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Combinatorial Synthesis of Benzimidazole-Azo-Phenol Derivatives as Antifungal Agents

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Abstract: A chemically diverse library of benzimidazole-azo-phenol derivatives was efficiently prepared and screened for their antifungal activities against five phytopathogenic fungi. Some compounds exhibited potent antifungal activities. As compared with a commercially available agricultural fungicide, hymexazol, especially compound **V-5** showed the most promising broad-spectrum antifungal activities against five phytopathogenic fungi. The EC_{50} values of **V-5** against *F. graminearum*, *A. solani*, *V. mali*, *B. cinerea*, and *C. lunata* were 0.09, 0.08, 0.06, 0.07, and 0.11 μmol/mL, respectively.

Keywords: Antifungal activity, benzimidazole-azo-phenol, phytopathogenic fungi, synthesis.

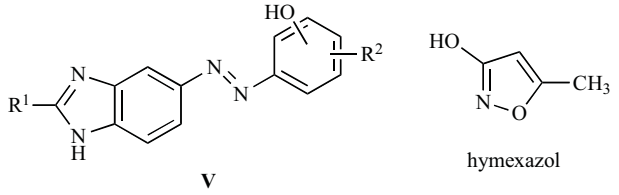
1. INTRODUCTION

The benzimidazole subunits, existing in many biologically active natural products and synthetic compounds, have recently gained widespread interest due to their key role in medically important species, such as those displaying anticancer activity [1,2], antimicrobial activity [3-5], inhibitors of hepatitis C virus NS5B polymerase [6], and p38 kinase inhibitory and anti-inflammatory activity [7]. On the other hand, phenol derivatives including those isolated from the plants displayed the antifungal activities. It suggested that the hydroxyl group of phenol analogs was required for their antifungal activities, that is, removal of the phenolic hydroxyl group would sharply lead to loss of activities [8-10]. To our knowledge, the 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 the meantime, combinatorial synthesis that makes possible to prepare a large number of compounds in a single process, has recently gained widespread interest [11-16]. In continuation of our program aimed at the discovery and development of compounds with superior bioactivity [17-20], here we combinatorially prepared a series of benzimidazole-phenol hybrids (**V**, Fig. **1**) as antifungal agents by incorporating the benzimidazole fragment with phenol moieties into a single molecular framework *via* the -N=N- bond.

2. RESULTS AND DISCUSSION

2.1. Chemistry

As shown in Scheme **1**, firstly, 1*H*-benzimidazoles (**I-1** and **I-2**) were prepared by the reaction of formic acid or acetic acid with *o*-phenylenediamine. Then nitrification reaction of **I-1** or **I-2** gave **II-1** or **II-2**, while 2-phenyl-5-nitro-1*H*-benzimidazole (**II-3**) was obtained by the reaction of benzoic acid with 4-nitro-*o*-phenylenediamine. Subsequently, reduction of **II-1**-**II-3** in the presence of $SnCl_2 \cdot 2H_2O$ afforded **III-1**-**III-3**. Finally, **III-1**-**III-3** reacted with concentrated hydrochloric acid and sodium nitrite at 0-5 ^oC to give the corresponding benzenediazonium chlorides, which further reacted with the different phenols (**IV-1**-**IV-10**) at 0-5 ^oC for 3-6 h to give benzimidazole-azo-phenol derivatives (**V-1**-**V-28**). The structures of the target compounds were well characterized by ^1H NMR, MS, and mp.



V hymexazol

Fig. (1). Chemical structures of benzimidazole-phenol hybrids (**V**) and hymexazol.

2.2. Antifungal Activities

As described in Table **1**, a series of benzimidazole-azo-phenol derivatives **V-1**-**V-28** were screened *in vitro* for their antifungal activities at 100 μg/mL against five phytopathogenic fungi such as *Fusarium graminearum*, *Alternaria solani*, *Valsa mali*, *Botrytis cinerea*, and *Curvularia lunata*. Hymexazol (Fig. **1**), a commercial agricultural fungicide, was used as a positive control. Compounds **V-4**-**V-6**, **V-10**, **V-11**, **V-18**, **V-22**, and **V-24**-**V-26** showed good antifungal activity against *A. solani*; compounds **V-4**, **V-5**, **V-8**-**V-11**, **V-19**-**V-22**, and **V-24**-**V-26** showed good antifungal activity against *V. mali*; compounds **V-5**, **V-6**, **V-10**, **V-11**, **V-19**, and **V-22**-**V-26** showed good antifungal activity against *C. lunata*; compounds **V-4** and **V-5** showed good antifungal activity against *B. cinerea*; compounds **V-4**, **V-5**, **V-8**, and **V-9** showed good antifungal activity against *F. graminearum*. Among them, compound **V-5** exhibited a good and broad-spectrum of antifungal

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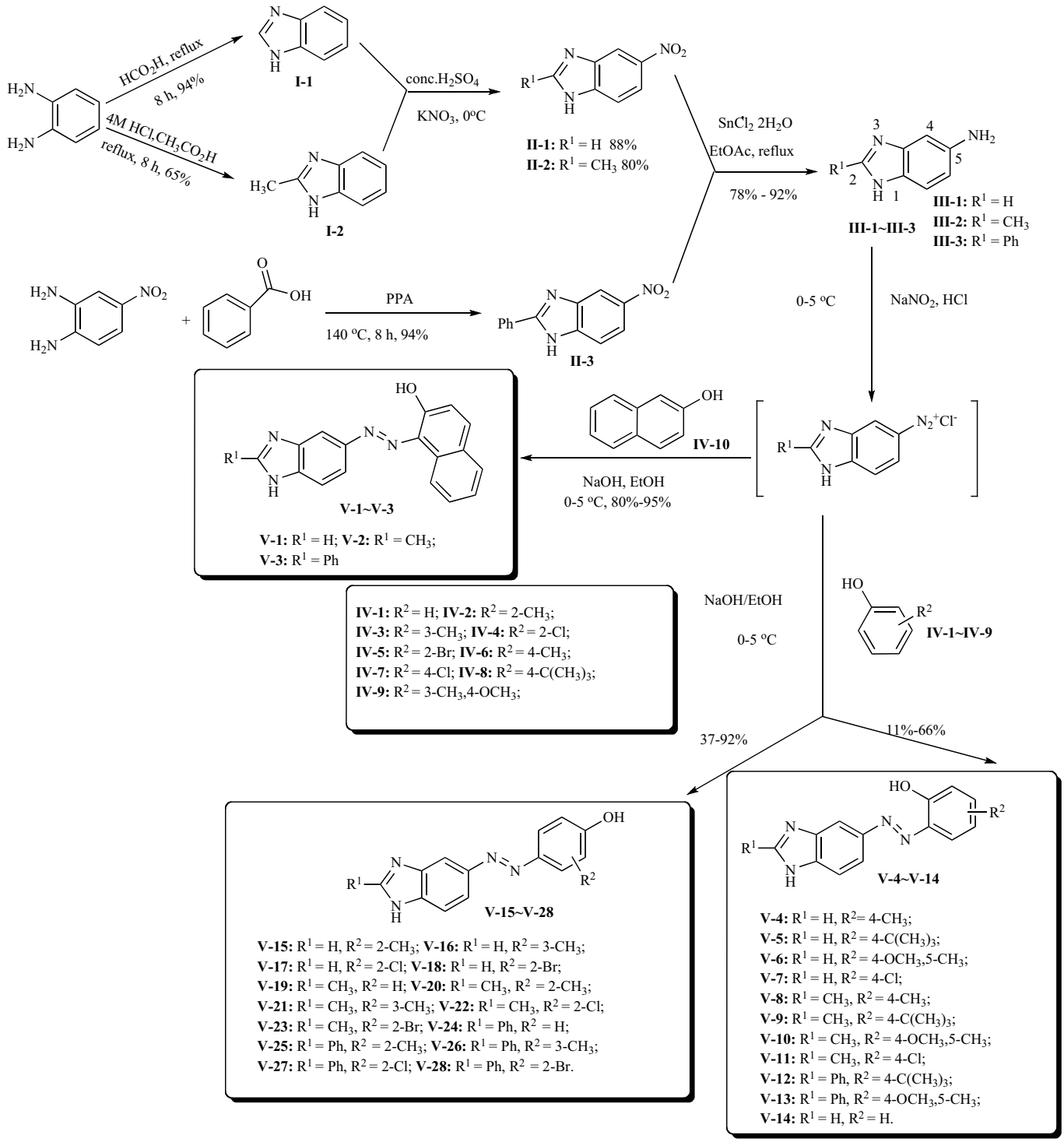

Scheme 1. The synthetic route of compounds **V-1**-**V-28**.

activities against five phytopathogenic fungi at the concentration of 100 μg/mL. The benzimidazole-azo-naphthol derivatives (**V-1**-**V-3**) generally exhibited less potent antifungal activities than benzimidazole-azo-phenol ones (**V-4**-**V-28**). To **V-4**-**V-14**, R^1 as the hydrogen atom is an important factor for their antifungal activities. For example, the inhibition rates of **V-9** and **V-12** at 100 μg/mL against *A. solani*, *V. mali*, *C. lunata, B. cinerea*, and *F. graminearum* were 40.2%/21.5%, 58.5%/29.4%, 39.2%/21.9%, 47.4%/21.5%, and 57.4%/3.9%, respectively; whereas the inhibition rates of **V-5** at 100 μg/mL against *A. solani*, *V. mali*, *C. lunata, B. cinerea*, and *F. graminearum* were 66.8%, 76.2%, 68.1%, 86%, and 84.6%, respectively. When R^1 = H, introduction of R^2 as the electron-donating groups could lead to the potent compounds (**V-4**-**V-6** *vs* **V-7**). To **V-15**-**V-28**, introduction of R^1 as the phenyl group, and R^2 as the hydrogen atom or the electron-donating groups could generally result in the potent compounds (**V-24**-**V-26**).

Table 1. Antifungal Activities of Compounds V-1-V-28 Against Five Phytopathogenic Fungi at 100 μg/mL[a]

Compounds	Antifungal Activities (Inhibition %)				
	A. solani	*V. mali*	*C. lunata*	*B. cinerea*	*F. graminearum*
V-1	13.6 (±0.8)	25.4 (±0.6)	8.1 (±0.7)	10.0 (±0.5)	5.4 (±0.7)
V-2	16.8 (±1.6)	35.0 (±1.1)	14.6 (±0.7)	13.8 (±0.5)	12.8 (±0.4)
V-3	25.2 (±0.8)	14.8 (±0.6)	1.2 (±0.7)	0.0 (±0.5)	1.4 (±0.4)
V-4	50.5 (±1.6)	70.6 (±0.5)	22.6 (±1.6)	73.1 (±0.6)	51.4 (±1.3)
V-5	66.8 (±0.8)	76.2 (±0.9)	68.1 (±1.2)	86.0 (±1.1)	84.6 (±0.6)
V-6	58.4 (±2.1)	47.7 (±0.5)	53.1 (±1.8)	15.6 (±1.1)	36.6 (±0.9)
V-7	33.2 (±0.8)	46.8 (±0.5)	44.1 (±1.2)	35.1 (±1.1)	41.0 (±0.4)
V-8	35.1 (±0.8)	57.9 (±0.5)	22.9 (±2.1)	42.5 (±1.0)	51.9 (±1.2)
V-9	40.2 (±1.6)	58.5 (±0.9)	39.2 (±1.6)	47.4 (±1.7)	57.4 (±0.6)
V-10	51.4 (±1.6)	52.9 (±0.5)	67.0 (±1.6)	21.4 (±2.0)	45.3 (±0.7)
V-11	54.7 (±1.6)	65.3 (±1.4)	74.0 (±1.0)	44.8 (±1.1)	31.7 (±0.7)
V-12	21.5 (±1.4)	29.4 (±1.8)	21.9 (±1.0)	21.5 (±1.2)	3.9 (±0.9)
V-13	25.2 (±1.6)	33.0 (±1.8)	20.8 (±1.0)	29.9 (±0.8)	11.5 (±0.7)
V-14	42.5 (±1.4)	49.7 (±1.8)	35.1 (±1.6)	24.7 (±0.5)	34.2 (±0.9)
V-15	41.6 (±0.8)	19.7 (±1.5)	42.0 (±1.6)	19.2 (±1.6)	17.1 (±0.9)
V-16	43.0 (±1.6)	26.5 (±1.0)	25.4 (±0.6)	18.1 (±0.8)	5.8 (±0.9)
V-17	19.2 (±1.6)	20.6 (±1.5)	31.2 (±1.4)	12.9 (±1.1)	6.8 (±0.8)
V-18	57.5 (±1.6)	26.7 (±1.0)	47.0 (±1.9)	24.8 (±1.6)	11.1 (±0.6)
V-19	38.3 (±2.4)	55.6 (±1.0)	75.7 (±1.2)	6.3 (±1.4)	22.8 (±0.6)
V-20	45.3 (±1.4)	67.5 (±1.5)	26.7 (±0.7)	30.1 (±0.5)	36.6 (±1.3)
V-21	35.5 (±1.4)	66.6 (±0.6)	34.0 (±1.9)	30.4 (±1.9)	36.4 (±0.6)
V-22	54.2 (±0.8)	57.6 (±1.9)	79.8 (±0.7)	33.6 (±0.5)	44.9 (±0.7)
V-23	41.1 (±1.4)	45.3 (±1.5)	72.9 (±0.7)	10.7 (±0.9)	18.9 (±0.7)
V-24	57.7 (±4.1)	60.8 (±0.6)	74.9 (±1.4)	32.0 (±1.4)	42.0 (±1.2)
V-25	70.1 (±0.8)	60.1 (±0.6)	81.8 (±0.0)	42.0 (±0.5)	38.5 (±0.4)
V-26	58.4 (±0.8)	49.8 (±0.0)	62.8 (±0.7)	22.6 (±0.5)	37.5 (±0.7)
V-27	29.5 (±0.8)	36.7 (±0.6)	44.9 (±0.7)	7.2 (±1.1)	2.1 (±0.7)
V-28	29.5 (±0.8)	41.5 (±0.6)	15.8 (±0.7)	5.7 (±0.5)	8.4 (±0.4)
Hym[b]	79.7 (±0.8)	43.5 (±0.9)	70.5 (±0.6)	79.9 (±0.6)	69.0 (±0.7)

[a]Values are means ± S.D. of three replicate.
[b]Hymexazol was used as a positive control.

Finally, the EC_{50} values of **V-5**, **V-6**, **V-11**, **V-24**, and **V-25** were further calculated against five phytopathogenic fungi as shown in Table **2**. Among them, compound **V-5** exhibited the most promising broad-spectrum antifungal activities against five fungi. The EC_{50} values of **V-5** against *F. graminearum*, *A. solani*, *V. mali*, *B. cinerea*, and *C. lunata* were 0.09, 0.08, 0.06, 0.07, and 0.11 μmol/mL, respectively. Whereas the EC_{50} values of hymexazol against *F. graminearum*, *A. solani*, *V. mali*, *B. cinerea*, and *C. lunata* were 0.42, 0.21, 1.33, 0.16, and 0.43 μmol/mL, respectively. It is noteworthy that **V-5** exhibited the most potent antifungal activity against *V. mali* with the EC_{50} value of 0.06 μmol/mL, which was more than 22-fold more potent than that of hymexazol (1.33 μmol/mL). In addition, **V-6**, **V-11**, **V-24**, and **V-25** were also showed more potent antifungal activity against *V. mali* and *C. lunata* as compared with hymexazol. For example, the EC_{50} values of **V-6**, **V-11**, **V-24**, and **V-25** against *V. mali* and *C. lunata* were 0.40/0.20, 0.25/0.20, 0.20/0.08, and 0.24/0.05 μmol/mL, respectively; whereas the EC_{50} values of hymexazol *V. mali* and *C. lunata* were 1.33, and 0.43 μmol/mL, respectively.

3. CONCLUSIONS

In summary, a chemically diverse library of benzimidazole-azo-phenol derivatives was efficiently prepared and screened for their antifungal activities against five phytopathogenic fungi. Some compounds exhibited

Table 2. EC_{50} Values of Compounds V-5, V-6, V-11, V-24, and V-25 Against Five Phytopathogenic Fungi

Compounds	EC_{50} (μmol/mL)[a]				
	A. solani	*V. mali*	*C. lunata*	*B. cinerea*	*F. graminearum*
hymexazol	0.21	1.33	0.43	0.16	0.42
V-5	0.08	0.06	0.11	0.07	0.09
V-6	0.13	0.40	0.20	-	-
V-11	0.28	0.25	0.20	-	-
V-24	0.24	0.20	0.08	-	-
V-25	0.13	0.24	0.05	-	-

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

potent antifungal activities. As compared with a commercially available agricultural fungicide, hymexazol, especially compound **V-5** showed the most promising broad-spectrum antifungal activities against five phytopathogenic fungi. It implied that **V-5** might be considered as a new promising lead candidate for further design and synthesis of agricultural fungicide.

4. MATERIALS AND METHODS

4.1. General Remarks

All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on a digital melting-point apparatus and were uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker Avance 400 MHz instrument in $CDCl_3$ or DMSO-d_6 using TMS (tetramethylsilane) as the internal standard. Electrospray iontrap mass spectrometry (ESI-TRAP-MS) was carried out with Bruker ESI-TRAP Esquire 6000 plus mass spectrometry instrument.

4.2. Synthesis of 1*H*-Benzimidazole (I-1)

A mixture of formic acid (11 mmol) and *o*-phenylenediamine (10 mmol) was refluxed for 8 h. Then the reaction mixture was cooled to room temperature and neutralized with 5N aq. NaOH. The precipitate was formed, filtered, and washed with water. Finally, 1*H*-benzimidazole (**I-1**) was obtained by recrystallization from water in 94% yield. White solid, m.p. 168-170°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.49 (s, 1H), 8.23 (s, 1H), 7.58-7.61 (m, 2H), 7.17-7.21 (m, 2H); MS (ESI): *m/z* (%) 119 ([M+H]$^+$, 100).

4.3. Synthesis of 2-Methyl-1*H*-benzimidazole (I-2)

A mixture of acetic acid (11 mmol) and *o*-phenylenediamine (10 mmol) in 9 mL of 4 N HCl was refluxed for 8 h. Then the reaction mixture was cooled to room temperature. The precipitate was formed, filtered, and washed with water. Finally, 2-methyl-1*H*-benzimidazole (**I-2**) was obtained by recrystallization from 10% aq. ethanol in 65% yield. White solid, m.p. 176-177°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 7.43-7.45 (m, 2H), 7.08-7.12 (m, 2H), 2.48 (s, 3H); MS (ESI): *m/z* (%) 133 ([M+H]$^+$, 100).

4.4. General Procedure for Synthesis of 5-Nitro-1*H*-Benzimidazole (II-1) and 2-Methyl-5-Nitro-1*H*-Benzimidazole (II-2)

To a mixture of compound **I-1**or **I-2** (7 mmol) in concentrated sulfuric acid (5 mL) at 0 °C, a solution of KNO_3 (7 mmol) in concentrated sulfuric acid (5 mL) was added dropwise for 20 min. After stirring continuously for 105 min, the reaction mixture was poured slowly to ice-water (100 mL) with stirring. The precipitated product was filtered, washed with cold water, dried over anhydrous Na_2SO_4, concentrated, and purified by PTLC to give product **II-1** in 88% yield or **II-2** in 80% yield.

*Data for **II-1***: Yellow solid, m.p. 202-204°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 1H), 8.52 (d, J = 2.0 Hz, 1H), 8.23 (dd, J = 8.8, 2.4 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H); MS (ESI): *m/z* (%) 164 ([M+H]$^+$, 100).

*Data for **II-2***: White solid, m.p. 222-224°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.92 (s, 1H), 8.36 (d, J = 2.4 Hz, 1H), 8.04 (dd, J = 8.8, 2.4 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 2.57 (s, 3H); MS (ESI): *m/z* (%) 178 ([M+H]$^+$, 100).

4.5. Synthesis of 2-Phenyl-5-Nitro-1*H*-Benzimidazole (II-3)

A mixture of 4-nitro-*o*-phenylenediamine (10 mmol), benzoic acid (11 mmol) and polyphosphoric acid (PPA, 20 g) was reacted at 140°C for 8 h. Then the reaction mixture was cooled to room temperature and neutralized with 5 N aq. NaOH. The resulting solid was filtered, and washed with water. Finally, 2-phenyl-5-nitro-1*H*-benzimidazole (**II-3**) was obtained by recrystallization from ethanol in 94% yield. White solid, m.p. 196-198°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.48 (d, J = 2.0 Hz, 1H), 8.26 (m, 2H), 8.10 (dd, J = 8.8, 2.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.54-7.63 (m, 3H); MS (ESI): *m/z* (%) 240 ([M+H]$^+$, 100).

4.6. General Procedure for Synthesis of Phenol-Azo-Benzimidazole Derivatives (V-1~V-28)

A mixture of compound **II-1**, **II-2 or II-3** (2 mmol), $SnCl_2 \cdot 2H_2O$ (10 mmol) in EtOAc (15 mL) was refluxed for 12 h. When the reaction was complete according to TLC analysis, the resulting reaction mixture was cooled to room

temperature. Subsequently, the reaction mixture was adjusted to pH 8-9 with saturated aq. $NaHCO_3$ (50 mL). Then the mixture was extracted with EtOAc (2 × 150 mL). The combined organic phase was washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated to afford compounds **III-1**, **III-2 or III-3**, which were used directly for the next step without further purification. To a mixture of 5-amino-1*H*-benzimidazole (**III-1**, **III-2 or III-3**, 2 mmol), water (5 mL) and concentrated HCl (12 mol/L, 0.51 mL) at 0°C, a solution of sodium nitrite ($NaNO_2$, 2.1 mmol) in water (10 mL) was added dropwise while maintaining the temperature below 5°C. After stirring for 20 min, a solution of diazonium chloride was prepared. Subsequently, a solution of diazonium chloride was added gradually to a mixture of phenols (**IV-1-IV-10**, 2 mmol), sodium hydroxide (NaOH, 2 mmol), ethanol (15 mL) and water (25 mL) at 0-5°C. After the addition of the above diazonium solution, the mixture was continued to stir for 3-6 h until a lot of precipitate was produced. The solid was collected, washed with water (3×10 mL), dried and purified by PTLC to give the target products **V-1~V-28**.

Data for ***V-1***: Dark red solid, yield: 80%, m.p. 204-206 °C; IR cm^{-1}: 3425, 3036, 2954, 2851, 1619, 1481, 1250, 1129, 824, 745; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 15.34 (s, 1H), 12.79 (s, 1H), 8.73 (d, J = 8.0 Hz, 1H), 8.40 (s, 1H), 8.24 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.87 (m, 2H), 7.79 (s, 1H), 7.65-7.69 (m, 1H), 7.47-7.51 (m, 1H), 7.13 (d, J = 9.2 Hz, 1H); MS (ESI): *m/z* (%) 289 ($[M+H]^+$, 100).

Data for ***V-2***: Red solid, yield: 86%, m.p. 152-154 °C; IR cm^{-1}: 3444, 3177, 3054, 2921, 2849, 1620, 1550, 1494, 1211, 1129, 825, 750; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 15.28(s, 1H), 12.55 (s, 1H), 8.73 (d, J = 8.4 Hz, 1H), 8.10 (s, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.83-7.90 (m, 2H), 7.62-7.69 (m, 2H), 7.47-7.51 (m, 1H), 7.14 (d, J = 9.2 Hz, 1H), 2.55 (s, 3H); MS (ESI): *m/z* (%) 302.3 ($[M+H]^+$, 100).

Data for ***V-3***: Dark red solid, yield: 95%, m.p. 182-184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 15.35 (s, 1H), 13.27 (s, 1H), 8.73 (d, J = 8.4 Hz, 1H), 8.22-8.24 (m, 3H), 7.99 (d, J = 9.2 Hz, 1H), 7.87-7.93 (m, 2H), 7.79 (s, 1H), 7.66-7.70 (m, 1H), 7.47-7.62 (m, 4H), 7.13 (d, J = 8.8 Hz, 1H); MS (ESI): *m/z* (%) 364.4 ($[M+H]^+$, 100).

Data for ***V-4***: Yellow solid, yield: 65%, m.p. 258-260 °C; IR cm^{-1}: 3443, 3022, 2959, 2867, 1624, 1595, 1498, 1284, 1136, 817; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.82 (s, 1H), 11.90 (s, 1H), 8.39 (s, 1H), 8.24 (s, 1H), 7.92 (dd, J = 8.8, 2.0 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.20 (dd, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 2.31 (s, 3H); MS (ESI): *m/z* (%) 352.2 ($[M+H]^+$, 100).

Data for ***V-5***: Yellow solid, yield: 63%, m.p. 196-198 °C; IR cm^{-1}: 3448, 3039, 2957, 2863, 1619, 1495, 1266, 1178, 834; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.35 (s, 1H), 8.41 (s, 1H), 8.25 (d, J = 1.6 Hz, 1H), 7.93 (dd, J = 8.8, 2.0 Hz, 1H), 7.79 (d, J =2.4 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.45 (dd, J = 8.4, 2.0 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 1.32 (s, 9H); MS (ESI): *m/z* (%) 295.3 ($[M+H]^+$, 100).

Data for ***V-6***: Dark red solid, yield: 51%, m.p. 210-212 °C; IR cm^{-1}: 3450, 3037, 2949, 1625, 1582, 1495, 1236, 1127, 868; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.80 (s, 1H), 10.91 (s, 1H), 8.32-8.38 (m, 2H), 7.92 (s, 1H), 7.73 (s, 1H), 7.30 (s, 1H), 6.89 (s, 1H), 3.82 (s, 3H), 2.20. (s, 3H); MS (ESI): *m/z* (%) 283.2 ($[M+H]^+$, 100).

Data for ***V-7***: Yellow solid, yield: 11%, m.p. 239-241 °C; IR cm^{-1}: 3415, 3023, 2956, 2867, 1622, 1585, 1482, 1285, 1183, 819, 656; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.40 (s, 1H), 8.29 (s, 1H), 7.95 (dd, J = 8.8, 2.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 7.40 (dd, J = 8.8, 2.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H); MS (ESI): *m/z* (%) 273.2 ($[M+H]^+$, 100).

Data for ***V-8***: Yellow solid, yield: 44%, m.p. 202-204 °C; IR cm^{-1}: 3388, 3025, 2974, 2866, 1624, 1625, 1593, 1498, 1279, 1025, 816; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.59 (s, 1H), 11.15 (s, 1H), 8.09 (d, J = 1.6 Hz, 1H), 7.83 (dd, J = 8.8, 2.0 Hz, 1H), 7.58-7.60 (m, 2H), 7.18 (dd, J = 8.8, 2.0 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 2.54 (s, 3H), 2.31 (s, 3H)); MS (ESI): *m/z* (%) 267.3 ($[M+H]^+$, 100).

Data for ***V-9***: Orange solid, yield: 28%, m.p. 186-88 °C; IR cm^{-1}: 3452, 3175, 2964, 2871, 1623, 1591, 1498, 1271, 1181, 822; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.56 (s, 1H), 11.41 (s, 1H), 8.10 (s, 1H), 7.83 (dd, J = 8.8, 2.0 Hz, 1H), 7.78 (d, J = 2.8 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.43 (dd, J = 8.8, 2.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 2.54 (s, 3H), 1.32 (s, 9H); MS (ESI): *m/z* (%) 309.3 ($[M+H]^+$, 100).

Data for ***V-10***: Red solid, yield: 45%, m.p. 194-196 °C; IR cm^{-1}: 3496, 3179, 2935, 2840, 1626, 1582, 1494, 1237, 1031, 874, 874; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, J = 1.6 Hz, 1H), 7.80 (dd, J = 8.4, 1.6 Hz, 1H), 7.61 (d, J = 8.8 Hz, 1H), 7.31 (s, 1H), 6.82 (s, 1H), 3.88 (s, 3H), 2.68 (s, 3H), 2.26 (s, 3H); MS (ESI): *m/z* (%) 297.3 ($[M+H]^+$, 100).

Data for ***V-11***: Orange solid, yield: 66%, m.p. 138-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.56 (s, 1H), 11.00 (s, 1H), 7.95 (s, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.72 (d, J = 8.8, 2.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 2.54 (s, 3H); MS (ESI): *m/z* (%) 287.2 ($[M+H]^+$, 100), 289.2 ($[M+H]^+$, 34).

Data for ***V-12***: Yellow solid, yield: 39%, m.p. 160-162 °C; IR cm^{-1}: 3415, 3061, 2960, 2867, 1622, 1503, 1498, 1265, 1180, 874; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.81 (s, 1H), 10.08 (s, 1H), 8.10-8.12 (m, 2H), 8.06 (s, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.87 (dd, J = 8.8, 1.6 Hz, 1H), 7.71(d, J = 8.8 Hz, 1H), 7.47-7.49 (m, 3H), 7.36 (dd, J = 8.4, 2.4 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 1.37 (s, 9H); MS (ESI): *m/z* (%) 370.4 ($[M+H]^+$, 100).

Data for ***V-13***: Yellow solid, yield: 64%, m.p. 116-118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.24-8.26 (m, 2H), 8.20 (d, J = 1.2 Hz, 1H), 7.92 (dd, J = 8.4, 2.0 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.53-7.63 (m, 3H), 7.31 (s, 1H), 6.90 (s, 1H); MS (ESI): *m/z* (%) 359.3 ($[M+H]^+$, 100).

Data for ***V-14***: Yellow solid, yield: 66%, m.p. 268-270 °C; IR cm^{-1}: 3434, 3174, 1583, 1464, 1271, 1139, 837; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.75 (s, 1H), 10.22 (s, 1H), 8.36 (s, 1H), 8.00-8.12 (m, 1H), 7.66-7.82 (m, 4H), 6.94 (d, J = 8.4 Hz, 2H); MS (ESI): *m/z* (%) 239.2 ($[M+H]^+$, 100).

Data for ***V-15***: Yellow solid, yield: 60%, m.p. 234-236 °C; IR cm^{-1}: 3442, 3272, 1614, 1591, 1474, 1266, 1126, 817; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.34 (s, 1H), 8.04 (s, 1H), 7.76 (dd, J = 8.4, 1.6 Hz, 1H), 7.69-7.71 (m, 2H), 7.73 (d, J = 8.8 Hz, 1H), 7.63 (dd, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 8.4

Hz, 1H), 2.22 (s, 3H); MS (ESI): *m/z* (%) 253.2 ([M+H]$^+$, 100).

Data for ***V-16***: Orange solid, yield: 61%, m.p. 270-272 ^{o}C; IR cm^{-1}: 3442, 3224, 1591, 1469, 1241, 1105, 849; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.72 (s, 1H), 10.08 (s, 1H), 8.36 (s, 1H), 7.99-8.11 (m, 1H), 7.78 (s, 2H), 7.61 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 8.8, 2.8 Hz, 1H), 2.65 (s, 3H); MS (ESI): *m/z* (%) 253.2 ([M+H]$^+$, 100).

Data for ***V-17***: Yellow solid, yield: 64%, m.p. 278-280 ^{o}C; IR cm^{-1}: 3425, 3278, 1621, 1590, 1484, 1297, 1139, 819, 711; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.79 (s, 1H), 11.03 (s, 1H), 8.38 (s, 1H), 8.10 (s, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.71-7.81 (m, 3H), 7.16 (d, J = 8.8 Hz, 1H); MS (ESI): *m/z* (%) 273.2 ([M+H]$^+$, 100).

Data for ***V-18***: Orange solid, yield: 40%, m.p. 270-272 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.75 (s, 1H), 8.37 (s, 1H), 8.09 (s, 1H), 8.04 (d, J = 2.0 Hz, 1H), 7.79-7.85 (m, 2H), 7.71 (s, 1H), 7.14 (d, J = 8.8 Hz, 1H); MS (ESI): *m/z* (%) 317.2 ([M+H]$^+$, 100), 319.2 ([M+H]$^+$, 92).

Data for ***V-19***: Yellow solid, yield: 61%, m.p. 264-266 ^{o}C; IR cm^{-1}: 3485, 3180, 3063, 2923, 1742, 1585, 1508, 1281, 1150, 840; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.49 (s, 1H), 10.19 (s, 1H), 7.70-7.81 (m, 5H), 6.93-6.96 (m, 2H), 2.53 (s, 3H); MS (ESI): *m/z* (%) 253.2 ([M+H]$^+$, 100).

Data for ***V-20***: Yellow solid, yield: 39%, m.p. 256-258 ^{o}C; IR cm^{-1}: 3380, 3059, 2924, 1595, 1502, 1274, 1029, 819; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.49 (s, 1H), 10.13 (s, 1H), 7.90 (s, 1H), 7.68-7.71 (m, 2H), 7.62 (dd, J = 8.4, 2.4 Hz, 1H), 7.55 (d, J = 2.4 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 2.53 (s, 3H), 2.22 (s, 3H); MS (ESI): *m/z* (%) 267.2 ([M+H]$^+$, 100).

Data for ***V-21***: Red solid, yield: 53%, m.p. 270-272 ^{o}C; IR cm^{-1}: 3439, 3193, 3063, 2921, 2829, 1736, 1587, 1446, 1244, 1021, 825; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.06 (s, 1H), 7.91 (d, J = 1.6 Hz, 1H), 7.70 (dd, J = 8.8, 1.6 Hz, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.69 (dd, J = 8.4, 2.8 Hz, 1H), 2.63 (s, 3H), 2.50 (s, 3H); MS (ESI): *m/z* (%) 267.2 ([M+H]$^+$, 100).

Data for ***V-22***: Yellow solid, yield: 37%, m.p. 196-197 ^{o}C; IR cm^{-1}: 3426, 3074, 2995, 2863, 1640, 1581, 1468, 1299, 1131, 829, 658; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.52 (s, 1H), 11.60 (s, 1H), 7.95 (s, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.8 Hz, 1H), 7.72 (dd, J = 8.4, 2.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 1.32 (s, 3H); MS (ESI): *m/z* (%) 287.2 ([M+H]$^+$, 100).

Data for ***V-23***: Yellow solid, yield: 37%, m.p. 142-144 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.54 (s, 1H), 11.05 (s, 1H), 8.03 (d, J = 2.0 Hz, 1H), 7.95 (s, 1H), 7.81 (dd, J = 8.8, 2.4 Hz, 1H), 7.72 (dd, J = 8.4, 1.6 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 2.54 (s, 3H); MS (ESI): *m/z* (%) 331.2 ([M+H]$^+$, 99), 333.2 ([M+H]$^+$, 100).

Data for ***V-24***: Orange solid, yield: 77%, m.p. 238-240 ^{o}C; IR cm^{-1}: 3404, 3061, 2923, 1586, 1503, 1242, 1143, 870; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.22 (s, 1H), 10.23(s, 1H), 8.21 (d, J = 7.2 Hz, 1H), 8.04 (s, 1H), 7.80-7.83 (m, 4H), 7.54-7.59 (m, 3H), 6.95 (d, J = 8.8 Hz, 2H). MS (ESI): *m/z* (%) 315.3 ([M+H]$^+$, 100).

Data for ***V-25***: Orange solid, yield: 92%, m.p. 192-194 ^{o}C; IR cm^{-1}: 3399, 3098, 2954, 2854, 1700, 1594, 1503, 1274, 1098, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.20 (s, 1H), 10.20 (s, 1H), 8.21-8.23 (m, 2H), 8.04 (s, 1H), 7.79 (dd, J = 8.4, 2.0 Hz, 1H), 7.72 (d, J = 1.6 Hz, 2H), 7.65-7.68 (m, 1H), 7.54-7.61 (m, 3H), 6.96 (d, J = 8.4 Hz, 1H), 2.24 (s, 3H); MS (ESI): *m/z* (%) 329.3 ([M+H]$^+$, 100).

Data for ***V-26***: Orange solid, yield: 83%, m.p. 184-185 ^{o}C; IR cm^{-1}: 3408, 3062, 2957, 2851, 1596, 1475, 1364, 1237, 1106, 862; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.21 (s, 1H), 10.08 (s, 1H), 8.21-8.23 (m, 2H), 8.03 (s, 1H), 7.80 (m, 1H), 7.73 (s, 1H), 7.53-7.65 (m, 4H), 6.80 (d, J = 2.4 Hz, 1H), 6.72 (dd, J = 8.4, 2.4 Hz, 1H), 2.66 (s, 3H); MS (ESI): *m/z* (%) 329.3 ([M+H]$^+$, 100).

Data for ***V-27***: Orange solid, yield: 44%, m.p. 156-158 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.27 (s, 1H), 8.21-8.23 (m, 2H), 8.08 (s, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.79-7.83 (m, 2H), 7.68-7.73 (m, 1H), 7.54-7.61 (m, 3H), 7.16 (d, J = 8.8 Hz, 1H); MS (ESI): *m/z* (%) 349.3 ([M+H]$^+$, 100), 351.3 ([M+H]$^+$, 33).

Data for ***V-28***: Yellow solid, yield: 62%, m.p. 220-222 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.31 (s, 1H), 8.22-8.24 (m, 2H), 8.05-8.07 (m, 2H), 7.80-7.85 (m, 2H), 7.71-7.74 (m, 1H), 7.54-7.61 (m, 3H), 7.13 (d, J = 8.4 Hz, 1H); MS (ESI): *m/z* (%) 393.3 ([M+H]$^+$, 99), 395.3 ([M+H]$^+$, 98).

4.7. Antifungal Activities Assay

A series of benzimidazole-azo-phenol derivatives **V-1-V-28** were screened *in vitro* for their antifungal activities against five phytopathogenic fungi by poisoned food technique. Five phytopathogenic fungi such as *Fusarium graminearum*, *Alternaria solani*, *Valsa mali*, *Botrytis cinerea*, and *Curvularia lunata*, were used for the assays. Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. Compounds **V-1-V-28** were dissolved in acetone before mixing with PDA, and the concentration of test compounds in the medium was fixed at 100 μg/mL. Subsequently, 50% effective concentration (EC_{50}) values of **V-5**, **V-6**, **V-11**, **V-24**, and **V-25** were further calculated. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at 28 ± 1°C for 5 days to get new mycelium for the antifungal assays, and 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 days. Acetone without any compounds mixed with PDA was served as a control, while hymexazol, a commercial agricultural fungicide, was used as a positive control. For each treatment, three replicates were conducted. The radial growths 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: Inhibition rate (%) = (C-T) × 100/C, where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA. Finally, the linear regressions of inhibition rates (%) versus seven concentrations of **V-5**, **V-6**, **V-11**, **V-24**, **V-25** and hymexazol were obtained, and the EC_{50} values were calculated against five phytopathogenic fungi.

CONFLICT OF INTEREST

The authors confirm that they do not have any conflicts of interest.

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