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Synthesis and investigation of the antibacterial activity and action mechanism of 1,3,4-oxadiazole thioether derivatives

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ABSTRACT

Various 1,3,4-oxadiazole thioether derivatives containing 2-chloro-5-methylene pyridine, 2-chloro-5-methylene thiazole, 3,4-dimethoxy-2-methylene pyridine, and *N,N*-dimethyl-2-ethylamino moieties were designed, synthesized, and assessed for their antibacterial activities against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) via the turbidimeter test in vitro. Preliminary bioassay results confirmed good antibacterial activities for most of these compounds. Among these substances, compound **6r** showed the best inhibitory effect against *Xoo*, and its half-maximal effective concentration (EC_{50}) value is 4.78 μg/mL, which is superior to that of commercial agent bismerthiazol (87.55 μg/mL). We then performed a label-free quantitative proteomic analysis of the response of *Xoo* to **6r**. A total of 1363 proteins were identified in the control and treatment groups. Upon treatment with the minimum inhibitory concentration, 349 proteins were found to be differentially expressed (fold changes > 1.5, $p < 0.05$), enriched, and may be involved in purine metabolism.

1. Introduction

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is a pathogenic Gram-negative bacterium in rice cultivation and can cause systemic infection by invading the rice xylem tissues either through wounds or stomata [1]. The incidence rate of rice bacterial leaf blight caused by *Xoo* ranges from 10% to 57% [2], whereas the damage caused by this disease ranges from 20% to 50% in Japan. Moreover, rice bacterial blight can reduce yield and affect grain quality by interfering with maturation [3]. The control of this disease relies mainly on chemical pesticides. Bismerthiazol is the main agent currently used to control rice bacterial leaf blight. However, the extensive use of this single bactericide may produce resistant individuals within a population and thus lead to control failure [4]. Hence, searching for novel, highly efficient antibacterial agents is a challenge in pesticide science.

1,3,4-Oxadiazole, a privileged five-membered heterocycle structure, affords its derivatives with a wide range of effective biological functions [5]. This structure has become a focus in medicinal and pesticide chemistry due to its significant biological properties against fungi [6] and bacteria [7]. Thioether compounds also display a broad spectrum of biological activities, such as antibacterial [8], antiviral [9], and anticancer activities [10].

In our previous work, we used gallic acid as the lead compound and synthesized a series of sulfone derivatives with high bioactivities against *Xoo*[11,12]. Another previous studies also reported several thioether derivatives containing the 1,3,4-oxadiazole moiety. These derivatives exhibited potent pesticidal activities, including antifungal [13], antibacterial [14], and antiviral effects [15]. We also offered a complete view of the proteomic changes in *Xanthomonas axonopodis* pv. *citri* in response to Fubianezuofeng [16]. Literature survey revealed that compounds containing 2-chloro-5-methylene pyridine, 2-chloro-5-methylene thiazole, 3,4-dimethoxy-2-methylene pyridine, and *N,N*-dimethyl-2-ethylamino moieties are commonly applied in pesticides or medicine because of their numerous biological activities, such as antifungal ($\mathbf{A_1}$, $\mathbf{A_3}$, $\mathbf{A_5}$, and $\mathbf{A_7}$) [17–20] and antibacterial ($\mathbf{A_4}$, $\mathbf{A_6}$, and $\mathbf{A_8}$) [19,21–23] in Fig. 1. However, the compounds containing these groups are not yet evaluated for their antibacterial activities against *Xoo*. Thus, these substructures were introduced separately to the heterocyclic system of thioether derivatives containing the 1,3,4-oxadiazole moiety in Fig. 2. This strategy was applied to construct promising molecules with antibacterial activity and to explore novel, highly efficient bioactive molecules. We also studied the effect of novel, highly efficient bioactive molecules on proteins against *Xoo*.

Abbreviations: Xoo, *Xanthomonas oryzae* pv. *oryzae*; HRMS, high-resolution mass spectrum; MIC, minimum inhibitory concentration; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; OD_{595}, optical density 595; iBAQ, intensity based absolute quantification; CC, cellular components; BP, biological processes; MF, molecular functions

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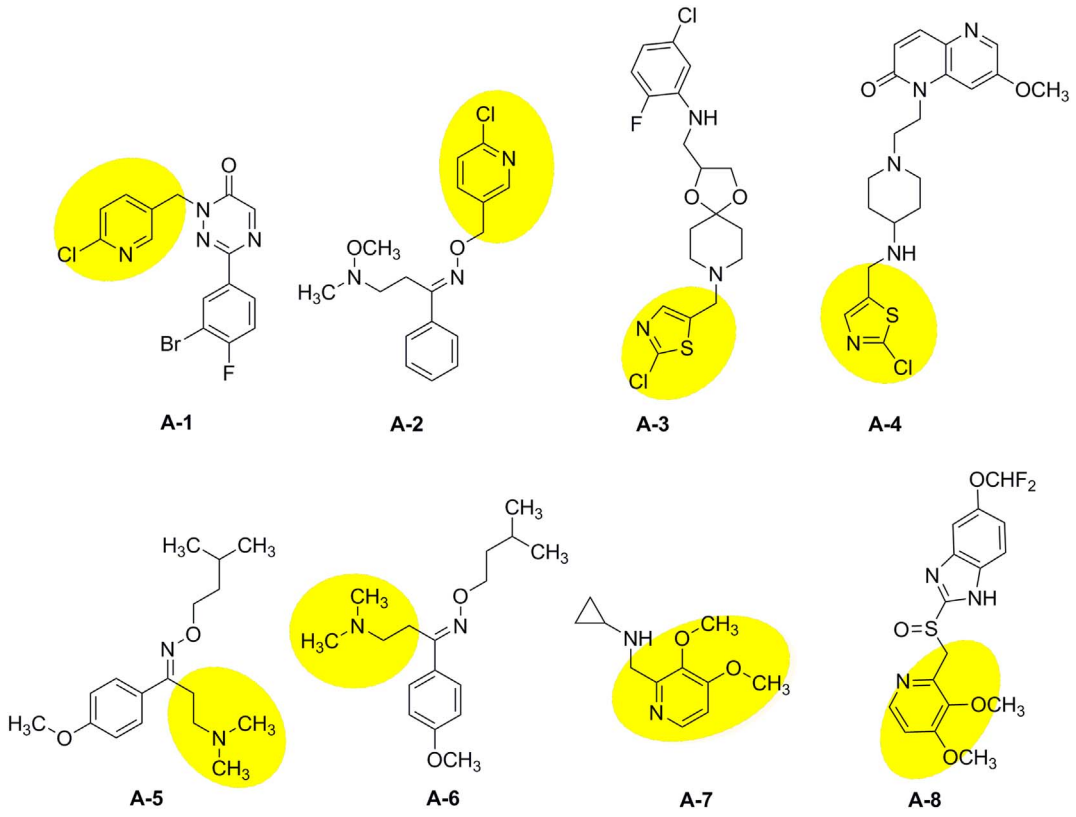

Fig. 1. Structures of the compounds A_1–A_8.

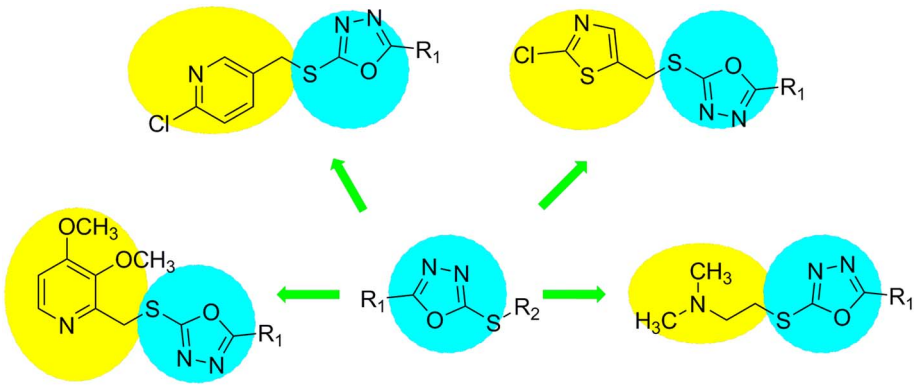

Fig. 2. Design strategy of the target compounds.

2. Materials and methods

2.1. Chemicals and instruments

All reagents and reactants were purchased from commercial suppliers. The melting points were then determined on a WRX-4 binocular microscope (Shanghai YiCe Apparatus & Equipment Co., Ltd.) (not corrected). ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectral analyses were performed on a JEOL ECX 500 NMR spectrometer (Japan) operated at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR at room temperature, and the internal standard was TMS. Infrared (IR) spectra were recorded with KBr using a Bruker VECTOR 22 spectrometer (Bruker, Karlsruhe, Germany). HRMS data were tested on Thermo Scientific Q Exactive (Thermo, Missouri, US). Meanwhile, MS (5600 Triple TOF MS) was coupled with a Nano-liquid Chromatogram (Eksigent, Dublin, CA, USA).

2.2. Chemistry

2.2.1. General procedure for preparing intermediate *4*

The structures and synthetic route for the target compounds (**6a–6ab**) are shown in Scheme 1. The important intermediate (5-substituted-1,3,4-oxadiazole-2-thiol (**4**)) was obtained by treating the starting material (substituted benzoic carboxylic acid (**1**) or 4-Cl-Ph-S(O)H (**2**)) through four steps, including esterification or substitution, hydrazidation, cyclization, and acidification [11,13,14,24–26].

2.2.2. General procedure for preparing target compounds *6a–6u*

A mixture of intermediates 5-substituted-1,3,4-oxadiazole-2-thiol (**4**) (1.2 mmol), halogen compound (**5**) (1.0 mmol), and potassium carbonate (1.4 mmol) in water was stirred for 2 h. The solvent was filtered, and the solid was washed by water to obtain the pure target compounds **6a–6u**. The data for the different compounds are as follows:

Data for **6a**. White solid, yield 91.0%, mp 101.9 °C–103.5 °C; IR