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ORIGINAL ARTICLE

Design, synthesis, crystal structure and in vitro antimicrobial activity of novel 1,2,4-triazolo[1,5-*a*]pyrimidine-containing quinazolinone derivatives

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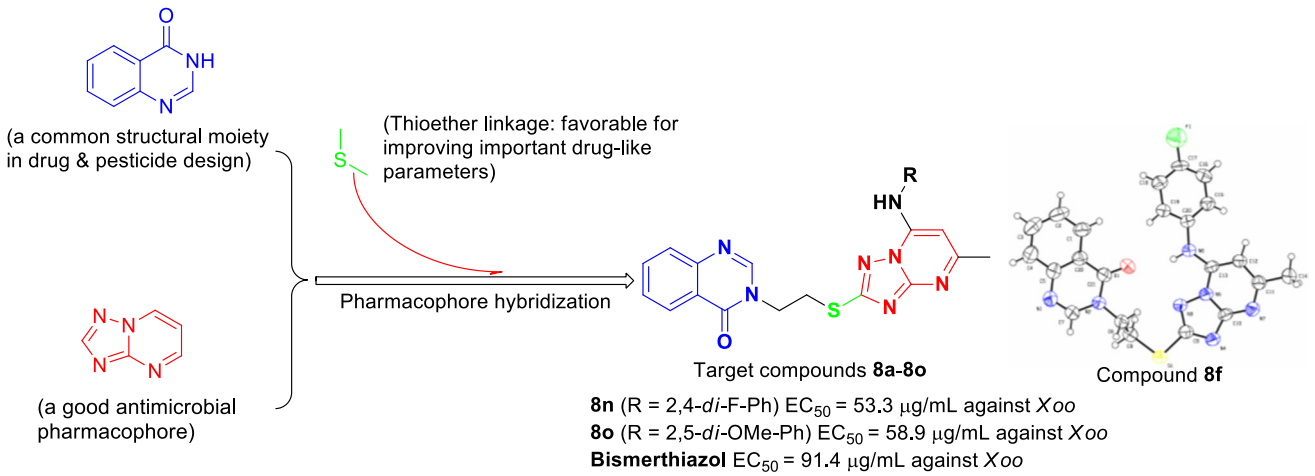
Abstract

A series of novel 1,2,4-triazolo[1,5-*a*]pyrimidine-containing quinazolin-4(3*H*)-one derivatives (**8a**–**8o**) were designed, synthesized and assessed for their in vitro antibacterial and antifungal activities in agriculture. All the title compounds were completely characterized via ^{1}H NMR, ^{13}C NMR, HRMS and IR spectroscopic data. In particular, the molecular structure of compound **8f** was further corroborated through a single-crystal X-ray diffraction measurement. The turbidimetric method revealed that some of the compounds displayed noticeable bactericidal potencies against the tested plant pathogenic bacteria. For example, compounds **8m**, **8n** and **8o** possessed higher antibacterial efficacies in vitro against *Xanthomonas oryzae* pv. *oryzae* with EC_{50} values of 69.0, 53.3 and 58.9 μg/mL, respectively, as compared with commercialized agrobactericide bismerthiazol (EC_{50} = 91.4 μg/mL). Additionally, compound **8m** displayed an EC_{50} value of 71.5 μg/mL toward *Xanthomonas axonopodis* pv. *citri*, comparable to control bismerthiazol (EC_{50} = 60.5 μg/mL). A preliminary structure–activity relationship (SAR) analysis was also conducted, based on the antibacterial results. Finally, some compounds were also found to have a certain antifungal efficacy in vitro at the concentration of 50 μg/mL.

Graphic abstract



Keywords Quinazolinone · 1,2,4-Triazolo[1,5-*a*]pyrimidine · Synthesis · Antimicrobial activity · Structure–activity relationship

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Extended author information available on the last page of the article

Introduction

Plant pathogenic bacteria of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas axonopodis* pv. *citri* (*Xac*) are responsible for rice bacterial blight and citrus bacterial canker, respectively, which lead to enormous economic loss to the farmers around the world each year [1, 2]. Bacterial blight of rice is one of the most deadly rice diseases, which had been reported to significantly reduce rice yield with an estimated loss of nearly 50% in some Asian countries [3]. Additionally, the phytobacteria of *Xoo* and *Rs* (*Ralstonia solanacearum*) ranked 4th and 2nd on the list of top 10 plant pathogenic bacteria, respectively [4]. On the other hand, plant pathogenic fungi remain the most serious plant disease from the perspective of crop protection [5]. Taking the fungus of *Verticillium dahliae* as an example, it had been reported that this pathogen could affect hundreds of herbaceous and woody host plants, also including some vegetables like tomatoes, potatoes, peppers and eggplants [6]. Although some agricultural antimicrobial agents are currently available for fighting against these plant diseases, the development of new, more effective and environment-friendly antimicrobial agents remains an extremely pressing task facing agricultural chemists, especially considering an increasing resistance of plant pathogens to the currently utilized antimicrobial agents in agriculture (thus leading to a significant reduction in the control efficiency of these agroantibiotics), high pesticide residues and concomitant environmental pollution [7, 8].

The quinazolin-4-one backbone as a common structural fragment in medicinal and pesticidal chemistry represents an important class of compounds with a wide range of bioactivities [9], such as antibacterial [10–12], antifungal [13], anti-viral [14] and anti-tumor [15] effects and so on. Several of quinazolinone-based derivatives have been on the market as drugs or pesticides for several years, including analgesics Diproqualone and Methaqualone (Fig. 1), sedatives Cloroqualone and Afloqualone, fungicides Fluquinconazole and Albaconazole, along with anticancer drugs Thymitaq and Raltitrexed. On the other hand, the 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives are the medicinally and pesticidally significant family of condensed heterocyclic compounds that were found as a core motif in a number of bioactive molecules, including agrofungicide Ametoctradin, antiplatelet drug Trapidil, and herbicides Metosulam and Pyroxsulam. In addition, some of 1,2,4-triazolo[1,5-*a*]pyrimidine-based derivatives were also found to demonstrate other biological activities, such as antibacterial [16], anticancer [17] and anti-tubercular effects [18].

It is well known that the 1,2,4-triazolo[1,5-*a*]pyrimidine heterocycle can be regarded as the fusion of a pyrimidine ring with a 1,2,4-triazole ring, from the perspective of the molecular structure. Based on the above-mentioned considerations and our long-standing interest in developing quinazolinone-1,2,4-triazole hybrids as agricultural antimicrobial agents [19–22], we thus synthesized a series of novel quinazolin-4(3*H*)-one derivatives containing a 1,2,4-triazolo[1,5-*a*]pyrimidine moiety through a thioether linkage (favorable for improving the drug-likeness [23]) using a pharmacophore hybridization strategy (with the advantages of overcoming the resistance, decreasing the toxicity and optimizing the pharmacokinetic profiles [24]) (Fig. 2), assessed their in vitro antimicrobial activities against several agriculturally important plant pathogenic bacteria and fungi, and conducted a preliminary structure–activity relationship analysis based on the antibacterial results. To our best knowledge, this was the first example of the preparation of quinazolinone-1,2,4-triazolo[1,5-*a*]pyrimidine hybrids with the aim of acting as antimicrobial agents in agriculture.

Results and discussion

Synthesis

The synthetic route of target compounds **8a**–**8o** was outlined in Scheme 1. Briefly, quinazolinone **2** [19] was firstly reacted with 1,2-dibromoethane in DMF with NaH as a base to furnish the corresponding alkyl bromide **3** [25], which was then subjected to thioetherification to give the intermediate **5** in 78% yield. After a cyclization reaction between **5** and ethyl acetoacetate in refluxing acetic acid, the key intermediate **6** was obtained in 72% yield. Once **6** in hand, it is readily converted into its chloride **7** after treatment with $POCl_3$. Finally, the desired target compounds **8a**–**8o** were readily obtained in 73–85% yield by reaction of intermediate **7** and various arylamines in ethanol at room temperature. All the target compounds were thoroughly characterized via ^{1}H NMR, ^{13}C NMR, HRMS and IR spectroscopic data.

Spectral and crystal structure analysis

Taking compound **8f** as a representative example, the intense signals observed at 3270 and 1667 cm^{-1} in the IR spectroscopy were ascribed to the presence of N–H and C=O groups, respectively. In its ^{1}H NMR spectrum, three singlets observed at 6.22, 8.29 and 10.02 ppm in DMSO-d_6 were assigned to the resonances of 6-CH of 1,2,4-triazolo[1,5-*a*]pyrimidine moiety, 2-CH of quinazolinone ring and NH functionality, respectively. In addition, three diagnostic signals (45.8, 29.7 and 24.7 ppm) were also observed in the far upfield region in the ^{13}C NMR spectrum, owing to the presence of three kinds of aliphatic CH protons within **8f**.

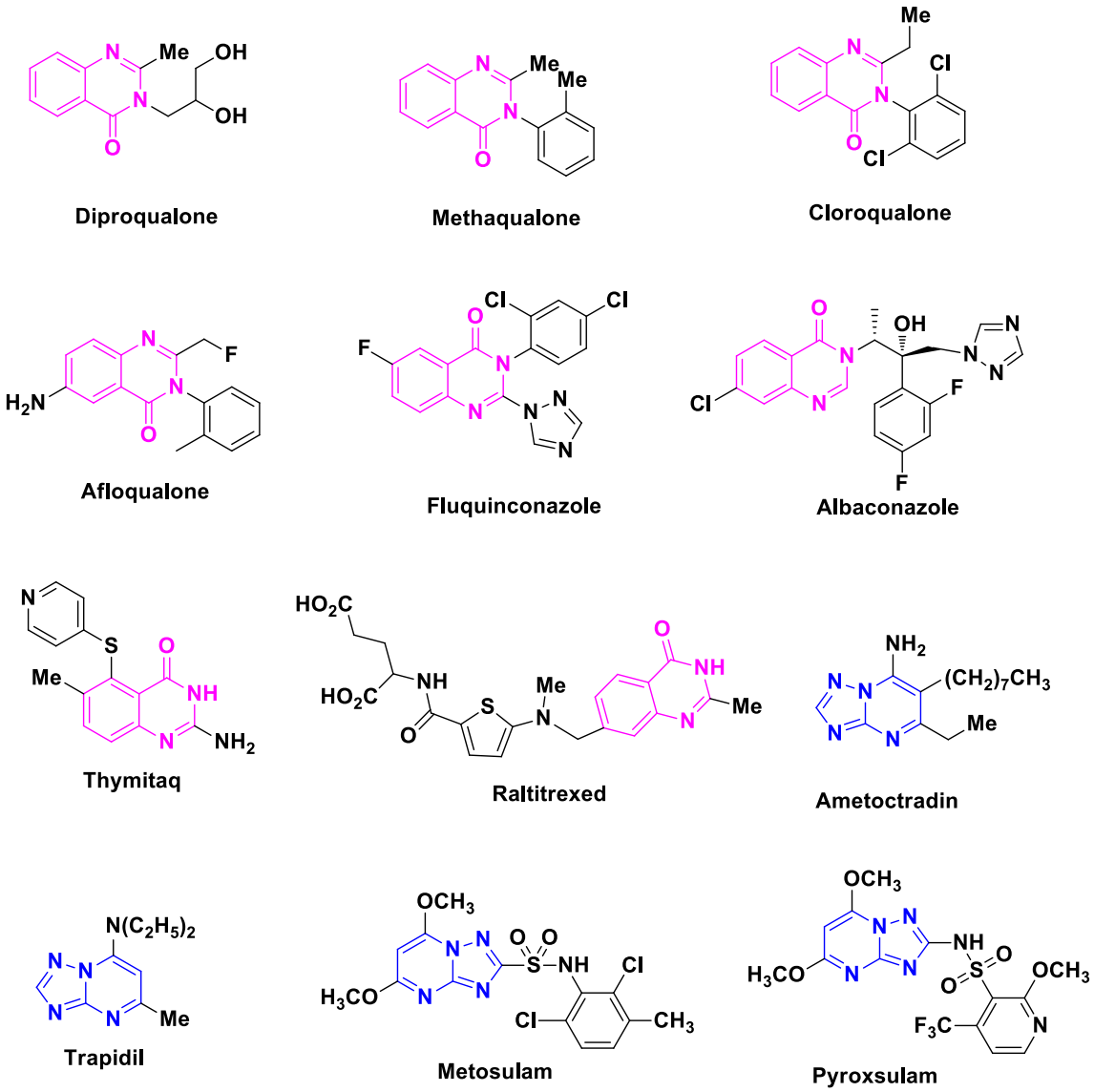


Fig. 1 Some of representative drug or pesticide molecules containing either the quinazolinone or 1,2,4-triazolo[1,5-*a*]pyrimidine moiety

Finally, high-resolution mass spectrum (HRMS) demonstrated a strong signal at $m/z = 448.1343$, coming from the chemical species of $[M+H]^+$.

A single crystal of compound **8f** suitable for X-ray diffraction analysis was obtained (Fig. 3) by slow evaporation of a CH_2Cl_2-EtOH (1/3, *v*/*v*) solution of **8f** at room temperature. Crystallographic data for compound **8f**: colorless crystal, $C_{22}H_{19}ON_7FS$, $M_r = 447.49$, monoclinic, space group $P2_1/c$; $a = 9.0313(4)$ Å, $b = 16.5322(5)$ Å, $c = 14.3290(5)$ Å; $\alpha = 90°$, $\beta = 102.549(4)°$, $\gamma = 90°$, $V = 2088.31(4)$ Å^3, $T = 293$ K, $Z = 4$, $D_c = 1.423$ g/cm^3, $F(000) = 948.0$, Reflections collected/Independent reflections = 3120/2322, Goodness of fit on $F^2 = 1.033$, Fine, $R1 = 0.0630$, $wR2 = 0.1906$. Crystallographic data for compound **8f** were deposited in the Cambridge Crystallographic Data Center (CCDC 1547339).

In vitro antibacterial activity

The turbidimetric method was employed to assess in vitro antibacterial activities of compounds **8a**–**8o** against three types of plant pathogenic bacteria *Xoo*, *Xac* and *Rs* (based on their particular significance in agricultural production [2, 4, 26]), with commercialized bactericides bismerthiazol (BMT) and thiodiazole-copper (TDC) as the positive control agents. As summarized in Table 1, several of the compounds were found to demonstrate comparable or higher bactericidal activities against the tested bacteria, after comparison with control BMT. For example, compounds **8l**, **8n** and **8o** showed the inhibition rates of 60.5%, 60.6% and 61.9% at 100 μg/mL against the bacterium *Xoo*, respectively, superior to that of control BMT (50.6%). Under the same concentration,

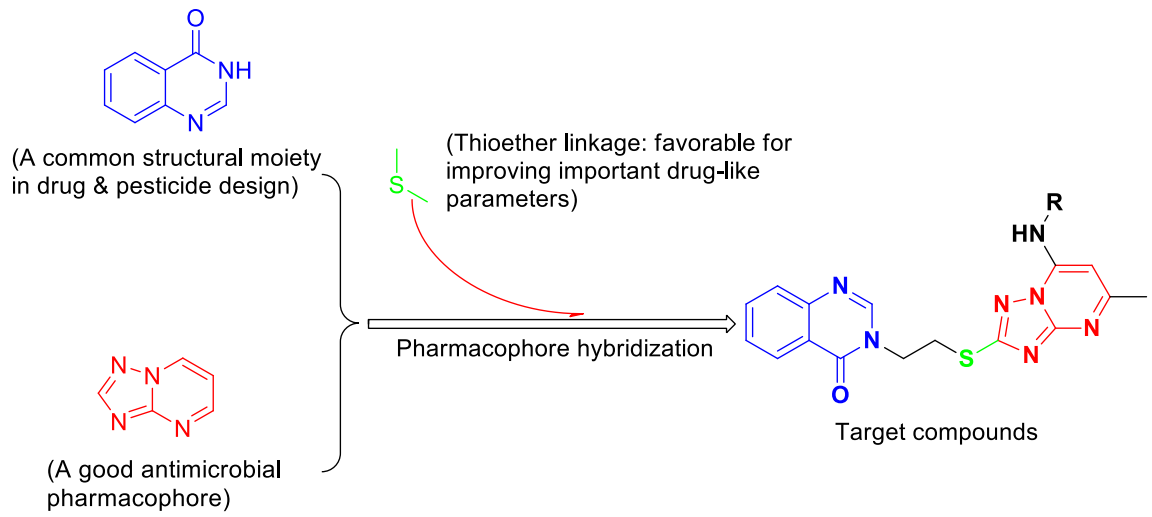
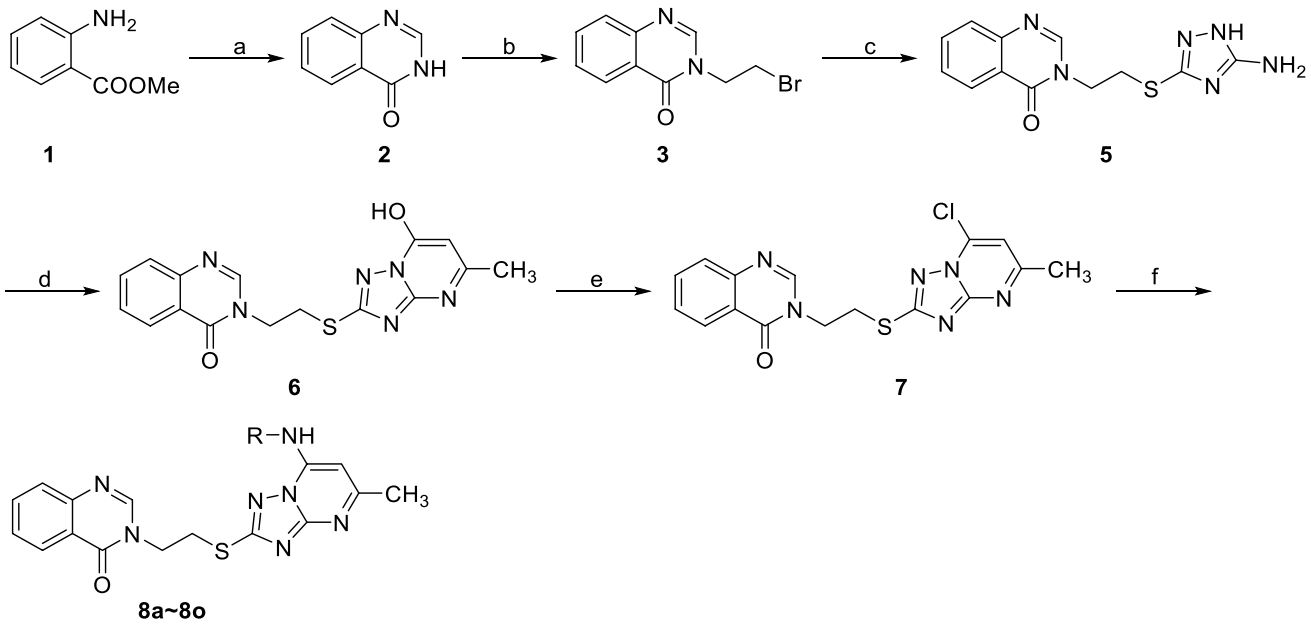


Fig. 2 Design strategy for target compounds in this work



8a: R = Ph; **8b**: R = 2-Cl-Ph; **8c**: R = 4-Cl-Ph; **8d**: R = 2-F-Ph; **8e**: R = 3-F-Ph;
8f: R = 4-F-Ph; **8g**: R = 4-CF_3-Ph; **8h**: R = 3-Br-Ph; **8i**: R = 2-CH_3-Ph; **8j**: R = 3-CH_3-Ph;
8k: R = 2-OCH_3-Ph; **8l**: R = 4-OCH_3-Ph; **8m**: R = 2,4-*di*-Cl-Ph; **8n**: R = 2,4-*di*-F-Ph; **8o**: R = 2,5-*di*-OCH_3-Ph.

Scheme 1. Reagents and conditions: (a) $HCONH_2$/HCOOH/reflux/41%; (b) 1,2-dibromoethane/NaH/DMF/59%; (c) 5-amino-1*H*-1,2,4-triazole-3-thiol(**4**)/DMF/NaOH/78%; (d) CH_3COCH_2COOEt/HOAc/reflux/72%; (e) $POCl_3$/reflux/40%; (f) RNH_2/EtOH/r.t./73~85%

Scheme 1 Synthesis of target compounds **8a**–**8o**

compounds **8l**, **8m**, **8n** and **8o** had the inhibition rates of 52.9%, 59.4%, 52.3% and 56.3% against the pathogen *Xac*, respectively, comparable to control BMT (64.3%). As for the bacterium *Rs*, only low to moderate bactericidal activities were observed for this series of compounds.

A preliminary structure–activity relationship (SAR) analysis was conducted based on the presented results in Table 1 and some conclusions could be drawn as follows: (a) An improved antibacterial efficiency was observed to different extents for target compounds **8a**–**8o** as compared with

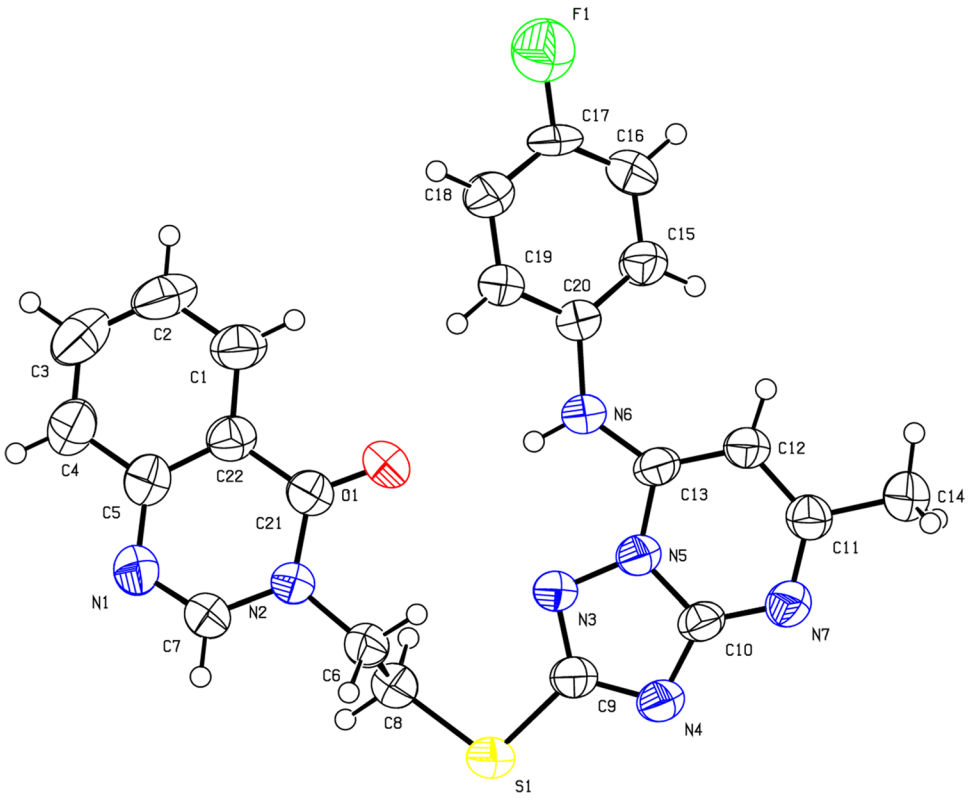


Fig. 3 X-ray crystal structure of target compound **8f**

intermediate **7**, indicating the indispensability of introducing the aniline moiety into final compounds; (b) For the anti-*Xoo* activity, the presence of halogen-containing substituents within the target compounds was favorable to the bactericidal efficiency (with an inhibition rate > 40% at 100 μg/mL in every case), except for the presence of monofluoro substituent (namely compounds **8d**, **8e** and **8f**); (c) Generally speaking, the presence of doubly substituted phenyl group (that is, compounds **8m**, **8n** and **8o**) was beneficial to the antibacterial activity of target compounds against *Xoo* and *Xac* (showing an inhibition rate > 50% at 100 μg/mL), after comparison with the rest of mono-substituted phenyl-containing compounds; (d) Compound **8o** with the 2,5-*di*-OCH_3-Ph group was found to exhibit a good broad-spectrum in vitro antibacterial activity against the tested bacteria.

Encouraged by the preliminary antibacterial results, EC_{50} values (half-maximal effective concentration) of several of the compounds against the bacteria *Xoo/Xac* were subsequently measured using the serial dilution method. As shown in Table 2, compounds **8m**, **8n** and **8o** had EC_{50} values of 69.0, 53.3 and 58.9 μg/mL against *Xoo*, respectively, considerably lower than that of control BMT (91.4 μg/mL). In addition, compound **8l** showed an EC_{50} value of 93.9 μg/mL toward this bacterium, similar to control BMT. Moreover, compound **8m** exhibited an EC_{50} value of 71.5 μg/mL against *Xac*, slightly less active than that of control BMT (60.5 μg/mL).

In vitro antifungal activity

In vitro fungicidal activities of target compounds **8a**–**8o** against three phytopathogenic fungi (namely *Gibberella zeae, Verticillium dahliae* and *Sclerotinia sclerotiorum)* were also evaluated via the mycelial growth rate method. As shown in Table 3, some of the compounds displayed a certain antifungal activity at 50 μg/mL. For example, compounds **8n** and **8o** had the inhibition rates of 34.0% and 23.4% against *V. dahliae*, respectively. Overall, antifungal potencies of this class of compounds were far from satisfying.

Conclusions

In summary, a series of novel quinazolin-4(3*H*)-one derivatives containing a 1,2,4-triazolo[1,5-*a*]pyrimidine moiety were designed, synthesized and evaluated as antimicrobial agents in agriculture. Among them, molecular structure of compound **8f** was clearly confirmed through single-crystal X-ray diffraction analysis. In vitro antibacterial assays indicated that compounds **8n** and **8o** possessed significantly better bactericidal activity against *Xoo*, than

Table 1 In vitro antibacterial activities of intermediate **7** and target compounds **8a**–**8o** against three phytopathogenic bacteria *Xoo*, *Xac* and *Rs* at 200 and 100 μg/mL

Compd.	Inhibition rate (%)[a]					
	Xoo[b]		*Xac*[c]		*Rs*[d]	
	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL
7	42.2 ± 1.3	25.0 ± 2.2	35.9 ± 1.8	21.6 ± 1.5	24.0 ± 1.4	12.2 ± 1.8
8a	37.5 ± 3.1	29.8 ± 1.2	38.3 ± 1.9	32.6 ± 4.8	36.2 ± 1.6	29.8 ± 3.6
8b	57.4 ± 2.2	42.9 ± 2.9	63.3 ± 3.3	37.2 ± 2.3	35.1 ± 5.3	11.3 ± 5.4
8c	61.8 ± 1.5	43.8 ± 4.8	59.7 ± 2.1	34.2 ± 2.5	31.2 ± 1.7	21.8 ± 4.5
8d	52.3 ± 1.7	29.3 ± 4.9	58.2 ± 4.4	37.7 ± 3.7	33.2 ± 2.9	24.9 ± 2.2
8e	57.1 ± 1.6	31.6 ± 1.2	51.6 ± 1.7	35.7 ± 1.8	37.3 ± 3.4	12.1 ± 4.3
8f	59.4 ± 2.8	35.3 ± 1.9	64.2 ± 5.0	43.8 ± 2.9	36.5 ± 3.5	27.5 ± 3.4
8g	65.6 ± 5.8	48.0 ± 4.3	66.1 ± 1.8	40.4 ± 2.4	40.7 ± 3.5	29.1 ± 5.3
8h	62.6 ± 1.1	40.2 ± 3.5	55.3 ± 4.6	36.9 ± 1.2	34.5 ± 3.1	23.7 ± 5.6
8i	64.3 ± 3.9	43.1 ± 2.4	56.5 ± 1.1	31.8 ± 1.6	40.6 ± 3.2	28.9 ± 3.0
8j	53.2 ± 5.3	36.8 ± 4.3	66.7 ± 1.3	34.0 ± 1.1	34.7 ± 4.4	22.4 ± 6.4
8k	68.5 ± 1.1	48.9 ± 6.3	58.5 ± 3.5	35.0 ± 4.3	44.8 ± 3.1	27.1 ± 4.2
8l	75.8 ± 2.8	60.5 ± 1.8	65.9 ± 1.8	52.9 ± 2.2	35.3 ± 3.8	24.9 ± 1.2
8m	71.0 ± 1.3	53.7 ± 2.2	75.4 ± 2.5	59.4 ± 1.9	28.1 ± 2.7	12.4 ± 5.1
8n	75.4 ± 1.3	60.6 ± 3.9	70.4 ± 1.8	52.3 ± 1.1	37.0 ± 5.4	12.6 ± 2.6
8o	77.2 ± 1.9	61.9 ± 1.2	69.6 ± 1.3	56.3 ± 4.4	39.5 ± 4.5	31.6 ± 3.6
BMT[e]	76.1 ± 2.3	50.6 ± 1.2	95.3 ± 2.9	64.3 ± 2.4	NT[f]	NT[f]
TDC[e]	NT[f]	NT[f]	NT[f]	NT[f]	54.8 ± 1.6	30.4 ± 1.7

[a]The average of three trials

[b]*Xoo* = *Xanthomonas oryzae* pv. *oryzae*

[c]*Xac* = *Xanthomonas axonopodis* pv. *citri*

[d]*Rs* = *Ralstonia solanacearum*

[e]Commercial agrobactericides bismerthiazol (BMT) and thiodiazole-copper (TDC) were used as positive control agents

[f]NT = Not tested

Table 2 EC_{50} values of several of the compounds against *Xoo* or *Xac*

Compd.	Tested bacteria	EC_{50} (μg/mL)	Toxic regression equation	*R*
8k	*Xoo*	101.8 ± 2.6	$y = 2.0473x + 0.8893$	0.9757
8l	*Xoo*	93.9 ± 2.4	$y = 1.9663x + 1.1216$	0.9816
8m	*Xoo*	69.0 ± 5.6	$y = 1.5858x + 2.0842$	0.9892
8n	*Xoo*	53.3 ± 2.5	$y = 1.7817x + 1.9246$	0.9910
8o	*Xoo*	58.9 ± 2.5	$y = 1.9964x + 1.4668$	0.9820
BMT[a]	*Xoo*	91.4 ± 3.1	$y = 1.5839x + 1.8942$	0.9847
8g	*Xac*	111.4 ± 3.1	$y = 1.8365x + 1.2410$	0.9858
8l	*Xac*	96.3 ± 3.3	$y = 2.0217x + 0.9900$	0.9758
8m	*Xac*	71.5 ± 5.6	$y = 1.7459x + 1.7629$	0.9732
8n	*Xac*	86.4 ± 3.8	$y = 1.8050x + 1.5083$	0.9849
8o	*Xac*	74.7 ± 2.4	$y = 1.7539x + 1.7148$	0.9943
BMT[a]	*Xac*	60.5 ± 3.5	$y = 2.1593x + 1.1529$	0.9744

Xoo = *Xanthomonas oryzae* pv. *oryzae*; *Xac* = *Xanthomonas axonopodis* pv. *citri*

[a]The commercial bactericide bismerthiazol (BMT) was used as the positive control

Table 3 In vitro antifungal activities of target compounds **8a**–**8o** at 50 μg/mL

Compd.	Inhibition rate (%)[a]		
	G. zeae[b]	*V. dahliae*[c]	*S. sclerotiorum*[d]
8a	0	15.0 ± 3.8	15.7 ± 3.2
8b	0	12.4 ± 2.4	12.6 ± 6.2
8c	0	18.4 ± 4.2	18.1 ± 2.2
8d	0	9.4 ± 4.3	17.4 ± 1.5
8e	0	0	9.7 ± 1.9
8f	17.9 ± 7.4	19.4 ± 1.6	10.2 ± 6.2
8g	15.1 ± 1.3	17.2 ± 1.9	21.9 ± 1.8
8h	0	16.5 ± 3.5	0
8i	12.8 ± 2.4	15.4 ± 3.9	19.0 ± 1.4
8j	0	0	12.4 ± 5.8
8k	0	0	16.2 ± 3.7
8l	0	0	21.8 ± 4.3
8m	0	18.6 ± 1.5	17.3 ± 2.8
8n	10.8 ± 4.5	34.0 ± 4.0	10.2 ± 4.9
8o	12.2 ± 5.8	23.4 ± 1.4	0
Hymexazol[e]	49.8 ± 2.4	86.1 ± 1.9	87.8 ± 3.1

[a]The average of three trials

[b]*G. Zeae* = *Gibberella zeae*

[c]*V. dahliae* = *Verticillium dahliae*

[d]*S. sclerotiorum* = *Sclerotinia sclerotiorum*

[e]The commercialized Hymexazol was used as a control agent

control bismerthiazol. This study showed the potential of 1,2,4-triazolo[1,5-*a*]pyrimidine-containing quinazolin-4-one derivatives as promising lead compounds for the development of more efficient agrobactericides.

Experimental

General

All the chemicals were purchased from commercial suppliers and used directly without further purification (unless stated otherwise). Melting points were determined and uncorrected on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). ^{1}H and ^{13}C NMR spectra were determined 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). The following abbreviations were employed in expressing multiplicity: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet. HRMS-ESI spectra were measured on a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer. The X-ray crystallographic data were collected using a Bruker Smart Apex CCD area detector diffractometer (Bruker, Germany) with Mo-Kα radiation. The software package SPSS 17.0 is developed by SPSS Inc.

Synthesis of intermediate 5

A mixture of 5-amino-1*H*-1,2,4-triazole-3-thiol **4** (1.38 g, 11.88 mmol) and 3-(2-bromoethyl)quinazolinone **3** (2.99 g, 11.88 mmol) was dissolved in DMF (20 mL) in the presence of NaOH (1.42 g, 35.6 mmol), stirred at room temperature for 1 h and then heated to 60 °C for 10 h. After completion of the reaction, ice water (15 mL) was added into the reaction mixture and the resultant precipitate was filtered, washed with water and dried under vacuum to give **5** as a white solid. Yield: 78.2%, mp 237–240 °C. ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 12.00 (s, 1H), 8.25 (s, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.6 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 6.09 (s, 2H), 4.26 (t, J = 6.3 Hz, 2H), 3.33 (t, J = 6.2 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 160.2, 157.5, 154.8, 148.1, 148.0, 134.4, 127.2, 127.0, 126.1, 121.6, 45.7, 29.8. HRMS (ESI) calcd for $[M+H]^+$ $C_{12}H_{13}N_6OS$: 289.0866, found: 289.0862.

Synthesis of intermediate 6

A mixture of intermediate **5** (3.00 g, 10.40 mmol) and ethyl acetoacetate (2.62 mL, 20.80 mmol) in acetic acid (20 mL) was heated to reflux and stirred for 8 h. The reaction mixture was then cooled to room temperature, and the resultant precipitate was filtered, washed with AcOH and EtOH, and dried to generate intermediate **6** as a white solid. Yield: 72.3%, mp 244–247 °C. ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 13.03 (s, 1H), 8.25 (s, 1H), 8.13 (d, J = 7.9 Hz, 1H), 7.78 (t, J = 7.6 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 5.74 (s, 1H), 4.35 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.2, 154.7, 151.0, 150.6, 148.0, 147.8, 134.3, 127.0, 126.9, 126.1, 121.4, 98.5, 45.8, 29.6, 18.5. HRMS (ESI) calcd for $[M+H]^+$ $C_{16}H_{15}N_6O_2S$: 355.0972, found: 355.0967.

Synthesis of intermediate 7

A mixture of intermediate **6** (3.68 g, 10.40 mmol) and $POCl_3$ (2.62 mL) was heated to reflux and stirred for 2.5 h. Excess $POCl_3$ was removed by distillation under reduced pressure; the residue was dumped into ice water and then neutralized with dilute NaOH solution. The crude product was extracted with CH_2Cl_2, evaporated and recrystallized with EtOH to afford **7** as a white solid. Yield: 40%, mp 158–160 °C; ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.25 (s, 1H), 8.12 (d,

J = 8.1 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.54 (d, J = 3.4 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.48 (s, 1H), 4.39 (t, J = 6.3 Hz, 2H), 3.66 (t, J = 5.8 Hz, 2H), 2.54 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 165.6, 165.1, 160.2, 155.4, 147.9, 147.7, 136.9, 134.2, 126.9, 126.8, 126.1, 121.4, 111.0, 45.9, 29.6, 24.3. HRMS (ESI) calcd for $[M+H]^+$ $C_{16}H_{14}ON_6ClS$: 373.0633, found: 373.0627.

General procedure for the synthesis of target compounds 8a–8o

A mixture of intermediate **7** (100 mg, 0.27 mmol) and appropriate arylamine (0.32 mmol) in EtOH (10 mL) was stirred at room temperature for 30 h. The crude product was collected by filtration, washed with EtOH and dried to give compounds **8a**–**8o**.

3-(2-((5-methyl-7-(phenylamino)-[1,2,4] triazolo[1,5-*a*] pyrimidin-2-yl)thio)ethyl)quinazolin-4(3*H*)-one (8a)

White solid, mp 204–206 °C, yield: 78.5%. IR (KBr, ν/cm^{-1}): 3274 (N–H), 1668 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.03 (s, 1H), 8.30 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 6.8 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 7.4 Hz, 2H), 7.44 (d, J = 7.4 Hz, 2H), 7.31 (t, J = 7.5 Hz, 1H), 6.31 (s, 1H), 4.42 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.9, 163.7, 160.2, 155.7, 148.1, 147.8, 144.8, 136.7, 134.2, 129.5, 127.0, 126.9, 126.1, 126.0, 124.3, 121.4, 89.2, 45.7, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{20}ON_7S$: 430.1445, found: 430.1439.

3-(2-((7-((2-chlorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8b)

White solid, mp 162–165 °C, yield: 81.6%. IR (KBr, ν/cm^{-1}): 3271 (N–H), 1672 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.06 (s, 1H), 8.32 (s, 1H), 8.15 (d, J = 6.4 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.51 (d, J = 6.4 Hz, 1H), 7.49 (d, J = 7.3 Hz, 1H), 7.45 (t, J = 8.7 Hz, 1H), 5.78 (s, 1H), 4.43 (t, J = 6.4 Hz, 2H), 3.65 (t, J = 5.9 Hz, 2H), 2.32 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.0, 163.9, 160.2, 155.7, 148.1, 147.9, 145.2, 134.3, 133.5, 131.3, 130.5, 129.7, 129.5, 128.7, 127.1, 126.1, 124.3, 121.5, 89.6, 45.7, 29.6, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7ClS$: 464.1055, found: 456.1051.

3-(2-((7-((4-chlorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8c)

White solid, mp 240–243 °C, yield: 80.3%; IR (KBr, ν/cm^{-1}): 3255 (N–H), 1678 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.09 (s, 1H), 8.29 (s, 1H), 8.13 (d, J = 8.6 Hz, 1H), 7.78 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.54 (d, J = 8.6 Hz, 2H), 7.51 (t, J = 6.9 Hz, 1H), 7.46 (d, J = 8.6 Hz, 2H), 6.36 (s, 1H), 4.41 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.1, 163.7 160.2, 155.7, 148.1, 147.8, 144.6, 135.8, 134.3, 130.0, 129.5, 127.0, 126.9, 126.0, 125.9, 121.4, 89.5, 45.8, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7ClS$: 464.1055, found: 464.1050.

3-(2-((7-((2-fluorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8d)

White solid, mp 166–169 °C; yield 74.3%; IR (KBr, ν/cm^{-1}): 3395 (N–H), 1660 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.04 (s, 1H), 8.31 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 6.9 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 6.8 Hz, 2H), 7.45 (t, J = 8.0 Hz, 2H), 7.34 (t, J = 6.3 Hz, 1H), 5.93 (d, J = 2.0 Hz, 1H), 4.42 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.0, 163.9, 160.2, 158.0, 156.0, 155.6, 148.2, 147.9, 145.2, 134.3, 128.9, 127.1, 126.9, 126.1, 125.5, 125.4, 123.9, 123.8, 121.5, 117.0 116.8, 89.5, 45.7, 29.7, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7FS$: 448.1350, found: 448.1353.

3-(2-((7-((3-fluorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8e)

White solid, mp 213–215 °C, yield: 73.6%. IR (KBr, ν/cm^{-1}): 3270 (N–H), 1663 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.14 (s, 1H), 8.29 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 7.2 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.13 (t, J = 7.4 Hz, 1H), 6.48 (s, 1H), 4.42 (t, J = 6.3 Hz, 2H), 3.66 (t, J = 6.3 Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.3, 163.8, 163.4, 161.5, 160.2, 155.7, 148.1, 147.8, 144.3, 138.8, 138.7, 134.3, 131.2, 131.1, 127.1, 126.9, 126.1, 121.5, 119.7, 112.6, 112.4, 111.0, 110.8, 89.9, 45.8, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7FS$: 448.1350, found: 448.1341.

3-(2-((7-((4-fluorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8f)

White solid, mp 193–196 °C, yield: 74.2%; IR (KBr, ν/cm^{-1}): 3270 (N–H), 1667 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.02 (s, 1H), 8.29 (s, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.79 (t, J = 8.2 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.51 (t, J = 6.9 Hz, 1H), 7.46 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 8.7 Hz, 2H), 6.22 (s, 1H), 4.41 (t, J = 6.1 Hz, 2H), 3.65 (t, J = 6.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.0, 163.7, 161.1, 160.2, 159.1, 155.7, 148.1, 147.9, 145.1, 134.3, 133.0, 127.1, 127.0, 126.9, 126.1, 121.5, 116.5, 116.3, 89.1, 45.8, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7FS$: 448.1350, found: 448.1343.

3-(2-((5-methyl-7-((4-(trifluoromethyl)phenyl) amino)-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8g)

White solid, mp > 250 °C, yield: 76.9%. IR (KBr, ν/cm^{-1}): 3231 (N–H), 1661 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.32 (s, 1H), 8.28 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.6 Hz, 2H), 7.77 (t, J = 6.9 Hz, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.4 Hz, 1H), 6.60 (s, 1H), 4.41 (t, J = 6.3 Hz, 2H), 3.66 (t, J = 6.6 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.4, 163.9, 160.2, 155.7, 148.1, 147.8, 143.9, 141.0, 134.3, 127.0, 126.9, 126.7, 126.1, 125.5, 125.3, 125.2, 125.1, 123.5, 121.4, 90.3, 45.8, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{19}ON_7F_3S$: 498.1318, found: 498.1314.

3-(2-((7-((3-bromophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8h)

White solid, mp 243–246 °C, yield: 77.8%. IR (KBr, ν/cm^{-1}): 3235 (N–H), 1669 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 10.12 (s, 1H), 8.29 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 8.1 Hz, 1H), 7.64 (s, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 5.7 Hz, 1H), 7.48 (t, J = 2.9 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 6.41 (s, 1H), 4.42 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.2, 163.8, 160.2, 155.7, 148.1, 147.8, 144.3, 138.6, 134.3, 131.4, 128.6, 127.1, 126.9, 126.7, 126.1, 122.8, 122.1, 121.5, 89.8, 45.8, 29.7, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{19}ON_7BrS$: 508.0550, found: 508.0545.

3-(2-((5-methyl-7-(*o*-tolylamino)-[1,2,4] triazolo[1,5-*a*] pyrimidin-2-yl)thio)ethyl)quinazolin-4(3*H*)-one (8i)

White solid, mp 215–217 °C, yield: 82.6%. IR (KBr, ν/cm^{-1}): 3377 (N–H), 1674 (C=O); ^{1}H NMR (125 MHz, DMSO-d_6, ppm) δ: 9.53 (s, 1H), 8.33 (s, 1H), 8.14 (d, J = 7.4 Hz, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 1H), 7.06 (t, J = 6.9 Hz, 1H), 5.85 (s, 1H), 4.43 (t, J = 6.3 Hz, 2H), 3.82 (s, 3H), 3.64 (t, J = 6.3 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.8, 163.7, 160.3, 155.6, 154.0, 148.1, 147.9, 145.2, 134.3, 128.6, 127.4, 127.1, 126.9, 126.1, 124.4, 121.5, 120.9, 112.6, 89.5, 55.7, 45.7, 29.6, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{22}ON_7S$: 444.1601, found: 444.1605.

3-(2-((5-methyl-7-(*m*-tolylamino)-[1,2,4] triazolo[1,5-*a*] pyrimidin-2-yl)thio)ethyl)quinazolin-4(3*H*)-one (8j)

White solid, mp 246–249 °C, yield: 84.7%. IR (KBr, ν/cm^{-1}): 3248 (N–H), 1670 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 9.95 (s, 1H), 8.31 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 8.6 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.23 (d, J = 7.4 Hz, 2H), 7.12 (d, J = 7.4 Hz, 1H), 6.29 (s, 1H), 4.43 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.35 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.9, 163.7, 160.2, 155.7, 148.1, 147.9, 144.9, 139.1, 136.6, 134.3, 129.4, 127.1, 126.9, 126.8, 126.1, 124.9, 121.5, 121.4, 89.3, 45.8, 29.7, 24.7, 21.0. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{22}ON_7S$: 444.1601, found: 444.1604.

3-(2-((7-((2-methoxyphenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8k)

White solid, mp 173–175 °C, yield: 82.4%. IR (KBr, ν/cm^{-1}): 3377 (N–H), 1674 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 9.54 (s, 1H), 8.33 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.37 (t, J = 5.7 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 5.85 (s, 1H), 4.43 (t, J = 6.3 Hz, 2H), 3.81 (s, 3H), 3.64 (t, J = 6.3 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.8, 163.7, 160.3, 155.6, 154.0, 148.2, 147.9, 145.2, 134.3, 128.6, 127.4, 127.1, 126.9, 126.1, 124.5, 121.5, 120.9, 112.6, 89.5, 55.7, 45.7, 29.6, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{22}O_2N_7S$: 460.1550, found: 460.1550.

3-(2-((7-((4-methoxyphenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8l)

White solid, mp 181–183 °C, yield: 72.8%. IR (KBr, ν/cm^{-1}): 3270 (N–H), 1683 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 9.89 (s, 1H), 8.30 (s, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.51 (t, J = 8.2 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 9.1 Hz, 2H), 6.09 (s, 1H), 4.42 (t, J = 6.4 Hz, 2H), 3.80 (s, 3H), 3.64 (t, J = 6.4 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.7, 163.6, 160.2, 157.7, 155.7, 148.2, 147.9, 145.6, 134.3, 129.1, 127.1, 126.9, 126.7, 126.1, 121.5, 114.8, 88.9, 55.4, 45.8, 29.6, 24.7. HRMS (ESI) calcd for $[M+H]^+$ $C_{23}H_{22}O_2N_7S$: 460.1550, found: 456.1559.

3-(2-((7-((2,4-dichlorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8m)

White solid, mp 210–213 °C, Yield: 75.7%. IR (KBr, ν/cm^{-1}): 3271 (N–H), 1655 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.81 (s, 2H), 8.16 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 3.4 Hz, 1H), 7.86 (t, J = 8.6 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.6 Hz, 1H), 7.59 (t, J = 4.6 Hz, 1H), 7.57 (d, J = 5.2 Hz, 1H), 6.05 (s, 1H), 4.48 (t, J = 5.7 Hz, 2H), 3.69 (t, J = 5.7 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.6, 161.0, 159.7, 152.8, 149.0, 146.3, 144.5, 135.0, 133.5, 132.4, 132.3, 130.9, 130.2, 129.0, 127.7, 126.5, 124.8, 120.9, 90.8, 46.3, 29.7, 22.3. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{18}ON_7Cl_2S$: 498.0665, found: 498.0655.

3-(2-((7-((2,4-difluorophenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8n)

White solid, mp 139–142 °C, yield: 84.5%. IR (KBr, ν/cm^{-1}): 3211 (N–H), 1692 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 9.98 (s, 1H), 8.31 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (t, J = 8.6 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.57 (t, J = 8.1 Hz, 1H), 7.52 (d, J = 6.9 Hz, 1H), 7.50 (d, J = 5.2 Hz, 1H), 7.24 (t, J = 8.5 Hz, 1H), 5.94 (s, 1H), 4.42 (t, J = 6.3 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 164.1, 164.0, 160.2, 156.3, 155.6, 148.2, 147.9, 145.4, 134.3, 130.6, 130.5, 127.1, 126.9, 126.1, 121.5, 120.6, 120.5, 112.6, 112.4, 105.5, 105.3, 89.5, 45.7, 29.7, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{18}ON_7F_2S$: 466.1256, found: 466.1260.

3-(2-((7-((2,5-dimethoxyphenyl)amino)-5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)thio)ethyl) quinazolin-4(3*H*)-one (8o)

Reddish brown solid, mp 202–205 °C, yield: 78.5%. IR (KBr, ν/cm^{-1}): 3367 (N–H), 1672 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 9.58 (s, 1H), 8.32 (s, 1H), 8.14 (d, J = 7.4 Hz, 1H), 7.79 (t, J = 8.9 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.14 (d, J = 9.7 Hz, 1H), 6.96 (d, J = 2.9 Hz, 1H), 6.95 (d, J = 3.4 Hz, 1H), 5.89 (s, 1H), 4.43 (t, J = 6.3 Hz, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.64 (t, J = 6.3 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 163.8, 163.6, 160.3, 155.5, 153.2, 148.2, 148.1, 147.9, 145.2, 134.3, 127.1, 126.9, 126.1, 125.0, 121.5, 113.5, 113.4 113.0, 89.9, 56.1, 55.6, 45.7, 29.6, 24.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{24}H_{24}O_3N_7S$: 490.1656, found: 490.1663.

In vitro antibacterial bioassay

In vitro antibacterial activities of intermediate **7** and target compounds **8a**–**8o** were tested against three types of plant pathogenic bacteria (*Xoo*, *Xac* and *Rs*) using the turbidimetric assay, which is the most frequently utilized method for the measurement of the agricultural bactericidal potency [27–31]. Tested compounds were initially prepared at two concentrations of 200 and 100 μg/mL. Pure DMSO in sterile distilled water was utilized as a blank control, and commercially available bactericides bismerthiazol (BMT) and thiodiazole-copper (TDC) were employed as positive control agents. 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/Xac/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 1–3 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 compound-treated NB. The I represented the inhibition ratio of tested compound against the bacterium.

Finally, antibacterial activities of compounds **8k**, **8l**, **8m**, **8n** and **8o** against *Xoo* and compounds **8g**, **8l**, **8m**, **8n** and **8o** against *Xac* were further determined under five different concentrations (namely 200, 100, 50, 25 and 12.5 μg/mL) to obtain their EC_{50} values, which were calculated by Probit analysis using the software package SPSS 17.0.

In vitro antifungal bioassay

In vitro antifungal activities of target compounds **8a**–**8o** were determined against three types of phytopathogenic fungi (namely, *G. zeae*, *V. dahliae* and *S. sclerotiorum*) using the mycelial growth rate method, which is the most common method for the evaluation of the agricultural fungicidal potency [8, 32–34]. A DMSO solution containing the tested compound was added into sterilized Petri dishes, which contained about 10 mL molten potato dextrose agar (PDA). Next, a 4 mm diameter of mycelial disk 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 every compound. In addition, pure DMSO and commercially available fungicide (Hymexazol) were utilized as negative and positive control agents, respectively.

The inhibition ratio (I) of tested compound was calculated 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 tested compound-treated PDA.

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