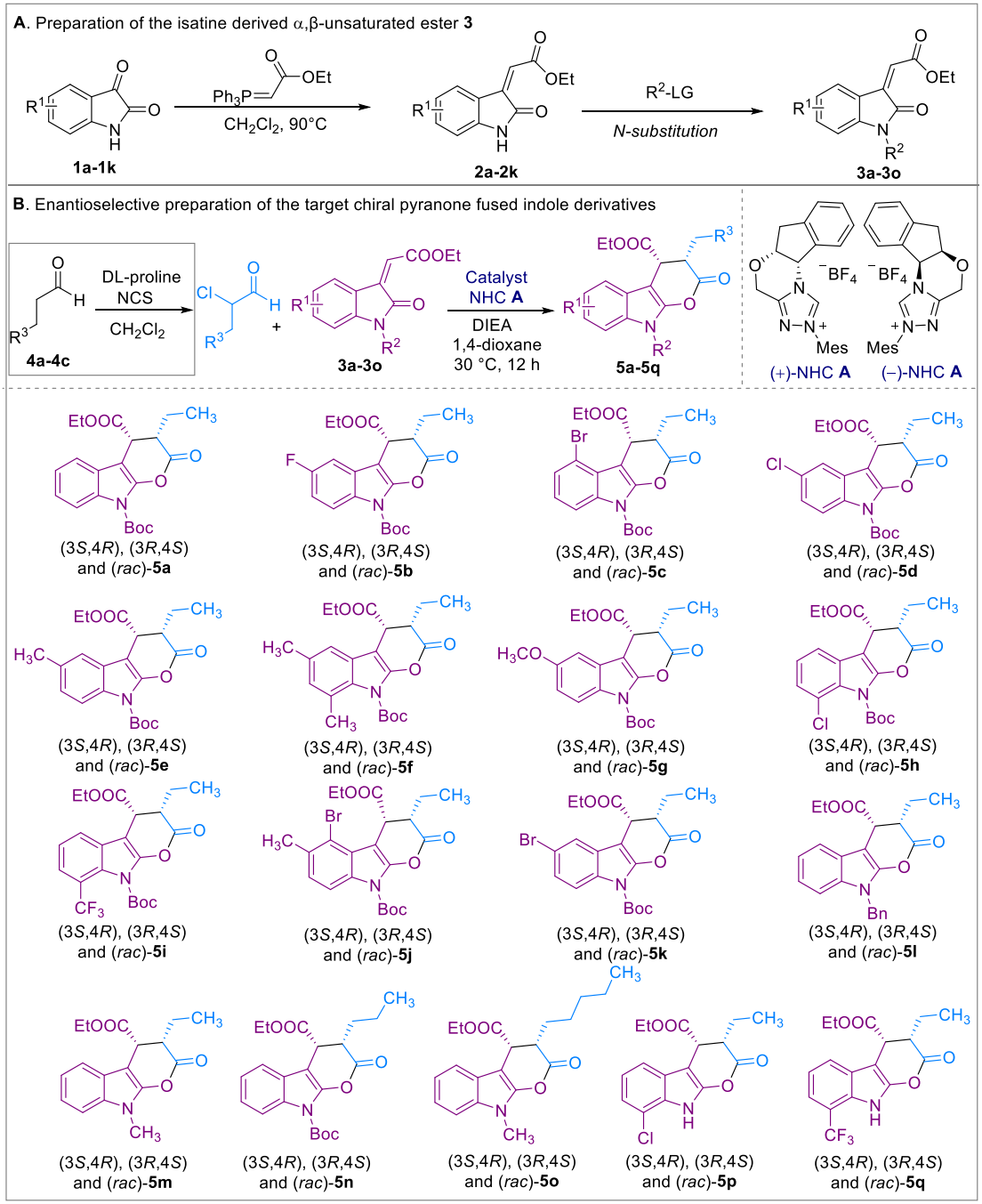

Figure 2. Synthesis of the chiral fused indole products **5a-5q**. Racemates of **5** were prepared by using a racemic mixture of the NHC catalyst **A**, while (3*R*,4*S*)-**5** and (3*S*,4*R*)-**5** were prepared by using (+)-NHC **A** and (−)-NHC **A** respectively.

Chemistry. The title fused indole derivatives (**5a-5q**) for antibacterial activities were readily prepared from aldehydes **4** and isatin derived α,β-unsaturated ester **3a**, which was obtained from a Wittig reaction and *N*-protection sequence (Figure 2A), as the starting materials (Figure 2B). The preparation involves a one-pot operation

developed within our laboratory through an enantioselective organocatalytic [4+2] annulations.[25] Currently, our focus was directed towards assessing the impact of various substituents on the biological activity of the resulting products **5**, in particular to study how the molecular chirality and functional moieties incorporated in the fused indole scaffold influenced the antibacterial activity. For instance, we introduced various substitutions (e.g. F, Cl, Br, CF_3 *etc*.) and substitution patterns into the pyranone fused indole core scaffold to study their inhibition activity against *Xoo*. We thereby synthesized over 50 fused indole compounds, encompassing their two optically pure (3*R,*4*S*)/(3*S,*4*R*) single enantiomers and racemic mixtures, with yields ranging from 21% to 98% (Figure 2). From the in vitro and in vivo anti-*Xoo* biological evaluations, five compounds, including (3*S,*4*R*)-**5b**, (3*S,*4*R*)-**5d** and (3*R,*4*S*)/(3*S,*4*R*)/(*rac*)-**5l** were identified with significant bactericidal activity. Comprehensive experimental data can be found in the **Supporting Information**.

***In Vitro* Antibacterial Bioassays and Structure-Activity Relationship (SAR).** A series of chiral indole derivatives containing different substituents in the pyranone moiety were synthesized, and the *in vitro* inhibitory activity of the target compounds on *Xoo* was studied. The antibacterial activity of these compounds was notably influenced by their stereoisomeric configurations (3*R,*4*S*)*,* (3*S,*4*R*)*,* (*rac*). For instance, at concentrations of 50.0 and 100 mg/L, single enantiomer (3*S,*4*R*)-**5b,** (3*S,*4*R*)-**5d**, (3*S,*4*R*)-**5m**, (3*S,*4*R*)-**5n**, (3*S,*4*R*)-**5p** exhibited significantly higher *in vitro* anti-*Xoo* activity compared to their corresponding (3*R,*4*S*)-configured stereoisomers, as well as their corresponding racemic mixtures (Table 1). Different substituents and substitution

patterns on the pyranone and indole moieties also exerted a significant influence in modulating their biological activity. For instance, the 5-F (**5b**) or 5-Cl (**5d**) groups on the benzene ring of indole significantly affected the anti-*Xoo* activity with obtained inhibition rates of 98.8% [(3*S,*4*R*)-**5b**] and 99.7% [(3*S,*4*R*)-**5d**], respectively when at the concentration of 100.0 mg/L, which are significantly higher than that of commercial drugs TC and BT. Introduction of 4-CF_3 unit on the indole ring showed a decrease of the activity to 60.8% [(3*S,*4*R*)-**5i**]. Furthermore, replacement of the ethyl group at the 3-position of the pyranone moiety with *n*-propyl (**5n**) or *n*-pentyl (**5o**) led to a significant enhancement in the inhibition rates (**5n** and **5o** *vs* **5a**), revealing an interesting modulation on the biological activity by the length of the carbon chain. Noteworthy is that the substitutions on the *N* atom of the indole moiety showed a significant impact on the activity. For instance, we were pleased to find that compounds (3*S,*4*R*)-**5l**, (3*R,*4*S*)-**5l** and (*rac*)-**5l** with *N*-benzyl group showed much superior antibacterial activities (99.1%, 98.1%, and 98.0% respectively at 50.0 mg/L) than that of compounds (3*S,*4*R*)/(3*R,*4*S*)/(*rac*)-**5a** bearing Boc (*tert*-butoxycarbonyl) unit on the *N* atom of indole moiety (59.0%, 20.8%, and 39.5% respectively at 50 mg/L).

Further evaluation of the bioactivity of compounds (3*S,*4*R*)-**5b**, (3*S,*4*R*)-**5d** and (3*S,*4*R*)/(3*R,*4*S*)/(*rac*)-**5l** were performed by EC_{50} tests. Gratifyingly, (3*S,*4*R*)-**5l** and (3*R,*4*S*)-**5l** with benzyl substitutions showed promising *in vitro* antibacterial potency with EC_{50} values calculated as 5.84 mg/L and 6.50 mg/L, respectively, which were both notably lower than their racemic mixture, (*rac*)-**5l** (11.5 mg/L). This could be

attributed to the antagonistic effect between different stereoisomers, resulting in a decreased activity of the racemate **5l**. It is worth mentioning that among all tested samples, (3*S*,4*R*)-**5l** exhibit the lowest EC_{50} (Table 2), which was superior than that of BT (27.8 mg/L) and TC (70.2 mg/L) under the same conditions. Therefore, compound (3*S*,4*R*)-**5l** was identified as the optimal molecule for further investigations.

Table 1. Preliminary antibacterial activities of title compounds anti-*Xoo* in Vitro

Compounds	*Xoo* Inhibition rate [%]		Compounds	*Xoo* Inhibition rate [%]	
	100 (mg/L)	50 (mg/L)		100 (mg/L)	50 (mg/L)
(3*S*,4*R*)-**5a**	77.6 ± 1.6	59.0 ± 6.5	(3*S*,4*R*)-**5j**	86.5 ± 1.1	54.1 ± 3.7
(3*R*,4*S*)-**5a**	35.8 ± 2.3	20.8 ± 7.0	(3*R*,4*S*)-**5j**	58.7 ± 6.0	19.0 ± 2.0
(*rac*)-**5a**	46.3 ± 5.3	39.5 ± 5.3	(*rac*)-**5j**	61.2 ± 1.1	38.7 ± 1.7
(3*S*,4*R*)-**5b**	98.9 ±2.0	98.5 ± 0.8	(3*S*,4*R*)-**5k**	84.9 ± 1.0	69.7 ± 5.6
(3*R*,4*S*)-**5b**	79.0 ± 0.6	65.1 ± 4.4	(3*R*,4*S*)-**5k**	66.9 ± 2.7	49.7 ± 0.7
(*rac*)-**5b**	94.9 ± 0.7	91.7 ± 0.2	(*rac*)-**5k**	72.1 ± 2.9	67.8 ± 2.1
(3*S*,4*R*)-**5c**	61.9 ±0.8	60.7 ± 0.6	(3*S*,4*R*)-**5l**	98.7 ± 3.3	99.1 ± 1.0
(3*R*,4*S*)-**5c**	47.9 ± 1.8	29.4 ± 1.1	(3*R*,4*S*)-**5l**	100.0 ± 1.7	98.1 ± 2.0
(*rac*)-**5c**	60.2 ± 0.8	42.1 ± 2.0	(*rac*)-**5l**	100.0 ± 0.9	98.0 ± 1.5
(3*S*,4*R*)-**5d**	99.7 ± 0.3	99.8 ± 0.8	(3*S*,4*R*)-**5m**	100.0 ± 1.9	85.2 ± 2.1
(3*R*,4*S*)-**5d**	86.1 ± 0.3	61.4 ± 0.5	(3*R*,4*S*)-**5m**	47.1 ± 2.8	16.1 ± 2.0
(*rac*)-**5d**	76.1 ± 0.4	59.0 ± 2.1	(*rac*)-**5m**	87.4 ± 0.9	75.0 ± 1.5
(3*S*,4*R*)-**5e**	88.8 ± 2.6	45.0 ± 3.5	(3*S*,4*R*)-**5n**	98.5 ± 0.4	70.9 ± 1.5
(3*R*,4*S*)-**5e**	64.2 ± 3.5	22.3 ± 1.7	(3*R*,4*S*)-**5n**	88.0 ± 1.2	70.8 ± 0.9
(*rac*)-**5e**	77.3 ± 0.7	34.7 ± 3.4	(*rac*)-**5n**	94.5 ± 1.2	69.4 ± 0.4
(3*S*,4*R*)-**5f**	84.9 ± 0.9	79.6 ± 0.2	(3*S*,4*R*)-**5o**	100.0 ± 0.1	73.6 ± 2.3
(3*R*,4*S*)-**5f**	68.6 ± 3.1	53.3 ± 1.9	(3*R*,4*S*)-**5o**	99.9 ± 0.1	71.7 ± 2.7
(*rac*)-**5f**	78.2 ± 0.6	73.7 ± 0.8	(*rac*)-**5o**	100.0 ± 0.3	78.3 ± 0.8
(3*S*,4*R*)-**5g**	85.0 ± 0.9	66.5 ± 2.2	(3*S*,4*R*)-**5p**	92.3 ± 0.7	88.8 ± 0.3
(3*R*,4*S*)-**5g**	57.7 ± 0.5	15.8 ± 0.4	(3*R*,4*S*)-**5p**	60.4 ± 0.9	43.9 ± 0.3
(*rac*)-**5g**	70.1 ± 1.3	27.4 ± 1.2	(*rac*)-**5p**	76.3 ± 1.9	66.0 ± 2.2
(3*S*,4*R*)-**5h**	84.9 ± 1.0	69.7 ± 5.6	(3*S*,4*R*)-**5q**	93.1 ± 0.4	90.9 ± 0.1
(3*R*,4*S*)-**5h**	66.9 ± 2.7	49.7 ± 0.7	(3*R*,4*S*)-**5q**	56.4 ± 2.8	47.0 ± 1.9
(*rac*)-**5h**	72.1 ± 2.9	67.8 ± 2.1	(*rac*)-**5q**	96.6 ± 0.3	85.6 ± 0.1
(3*S*,4*R*)-**5i**	60.8 ± 3.7	48.7 ± 1.3	TC	68.1 ± 1.2	37.4 ± 4.6
(3*R*,4*S*)- **5i**	41.3 ± 4.0	26.2 ± 1.1	BT	91.2 ± 0.2	89.1 ± 0.8
(*rac*)-**5i**	54.4 ± 1.2	36.2 ± 1.0			

Table 2. EC_{50} Values against *Xoo* of the target compounds (3*S*,4*R*)-**5b**, (3*S*,4*R*)-**5d**, (3*S*,4*R*)-**5l**, (3*R*,4*S*)-**5l**, and (*rac*)-**5l**

Compounds	Regression equation	EC_{50} (mg/L)	R^2
(3*S*,4*R*)-**5b**	$y = 0.0361x + 0.1734$	9.05	0.92
(3*S*,4*R*)-**5d**	$y = 0.0360x + 0.1504$	9.71	0.92
(3*S*,4*R*)-**5l**	$y = 0.0780x + 0.0445$	5.84	0.99

(3*R*,4*S*)-**5l**	$y = 0.0808x - 0.0256$	6.50	0.99
(*rac*)-**5l**	$y = 0.0406x + 0.0314$	11.5	0.96
BT	$y = 0.0179x + 0.0016$	27.8	0.99
TC	$y = 0.0067x + 0.0300$	70.1	0.98

***In Vivo* Bioassay against Rice Bacterial Leaf Blight.** *In vivo* experiments were conducted to further evaluate the potential application of compound (3*S,*4*R*)-**5l** in controlling rice bacterial leaf blight. As illustrated in Table 3 and Figure 3, compound (3*S,*4*R*)-**5l** demonstrated substantial therapeutic efficacy against the disease with a control efficiency of 55.3% at a concentration of 200 mg/L, which was superior than commercial bactericides, BT (53.7%) and TC (47.2%). Furthermore, (3*S,*4*R*)-**5l** also exhibited a notable protective effect, resulting in a slightly higher prevention and treatment efficiency of 52.0% at a concentration of 200 mg/L than that of BT (50.4%) and TC (48.8%). By consideration of its intriguing curative and protective effects, the (3*S,*4*R*)-**5l** holds significant promise as a potential lead compound for further pesticide development against *Xoo* infections.

Table 3. Curative and protective activities of compound (3*S,*4*R*)-**5l** against *Xoo* at 200 mg/L under greenhouse conditions

Compounds	Morbidity (%)	Curative activity (14 days after spraying)		Protective activity (14 days after spraying)	
		Disease index	Control efficiency (%)[b]	Disease index	Control efficiency (%)[b]
(3*S*,4*R*)-**5l**	100	40.7	55.3	43.7	52.0
BT	100	42.2	53.7	45.2	50.4
TC	100	48.2	47.2	46.7	48.8
CK[a]	100	91.1	/	91.1	/

[a] Negative control. [b] Statistical analysis was conducted by the ANOVA method under the condition of equal variances assumed ($P > 0.05$) and equal variances not assumed ($P < 0.05$).

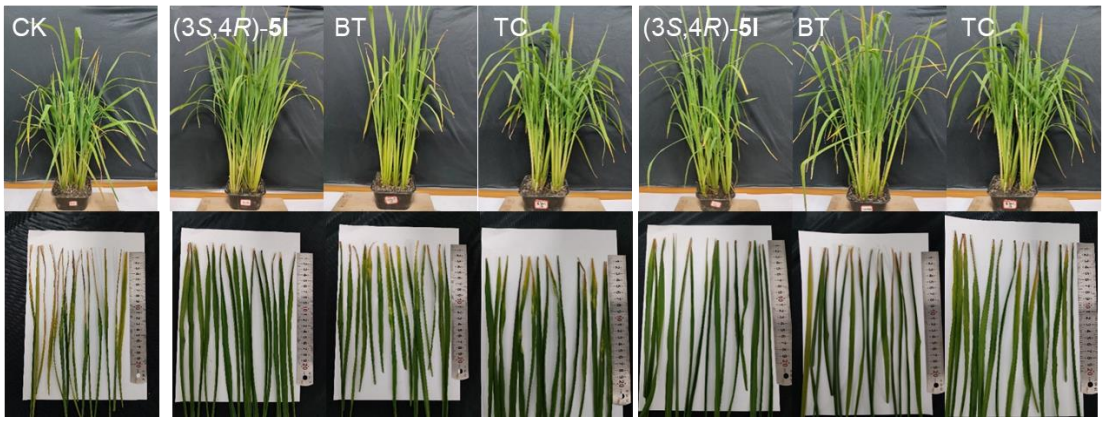

Figure 3. Curative and protective activities of compound (3*S*,4*R*)-**5l** anti-*Xoo* at 200 mg/L.

Molecular Docking. Dihydropteroate synthase (DHPS), as an essential enzyme, is pivotal in the biosynthesis of folic acid which is important for the preparation of bacterial nucleotide.[12,44] In order to gain insights into the binding interaction between different single enantiomers and *Xoo*DHPS, we conducted molecular docking analysis using the Schrödinger software, as illustrated in Figure 4. Owing to their superior biological activity, compounds (3*S*,4*R*)-**5l** and (3*R*,4*S*)-**5l** were selected as the primary candidates for docking studies to show their activity differences. The results showed that (3*S*,4*R*)-**5l** formed hydrophobic interactions with residues VAL443 and PHE440 of *Xoo*DHPS. Meanwhile, the oxygen atom in the ester group of (3*S*,4*R*)-**5l** resulted in a hydrogen bond with the residue GLU437 at distance of 1.79Å. On the other hand, hydrophobic interactions between (3*R*,4*S*)-**5l** with residues PHE564 and VAL443 of *Xoo*DHPS were also observed, while (3*R*,4*S*)-**5l** formed a hydrogen bond with LYS513 at distance of 2.40Å. The notable longer hydrogen bond between (3*R*,4*S*)-**5l** and LYS513 indicated a weaker interaction than that formed by (3*S*,4*R*)-**5l** and the residue GLU43, probably leading to a lower anti-bactericidal activity of (3*R*,4*S*)-**5l** in comparison to its enantiomer (3*S*,4*R*)-**5l**. The difference of these two enantiomers on

the binding affinity with *Xoo*DHPS was further verified by XP docking and MM-GBSA results, wherein (3*S*,4*R*)-**5l** showed a XP GScore of -3.08 and an MM-GBSA score of -20.0 kcal/mol, while a XP GScore of -1.84 and an MM-GBSA score of -22.4 kcal/mol were calculated for the other enantiomer (3*R*,4*S*)-**5l**. The relatively lower (3*S*,4*R*)-**5l** docking score (-3.08) than (3*R*,4*S*)-**5l** (-1.84) revealed a more intense binding interaction between (3*S*,4*R*)-**5l** and *Xoo*DHPS.

Table 4. XP and MM-GBSA result.

Compound	Target	XP GScore	MM-GBSA dG Bind (kcal/mol)
(3*S*,4*R*)-**5l**	DHPS	-3.08	-20.0
(3*R*,4*S*)-**5l**	DHPS	-1.84	-22.4

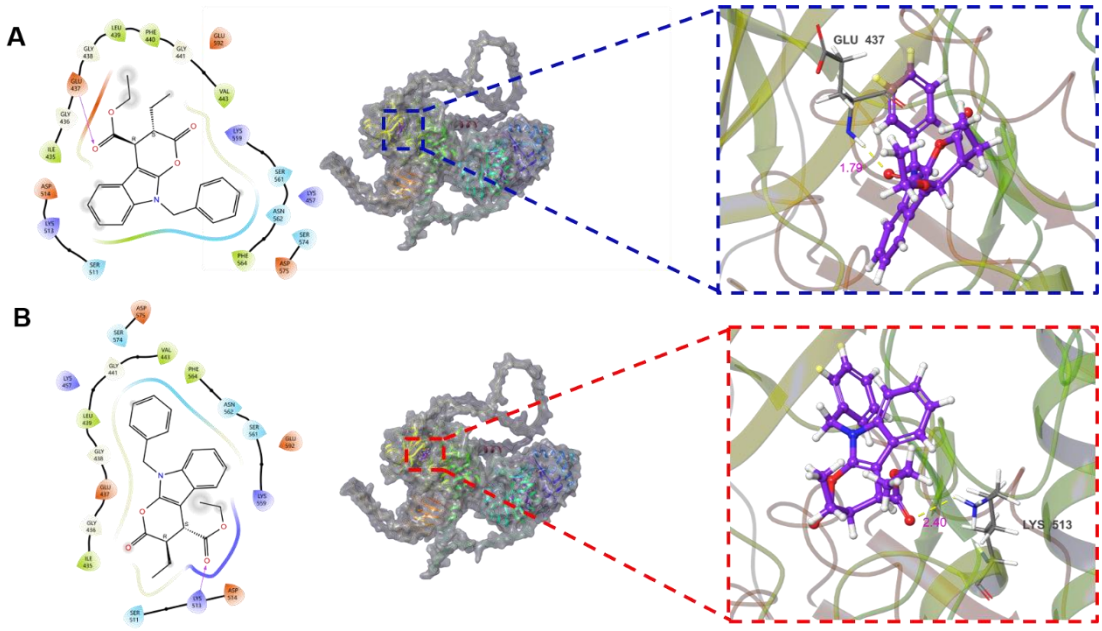

Figure 4. Molecular docking of compounds (3*S*,4*R*)-**5l** and (3*R*,4*S*)-**5l**. (A) Computational binding modes of (3*S*,4*R*)-**5l**-*Xoo*DHPS**.** (B) Computational binding modes of (3*R*,4*S*)-**5l**-*Xoo*DHPS**.** The hydrogen bond is depicted as yellow dashed lines.

In summary, we have successfully prepared a novel category of chiral pyranone fused indole scaffolds that includes their two single enantiomers and racemate and evaluated their antibacterial activities against *Xoo*. *In vitro* anti-*Xoo* bioassay results

showed that compounds (3*S,*4*R*)-**5b**, (3*S,*4*R*)-**5d**, (3*S,*4*R*)-**5l**, (3*R,*4*S*)-**5l** and (*rac*)-**5l** exhibited superior inhibition efficiency against *Xoo* to that of commercial antibacterial agents, BT and TC, with EC_{50} values of 9.05, 9.71, 5.84, 6.50, and 11.5 mg/L, respectively versus the positive controls with BT (EC_{50} = 27.8 mg/L) and TC (EC_{50} = 70.1 mg/L). Moreover, the anti-*Xoo* curative and protective activities of the optimal compound (3*S,*4*R*)-**5l** was studied by the *in vivo* tests, demonstrating notable control efficiency of 55.3% (curative activity) and 52.0% (protective activity) at 200 mg/L concentration, which slightly surpass the positive control with TC and BT. Molecular docking studies between (3*S,*4*R*)-, (3*R,*4*S*)-**5l** and DHPS shed preliminary insights on the distinct anti-*Xoo* activity influenced by the chirality of these compounds, wherein (3*S,*4*R*)-**5l** established more intense interactions with *Xoo*DHPS. This study underscores the fundamental impact of the stereochemical course of chiral compounds on their antibacterial activities against *Xoo*. A wide-ranging exploration with the developed pyranone fused indole derivatives as promising lead structures for the development of chiral bactericides and other agrochemicals could be anticipated.

SUPPORTING INFORMATION

All physical data, biological assay methods, high-resolution spectra, and NMR data of compounds **5a-5q** are presented in **Supporting Information**.

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Notes

The authors declare no competing financial interest.

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Design and enantioselective synthesis of chiral pyranone fused indole derivatives with antibacterial activities against *Xanthomonas oryzae* pv. *oryzae* for protection of rice.