A New Polyketide from the Endophytic Fungus *Penicillium chermesinum*

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ABSTRACT

*A new polyketide derivative, 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6H-benzo[c]chromen-6-one (**1**), was isolated from the endophytic fungus* Penicillium chermesinum*. The structure was established on the basis of UV, IR, HR-ESI MS, and 1D and 2D NMR experiments. The cytotoxicity against four cancer cell lines (HuCCA-1, HepG2, A-549, and MOLT-3) of compound **1** are 14.94-115.71 µM.*

Keywords: Penicillium chermesinum*; natural products; polyketide; endophytic fungi; cytotoxicity*

ABSTRAK

Senyawa baru turunan poliketida diisolasi dari jamur endofitik Penicillium chermesinum*. Struktur senyawa ditentukan menggunakan analisis spektroskopi, UV, IR, HR-ESI MS, dan 1- dan 2-dimensi NMR. Berdasarkan analisis spektra, senyawa **1** dinyatakan sebagai 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6H-benzo[c]chromen-6-one. Senyawa **1** menunjukkan aktivitas sitotoksisitas lemah terhadap sel kanker (HuCCA-1, HepG2, A-549, dan MOLT-3) dengan IC_{50} sebesar 14,94-115,71 µM.*

Kata Kunci: Penicillium chermesinum*; bahan alam; poliketida; jamur endofitik; sitotoksisitas*

INTRODUCTION

The endophytic fungi are microorganisms which live on the higher plants and have symbiotic to the pathogenic relationship to host plants [1-3]. Chemical substances can be produced by the endophytic fungi to protect the host plants from insects and other animals in the environment [4]. The endophytic fungi are recently considered as a source of bioactive compounds, which possess unique structures and have specific biological activities such as antimicrobial, antiviral, anticancer, antioxidant, neuroprotective, and antifungal activities [5-9], in drug discovery and development.

Metabolites of endophytic fungi and their specific biological activities have been reported. Ethyl acetate extract of three endophytic fungi (*Physalospora* sp., *Crataegus monogyna*, and *Plectophomella* sp.) led to the isolation of four compounds (Cytochalasins E and K, (-)-Mycorrhizin A, and Radicidin), which exhibited antifungal, antibacterial activities, and herbicidal properties [10]. Ethyl acetate extracts of *Aspergillus* sp., *Aspergillus peyronelii*, *Aspergillus niger*, and *Chaetomium* sp. strain from *Eugenia jambolana* exhibited highest antioxidant activity (50-80%) and their total phenol values ranging from 58-60 mg/g GAE [11].

Polyketide is widely distributed in the plants, fungi, and microorganisms. Long chain polyketides have been isolated from *Amphidinum* spp., a genus of symbiotic marine *Dinoflagellates* [12]. Other polyketides were isolated from endophytic fungi which isolated from mangrove and medicinal plants [13-15]. They have been reported to exhibited antibacterial, anticancer, and antifungal activities [16-19]. Recently, we reported the isolation of four new polyketides, penicilliumulides A-D, one new eremophilane sesquiterpene together with three known compounds, TMC-264, PR-toxin, and eremofortine C, from the mangrove derived endophytic fungus *Penicillium chermesinum*. TMC-264 and PR-toxin exhibited selective cytotoxic properties against the cancer cell lines [20]. As a continuation of our studies, we re-cultivated the endophytic fungus *P. chermesinum* aiming to isolate the other minor bioactive metabolites.

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EXPERIMENTAL SECTION

Materials

The endophytic fungus *P. chermesinum* was isolated from a healthy root of a mangrove tree, *Hertiera littoralis*, which was collected in November 2012 from the Mangrove Forest Learning and Development Center 2, Samut Sakhon Province, Thailand. Potato Dextrose (PDA) and Potato Dextrose Broth (PDB) (HiMedia) were used for cultivation of the endophytic fungus *P. chermesinum*. Column chromatography was performed with Sephadex LH-20.

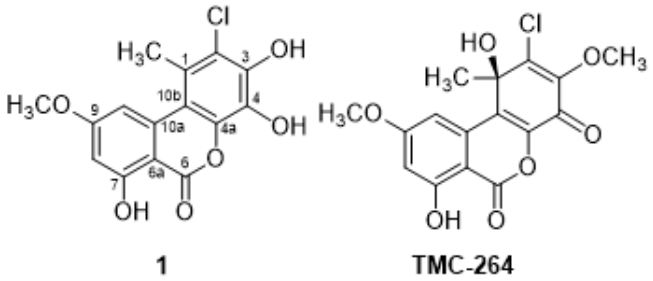
Instrumentation

^{1}H-, ^{13}C- and 2D-NMR spectra were obtained using a Bruker AVANCE 600 spectrometer with TMS as an internal standard. ESITOF-MS were determined using a Bruker MicroTOF$_{LC}$ spectrometer. FTIR spectra were recorded with a universal attenuated total reflectance (UATR) attachment on a Perkin–Elmer Spectrum One spectrometer. The UV-Vis spectra were measured by a Shimadzu UV-1700 Pharma Spec spectrophotometer. High-performance liquid chromatography (HPLC) was carried out on a Waters 1525 Binary HPLC Pump and Waters 2998 Photodiode Array Detector.

Procedure

Cultivation, extraction, and isolation of bioactive compound from the endophytic fungus P. chermesinum

The endophytic fungus *P. chermesinum* was grown on PDA for nine days and then cultivated into 1L Erlenmeyer flasks (20 flasks), each flask containing 250 mL of PDB, and incubated for 31 days at room temperature. The culture was filtered through filter paper (Whatman 41) and the mycelia and broth were handled separately. The broth was extracted with equal volume of ethyl acetate three times, and then the organic fractions were combined and dried under reduced pressure to afford a crude extract (1.1 g). The ethyl acetate extract was first subjected on Sephadex



1 **TMC-264**

Fig 1. Structures of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**) and TMC-264

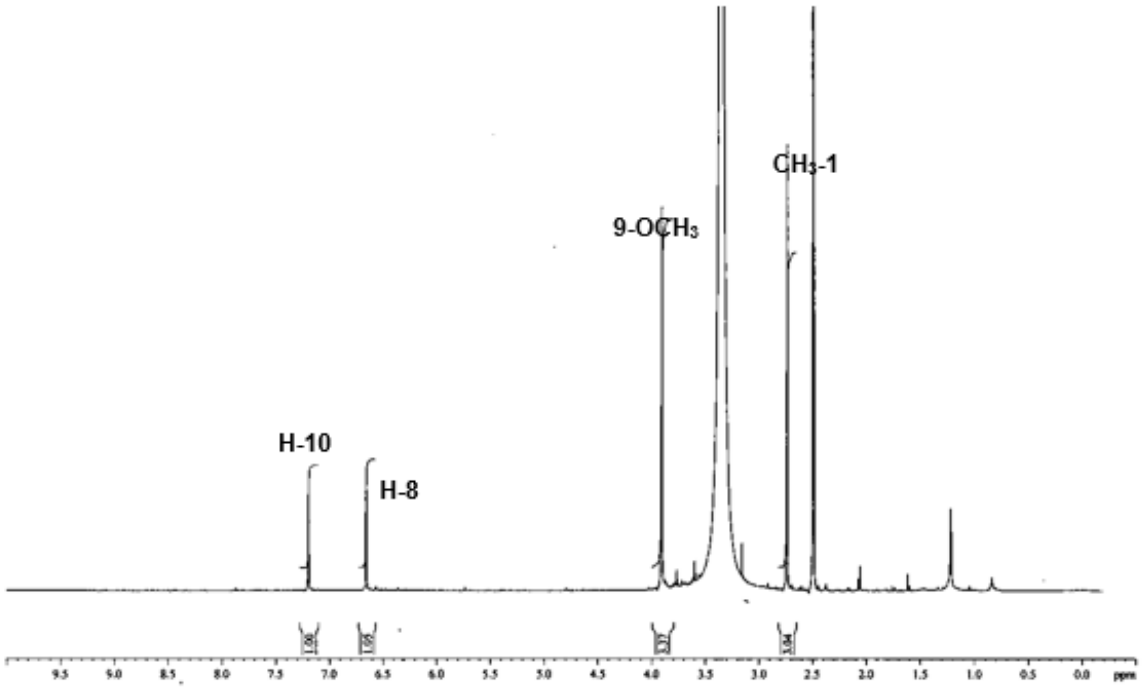


Fig 2. ^{1}H-NMR (DMSO-d_6) spectrum of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**)

LH 20 (3 x 120 cm), eluted with 100% MeOH, to give seven fractions (F1-F7). Fraction F4 was subjected to RP-HPLC (HiChrome 5 C18 column, 21.2 x 250 mm, gradient system MeOH/H_2O from 35 to 100% in 60 min, a flow rate 10 mL min^{-1}) to give ten fractions (G1-G10). Fraction G9 was identified as 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**, 5.3 mg). The structure **1** was elucidated by analysis of UV spectrophotometer, IR spectrometer, HR-ESI MS, and 1D and 2D NMR experiments.

Cytotoxicity evaluation of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6H-benzo[c]chromen-6-one (1)

Cytotoxic activities were evaluated at Chulabhorn Research Institute, Bangkok, Thailand. The test against HuCCA-1 (human chlolangiocarcinoma), HepG2 (hepatocarcinoma), and A549 (human lung cancer) cell lines were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [22], whereas the cytotoxicity towards MOLT-3 (acute lymphoblastic leukemia) cell line was performed by the 2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT) assay [23].

2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**) : Green powder; IR (ATR) (cm^{-1}) : 3419, 2925, 2850, 1664, 1598, 1321, 1230, 1162, 794, 714. UV λ_{max} MeOH (log ε) : 340 (3.9), 290 (4.0), 261 (4.5), 237 (4.4). ^1H and ^{13}C NMR (Table 1). ESI-TOF MS : *m/z* 321.0173 $[M-H]^-$ (calcd for $C_{15}H_{10}ClO_6$, 321.0171).

RESULT AND DISCUSSION

The ethyl acetate extract of endophytic fungus *P. chermesinum* was subjected to chromatography on Sephadex LH-20 and semipreparative RP-18 HPLC to provide a new polyketide derivate, 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**).

Table 1. ^1H (600 MHz) and ^{13}C (150MHz) NMR data of 2-chloro-3,4,7-trihydroxy-9 methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (1)

Position	**1** (DMSO-d_6)	
	δ_C, type	δ_H, multi. (*J* in Hz)
1	124.1, C	–
2	119.9, C	–
3	144.5[a], C	–
4	131.7[a], C	–
4a	139.9[a], C	–
6	164.0, C	–
6a	98.9, C	–
7	163.9, C	–
8	99.7, CH	6.66, d (1.8)
9	166.0, C	–
10	104.8, CH	7.19, d (1.8)
10a	137.4, C	–
10b	110.1, C	–
1-CH_3	20.1, CH_3	2.74, s
9-OCH_3	55.9, CH_3	3.91, s

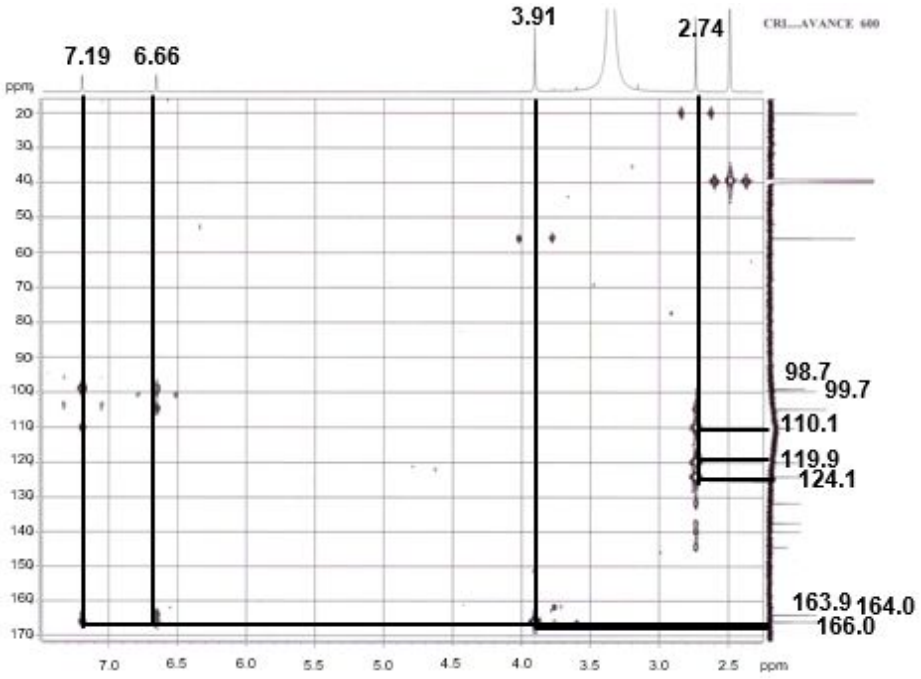


Fig 3. HMBC spectrum of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6H benzo[c]chromen-6-one (1)

Table 2. Cytotoxic activity of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H* benzo[*c*]chromen-6-one (**1**)

Compound	Cytotoxic activity (IC_{50}, μM); mean ± s.d., n = 3			
	HuCCA-1	HepG2	A549	MOLT-3
1	56.39±1.73	55.06±2.48	115.71±5.53	14.94±1.15
Doxorubicin§	1.24±4.35	1.43±4.38	0.84±0.09	0.14±0.01

§Standard drugs, HuCCA-1 = human cholangiocarcinoma cell line; HepG2 = human hepatocellular carcinoma cell line; A549 = human lung adenocarcinoma cell line; and MOLT-3 = human acute T-lymphoblastic leukemia cell line

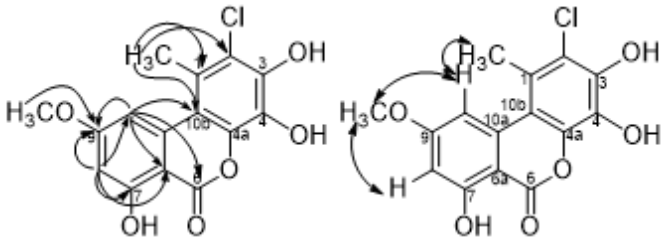


Fig 4. (a) Selected HMBC and (b) NOESY correlations of 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one (**1**)

Compound **1** (Fig. 1) was obtained as a green powder. The molecular formula $C_{15}H_{11}ClO_6$ was determined from ESI-TOF MS at *m/z* 321.0173 $(M-H)^-$, indicating ten degrees of unsaturation. Its IR spectrum suggested the presence of hydroxyl (3419 cm^{-1}) and a δ lactone (1664 cm^{-1}) groups.

The ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) (Fig. 2) of compound **1** showed signals of *meta*-coupled aromatic protons at δ_H 6.66 and 7.19 (each d, J = 1.8 Hz), aromatic methyl protons at δ_H 2.74, and an aromatic methoxy group at δ_H 3.91. Detailed of ^{1}H-NMR was shown in Table 1. The ^{1}H-NMR of **1** was similar to those of TMC-264, which was also obtained from the fungus *P. chermesinum* [21], except for the absence of the methoxy group at C-3 in **1**. The chlorine substituent in compound **1** was placed at C-2 because of the HMBC correlation from 1-CH_3 to C-2 (δ_C 119.9) (Fig. 4) and its structural analog to TMC-264. The downfield shift of methyl protons and quaternary carbon at C-1 (δ_H 2.74, δ_c 124.1), together with the MS data in **1** indicated that the quinol moiety in TMC-264 arranged to an aromatic ring in **1**.

The ^{13}C-NMR (Table 1) and DEPT spectra of compound **1** revealed the presence of two methyls, two methines, and eleven quaternary carbons including one corresponding to a δ-lactone carbonyl at δ_C164.0. The placement of CH_3 at C-1, OH at C-7, and OMe at C-9 were determined by analysis of the HMBC spectrum (Fig. 3 and 4) as follows: from 9-OCH_3 (δ_H 3.91),H-10 (δ_H7.19), and H-8 (δ_H6.66) to C-9 (δ_C166.0); H-8 (δ_H 6.66) to C-9 (δ_C166.0); and 1-CH_3 (δ_H 2.74) to C-1 (δ_C 124.1), C-2 (δ_C 119.9), and C-10b (δ_C 110.1); and H-8 (δ_H 6.66) to C-7 (δ_C 163.9) as well as the NOESY correlations (Fig. 4) of H-8/H-10/9-OCH_3 and H-10/1-Me. Therefore, compound **1** was identified as 2-chloro-3,4,7-trihydroxy-9-metoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one shown in Fig. 1.

Cytotoxicity of compound **1** was evaluated against four cancer cell lines, including HuCCA-1, HepG2, A549, and MOLT-3 cell lines, as shown in Table 2. Compound **1** was inactive or exhibited only weak cytotoxic activity against HuCCA-1, HepG2, A549, with IC_{50} values of 56.39, 55.06, and 115.71 μM, respectively. However, compound **1** exhibited selective cytotoxic activity towards the MOLT-3 cell line, with an IC_{50} value of 14.94 μM.

CONCLUSION

A new polyketide derivative (**1**) was isolated from the broth of the endophytic fungus *P. chermesinum*. Base on spectra analysis (UV, IR, HR-ESI MS, 1-D and 2-D NMR), it was determined as 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[*c*]chromen-6-one and exhibited weak cytotoxic activity toward HuCCA-1, HepG2, A549, and MOLT-3 cell lines.

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