SUPPLEMENTARY MATERIAL

Synthesis and bioactivities of Phenazine-1-carboxylic piperazine derivatives

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**Abstract:** Phenazine-1-carboxylic acid (PCA) as a natural product which has significant inhibition effects against many soil-borne fungal phytopathogens in agricultural application and has been registered in China as the fungicide against rice sheath blight. In order to find new higher fungicidal activities lead compounds and develop new eco-friendly agrochemicals, we introduced substructure piperazines which also have high biological activity into PCA, designed and synthesized a series of phenazine-1-carboxylic piperazine derivatives, and their structures were confirmed by \(^1\)H NMR and HRMS. Most compounds exhibited certain *in vitro* fungicidal activities. In particular, Compounds 5r exhibited the activity against all the tested pathogenic fungi, such as *Rhizoctonia solani*, *Alternaria solani*, *Fusarium oxysporum*, *Fusarium graminearum*, *Pyricularia oryzae* Carygro, with the EC\(_{50}\) value of 24.6μM, 42.9μM, 73.7μM, 73.8μM, 34.2μM, respectively, more potent activities than PCA (33.2μM, 81.5μM, 186.5μM, 176.4μM, 37.3μM). This result provided a highly active lead compound for the further structure optimization design.

**Keywords:** phenazine-1-carboxylic acid; piperazine; fungicidal activity; derivatives; synthesis
General chemistry method

Chemicals and solvents were purchased from commercial suppliers and were used without further purification. Solvents and reagents were abbreviated as follow: acetonitrile (CHCl₃), petroleum ether, dichloromethane (DCM), dimethylformamide (DMF), ethyl acetate (EtOAc), methanol, hydrochloric acid (HCl), sodium sulfate (Na₂SO₄), kalium carbonate (K₂CO₃). All fungi were obtained from the School of Agricultural, Yangtze University. The melting points were determined on a WRR melting point apparatus (Shanghai Jingke Industrial Co. Ltd., PR China) and modified. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Qingdao Marine Chemical Ltd., P. R. China). Column chromatography (CC) was performed over silica gel (200-300 mesh, Qingdao Marine Chemical Ltd.). ¹H NMR spectrum were recorded in CDCl₃ solution on a Bruker 600 MHz and 400 MHz spectrometer (Bruker Co., Switzerland), using tetramethyl silane (TMS) as an internal standard, and chemical shift values (δ) were given in parts per million (ppm). The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple. MS data were obtained using aAPEX IV Fourier-Transform Mass Spectrometry (Bruker).

General procedure

Synthesis of monosubstituted piperazines

Piperazine is mixed by 20 mmol in acetonitrile solution at reflux temperature, with the 30mL of dry acetonitrile and 15 mmol of K₂CO₃ as base, then added 10 mmol of RCH₂Cl with 20 mL of dry acetonitrile. The reaction was stirred for 6 h at reflux temperature. The mixture was decompressed and filtered, and filter cake was washed by use water for three three times. Generally, the Compounds of mixture was purified by silica column chromatography (methanol: ethyl acetate = 1: 5) and used in next step (Yarosh HL et al. 2007).

Synthesis of phenazine-1-carbonyl chloride

Mixed 10 mmol of phenazine-1-carboxylic acid, 0.1 mmol of DMF and 30 mL of dry DCM, cooled at 0°C, then added a solution of 15 mmol of oxalyl chloride in 20 mL of dry DCM. The reaction was stirred at reflux temperature for 8 h. The mixture was evaporated under vacuum to obtain phenazine-1-carbonyl chloride, which was dissolved in 10 mL of dry DCM and used in next step without purification (Xiong et al. 2017; Zhu et al. 2018).

Synthesis of Phenazine-1-carboxylic piperazine derivatives (5a – 5r)
10 mmol of purified compounds, monosubstituted piperazines, was dissolved in 20 mL of dry DCM, using triethylamine as base at room temperature. And then, phenazine-1-carbonyl chloride which was dissolved in 10 mL of dry DCM was dropped in slowly. The reaction was stirred at room temperature for 4 h. The mixture was extracted for three times with saturated K$_2$CO$_3$ solution, retaining the phase of DCM. Generally, the afforded crude was purified by silica column chromatography (dichloromethane : ethylacetate = 3 : 1) to give pure compound 5a – 5r (Sudha et al. 2017).

**(4-(2-methylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5a)**

Figure S1

![Chemical structure of 5a](image.png)

Yellow solid; yield: 46.00% ; m.p. 167.2-168.1 °C. 1H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.33 – 8.23 (m, 3H, phenazine-H), 7.92 – 7.82 (m, 4H, phenazine-H), 7.23 (d, $J = 7.2$, 1H, benzene-H), 7.12-7.18 (m, 3H, benzene-H), 4.09 (s, 1H, CH2), 3.98 (s, 1H, CH2), 3.52 (s, 2H, piperazine-H), 3.18 (m, 2H, piperazine-H), 2.77 (dd, $J = 7.2$, 3.6 Hz, 1H, piperazine-H), 2.72 – 2.57 (m, 1H, piperazine-H), 2.42 (dd, $J = 7.5$, 3.6 Hz, 1H, piperazine-H), 2.38 (s, 3H, CH3), 2.29 – 2.18 (m, 1H, piperazine-H). HRMS calcd for C$_{25}$H$_{24}$N$_4$O (M+H)$^+$: 397.2023, found 397.2023.

**(4-(3-methylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5b)**

Figure S2

![Chemical structure of 5b](image.png)

Yellow solid; yield: 31.33 % ; m.p. 144.3-145.7 °C. 1H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.29 (dd, $J = 8.4$, 1.4 Hz, 1H, phenazine-H), 8.25 (dd, $J = 8.7$, 1.2 Hz, 1H, phenazine-H), 8.24 – 8.20 (m, 1H, phenazine-H), 7.91 – 7.81 (m, 4H, phenazine-H), 7.21 (t, $J = 7.5$ Hz, 1H, benzene-H), 7.10 (dd, $J = 16.8$, 9.0 Hz, 3H, benzene-H), 4.14 (s, 1H, CH2), 3.97 (s, 1H, CH2), 3.54 (s, 2H, piperazine-H),
3.30 – 3.13 (m, 2H, piperazine-H), 2.85 – 2.74 (m, 1H, piperazine-H), 2.68 – 2.56 (m, 1H, piperazine-H), 2.47 – 2.39 (m, 1H, piperazine-H), 2.34 (s, 3H, CH3), 2.30 – 2.16 (m, 1H, piperazine-H). HRMS calcd for C25H24N4O (M+H)⁺: 397.2023, found 397.2023.

**(4-(4-methylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5c)**

Figure S3

Yellow solid; yield: 43.67 %; m.p, 168.3-169.8°C. 1H-NMR (600 MHz, CDCl3) δ 8.29 (dd, J = 8.4, 1.2 Hz, 1H, phenazine-H), 8.27 – 8.24 (m, 1H, phenazine-H), 8.24 – 8.19 (m, 1H, phenazine-H), 7.86 (m, J = 13.2, 6.6, 1.2 Hz, 4H, phenazine-H), 7.19 (d, J = 7.8 Hz, 2H, benzene-H), 7.13 (d, J = 7.8 Hz, 2H, benzene-H), 4.13 (s, 1H, CH2), 3.97 (d, J = 6.7 Hz, 1H, CH2), 3.53 (s, 2H, piperazine-H), 3.28 – 3.13 (m, 2H, piperazine-H), 2.82 – 2.71 (m, 1H, piperazine-H), 2.68 – 2.59 (m, 1H, piperazine-H), 2.42 (m, J = 10.2, 6.6, 3.3 Hz, 1H, piperazine-H), 2.34 (s, 3H, CH3). HRMS calcd for C25H24N4O (M+H)⁺: 397.2023, found 397.2023.

**(4-(4-isopropylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5d)**

Figure S4

Yellow solid; yield: 30.33 %; m.p, 139.2-140.3°C. 1H-NMR (400 MHz, CDCl3) δ 8.34 – 8.20 (m, 3H, phenazine-H), 7.94 – 7.81 (m, 4H, phenazine-H), 7.23 (d, J = 8.0 Hz, 2H, benzene-H), 7.16 (d, J = 8.0 Hz, 2H, benzene-H), 4.14 (s, 1H, 4-ethylbenzy-CH), 3.99 (s, 1H, Ar-CH), 3.56 (s, 2H, piperazine-H), 3.21 (d, J = 3.6 Hz, 2H, methyl-CH2), 2.78 (s, 1H, piperazine-H), 2.65 (dd, J = 7.6 Hz, 2H, piperazine-H), 2.44 (s, 1H, piperazine-H), 2.26 (s, 1H, piperazine-H), 1.24 (t, J = 7.5 Hz, 3H, CH3). HRMS calcd for C27H28N4O (M+H)⁺: 425.2335, found 425.2336.

**(4-(4-isopropylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5e)**
Yellow solid; yield: 30.33 % ; m.p, 139.2-140.3°C. $^1$H-NMR (600 MHz, CDCl$_3$) δ 8.29 (dd, J = 8.4, 1.5 Hz, 1H, phenazine-H), 8.27 – 8.22 (m, 2H, phenazine-H), 7.92 – 7.82 (m, 4H, phenazine-H), 7.23 (d, J = 8.1 Hz, 2H, benzene-H), 7.18 (d, J = 8.1 Hz, 2H, benzene-H), 4.14 (d, J = 7.2 Hz, 1H, CH$_2$), 3.97 (s, 1H, CH$_2$), 3.54 (s, 2H, piperazine-H), 3.20 (m, 2H, piperazine-H), 2.95 – 2.82 (m, 1H, piperazine-H), 2.77 (s, 1H, piperazine-H), 2.64 (s, 1H, piperazine-H), 2.43 (s, 1H, piperazine-H), 2.25 (s, 1H, piperazine-H), 1.25 (d, J = 6.9 Hz, 6H, CH$_3$). HRMS calcd for C$_{27}$H$_{28}$N$_4$O (M+H)$^+$: 425.2335, found 425.2336.

(4-(4-methoxybenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5f)

Yellow solid; yield: 45.00%; m.p, 155.6-156.8°C. $^1$H-NMR (600 MHz, CDCl$_3$) δ 8.33 – 8.17 (m, 3H, phenazine-H), 7.93 – 7.80 (m, 4H, phenazine-H), 7.22 (d, J = 8.4 Hz, 2H, benzene-H), 6.86 (d, J = 8.6 Hz, 2H, benzene-H), 4.23 – 4.09 (m, 1H, CH$_2$), 3.96 (dd, J = 9.9, 6.6 Hz, 1H, CH$_3$), 3.81 (s, 3H, OCH$_3$), 3.53 (s, 2H, piperazine-H), 3.21 (dd, J = 9.6, 5.4 Hz, 2H, piperazine-H), 2.83 – 2.71 (m, 1H, piperazine-H), 2.64 (dd, J = 9.1, 5.4 Hz, 1H, piperazine-H), 2.48 – 2.36 (m, 1H, piperazine-H), 2.25 (d, J = 10.8, 4.2 Hz, 1H, piperazine-H). HRMS calcd for C$_{25}$H$_{24}$N$_4$O$_2$ (M+H)$^+$: 413.1967, found 413.1972.

(4-(2-fluorobenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5g)
Yellow solid; yield: 21.67 % ; m.p, 150.5-152.8°C. 1H-NMR (600 MHz, CDCl3) δ 8.29 (dd, J = 8.4, 1.5 Hz, 1H, phenazine-H), 8.27 – 8.22 (m, 2H, phenazine-H), 7.94 – 7.80 (m, 4H, phenazine-H), 7.44 (dd, J = 7.5, 1.2 Hz, 1H, benzene-H), 7.35 (d, J = 7.7 Hz, 1H, benzene-H), 7.26 – 7.15 (m, 2H, benzene-H), 4.23 – 4.09 (m, 1H, CH2), 3.99 (d, J = 6.9 Hz, 1H, CH2), 3.77 – 3.63 (m, 2H), 3.30 – 3.10 (m, 2H, piperazine-H), 2.85 (m, 1H, piperazine-H), 2.78 – 2.66 (m, 1H, piperazine-H), 2.50 (m, 1H, piperazine-H), 2.37 – 2.27 (m, 1H, piperazine-H). HRMS calcld for C24H21FN4O (M+H)⁺: 401.1771, found 401.1772.

(4-(2-chlorobenzyl)piprazin-1-yl)(phenazin-1-yl)methanone(5h)
Figure S8

Yellow solid; yield: 46.00 % ; m.p, 154.3-155.9°C. 1H-NMR (600 MHz, CDCl3) δ 8.29 (dd, J = 8.4, 1.5 Hz, 1H, phenazine-H), 8.27 – 8.22 (m, 2H, phenazine-H), 7.92 – 7.82 (m, 4H, phenazine-H), 7.44 (dd, J = 7.5, 1.2 Hz, 1H, benzene-H), 7.35 (d, J = 7.8 Hz, 1H, benzene-H), 7.26 – 7.15 (m, 2H,benzene-H), 4.19 – 4.09 (m, 1H, CH2), 3.99 (d, J =6.9Hz, 1H, CH2), 3.76 – 3.63 (m, 2H, piperazine-H), 3.29 – 3.13 (m, 2H, piperazine-H), 2.85 (m, 1H, piperazine-H), 2.78 – 2.65 (m, 1H, piperazine-H), 2.50 (m, 1H, piperazine-H), 2.40 – 2.25 (m, 1H, piperazine-H). HRMS calcld for C24H21ClN4O (M+H)⁺: 417.1478, found 417.1477.

(4-(3-chlorobenzyl)piprazin-1-yl)(phenazin-1-yl)methanone(5i)
Figure S9
Yellow solid; yield: 50.67 %; m.p. 136.2-137.5°C. $^1$H-NMR (600 MHz, CDCl$_3$) δ 8.29 (dd, $J = 8.4$, 1.2 Hz, 1H, phenazine-H), 8.27 − 8.24 (m, 1H, phenazine-H), 8.24 − 8.19 (m, 1H, phenazine-H), 7.86 (m, 4H, phenazine-H), 7.19 (d, $J = 7.8$ Hz, 2H, benzene-H), 7.13 (d, $J = 7.8$ Hz, 2H, benzene-H), 4.13 (s, 1H, CH$_2$), 3.97 (d, $J = 6.6$ Hz, 1H, CH$_2$), 3.53 (s, 2H, piperazine-H), 3.28 − 3.13 (m, 2H, piperazine-H), 2.82 − 2.71 (m, 1H, piperazine-H), 2.68 − 2.59 (m, 1H, piperazine-H), 2.42 (m, 1H, piperazine-H), 2.30 − 2.21 (m, 1H, piperazine-H). HRMS calcd for C$_{24}$H$_{21}$ClN$_4$O ($M$+H)$^+$: 417.1478, found 417.1477.

(4-(4-chlorobenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5j)

Figure S10

Yellow solid; yield: 66.00 %; m.p. 163.2-164.1°C. $^1$H-NMR (600 MHz, CDCl$_3$) δ: 8.29 (d, $J = 8.4$ Hz, 1H, phenazine-H), 8.25 (d, $J = 8.4$ Hz, 1H, phenazine-H), 8.16 (d, $J = 8.4$ Hz, 1H, phenazine-H), 7.91 − 7.82 (m, 4H, phenazine-H), 7.36 (s, 1H, benzene-H), 7.13 (d, $J = 7.5$ Hz, 1H, benzene-H), 7.05 (d, $J = 9.3$Hz, 2H, benzene-H), 4.17 (s, 1H, CH$_2$), 3.94 (s, 1H, CH$_2$), 3.67 (s, 2H, piperazine-H), 3.21 (t, $J = 3.6$ Hz, 2H, piperazine-H), 2.88 − 2.78 (m, 1H, piperazine-H), 2.70 (dd, $J = 9.3$, 5.4 Hz, 1H, piperazine-H), 2.51-2.46 (m, 1H, piperazine-H), 2.39 − 2.24 (m, 1H, piperazine-H). HRMS calcd for C$_{24}$H$_{21}$ClN$_4$O ($M$+H)$^+$: 417.1478, found 417.1477.

(4-(3-nitrobenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5k)

Figure S11
Yellow solid; yield: 39.67 %; m.p, 125.4-127.3°C. $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.29 (d, $J = 8.4$ Hz, 1H, benzene-H), 8.27 – 8.19 (m, 3H, phenazine-H), 8.12 (d, $J = 8.1$ Hz, 1H, benzene-H), 7.93 – 7.80 (m, 4H, phenazine-H), 7.67 (d, $J = 7.2$ Hz, 1H, benzene-H), 7.49 (t, $J = 7.8$ Hz, 1H, benzene-H), 4.17 (s, 1H, CH$_2$), 3.97 (s, 1H, CH$_2$), 3.66 (s, 2H, piperazine-H), 3.23 (s, 2H, piperazine-H), 2.73 (d, $J = 69.6$ Hz, 2H, piperazine-H), 2.44 (s, 1H, piperazine-H), 2.28 (s, 1H, piperazine-H). HRMS calcd for C$_{24}$H$_{21}$N$_5$O$_3$ (M+H)$^+$: 428.1718, found 428.1717.

(4-(3-trifluoromethylbenzyl)piperazin-1-yl)(phenazin-1-yl)methanone (5l)

Figure S12

[Chemical structure image]

Yellow solid; yield: 41.33 %; m.p, 169.1-170.3°C. $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.34 – 8.19 (m, 3H, phenazine-H), 7.97 – 7.77 (m, 4H, phenazine-H), 7.60 (s, 1H, benzene-H), 7.53 (d, $J = 7.5$ Hz, 2H, benzene-H), 7.48 – 7.38 (m, 1H, benzene-H), 4.31 – 4.08 (m, 1H, CH$_2$), 3.99 (s, 1H, CH$_2$), 3.62 (s, 2H, piperazine-H), 3.32 – 3.14 (m, 2H, piperazine-H), 2.85 – 2.72 (m, 1H, piperazine-H), 2.66 (s, 1H, piperazine-H), 2.42 (d, $J = 6.3$ Hz, 1H, piperazine-H), 2.27 (d, $J = 4.2$ Hz, 1H, piperazine-H). HRMS calcd for C$_{24}$H$_{21}$N$_5$O$_3$ (M+H)$^+$: 451.1732, found 451.174.

(4-((6-chloropyridin-3-yl)methyl)piperazin-1-yl)(phenazin-1-yl)methanone (5m)

Figure S13

[Chemical structure image]

Yellow solid; yield: 54.67 %; m.p, 152.4-154.3°C. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 8.30 (d, $J = 2.8$ Hz, 1H, pyridine-H), 8.30 – 8.18 (m, 3H, phenazine-H), 7.92 – 7.80 (m, 4H, phenazine-H), 7.65 (dd, $J = 8.0$, 2.0 Hz, 1H, pyridine-H), 7.28 (d, $J = 3.2$ Hz, 1H, pyridine-H), 4.13 (d, $J = 14.4$ Hz, 1H, CH$_2$), 3.93 (dd, $J = 10.0$, 6.8 Hz, 1H, CH$_2$), 3.54 (d, $J = 12.8$ Hz, 2H, piperazine-H), 3.27 – 3.11 (m, 2H, piperazine-H), 2.83 – 2.69 (m, 1H, piperazine-H), 2.64 (dd, $J = 9.2$, 5.2 Hz, 1H,
piperazine-H), 2.48 – 2.33 (m, 1H, piperazine-H), 2.34 – 2.18 (m, 1H, piperazine-H). HRMS calcd for C23H21N5O (M+H)⁺: 384.1813, found 384.1819.

(4-(naphthalene-2-ylmethy)piperazin-1-yl)(phenazin-1-yl)methanone (5n)
Figure S14

Yellow solid; yield: 54.67 % ; m.p, 145.3-156.9°C. 1H-NMR (400 MHz, CDCl3) δ 8.31 (d, J = 2.0 Hz, 1H, naphthalene-H), 8.27 (m, 3H, phenazine-H), 7.93 – 7.86 (m, 4H, phenazine-H), 7.86 – 7.83 (m, 1H, naphthalene-H), 7.80 (d, J = 8.0 Hz, 1H, naphthalene-H), 7.56 – 7.46 (m, 2H, naphthalene-H), 7.40 (m, 2H naphthalene-H), 4.13 (d, J = 7.2 Hz, 1H, CH2), 4.00 (s, 1H, CH2), 3.99 (d, J = 2.4 Hz, 2H, naphthalene-H), 3.20 (d, J = 3.6 Hz, 2H, piperazine-H), 2.87 (s, 1H, piperazine-H), 2.74 (s, 1H, piperazine-H), 2.59 – 2.43 (m, 1H, piperazine-H), 2.32 (dd, J = 17.6, 12.8 Hz, 1H, piperazine-H). HRMS calcd for C28H24N4O (M+H)⁺: 433.2023, found 433.2013.

phenazin-1-yl(4-phenethylpiperazin-1-yl)methanone(5o)
Figure S15.

Yellow solid; yield: 79.00 % ; m.p, 190.5-192.6°C. 1H-NMR (600 MHz, CDCl3) δ 8.29 (d, J = 8.5 Hz, 1H, phenazine-H), 8.25 (d, J = 8.5 Hz, 1H, phenazine-H), 8.16 (d, J = 8.5 Hz, 1H, phenazine-H), 7.92 – 7.81 (m, 4H, phenazine-H), 7.36 (s, 1H, benzene-H), 7.28 (s, 2H, benzene-H), 7.13 (d, J = 7.5 Hz, 1H, benzene-H), 7.05 (d, J = 9.2 Hz, 1H, benzene-H), 4.17 (s, 1H, CH2-toluene), 3.94 (s, 1H, CH2-toluene), 3.67 (s, 2H, piperazine-H), 3.21 (t, J = 3.6 Hz, 2H, piperazine-H), 2.87 – 2.78 (m, 1H, piperazine-H), 2.70 (dd, J = 9.3, 5.4 Hz, 1H, piperazine-H), 2.48 (m, 1H, piperazine-H), 2.34 – 2.26 (m, 1H, piperazine-H), 1.27 (s, 2H CH2-benzene). HRMS calcd for C25H24N4O (M+H)⁺: 397.2024, found 397.2023.
**Phenazin-1-yl(3-phenyl)piperazin-1-yl)methanone (5p)**

Figure S16

Yellow solid; yield: 26.00 %; m.p. 116.8-117.8°C. ¹H-NMR (400 MHz, CDCl₃) δ 8.29 (m, 3H, phenazine-H), 7.93 – 7.82 (m, 4H, phenazine-H), 7.31 (s, 1H benzene-H), 7.27 (s, 1H benzene-H), 7.20 (t, J = 6.8 Hz, 3H benzene-H), 4.14 (s, 1H, benzene-H), 4.00 (s, 1H, benzene-H), 3.23 (s, 2H, piperazine-H), 2.77 (s, 1H, piperazine-H), 2.70 – 2.64 (m, 2H, piperazine-H), 2.44 (s, 2H, piperazine-H), 2.26 (s, 1H, piperazine-H), 1.85 (s, 2H, CH₂-benzene), 1.60 (s, 2H, CH₂-toluene).

HRMS calcd for C₂₆H₂₆N₄O (M+H)⁺: 411.2179, found 411.2173.

**Phenazin-1-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone (5q)**

Figure S17

Yellow solid; yield: 33.67 %; m.p. 145.3-146.9°C. ¹H-NMR (400 MHz, CDCl₃) δ 8.35 – 8.21 (m, 3H phenazine-H), 7.94 – 7.80 (m, 4H, phenazine-H), 7.30 – 7.26 (m, 3H, benzene-H), 6.97 (t, J = 7.6 Hz, 1H, benzene-H), 6.93 – 6.87 (m, 2H, benzene-H), 4.19 (s, 1H, CH₂-anisole), 4.14 (t, J = 5.6 Hz, 2H, phenol-H), 4.04 – 3.91 (m, 1H, CH₂-anisole), 3.35 – 3.15 (m, 2H, piperazine-H), 3.01 – 2.85 (m, 3H, piperazine-H), 2.81 (s, 1H, piperazine-H), 2.61 (s, 1H, piperazine-H), 2.43 (s, 1H, piperazine-H). HRMS calcd for C₂₅H₂₄N₄O₂ (M+H)⁺: 413.1972, found 413.1966.

**(4-(2-morpholinoethyl)piperazin-1-yl)(phenazin-1-yl)methanone (5r)**

Figure S18
Yellow solid; yield: 29.00 %; m.p, 137.8-138.7℃. 1H-NMR (600 MHz, CDCl₃) δ 8.36 – 8.22 (m, 3H, phenazine-H), 7.95 – 7.83 (m, 4H, phenazine-H), 4.14 (d, J = 7.2 Hz, 1H, CH2-methylmorpholine), 3.98 (dd, J = 10.0, 6.8 Hz, 1H, CH2-methylmorpholine), 3.72 (s, 4H, morpholine-H), 3.24 (dd, J = 13.2, 6.8 Hz, 2H, piperazine-H), 2.90 – 2.78 (m, 1H, piperazine-H), 2.70 (s, 1H, piperazine-H), 2.66 – 2.37 (m, 8H, morpholine-H), 2.32 (s, 1H, piperazine-H). HRMS calcd for C23H27N5O (M+H)⁺: 406.2238, found 406.2230.

**Spectra characters of target compounds**

Structures of the target compounds (5a-5r) were confirmed on basis of their spectroscopic data. In the 1H NMR spectra, the protons at C-2, C-3 and C-4 of phenazine appeared as a multiplet range from δ 8.35 to 8.20 ppm, the protons at C-6, C-7, C-8 and C-9 of phenazine appeared as a multiplet range from δ 7.95 to 7.80 ppm. The two protons of CH₂ appeared as singlet near δ 4.00 ppm. The 1H NMR and HR-MS data of the target compounds 5a to 5r are presented in more detail in the “Supplementary Material”.

**Bioassays of fungicidal activities**

All synthesized compounds were screened for their antifungal activities *in vitro* against five fungi, including *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria solani*, *Fusarium graminearum* and *Pyricularia oryzae Cavgra* by using the mycelium growth rate method (Shentu et al. 2014). The synthesized compounds were dissolved in acetone (1 mL), then diluted with aqueous 1% Tween 80 as solutions which were then added to sterile potato dextrose agar (PDA, 49 mL). The compounds were tested at a concentration of 200 μM (the concentration of PCA = 44.8 mg/L) for primary screening. Before the antifungal assays were conducted, All fungi species were cultivated in PDA at 28 ± 1 °C for 2 hours to produce new mycelium. Next, mycelia dishes of about 7 mm diameter were cut from the culture medium and were picked up with a sterilized inoculating needle and inoculated in the center of the PDA plates. The inoculated plates were incubated at 28 ± 1 °C for 24 hours. Acetone in sterile aqueous 1 % Tween 80 was served as the
negative control, whereas the PCA was assessed under the same conditions as positive controls. Each sample was screened in three replicates and each colony diameter of the three replicates was measured four times by the cross-bracketing method. The data was statistically analysed and the corrected inhibitory rates (I) were calculated using the following formula:

$$I(\%) = \frac{[(C - T) \times (C - 7\, \text{mm})]}{100}$$

(where C represents the average diameter of mycelia in the blank test, T represents the average diameter of mycelia on treated PDA media.)

The results of the antifungal tests can be found summarized in Table 1.

The assay of the fungicidal activity was tested by hyphae growth velocity (Shentu et al. 2014). The sample caused an effective concentration of 50% inhibition of mycelial growth (EC$_{50}$) was determined. The commercial fungicidal agent phenazine-1-carboxylic acid was used as positive control. Mycelial discs (7 mm in diameter) of phytopathogenic fungi grown on PDA were cut from the margins of the colony and placed on the same medium containing different concentrations of the sample. A negative control was maintained with sterile water mixed with PDA medium. Each treatment had three replicates. The diameter of colony growth was measured when the fungal growth in the control had completely covered the petri dishes. The inhibition percentage of mycelial growth was calculated as follows:

$$\text{Mycelial growth inhibition (\%) } = \frac{(D_a - D_b)}{D_a} \times 100$$

(where $D_a$ represents control colony diameter and $D_b$ represents treated colony diameter. The colony diameter is in millimeters.)

All statistical analysis was performed using EXCEL 2010 software. The log dose-response curves allowed determination of the EC$_{50}$ for the fungi bioassay according to probit analysis. The 95% confidence limits for the range of EC$_{50}$ values were determined by the least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration. The EC$_{50}$ values of 5r were calculated and can be found in Table 2.

**Spectra of target compounds**

*Spectra of compound 5a*

$^1H$ NMR spectrum

Figure S19
**HRMS Spectrum**

Figure S20

SQBQ001 #67  RT: 0.66  AV: 1  NL: 1.97E10
T: FTMS + p ESI Full ms [100.00-1500.00]

**Spectra of compound 5b**

\(^1\)H NMR spectrum

Figure S21
**Spectra of compound 5c**

\(^1\)H NMR spectrum

Figure S23
**HRMS Spectrum**

Figure S24

**Spectra of compound 5d**

$^1H$ NMR spectrum

Figure S25
HRMS Spectrum

Figure S26

SQBQ005 #259 RT: 2.57 AV: 1 NL: 1.75E10
T: FTMS + p ESI Full ms [100.00-1500.00]

425.2355

Spectra of compound 5e

$^1$H NMR spectrum

Figure S27
HRMS Spectrum
Figure S28

SQBQ004 #273  RT: 3.50  AV: 1  NL: 9.31E7
T: FTMS + p ESI Full ms [100.00-1500.00]

Spectra of compound 5f

$^1H$ NMR spectrum

Figure S29
**HRMS Spectrum**

Figure S30

SQBQ06_170703113925 #87 RT: 0.91 AV: 1 NL: 3.06E8
T: FTMS + p ESI Full ms [100.00-1500.00]

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**Spectra of compound 5g**

$^1H$ NMR spectrum

Figure S31
**Spectra of compound 5h**

*1H NMR spectrum*

Figure S33
HRMS Spectrum

Figure S34

SQBQ009 #73  RT: 0.72  AV: 1  NL: 1.78E10
T: FTMS + p ESI Full ms [100.00-1500.00]

417.1478

419.1443

420.1463

Spectra of compound 5i

$^1$H NMR spectrum

Figure S35
HRMS Spectrum
Figure S36

Spectra of compound 5j

$^1$H NMR spectrum

Figure S37
HRMS Spectrum

Figure S38

Spectra of compound 5k

\(^1\)H NMR spectrum

Figure S39
Spectra of compound 5l

$^1$H NMR spectrum

Figure S41
HRMS Spectrum
Figure S42
SQBQ015 #321  RT: 4.27  AV: 1  NL: 4.93E7
T: FTMS + p ESI Full ms [100.00-1500.00]

Spectra of compound 5m
$^1$H NMR spectrum
Figure S43
**HRMS Spectrum**

Figure S44

SQBQ016_170703114618 #317 RT: 3.30 AV: 1 NL: 1.00E7
T: FTMS + p ESI Full ms [100.00-1500.00]

**Spectra of compound 5n**

$^1$H NMR spectrum

Figure S45
HRMS Spectrum

Figure S46

Spectra of compound 5o

$^1$H NMR spectrum
HRMS Spectrum

Figure S48

SQB0012 #69  RT: 0.68  AV: 1  NL: 2.46E10
T: FTMS + p ESI Full ms [100.00-1500.00]

Spectra of compound 5p

$^1$H NMR spectrum

Figure S49
HRMS Spectrum

Figure S50

SQBQ013 #255  RT: 3.31  AV: 1  NL: 1.52E8
T: FTMS + p ESI Full ms [100.00-1500.00]

$^1$H NMR spectrum

Figure S51
HRMS Spectrum

Figure S52

SQBQ020 #283 RT: 3.72 AV: 1 NL: 8.81E7
T: FTMS + p ESI Full ms [100.00-1500.00]

413.1966

Spectra of compound 5q

\(^1\)H NMR spectrum

Figure S53
HRMS Spectrum

Figure S54

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