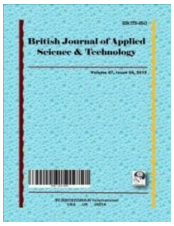

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Synthesis of Novel Imidazole Derivatives Based on Camalexin Scaffold and Anti-fungal Activity against Rice Blast

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Authors' contributions

This work was carried out in collaboration between all authors. Author OHK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MI and TH carried out the chemical synthesis and bioassay. Authors YY managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Rice blast disease (RBD), which is caused by the pathogenic fungi of *Magnaporthe oryzae* is the most devastating diseases of cultivated rice (*Oryza sativa* L) Although many strategies have been applied to control the RBD, chemical anti-fungal agents are the most effective solutions to minimize the severity of RBD. The aim of this study is to explore new anti-fungal agents for RBD control.
Study Design: Natural products are important source of biological active compounds. We used camalexin (3-thiazol-2'-yl-indole), which is a well known phytoalexin of *Arabidopsis thaliana*. In the present work, we designed new imidazoles based on camalexin as a molecular scaffold and the anti-fungal activity of the synthesized compounds against *Magnaporthe oryzae* were determined.
Place and Duration of Study: All the experiments were conducted from October 2014 to March 2015 at Akita Prefectural University, Japan.
Methodology: A series of new imidazole derivatives was designed and synthesized based on the camalexin as a molecular scaffold. The antifungal activity of the synthesized compounds against *Magnaporthe oryzae* was determined by using *in vitro* mycelial growth inhibition tests.

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Results: Among 3 newly synthesized camalexin based imidazole compounds, we found that 3-[3-(*1H*-imidazol-1-yl)propyl]-1H-Indole **(2c)** exhibits potent inhibitory activity with an IC_{50} value approximately 7.8±1.2 μM.
Conclusion: We discovered a new lead compound with potent activity for rice blast control.

Keywords: Camalexin; antifungal agent; imidazole derivatives; rice blast.

1. INTRODUCTION

Biotic and abiotic stresses are major external stimuli which greatly affect the growth of plants. Unfavorable temperature, drought, chemical pollutions are major abiotic stresses. Among the biotic stresses, pathogen infection is one of the most severe stresses. Rice blast diseases (RBD) caused by pathogenic fungi of *Magnaporthe oryzae* is a leading constraint in world's rice production [1-3]. The high incidence of plant mortality and the lack of effective control methods make it responsible for billions of dollars losses worldwide each year.

To control the RBD, tremendous efforts have been made and various management strategies have been applied [4], including controlled use of nitrogen fertilizers, application of silica and flooding of paddy fields [4]. Currently, chemical fungicide is the most common solution effectively to minimize the severity of RBD. Many systemic fungicides with different mode of actions have been used for RBD control. Anti-fungal compounds [5], melanin inhibitors [6], ergo sterol biosynthesis inhibitor (EBI) and other organic compounds have been widely used [7]. With the increasing use of chemical fungicides, the appearance of pathogens that resistant to existing fungicides raised new challenges for diseases control in agricultural industry. Hence, development new anti-microbial agent for RBD control is of significant importance in modern agricultural industry.

Different from vertebrate, the cell order and integrity of fungal membrane are significantly important in fungi. Hence, chemicals that directly or indirectly target fungal membranes functions or their components synthesis are quite effective to cause the lethality of the fungi thereby controlling the fungal disease. Some of these anti-fungal compounds affect the synthesis of specific membrane components (e.g., sterol biosynthesis inhibitors) are among the most effective fungicides.

To meet the demands for plant diseases control, variety strategies have been applied for fungicides development. Natural products are useful sources of novel chemical structures for development of new fungicides [8-11]. Until now, major advances in modern medicine and the pharmaceutical industry were driven by natural products or their derivatives [12]. Many experts believe that using natural products as leads is a feasible way to find the core structure of biological active compounds in drug discovery [13].

Based on these facts, using the core structure of natural products which display anti-microbial activity is a straightforward approach to explore new agents for RBD control. Phytoalexins are heterogeneous low molecular mass secondary metabolites with antimicrobial activity at the infection site [14,15].

The biosynthesis of phytoalexins is commonly regulated by a complex mechanism of plant defense signal transduction networks. The plant hormones, jasmonic acid, salicylic acid and ethylene, are important signal mediators that involve in plant defense response and phytoalexins synthesis [16]. These plant hormones activate the expression of defense genes, causing rapidly accumulation of phytoalexins at the areas of pathogen infection [17]. Indeed, the structural diversity of phytoalexins are in different plant species and most of the phytoalexins are belong to terpenoids [18], glycosteroids and alkaloids [19].

One of the well-known phytoalexin is camalexin (3-thiazol-2'-yl-indole, the structure is shown in Fig. 1), a primary phytoalexin of Arabidopsis thaliana [20], is induced by a great variety of plant pathogens. The biosynthesis and the regulation of the biosynthesis of camalexin have been studied extensively [21,22]. It has been demonstrated that camalexin is biosynthesized from tryptophan [23] and its biosynthesis involves the cytochrome P450 enzymes CYP79B2 [24] and CYP71B15 (PAD3) [25]. Accumulation of camalexin upon pathogen infection, as well as its anti-microbial nature supports the role in disease resistance [26].

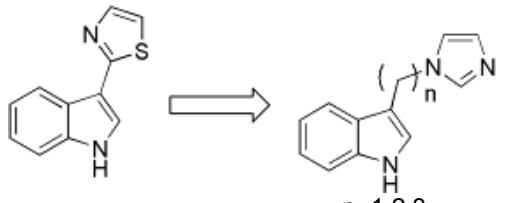


Fig. 1. Design of new imidazole derivatives based on camalexin scaffold

To explore new agents for RBD control, we report the synthesis of new compounds based on the camalexin scaffold. We design the target compound with an indole skeleton. On the other hand, the thiazol moiety was replaced by an imidazole group with a spacer structure as shown in Fig. 1. The biological activity of synthesized compounds against *Magnaporthe oryzae* was evaluated by using in vitro mycelial growth inhibition tests.

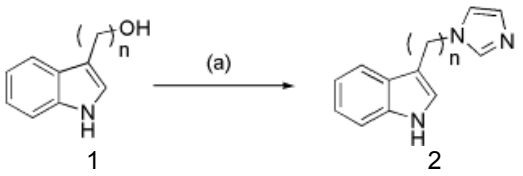
2. METERIALS AND METHODS

2.1 General

Stock solutions of all the test compound were dissolved in DMSO at a concentration of 100 mM and stocked at −30°C. Other reagents were of the highest grade and purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). 1H-NMR spectra were recorded with a JEOL ECP-400 spectrometer (Tokyo, Japan), chemical shifts being expressed in ppm downfield from TMS as an internal standard. High resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectra (ESI-FTICR) were recorded on an Exactive MS System (Thermo Fisher Scientific, Waltham, MA, USA).

2.2 Chemical Synthesis

Preparation of 3-(1H-imidazol-1-ylmethyl)-1H-Indole (2a) was carried out by a method as previously described [11]. A solution of 1H-indole-3-methanol (1a; 294 mg, 2 mmol) and CDI (420 mg, 2.6 mmol, 1.3 equiv) in anhyd MeCN (5 ml) was refuxed for 5 h (the reactions were monitored by TLC and terminated after complete consumption of the hydroxyl compound) the reaction mixture was diluted with cold water (20 ml) and the resulting precipitate was collected, washed with water and dried under vacuum at 40-50ºC. Recristalization were carried out by using anhyd MeCN to give pure 3-(1H-imidazol-1-ylmethyl)-1H-Indole (2a) (Yield 92%), 1H NMR(CDCl3), δ: 5.31 (2H, s), 6.96 (1H, s), 7.05 (1H, s), 7.13 (1H, t, J=7.1), 7.18 (1H, d, J=2.3), 7.23(1H, d, J=8.2), 7.43 (2H, q, J1=8.1, J2=12.0), 7.59(1H, s), 8.23(1H, s).



n=1: **1a**
n=2: **1b**
n=3: **1c**

n=1: **2a**
n=2: **2b**
n=3: **2c**

Scheme 1. Synthesis of target compounds: reagents and conditions: (a), CDI; CH3CN (anhydride), reflux, 5 hour

Compound 2b and 2c were prepared in a similar way by using different alcohols as a starting material as shown below.

3-[2-(1H-imidazol-1-yl)ethyl]-1H-Indole (2b). (Yield 90%), 1H NMR (CDCl3), δ: 3.28 (2H, t, J=7.1), 4.68 (2H, t, J=7.1), 7.06(1H, t, J=1.3), 7.10 (1H, d, J=2.3), 7.16 (1H, d, J=0.92), 7.18-7.25 (1H, m), 7.39-7.41 (2H, m), 7.64 (1H, d, J=8.0), 8.11 (2H, d, J=6.4). 2c were prepared in a similar way by using corresponding indole derivatives.

3-[3-(1H-imidazol-1-yl)propyl]-1H-Indole (2c). (Yield 94%), 1H NMR (CDCl3), δ: 2.20-2.27 (2H, m), 2.94 (2H, t, J=7.5), 4.48 (2H, t, J=6.5), 7.03 (1H, d, J=2.3), 7.07 (1H, q, J1=0.92, J2=1.6), 7.13 (1H, t, J=0.92), 7.15-7.23 (1H, m), 7.38-7.39 (2H, m), 7.60 (1H, d, J=8.2), 8.05 (2H, d, J=23.8).

2.3 *Magnaporthe-oryzae* Strain

Pathogenic fungi of Rice blast (*Magnaporthe oryzae*) isolate designated APU00-093A (race 007.0) was obtained by mono-spore isolation from diseased rice panicle on the paddy field of Akita Prefecture Japan in 2000. This isolate was kept on potato dextrose agar at 15ºC.

2.4 Antifungal Activity Assay

Poisoned food technique was performed to investigate antifungal effect of test compounds against *Magnaporthe oryzae*. The Mycelial

growth inhibition tests were carried out. Each test compound dissolved and diluted in DMSO was added to potato sucrose agar (PSA) medium (kept at 50ºC after autoclaving) to the appropriate concentration. The final concentration of DMSO of each medium was 0.1%. Three mycelial pellet (1mm in diameter) of *Magnaporthe oryzae* pre-cultured on potato dextrose agar (PDA) medium were placed on the PSA medium containing the given concentrations of the test compound. The diameter of the mycelial mat of *Magnaporthe oryzae* was measured when the diameter of each corresponding untreated control reached about 20-30 mm. Concentration for 50% inhibition (IC_{50}, μM) of mycelial growth was calculated by the linear regression formula obtained from the logarithm of concentration and the inhibition rate at each concentration against untreated control. All experiments were carried out in triplicate and the report data represents average values.

3. RESULTS AND DISCUSSION

3.1 Chemistry

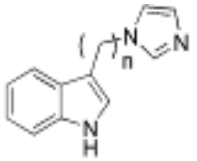
Method for synthesis target compounds is shown in Scheme 1 in a condition as previously reported [27]. 3-Indolemethanol (1a) and its derivatives (2b,2c) were used as starting materials. Compound 2a was prepared by reacting compound 1a with 1.3 equivalent of 1, 1'-carbonylbis-1H-imidazole in anhydride acetonitrile under a condition of reflux for 5 hours. The reaction was monitored by using TLC until the starting material 1a is disappeared. Compound 2b and 2c were prepared in a similar way by using 1b and 1c as starting materials.

3.2 Biology

The chemical structures of the test compounds were listed in Table 1. The concentration of all the test compounds were adjusted to a final concentration of 100 μM in potato sucrose agar (PSA) medium while propiconazole was used as a positive control. The key structure were the indole moiety and an imidazole moiety in this synthetic series. Thus, we introduced three kinds of chain structure with different carbon atoms. As shown in Table 1, introducing single carbon atom (2a) exhibited antifungal activity with a fungal growth rate at approximately 48.2±4.2%. When two carbon atoms were introduced (2b), we found that the antifungal activity was significantly decreased. The fungal growth rate was found approximately 85.6±8.2% compared to none chemical treated control. Interestingly, when three carbon atoms chain was introduced (**2c**), we found that the antifungal activity was enhanced greatly. The fungal growth rate was found approximately 18.2±1.6%.

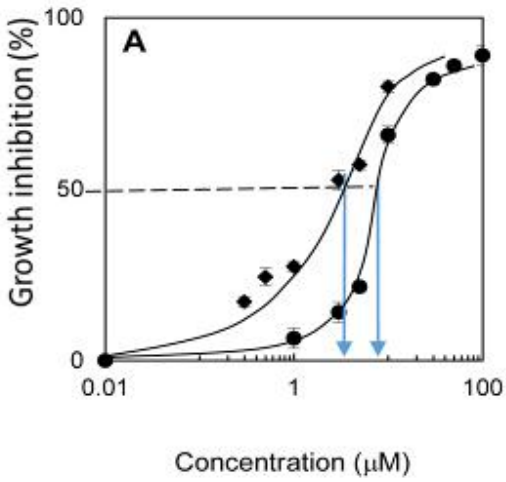
Next, we used compound **2c** to determine the dose-dependent effect of antifungal activity against *Magnaporthe oryzae* of this synthetic series. As shown in Fig. 2, compound **2c** inhibits *Magnaporthe oryzae* growth in a dose dependent manner. The IC_{50} was found approximately 7.8±1.2 μM. while the IC_{50} of the positive control of propiconazole was found approximately 3.7±0.2 μM in our assay system.

Table 1. Antifungal activity of test compounds



Compound No.	n	Fungal growth (%)*
2a	1	48.2±4.2
2b	2	85.6±8.2
2c	3	18.2±1.6
Propiconazole		3.3±2.8

**The concentration of all the test compounds and positive control Propiconazole were 100 μM. Fungal growth was determined by measure the mycelial diameter of none chemical treated line as 100%. All the experiment were done three times to establish the repeatability*



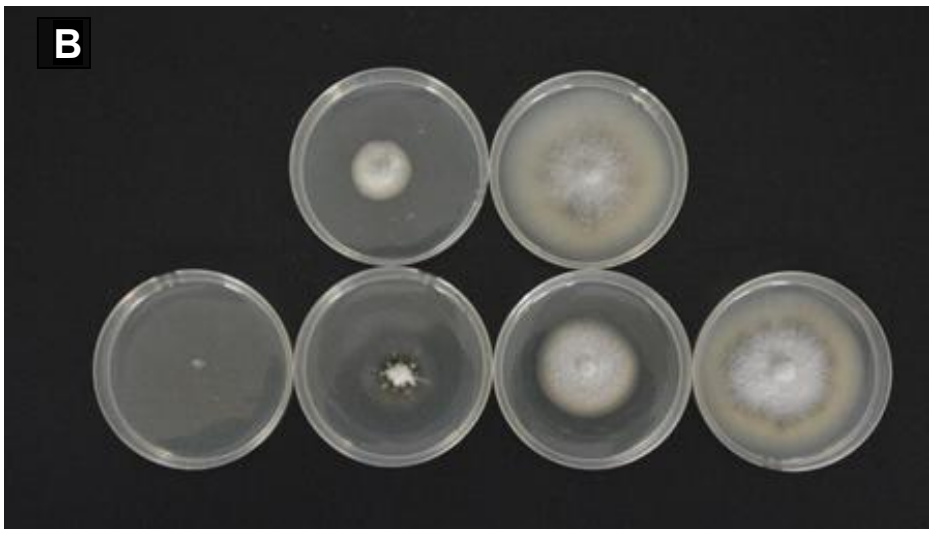


Fig. 2. Antifungal activity of compound 2c against *Magnaporthe oryzae*. (A) Does dependent effect of 2c on growth of *Magnaporthe oryzae*, filled cycle: compound 2c; filled diamond: propiconazole. (B) Effect of compound 2c on growth of *Magnaporthe oryzae*, upper left: 10 μM propiconazole, upper right: control; lower left. 1 mM 2c, lower second from left: 100 μM 2c, lower second from right, 10 μM 2c, lower right, 1 μM. All the experiments were taken three times to establish the repeatability.

4. CONCLUSION

In the present work, we synthesized a series of new imidazole derivatives based on the camalexin (3-thiazol-2'-yl-indole) scaffold. Camalexin is a primary phytoalexin of Arabidopsis thaliana that display antifungal activity against many kinds of pathogenic fungi. Evaluation the antifungal activity of newly synthesized imidazole derivatives against *Magnaporthe oryzae* indicated that the carbon number of the chain structure between indole and imidazole moiety significantly influence the antifungal activity. Among the synthesized compounds, compound **2c** exhibits potent antifungal activity with an IC_{50} value approximately 7.8±1.2 μM. The antifungal potency was found approximately half of that of propiconazole. We expect further structure-activity relationship studies may provide in slight information about the structural requirements of this synthetic series on antifungal activity against rice blast pathogen *Magnaporthe oryzae*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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