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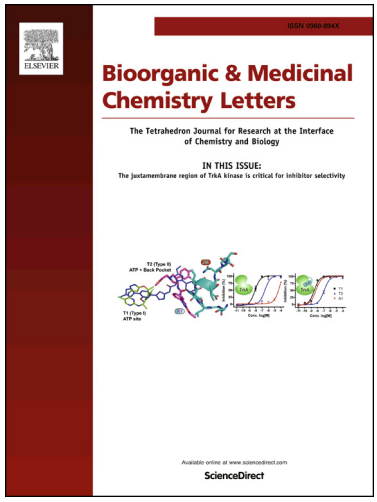

Synthesis and bioactivities of Phenazine-1-carboxylic acid derivatives based on the modification of PCA carboxyl group

Zhipeng Xiong, Junfan Niu, Hao Liu, Zhihong Xu, Junkai Li, Qinglai Wu

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Synthesis and bioactivities of Phenazine-1-carboxylic acid derivatives based on the modification of PCA carboxyl group

Zhipeng Xiong, Junfan Niu, Hao Liu, Zhihong Xu, Junkai Li*, Qinglai Wu*

School of Agricultural, Yangtze University, Jingmi Road 88, Jingzhou 434025, China

*Corresponding author. Tel/Fax: +86 716-8066314.

E-mail address: wql106@163.com (Qinglai Wu).

*Corresponding author. Tel/Fax: +86 716-8066314.

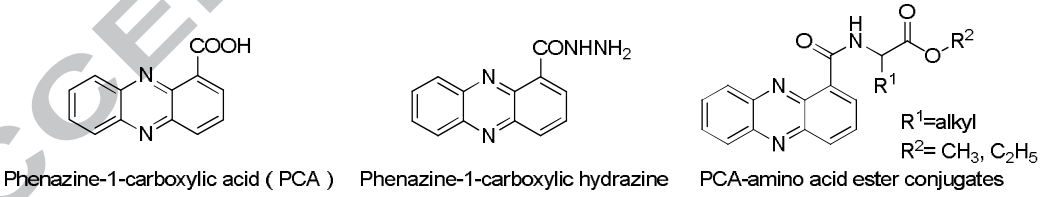
E-mail address: junkaili@sina.com (Junkai Li).

Abstract: Phenazine-1-carboxylic acid (PCA) as a natural product widely exists in microbial metabolites of *Pseudomonads* and *Streptomycetes* and has been registered for the fungicide against rice sheath blight in China. To find higher fungicidal activities compounds and study the effects on fungicidal activities after changing the carboxyl group of PCA, we synthesized a series of PCA derivatives by modifying the carboxyl group of PCA and their structures were confirmed by ^{1}H NMR and HRMS. Most compounds exhibited significant fungicidal activities *in vitro*. In particular, compound **6** exhibited inhibition effect against *Rhizoctonia solani* with EC_{50} values of 4.35 mg/L and compound **3b** exhibited effect against *Fusarium graminearum* with EC_{50} values of 8.30 mg/L, compared to the positive control PCA with its EC_{50} values of 7.88 mg/L (*Rhizoctonia solani*) and 127.28 mg/L (*Fusarium graminearum*), respectively. The results indicated that the carboxyl group of PCA could be modified to be amide group, acylhydrazine group, ester group, methyl, hydroxymethyl, chloromethyl and ether group *et.al*. And appropriate modifications on carboxyl group of PCA were useful to extend the fungicidal scope.

Keywords: phenazine-1-carboxylic acid; carboxyl group; fungicidal activity; derivatives; synthesis

Rice and wheat are the most important crops in the world. *Rhizoctonia solani Kuhn* and *Fusarium graminearum*, the pathogens that cause sheath blight of rice and *Fusariumu* head blight of wheat, are the most devastating pathogenic funguses infecting rice and wheat.[1-5] According to the statistics, the funguses can reduce the grain yield and quality of crops.[6-12] Unfortunately, the control of crop diseases caused by *Rhizoctonia solani Kuhn* and *Fusarium graminearum* is still a hard problem[12,13] and the intervention of agrochemicals is the most effective method at present.[14,15] However, the current chemical-control agents were not fully effective in inhibition of *Rhizoctonia solani Kuhn* and *Fusarium graminearum*.[14,15] Hence, it is necessary to develop more effective novel agents to replace the conventional agrochemicals.[15, 16]

As all we know, it is an effective method to develop new green agrochemicals by introducing known active sub-structures in natural products. Phenazine-1-carboxylic acid (PCA) (**Figure1**) is a very important compound widely existed in microbial metabolites of *Pseudomonads* and *Streptomycetes,* which has been proved having antifungal, antitumor and antileukemia effects.[17,18] In recent years, PCA can be produced by several *pseudomonads* and be of great significance in agricultural application because of inhibition effects against several soil-borne fungal phytopathogens.[19-22] It has been registered as the biofungicide "Shenqinbactin" in China and is noted for its high fungicidal efficiency, low toxicity to human and animals, friendliness to environment and improvement of crop production.[23-27] Phenazine-1-carboxylic hydrazine (**Figure1**), one analogue of PCA, is also derived from metabolites of *Pseudomonads* and has shown potent fungicidal activities against *Botrytis cinerea*, *Powdery mildew*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora capsici* and *Sclerotinia sclerotiorum* with the EC_{50} value of 0.56 mg/L, 0.85 mg/L, 0.28 mg/L, 1.88 mg/L, 1.20 mg/L and 1.52 mg/L, respectively.[28] Phenazine-1-carboxylic esters have been reported having excellent fungicidal activities against *magnaporthe oryzae*.[29] In our previous studies, we have synthesized a series of PCA-amino acid ester conjugates (**Figure1**) by conjugating PCA with different amino-acid esters and found that some of them showed better results against *Rhizoctonia solani Kuhn* than PCA.[30]



Phenazine-1-carboxylic acid (PCA) Phenazine-1-carboxylic hydrazine PCA-amino acid ester conjugates

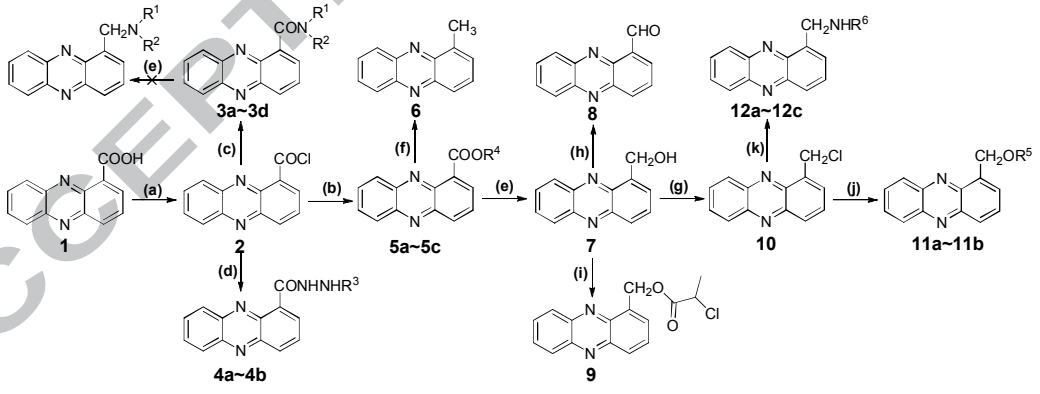
Figure 1. The structures of PCA and its analogues.

In consideration of the excellent fungicidal activities of the above compounds and the structural feature of PCA, we synthesized a series of PCA derivatives by modifying the carboxyl group of PCA and the fungicidal activities of all the synthesized compounds were tested. In the present study, we found potent fungicidal

activities compounds and discussed the primary structure-activity relationship.

Synthesis of the PCA derivatives was achieved in Scheme 1. Treatment of PCA with oxalyl chloride at reflux temperature in CH_2Cl_2 solution afforded intermediate **2** after the evaporation of CH_2Cl_2;[30] Subjecting **2** to the corresponding amines led to **3a-3d**[31,32] and to the hydrazides led to **4a-4b**.[28] Compounds **2** was treated with the corresponding alcohols to afford the esters **5a-5c**;[29] Reduction of PCA methyl ester (**5a**) or ethyl ester (**5b**) with lithium aluminum hydride afforded phenazin-1-ylmethanol **7**,[33] which was reacted with 2-chloropropanoyl-chloride in CH_2Cl_2 led to phenazin-1-ylmethyl-2-chloropropanoate **9** or with thionyl chloride afforded 1-(chloromethyl)-phenazine **10**.[34] Meanwhile, Compounds **6** was obtained by the reduction of compounds **5a-5b** with aluminum chloride as catalyst.[35] Oxidization of phenazin-1-ylmethanol **7** with potassium dichromate in acetone afforded phenazine-1-carbaldehyde **8**.[36]

Treating 1-(chloromethyl)-phenazine **10** with sodium alkoxide led to the corresponding 1-(alkoxymethyl)-phenazines **11a-11b** or with amines gave 1-(phenazin-1-yl)-methanamines **12a-12c**.[34] (Analysis data of all the synthesized compounds are available in Supplementary Information).



Scheme 1 Synthetic route of target compounds. Reagents and conditions: (a) Oxalyl chloride, CH_2Cl_2, DMF, reflux, 8h; (b) Alcohol, 20℃ to reflux, 30min; (c) Amine, CH_2Cl_2, 0℃ to reflux,

2h; (d) Amines, CH_2Cl_2, 0℃ to room temperature, 4h; (e) $LiAlH_4$, THF, 0℃ to reflux, 4h; (f) $AlCl_3$, $LiAlH_4$, THF, 0℃ to reflux, 8h; (g) $SOCl_2$, CH_2Cl_2, reflux, 6h; (h) $K_2Cr_2O_7$, 20%H_2SO_4, actone, room temperature to reflux, 1h; (i) Chloroacetyl chloride, CH_2Cl_2, 0℃ to reflux, 2h; (j) Sodium alkoxide, alcohol, 60~80℃, 3h; (k) Amine, CH_2Cl_2, room temperature to reflux, 6h.

In a standard primary screen, all the synthesized compounds **3a-12c** were evaluated for their fungicidal activity *in vitro* against *Rhizoctonia solani, Fusarium graminearum, Walnut root rot, Rhizoctonia solani Kühn*, and *Sclerotinia sclerotiorum (Lib.) de Bary* by using a plate method (PDA medium).[30,37] The results of preliminary bioassay showed that all compounds exhibited certain fungicidal activities against the pathogenic fungi at the concentration of 50 mg/L. In particular, compound **3a**, **3b**, **4a**, **5a**, **5b**, **6**, **7**, **10** and **11b** exhibited bioactivity more potent than PCA against *Rhizoctonia solani* and *Fusarium graminearum*, and the result indicated that the carboxyl group of PCA could be modified to amide group, acylhydrazine group, ester group, methyl, hydroxymethyl, chloromethyl and ether group. By comparing the structures of all compounds, it was found that the derivatization of the PCA with CH_3 and CH_2OH instead of the groups with large steric hindrance enhanced the activities. When comparing the inhibitory activity of the amides derivatives, the bioassay results showed the activities rank as **3a>3b>**PCA**>3c>3d** and the hydrophobicity of the amines played an important role in fungicidal activities. The activities enhanced as the hydrophobicity of the amines increased appropriately among a certain level. Compounds **3a**, **11b** exhibited the most potent fungicidal activities against *Rhizoctonia solani* with the inhibitory rates of 96.6%, 98.6% at the concentration of 50 mg/L, and better than PCA. In addition, the fungicidal activities increased significantly when carboxyl group of PCA was modified to be amide group or ether group, which provided helpful research ideas for the design of new members of this type derivatives as well as biological studies of these molecules and their analogues. Interestingly, PCA had poor fungicidal activity against *Fusarium graminearum*, but most of the compounds exhibited better fungicidal activities against *Fusarium graminearum* than PCA. In particular, compound **3a**, **3b** exhibited the most potent bioactivity against *Fusarium graminearum* with the inhibitory rate of 100% at the concentration of 50 mg/L, which indicated that the appropriate modifications on

carboxyl group of PCA were useful to extend the fungicidal scope and the derivatives with amide groups showed a good prospect in developing new fungicidal agents.

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

Compound*	*Rhizoctonia solani* (%)	*Fusarium graminearum* (%)	*Walnut root rot* (%)	*Rhizoctonia solani Kühn* (%)	*Sclerotinia sclerotiorum* (%)
PCA	83.3	32.9	66.9	50.9	11.6
3a	96.6	100	68.5	57.9	46.3
3b	83.5	100	58.2	57.1	45.5
3c	75.0	52.7	37.3	36.4	23.2
3d	70.6	39.0	33.3	22.0	2.5
4a	89.8	58.9	34.7	28.2	7.5
4b	70.1	68.5	26.0	29.5	40.5
5a	88.8	50.7	33.3	31.2	10.8
5b	83.9	34.3	34.0	29.5	11.6
5c	54.4	38.4	26.8	26.0	5.8
6	93.3	75.3	53.8	37.2	20.7
7	88.8	85.0	59.7	47.6	28.1
8	76.5	45.2	62.7	52.8	12.4
9	30.9	17.8	30.3	39.5	8.3
10	85.4	58.9	44.3	38.1	26.5
11a	79.4	63.7	39.1	37.3	14.9
11b	98.6	49.3	41.3	33.8	12.4
12a	77.2	33.6	45.0	40.7	26.5
12b	81.3	35.6	47.8	52.2	33.8
12c	72.6	29.5	42.3	38.9	21.6

*at the concentration of 50 mg/L

Compounds **3a**, **3b**, **6** and **7** were chosen to determine EC_{50} values[30,37] against *Rhizoctonia solani* and *Fusarium graminearum,* because these compounds showed fungicidal activities with the inhibitory rate of over 75% against the two pathogenic fungi at the concentration of 50 mg/L. Compound **11b** was also chosen to determine EC_{50} value because of its best fungicidal activities against *Rhizoctonia solani*. The results were shown in Table 2. Especially, compound **6** exhibited the best activity among all the target compounds against *Rhizoctonia solani* with the EC_{50} value of 4.35 mg/L and compound **3b** exhibited the best activity among all the target compounds against *Fusarium graminearum* with the EC_{50} value of 8.30 mg/L. Their

activities were more potent than that of the positive control PCA with its EC_{50} values of 7.88 mg/L (*Rhizoctonia solani*) and 127.28 mg/L (*Fusarium graminearum*), respectively.

Table 2
Fungicidal activities of **3a**, **3b**, **6** and **7** against *Rhizoctonia solani* and *Fusarium graminearum in vitro*

Compound	*Rhizoctonia solani*	*Fusarium graminearum*
	EC_{50} (mg/L)	
PCA	7.88±1.82	127.28±5.86
3a	6.66±0.72	12.67±1.63
3b	7.09±0.75	8.30±1.35
6	4.35±0.83	21.44±3.36
7	9.98±1.64	41.82±4.34
11b	8.68±1.26	51.36±3.21

In summary, a series of PCA derivatives were designed, synthesized and their structures were confirmed by ^{1}HNMR spectrum and elemental analysis measured with HRMS. All the compounds exhibited excellent fungicidal activities *in vitro*. It is observed that the carboxyl group of PCA could be modified to be amide group, acylhydrazine group, ester group, methyl, hydroxymethyl, chloromethyl and ether group. In addition, the derivatization of the PCA with CH_3 and CH_2OH instead of the groups with large steric hindrance enhanced the activities. The structure–activity analysis of the amides revealed that the hydrophobicity of the amines seemed to be crucial for the fungicidal activities. And appropriate modifications on carboxyl group of PCA were useful to extend the fungicidal scope.

Acknowledgments

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References and notes

1. Broglie, K.; Chet, I.; Holliday, M.; Cressman, R.; Biddle, P.; Knowlton, S.; Mauvais, C. J.; Broglie, R. *Science*.**1991**, 254, 1194.
2. Lei, D.; Lin, R.; Yin, C.; Li, P.; Zheng, A. *J. Proteome Res.***2014**, 13(7), 3277.
3. Arra, Y.; Duraisamy, L.; Vellaichamy, P.; Mangrauthia, S. K.; Prasad, M. S.; Donempudi, K.; Maganti, S. M.; Raman, M. S.; Gouri, S. L. *J. Phytopathol.***2015**, 163(6), 465.
4. Manstretta, V.; Gourdain, E.; Rossi, V. *Eur. J. Plant Pathol.***2015**, 143(4),1.
5. Jiang, C.; Zhang, S.; Zhang, Q.; Tao, Y.; Xu, J. *Environ.Microbiol.***2014**, 17(4), 1245.
6. Singh, A. K.; Singh, V. K.; Singh, A.; Ellur, R. K.; Pandian, R. T. P.; Krishnan, S. G.; Singh, U. D.; Nagarajan, M.; Vinod, K. K.; Prabhu, K. V. *Euphytica*. **2015**, 203(1), 97.
7. Zhou, Q.; Chen, Y.; Yang, Y.; Ahmed, H. U.; Hwang, S. F.; Strelkov, S. E. *Crop Prot.***2014**, 59(59), 71.
8. Salgado, J. D.; Madden, L. V.; Paul,P. *Phytopathology.* **2015**, 105(3), 295.
9. Tembo, L.; Asea, G.; Gibson, P. T.; Okori, P. *Plant Breeding*. **2016**, 135(4), 446.
10. Ntushelo, K. *Acta Biol. Hung.* **2016**, 67(2), 220.
11. Quesadaocampo, L. M.; Alhaddad, J.; Scruggs, A. C.; Buell, C. R.; Trail, F. *Phytopathology.* **2016**, 106(8), 920.
12. Prasad, B.; Eizenga, G. C. *Plant Dis.* **2008**, 92, 1503.
13. Okubara, P. A.; Dickman, M. B.; Blechl, A. E. *Plant Sci.* **2014**, 228, 61.
14. Dweba, C. C.; Figlan, S.; Shimelis, H. A.; Motaung, T. E.; Sydenham, S.; Mwadzingeni, L.; Tsilo, T.J. *Crop Prot.* **2016**,91, 114.
15. Peng, D.; Li, S.; Wang, J.; Chen, C.; Zhou, M. *Pest Manag. Sci.* **2014**, 70(2), 258.
16. Goswamir, R. S.; Kistler, H. C. *Mol. Plant Pathol.***2004**, 5(6), 515.
17. Hu, H. B.; Xu, Y. Q.; Chen, F.; Xue, H. Z.; Hur, B. K. *J. Microbiol. Biotechnol.* **2005**, 15, 86.
18. Laursen, J. B.; Nielsen, J. *Chem. Rev.* **2004**, *104,* 1663.
19. Padaria, J. C.; Tarafdar, A.; Raipuria, R.; Lone, S. A.; Gahlot, P.; Shakil, N. A.; Kumar, J. *J. Basic Microb.* **2016**, 56(9), 999.
20. Xu, S.; Pan, X.; Luo, J.; Wu, J.; Zhou, Z.; Liang, X.; He, Y.; Zhou, M. *Pest.Biochem. Phys.* **2014**,117, 39.
21. Pan, X.; Wu, J.; Xu, S.; Duan, Y.; Zhou, M. *Phytopathology*. **2017**, 107(2), 163.
22. Arseneault, T.; Goyer, C.; Filion, M. *Phytopathology.* **2013**, 103(10), 995.
23. Wen, G. Y.; Zhong, H.; Zhong, H. Y.; Xu, Y. Q.; Yang, X. *Plant Prot*. **2008**, 34, 143.
24. He, L.; Xu, Y. Q.; Zhong, X. H. *Biotechnol. Bioeng.* **2008**, 100, 250.
25. Li, Y. Q.; Jiang, H. X.; Xu, Y. Q.; Zhong, X. H. *Appl. Microbiol. Biotechonl.* **2008**, 77, 1207.
26. Su, J. J.; Zhou, Q.; Zhong, H. Y.; Li, Y. Q.; Huang, X. Q.; Xu, Y. Q. *Bioresour. Technol.* **2010**, 101, 4089.
27. Zhou, Q.; Su, J. J.; Jiang, H. X.; Huang, X. Q.; Xu, Y. Q. *Appl. Microbiol. Biotechonl.* **2010**, 86, 1761.
28. Chin-A-Woeng, T. F. C.; Bloemberg, G. V.; van der Bij, A. J.; van der Drift, K. M. G. M.; Schripsema, J.; Kroon, B.; Scheffer, R. J.; Keel, C.; Bakker, P. A. H. M.; Tichy, H. V.; de Bruijn, F. J.; Thomas-Oates, J. E.; Lugtenberg, B. J. J. *Mol. Plant-Microbe In*. **1998**, 11, 1069.

29. Li, B.; Lu, L.; Sun, Q.; Zhu, D. Q.; Li, Z. N.; Wang G. CN105418518. **2016**-03-23
30. Niu, J. F.; Chen, J.; Xu, Z. H.; Zhu, X.; Wu, Q. L.; Li, J. K. *Bioorg. Med. Chem. Lett.* **2016**, 26, 5384.
31. Jayatilake, G. S.; Thornton, M. P.; Leonard, A. C.; Grimwade, J. E.; Baker, B. J. *J. Nat. Prod.* **1996**, 59, 293.
32. Palchykovska, L. G.; Vasylchenko, O.V. V.; Platonov, M. O.; Kostina, V. G.; Babkina, M. M.; Tarasov, O. A.; Starosyla, D. B.; Samijlenko, S. P.; Rybalko, S. L.; Deriabin, O. M.; Hovorun, D. M. *Biopolym. Cell.* **2012**, 28, 477
33. Shlyakhtina, N. I.;Safronov, A. V.;Sevryugina, Y. V.;Jalisatgi, S. S.; Hawthorne, M. F. *J. Organomet. Chem.* **2015**, 798, 234.
34. Kettle, J. G.; Alwan, H.; Bista, M.; Breed, J.; Davies, N. L.; Eckersley, K.; Fillery, S.; Foote, K. M.; Goodwin, L.; Jones, D. R.; Kack, H.; Lau, A.; Nissink, J. W. M.; Read, J.; Scott, J. S.; Taylor, B.; Walker, G.; Wissler, L.; Wylot, M. *J. Med. Chem.* **2016**, 59, 2346.
35. Hicks, J. L.; Heys, J. R. *J. Lab. Compd.* **1981**, 18, 1457.
36. Hassan, M.; Alhakimi, A. N.; Alahmadi, M. D. *S. Afr. J. Chem.* **2011**, 64, 237.
37. Shentu, X. P.; Zhan, X. H.; Ma, Z.; Yu, X. P.; Zhang, C. X. *Braz. J. Microbiol.* **2014**, 45, 248.

Graphical abstract

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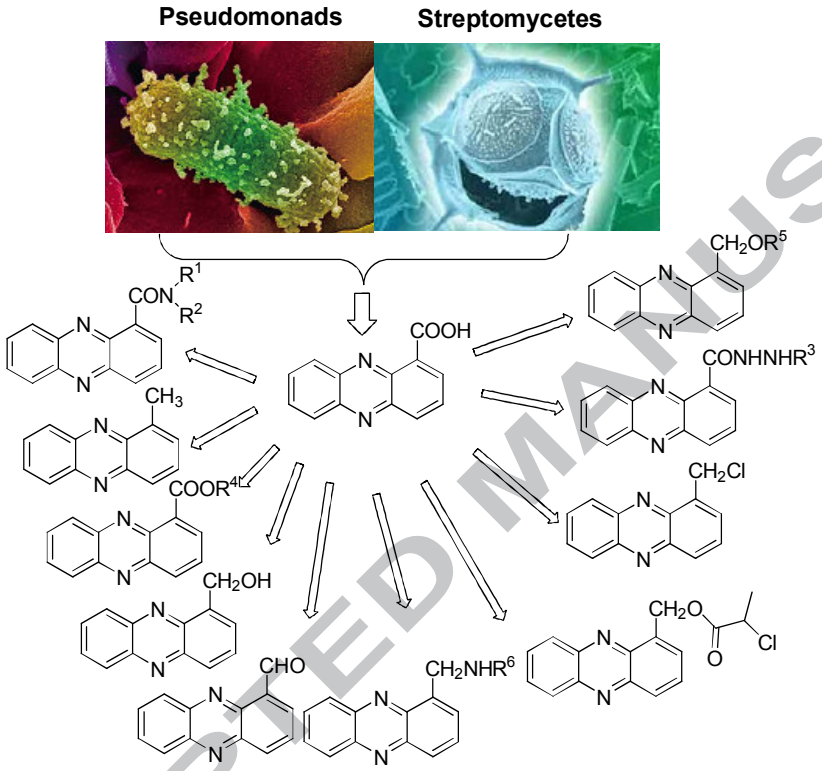
Zhipeng Xiong, Hao Liu, Junfan Niu, Zhihong Xu, Junkai Li*, Qinglai Wu*
School of Agricultural, Yangtze University, Jingmi Road 88, Jingzhou 434025, China



The synthesis and bioactivities of Phenazine-1-carboxylic acid derivatives based on the modification of PCA carboxyl group were studied systematically. To find higher fungicidal activities compounds and study the effects on fungicidal activities after changing the carboxyl group of PCA, we synthesized a series of Phenazine-1-carboxylic acid derivatives by modifying the carboxyl group of PCA as much as possible. The results of structure–activity analysis indicated that the carboxyl group of PCA could be modified to be amide group, acylhydrazine group, ester group, methyl, hydroxymethyl, chloromethyl and ether group *et.al.* And appropriate modification on carboxyl group of PCA was useful to extending the fungicidal scope.