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Phenazine-1-carboxylic acid derivatives: Design, synthesis and biological evaluation against *Rhizoctonia solani* Kuhn

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ABSTRACT

Rhizoctonia solani Kuhn is the pathogen that causes sheath blight and results in significant yield reduction in rice and in nearly 50 other crops. In order to develop a new fungicide effective against this pathogen, a series of structurally diverse phenazine-1-carboxylic acid derivatives, **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j**, and **2k**, were designed, synthesized and evaluated for their antifungal activity. The two most active compounds **2i** and **2j** were selected as lead compounds for further antifungal research.

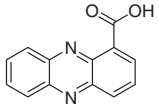
Rice is one of the major staple crops consumed by almost half of the world's population, particularly in East, Southeast and South Asia.[1,2] *Rhizoctonia solani* Kuhn, the pathogen that causes sheath blight, is one of the most devastating pathogenic fungi affecting rice.[3–5] In addition to rice, this fungus can infect crops of nearly 50 other species, including barley, lettuce, tomato, sorghum, and maize. Sheath blight infection causes significant decreases in rice yield; for example, a yield reduction of 40% was recorded in a rice crop with the highest inoculum density.[6] In the United States, a yield loss of 50% was reported when susceptible cultivars were planted.[7] In China, sheath blight disease affects about 15–20 million hectares and causes a yield loss of 6 million tons of rice grain per year.[4,8] For these reasons, development of an efficient method is urgent for the suppression of *R. solani* infection.

In recent years, significant research effort has been directed towards the screening of biocontrol agents and chemical compounds against *R. solani*, many of which are now considered to be promising.[9–15] In the case of biofungicides, high efficiency and low toxicity are necessary requirement. Phenazine-1-carboxylic acid (PCA, **1**, Scheme 1), one of the research focuses of our research group, is produced by several plant growth-promoting rhizosphere (PGPR) pseudomonads and has proven effective against several soil-borne fungal phytopathogens that are of great agriculture significance.[16–18] PCA has been registered as the biofungicide 'Shenqinbactin' in China and is noted for its high fungicidal efficiency, low toxicity to humans and animals, environmental friendliness, and improvement of crop production.[19–23] Our current aim is to identify new and more efficient antifungal compounds. In the present study, we describe the design and synthesis of PCA derivatives modified on the 1-position of PCA, taking advantage of the high chemical reactivity of carboxylic acid with other reagents such as amines and alcohols.

We investigate the antifungal activities of the derived compounds on *R. solani* as well as preliminary SAR studies.

PCA was isolated from metabolites extracted from genetically modified *Pseudomonas* sp. M18 which produces much larger amounts of PCA than the wild type, using the purification procedure previously described.[21,24,25] A series of PCA derivatives (**2a**–**2k**, Scheme 2) were then designed with respect to structure and chemical diversity. Different modifications, such as addition of linear chain groups, cyclic hydrocarbons, or aromatic groups, and changes in hydrophilicity or hydrophobicity, were all taken into consideration.

Scheme 3 outlines the general synthesis of PCA derivatives **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j** and **2k**. Treatment of PCA isolated



Scheme 1. PCA structure.

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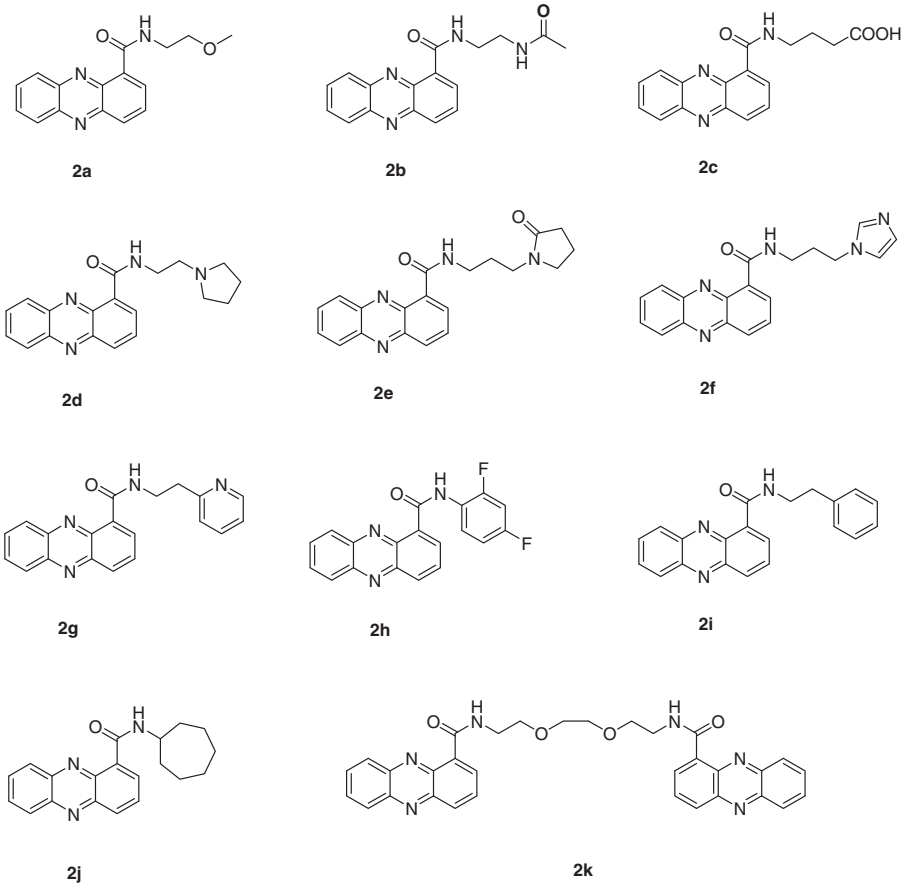
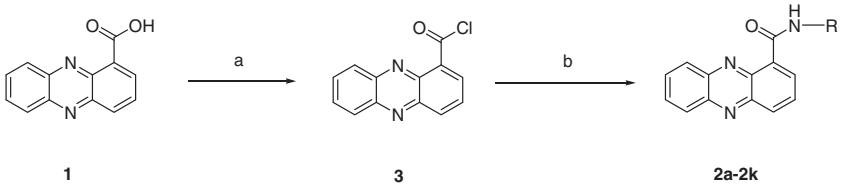


Scheme 2. PCA derivatives: **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j**, and **2k**.



Scheme 3. Preparation of target compounds **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j**, and **2k**. Reagents and conditions: (a) $SOCl_2$, reflux, 6 h; (b) RNH_2 (**2a**–**2j** 1 equiv, **2k** 0.5 equiv), triethylamine (4 equiv), CH_2Cl_2, 0 °C to room temperature, overnight.

from genetically modified *Pseudomonas* sp. M18 with $SOCl_2$ at reflux temperature afforded intermediate **3** after the evaporation of $SOCl_2$. The target compounds were then synthesized by adding corresponding amines to intermediate **3** as a CH_2Cl_2 solution, utilizing triethylamine as a base at 0 °C (analysis data of target compounds are presented in Supplementary data).

All target compounds were screened for their antifungal activity against *R. solani*. Concentration effective for growth inhibition was tested by a plate method (PDA medium, procedures are described in Supplementary data). Authentic PCA was used as a positive control. The inhibitory concentration (IC_{50}) values (in μM) are presented in Table 1.

For *R. solani*, the biological results were relatively heterogeneous ($0.003 < IC_{50}$ (μM) < 0.246). Compounds bearing a cyclic side chain with high hydrophobicity (**2i** and **2j**) exhibited a significant level of activity, with IC_{50} values 8- to 23-fold lower than that of PCA itself (**1**, $IC_{50} = 0.068$ μM). Compounds bearing a linear side chain with high hydrophilicity (**2b** and **2c**) were less active (IC_{50} values of 0.123 and 0.246 μM, respectively). Replacement of phenyl group (**2i**, $IC_{50} = 0.008$ μM) by a pyridinyl group (**2g**, $IC_{50} = 0.023$ μM) led to weaker inhibition (compared with **2i**, but

Table 1
Antifungal evaluation of PCA derivatives

Compound	*Rhizoctonia solani* Kuhn IC_{50} (μM)
1 (positive control)	0.068
2a	0.079
2b	0.123
2c	0.246
2d	0.087
2e	0.074
2f	0.084
2g	0.023
2h	0.075
2i	0.008
2j	0.003
2k	0.051

still more active than PCA). Other derivatives (**2a**, **2d**, **2e**, **2f**, **2h**, and **2k**) showed nearly the same IC_{50} values (0.079, 0.087, 0.074, 0.084, 0.075 and 0.051 μM, respectively) as that of PCA (**1**, IC_{50} = 0.068 μM).

Compound **2i** and **2j** had the highest activity while **2b** and **2c** had the lowest. Since **2i** and **2j** have the highest hydrophobicity, while **2b** and **2c** have the lowest, hydrophobicity can be assumed to be one of the most important factors for enhancing the antifungal activity of PCA derivatives against *R. solani*. The supposition is supported by the comparison of **2g** and **2i**, because **2i** is more active (three times) than **2g**, even though they differ structurally by only one atom (carbon vs nitrogen) on the aromatic group of the side chain. At the same time, we can speculate from the biological results that compounds bearing a cyclic group are more active than those with a linear group (activity: **2j**, **2h** > **2a**, **2b**, **2c**; other compounds bear both linear and cyclic groups).

In conclusion, we have successfully prepared a series of structurally diverse PCA derivatives, some of which exhibited significantly higher levels of antifungal activity against *Rhizoctonia solani* Kuhn than did PCA itself. Compound **2i** and **2j** were screened as lead compounds for further antifungal research on PCA derivatives and their activities indicate them to be potential candidates for development as fungicides.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.050.

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