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Synthesis of diaryl-azo derivatives as potential antifungal agents

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ABSTRACT

As compared with a commercially available agricultural fungicide hymexazol, some phenyl-azo phenol derivatives (e.g., **4a**, **4b**, **4f**, **4n**, **4q**, **4u**, and **4v**) exhibited the more promising and pronounced antifungal activities in vitro against seven phytopathogenic fungi. It seemed that 4-((un)substituted phenylazo)-phenol and 4-((un)substituted phenylazo)-3-methylphenol might be considered as new lead structures for further design of agricultural fungicides.

Many phenol derivatives (**I**, Fig. 1) including those isolated from the plants exhibited the good antifungal activity, and it suggested that the hydroxyl group of phenol analogs was required for their antifungal activity through the structure–activity relationship (SAR) study, that is, removal of the phenolic hydroxyl group would sharply lead to loss of activity.[1–4] In the meantime, it was well-known that azo compounds (**II**, Fig. 1), besides their use as dyes, showed antibacterial and antifungal activities.[5–7]

Bioisosterism is an effective and useful strategy for molecular modification and drug design.[8] In light of the above interesting biological activities, and in continuation of our program aimed at the discovery and development of bioactive molecules,[9–11] we wanted to prepare phenyl-azo-phenol derivatives (**4**, Fig. 1) as antifungal agents by combining the azo moiety with the phenolic hydroxyl group. To the best of our knowledge, little attention has been paid to the antifungal activities of simple phenyl-azo phenol derivatives against phytopathogenic fungi. Meanwhile, phytopathogenic fungi, which are hard to control, easily infect many crops, therefore the development of bioactive compounds for effective control of those agricultural diseases is highly desirable. In this Letter 23 phenyl-azo phenol derivatives (**4a–w**, Scheme 1) were designed, synthesized and evaluated in vitro for their antifungal activities against seven phytopathogenic fungi. The SAR of these compounds was also preliminarily investigated.

As shown in Scheme 1, phenylamine derivatives (**1a–f**) firstly reacted with concentrated hydrochloric acid and sodium nitrite at 0–5 °C to give the corresponding benzenediazonium chlorides (**2a–f**), which were used directly for the next step without further purification. Then the intermediates **2a–f** further reacted with phenols (**3a–e**) at 0–5 °C for 3–6 h to produce phenyl-azo phenol derivatives (**4a–w**).[12] The structures of the target compounds were well characterized by ^{1}H NMR, MS, and mp (see Supplementary data). The purities of the target compounds were all larger than 95% measured with RP-HPLC.

The antifungal activities of the 23 phenyl-azo phenol derivatives (**4a–w**) against seven phytopathogenic fungi (i.e., *Fusarium graminearum*, *Alternaria alternata*, *Bipolaris sorokinianum*, *Pyricularia oryzae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *cucumarinum*, and *Alternaria brassicae*) were investigated in vitro by poisoned food technique.[9,13] Hymexazol, a commercially available agricultural fungicide, was used as a positive control.

As outlined in Table 1, among of all the derivatives, compounds **4a**, **4b**, **4e**, **4f**, **4i**, **4j**, **4n**, **4q**, **4u**, and **4v** exhibited a good and broad spectrum of antifungal activities against the seven phytopathogenic fungi tested at the concentration of 100 μg/mL. It demonstrated that the hydroxyl group at the 4-position on the right

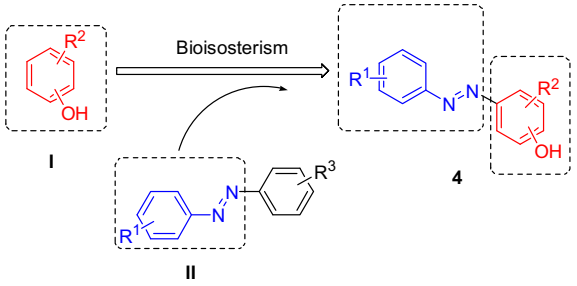

Figure 1. Design strategy of the target compounds **4**.

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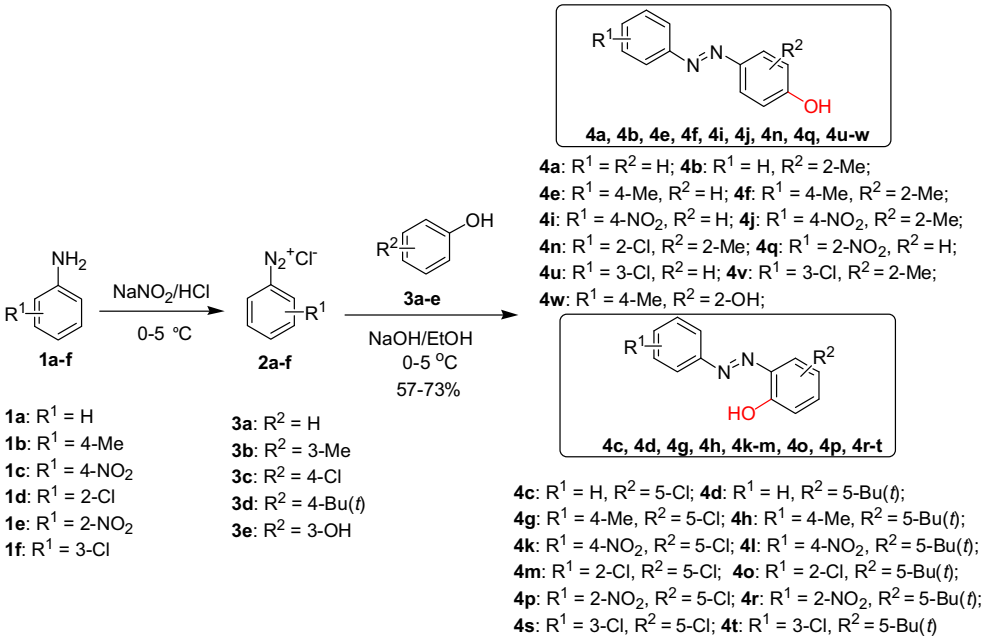

Scheme 1. The synthetic route of compounds **4a**–**w**.

phenyl ring of **4** was a very important factor for their antifungal activities. Once the hydroxyl group was introduced at the 2-position on the right phenyl ring of **4**, the antifungal activities of the corresponding derivatives were reduced even if other substituents were present on the left phenyl ring of **4**. For example, compounds **4c**, **4d**, **4g**, **4h**, **4k**–**m**, **4o**, **4p** and **4r**–**t**, all bearing 2-hydroxyl group on the right phenyl ring, showed inhibition rates against the seven phytopathogenic fungi less than 45%. Meanwhile, if methyl and hydroxyl groups were introduced at the 2- and 4-positions on the right phenyl ring of **4**, respectively, the corresponding

Table 1
Antifungal activities of compounds **4a**–**w**

Compd	Concn (μg/mL)	Antifungal activities (inhibition%)						
		F. graminearum	*P. oryzae*	*F. oxysporium* f. sp. *vasinfectum*	*A. alternata*	*A. brassicae*	*B. sorokinianum*	*F. oxysporium* f. sp. *cucumarinum*
4a	100	72.37 (±0.67)	100 (±0)	86.67 (±0)	95.43 (±0)	93.27 (±0.96)	86.84 (±1.20)	97.38 (±0)
	50	63.03 (±0.38)	87.27 (±1.16)	85.62 (±1.11)	83.51 (±1.61)	76.22 (±1.49)	77.65 (±1.18)	73.89 (±2.61)
4b	100	71.02 (±0.67)	93.73 (±0)	85.33 (±1.33)	100 (±0)	94.23 (±0)	91.15 (±1.38)	93.46 (±0)
	50	65.82 (±0.77)	92.06 (±0.67)	83.85 (±1.28)	90.06 (±1.22)	89.67 (±1.13)	83.53 (±1.18)	90.08 (±0.75)
4c	100	44.47 (±0.39)	7.89 (±1.20)	0 (±1.54)	5.25 (±1.14)	6.73 (±0.96)	1.44 (±1.83)	8.38 (±1.31)
4d	100	21.16 (±0.67)	21.53 (±1.38)	3.20 (±1.54)	9.82 (±1.14)	19.81 (±0.56)	12.20 (±0.69)	14.14 (±0.76)
4e	100	45.01 (±1.70)	79.19 (±1.38)	70.67 (±0)	79.45 (±0)	73.08 (±0.56)	70.57 (±1.38)	77.74 (±1.31)
4f	100	56.06 (±0.39)	86.84 (±1.20)	82.33 (±1.33)	92.81 (±0)	78.85 (±1.11)	79.19 (±0.69)	92.15 (±1.31)
	50	54.78 (±0)	80.79 (±1.34)	75.00 (±1.28)	86.32 (±0)	75.05 (±1.13)	69.41 (±0)	87.47 (±0.75)
4g	100	8.36 (±0)	2.63 (±0.69)	0 (±1.54)	11.42 (±0.66)	2.88 (±0.96)	15.55 (±0.69)	10.47 (±0.76)
4h	100	36.93 (±0.39)	16.75 (±0.69)	0 (±1.33)	5.25 (±1.14)	7.12 (±0.56)	1.44 (±0.69)	14.40 (±1.51)
4i	100	43.40 (±0)	66.99 (±0.69)	52.53 (±0.77)	69.63 (±1.14)	63.46 (±0.56)	68.18 (±1.38)	80.37 (±1.31)
4j	100	41.64 (±1.03)	70.81 (±0.69)	45.86 (±0.77)	69.86 (±0.66)	68.27 (±0)	58.61 (±1.38)	67.80 (±2.00)
4k	100	0.26 (±0)	3.11 (±1.20)	0 (±1.33)	2.97 (±1.14)	2.88 (±0.96)	13.88 (±1.20)	12.30 (±1.31)
4l	100	3.64 (±0)	0.72 (±1.20)	0 (±1.33)	1.14 (±1.32)	0.96 (±0.96)	4.78 (±0.69)	10.99 (±1.31)
4m	100	9.03 (±0.67)	0.72 (±1.20)	0 (±1.33)	2.51 (±1.32)	8.08 (±0.56)	3.59 (±0.69)	14.40 (±1.51)
4n	100	61.50 (±0.67)	88.04 (±0)	82.33 (±0.77)	92.12 (±0.66)	72.12 (±0.96)	86.36 (±1.38)	90.31 (±2.00)
	50	60.11 (±0.66)	86.81 (±1.34)	80.30 (±1.11)	90.91 (±0.61)	70.71 (±0.97)	80.47 (±1.36)	84.33 (±1.31)
4o	100	9.03 (±0)	10.29 (±1.20)	0 (±1.33)	1.83 (±1.14)	5.38 (±0.56)	0.72 (±0)	3.93 (±1.51)
4p	100	33.02 (±0.67)	27.03 (±1.20)	8.53 (±0.77)	31.05 (±0.66)	19.81 (±0.96)	13.40 (±1.38)	1.31 (±0.76)
4q	100	61.77 (±1.03)	85.65 (±0)	68.80 (±0.77)	79.45 (±0)	78.85 (±0.56)	62.44 (±1.83)	78.27 (±0.76)
	50	53.12 (±1.33)	69.91 (±2.31)	56.86 (±1.11)	68.29 (±0)	73.68 (±0.97)	55.29 (±0)	71.28 (±0)
4r	100	24.93 (±0.78)	12.68 (±1.20)	0 (±1.54)	12.10 (±1.14)	10.19 (±1.11)	11.96 (±1.38)	6.54 (±1.51)
4s	100	31.75 (±0.39)	16.96 (±0.73)	22.82 (±1.12)	23.18 (±0.64)	16.10 (±1.52)	17.46 (±1.20)	25.39 (±1.31)
4t	100	7.46 (±1.04)	7.59 (±0.73)	2.68 (±1.12)	8.83 (±1.69)	13.92 (±1.52)	4.78 (±1.38)	7.59 (±0.76)
4u	100	71.51 (±0.68)	89.30 (±0.73)	89.91 (±1.29)	90.41 (±1.27)	83.12 (±0.99)	88.12 (±0.69)	89.70 (±0.76)
	50	65.69 (±1.02)	87.27 (±1.16)	88.27 (±1.28)	89.01 (±0.61)	79.92 (±1.13)	86.35 (±0.68)	88.77 (±1.51)
4v	100	61.74 (±0.39)	90.57 (±1.46)	82.55 (±0.65)	89.62 (±1.69)	74.55 (±0.57)	83.97 (±0.69)	85.60 (±1.31)
	50	50.74 (±0.77)	79.86 (±1.34)	77.87 (±0)	84.14 (±1.06)	73.68 (±0.97)	77.65 (±1.18)	84.33 (±0)
4w	100	40.98 (±1.36)	27.09 (±1.46)	27.96 (±1.71)	35.98 (±1.91)	37.97 (±1.52)	25.12 (±1.38)	25.39 (±1.31)
Hym	100	66.54 (±0.39)	74.22 (±1.20)	61.74 (±1.33)	71.61 (±0.66)	55.34 (±0.96)	56.46 (±0.69)	65.18 (±1.51)
	50	54.45 (±1.15)	53.70 (±2.31)	58.63 (±0.64)	64.29 (±2.20)	46.98 (±0.56)	43.53 (±2.35)	52.48 (±1.51)

Table 2
The EC_{50} values of seven potential compounds

Compd	EC_{50}[a] (μg/mL)						
	F. graminearum	*P. oryzae*	*F. oxysporium* f. sp. *vasinfectum*	*A. alternata*	*A. brassicae*	*B. sorokinianum*	*F. oxysporium* f. sp. *cucumarinum*
4a	29.51	15.15	18.41	12.51	18.46	15.01	26.89
4b	25.02	9.72	14.98	9.32	9.42	13.08	23.05
4f	21.50	10.51	19.58	8.11	11.95	28.14	16.00
4n	25.77	13.56	21.85	11.67	13.52	17.36	18.12
4q	47.51	27.25	49.14	20.78	14.60	35.66	24.18
4u	28.62	12.38	18.14	6.96	9.46	8.54	13.57
4v	30.93	13.45	14.15	11.19	11.88	17.80	18.85
Hym	36.12	22.34	33.50	25.10	65.71	54.07	44.47

[a] 50% Effective concentration: concentration of compound that inhibits the fungi growth by 50%.

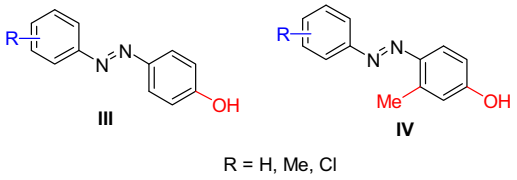

Figure 2. The potential lead structures **III** and **IV**.

compounds still exhibited potent activities (e.g., **4b**, **4f**, **4j**, **4n**, and **4v**). On the contrary, introduction of another hydroxyl group at the 2-position on the right phenyl ring of **4e** gave **4w**, the corresponding antifungal activities of which were decreased sharply as compared with **4e**. For example, the inhibition rates of **4e** and **4w** at 100 μg/mL against *F. graminearum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *A. alternata*, *A. brassicae*, *B. sorokinianum*, and *F. oxysporum* f. sp. *cucumarinum* were 45.01%/40.98%, 79.19%/27.09%, 70.67%/27.96%, 79.45%/35.98%, 73.08%/37.97%, 70.57%/25.12%, and 77.74%/25.39%, respectively. Subsequently, compounds **4a**, **4b**, **4f**, **4n**, **4q**, **4u**, and **4v** showing the higher inhibition rates at 100 μg/mL were further bioassayed at 50 μg/mL. The results showed that the seven compounds still displayed the more potent antifungal activities than the commercially available agricultural fungicide hymexazol (Table 1).

Finally, EC_{50} values of **4a**, **4b**, **4f**, **4n**, **4q**, **4u**, and **4v** were calculated for seven phytopathogenic fungi. As shown in Table 2, compounds **4a**, **4b**, **4f**, **4n**, **4u**, and **4v** were all more potent than hymexazol. Compound **4f** showed the most potent antifungal activity against *F. graminearum* with EC_{50} value of 21.50 μg/mL; compound **4b** exhibited the most potent antifungal activity against *P. oryzae* with EC_{50} value of 9.72 μg/mL; compound **4v** showed the most potent antifungal activity against *F. oxysporum* f. sp. *vasinfectum* with EC_{50} value of 14.15 μg/mL; compound **4u** was the most potent derivative against *A. alternata* with EC_{50} value of 6.96 μg/mL, which was more than threefold more potent than hymexazol; compounds **4b** and **4u** displayed the most potent antifungal activity against *A. brassicae* with EC_{50} values of 9.42 and 9.46 μg/mL, respectively, which were nearly sevenfold more potent than hymexazol; compound **4u** showed the most potent antifungal activity against *B. sorokinianum* with EC_{50} value of 8.54 μg/mL, which was more than sixfold more potent than hymexazol; compound **4u** exhibited the most potent antifungal activity against *F. oxysporum* f. sp. *cucumarinum* with EC_{50} value of 13.57 μg/mL, which was more than threefold more potent than hymexazol.

Obviously, introduction of 3-chloro group on the left phenyl ring of **4a** led to the more potent compound **4u**, and generally introduction of a nitro group on the left phenyl ring of **4a** led to less potent compounds **4i** and **4q**; introduction of the 4-nitro group on the left phenyl ring of **4b** gave less potent compound **4j**. Therefore, according to the preliminary SAR study, the potential lead structures (**III** and **IV**, R **=** H, Me, and Cl) for phenyl-azo phenol derivatives were shown in Figure 2.

In conclusion, 23 phenyl-azo phenol derivatives were synthesized and evaluated in vitro for their antifungal activities against seven phytopathogenic fungi. Among of all the derivatives, some compounds showed good antifungal activities at the concentrations of 100 and 50 μg/mL. Especially compounds **4a**, **4b**, **4f**, **4n**, **4q**, **4u**, and **4v** exhibited the more promising and pronounced antifungal activities than hymexazol, a commercially available agricultural fungicide. It indicated that 4-((un)substituted phenylazo)-phenol (**III**) and 4-((un)substituted phenylazo)-3-methylphenol (**IV**) might be considered as new promising lead candidates for further design and synthesis of agricultural fungicides.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.048.

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