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NOTE

Inhibition of indoleamine 2,3-dioxygenase by thielavin derivatives from a soil fungus, *Coniochaeta* sp. 10F058

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Indoleamine 2,3-dioxygenase (IDO) is an intracellular monomeric heme-containing protein that catalyzes the initial step of tryptophan catabolism via the kynurenine pathway (KP).[1] The IDO product, *N*-formylkynurenine, is further metabolized along this pathway to generate several biologically active molecules collectively called kynurenine and is subsequently converted to a range of metabolites including excitotoxin quinolinic acid.[2] Dysregulation of the KP, mainly associated with the elevation of IDO activity and quinolinic acid production, has been implicated in the pathogenesis of neuroinflammatory, neurodegenerative disorders (Alzheimer's disease), depression, age-related cataract, and HIV encephalitis.[3–5] In addition, IDO is associated with immunosuppression and immunotolerance.[6] IDO-expressing cancer cells use KP transformation to reduce tryptophan concentrations in their microenvironments to the levels that prevent T-lymphocyte proliferation.[7] Some catabolites in the KP are toxic to T cells and further inhibit their action.[8] A growing body of clinical data has shown that many primary tumor cell lines obtained from patients overexpress IDO, and this strongly correlates with a poor prognosis for survival.[9,10] Thus, IDO has emerged as a promising molecular target of new therapeutic agents for treating neurological disorders, cancer as well as other diseases characterized by pathological tryptophan metabolism. Several studies have provided proof-of-principle demonstration for the potential value of IDO inhibitors in cancer treatment.[11,12]

In this regard, we have commenced a screening program to identify and develop new IDO inhibitors derived from microbial metabolites. Recently, we reported the identification of a novel IDO inhibitor, benzomalvin E, isolated from fungal metabolites.[13] In the course of our screening for IDO inhibitors, new benzoate trimer, named thielavin Q (**1**) together with two known thielavin F (**2**) and B (**3**), were isolated from the fermentation broth of *Coniochaeta* sp. 10F058 (Figure 1). In this paper, we describe the fermentation, isolation, structure determination, and biological activity of thielavins. A fungal strain, 10F058, was isolated from a soil sample of Ochang in Korea and was identified on the basis of the rRNA sequences and morphological evaluation. A GenBank search with the 26S rRNA gene of 10F058 indicated *Coniochaeta* sp. MAB-2010a (HQ829070) and *Coniochaeta ligniaria* (AY198390) as the closest matches, both of them showing sequence identities of 100%. Therefore, the fungal strain 10F058 was identified and named as a *Coniochaeta* sp. 10F058. 10F058 was grown on the potato dextrose agar medium for 7 days and was then inoculated into a 500-ml Erlenmeyer flask containing 75 ml of seed culture medium potato dextrose broth (24 g l^{-1} potato dextrose; BD Bioscience, San Jose, CA, USA). Incubation was carried out at 28 °C for 3 days on a rotary shaker operating at 135 r.p.m. This seed medium (150 ml) was transferred to 9 l of the same production medium in a two 14-l jar fermentor. The fermentation was carried out at 28 °C for 6 days with agitation at 165 r.p.m. and an air flow of 10 l min^{-1}. The culture broth (18 l) was filtered and extracted three times with an equal volume of EtOAc, and the EtOAc layer was concentrated *in vacuo.* The EtOAc extract (2.3 g) was separated using silica gel column chromatography using a gradient of $CHCl_3$-MeOH (from 20:1 to 0:1) to yield five fractions (Frs. 1 ~ 5). The active fraction 2 (315 mg) was subjected to reversed-phase C_{18} flash column chromatography using a stepwise gradient of MeOH/H_2O (from 20/80, 40/60, 60/40, 80/20 to 100/0; 700 ml for each step) to yield five fractions (Frs. 2–1 ~ 2–5). The active fraction 2–4 (81.0 mg) eluted with MeOH/H_2O (80/20) as purified by semipreparative reverse phase HPLC using an isocratic solvent system

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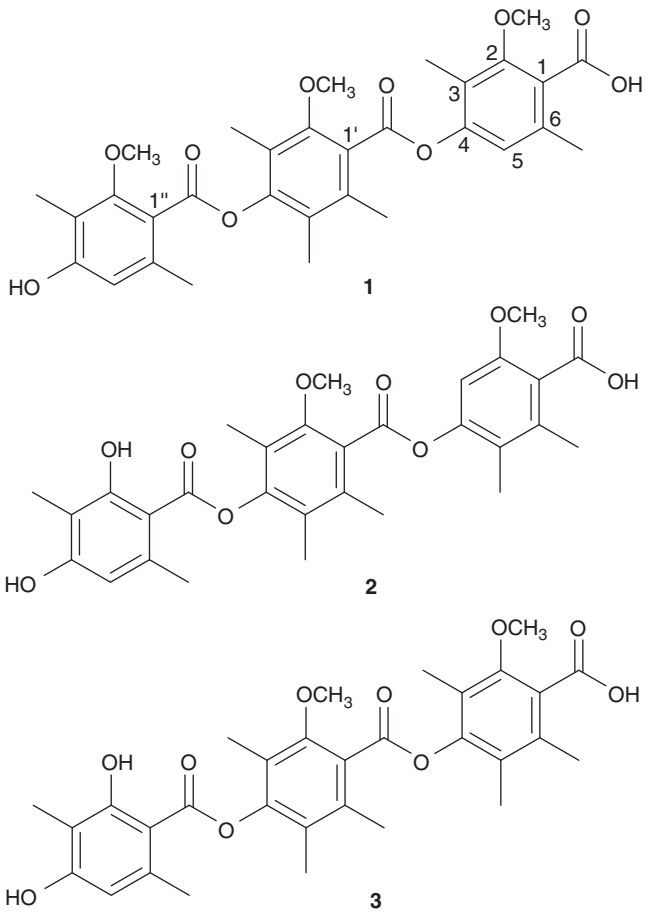

Figure 1 The structures of thielavins Q (**1**), F (**2**) and B (**3**).

of MeOH/H_2O (78/22) to yield compound **1** (t_R 25.5 min, 3.5 mg), **2** (t_R 32.7 min, 3.9 mg), and **3** (t_R 38.9 min, 4.9 mg).

Compound **1** was as a white amorphous powder, and its molecular formula was determined to be $C_{31}H_{34}O_{10}$ by HRESIMS analysis (*m/z* 565.2075 $[M\text{-}H]^-$, calcd for $C_{30}H_{31}O_{10}$, 565.2074; see Supplementary Table). Compound **1** also displayed the IR spectrum [(KBr) ν_{max}] at 3426, 1734, 1666, 1451, 1402, 1376, 1172, and 1075 cm^{-1} (see Supplementary Table). The NMR data (Table 1) showed resonances characteristic for benzoate trimer, termed thielavins,[14] consisting of seven methyls, three methoxys, two aromatics, sixteen quaternary, and three carbonyl carbons. The structure of **1** was elucidated as follows: ^1H and ^{13}C NMR spectroscopic data (see Supplementary Figures S1–S3) for **1** are shown in Table 1 and the structural information on **1** was further obtained by a series of 2D NMR analyses, such as COSY, HMQC, HMBC, and ROESY spectra (see Supplementary Figures S4–S7). Analyses of ^1H–^{13}C long-range correlations in the HMBC spectrum revealed three partial structures. The HMBC correlation peaks, aromatic proton (H-5/C-1, C-3, and C-4), two methyl protons (CH_3-3/C-2, C-3, and C-4 and CH_3-6/C-1, C-5 and C-6), and a methoxy proton 2-OCH_3 (δ_H 3.93) was long-range coupled to C-2. These results revealed the presence of 2-methoxy-3,6-dimethylbenzene moiety shown in Figure 2. The second benzene ring was proven by long-range correlations from three methyl protons ((CH_3-3′/C-2′, C-3′, and C-4′), (CH_3-5′/C-4′, C-5′, and C-6′) and (CH_3-6′/C-1′, C-5′, and C-6′)). The long-range correlation from 2′-OCH_3 (δ_H 3.90) to C-2′ indicated that the methoxy group was attached to C-2′ of the second benzene ring. The HMBC correlation

Table 1 ^1H (900 MHz) and ^{13}C (225 MHz) NMR data for thielavin Q (1) in $CDCl_3$

	*Thielavin Q (**1**)*	
Position	δ_C	δ_H, *mult*
1-Carbonyl	169.1	
1	123.6	
2	157.6	
2-OCH_3	62.4	3.93, s
3	122.5	
3-CH_3	9.6	2.29, s
4	151.6	
5	120.5	6.96.s
6	137.5	
6-CH_3	20.5	2.54, s
1′-Carbonyl	166.5	
1′	126.3	
2′	154.0	
2′-OCH_3	62.3	3.90, s
3′	122.4	
3′-CH_3	10.3	2.31, s
4′	150.3	
5′	132.8	
5′-CH_3	13.0	2.27, s
6′	126.0	
6′-CH_3	17.0	2.42, s
1″-Carbonyl	166.0	
1″	119.4	
2″	158.3	
2″-OCH_3	62.1	3.88, s
3″	115.4	
3″-CH_3	8.8	2.24, s
4″	156.6	
5″	113.2	6.55, s
6″	136.4	
6″-CH_3	20.2	2.46, s

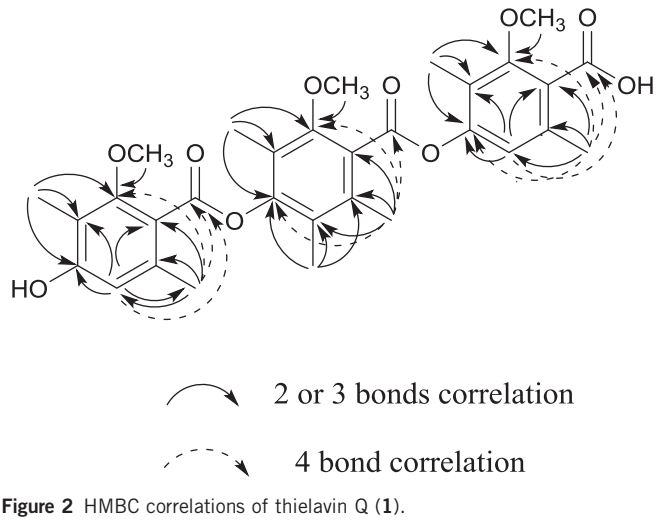

Figure 2 HMBC correlations of thielavin Q (**1**).

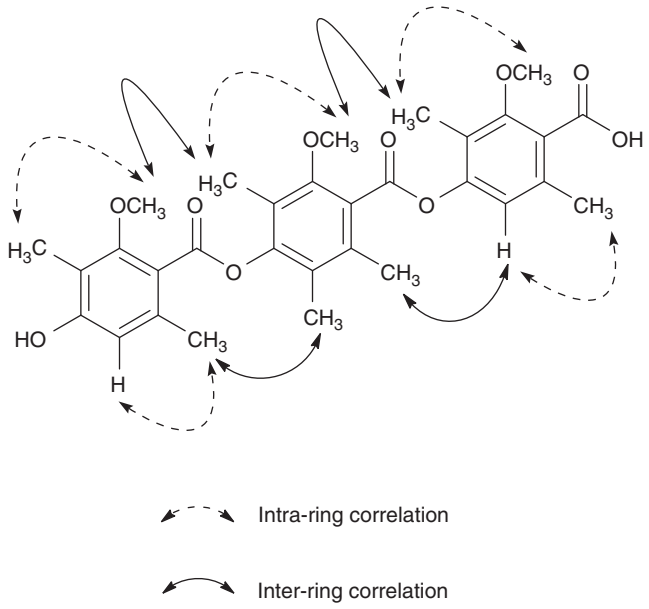

Figure 3 Key ROESY correlations of thielavin Q (**1**).

peaks aromatic proton (H-5″/C-1″, C-3″, C-4″, and CH_3-6″) and two methyl protons ((CH_3-3″/C-2″, C-3″, and C-4″) and (CH_3-6″/C-1″, C-5″, and C-6″)) gave proof for the third benzene ring. In addition, the long-range correlation from 2″-OCH_3 (δ_H 3.88) to C-2″ also indicated that the methoxy group was attached to C-2″ position. The HMBC spectra gave many clear 4-bond correlations, although these signals were weaker than those of the 2- or 3-bond correlations (Figure 2). The 4-bond HMBC correlations were observed (H-5 and CH_3-6/δ_C 169.1, CH_3-6′/δ_C 166.5, H-5″ and CH_3-6″/δ_C 166.0). These spectral data indicated that the three carbonyl groups were attached to C-1, C-1′, and C-1″, respectively. This led to a conclusion that the compound consists of three connected benzoic acid units. The sequence of these benzoic acid units was determined by the analysis of ROESY. The ROESY spectrum showed both intra-ring correlations (2-OCH_3/CH_3-3, CH_3-6/H-5, 2′-OCH_3/CH_3-3′, 2″-OCH_3/CH_3-3″, and CH_3-6″/H-5″) and inter-ring correlations (H-5/CH_3-6′, CH_3-3/2″-OCH_3, CH_3-5′/CH_3-6″, and CH_3-3′/2″-OCH_3) (Figure 3). Thus, the structure of **1** was determined to be 4-[4′-(2″-methoxy-4″-hydroxy-3″,6″-dimethylbenzoyloxy)-3′,5′,6′-trimethyl-2′-methoxy-benzoyloxy]-2-methoxy-3,6-dimethylbenzoic acid as shown in Figure 1. Compounds **2** and **3** were identified as thielavin F (**2**) and B (**3**) by the comparison of their NMR and MS data (Supplementary Figures S8–S11) with those in the literature.[14] Compounds **1**, **2**, and **3** were evaluated for their inhibitory activity against IDO. Thielavin Q (**1**), thielavin F (**2**), and thielavin B (**3**) inhibited the activity of IDO in a dose-dependent manner, with IC_{50} values of 26.5 ± 1.3, 14.5 ± 1.1, and $21.2 \pm 1.1\,\mu M$, respectively. A known IDO inhibitor, menadione ($IC_{50} = 3.7 \pm 0.5\,\mu M$) was employed as a positive control in the assay.

Thielavin derivatives have been reported as the inhibitors of prostaglandin biosynthesis and phospholipase A_2.[15,16] The thielavins have also shown inhibitory activity against telomerase, phospholipase C, and glucose-6-phosphatase.[14,17,18] In addition, recently thielavin B methyl ester exhibited moderate cytotoxicity.[19] Structurally related compounds, such as gyrophoric acid[20] and amidepsines[21] have been reported as inhibitors against cancer cell growth and diacylglycerol acyltransferase, respectively.

To our knowledge, our study is the first one showing that thielavins have IDO inhibitory activity. Further investigation and optimization of thielavins might lead to the finding of new IDO inhibitors potentially useful in the treatment of cancer and neurological disorders.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)