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Benzofurazan derivatives as antifungal agents against phytopathogenic fungi

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ABSTRACT

A series of benzofurazan derivatives were prepared and evaluated for their biological activities against four important phytopathogenic fungi, namely, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium graminearum* and *Phytophthora capsici*, using the mycelium growth inhibition method. The structures of these compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. *N*-(3-chloro-4-fluorophenyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (**A3**) displayed the maximum antifungal activity against *R. solani* (IC_{50} = 1.91 μg/mL), which is close to that of the positive control Carbendazim (IC_{50} = 1.42 μg/mL). For other benzofurazan derivatives with nitro group at R^4 position (**A** series), 9 out of 30 compounds exhibited high antifungal effect against strain *R. solani*, with IC_{50} values less than 5 μg/mL. Most of the derivatives with substituents at R^2 and R^3 positions (**B** series) displayed moderate growth inhibition against *S. sclerotiorum* (IC_{50} < 25 μg/mL). Also, several benzofuran derivatives with nitro group at R^4 position and another conjugated aromatic ring at the R^1 position of the phenyl ring displayed high antifungal capability against strain *R. solani*. Compounds with substituents at R^2 and R^3 position had moderate efficacy against strain *S. sclerotiorum*.

1. Introduction

Pathogenic fungi and insect pests caused over 30% yield losses in major crops [1]. To increase yields and provide healthy crops, application of agrochemicals has become an established part of modern agriculture. For complicated plant disease situation, the combined use of different antifungal agents is common (ref). Consequently, fungicide-resistant pathogens have been selected and evolved. Therefore, there is a need for novel antifungal compounds with new mechanism of action and lower application dosage.

During screening of our in house compound library for biologically active components targeting plant pathogens, the benzofuroxan scaffold (Fig. 1) was identified to exhibit good *in vitro* activity against four important phytopathogenic fungi. Further structural modification revealed benzofuroxan derivatives with nitro at the R^4 position and small sized amino group at R^1 position resulted in candidates displaying significant *in vitro* and *in vivo* antifungal activity [2]. Unlike furoxan (Fig. 1), the benzofuroxans were considered to be devoid of the nitric oxide (NO) releasing capability [3]. Therefore, the reported antifungal activity should not relate to the NO biological function. Alternatively, the benzofuroxan system is highly electron deficient and nucleophile sensitive, especially when the phenyl R^4 position contains a nitro group. Nucleophiles like thiol or hydroxyl would readily attack the nitro *para* position to form the so called Meisenheimer-type complex intermediate, which quickly undergoes elimination reaction to provide a more stable benzofuroxan [4]. Previous studies have also established that substituents *ortho* to nitro would considerably diminish the Meisenheimer-type complex formation [5]. Whether the compound electrophilicity is involved in the antifungal activity should be explored and addressed. We anticipate that removal of the electron negative oxygen atom, transformation to benzofurazan (Fig. 1), will render the whole conjugated system less electron-deficient. Alternatively, the benzofurazan derivatives have been reported to possess a variety of bioactivity, such as anti-protozoa, antibacterial and calcium channel modulated property [6–10]. Given the structural similarity of benzofuroxan and benzofurazan,

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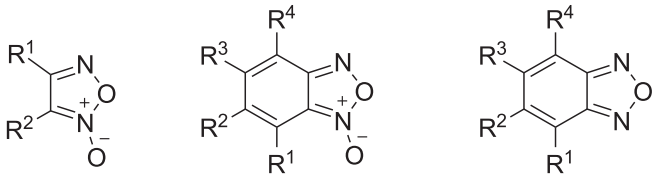

Fig. 1. Chemical structures of furoxan, benzofuroxan and benzofurazan.

we investigated the antifungal aspect of benzofurazan. In this paper, series of benzofurazan compounds were prepared, and the *in vitro* antifungal activities were evaluated against four important plant pathogen strains including *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, and *Phytophthora capsici*. These four strains of fungi are representative in their biological species, and caused a wide range of significant plant disease on different crops. For example, *F. graminearum*, also known by the name of teleomorph *Gibberella zeae*, is a plant pathogen that causes fusarium head blight on wheat and barley [11]; *R. solani* is a plant pathogenic fungus with a wide host range and worldwide distribution, also one of the fungi responsible for Brown patch (a turfgrass disease), as well as black scurf of potatoes, bare patch of cereals, root rot of sugar beet, belly rot of cucumber, sheath blight of rice, and many other pathogenic conditions [12].

2. Chemistry

To investigate whether the nitrogen–oxygen coordinate bond in benzofuroxan is necessary for antifungal activity, the corresponding benzofurazans were synthesized. Specifically, the benzene ring at the R^1 position (Fig. 1) was first chosen for modification (**A18**–**A26**, **B2**–**B4 and B10**). Further change with other conjugated heterocyclics, such as azoles and thiophene [13–16], which are common structural elements in compounds with antifungal activity were investigated. All new benzofurazan compounds were prepared using the synthetic routes shown in Schemes 1 and 2. 4-Chloro-7-nitrobenzo[c][1,2,5]oxadiazole(A1) was prepared from commercially available 2,6-dichloroaniline employing the reported synthesis procedure [17]. Then nucleophilic substitution reaction of compound **A1** with different amines was carried in a sealed tube at 100 °C to provide **A2**–**A6**. To prepare **A7**–**A8**, a nitrogen atmosphere and low temperature reaction condition was used for the pyrazole and 1,2,4-triazole substrates. Treating **A1** with thiols using sodium methoxide as base afforded **A9**–**A12** in good yield (>90%).

The key intermediate 5-chloro-4-fluoro-2-nitroaniline (**1**) was obtained from commercially available 3-chloro-4-fluoroaniline using the reported procedure [2]. Nucleophilic substitution reaction of compound **1** with sodium methoxide gave compound **2** in 90% yield. Based on ^{1}H NMR analysis, only the C5-substituted product was obtained. Treatment of compound **2** with sodium hypochlorite and 0.25% (w/v) KOH in ethanol afforded 5-ethoxy-6-methoxybenzofuroxan (**3**) in 53% yield. The other three benzofurazans (4-2, 4-3, 4-4) were prepared according to our previously reported procedure [2]. Reduction of these benzofuroxans with triphenylphosphine under reflux in dichloromethane afforded the target compounds **B1**–**B4**. The remaining listed compounds in Tables 1–3 (**A13**–**A30**, **B5**–**B10** and **C1**–**C5**) were prepared according to previously reported methods [18–24,27]. All compounds were analyzed by high-pressure liquid chromatography to ensure the purity (>95%) before submission for biological evaluation.

3. Antifungal activity

The antifungal activities of the synthetic molecules, expressed as IC_{50} (median inhibitory concentration) values, were determined using the mycelia growth inhibitory rate method. The results are shown in Tables 1–3.

The benzofurazan derivative **A3**, with the 3-chloro-4-fluoroaniline substituent at the R^1 position, showed a broad spectrum of antifungal activity against all four tested fungi phytopathogens. Its IC_{50} value against *R. solani* was 1.91 μg/mL, which was close to that of the positive control Carbendazim (IC_{50} = 1.42 μg/mL). Most of the other compounds with different aniline substitutions at the R^1 position also displayed high potency against *R. solani*. Especially, the 4-bromoaniline derivative **A4** (IC_{50} = 2.03 μg/mL) and corresponding chloro analogue **A28** (IC_{50} = 3.87 μg/mL) displayed inhibitory potency similar to that of carbendazim. Compounds **A20**–**A24**, with pyrrolidine, piperidine, piperazine and morpholine at the R^1 position, displayed weak antifungal activity with IC_{50} value higher than 25 μg/mL against all four tested fungi phytopathogens. Replacement of the above aliphatic amine with aromatic triazole and pyrazole (compounds **A7**–**A8**) improved the activity, with IC_{50} values 3.11–22.29 μg/mL against *R. solani*, *S. sclerotiorum* and *F. graminearum Sehw.* This suggested that the hetero-aromatic azole group has a great contribution on compound antifungal activity. Finally, **A10**–**A12** and **A19**, with different thiols at position 4 exhibited low IC_{50} (<5 μg/mL) against *R. solani*. These findings indicated that the presence of another conjugated system at the R^1 position is favorable for the antifungal activity of type **A** benzofurazan derivatives, and they may serve as new leads for the development of potentially useful antifungal agents against *R. solani*.

In the case of series **B** of benzofurazan derivatives, with substituents variation at the R^2 and R^3 positions, whereas both R^1 and R^4 positions are hydrogen atoms, seven out of ten compounds (**B2**–**B4** and **B6**–**B9**) exhibited potency against *S. sclerotiorum* (IC_{50} = 13.18–24.41 μg/mL), and their antifungal activity against the other three fungi phytopathogens was weak (IC_{50} > 25 μg/mL). Replacement of a chlorine atom at R^2 position with a fluorine atom increased the IC_{50} value from 8.54 μg/mL (for **B2**) to >25 μg/mL (for **B4**) against *R. solani*, indicating a chlorine atom at R^2 position was more appropriate than a fluorine atom. Moreover, the same trend was confirmed with **B3** (IC_{50} = 16.64 μg/mL) versus **B10** (IC_{50} > 25 μg/mL) or **B8** (IC_{50} = 15.71 μg/mL) versus **B9** (IC_{50} = 24.41 μg/mL) against *S. sclerotiorum*. In general, this series of compounds demonstrated better antifungal activity against *S. sclerotiorum* as compared with the other three fungi phytopathogens.

Series **C** benzofurazan derivatives exhibited weak antifungal activity (IC_{50} values >25 μg/mL). Compound **A13** had potency against *R. solani*, *S. sclerotiorum* and *F. graminearum Sehw* (IC_{50} = 10.07–20.82 μg/mL), while compound **C1** barely exhibited antifungal activity. When comparing the activity of **C4**–**C5** with the corresponding **A15** and **A18** (IC_{50} values >25 μg/mL), it was concluded that substitution of the R^4 nitro group with another electron-withdrawing benzenesulfonyl substituent significantly reduced the antifungal activity. The above results suggested that the nitro at R^4 position was highly important for the antifungal activity of the compounds.

To further understand the selectivity of these prepared compounds, the most potent compound **A3** was evaluated against invasive fungal pathogen *Candida albicans* and the human normal liver cell line HL-7702. At 25 μg/mL. The percentage of inhibition against *C. albicans* CMCC(F)98001 is 48.18 ± 2.53, and 84.8% of human normal liver cell HL-7702 was inhibited. Considering the IC_{50} of **A3** against phytopathogenic fungi *R. solani* is 1.91 ± 0.14 μg/mL, these results demonstrated that compound **A3** exhibited a good selectivity against the phytopathogenic fungi.