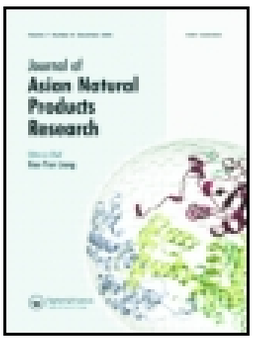

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Synthesis and antifungal evaluation of PCA amide analogues

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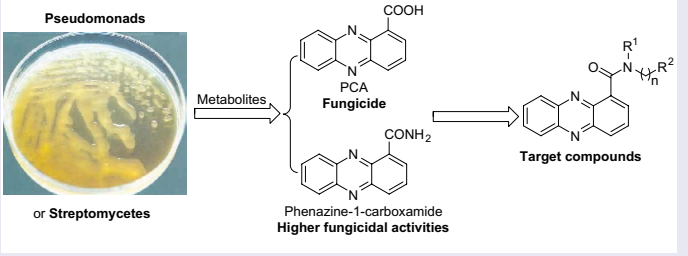
ABSTRACT

To improve the physical and chemical properties of phenazine-1-carboxylic acid (PCA) and find higher antifungal compounds, a series of PCA amide analogues were designed and synthesized and their structures were confirmed by ^{1}H NMR, HRMS, and X-ray. Most compounds showed some antifungal activities *in vitro*. Particularly, compound **3d** exhibited inhibition effect against *Pyriculariaoryzac Cavgra* with EC_{50} value of 28.7 μM and compound **3q** exhibited effect against *Rhizoctonia solani* with EC_{50} value of 24.5 μM, more potently active than that of the positive control PCA with its EC_{50} values of 37.3 μM (*Pyriculariaoryzac Cavgra*) and 33.2 μM (*Rhizoctonia solani*), respectively.



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Phenazine-1-carboxylic acid (PCA); amide; analogue; antifungal activity; synthesis

1. Introduction

Phenazine-1-carboxylic acid (PCA) (Figure 1) is a very promising natural product occurring in microbial metabolites of *Streptomycetes* and *Pseudomonads*, which has been proved having antifungal, antitumor, and antileukemia effects [1,2]. Particularly, in recent years, PCA has received significant attention extensively because of its remarkable inhibition effects against many soil-borne fungal phytopathogens in agricultural application [3–6]. It has been registered as the biofungicide "Shenqinbactin" in China and is noted for its high antifungal efficiency, low toxicity to human and animals, friendliness to environment, and improvement of crop production [7–11]. But PCA is difficult to dissolve in any solvents

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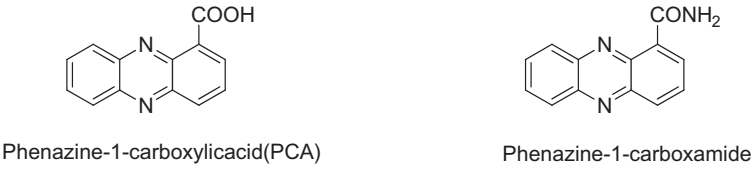


Figure 1. The structures of PCA and phenazine-1-carboxamide.

whcich cause it to become hard to be absorbed by the object and produce suitable dosage form [12]. As a very important class of PCA's derivatives, phenazine-1-carboxamines (Figure 1) also have excellent antifungal activities [13–17], such as phenazine-1-carboxamide, which showed more potent activity against *Rhizoctonia solani* than Carbendazim and equal to Rifamycin against *Xanthomonas oryzae pv. oryzae* at the concentration of 5 mg/L [13].

It's well known that the appropriate oil–water amphiphilicity of a compound is a very important factor to influence its efficacy [18,19]. To improve the physical and chemical properties of phenazine-1-carboxylic acid (PCA) and find higher antifungal compounds, we have designed and synthesized a series of PCA amide analogues, including fifteen novel compounds and three known compounds (**3a** [16], **3p** [14] and **3q** [20]). Different amines, such as aromatic amines, heterocyclic amines, aryl aliphatic amines and diamines, were taken into consideration respect to structure and chemical diversity. The antifungal activities of all the synthesized compounds were tested and the primary structure–activity relationship was discussed.

2. Results and discussion

Synthesis of the phenazine-1-carboxamines was achieved in Scheme 1. PCA was treated with oxalyl chloride at reflux temperature in CH_2Cl_2 solution for 8 hours, and intermediate **2** was afforded after the evaporation of CH_2Cl_2 [21,22]. **2** was allowed to react with the corresponding amines to give **3a–3r** [14,16]. (Analysis data of all the synthesized compounds are available in Supplementary Information).

To determine the stereo structure of the target compounds, a single crystal of the target compound **3p** (n = 2, R^1 = H, R^2 = Ph) was prepared by slow evaporation of a solution of compound **3p** in ethyl acetate at room temperature. As shown in Figure 2, the crystal data for **3p**: triclinic, space group P-1, a = 11.283(2) Å, b = 11.499(2) Å, c = 14.726(3) Å, a = 68.211(3)°, b = 74.173(3)°, g = 76.611(3)°, V = 1688.6(6) Å^3, Z = 2, T = 296(2) K, μ(Mo)=0.085 mm^{-1}, $D_{calcd.}$ = 1.323 Mg/m^{3,12}124 reflections measured (1.522 ≤ 2Θ ≤ 25.499°), 6207 unique (R(int) = 0.0269) which were used in all calculations. The final R_1 was 0.0520 (I > 2 sigma (I)) and wR_2 was 0.1541. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, and the deposition number was CCDC 1563916.

The antifungal activities of all target compounds *in vitro* were screened against *Rhizoctonia solani, Fusarium oxysporum, Alternaria solani, Fusarium graminearum*, and *Pyricularia oryzac* Cavgra [23,24], and the Log K_{ow} value of all target compounds were calculated by the ALOGPS 2.1 program, as shown in Table 1. The preliminary bioassay results showed that all compounds exhibited moderate antifungal activities against the pathogenic fungi at the concentration of 200 μM (this concentration of PCA equal to 44.8 mg/L). In particular, compounds **3d** and **3 g** showed great antifungal activities against *Pyricularia oryzac* Cavgra

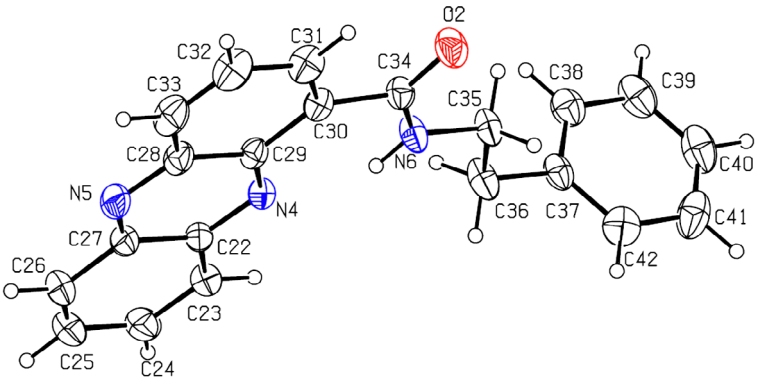


Figure 2. The X-ray crystal structure of **3p**.

with inhibition rate of more than 85%, and compounds **3 m** and **3q** exhibited excellent antifungal activities against *Rhizoctonia solani* with inhibition rate of more than 90%. When n = 0, compared to **3a** (R^1 = H, R^2 = Ph), R^2 is substituted phenyl or heterocyclic group, such as pyridinyl, isoxazolyl, and benzothiazolyl, or the H atom of NH substituted by CH_3 or $CH_2COOC_2H_5$, the majority of the tested antifungal activities increased to some degree, especially compounds **3d** showed the best activity (94.5%) against *Pyricularia oryzac* Cavgra which was higher than PCA .The results showed that the value of "n" also affected the activities of the target compounds. N = 0 (**3a, 3b, 3c**) and n = 1 (**3j, 3 k, 3 l**) have equivalent antifungal activities, however when n increased to 2, the compounds (**3p**) exhibited the best activities, and the antifungal activities declined as n = 3 (**3r**). Significantly, the F atom of aryl group can help to increase the antifungal activities, such as **3d, 3 m** and **3n**. Especially compound **3 m** showed remarkable activity against *Pyricularia oryzac* Cavgra with inhibition rate of 90.6%. In the halogenated compounds, the *ortho*-position substituted by halogen (**3j, 3 m**) was beneficial to increase their antifungal activities.

Meanwhile, it was another very important factor in the influence factors of antifungal activities against *Rhizoctonia solani*, hydrophilicity. In the compounds with "n = 1" (**3j**~**3o**), the Log K_{ow} value of compounds plays very important role in antifungal activities, the Log K_{ow} value showed **3 m** < **3n** < **3j** < **3 l** < **3 k** < **3o**, the antifungal activities against *Rhizoctonia solani* exhibited **3 m** > **3n** > **3j** > **3 l** > **3 k** > **3o**, as shown in Figure 3. Compound **3q** with "n = 2" has the higher hydrophilicity than compound **3p**, and showed better antifungal activities against *Rhizoctonia solani* than compound **3p** in which the value of "n" is 2 also.

These compounds, such as **3d**, **3 g**, **3 m**, and **3q**, with inhibitory activities over 85% at the concentration of 200 μM, were chosen to determine EC_{50} values [23,24]. The results were shown in Table 2. Compounds **3d** and **3 g** showed the antifungal activities against *Pyricularia oryzac* Cavgra with the EC_{50} values of 28.7 and 89.3 μM. Compounds **3 m** and **3q** exhibited the antifungal activities against *Rhizoctonia solani* with the EC_{50} values of 39.6 μM and 24.5 μM. Especially, compound **3d** against *Pyricularia oryzac* Cavgra and **3q** against *Rhizoctonia solani* were more potently active than that of the positive control PCA with its EC_{50} values of 33.2 μM (*Rhizoctonia solani*) and 37.3 μM (*Fusarium graminearum*), respectively.

In this study, a series of phenazine-1-carboxamines were designed, synthesized, and their structures were confirmed by ^{1}HNMR spectrum, elemental analysis measured with

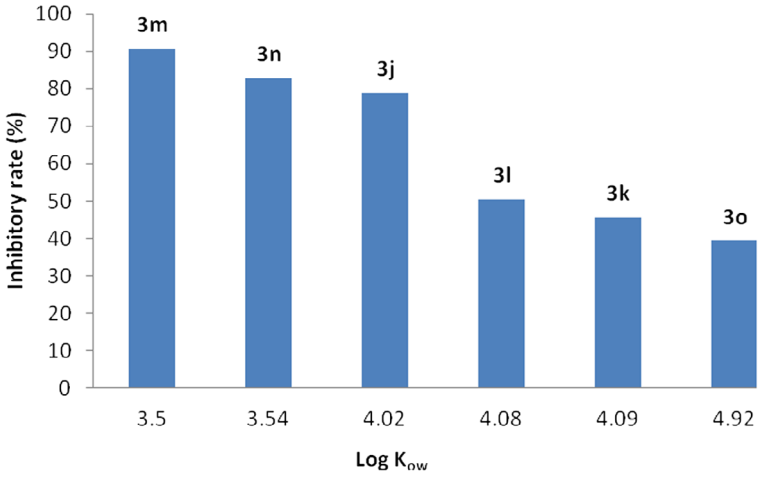
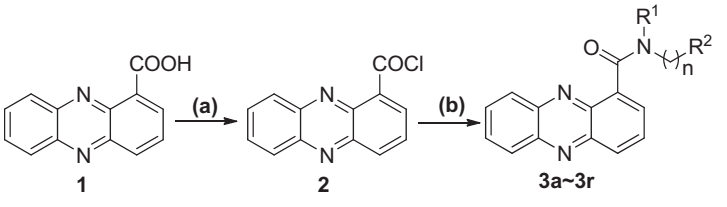


Figure 3. Relationship between Log K_{ow} of 3j~3o and inhibitory rates against *Rhizoctonia solani*.



3a: n=0, R^1=H, R^2=Ph

3b: n=0, R^1=H, R^2=PhOPh

3c: n=0, R^1=H, R^2=2-Cl-4-CF_3Ph

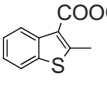
3d: n=0, R^1=CH_3, R^2=2-FPh

3e: n=0, R^1=$CH_2COOC_2H_5$, R^2=Ph

3f: n=0, R^1=H, R^2=4-(2-ClPy)

3g: n=0, R^1=H, R^2=4-(3-ClPy)

3h: n=0, R^1=H, R^2=3-(5-CH_3isoxazolyl)

3i: n=0, R^1=H, R^2= 

3j: n=1, R^1=H, R^2=2-ClPh

3k: n=1, R^1=H, R^2=3-ClPh

3l: n=1, R^1=H, R^2=4-ClPh

3m: n=1, R^1=H, R^2=2-FPh

3n: n=1, R^1=H, R^2=3-FPh

3o: n=1, R^1=H, R^2=$PhCHCH_3$

3p: n=2, R^1=H, R^2=Ph

3q: n=2, R^1=H, R^2=NH_2

3r: n=3, R^1=H, R^2=Ph

Scheme 1. Synthetic route of target compounds. Reagents and conditions: (a) Oxalyl chloride, CH_2Cl_2, DMF, reflux, 8 h; (b) Amine, CH_2Cl_2, 0 °C to reflux, 2 h.

HRMS and stereo structure determined by X-ray. All the target compounds showed the antifungal activities *in vitro*. It is observed that "*n*" increased to 2 or R^2 is substituted phenyl and heterocyclic group or the H atom of NH substituted by alkyl *et al*, the majority of the

Table 1. Inhibitory rates of PCA derivatives against five pathogenic fungi *in vitro*.

Compound*	Log K_{ow}	*R. solani* (%)	*F. oxysporum* (%)	*A. solani* (%)	*P. Oryzac Cavgra* (%)	*F. graminearum* (%)
PCA	1.59	93.3	85.2	81.3	93.6	49.6
3a	3.76	53.6	10.0	31.8	23.0	15.3
3b	5.09	79.8	15.0	34.6	35.8	19.7
3c	5.10	73.0	30.7	46.1	64.4	10.0
3d	3.58	74.3	23.9	53.0	**94.5**	37.5
3e	3.63	56.8	18.3	32.0	47.9	25.8
3f	3.73	56.8	17.1	32.9	44.4	10.0
3g	3.43	59.4	10.0	32.9	**85.2**	18.8
3h	3.17	78.2	18.3	35.2	26.4	17.0
3i	4.51	49.7	22.5	45.9	53.8	21.6
3j	4.02	79.0	10.0	46.1	33.3	16.4
3k	4.09	45.7	15.9	41.8	33.3	19.4
3l	4.08	50.4	18.3	34.6	23.0	18.8
3m	3.50	**90.6**	14.1	39.8	74.3	20.5
3n	3.54	82.8	13.6	39.8	33.3	10.0
3o	4.92	39.4	14.7	38.6	29.8	24.6
3p	3.89	61.3	18.3	47.8	58.0	34.6
3q	1.41	**95.8**	16.5	43.8	61.5	24.6
3r	4.30	58.1	17.7	38.6	29.8	17.6

*at the concentration of 200 μM; "Log K_{ow}" was calculated by the ALOGPS 2.1 program.

Table 2. Antifungal activities of **3a**, **3b**, **6** and **7** against *Rhizoctonia solani* and *Pyricularia oryzac* Cavgra *in vitro.*

	Rhizoctonia solani	*Pyricularia oryzac* Cavgra
Compound	EC_{50} (μM)	
PCA	33.2	37.3
3d	NT	28.7
3g	NT	89.3
3m	39.6	NT
3q	24.5	NT

tested antifungal activities increased. Especially when R^2 is F atom substituted aryl group, they can increase the antifungal activities remarkably. In the halogenated compounds, the ortho-position substituted by halogen was beneficial to increase their antifungal activities, and the oil–water amphiphilicity of the target compounds was another very important factor of antifungal activities against *Rhizoctonia solani*.

3. Experimental

3.1. General experimental procedures

The melting points are determined on a WRR melting point apparatus (Shanghai Jingke Industrial Co. Ltd.,Shanghai, China) and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ solution on Bruker 600 MHz spectrometer (Bruker Co., Switzerland) and Bruker 400 MHz spectrometer (Bruker Co., Switzerland). MS data were obtained using a APEX IV Fourier Transform Mass Spectrometry (Bruker Co., Switzerland). Chemicals and solvents were obtained from Sinopharm Chemical Reagent Co.,Ltd (Beijing, China) and were used without further purification. All fungi were obtained from the School of Agricultural,

Yangtze University (Jingzhou, China). Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Qingdao Marine Chemical Ltd., Qingdao, China).

3.2. General procedure for synthesis of phenazine-1-carbonyl chloride

To a suspension of 10 mmol of phenazine-1-carboxylic acid and 0.1 mmol of DMF in 30 ml of dry DCM, cooled at 0 °C, a solution of 15 mmol of oxalyl chloride in 20 ml of dry DCM was added. The reaction was stirred at reflux temperature for 12 h. The mixture was evaporated under vacuum; the residue was dissolved in 10 ml of dry DCM and used in next step without purification.

3.3. General procedure for synthesis of PCA amide analogues 3a–3r

To a suspension of 20 mmol of the proper amides and 15 mmol of Et_3 N in 30 ml of dry DCM, cooled at 0 °C, the solution of phenazine-1-carbonyl chloride (10 mmol) in 10 ml of dry DCM prepared in step 1) was added. The reaction was stirred at reflux temperature for 4~6 h. The mixture was then quenched with water and 5% Na_2CO_3 aqueous solution, dried over Na_2SO_4, filtered and concentrated *in vacuo.* The afforded crude was purified by recrystallizing from the solution of EtOAc–DCM (1:1) to give pure compounds **3a–3r.**

3.3.1. N-Phenylphenazine-1-carboxamide (3a)

Yellow solid in 86% yield [16]; ^{1}H NMR (600 MHz, $CDCl_3$): δ 13.31 (s, 1H), 9.05 (dd, J = 7.2, 1.1 Hz, 1H), 8.39 (dd, J = 8.6, 1.1 Hz, 1H), 8.25 (dd, J = 8.6, 7.2 Hz, 2H), 7.74–8.12 (m, 5H), 7.46 (t, J = 7.8 Hz, 2H), 7.03–7.32 (m, 1H). HRESIMS: *m/z* 300.1136 $[M + H]^+$ (calcd for $C_{19}H_{14}N_3O$, 300.1131).

3.3.2. N-(4-Phenoxyphenyl)phenazine-1-carboxamide (3b)

Orange yellow solid in 94% yield; m.p.: 193.4–196.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 13.29 (s, 1H), 9.05 (d, J = 7.2 Hz, 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.81–8.02 (m, 5H), 7.32–7.41 (m, 2H), 7.09–7.18 (m, 3H), 7.07 (t, J = 8.4 Hz, 2H). HRESIMS: *m/z* 392.1393 $[M + H]^+$ (calcd for $C_{25}H_{18}N_3O_2$, 392.1394).

3.3.3. N-(2-Chloro-4-(trifluoromethyl)phenyl)phenazine-1-carboxamide (3c)

Yellow solid in 94% yield; m.p. 201.5–203.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.50 (s, 1H), 9.15 (s, 1H), 9.05 (t, J = 7.2 Hz, 1H), 8.44 (t, J = 8.4 Hz, 1H), 8.34–8.41 (m, 1H), 8.25–8.32 (m, 1H), 7.99 (t, J = 8.4 Hz, 1H), 7.88–7.96 (m, 2H), 7.61 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 8.4, 1.2 Hz, 1H). HRESIMS: *m/z* 402.0617 $[M + H]^+$ (calcd for $C_{20}H_{12}N_3OF_3Cl$, 402.0616).

3.3.4. N-(2-Fluorophenyl)-N-methylphenazine-1-carboxamide (3d)

Yellow solid in 93% yield; m.p. 120.1–122.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (t, J = 7.6 Hz, 1H), 8.17 (t, J = 7.6 Hz, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.80–7.98 (m, 3H), 7.70 (t, J = 8.8 Hz, 1H), 6.94 (dd, J = 8.4, 1.2 Hz, 3H), 6.52 (dd, J = 8.4, 1.2 Hz, 1H), 3.66 (s, 3H). HRESIMS: *m/z* 332.1193 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OF$, 332.1194).

3.3.5. Ethyl 2-(N-phenylphenazine-1-carboxamido)acetate (3e)

Yellow solid in 91% yield; m.p. 129.9-132.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32–8.38 (m, 1H), 8.15–8.21 (m, 1H), 8.11 (dd, J = 8.8, 1.2 Hz, 1H), 7.77–7.92 (m, 3H), 7.62–7.71 (m, 1H), 7.19 – 7.29 (m, 2H), 6.78–7.02 (m, 3H), 4.44–5.66 (m, 2H), 4.34 (q, J = 7.2 Hz, 2H), 1.38 (t, J = 7.2 Hz, 3H). HRESIMS: m/z 386.1500 $[M + H]^+$ (calcd for $C_{23}H_{20}N_3O_3$, 386.1499).

3.3.6. N-(2-Chloropyridin-4-yl)phenazine-1-carboxamide (3f)

Yellow solid in 96% yield; m.p. 242.2–243.7 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.78 (s, 1H), 9.04 (dd, J = 7.2, 1.2 Hz, 1H), 8.49 (dd, J = 8.4, 1.2 Hz, 1H), 8.38 (d, J = 5.4 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.91–8.17 (m, 4H), 7.73 (dd, J = 5.4, 1.8 Hz, 1H). HRESIMS: m/z 335.0695 $[M + H]^+$ (calcd for $C_{18}H_{12}N_4OCl$, 335.0694).

3.3.7. N-(3-Chloropyridin-4-yl)phenazine-1-carboxamide (3g)

Yellow solid in 94% yield; m.p. 206.2–208.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.52 (s, 1H), 9.12 (dd, J = 8.1, 1.5 Hz, 1H), 9.05 (dd, J = 7.1, 1.5 Hz, 1H), 8.38–8.53 (m, 2H), 8.29 (t, J = 9.3 Hz, 1H), 8.21 (dd, J = 7.1, 1.5 Hz, 1H), 7.86–8.07 (m, 3H), 7.37 (dd, J = 8.1, 1.5 Hz, 1H). HRESIMS: m/z 335.0695 $[M + H]^+$ (calcd for $C_{18}H_{12}N_4OCl$, 335.0694).

3.3.8. N-(5-Methylisoxazol-3-yl)phenazine-1-carboxamide (3h)

Yellow solid in 95% yield; m.p.>250 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.88 (s, 1H), 9.07 (d, J = 7.2 Hz, 1H), 8.50 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.89–8.12 (m, 3H), 6.98 (s, 1H), 2.51 (s, 3H). HRESIMS: m/z 305.1032 $[M + H]^+$ (calcd for $C_{17}H_{13}N_4O_2$, 305.1033).

3.3.9. Methyl 2-(phenazine-1-carboxamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (3i)

Red solid in 78% yield; m.p. 199.1–200.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.91 (s, 1H), 9.01– 9.15 (m, 2H), 8.47 (dd, J = 8.8, 1.6 Hz, 1H), 8.25–8.34 (m, 1H), 7.90–8.04 (m, 3H), 4.03 (s, 3H), 2.88 (t, J = 4.8 Hz, 2H), 2.76 (t, J = 4.8 Hz, 2H), 1.87 (t, J = 3.2 Hz, 4H). HRESIMS: m/z 418.1216 $[M + H]^+$ (calcd for $C_{23}H_{20}N_3O_3S$, 418.1220).

3.3.10. N-(2-Chlorobenzyl)phenazine-1-carboxamide (3j)

Yellow solid in 97% yield; m.p. 168.4–170.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.60 (s, 1H), 9.03 (dd, J = 7.2, 1.6 Hz, 1H), 8.40 (dd, J = 8.8, 1.6 Hz, 1H), 8.25–8.34 (m, 1H), 8.16–8.24 (m, 1H), 7.87–8.02 (m, 3H), 7.67 (dd, J = 7.2, 2.4 Hz, 1H), 7.40–7.51 (m, 1H), 7.26–7.31 (m, 2H), 5.00 (d, J = 6.0 Hz, 2H). HRESIMS: m/z 348.0895 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OCl$, 348.0898).

3.3.11. N-(3-Chlorobenzyl)phenazine-1-carboxamide (3k)

Yellow solid in 95% yield; m.p. 167.1–168.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.59 (s, 1H), 9.03 (dd, J = 7.2, 1.6 Hz, 1H), 8.39 (dd, J = 8.8, 1.6 Hz, 1H), 8.24–8.33 (m, 1H), 8.13–8.24 (m, 1H), 7.79–8.04 (m, 3H), 7.58–7.79 (m, 1H), 7.38–7.58 (m, 1H), 7.20–7.38 (m, 2H), 4.99 (d, J = 6.0 Hz, 2H). HRESIMS: m/z 348.0896 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OCl$, 348.0898).

3.3.12. N-(4-Chlorobenzyl)phenazine-1-carboxamide (3l)

Yellow solid in 94% yield; m.p. 159.3–160.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.31 (d, J = 9.6 Hz, 1H), 9.01–9.12 (m, 1H), 8.35–8.48 (m, 1H), 8.19–8.34 (m, 1H), 7.96–8.11 (m, 2H), 7.78–7.96 (m, 2H), 7.48 (t, J = 8.0 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 4.89 (t, J = 4.8 Hz, 2H). HRESIMS: m/z 348.0896 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OCl$, 348.0898).

3.3.13. N-(2-Fluorobenzyl)phenazine-1-carboxamide (3 m)

Yellow solid in 92% yield; m.p. 136.3–138.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.58 (s, 1H), 9.04 (dd, J = 7.2, 1.2 Hz, 1H), 8.42 (dd, J = 8.8, 1.2 Hz, 1H), 8.27–8.34 (m, 1H), 8.14–8.23 (m, 1H), 7.90–8.05 (m, 3H), 7.55–7.66 (m, 1H), 7.29–7.36 (m, 1H), 7.13–7.21 (m, 2H), 4.97 (d, J = 5.6 Hz, 2H). HRESIMS: m/z 332.1191 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OF$, 332.1194).

3.3.14. N-(3-Fluorobenzyl)phenazine-1-carboxamide (3n)

Yellow solid in 93% yield; m.p. 161.0–163.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 9.06 (dd, J = 7.2, 1.6 Hz, 1H), 8.44 (dd, J = 8.8, 1.6 Hz, 1H), 8.26–8.35 (m, 1H), 8.06–8.14 (m, 1H), 8.01 (dd, J = 8.8, 7.2 Hz, 1H), 7.86–7.96 (m, 2H), 7.28–7.43 (m, 3H), 6.99–7.10 (m, 1H), 4.93 (d, J = 5.6 Hz, 2H). HRESIMS: m/z 332.1187 $[M + H]^+$ (calcd for $C_{20}H_{15}N_3OF$, 332.1194).

3.3.15. N-(1-Phenylethyl)phenazine-1-carboxamide (3o)

Yellow solid in 91% yield; m.p. 161.6–163.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.56 (d, J = 6.8 Hz, 1H), 9.02 (dd, J = 7.2, 1.6 Hz, 1H), 8.40 (dd, J = 8.8, 1.6 Hz, 1H), 8.25–8.33 (m, 1H), 8.07– 8.14 (m, 1H), 7.90–8.01 (m, 3H), 7.61 (d, J = 7.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.34 (t, J = 7.6 Hz, 1H), 5.45–5.64 (m, 1H), 1.80 (d, J = 6.8 Hz, 3H). HRESIMS: m/z 328.1438 $[M + H]^+$ (calcd for $C_{21}H_{18}N_3O$, 328.1444).

3.3.16. N-Phenethylphenazine-1-carboxamide (3p)

Yellow solid in 89% yield [14]; m.p. 110.5–112.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.03 (s, 1H), 9.03 (dd, J = 7.2, 1.6 Hz, 1H), 8.38 (dd, J = 8.8, 1.6 Hz, 1H), 8.25 (dd, J = 8.4, 1.2 Hz, 1H), 7.97 (dd, J = 8.4, 7.2 Hz, 1H), 7.78–7.93 (m, 2H), 7.63–7.69 (m, 1H), 7.26–7.43 (m, 5H), 4.03-4.08 (m, 2H), 3.12 (t, J = 6.8 Hz, 2H). HRESIMS: m/z 328.1441 $[M + H]^+$ (calcd for $C_{21}H_{18}N_3O$, 328.1444).

3.3.17. N-(2-Aminoethyl)phenazine-1-carboxamide (3q)

Yellow solid in 65% yield [20]; m.p. 171.9–173.7 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.22 (s, 1H), 8.99 (dd, J = 7.2, 1.2 Hz, 1H), 8.38 (dd, J = 8.4, 1.2 Hz, 1H), 8.22–8.30 (m, 2H), 7.96 (dd, J = 8.4, 7.2 Hz, 1H), 7.88–7.94 (m, 2H), 3.73-3.81 (m, 2H), 3.12 (t, J = 6.0 Hz, 2H). HRESIMS: m/z 267.1241 $[M + H]^+$ (calcd for $C_{15}H_{15}N_4O$, 267.1243).

3.3.18. N-(3-Phenylpropyl)phenazine-1-carboxamide (3r)

Yellow solid in 87% yield; m.p. 124.0–125.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.08 (s, 1H), 9.04 (dd, J = 7.2, 1.6 Hz, 1H), 8.41 (dd, J = 8.8, 1.6 Hz, 1H), 8.29–8.35 (m, 1H), 8.19–8.25 (m, 1H), 7.92–8.03 (m, 3H), 7.29–7.35 (m, 4H), 7.18–7.25 (m, 1H), 3.73-3.82 (m, 2H), 2.92 (t, J = 8.0 Hz, 2H), 2.12– 2.22 (m, 2H). HRESIMS: m/z 342.1596 $[M + H]^+$ (calcd for $C_{22}H_{20}N_3O$, 342.1601).

3.4. Bioassays of fungicidal activities

The *in vitro* fungicidal activities of all target compounds against *Rhizoctonia solani*, *Fusarium graminearum*, *Walnut root rot*, *Rhizoctonia solani* Kühn, and *Sclerotinia sclerotiorum* were evaluated and screened by the mycelium growth rate method [23,24]. A quantity of 5 ml of prepared solutions containing the compounds was added to the sterile potato dextrose agar (PDA, 45 ml), and the compounds were tested at a concentration of 200 μM. Acetone in sterile aqueous 1% Tween 60 served as the negative control. Before antifungal assaying, all fungi species were cultivated for 2–5 days in PDA at 25 ± 1 °C to produce new mycelium. Then, the culture medium was cut into mycelia dishes of about 6 mm diameter which were picked up with a sterilized inoculating needle to inoculate in the center of the PDA plates. Every treatment with three replicates was incubated at 25 ± 1 °C in a bioclean environment for 2–7 days from different fungi. The hypha diameter was measured four times by the cross-bracketing method, and the data were statistically analyzed. The inhibitory rates (I) of the target compounds were calculated by the following formula: $I\ (\%) = [(C - T)/(C - 6)] \times 100$, where C represents the average diameter (mm) of mycelia on untreated PDA, and T represents the average diameter (mm) of fungi on treated PDA.

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Disclosure statement

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