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Design, Synthesis, Crystal Structure, and Antimicrobial Evaluation of 6-Fluoroquinazolinylpiperidinyl-Containing 1,2,4-Triazole Mannich Base Derivatives against Phytopathogenic Bacteria and Fungi

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Abstract: A total of twenty 1,2,4-triazole Mannich base derivatives bearing the 6-fluoroquinazolinylpiperidinyl moiety were designed, synthesized, and evaluated as antimicrobial agents against phytopathogenic bacteria and fungi, according to molecular hybridization strategy. Of note, the structure of target compound **4h** was clearly confirmed through single crystal X-ray diffraction analysis. The turbidimetric assays indicated that some compounds exhibited excellent antibacterial efficacies in vitro against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). For example, compounds **4c**, **4f**, **4j**, and **7j** had EC_{50} values of 23.6, 18.8, 23.4, and 24.3 μg/mL, respectively, far superior to that of agrobactericide bismerthiazol (EC_{50} = 92.4 μg/mL). Particularly, compound **4f** demonstrated a potent anti-*Xoo* activity, approximately 5-fold more active than bismerthiazol. Moreover, in vivo assays showed excellent protective and curative activities of compound **4f** against rice bacterial blight, having the potential as an alternative bactericide for controlling *Xoo*. The structure-activity relationship analysis showed a good pesticide-likeness concerning compound **4f**, following Tice's criteria. The anti-*Xoo* mechanism of compound **4f** was preliminarily explored by SEM measurements in living bacteria. Finally, several compounds also exhibited good antifungal activities in vitro against *Gibberella zeae* at 50 μg/mL. In short, the presented work showed the potential of 6-fluoroquinazolinylpiperidinyl-containing 1,2,4-triazole Mannich base derivatives as effective bactericides for controlling *Xoo*.

Keywords: 6-fluoroquinazoline, 1,2,4-triazole Mannich base, antimicrobial evaluation, structure-activity relationship (SAR)

1. Introduction

Plant bacterial diseases lead to enormous yield and economic losses in agricultural production every year.[1–3] For example, rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered as one of the most destructive bacterial diseases that can adversely affect rice plants during the whole growth stage. The pathogen of *Xoo* infects rice leaves mainly through the hydathodes at the leaf tip, leaf margins as well as the wounded leaves or roots, colonizes the intercellular spaces and enters the xylem vessels.[1] It had been reported that rice bacterial blight reduced the rice yield by ~50% in some Asian countries.[4] Of note, the pathogen of *Xoo* ranked 4th on the list of top 10 plant pathogenic bacteria because of its particular significance in agriculture.[1] Moreover, citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*) is a devastating bacterial disease, which leads to lesions on the leaves, fruits & stems and thus gives rise to serious damage to the whole citrus industry.[5,6] Additionally, *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is the causal agent of bacterial canker in kiwifruit and does huge damage to the international kiwifruit production.[7,8] On the other hand, it is well-known to us that fungal disease of the crops is a major threat to global agricultural production all the time, from the viewpoint of plant protection.[9] For example, the fungus of *Gibberella zeae* is capable of infecting a wide range of crop plants (including wheat and barley), and causing head blight and rot diseases around the world.[10,11] Although some agricultural antimicrobial agents (like bismerthiazol, thiodiazole-copper, copper quinolate, zinc thiazole, hymexazol, carbendazim, and thiophanate-methyl *et al*.) are now available

for fighting against the aforementioned pathogens, the ever-increasing resistances of them to currently-utilized antimicrobial agents[12,13] and their adverse environmental impacts posed by the abuse of these agrochemicals make it very urgent for the development of new agricultural antimicrobial agents with novel structures and improved performances.

Heterocyclic compounds, as the essential fragment of numerous commercialized drugs and pesticides, play a crucial role in the process of developing medical and agricultural antibiotics. Among them, the 1,2,4-triazole derivatives exhibit unique derivatization advantages since this backbone is capable of having the substituents not only at the 3- and 5-position but also at the 4-position, over its 1,3,4-oxadiazole and 1,3,4-thiadiazole couterparts. The introduction of additional substituent to 4-position of the 1,2,4-triazole ring can be utilized to optimize the physicochemical features and modulate the pharmacodynamics of target molecules.[14] In particular, the 1,2,4-triazole-5(4*H*)-thione Mannich base derivatives were found to exhibit a wide variety of bioactivities, including antibacterial,[15] antifungal,[16,17] anticancer,[18] antitubercular,[19] anti-inflammatory,[20] and antiprotozoal[21] effects (Figure 1). On the other hand, 6-fluoroquinazoline derivatives are being attracting more and more attention from medicinal and agrochemical chemists owing to their various biological activities, such as antibacterial,[22] antifungal,[23] anti-TMV (tobacco mosaic virus),[24] anti-HBV (hepatitis B virus),[25] anticancer,[26] antiproliferative,[27] and anticonvulsant[28] effects (Figure 1). Numerous studies had shown that the presence of piperidinyl linkage within small molecules was essential for the observed bioactivity, which is

also witnessed by their high prevalence in both drug and pesticide molecules (including dopamine antagonist Clebopride, narcotic analgesic Fentanyl, antiallergic drug Astemizole, and agrofungicide Oxathiapiprolin). Very interestingly, a statistical analysis conducted by Njardarson and co-workers showed that the piperidine heterocycle was the most prevalent nitrogen ring system among 640 drugs bearing a nitrogen heterocycle (approved by FDA before the year 2014),[29] followed by pyridine and piperazine rings, respectively.

Insert Figure 1

In our previous work,[30] we synthesized a small group of 1,2,4-triazole-5-thione Mannich base-quinazolinone hybrids (compound **I**, Figure 2) and evaluated their antibacterial and antifungal activities in agriculture. Unfortunately, the most potent compound in this series was found to possess only moderate bactericidal efficacy (~50% inhibiton rate) against *Xoo* at 100 μg/mL. After that, our group reported the antimicrobial capabilities of quinazolinylpiperidinyl-containing 1,2,4-triazole thioether acetamide derivatives (compound **II**).[12] Delightedly, nearly all the target compounds (except for two of them) exhibited comparable or even higher antibacterial potencies against *Xoo* (the best compound inhibited *Xoo* with EC_{50} = 34.5 μg/mL in vitro) after comparison with control agent bismerthiazol. Very recently, we prepared a series of 6-fluoroquinazolinylpiperidinyl-bearing 1,3,4-oxadiazole thioether derivatives (compound **III**) and tested their antibacterial & antifungal activities in vitro.[22] Notably, four of them demonstrated significantly better bactericidal activities against *Xoo* (the best compound suppressed *Xoo* with EC_{50} =

26.0 μg/mL in vitro) than control bismerthiazol. However, the search of easy-to-prepare, tunable molecular motifs that allow comprehensive structure-activity relationship studies with the purpose of improving the bioactivity is crucial to develop new agrochemicals in the future.

Insert Figure 2

Based on the above-mentioned considerations, herein we designed and synthesized a series of 6-fluoroquinazolinylpiperidinyl-bearing 1,2,4-triazole-5-thione Mannich base derivatives by the molecular hybridization approach (favorable for exerting synergistic effects on the bioactivity,[31,32] Figure 3). All the target compounds were assessed in detail for their in vitro antibacterial and antifungal efficacies, against three types of phytopathogenic bacteria and six types of phytopathogenic fungi of great agricultural significance. Moreover, in vivo anti-*Xoo* activity was further tested for highly-active compound **4f**, in order to evaluate its practical application for the prevention and control of rice bacterial blight. Finally, anti-*Xoo* mechanism of this compound was preliminary explored using scanning electron microscopy (SEM) technique.

Insert Figure 3

2. Materials and Methods

2.1. Instruments, Chemicals and Plant Pathogens

Melting points were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and uncorrected. ^{1}H and ^{13}C NMR data were collected on a Bruker Avance III 400 MHz NMR spectrometer at 298 K using DMSO-d_6 as a

123 solvent and TMS as an internal standard, and chemical shift (δ) was expressed in parts
124 per million (ppm). The following abbreviations were used in expressing the
125 multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad
126 peak. High resolution mass spectra (HRMS-ESI) were recorded on a Thermo
127 Scientific Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer. IR spectra
128 were recorded on a Thermo Scientific Nicolet iS50 FT-IR spectrometer. Elemental
129 analyses were determined by an Elementar Vario-III elemental analyzer. SEM images
130 were visualized and obtained using a Nova NanoSEM 450. The X-ray
131 crystallographic data were collected using a Bruker Smart Apex CCD area detector
132 diffractometer (Bruker, Germany) with Mo-Kα radiation. All the chemicals were
133 purchased from commercial suppliers and directly used without further purification
134 (unless stated otherwise).
135 All the strains of bacteria and fungi tested were provided by the Laboratory of Plant
136 Disease Control at Guizhou University.
137

138 **2.2. In Vitro Antibacterial Bioassay**

139 In vitro antibacterial activities of target compounds **4a**–**4j** and **7a–7j** were determined
140 against three plant pathogenic bacteria (*Xoo*, *Xac*, and *Psa*), according to the classical
141 turbidimetric method.[12,33,34] The tested compounds were prepared at two
142 concentrations of 100 and 50 μg/mL. Pure DMSO in sterile distilled water was used
143 as a blank control, and commercially available agrobactericides bismerthiazol (BMT)
144 and thiodiazole-copper (TDC) were used as positive control agents (100 and 50

145 μg/mL). About 40 μL of solvent NB (3.0 g of beef extract, 5.0 g of peptone, 1.0 g of
146 yeast powder, 10.0 g of glucose, 1000 mL of distilled water, pH = 7.0−7.2) containing
147 the bacterium *Xoo*, *Xac* or *Psa* was added to the mixed solvent system including 4 mL
148 of solvent NB and 1 mL of 0.1% Tween-20 containing tested compound or
149 BMT/TDC. The above test tube was incubated at 28 ± 1 °C and continuously shaken
150 at 180 rpm for one to three days. The bacterial growth was monitored by measuring
151 the optical density at 595 nm (OD_{595}), given by $turbidity_{corrected\ values} = OD_{bacterium} -$
152 $OD_{no\ bacterium}$, $I = (C_{tur} - T_{tur})/C_{tur} \times 100\%$. The C_{tur} represented the corrected turbidity
153 value of bacterial growth of untreated NB (blank control), and T_{tur} denoted the
154 corrected turbidity value of bacterial growth of tested compound-treated NB. The I
155 represented inhibition ratio of tested compound against the bacterium.

156 Subsequently, antibacterial potencies of target compounds **4a**–**4j** and **7a–7j** were
157 further measured against *Xoo* under five different concentrations (namely 100, 50, 25,
158 12.5, and 6.25 μg/mL) to obtain their EC_{50} values, which were statistically determined
159 by Probit analysis using the software package SPSS 17.0.

160

161 **2.3. In vivo Bioassay against Rice Bacterial Leaf Blight**

162 The curative and protection activities in potted plants of compound **4f** against rice
163 bacterial blight were determined, based on the previously-reported methods with
164 slight modifications.[34,35] Bismerthiazol (20% wettable powder) and thiodiazole copper
165 (20% suspending agent), commercialized agrobactericides for controlling rice
166 bacterial leaf blight, were bought from the market and used as positive control agents.

The curative activity for weakening rice bacterial leaf blight was tested under the controlled conditions in a vertical incubator. After sowing rice seeds of variety "Fengyouxiangzhan" about 8~10 weeks, rice leaves were then inoculated with *Xoo*, which was incubated at the logarithmic growth by use of sterilized scissors. One day after the inoculation, the solution of compound **4f**, BMT, and TDC at 200 μg/mL were uniformly sprayed into rice leaves, respectively. Meanwhile, distilled water was also uniformly sprayed as the negative control. Subsequently, all the inoculated rice plants were placed in a plant growth incubator (28 °C and 90% RH). At 14 days after spraying, the disease index of the inoculated rice leaves was calculated. Likewise, the protective activity in potted plants for weakening rice bacterial leaf blight was also determined under the controlled conditions. After sowing rice seeds of variety "Fengyouxiangzhan" about 8~10 weeks, the solution of compound **4f**, BMT and TDC at 200 μg/mL were uniformly sprayed onto rice leaves until dripping down. Meanwhile, distilled water was also uniformly sprayed into the negative control plants. One day after the spraying, *Xoo*, which was incubated at the logarithmic growth, was inoculated on rice leaves through sterilized scissors. All the inoculated rice plants were placed in a plant growth incubator (28 °C and 90% RH). At 14 days after the inoculation, the disease index of inoculated rice leaves was measured. The control efficiencies I (%) for the curative and protection activities were calculated using the following equation. In this equation, C is the disease index of the negative control and T is the disease index of the treatment group.

$$I\,(\%) = (C - T)/C \times 100\%$$

189

190 **2.4. Scanning Electron Microscopy (SEM)**[36]

191 In this assay, 1.50 mL of *Xoo* cell suspension within the logarithmic growth phase
192 was centrifuged and washed with PBS buffer (pH = 7.2) three times, then
193 re-suspended them with 1.5 mL PBS buffer. Subsequently, these *Xoo* cells were
194 incubated with compound **4f** at two different concentrations of 50.0 and 100.0 μg/mL
195 for 8 h, respectively. Meanwhile, an equiamount of DMSO was also used as a blank
196 control. After that, these samples were washed three times with PBS buffer (pH = 7.2).
197 Next, these *Xoo* cells were immobilized using 2.5% glutaraldehyde solution for 8 h at
198 4 °C, followed by dehydration with graded ethanol solution and absolute *tert*-butanol,
199 respectively. Finally, the samples were freezing dried, coated with gold and visualized
200 using Nova NanoSEM 450.

201

202 **2.5. In vitro Antifungal Bioassay**

203 The mycelial growth rate method[13,37] was employed to assess in vitro fungicidal
204 activities of target compounds against six phytopathogenic fungi, including
205 *Gibberella zeae*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Gloeosporium*
206 *fructigenum*, *Cytospora mandshurica*, and *Colletotrichum gloeosporioides*. A DMSO
207 solution of the tested compound was added into sterilized Petri dishes, which
208 contained about 10 mL molten potato dextrose agar (PDA). Next, a 4.0 mm-diameter
209 of mycelial plug was cut from the fungal colony and placed at the center of PDA plate
210 at 28 ± 1 °C for three to six days. Antifungal assays were performed in triplicate for

each compound. In addition, pure DMSO and commercialized agrofungicide hymexazol were also tested as negative and positive controls, respectively.

The inhibition ratio (I) of the tested compound was calculated using the following formula:

$$I = (C-T)/(C-0.4) \times 100\%$$

In the formula, the C represented the average mycelial diameter of negative control, and T represented the average mycelial diameter of the tested compound-treated PDA.

2.6. Preparation of Intermediates 2, 3, 5, and 6

2.6.1. Synthesis of Intermediate 2

Acylhydrazine **1**[22] (500 mg, 1.73 mmol) and phenyl isothiocyanate (0.25 mL, 2.07 mmol) were added to EtOH (20 mL), and the above mixture was heated to reflux for 6 h. After cooling down to room temperature, the formed precipitate was filtered, washed with ethanol three times and dried to give amidothiourea **2**.

2-(1-(6-fluoroquinazolin-4-yl)piperidine-4-carbonyl)-N-phenylhydrazinecarbothioamide (**2**). Yield: 90%, m.p. 187–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 9.98 (br, 1H), 9.58 (br, 2H), 8.64 (s, 1H), 7.92–7.88 (m, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.34 (t, J = 8.0 Hz, 2H), 7.17 (t, J = 8.0 Hz, 1H), 4.28 (d, J = 16.0 Hz, 2H), 3.21 (t, J = 12.0 Hz, 2H), 2.64–2.59 (m, 1H), 1.98 (d, J = 8.0 Hz, 2H), 1.86–1.78 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 180.8, 173.9, 163.8 (d, $^4J_{C\text{-}F}$ = 4.0 Hz), 158.5 (d, $^1J_{C\text{-}F}$ = 243.0 Hz), 153.3 (d, $^4J_{C\text{-}F}$ =

233 2.0 Hz), 148.4, 139.2, 131.0 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz), 130.0, 128.1, 126.0, 122.5 (d, $^{2}J_{C\text{-}F}$ =
234 25.0 Hz), 116.6 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz), 109.4 (d, $^{2}J_{C\text{-}F}$ = 23.0 Hz), 56.1, 48.8, 27.9.
235 ESI-HRMS *m*/*z*: $[M + H]^+$ calcd for $C_{21}H_{22}FN_6OS$: 425.1554; found: 425.1538.
236
237 **2.6.2. Synthesis of Intermediate 3**
238 Amidothiourea **2** (500 mg, 1.18 mmol) was added to an aqueous solution of 5%
239 potassium carbonate (15 mL), and the above mixture was heated to reflux for 6 h.
240 After cooling down to room temperature, the reaction system was neutralized to pH =
241 7.0 with a 10% HCl solution, and the resultant precipitate was filtered, washed with
242 water and dried to give the key 1,2,4-triazole-5-thione **3**.
243 *3-(1-(6-fluoroquinazolin-4-yl)piperidin-4-yl)-4-phenyl-1H-1,2,4-triazole-5(4H)-thione*
244 (**3**). Yield: 94%, m.p. 162–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 13.82 (br,
245 1H), 8.60 (s, 1H), 7.89–7.86 (m, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.64–7.57 (m, 4H),
246 7.51–7.49 (m, 2H), 4.17 (d, J = 12.0 Hz, 2H), 3.15–3.09 (m, 2H), 2.87–2.81 (m, 1H),
247 1.88–1.81 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 167.9, 163.5 (d, $^{4}J_{C\text{-}F}$ =
248 5.0 Hz), 158.5 (d, $^{1}J_{C\text{-}F}$ = 243.0 Hz), 154.5, 153.2 (d, $^{4}J_{C\text{-}F}$ = 2.0 Hz), 148.4, 133.8,
249 131.0 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz), 129.7, 129.6, 128.5, 122.4 (d, $^{2}J_{C\text{-}F}$ = 25.0 Hz), 116.5 (d,
250 $^{3}J_{C\text{-}F}$ = 8.0 Hz), 109.3 (d, $^{2}J_{C\text{-}F}$ = 24.0 Hz), 48.4, 32.5, 28.8. ESI-HRMS *m*/*z*: $[M + H]^+$
251 calcd for $C_{21}H_{20}FN_6S$: 407.1449; found: 407.1446.
252
253 **2.6.3. Synthesis of Intermediate 5**
254 Amidothiourea **5** was synthesized in a similar manner to amidothiourea **2**, using

4-(trifluoromethyl)phenyl isothiocyanate in place of phenyl isothiocyanate.

2-(1-(6-fluoroquinazolin-4-yl)piperidine-4-carbonyl)-N-(4-(trifluoromethyl)phenyl)hydrazinecarbothioamide (**5**). Yield: 93%, m.p. 163–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.05 (br, 1H), 9.87 (br, 2H), 8.65 (s, 1H), 7.92–7.89 (m, 1H), 7.79–7.74 (m, 3H), 7.72–7.68 (m, 3H), 4.29 (d, J = 12.0 Hz, 2H), 3.23 (t, J = 12.0 Hz, 2H), 2.72–2.58 (m, 1H), 1.99 (d, J = 8.0 Hz, 2H), 1.88–1.83 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 180.9, 174.0, 163.8 (d, $^{4}J_{C\text{-}F}$ = 5.0 Hz), 158.5 (d, $^{1}J_{C\text{-}F}$ = 244.0 Hz), 153.3 (d, $^{4}J_{C\text{-}F}$ = 2.0 Hz), 148.4, 143.1, 131.0 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz), 128.4, 125.7 (q, $^{2}J_{C\text{-}F}$ = 32.0 Hz), 125.2 (q, $^{3}J_{C\text{-}F}$ = 4.0 Hz), 123.3 (q, $^{1}J_{C\text{-}F}$ = 271.0 Hz), 122.5 (d, $^{2}J_{C\text{-}F}$ = 25.0 Hz), 116.5 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz) 109.4 (d, $^{2}J_{C\text{-}F}$ = 23.0 Hz), 56.1, 48.8, 27.9. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{22}H_{21}F_4N_6OS$: 493.1428; found: 493.1408.

2.6.4. Synthesis of Intermediate 6

Similar to the preparation of 1,2,4-triazole-5-thione **3**.

3-(1-(6-fluoroquinazolin-4-yl)piperidin-4-yl)-4-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5(4H)-thione (**6**). Yield: 95%, m.p. 155–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 13.71 (br, 1H), 8.60 (s, 1H), 8.01 (d, J = 8.0 Hz, 2H), 7.89–7.86 (m, 1H), 7.80 (d, J = 4.0 Hz, 2H), 7.74 (t, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 4.17 (d, J = 12.0 Hz, 2H), 3.19–3.13 (m, 2H), 2.90–2.84 (m, 1H), 1.87–1.83 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 167.8, 163.5 (d, $^{4}J_{C\text{-}F}$ = 4.0 Hz), 158.5 (d, $^{1}J_{C\text{-}F}$ = 244.0 Hz), 154.3, 153.2 (d, $^{4}J_{C\text{-}F}$ = 2.0 Hz), 148.4, 137.5, 131.0 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz), 129.9 (q, $^{2}J_{C\text{-}F}$ = 32.0 Hz), 129.8, 126.8 (q, $^{3}J_{C\text{-}F}$ = 4.0 Hz), 123.9 (q, $^{1}J_{C\text{-}F}$ = 272.0

277 Hz), 122.4 (d, $^{2}J_{C\text{-}F}$ = 24.0 Hz), 116.5 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz), 109.3 (d, $^{2}J_{C\text{-}F}$ = 23.0 Hz),
278 48.3, 32.3, 28.8. ESI-HRMS *m*/*z*: $[M + H]^+$ calcd for $C_{22}H_{19}F_4N_6S$: 475.1323; found:
279 475.1306.
280
281 **2.7. General Procedures for the Synthesis of Target Compounds 4a–4j and 7a–7j**
282 A mixture of 1,2,4-triazole-5-thiones **3** or **6** (1.0 mmol) and 37% formaldehyde
283 solution (1.5 mmol) in ethanol (15 mL) were firstly stirred at room temperature for
284 0.5 h, and the appropriate secondary amine (1.2 mmol) was then added and
285 continuously stirred for 6~12 h. After the filtration, the crude product was dried and
286 recrystallized with dichloromethane/petroleum ether (1.0%, v/v) to afford target
287 compounds **4a–4j** and **7a–7j**.
288
289 **2.8. Crystal Structure of Compound 4h**
290 Single crystals of compound **4h** suitable for X-ray diffraction analysis were obtained
291 by slow evaporation of a mixed DMF/EtOH (5.0%, v/v) solution of **4h** at room
292 temperature. The crystal structure (Figure 4) revealed that compound **4h** formed a 1:1
293 complex with water molecule through a hydrogen bond, involving water O–H and
294 1-position nitrogen atom of the quinazoline backbone. Crystal data for complex
295 **4h**·H_2O (CCDC 1936090): Colorless crystal, $C_{28}H_{31}FN_8OS{\cdot}H_2O$, M_r = 564.68,
296 triclinic, space group *P*-1, *a* = 8.600(6) Å, *b* = 9.829(7) Å, *c* = 18.434(13) Å; α =
297 75.578(19)°, β = 87.105(18)°, γ = 64.757(16)°; *V* = 1362.1(17) Å^3, T = 296 K, *Z* = 2,
298 *Dc* = 1.377 g/cm^3, *F*(000) = 596.0, reflections collected/independent reflections =

299 4789/2188, goodness-of-fit on $F^2 = 1.015$, $R = 0.0642$, $wR2 = 0.1447$.

300 Insert Figure 4

301 # 3. Results and Discussion

302 ## 3.1. Synthesis

303 Target compounds **4a**–**4j** were prepared in a consecutive seven-step reaction (as
304 depicted in Scheme 1), starting from 2-amino-5-fluorobenzoic acid. Briefly,
305 acylhydrazine **1** was firstly reacted with phenyl isothiocyanate in refluxing ethanol to
306 generate amidothiourea **2** in 90% yield, which then underwent a ring-closure reaction
307 under basic conditions and followed by the neturalization to give
308 1,2,4-triazole-5-thione **3** in 94% yield. Next, intermediate **3**, formaldehyde solution
309 along with various secondary amines in ethanol solution were reacted at room
310 temperature to afford target compounds **4a**–**4j**. Some previous studies have shown
311 that the prescence of a trifluoromethyl group within bioactive compounds was capable
312 of improving the bioactivity.[18,38] Thus, we also synthesized another class of target
313 compounds **7a**–**7j** containing a 4-trifluoromethylphenyl group at the 4-position of the
314 1,2,4-triazole-5-thione ring for comparison. It should be noted that no time-consuming
315 and laborious column chromatographic separation was required throughout the entire
316 reaction process (only through simple filtration, washing or recrystallization steps)
317 and good to excellent yields for all the intermediates and target compounds were
318 obtained, which were particularly important for the preparation of agrochemicals.

319 Insert Scheme 1

320

3.2. In Vitro Antibacterial Activity

Antibacterial activities of target compounds **4a**–**4j** and **7a**–**7j** against three bacteria *Xoo*, *Xac*, and *Psa* were evaluated in vitro by means of the classical turbidimetric method, using commercialized agrobactericides bismerthiazol (BMT) and thiodiazole copper (TDC) as positive control agents. As summarized in Table 1, all the target compounds (except for compounds **4d** and **7d**) did exhibit a significantly higher bactericidal potency against *Xoo* under two tested concentrations, as compared to control BMT. In particular, compounds **4a**, **4c**, **4e**, **4f**, **4h**, **4j**, **7c**, **7e**, **7i**, and **7j** were found to possess the inhibition rates 100%, 100%, 99.5%, 100%, 96.2%, 100%, 100%, 96.6%, 96.1%, and 100% towards this bacterium at 100 μg/mL, respectively, which were considerably superior to that of control BMT (56.3%). In sharp contrast to *Xoo*, only compound **7f** across the series displayed comparable inhibition efficacies against *Xac*, to those of control BMT. In addition, nine of them (including compounds **4b**, **4j**, **7b**, **7c**, **7d**, **7f**, **7g**, **7i**, and **7j**) demonstrated similar bactericidal efficacies (>55%) agasint the bacterium *Psa* at 100 μg/mL, to those of control agents BMT (59.6%) and TDC (52.6%). The above observations indicated that 1,2,4-triazole Mannich base derivatives bearing a 6-fluoroquinazolinylpiperidinyl moiety had a good antibacterial selectivity towards *Xoo* over *Xac* and *Psa*.

Insert Table 1

Inspired by the preliminary antibacterial results shown in Table 1, EC_{50} values (effective concentration for 50% inhibition activity) of these compounds against the

bacterium *Xoo* were further determined utilizing the serial dilution method. As shown in Table 2, all the compounds were found to possess lower EC_{50} values than control BMT. In particular, compounds **4a**, **4b**, **4c**, **4e**, **4f**, **4j**, **7c**, **7e**, **7i**, and **7j** had EC_{50} values of 28.6, 30.2, 23.6, 28.0, 18.8, 23.4, 27.1, 27.9, 28.5, and 24.3 μg/mL, respectively, over 3-fold more potent than BMT (EC_{50} = 92.4 μg/mL). In other words, half of this class of compounds demonstrated far better bactericidal capabilities than commericialized bactericide BMT.

Insert Table 2

3.3. Structure–Activity Relationship (SAR) Analysis for Target Compounds against *Xoo*

Initially, we tested inhibition activities of compounds **4b**, **4c**, and **4d** against *Xoo*, having the pyrrolidine, piperidine and diethylamine moieties, respectively. Clearly, compound **4d** with a conformationally-flexible acyclic diethylamine group displayed the weakest bactericidal activity among these three compounds, with EC_{50} values of 30.2, 23.6, and 72.0 μg/mL for compounds **4b**, **4c**, and **4d**, respectively. The same finding also held true for compounds 7**b**, **7c**, and **7d**, which contained a 4-CF_3-phenyl substituent on the 1,2,4-triazole-5-thione ring. Thus, the subsequent structural optimizations for preparing the final Mannich base derivatives were mainly limited to conformationally-rigid cyclic secondary amines, including morpholine, pyrrolidine, piperidine, and various *N*-substituted piperazine derivatives. Overall, the presence of a strong electron-withdrawing -CF_3 group was detrimental or at least unhelpful to the

anti-*Xoo* activity for the final compounds, with the exception of compounds **4i** and **7i** (EC_{50} = 49.1 *vs*. 28.5 μg/mL). As far as the heterocycles containing a single nitrogen atom were concerned, Mannich bases with a piperidine ring were identified as the best bactericides, followed by those having the morpholine and pyrrolidine rings (**4c** > **4a** ≈ **4b** and **7c** > **7a** > **7b**). As for piperazine-derived Mannich bases, the presence of weak electron-donating groups (such as compounds **4e**/-CH_3, **4f**/-CH_2CH_3, and **4j**/-Ph) on the piperazine ring proved to be superior to those bearing the electron-withdrawing groups (like compounds **4h**/-$COCH_3$ and **4i**/-Boc). However, the existence of sterically-bulky and weakly electron-donating *iso*-propyl group did not contribute to the improvement of anti-*Xoo* activity, as exemplified by compounds **4g** and **7g**. Among the whole series of compounds, Mannich base **4f** with a *N*-ethyl piperazine moiety demonstrated the highest anti-*Xoo* activity with an EC_{50} value of 18.8 μg/mL, nearly 5-fold more potent than control BMT.

Given the particular importance of physicochemical properties in the development of new pesticides,[31,34,39,40] five parameters (namely, molecular weight (MW), octanol/water partition coefficient (cLog*P*), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), and number of rotatable bonds (ROTB)) for target compounds **4a**−**4j** and **7a**−**7j** were obtained from the softwares of ChemBioOffice 2010 and/or Molinspiration Cheminformatics (see Table 2). Generally speaking, a promising pesticide candidate should meet the following requirements (also known as Tice's criteria[41]): (a) a cLog*P* value between 0 and 5; (b) a MW magnitude ranging from 150 to 500; (c) a HBA number between 1 and 8; (d)

HBD number ≤ 2; (e) ROTB number <12. Obviously, the most potent compound **4f** (having a relatively hydrophilic nature of cLog*P* = 3.17 relative to other compounds) satisfied all the above listed requirements, except for molecular weight descriptor (MW = 532.3, slightly larger than upper limit of 500 required by Tice's criteria). On the basis of these physicochemical features, this compound thus possessed a good pesticide-likeness suitable for further studies.

3.4. In Vivo Bioassay against Rice Bacterial Leaf Blight

In order to examine the practical potential of highly-active compound **4f**, in vivo bioassays against rice bacterial leaf blight were also carried out. As displayed in Table 3 and Figure 5, the obtained results revealed that compound **4f** possessed an excellent protection activity against *Xoo* at 200 μg/mL (with a control efficiency of 56.23%), better than those of BMT (43.45%) and TDC (45.34%). Furthermore, compound **4f** was also found to have an outstanding curative activity against *Xoo* at 200 μg/mL (with a control effect of 52.30%), higher than those of BMT (46.80%) and TDC (42.43%). These findings implied that compound **4f** had a good application perspective as an alternative bactercide for the prevention and control of *Xoo*.

Insert Table 3

Insert Figure 5

3.5. SEM Studies on Cell Morphology

In order to explore the possible antibacterial mechanism, the morphological

409 modifications of the bacterium *Xoo* in the presence of highly-active compound **4f**
410 were monitored through the SEM technique. As seen in Figure 6a, the untreated
411 bacteria showed the form of elongated cells, having a firm surface and a well-shaped
412 appearance. After treatment with 50 μg/mL of compound **4f** for 8 h, the appearance
413 was altered significantly. The size of *Xoo* was considerably reduced, and surface
414 collapses (accompanied by lots of pore-like structures) on the cell membranes were
415 clearly observed (Figure 6b). As the concentration rose to 100 μg/mL, more
416 accentuated morphological changes and few intact cells were simultaneously observed
417 (Figure 6c). These observations implied that compound **4f** changed the permeability
418 of cell membranes, possibly leading to the leakage of the bacterial contents to the
419 extracellular environment.

420 Insert Figure 6

421

422 **3.6. In Vitro Antifungal Activity**

423 Antifungal activities of target compounds **4a**−**4j** and **7a**−**7j** were also assessed in vitro
424 against six types of fungal strains, according to the mycelial growth rate method. As
425 summarized in Table 4, most of the target compounds failed to exhibit notable
426 fungicidal activities at 50 μg/mL. However, four compounds (**7d**, **7e**, **7g**, and **7i**)
427 revealed comparable antifungal efficiencies agaist *G. zeae* to control hymexazol
428 (55.0%), having the inhibition rates of 46.2%, 45.6%, 45.0%, and 45.6%, respectively.
429 In addition, compound **7i** effectively suppressed the growth of *V. dahlia* at 50 μg/mL,
430 with the inhibition rate of 51.3%.

Insert Table 4

In summary, a series of easy-to-make and structurally tunable 6-fluoroquinazolinylpiperidinyl-containing 1,2,4-triazole Mannich base derivatives were synthesized and assessed as antimicrobial agents in agriculture. Among them, the structure of compound **4h** was unambiguously confirmed by single crystal X-ray diffraction analysis. The bioassays indicated that nearly all the target compounds exhibited better inhibition activites against *Xoo* in vitro, relative to control bismerthiazol. In particular, compounds **4c**, **4f**, **4j**, and **7j** possessed EC_{50} values ranging from 18.8 to 24.3 μg/mL, considerably superior to that of commercialized bactericide bismerthiazol (92.4 μg/mL). Moreover, the best compound **4f** demonstrated excellent protective and curative activities in vivo against rice bacterial leaf blight caused by *Xoo*. The SEM measurements revealed that compound **4f** significantly changed bacterial cellular morphologies after interaction with *Xoo*. Taken together, the present work showed the potential of 6-fluoroquinazolinylpiperidinyl-containing 1,2,4-triazole Mannich base derivatives as a promising molecular platform for developing effective bactericides to control *Xoo*.

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448 ## Supporting Information

449 Characterization data and original spectral files for intermediates **2**, **3**, **5** & **6** as well as
450 target compounds **4a**–**4j** & **7a–7j** (including ^{1}H & ^{13}C NMR) are available free of
451 charge on the ACS Publications website.

452

453 ## Conflict of interest

454 The authors declare no competing financial interest.

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Figure Captions

629 **Figure 1.** Several representative bioactive molecules containing a
630 1,2,4-triazole-5-thione Mannich base or a 6-fluoroquinazoline moiety or a
631 piperidinyl linkage.

632 **Figure 2.** Previously-reported three classes of antibacterial compounds by our group.

633 **Figure 3.** Design strategy of target compounds in this work.

634 **Figure 4.** X-ray crystal structure of **4h**·H_2O complex.

635 **Figure 5.** Curative and protection activities of compound **4f** against rice bacterial leaf
636 blight under greenhouse conditions at 200 μg/mL, with **BMT** and **TDC** as
637 the positive control agents.

638 **Figure 6.** SEM images for *Xoo* after being incubated with different concentrations of
639 compound **4f**: (a) 0 μg/mL; (b) 50.0 μg/mL; (c) 100.0 μg/mL.

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Tables

Table 1. Antibacterial Activities of Target Compounds **4a−4j** and **7a−7j** against Phytopathogenic Bacteria *Xoo*, *Xac,* and *Psa* in Vitro.

Compd.	Inhibition rate[a] (%)					
	Xoo		*Xac*		*Psa*	
	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL	100 μg/mL	50 μg/mL
4a	100.0 ± 0.0	63.7 ± 0.7	40.4 ± 3.3	29.4 ± 4.1	46.3 ± 3.1	32.2 ± 4.7
4b	93.6 ± 1.2	60.3 ± 2.6	44.6 ± 3.1	20.0 ± 3.4	55.9 ± 2.1	36.2 ± 0.6
4c	100.0 ± 0.0	68.1 ± 1.2	33.6 ± 3.3	14.0 ± 2.7	41.5 ± 1.6	26.9 ± 1.8
4d	58.1 ± 3.2	39.6 ± 2.9	38.5 ± 4.0	24.0 ± 5.2	32.3 ± 2.0	16.6 ± 3.1
4e	99.5 ± 0.3	69.3 ± 3.7	18.0 ± 2.2	7.5 ± 0.9	41.7 ± 3.2	31.7 ± 2.6
4f	100.0 ± 0.0	90.3 ± 2.6	36.9 ± 1.0	26.4 ± 3.9	44.0 ± 4.4	30.4 ± 0.7
4g	91.2 ± 1.2	55.2 ± 2.7	24.6 ± 2.6	12.9 ± 3.6	46.6 ± 2.4	34.0 ± 2.9
4h	96.2 ± 2.7	57.2 ± 1.9	44.0 ± 3.9	15.5 ± 5.0	45.7 ± 2.2	33.7 ± 1.7
4i	74.5 ± 0.9	46.5 ± 1.4	16.5 ± 3.8	15.8 ± 2.9	39.8 ± 2.0	23.2 ± 5.4
4j	100.0 ± 0.0	74.0 ± 2.6	49.6 ± 3.3	23.7 ± 3.2	62.7 ± 1.2	43.4 ± 1.7
7a	84.4 ± 2.1	51.0 ± 1.2	44.9 ± 1.6	23.5 ± 2.6	46.6 ± 0.6	26.8 ± 1.5
7b	69.4 ± 2.4	45.7 ± 2.1	53.8 ± 3.5	33.8 ± 4.9	56.3 ± 3.5	45.4 ± 2.0
7c	100.0 ± 0.0	70.8 ± 1.5	48.2 ± 2.1	29.7 ± 1.2	62.6 ± 0.5	36.6± 3.1
7d	69.7 ± 3.2	39.4 ± 2.0	50.0 ± 2.4	31.3 ± 3.3	55.0 ± 0.6	27.4 ± 1.3
7e	96.6 ± 2.3	61.8 ± 1.9	25.7 ± 4.6	16.1 ± 3.8	46.0 ± 1.9	36.4 ± 4.5
7f	92.4 ± 2.5	55.4 ± 1.0	64.5 ± 1.6	39.0 ± 0.9	57.3 ± 0.8	41.5 ± 2.0
7g	80.4 ± 0.5	48.9 ± 2.3	43.4 ± 0.3	33.2 ± 1.8	60.4 ± 2.2	46.1 ± 3.5
7h	79.6 ± 1.4	56.3 ± 2.2	50.3 ± 0.9	39.4 ± 3.7	50.8 ± 1.4	36.8 ± 1.8
7i	96.1 ± 1.2	60.6 ± 2.2	28.9 ± 1.8	18.6 ± 3.6	67.1 ± 3.6	46.3 ± 4.2
7j	100.0 ± 0.0	68.0 ± 3.7	48.7 ± 2.2	24.8 ± 3.8	63.0 ± 3.2	41.0 ± 3.9
BMT[b]	56.3 ± 3.1	35.8 ± 2.9	61.4 ± 3.0	33.9 ± 1.5	59.6 ± 2.9	37.5 ± 4.0
TDC[b]	NT[c]	NT[c]	NT[c]	NT[c]	52.6 ± 1.0	38.2 ± 2.3

[a] The average of three trials. [b] Commercial bactericides bismerthiazol (BMT) and thiodiazole-copper (TDC) were used as positive control agents. [c] NT = not tested.

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673 **Table 2.** EC_{50} Values of Target Compounds **4a−4j** and **7a−7j** against *Xoo* and Their Physicochemical
674 Properties.

compd.	toxic regression equation	r	EC_{50} (μg/mL)	MW[a]	cLog$P$[b]	HBA[c]	HBD[d]	ROTB[e]
4a	$y = 2.8108x + 0.9078$	0.9437	28.6 ± 1.3	505.2	3.52	8	0	5
4b	$y = 2.3105x + 1.5819$	0.9489	30.2 ± 2.9	489.2	4.17	7	0	5
4c	$y = 2.6523x + 1.3572$	0.9371	23.6 ± 0.9	503.2	4.73	7	0	5
4d	$y = 1.7156x + 1.8132$	0.9990	72.0 ± 5.3	491.2	4.48	7	0	7
4e	$y = 2.7736x + 0.9859$	0.9733	28.0 ± 0.3	518.2	2.64	8	0	5
4f	$y = 3.0787x + 1.0768$	0.9947	18.8 ± 0.8	532.3	3.17	8	0	6
4g	$y = 2.1654x + 1.7149$	0.9649	32.9 ± 1.0	546.3	3.48	8	0	6
4h	$y = 2.4242x + 1.2733$	0.9614	34.5 ± 1.7	546.2	3.03	9	0	5
4i	$y = 2.0924x + 1.4625$	0.9969	49.1 ± 1.1	604.3	5.43	10	0	7
4j	$y = 2.8715x + 1.0671$	0.9672	23.4 ± 1.0	580.3	5.51	8	0	6
7a	$y = 2.0361x + 1.7603$	0.9770	39.0 ± 1.0	573.2	4.60	8	0	6
7b	$y = 1.7106x + 2.0700$	0.9979	51.6 ± 3.6	557.2	5.26	7	0	6
7c	$y = 2.5111x + 1.4028$	0.9823	27.1 ± 1.2	571.2	5.82	7	0	6
7d	$y = 1.9767x + 1.4055$	0.9835	65.8 ± 4.1	559.2	5.56	7	0	8
7e	$y = 2.4965x + 1.3899$	0.9512	27.9 ± 0.6	586.2	3.73	8	0	6
7f	$y = 2.3127x + 1.5349$	0.9629	31.5 ± 1.3	600.2	4.26	8	0	7
7g	$y = 2.0060x + 1.7071$	0.9809	43.8 ± 1.8	614.3	4.56	8	0	7
7h	$y = 2.0392x + 1.6692$	0.9993	43.0 ± 1.4	614.2	4.12	9	0	6
7i	$y = 2.5510x + 1.2876$	0.9433	28.5 ± 1.0	672.3	6.51	10	0	8
7j	$y = 2.6608x + 1.3110$	0.9502	24.3 ± 0.7	648.2	6.59	8	0	7
BMT[f]	$y = 1.4580x + 2.1340$	0.9862	92.4 ± 3.1	278.0	−1.46	6	4	4

675 [a]Molecular Weight. [b]Logarithm of the partition coefficient between *n*-octanol and water (calculated using the
676 ChemBioOffice 2010). [c]Number of hydrogen-bond acceptors. [d]Number of hydrogen-bond donors. [e]Number of
677 rotatable bonds (determined using the Molinspiration Cheminformatics software). [f]Commercialized agrobactericide
678 bismerthiazol.

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Table 3. Protection and Curative Activities of Compound **4f** against Rice Bacterial Leaf Blight under Greenhouse Conditions at 200 μg/mL in Vivo.

treatment	Protection activity (14 days after spraying)			Curative activity (14 days after spraying)		
	morbidity (%)	disease index (%)	control efficiency (%)[b]	morbidity (%)	disease index (%)	control efficiency (%)[b]
4f	100	33.33	56.23	100	36.11	52.30
BMT	100	43.06	43.45	100	40.28	46.80
TDC	100	41.62	45.34	100	43.59	42.43
CK[a]	100	76.15	/	100	75.71	/

[a] Negative control. [b] Statistical analysis was conducted by ANOVA method under the condition of equal variances assumed ($P > 0.05$) and equal variances not assumed ($P < 0.05$). Different uppercase letters indicate the values of protection activity with significant difference among different treatment groups at $P < 0.05$.

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715 **Table 4.** Antifungal Activities of Target Compounds **4a−4j** and **7a−7j** at 50 μg/mL in Vitro.

compd.	Inhibition rate[a] (%)					
	GZ[c]	VD[d]	SS[e]	GF[f]	CM[g]	CG[h]
4a	20.5 ± 1.1	35.4 ± 2.6	13.6 ± 2.5	11.3 ± 1.0	7.8 ± 0.1	0
4b	19.9 ± 1.7	28.8 ± 2.4	15.6 ± 3.6	9.3 ± 2.1	16.2 ± 1.2	15.1 ± 2.9
4c	15.8 ± 3.3	26.1 ± 1.0	12.6 ± 2.2	15.3± 0.9	0	0
4d	21.6 ± 1.7	35.0 ± 3.2	18.7 ± 4.2	8.6 ± 2.4	11.0 ± 1.1	13.8 ± 1.5
4e	20.5 ± 1.1	31.8 ± 4.4	15.7 ± 1.1	9.9 ± 3.8	9.1 ± 2.1	11.8 ± 3.3
4f	37.4 ± 2.3	35.4 ± 2.6	14.1 ± 2.2	18.7± 1.2	0	5.9 ± 0.1
4g	19.3 ± 1.4	34.1 ± 2.4	10.1 ± 3.7	6.0 ± 3.4	7.1 ± 3.0	0
4h	12.3 ± 2.9	27.0 ± 3.7	11.1 ± 3.1	11.3± 3.0	7.8 ± 3.8	6.6 ± 3.0
4i	8.8 ± 1.6	18.1 ± 0.9	9.1 ± 0.1	18.7 ± 1.5	5.2 ± 4.0	0
4j	11.7 ± 1.0	20.8 ± 3.1	8.1 ± 0.8	17.3 ± 2.7	6.5 ± 2.9	0
7a	25.7 ± 0.7	31.4 ± 1.6	15.1 ± 2.4	14.7 ± 1.2	8.4 ± 2.2	9.9 ± 0.1
7b	34.5 ± 2.5	36.7 ± 2.4	20.7 ± 3.3	19.3 ± 2.0	17.5 ± 1.8	14.5 ± 2.1
7c	29.8 ± 2.6	31.0 ± 2.3	16.2 ± 0.9	11.3± 4.5	13.0 ± 1.4	7.9 ± 2.0
7d	46.2 ± 1.8	39.8 ± 1.7	18.2 ± 3.8	14.7 ± 0.9	15.6 ± 0.2	11.8 ± 1.3
7e	45.6 ± 2.4	38.9 ± 2.0	20.2 ± 3.0	20.6 ± 2.6	11.0 ± 1.1	13.8 ± 1.8
7f	29.2 ± 0.6	32.8 ± 2.4	14.1 ± 2.1	17.3 ± 2.7	15.6 ± 3.2	9.9 ± 1.9
7g	45.0 ± 3.6	39.9 ± 4.5	21.7 ± 0.9	20.7 ± 1.2	12.3 ± 1.4	15.8 ± 2.0
7h	20.5 ± 0.7	31.0 ± 1.7	16.1 ± 4.4	19.3 ± 4.3	7.2 ± 2.3	0
7i	45.6 ± 2.7	51.3 ± 2.3	22.2 ± 2.1	28.0 ± 1.4	19.5 ± 1.8	19.1 ± 2.8
7j	24.5 ± 2.6	22.5 ± 2.0	18.1 ± 4.3	23.3 ± 3.7	16.9 ± 1.0	9.2 ± 2.5
Hymexazol[b]	55.0 ± 2.9	80.1 ± 2.5	72.2 ± 2.0	50.6 ± 3.1	64.9 ± 2.3	72.4 ± 1.2

716 [a]The average of three trials. [b]Commercialized agrofungicide Hymexazol. [c]GZ = *Gibberella zeae*; [d]VD =
717 *Verticillium dahliae*; [e]SS = *Sclerotinia sclerotiorum*; [f]GF = *Gloeosporium fructigenum*; [g]CM = *Cytospora*
718 *mandshurica*; [h]CG = *Colletotrichum gloeosporioides*.

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Figures

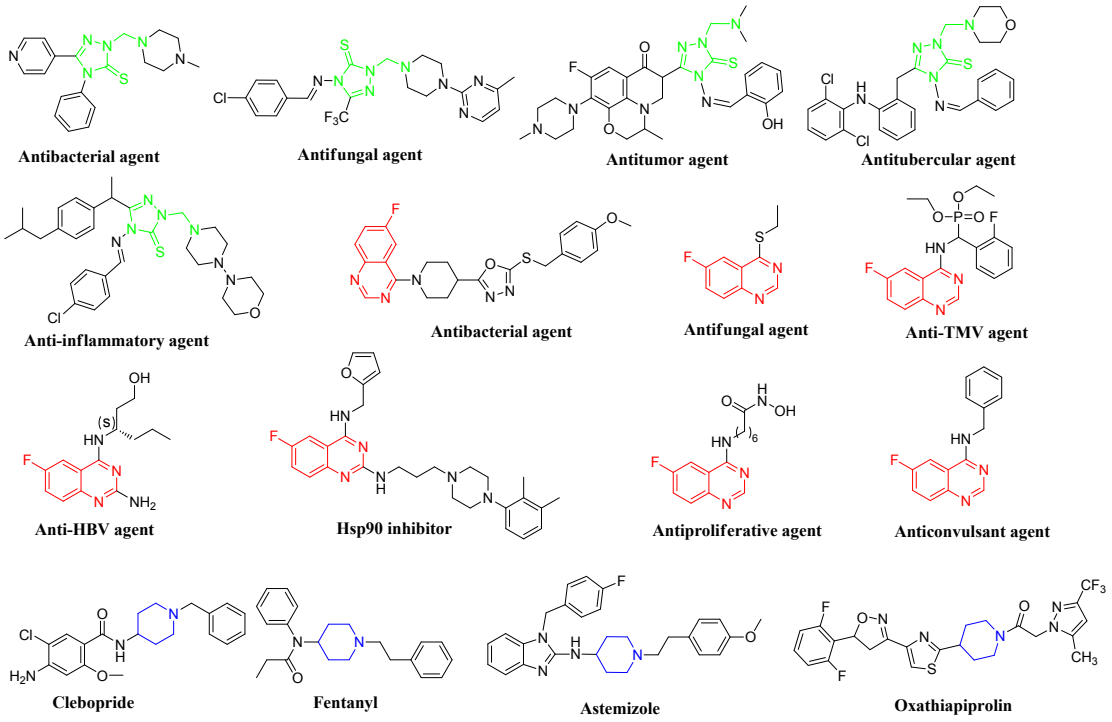


Figure 1. Several representative bioactive molecules containing a 1,2,4-triazole-5-thione Mannich base or a 6-fluoroquinazoline moiety or a piperidinyl linkage.

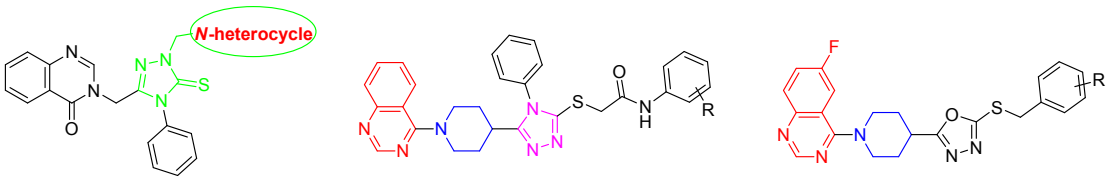


Figure 2. Previously-reported three classes of antibacterial compounds by our group.

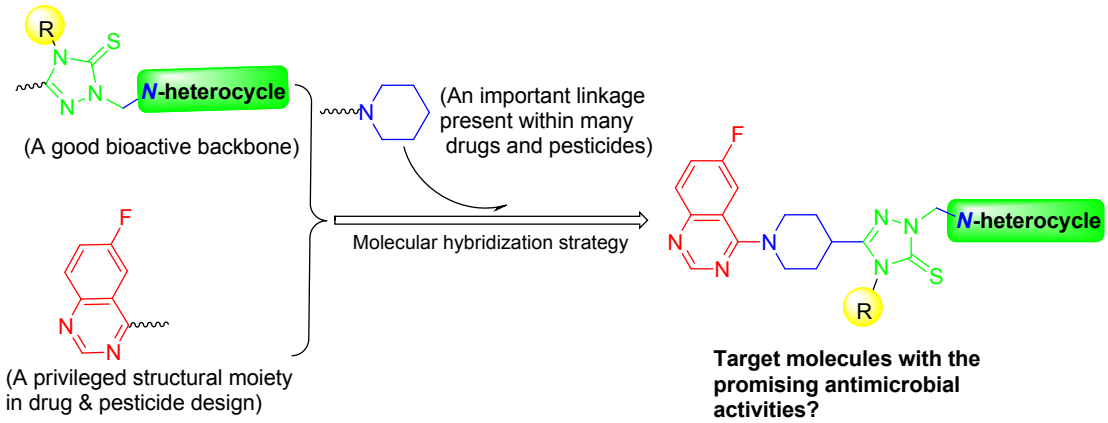


Figure 3. Design strategy of target compounds in this work.

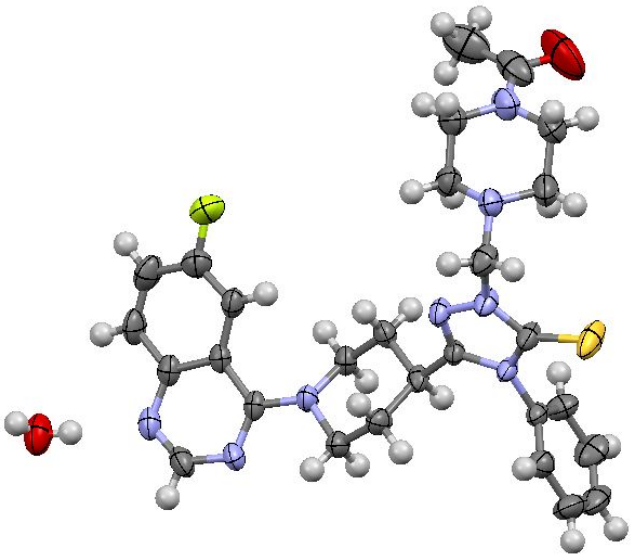
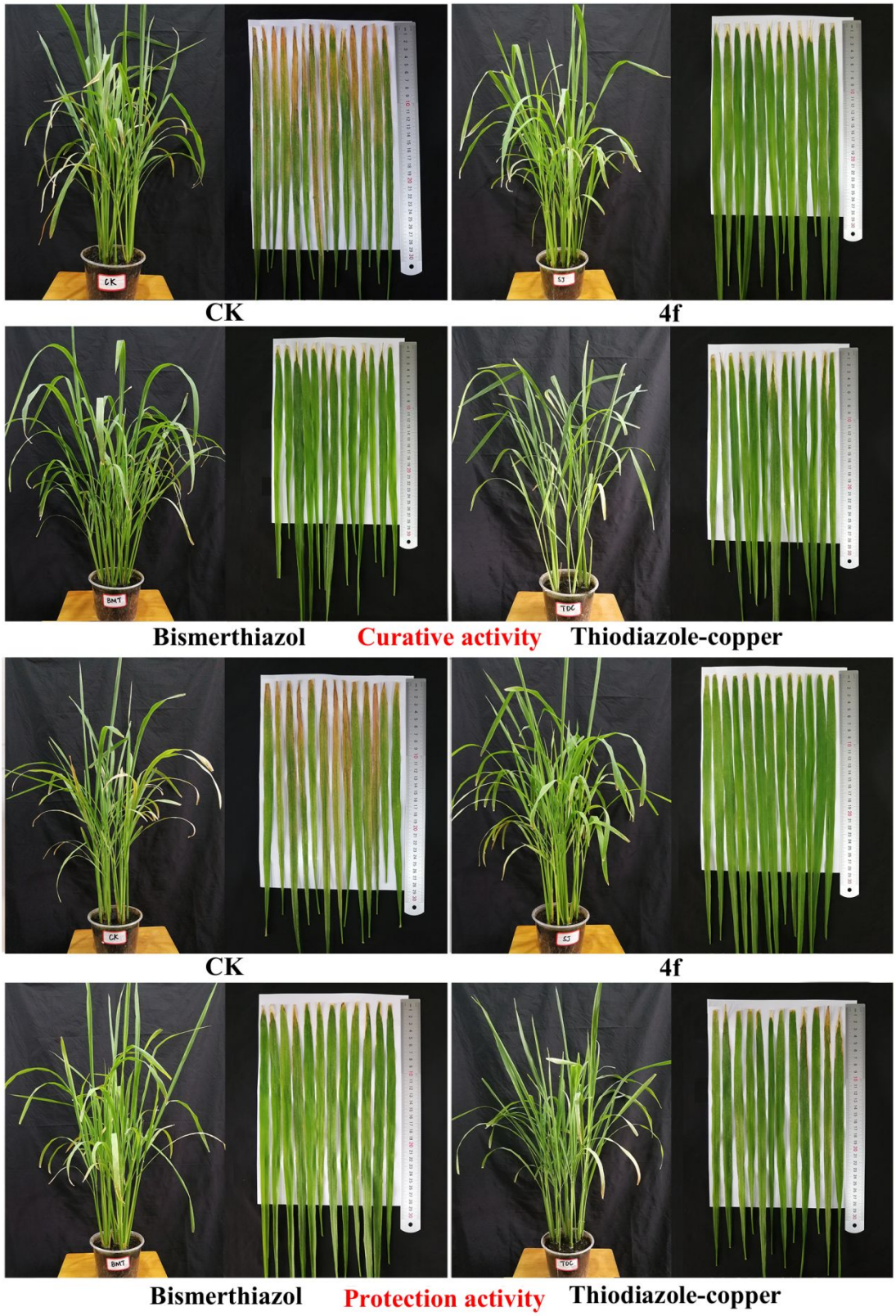


Figure 4. X-ray crystal structure of **4h**·H_2O complex.



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826 **Figure 5.** Curative and protection activities of compound **4f** against rice bacterial leaf blight under
827 greenhouse conditions at 200 μg/mL, with **BMT** and **TDC** as the positive control agents.

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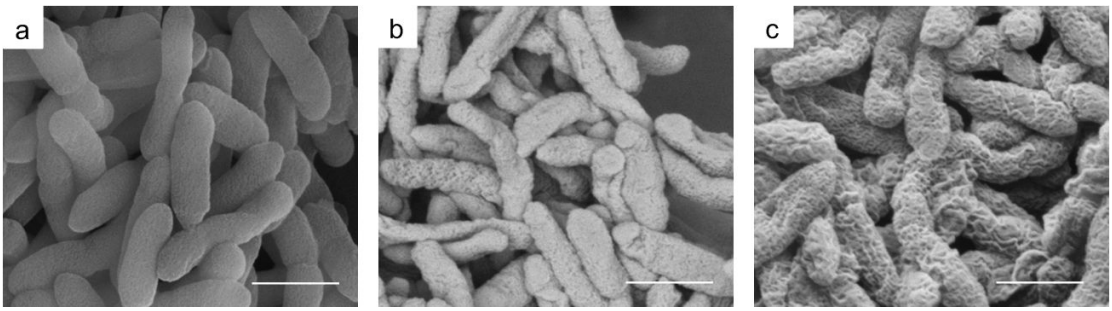
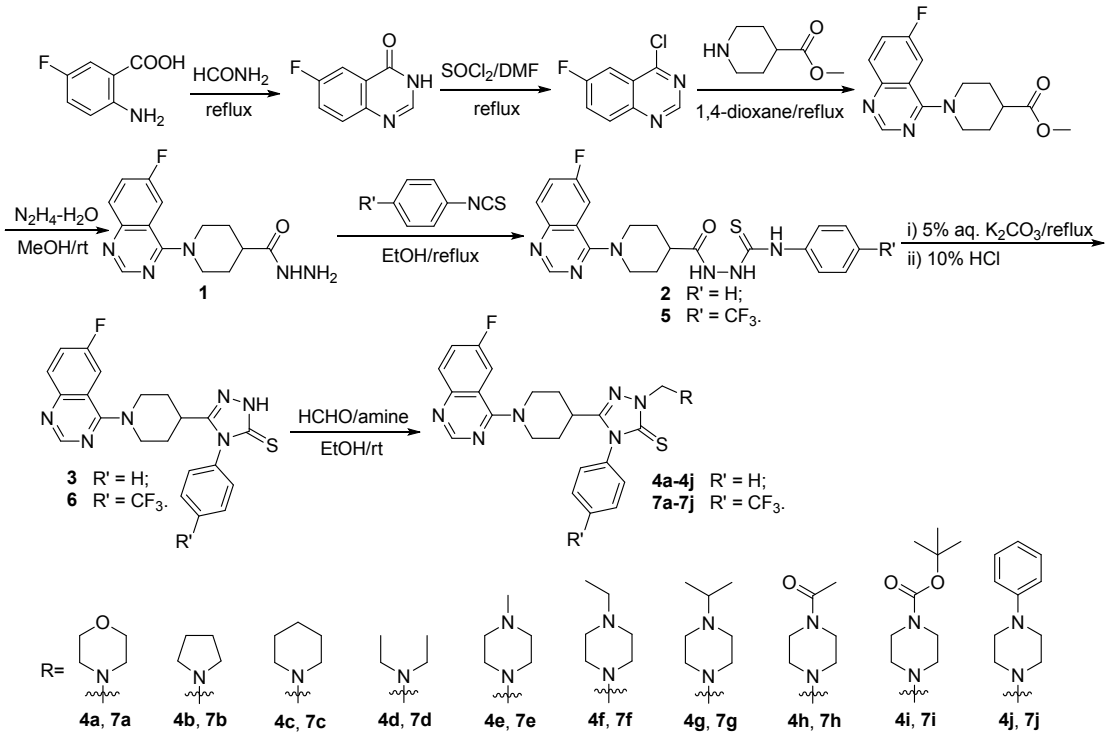
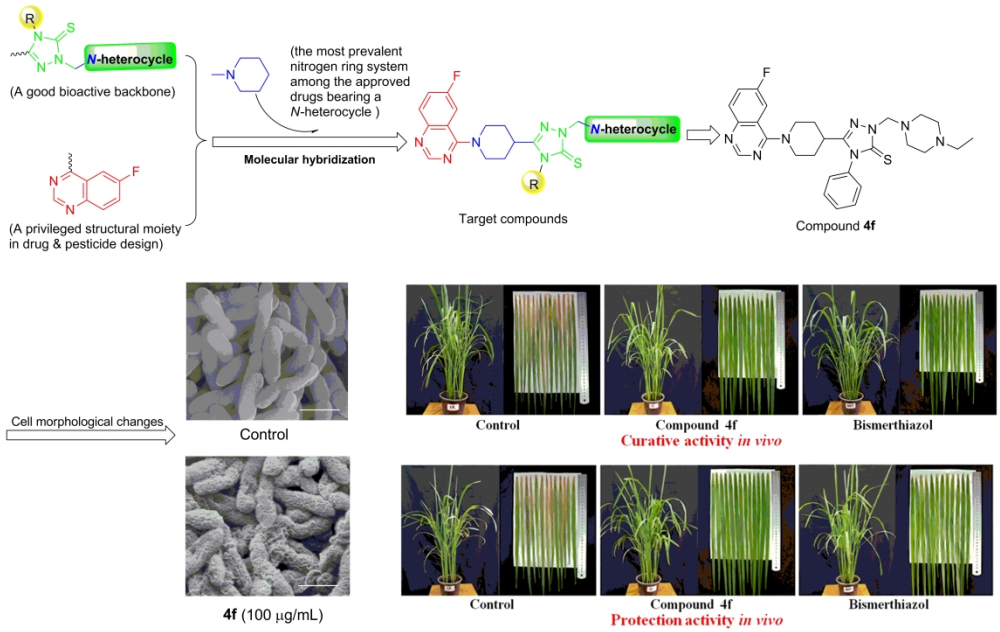


Figure 6. SEM images for *Xoo* after being incubated with different concentrations of compound **4f**: (a) 0 μg/mL; (b) 50.0 μg/mL; (c) 100.0 μg/mL. Scale bar: 1.0 μm.



Scheme 1. The synthesis of target compounds **4a–4j** and **7a–7j**.



232x146mm (600 x 600 DPI)