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Synthesis and bioactivity of novel sulfone derivatives containing 2,4-dichlorophenyl substituted 1,3,4-oxadiazole/thiadiazole moiety as chitinase inhibitors

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ABSTRACT

The paper reported the synthesis and antifungal properties and mechanism of action of a series of 2-substituted methylthio-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole/thiadiazole and their corresponding sulfones. The preliminary biological test showed these compounds exhibit moderate to good antifungal activity. Particularly, the compounds **7g** and **7c** inhibited mycelia growth by approximately 50% (EC_{50}) at 2.6–59.2 μg/mL and 17.2–54.4 μg/mL respectively against nine kinds of fungi. The extent of inhibition induced by **7c** on *Rhizoctonia solani* and underlying mechanism of action were studied *in vitro*. Docking simulation was performed to position selected compounds into the active site of family 18 chitinases. Variation in D-GlcNAc content and chitinase activity indicated that **7c** can act as chitinase inhibitor for controlling fungal pathogens in plants.

1. Introduction

Chitin, a polymer of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc), is an essential structural component of fungal cell walls [1]. Family 18 chitinases which degrade this linear polymer, play key roles in the life cycles of pathogenic fungi [2]. Being *endo*-chitinases, they are able to cleave random β-(1,4)-glycosidic bonds within the oligosaccharide chain leading to oligosaccharide products of varying lengths [3,4]. Therefore, certain small-molecule chitinase inhibitors may serve as potential biocontrol agents, e.g. allosamidin [5], argifin [6] and argadin [7] are associated with chitinase inhibition activity. The first chitinase inhibitor allosamidin was isolated from the mycelial extract of *Streptomyces* sp. No. 1713 [8]. It must be noted that chitin turnover is regulated by two enzymes, chitin synthase and chitinase. Polyoxins, which are commercially produced through fermentation, interfere with the fungal cell wall synthesis and the insect ecdysis *in vivo* by inhibiting chitin synthase [9]. Polyoxin B is often used to control plant fungal pathogens whereas polyoxin D is marketed as the Zn salt to control rice sheath blight *Rhizoctonia solani*. Chitinase hydrolyzes chitin into oligomer of *N*-acetylglucosamine and is the principal enzyme for the ecdysis of insects. This enzyme has also been viewed by various workers as an attractive target for the development of fungicides and insecticides [10]. Allosamidin, a competitive inhibitor of the fungal chitinase CiX1 from *Coccidioidesimmitis,* has a Ki value of 60 nM [11]. Allosamidin and other related products of natural origin are unfortunately restricted in use by their limited availability and high cost. Consequently, the development of new antifungal agents has become an absolute necessity for agricultural application.

On the other hand, sulfone derivatives provide example of an important class of bioactive compounds with wide spectrum of activities. They are employed as anti-inflammatory [12], anti-infective [13] and anti-HIV-1 [14] agents for pharmaceutical application or insecticides [15], herbicides [16] and fungicide [17] for crop protection. 1,3,4-oxadiazole/thiadiazole scaffold is an important pharmacophore in agricultural science and compounds bearing this moiety often display antifungal [18], herbicidal [19] and insecticidal [20] activities.

In our previous work we had demonstrated antifungal activities of a series of 2-substituted sulfonyl/sulfoxide-5-(trimethoxy phenyl)-1,3,4-thiadiazole/oxadiazole derivatives [21] and some *s*-substituted 6-fluoro-4-alkyl(aryl)-thioquinazoline derivatives [22]. Some of the studied compounds could completely inhibit *Botrytis cinerea* and *Sclerotinia sclerotiorum* at 50 μg/mL under laboratory conditions. More interestingly, the preliminary mechanistic studies indicated that these compounds can influence

Abbreviations: ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; *G. zeae*, *Gibberella zeae*; *C. mandshurica*, *Cytospora mandshurica*; *F. oxysporum*, *Fusarium oxysporum*; *P. infestans*, *Phytophthora infestans*; *R. solani*, *Rhizoctonia solani*; *T. cucumeris*, *Thanatephorus cucumeris*; *C. gloeosporioides*, *Colletotrichum gloeosporioides*; *B. cinerea*, *Botrytis cinerea*; *S. sclerotiorum*, *Sclerotinia sclerotiorum*; D-GlcNAc content, D-acetylglucosamine.

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chitinases activity. Since incorporation of 2,4-dichlorophenyl group into parent structure often leads to the generation of products with enhanced fungicidal activity, commercial agents such as diniconazole, hexaconazole, bromuconazole, fluquinconazole, and tetraconazole have been extensively employed in recent times for the control of the plant disease in agriculture [23]. To aid the development of potent, drug-like, and readily available chitinase inhibitors, we developed herein a series of new sulfones bearing 2,4-dichlorophenyl substituted 1,3,4-thiadiazole/oxadiazole moieties and evaluated them for their fungicidal activities. The potential mechanism by which the title compounds inhibited the fungal growth was ascertained and docking simulation was performed to position selected compounds into the active site of family 18 chitinases 2A3E. The compounds **5g**, **7a**, **7b**, **7c, 7g**, **7m** and **8g** displayed good antifungal activities at 50 μg/mL; **7g** and **7c** could inhibit mycelia growth by approximately 50% (EC_{50}) at 2.6–59.2 and 17.2–54.4 μg/mL *in vitro* against 9 kinds of fungi. The extent of inhibition induced by **7c** on *R. solani* and underlying mechanism of action were studied *in vitro* by mycelial growth rate method. After treating *R. solani* with **7c**, the spore bourgeon was inhibited and permeability of the cell membrane increased with the malformation of the hypha and condensation of its endosome. The mycelial reducing sugar content in *R. solani* changed more or less in a *V*-shape, the D-GlcNAc content, chitinase activity, mycelial pyruvate content and soluble protein content showed declining tendency. The changes in D-GlcNAc content and chitinase activity indicated that **7c** could inhibit the activity of chitinase in the cell of *R. solani*.

2. Materials and methods

2.1. General

Unless otherwise indicated, all common reagents and solvents were used as obtained from commercial supplies without further purifications. The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer in KBr disk. ^1H and ^{13}C NMR (solvent $CDCl_3$ or DMSO-d_6) spectra were recorded on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Analytical TLC was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. The optical density (OD) was determined on WFZUV-2100 360 UV–Vis Spectrophotometer. The conductivity was done by DDS-11A Conductivity Meter. The microphotograph of the hyphal morphology was taken from an Olympus Microscope. Guangkuling, a water mixture of 0.5% hymexazol and 2.5% metalaxyl as active ingredients, was prepared by Center for Research and Development of Fine Chemicals Guizhou Province, China.

2.2. Preparation of 2-substituted methylthio-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (5a–5m)/thiadiazole (6a–6i)

A 50 mL round-bottomed flask equipped with a magnetic stirrer was charged with **3**/**4**(1.5 mmol) and 3 mL (3%, w/w) of sodium hydroxide solution. The mixture was dissolved in 20 mL of distilled water. The flask was stirred at room temperature for 15 min, and then halide (1.5 mmol) and indium tribromide (0.15 mmol) were added to the reaction mixture and stirred at room temperature for 5 h. The mixture was filtered and the white solid obtained was washed with 5% Na_2CO_3 solution and distilled water, dried under vacuum, and recrystallized from ethanol to give compounds **5(5a–5m)**/**6(6a–6i)**.

2.3. Preparation of 2-substituted sulfonyl-5-(2,4-dichlorophenyl)-1,3,4-thiadiazole (7a–7m)/oxadiazole (8a–8i)

To a 100 mL, three-necked, round-bottomed flask equipped with a magnetic stirrer were added 2-substituted methylthio-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole/thiadiazole (0.43 mmol), acetic acid (10 mL) and $Na_2WO_4{\cdot}2H_2O_2$ (0.022 mmol). The resulting solution was stirred for 5 min, then 30% H_2O_2 (2.58 mmol) was slowly added, heated to 55 °C and the reaction was continued for 50 min. After cooling to room temperature, the mixture was neutralized by 5% sodium hydroxide to a pH of 7.0, extracted with chloroform (3 × 30 mL), dried over anhydrous magnesium sulfate, and separated on silica column with ethyl acetate/petroleum ether (v/v = 1:3) to give pure products **7(7a–7m)**/**8(8a–8i)**.

2.3.1. 2-(allylsulfonyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole(7c)

White solid; mp 77–79 °C; yield 84.5%; ^1H NMR(500 MHz, $CDCl_3$) δ: 4.30 (d, 2H, J = 7.45 Hz, CH_2–SO_2), 5.49–5.57 (m, 2H, CH=CH_2), 5.90–5.97(m, 1H, CH=CH_2), 7.27–8.02 (m, 3H, benzyl-H); ^{13}C NMR(125 MHz, $CDCl_3$) δ: 164.38, 161.68, 139.88, 134.76, 132.48, 131.65, 128.05, 127.73, 121.95, 120.07, 59.96; IR (KBr) cm^{-1}: 3023, 1591, 1556, 1471, 1423, 1348, 1238, 1145; Anal. Calcd for $C_{11}H_8Cl_2N_2O_3S$: C 41.39, H 2.53, N 8.78; found C 41.58, H 2.32, N 8.87.

2.3.2. 2-(methylsulfonyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole(7g)

White solid; mp 103–104 °C; yield 85.1%; ^1H NMR(500 MHz, $CDCl_3$) δ: 3.53 (s, 3H, CH_3), 7.45–8.01 (m, 3H, benzyl-H); ^{13}C NMR(125 MHz, $CDCl_3$) δ: 167.01, 165.71, 138.11, 133.84, 131.67, 131.14, 127.61, 122.98, 18.83.; IR (KBr) cm^{-1}: 3041, 2966, 1587, 1560, 1456, 1406, 1350, 1157; Anal. Calcd for $C_9H_6Cl_2N_2O_3S$: C 36.88, H 2.06, N 9.56; found C 37.10, H 2.12, N 9.90.

2.4. In vitro antifungal activity

The antifungal activity of all synthesized compounds was tested against three pathogenic fungi, *Gibberella zeae*, *Fusarium oxysporum*, and *Cytospora mandshurica* by the poison plate technique [24].

Compounds were dissolved in 1 mL dimethyl sulfoxide before mixing with 90 mL potato dextrose agar (PDA). The compounds were tested at a concentration of 50 μg/mL. All kinds of fungi were incubated in PDA at 27 ± 1 °C for 4 days to get new mycelium for antifungal assay. Then mycelia dishes of approximately 4 mm diameter were cut from culture medium and one of them was picked up with a sterilized inoculation needle and inoculated in the center of PDA plate aseptically. The inoculated plates were incubated at 27 ± 1 °C for 5 days. Acetone in sterile distilled water served as control, while thiophanate-methyl, hymexazol and myclobutanil acted as reference agents. For each treatment, three replicates were conducted. The radial growth of the fungal colonies was measured and the data were statistically analyzed. The inhibiting effects of the test compounds *in vitro* on these fungi were calculated by the formula: $I(\%) = [(C - T)/(C - 0.4)]^*100$, where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA while I means the inhibition rate.

The compound **7c** was tested against nine pathogenic fungi namely *G. zeae*, *F. oxysporum*, *C. mandshurica*, *Phytophthora infestans*, *R. solani*, *Thanatephorus cucumeris*, *Colletotrichum gloeosporioides*, *B. cinerea* and *S. sclerotiorum* at different concentrations of 200, 100, 50, 25, 12.5, 6.25, 0 μg/mL, 50, 25, 12.5, 6.25, 3.125, 0 μg/mL or 25, 12.5, 5, 2.5, 1, 0.5, 0 μg/mL. The EC_{50} values were estimated statistically by probit analysis with the help of probit package of SPSS software using a personal computer. The average EC_{50} (μg/mL) was taken (effective dose for 50% inhibition μg/mL)