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ORIGINAL ARTICLE

Synthesis of novel quinazolin-4(3*H*)-one derivatives containing the 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine moiety as effective agricultural bactericides against the pathogen *Xanthomonas oryzae* pv. *oryzae*

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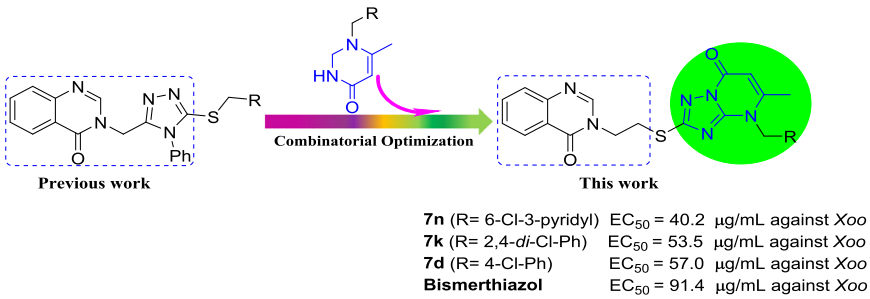
Abstract A series of novel quinazolin-4-one derivatives (**7a**–**7n**) bearing the 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine moiety were designed, synthesized and evaluated for their inhibition activities against phytopathogenic bacteria and fungi in vitro. All of the target compounds were fully characterized through ^{1}H NMR, ^{13}C NMR, HRMS and IR spectra. Among these compounds, the structure of compound **7e** was unambiguously confirmed via single-crystal X-ray diffraction analysis. The turbidimetric assays indicated that compounds **7b**, **7d**, **7g**, **7k** and **7n** exhibited much more potent inhibition activities against the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), relative to control Bismerthiazol. Moreover, antibacterial activities of compounds **7j**, **7k** and **7n** against the pathogen *Xanthomonas axonopodis* pv. *citri* (*Xac*) were comparable to that of control Bismerthiazol. As for the pathogen *Ralstonia solanacearum* (*Rs*), only compounds **7g** and **7i** demonstrated inhibition activities similar to control Thiadiazole-copper. Moreover, this class of compounds did not display inhibition activity against three fungi tested. The above findings indicated that quinazolin-4-one derivatives containing the 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine moiety have a potential as promising candidates for the development of new and more efficient agricultural bactericides.

Graphical Abstract



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Keywords Quinazolinone · 1,2,4-Triazolo[1,5-*a*]pyrimidine · Synthesis · Antibacterial activity

Introduction

The phytobacteria of *Xoo* and *Xac* are two types of Gram-negative pathogenic bacteria, which cause rice bacterial leaf blight and citrus bacterial canker, respectively, and therefore lead to huge economic losses to global agricultural produc-

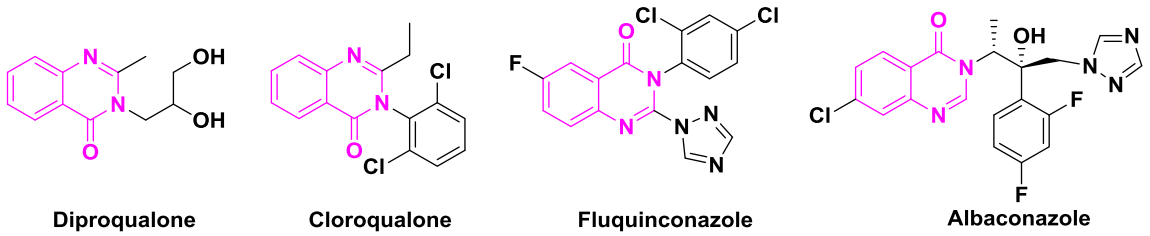


Fig. 1 Chemical structures of some commercial medicine/pesticide molecules containing the quinazolin-4-one moiety

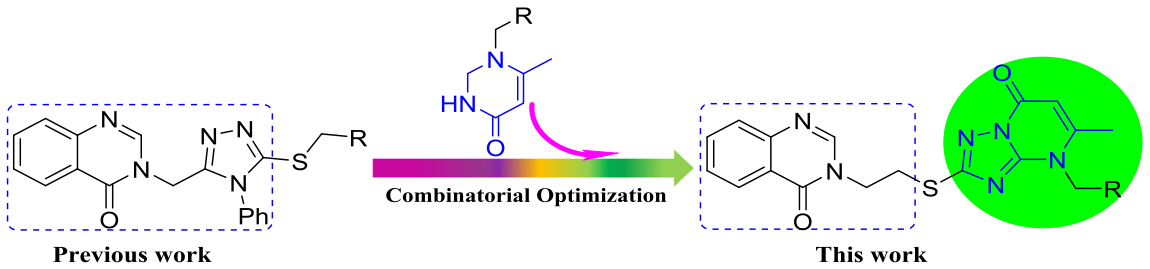


Fig. 2 Design strategy for target compounds **7a**–**7n** in this work

tion every year [1,2]. Taking the pathogen *Xoo* as an example, it invades via the vascular system and then colonizes the intercellular spaces of the parenchyma tissue [3], generally bringing about production losses by up to 50% [4]. Moreover, the bacterium *Xac* is typically spread through windblown rain and enters the host plants via stomata and/or wounds [5]. Although some agricultural bactericides are currently available for fighting against the above pathogenic phytobacteria, new and more efficient antibacterial agents are still extremely demanded, considering agents-associated toxicity and ceaseless evolution of antibiotic-resistant bacteria.

Quinazolin-4(3*H*)-one, found in more than 200 naturally occurring alkaloids [6], constitutes a significant class of compounds with diverse therapeutic and pharmacological properties such as antibacterial [7,8], antifungal [9] and antiviral activities [10]. Some commercial medicine/pesticide molecules also contain the quinazolin-4-one backbone, including the analgesic Diproqualone, the sedative Cloroqualone, the agrofungicide Fluquinconazole and the fungicide Albaconazole (Fig. 1). On the other hand, 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine derivatives also display a wide range of bioactivities, such as antiherpetic [11], antileishmanial [12], antiparasital [13], antimalarial [14] and antitumor [15] activities.

From a structural perspective, the fused heterocycle 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one can be considered as the annulation of pyrimidin-4-one moiety to 1,2,4-triazole ring. Based on all of the above considerations and our continuing interest in searching for efficient 1,2,4-triazole-quinazoline/quinazolinone hybrid derivatives as agricultural antimicrobial agents [16–19], herein a series of novel quinazolin-4(3*H*)-one derivatives (**7a**–**7n**) containing the 7-oxo-1,2,4-triazolo[1,5-*a*]- pyrimidine moiety were designed, synthesized based on the "combinatorial optimization" method [20,21] (Fig. 2) and evaluated for their inhibition activities in vitro against selected common pathogenic phytobacteria and phytofungi.

Materials and methods

All the chemicals were obtained from commercial suppliers and used without further purification (unless stated otherwise). 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 using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded 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). Multiplicity abbreviations used for the chemical shifts are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. HRMS-ESI spectra were recorded on a Thermo Scientific Q Exactive series. X-ray crystallographic data were collected on a Bruker Smart Apex CCD area detector diffractometer (Bruker, Germany) using Mo-Kα radiation. The software package SPSS 17.0 is developed by SPSS Inc., which was downloaded from http://www.126xiazai.com/fileview_715650.html.

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** [22] (3.01 g, 11.88 mmol) dissolved in DMF (20 mL) in the

presence of NaOH (1.42 g, 35.6 mmol) was stirred at room temperature for 1 h and then heated at 60 °C for 10 h. After completion of the reaction indicated by the TLC analysis, ice water (15 mL) was added into the reaction mixture and the resulting 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 formed precipitate was filtered, washed with AcOH and EtOH, and then dried to give **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.

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

A mixture of intermediate **6** (142 mg, 0.40 mmol) and an appropriate chlorinated compound (0.44 mmol) dissolved in CH_3COCH_3 (45 mL) in the presence of K_2CO_3 (83 mg, 0.6 mmol) was heated to reflux and stirred for 12−15 h. After cooling the reaction mixture to room temperature, the pure compounds were separated by flash column chromatography (petroleum ether/ethyl acetate = 3/1−3/2, v/v) to afford **7a**–**7n**.

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

White solid, mp 205 − 208 °C, yield: 53.2%. IR (KBr, ν/cm^{-1}): 1704 (C=O), 1666 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.25 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 6.9 Hz, 1H), 7.24 (d, J = 7.5 Hz, 2H), 5.96 (s, 1H), 5.36 (s, 2H), 4.35 (t, J = 6.3 Hz, 2H), 3.58 (t, J = 6.3 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.2, 153.7, 152.7, 151.6, 148.0, 147.8, 135.3, 134.3, 128.9, 127.8, 127.0, 126.9, 126.4, 126.0, 121.4, 100.9, 50.2, 45.6, 29.7, 18.2; HRMS (ESI) calcd for $[M + H]^+C_{23}H_{21}N_6O_2S$: 445.1441, found: 445.1434.

*3-(2-((5-Methyl-4-(4-nitrobenzyl)-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (**7b**)*

White solid, mp 178 − 181 °C, yield: 41.9%. IR (KBr, ν/cm^{-1}): 1713 (C=O), 1674 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.21 (s, 1H), 8.20 (d, J = 9.2 Hz, 2H), 8.13 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.52 (t, J = 9.2 Hz, 3H), 6.00 (s, 1H), 5.51 (s, 2H), 4.33 (t, J = 6.3Hz, 2H), 3.56 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.1, 160.2, 153.9, 152.6, 151.5, 148.1, 147.8, 147.1, 143.0, 134.4, 127.8, 127.1, 127.0, 126.1, 124.1, 121.4, 101.2, 49.8, 45.7, 29.8, 18.3; HRMS (ESI) calcd for $[M + H]^+C_{23}H_{20}N_7O_4S$: 490.1292, found: 490.1286.

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

White solid, mp 198 − 201 °C, yield: 42.2%. IR (KBr, ν/cm^{-1}): 1704 (C=O), 1672 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.20 (s, 1H), 8.14 (d, J = 6.9 Hz, 1H), 7.80 (t, J = 6.9 Hz, 1H), 7.60–7.52 (m, 3H), 7.35 (t, J = 6.9 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 6.93 (d, J = 7.5 Hz, 1H), 6.04 (s, 1H), 5.33 (s, 2H), 4.33 (t, J = 6.3 Hz, 2H), 3.54 (t, J = 6.3 Hz, 2H), 2.24 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.1, 153.8, 152.4, 151.4, 147.9, 147.8, 134.3, 132.3, 131.1, 129.7, 129.5, 127.9, 127.0, 126.9, 126.6, 126.0, 121.4, 101.2, 48.1, 45.7, 29.7, 17.9; HRMS (ESI) calcd for $[M + H]^+C_{23}H_{20}N_6O_2SCl$: 479.1052, found: 479.1046.

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

White solid, mp 204 − 207 °C, yield: 35.7%. IR (KBr, ν/cm^{-1}): 1714 (C=O), 1674 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.25 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.41 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 5.96 (s, 1H), 5.35 (s, 2H), 4.35 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.1, 160.3, 153.8, 152.7, 151.6, 148.1, 147.8, 134.4, 132.5, 128.9, 128.8, 128.6,

127.1, 127.0, 126.1, 121.4, 101.1, 49.6, 45.7, 29.8, 18.2; HRMS (ESI) calcd for $[M+H]^+C_{23}H_{20}N_6O_2SCl$: 479.1052, found: 479.1047.

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

White solid, mp 196 − 199 °C, yield: 45.8%. IR (KBr, ν/cm^{-1}): 1710 (C=O), 1674 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.22 (s, 1H), 8.13 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 6.9 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.27 (t, J = 8.6 Hz, 1H), 7.16–7.10 (m, 2H), 6.00 (s, 1H), 5.38 (s, 2H), 4.34 (t, J = 6.3 Hz, 2H), 3.56 (t, J = 6.3 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.4, 160.2, 153.7, 152.5, 151.5, 148.0, 147.8, 134.3, 130.1, 130.0, 128.0, 127.0, 126.9, 126.0, 125.1, 125.0, 122.2, 122.1, 121.4, 115.7, 115.5, 101.0, 45.6, 44.7, 29.7, 18.0; HRMS (ESI) calcd for $[M+H]^+C_{23}H_{20}FN_6O_2S$: 463.1347, found: 463.1343.

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

White solid, mp 193 − 195 °C, yield: 42.3%. IR (KBr, ν/cm^{-1}): 1712 (C=O), 1647 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.25 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 7.79 (t, J = 7.0 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.53 (t, J = 6.9 Hz, 1H), 7.41 (s, 1H), 7.38-7.37 (m, 2H), 7.19 (t, J = 5.8 Hz, 1H), 5.97 (s, 1H), 5.37 (s, 2H), 4.35 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 160.9, 160.2, 153.8, 152.7, 151.4, 148.0, 147.8, 137.9, 134.3, 133.6, 130.8, 127.9, 127.1, 127.0, 126.5, 126.0, 125.1, 121.4, 101.1, 49.6, 45.6, 29.8, 18.2; HRMS (ESI) calcd for $[M+H]^+C_{23}H_{20}FN_6O_2S$: 463.1347, found: 463.1354.

*3-(2-((4-(4-Fluorobenzyl)-5-methyl-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (***7g***)*

White solid, mp 204 − 207 °C, yield: 51.8%. IR (KBr, ν/cm^{-1}): 1703 (C=O), 1678 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.26 (s, 1H), 8.13 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.32 (d, J = 4.1 Hz, 2H), 7.18 (t, J = 8.6 Hz, 2H), 5.95 (s, 1H), 5.34 (s, 2H), 4.36 (t, J = 6.3 Hz, 2H), 3.58 (t, J = 6.3 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 162.6, 161.0, 160.2, 153.7, 152.6, 151.5, 148.0, 147.8, 134.3, 131.6, 131.5, 128.9, 128.8, 127.0, 126.9, 126.0, 121.4, 115.8, 115.6, 101.0, 49.5, 45.7, 29.8, 18.2; HRMS (ESI) calcd for $[M+H]^+C_{23}H_{20}FN_6O_2S$: 463.1347, found: 463.1342.

*3-(2-((5-Methyl-4-(3-methylbenzyl)-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (***7h***)*

White solid, mp 183 − 186 °C, yield: 54.6%. IR (KBr, ν/cm^{-1}): 1709 (C=O), 1671 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.24 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.05 (s, 1H), 6.99 (d, J = 7.5 Hz, 1H), 5.95 (s, 1H), 5.31 (s, 2H), 4.36 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.26 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.2, 153.8, 152.7, 151.7, 148.0, 147.8, 138.3, 135.2, 134.3, 128.8, 128.5, 127.1, 127.0, 126.8, 126.0, 123.4, 121.4, 100.9, 50.2, 45.7, 29.7, 21.0, 18.2; HRMS (ESI) calcd for $[M+H]^+C_{24}H_{23}N_6O_2S$: 459.1598, found: 459.1592.

*3-(2-((5-Methyl-4-(4-methylbenzyl)-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (***7i***)*

White solid, mp 195 − 197 °C, yield: 56.4%. IR (KBr, ν/cm^{-1}): 1714 (C=O), 1673 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.25 (s, 1H), 8.13 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 5.95 (s, 1H), 5.30 (s, 2H), 4.36 (t, J = 6.3 Hz, 2H), 3.58 (t, J = 6.3 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.2, 153.7, 152.7, 151.7, 148.0, 147.8, 137.1, 134.3, 132.3, 129.5, 127.0, 126.9, 126.5, 126.0, 121.4, 100.8, 50.0, 45.7, 29.7, 20.7, 18.2; HRMS (ESI) calcd for $[M+H]^+C_{24}H_{23}N_6O_2S$: 459.1598, found: 459.1592.

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

White solid, mp 171 − 174 °C, yield: 62.8%. IR (KBr, ν/cm^{-1}): 1705 (C=O), 1677 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.24 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.83 (s, 1H), 6.73 (d, J = 7.5 Hz, 1H), 5.96 (s, 1H), 5.32 (s, 2H), 4.36 (t, J = 6.3 Hz, 2H), 3.72 (s, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.1, 160.2, 159.6, 153.8, 152.7, 151.7, 148.1, 147.8, 136.9, 134.4, 130.2, 127.1, 127.0, 126.1, 121.4, 118.3, 113.1, 112.4, 100.9, 55.1, 50.1, 45.7, 29.8, 18.2;

HRMS (ESI) calcd for $[M + H]^+C_{24}H_{23}N_6O_3S$: 475.1547, found: 475.1542.

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

White solid, mp 118−121 °C, yield: 44.5%. IR (KBr, ν/cm^{-1}): 1709 (C=O), 1673 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.21 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.81–7.78 (m, 1H), 7.73 (d, J = 2.3 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.33–7.31 (m, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.04 (s, 1H), 5.30 (s, 2H), 4.32 (t, J = 6.3 Hz, 2H), 3.54 (t, J = 6.3 Hz, 2H), 2.18 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.0, 160.1, 153.8, 152.4, 151.3, 147.9, 147.8, 134.3, 133.2, 132.2, 131.6, 129.2, 128.3, 128.0, 127.1, 127.0, 126.0, 121.4, 101.3, 47.8, 45.7, 29.7, 17.9; HRMS (ESI) calcd for $[M + H]^+C_{23}H_{19}N_6O_2SCl_2$: 513.0662, found: 513.0658.

*3-(2-((4-(2,6-Dichlorobenzyl)-5-methyl-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (***7l***)*

White solid, mp 129−131 °C, yield: 54.5%; IR (KBr, ν/cm^{-1}): 1711 (C=O), 1670 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.15 (s, 1H), 8.13 (s, 1H), 7.80 (t, J = 7.5 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.37 (t, J = 8.1 Hz, 1H), 6.01 (s, 1H), 5.55 (s, 2H), 4.23 (t, J = 6.3 Hz, 2H), 3.48 (t, J = 6.3 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 160.9, 160.2, 153.7, 152.6, 151.9, 148.0, 147.8, 135.0, 134.3, 130.7, 130.1, 129.3, 127.1, 127.0, 126.0, 121.4, 100.9, 47.4, 45.6, 29.9, 19.0; HRMS (ESI) calcd for $[M + H]^+C_{23}H_{19}N_6O_2SCl_2$: 513.0662, found: 513.0657.

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

White solid, mp 115 − 117 °C, yield: 39.5%. IR (KBr, ν/cm^{-1}): 1697 (C=O), 1640 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.28 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.83 (s, 1H), 7.79 (t, J = 6.9 Hz, 1H), 7.60 (d, J = 8.1 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 5.95 (s, 1H), 5.48 (s, 2H), 4.39 (t, J = 6.3 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 161.1, 160.3, 153.7, 151.8, 151.5, 151.1, 148.2, 147.9, 141.8, 134.4, 134.3, 127.1, 127.0, 126.1, 121.4, 101.2, 45.7, 43.2, 30.0, 18.3; HRMS (ESI) calcd for$[M + H]^+C_{20}H_{17}N_7O_2S_2Cl$: 486.0568, found: 486.0561.

*3-(2-((4-((6-Chloropyridin-3-yl)methyl)-5-methyl-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)ethyl)quinazolin-4(3H)-one (***7n***)*

White solid, mp 158−161 °C, yield: 55.1%. IR (KBr, ν/cm^{-1}): 1711 (C=O), 1675 (C=O);^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ: 8.42 (s, 1H), 8.25 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 7.79 (t, J = 7.5 Hz, 2H), 7.60 (d, J = 8.6 Hz, 1H), 7.54–7.48 (m, 2H), 5.96 (s, 1H), 5.39 (s, 2H), 4.35 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.3 Hz, 2H), 2.31 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm) δ: 160.9, 160.2, 153.8, 152.6, 151.3, 149.8, 148.7, 148.1, 147.8, 138.6, 134.3, 130.7, 127.1, 127.0, 126.0, 124.4, 121.4, 101.2, 47.4, 45.6, 29.8, 18.3; HRMS (ESI) calcd for $[M + H]^+C_{22}H_{19}N_7O_2SCl$: 480.1004, found: 480.1000.

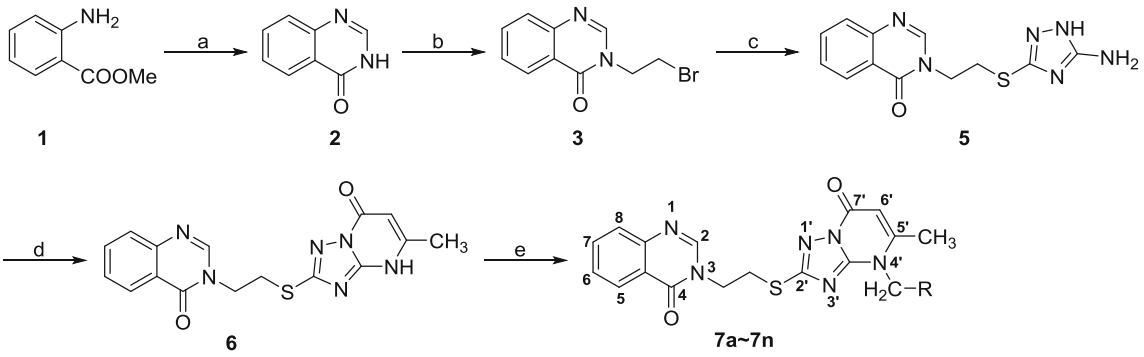
Antibacterial bioassay

Antibacterial activities of target compounds **7a**–**7n** were determined against three phytopathogenic bacteria (*Xoo*, *Xac* and *Rs*) based on a turbidimetric method [23–25]. Tested compounds were prepared at two concentrations of 200 and 100 μg/mL. Pure DMSO in sterile distilled water was used as blank control, and commercially available bactericide bismerthiazol (BMT) and thiadiazole-copper (TDC) were used as positive controls. 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 three days. The bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}), given by $\text{turbidity}_{\text{corrected value}} = OD_{\text{bacterium}} - OD_{\text{no bacterium}}$, $I = (C_{\text{tur}} - T_{\text{tur}})/C_{\text{tur}} \times 100\%$. 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.

Finally, antibacterial activities of target compounds **7a**–**7n** (against the *Xoo*) and **7b**, **7d**, **7j**, **7k** and **7n** (against the *Xac*) were determined at five different concentrations (200, 100, 50, 25 and 12.5 μg/mL) to obtain their EC_{50} values, which were determined statistically by Probit analysis with the software package SPSS 17.0 [16,24].

Antifungal bioassay

Mycelial growth rate method [26,27] was utilized to assess the antifungal activities of target compounds **7a**–**7n** against three phytopathogenic fungi (*G. zeae*, *V. dahliae* and *S.*



7a: R = Ph; **7b**: R = 4-NO_2-Ph; **7c**: R = 2-Cl-Ph; **7d**: R = 4-Cl-Ph; **7e**: R = 2-F-Ph;
7f: R = 3-F-Ph; **7g**: R = 4-F-Ph; **7h**: R = 3-CH_3-Ph; **7i**: R = 4-CH_3-Ph; **7j**: R = 3-OCH_3-Ph;
7k: R = 2,4-*di*-Cl-Ph; **7l**: R = 2,6-*di*-Cl-Ph; **7m**: R = 2-Cl-5-thiazolyl; **7n**: R = 6-Cl-3-pyridyl.

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) RCH_2Cl/K_2CO_3/CH_3COCH_3/reflux/36~63%.

Scheme 1 Synthesis of target compounds **7a**–**7n**

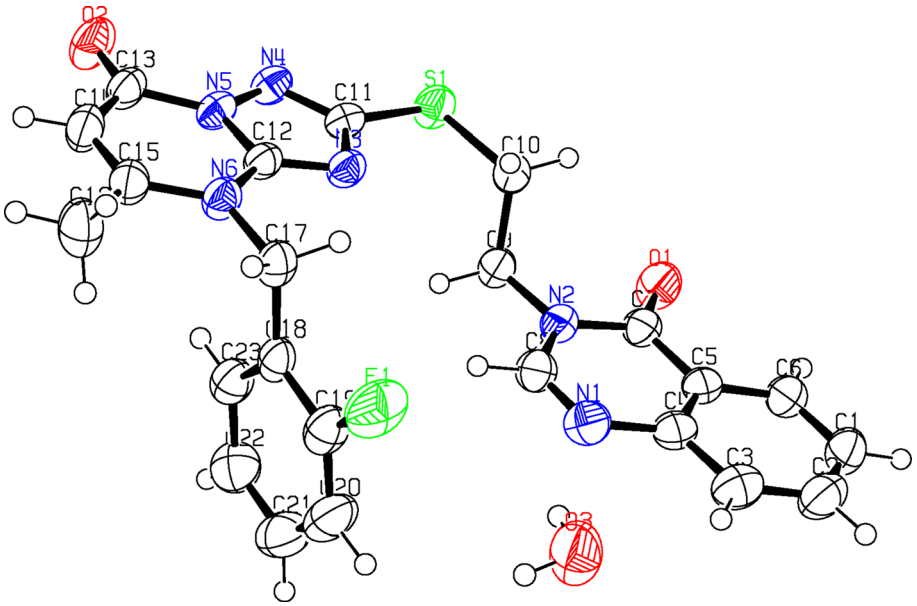


Fig. 3 Crystal structure of the adduct **7e**·H_2O

sclerotiorum). DMSO solution of the tested compound was added into sterilized Petri dishes, which contained about 10 mL molten potato dextrose agar (PDA). Subsequently, a 4-mm-diameter 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 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 tested compound-treated PDA.

Results and discussion

Synthesis

The synthetic route of target compounds **7a**–**7n** is summarized in Scheme 1. Briefly, quinazolin-4-one **2** [16] was reacted with 1,2-dibromoethane in DMF-NaH to give 3-(2-bromoethyl)quinazolinone **3** [22], which was then subjected to a thioetherification reaction with 5-amino-1*H*-1,2,4-

Table 1 Antibacterial activities of target compounds **7a**–**7n** and intermediate **6** against three phytopathogenic bacteria *Xoo*, *Xac* and *Rs*

Compd.	Inhibition rate (%)[a]					
	Xoo		*Xac*		*Rs*	
	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL	200 μg/mL	100 μg/mL
6	51.9 ± 1.0	39.0 ± 1.3	40.1 ± 4.1	22.5 ± 1.6	28.7 ± 2.7	18.7 ± 3.9
7a	74.8 ± 1.9	61.9 ± 4.6	61.0 ± 2.1	43.7 ± 4.1	43.6 ± 2.7	34.8 ± 3.6
7b	88.4 ± 1.8	63.2 ± 3.0	60.8 ± 3.3	48.0 ± 1.9	30.7 ± 2.4	15.0 ± 3.4
7c	65.5 ± 1.3	28.2 ± 4.7	52.3 ± 3.6	33.3 ± 3.9	40.0 ± 3.0	32.0 ± 4.4
7d	91.6 ± 2.0	52.3 ± 2.5	65.7 ± 3.1	43.3 ± 2.8	35.0 ± 1.9	27.6 ± 2.9
7e	90.3 ± 2.2	45.2 ± 3.5	61.1 ± 3.5	44.5 ± 2.5	33.4 ± 5.0	18.1 ± 3.7
7f	46.6 ± 3.1	37.0 ± 1.1	56.8 ± 2.7	40.4 ± 4.9	33.6 ± 2.6	26.6 ± 2.2
7g	66.7 ± 1.7	57.1 ± 2.9	68.7 ± 1.8	51.3 ± 1.8	48.6 ± 3.1	34.1 ± 4.8
7h	76.2 ± 2.7	42.2 ± 3.5	62.3 ± 3.9	48.5 ± 2.4	19.1 ± 3.5	0
7i	65.2 ± 2.4	47.0 ± 3.9	54.0 ± 2.5	39.4 ± 4.6	51.0 ± 2.1	33.4 ± 2.5
7j	76.1 ± 2.9	60.2 ± 1.7	70.6 ± 2.4	55.7 ± 3.0	45.9 ± 3.5	20.7 ± 3.2
7k	83.3 ± 3.9	81.6 ± 3.4	70.7 ± 1.6	61.2 ± 2.1	37.3 ± 2.5	16.7 ± 3.1
7l	72.9 ± 1.7	43.0 ± 2.3	62.0 ± 1.5	37.6 ± 1.1	30.8 ± 3.9	23.7 ± 2.8
7m	70.6 ± 1.5	51.1 ± 2.7	56.7 ± 3.6	40.9 ± 4.4	46.3 ± 4.2	28.6 ± 1.5
7n	99.8 ± 1.2	95.4 ± 1.4	70.0 ± 4.3	50.1 ± 2.4	33.1 ± 5.9	27.7 ± 1.6
BMT[b]	76.1 ± 2.3	50.6 ± 1.2	95.3 ± 2.9	64.3 ± 2.4	NT[c]	NT[c]
TDC[b]	NT[c]	NT[c]	NT[c]	NT[c]	54.8 ± 1.6	30.4 ± 1.7

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

triazole-3-thiol in DMF-NaOH to generate the intermediate **5** in 78% yield. After a cyclization reaction between **5** and ethyl acetoacetate in refluxing HOAc, the key intermediate **6** was obtained in 72% yield. Finally, intermediate **6** and a substituted benzyl chloride were reacted in $CH_3COCH_3-K_2CO_3$ to afford target compounds **7a**–**7n**. All of the target compounds were fully characterized through ^{1}H NMR, ^{13}C NMR, HRMS and IR spectra. It should be noted that the alkylation of **6** occurred at the 4-position nitrogen atom of the 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one heterocycle (instead of 7-position oxygen atom), which was clearly confirmed by the following crystal structure.

Spectral and single-crystal X-ray diffraction analysis

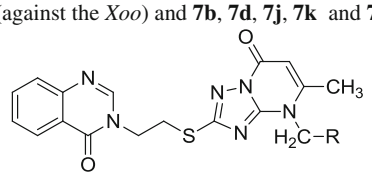
Taking compound **7e** as a representative example, the strong signals at 1710 and 1674 cm^{-1} in the IR spectrum were due to the presence of two C=O functionalities. In the ^{1}H NMR spectrum, the three singlets at 5.38, 6.00 and 8.22 ppm were assigned to the protons at the benzylic CH_2, 6-CH of 1,2,4-triazolo[1,5-*a*]pyrimidin-7-one and 2-CH of quinazolinone, respectively. Additionally, two signals at 161.0 and 160.4 ppm in the ^{13}C NMR spectrum correspond to two C=O functionalities in **7e**. Finally, high-resolution mass spectrum (HRMS) of compound **7e** displayed an intense signal at m/z = 463.1343, corresponding to the protonated pseudo-molecular ion of $[M + H]^+$.

Single crystal of compound **7e**·H_2O suitable for X-ray diffraction analysis was obtained (Fig. 3) by slow evaporation of a CH_2Cl_2-EtOH (1/2, v/v) solution of **7e** at room temperature. Crystal data for **7e**·H_2O are as follows: yellow crystal, $C_{23}H_{21}FN_6O_3S$, M_r = 480.52, triclinic, space group $P-1$; a = 9.511(11), b = 10.033(11), c = 11.726(13) (Å); α = 82.776(12), β = 88.549(12), γ = 86.401(12), V = 1108(2)Å^3, T = 296K, Z = 2, Dc = 1.441g/cm^3, $F(000)$ = 500, reflections collected/independent reflections = 3879/3585, goodness of fit on F^2 = 1.043, fine, $R1$ = 0.0317, $wR2$ = 0.0956. Crystallographic data of compound **7e** have been deposited in the Cambridge Crystallographic Data Center (CCDC 1546024).

Antibacterial activity

A turbidimetric method [23–25] was conducted to assess the antibacterial activities of compounds **7a**–**7n** against three pathogenic phytobacteria *Xoo*, *Xac* and *Rs* in vitro. Moreover, the commercial bactericides bismerthiazol (BMT) and thiadiazole-copper (TDC) were employed as control agents. As shown in Table 1, more than half of the target

Table 2 EC_{50} values of compounds **7a–7n** (against the *Xoo*) and **7b**, **7d**, **7j**, **7k** and **7n** (against the *Xac*)



Compd.	*R*	Tested bacterium	EC_{50} (μg/mL)	Toxic regression equation	*R*
7a	Ph	*Xoo*	121.4 ± 3.3	$y = 1.9663x + 0.9018$	0.9863
7b	4-NO_2-Ph	*Xoo*	72.1 ± 3.1	$y = 1.7979x + 1.6929$	0.9945
7c	2-Cl-Ph	*Xoo*	83.6 ± 1.6	$y = 1.7704x + 1.5965$	0.9636
7d	4-Cl-Ph	*Xoo*	57.0 ± 2.2	$y = 1.6968x + 2.0209$	0.9774
7e	2-F-Ph	*Xoo*	81.7 ± 1.8	$y = 1.6537x + 1.8377$	0.9629
7f	3-F-Ph	*Xoo*	95.1 ± 1.4	$y = 1.7652x + 1.5080$	0.9978
7g	4-F-Ph	*Xoo*	67.8 ± 2.7	$y = 1.7771x + 1.7460$	0.9968
7h	3-CH_3-Ph	*Xoo*	113.9 ± 1.1	$y = 1.5285x + 1.8565$	0.9702
7i	4-CH_3-Ph	*Xoo*	130.8 ± 1.9	$y = 1.6085x + 1.5953$	0.9904
7j	3-OCH_3-Ph	*Xoo*	95.1 ± 2.7	$y = 1.5016x + 2.0297$	0.9971
7k	2,4-*di*-Cl-Ph	*Xoo*	53.5 ± 2.9	$y = 2.3827x + 0.8818$	0.9743
7l	2,6-*di*-Cl-Ph	*Xoo*	109.3 ± 2.3	$y = 1.8680x + 1.1917$	0.9938
7m	2-Cl-5-thiazolyl	*Xoo*	104.4 ± 4.7	$y = 1.8280x + 1.3096$	0.9944
7n	6-Cl-3-pyridyl	*Xoo*	40.2 ± 2.4	$y = 1.7714x + 2.1592$	0.9949
BMT[a]	–	*Xoo*	91.4 ± 3.1	$y = 1.5839x + 1.8942$	0.9847
7b	4-NO_2-Ph	*Xac*	77.4 ± 2.9	$y = 1.5658x + 2.0425$	0.9887
7d	4-Cl-Ph	*Xac*	81.8 ± 2.0	$y = 1.7040x + 1.7410$	0.9831
7j	3-OCH_3-Ph	*Xac*	56.9 ± 2.3	$y = 1.7055x + 2.0070$	0.9705
7k	2,4-*di*-Cl-Ph	*Xac*	54.6 ± 1.5	$y = 1.5129x + 2.3720$	0.9886
7n	6-Cl-3-pyridyl	*Xac*	67.8 ± 3.7	$y = 1.7085x + 1.8715$	0.9771
BMT[a]	–	*Xac*	60.5 ± 3.5	$y = 2.1593x + 1.1529$	0.9744

[a] The commercial agricultural bactericide bismerthiazol (BMT) was used as control agent

compounds were found to have comparable or even better inhibition activities against the pathogen *Xoo* at 200 and 100 μg/mL, relative to control BMT. Additionally, compounds **7j** and **7k** showed appreciable antibacterial activities against the bacterium *Xac* at 100 μg/mL, similar to that of control BMT. In stark contrast to the phytobacteria *Xoo* and *Xac*, almost all the target compounds did not demonstrate noticeable inhibitory activity toward the pathogen *Rs*, except for compounds **7g** and **7i**. Lastly, compared with intermediate **6**, most of the target compounds possessed remarkably improved antibacterial activities against the pathogenic bacteria *Xoo* and *Xac*, which proved the necessity of the introduction of substituted benzyl group into the target molecules.

Encouraged by the above experimental results, the EC_{50} (half-maximal effective concentration) values of compounds **7a–7n** (against the *Xoo*) and **7b**, **7d**, **7j**, **7k** and **7n** (against the *Xac*) were further determined using the serial dilution method (200, 100, 50, 25 and 12.5 μg/mL). As displayed in Table 2, a vast majority of target compounds exhibited comparable or much better EC_{50} values relative to control BMT. On the whole, the presence of electron-withdrawing substitutions helped to enhance their antibacterial activities against the *Xoo*, such as seen in compounds **7b** (4-NO_2-Ph), **7d** (4-Cl-Ph), **7g** (4-F-Ph) and **7k** (2,4-*di*-Cl-Ph) with EC_{50} values of 72.1, 57.0, 67.8 and 53.5 μg/mL, respectively, compared to control BMT (91.4 μg/mL). Notably, compound **7n** bearing the heterocyclic 6-Cl-3-pyridyl group displayed the strongest inhibition activity (EC_{50} = 40.2 μg/mL) among this class of compounds, which may be due to the extra contribution from hydrogen-bonding interaction between the pyridine nitrogen atom and some specific proteins within the *Xoo*. Furthermore, the position of electron-withdrawing groups on the benzene ring also produced a remarkable effect on the inhibition activity, such as compounds **7d** versus **7c**, **7g** versus **7f** and **7k** versus **7l**. In other words, the halogen substitution at the *para* position of the benzene ring exhibited better activity than their *ortho*- and *meta*-position counterparts, implying that steric hindrance could result in reduced antibacterial activity. As for the pathogen *Xac*, com-

Table 3 Antifungal activities of target compounds **7a**–**7n** at 50 μg/mL

Compd.	Inhibition rate (%)[a]		
	G. zeae	*V. dahliae*	*S. sclerotiorum*
7a	12.3 ± 1.6	10.9 ± 1.8	0
7b	0	0	11.7 ± 2.5
7c	0	0	0
7d	13.2 ± 1.3	21.1 ± 3.4	12.2 ± 1.2
7e	0	22.7 ± 1.4	10.7 ± 2.2
7f	15.0 ± 1.2	0	0
7g	12.7 ± 1.7	14.5 ± 3.5	15.1 ± 1.8
7h	0	0	0
7i	11.1 ± 2.8	22.1 ± 2.7	11.8 ± 3.3
7j	0	19.1 ± 2.1	0
7k	0	0	11.1 ± 3.1
7l	12.8 ± 1.8	15.5 ± 1.9	14.1 ± 1.4
7m	14.1 ± 1.6	18.5 ± 3.8	35.9 ± 1.7
7n	25.0 ± 3.1	26.8 ± 2.1	0
Hymexazol[b]	49.8 ± 2.4	86.1 ± 1.9	87.8 ± 3.1

[a] The average of three trials
[b] The commercial agricultural fungicide (hymexazol) was used as control agent

pounds **7j**, **7k** and **7n** had EC_{50} values of 56.9, 54.6 and 67.8 μg/mL, respectively, which were similar to control BMT (60.5 μg/mL).

Antifungal activity

Finally, antifungal activities of compounds **7a**–**7n** against three pathogenic phytofungi (*Gibberella zeae, Verticillium dahliae* and *Sclerotinia sclerotiorum)* were also assessed via the mycelial growth rate method [26,27]. Unfortunately, all of the target compounds did not show any noticeable inhibition activity against the above fungi at 50 μg/mL (Table 3).

Conclusion

In summary, a series of novel quinazolin-4-one derivatives containing a 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine moiety were synthesized, and the structure of compound **7e** was further determined via single-crystal X-ray crystallography. The obtained results indicate that some of the target compounds possess far more potent antibacterial activities against the pathogenic phytobacterium *Xoo*, relative to commercial bactericide bismerthiazol. The above findings demonstrate that quinazolin-4(3*H*)-one derivatives bearing a 7-oxo-1,2,4-triazolo[1,5-*a*]pyrimidine moiety are promising candidates for the development of new agrobactericides against the bacterium *Xoo*.

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