New Axially Chiral Molecular Scaffolds with Antibacterial Activities against *Xanthomonas oryzae* pv. *oryzae* for Protection of Rice

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ABSTRACT: A new class of axially chiral thiazine molecules were constructed and showed promising antibacterial activities against plant pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The axial chiralities of these compounds (*R* or *S*-atropisomer) showed clear impacts on the *in vitro* inhibitory activities against *Xoo*. An optimal molecule of this class with (*S*)-axially chiral configuration was identified to exhibit inhibitory activity against *Xoo* with an EC_{50} value of 4.18 μg/mL. This inhibition efficiency is superior to two commercial antibacterial agrochemicals thiodiazole-copper (TC) and bismerthiazol (BT) as the positive controls. This hit compound also performed better than the controls in our *in vivo* studies. Preliminary mechanistic studies *via* Scanning Electron Microscopy (SEM) images showed that our hit compound at the concentration of 10 μg/mL destroyed the bacterial integrity of *Xoo*. Label-free quantitative proteomics analysis indicated that a total of 366 differentially expressed proteins of the rice plants were significantly influenced in the presence of our hit molecule.

KEYWORDS: *axial chirality; thiazine; atropisomer; anti-bacterial; Xoo.*

INTRODUCTION

The protection of agricultural plants from diseases caused by bacterial infections is an endless battle of both fundamental and practical significance. The problems are often complicated involving many disciplines; and solutions based on physical, biological, and chemical strategies are all used.[1-2] Each of these strategies has its own merits and limitations, and therefore advancements in all these three domains continue to be critical. In particular, the use of chemicals constitutes a sizable share in modern plant disease management. Many organic molecules and metal compounds, such as azoxystrobin,[3] kasugamycin,[4] and Bordeaux mixture,[5] have been widely used to treat fungal and / or bacterial infections on various plants.

Among these bacterial-related plant diseases, rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) remains a most difficult one with potentially big damages.[6] To date, management of rice bacterial leaf blight is primarily achieved through integration of planting techniques, or with the use of disease resistant rice plants as a most effective manner.[7-8] Antimicrobial chemical agents, such as bismerthiazol[9] and Zinc thiazole,[10-11] have also been used to control *Xoo* infections. Somewhat unfortunately, the efficacies of these metal or organic chemical agents are largely unsatisfactory as of today.[12-13] There is a strong need to search for effective chemical entities to cure or prevent *Xoo* infections on rice plants.

Our entry to this problem was directed toward designing new chiral molecules[14] for agricultural applications. Agrochemicals containing one or multiple stereocenters (chiral centers) is showing an increasing presence on the markets.[14-16] The enantiomers

/ stereoisomers often have different efficacies and / or environmental impacts. Typically, one of the two enantiomers (in the case of a racemate) performs better in terms of both efficiency and side effects. Most of these chiral agrochemicals are still used as a mixture of stereoisomers (e.g., racemate of two enantiomers of mixture of multiple diastereomers) to date. On the other hand, with the fast development of chiral synthesis techniques,[17-22] one can expect to see commercialization of a bigger number of optically enriched single stereoisomer agrochemicals. It has now well accepted that the development of chiral agrochemicals can significantly contribute to new and greener plant protection solutions. Of the numerous chiral agrochemicals being commercialized or under development, the vast majorities are central-chiral molecules (e.g., molecules with an atom as the stereogenic center) (Figure 1a). In contrast, other types of chiral molecules, such as those based on axial chirality (Figure 1b), are barely explored as pesticides for plant protections.[23-27] Axial chirality is a common phenomenon observed in living systems, including natural products isolated from plants.[28-31] Multiple human medicines approved by FDA (U.S. Food and Drug Administration) are racemizing atropisomeric (axially chiral) molecules,[32] such as Dabrafenib,[33] Eszopiclone[34-35] and Afatinib.[36-37] A much bigger number of axially chiral molecules as potential drug candidates are under active studies at different stages of development.[32,38-41] The relatively rapid development of axially chiral molecules as human medicines does not seem to influence much of the landscape of the pesticide research field. A representative example of the rather limited studies of axially chiral pesticide is the herbicide Metolachlor (Figure 1b).[27] The chiral C-N axis presented in Metolachlor was found to

have little influence on the efficacy[42-43] but different toxicity on aquatic organisms.[44-45] Other studies of axially chiral molecules as potential pesticides mainly involve isolated natural products bearing chiral axis, such as Laurokamurol and Ustilaginoidin.[46-47] Overall, very few studies or little background knowledge are available in current literature concerning axially chiral pesticides. There are very few deliberate designs of axially chiral molecules for agricultural uses. Part of the reason, to the best of our understanding, is due to the costs/challenges for access to such axially chiral molecules. On the other hand, in the recent one-to-two decades, with explosive development on asymmetric catalysis and chemical synthesis, synthesis of axially chiral molecules with low cost becomes feasible. We therefore believe that it is now a critical time to start serious evaluation of axially chiral molecules for plant diseases, especially those such as *Xoo* infection that currently does not have an effective chemical solution.

Our strategy and key findings are briefed in Figure 1c. We started with two building blocks (**1a** and **2a-m**) that are readily available and contain typical functional moieties found in pesticides and other bioactive molecules. The first building block (**1a**) is an ynal that after reactions become an α,β-unsaturated amide moiety (as in **3a**-**m**). Similar unsaturated amide groups are found in pesticides such as Maleic hydrazide and Nicobifen.[48-50] The second building block (**2a**-**m**) is an acylated thiourea that after reaction can form a heterocycle (a nitrogen and sulfur atoms-containing thiazine derivative) connecting to multiple heteroatom. Heterocycles of this type are common moieties in bactericides such as Cefradine and Omonasteine. The substituents (R, R', and R") on both building blocks can be easily modified to offer tunable structures. The

two building blocks can react with each other enabled by N-heterocyclic carbene (NHC) organocatalysts studied in our labs and others.[51-52] Importantly, this organocatalytic reaction (Equation 1) creates a new carbon-nitrogen (C-N) bond as a chiral axis, leading to a novel type of axially chiral molecules (**3a**-**m**). The axial chirality of most of these molecules (*R* or *S*-atropisomer) shows a clear impact on the *in vitro* inhibitory activities of these molecules against *Xoo*. For example, for a best-performing molecule [(*S*)-**3a**] with *Xoo* inhibition rate at about 92%, the corresponding enantiomer [(*R*)-**3a**] exhibits a much lower inhibition rate at around 52% under otherwise identical experimental conditions (Figure 1c). The EC_{50} of (*S*)-**3a** (4.18 μg/mL) of the *in vitro* studies is better than those of commercial pesticides (thiodiazole-copper and bismerthiazol). This lead compound (*S*)-**3a** also shows promising *in vivo* curative and protective effects on rice plants, compared to blank controls and commercial pesticides. These promising results clearly shows the effects of axial chirality on antimicrobial activities against *Xoo* for rice protection. That information obtained here with this new class of axially chiral molecules is unprecedented, and has potentials for probing new mechanisms of actions. It provides conceptual insights for further explorations of axially chiral molecules for agricultural applications.

Figure 1

MATERIALS AND METHODS

Instruments and Chemicals. Commercially available materials were purchased from J&K Scientific and Energy Chemical. NMR spectra were obtained by JEOL-

ECX-500 (500 MHz) or Bruker ASCEND 400 (400 MHz) spectrometer. Scanning electron microscope images were observed by Nova Nano SEM 450.

Synthesis of Axially Chiral Compounds (3a-m).

The synthetic scheme (Equation 2) and structures of compounds **3a**-**m** are illustrated in Figure 2. Th e synthetic procedures and product characterizations follow a recent report from our own laboratories.[53] A typical procedure is as the following. Chiral NHC pre-catalyst (+)-**A** (0.02 mmol, 0.2 equiv., 8.4 mg) was added into a 4.0 mL vial equipped with a magnetic stir bar, then DMAP (0.1 mmol, 1.0 equiv., 12.2 mg), $Sc(OTf)_3$ (0.02 mmol, 0.2 equiv., 9.8 mg), 5 Å molecular sieves (150.0 mg), DQ (0.3 mmol, 3.0 equiv., 122.4 mg) and substituted thiourea **2a** (0.1 mmol) were added. After that, furan (2.0 mL) and ynal **1a** (0.3 mmol) was added and the reaction mixture was allowed to stir for 12 hours at 30 ºC. After consumption of the thiourea substrate **2**, the reaction mixture was directly subjected to column chromatography on silica gel (20:1 hexanes / EtOAc) to afford the desired product (*S*)-**3a**. The other enantiomer (*R*)-**3a** was prepared by using the NHC catalyst with the opposite chirality [(-)-**A**] as the catalyst under an otherwise identical condition. The racemic form of the compound [(±)-**3a**] was prepared by using a racemic mixture of the NHC catalyst. Each of the compound were characterized by ^{1}H, ^{13}C, ^{19}F NMR, as documented in the earlier report.[53]

Figure 2

Methods for antibacterial activities against *Xoo*.

The *in vitro* and *in vivo* experiment against *Xoo*, scanning electron microscope analysis and label-free quantitative proteomics analysis were carried out,[54-59] and more

details could be seen in Supporting Information.

RESULTS AND DISCUSSIONS

Chemistry

The model axially chiral compounds for antimicrobial activities (**3a**-**3m**) were readily synthesized from acylthioureas and ynals as the starting materials (Figure 2, Equation 2). The synthesis involves a catalytic one-pot operation developed in our laboratories earlier[53]. Unlike our previous report[53] that concerns new catalysis concept and reaction designs, here in the present study we focused on how the axial chirality and the functional groups presented in the molecules influence the bioactivities. In particular, we introduced different substituent and substitution patterns into the structure of the compound **3** to investigate their antibacterial activities against *Xoo* (Figure 2). Both enantiomers of the target molecules were prepared and evaluated. Substituents commonly found to improve bioactivities in pesticides (such as F, Cl, Br) were found in our study to give high antimicrobial activities against *Xoo* (compound **3a**-**3c**, **3h**, **3i**). Meanwhile, electron-withdrawing group (NO_2) or electron-donating group (CH_3O) were also installed on the phenyl group to explore the influence of the electronic effect on target compounds (**3d**-**3f**). Although a comprehensive structure-activity relationship cannot be established at this point, our chemistry designs do provide sufficient insights for further development. For the first time, the chemical entities and their unique spatial arrangements induced by the axial chirality were found to have drastic influence on the antimicrobial activities against *Xoo* that infects rice plants.

***In Vitro* Antibacterial Bioassays.**

A series of axially chiral compounds (*R* or *S*-atropisomer) were prepared to investigate the *in vitro* inhibitory activities against *Xoo*. The agricultural antibiotic agents thiodiazole-copper (TC) and bismerthiazol (BT) were used as the positive controls. Among the compounds evaluated (Table 1), (*S*)-**3a** was found to exhibit the best antibacterial activity against *Xoo* at the concentrations of 50 and 100 μg/mL. The stereo-configurations (*R*, *S*, *rac*) of the compounds were found to significantly affect their antimicrobial activities. For example, the inhibition rate of (*S*)-**3a** was much better than its enantiomer (*R*)-**3a** and the corresponding racemic mixture [(*rac)*-**3a**]. Additional bioactivity studies with (*S*)-**3a** revealed an EC_{50} value of 4.18 μg/mL, which was superior to those of BT (16.22 μg/mL) and TC (37.35 μg/mL) under the same conditions (Table 1b). Different substituents or substitution patterns also have significant influence on the antibacterial activities of the compounds. For instance, when the Br group of **3a** was moved from the *ortho*- to the *meta*- (**3b**) or *para*-position (**3c**) on the benzene ring of the benzoyl group, a clear decrease on activities were observed. Replacing the *o*-Br group of (*S*)-**3a** with an electron-donating methoxy unit (to get compound **3d**) led to a moderate activity decrease. Multiple other substituents and substitution patterns commonly evaluated for drug and pesticide developments were also studied, as exemplified by compounds **3e**-**m**. Notably, compound (*S*)-**3e** was found to have an inhibition rate of 53.90% at a concentration of 50 μg/mL and a EC_{50} value of 17.75 μg/mL that is comparable to the positive controls using commercial pesticides (Table 1). As a technical note, as the solubility of these compounds is

relatively low in the experimental medium, the observed inhibition rates at the concentrations of 50 and 100 μg/mL are likely underestimated (Table 1). The EC_{50} values (Table 1b) of the compounds in comparison with the positive controls shall be relatively accurate.

Table 1

***In Vivo* Bioassay against Rice Bacterial Leaf Blight.**

To further evaluate the potential applications of (*S*)-**3a** against rice bacterial leaf blight, *in vivo* experiments were carried out. As shown in Figure 3, (*S*)-**3a** displayed a promising *in vivo* curative activity against this disease with control efficiencies of 45.90% at 200 μg/mL, which was better than that of TC (37.22%). For the protective activity, compound (*S*)-**3a** exerted a control efficiency of 53.70% that was superior to those of BT (20.02%) and TC (47.26%). Results from both *in vitro* and *in vivo* studies suggest that this type of axially chiral thiazine derivatives are a class of new scaffolds worth of further explorations as pesticides to fight against bacterial infections and other plant diseases.

Figure 3

Scanning Electron Microscopy (SEM).

SEM images were used to detect the morphological variations of pathogens after treatments with compounds (*S*)-**3a** and TC (10 μg/mL, 5 μg/mL) (Figure 4) that expressed in a concentration-dependent manner. The morphology of pathogen *Xoo* was changed from intact to partially damaged or distorted after treatments of (*S*)-**3a** (10 μg/mL, 5 μg/mL). Comparing with the positive control (of TC), the morphology of

bacteria showed more deformity, corrugations and cracking with the increase of the drug dosages of (*S*)-**3a**. Observations of the SEM analysis suggested compound (*S*)-**3a** could cause deformation of the cell membranes for *Xoo*. This disruption of cell membranes likely contributes to (part of) the activities observed from the *in vitro* and *in vivo* studies.

Figure 4

Label-Free Quantitative Proteomics Analysis.

We obtained a label-free quantitative proteomic profile[60] by treating rice plant with *Xoo* and compound (*S*)-**3a** successively (Figure 5), which can derive a preliminary understanding of the adjusting effect and antibacterial mechanism caused by our axially chiral thiazine derivatives. From the rice plant treated with *Xoo* and blank control, approximately 5797 proteins (5620 + 139; Figure 5a) were initially excavated. From the plant treated with *Xoo* and compound (*S*)-**3a**, the same analysis revealed 5658 (5620 + 38) proteins. Among these proteins, 5620 (96.9%) of them were commonly observed from plants treated with blank control and our compound respectively. Comparative proteomic analysis ((*S*)-**3a**/CK), 366 proteins (97 + 269; Figure 5b) were revealed to be differently expressed when triggered by compound (*S*)-**3a**. Under the screening conditions of multiple change >1.5 and $P < 0.05$, 97 proteins were up-regulated and 269 proteins were down-regulated.

The biological functions of the different expressed proteins were detected by three main GO categories (Figure 5d). The results of Biological Process (BP) analysis showed that these proteins were mainly related to metabolic process, cellular process,

biological regulation, localization, and response to stimulus. For the Molecular Function (MF), these proteins were involved in multiple biological actions such as catalytic activity, binding, and transporter activity. Meanwhile, Cellular Component (CC) analysis presented that most of the differentially expressed proteins were concentrated in the cell, the cell part, the organelle and the membrane.

Compound (*S*)-**3a** induced different expressions of the multiple proteins, which reveals the reasonable action pathways though the KEGG pathway enrichment bubble plot chart (Figure 5e). Galactose metabolism is one of the main metabolic pathways. It's well known that galactose metabolism plays significant roles in the bioactivities of malate dehydrogenase and isocitrate lyase, which is crucial in multifarious physiological processes incorporating the metabolisms of fat, glucose, and energy. It's also observed that the citrate, malic enzyme, and glyoxylic acid in the glyoxylate and diacid metabolism pathways are down-regulated, which might result in the indirect up-regulation of the Cytochrome P450 that plays significant roles in the nitrogen metabolism pathway. The up-regulation of the Cytochrome P450 can cause the death of the bacteria via improvement of the self-protective ability of the plants. Moreover, the amount of the peroxidase is significantly increased and can strengthen the plant entities to help resist various plant pathogens.

Figure 5

In summary, we have developed a new class of axially chiral thiazine derivatives with antibacterial activities against plant pathogen *Xoo*. Among these molecules, compound (*S*)-**3a** displayed potent inhibition effects with an EC_{50} value of 4.18 μg/mL

that are better than commercial antibacterial agents (BT and TC). *In vivo* studies revealed promising protective activities of (*S*)-**3a** against rice bacterial blight with a control efficiency of 53.70%, which is better than those for BT and TC. The chiralities of these molecules have profound effect on the corresponding antibacterial activities. Preliminary mechanistic studies *via* SEM images showed that the hit compound (*S*)-**3a** could clearly destroy the bacterial integrity of *Xoo*. Label-free quantitative proteomics analysis indicated that a total of 366 proteins were differentially expressed in plant cells as induced by compound (*S*)-**3a**. Our study opens a previously unexplored window in developing axially chiral molecules as effective cures of *Xoo* infections on rice plant. Insights generated from present and future studies in the direction of bioactive axially chiral scaffolds shall likely benefit the area of plant protection beyond *Xoo* infections. Ongoing studies in our laboratories include further structural optimizations and mechanistic evaluations.

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Notes

The authors confirm that this article content has no conflict of interest.

ABBREVIATIONS

Xoo, *Xanthomonas oryzae* pv. *oryzae*; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EC_{50}, the half maximal effective concentrations; TC, Thiodiazole copper; BT, bismerthiazol; DMAP, 4-Dimethylaminopyridine; SEM, Scanning Electron Microscopy.

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Figure Captions

Figure 1 Designing new axially chiral molecule scaffolds for potential treatment of rice plant *Xoo* infections.

Figure 2 Synthesis and structures of the axially chiral molecules.

Figure 3 Curative and protective activities of (*S*)-**3a** against rice bacterial blight at 200 μg/mL. BT and TC were the positive controls.

Figure 4 SEM images for *Xoo* after incubation in various dosages of (*S*)-**3a** or thiodiazole-copper. (*S*)-**3a**: (a) 0 μg/mL, (b) 10 μg/mL, (c) 5 μg/mL; thiodiazole-copper: (d) 10 μg/mL, (e) 5 μg/mL. Scale bars for (a)–(e) are 2000 nm.

Figure 5 (a) Venn diagrams for proteome comparison on control and treatment groups; (b) histogram of the number distribution of differentially expressed proteins in different comparison groups [(*S*)-**3a**/CK]; (c) volcano plot of differentially expressed proteins [(*S*)-**3a**/CK]; (d) Differentially expressed proteins in control and treatment groups; (e) KEGG pathway enrichment bubble plot of differentially expressed proteins

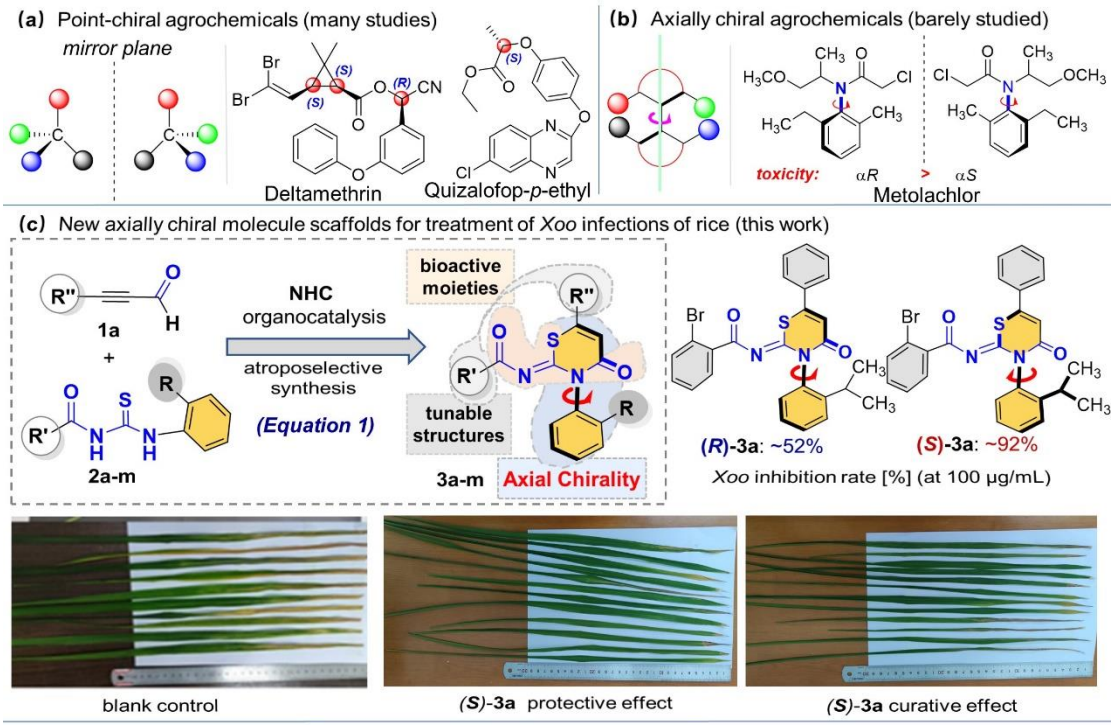
Table 1 *In vitro* antibacterial activities of title compounds against pathogens *Xoo*

(a) Preliminary antibacterial activities of title compounds against pathogens *Xoo in Vitro*

Compounds	*Xoo* inhibition rate [%]		Compounds	*Xoo* inhibition rate [%]	
	100 μg/mL	50 μg/mL		100 μg/mL	50 μg/mL
(*R*)-**3a**	52.25 ± 5.57	28.87 ± 8.01	(*R*)-**3h**	9.04 ± 9.09	14.04 ± 5.51
(*S*)-**3a**	92.16 ± 9.38	58.91 ± 3.61	(*S*)-**3h**	35.02 ± 7.57	18.41 ± 3.12
(*rac*)-**3a**	70.18 ± 7.67	31.52 ± 5.45	(*rac*)-**3h**	20.18 ± 1.68	11.64 ± 3.18
(*R*)-**3b**	37.96 ± 2.86	32.67 ± 8.21	(*R*)-**3i**	44.26 ± 7.51	7.65 ± 3.59
(*S*)-**3b**	18.28 ± 9.15	2.95 ± 9.73	(*S*)-**3i**	47.16 ± 8.84	31.43 ± 2.37
(*rac*)-**3b**	77.33 ± 1.62	46.06 ± 3.69	(*rac*)-**3i**	19.28 ± 5.30	36.27 ± 3.64
(*R*)-**3c**	25.83 ± 3.51	4.40 ± 2.47	(*R*)-**3j**	47.85 ± 1.55	21.11 ± 2.21
(*S*)-**3c**	34.07 ± 3.58	15.84 ± 7.24	(*S*)-**3j**	68.28 ± 8.15	32.95 ± 4.73
(*rac*)-**3c**	31.57 ± 9.16	26.77 ± 6.18	(*rac*)-**3j**	51.33 ± 2.62	16.06 ± 3.69
(*R*)-**3d**	74.28 ± 5.44	23.88 ± 4.76	(*R*)-**3k**	46.47 ± 9.54	5.65 ± 4.84
(*S*)-**3d**	86.76 ± 2.62	47.00 ± 1.90	(*S*)-**3k**	39.73 ± 8.59	9.95 ± 3.96
(*rac*)-**3d**	42.01 ± 3.92	34.47 ± 2.67	(*rac*)-**3k**	13.63 ± 6.98	4.17 ± 2.08
(*R*)-**3e**	54.75 ± 4.71	23.78 ± 4.39	(*R*)-**3l**	37.39 ± 0.68	15.83 ± 9.19
(*S*)-**3e**	68.23 ± 5.38	53.90 ± 2.29	(*S*)-**3l**	45.56 ± 4.47	15.97 ± 7.46
(*rac*)-**3e**	65.48 ± 2.78	62.19 ± 6.42	(*rac*)-**3l**	18.32 ± 6.52	27.25 ± 3.15
(*R*)-**3f**	44.96 ± 7.32	25.42 ± 1.65	(*R*)-**3m**	32.47 ± 9.57	23.08 ± 6.54
(*S*)-**3f**	37.96 ± 1.95	37.11 ± 4.14	(*S*)-**3m**	38.11 ± 6.59	34.42 ± 3.20
(*rac*)-**3f**	40.56 ± 9.94	36.11 ± 1.65	(*rac*)-**3m**	22.58 ± 9.02	11.54 ± 2.33
(*R*)-**3g**	28.72 ± 2.34	17.78 ± 1.59	TC	87.79 ± 4.69	53.45 ± 2.11
(S)-**3g**	17.48 ± 3.70	19.88 ± 8.26	BT	98.30 ± 2.19	79.53 ± 1.11
(*rac*)-**3g**	21.88 ± 1.23	18.11 ± 5.11	/	/	/

(b) EC_{50} values of target compounds(*S*)-**3a**, (*S*)-**3d** and (*S*)-**3e** against *Xoo*

Compounds	***Xoo* inhibition rate [%]**		
	regression equation	EC_{50} (μg/mL)	R^2
(*S*)-**3a**	y = 0.5285x + 4.6729	4.18	0.9257
(*S*)-**3d**	y = 0.4730x + 4.1628	58.88	0.9165
(*S*)-**3e**	y = 0.9932x + 3.7594	17.75	0.9677
BT	y = 2.5275x + 1.9419	16.22	0.9514
TC	y = 0.4267x + 4.3291	37.35	0.9758



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490 **Figure 1** Designing new axially chiral molecule scaffolds for potential treatment of rice plant *Xoo*

491 infections.

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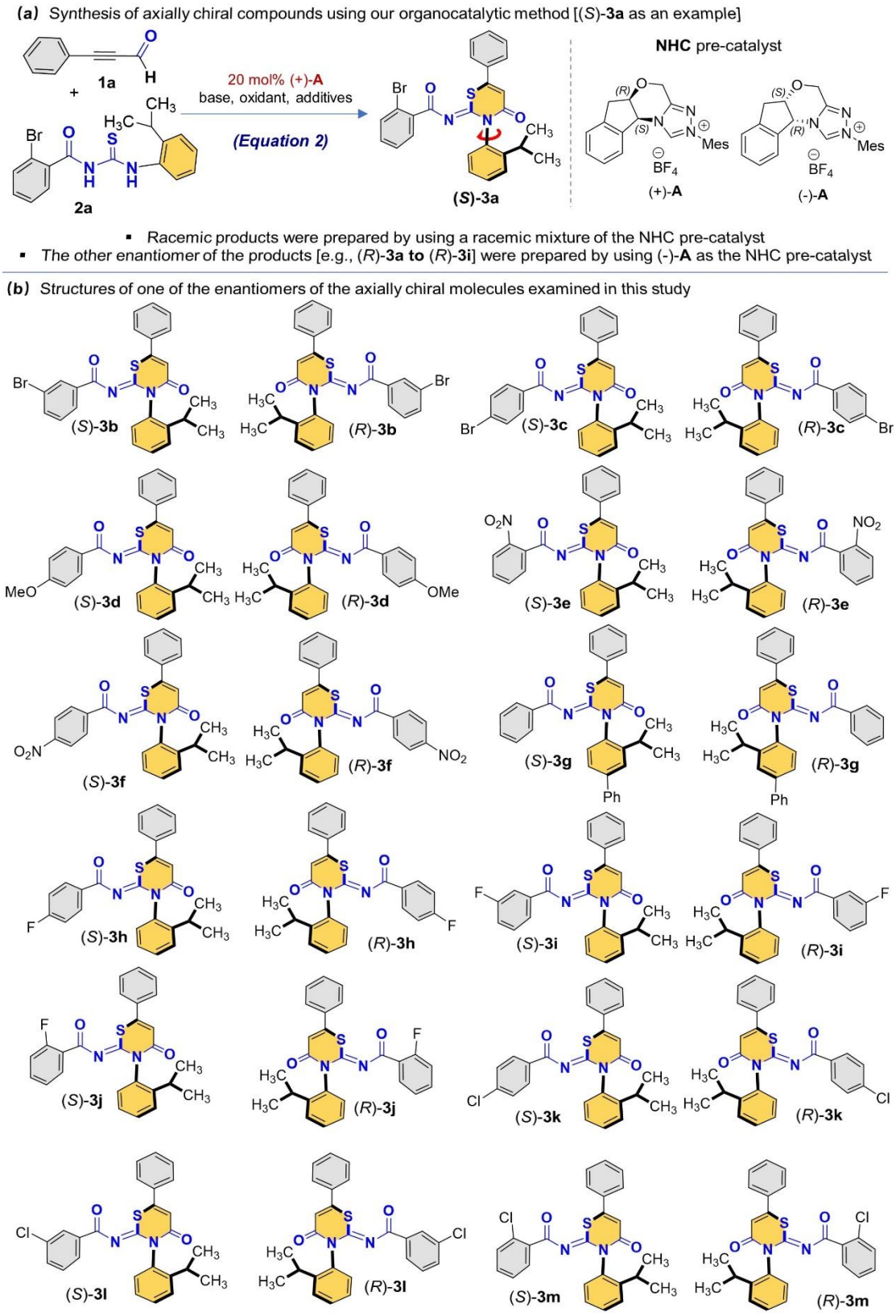

Figure 2 Synthesis and structures of the axially chiral molecules.

Compounds	Morbidity(%)	Curative activity (14 day after spraying)		Protective activity (14 day after spraying)	
		disease index	control efficiency(%)[b]	disease index	control efficiency(%)[b]
(*S*)-**3a**	100	45.80	45.90	39.20	53.70
TC	100	53.17	37.22	67.74	20.02
BT	100	36.67	56.71	44.67	47.26
CK[a]	100	84.70	/	84.70	/

[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).

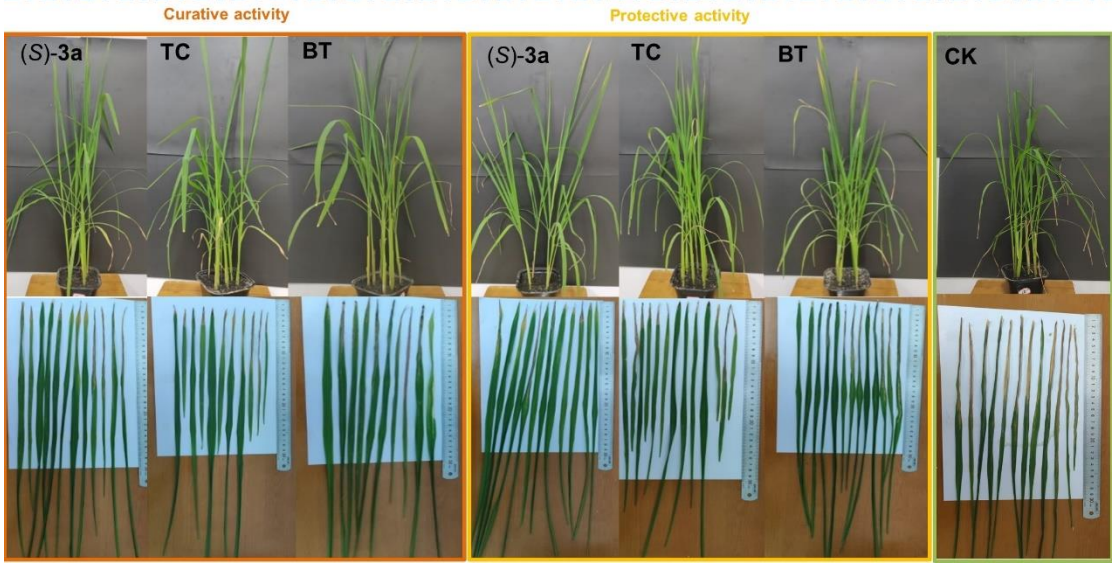

Figure 3 Curative and protective activities of (*S*)-**3a** against rice bacterial blight at 200 μg/mL. BT and TC were the positive controls.

500 

501 **Figure 4** SEM images for *Xoo* after incubation in various dosages of (*S*)-**3a** or

502 thiodiazole-copper. (*S*)-**3a**: (a) 0 μg/mL, (b) 10 μg/mL, (c) 5 μg/mL; thiodiazole-copper:

503 (d) 10 μg/mL, (e) 5 μg/mL. Scale bars for (a)–(e) are 2000 nm.

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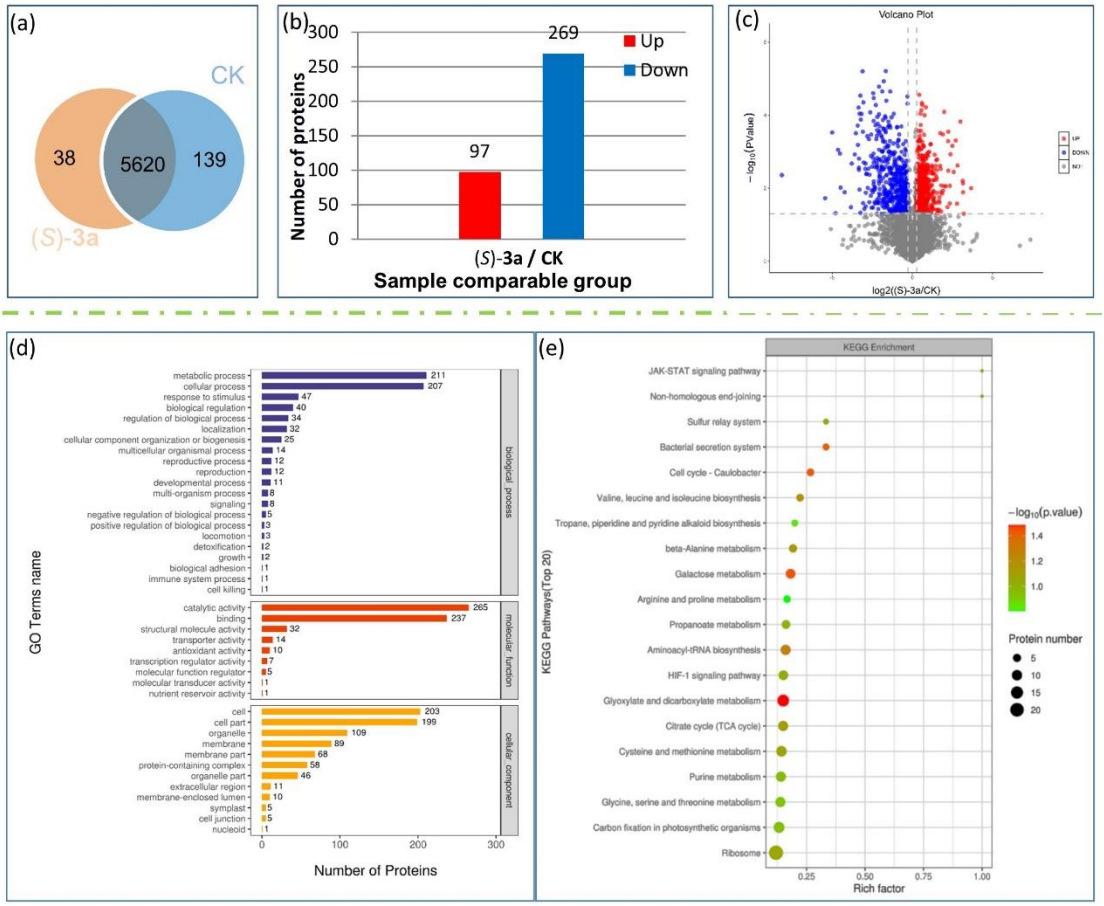

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506 **Figure 5** (a) Venn diagrams for proteome comparison on control and treatment groups;

507 (b) histogram of the number distribution of differentially expressed proteins in different

508 comparison groups [(*S*)-**3a**/CK]; (c) volcano plot of differentially expressed proteins

509 [(*S*)-**3a**/CK]; (d) Differentially expressed proteins in control and treatment groups; (e)

510 KEGG pathway enrichment bubble plot of differentially expressed proteins.

511 **TOC Graphic:**