Antifungal Activity of some Diaryl Ethers

Hui XU,*[,a] Kun-Zhong JIAN,[b] Qiu GUAN,[a] Fei YE,[a] and Min LV*[,a,b]

[a] *Lab of Pharmaceutical Synthesis, College of Sciences, Northwest A&F University; and* [b] *College of Plant Protection, Northwest A&F University; Yangling 712100, China.*

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Several diaryl ethers were synthesized and tested *in vitro* against seven phytopathogenic fungi, namely *Fusarium graminearum*, *Alternaria alternate*, *Helminthosporium sorokinianum*, *Pyricularia oryzae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *cucumarinum* and *Alternaria brassicae*. Compared to a commercial agricultural fungicide, hymexazol, especially compounds a, b, e, g and k were found to be more effective at 50 μg/ml against *F. graminearum*, *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *cucumarinum*. Meantime, some structure–activity relationships were also observed.

Key words diaryl ether; antifungal activity; phytopathogenic fungi

Recently many reports have been demonstrated that the diaryl ether scaffolds are found in a number of natural products and biologically important molecules, which elicit pharmacologically outstanding activities, *e.g.*, antimitotic, immunosuppressive and antibiotic activity,[1—3] and are used in the control of weeds.[4,5] However, to the best of our knowledge, little attention has been paid to the antifungal activity of the single diaryl ethers, with low-molecular weight. Meanwhile, many crops are easily infected by phytopathogenic fungi, which are hard to control, therefore the development of very bioactive compounds for control of those agricultural diseases is highly desirable. As part of our program aimed at the discovery and development of bioactive molecules,[6—8] here we report the synthesis and antifungal activity of some single diaryl ethers with various functional groups.

Results and Discussion

All the fourteen compounds of diaryl ethers **a**—**n**, as shown in Fig. 1, were tested *in vitro* at the concentrations of 50 and 500 μg/ml for their antifungal activity against seven phytopathogenic fungi, namely *Fusarium graminearum*, *Alternaria alternate*, *Helminthosporium sorokinianum*, *Pyricularia oryzae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *cucumarinum* and *Alternaria brassicae*. The results of preliminary bioassays were compared with those of a commercial agricultural fungicide, hymexazol. As indicated in Table 1, most of the diaryl ethers showed certain antifungal activity as potent as hymexazol against the tested fungi at 500 μg/ml. For example, the compounds **a**, **b**, **e**, and **k** inhibited the growth of *F. graminearum* at 87.4%, 92.8%, 92.4%, and 94.0%, respectively; compounds **a**, **b**, and **k** inhibited the growth of *A. alternate* at 83.8%, 81.9%, and 95.3%, respectively; compounds **a**, **b**, **i**, **k**, and **n** inhibited the growth of *H. sorokinianum* at 87.2%, 89.5%, 88.7%, 95.0%, and 85.5%, respectively; compounds **a**, **k**, and **n** inhibited the growth of *P. oryzae* at 81.9%, 92.0%, and 82.8%, respectively; compound **a** inhibited the growth of *F. oxysporum* f. sp. *vasinfectum* at 83.4%; compound **k** inhibited the growth of *A. brassicae* at 87.9%.

But it is noteworthy that at 50 μg/ml some antifungal activity of several diaryl ethers were more potent than that of hymexazol. For example, the compounds **b**, **c**, **e**, **h**, **i**, **j**, and **k** inhibited the growth of *F. graminearum* at 79.5%, 63.0%, 62.8%, 66.2%, 65.2%, 59.7%, and 64.9%, respectively; the compounds **b**, **c**, **i**, **j**, and **n** inhibited the growth of *H. sorokinianum* at 66.0%, 74.3%, 71.1%, 40.9%, and 58.5%, respectively; the compounds **b**, **d**, **e**, **i**, **k**, and **n** inhibited the growth of *F. oxysporum* f. sp. *vasinfectum* at 47.5%, 57.6%, 65.1%, 59.7%, 70.1%, and 53.2%, respectively; the compounds **b**, **c**, **d**, **e**, **i**, **j**, **k**, and **n** inhibited the growth of *F. oxysporum* f. sp. *cucumarinum* at 38.8%, 34.1%, 38.0%, 57.9%, 48.0%, 44.9%, 55.6%, and 50.2%, respectively. Especially the percentage inhibitions of compounds **a**/**g** on the growth of *F. graminearum*, *H. sorokinianum*, *F. oxysporum* f. sp. *vasinfectum*, and *F. oxysporum* f. sp. *cucumarinum* were 71.6%/79.6%, 73.0%/56.2%, 81.8%/52.5%, and 61.2%/48.8%, respectively, which were higher than those of hymexazol (38.7% against *F. graminearum*, 37.1% against *H. sorokini-*

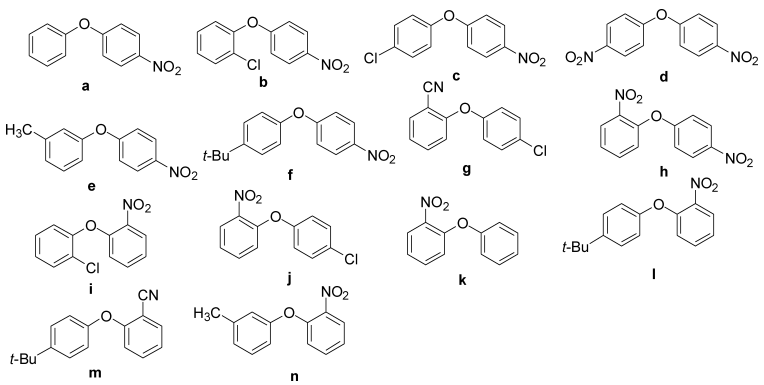

Fig. 1. Structures of Different Diaryl Ethers **a**—**n**

* To whom correspondence should be addressed. e-mail: orgxuhui@nwsuaf.edu.cn; lvmin@nwsuaf.edu.cn

Table 1. Antifungal Activity of Diaryl Ethers (**a**—**n**) against Phytopathogenic Fungi *in Vitro*

Compds.	Conc. (μg/ml)	Inhibition rate (%)[a]						
		Fusarium graminearum	*Alternaria alternata*	*Helminthosporium sorokinianum*	*Pyricularia oryzae*	*Fusarium oxysporum* f. sp. *vasinfectum*	*Fusarium oxysporum* f. sp. *cucumarinum*	*Alternaria brassicae*
a	500	87.4 (±0.8)	83.8 (±1.4)	87.2 (±1.7)	81.9 (±0.8)	83.4 (±5.7)	72.9 (±1.3)	76.0 (±0)
	50	71.6 (±0.1)	66.8 (±1.8)	73.0 (±2.9)	48.0 (±0.8)	81.8 (±8.1)	61.2 (±0)	55.5 (±2.5)
b	500	92.8 (±0.7)	81.9 (±1.1)	89.5 (±0.3)	77.5 (±2.7)	55.6 (±2.0)	75.5 (±3.9)	65.3 (±2.7)
	50	79.5 (±3.0)	60.4 (±2.0)	66.0 (±2.3)	47.8 (±6.6)	47.5 (±2.0)	38.8 (±1.2)	36.0 (±0)
c	500	66.8 (±0.4)	61.3 (±0.2)	78.1 (±0.2)	63.8 (±4.7)	43.4 (±0)	38.0 (±2.6)	47.2 (±1.9)
	50	63.0 (±2.8)	47.4 (±2.6)	74.3 (±1.0)	47.6 (±0.5)	41.4 (±0)	34.1 (±3.9)	42.7 (±0.6)
d	500	3.1 (±0.9)	19.8 (±5.2)	20.4 (±0.3)	36.6 (±7.4)	63.6 (±4.0)	50.9 (±1.2)	75.2 (±1.3)
	50	13.6 (±2.7)	15.0 (±4.9)	16.7 (±0.4)	1.9 (±3.8)	57.6 (±6.1)	38.0 (±2.1)	36.8 (±1.3)
e	500	92.4 (±1.0)	61.2 (±1.2)	44.4 (±4.9)	60.3 (±5.3)	74.3 (±1.0)	64.6 (±1.2)	52.8 (±2.3)
	50	62.8 (±5.3)	48.4 (±1.9)	32.0 (±4.9)	39.2 (±6.0)	65.1 (±2.5)	57.9 (±1.2)	37.3 (±2.3)
f	500	56.0 (±4.6)	27.7 (±2.2)	21.6 (±0)	40.4 (±1.5)	67.7 (±4.0)	10.3 (±6.4)	32.0 (±1.3)
	50	55.6 (±3.0)	25.5 (±5.9)	23.5 (±4.2)	32.0 (±4.5)	14.1 (±1.0)	3.6 (±1.2)	32.0 (±1.3)
g	500	83.7 (±1.2)	78.0 (±1.2)	68.0 (±2.4)	77.7 (±2.3)	57.0 (±3.8)	54.3 (±3.2)	74.1 (±3.3)
	50	79.6 (±0.7)	55.9 (±0.6)	56.2 (±4.6)	59.7 (±6.1)	52.5 (±6.7)	48.8 (±0.6)	56.8 (±0.6)
h	500	68.7 (±2.7)	56.3 (±5.6)	42.2 (±2.4)	47.1 (±0.9)	42.7 (±3.0)	33.3 (±0)	49.6 (±0)
	50	66.2 (±1.2)	49.3 (±0.6)	35.3 (±0)	31.4 (±1.5)	19.3 (±6.9)	9.3 (±4.4)	8.5 (±4.8)
i	500	78.6 (±0.7)	74.0 (±3.3)	88.7 (±0)	71.6 (±2.6)	58.0 (±4.6)	53.8 (±4.5)	76.2 (±1.4)
	50	65.2 (±0.7)	52.8 (±1.3)	71.1 (±9.4)	51.0 (±5.2)	59.7 (±4.1)	48.0 (±1.1)	64.5 (±2.6)
j	500	73.6 (±1.9)	71.7 (±0)	79.9 (±7.1)	69.0 (±4.9)	47.5 (±5.0)	50.2 (±2.5)	74.3 (±2.4)
	50	59.7 (±2.4)	51.8 (±0)	40.9 (±9.4)	48.3 (±5.2)	40.3 (±2.3)	44.9 (±4.5)	61.0 (±5.3)
k	500	94.0 (±0)	95.3 (±4.8)	95.0 (±1.8)	92.0 (±6.5)	70.9 (±2.0)	65.3 (±0)	87.9 (±2.6)
	50	64.9 (±0)	62.2 (±3.5)	30.2 (±5.7)	58.0 (±2.2)	70.1 (±1.1)	55.6 (±4.5)	79.0 (±4.2)
l	500	52.7 (±1.9)	53.3 (±1.4)	50.3 (±3.2)	44.8 (±3.4)	11.2 (±3.0)	17.3 (±5.8)	37.4 (±2.6)
	50	38.8 (±1.5)	33.0 (±7.4)	38.7 (±0.9)	37.9 (±6.9)	19.3 (±9.3)	8.4 (±2.5)	38.4 (±0)
m	500	56.0 (±3.7)	51.0 (±5.8)	42.8 (±8.5)	43.1 (±1.7)	44.3 (±0)	33.3 (±2.2)	53.3 (±3.5)
	50	38.3 (±3.1)	35.8 (±2.7)	38.4 (±11.7)	43.7 (±3.3)	20.1 (±0)	20.4 (±2.7)	43.5 (±3.3)
n	500	70.1 (±4.5)	74.5 (±4.0)	85.5 (±0.9)	82.8 (±0)	46.7 (±7.1)	46.0 (±0)	73.9 (±4.8)
	50	38.8 (±3.2)	49.0 (±2.8)	58.5 (±0)	56.3 (±1.6)	53.2 (±2.3)	50.2 (±2.5)	58.9 (±0.7)
Hy[b]	500	99.4 (±0.8)	100 (±0)	100 (±0)	100 (±0)	92.3 (±1.1)	91.5 (±3.0)	100 (±0)
	50	38.7 (±1.7)	82.3 (±0)	37.1 (±0)	66.4 (±1.8)	45.8 (±1.0)	19.8 (±0.8)	89.0 (±1.2)
Acetone[c]		0	0	0	0	0	0	0

a) Values are means of three experiments, standard deviation is given in parentheses; *b*) hymexazole as a reference compound; *c*) control.

anum, 45.8% against *F. oxysporum* f. sp. *vasinfectum*, and 19.8% against *F. oxysporum* f. sp. *cucumarinum* at 50 μg/ml).

From this comparative study, it is possible to draw some structure–activity relationships as shown in Table 1. The percentage inhibitions of compounds 4-(phenoxy)nitrobenzene (**a**)/2-(phenoxy)nitrobenzene (**k**) on the growth of *F. graminearum*, *A. alternate*, *H. sorokinianum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *F. oxysporum* f. sp. *cucumarinum* and *A. brassicae* at 50 μg/ml were 71.6%/64.9%, 66.8%/62.2%, 73.0%/30.2%, 48.0%/58.0%, 81.8%/70.1%, 61.2%/55.6%, and 55.5%/79.0%, respectively. Whereas the percentage inhibitions of compounds 4-(4′-nitrophenoxy)nitrobenzene (**d**)/2-(4′-nitrophenoxy)nitrobenzene (**h**), which have two nitro groups in their chemical structures, on the growth of the above tested seven phytopathogenic fungi at 50 μg/ml were 13.6%/66.2%, 15.0%/49.3%, 16.7%/35.3%, 1.9%/31.4%, 57.6%/19.3%, 38.0%/9.3%, and 36.8%/8.5%, respectively. That is, introducing more than one nitro group on the phenyl ring of diaryl ethers will afford less active compounds. Meanwhile, the antifungal activity of 4-(phenoxy)nitrobenzene (**a**) having *para*-nitro group on the phenyl ring, were more potent than that of 2-(phenoxy)nitrobenzene (**k**) having *ortho*-nitro group on the phenyl ring against *F. graminearum*, *A. alternate*, *H. sorokinianum*, *F. oxysporum* f. sp. *vasinfectum*, and *F. oxysporum* f. sp. *cucumarinum* at 50 μg/ml.

Interestingly, compounds 4-(4′-*tert*-butylphenoxy)nitrobenzene (**f**)/2-(4′-*tert*-butylphenoxy)nitrobenzene (**l**)/2-(4′-*tert*-butylphenoxy)benzonitrile (**m**), having the same *tert*-butyl group on the phenyl ring, generally showed very low activity at 50 and 500 μg/ml against the seven tested phytopathogenic fungi.

It was also found that the compounds 4-(phenoxy)nitrobenzene (**a**)/4-(2′-chlorophenoxy)nitrobenzene (**b**)/4-(4′-chlorophenoxy)nitrobenzene (**c**), and 2-(phenoxy)nitrobenzene (**k**)/2-(2′-chlorophenoxy)nitrobenzene (**i**)/2-(4′-chlorophenoxy)nitrobenzene (**j**) at 50 μg/ml inhibited the growth of *F. graminearum* at 71.6%/79.5%/63.0%, and 64.9%/65.2%/59.7%, respectively, therefore introducing chloro group at the *ortho* position on the phenyl ring of compounds **a** and **k** could give more potent compounds than those having *para*-chloro group on the phenyl ring against *F. graminearum*. Especially 4-(phenoxy)nitrobenzene (**a**), the percentage inhibition of which was 81.8% at 50 μg/ml against *F. oxysporum* f. sp. *vasinfectum*, was the most potent one among all the tested compounds.

Figure 2 shows the inhibition of mycelial growth of isolated hypha of *F. graminearum* by compound **k** at different concentrations (7.8, 31.3, 62.5, 250, 500 μg/ml) as compared to the control *in vitro*. Almost complete inhibition of mycelial growth was observed at 500 and 250 μg/ml concentrations as compared to the control (full growth).

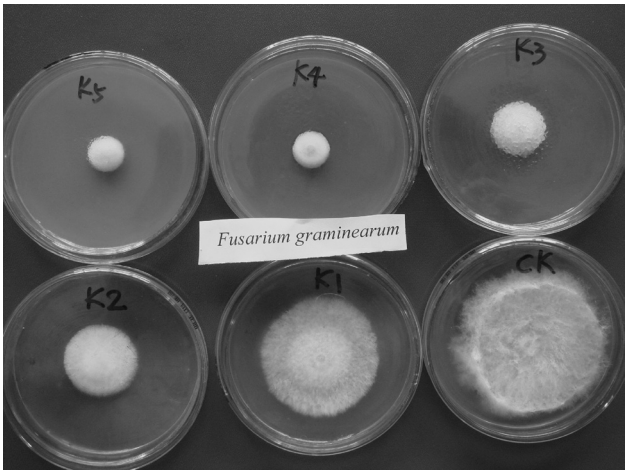

Fig. 2. Effects of Different Concentrations of Compound **k** on the Growth of *F. graminearum*

The concentrations of **CK**, **k1**, **k2**, **k3**, **k4** and **k5** are 0, 7.8, 31.3, 62.5, 250 and 500 μg/ml, respectively.

Conclusion

In conclusion, antifungal activity of the fourteen simple diaryl ethers were tested *in vitro* against seven phytopathogenic fungi. Some compounds **a**, **b**, **e**, **g**, **h**, **i**, **j**, **k** and **n** showed promising and good activity against certain phytopathogenic fungi at 50 μg/ml as displayed in Table 1. Furthermore, new analogues of the diaryl ethers will be designed in our research group in order to obtain the more potent compounds.

Experimental

Diaryl ethers **a**—**n** (Fig. 1) were prepared using our previously published method.[8] All the compounds were characterized by proton nuclear magnetic resonance (^{1}H-NMR), high resolution mass spectrometry (HR-MS) or electron ionization mass spectrometry (EI-MS), and melting point.[8]

The Typical Spectral Data of Compound **e**: White solid, mp 61.9—62.2 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.78 (3H, s), 6.88 (2H, m), 6.99 (2H, d, J=9.2 Hz), 7.05 (1H, d, J=8.0 Hz), 7.29 (1H, t, J=8.0 Hz), 8.18 (2H, d, J=9.2 Hz); HR-MS *m*/*z*: 230.0811 $[M+NH_4]^+$, Calcd 230.0812.

Compound **g**: White solid, mp 85.0—85.7 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.85 (1H, d, J=8.8 Hz), 7.01 (2H, m), 7.14 (1H, t, J=7.2 Hz), 7.35 (2H, m), 7.47 (1H, m), 7.66 (1H, dd, J=8.0, 2.0 Hz); HR-MS *m*/*z*: 247.0633 $[M+NH_4]^+$, Calcd 247.0627.

Antifungal Assay Seven phytopathogenic fungi, namely *F. graminearum*, *A. alternate*, *H. sorokinianum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *F. oxysporum* f. sp. *cucumarinum* and *A. brassicae*, were used for biological assays. The synthesized diaryl ethers **a**—**n** were screened *in vitro* for their antifungal activity against these fungi by Poisoned Food Technique.[9] Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. The test compounds were dissolved in acetone before mixing with PDA, and the final concentrations of the compounds in the medium were fixed at 50 and 500 μg/ml. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at 28±1 °C for 5 d to get new mycelium for the antifungal assays, then a mycelia disk of approximately 5 mm diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA Petri dishes. The inoculated Petri dishes were incubated at 28±1 °C for 4 d. Acetone without any compounds mixed with PDA served as control, while hymexazole, a commercial agricultural fungicide, severed as positive control. For each treatment, three replicates were conducted. The radial growth of the fungal colonies were measured and the data were statistically analyzed. The inhibitory effects of the test compounds on these fungi *in vitro* were calculated by the formula:

$$\text{inhibition rate (\%)} = (C - T) \times 100 / C$$

where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA.

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