Antibacterial and Antifungal Activities of 2-(substituted ether)-5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole Derivatives

Teng-Teng Zhang,[†] Pei-Yi Wang,*[†] Jian Zhou, Wu-Bin Shao, He-Shu Fang, Xiang Zhou, and Zhi-Bing Wu*

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for R&D of Fine Chemicals of Guizhou University, Guiyang, Huaxi District, 550025, China

*E-mail: pywang888@126.com; wzb1171@163.com

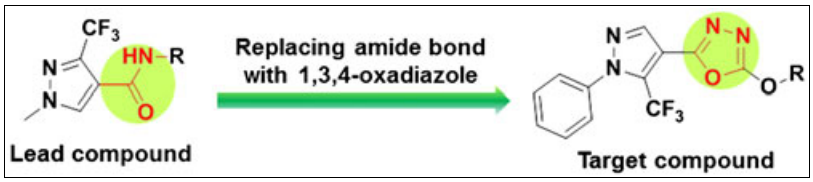
[†]These two authors contribute equally to this work.

Additional Supporting Information may be found in the online version of this article.

Received August 14, 2016

DOI 10.1002/jhet.2820

Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



By replacing the amide bond into 1,3,4-oxadiazole moiety, a series of 1-phenyl-5-(trifluoromethyl)-1H-pyrazole derivatives bearing 1,3,4-oxadiazole were synthesized and evaluated their antibacterial and antifungal activity. The bioassay results revealed that compounds 7a and 7b showed the strongest antibacterial activity toward pathogen Xanthomonas oryzae pv. oryzae with the EC50 values of 15.0 and 6.4 μg/mL, respectively; compound 6a exhibited comprehensive antifungal activity toward six kinds of fungi; compound 6f could selectively inhibit the growth of Sclertinia sclerotiorum and Rhizoctonia solani with the inhibition rates of 82.5 and 80.3% at the concentrate of 100 μg/mL, respectively; compound 7b exerted good antifungal activity toward Fusarium oxysporum, Cytospora mandshurica, and Rhizoctonia solani with the inhibition rates of 70.8, 69.5, and 71.5%, respectively. The results suggested that this kind of compounds could be further studied as promising antimicrobial agents.

J. Heterocyclic Chem., **00,** 00 (2017).

INTRODUCTION

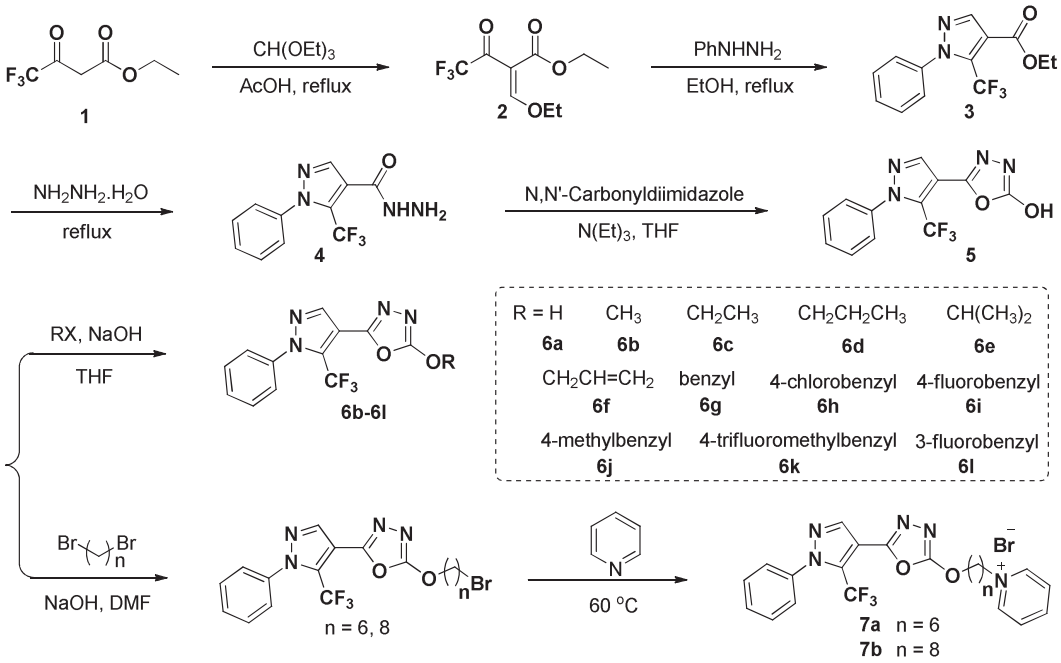
Compounds bearing the pyrazole moiety have exposed powerful applications in the development of pharmaceuticals and agrochemicals for their extensive bioactivities, such as antitumor, anti-inflammatory, insecticidal, herbicidal, antifungal, and antibacterial activities [1–16]. In the past decades, a series of pyrazole derivatives had been commercialized, including pyazosulfuron-ethyl (herbicide), [sedaxane, fluxapyroxad, penflufen, pyrametostrobin, fenpyrazamine, pyraclostrobin, rabenzazole, penthiopyrad, furametpyr, bixafen] (fungicides), and chlorantraniliprole (insecticide). Because pyrazole compounds play a crucial role in the development of pesticides, considerable works, and efforts based on this skeleton are still going on [17–20]. For example, Fang and co-workers reported a series of pyrazole oxime derivatives containing a 2-chloro-5-thiazolyl moiety exhibiting good antifungal activities toward *Alternaria solani* and *Cercosporaa rachidicola* [21]. Padmavathi and co-workers had evaluated the antifungal activity of a series of thiazolyl pyrazoles, and found that nitro-substituted thiazolyl pyrazole exerted significant antifungal activity toward *Aspergillus niger* [22]. Obviously, compounds containing pyrazole groups can be explored as the promising and preferred agents in the development of novel high-efficient pesticide.

In our previous work, we have demonstrated 3-(or 5-) trifluoromethyl-1*H*-pyrazole-4-carboxamide derivatives exhibited antifungal and antibacterial activities toward pathogenic bacteria and fungi [23,24]. Herein, the amide bond was replaced by 1,3,4-oxadiazole, which was verified as one of the key functional substructures in the exploration and construction of new agrochemicals [25–28]. Meanwhile, pyridinium scaffolds can modulate the physical properties of a molecule, and bioactivities are often enhanced by introducing the pyridinium moieties into target compounds [29,30]. Therefore, a series of 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole derivatives bearing 1,3,4-oxadiazole (or both 1,3,4-oxadiazole and pyridinium groups) were constructed and evaluated their antibacterial and antifungal activities.

RESULTS AND DISCUSSION

Chemistry. The synthesis and structures of target compounds (**6a–6l**, **7a–7b**) are shown in Scheme 1. Briefly,

Scheme 1. Synthetic route of the target compounds (**6a–6l**, **7a**, **7b**).



the crucial intermediate 5-(1-phenyl-5-(trifluoromethyl) -1*H*-pyrazol-4-yl)-1,3,4-oxadiazol-2-ol (**5**) was obtained by treating the starting material ethyltrifluoroacetoacetate **1** with four steps including condensation, cyclocondensation, hydrazidation, and cyclization [28]. A subsequent reaction with halogenated reagents provided the target molecules (**6b–6l**). While compounds **7a** and **7b** were synthesized via incubating the bromide-tailored intermediates in pyridine at 60°C [29,30]. All the structures were characterized by IR, ^{1}H NMR (Figure S1–S14, supplementary data), ^{13}C NMR, MS, and elemental analysis. In the ^{1}H NMR spectra, the singlet peak at 8.17–8.35 ppm is the proton signal for the pyrazole ring of compounds (**6a–6 l**); while the chemical shift of the pyrazole proton of compounds **7a** and **7b** appears at 7.98 and 7.93 ppm, respectively.

Bioactivities. ***Antibacterial activity.*** Turbidmeter test [31,32] was employed to evaluate their antibacterial activity against pathogen *Xanthomonas oryzae pv. oryzae* (*Xoo*), which was regarded as one of the most serious threats to rice and could result in huge economic losses in rice-growing countries [33–35]. As a comparison of the bioactivity, the commercial agricultural antibacterial agents bismerthiazol (**BT**) and thiodiazole copper (**TC**) were exploited. The preliminary bioassays indicated that most of the target compounds showed certain inhibition bioactivity against *Xoo* in the dosage of 200 or 100 μg/mL (Table 1). Among them, compound **6a** exhibited good antibacterial activity with the inhibition rate of 72.0% at the concentrate of 200 μg/mL, which was comparable with that of **BT** (72.1%); compounds **7a** and **7b** exerted the strongest antibacterial effect toward pathogen *Xoo* with the inhibition rate up to 100% even at the concentrate of 100 μg/mL. Further studies revealed that EC_{50} values of compounds 7**a** and **7b** against *Xoo* were 15.0 and 6.4 μg/mL (Table 2), respectively, which were lower than that of the control agents **BT** (92.6 μg/mL) and **TC** (121.8 μg/mL), suggesting that the two compounds could be further studied as promising antibacterial agents.

The preliminary structure–activity relationship (SAR) was illuminated in accordance with the antibacterial effect against *Xoo*. It was worth noting that the substituents on 2-position of 1,3,4-oxadiazole showed a significant impact on the antibacterial activity. For compounds **6a–6l**, the bioactivity was decreased when a sterically hindered group was introduced on the hydroxyl in comparison of that of compound **6a** (R = H, 72.0%, 200 μg/mL), indicating that a large substituent (R) was not favorable to the bioactivity. The substituents on the benzyl group also affected the antibacterial activity. For example, the order of activities followed (H, **6g**, 52.2%, 200 μg/mL) > (4-Cl, 4-F, 4-CH_3, 4-CF_3, 3-F, **6h–6l**, <44.9%, 200 μg/mL), suggesting a substituent on the benzyl moiety was not good for the bioactivity. The bioassay result also revealed that the electronic effect of substituents on the benzyl group exhibited the effect toward the bioactivity. For example, an electron-donating group (−CH_3, **6j**, 40.2%, 200 μg/mL) on the benzyl moiety exerted higher antibacterial activity than that of other electron-withdrawing groups (4-Cl, **6h**, 22.4%; 4-F,

Table 1

Inhibition effect of target compounds (**6a–6l**, **7a–7b**) against *Xoo*.

Compound	Inhibition rate (%)		Compound	Inhibition rate (%)	
	200 μg/mL	100 μg/mL		200 μg/mL	100 μg/mL
6a	72.0 ± 1.7	39.0 ± 1.7	**6i**	32.3 ± 1.7	10.9 ± 2.4
6b	43.3 ± 1.5	32.4 ± 2.2	**6j**	40.2 ± 0.9	14.6 ± 0.9
6c	40.3 ± 0.8	21.4 ± 3.1	**6k**	22.1 ± 2.5	10.4 ± 3.2
6d	42.8 ± 3.4	16.7 ± 3.6	**6l**	44.9 ± 1.7	10.8 ± 0.6
6e	41.1 ± 1.8	9.3 ± 1.8	**7a**	100	100
6f	37.3 ± 3.2	33.2 ± 1.5	**7b**	100	100
6g	52.2 ± 3.1	41.4 ± 2.8	**BT**	72.1 ± 0.7	53.7 ± 1.2
6h	22.4 ± 2.5	10.3 ± 3.2	**TC**	64.2 ± 2.8	43.1 ± 3.2

Table 2

EC_{50} values of **7a** and **7b** against pathogen *Xoo*.

Compound	Regression equation	EC_{50} (μg/mL)	r
7a	y = 2.186x + 2.429	15.0 ± 1.3	0.94
7b	y = 1.646x + 3.676	6.4 ± 2.7	0.99
BT	y = 1.499x + 2.052	92.6 ± 2.1	0.98
TC	y = 1.540x + 1.788	121.8 ± 3.6	0.98

6i, 32.3%; 4-CF_3, **6k**, 22.1%). For compounds **7a** and **7b**, the bioactivity was enhanced with the increase in the alkyl chains, suggesting that even slight changes in the ratio between hydrophobic and hydrophilic parts will affect their bioactivities [30].

Fungicidal activity. The poison plate technique [36,37] was used to examine the antifungal activity of the target compounds (**6a–6l**, **7a–7b**) against six kinds of phytopathogenic fungi, *Gibberella zeae* (*G. zeae*), *Fusarium oxysporum* (*F. oxysporum*), *Cytospora mandshurica* (*C. mandshurica*), *Phytophthora infestans* (*P. infestans*), *Sclertinia sclerotiorum* (*S. sclerotiorum*), and *Rhizoctonia solani* (*R. solani*) at the concentrate of 100 μg/mL. While the hymexzaol and carbendazim were chosen as the positive control for the comparison of the bioactivity. As shown in Table 3, the bioassay results revealed that most of the title compounds exhibited a certain degree of antifungal activities toward the six kinds of phytopathogenic fungi. Among them, compound **6a** exhibited comprehensive antifungal activity against six kinds of fungi, which was comparable with that of hymexazol; compound **6f** could selectively inhibit the growth of *S. sclerotiorum* and *R. solani* with the inhibition rates of 82.5 and 80.3% at the concentrate of 100 μg/mL, respectively; compound **7b** exerted good antifungal activity toward *F. oxysporum*, *C. mandshurica*, and *R. solani* with the inhibition rates of 70.8, 69.5, and 71.5%, respectively. Given the above results, this kind of compounds could be further investigated as promising antifungal structures.

Table 3

Inhibition effect of title compounds toward phytopathogenic fungi at 100 μg/mL.

Compound	Inhibition (%)					
	G. zeae	*F. oxysporum*	*C. mandshurica*	*P. infestans*	*S. sclerotiorum*	*R. solani*
6a	55.1 ± 2.4	67.6 ± 3.5	54.9 ± 2.3	64.7 ± 2.9	61.4 ± 2.2	73.0 ± 3.4
6b	29.9 ± 2.0	24.7 ± 1.9	21.0 ± 1.7	19.0 ± 1.5	5.8 ± 1.3	21.6 ± 1.6
6c	57.7 ± 2.5	46.3 ± 2.1	35.1 ± 1.8	52.6 ± 2.4	51.4 ± 2.0	62.1 ± 2.6
6d	34.1 ± 1.7	26.4 ± 1.7	19.2 ± 1.6	38.4 ± 2.0	55.3 ± 2.0	55.5 ± 2.4
6e	58.3 ± 2.3	40.5 ± 2.1	22.9 ± 1.7	39.1 ± 2.0	59.2 ± 2.4	56.4 ± 2.5
6f	40.5 ± 1.8	30.1 ± 1,9	44.2 ± 1.9	38.8 ± 2.2	82.5 ± 4.0	80.3 ± 4.0
6g	4.2 ± 1.3	9.8 ± 1.6	4.3 ± 1.4	18 ± 1.8	8.9 ± 1.3	5.0 ± 1.4
6h	8.8 ± 1.5	15.5 ± 1.6	5.8 ± 1.4	14.9 ± 1.8	5.6 ± 1.3	5.3 ± 1.5
6i	6.5 ± 1.5	15.6 ± 1.6	7.9 ± 1.4	12.0 ± 1.4	14.4 ± 1.3	7.5 ± 1.4
6j	9.1 ± 1.4	10.8 ± 1.7	6.4 ± 1.4	15.9 ± 1.7	6.1 ± 1.3	14.1 ± 1.5
6k	4.2 ± 1.3	12.2 ± 1.6	9.5 ± 1.4	12.1 ± 1.6	4.2 ± 1.3	5.6 ± 1.4
6l	2.7 ± 1.4	9.5 ± 1.6	6.1 ± 1.4	9.3 ± 1.8	5.8 ± 1.3	5.0 ± 1.4
7a	39.2 ± 1.3	53.0 ± 5.3	5.6 ± 2.4	13.7 ± 2.2	0	57.0 ± 1.8
7b	39.8 ± 1.6	70.8 ± 1.5	69.5 ± 3.7	43.2 ± 4.5	0	71.5 ± 1.5
Hymexazol	72.2 ± 3.6	62.1 ± 2.5	66.1 ± 2.1	63.7 ± 3.0	51.3 ± 1.9	74.7 ± 1.1
Carbendazim	100	100	100	100	100	100

CONCLUSIONS

In summary, a series of 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole derivatives bearing 1,3,4-oxadiazole were synthesized and designed via replacing the amide bond into 1,3,4-oxadiazole moiety, and their antibacterial and antifungal activity were screened via turbidmeter test and the poison plate technique, respectively. The bioassay results revealed that compounds **7a** and **7b** showed the strongest antibacterial activity toward pathogen *Xoo* with the EC_{50} values of 15.0 and 6.4 μg/mL, respectively; compound **6a** exhibited comprehensive antifungal activity toward six kinds of fungi, which was comparable with that of hymexazol; compound **6f** could selectively inhibit the growth of *S. sclerotiorum*, and *R. solani* with the inhibition rates of 82.5, and 80.3% at the concentrate of 100 μg/mL, respectively; compound **7b** exerted good antifungal activity toward *F. oxysporum*, *C. mandshurica*, and *R. solani* with the inhibition rates of 70.8, 69.5, and 71.5%, respectively. The results indicated that this kind of structures could be further studied as promising antimicrobial agents.

EXPERIMENTAL

Instruments. Melting points of the compounds were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). The IR spectra were recorded on a Bruker VECTOR 22 spectrometer. Elemental analysis was performed on an Elementar Vario-III CHN analyzer (Hanau, Germany). NMR spectra were obtained by using a JEOL-ECX-500 apparatus. Chemical shifts were reported in parts per million (ppm) down field from TMS with the solvent resonance as the internal standard. Coupling constants (*J*) were reported in Hz and referred to apparent peak multiplications. MS were recorded on an Agilent ESI-MSD Trap (VL) mass instrument.

General synthetic procedures for the target compounds (6a). Intermediate **4** (14.80 mmol) was dissolved in a mixture of THF (20.0 mL), and carbonyldimidazole (17.76 mmol), and then triethylamine (6.0 mL) was added. The mixture was stirred at room temperature for 6 h. After that, THF was removed under reduce pressure. The crude residue was further purified by flash column chromatography on a silica gel using CH_2Cl_2 and CH_3OH (100:1) as the eluant to afford the desired product (**6a**).

5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazol-2-ol (6a). This compound was obtained as white solid; yield 90.8%, m.p. 81 ~ 83°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.75 (s, 1H, NH), 8.33 (s, 1H, pyrazole-H), 7.65–7.48 (m, 5H, benzene-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.9, 154.2, 141.1, 138.8, 130.9, 129.9, 126.6, 119.3 (q, J = 270.9 Hz, CF_3), 108.0; IR (KBr) v: 3446, 3108, 3032, 1784, 1751, 1647, 1595, 1560, 1498, 1432, 1406, 1292, 1149, 952, 920, 771, 694, 661 cm^{-1}; MS(ESI) m/z 297 [M + H]$^+$; *Anal.* Calcd. for $C_{12}H_7F_3N_4O_2$: C, 48.66; H, 2.38; N, 18.91. Found: C, 48.85; H,2.49; N, 19.17.

General synthetic procedures for the target compounds (6b–6l). Intermediate **5** (1.01 mmol) was dissolved in a mixture of THF (3.0 mL), H_2O (3.0 mL), and NaOH (1.11 mmol). Then, the halogenated agent (1.31 mmol) was added and stirred at room temperature for 3 h. After that, THF was removed under reduce pressure, and 30 mL CH_2Cl_2 was added into the mixture. Finally, the organic layer was washed by water, dried with anhydrous sodium sulfate, filtered, and followed by the removal of the solvent under vacuum. The crude residue was further purified by flash column chromatography on a silica gel using ethyl acetate and petroleum ether (1:10) as the eluant to afford the desired product **(6b–6l)**.

2-methoxy-5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (6b). This compound was obtained as white solid; yield 87.1%; m. p. 121 ~ 122°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.64–7.52 (m, 5H, benzene-H), 3.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.3, 146.0, 140.6, 138.9, 130.9, 129.9, 126.6, 119.4 (q, J = 270.6 Hz, CF_3), 109.2, 33.1; IR (KBr) v: 3105, 3073, 3021, 2957, 2921, 2849, 1784, 1653, 1604, 1540, 1496, 1463, 1404, 1296, 1195, 1136, 970, 879, 769, 743, 696, 664 cm^{-1}; MS(ESI) m/z 311 [M + H]$^+$; *Anal.* Calcd. for $C_{13}H_9F_3N_4O_2$: C, 50.33; H, 2.92; N, 18.06. Found: C, 50.68; H, 3.28; N, 18.03.

2-ethyloxy-5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (6c). This compound was obtained as canary yellow oily liquid; yield 85.8%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.71–7.48 (m, 5H, benzene-H), 3.75 (q, J = 7.2 Hz, 2H, CH_2), 1.26 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.8, 146.2, 140.6, 138.9, 130.9, 129.9, 126.6, 119.4 (q, J = 270.6 Hz, CF_3), 109.2, 41.2, 13.6; IR (KBr) v: 3124, 3072, 2984, 2940, 2854, 1790, 1646, 1596, 1558, 1532, 1505, 1304, 1187, 1143, 973, 953, 769, 746, 695, 658 cm^{-1}; MS(ESI) m/z 325 [M + H]$^+$; *Anal.* Calcd. for $C_{14}H_{11}F_3N_4O_2$: C, 51.86; H, 3.42; N, 17.28. Found: C, 51.96; H, 3.80; N, 17.06.

2-propyloxy-5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (6d). This compound was obtained as white solid; yield 86.7%, m. p. 59 ~ 60°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.59–7.53 (m, 5H, benzene-H), 3.68 (t, J = 6.8 Hz, 2H, O-CH_2), 1.73–1.65 (m, 2H, CH_2), 0.87 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1, 146.2, 140.7, 138.9, 130.9, 129.9, 126.6, 119.4 (q, J = 270.3 Hz, CF_3), 109.2, 47.5, 21.5, 11.2; IR (KBr) v: 3103, 2970, 2939, 2880, 1780, 1646, 1594, 1500, 1463, 1405, 1302, 1181, 999, 967, 770, 746, 697 cm^{-1}; MS(ESI) m/z 339 [M + H] $^+$; *Anal.* Calcd. for $C_{15}H_{13}F_3N_4O_2$: C, 53.26; H, 3.87; N, 16.56. Found: C, 53.51; H, 4.01; N, 16.32.

2-isopropyloxy-5-(1-phenyl-5-(trifluoromethyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (6e). This compound was obtained as white solid; yield 86.2%, m. p. 61 ~ 62°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H, pyrazole-H), 7.59–7.53 (m, 5H, benzene-H), 4.30–4.24 (m, 1H, CH), 1.30 (d, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.2, 146.2, 140.6, 138.9, 130.8, 129.9, 126.6, 119.4 (q, J = 270.3 Hz, CF_3), 109.3, 48.5, 21.0; IR (KBr) v: 3104, 3029, 2987, 2939, 2877, 2850, 1779, 1646, 1596, 1501, 1465, 1457, 1405, 1302, 1190, 963, 918, 766, 743, 693 cm^{-1}; MS(ESI) m/z 339 [M + H]$^+$; *Anal.* Calcd. for $C_{15}H_{13}F_3N_4O_2$: C, 53.26; H, 3.87; N, 16.56. Found: C, 53.31; H, 4.10; N, 16.61.

2-allyloxy-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6f). This compound was obtained as canary yellow solid; yield 81.0%, m. p. 77 ~ 78°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.60–7.52 (m, 5H, benzene-H), 5.91 (ddd, J_1 = 22.4, J_2 = 10.6, J_3 = 5.4 Hz, 1H, CH=), 5.25 (d, J = 9.1 Hz, 2H, O-CH_2), 4.37 (d, J = 5.3 Hz, 2H, =CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.4, 146.3, 140.7, 138.9, 133.1, 130.9, 129.9, 126.6, 119.9, 119.4 (q, J = 270.6 Hz, CF_3), 109.1, 48.2; IR (KBr) *v*: 3106, 3022, 2959, 2927, 2853, 1781, 1646, 1595, 1499, 1465, 1419, 1405, 1303, 1183, 968, 928, 770, 696 cm^{-1}; MS(ESI) m/z 337 [M + H] $^+$. *Anal.* Calcd. for $C_{15}H_{11}F_3N_4O_2$: C, 53.58; H, 3.30; N, 16.66. Found: C, 53.30; H, 3.72; N, 16.22.

2-benyloxy-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6g). This compound was obtained as white solid; yield 84.3%, m. p. 98 ~ 99°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.59–7.52 (m, 5H, benzene-H), 7.42–7.28 (m, 5H, benzyl-H), 4.96 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1, 146.5, 140.7, 138.9, 135.8, 130.9, 129.9, 129.2, 128.5, 128.2, 126.6, 119.4 (q, J = 270.1 Hz, CF_3), 109.1, 49.5; IR (KBr) *v*: 3116, 3031, 2962, 2927, 2852, 1781, 1642, 1596, 1501, 1465, 1452, 1419, 1305, 1189, 970, 959, 764, 747, 686 cm^{-1}; MS(ESI) m/z 387 [M + H]$^+$. *Anal.* Calcd. for $C_{19}H_{13}F_3N_4O_2$: C, 59.07; H, 3.39; N, 14.50. Found: C, 58.94; H, 3.75; N, 14.05.

2-((4-chlorobenzyl)oxy)-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6h). This compound was obtained as canary yellow solid; yield 85.2%, m. p. 80 ~ 82°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H, pyrazole-H), 7.63–7.50 (m, 5H, benzene-H), 7.43 (d, J = 8.4 Hz, 2H, 4-Cl-benzyl-H), 7.37 (d, J = 8.4 Hz, 2H, 4-Cl-benzyl-H), 4.96 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1, 146.6, 140.7, 138.9, 134.8, 133.2, 130.9, 130.1, 129.9, 129.2, 126.6, 119.4 (q, J = 270.6 Hz, CF_3), 109.1, 48.7; IR (KBr) *v*: 3086, 3059, 3035, 2923, 2850, 1792, 1653, 1596, 1503, 1492, 1465, 1437, 1302, 1181, 970, 925, 765, 747, 690 cm^{-1}; MS(ESI) m/z 421 [M + H]$^+$. *Anal.* Calcd. for $C_{19}H_{12}ClF_3N_4O_2$: C, 54.23; H, 2.87; N, 13.32. Found: C, 54.62; H, 3.25; N, 12.87.

2-((4-fluorobenzyl)oxy)-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6i). A light yellow liquid, yield 83.4%, ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 (s, 1H, pyrazole-H), 7.59–7.47 (m, 5H, benzene-H), 7.43 (dd, J_1 = 8.6, J_2 = 5.3 Hz, 2H, 4-F-benzyl-H), 7.10 (t, J = 8.8 Hz, 2H, 4-F-benzyl-H), 4.95 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.19 (d, J = 243.8 Hz, benzyl C), 153.21, 146.65, 140.76, 138.95, 132.76 (d, J = 2.6 Hz, benzyl C), 131.89 (d, J = 8.3 Hz, benzyl C), 130.87, 129.95, 126.65, 119.41 (q, J = 270.1 Hz, CF_3), 116.09 (d, J = 21.3 Hz, benzyl C), 109.11, 48.71; IR (KBr) *v*: 3072, 3046, 2947, 2923, 2852, 1784, 1653, 1598, 1559, 1506, 1472, 1433, 1325, 1179, 989, 959, 771, 694 cm^{-1}; MS(ESI) m/z 405 [M + H] $^+$; *Anal.* Calcd. for $C_{19}H_{12}F_4N_4O_2$: C, 56.44; H, 2.99; N, 13.86. Found: C, 56.70; H, 2.92; N, 14.10.

2-((4-methylbenzyl)oxy)-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6j). This compound was obtained as white solid; yield 83.1%, m. p. 71 ~ 72°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H, pyrazole-H), 7.60–7.50 (m, 5H, benzene-H), 7.22 (d, J = 7.9 Hz, 2H, 4-CH_3-benzyl-H), 7.15 (d, J = 7.9 Hz, 2H, 4-CH_3-benzyl-H), 4.90 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.2, 146.5, 140.7, 138.9, 137.8, 132.8, 130.8, 129.9, 129.7, 128.2, 126.6, 119.4 (q, J = 270.3 Hz, CF_3), 109.1, 49.3, 21.2; IR (KBr) *v*: 3121, 3059, 3027, 2932, 2856, 1786, 1643, 1595, 1502, 1458, 1428, 1410, 1302, 1191, 973, 936, 772, 750, 695 cm^{-1}; MS (ESI) m/z 401 [M + H]$^+$; *Anal.* Calcd. for $C_{20}H_{15}F_3N_4O_2$: C, 60.00; H, 3.78; N, 13.99. Found: C, 59.90; H, 3.98; N, 14.24.

2-((trifluoromethylbenzyl)oxy)-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6k). This compound was obtained as canary yellow solid; yield 78.1%, m. p. 81 ~ 82°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, pyrazole-H), 7.73 (d, J = 8.1 Hz, 2H, benzyl-H), 7.61–7.50 (m, 7H, benzene H & benzyl-H), 5.08 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.2, 146.8, 140.8, 140.6, 138.9, 130.9, 129.9, 128.9, 126.6, 126.1, 124.7 (q, J = 272.4 Hz, benzyl-CF_3), 119.4 (q, J = 270.7 Hz, pyrazole-CF_3), 109.1, 48.9; IR (KBr) *v*: 3067, 3048, 2953, 2927, 2854, 1792, 1653, 1599, 1559, 1501, 1472, 1419, 1325, 1169, 998, 969, 761, 747, 684, 668 cm^{-1}; MS(ESI) m/z 455 [M + H]$^+$; *Anal.* Calcd. for $C_{20}H_{12}F_6N_4O_2$: C, 52.87; H, 2.66; N, 12.33. Found: C, 52.77; H, 3.07; N, 12.25.

2-((3-fluorobenzyl)oxy)-5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (6l). A white solid, yield 79.7%, m. p. 96 ~ 98°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.60–7.51 (m, 5H), 7.40 (td, J_1 = 8.3, J_2 = 6.4 Hz, 1H), 7.19 (dd, J_1 = 9.6, J_2 = 4.4 Hz, 2H), 7.14 (td, J_1 = 9.1, J_2 = 2.6 Hz, 1H), 4.99 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.60 (d, J = 242.5 Hz, benzyl-C), 153.12, 146.71, 140.77, 139.83 (d, J = 7.9 Hz, benzyl-C), 138.95, 131.15 (d, J = 8.5 Hz, benzyl-C), 130.90, 129.95, 126.66, 125.80 (d, J = 2.2 Hz, benzyl-C), 119.43 (q, J = 270.0 Hz, CF_3), 116.59 (d, J = 22.3 Hz, benzyl-C), 115.36 (d, J = 21.3 Hz, benzyl-C), 109.08, 48.89; IR (KBr) *v*: 3118, 3081, 2963, 2930, 2852, 1783, 1645, 1593, 1501, 1486, 1449, 1419, 1306, 1189, 971, 946, 763, 748, 681 cm^{-1}; MS(ESI) m/z 405 [M + H]$^+$; *Anal.* Calcd. for $C_{19}H_{12}F_4N_4O_2$: C, 56.44; H, 2.99; N, 13.86. Found: C, 56.82; H, 3.27; N, 13.84.

General synthetic Procedures for the target compounds (7a–7b). Intermediate **5** (1.01 mmol) was dissolved in a mixture of DMF (10.0 mL), and NaOH (1.21 mmol). Then the dibromo-substituted alkane (1.51 mmol) was added and stirred at room temperature for 5 h. After that, 30 mL ethyl acetate was added into the mixture. Later, the organic layer was washed by saturated NH_4Cl, water, dried with anhydrous sodium sulfate, filtered, and followed by the removal of the solvent under vacuum. The crude product was incubated in pyridine at 60°C for 12 h. After that, the extra pyridine was removed under reduced pressure. The crude residue was further purified by flash column chromatography on a silica gel using CH_2Cl_2 and CH_3OH (6:1) as the eluant to afford the desired product **(7a–7b)**.

1-(6-((5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)oxy)hexyl)pyridin-1-ium bromide (7a). A white liquid; yield 55.0%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (d, J = 10.0 Hz, 2H, pyridine-H), 8.55 (t, J = 7.5 Hz, 1H, pyridine-H), 8.12 (t, J = 5.0 Hz, 2H, pyridine-H), 7.98 (s, 1H, pyrazole-H), 7.32–7.42 (m, 5H, phenyl-H), 4.85 (t, J = 7.5 Hz, 2H, CH_2-N), 3.68 (t, J = 7.5 Hz, 2H, CH_2-O), 1.97–2.02 (m, 2H, CH_2), 1.67–1.73 (m, 2H, CH_2), 1.35–1.38 (m, 4H, 2CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.3, 146.6, 145.8, 144.9, 140.0, 138.8, 130.1, 129.3, 128.8, 125.8, 120.1, 117.9, 109.0, 61.8, 45.8, 31.6, 27.8, 25.7, 25.3. MS(ESI): m/z 458 [M-Br] $^+$; *Anal.* Calcd. for $C_{23}H_{23}BrF_3N_5O_2$: C, 51.31; H, 4.31; N, 13.01. Found: C, 50.92; H, 4.51; N, 12.65.

1-(7-((5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)oxy)heptyl)pyridin-1-ium bromide (7b). A white liquid; yield 45.1%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.34 (d, J = 5.0 Hz, 2H, pyridine-H), 8.47 (t, J = 7.5 Hz, 1H, pyridine-H), 8.07 (t, J = 5.0 Hz, 2H, pyridine-H), 7.92 (s, 1H, pyrazole-H), 7.28–7.38 (m, 5H, phenyl-H), 4.82 (t, J = 7.5 Hz, 2H, CH_2-N), 3.62 (t, J = 7.5 Hz, 2H, CH_2-O), 1.89–1.95 (m, 2H, CH_2), 1.61–1.63 (m, 2H, CH_2), 1.19–1.26 (m, 4H, $2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.2, 146.4, 145.6, 145.0, 139.9, 138.7, 130.3, 130.1, 130.0, 129.2, 128.7, 125.8, 120.0, 117.9, 109.0, 61.9, 45.9, 31.7, 28.7, 28.5, 27.9, 26.0, 25.8; MS (ESI): m/z 486 $[M\text{-}Br]^+$; *Anal.* Calcd. for $C_{25}H_{27}BrF_3N_5O_2$: C, 53.01; H, 4.80; N, 12.36. Found: C, 53.32; H, 4.69; N, 12.18.

In vitro antibacterial bioassay (turbidimeter test). In our study, all the synthesized target compounds were evaluated for their antibacterial activities against *Xoo* by the turbidimeter test *in vitro*. Dimethylsulfoxide in sterile distilled water served as a blank control, Bismerthiazol and Thiodiazole Copper served as the positive controls. Approximately 40 μL of solvent NB (1.5 g beef extract, 2.5 g peptone, 0.5 g yeast powder, 5.0 g glucose, and 500 mL distilled water; pH = 7.0–7.2) containing *Xoo*, incubated on the phase of logarithmic growth, was added to 5 mL of solvent NB containing the test compounds and positive control. The inoculated test tubes were incubated at 28 ± 1°C and continuously shaken at 180 rpm for 24–48 h until the bacteria were incubated on the logarithmic growth phase. The growth of the cultures was monitored on a microplate reader by measuring the optical density at 595 nm (OD_{595}) given by turbiditycorrected values = $OD_{\text{bacterial wilt}} - OD_{\text{no bacterial wilt}}$, and the inhibition rate I was calculated by $I = (C - T)/C \times 100\%$. C is the corrected turbidity values of bacterial growth on untreated NB (blank control), and T is the corrected turbidity values of bacterial growth on treated NB. The results of antibacterial activities (expressed by EC_{50}) against *Xoo* was calculated with SPSS 17.0 software. The experiment was repeated three times.

Antifungal activities assay. All title compounds were dissolved in DMSO (1.0 mL) and then added into 9.0 mL sterilized water containing Tween 20 (1%) before mixing with potato dextrose agar (PDA, 90.0 mL). The compounds were tested at a concentration of 100 μg/mL. The stock solution was transferred into three 9 cm diameter of Petri dishes evenly. Then, mycelia dishes of approximately 4 mm diameter were cut from the culture medium and inoculated in the middle of the PDA plate aseptically. The inoculated plates were incubated at 27 ± 1°C for 5 days. DMSO in sterile distilled water was used as the negative control, whereas hymexazol and carbendazim served as the positive control. Each treatment condition consisted of three replicates. Radial growth of the fungal colonies was measured, and the data were statistically analyzed. Inhibitory effects on these fungi were calculated by the formula $I = [(C - T)/(C - 0.4)] \times 100\%$, where C represents the diameter of fungal growth on untreated PDA, T represents the diameter of fungi on treated PDA, and I represents the inhibition rate.

Acknowledgments. We acknowledge the financial support of the Key Technologies R&D Program (2014BAD23B01), National Natural Science Foundation of China (21372052, 21462011), the Research Project of Chinese Ministry of Education (213033A, 20135201110005), and Scientific Research Foundation for the Introduced Talents of Guizhou University 2015 [34].

REFERENCES AND NOTES

[1] Bertuzzi, G.; Locatelli, E.; Colecchia, D.; Calandro, P.; Bonini, B. F.; Chandanshive, J. Z.; Mazzanti, A.; Zani, P.; Chiariello, M.; Franchini, M. C. Eur J Med Chem 2016, 117, 1.

[2] Reddy, T. S.; Reddy, V. G.; Kulhari, H.; Shukla, R.; Kamal, A.; Bansal, V. Eur J Med Chem 2016, 117, 157.

[3] Thore, S. N.; Gupta, S. V.; Baheti, K. G. J Saudi Chem Soc 2016, 20, 259.

[4] Zaki, R. M.; El-Ossaily, Y. A.; Geies, A. A. Med Chem Res 2016, 25, 893.

[5] Deng, X. L.; Xie, J.; Li, Y. Q.; Yuan, D. K.; Hu, X. P.; Zhang, L.; Wang, Q. M.; Chi, M.; Yang, X. L. Chin Chem Lett 2016, 27, 566.

[6] Wang, Y.; Cheng, F. X.; Yuan, X. L.; Tang, W. J.; Shi, J. B.; Liao, C. Z.; Liu, X. H. Eur J Med Chem 2016, 112, 231.

[7] Chen, Y. Y.; Wu, X. Q.; Tang, W. J.; Shi, J. B.; Li, J.; Liu, X. H. Eur J Med Chem 2016, 110, 65.

[8] Dai, H.; Huang, J. H.; Jin, Z. C.; Cheng, X. Y.; Huang, K. W.; Ling, Y.; Wang, Q. M.; Shi, Y. J Chinese J Org Chem 2015, 35, 2617.

[9] Wang, B. L.; Zhang, L.Y.; Zhan, Y. Z.; Zhang, Y.; Zhang, X.; Wang, L. Z.; Li, Z. M. J Fluorine Chem 2016, 184, 36.

[10] Tong, X.; Chen, R.; Zhang, T. T.; Han, Y.; Tang, W. J.; Liu, X. H. Bioorg Med Chem 2015, 23, 515.

[11] Shi, J. B.; Tang, W. J.; Qi, X. B.; Li, R.; Liu, X. H. Eur J Med Chem 2015, 90, 889.

[12] Cheng, Q.; Jia, Y. M.; Cheng, F. H.; Liu, X. H. Lett Drug Des Discov 2014, 11, 572.

[13] Wu, X. Q.; Huang, C.; Jia, Y. M.; Song, B. A.; Li, J.; Liu, X. H. Eur J Med Chem 2014, 74, 717.

[14] Ma, H. J.; Zhang, J. H.; Xia, X. D.; Kang, J.; Li, J. H. Pest Manag Sci, 2015, 71, 1189.

[15] Abrigach, F.; Bouchal, B.; Riant, O.; Mace, Y.; Takfaoui, A.; Radi, S.; Oussaid, A.; Bellaoui, M.; Touzani, R. Med Chem 2016, 12, 83.

[16] Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. J Med Chem 2004, 47, 3693.

[17] Abdallah, M. A.; Gomha, S. M.; Abdelaziz, M. R.; Serag, N. S. E. Heterocycles 2016, 92, 649.

[18] Mady, M. F.; Saleh, T. S.; El-Kateb, A. A.; Abd El-Rahman, N. M.; Abd El-Moez, S. I. Res Chem Intermedia 2016, 42, 753.

[19] Seelam, N.; Shrivastava, S. P. J Saudi Chem Soc 2016, 20, 33.

[20] Mert, S.; Kasimogullari, R.; Ica, T.; Colak, F.; Altun, A.; Ok, S. Eur J Med Chem 2014, 78, 86.

[21] Dai, H.; Li, Y. Q.; Du, D.; Qin, X.; Zhang, X.; Yu, H. B.; Fang, J. X. J Agric Food Chem 2008, 56, 10805.

[22] Basha, S. S.; Divya, K.; Padmaja, A.; Padmavathi, V. Res Chem Intermed 2015, 41, 10067.

[23] Wu, Z. B.; Hu, D. Y.; Kuang, J. Q.; Cai, H.; Wu, S. X.; Xue, W. Molecules 2012, 17, 14205.

[24] Wu, Z. B.; Wu, S. X.; Ye, Y. Q.; Zhou, X.; Wang, P. Y.; Xue, W.; Hu, D. Y. J Heterocyclic Chem 2015, DOI 10.1002/jhet.

[25] Bhat, M. A.; Al-Omar, M. A.; Siddiqui, N. Med Chem Res 2013, 22, 4455.

[26] Li, P.; Shi, L.; Yang, X.; Yang, L.; Chen, X.; Wu, F.; Shi, Q.; Xu, W. M.; He, M.; Hu, D. Y.; Song, B. A. Bioorg Med Chem Lett 2014, 24, 1677.

[27] Desai, N. C.; Dodiya, A. M.; Rajpara, K. M.; Rupala, Y. M. J Saudi Chem Soc 2014, 18, 255.

[28] Xu, W. M.; Li, S. Z.; He, M.; Yang, S.; Li, X. Y.; Li, P. Bioorg Med Chem Lett 2013, 23, 5821.

[29] Wang, P. Y.; Zhou, L.; Zhou, J.; Wu, Z. B.; Xue, W.; Song, B. A.; Yang, S. Bioorg Med Chem Lett 2016, 26, 1214.

[30] Wang, P. Y.; Gao, M. N.; Zhou, L.; Wu, Z. B.; Hu, D. Y.; Hu, J.; Yang, S. Bioorg Med Chem Lett 2016, 26, 1136.

[31] Paw, D.; Thomas, R.; Laura, K.; Karina, N.; Thomas, A. M. Int J Food Microbiol 1994, 23, 391.

[32] Wang, X.; Yin, J.; Shi, L.; Zhang, G.; Song, B. A. Eur J Med Chem 2014, 77, 65.

[33] Li, P.; Shi, L.; Gao, M.; Yang, X.; Xue, W.; Jin, L. H.; Hu, D. Y.; Song, B. A. Bioorg Med Chem Lett 2015, 25, 481.

[34] Chen, Y.; Yang, X.; Gu, C. Y.; Zhang, A. F.; Zhang, Y.; Wang, W. X.; Gao, T. C; Yao, J.; Yuan, S. K. Ann Appl Biol 2015, 166, 129.

[35] Perumalsamy, S.; Bharani, M.; Sudha, M.; Nagarajan, P.; Arul, L.; Saraswathi, R.; Balasubramanian, P.; Ramalingam, J. Plant Breeding 2010, 129, 400.

[36] Chattapadhyay, T. K.; Dureja, P. J Agric Food Chem 2006, 54, 2129.

[37] Wu, J.; Shi, Q.; Chen, Z.; He, M.; Jin, L. H.; Hu, D. Y. Molecules 2012, 17, 5139.