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Synthesis and biological activities of novel quinazolinone derivatives containing a 1,2,4-triazolylthioether moiety

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A series of novel quinazolinone derivatives containing a 1,2,4-triazolylthioether moiety were synthesised and their antimicrobial activities were evaluated. All the target compounds were characterised by ^{1}H NMR, ^{13}C NMR, ESI-MS, IR and elemental analyses. The single crystal structure of 3-((5-((2-fluorobenzyl)thio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-one (*VIIi*) was also determined. The preliminary bioassays indicated that some of the target compounds possessed good antimicrobial activities. For example, 3-((4-phenyl-5-((4-(trifluoromethyl)benzyl)thio)-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-one (*VIIs*) exhibited the best inhibitory effect against *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas axonopodis* pv. *citri* with the half-effective concentration (EC_{50}) values of 47.6 μg mL^{-1} and 22.1 μg mL^{-1}, respectively, which were superior to the commercial bactericide, bismerthiazol. Meanwhile, 3-((5-((4-chlorobenzyl)thio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-one (*VIIh*) exhibited better fungicidal activities against *Pellicularia sasakii* and *Colletotrichum capsici* at the concentration of 50 μg mL^{-1}, in comparison with the commercial fungicide, hymexazol.

Keywords: quinazolinone, 1,2,4-triazolylthioether, antimicrobial activity, synthesis

Introduction

Many plant pathogenic bacteria, such as *Xanthomonas oryzae* pv. *oryzae* (XOO) and *Xanthomonas axonopodis* pv. *citri* (XAC), are difficult to control in the agricultural production process. The high loss-rate in bacteria-infected plants and the lack of effective control methods cause an enormous economic loss to farmers worldwide every year. The traditional bactericides currently available are not very effective, and frequent use of these bactericides has resulted in the development of bacterial resistance to them. Like pathogenic bacteria, phytopathogenic fungi also cause severe yield losses and a reduction in the quality of agricultural products every year. Accordingly, the development of new antibacterial and antifungal agents represents a major challenge in the field of pesticide chemistry.

Many quinazolin-4(3*H*)-one derivatives have attracted growing attention in recent years due to their various biological activities, such as antibacterial (Panneerselvam et al., 2009; Shi et al., 2013; Dixit et al., 2015), antifungal (Bartroli et al., 1998; Wang et al., 2013; El-Hashash et al., 2015), antimalarial (Zhu et al., 2010), antiviral (Ma et al., 2014), antitumour (Cao et al., 2005) and anticonvulsant activities (Jatav et al., 2008). Recently, Asker's study indicated that an *N*-substituted-2-methyl quinazolin-4(3*H*)-one derivative bearing 1,3,4-thiadiazole and 2-azetidinone moieties (compound A, Fig. 1) exhibited a broad spectrum of antimicrobial activity (Asker et al., 2014). In addition, 1,2,4-triazole derivatives (as another important heterocyclic compounds) have attracted attention in the

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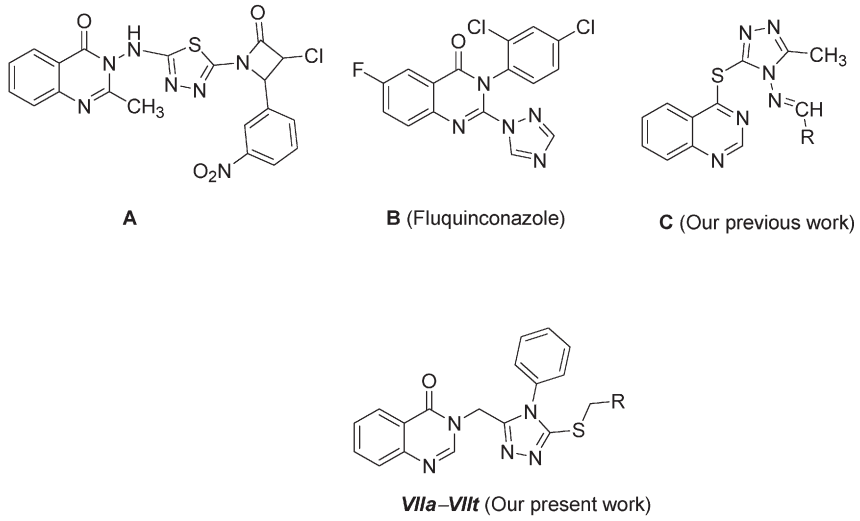

Fig. 1. Chemical structures of compounds A, B, C and *VIIa–VIIt*.

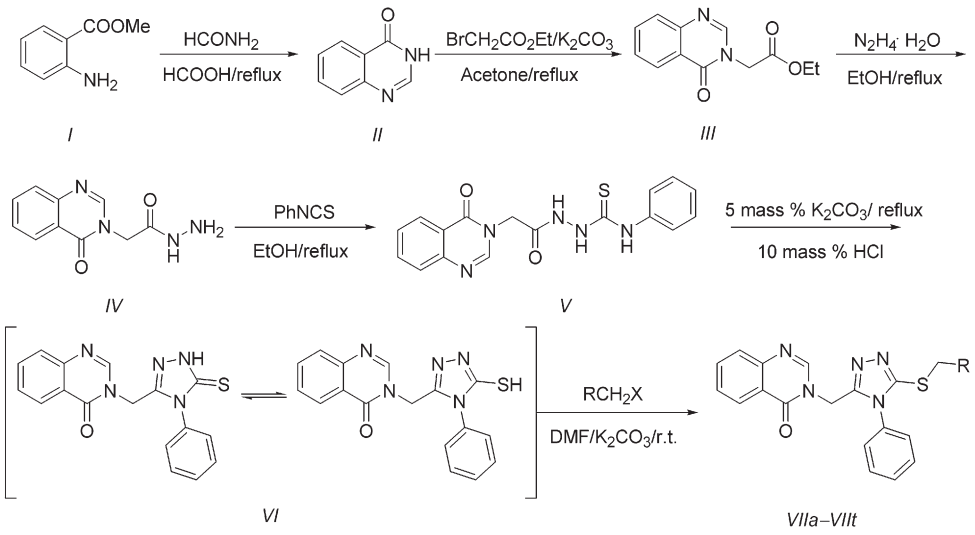

Fig. 2. Synthesis of target compounds *VIIa–VIIt*.

development of new antibacterial or antifungal agents (Holla et al., 2000; Turan-Zitouni et al., 2005; Cao et al., 2008; Hasan et al., 2011; Plech et al., 2013; Zhang et al., 2014). Fluquinconazole (compound B, Fig. 1), as the best-known example of a combination of quinazolinone backbone with 1,2,4-triazole heterocycle, has been used extensively as an agricultural fungicide for crop protection. Moreover, previous studies indicated that the introduction of a thioether unit into pesticide molecules was helpful in improving their bioactivities (Gülerman et al., 2001; Shin et al., 2001; Patil et al., 2010). However, to the best of our knowledge, no example of the integration of a quinazolin-4(3*H*)-one backbone with 1,2,4-triazolylthioether into a single molecule for the development of new agrochemicals has been reported to date. In a previous work (Liu et al., 2013), a series of quinazoline derivatives containing 1,2,4-triazole Schiff-base unit (compounds C, Fig. 1) were synthesised and some compounds were found to exhibit a certain fungicidal activity against the tested fungi. On the basis of the above considerations and in a continuing search for highly active antimicrobial agents, in the present study a series of novel quinazolinone derivatives containing 1,2,4-triazolylthioether moiety were designed and synthesised in accordance with the principle of the combina-

Table 1. Physical properties and analytical data for target compounds *VIIa–VIIt*

Compound	Formula	Appearance	M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p.
				C	H	N	%	℃
VIIa	$C_{24}H_{19}N_5OS$	Colourless solid	425.13	67.74	4.50	16.46	71.6	188–190
				67.79	4.93	16.86		
VIIb	$C_{28}H_{27}N_5OS$	White solid	481.19	69.83	5.65	14.54	58.0	175–178
				69.57	6.01	14.87		
VIIc	$C_{24}H_{18}N_6O_3S$	Yellow solid	470.12	61.27	3.86	17.86	50.0	188–199
				60.88	4.14	18.06		
VIId	$C_{24}H_{17}Cl_2N_5OS$	Colourless solid	493.05	58.30	3.47	14.17	90.4	> 250
				58.12	3.68	14.55		
VIIe	$C_{24}H_{14}F_5N_5OS$	White solid	515.08	55.92	2.74	13.59	60.1	127–128
				55.90	3.00	13.94		
VIIf	$C_{24}H_{18}ClN_5OS$	Colourless solid	459.05	62.67	3.94	15.23	72.9	211–213
				62.87	4.20	15.58		
VIIg	$C_{24}H_{18}ClN_5OS$	Yellow solid	459.05	62.67	3.94	15.23	72.9	150−151
				62.79	4.20	15.57		
VIIh	$C_{24}H_{18}ClN_5OS$	White solid	459.05	62.67	3.94	15.23	75.8	145–146
				62.74	4.35	15.46		
VIIi	$C_{24}H_{18}FN_5OS$	Colourless solid	443.12	65.00	4.09	15.79	75.6	200–202
				65.38	4.34	16.14		
VIIj	$C_{24}H_{18}FN_5OS$	White solid	443.12	65.00	4.09	15.79	90.7	168–169
				65.13	4.07	16.14		
VIIk	$C_{24}H_{18}FN_5OS$	White solid	443.12	65.00	4.09	15.79	58.4	134–136
				65.40	4.36	16.20		
VIIl	$C_{25}H_{21}N_5OS$	Yellow solid	439.15	68.32	4.82	15.93	89.0	210–213
				68.69	5.09	16.26		
VIIm	$C_{25}H_{21}N_5OS$	Yellow solid	439.15	68.32	4.82	15.93	69.8	146–148
				68.66	5.19	16.32		
VIIn	$C_{25}H_{21}N_5OS$	White solid	439.15	68.32	4.82	15.93	83.7	153–154
				68.60	5.23	16.30		
VIIo	$C_{25}H_{21}N_5O_2S$	White solid	455.14	65.92	4.65	15.37	92.0	162–164
				66.30	4.67	15.75		
VIIp	$C_{25}H_{21}N_5O_2S$	Colourless solid	455.14	65.92	4.65	15.37	70.1	151–153
				66.17	5.04	15.76		
VIIq	$C_{25}H_{18}F_3N_5OS$	White solid	493.12	60.84	3.68	14.19	92.7	180–181
				60.47	4.05	14.21		
VIIr	$C_{25}H_{18}F_3N_5OS$	White solid	493.12	60.84	3.68	14.19	86.7	116–119
				61.13	4.06	14.58		
VIIs	$C_{25}H_{18}F_3N_5OS$	White solid	493.12	60.84	3.68	14.19	67.9	98–101
				60.58	4.02	14.52		
VIIt	$C_{23}H_{17}ClN_6OS$	Brown solid	460.09	59.93	3.72	18.23	70.0	132–134
				59.57	3.85	18.41		

tion of bioactive substructures (Fig. 2). The biological test indicated that some of the target compounds possessed good antibacterial activities against XOO and XAC.

Experimental

All the solvents and materials were of analytical grade and used without further purification. Melting points were uncorrected and determined on an XT-4 binocular microscope (Beijing Tech Instrument, China); the IR spectra were recorded on an IR Prestige-21 spectrometer (Shimadzu, Japan) using the KBr disk technique. The ^{1}H and ^{13}C NMR spectra were measured on a JEOL-ECX 500 NMR spectrometer (JEOL, Japan) at ambient temperature using TMS as an internal standard and chemical shifts δ are expressed in parts per million (ppm). Mass spectra were recorded on an Agilent LC/MSD Trap VL spectrometer (Agilent, USA). Elemental analysis was performed on an Elementar Vario-III CHN analyser (Elementar, Germany). The X-ray data for compound *VIIi* were collected using a Bruker Smart Apex CCD area detector diffractometer (Bruker, Germany) with Mo-K_α radiation. The progress of the reactions was monitored by TLC performed on silica gel GF254. Intermediates *II*, *III* and *IV* were prepared as previously described in the literature (Sun et al., 2013; Mali et al., 2009).

Synthesis of 2-(2-(4-oxoquinazolin-3(4H)-yl) acetyl)-N-phenylhydrazinecarbothioamide (V)

Compound *IV* (2.29 mmol) and phenylisothiocyanate (2.52 mmol) were dissolved in ethanol (20 mL)

and heated to reflux for 3 h. Subsequently, the reaction mixture was cooled to ambient temperature and the precipitate thus formed was separated by filtration, washed with ethanol and dried to afford intermediate *V*. White solid; yield, 83.9 %; m.p.: 218–220 ℃; ^{1}H NMR (DMSO-d_6, 500 MHz), δ: 4.74 (s, 2H, QuCH$_2$), 7.15 (d, J = 7.5 Hz, 1H, ArH), 7.32 (t, J = 7.5 Hz, 2H, ArH), 7.46 (d, J = 7.5 Hz, 2H, ArH), 7.54 (t, J = 7.2 Hz, 1H, QuH), 7.68 (d, J = 8.0 Hz, 1H, QuH), 7.83 (t, J = 7.7 Hz, 1H, QuH), 8.10 (d, J = 8.0 Hz, 1H, QuH), 8.30 (s, 1H, QuH), 9.42 (s, 1H, NH), 9.78 (s, 1H, NH), 10.53 (s, 1H, NH).

Synthesis of 3-((5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl)quinazolin-4(3H)-one (VI)

Compound *V* (1.87 mmol) was slowly added to 5 mass % aqueous potassium carbonate solution (20 mL) and heated to reflux for 6 h. After cooling to ambient temperature, the resulting solid was removed by filtration and the filtrate was neutralised with dilute HCl to pH 7. The precipitate was separated by filtration, washed with water, dried and recrystallized with DMF/H$_2$O (1 : 1, vol.) to give *VI*. White solid; yield, 50.0 %; m.p.: 260–261 ℃; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 5.05 (s, 2H, QuCH$_2$), 7.49 (d, J = 6.9 Hz, 2H, ArH), 7.54–7.59 (m, 4H, ArH & QuH), 7.69 (d, J = 8.0 Hz, 1H, QuH), 7.86 (t, J = 7.4 Hz, 1H, QuH), 8.10 (d, J = 6.9 Hz, 1H, QuH), 8.27 (s, 1H, QuH), 13.91 (s, 1H, NH).

General synthetic procedure for target compounds VIIa–VIIt

Substituted benzyl halide or 2-chloro-5-chloromethylpyridine (0.6 mmol) was added to a solution of compound *VI* (0.5 mmol) in DMF (10 mL) at ambient temperature, then potassium carbonate (1 mmol) was added. The resulting mixture was stirred at ambient temperature for several hours. Upon completion of the reaction as indicated by TLC (CH$_2$Cl$_2$/CH$_3$OH, 5 : 1, vol.), the reaction mixture was poured into cold water and the resulting precipitate was separated by filtration, washed with water and recrystallized from CH$_2$Cl$_2$/CH$_3$CH$_2$OH (1 : 15, vol.) to afford the title compounds. The physical properties and analytical data of these compounds are listed in Table 1, and the corresponding spectral data are shown in Table 2.

Antibacterial and antifungal activity

The antibacterial activities of compounds *VIIa–VIIt* against tobacco bacterial wilt (*Ralstonia solanacearum* strain MR111, Guizhou University, China), *Xanthomonas axonopodis* pv. *citri* (strain 29-1, Shanghai Jiao Tong University, China) and *Xanthomonas oryzae* pv. *oryzae* (strain PXO99A, Nanjing Agricultural University, China) were evaluated using the turbidimeter test (Xu et al., 2012).

The antifungal activities of compounds *VIIa–VIIt* against six phytopathogenic fungi: *Gibberella zeae* (*G. zeae*), *Cytospora mandshurica* (*C. mandshurica*), *Phytophthora infestans* (*P. infestans*), *Pellicularia sasakii* (*P. sasakii*), *Colletotrichum capsici* (*C. capsici*) and *Gloeosporium fructigenum* (*G. fructigenum*), stored in Guizhou University, were evaluated using the mycelium growth-rate method (Liu et al., 2008).

Results and discussion

Synthesis

The synthesis of target compounds *VIIa–VIIt* is shown in Fig. 2. First, quinazolin-4-one (*II*) was synthesised by the condensation of methyl *o*-aminobenzoate with formamide in refluxing formic acid (Sun et al., 2013). The treatment of *II* with ethyl bromoacetate in the presence of K$_2$CO$_3$ in refluxing acetone afforded ethyl 2-(4-oxoquinazolin-3(4*H*)-yl) acetate (*III*) (Mali et al., 2009). The further reaction of *III* with hydrazine hydrate in refluxing ethanol afforded 2-(4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (*IV*) (Mali et al., 2009), which was subsequently allowed to react with phenylisothiocyanate to generate 2-(2-(4-oxoquinazolin-3(4*H*)-yl)acetyl)-*N*-phenylhydrazinecarbothioamide (*V*). The 5 mass % aqueous potassium carbonate solution of *V* was heated to reflux for 6 h and then neutralised with 10 mass % HCl; the resulting 3-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-one (*VI*) was obtained with a 50 % yield. Finally, target compounds *VIIa–VIIt* were readily obtained by the reaction of *VI* with substituted benzyl halide or 2-chloro-5-chloromethylpyridine in DMF with K$_2$CO$_3$ as catalyst at ambient temperature.

The structures of target compounds (*VIIa–VIIt*) were characterised using ^{1}H NMR, ^{13}C NMR, MS, IR and elemental analyses. In the ^{1}H NMR spectra of *VIIa–VIIt*, one singlet at δ 5.22–5.15 was attributed to the signal of the CH$_2$ protons linked to the quinazolinone ring, and the other CH$_2$ protons of the —SCH$_2$— fragment appeared at δ 4.69–4.36 as a singlet. The chemical shift at around δ 160.0 in the ^{13}C NMR spectra of target compounds showed the existence of C═O group of the quinazolinone ring. The IR spectra of *VIIa–VIIt* also displayed characteristic bands at 1689–1672 cm^{-1}, attributed to the stretching vibration of the quinazolinone C═O group. All the target compounds were confirmed using ESI-MS, which displayed the existence of [M + H]$^+$ and [M + Na]$^+$ peaks. The structure of *VIIi* was also confirmed using the X-ray single-crystal diffraction method (Fig. 3 and Table 3). The crystallographic data for this compound were deposited in the Cambridge Crystallographic Data Centre, no. CCDC-1036531.

Table 2. Spectral data of target compounds *VIIa–VIIt*

Compound	Spectral data
VIIa	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N),1683 (C═O), 3053 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.41 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 7.09 (d, J = 7.4 Hz, 2H, ArH), 7.23–7.28 (m, 5H, ArH), 7.40 (t, J = 7.4 Hz, 2H, ArH), 7.44–7.48 (m, 2H, ArH), 7.71 (d, J = 7.5 Hz, 1H, ArH), 7.76 (t, J = 7.0 Hz, 1H, ArH), 8.14 (d, J = 8.6 Hz, 1H, ArH), 8.15 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 37.4, 39.6, 121.8, 126.7, 127.1, 127.6, 127.7, 127.9, 128.8, 129.2, 130.1, 130.6, 132.2, 134.6, 136.3, 145.8, 147.9, 151.0, 153.0, 160.3 MS (ESI) m/z: 426.3 ($[M + H]^+$), 448.3 ($[M + Na]^+$)
VIIb	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1685 (C═O), 2962 (CH_2) ^{1}H NMR ($CDCl_3$), δ: 1.27 (s, 9H, CH_3), 4.38 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2), 7.04 (d, J = 8.0 Hz, 2H, ArH), 7.19 (d, J = 8.0 Hz, 2H, ArH), 7.28 (d, J = 8.6 Hz, 2H, ArH), 7.38 (t, J = 7.7 Hz, 2H, ArH), 7.42–7.47 (m, 2H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.75 (t, J = 7.0 Hz, 1H, ArH), 8.14 (d, J = 8.0 Hz, 1H, ArH), 8.16 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 31.4, 34.6, 37.3, 39.5, 121.8, 125.7, 126.8, 127.1, 127.6, 127.8, 128.9, 130.0, 130.5, 132.2, 133.3, 134.6, 145.7, 147.9, 150.9, 151.0, 153.2, 160.2 MS (ESI) m/z: 482.4 ($[M + H]^+$), 504.4 ($[M + Na]^+$)
VIIc	IR, $\tilde{\nu}$/cm^{-1}: 1165 (C—S), 1610 (C═N), 1672 (C═O), 3076 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.47 (s, 2H, SCH_2), 5.15 (s, 2H, CH_2), 7.20 (d, J = 7.4 Hz, 2H, ArH), 7.46–7.52 (m, 4H, ArH), 7.55 (d, J = 8.6 Hz, 2H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 6.9 Hz, 1H, ArH), 8.12–8.15 (m, 4H, ArH) ^{13}C NMR ($CDCl_3$), δ: 35.8, 39.8, 121.8, 123.9, 126.7, 127.0, 127.7, 127.8, 130.2, 130.3, 130.8, 132.0, 134.7, 144.4, 145.8, 147.4, 148.0, 151.4, 152.0, 160.4 MS (ESI) m/z: 471.3 ($[M + H]^+$), 493.3 ($[M + Na]^+$)
VIId	IR, $\tilde{\nu}$/cm^{-1}: 1238 (C—S), 1608 (C═N), 1683 (C═O), 2937 (CH_2) ^{1}H NMR ($CDCl_3$), δ: 4.69 (s, 2H, SCH_2), 5.22 (s, 2H, CH_2), 7.13 (t, J = 8.0 Hz, 1H, ArH), 7.22 (d, J = 7.4 Hz, 2H, ArH), 7.24−7.26 (m, 2H, ArH), 7.40–7.49 (m, 4H, ArH), 7.72 (d, J = 7.4 Hz, 1H, ArH), 7.76 (t, J = 7.5 Hz, 1H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.19 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 33.6, 39.7, 121.8, 126.8, 127.1, 127.6, 127.8, 128.5, 129.6, 130.1, 130.6, 132.1, 132.2, 134.6, 136.2, 145.8, 147.9, 151.4, 152.4, 160.3 MS (ESI) m/z: 494.2 ($[M + H]^+$), 516.2 ($[M + Na]^+$)
VIIe	IR, $\tilde{\nu}$/cm^{-1}: 1170 (C—S), 1612 (C═N), 1676 (C═O), 3066 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.41 (s, 2H, SCH_2), 5.19 (s, 2H, CH_2), 7.29 (d, J = 6.8 Hz, 2H, ArH), 7.47–7.53 (m, 4H, ArH), 7.72 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 7.4 Hz, 1H, ArH), 8.16 (d, J = 8.0 Hz, 1H, ArH), 8.18 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 24.0, 39.8, 121.8, 126.7, 127.0, 127.6, 127.8, 130.3, 130.8, 132.0, 134.7, 145.8, 148.0, 151.2, 151.7, 160.4 MS (ESI) m/z: 516.3 ($[M + H]^+$), 538.3 ($[M + Na]^+$)
VIIf	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1674 (C═O), 3051 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.53 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 7.12 (d, J = 8.0 Hz, 2H, ArH), 7.15–7.21 (m, 2H, ArH), 7.33 (d, J = 7.5 Hz, 1H, ArH), 7.42 (t, J = 7.5 Hz, 2H, ArH), 7.44–7.48 (m, 3H, ArH), 7.70 (d, J = 8.0 Hz, 1H, ArH), 7.69–7.77 (m, 1H, ArH), 8.14–8.15 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 35.0, 39.7, 121.8, 126.8, 127.0, 127.1, 127.6, 127.8, 129.4, 129.7, 130.2, 130.6, 131.6, 132.1, 134.4, 134.5, 134.7, 145.8, 147.9, 151.2, 153.0, 160.3 MS (ESI) m/z: 460.3 ($[M + H]^+$), 482.3 ($[M + Na]^+$)
VIIg	IR, $\tilde{\nu}$/cm^{-1}: 1168 (C—S), 1610 (C═N), 1682 (C═O), 3051 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.37 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 7.14 (d, J = 7.0 Hz, 2H, ArH), 7.19–7.22 (m, 3H, ArH), 7.29 (s, 1H, ArH), 7.43–7.49 (m, 4H, ArH), 7.71 (d, J = 7.5 Hz, 1H, ArH), 7.76 (t, J = 7.0 Hz, 1H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.16 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.6, 39.7, 121.8, 126.8, 127.0, 127.5, 127.6, 127.8, 128.1, 129.3, 130.0, 130.2, 130.7, 132.1, 134.5, 134.7, 138.5, 145.8, 147.9, 151.2, 152.6, 160.3 MS (ESI) m/z: 460.3 ($[M + H]^+$), 482.3 ($[M + Na]^+$)
VIIh	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1676 (C═O), 3061 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.37 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 7.13 (d, J = 8.0 Hz, 2H, ArH), 7.22–7.24 (m, 4H, ArH), 7.42–7.49 (m, 4H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 7.5 Hz, 1H, ArH), 8.14–8.15 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.4, 39.7, 121.8, 126.7, 127.0, 127.6, 127.8, 128.9, 130.2, 130.6, 130.6, 132.1, 133.7, 134.5, 135.1, 145.8, 147.9, 151.2, 152.7, 160.3 MS (ESI) m/z: 460.3 ($[M + H]^+$), 482.3 ($[M + Na]^+$)
VIIi	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1676 (C═O), 3055 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.44 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 6.99 (t, J = 9.2 Hz, 1H, ArH), 7.04 (d, J = 7.7 Hz, 1H, ArH), 7.14 (d, J = 8.0 Hz, 2H, ArH), 7.21–7.24 (m, 1H, ArH), 7.39–7.49 (m, 5H, ArH), 7.70 (d, J = 7.4 Hz, 1H, ArH), 7.76 (t, J = 7.5 Hz, 1H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.16 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 30.4, 39.7, 115.5, 115.6, 121.8, 123.8, 123.9, 124.3, 124.4, 126.7, 127.0, 127.6, 127.8, 129.8, 129.9, 130.2, 130.6, 131.5, 131.6, 132.1, 134.6, 145.8, 147.9, 151.2, 152.8, 160.0, 160.3, 162.0 MS (ESI) m/z: 444.3 ($[M + H]^+$), 466.3 ($[M + Na]^+$)

Table 2. (continued)

Compound	Spectral data
VIIj	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1685 (C═O), 3062 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.40 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 6.94 (t, J = 8.3 Hz, 1H, ArH), 7.04 (d, J = 9.7 Hz, 1H, ArH), 7.09 (d, J = 7.4 Hz, 1H, ArH), 7.15 (d, J = 6.3 Hz, 2H, ArH), 7.20–7.23 (m, 1H, ArH), 7.44–7.49 (m, 4H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.75 (t, J = 7.7 Hz, 1H, ArH), 8.13–8.15 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.5, 39.6, 114.7, 114.9, 116.0, 116.2, 121.7, 124.8, 124.9, 126.6, 126.9, 127.5, 127.7, 130.2, 130.3, 130.6, 132.0, 134.6, 138.7, 138.8, 145.7, 147.8, 151.1, 152.6, 160.2, 161.7, 163.7 MS (ESI) m/z: 444.3 ($[M + H]^+$), 466.3 ($[M + Na]^+$)
VIIk	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1674 (C═O), 3062 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.39 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 6.95 (t, J = 8.6 Hz, 2H, ArH), 7.16 (d, J = 7.4 Hz, 2H, ArH), 7.27–7.30 (m, 2H, ArH), 7.42–7.50 (m, 4H, ArH), 7.71 (d, J = 7.4 Hz, 1H, ArH), 7.76 (t, J = 8.0 Hz, 1H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.16 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.4, 39.7, 115.6, 115.7, 121.8, 126.7, 127.0, 127.6, 127.8, 130.2, 130.6, 130.9, 131.0, 132.1, 132.2, 134.7, 145.8, 147.9, 151.1, 152.8, 160.3, 161.4, 163.4 MS (ESI) m/z: 444.3 ($[M + H]^+$), 466.3 ($[M + Na]^+$)
VIIl	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1608 (C═N), 1676 (C═O), 3045 (ArH) ^{1}H NMR ($CDCl_3$), δ: 2.27 (s, 3H, CH_3), 4.42 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2), 7.06 (d, J = 8.0 Hz, 2H, ArH), 7.10 (t, J = 8.3 Hz, 2H, ArH), 7.15 (t, J = 7.4 Hz, 1H, ArH), 7.20 (d, J = 7.4 Hz, 1H, ArH), 7.39 (t, J = 7.7 Hz, 2H, ArH), 7.43−7.49 (m, 2H, ArH), 7.72 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 8.0 Hz, 1H, ArH), 8.14 (d, J = 8.0 Hz, 1H, ArH), 8.15 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 19.1, 35.9, 39.6, 121.8, 126.4, 126.8, 127.1, 127.6, 127.8, 128.3, 130.0, 130.2, 130.5, 130.7, 132.2, 133.8, 134.6, 137.2, 145.8, 147.9, 151.0, 153.1, 160.3 MS (ESI) m/z: 440.3 ($[M + H]^+$), 462.3 ($[M + Na]^+$)
VIIm	IR, $\tilde{\nu}$/cm^{-1}: 1178 (C—S), 1610 (C═N), 1683 (C═O), 3035 (ArH) ^{1}H NMR ($CDCl_3$), δ: 2.29 (s, 3H, CH_3), 4.38 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2), 7.04–7.10 (m, 5H, ArH), 7.15 (t, J = 7.7 Hz, 1H, ArH), 7.41 (t, J = 7.4 Hz, 2H, ArH), 7.45–7.49 (m, 2H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 6.9 Hz, 1H, ArH), 8.15 (d, J = 7.5 Hz, 1H, ArH), 8.16 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 21.4, 37.4, 39.6, 121.8, 126.3, 126.8, 127.1, 127.6, 127.8, 128.7, 129.9, 130.1, 130.6, 132.2, 133.1, 134.6, 136.1, 138.5, 145.8, 147.9, 151.0, 153.2, 160.3 MS (ESI) m/z: 440.3 ($[M + H]^+$), 462.3 ($[M + Na]^+$)
VIIn	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1610 (C═N), 1687 (C═O), 3059 (ArH) ^{1}H NMR ($CDCl_3$), δ: 2.29 (s, 3H, CH_3), 4.38 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2), 7.05–7.08 (m, 4H, ArH), 7.18 (d, J = 8.0 Hz, 2H, ArH), 7.40 (t, J = 7.7 Hz, 2H, ArH), 7.44–7.49 (m, 2H, ArH), 7.71 (d, J = 7.4 Hz, 1H, ArH), 7.76 (t, J = 6.9 Hz, 1H, ArH), 8.14 (d, J = 7.0 Hz, 1H, ArH), 8.15 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 21.2, 37.2, 39.5, 121.8, 126.8, 127.1, 127.6, 127.8, 129.1, 129.4, 130.1, 130.5, 132.2, 133.2, 136.6, 137.7, 145.8, 147.9, 151.0, 153.2, 160.3 MS (ESI) m/z: 440.3 ($[M + H]^+$), 462.3 ($[M + Na]^+$)
VIIo	IR, $\tilde{\nu}$/cm^{-1}: 1153 (C—S), 1610 (C═N), 1683 (C═O), 3001 (ArH) ^{1}H NMR ($CDCl_3$), δ: 3.74 (s, 3H, OCH_3), 4.37 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 6.78 (d, J = 8.0 Hz, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, J = 8.0 Hz, 1H, ArH), 7.10 (d, J = 8.0 Hz, 2H, ArH), 7.17 (t, J = 7.7 Hz, 1H, ArH), 7.40 (t, J = 8.0 Hz, 2H, ArH), 7.44–7.48 (m, 2H, ArH), 7.70 (d, J = 7.4 Hz, 1H, ArH), 7.75 (t, J = 7.0 Hz, 1H, ArH), 8.12–8.14 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 37.4, 39.6, 55.3, 113.7, 114.5, 121.5, 121.8, 126.7, 127.1, 127.6, 127.8, 129.8, 130.1, 130.5, 132.2, 134.6, 137.7, 145.8, 147.9, 151.0, 153.0, 159.8, 160.3 MS (ESI) m/z: 456.3 ($[M + H]^+$), 478.3 ($[M + Na]^+$)
VIIp	IR, $\tilde{\nu}$/cm^{-1}: 1172 (C—S), 1608 (C═N), 1683 (C═O), 3051 (ArH) ^{1}H NMR ($CDCl_3$), δ: 3.75 (s, 3H, OCH_3), 4.36 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2), 6.79 (d, J = 8.6 Hz, 2H, ArH), 7.11 (d, J = 8.0 Hz, 2H, ArH), 7.21 (d, J = 9.2 Hz, 2H, ArH), 7.40 (t, J = 8.0 Hz, 2H, ArH), 7.43–7.38 (m, 2H, ArH), 7.70 (d, J = 7.4 Hz, 1H, ArH), 7.74 (t, J = 7.0 Hz, 1H, ArH), 8.13 (d, J = 8.0 Hz, 1H, ArH), 8.14 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.9, 39.6, 55.4, 114.1, 121.8, 126.7, 127.1, 127.6, 127.8, 128.2, 130.1, 130.4, 130.5, 132.2, 134.6, 145.8, 147.9, 151.0, 153.2, 159.3, 160.3 MS (ESI) m/z: 456.3 ($[M + H]^+$),478.3 ($[M + Na]^+$)
VIIq	IR, $\tilde{\nu}$/cm^{-1}: 1170 (C—S), 1614 (C═N), 1689 (C═O), 3062 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.63 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2), 7.16 (d, J = 8.0 Hz, 2H, ArH), 7.36 (t, J = 7.7 Hz, 1H, ArH), 7.42–7.50 (m, 5H, ArH), 7.61 (d, J = 7.4 Hz, 1H, ArH), 7.67–7.72 (m, 2H, ArH, 7.75–7.78 (m, 1H, ArH), 8.16 (d, J = 8.0 Hz, 1H, ArH), 8.17 (s, 1H, ArH) ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 33.3, 39.6, 121.8, 123.1, 125.2, 126.2, 126.3, 126.7, 127.0, 127.6, 127.8, 130.2, 130.7, 132.0, 132.3, 132.4, 134.7, 135.3, 145.8, 148.0, 151.3, 153.0, 160.4 MS (ESI) m/z: 494.3 ($[M + H]^+$), 516.3 ($[M + Na]^+$)

Table 2. (continued)

Compound	Spectral data
VIIr	IR, $\tilde{\nu}$/cm^{-1}: 1166 (C—S), 1612 (C═N), 1683 (C═O), 3062 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.45 (s, 2H, SCH_2), 5.16 (s, 2H, CH_2), 7.13 (d, J = 7.0 Hz, 2H, ArH), 7.39 (t, J = 7.7 Hz, 1H, ArH), 7.42–7.55 (m, 7H, ArH), 7.71 (d, J = 8.0 Hz, 1H, ArH), 7.76 (t, J = 7.0 Hz, 1H, ArH), 8.12–8.14 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.6, 39.7, 121.8, 122.9, 124.6, 124.7, 125.9, 126.7, 127.0, 127.6, 127.8, 129.2, 130.2, 130.7, 132.0, 132.7, 134.7, 137.6, 145.8, 147.9, 151.2, 152.4, 160.3 MS (ESI) m/z: 494.3 ($[M + H]^+$), 516.3 ($[M + Na]^+$)
VIIs	IR, $\tilde{\nu}$/cm^{-1}: 1165 (C—S), 1612 (C═N), 1683 (C═O), 3051 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.44 (s, 2H, SCH_2), 5.16 (s, 2H, CH_2), 7.14 (d, J = 7.5 Hz, 2H, ArH), 7.42–7.45 (m, 4H, ArH), 7.46–7.49 (m, 2H, ArH), 7.53 (d, J = 8.0 Hz, 2H, ArH), 7.71 (d, J = 6.9 Hz, 1H, ArH), 7.76 (t, J = 7.5 Hz, 1H, ArH), 8.13−8.16 (m, 2H, ArH) ^{13}C NMR ($CDCl_3$), δ: 36.4, 39.7, 121.8, 125.6, 125.7, 126.7, 127.0, 127.6, 127.8, 129.6, 130.2, 130.7, 131.1, 132.0, 134.7, 140.8, 145.8, 147.9, 151.3, 152.4, 160.4 MS (ESI) m/z: 494.3 ($[M + H]^+$),516.3 ($[M + Na]^+$)
VIIt	IR, $\tilde{\nu}$/cm^{-1}: 1165 (C—S), 1608 (C═N), 1683 (C═O), 3064 (ArH) ^{1}H NMR ($CDCl_3$), δ: 4.38 (s, 2H, SCH_2), 5.16 (s, 2H, CH_2), 7.21 (d, J = 7.0 Hz, 2H, ArH), 7.25 (d, J = 8.0 Hz, 1H, ArH), 7.47–7.53 (m, 4H, ArH), 7.70–7.78 (m, 3H, ArH), 8.14–8.16 (m, 2H, ArH), 8.35 (d, J = 2.3 Hz, 1H, ArH) ^{13}C NMR ($CDCl_3$), δ: 32.9, 39.8, 121.8, 124.2, 126.7, 126.9, 127.6, 127.8, 130.3, 130.8, 131.9, 131.9, 134.7, 139.8, 145.8, 148.0, 150.1, 150.8, 151.4, 152.0, 160.4 MS (ESI) m/z: 461.3 ($[M + H]^+$), 483.3 ($[M + Na]^+$)

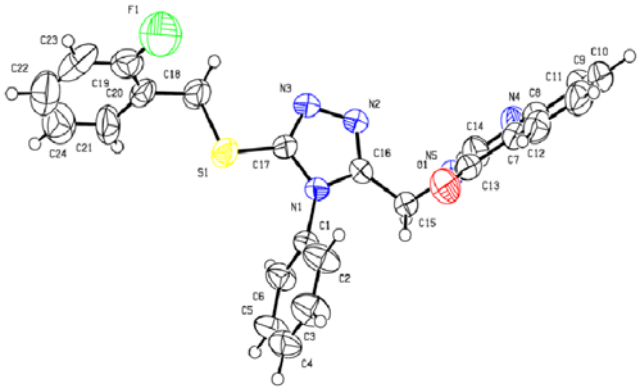

Fig. 3. Crystal structure of compound *VIIi*.

Antibacterial activity and structure-activity relationship

The commercial agricultural bactericides, thiodiazole-copper and bismerthiazol, were used as positive controls (Table 4). Some of the compounds with good antibacterial activities were tested at five double-declining concentrations (80 μg mL^{-1}, 40 μg mL^{-1}, 20 μg mL^{-1}, 10 μg mL^{-1} and 5.0 μg mL^{-1}) in order to obtain their corresponding EC_{50} values. The average EC_{50} was calculated from at least three separate analyses of growth inhibition using the software package SPSS 17.0. The screening results are summarised in Tables 5 and 6.

As shown in Table 4, the biological tests in vitro indicated that some compounds possessed good antibacterial activities at a concentration of 200 μg mL^{-1}. For example, the inhibition rates of compounds *VIIg*, *VIIh* and *VIIs* against XOO at 200 μg mL^{-1} were 99.2 %, 99.8 % and 99.9 %, respectively, which were even better than those of the positive controls, thiodiazole-copper and bismerthiazol. In addition, compounds *VIIb* and *VIIs* exhibited 53.0 % and 99.9 % inhibition rates against at 100 μg mL^{-1}, better than that of thiodiazole-copper (17.0 %). Table 5 shows that compounds *VIIh* and *VIIs* exhibited potent activities against with EC_{50} values of 88.5 μg mL^{-1} and 47.6 μg mL^{-1}, respectively, implying that these two compounds had higher inhibition activities against XOO than bismerthiazol (92.6 μg mL^{-1}). Table 6 shows that compounds *VIIb* and *VIIs* exhibited significant activities against XAC, with EC_{50} values of 82.1 μg mL^{-1} and 22.1 μg mL^{-1}, respectively, suggesting that *VIIs* had a higher inhibition activity against XAC than bismerthiazol (EC_{50} = 58.2 μg mL^{-1}).

A preliminary analysis of structure-activity relationship was conducted based on the results given in Table 4. Out of the target compounds, compound *VIIs* (having 4-CF_3 on the phenyl ring) was the best antibacterial agent against XOO and XAC. The presence of a strong electron-donating substitution (—OCH_3) on the phenyl ring (viz. *VIIo* and *VIIp*) was found to exhibit no activity against tobacco bacterial wilt. As far as XOO was concerned, the presence of —F or —Cl substitution on the 4-position of phenyl ring was beneficial to the activity, in comparison with that of the halogen at the 2- or 3-position of phenyl ring. In addition, the existence of a weak electron-donating substitution (*t*-Bu) or a strong electron-withdrawing substitution (—CF_3) at the 4-position of phenyl ring was favourable for an inhibition effect against XAC. The results indicated that quinazolinone derivatives con-

Table 3. Crystallographic data and structural refinements for compound *VIIi*

Empirical formula	$C_{24}H_{18}FN_5OS$	$D_{calc}/(g\ cm^{-3})$	1.416
Formula mass	443.49	$2\theta/^\circ$	1.55–25.00
Crystal system	Triclinic, colourless crystal	M_μ/mm^{-1}	0.193
Space group	P–1	Unique reflections	3557
a/Å	8.884(9)	Observed reflections	2331
b/Å	9.123(10)	Parameters	284
c/Å	13.739(14)	R_{int}	0.0430
$\alpha/^\circ$	87.870(15)	$R[I > 2\sigma(I)]$	0.0704
$\beta/^\circ$	73.215(13)	$wR[I > 2\sigma(I)]$	0.2671
$\gamma/^\circ$	77.362(14)	R(all data)	0.1123
V/Å^3	1039.9(19)	wR(all data)	0.2136
Z/Å^3	2	GOF on F_2	1.126
Crystal size/mm^3	0.31 × 0.26 × 0.22	$F(000)$	460

Table 4. Antibacterial activities of compounds *VIIa*–*VIIt* against XOO, XAC and tobacco bacterial wilt

Compound	Inhibition rate[a]/%					
	XOO		XAC		Tobacco bacterial wilt	
	200 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	100 μg mL^{-1}
VIIa	30.1 ± 2.5	24.0 ± 0.9	44.5 ± 2.3	23.4 ± 1.3	37.3 ± 1.0	19.1 ± 1.0
VIIb	52.7 ± 1.3	38.0 ± 2.6	85.3 ± 1.7	53.0 ± 2.9	42.5 ± 1.3	29.0 ± 2.7
VIIc	48.5 ± 1.2	26.3 ± 3.7	7.2 ± 3.4	3.0 ± 2.1	45.4 ± 2.5	25.2 ± 2.9
VIId	41.9 ± 3.6	24.4 ± 2.8	36.1 ± 1.5	7.2 ± 4.9	27.1 ± 3.0	24.5 ± 1.6
VIIe	20.2 ± 3.8	18.3 ± 4.1	22.1 ± 2.6	11.8 ± 2.7	36.5 ± 1.5	36.0 ± 3.2
VIIf	37.2 ± 6.9	33.3 ± 1.8	30.0 ± 3.0	6.1 ± 1.6	50.8 ± 1.6	34.5 ± 2.8
VIIg	99.2 ± 2.3	45.5 ± 7.4	3.2 ± 7.1	0	41.3 ± 2.8	33.0 ± 1.3
VIIh	99.8 ± 2.0	59.4 ± 7.2	13.0 ± 2.9	5.1 ± 3.6	37.2 ± 1.4	22.0 ± 2.6
VIIi	35.4 ± 2.6	10.1 ± 3.7	25.3 ± 3.8	12.0 ± 2.8	31.4 ± 2.9	29.0 ± 2.9
VIIj	44.0 ± 2.3	26.8 ± 1.6	10.9 ± 1.2	4.2 ± 4.3	39.2 ± 3.8	18.3 ± 1.2
VIIk	60.5 ± 4.6	24.3 ± 2.2	23.2 ± 2.8	10.0 ± 3.0	56.0 ± 1.1	40.0 ± 3.9
VIIl	30.1 ± 4.6	29.6 ± 3.3	14.3 ± 1.0	5.0 ± 3.7	29.4 ± 1.4	28.2 ± 2.9
VIIm	62.5 ± 3.5	32.1 ± 2.1	42.4 ± 2.0	30.3 ± 1.0	26.1 ± 2.2	25.8 ± 1.1
VIIn	53.4 ± 2.2	25.7 ± 6.5	27.2 ± 1.8	20.0 ± 2.2	14.0 ± 2.7	4.8 ± 6.6
VIIo	42.5 ± 3.1	32.2 ± 2.0	38.5 ± 1.4	11.3 ± 5.9	0	0
VIIp	45.5 ± 1.2	32.3 ± 5.1	42.2 ± 2.6	16.1 ± 1.7	10.0 ± 3.8	6.1 ± 2.7
VIIq	84.1 ± 3.6	32.4 ± 3.0	32.0 ± 2.8	9.9 ± 1.2	32.3 ± 1.8	24.2 ± 0.4
VIIr	52.1 ± 3.8	48.6 ± 1.0	45.3 ± 2.8	18.1 ± 2.3	27.1 ± 1.9	16.0 ± 3.8
VIIs	99.9 ± 1.2	87.4 ± 6.8	99.9 ± 2.8	99.9 ± 1.1	35.0 ± 2.6	15.0 ± 1.1
VIIt	25.4 ± 3.3	17.7 ± 3.5	50.1 ± 2.9	29.3 ± 1.1	38.0 ± 1.8	29.9 ± 2.4
Thiodiazole-copper[b]	35.3 ± 1.8	29.1 ± 2.0	45.1 ± 3.0	17.0 ± 2.3	50.0 ± 1.2	30.2 ± 2.1
Bismerthiazol[b]	71.9 ± 0.6	54.1 ± 1.2	99.9 ± 0.6	67.1 ± 6.8	–	–

a) Average of three replicates; *b*) the commercial agricultural bactericides, thiodiazole-copper and bismerthiazol, were used in a comparison of antibacterial activity.

Table 5. Inhibitory effect of *VIIg*, *VIIh* and *VIIs* against XOO[a]

Compound	R	Inhibition/%		Toxic regression equation	r	$EC_{50}/(\mu g\ mL^{-1})$
		200 μg mL^{-1}	100 μg mL^{-1}			
VIIg	3-Cl-Ph	99.2 ± 2.3	45.5 ± 7.4	$y = 0.9155x + 3.0944$	0.9908	120.6 ± 1.7
VIIh	4-Cl-Ph	99.8 ± 2.0	59.4 ± 7.2	$y = 1.2915x + 2.4855$	0.9916	88.5 ± 2.5
VIIs	4-CF_3-Ph	99.9 ± 1.2	87.4 ± 6.8	$y = 1.3156x + 2.7924$	0.9933	47.6 ± 0.1
Bismerthiazol[b]		71.9 ± 0.6	54.1 ± 1.2	$y = 1.4990x + 2.0520$	0.9800	92.6 ± 2.1

a) Average of three replicates; *b*) the commercial agricultural bactericide bismerthiazol was used for comparison of antibacterial activity.

Table 6. Inhibitory effect of *VIIb* and *VIIs* against XAC[a]

Compound	R	Inhibition/%		Toxic regression equation	r	EC_{50}/(μg mL^{-1})
		200 μg mL^{-1}	100 μg mL^{-1}			
VIIb	4-*t*-Bu-Ph	85.3 ± 1.7	53.0 ± 2.9	$y = 0.7844x + 3.4908$	0.9944	82.1 ± 2.4
VIIs	4-CF_3-Ph	99.9 ± 2.8	99.9 ± 1.1	$y = 1.5547x + 2.9105$	0.9924	22.1 ± 0.3
Bismerthiazol[b]		99.9 ± 0.6	67.1 ± 6.8	$y = 1.6100x + 2.1500$	0.9856	58.2 ± 2.7

a) Average of three replicates; *b*) commercial agricultural bactericide bismerthiazol was used for comparison of antibacterial activity.

Table 7. Fungicidal activities of *VIIa–VIIt* at a concentration of 50 μg mL^{-1}

Compound	Inhibition rate/%[a]					
	G. zeae	*C. mandshurica*	*P. infestans*	*P. sasakii*	*C. capsici*	*G. fructigenum*
VIIa	21.5 ± 2.7	11.5 ± 2.4	10.4 ± 0.4	28.8 ± 1.2	46.7 ± 0.4	27.3 ± 2.0
VIIb	4.2 ± 1.2	7.9 ± 1.2	16.3 ± 6.5	23.7 ± 0.4	24.3 ± 1.3	15.9 ± 1.7
VIIc	17.6 ± 1.9	8.2 ± 1.6	15.3 ± 2.3	33.1 ± 1.5	28.3 ± 0.8	29.4 ± 1.3
VIId	2.9 ± 0.7	6.9 ± 0.9	8.1 ± 1.3	4.5 ± 0.4	24.6 ± 1.4	32.3 ± 0.5
VIIe	21.8 ± 2.4	7.9 ± 0.4	23.1 ± 3.3	51.3 ± 0.1	43.6 ± 0.8	37.2 ± 0.8
VIIf	10.9 ± 1.2	15.5 ± 1.6	9.1 ± 0.8	5.7 ± 0.1	23.7 ± 0.1	41.4 ± 0.5
VIIg	17.9 ± 1.8	27.5 ± 0.4	10.7 ± 1.6	3.9 ± 0.1	14.4 ± 1.0	28.0 ± 1.0
VIIh	29.2 ± 1.6	46.4 ± 0.9	29.7 ± 2.2	65.9 ± 1.8	65.1 ± 1.3	47.9 ± 0.8
VIIi	3.8 ± 2.0	11.9 ± 1.6	11.1 ± 0.8	18.8 ± 1.9	29.7 ± 1.0	25.2 ± 1.0
VIIj	13.5 ± 2.7	10.2 ± 2.1	25.4 ± 3.6	18.8 ± 1.2	50.4 ± 1.0	31.6 ± 2.1
VIIk	8.0 ± 0.4	26.3 ± 3.2	17.3 ± 2.0	75.4 ± 1.4	40.5 ± 0.4	45.7 ± 1.7
VIIl	2.9 ± 0.7	30.7 ± 1.6	29.1 ± 0.4	15.4 ± 1.1	54.9 ± 0.1	21.3 ± 0.8
VIIm	10.9 ± 0.9	15.1 ± 1.6	10.7 ± 0.4	29.7 ± 0.6	18.4 ± 0.6	42.2 ± 0.5
VIIn	47.1 ± 2.8	18.7 ± 1.2	33.2 ± 1.6	19.1 ± 0.4	39.6 ± 1.3	27.3 ± 1.0
VIIo	14.4 ± 2.0	27.3 ± 2.4	14.9 ± 1.6	30.4 ± 3.7	25.6 ± 1.1	20.7 ± 1.6
VIIp	16.7 ± 3.5	13.2 ± 2.9	15.6 ± 2.0	36.9 ± 1.8	36.1 ± 3.0	16.7 ± 1.0
VIIq	10.3 ± 1.9	26.6 ± 0.9	7.2 ± 0.8	26.2 ± 0.1	21.3 ± 4.0	16.2 ± 2.5
VIIr	50.3 ± 1.6	39.1 ± 3.2	30.9 ± 2.5	43.4 ± 2.2	43.8 ± 2.6	43.5 ± 1.5
VIIs	26.3 ± 3.9	23.7 ± 2.0	32.9 ± 2.5	47.6 ± 2.3	56.2 ± 2.0	35.5 ± 1.0
VIIt	12.2 ± 0.9	27.9 ± 0.9	11.1 ± 0.8	39.9 ± 1.5	21.9 ± 0.8	7.3 ± 1.0
Hymexazol[b]	55.5 ± 3.9	49.6 ± 7.8	68.2 ± 2.4	51.2 ± 3.9	45.0 ± 7.8	58.2 ± 2.4

a) Average of three replicates; *b*) commercial agricultural fungicide hymexazol was used for comparison of antifungal activity.

taining 1,2,4-triazolylthioether unit exhibited a certain antibacterial activity. One possible explanation is that flexible methylene spacers among quinazolinone, 1,2,4-triazolylthioether moiety and substituted aromatic ring allowed target compounds to bind to the protein cavity of the pathogenic bacteria with lower binding energy.

Antifungal activity and structure-activity relationship

The antifungal results thus obtained were compared with those obtained from hymexazol (Table 7), a commercial fungicide with a broad-spectrum activity. The target compounds *VIIa–VIIt* against *G. zeae*, *C. mandshurica*, *P. infestans*, *P. sasakii*, *C. capsici* and *G. fructigenum* displayed inhibition rates ranging from 2.9 % to 50.3 %, 6.9 % to 46.4 %, 7.2 % to 33.2 %, 3.9 % to 75.4 %, 14.4 % to 65.1 %, 7.3 % to 47.9 %, respectively, at 50 μg mL^{-1}. The inhibition rates of 50 μg mL^{-1} of hymexazol on the above fungi were 55.5 %, 49.6 %, 68.2 %, 51.2 %, 45.0 % and 58.2 %, respectively. Notably, compound *VIIr* exhibited an activity comparable with hymexazol against *G. zeae* (50.3 % vs 55.5 % inhibition). In addition, compounds *VIIe*, *VIIh* and *VIIk* exhibited better antifungal activities against *P. sasakii*, with inhibition rates of 51.3 %, 65.9 % and 75.4 %, than that of hymexazol (51.2 % inhibition). Compounds *VIIh*, *VIIl* and *VIIs* possessed stronger antifungal activities against *C. capsici*, with inhibition rates of 65.1 %, 54.9 % and 56.2 %, than hymexazol (45.0 % inhibition). Out of all the target compounds, compound *VIIh* (R = 4-Cl-Ph) had the broadest fungicidal spectrum, with an inhibition rate of more than 45 % against four kinds of fungi. Additionally, out of the six kinds of fungi tested, the compounds evaluated displayed the best inhibitory activity against *C. capsici*, and seven of target compounds demonstrated an activity similar to or better than that of hymexazol.

The structure-activity relationship analysis indicated that the compound with 4-Cl-Ph substitution (*VIIh*) had a clear advantage over those substituted

at the 2- (*VIIf*) or 3-position (*VIIg*) against all the fungi tested. Similar phenomena were also observed for those having —F substitution on the phenyl ring, but only effective against *C. mandshurica*, *P. sasakii* and *G. fructigenum*. As far as the strong electron-withdrawing substitution (—CF_3) was concerned, the substitution at the 3-position (*VIIr*) displayed more favourable activities towards most of the fungi tested, relative to its 2- and 4-isomers (*VIIq* and *VIIs*). The position of the substitution (especially for —Cl, —F and —CF_3) on the phenyl ring had a significant effect on their antifungal activities.

Conclusions

A series of novel quinazolinone derivatives containing a 1,2,4-triazolylthioether unit were synthesised and their antimicrobial activities were preliminarily evaluated in vitro. Out of this series of compounds, compound *VIIs* exhibited the highest antibacterial activities against *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas oryzae* pv. *oryzae* with EC_{50} values of 47.6 μg mL^{-1} and 22.1 μg mL^{-1}, respectively, which was superior to that of commercial bactericide, bismerthiazol. To the best of our knowledge, this is the first example of the use of quinazolinone derivatives bearing 1,2,4-triazolylthioether moiety as potential antimicrobial agents in agricultural applications.

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