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Synthesis of novel 1,2,4-triazole derivatives containing the quinazolinylpiperidinyl moiety and *N*-(substituted phenyl)acetamide group as efficient bactericides against the phytopathogenic bacterium *Xanthomonas oryzae* pv. *oryzae*†

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A series of novel 1,2,4-triazole derivatives (**7a–7p**) containing the quinazolinylpiperidinyl moiety and *N*-(substituted phenyl)acetamide group were designed, synthesized and evaluated for their antimicrobial activities *in vitro*. These compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, HRMS and IR spectra. Notably, the structure of compound **7p** was further confirmed through the single-crystal X-ray diffraction method. The obtained bioassay results indicated that most of these compounds exhibited good to excellent antibacterial activities against the rice bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). For example, compounds **7e**, **7g**, **7n**, **7l**, **7i**, **7k**, **7a** and **7h** had EC_{50} (half-maximal effective concentration) values of 34.5, 38.3, 39.0, 46.0, 47.5, 54.6, 55.0 and 58.2 μg mL^{-1} against the bacterium, respectively, which were significantly lower than the control agent Bismerthiazol (85.6 μg mL^{-1}). Additionally, antifungal experiments demonstrated that all the compounds did possess weak inhibition capabilities against three phytopathogenic fungi at 50 μg mL^{-1}, except in the cases of compounds **7e** and **7p** against the fungus *Gibberella zeae*. The above experimental results proved that 1,2,4-triazole derivatives bearing both a quinazolinylpiperidinyl fragment and *N*-(substituted phenyl)acetamide unit are promising candidates for the development of new agricultural bactericides against the pathogenic bacterium *Xoo*, deserving further investigation in the future.

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Introduction

Xanthomonas oryzae pv. *oryzae* (*Xoo*) and *Ralstonia solanacearum* (*Rs*) are pathogenic bacteria for rice bacterial leaf blight and tobacco bacterial wilt, respectively, which cause serious harm to global agricultural products and give rise to huge economic losses to farmers all over the world.[1] Taking the phytobacterium *Xoo* as an example, it is one of the most destructive bacterial diseases for the rice crop, which occurs throughout all the growth stages of rice and triggers bacterial blight by invading vascular tissues,[2] generally bringing about production losses by up to 50%.[3] Additionally, the phytobacterium *Rs* is also one kind of highly devastating and widespread soil-borne plant pathogen.[4] The most typical symptoms of tobacco plants infected by this pathogen are rapid yellowing and wilting of tobacco leaves.[5] Although some commercial antibactericidal agents (*i.e.*, Bismerthiazol and Thiodiazole copper) are currently available on the market for fighting against these two bacterical diseases, however, poor efficiency, high phytotoxicities, high residue levels, adverse effects on the natural environment and growing problems of antibacterial resistance related with the utilization of these bactericides are attracting increasing attention from agricultural chemists. Therefore, the search of new and more efficient antibacterial agents still remains an urgent task in the agrochemical field.

1,2,4-Triazole derivatives are a class of important nitrogen-containing heterocyclic compounds, and many of them displayed remarkable antibacterial activities.[6–8] Some marketed agrofungicides also contain the ring of 1,2,4-triazole, such as Triadimefon, Triadimenol, Diniconazole, Flusilazole and Difenoconazole (Fig. 1). Moreover, quinazoline and quinazolinone derivatives have attracted a great deal of attention from medicinal chemists because of their diverse biological activities, such as anticancer,[9] anticonvulsant,[10] anti-leishmanial & anti-proliferative,[11] analgesic and anti-inflammatory activities.[12] Until now, the method of "active substructure combination" is

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† Electronic supplementary information (ESI) available: Characterization data (including melting point, ^{1}H & ^{13}C NMR, HRMS and IR) of target compounds **7a–7p** and spectra (^{1}H & ^{13}C NMR, HRMS) of intermediates **2–5** as well as target compounds. CCDC 1479247. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ra04819j

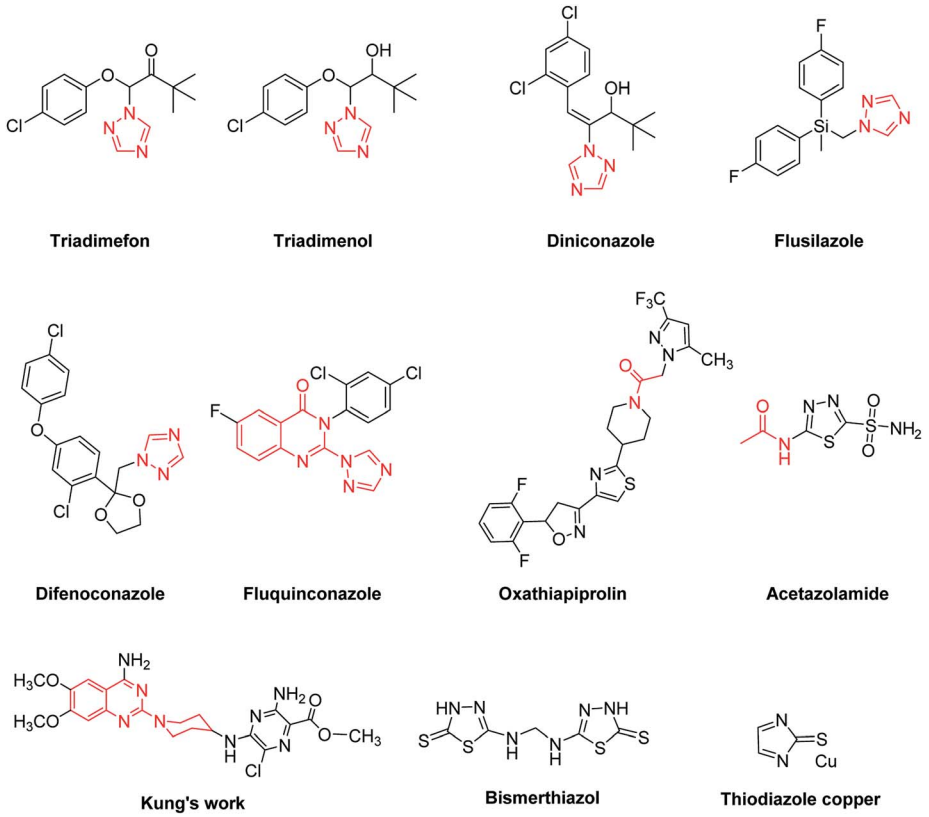


Fig. 1 Chemical structures of some bioactive molecules.

still quite useful for the development of new bioactive molecules,[13,14] because it can provide multiple binding sites within a single molecule to interact with target proteins of pathogenic microorganisms. Fluquinconazole, a well-known agrofungicide, is just one good example of ingenious combination of quinazolinone and 1,2,4-triazole heterocycles. Moreover, the moiety of acetamide often occurs in bioactive molecules, such as fungicide Oxathiapiprolin and carbonic anhydrase inhibitor Acetazolamide.

In 1999, the findings made by Kung *et al.* indicated that both quinazoline ring and piperidinyl linker in target compounds were indespensible for achieving effective antibacterial activities.[15] Based on the above-mentioned considerations and our continuing interest in the development of 1,2,4-triazole-quinazoline/quinazolinone hybrid derivatives as effective antimicrobial agents,[16–20] herein we introduced various *N*-(substituted phenyl) acetamide groups into quinazolinylpiperidinyl-modified 1,2,4-triazole ring (Fig. 2) and evaluated their inhibition activities against some important phytopathogenic bacteria and fungi.

Results and discussion

Chemistry and spectral analysis

The synthetic route of target compounds **7a–7p** was depicted in Scheme 1. Briefly, quinazolin-4-one[21] as starting material was reacted with $SOCl_2$ to give 4-chloroquinazoline **1**,[22] which was then subjected to substitution reaction with methyl 4-piperidinecarboxylate to generate ester **2**. Subsequently, the ester **2** was converted into the corresponding hydrazide **3** through reaction with $N_2H_4 \cdot H_2O$. Once **3** in hand, it was reacted with phenyl isothiocyanate in ethanol to afford amidothiourea **4**. After a ring closure reaction under basic conditions, triazole **5**

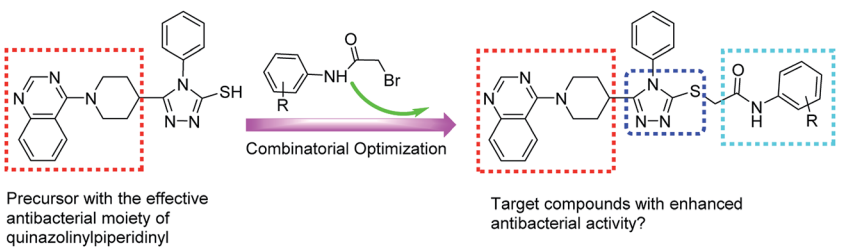
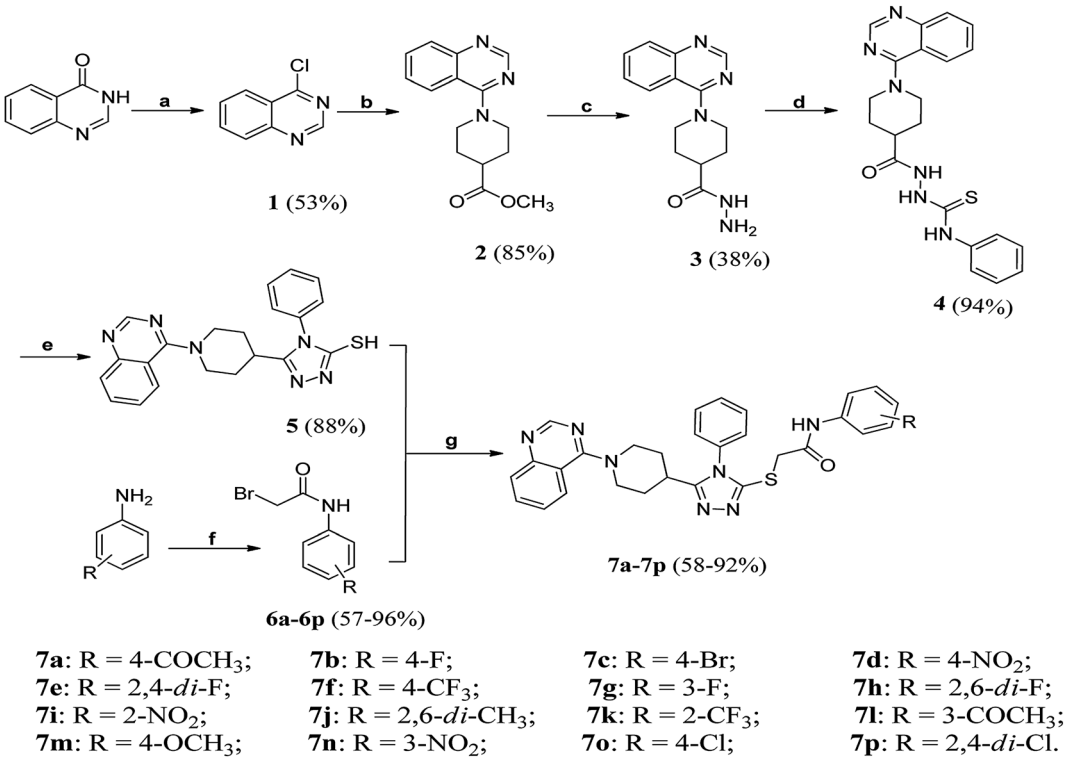


Fig. 2 Design strategy for target compounds **7a–7p**.



7a: R = 4-$COCH_3$; **7b**: R = 4-F; **7c**: R = 4-Br; **7d**: R = 4-NO_2;
7e: R = 2,4-*di*-F; **7f**: R = 4-CF_3; **7g**: R = 3-F; **7h**: R = 2,6-*di*-F;
7i: R = 2-NO_2; **7j**: R = 2,6-*di*-CH_3; **7k**: R = 2-CF_3; **7l**: R = 3-$COCH_3$;
7m: R = 4-OCH_3; **7n**: R = 3-NO_2; **7o**: R = 4-Cl; **7p**: R = 2,4-*di*-Cl.

Scheme 1 The synthesis of target compounds **7a–7p**. Reagents and conditions: (a) $SOCl_2$/DCE/DMF, 140 °C, 5 h; (b) methyl 4-piperidinecarboxylate/1,4-dioxane, r.t. 5 h to 130 °C, 4 h; (c) hydrazine hydrate/MeOH, r.t. 13 h; (d) PhNCS/EtOH, 100 °C, 5 h; (e) (1) 10% K_2CO_3, 110 °C, 5 h, (2) dilute HCl, pH = 7.0; (f) $BrCH_2COBr/Et_3N$/1,4-dioxane, r.t. 5 h; (g) CH_3COCH_3/K_2CO_3, 40 °C, 2–4 h.

was obtained in good yield. Finally, the triazole **5** and various *N*-(substituted phenyl)bromoacetamide derivatives (**6a–6p**) were reacted under CH_3COCH_3/K_2CO_3 system to give target compounds **7a–7p**. Taking **7p** as an example, the strong peak at 1688 cm^{-1} showed the presence of amide C=O functionality. In the DMSO-d_6 solution of compound **7p**, the singlet at 4.12 ppm was attributed to SCH_2 proton resonance. The appearance of a broad peak around 10.01 ppm was assigned to amide NH, which rapidly vanished after D_2O exchange. Additionally, a distinct singlet at 8.53 ppm was seen, resulting from the resonance of the position-2 (sp^2 methine) of the quinazoline ring. In the ^{13}C NMR spectrum, the C=O carbon signal was found at 164.2 ppm. The high-resolution mass spectrum (HRMS) of **7p** demonstrated an intense peak at m/z = 590.1293, attributed to the species of $[M + H]^+$.

Crystal structure analysis

Fortunately, single-crystal of compound **7p** suitable for X-ray diffraction analysis (Fig. 3) was obtained by slow evaporation of CH_2Cl_2–EtOH (2/1, v/v) solution of **7p** at room temperature. Crystallographic parameters of **7p**: colorless crystal, $C_{29}H_{25}Cl_2N_7OS$, M_r = 590.52, triclinic, space group $P\bar{1}$, a = 8.7640(5), b = 10.6660(5), c = 15.4487(9) (Å); α = 74.316(5), β = 82.742(5), γ = 82.883(4); V = 1373.09(13) $Å^3$, T = 293 K, Z = 1, Dc = 0.714 g cm^{-3}, $F(000)$ = 306, reflections collected/independent reflections = 4826/3391, goodness-of-fit on F^2 = 1.035, fine, R_1 = 0.0447, wR_2 = 0.1112. Crystallographic data for compound **7p** (CCDC 1479247†).

Antibacterial activity

The turbidimetric assay[23–25] was conducted to evaluate antibacterial activities of target compounds **7a–7p** and intermediate **5** against two phytopathogenic bacteria *Xoo* and *Rs*. Meanwhile, two commercial agricultural bactericides (namely Bismerthiazol (BMT) and Thiodiazole copper (TDC)) were utilized as the control agents. As shown in Table 1, most of the target compounds exhibited comparable or even better antibacterial activity against the pathogen *Xoo*. For example, compounds **7e**, **7g**, **7l**, **7n** and **7h** had the inhibition rate of 77.8%, 66.7%, 66.9%, 65.3% and 64.4% against this bacterium at 100 μg mL^{-1}, respectively, which was more active than the control agent BMT (54.1%). Moreover, compounds **7a**, **7b**, **7c**, **7d**, **7f**, **7i**, **7k**, **7m** and

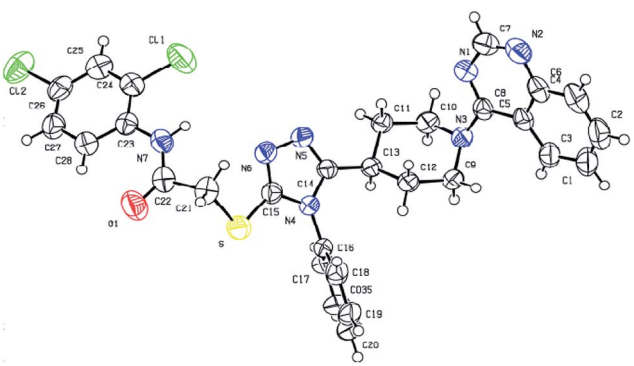


Fig. 3 Crystal structure of compound **7p**.

Table 1 Antibacterial activities of intermediate **5** and target compounds **7a–7p** against two phytopathogenic bacteria *Xoo* and *Rs*

	Inhibition rate[a] (%)			
	Xoo		*Rs*	
Compd	200 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	100 μg mL^{-1}
5	32.0 ± 0.2	17.9 ± 1.3	17.3 ± 3.1	16.6 ± 2.2
7a	77.4 ± 1.0	55.8 ± 2.6	80.0 ± 2.5	48.9 ± 2.1
7b	77.5 ± 3.7	54.0 ± 3.3	44.6 ± 1.9	25.4 ± 2.6
7c	74.7 ± 2.4	63.8 ± 0.9	55.5 ± 4.2	24.1 ± 4.7
7d	72.2 ± 2.7	61.6 ± 4.6	39.0 ± 4.2	24.3 ± 1.4
7e	88.9 ± 5.3	77.8 ± 1.3	23.0 ± 0.5	17.9 ± 0.9
7f	72.7 ± 3.3	58.6 ± 0.9	58.9 ± 0.7	41.4 ± 1.2
7g	83.7 ± 2.3	66.7 ± 2.5	42.0 ± 1.4	20.6 ± 0.7
7h	69.9 ± 1.1	64.4 ± 1.8	48.5 ± 3.1	35.2 ± 1.0
7i	74.0 ± 2.2	61.6 ± 6.4	100 ± 1.5	84.4 ± 4.5
7j	40.1 ± 1.7	27.2 ± 1.3	41.0 ± 2.3	23.2 ± 2.2
7k	76.1 ± 1.2	63.5 ± 1.9	52.8 ± 9.1	46.1 ± 0.1
7l	75.8 ± 3.2	66.9 ± 4.7	26.6 ± 0.7	18.8 ± 0.2
7m	67.6 ± 2.2	54.6 ± 0.9	39.3 ± 2.1	25.3 ± 3.6
7n	73.2 ± 1.7	65.3 ± 3.8	67.0 ± 2.7	40.7 ± 5.6
7o	62.4 ± 1.2	48.4 ± 0.6	44.4 ± 0.2	34.8 ± 5.3
7p	66.7 ± 1.3	55.3 ± 1.1	55.6 ± 1.2	52.4 ± 3.1
BMT[b]	69.4 ± 1.6	54.1 ± 0.8	81.9 ± 3.3	34.1 ± 5.2
TDC[b]	NT[c]	NT[c]	48.8 ± 0.6	26.0 ± 0.9

[a] The average of three trials. [b] Commercial agricultural bactericides Bismerthiazol (BMT) and Thiodiazole copper (TDC) were used as control. [c] NT = not tested.

Table 2 EC_{50} values of intermediate **5** and target compounds **7a–7p** against the pathogen *Xoo*

Compd	EC_{50}[a] (μg mL^{-1})	Toxic regression equation	Correlation coefficient (r)
5	332.9 ± 12.2	$y = 1.6086x + 0.9426$	0.9552
7a	55.0 ± 3.7	$y = 1.1005x + 3.0844$	0.9791
7b	75.8 ± 1.1	$y = 1.7358x + 1.7373$	0.9823
7c	78.6 ± 2.3	$y = 1.9210x + 1.3585$	0.9922
7d	77.4 ± 1.0	$y = 1.6224x + 1.9359$	0.9945
7e	34.5 ± 1.6	$y = 1.5664x + 2.5902$	0.9939
7f	85.7 ± 4.3	$y = 1.7409x + 1.6347$	0.9931
7g	38.3 ± 0.4	$y = 1.2438x + 3.0307$	0.9853
7h	58.2 ± 3.9	$y = 1.0924x + 3.0723$	0.9836
7i	47.5 ± 2.5	$y = 1.0712x + 3.2042$	0.9808
7j	233.0 ± 0.6	$y = 1.6841x + 1.0132$	0.9750
7k	54.6 ± 2.3	$y = 1.2129x + 2.8932$	0.9955
7l	46.0 ± 3.4	$y = 1.2846x + 2.8637$	0.9476
7m	93.7 ± 2.3	$y = 1.6639x + 1.7189$	0.9948
7n	39.0 ± 1.4	$y = 0.9623x + 3.4693$	0.9854
7o	116.0 ± 2.5	$y = 1.8645x + 1.1506$	0.9926
7p	75.1 ± 1.8	$y = 1.0959x + 2.9444$	0.9945
BMT[b]	85.6 ± 1.6	$y = 1.5786x + 1.9496$	0.9852

[a] The average of three trials. [b] The commercial bactericide Bismerthiazol (BMT) was used as control agent.

7p were found to possess similar inhibition capability against the pathogen *Xoo* (relative to BMT), at 200 and 100 μg mL^{-1}. Importantly, intermediate **5** only exhibited very weak antibacterial activity towards this bacterium (with the inhibition rate of 32.0% and 17.9% at the above two concentrations, respectively), which adequately proved the necessarity for introduction of *N*-(substituted phenyl)acetamide group into target compounds and the rationality of our initial design strategy depicted in Fig. 2. Different with the bacterium *Xoo*, a large majority of the target compounds did not have noticeable antibacterial activity against the pathogen *Rs*, excluding compounds **7a**, **7i**, **7n** and **7p**. Notably, 2-nitro substituted compound **7i** was found to possess a potent inhibition activity against the bacteriumn *Rs* (84.4%) even at 100 μg mL^{-1}, which was far better than two control agents BMT and TDC (34.1% and 26.0%, respectively).

Inspired by the above experimental results, the EC_{50} (half-maximal effective concentration) values of compounds **7a–7p** and intermediate **5** against the pathogen *Xoo* were further determined. As shown in Table 2, nearly all of the target compounds did exhibit comparable or much lower EC_{50} values relative to control BMT, except compounds **7j**, **7o** and intermediate **5**. Among them, compounds **7e**, **7g**, **7n**, **7l**, **7i**, **7k** and **7a** had an EC_{50} value of 34.5, 38.3, 39.0, 46.0, 47.5, 54.6 and 55.0 μg mL^{-1}, respectively, which was obviously better than control BMT (85.6 μg mL^{-1}). A preliminary structure–activity relationship analysis was conducted, which showed that the presence of electron-donating substituents on the benzene ring (*e.g.*, 4-OCH_3 and 2,6-di-CH_3) was unfavorable for their antibacterial activities against the pathogen *Xoo*. For example, compound **7j** incorporating electron-donating and sterically hindered 2,6-dimethylphenyl substitution gave the weakest activity (EC_{50} = 233.0 μg mL^{-1}) among this class of compounds. On the whole, the presence of strongly electron-withdrawing substituents (such as 2,4-di-F, 3-F, 3-NO_2, 3-$COCH_3$, 2-NO_2, 2-CF_3 and 4-$COCH_3$) helped to enhance antibacterial activity against the pathogen *Xoo*. Interestingly, the position of the substitutions (concerning these electron-withdrawing substitutions) in the benzene ring also produced a remarkable influence on their antibacterial activities against the *Xoo*, such as compounds **7e** *vs.* **7h**, **7g** *vs.* **7b**, **7n** *vs.* **7d** and **7k** *vs.* **7f**.

Compared with some of the arylimine derivatives containing the 3-aminoethyl-2-[(*p*-trifluoromethoxy)anilino]-4(3*H*)-quinazolinone moiety reported by Song *et al.*,[24] compounds **7e**, **7g** and **7n** had weaker but comparable activities against the *Xoo* on the basis of their EC_{50} values. Notably, relative to some quinazoline derivatives containing the 1,2,4-triazolylthioether moiety reported by our group,[16] the above three compounds exhibited significantly better antibacterial activities against the *Xoo*.

Antifungal activity

The mycelial growth rate method[24,26] was utilized to assess antifungal activities of target compounds **7a–7p** against three phytopathogenic fungi (namely *Phytophthora infestans*, *Gibberella zeae* and *Verticillium dahliae*). As displayed in Table 3, all the compounds were proven to have no noticeable inhibition activity against the above fungi at 50 μg mL^{-1}, except that moderate inhibition activities were found for compounds **7e** and **7p** against the fungus *G. zeae*.

Table 3 Antifungal activities of target compounds **7a–7p** at 50 μg mL^{-1}

Compd	Inhibition rate[a] (%)		
	P. infestans	*G. zeae*	*V. dahliae*
7a	0	14.4 ± 6.7	6.1 ± 2.4
7b	3.7 ± 1.8	19.3 ± 2.5	22.0 ± 2.4
7c	0	0	14.1 ± 2.0
7d	0	12.3 ± 2.5	0
7e	7.4 ± 1.8	30.9 ± 4.4	26.7 ± 2.0
7f	3.1 ± 2.1	19.3 ± 5.1	16.0 ± 2.0
7g	3.1 ± 1.1	7.0 ± 1.3	7.6 ± 2.4
7h	4.3 ± 1.0	14.2 ± 0.8	15.2 ± 2.0
7i	8.0 ± 7.0	19.1 ± 0.8	32.3 ± 2.0
7j	15.1 ± 2.2	11.9 ± 6.2	4.2 ± 2.0
7k	7.4 ± 3.2	9.2 ± 2.6	18.2 ± 2.4
7l	0	0	21.9 ± 2.4
7m	5.2 ± 0.2	14.8 ± 6.9	22.5 ± 2.4
7n	0	14.8 ± 4.9	6.0 ± 2.0
7o	11.1 ± 0.4	18.9 ± 0.6	30.3 ± 2.6
7p	11.6 ± 4.1	36.7 ± 0.7	37.9 ± 2.0
Hymexazol[b]	69.2 ± 3.2	48.4 ± 2.4	84.7 ± 0.8

[a] The average of three trials. [b] The commercial fungicide Hymexazol was used as control agent.

Experimental

Instruments

All the chemicals were obtained from commercial suppliers and used without further purification (unless otherwise stated). Melting points were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). IR spectra were recorded on a Shimadzu IR Prestige-21 spectrometer in KBr disk. ^{1}H and ^{13}C NMR spectra were measured on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard and chemical shift (δ) was expressed in parts per million (ppm). HRMS-ESI spectra were recorded on Thermo Scientific Q Exactive series. The X-ray crystallographic data were collected based on a Bruker Smart Apex CCD area detector diffractometer (Bruker, Germany) with Mo-Kα radiation.

Synthesis of intermediate 2

The 1,4-dioxane solution (20 mL) of newly-prepared 4-chloroquinazoline **1** (1.0 g, 6.08 mmol) and methyl 4-piperidinecarboxylate (0.83 mL, 6.08 mmol) was stirred for 5 h at room temperature and then heated at reflux for 4 h. After cooling to room temperature, the formed precipitate was filtrated off, washed with 1,4-dioxane and dried to give **2** (1.4 g) as a white solid. Yield: 85%; mp 181–182 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 8.79 (s, 1H), 8.12 (d, J = 10.0 Hz, 1H), 7.98 (t, J = 5.0 Hz, 1H), 7.93 (d, J = 10.0 Hz, 1H), 7.67 (t, J = 5.0 Hz, 1H), 4.59 (d, J = 15.0 Hz, 2H), 3.67 (d, J = 10.0 Hz, 2H), 3.61 (s, 3H), 2.92–2.86 (m, 1H), 2.08–2.04 (m, 2H), 1.84–1.76 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): 174.5, 162.6, 148.9, 140.8, 136.1, 127.9, 127.8, 127.7, 119.7, 112.6, 52.3, 49.0, 28.3; ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{15}H_{18}N_3O_2$: 272.1394; found: 272.1394.

Synthesis of intermediate 3

A mixture of ester **2** (300 mg, 1.11 mmol) and hydrazine hydrate (1 mL) in methanol (3 mL) was stirred at room temperature for 13 h, and the formed white precipitate was filtered off, washed with methanol and dried to afford **3** (114 mg). Yield: 38%; mp 173–175 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 9.03 (s, 1H), 8.58 (s, 1H), 7.93 (d, J = 10.0 Hz, 1H), 7.78–7.75 (m, 2H), 7.53–7.49 (m, 1H), 4.26 (d, J = 15.0 Hz, 2H), 4.16 (s, 2H), 3.16–3.10 (m, 2H), 2.44–2.37 (m, 1H), 1.79–1.74 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): 174.0, 164.4, 154.2, 151.9, 133.2, 128.5, 126.1, 125.8, 116.4, 49.5, 28.8; ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{14}H_{18}N_5O$: 272.1506; found: 272.1505.

Synthesis of intermediate 4

Intermediate **3** (304 mg, 1.12 mmol) and phenyl isothiocyanate (0.16 mL, 1.34 mmol) were added to EtOH (12 mL), and the above mixture was heated at reflux for 5 h. After cooling to room temperature, the formed solid was filtered off, washed with ethanol and dried to generate **4** (428 mg) as a white solid. Yield: 94%, mp 195–196 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 9.94 (s, 1H), 9.54 (s, 1H), 8.59 (s, 1H), 7.97 (d, J = 10.0 Hz, 1H), 7.80–7.76 (m, 2H), 7.53–7.12 (m, 6H), 4.28 (d, J = 10.0 Hz, 2H), 3.16 (t, J = 10.0 Hz, 2H), 2.61–2.56 (m, 1H), 1.94–1.75 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): 174.3, 144.7, 144.1, 139.7, 137.7, 135.0, 134.5, 131.3, 130.8, 129.8, 129.2, 128.5, 126.2, 122.2, 49.5, 28.5; ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{21}H_{23}N_6OS$: 407.1649; found: 407.1651.

Synthesis of intermediate 5

An aqueous solution of potassium carbonate (10%, 4 mL) containing **4** (100 mg, 0.26 mmol) was heated to reflux for 5 h. After cooling to room temperature, the above solution was neutralized with dilute HCl to pH = 7.0, and the resulted precipitate was filtered off, washed with water and dried to give triazole **5** (88 mg) as a white solid. Yield: 88%, mp 142–144 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 13.70 (s, 1H), 8.55 (s, 1H), 7.89 (d, J = 5.0 Hz, 1H), 7.76–7.75 (m, 2H), 7.57–7.53 (m, 3H), 7.49–7.44 (m, 3H), 4.16 (d, J = 10.0 Hz, 2H), 3.10–3.05 (m, 2H), 2.82–2.76 (m, 1H), 1.83–1.79 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): 168.4, 164.2, 155.0, 154.1, 151.8, 134.3, 133.2, 130.2, 130.1, 129.1, 128.5, 126.1, 125.8, 116.4, 49.0, 33.0, 29.5; ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{21}H_{21}N_6S$: 389.1543; found: 389.1545.

General procedure for the synthesis of intermediates 6a–6p

Intermediates **6a–6p** were synthesized using a modified method reported by Barker *et al.*[27] A mixture of substituted aniline (3.11 mmol) and dry triethylamine (1.5 mL) in dry 1,4-dioxane (6 mL) was stirred at 0 °C for 5 min, and then bromoacetyl bromide (10.54 mmol) dissolved in the dried dioxane (6 mL) was added dropwise to the above solution and continued to stir for 5 h at room temperature. Finally, the reaction mixture was poured into cold water and the resulted precipitate was filtered off, washed with water and dried to afford amides **6a–6p** in 57–96% yield.

General procedure for the synthesis of target compounds 7a–7p

A mixture of triazole **5** (50 mg, 0.13 mmol), potassium carbonate (18 mg, 0.13 mmol) and the corresponding amide intermediates **6a–6p** (0.13 mmol) was added to acetone (4 mL) and then stirred at 40 °C until the reaction was complete as indicated by TLC (2–4 h). Subsequently, the reaction mixture was poured into cold water and the resulted precipitate was filtered off, washed with water and dried to give compounds **7a–7p** in 58–92% yield.

Antibacterial bioassay

Antibacterial activities of target compounds **7a–7p** were determined against two phytopathogenic bacteria (*Xoo* and *Rs*), based on the turbidimetric method.[23] Firstly, the tested compounds were prepared with two concentrations of 200 and 100 μg mL^{-1}. Pure DMSO in sterile distilled water was used as blank control, and commercially available bactericide Bismerthiazol (BMT) and Thiodiazole copper (TDC) were used as positive control. About 40 μL of solvent NB (3 g of beef extract, 5 g of peptone, 1 g of yeast powder, 10 g of glucose, 1 L of distilled water, pH = 7.0–7.2) containing the bacterium *Xoo* or *Rs* was added to the mixed solvent system including 4 mL of solvent NB and 1 mL of 0.1% Tween-20 containing tested compound or BMT/TDC. The above test tube was incubated at 30 ± 1 °C and continuously shaken at 180 rpm for three days. The bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}), given by $\text{turbidity}_{\text{corrected values}} = OD_{\text{bacterium}} - OD_{\text{no bacterium}}$, $I = (C_{\text{tur}} - T_{\text{tur}})/C_{\text{tur}} \times 100\%$. The C_{tur} represented the corrected turbidity value of bacterial growth of untreated NB (blank control), and T_{tur} represented the corrected turbidity value of bacterial growth of tested compound-treated NB. The I represented the inhibition rate of tested compound against the bacterium.

Secondly, antibacterial activities of target compounds **7a–7p** were assessed against the pathogen *Xoo* under five different concentrations (namely 200, 100, 50, 25 and 12.5 μg mL^{-1}) to obtain their EC_{50} values, which were statistically determined by probit analysis with the probit package of the SPSS 17.0 software.

Antifungal bioassay

Mycelial growth rate method[26] was utilized to assess antifungal activities of target compounds against three phytopathogenic fungi (namely *P. infestans*, *G. zeae* and *V. dahliae*). The tested compounds were prepared at 50 μg mL^{-1} in DMSO solution. The above solution was added into sterilized Petri dishes, which contained about 10 mL molten potato dextrose agar (PDA). Subsequently, a 4 mm-diameter of mycelial plug was cut from the fungal colony and placed at the center of PDA plate at 28 ± 1 °C for 4 days. Antifungal assays were conducted in triplicate for each compound. Additionally, pure DMSO and commercially available fungicide (Hymexazol) were utilized as negative and positive control, respectively.

The inhibition rate (I) of the tested compound was determined based on the following formula:

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

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

Conclusion

In conclusion, a class of novel 1,2,4-triazole derivatives containing both quinazolinylpiperidinyl moiety and *N*-(substituted phenyl)acetamide group were designed and synthesized as agricultural bactericides. The bioassay results showed that some of the target compounds possessed good to excellent antibacterial activities against phytopathogenic bacterium *Xoo*. For example, compounds **7e**, **7g**, **7i**, **7l** and **7n** had EC_{50} values of 34.5–47.5 μg mL^{-1}, which was significantly better than commercial bactericide Bismerthiazol (85.6 μg mL^{-1}). The above studies proved that 1,2,4-triazole derivatives containing quinazolinylpiperidinyl heterocycle and acetamide unit were promising candidates for the development of new and more efficient agricultural bactericides against the pathogen *Xoo*, deserving further investigation in the future.

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