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Synthesis and antifungal activities of 3-substituted phthalide derivatives

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Abstract: In order to obtain novel bioactive compounds with significant antifungal activities, two series of 3-substituted phthalide derivatives were designed and synthesized via reduction, bromine substitution, and etherification. In addition, the antifungal activities of all target compounds against nine phytopathogenic fungi *in vitro* were tested by using the mycelial growth rate method at the concentration of 50 μg mL^{-1}. Preliminary bioassay tests showed that some compounds exhibited more potent antifungal activities as compared with hymexazol. The preliminary structure-activity relationships (SARs) of all target compounds were also investigated.

Keywords: antifungal activity; phthalide derivatives; phytopathogenic fungi; synthesis.

1 Introduction

Phytopathogenic fungi easily infect many crops and are often hard to control. Therefore, the development of new compounds that effectively inhibit these agricultural diseases is still highly desirable. Phthalide is an important heterocyclic scaffold found in several plants [1]. Molecules containing this pharmacophore exhibit a broad spectrum of biological activities [2], such as antioxidant [3], analgesic [4], antithrombotic [5], antiplatelet [6], insecticidal [7–9], bactericidal [10], antifungal [11], and anti-HIV [12, 13] activities. For example, 3-*n*-butylphthalide (NBP) (Fig. 1), an antiplatelet drug, which was approved in 2002 by the State Food and Drug Administration of China for the clinical treatment of acute ischemic stroke [14]. Mycophenolic acid (MPA) is in clinical trial for the prevention and reversal of transplant rejection and as an anticancer drug [15]. Corollosporine is a new phthalide derivative from the marine fungus *Corollospora maritima* which exhibits concentration-dependent antibacterial activity against *Staphylococcus aureus* and other microorganisms [16]. (±)-Chrycolide is isolated from the leaves and stem of a popular vegetable *Chrysanthemum coronarium*, which shows plant growth inhibiting activity [17].

Encouraged by the numerous pharmacological activities of phthalide derivatives, and in continuation of our ongoing work on the discovery and development of compounds with superior antifungal activities [18, 19], we prepared two series of 3-substitued phthalide derivatives and evaluated their antifungal activities against nine phytopathogenic fungi *in vitro*.

2 Results and discussion

As shown in Scheme 1, isobenzofuran-1(3*H*)-one (**1**) was prepared by the reduction of phthalic anhydride with sodium borohydride ($NaBH_4$), followed by cyclization in 3 N hydrochloric acid (HCl) at room temperature. Compound **1** was then subjected to radical bromination in the presence of N-bromosuccinimide/azobisisobutyronitrile (NBS/AIBN) to give the key intermediate **2**. Finally, two series of 3-substituted phthalide derivatives (**3a–j** and **4a–m**) were synthesized from intermediate **2** by Williamson etherification reaction in the presence of potassium carbonate (K_2CO_3). To our surprise, 5-methyl-2-(3-oxo-1,3-dihydroisobenzofuran-1-yl)-3-(3-oxo-1,3-dihydroisobenzofuran-1-yloxy)is-oxazol bromide (**3j**) was obtained when compound **2** reacted with the nucleophile 5-methylisoxazol-3-ol (**5**) under the same conditions. Closer scrutiny of this reaction revealed that it was completely determined by the reaction time. If the reaction time was prolonged to 12 h, compound **3i** was immediately and completely transformed into the unexpected quaternary salt **3j** [20]. The structures of all target compounds were characterized by

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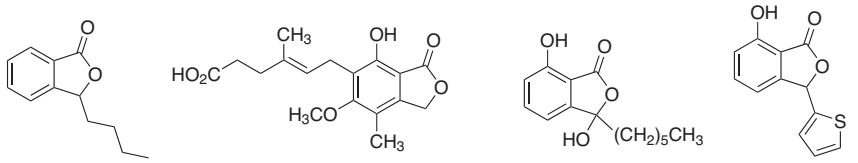
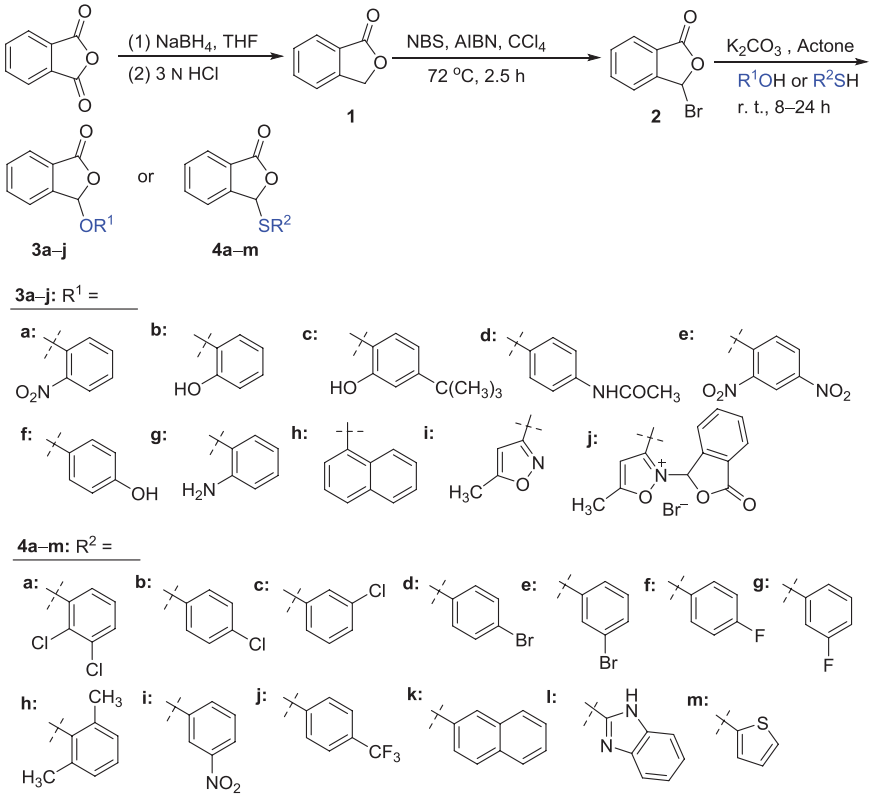
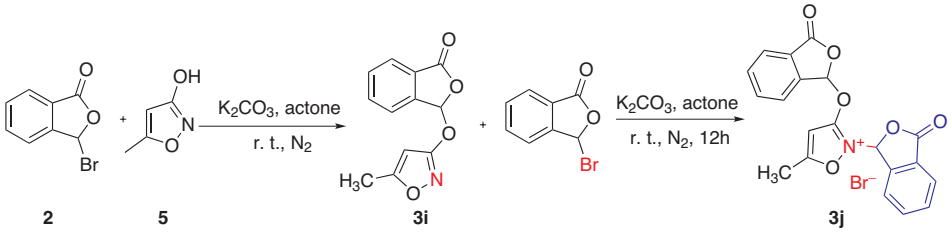


Fig. 1: Selected biologically important compounds containing a phthalide scaffold.



Scheme 1: Synthetic route of the preparation of phthalide derivatives **3a–j** and **4a–m**.



Scheme 2: The conversion of compound **3i–j**.

^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and high-resolution mass spectrometry.

The antifungal activities of 3-substituted phthalide derivatives **3a–j** and **4a–m** against nine phytopathogenic fungi [i.e. *Fusarium solani* (FS), *Botryosphaeria berengriana f.* sp. *Piricola* (BP), *Curvularia lunata* (CL), *Fusarium oxysporum schlecht. f.* sp. *C. maxima* (FM), *Fusarium graminearum* (FG), *Alternaria alternata* (AA), *Pyricularia oryzae* (PO), *Fusarium oxysporum f.* sp. *Vasinfectum* (FV), and *Alternaria brassicae* (AB)] were investigated at a

Table 1: Antifungal activities of compounds at 50 μg mL^{-1}.

Compounds	Antifungal activities (inhibition % ± SE)[a]								
	FS	FV	FM	FG	CL	BP	AA	PO	AB
3a	5 (±1)	9 (±1)	8 (±3)	10 (±1)	–	9 (±1)	6 (±1)	16 (±1)	12 (±1)
3b	45 (±2)	38 (±2)	28 (±1)	48 (±1)	38 (±1)	42 (±1)	31 (±2)	22 (±1)	29 (±2)
3c	43 (±3)	34 (±2)	47 (±2)	49 (±2)	48 (±4)	48 (±4)	43 (±1)	41 (±1)	47 (±1)
3d	14 (±1)	27 (±1)	27 (±2)	40 (±1)	29 (±1)	34 (±1)	26 (±1)	18 (±3)	20 (±1)
3e	12 (±3)	29 (±1)	33 (±1)	62 (±2)	35 (±3)	43 (±2)	26 (±2)	29 (±3)	30 (±1)
3f	58 (±1)	31 (±1)	30 (±1)	52 (±1)	32 (±1)	53 (±2)	38 (±1)	37 (±1)	32 (±1)
3g	46 (±2)	34 (±1)	32 (±1)	22 (±1)	41 (±2)	52 (±2)	24 (±3)	21 (±4)	38 (±1)
3h	2 (±2)	15 (±3)	39 (±2)	33 (±1)	22 (±2)	19 (±2)	15 (±2)	7 (±1)	24 (±1)
3i	53 (±1)	37 (±2)	36 (±2)	19 (±1)	28 (±1)	45 (±1)	54 (±1)	44 (±1)	32 (±2)
3j	34 (±1)	43 (±1)	44 (±1)	43 (±1)	43 (±1)	41 (±2)	20 (±3)	23 (±3)	35 (±1)
4a	10 (±2)	25 (±1)	29 (±1)	44 (±3)	30 (±1)	44 (±1)	28 (±1)	27 (±1)	29 (±1)
4b	17 (±1)	9 (±1)	12 (±1)	1 (±1)	22 (±2)	28 (±1)	5 (±1)	22 (±1)	15 (±1)
4c	44 (±2)	38 (±2)	35 (±1)	39 (±1)	56 (±2)	41 (±1)	50 (±1)	45 (±1)	50 (±2)
4d	2 (±2)	10 (±2)	10 (±1)	–	20 (±1)	14 (±2)	19 (±2)	18 (±3)	13 (±2)
4e	1 (±1)	13 (±1)	17 (±1)	8 (±1)	33 (±1)	13 (±2)	8 (±1)	13 (±3)	16 (±1)
4f	17 (±1)	18 (±1)	30 (±1)	16 (±3)	30 (±1)	13 (±2)	23 (±1)	24 (±1)	22 (±1)
4g	4 (±1)	15 (±1)	18 (±2)	9 (±1)	25 (±1)	20 (±2)	23 (±2)	17 (±1)	29 (±6)
4h	15 (±2)	8 (±4)	21 (±3)	5 (±2)	33 (±1)	–	20 (±1)	20 (±4)	22 (±1)
4i	29 (±2)	25 (±1)	24 (±1)	33 (±1)	33 (±1)	22 (±2)	19 (±1)	22 (±3)	31 (±1)
4j	52 (±2)	37 (±1)	50 (±1)	39 (±1)	55 (±1)	50 (±3)	43 (±1)	45 (±1)	45 (±1)
4k	51 (±1)	39 (±1)	47 (±2)	43 (±1)	61 (±1)	33 (±1)	42 (±1)	50 (±4)	47 (±1)
4l	23 (±2)	17 (±3)	18 (±1)	26 (±4)	31 (±1)	20 (±2)	11 (±2)	19 (±1)	21 (±2)
4m	31 (±2)	39 (±1)	43 (±1)	44 (±1)	42 (±1)	27 (±1)	33 (±1)	30 (±1)	36 (±2)
Hym[b]	42 (±3)	37 (±1)	51 (±1)	52 (±1)	40 (±3)	31 (±1)	69 (±1)	69 (±3)	53 (±1)

[a]Values are the mean ± SE of three replicates. [b]Hym, hymexazol.

concentration of 50 μg mL^{-1} *in vitro* by the poisoned food technique [21]. Hymexazol, a commercially available agricultural fungicide, was used as a positive control at the same concentration (50 μg mL^{-1}).

As is shown in Table 1, the results revealed that most of derivatives displayed certain inhibitory effects on the growth of the tested phytopathogenic fungi. Among all the synthesized compounds, ten derivatives **3b**, **3c**, **3e**, **3f**, **3g**, **3i**, **3j**, **4a**, **4c**, and **4j** (42, 48, 43, 53, 52, 45, 41, 44, 41, 50%, respectively) generally exhibited more pronounced antifungal activity against BP than hymexazol (31%). This finding indicated that the synthesized 3-substituted phthalide derivatives could represent a new structure skeleton for inhibiting BP. Compounds **3b**, **3c**, **3f**, **3g**, **3i**, **4c**, **4j**, and **4k** (45, 43, 58, 46, 53, 44, 52, 51%, respectively) showed relatively higher activity against FS than hymexazol (42%). Preliminary structure-activity relationships (SARs) presumed that introduction electron-donating groups -OH and -NH_2 on the benzene ring could enhance their antifungal activity against FS, probably because they form a hydrogen bond with the active site of the enzyme in the fungus leading to metabolic system disorder [22]. For FV strains, compounds **3b**, **3j**, **4c**, **4j**, **4k**, and **4m** (38, 43, 38, 37, 39, 39%, respectively) displayed slightly higher inhibition rates than hymexazol (37%). Toward FG strains, the introduction of two nitrogen dioxide (-NO_2) group or -OH group on the benzene ring give the compounds **3e** (62%) and **3f** (52%), which show better activities than hymexazol (52%). For CL strains, seven compounds **3c** (48%), **3g** (41%), **3j** (43%), **4c** (56%), **4j** (55%), **4k** (61%), and **4m** (42%) display more potent antifungal activities than hymexazol (40%). In addition, all compounds showed poor fungicidal activities against the four tested fungi (FM, AA, PO, and AB) except compounds **3c**, **3i**, **4c**, **4j**, and **4k**. Based on these results compounds **3c, 4c, 4j**, and **4k** provide broad-spectrum antifungal activities to the tested fungi and could be taken under consideration for further research.

3 Conclusion

In conclusion, 24 3-substituted phthalide derivatives were designed and synthesized via reduction, bromine substitution and Williams etherification, and evaluated *in vitro* for

their antifungal activities against nine phytopathogenic fungi at the concentration of 50 μg mL^{-1}. Among these derivatives, four compounds **3c**, **4c**, **4j**, and **4k** generally exhibit broad-spectrum antifungal activities as compared with the commercially available agricultural fungicide hymexazol. This clearly demonstrates that the introduction of appropriate thioether and oxyether substituents on the 3-position of phthalide leads to more potent antifungal derivatives.

4 Experimental section

4.1 General information

All reagents and solvents were of reagent grade or purified according to standard methods before use. Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were used with silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., China). Melting points (m. p.) were determined on a digital m.p. apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance NEO 600 MHz and 150 MHz instruments, respectively, using TMS as the internal standard and $CDCl_3$ or DMSO-d_6 as the solvent. High-resolution mass spectra (HRMS) were carried out with an APEX II Bruker 4.7T AS instrument.

4.1.1 Synthesis of isobenzofuran-1(3*H*)-one (1)

Phthalic anhydride (10.0 g, 67.5 mmol) was dissolved in tetrahydrofuran (THF) (200 mL) then sodium borohydride ($NaBH_4$) (2.6 g, 67.5 mmol) was added in portions at $T = 0$–5°C. The resulting mixture was stirred for 13 h at room temperature. The reaction mixture was acidified with 3 N HCl to pH = 1 and stirred for an additional 10.5 h. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate (2 × 250 mL), which was then washed with 10% K_2CO_3 aqueous solution (3 × 100 mL), brine (150 mL), and dried over sodium sulfate (Na_2SO_4). The solvent was evaporated to dryness under reduced pressure to give compound **1** (7.4 g, 81.9%) as a white solid; m. p. 74–75°C (lit. [23]: 72–73°C).

4.1.2 Synthesis of 3-bromoisobenzofuran-1(3*H*)-one (2)

Isobenzofuran-1(3*H*)-one (6.6 g, 49.6 mmol), NBS (9.7 g, 54.5 mmol) and AIBN (0.8 g, 4.9 mmol) were diluted in dry carbon tetrachloride (CCl_4) (200 mL) and heated to 72°C for 2.5 h. The mixture was cooled to room temperature overnight, filtered, and the residue was washed with petroleum ether. The filtrate was concentrated *in vacuo* to provide the crude product, which was recrystallized from cyclohexane to give **2** (8.6 g, 81.8%) as a light brown solid; m. p. 79–82°C (lit. [24]: 76–78°C). – ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.65$ (d, 1H, $J = 7.2$ Hz), 7.80–7.77 (m, 1H), 7.64–7.61 (m, 2H), 7.41 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 167.3$, 148.8, 135.2, 130.9, 125.9, 124.0, 123.5, 74.6.

4.2 General procedure for the synthesis of compounds 3a–j and 4a–m

In a 50 mL round-bottom flask containing 5 mL acetone, anhydrous K_2CO_3 (0.5 mmol), compound **2** (0.5 mmol) and substituted phenol or benzenethiol (0.5 mmol) were added and reacted at room temperature under N_2. When the reaction was complete (TLC control), the organic solvent was removed, followed by addition of water (20 mL). The solution was extracted with ethyl acetate (EtOAc) (3 × 30 mL). Finally, the resulting organic phases was washed with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude material was purified by silica gel column chromatography to give desired products **3a–j** and **4a–m**, which were characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

4.2.1 (*R*/*S*)-3-(2-nitrophenoxy)isobenzofuran-1(3*H*)-one (3a)

Yield: 50.1%, white solid; m. p. 192–194°C. – ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 8.03$–7.93 (m, 3H), 7.89–7.79 (m, 3H), 7.73 (dd, 1H, $J = 8.4$ Hz, 1.2 Hz), 7.42 (s, 1H), 7.41–7.37 (m, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 168.0$, 149.0, 144.4, 141.1, 136.0, 135.3, 132.3, 126.1, 125.8, 125.1, 124.6, 119.1, 100.1. – HRMS [(+)-(electrospray ionization [ESI])]: $m/z = 294.0376$ (calcd. 294.0378 for $C_{14}H_9NO_5Na$, $[M + Na]^+$).

4.2.2 (*R*/*S*)-3-(2-hydroxyphenoxy)isobenzofuran-1(3*H*)-one (3b)

Yield: 55.6%, white solid; m. p. 128–130°C. – ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 9.50$ (s, 1H, OH), 7.89–7.85 (m, 3H), 7.73–7.29 (m, 1H), 7.69–7.18 (dd, 1H, $J = 6.4$ Hz, 1.2 Hz), 7.10 (s, 1H), 6.97–6.93 (m, 1H), 6.90 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 6.79–6.75 (m, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 168.5$, 148.5, 145.2, 144.5, 135.5, 131.9, 126.7, 125.4,

125.2, 125.1, 119.7, 119.6, 117.4, 101.2. – HRMS [(+)-(ESI)]: m/z = 243.0660 (calcd. 243.0657 for $C_{14}H_{11}O_4$, $[M+H]^+$).

4.2.3 (*R*/*S*)-3-(4-*tert*-butyl-2-hydroxyphenoxy) isobenzofuran-1(3*H*)-one (3c)

Yield: 62.5%, white solid; m. p. 135–139°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.40 (s, 1H, OH), 7.90–7.89 (m, 3H), 7.77–7.73 (m, 1H), 7.15 (d, 1H, J = 7.6 Hz), 7.08 (s, 1H), 6.95 (d, 1H, J = 6.4 Hz), 6.84 (dd, 1H, J = 8.4 Hz, 2.0 Hz), 1.24 (s, 9H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.5, 147.9, 147.8, 145.2, 142.3, 135.5, 131.8, 126.7, 125.4, 125.0, 119.3, 116.4, 114.5, 101.6, 34.5, 31.7. – HRMS [(+)-(ESI)]: m/z = 299.1285 (calcd. 299.1283 for $C_{18}H_{19}O_4$, $[M+H]^+$).

4.2.4 (*R*/*S*)-*N*-(4-(3-oxo-1,3-dihydroisobenzofuran-1-yloxy)phenyl)acetamide (3d)

Yield: 54.8%, white solid; m. p. 194–196°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.99 (s, 1H, NH), 7.95–7.89 (m, 3H), 7.78–7.76 (m, 1H), 7.60 (d, 2H, J = 8.4 Hz), 7.25 (s, 1H), 7.17 (d, 2H, J = 8.4 Hz), 2.03 (s, 3H, $COCH_3$). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.5, 168.4, 152.0, 145.0, 135.7, 135.6, 132.01, 132.00, 126.4, 125.6, 125.0, 120.8, 117.7, 100.1, 24.4. – HRMS [(+)-(ESI)]: m/z = 284.0927 (calcd. 284.0923 for $C_{16}H_{14}NO_4$, $[M+H]^+$).

4.2.5 (*R*/*S*)-3-(2,4-dinitrophenoxy)isobenzofuran-1(3*H*)-one (3e)

Yield: 58.7%, white solid; m. p. 181–182°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.87 (d, 1H, J = 2.8 Hz), 8.71 (dd, 1H, J = 9.2 Hz, 2.0 Hz), 8.02–7.99 (m, 1H), 7.97–7.93 (m, 2H), 7.90 (d, 1H, J = 7.6 Hz), 7.85 (td, 1H, J = 8.8 Hz, 0.8 Hz), 7.61 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.7, 153.5, 143.8, 142.3, 140.0, 136.2, 132.6, 130.3, 126.0, 125.8, 125.2, 121.9, 118.8, 99.2. – HRMS [(+)-(ESI)]: m/z = 317.0334 (calcd. 317.0332 for $C_{14}H_9N_2O_7$, $[M+H]^+$).

4.2.6 (*R*/*S*)-3-(4-hydroxyphenoxy)isobenzofuran-1(3*H*)-one (3f)

Yield: 83.9%; m. p. 183–185°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.28 (s, 1H, OH), 7.92–7.87 (m, 3H), 7.75–7.74 (m, 1H), 7.05 (d, 2H, J = 8.4 Hz), 6.72 (d, 2H, J = 8.4 Hz), 6.56 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.4, 154.0, 150.2, 149.0, 145.1, 135.5, 131.8, 126.5, 125.4, 119.1, 116.1, 101.0. – HRMS [(+)-(ESI)]: m/z = 243.0659 (calcd. 243.0656 for $C_{14}H_{11}O_4$, $[M+H]^+$).

4.2.7 (*R*/*S*)-3-(3-aminophenoxy)isobenzofuran-1(3*H*)-one (3g)

Yield: 68.5%, white solid; m. p. 85–87°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.90 (d, 1H, J = 7.2 Hz), 7.76 (d, 1H, J = 7.2 Hz), 7.71 (d, 1H, J = 7.2 Hz), 7.64 (d, 1H, J = 7.2 Hz), 7.56 (d, 1H, J = 7.2 Hz), 7.05 (t, 1H, J = 7.8 Hz), 6.99 (t, 1H, J = 7.8 Hz), 6.92 (s, 1H), 6.87 (d, 1H, J = 7.6 Hz). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 169.8, 155.7, 142.4, 133.7, 132.8, 131.2, 130.2, 125.7, 125.1, 124.5, 121.9, 117.0, 109.7, 95.5. – HRMS [(+)-(ESI)]: m/z = 242.0819 (calcd. 242.0817 for $C_{14}H_{12}NO_3$, $[M+H]^+$).

4.2.8 (*R*/*S*)-3-(naphthalen-1-yloxy)isobenzofuran-1(3*H*)-one (3h)

Yield: 88.1%, white solid; m. p. 153–155°C. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.07 (d, 1H, J = 8.4 Hz), 8.01 (d, 2H, J = 8.4 Hz), 7.98 (t, 2H, J = 7.2 Hz), 7.83 (t, 1H, J = 7.2 Hz), 7.73 (d, 1H, J = 7.8 Hz), 7.59–7.53 (m, 3H), 7.50 (d, 1H, J = 7.8 Hz), 7.47 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.3, 152.3, 145.1, 135.8, 134.6, 132.0, 128.1, 127.2, 126.3, 125.5, 125.0, 123.4, 121.7, 110.2, 100.1. – HRMS [(+)-(ESI)]: m/z = 277.0869 (calcd. 277.0865 for $C_{18}H_{13}O_3$, $[M+H]^+$).

4.2.9 (*R*/*S*)-3-(5-methylisoxazol-3-yloxy)isobenzofuran-1(3*H*)-one (3i)

Yield: 20.3%, white solid; m. p. 153–154°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.99 (d, 1H, J = 7.8 Hz), 7.80–7.78 (m, 1H), 7.70 (t, 1H, J = 7.8 Hz), 7.63 (d, 1H, J = 7.2 Hz), 7.28 (s, 1H), 5.56 (s, 1H), 2.14 (s, 3H, CH_3). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.7, 170.9, 167.9, 142.2, 134.8, 131.1, 127.2, 125.9, 123.2, 97.9, 82.3, 13.7. – HRMS [(+)-(ESI)]: m/z = 232.0612 (calcd. 232.0610 for $C_{12}H_{10}NO_4$, $[M+H]^+$).

4.2.10 (*R*/*S*)-5-methyl-2-(3-oxo-1,3-dihydroisobenzofuran-1-yl)-3-(3-oxo-1,3-dihydroisobenzofuran-1-yloxy) isoxazol bromide (3j)

Yield: 40.2%, white solid; m. p. 161–164°C. – ^{1}HNMR (600 MHz, $CDCl_3$): δ = 8.00–7.99 (m, 1H), 7.95 (d, 1H, J = 7.8 Hz), 7.92–7.90 (m, 1H), 7.86–7.80 (m, 4H), 7.77 (t, 1H, J = 7.2 Hz), 7.69 (s, 1H), 6.12 (s, 1H), 2.22 (s, 3H). – ^{13}CNMR

(150 MHz, DMSO-d_6) δ = 168.0, 167.2, 166.6, 165.3, 165.0, 144.3, 135.8, 133.5, 132.8, 132.1, 131.0, 130.5, 130.5, 129.5, 125.8, 125.7, 124.8, 107.4, 93.9, 18.7. – HRMS [(+)-(ESI)]: m/z = 364.0846 (calcd. 364.0849 for $C_{20}H_{14}NO_6^+$, $[M]^+$).

4.2.11 (*R*/*S*)-3-(2,3-dichlorophenylthio)isobenzofuran-1(3*H*)-one (4a)

Yield: 89.1%, white solid; m. p. 162–163°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.92 (d, 1H, J = 7.8 Hz), 7.77–7.70 (m, 3H), 7.62 (t, 1H, J = 7.8 Hz), 7.42 (d, 1H, J = 8.4 Hz), 7.24 (d, 1H, J = 7.8 Hz), 6.84 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.8, 145.1, 134.6, 134.4, 133.8, 133.1, 130.6, 130.5, 130.0, 127.8, 126.1, 125.8, 123.5, 84.9. – HRMS [(+)-(ESI)]: m/z = 310.9702 (calcd. 310.9700 for $C_{14}H_9Cl_2O_2S$, $[M+H]^+$).

4.2.12 (*R*/*S*)-3-(4-chlorophenylthio)isobenzofuran-1(3*H*)-one (4b)

Yield: 70.5%, white solid; m. p. 116–118°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83 (d, 1H, J = 7.2 Hz), 7.73 (t, 1H, J = 6.6 Hz), 7.67 (d, 1H, J = 7.8 Hz), 7.56 (t, 1H, J = 7.2 Hz), 7.45–7.43 (m, 2H), 7.26–7.24 (m, 2H), 6.70 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.9, 145.8, 135.5, 135.2, 134.4, 130.1, 129.2, 128.4, 126.2, 125.6, 123.3, 86.1. – HRMS [(+)-(ESI)]: m/z = 277.0092 (calcd. 277.0090 for $C_{14}H_{10}O_2SCl$, $[M+H]^+$).

4.2.13 (*R*/*S*)-3-(2-chlorophenylthio)isobenzofuran-1(3*H*)-one (4c)

Yield: 65.7%, white solid; m. p. 85–88°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.86 (d, 1H, J = 7.8 Hz), 7.77–7.74 (m, 1H), 7.68 (d, 1H, J = 7.2 Hz), 7.58 (t, 1H, J = 7.2 Hz), 7.53 (s, 1H), 7.46 (dd, 1H, J = 7.8, 1.2 Hz), 7.30–7.29 (m, 1H), 7.26 (t, 1H, J = 7.8 Hz), 6.71 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.9, 145.6, 134.7, 134.4, 132.8, 132.6, 131.3, 130.1, 129.0, 126.2, 125.6, 123.4, 86.1. – HRMS [(+)-(ESI)]: m/z = 277.0091 (calcd. 277.0090 for $C_{14}H_{10}O_2SCl$, $[M+H]^+$).

4.2.14 (*R*/*S*)-3-(4-bromophenylthio)isobenzofuran-1(3*H*)-one (4d)

Yield: 80.6%, white solid; m. p. 139–141°C (lit. [25]: 142–144°C). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.84 (d, 1H, J = 7.8 Hz), 7.75 (t, 1H, J = 7.8 Hz), 7.67 (d, 1H, J = 7.8Hz), 7.56 (t, 1H, J = 7.2 Hz), 7.42–7.37 (m, 4H), 6.71 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.9, 145.8, 135.3, 134.4, 132.2, 129.2, 126.2, 125.6, 123.6, 123.3, 86.0. – HRMS [(+)-(ESI)]: m/z = 320.9590 (calcd. 320.9586 for $C_{14}H_{10}O_2SBr$, $[M+H]^+$).

4.2.15 (*R*/*S*)-3-(3-bromophenylthio)isobenzofuran-1(3*H*)-one (4e)

Yield: 81.0%, white solid; m. p. 110–112°C (lit. [25]: 114–116°C). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.84 (d, 1H, J = 7.2 Hz), 7.73 (t, 1H, J = 7.2 Hz), 7.65–7.64 (m, 2H), 7.56 (t, 1H, J = 7.2 Hz), 7.49–7.47 (m, 1H), 7.43–7.42 (m, 1H), 7.18 (t, 1H, J = 7.8 Hz), 6.73 (s, 1H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.9, 145.6, 135.6, 134.4, 132.8, 131.9, 131.8, 130.4, 130.3, 126.2, 125.6, 123.4, 122.6, 86.1. – HRMS [(+)-(ESI)]: m/z = 320.9591 (calcd. 320.9586 for $C_{14}H_{10}O_2SBr$, $[M+H]^+$).

4.2.16 (*R*/*S*)-3-[(4-Fluorophenyl)thio]isobenzofuran-1(3*H*)-one (4f)

Yield: 83.2%, white solid; m. p. 103–105°C (lit. [25]: 112–114°C). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.77 (d, 1H, J = 7.8 Hz), 7.72–7.69 (m, 2H), 7.65 (d, 1H, J = 7.8 Hz), 7.51 (t, 1H, J = 7.2 Hz), 7.46–7.44 (m, 2H), 6.95–6.92 (m, 1H), 6.65 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.9, 164.3, 162.6, 146.0, 136.8, 136.7, 134.3, 130.0, 126.2, 125.4, 124.6, 123.3, 116.2, 86.2. – HRMS [(+)-(ESI)]: m/z = 261.0390 (calcd. 261.0386 for $C_{14}H_{10}O_2FS$, $[M+H]^+$).

4.2.17 (*R*/*S*)-3-[(3-Fluorophenyl)thio]isobenzofuran-1(3*H*)-one (4g)

Yield: 72.5%, white solid; m. p. 91–94°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.86 (d, 1H, J = 7.2 Hz), 7.76–7.74 (m, 1H), 7.68 (d, 1H, J = 7.8 Hz), 7.58 (t, 1H, J = 7.2 Hz), 7.35–7.34 (m, 1H), 7.30–7.26 (m, 2H), 7.04–7.00 (m, 1H), 6.77 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.9, 163.2, 161.6, 145.6, 134.4, 132.7, 130.4, 128.8, 126.2, 125.6, 123.3, 120.0, 116.0, 86.0. – HRMS [(+)-(ESI)]: m/z = 261.0387 (calcd. 261.0386 for $C_{14}H_{10}O_2FS$, $[M+H]^+$).

4.2.18 (*R*/*S*)-3-(2,6-dimethylphenylthio)isobenzofuran-1(3*H*)-one (4h)

Yield: 85.4%, white solid; m. p. 128–130°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.93 (d, 1H, J = 7.2 Hz), 7.76–7.73 (m, 2H), 7.62–7.59 (m, 1H), 7.24–7.22 (m, 1H), 7.20–7.19 (m, 2H), 6.51 (s, 1H), 2.68 (s, 6H). – ^{13}C NMR (150 MHz, $CDCl_3$):

δ = 169.2, 146.4, 143.9, 134.3, 130.1, 129.8, 129.8, 128.6, 126.1, 125.6, 123.3, 88.1, 22.4. – HRMS [(+)-(ESI)]: m/z = 271.0792 (calcd. 271.0790 for $C_{16}H_{15}O_2S$, $[M+H]^+$)

4.2.19 (*R*/*S*)-3-(3-nitrophenylthio)isobenzofuran-1(3*H*)-one (4i)

Yield: 87.6%, white solid; m. p. 177–178°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.19 (dd, 2H, J = 7.2 Hz, 2.4 Hz), 7.93 (d, 1H, J = 7.8 Hz), 7.80–7.77 (m, 1H), 7.72–7.68 (m, 3H), 7.64 (t, 1H, J = 7.8 Hz), 6.89 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.5, 147.3, 145.0, 141.0, 134.7, 131.3, 130.7, 126.1, 126.0, 124.1, 123.3, 84.8. – HRMS [(+)-(ESI)]: m/z = 287.0257 (calcd. 287.0253 for $C_{14}H_{10}NO_4S$, $[M+H]^+$).

4.2.20 (*R*/*S*)-3-(4-(trifluoromethyl)phenylthio) isobenzofuran-1(3*H*)-one (4j)

Yield: 88.2%, white solid; m. p. 112–113°C (lit. [26]: mp 114.0–114.6°C). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.89 (d, 1H, J = 7.8 Hz), 7.77 (td, 1H, J = 7.8 Hz, 1.2 Hz), 7.69–7.67 (m, 3H), 7.60–7.56 (m, 3H), 6.81 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.8, 145.5, 136.1, 134.5, 132.4, 130.4, 126.2, 125.9, 125.7, 123.3, 85.6. – HRMS [(+)-(ESI)]: m/z = 311.0348 (calcd. 311.0353 for $C_{15}H_{10}F_3O_2S$, $[M+H]^+$).

4.2.21 (*R*/*S*)-3-(naphthalen-2-ylthio)isobenzofuran-1(3*H*)-one (4k)

Yield: 68.5%, white solid; m. p. 99–101°C (lit. [26]: 97.5–97.8°C). – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.05 (s, 1H), 7.79–7.70 (m, 6H), 7.56 (dd, 1H, J = 8.4 Hz, 1.8 Hz), 7.50–7.48 (m, 3H), 6.80 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.1, 146.0, 134.3, 133.4, 133.0, 132.9, 130.1, 130.0, 128.7, 127.9, 127.8, 127.7, 126.9, 126.7, 126.2, 125.5, 123.4, 86.7. – HRMS [(+)-(ESI)]: m/z = 293.0636 (calcd. 293.0641 for $C_{18}H_{13}O_2S$, $[M+H]^+$).

4.2.22 (*R*/*S*)-3-(1*H*-benzo[d]imidazol-2-ylthio) isobenzofuran-1(3*H*)-one (4l)

Yield: 64.1%, white solid; m. p. 86–88°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.94 (d, 1H, J = 7.8 Hz), 7.77–7.74 (m, 1H), 7.71 (d, 1H, J = 8.4 Hz), 7.64–7.61 (m, 3H), 7.31–7.30 (m, 2H), 7.23 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.7, 144.9, 144.1, 135.0, 130.8, 125.9, 125.7, 123.5, 123.4, 84.9. – HRMS [(+)-(ESI)]: m/z = 283.0549 (calcd. 283.0544 for $C_{15}H_{11}N_2O_2S$, $[M+H]^+$).

4.2.23 (*R*/*S*)-3-(thiophen-2-ylthio)isobenzofuran-1(3*H*)-one (4m)

Yield: 62.3%, white solid; m. p. 99–101°C. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.73–7.69 (m, 2H), 7.65 (dd, 1H, J = 7.8 Hz, 0.6 Hz), 7.50 (t, 1H, J = 7.2 Hz), 7.32 (dd, 1H, J = 7.2 Hz, 1.2 Hz), 7.12 (dd, 1H, J = 3.6 Hz, 1.2 Hz), 6.89–6.88 (m, 1H), 6.55 (s, 1H). – ^{13}C NMR (150 MHz, $CDCl_3$) δ = 168.9, 145.8, 137.6, 134.3, 132.2, 130.0, 127.7, 126.2, 125.3, 123.4, 86.2. – HRMS [(+)-(ESI)]: m/z = 249.0054 (calcd. 249.0049 for $C_{12}H_9O_2S_2$, $[M+H]^+$).

4.3 Biological assay

Twenty-four phthalide derivatives (**3a–j**, **4a–m**) were screened *in vitro* for their antifungal activities against nine phytopathogenic fungi (i.e. FS, BP, CL, FM, FG, AA, PO, FV, and AB). Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. Compounds **3a–j** and **4a–m** were dissolved in acetone before mixing with PDA, and the concentration of test compounds in the medium was fixed at 50 μg mL^{-1}. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at T = 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 addition of any compound mixed with PDA served as the control. Hymexazol, a commercially available agricultural fungicide, was used at 50 μg mL^{-1} 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 the fungi *in vitro* were calculated by the formula:

$$\text{Inhibition rate (\%)} = [(C - T) \times 100]\, C^{-1}$$

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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Graphical synopsis

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