J. Pestic. Sci. **49**(3), 159–167 (2024)
DOI: 10.1584/jpestics.D24-014

JOURNAL OF PESTICIDE SCIENCE

Regular Article

Synthesis and biological evaluation of burnettiene A derivatives enabling discovery of novel fungicide candidates

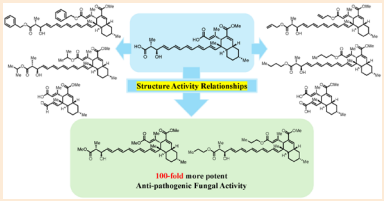
Aoi Kimishima,[1,2,†] Atsuka Nishitomi,[1,†] Iori Tsuruoka,[1,†] Masako Honsho,[1,2] Sota Negami,[1] Sota Honma,[1,2] Katsuyuki Sakai,[1] Toshiyuki Tokiwa,[2] Hiroki Kojima,[1,2] Kenichi Nonaka,[1,2] Shin-ichi Fuji,[3] Takumi Chinen,[4] Takeo Usui[4] and Yukihiro Asami[1,2,*]

[1] *Graduate School of Infection Control Sciences, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan*
[2] *Ōmura Satoshi Memorial Institute, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo, 108–8641, Japan*
[3] *Faculty of Bioresource Sciences, Akita Prefectural University, 241–438 Nakanokaidoubatanishi, Shimoshinjo, Akita, Akita 010–0195, Japan*
[4] *Graduate School of Life and Environmental Sciences, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki 305–8572, Japan*

(Received March 26, 2024; Accepted May 27, 2024)

Supplementary material

An antifungal polyene-decalin polyketide natural product, burnettiene A (**1**) has been re-discovered from the culture broth of *Lecanicillium primulinum* (current name: *Flavocillium primulinum*) FKI-6715 strain utilizing our original multidrug-sensitive yeast system. This polyene-decalin polyketide natural product was originally isolated from *Aspergillus burnettii*. The antifungal activity of **1** against *Candida albicans* has been reported. However, only one fungal species for the antifungal activity of **1** has been revealed, and details of the antifungal activity against other pathogenic fungus remain unknown. After extensive screening for antifungal activity, we found that **1** exhibits broad antifungal activity against pathogenic plant fungi, including *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Pyricularia oryzae*, *Leptosphaeria maculans*, and *Rhizoctonia solani*. Furthermore, we synthesized 12 derivatives from **1** and evaluated their antifungal activity to reveal the detailed structure–activity relationship. The methyl ester derivative showed antifungal activity against *Saccharomyces cerevisiae* 12geneΔ0HSR-iERG6 100-fold more potent than that of **1**. Our research indicates that **1** would be a promising natural product as a new fungicidal candidate and the methyl ester derivative especially has great potential.



Keywords: multidrug-sensitive budding yeast, natural product, derivatization, pathogenic plant fungi.

Introduction

We have been searching for new fungicide candidates from secondary metabolites produced by microorganisms using multidrug-sensitive yeast, *Saccharomyces cerevisiae* 12geneΔ0HSR-iERG6, as our test model.[1,2] Using this system, we have been able to identify several new natural products with anti-microbial activities.[3–7] Among them, we have recently discovered a novel fungicide (sakurafusariene) against rice blast caused by *Pyricularia oryzae*.[6] Sakurafusariene displayed extensive antifungal activity after screening of overlooked natural products utilizing the multidrug-sensitive yeast system. In this manner, we have displayed the utility of our strategy based on the screening method using the multidrug-sensitive yeast and extensive biological evaluation to discover the hidden antifungal activity. As a result, we re-discovered burnettiene A (**1**)[8] (Fig. 1), which was originally isolated as an antifungal compound.[8] However, only one fungal specie for the antifungal activity of **1** has been revealed and the detailed antifungal activity against other pathogenic fungus remains unknown. Pathogenic plant fungi threaten global food security for people all over the world.[9] Additionally, pathogenic plant fungal diseases cause tremendous damage to the crop products comparable to enough food annually for 600 million people and there is growing resistance to current fungicides.[10] Furthermore, food demand has been increasing due to population growth and economic devel-

* To whom correspondence should be addressed.
E-mail: yasami@lisci.kitasato-u.ac.jp
† These authors contributed equally to this work.
Published online October 4, 2024

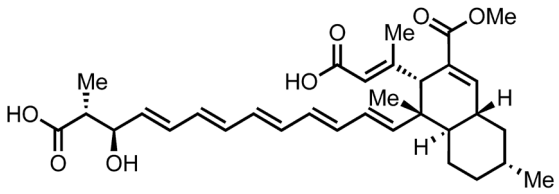


Fig. 1. Structure of burnettiene A (**1**).

opment.[11] Therefore, there is need for novel fungicides to stabilize and increase crop supply. In this study, we re-discovered great potential of **1** as a new anti-fungicide candidate and synthesized 12 derivatives. Furthermore, we evaluated antifungal activity of derivatives, enabling us to verify the structure–activity relationship and discover a great fungicide candidate.

Materials and methods

1. General experimental procedures

High- and low-resolution mass spectra were obtained using an AB Sciex QSTAR Hybrid LC/MS/MS Systems (AB Sciex, Framingham, MA, USA) and JEOL JMS-T100LP (JEOL, Tokyo, Japan). NMR spectra were measured using a Varian XL-400 spectrometer (Agilent Technologies, CA, USA) with ^{1}H NMR and ^{13}C NMR obtained at 400 MHz and 100 MHz, JEOL JNM-ECA-500 (JEOL, Tokyo, Japan) with ^{1}H NMR and ^{13}C NMR obtained at 500 MHz and 125 MHz, and Bruker AV ANCE III HD600 (Bruker, Massachusetts, USA) with ^{1}H NMR and ^{13}C NMR obtained at 600 MHz and 150 MHz in DMSO-d_6 and $CDCl_3$. The chemical shifts are reported in ppm and referenced to DMSO-d_6 (2.50 ppm) in the ^{1}H NMR spectra and DMSO-d_6 (39.52 ppm) in the ^{13}C NMR, and $CDCl_3$ (7.26 ppm) in the ^{1}H NMR spectra and $CDCl_3$ (77.16 ppm) in the ^{13}C NMR.

2. Antifungal activity evaluation

S. cerevisiae 12geneΔ0HSR-iERG6, *P. oryzae* APU15-60A (Quinone outside inhibitors (QoI)-sensitive strain),[12] *P. oryzae* APU15-63A (QoI-resistant strain),[12] *C. gloeosporioides* MAFF-237219,[13] *L. maculans* MAFF-726728,[14] *B. cinerea* MAFF-306820,[15] and *R. solani* MAFF-237699[16] were used as test organisms. Antifungal activity was evaluated by disc diffusion method.

Results and discussion

1. Antifungal activity screening

We screened compound **1** for antifungal activity against several pathogenic fungi. We found that **1** exhibits broad antifungal activity against plant pathogenic fungi including *C. gloeosporioides*, *B. cinerea*, *P. oryzae, L. maculans*, and *R. solani* (Table 1). These plant pathogenic fungi cause serious damage to wide variety of crops such as rice, vegetables, and fruits. To our delight, **1** showed potent antifungal activity against *C. gloeosporioides* and *B. cinerea* at a level comparable to the potent and broad-spectrum antifungal compound, amphotericin B. Quinone outside inhibitors (QoI) such as kresoxim-methyl are common fungicides for *P. oryzae*. However, widespread distribution of QoI-resistsnce *P. oryzae* is serious problem in rice-growing areas. Compound **1** are effective against both QoI-sensitive and resistant *P. oryzae* strains, showing great potential to become a new lead fungicide, which prompted us to explore **1** as a new antifungal candidate. Therefore, we decided to synthesize derivatives from **1** to verify the structure activity relationship and create more potent analogs.

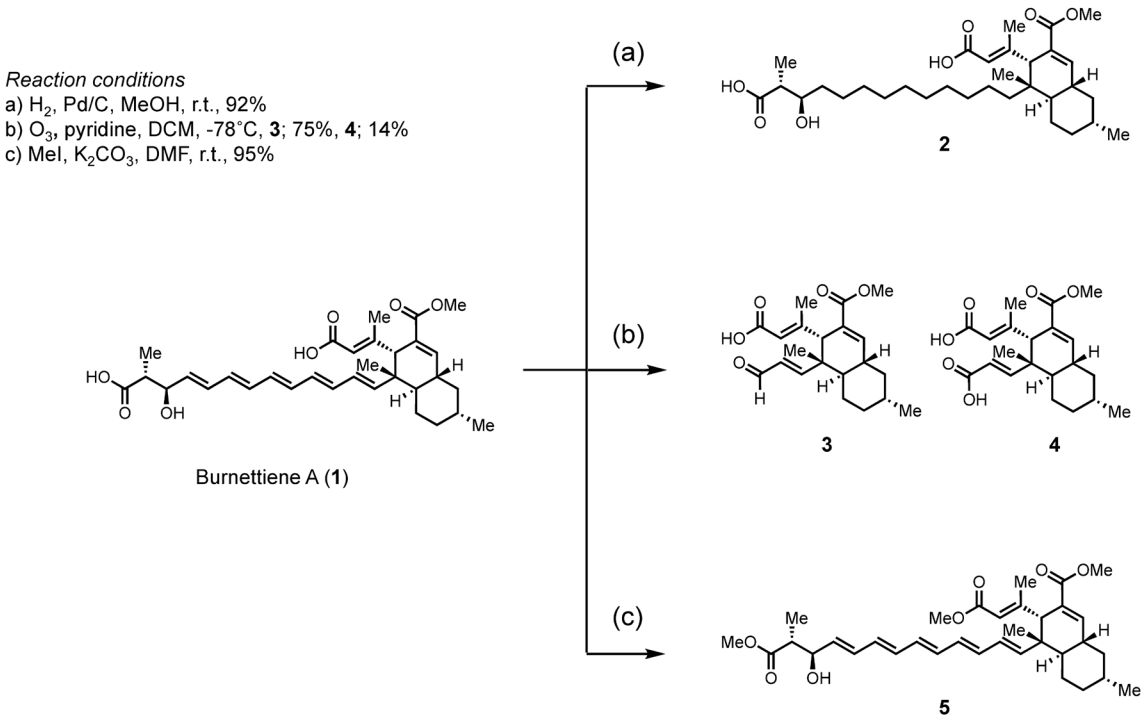
*2. Preliminary structure activity relationship of **1***

To verify the preliminary structure activity relationship of **1**, we first decided to functionalize the characteristic functional groups such as the polyene and carboxylic groups moieties in **1** (Scheme 1). Considering the instability of **1**, hydrogenation with H_2 and Pd/C was conducted to afforded saturated compound **2**. We sought the structure–activity relationship about the decalin moiety and ozonolysis of **1** in the presence of pyridine, provided aldehyde derivative **3** and acid **4**. Next, our attention was shifted to derivatization of carboxylic groups in **1** and we tried amidation and esterification conditions using condensation agents. However, these conditions were unfruitful and even a mild methyl ester formation reagent, $TMSCHN_2$ was not successful. We encountered a similar problem in derivatization of a polyene natural product, sakurafusariene, whose low reactivity would attribute to its conjugation stabilization from the polyene moiety.[9] Eventually, alkylation conditions using electrophiles and K_2CO_3 were chosen for the synthesis of sakurafusariene ester de-

Table 1. Antifungal activity of **1** against phytopathogenic fungi.

Strain	Inhibition zone diameters (mm)							
	1 (μg/disk)						AMPH	KXM
	100	30	10	3	1	0.3	1	0.3
Pyricularia oryzae APU15-60A	12.7	11.5	8.5	—	—	N.T.	12.4	18.2
Pyricularia oryzae APU15-63A	13.4	13.1	10.3	—	—	N.T.	14.6	—
Colletotrichum gloeosporioides MAFF-237219	14.6/28.5	12.5/28.1	11.8/27.4	10.5/24.5	18.2	—	12.3	N.T.
Leptosphaeria maculans MAFF-726728	13.9	9.0	—	—	—	N.T.	13.0	N.T.
Botrytis cinerea MAFF-306820	15.1	13.3	12.9	12.2	11.6	—	8.4	N.T.
Rhizoctonia solani MAFF-237699	7.2	—	—	—	—	N.T.	8.2	N.T.

AMPH, Amphotericin B; KXM, Kresoxim-methyl, N.T., Not tested; —, No inhibition; Inner diameter/outer diameter, *Pyricularia oryzae* APU15-60A, Susceptible to QoI; *Pyricularia oryzae* APU15-63A, Resistance to QoI.



Scheme 1. Synthesis of Derivatives-**1**. a) H_2, Pd/C, MeOH, r.t., 92.0%, b) O_3, pyridine, DCM, −78.0°C, **3**; 75.0%, **4**; 14.0%, c) MeI, K_2CO_3, DMF, r.t., 95.0%.

rivatives, which prompted us to carry out alkylation of **1**. Methylation of the carboxylic groups in **1** utilizing MeI and K_2CO_3 proceeded to furnish methyl ester **5** as we expected. We evaluated the antifungal activity of those preliminary five derivatives against the multidrug-sensitive budding yeast (Table 2). The parent compound shows antifungal activity at 3 μg/disk, whereas saturated compound **2** did not show antifungal activity even at 100 μg/disk. Aldehyde derivative **3** and acid **4** retain antifungal activity, suggesting that the polyene moiety would not be an essential functional group, but the saturated side chain in **2** might negatively influence the antifungal activity due to its hydrophobicity or flexibility. To our delight, methyl ester derivative **5** exhibits a 100-fold increase in antifungal activity compared to **1**, driving us to synthesis more ester derivatives.

3. Synthesis of ester derivatives

Methyl ester **5** was found to be a promising derivative and we synthesized several ester derivatives (Scheme 2). In terms of length of the alkyl group, ethyl, propionyl, and butyl esters were synthesized (**6–8**). To verify the influence of unsaturated and branched functional groups to the antifungal activity, allyl, benzyl, propargyl, isopropyl, and isobutyl esters were also derivatized from **1** (**9–13**).

4. Antifungal activity evaluation of derivatives

With 12 derivatives in hand, we evaluated the antifungal activity of them against QoI-sensitive *P. oryzae*, QoI-resistant *P. oryzae*, *C. gloeosporioides*, *L. maculans*, *B. cinerea*, and *R. solani*. Normal alkyl esters such as methyl, ethyl, and propyl exhibited potent antifungal activity against QoI-sensitive *P. oryzae* more

Table 2. Antifungal activity of **1**–**5** against *Saccharomyces cerevisiae* 12geneΔ0HSR-iERG6.

Compound	Inhibition zone diameter (mm)								
	μg/disk								
	100	30	10	3	1	0.3	0.1	0.03	0.01
1	19.3	12.2	11.8	9.2	—	—	—	N.T.	N.T.
2	—	—	—	—	—	—	—	N.T.	N.T.
3	9.3	8.2	—	—	—	—	—	N.T.	N.T.
4	10.0	10.3	—	—	—	—	—	N.T.	N.T.
5	16.0	15.6	12.3	11.0	10.9	9.1	7.7	6.5	—
AMPH	N.T.	N.T.	N.T.	N.T.	11.6	N.T.	N.T.	N.T.	N.T.

AMPH, Amphotericin B, N.T., Not tested; —, No inhibition.

Burnettiene A (**1**)

Scheme 2 . Synthesis of Ester Derivatives
6; R = Ethyl (45% yield)
7; R = Propyl (26% yield)
8; R = Butyl (21% yield)
9; R = Allyl (22% yield)
10; R = Benzyl (34% yield)
11; R = Propargyl (32% yield)
12; R = Isopopyl (28% yield)
13; R = Isobutyl (7% yield)

Scheme 2. Synthesis of Ester Derivatives. **6**; R=Ethyl (45.0% yield), **7**; R=Propyl (26.0% yield), **8**; R=Butyl (21.0% yield), **9**; R=Allyl (22.0% yield), **10**; R=Benzyl (34.0% yield), **11**; R=Propargyl (32.0% yield), **12**; R=Isopropyl (28.0% yield), **13**; R=Isobutyl (7.0% yield).

than 10 to 30-folds compared to the parent compound (Table 3). Interestingly, aldehyde derivative **3**, which did not show significant antifungal activity against the multidrug-sensitive budding yeast, displayed great potency against QoI-sensitive *P. oryzae*. Additionally, propargyl ester **11** also showed potent antifungal activity, which would provide a great opportunity to synthesize novel derivatives utilizing click chemistry.[17] Benzyl ester **10** and isobutyl **13** retained antifungal activity against QoI-resistance *P. oryzae*, suggesting that branch carbon chains attached to a methylene group would be a better functional group than the other groups. In the case of *C. gloeosporioides*, all derivatives showed weak antifungal activity compared to the parent compound, but the reduction product **2**, which was not effective against the multidrug-sensitive budding yeast, exhibited slight better efficacy than the other derivatives. Furthermore, **2** was found to retain antifungal activity even against *L. maculans* comparable to **1**, which would lead us to discover more stable derivatives based on **2**. The antifungal activity of **5** against *B. cinerea* was comparably effective to that of the parent compound, and alkyl derivatives such as **6** and **12** maintained some effectiveness. Overall, esterification of **1** facilitated the discovery of novel antifungal derivatives. Methyl ester **5**, especially displayed antifungal activity against QoI-sensitive *P. oryzae* and *B. cinerea*. Benzyl ester **10** exhibited good antifungal activity against QoI-resistance *P. oryzae*; introduction of substituents on the aromatic ring might open up possibility for more potent antifungal derivatives. Intriguingly, reduction derivative **2** is relatively active against *C. gloeosporioides* and *L. maculans*, representing new insight into more stable derivatives.

Conclusions

We have re-discovered a new antifungal natural product, burnettiene A (**1**) from the culture broth of *Flavocillium primulinum* FKI-6715 strain using a multidrug-sensitive budding yeast screening system.[3] After extensive antifungal activity screening to uncover hidden antifungal activity, **1** was found to exhibit broad antifungal activity against pathogenic plant fungi. This result prompted us to synthesize new derivatives and evaluated the antifungal activity of them to verify the structure activity relationship. As a first approach, we synthesized four derivatives focused on the characteristic functional groups of **1** and evaluated the antifungal activity of them against the multidrug-sensitive budding yeast, indicating the preliminary structure activity relationship and methyl ester **5** exhibited a 100-fold increase in antifungal activity compared to **1**. Based on this finding, we derivatized the natural product to several ester derivatives. With 12 derivatives in hand, we evaluated antifungal activity against pathogenic plant fungi such as *C. gloeosporioides*, *B. cinerea*, *P. oryzae, L. maculans*, and *R. solani*. We obtained valuable knowledge about the structure activity relationship. Notably, methyl ester **5**, showed more potent antifungal activity against QoI-sensitive *P. oryzae* than **1**. Benzyl ester **10** and isobutyl **13** exhibited good antifungal activity against QoI-resistance *P. oryzae*. Moreover, reduction derivative **2**, which does not show antifungal activity against the multidrug-sensitive budding yeast, was relatively active against *C. gloeosporioides* and *L. maculans*. Therefore, our study enabled us to discover appropriate seed compounds according to pathogenic plant fungi and showed the utility of our strategy based on the multidrug-sensitive yeast screening system and chemical synthesis. We are currently carrying out synthesis of new derivatives based on each seed compound and planning to evaluate the ant-fungal activity of them *in vivo* model.

Associated content

Preparation of the compounds

Burnettiene A (1)

Burnettiene A (**1**) used for derivatization was prepared from the cultured broth of *F. primulinum* FKI-6715 strain as shown in supporting information.

Saturated derivative 2

To a solution of **1** (6.0 mg, 11.1 μmol) in MeOH (0.7 mL, 15.9 mM), 10% Pd/C (1.0 mg, 0.9 mmol) was added and stirred at room temperature under a H_2 atmosphere for 1 hr. The reaction mixture was filtered with celite and concentrated under reduced pressure. The residue was purified by prep. TLC to obtain **2** (5.6 mg, 10.2 μmol, 92.0%). $[\alpha]_D^{23}$ −77.5 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 243 (6,964), 377 (1,535). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.75 (s, 1H), 5.42 (s, 1H), 3.78 (s, 1H), 3.60 (s, 3H),

Table 3. Antifungal activity of **1**–**13** against phytopathogenic fungi.

Strain	Compound	Inhibition zone diameter (mm) μg/disk						Strain	Compound	Inhibition zone diameter (mm) μg/disk				
		100	30	10	3	1	0.3			100	30	10	3	1
Pyricularia oryzae APU15-60A (QoIS)	**1**	11.6	10.9	8.9	—	—	—	*Pyricularia oryzae* APU15-63A (QoIR)	**1**	12.8	11.9	9.5	—	—
	2	11.1	8.2	7.1	—	—	—		**2**	8.2	—	—	—	—
	3	9.0	8.7	7.7	7.2	7.0	—		**3**	11.9	10.5	—	—	—
	4	13.2	10.9	10.5	—	—	N.T.		**4**	13.9	—	—	—	—
	5	13.7	11.4	12.2	10.4	8.4	7.1		**5**	14.8	9.3	—	—	—
	6	12.5	11.5	10.3	10.4	9.8	—		**6**	12.9	—	—	—	—
	7	14.0	12.4	10.5	9.4	10.3	7.8		**7**	11.7	—	—	—	—
	8	14.6	11.6	—	—	—	N.T.		**8**	14.4	—	—	—	—
	9	14.5	12.0	—	—	—	N.T.		**9**	16.9	—	—	—	—
	10	11.8	11.3	11.5	—	—	N.T.		**10**	9.0	8.2	6.3	—	—
	11	14.1	11.0	9.8	9.6	—	N.T.		**11**	13.4	—	—	—	—
	12	14.1	11.6	10.6	—	—	N.T.		**12**	12.9	—	—	—	—
	13	14.1	—	—	—	—	N.T.		**13**	13.9	11.5	10.9	—	—
	AMPH	N.T.	N.T.	N.T.	N.T.	10.4	N.T.		AMPH	N.T.	N.T.	N.T.	N.T.	10.3
	KXM	N.T.	N.T.	N.T.	N.T.	N.T.	21.5		KXM	N.T.	N.T.	N.T.	N.T.	—
Colletotrichum gloeosporioides MAFF-237219	**1**	19.9	18.3	16.9	13.0	12.5	N.T.	*Botrytis cinerea* MAFF-306820	**1**	15.0	13.3	11.5	10.2	9.1
	2	10.5	9.9	—	—	—	N.T.		**2**	11.6	—	—	—	—
	3	9.1	—	—	—	—	N.T.		**3**	9.9	—	—	—	—
	4	—	—	—	—	—	N.T.		**4**	9.6	—	—	—	—
	5	14.0	—	—	—	—	N.T.		**5**	11.1	10.1	9.1	9.0	—
	6	—	—	—	—	—	N.T.		**6**	10.1	9.5	9.4	—	—
	7	17.7	—	—	—	—	N.T.		7	—	—	—	—	—
	8	18.4	—	—	—	—	N.T.		**8**	9.8	9.3	—	—	—
	9	—	—	—	—	—	N.T.		**9**	7.7	—	—	—	—
	10	—	—	—	—	—	N.T.		**10**	9.9	8.6	—	—	—
	11	14.3	—	—	—	—	N.T.		**11**	9.8	8.5	—	—	—
	12	14.2	—	—	—	—	N.T.		**12**	10.6	9.9	9.9	—	—
	13	15.9	—	—	—	—	N.T.		**13**	10.7	9.3	—	—	—
	AMPH	N.T.	N.T.	N.T.	N.T.	13.3	N.T.		AMPH	N.T.	N.T.	N.T.	N.T.	10.2
Leptosphaeria maculans MAFF-726728	**1**	12.7	10.5	8.9	—	—	N.T.	*Rizoctonia solani* MAFF-237699	**1**	—	—	—	—	—
	2	8.7	8.6	8.1	—	—	N.T.		**2**	—	—	—	—	—
	3	8.3	—	—	—	—	N.T.		**3**	—	—	—	—	—
	4	—	—	—	—	—	N.T.		**4**	—	—	—	—	—
	5	—	—	—	—	—	N.T.		**5**	—	—	—	—	—
	6	—	—	—	—	—	N.T.		**6**	—	—	—	—	—
	7	—	—	—	—	—	N.T.		**7**	—	—	—	—	—
	8	—	—	—	—	—	N.T.		**8**	—	—	—	—	—
	9	—	—	—	—	—	N.T.		**9**	—	—	—	—	—
	10	—	—	—	—	—	N.T.		**10**	—	—	—	—	—
	11	—	—	—	—	—	N.T.		**11**	—	—	—	—	—
	12	10.3	—	—	—	—	N.T.		**12**	—	—	—	—	—
	13	9.9	8.1	—	—	—	N.T.		**13**	—	—	—	—	—
	AMPH	N.T.	N.T.	N.T.	N.T.	12.8	N.T.		AMPH	N.T.	N.T.	N.T.	N.T.	8.5

AMPH, Amphotericin B; KXM, Kresoxim-methyl, N.T., Not tested; —, No inhibition, *Pyricularia oryzae* APU15-60A, Susceptible to QoI; *Pyricularia oryzae* APU15-63A, Resistance to QoI.

2.80 (s, 1H), 2.32 (m, 1H), 2.13 (s, 3H), 1.96 (m, 1H), 1.93 (m, 1H), 1.75 (m, 1H), 1.50 (m, 1H), 1.40 (m, 2H), 1.37 (m, 1H), 1.19–1.27 (complex m, 18H), 1.23 (m, 1H), 0.96 (d, J=7.0 Hz, 3H), 0.90 (d, J=6.6 Hz, 3H), 0.81–1.11 (complex m, 3H), 0.75 (s, 3H); and ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.55, 167.36, 166.54, 154.79, 142.83, 130.24, 120.30, 79.24, 71.66, 54.62, 51.63, 45.90, 40.47, 38.55, 38.30, 36.89, 35.39, 33.34, 32.83, 32.28, 30.22, 29.18, 29.10, 29.00, 28.64, 25.45, 25.21, 23.44, 22.32, 21.56, 18.43, 12.87. ESI-MS m/z 547.3648 $[M-H]^-$ (Calcd. for $C_{32}H_{51}O_7$, m/z 547.3635 $[M-H]^-$).

Aldehyde derivative 3 and acid derivative 4

To a solution of **1** (10.0 mg, 18.6 μmol) in pyridine (7.4 μL, 92.9 μmol) and dichloromethane (740.0 μL, 25.0 mM) at −78.0°C, O_3 was bubbled through the solution for few minutes. The reaction mixture was allowed to warm to room temperature and then 1N HCl aq. was added. The resulting mixture was extracted with $CHCl_3$, then the organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by prep. TLC ($CHCl_3$/MeOH=5:1) to obtain **3** (5.0 mg, 13.9 μmol, 75.0%) and **4** (1.0 mg, 2.7 μmol, 14.0%). Physicochemical properties of **3**: $[\alpha]_D^{23}$ −146.6 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 242 (12,463), 279 (8,104), 323 (3,242); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (d, J=11.4 Hz, 1H), 6.95 (s, 1H), 6.49 (d, J=24.0 Hz, 1H). 6.20 (dd, J=24.0, 11.4 Hz, 1H), 5.59 (s, 1H), 3.70 (s, 3H), 3.07 (s, 1H), 2.23 (d, J=1.36 Hz, 3H), 1.99 (m, 1H), 1.95 (m, 1H), 1.78 (m, 1H), 1.53 (m, 1H), 1.39 (m, 1H), 1.23 (m, 1H), 1.10 (s, 3H), 0.96 (d, J=6.6 Hz, 3H), 0.85–1.07 (complex m, 3H); and ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.86, 170.73, 166.81, 164.77, 152.12, 143.76, 132.16, 129.31, 118.76, 56.40, 52.09, 42.89, 40.70, 40.14, 38.62, 35.32, 33.48, 29.84, 26.87, 22.31, 18.19. $[M+NH_4]^+$ (Calcd. for $C_{21}H_{32}NO_5$, m/z 378.2280 $[M+NH_4]^+$). Physicochemical properties of **4**: $[\alpha]_D^{23}$ −149.3 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 242 (11,022), 260 (7,862), 310 (6,169); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.72 (d, J=16.7 Hz 1H), 5.86 (d, J=16.7 Hz, 1H), 5.55 (s, 1H), 3.69 (s, 3H), 3.03 (s, 1H), 2.17 (s, 3H), 1.96 (m, 1H), 1.93 (m, 1H), 1.75 (m, 1H), 1.50 (m, 1H), 1.37 (m, 1H), 1.23 (m, 1H), 1.07 (s, 3H), 0.94 (d, J=6.5 Hz, 3H), 0.79–1.03 (complex m, 3H); and ^{13}C NMR (150 MHz, $CDCl_3$) δ 207.14, 172.11, 166.92, 162.67, 158.13, 143.74, 129.47, 120.16, 118.84, 56.41, 52.02, 42.40, 40.73, 39.94, 38.75, 35.31, 33.55, 31.06, 26.88, 22.34, 18.00. ESI-MS m/z 394.2227 $[M+NH_4]^+$ (Calcd. for $C_{21}H_{32}NO_6$, m/z 394.2230 $[M+NH_4]^+$).

General method for preparation of alkyl derivatives 5 and 11

To a solution of **1** (10.0 mg, 18.6 μmol) in DMF (740.0 μL, 25.0 mM) was added alkyl halide (92.9 μmol) and K_2CO_3 (12.7 mg, 92.9 μmol) at room temperature and stirred for 1 hr. The reaction mixture was quenched with brine and diluted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by prep. TLC to obtain alkyl derivatives.

Methyl ester 5

Burnettiene A methyl ester **5** was prepared by added iodomethane (5.7 μL, 92.9 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=1:1) to obtain **5** (10.0 mg, 17.7 μmol, 95.0%). $[\alpha]_D^{23}$ −235.1 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (10,987), 310 (21,690), 324 (39,643), 339 (56,915), 357 (55,669). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.10–6.35 (complex m, 8H), 5.65 (dd, J=14.9, 7.3 Hz, 1H), 5.48 (d, J=1.5 Hz, 1H), 5.34 (d, J=15.4 Hz, 1H), 4.26 (t, J=10.1 Hz, 1H), 3.71 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 2.96 (s, 1H), 2.58 (m, 1H), 2.22 (d, J=1.6 Hz, 3H), 1.86–1.97 (complex m, 2H), 1.73 (m, 1H), 1.44 (m, 1H), 1.23–1.28 (complex m, 2H), 1.02 (s, 3H), 1.16 (d, J=7.2, 3H), 0.93 (d, J=6.6 Hz, 3H), 0.85–1.00 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 167.27, 167.18, 161.82, 143.59, 142.47, 134.19, 133.99, 133.75, 132.92, 132.75, 132.43, 131.72, 131.62, 130.09, 129.25, 118.09, 74.60, 56.87, 52.01, 51.87, 51.03, 45.73, 41.82, 40.93, 40.76, 39.01, 35.60, 33.60, 29.83, 26.78, 22.41, 18.40, 14.24. ESI-MS m/z 584.3582 $[M+NH_4]^+$ (Calcd. for $C_{34}H_{50}NO_7$, m/z 584.3587 $[M+NH_4]^+$).

Propargyl ester 11

Burnettiene A propargyl ester **11** was prepared by added propargyl bromide (7.0 μL, 92.9 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=2:1) to obtain **11** (3.6 mg, 5.9 μmol, 32.0%). $[\alpha]_D^{23}$ −303.9 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (12,717), 310 (23,713), 324 (40,607), 339 (53,323), 358 (53,323). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.10–6.36 (complex m, 8H), 5.66 (dd, J=14.9, 7.1 Hz, 1H), 5.51 (s, 1H), 5.33 (d, J=15.2 Hz, 1H), 4.69–4.72 (complex m, 4H), 4.30 (t, J=14.3 Hz, 1H), 3.67 (s, 3H), 2.97 (s, 1H), 2.63 (m, 1H), 2.49 (d, J=2.4 Hz, 1H), 2.47 (d, J=2.4 Hz, 1H), 2.23 (d, J=1.4 Hz, 3H), 1.86–1.97 (complex m, 2H), 1.73 (m, 1H), 1.40–1.50 (complex m, 2H), 1.22 (m, 1H), 1.18 (d, J=7.2 Hz, 3H), 1.03 (s, 3H), 0.94 (d, J=6.5 Hz, 3H), 0.81–1.00 (complex m, 3H); and ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.01, 174.66, 167,20, 165.70, 143.85, 142.34, 139.84, 134.35, 134.07, 133.75, 133.15, 132.45, 132.40, 131.78, 131.55, 129.92, 129.33, 78.33, 77.58, 75.20, 74.75, 74.55, 57.01, 52.29, 51.91, 51.42, 45.74, 41.91, 40.85, 40.76 38.99, 35.53, 33.60, 29.84, 26.76, 22.40, 18.44, 14.03. ESI-MS m/z 632.3589 $[M+NH_4]^+$ (Calcd. for $C_{38}H_{50}NO_7$, m/z 632.3587 $[M+NH_4]^+$).

General method for preparation of alkyl derivatives 6–10 and 11–13

To a solution of **1** (20.0 mg, 37.2 μmol) in DMF (740.0 μL, 50.0 mM) was added alkyl halide (186.0 μmol) and K_2CO_3 (25.7 mg, 186.0 μmol) at room temperature. The mixture was stirred for 1 hr, then the reaction mixture was quenched with brine and diluted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by prep. TLC to obtain alkyl derivatives.

Ethly ester 6

Burnettiene A ethyl ester **6** was prepared by added iodoethane (14.8 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=2:1) to obtain **6** (10.0 mg, 16.8 μmol, 45.0%). $[\alpha]_D^{23}$ −196.3 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (16,391), 296 (17,106), 311 (24,065), 324 (34,537), 339 (41,626), 357

(40,326), 386 (8,130), 411 (6,439). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.12–6.32 (complex m, 8H), 5.65 (dd, J=14.8, 6.5 Hz, 1H), 5.45 (s, 1H), 5.35 (d, J=15.2 Hz, 1H), 4.26 (m, 1H), 4.09–4.19 (complex m, 4H), 3.67 (s, 3H), 2.95 (s, 1H), 2.56 (m, 1H), 2.20 (s, 3H), 1.90–1.97 (complex m, 2H), 1.73 (m, 1H), 1.47 (m, 1H), 1.23–1.34 (complex m, 8H), 1.02 (s, 3H), 1.16 (d, J=7.1 Hz, 3H), 0.95 (d, J=6.8 Hz, 3H), 0.79–1.02 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.64, 167.33, 166.88, 143.60, 142.53, 134.11, 133.95, 133.75, 132.87, 132.78, 132.45, 131.70, 131.66, 130.11, 129.22, 118.57, 74.56, 60.86, 59.84, 56.89, 51.88, 45.76, 41.84, 40.94, 40.74, 39.04, 35.60, 33.62, 29.84, 26.79, 22.83, 22.42, 18.42, 14.45, 14.34, 14.25. ESI-MS m/z 612.3903 $[M+NH_4]^+$ (Calcd. for $C_{36}H_{54}NO_7$, m/z 612.3900 $[M+NH_4]^+$).

Propyl ester 7

Burnettiene A propyl ester **7** was prepared by added 1-iodopropane (21.0 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=2:1) to obtain **7** (6.0 mg, 9.6 μmol, 26.0%). $[\alpha]_D^{23}$ −241.1 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (16,058), 296 (16,680), 311 (24,460), 324 (36,908), 339 (46,741), 357 (45,745), 390 (9,958), 411 (7,967). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.10–6.35 (complex m, 8H), 5.66 (dd, J=14.9, 7.0 Hz, 1H), 5.46 (s, 1H), 5.35 (d, J=15.2 Hz, 1H), 4.25 (t, J=13.7 Hz, 1H), 4.00–4.09 (complex m, 4H), 3.67 (s, 3H), 2.95 (s, 1H), 2.57 (m, 1H), 2.20 (s, 3H), 1.87–1.96 (complex m, 2H), 1.63–1.72 (complex m, 5H), 1.39–1.49 (complex m, 2H), 1.21 (m, 1H), 1.02 (s, 3H), 1.16 (d, J=7.2 Hz, 3H), 0.96 (d, J=3.2 Hz, 3H), 0.94(d, J=2.8 Hz, 3H), 0.92 (d, J=2.0 Hz, 3H), 0.78–0.99 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.69, 167.32, 167.31, 166.98, 143.60, 142.52, 134.07, 133.92, 133.73, 132.91, 132.74, 132.45, 131.69, 130.09, 129.20, 118.67, 74.51, 66.43, 65.57, 56.89, 51.86, 45.82, 41.82, 41.03, 39.04, 35.62, 33.60, 32.05, 31.81, 29.82, 26.78, 23.92, 22.42, 22.15, 18.41, 14.26, 10.63, 10.49. ESI-MS m/z 640.4220 $[M+NH_4]^+$ (Calcd. for $C_{38}H_{58}NO_7$, m/z 640.4213 $[M+NH_4]^+$).

Butyl ester 8

Burnettiene A butyl ester **8** was prepared by added 1-iodobutane (21.0 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC to obtain **8** (5.1 mg, 7.8 μmol, 21.0%). $[\alpha]_D^{23}$ −243.6 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (14,569), 296 (15,545), 310 (22,504), 324 (32,781), 339 (39,936), 357 (40,001), 390 (11,773), 411 (9,691). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.12–6.33 (complex m, 8H), 5.66 (dd, J=14.7, 7.1 Hz, 1H), 5.46 (s, 1H), 5.35 (d, J=15.4 Hz, 1H), 4.25 (t, J=13.8 Hz, 1H), 4.03–4.17 (complex m, 4H), 3.67 (s, 3H), 2.96 (s, 1H), 2.57 (m, 1H), 2.20 (s, 3H), 1.88–1.97 (complex m, 2H), 1.73 (m, 1H), 1.59–1.66 (complex m, 4H), 1.34–1.49 (complex m, 6H), 1.23 (m, 1H), 1.17 (d, J=7.2, 3H), 1.02 (s, 3H), 0.91–0.98 (complex m, 9H), 0.79–0.95 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.71, 168.85, 167.33, 167.01, 143.61, 142.54, 134.10, 133.93, 133.74, 132.89, 132.74, 132.45, 131.70, 131.68, 130.09, 129.21, 118.67, 74.52, 64.74, 63.91, 56.89, 51.87, 45.79, 41.83, 40.96, 40.77, 39.05, 35.63, 33.61, 30.86, 30.77, 29.84, 26.80, 22.83, 22.43, 19.42, 19.25, 18.42, 13.94, 13.83. ESI-MS m/z 668.4529 $[M+NH_4]^+$ (Calcd. for $C_{40}H_{62}NO_7$, m/z 668.4526 $[M+NH_4]^+$).

Allyl ester 9

Burnettiene A allyl ester **9** was prepared by added allyl bromide (15.9 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=2:1) to obtain **9** (5.0 mg, 8.1 μmol, 22.0%). $[\alpha]_D^{23}$ −285.7 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 242 (13,666), 310 (26,404), 323 (49,468), 339 (74,141), 357 (70,864). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.11–6.35 (complex m, 8H), 5.88–5.99 (complex m, 2H), 5.66 (dd, J=14.9, 7.2 Hz, 1H), 5.50 (s, 1H), 5.35 (d, J=15.3 Hz, 1H), 5.34 (brs, 1H), 5.31 (brs 1H), 5.25 (s, 1H), 5.23 (s, 1H), 4.55–4.64 (complex m, 4H), 4.28 (t, J=13.9 Hz, 1H), 3.66 (s, 3H), 2.96 (s, 1H), 2.61 (m, 1H), 2.22 (s, 3H), 1.87–1.96 (complex m, 2H), 1.73 (m, 1H), 1.41–1.51 (complex m, 2H), 1.24 (m, 1H), 1.18 (d, J=7.2 Hz, 3H), 1.02 (s, 3H), 0.93 (d, J=6.6 Hz, 3H), 0.84–0.99 (complex m, 3H); and ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.26, 167.28, 166.40, 162.21, 143.70, 142.45, 134.19, 133.98, 133.74, 132.93, 132.80, 132.72, 132.44, 132.06, 131.73, 131.62, 130.01, 129.25, 118.56, 118.19, 118.09, 74.57, 65.46, 64.76, 56.90, 51.89, 45.82, 41.84, 40.89, 40.74, 38.99, 35.56, 32.06, 26.81, 22.83, 22.41, 18.40, 14.25. ESI-MS m/z 636.3903 $[M+NH_4]^+$ (Calcd. for $C_{38}H_{54}NO_7$, m/z 636.3900 $[M+NH_4]^+$).

Benzyl ester 10

Burnettiene A benzyl ester **10** was prepared by added benzyl bromide (21.9 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC (n-hexane/EtOAc=2:1) to obtain **10** (9.2 mg, 12.8 μmol, 34.0%). $[\alpha]_D^{23}$ −259.2 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (19,468), 311 (29,945), 324 (47,198), 340 (65,661), 358 (65,158), 392 (13,290), 414 (10,848). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.40 (complex m, 10H), 6.89 (s, 1H), 6.11–6.34 (complex m, 8H), 5.65 (dd, J=14.8, 7.1 Hz, 1H), 5.53 (s, 1H), 5.35 (d, J=15.4 Hz, 1H), 5.10–5.18 (complex m, 4H), 4.29 (t, J=13.0 Hz, 1H), 3.66 (s, 3H), 2.98 (s, 1H), 2.65 (m, 1H), 2.24 (d, J=1.6 Hz, 3H), 1.88–1.94 (complex m, 2H), 1.73 (m, 1H), 1.41–1.47 (complex m, 2H), 1.22 (m, 1H), 1.19 (d, J=7.2 Hz, 3H), 1.03 (s, 3H), 0.93 (d, J=6.5 Hz, 3H), 0.80–0.98 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.34, 167.27, 166.58, 162.26, 143.73, 142.46, 136.45, 135.88, 134.18, 133.97, 133.73, 132.85, 132.72, 132.45, 131.73, 131.62, 129.99, 129.25, 128.72 (2C), 128.65 (2C), 128.47 (2C), 128.40, 128.27 (2C), 128.23, 118.14, 74.52, 66.58, 65.77, 56.97, 51.87, 45.85, 41.88, 40.88, 40.77, 39.02, 35.56, 33.59, 28.83, 26.76, 22.39, 18.43, 14.16. ESI-MS m/z 736.4221 $[M+NH_4]^+$ (Calcd. for $C_{46}H_{58}NO_7$, m/z 736.4213 $[M+NH_4]^+$).

Isopropyl ester 12

Burnettiene A isopropyl ester **12** was prepared by added isopropyl iodide (18.6 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC to obtain **12** (6.4 mg, 10.3 μmol, 28.0%). $[\alpha]_D^{23}$ −288.2 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 241 (24,833), 311 (41,078),

324 (65,786), 339 (87,072), 357 (86,201), 390 (19,481), 412 (16,200). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.09–6.35 (complex m, 8H), 5.66 (dd, J=14.7, 7.0 Hz, 1H), 5.41 (s, 1H), 5.36 (d, J=15.4 Hz, 1H), 4.97–5.08 (complex m, 2H), 4.25 (t, J=13.0 Hz, 1H), 3.67 (s, 3H), 2.94 (s, 1H), 2.52 (m, 1H), 2.19 (s, 3H), 1.86–1.96 (complex m, 2H), 1.72 (m, 1H), 1.40–1.49 (complex m, 2H), 1.22–1.29 (complex m, 13H), 1.15 (d, J=7.2 Hz, 3H), 1.01 (s, 3H), 0.94 (d, J=6.4 Hz, 3H), 0.79–0.99 (complex m, 3H); and ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.17, 168.07, 167.37, 166.48, 143.60, 142.57, 139.43, 134.55, 133.90, 132.98, 132.51, 131.67, 130.10, 129.18, 128.01, 127.45, 119.15, 74.50, 68.26, 67.89, 67.09, 56.88, 51.88, 45.80, 41.84, 40.93, 40.71, 39.05, 35.61, 33.62, 29.83, 26.79, 22.43, 22.14, 22.08, 18.42, 14.20, 12.91. ESI-MS m/z 640.4207 $[M+NH_4]^+$ (Calcd. for $C_{38}H_{58}NO_7$, m/z 640.4213 $[M+NH_4]^+$).

Isobutyl ester 13

Burnettiene A isobutyl ester **13** was prepared by added 1-iodo-2-methylpropane (21.4 μL, 186.0 μmol) according to the general method for preparation of alkyl derivatives. The residue was purified by prep. TLC to obtain **13** (1.6 mg, 2.5 μmol, 7.0%). $[\alpha]_D^{23}$ −306.1 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ε) 242 (20,423), 310 (40,261), 324 (73,822), 339 (108,034), 357 (105,238). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.11–6.34 (complex m, 8H), 5.67 (dd, J=15.1, 7.1 Hz, 1H), 5.36 (d, J=15.4 Hz, 1H), 5.47 (s, 1H), 4.27 (t, J=14.2 Hz, 1H), 3.83–3.90 (complex m, 4H), 3.67 (s, 3H), 2.96 (s, 1H), 2.59 (m, 1H), 2.20 (s, 3H), 1.89–1.98 (complex m, 4H), 1.74 (m, 1H), 1.40–1.50 (complex m, 2H), 1.23 (m, 1H), 1.18 (d, J=7.1 Hz, 3H), 1.02 (s, 3H), 0.92–0.95 (complex m, 15H), 0.79–1.00 (complex m, 3H); and ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.61, 167.33, 167.02, 160.81, 143.62, 143.19, 139.63, 138.31, 136.57, 135.32, 134.58, 132.53, 132.48, 131.71, 130.07, 129.23, 127.93, 127.05, 118.79, 70.86, 70.22, 56.92, 51.88, 41.91, 41.00, 40.81, 39.07, 35.67, 33.61, 29.84 (2C), 28.01, 27.86, 26.82, 22.45, 19.39(2C), 19.34, 18.43, 12.95. ESI-MS m/z 668.4523 $[M+NH_4]^+$ (Calcd. for $C_{40}H_{62}NO_7$, m/z 668.4526 $[M+NH_4]^+$).

Acknowledgements

We are grateful to Distinguished Emeritus Professor S. Ōmura (Kitasato University, Tokyo, Japan) for his helpful support and valuable suggestions. Further, we are grateful to Dr. K. Nagai, Ms. R. Seki, and Ms. N. Sato, School of Pharmacy, Kitasato University for the measurements of mass and NMR spectra. We are also grateful to Dr. Andrew Piggott from Macquarie University for providing us with the burnettiene A NMR data. Further, we are also grateful to Dr. T. Nogawa, and Dr. H. Koshino (RIKEN) for suggestions concerning the structural identification of burnettiene A.

Funding Sources

This study was partially supported by the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS), the Japan Agency for Medical Research & Development (AMED) under Grant Number JP21am0101096 (Phase I), JP22ama121035 (Phase II), and Grant-in-Aid for Scientific Research (KAKENHI C) 21K06616.

Author Contributions

A. K., A. N., and I. T. contributed equally. Conceptualization: A. K.; Funding acquisition: A. Y.; Investigation: A. K., K. S., M. H., K. N., T. T., H. K., and K. N.; Project administration: Y. A.; Resources: S. F., T. C. and T. U.; Supervision: Y. A. and T. U.; Writing – original draft: A. K.; Writing – review & editing: all authors.

Electronic supplementary materials

The online version of this article contains supplementary materials, which are available at https://www.jstage.jst.go.jp/browse/jpestics/.

Supporting Information

^{1}H-NMR and ^{13}C-NMR spectrum of burnettiene A derivatives **2**–**13**. Preparation method for burnettiene A (**1**).

References

1) T. Chinen, Y. Ota, Y. Nagumo, H. Masumoto and T. Usui: Construction of multidrug-sensitive yeast with high sporulation efficiency. *Biosci. Biotechnol. Biochem.* **75**, 1588–1593 (2011).
2) T. Chinen, Y. Nagumo and T. Usui: Construction of a genetic analysis-available multidrug sensitive yeast strain by disruption of the drug efflux system and conditional repression of the membrane barrier system. *J. Gen. Appl. Microbiol.* **60**, 160–162 (2014).
3) K. Sakai, T. Hirose, M. Iwatsuki, T. Chinen, T. Kimura, T. Suga, K. Nonaka, T. Nakashima, T. Sunazuka, T. Usui, Y. Asami, S. Ōmura and K. Shiomi: Pestynol, an antifungal compound discovered using a *Saccharomyces cerevisiae* 12geneΔ0HSR-iERG6-based assay. *J. Nat. Prod.* **81**, 1604–1609 (2018).
4) K. Sakai, Y. Unten, M. Iwatsuki, H. Matsuo, W. Fukasawa, T. Hirose, T. Chinen, K. Nonaka, T. Nakashima, T. Sunazuka, T. Usui, M. Murai, H. Miyoshi, Y. Asami, S. Ōmura and K. Shiomi: Fusaramin, an antimitochondrial compound produced by *Fusarium* sp., discovered using multidrug-sensitive *Saccharomyces cerevisiae*. *J. Antibiot. (Tokyo)* **72**, 645–652 (2019).
5) K. Sakai, Y. Unten, A. Kimishima, K. Nonaka, T. Chinen, K. Sakai, T. Usui, K. Shiomi, M. Iwatsuki, M. Murai, H. Miyoshi, Y. Asami and S. Ōmura: Traminines A and B, produced by *Fusarium concentricum*, inhibit oxidative phosphorylation in *Saccharomyces cerevisiae* mitochondria. *J. Ind. Microbiol. Biotechnol.* **48**, 9–10 (2021).
6) A. Kimishima, Y. Ono, K. Sakai, K. Sakai, M. Honsho, K. Naher, T. Tokiwa, H. Kojima, M. Higo, K. Nonaka, M. Iwatsuki, S. Fuji, T. Chinen, T. Usui and Y. Asami: A combination strategy of multidrug-sensitive budding yeast and chemical modifications enabling to find a new overlooked antifungal compound, sakurafusariene, from In-house fractionated library. *J. Agric. Food Chem.* **71**, 3219–3224 (2023).
7) A. Kimishima, D. Hagimoto, M. Honsho, K. Sakai, S. Honma, S. Fuji, M. Iwatsuki, T. Tokiwa, K. Nonaka, T. Chinen, T. Usui and Y. Asami: Total synthesis of fusaramin, enabling stereochemical elucidation, structure–activity relationship, and uncovering the hidden antimicrobial activity against plant pathogenic fungi. *Org. Lett.* **26**, 597–601 (2024).
8) I. Roux, S. Bowles, J. A. Kalaitzis, D. Vuong, E. Lacey, Y.-H. Chooi and A. M. Piggott: Characterisation and heterologous biosynthesis of burnettiene A, a new polyene-decalin polyketide from *Aspergillus burnettii*. *Org. Biomol. Chem.* **19**, 9506–9513 (2021).
9) S. V. Avery, I. Singleton, N. Magan and G. H. Goldman: The fungal threat to global food security. *Fungal Biol.* **123**, 555–557 (2019).
10) R. N. Silva, V. N. Monteiro, A. S. Steindorff, E. V. Gomes, E. F.

Noronha and C. J. Ulhoa: Trichoderma/pathogen/plant interaction in pre-harvest food security. *Fungal Biol.* **123**, 565–583 (2019).
11) M. C. Fisher, N. J. Hawkins, D. Sanglard and S. J. Gurr: Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* **360**, 739–742 (2018).
12) S. A. Shahriar, A. Imtiaz, B. Hossain, A. Husna and N. K. Eaty: Rice blast disease. *Annu. Res. Rev. Biol.* **35**, 50–64 (2020).
13) B. S. Weir, P. R. Johnston and U. Damm: The *Colletotrichum gloeosporioides* species complex. *Stud. Mycol.* **73**, 115–180 (2012).
14) A. H. K. Robin, R. Laila, M. Abuyusuf, J. Park and I. Nou: *Leptosphaeria maculans* alters glucosinolate accumulation and expression of aliphatic and indolic glucosinolate biosynthesis genes in blackleg disease-resistant and -susceptible cabbage lines at the seedling stage. *Front. Plant Sci.* **11**, 1134 (2020).
15) N. Cheung, L. Tian, X. Liu and X. Li: The destructive fungal pathogen *Botrytis cinerea*-insights from genes studied with mutant analysis. *Pathogens* **9**, 923 (2020).
16) N. A. Anderson: The genetics and pathology of *Rhizoctonia solani*. *Annu. Rev. Phytopathol.* **20**, 329–347 (1982).
17) N. K. Devaraj and M. G. Finn: Introduction: click chemistry. *Chem. Rev.* **121**, 6697–6698 (2021).