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Design, synthesis and fungicidal evaluation of novel pyraclostrobin analogues

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ABSTRACT

A series of novel pyraclostrobin derivatives were designed and prepared as antifungal agents. Their antifungal activities were tested in vitro with five important phytopathogenic fungi, namely, *Batrylis cinerea*, *Phytophthora capsici*, *Fusarium sulphureum*, *Gloeosporium pestis* and *Sclerotinia sclerotiorum* using the mycelium growth inhibition method. Among these compounds, **5s** displayed IC_{50} value of 0.57 μg/mL against *Batrylis cinerea* and **5k-II** displayed IC_{50} value of 0.43 μg/mL against *Sclerotinia sclerotiorum*, which were close to that of the positive control pyraclostrobin (0.18 μg/mL and 0.15 μg/mL). Other compounds **5f**, **5k-II**, **5j**, **5m** and **5s** also exhibited strong antifungal activity. Further enzymatic assay demonstrated compound **5s** inhibited porcine bc_1 complex with IC_{50} value of 0.95 μM. The statistical results from an integrated computational pipeline demonstrated the predicted total binding free energy for compound **5s** is the highest. Consequently, compound **5s** with the biphenyl-4-methoxyl side chain could serve as a new motif as inhibitors of bc_1 complex and deserve to be further investigated.

1. Introduction

Pyraclostrobin (Fig. 1) belongs to the strobilurin type of fungicide.[1] Pyraclostrobin was believed to bind to the Q_o site of a membrane-bound homodimeric cytochrome bc_1 complex (EC 1.10.2.2, also known as complex III of mitochondrial respiration) and blocked the electron transfer between cytochrome b and cytochrome c1, resulting in the inhibition of the mitochondrial respiration chain and the reduced production of ATP which is essential for the proper function of fungal cell.[2] Ever since being introduced to the market by BASF,[3] pyraclostrobin has been playing critical roles in crop protection. After extensive application for over two decades, fungi phytopathogens with moderate to high resistance to pyraclostrobin have been reported.[4,5] Therefore, development of pyraclostrobin analogues for the treatment of resistant pathogens has been the efforts for the agricultural chemists.

As shown in Fig. 1, the structure of pyraclostrobin could be divided into three parts: the pharmacophore, the phenyl bridge and the side chain. The pharmacophore moiety exists in a number of highly potent strobilurin type fungicides,[6–17] variation at the side chain is observed in several marketed agents, while modification of the phenyl linker is rarely reported.[18–21] Considering phenyl is commonly replaced by its bioisostere pyridinyl, and the synthesis of *N*-ortho substituted pyridine analogues could be easily achieved by nucleophilic aromatic substitution reaction, we herein designed and synthesized a series of pyraclostrobin derivatives bearing the pyridinyl linker, and the side chain was accordingly investigated. The fungicidal activities of these compounds were evaluated against five important plant pathogen strains including *Batrylis cinerea*, *Phytophthora capsici*, *Fusarium sulphureum*, *Gloeosporium pestis* and *Sclerotinia sclerotiorum* in vitro, and the inhibitory capability against porcine bc_1 complex were evaluated. Finally, the structure-activity relationships (SARs) were also analyzed using integrated computational strategy including molecular docking, MM/GBSA binding free energy calculation and binding free energy decomposition.

2. Results and discussion

2.1. Chemistry

The synthesis of the target molecules **5a–u** and **6a–b** was started from the commercially available 2-chloro-3-nitropyridine

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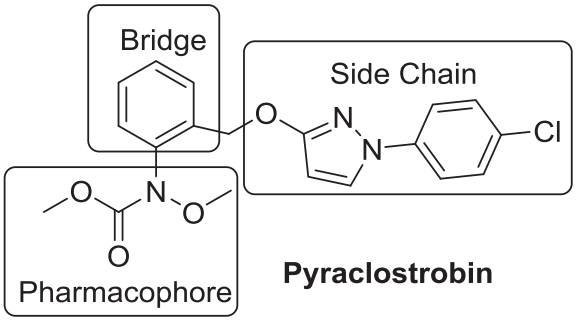
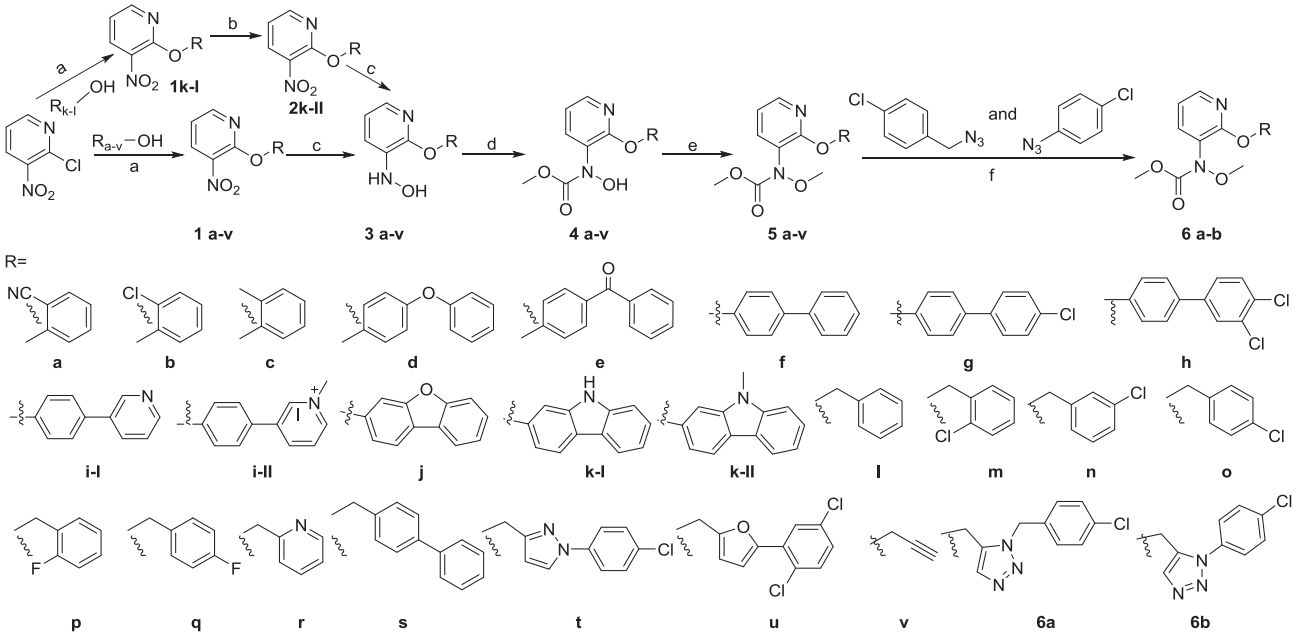


Fig. 1. Chemical structure of pyraclostrobin.

(Scheme 1). To prepare **1a**–**v**, the essential reaction intermediates 4′-chloro-(1,1′-biphenyl)-4-ol, 3′,4′-dichloro-(1,1′-biphenyl)-4-ol and 4-(pyridin-3-yl)phenol were prepared by Suzuki coupling reactions according to reported procedures.[22,23] Reaction intermediate (1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)methanol was synthesized employing the reported method through a three-step procedure.[24] Reduction of the commercially available 5-(2,5-dichlorophenyl)furan-2-carbaldehyde using $NaBH_4$ afforded the alcohol intermediate (5-(2,5-dichlorophenyl)furan-2-yl)methanol. All other side chain fragments were from commercial sources. Nucleophilic aromatic substitution reaction of 2-chloro-3-nitropyridine with the above reaction intermediates using different base afforded the corresponding products **1a**–**v**. Treatment of **1k** with NaH in anhydrous THF followed by addition of iodomethane provided intermediate **2k**. Reduction of **1a**–**j**, **1l**–**v** and **2k** using stannous chloride in the presence of sodium acetate afforded the crude product hydroxylamines **3a**–**v**, which were immediately treated with methyl chloroformate at −10 °C to give the corresponding **4a**–**v** in good yields (60–83%). Methylation of **4a**–**v** with iodomethane in a sealed tube under basic condition (potassium carbonate in acetone) afforded the target molecules **5a**–**v** in 66–91% yields. The quaternary ammonium salt **5i** was obtained at the methylation step. Compounds **6a**–**b** were prepared through the classical click chemistry (copper-catalyzed azide–alkyne cycloaddition). Treatment of **5v** with the two azido compounds 1-(azidomethyl)-4-chlorobenzene and 1-azido-4-chlorobenzene[25,26] in the presence of hydrazine hydrate and copper(II) sulfate gave **6a**–**b**. All prepared compounds were analyzed by high-pressure liquid chromatography to ensure the purity (>98%) before submission for biological evaluation.

2.2. Antifungal activity

As shown in Table 1, the prepared twenty-three target compounds were divided into two different types. For type I compounds, the aryl ring is directly attached to the pyridone oxygen, while type IA (**5a**–**c**) distinguished from IB (**5d**–**k**) by the presence of only one aryl ring at the side chain. For type II compounds, the pyridone oxygen is attached to the aromatic ring through a methylene linker. In term of antifungal activity, all type IA compounds displayed poor antifungal activity. By comparison, Type IB compounds generally displayed improved antifungal activity. Specifically, compound **5k-II**, with *N*-methylcarbazole at the side chain, exhibited a broad spectrum of antifungal activity against all five tested phytopathogens. The inhibition rate (25 μg/mL) against *Fusarium sulphureum* and *Sclerotinia sclerotiorum* were 73% and 98%, comparable to that of the positive control pyraclostrobin (78% and 100%). Replacement of one or two hydrogen atoms with chlorine at the side chain lead to significantly decreased inhibition percentage, from 100% (**5f**) to 31% (**5h**) against *Sclerotinia sclerotiorum*, suggesting the chlorine atom on the side chain of type IB compounds could not be accommodated. Compound **5i**, with the quaternary ammonium side chain, exhibited less than 10% antifungal activity against all five tested fungi phytopathogens; while high inhibitory activity of **5f** and **5k-II** concluded that the electron rich hydrophobic side chain is favorable for the compounds antifungal activity. Compounds with benzyloxyl group at the 2-position of the pyridine ring were grouped as typeⅡ, while type IIA (**5l**–**r**) with a single aryl ring at the side chain distinguished from type IIB (**5s**–**5u**, **6a-ab**) with biaryl rings. As shown in Table 1, nine out of twelve typeⅡ compounds displayed moderate to good antifungal activity. Compound **5m** demonstrated the highest inhibitory potency against all tested fungi phytopathogens (inhibition rate between 43 and 100% at 25 μg/mL) in this group. Compound **5b**



Scheme 1. Synthetic route of the target molecules **5a**–**v** and **6a**–**b**.