Synthesis of Imidazole and Indole Hybrid Molecules and Antifungal Activity against Rice Blast

Tomio Sumiya, Mai Ishigaki, and Keimei Oh

Abstract—**Azole and indole are parent substances of many natural and synthetic compounds with significant biological activity. However, the biological activity of the indole and azole conjugates are lack of investigation. In the present work, a series of hybrid molecules with imidazole and indole moiety were designed by using camalexin as a molecular scaffold. Compounds with different length of the carboxylic acid (4a-4f) were prepared. The antifungal activity of this synthetic series together with the ethyl esters analogues (3a-3f) against *Magnaporthe oryzae* were determined by using agar cup plate assay. Data obtained from the structure-activity relationship studies indicated that the ester analogues displayed antifungal activity against *Magnaporthe oryzae while* the carboxylic acid derivatives did not. This result indicated that the carboxylic acid ethyl ester moiety is important to antifungal activity. Among all the synthesized compounds, we found that, at a concentration of 100 μM, compound 3c displays the most potent inhibition activity with 38.8±2.5% on the inhibition of the diameter of the mycelial mat of *Magnaporthe oryzae* while the positive control of propiconazole (10 μM) was found 39.3±2.9%.**

Index Terms—**Antifungal activity, imidazole, indole, rice blast.**

I. INTRODUCTION

There are tremendous needs to control pathogenic microbes for health care and in agricultural industry. The chemicals that either kill or display inhibitory activity on microbial growth are currently the major solutions for pathogenic microbe control [1].

Rice blast disease (RBD) which is caused by the pathogenic fungi of *M. oryzae,* is a leading constraint in world rice production [2]. The high incidence of plant mortality and the lack of effective control methods cause billions of dollars in losses worldwide each year. Hence, great efforts and various management strategies have been made to control the RBD [3] inimize the severity of RBD [4].

Some fungicides are targeting membranes functions due to the cell order and integrity of microbial membrane are significantly important. Chemicals that directly or indirectly target membranes functions or their components synthesis are quite effective to cause the lethality of the microbes thereby controlling the microbial disease.

Several chemical structures have been found effective on killing or inhibiting the microbial growth which including indole [5] and azole derivatives [6]. Some indole derivatives has been found with broad spectrum of antimicrobial activity [5]. Many indole scaffold antimicrobial agents have been well investigated [7]-[9]. On the other hand, azole derivatives are also well known antimicrobial agents [6]. The action mechanism of azoles is believed that they targeting the P450 enzymes involved in the biosynthesis of sterol [10]. It is known that azole derivatives have widespread ability to function as cytochrome P450 inhibitors [11], apparently due to the intrinsic affinity of the nitrogen electron pair in heterocyclic molecules for the prosthetic heme iron [12]. The azoles thus bind not only to lipophilic regions of the protein, they also simultaneously bind to the prosthetic heme iron [13] thereby prevent the enzyme-substrate interactions. There are many azole derivatives which serve as potent fungicidal agent and some of which are commercially available [14]-[16].

In spite of the antimicrobial activity of indole and azole derivatives have been investigated extensively, the biological activity of the conjugates of these core structure against pathogenic fungi is lack of investigation. In the present work, we designed an indole-imidazole conjugate using Camalexin (3-thiazol-2'-yl-indole) (Fig. 1), the end product of tryptophan pathways and the major phytoalexin of *Arabidopsis*. We report herein the synthesis and biological evaluation of the fungicide activity of indole-imidazole conjugate compounds against *M. oryzae.*

n -, 3; 4; 5; 6; 9; 10
R: H; Et
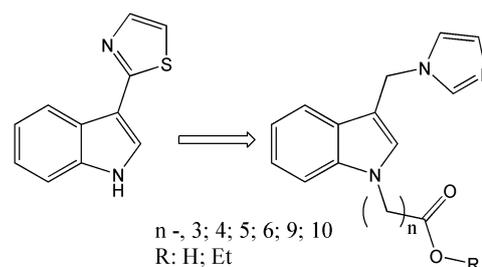
Fig. 1. Molecular design of indole-imidazole conjugates based on Camalexin scaffold.

II. MATERIALS AND METHODS

A. 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 down TMS as an internal standard. High resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectra (ESI were recorded on an Exactive MS System (Thermo Fisher Scientific, Waltham, MA, USA).

B. Chemical Synthesis

Preparation of *3-(1H-imidazol)-1-Indole (2)* was carried out by a method as previously described [17]. 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 (5ml) was stirring at room temperature for 5 h. The reaction mixture was worked up and diluted with cold water (20 ml) and the resulting precipitate was collected, washed with water and dried under vacuum at 40-50°C. Recrystallization were carried out by using anhydride MeCN to give pure 3-(1H-imidazo- 1-ylmethyl)-1H-Indole (**2**) (Yield 92%), ^{1}H NMR(CDCl$_3$), 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,J2=12.0), 7.59(1H, s), 8.23(1H, s).

Preparation of *4-(3-Imidazol-1-ylmethyl-indol-1-yl)-butyric acid ethyl ester (3a)* was carried out by a method as we previously described [18]. Stirring 40 mg of NaH (approximately 60% pure, 1 mmol) in a suspension of anhydride DMF (2 ml) in a 50 ml flask on ice. A solution of 3-(1H-imidazol-1-Indole in anhydride DMF (containing 197 mg, 1 mmol) was added dropwise and stirred for 30 min at room temperature. Then, 4-bromobutric acid ethyl ester (187 mg, 1 mol) was added dropwise and stirred for 5 hour at room temperature. The reaction was quenched by added H$_2$O. The DMF was removed by extraction of target compound with three times of chloroform (20 ml). The organic layer was combined and dried with anhydride Na$_2$SO$_4$. The solvent was removed by concentration under reduced pressure gave 67 mg of **3a**. Yield: 40.3 % . ^{1}H NMR(CDCl$_3$). δ: 1.21-1.25 (m, 3H), 2.13-2.19 (m, 2H), 2.29 (*t*, *J*=6.18 Hz, 2H), 4.08-4.13 (m, 2H), 4.18-4.21 (m, 2H), 5.31-5.35 (m, 2H), 7.00 (s, 1H), 7.09-7.14 (m, 3H), 7.24-7.26 (m, 1H), 7.40 (q, *J*$_1$=8.01, *J*$_2$=15.0 Hz, 2H), 7.78 (d, *J*=10.1 Hz, 1H). The HRMS-ESI calculated for C$_{12}$H$_{22}$N$_3$ [M+H]$^+$ was 312.1712, and 312.1707 was the experimental value.

Other compounds were prepared in a similar way gave yield from 48% to 62%.

5-(3-Imidazol-1-ylmethyl-indol-1-yl)-pentanoic acid ethyl ester (3b) Yield: 74.0 %, ^{1}H NMR(CDCl$_3$). δ: 1.21-1.25 (m, 3H), 1.57-1.68 (m, 2H), 1.84-1.91 (m, 2H), 2.30-2.33 (t, *J*=7.33 Hz, 2H), 4.08-4.14 (m, 4H), 5.30 (s, 2H), 6.97 (t, *J*=1.14, 1H), 7.06-7.13 (m, 3H), 7.23-7.27 (m, 1H), 7.34 (dd, *J*=0.92, 7.33 Hz, 1H), 7.42 (dd, *J*=0.69, 7.33 Hz, 1H), 7.70 (d, *J*=6.87 Hz, 1H). The HRMS-ESI calculated for C$_{12}$H$_{24}$N$_3$ [M+H]$^+$ was 326.1869, and 326.1863 was the experimental value.

6-(3-Imidazol-1-ylmethyl-indol-1-yl)-hexanoic acid ethyl ester (3c). Yield: 66.8 %. ^{1}H NMR(CDCl$_3$). δ: 1.20-1.24 (m, 3H), 1.30-1.37 (m, 2H), 1.61-1.69 (m, 2H), 1.80-1.88 (m, 2H), 2.27 (t, *J*=7.33 Hz, 2H), 4.07-4.13 (m, 4H), 5.27 (s, 2H), 6.95 (d, *J*=1.37, 1H), 7.03 (d, *J*=0.69, 1H), 7.06 (s, 1H), 7.10 (t, *J*=7.10 Hz, 1H), 7.21-7.27 (m, 1H), 7.33 (d, *J*=8.24 Hz, 1H), 7.42 (d, *J*=7.79 Hz, 1H), 7.59 (s, 1H). The HRMS-ESI calculated for C$_{12}$H$_{26}$N$_3$ [M+H]$^+$ was 340.2025, and 340.2020 was the experimental value.

7-(3-Imidazol-1-ylmethyl-indol-1-yl)-heptanoic acid ethyl ester (3d). Yield: 85.6 %. ^{1}H NMR(CDCl$_3$). δ: 1.22-1.26 (m, 3H), 1.33-1.34 (m, 4H), 1.57-1.62 (m, 2H), 1.76-1.87 (m, 2H), 2.25-2.29 (m, 2H), 4.08-4.14 (m, 4H), 5.31 (d, 2H), 6.97-6.98 (m, 1H), 7.06-7.13 (m, 3H), 7.22-7.27 (m, 1H), 7.34 (d, *J*=8.24 Hz, 1H), 7.42 (d, *J*=8.01 Hz, 1H), 7.71 (d, *J*=12.59 Hz, 1H). The HRMS-ESI calculated for C$_{12}$H$_{28}$N$_3$ [M+H]$^+$ was 354.2182, and 354.2176 was the experimental value.

10-(3-Imidazol-1-ylmethyl-indol-1-yl)-decanoic acid ethyl ester (3e). Yield: 30.1 %. ^{1}H NMR(CDCl$_3$). δ: 1.25-1.30 (m, 13H), 1.60 (t, *J*=6.18 Hz, 2H), 1.82 (t, *J*=5.61 Hz, 2H), 2.25-2.29 (m, 2H), 4.07-4.15 (m, 4H), 5.30 (s, 2H), 6.98 (s, 1H), 7.08-7.13 (m, 3H), 7.22-7.26 (m, 1H), 7.35 (d, *J*=8.24 Hz, 1H), 7.39-7.43 (m, 1H), 7.74 (s, 1H). The HRMS-ESI calculated for C$_{12}$H$_{34}$N$_3$ [M+H]$^+$ was 396.2651, and 396.2646 was the experimental value.

11-(3-Imidazol-1-ylmethyl-indol-1-yl)-undecanoic acid ethyl ester (3f). Yield: 63.8 % . ^{1}H NMR(CDCl$_3$). δ: 1.23-1.30 (m, 15H), 1.60 (t, *J*=6.18 Hz, 2H), 1.82 (t, *J*=6.07 Hz, 2H), 2.26-2.30 (m, 2H), 4.08-4.15 (m, 4H), 5.33 (s, 2H), 6.99 (s, 1H), 7.09-7.13 (m, 3H), 7.23-7.27 (m, 1H), 7.36 (d, *J*=8.24 Hz, 1H), 7.42 (d, *J*=7.90 Hz, 1H), 7.80 (s, 1H). The HRMS-ESI calculated for C$_{12}$H$_{36}$N$_3$ [M+H]$^+$ was 410.2808, and 410.2802 was the experimental value.

Preparation of *4-(3-imidazol-1-ylmethyl-indol-1-yl)-butyric acid (4a)* was carried out by hydrolysis the corresponding ethyl esters in a basic condition using KOH in a solution of MeOH and H$_2$O (1M KOH, 20 mL, MeOH : H$_2$O = 1 : 1). The reaction mixture was allowed stirring for 3 - 4 h. The reaction was worked up by acidified (pH 2 using 1N HCl) and the target compound was extracted by ethyl acetate. The organic layers were combined and dried with Na$_2$SO$_4$. The solvent was removed in a reduced pressure. The resulting oil was purified by silica gel column chromatography and PTLC gave pure **4a**. Yield: 13.0 %, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 2.10-2.22 (m, 2H), 3.37 (s, 1H), 4.22-4.26 (t, *J*=6.9 Hz, 2H), 5.39 (s, 2H), 6.95 (s, 1H), 7.03 -7.21 (m, 4H), 7.40-7.49 (m, 4H), 7.78 (s, 1H). The HRMS-ESI calculated for C$_{16}$H$_{17}$N$_3$O$_2$[M-H]$^-$ was 282.1243, and 282.1245 was the experimental value. Other compounds were prepared in a similar way gave **4b**-**4e**.

5-(3-Imidazol-1-ylmethyl-indol-1-yl)-pentanoic acid (4b). Yield: 14.5 %, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 1.57-1.65 (m, 2H), 1.84-1.96 (m, 2H), 2.24-2.27 (t, *J*=7.3 Hz, 2H), 3.32-3.34 (m, 2H), 4.18-4.22 (t, *J*=7.1 Hz, 2H), 5.38 (s, 2H), 6.97 (s, 1H), 7.03-7.07 (m, 1H), 7.15-7.21 (m, 1H), 7.39-7.45 (m, 4H), 7.81 (s, 1H). The HRMS-ESI calculated for C$_{17}$H$_{19}$N$_3$O$_2$[M-H]$^-$ was 296.1399, and 296.1398 was the experimental value.

6-(3-Imidazol-1-ylmethyl-indol-1-yl)-hexanoic acid (4c). Yield: 8.9 %, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 1.58-1.64 (m, 2H), 1.83-1.90 (m, 2H), 2.20-2.23 (m, 2H), 4.21-4.24 (m, 2H), 5.39 (s, 2H), 6.98-7.07 (m, 3H), 7.18-7.23 (m, 3H), 7.41-7.46 (m, 4H), 7.74 (s, 1H). The HRMS-ESI calculated for C$_{18}$H$_{21}$N$_3$O$_2$ [M-H]$^-$ was 310.1556, and 310.1556 was the experimental value.

7-(3-Imidazol-1-ylmethyl-indol-1-yl)-heptanoic acid (4d). Yield: 11.0 %, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 1.30-1.39 (m, 5H), 1.53-1.61 (m, 2H), 1.82-1.89 (m, 2H), 2.20-2.24 (t, *J*=7.3 Hz, 2H), 4.17-4.21 (t, *J*=6.9 Hz, 2H), 5.40 (s, 2H), 7.00 (s, 1H), 7.04-7.07 (m, 1H), 7.18-7.22 (m, 2H), 7.38-7.47 (m, 3H), 7.85 (s, 1H). The HRMS-ESI calculated for C$_{19}$H$_{23}$N$_3$O$_2$[M-H]$^-$ was 324.1712, and 324.1713 was the experimental value.
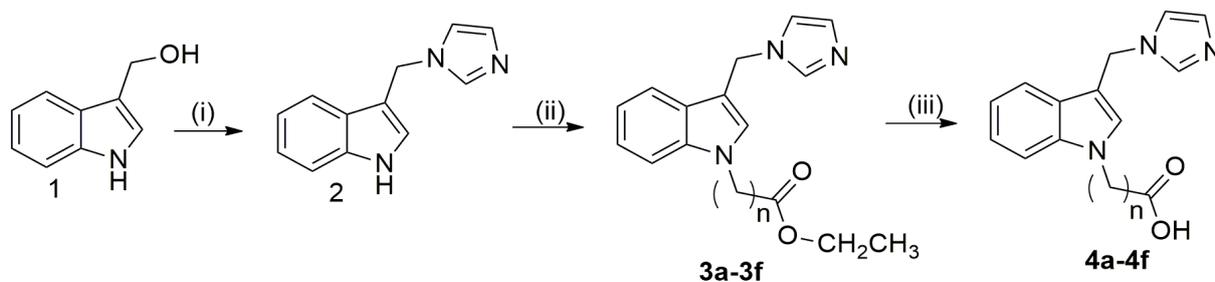
Conditions: (i) CDI, MeCN, r.t., 5 h.(ii)1, NaH, DMF, 2, Br(CH$_2$)nCOOEt.(iii) KOH
Scheme 1. General method of synthesis the target compounds

10-(3-Imidazol-1-ylmethyl-indol-1-yl)-decanoic acid (*4e*). Yield: 4.2 %, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 1.30-1.36 (m, 5H), 1.57-1.60 (m, 2H), 1.83-1.93 (m, 5H), 3.32-3.34 (m, 2H), 4.17-4.21 (t, *J*=7.1 Hz, 2H), 5.38 (s, 2H), 6.95 (s, 1H), 7.03-7.13 (m, 1H), 7.18-7.22 (m, 2H), 7.37-7.47 (m, 4H), 7.74 (s,1H). The HRMS-ESI calculated for C$_{22}$H$_{29}$N$_3$O$_2$[M-H]$^-$ was 366.2182, and 366.2176 was the experimental value.

11-(3-Imidazol-1-ylmethyl-indol-1-yl)-undecanoic acid (*4f*). Yield: 5.7%, oil, ^{1}H NMR (400 MHz, MeOH-D$_4$) δ: 1.27-1.30 (m, 4H), 1.56-1.61 (m, 3H), 1.83-1.86 (m, 3H), 2.21-2.25 (m, 4H), 3.32-3.34 (m, 2H), 4.17-4.21 (m, 2H), 5.39 (s, 2H), 6.97 (s, 1H), 7.04-7.08 (m, 2H), 7.16-7.22 (m, 3H), 7.37 (s, 1H), 7.41-7.48 (m, 3H), 7.79 (s, 1H). The HRMS-ESI calculated for C$_{23}$H$_{31}$N$_3$O$_2$[M-H]$^-$ was 380.2338, and 380.2335 was the experimental value.

C. Magnaporthe oryzae Strain and Bioassay

Rice blast isolate designated as APU00-093A (race 007.0), was obtained by mono-spore isolation from diseased rice panicle on a paddy field from the Akita Prefecture, Japan, in 2000 was kindly provided from Dr. S. Fuji of Akita Prefectural University. This isolate was kept on Potato Dextrose Agar (PDA) at 15 ℃. Determine the fungicidal activity of the test compounds was based on a method as we described previously [19].

III. RESULTS AND DISCUSSION

A. Chemistry

Method for synthesis the target compounds is shown in Scheme 1 in a condition as previously reported [8]. 3-Indolemethanol (**1**) was used as starting materials. Compound **2** was prepared by reacting compound 1 with 1.3 equivalent of 1, 1' carbonylbis-1H-imidazole in anhydride acetonitrile at room temperature for 5 hours. The reaction was monitored by using TLC until the starting material 1 is disappeared. Compound **2** was purified by recrystallization of crude compound **2**. The target compounds **3a-3f** were prepared by using NaH in DMF following adding ω-bromo-carboxlic acid ethyl ester based on a method as previously reported [18].

B. Biology

To determine the antifungal activity of the synthesized compounds against *M. oryzae,* Mycelial growth inhibition tests were carried out [19]. The concentration of all the test compounds were adjusted to a final concentration of 100 μM in PSA medium, while propiconazole (10 μM) was used as a positive control.

TABLE I: FUNGICIDAL ACTIVITY OF ETHYL ESTER DERIVATIVES
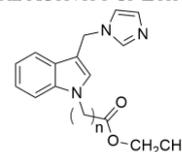
Com. No.	*n*	Inhibition Activity (%)
3a	3	6.16±1.1
3b	4	21.2±2.4
3c	5	38.8±2.5
3d	6	36.5±4.6
3e	9	18.3±2.9
3f	10	5.53±1.5
Propiconazole		39.3±2.9
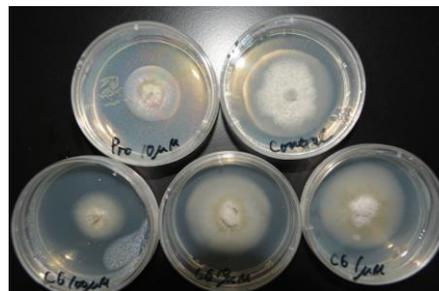
Fig. 2. Effect of compound 3c on growth of *M. oryzae,* upper left: 10 M; propiconazole, upper right: control; lower left. 0.1 mM **3c**, lower middle: 10 M **3c**, lower right, 1 M **3c**, All the experiments were taken three times to establish the repeatability.

Table I shows the chemical structure and the antifungal activity of the ethyl ester derivatives (**3a-3f**).We found that the inhibition activity of butric acid ethyl ester (**3a**) was approximately 6.16±1.1. Increasing the carbon number of the carboxylic acid side chain enhanced the inhibitory activity with 21.2±2.4, 38.8±2.5 and 36.5±4.6% for those analogues of pentanic acid ethyl ester (**3b**), hexanic acid ethyl ester (**3c**) and heptanic acid ethyl ester (**3d**), respectively. In contrast, the inhibitory activity of the positive control of propiconazole (10 mM) was found approximately 39.3±2.9%. this result indicated that compound **3c** displayed inhibitory activity against M. oryzae is approximately one tenth of the propiconazole.

Fig. 2 displayed the growth of Magnaporthe oryzae in the absence and presence of compound **3c**. Data obtained clearly indicated that compound **3c** inhibits the Magnaporthe oryzae growth in our assay system. This result suggested that the modifications that may allow us to find a new series of

antifungal agent against M. oryzae.

Table II shows the chemical structure and the antifungal activity of the carboxylic acid derivatives (**4a-4f**). We found that the inhibition activity decreased significantly. This result indicated that carboxylic acid ester moiety is important for antifungal activity against *M. oryzae* in this synthetic series.

compound **3c** is a good candidate for further chemical

TABLE II: FUNGICIDAL ACTIVITY OF CARBOXYLIC ACID DERIVATIVES
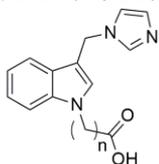
Com. No.	n	Inhibition Activity (%)
4a	3	4.15 ±2.5
4b	4	2.59 ±3.0
4c	5	2.22 ±3.9
4d	6	5.49 ±2.0
4e	9	3.22 ±4.0
4f	10	4.80 ±4.2
Propiconazole		39.3 ±2.9

IV. CONCLUSIONS

To determine the antifungal activity of indole and imidazole conjugate against *M. oryzae,* we designed a series of new compounds based on the Camalexin scaffold. The ethyl ester analogues displayed antifungal activity against *M. oryzae.* We found that compound **3c** displayed the most potent inhibitory activity against *M. oryzae* Data obtained in the present work indicated that the carboxlic acid analogues did not exhibit inhibitory activity against *M. oryzae* This result suggested that the carboxlic acid ester moiety is important for antifungal activity. We expect further chemical modification of this synthetic series may allow us to discover new compounds for *M. oryzae* control.

ACKNOWLEDGMENT

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