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## Thielavins: Tuned Biosynthesis and LR-HSQMBC for Structure Elucidation

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### Abstract

A series of thielavins I, V, and Q (**1-3**) and the previously undescribed thielavin Z<sub>8</sub> (**4**) were isolated from cultures of a fungal *Shiraia*-like sp. (strain MSX60519) that were grown under a suite of media and light conditions, with enhanced biosynthesis noted using rice as a substrate with 12:12 h light:dark cycles. Conversely, oatmeal medium and continuous white LED light exposure negatively affected the production of these compounds, at least by strain MSX60519. The structure of **4** was determined using NMR spectroscopic data and mass fragmentation patterns. Of note, the utility of LR-HSQMBC and NOESY NMR experiments in the structural elucidation of these hydrogen-deficient natural products was demonstrated. Compounds **1-4** exhibited cytotoxic activity at the micromolar level against human breast, ovarian, and melanoma cancer cell lines.

### Keywords

Thielavins; LR-HSQMBC NMR; *Shiraia*-like sp.; Culture medium

### Introduction

Recently, we evaluated the effect of different fermentation conditions, including varying both culture media and light exposure, on the biosynthesis of perylenequinones, specifically hypocrellins and hypomycesins, by the fungus *Shiraia*-like sp. (strain MSX60519) [1, 2]. We showed that the production of these fungal metabolites could be tuned based on media and

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Supporting Information

<sup>1</sup>H NMR, <sup>13</sup>C NMR and (+)-HRESIMS data for compounds **1-4**. 2D NMR data (HSQC and HMBC) for the new compound (**4**). MS fragmentation patterns of **1-4**. LR-HSQMBC NMR for **1** & **4** and NOESY NMR for **3** & **4**. The 1D and 2D NMR spectra for **1-4** were deposited in Harvard Dataverse and can be freely accessed through <https://doi.org/10.7910/DVN/QF3WOJ>.

light exposure. For instance, continuous LED exposure led to enhanced production of hypocrellins on rice medium vs hypomyces on oatmeal medium [1]. Previously, those classes of secondary metabolites had not been reported from the same fungus, and it was interesting to see how their biosynthesis could be modulated.

As part of follow-up work, the production of non-erythrin secondary metabolites by cultures grown on rice and incubated under 12:12 h light:dark cycles was observed. As described herein, these minor constituents were identified as thielavins I (**1**), V (**2**), and Q (**3**), along with the previously undescribed thielavin Z<sub>8</sub> (**4**). Thielavins are a group of polyphenolic fungal secondary metabolites composed of two or more monocyclic aromatic units linked by ester bonds. A wide-range of biological properties have been reported for thielavins, and these include antimicrobial [3, 4], antihyperglycemic [5, 6], antifouling [7], cytotoxic [8-10], and herbicidal activities [11], inhibition of prostaglandin biosynthesis [12, 13], and inhibition of indoleamine 2,3-dioxygenase [14]. Given the distinct conditions used to generate **1-4** and the broad range of biological activities, we felt compelled to explore these compounds further.

Despite the description of many thielavins and thielavin-like secondary metabolites since their discovery in 1981 [12, 15], various challenges are associated with their structure elucidation. This is due to the sparsity of <sup>1</sup>H-<sup>1</sup>H coupling, the low hydrogen to carbon ratio, and the abundance of fully-substituted carbons (e.g., ranging from 16 to 19 in **1-4**). Data derived from standard COSY and HMBC NMR experiments may not be enough to derive the full structural assignments of these compounds, leading to at least some incorrect assignments in the literature [3, 16]. As such, the sequence of aromatic units in thielavins have been assigned based on MS fragmentation patterns [7, 8, 17], and the chemical shifts of ester carbonyls were arbitrarily assigned for most thielavins [5, 7, 8]. Given the prominence of NMR spectroscopy for establishing molecular connectivity [18, 19], the isolated thielavins (**1-4**) were used to test the power of a relatively new NMR experiment, i.e. long-range heteronuclear single quantum multiple bond correlation (LR-HSQMBC), coupled with NOESY experiments, to assign the full structures of these hydrogen-deficient compounds.

## Result and discussion

There were a few key goals with these studies. First, we were intrigued with the ability to tune the biosynthesis of distinct fungal metabolites by varying the media and the light source when fermenting *Shiraia*-like sp. (strain MSX60519). In addition, while thielavins are known in the literature, their structure elucidation by NMR can be challenging, especially due to the relatively low number of hydrogens. As such, we strove to evaluate the usefulness of LR-HSQMBC NMR experiments, coupled with NOESY experiments, to fully assign the structures of these molecules. Finally, the biological activity of **1-4** was evaluated against a suite of human cancer cell lines.

### The effect of growth medium and light on the production of thielavins

The *Shiraia*-like sp. (strain MSX60519) was grown on three different grain-based media: rice, Cheerios [20], and breakfast oatmeal (old fashioned Quaker oats). As previously

described [1], these cultures were incubated under three different light conditions in triplicates: 12:12 h light:dark cycles, continuous LED light, and complete darkness. The relative abundance of **1-4** (Fig. 1) were evaluated using UPLC-HRESIMS data from the resulting extracts. Cultures grown on rice medium under 12:12 h light:dark cycles showed the highest abundance of **1-4** (Fig. 2), suggesting rice as the best medium for the production of thielavins, while oatmeal medium showed the lowest abundance of these compounds. On the other hand, exposure to continuous LED light negatively affected the biosynthesis of thielavins, regardless of culture media (Fig. 2). Interestingly, the production of three distinct classes of compounds (i.e., hypocrellins, hypomyces, and thielavins) from *Shiraia*-like sp. was affected differently by the culture medium and light exposure [1]. Enhanced production of hypocrellins was observed in cultures grown on rice and incubated under light:dark cycles or continuous LED light, while hypomyces preferred oatmeal medium and LED light incubation [1]. The optimal growth conditions for enhanced production of thielavins have not been reported extensively in the literature, and with this study, thielavins showed higher abundance in rice-fermentation cultures incubated with regular light:dark cycles. While it is well appreciated that secondary metabolite production in fungi is sensitive to fermentation conditions [21], biosynthesis experiments with strain MSX60519 were particularly notable in this regard.

### Structure elucidation of 1-4

Compound **1** was obtained as white amorphous powder. The HRESIMS and NMR data of **1** suggested that it belonged to the thielavin class of natural products (Figs. S1-S2 and Table S1), which our team had studied previously [8]. For instance, key features apparent in the  $^{13}\text{C}$  NMR spectrum included the presence of two ester carbonyl moieties, 18 aromatic carbons, and 8 aryl methyls. In addition, the HMBC correlations exhibited by methyl groups and aromatic hydrogens (Fig. S3) suggested that **1** was thielavin I, which was reported previously from the fungus *Chaetomium carinthiacum* [5]. However, the collected and reported  $^1\text{H}$  and  $^{13}\text{C}$  NMR data did not align perfectly (Table S2), possibly due to differences in referencing based on residual solvent signals. Moreover, as discussed below, we were able to more fully assign the structure, and those data are reported in comparison to the literature, to facilitate the identification of this compound in the future (see Tables S1 and S2).

While these oligomeric molecules may appear relatively simple, there are several challenges to assigning their structures completely. To start, the structural similarities between the aromatic units within and/or between thielavins results in only subtle differences, typically the presence, absence, or variable position of a methyl moiety. This is compounded by the fact that they are hydrogen deficient, and this renders the COSY NMR spectrum (and even coupling constants from the  $^1\text{H}$  NMR spectrum) of limited value due to the lack of  $^1\text{H}$ - $^1\text{H}$  coupling. Moreover, even with typical heteronuclear experiments, insufficient HMBC correlations could lead to erroneous structural assignments, as was the case with thielavins Q and R [3, 16].

To address these challenges, the usefulness of the LR-HSQMBC NMR experiment was evaluated. As the name indicates, LR-HSQMBC is a recently developed NMR experiment

that provides long-range correlation data across 4-, 5-, and even 6-bond heteronuclear couplings [22], and it has been used to enhance the structure elucidation of hydrogen-deficient molecules [23-25]. The LR-HSQMBC spectrum of **1** (Fig. S4) showed correlations of H-5, H<sub>3</sub>-10, H<sub>3</sub>-8', and H<sub>3</sub>-9' with the carbonyl at C-7 ( $\delta_C$  171.2), which confirmed the connection between rings A and B (Fig. 3). The connection between rings B and C was supported by the LR-HSQMBC correlations of H<sub>3</sub>-10', H<sub>3</sub>-7'', and H<sub>3</sub>-9'' with the carbonyl at C-7' ( $\delta_C$  169.5). Thus, the LR-HSQMBC experiment of **1** confirmed the sequence of the rings and the assignment of the two separate carbonyl moieties (i.e., C-7 and C-7') (Table S1); this was further evidence that the assignments for this compound reported previously could be improved (Table S2) [5]. Additionally, the HRESIMS fragmentation data of **1** were supportive of the sequence of the rings (Fig. S14).

Compound **2** was a white powder with a molecular formula C<sub>27</sub>H<sub>28</sub>O<sub>8</sub> of as deduced by HRESIMS (Fig. S1). <sup>1</sup>H and <sup>13</sup>C NMR spectra collected in CD<sub>3</sub>OD, identified **2** as thielavin V (Fig. S5), which was reported previously from a *Setophoma* sp. [17]. Thielavin V (**2**) differs from **1** by the lack of a 9''-CH<sub>3</sub> and the presence of an extra aromatic hydrogen at the 6'' position. Table S3 compares the collected and literature NMR data of **2**, and the HRESIMS fragmentation data of **2** were supportive of this structural assignment (Fig. S14).

Compound **3** was isolated as a white amorphous powder. The molecular formula of **3** was deduced by HRESIMS data (Fig. S1) and 1D and 2D NMR data (Figs. S6-S8), indicating that **3** matched the structure of thielavin Q. This was first reported in 2011, and the structure was later revised in 2013 based on new HMBC correlations [3, 16]. The previously reported <sup>1</sup>H and <sup>13</sup>C NMR data of thielavin Q (**3**) were measured in two different solvents (CD<sub>3</sub>OD for <sup>1</sup>H and a 9.75 to 0.25 mixture of CDCl<sub>3</sub>/CD<sub>3</sub>OD for <sup>13</sup>C). Herein, we report the <sup>1</sup>H and <sup>13</sup>C NMR data for **3** in CDCl<sub>3</sub> for more convenient identification of this compound in the future (Table S1). In addition, the HRESIMS fragmentation data of **1** were supportive of this structural assignment (Fig. S14).

The molecular formula of **4** was deduced as C<sub>29</sub>H<sub>32</sub>O<sub>8</sub> by HRESIMS data (Fig. S1), suggesting the same index of hydrogen deficiency (i.e., 14) as observed for **1-3**. The <sup>1</sup>H and <sup>13</sup>C NMR data of **4** indicated nine aryl methyls, one aromatic hydrogen, and four hydroxy groups (Table 1 and Fig. S9). The two chelated and exchangeable hydrogens at  $\delta_H$  11.59 (2-OH) and 11.42 (2'-OH) suggested a hydroxy group  $\beta$  to each ester (i.e., C-7 and C-7'). The HMBC correlations of three methyl groups [H<sub>3</sub>-8 ( $\delta_H$  2.18) to C-2, C-3, and C-4; H<sub>3</sub>-9 ( $\delta_H$  2.23) to C-4, C-5, and C-6; and H<sub>3</sub>-10 ( $\delta_H$  2.67) to C-1, C-5, and C-6] and the two hydroxys [2-OH ( $\delta_H$  11.59) to C-1, C-2, and C-3; and 4-OH ( $\delta_H$  5.30) to C-3, C-4, and C-5] indicated the presence of a 2,4-dioxygenated-3,5,6-trimethylbenzoyl ring (i.e., ring A). Similarly, the HMBC correlations of three methyl groups [H<sub>3</sub>-8' ( $\delta_H$  2.13) to C-2', C-3', and C-4'; H<sub>3</sub>-9' ( $\delta_H$  2.14) to C-4', C-5', and C-6'; and H<sub>3</sub>-10' ( $\delta_H$  2.70) to C-1', C-5', and C-6'] and a chelated hydroxy [2'-OH ( $\delta_H$  11.42) to C-1', C-2', and C-3'] confirmed the structure of ring B as a 2',4'-dioxygenated-3',5',6'-trimethylbenzoyl moiety (Figs. 4 and S11). The third aromatic ring (i.e., ring C) was found to have three methyl groups attached to C-2'', C-5'', and C-6'' based on HMBC correlations (Fig. 4), in addition to the oxygenated carbon at C-1'' and a hydroxylated carbon at C-3''. The oxygenated C-4' in ring B was linked with C-1 in ring A through an ester linkage, while another ester bond formed the connection of

ring B at C-1' with ring C at C-1'. The LR-HSQMBC spectrum of **4** showed four-bond heteronuclear couplings of H<sub>3</sub>-10 and H<sub>3</sub>-10' with the C-7 and C-7' carbonyls, respectively (Figs. S12-S13). However, the correlations of CH<sub>3</sub>-8', -9', -7'', and -9'' with the ester linkages was not very clear, which might be attributed to the paucity of sample, relative to what was used for the same experiment on **1**. Compound **4** was ascribed the trivial name thielavin Z<sub>8</sub>.

In the literature, NOESY NMR data were not typically collected for thielavins, probably because these molecules lack asymmetric centers. However, NOESY experiments can be used to facilitate the structure elucidation of these compounds, as shown for **3** and **4** (Fig. 5). The NOESY spectra of these two compounds showed intra-ring and inter-ring correlations among the methyl groups, aromatic hydrogens, and/or hydroxy groups attached to rings A, B, and C (Figs. S15 and S16), confirming the positions of the aromatic substituents in **3** and **4** (Fig. 5). Compound **4**, for instance, showed NOESY correlations between 2-OH/H<sub>3</sub>-8, H<sub>3</sub>-8/4-OH, 4-OH/H<sub>3</sub>-9, and H<sub>3</sub>-9/H<sub>3</sub>-10 in ring A. Similar correlations were exhibited by the substituents attached to rings B and C (Fig. 5). On the other hand, inter-ring NOESY correlations between H<sub>3</sub>-10/H<sub>3</sub>-8' and H<sub>3</sub>-10'/H<sub>3</sub>-7'' in **4** confirmed the sequence of rings A, B, and C, and these conclusions were confirmed via MS fragmentation patterns (Fig. S14). Overall, while somewhat non-traditional, NOESY correlations can be used as an orthogonal means for confirming the structures of thielavins.

## Cytotoxic activities of thielavins 1-4.

The cytotoxic activities of **1-4** were evaluated against three cancer cell lines, including MDA-MB-231 (human breast cancer), OVCAR3 (human ovarian cancer), and MDA-MB-435 (human melanoma cancer). Compounds **1-4** exhibited moderate cytotoxic activities, ranging between 8-24  $\mu$ M (Table 2). The difference between **1** and **2** is only a single methyl group in ring C, suggesting this may improve cytotoxicity in the latter, albeit only slightly.

This study brought to light three new aspects to the thielavin literature. First, we showed the effect of culture medium and light exposure on the production of thielavins by *Shiraia*-like sp., suggesting that fermentation on rice under 12:12 h light:dark cycles enhanced biosynthesis of these compounds. We also expanded the number of thielavins by the identification of thielavin Z<sub>8</sub> (**4**). Perhaps more importantly, we showed how LR-HSQMBC and NOESY NMR experiments can be used in a mutually supportive manner to enhance the structural assignments of these hydrogen deficient molecules.

## Materials and methods

### General experimental procedures

Ultraviolet (UV) spectra were measured using a Varian Cary 100 Bio UV-Vis spectrophotometer (Varian Inc.). 1D and 2D NMR data were obtained using an Agilent 700 MHz NMR spectrometer equipped with a cryoprobe, or a JEOL ECA-500 NMR spectrometer operating at 500 MHz, or a JEOL ECS-400 spectrometer operating at 400 MHz that is equipped with a high-sensitivity JEOL Royal probe and a 24-slot autosampler. Residual solvent signals were used for referencing the NMR spectra. UPLC-HRESIMS data

for the culture extracts and the pure compounds **1-4** were collected via a Thermo Fisher Scientific Q Exactive Plus mass spectrometer equipped with an electrospray ionization source (ESI) and connected to a Waters Acquity UPLC system with a BEH Shield RP18 column (Waters, 1.7  $\mu\text{m}$ ; 50  $\times$  2.1 mm) that was heated to 40 °C. The mobile phase consisted of CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% formic acid) using a gradient system of 15:85 to 100:0 over 10 min at a flow rate of 0.3 mL/min. MS data were collected from  $m/z$  150 to 2000, while alternating between positive and negative modes. Analytical and preparative HPLC experiments were carried out using a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector (PDA). Flash chromatography was carried out using a Teledyne ISCO CombiFlash Rf 200 that was equipped with UV and evaporative light-scattering detectors and using Silica Gold columns.

### Fungal identification, fermentation, and isolation

Fungal strain MSX60519 was previously identified as a *Shiraia*-like sp. in the family Shiraiaaceae, Pleosporales, Dothideomycetes, Ascomycota, as detailed recently [1]. A live culture of this strain is accessioned at Mycosynthetix, Inc. (Hillsborough, NC, USA). The ITS sequences were deposited in GenBank under accession numbers: [MN970609](#) and [MN970610](#).

The media and fermentation studies were described recently [1]. In brief, cultures of strain MSX60519 were grown on three different grain-based media [i.e., rice, Cheerios [20], and breakfast oatmeal (old fashioned Quaker oats)] using nine cultures per medium, where three were incubated under 12:12 h light:dark cycles, three were placed under white light-emitting diode (LED) lamps (Ustellar, flexible LED strip lights; 24W) to provide continuous light exposure, and three cultures were incubated in complete darkness. Statistical analysis was carried out using GraphPad Prism (GraphPad Software, La Jolla, CA), and comparisons were made using one-way ANOVA followed by Tukey post-test.

The organic extracts of rice-fermentation cultures incubated under 12:12 h light:dark cycles were defatted and then subjected to fractionation via normal-phase flash chromatography to afford four fractions. The second flash chromatography fraction of each extract was subjected to preparative HPLC over a Phenomenex Synergi C<sub>12</sub> preparative column using an isocratic system of 60:40 of CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% formic acid) for 45 min at a flow rate of 21.2 mL/min to yield thielavin V (**2**, 1.3 mg), thielavin Q (**3**, 1.1 mg), thielavin I (**1**, 10.8 mg), and thielavin Z<sub>8</sub> (**4**, 1.1 mg).

#### Thielavin I (**1**)

Compound **1** was isolated as a white amorphous powder; <sup>1</sup>H NMR (500 MHz, pyridine-*d*<sub>5</sub>) and <sup>13</sup>C NMR (125 MHz, pyridine-*d*<sub>5</sub>) (see Table S1); HRESIMS  $m/z$  495.1999 [M + H]<sup>+</sup> (calcd. for C<sub>28</sub>H<sub>31</sub>O<sub>8</sub>, 495.2019).

#### Thielavin V (**2**)

Compound **2** was isolated as a white amorphous powder; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) (see Table S1); HRESIMS  $m/z$  481.1844 [M + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>29</sub>O<sub>8</sub>, 481.1862).

### Thielavin Q (3)

Compound **3** was isolated as a white amorphous powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 315 (3.69), 276 (4.13), 217 (4.44), 211 (4.38) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 700 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 175 MHz) (see Table S1); HRESIMS  $m/z$  467.1691  $[\text{M} + \text{H}]^+$  (calcd. for  $\text{C}_{26}\text{H}_{27}\text{O}_8$ , 467.1706).

### Thielavin Z<sub>8</sub> (4)

Compound **4** was isolated as a white amorphous powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 320 (3.75), 278 (4.16), 217 (4.55) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 700 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 175 MHz) (see Table 1); HRESIMS  $m/z$  509.2156  $[\text{M} + \text{H}]^+$  (calcd. for  $\text{C}_{29}\text{H}_{33}\text{O}_8$ , 509.2175).

### Cytotoxicity Assay

To evaluate the cytotoxic activity of **1-4**, human melanoma cancer cells MDA-MB-435, human breast cancer cells MDA-MB-231, and human ovarian cancer cells OVCAR3, were purchased from the American Type Culture Collection (Manassas, VA). The cell line was propagated at 37 °C in 5%  $\text{CO}_2$  in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ). Cells in log phase growth were harvested by trypsinization followed by two washes to remove all traces of enzyme. A total of 5,000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96, Falcon) and incubated overnight (37 °C in 5%  $\text{CO}_2$ ). Samples dissolved in DMSO were then diluted and added to the appropriate wells. The cells were incubated in the presence of test substance for 72 h at 37 °C and evaluated for viability with a commercial absorbance assay (CellTiter-Blue Cell Viability Assay, Promega Corp, Madison, WI) that measured viable cells.  $\text{IC}_{50}$  values are expressed in  $\mu\text{M}$  relative to the solvent (DMSO) control; taxol (paclitaxel) was used as a positive control.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The authors declare the following competing financial interest(s): Nicholas Oberlies declares that he is a member of the Scientific Advisory Board of Mycosynthetix, Inc.

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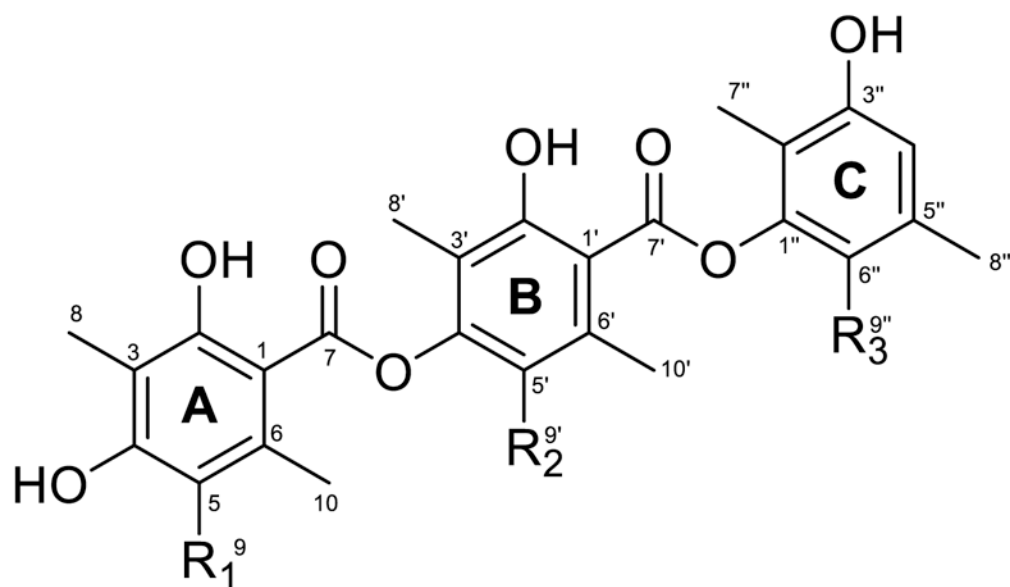
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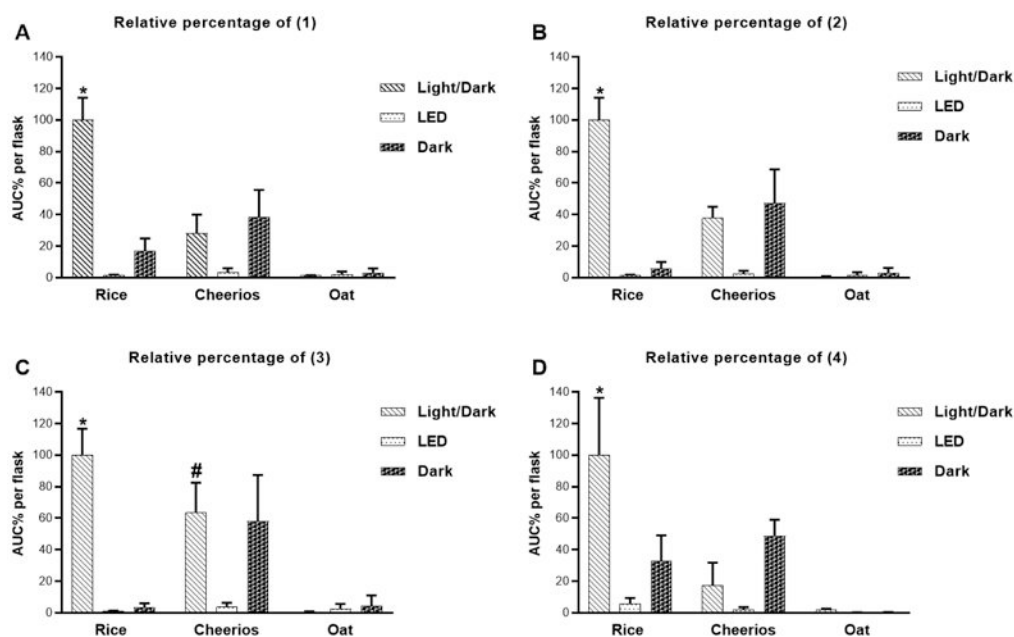
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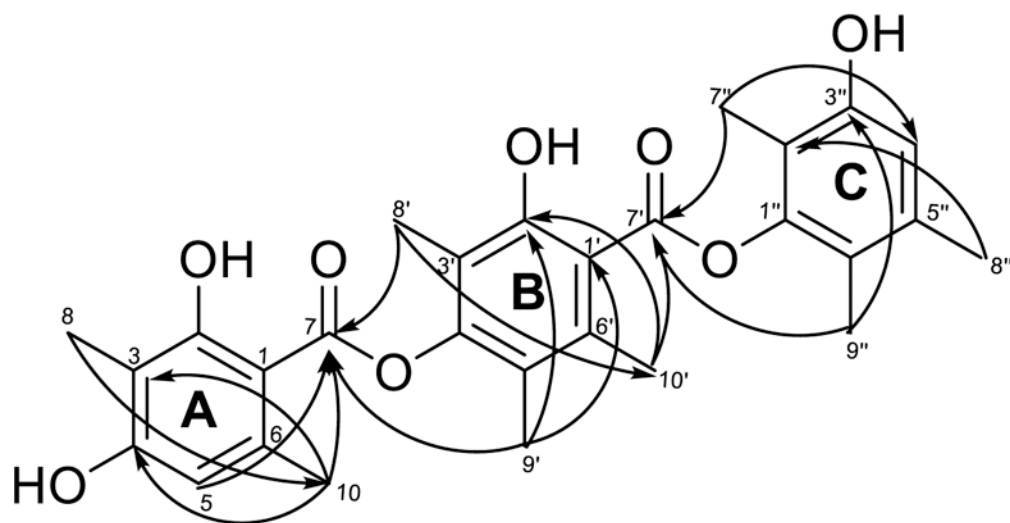
Compound	$R_1$	$R_2$	$R_3$
1	H	$CH_3$	$CH_3$
2	H	$CH_3$	H
3	H	H	H
4	$CH_3$	$CH_3$	$CH_3$

**Fig. 1.**  
Structures of compounds 1-4.

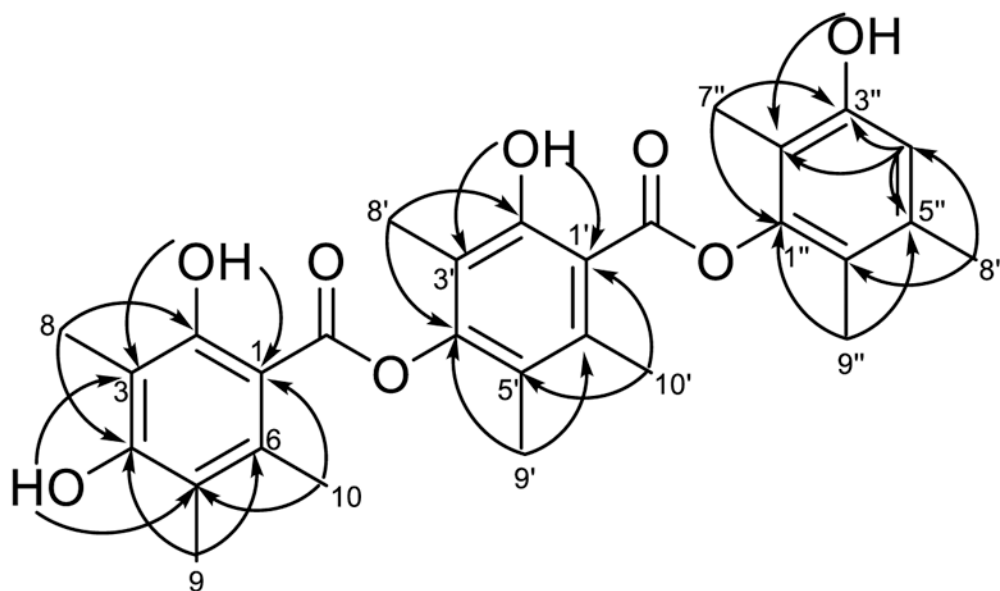


**Fig. 2.**

Panels A-D show the relative percentages of **1-4**, respectively, across cultures grown on rice, Cheerios, and oatmeal media under 12:12 h light:dark cycles, continuous LED, or in darkness. The relative percentages were measured by LC-HRMS in three biological replicates and multiplied by the extract weight, and then normalized according to the extract with highest abundance. \* Indicates significantly higher productions of **1-4** by cultures grown on rice under 12:12 h light:dark cycles as compared to other growth conditions ( $p < 0.05$ ). # Indicates statistically no difference in thielavin production as compared to culture grown rice under 12:12 h light:dark cycles. Data are presented as mean  $\pm$  SD.



**Fig. 3.**  
Key LR-HSQC correlations of compound **1**.



**Fig. 4.**  
Key HMBC correlations of compound 4.



**Table 1.**<sup>1</sup>H (700 MHz) and <sup>13</sup>C (175 MHz) NMR data of **4** in CDCl<sub>3</sub>.

no.	Ring A		no.	Ring B		no.	Ring C	
	$\delta_C$ , type	$\delta_H$		$\delta_C$ , type	$\delta_H$		$\delta_C$ , type	$\delta_H$
1	104.6, C		1'	110.3, C		1''	148.4, C	
2	161.5, C		2'	160.7, C		2''	114.0, C	
3	107.8, C		3'	117.0, C		3''	152.0, C	
4	158.0, C		4'	152.9, C		4''	115.0, C	6.61, s
5	115.7, C		5'	138.4 or 138.5		5''	120.8, C	
6	138.4 or 138.5, C		6'	121.4, C		6''	136.0, C	
7	170.5, C		7'	170.5, C		7''	9.8, CH <sub>3</sub>	2.07, s
8	8.2, CH <sub>3</sub>	2.18, s	8'	10.0, CH <sub>3</sub>	2.13, s	8''	20.1, CH <sub>3</sub>	2.26, s
9	12.2, CH <sub>3</sub>	2.23, s	9'	13.7, CH <sub>3</sub>	2.14, s	9''	12.8, CH <sub>3</sub>	2.03, s
10	19.5, CH <sub>3</sub>	2.67, s	10'	19.6, CH <sub>3</sub>	2.70, s	3''-OH		4.62, s
2-OH		11.59, s	2'-OH		11.42, s			
4-OH		5.30, s						

**Table 2.**IC<sub>50</sub> ( $\mu$ M) values of **1-4** against three human cancer cell lines

Compound	MDA-MB-231	OVCAR3	MDA-MB-435
1	24.1	10.6	12.4
2	8.9	4.5	7.8
3	13.8	14.7	18.6
4	14.3	8.2	18.1
taxol	0.6	1.8	0.3

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