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Cytotoxic and Cell Proliferation Activities of Isocoumarin Derivatives Isolated from *Phialocephala scopiformis* FC-1873

Yuji Higashira[1], Daigo Wakana[1], Yuji Doi[1], Hisashi Takeda[1], Nobutomo Ikarashi[1], Satoshi Kitaoka[1,2], Fumiaki Sato[1,3], Hiyori Itagaki[4,5], Tsuyoshi Hosoya[4], Tomoo Hosoe[1*]

[1]School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan
[2]Faculty of Pharmacy and Pharmaceutical Sciences, School of Pharmacy, Josai University, Saitama, Japan
[3]Faculty of Pharmacy, Juntendo University, Chiba, Japan
[4]Department of Botany, National Museum of Nature and Science, Ibaraki, Japan
[5]River Museum of Saitama, Saitama, Japan
Email: *hosoe@hoshi.ac.jp

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Abstract

Phialocephala scopiformis (Ascomycota, Helotiales, Mollisiaceae) are fungi known as both saprophytes and endophytes, forming small apothecia (1 - 3 mm in diameter) on decaying wood. In this study, three novel isocoumarin derivatives (+)-phaeosphaerin A (**1**), (+)-phaeosphaerin B (**2**), and (*S*)-6-demethylkigelin (**3**) were isolated from *Phialocephala scopiformis* FC-1873 collected in Japan, along with two known isocoumarins, lignicol (**4**) and 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**). The cytotoxicity of **1** and **4** were evaluated against HL-60, PANK-1, HepG2, HT-29, and T98G cell lines, as well as their effects on cell proliferation in the skin keratinocyte cell line HaCaT. These findings highlight the potential of mollisioid fungi as a source of novel bioactive compounds.

Keywords

(+)-Phaeosphaerin A, (*S*)-6-Demethylkigelin, Cytotoxicity, Cell Proliferation Effect, *Phialocephala scopiformis*

1. Introduction

Phialocephala scopiformis is a fungus that forms small disc-shaped apothecia on decaying wood and widely distributed in temperate region. The fungus is also frequently isolated from living conifer needles as an endophyte that inhabits inside healthy plant tissues without causing apparent symptoms to the host plant [1] [2].

Infection of *P. scopiformis* affects the pest by producing rugulosin and gives a significant benefit to the host [1] [2].

Phialocephala is a member of "mollisioid fungi", morphologically and phylogenetically related with *Mollisia* [1]. *Mollisia* is also known to be a rich source of bioactive compounds. Zargozic acid derivatives F-10863B, F-10863C, F-10863D and zargozic acid D3 isolated from *Mollisia* sp. SANK 10294 are potent inhibitors of squalene synthase [3], benesudon isolated from *M. benesuada* A226-93 [4] and mollisianitrile isolated from *Mollisia* sp. A59-96 [5] show antimicrobial, cytotoxic and phytotoxic activities, chlorinated cyclopentane derivatives KS-504a-d isolated from *Mollisia ventosa* KAC-1148 are inhibitors of Ca^{2+} and calmodulin-dependent phosphodiesterase [6] and A11-99-1 isolated from *Mollisia melaleuca* A11-99 shows inhibition of TNF-a promoter activity [7], and chlorinated pyrrole derivatives isolated from *Mollisia* sp. SCSIO41409 is showed antimicrobial and cytotoxic activities [8]. Therefore, discovery of new bioactive compounds from mollisioid fungi was expected.

In this study, we isolated new isocoumarin derivatives (+)-phaeosphaerin A (**1**), (+)-phaeosphaerin B (**2**) and (*S*)-6-demethylkigelin (**3**) and together with known isocoumarin derivatives lignicol (**4**) [9] and 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**) [10] from *Phialocephala scopiformis* FC-1873 collected in Japan. These compounds are classified as isocoumarins, and cytotoxicities were reported in many isocoumarins [11] [12]. Therefore, we investigated the cytotoxicity against promyelocytic leukemia cell lines HL-60, pancreatic carcinoma cell lines PANK-1, hepatocellular carcinoma cell lines HepG2, Colon Adenocarcinoma cell lines HT-29, glioblastoma multiforma tumor cell lines T98G and cell proliferation activity against skin keratinocyte cell line HaCaT of **1** and **4**.

2. Materials and Methods

2.1. Experimental Instruments

For column chromatography, sephadex LH-20 (GE Healthcare, Chicago, IL, USA) was used. MPLC for isolation of compounds, HPLC pump SSC-3160 (Senshu Science, Tokyo, Japan), RI-UV detector YRU-883 (Yamazen, Osaka, Japan), glass column Ultra pack ODS-S-50B (26 × 300 mm, Yamazen), and chromatographic condition as below; flow rate: 8 mL/min, detected by RI-UV detector. HPLC for isolation of compounds, HPLC pump LC-20AT (Shimadzu, Kyoto, Japan), UV detector SPD-20A (Shimadzu), column oven CO-965 (JASCO, Tokyo, Japan) and HPLC column Inertsustain C18 (5 μm, 10 × 250 mm), and chromatographic condition as below; flow rate: 2 mL/min, detected wavelength; 210 nm. NMR spectra were measured by ECAII 600 spectrometer (JEOL, Tokyo, Japan). The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. Optical rotation measured by DIP-10000 polarimeter (JASCO), UV spectra measured by Ultrospec 2100 pro spectrophotometer, and CD spectra measured by J-820 circular dichroism spectrometer (JASCO). Microplate reader was used as MPR-A100 (AS ONE, Osaka, Japan). ESI-MS were measured at negative mode on T-100LP mass spec-

trometer (JASCO).

2.2. Fungal Material

Fungal specimen of *P. scopiformis* was collected from decaying wood of *Viburnum* sp. at Mt. Kasho, Gunma Pref. on 18 May 2009. The isolate (FC-1873) was obtained from ascospores discharged from fresh apothecium on a potato dextrose agar (PDA) and stored at 10˚C in the dark. Dried specimen is conserved in the fungarium in National Museum of Nature and Science (TNS F-25559).

2.3. Cultivation and Isolation

Rice medium was prepared that polished rice 100 g and purified water 105 g was added to Roux flask and sterilized by autoclave at 121˚C for 20 min. Mycelia of *P. scopiformis* FC-1873 cut from the developed colony on PDA was inoculated to 10 × Roux flasks obtain rice medium and cultivated for 25˚C for 3 weeks. After cultivation, methanol 500 mL was added to each Roux flask and extracted overnight. This operation was repeated twice to obtain methanol extract 17.42 g.

This methanol extract was suspended by water and extracted with ethyl acetate and 1-butanol, in turn. The 1.0 g of ethyl acetate extract (4.8 g) was chromatographed on LH-20 with the mobile phase using 200 mL of hexane-chloroform 1:4, 200 mL of chloroform-acetone 3:2, 200 mL of chloroform-acetone 1:4, 200 mL of acetone and 500 mL of methanol to obtain 10 fractions. The fourth fraction (123.3 mg) was purified by MPLC (mobile phase: 25% acetonitrile) and HPLC (mobile phase: 25% acetonitrile) to get (*S*)-6-demethylkigelin (**3**, 2.1 mg). The sixth fraction (91.0 mg) was separated by MPLC (mobile phase: 25% acetonitrile) to obtain (+)-phaeospharin A (**1**, 18.8 mg) together with three fractions (fr. 6A: 19.8 mg, fr. 6B: 17.0 mg, fr. 6C: 11.7 mg). Fr. 6A was purified by HPLC (mobile phase: 25% acetonitrile) to get lignicol (**4**, 15.2 mg). Fr. 6B was purified by HPLC (mobile phase: 30% acetonitrile) to obtain (+)-phaeospharin B (**2**, 5.3 mg), and Fr. 6C was purified by HPLC (mobile phase: 40% acetonitrile) to obtain 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**, 8.6 mg).

(+)-phaeospharin A (**1**)

Colorless needles. $[\alpha]_D$ +12.9˚ (c 0.485, MeOH). ESI-MS (negative mode) m/z: 239.0538 calcd. 239.0561 for $C_{11}H_{11}O_6[M\text{-}H]^-$. UV (MeOH) λ_{max} nm (log ε): 220 (2.23), 273 (1.22), 305 (0.51). The ^{1}H- and ^{13}C-NMR data showed in Table 1.

(+)-phaeospharin B (**2**)

Brown solid. $[\alpha]_D$ +5.8˚ (c 0.485, MeOH). ESI-MS (negative mode) m/z: 273.0166 calcd. 273.0173 for $C_{11}H_{10}O_6Cl[M\text{-}H]^-$. UV (MeOH) λ_{max} nm (log ε): 226 (2.45), 273 (0.834), 318 (0.612). The ^{1}H- and ^{13}C-NMR data showed in Table 1.

(*S*)-demethylkigelin (**3**)

Brown solid. $[\alpha]_D$ +45.7˚ (c 0.105, MeOH). ESI-MS (negative mode) m/z: 223.0571 calcd. 239.0612 for $C_{11}H_{11}O_5[M\text{-}H]^-$. UV (MeOH) λ_{max} nm (log ε): 220 (1.21), 274 (0.77), 299 (0.25). The ^{1}H- and ^{13}C-NMR data showed in Table 1.

6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**)

Pale brown solid. RSI-MS (negative mode): m/z 205.0501 calcd. 205.0506 for $C_{11}H_9O_4[M\text{-}H]^-$, ^{1}H-NMR (DMSO-$d_6$) δ 11.02 (s, 1H, 8-OH), δ 6.62 (s, 1H, 4-H), δ 6.38 (s, 1H, 7-H), δ 2.23 (s, 3H, 3-Me), δ 2.06 (s, 3H, 5-Me) ppm. ^{13}C-NMR (DMSO-d_6) δ 166.1 (C1), δ 163.8 (C6), δ 160.7 (C8), δ 153.6 (C3), δ 137.0 (C4a), δ 109.3 (C5), δ 100.8 (C7), δ 97.5 (C8a), δ 19.1 (C3-Me), δ 9.9 (C5-Me) ppm.

Table 1. ^{1}H- and ^{13}C-NMR data of **1**-**3**.

	(+)-phaeosphaerin A (**1**)*a		(+)-phaeosphaerin B (**2**)*a		(S)-demethylkigelin (**3**)*b	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	171.4		171.1		171.0	
3	79.9	4.61 qδ (6.6, 2.1)	79.6	4.61 brq (6.6)	76.7	4.69 δqδ (11.7, 6.2, 3.4)
4	67.9	4.40 δ (2.1)	64.8	4.81 brs	34.6	2.81 $\delta\delta$ (16.1, 11.7)
						2.91 $\delta\delta$ (16.1, 3.4)
4a	139.1		135.7		136.9	
5	109.1	6.46 s	112.6		107.3	6.34 s
6	159.7		155.1		157.9	
7	136.4		137.1		134.5	
8	157.4		156.0		157.2	
8a	100.8		101.8		101.9	
3-CH_3	16.3	1.47 δ (6.6)	16.5	1.52 δ (6.6)	20.8	1.44 δ (6.2)
7-OCH_3	61.0	3.83 s	61.1	3.87 s	60.5	3.80 s

*a: measured at methanol-d_4, *b: measured at acetone-d_6.

2.4. Cell Lines and Cell Culture

Human hepatoma cells (HepG2) were obtained from the National Institutes of Bio-medical Innovation, Health, and Nutrition (Osaka, Japan). Human promyelocytic leukemia cells (HL60), human pancreatic carcinoma cells (PANC-1), and human glioblastoma multiforme tumor cells (T98G) were obtained from Riken BioResource Research Center (Ibaraki, Japan). Human colon adenocarcinoma cells (HT29) were obtained from KAC Co., Ltd. (Kyoto, Japan).

Cells were cultured in Dulbecco's modified Eagle medium (DMEM: Nacalai Tesque, Inc., Kyoto, Japan) or RPMI 1640 (Nacalai Tesque, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Merck, Darmstadt, Germany) and 1% Antibiotic-Antimycotic Mixed Stock Solution (Nacalai Tesque, Inc.) at 37˚C in a humid incubator containing ambient air supplemented with 5% CO_2.

Human epidermal keratinocytes (HaCaT) were provided from Cell line service (Eppelheim, Germany). HaCaT cell lines were cultured in DMEM containing potassium penicillin G, streptomycin, and 10% fetal bovine serum.

2.5. Cytotoxicity against Human Cancer Cell Lines

Inhibition of cell growth for **1** and **4** was determined by WST-1 assay (Takara-Bio,

Shiga, Japan). The cell lines (HL-60, PANC-1, HepG2, HT-29 and T98G) were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum at 37˚C in humidified air containing 5% CO_2. The cells were distributed at proper density (HL-60: 1.0×10^4 cells/well, PANC-1: 2.0×10^4 cells/well, HepG2: 1.0×10^4 cells/well, HT-29: 2.0×10^4 cells/well, and T98G: 0.2×10^4 cells/well) in 96-well plates and incubated for 48 hr. Compound **1** and **4** (Concentrations adjusted to 3.125, 6.25, 12.5, 25 and 50 μM each) were added and incubated for 24 hr. On the addition of WST-1 solution (10 μL each), the suspensions were further incubated for 2 hr under the same condition. The UV absorbance was measured at 450 nm.

2.6. Cell Proliferation Activity against Human Epidermal Keratinocyte Line

Effect of cell growth for **1** and **4** was determined by WST-1 assay. The cell (HaCaT) was cultured in DMEM containing potassium penicillin G, streptomycin, and 10% fetal bovine serum at 37˚C in humidified air containing 5% CO_2. The cells were distributed at proper density (1.0×10^4 cells/well) in 96-well plates and incubated for 24 hr. The cells transfer to serum-free medium and further incubated for 24 hr. compound **1** and **4** (Concentrations adjusted to 0.78, 1.56, 3.125 and 6.25 μM each) were added and incubated for 24 hr. On the addition of WST-1 solution (10 μL), the suspensions were further incubated for 2 hr under the same condition. The UV absorbance was measured at 450 nm.

2.7. Statistical Analysis

Statistical analysis was performed using the multcomp package in R, version 4.1.0 [13]. Where appropriate, values are expressed as mean ± standard deviation (SD). Comparisons to the respective negative controls were conducted using Dunnett's multiple comparisons test. Values of $p < 0.05$ were considered statistically significant.

3. Results

Phialocephala scopiformis FC-1873 was cultured on rice medium at 25˚C for 3 weeks, and extracted with methanol. The methanol extract was purified by liquid-liquid extraction and various HPLC techniques to obtain new isocoumarin derivatives (+)-phaeosphaerin A (**1**), (+)-phaeosphaerin B (**2**) and (*S*)-6-demethylkigelin (**3**) and together with known isocoumarin derivatives lignicol (**4**) [9] and 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**) [10] (**Figure 1**).

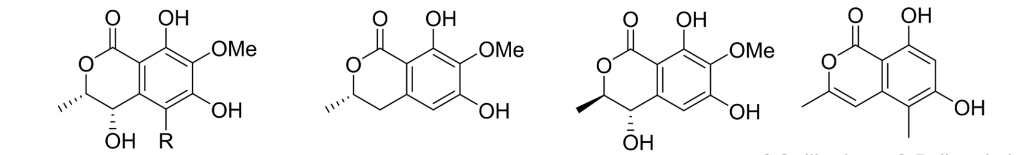

Figure 1. The chemical compounds isolated from *Phialocephala scopiformis* FC-1873.

3.1. Characterization of Isolated Compounds

The molecular formula of **1** was determined at $C_{11}H_{12}O_6$ by HR-ESI-MS in negative mode. The ^{1}H-NMR spectrum showed an aromatic proton (δ_H 6.46, s), two oxygenated methine protons (δ_H 4.61, dq, J = 6.6, 2.1 Hz and δ4.30, brd, J = 2.1 Hz), a methyl group (δ_H 1.47, d, J = 6.6 Hz) and a methoxy group (δ_H 3.83, s). ^{13}C-NMR spectrum showed seven sp^2 carbons including an ester carbon (δc 171.4), two oxygenated methine carbons (δc 67.9 and δc 79.9), a methyl carbon (δc 16.3) and a methoxy carbon (δc 61.0) (Table 1).

These data were good agreement to that of phaeosphaerin A [14]. The detailed analysis of 2D-NMR spectra supported this structure (Figure 2). However, the optical rotation value of **1** was opposite polarity in compare to that of phaeosphaerin A (**1**: +12.9˚ in methanol, phaeosphaerin A: −15.0˚ [14]). Therefore, (+)-phaeosphaerin A (**1**) was an enantiomer of phaeosphaerin A and the absolute configurations of **1** were determined to be 3*S*, 4*S*.

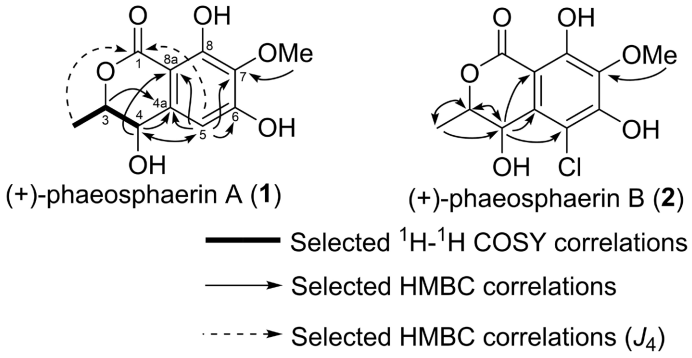

Figure 2. 2D-NMR correlations of **1** and **2**.

The molecular formula of **2** was decided to $C_{11}H_{11}O_6Cl$ by HR-ESI-MS, one hydrogen atom less and one chlorine atom more than **1**. The molecular formula and ^{1}H- and ^{13}C-NMR data (Table 1) of **2** were good agreement to phaeosphaerin B [14]. The detailed analysis of 2D-NMR spectra was supported this structure (Figure 2). However, the optical rotation value of **2** was opposite polarity in compare to that of phaeosphaerin B (**2**: +5.8˚ in methanol, phaeosphaerin B: −6.0˚). Therefore, **2** was an enantiomer of phaeosphaerin B and the absolute configurations of **2** were determined to be 3*S*, 4*S*.

Compound **3** showed the molecular formula as $C_{11}H_{12}O_5$ by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR data were match to that of 6-demethylkigelin [15], but the optical rotation value of **3** showed opposite sign to 6-demethylkigelin (**3**: $[\alpha]_D$ = +45˚, 6-demethylkigelin: $[\alpha]_D$ = −33˚ [15]). Therefore, the structure of **3** was determined as (*S*)-6-demethylkigelin (**3**), enantiomer of 6-demethylkigelin.

3.2. Effect of the Compounds on the Cytotoxicity of Humann Cancer Cell Lines

The cytotoxicity of (+)-phaeosphaerin A (**1**) and lignicol (**4**) against HL-60,

PANC-1, HepG2, HT-29 and T98G were investigated because many naturally occurred isocoumarin derivatives showed cytotoxicity [11] [12]. These cell lines were treated by **1** and **4** at concentration of 3.125, 6.25, 12.5, 25 and 50 μM and cultivated under 5% CO_2 at 37˚C for 24 hr. After cultivation, cytotoxicity of **1** and **4** was measured by WST assay. In the results, **1** and **4** showed weak cytotoxicity against only HL-60 (**Figure 3**).

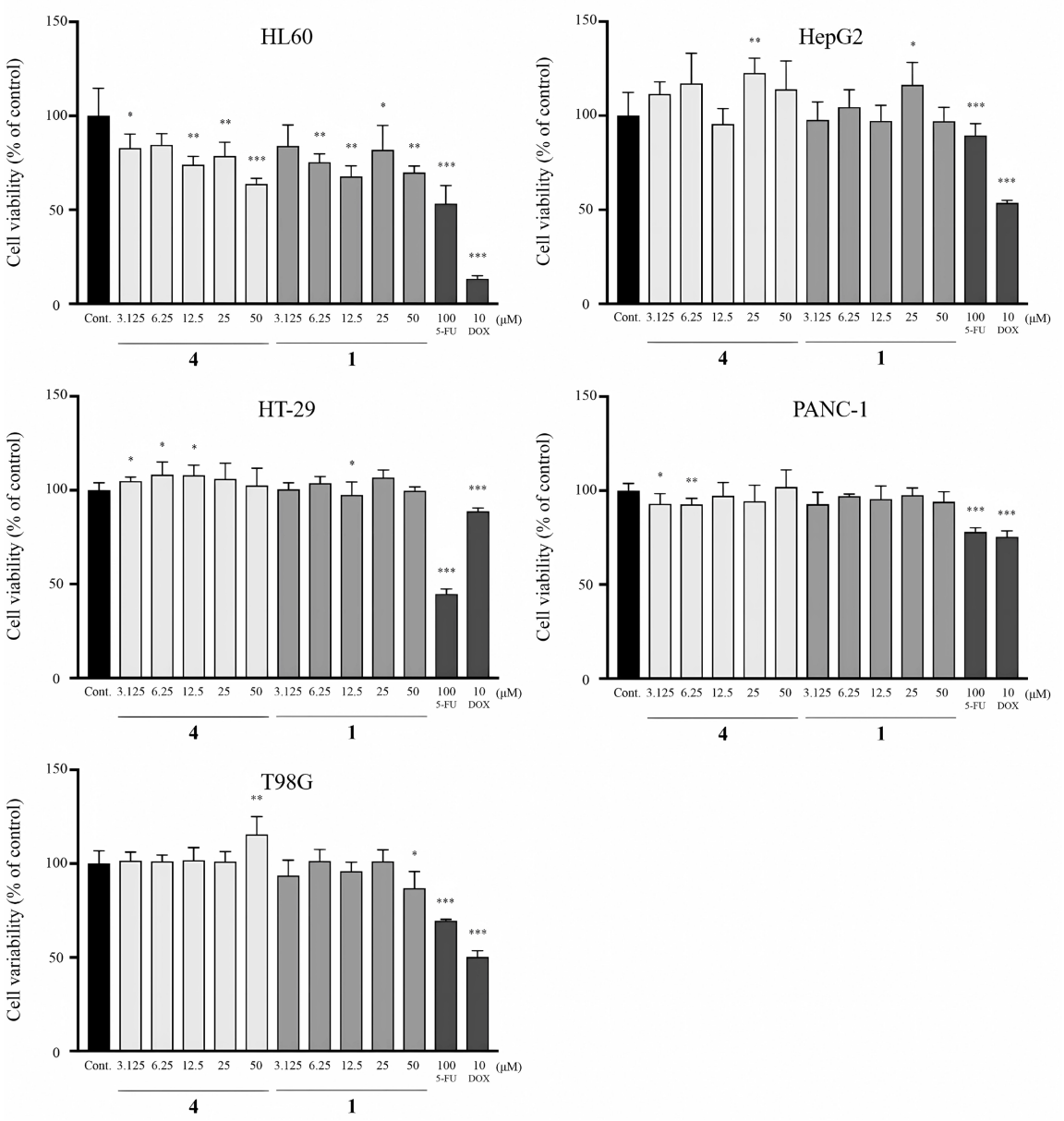

Figure 3. Cytotoxicity of **1** and **4** against HL60, HepG2, HT-29, PANC-1 and T98G cell lines.

The cytotoxicity of **1** and **4** isolated from *Phialocephala scopiformis* FC-1873 against these cell lines were tested by WST-1 assay. Each cell lines were preincubated for 48 hr, and incubated for 24 hr treated with **1** and **4** (conc. 3.125, 6.25, 12.5, 25 and 50 μM). The absorbance at 450 nm was measured after added WST-

1 reagent. The Smirnov-Grubbs test was used to evaluate the outliers (a < 0.05). Data are mean ± SD (n = 3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by student's *t*-test).

3.3. Cell Proliferation Effect of 1 and 4 against HaCaT Cell Line

The cell proliferative effects of (+)-phaeosphaerin A (**1**) and lignicol (**4**) on human noncancer cells (HaCaT) were also examined.

HaCaT cells were seeded to 96 well plate and preincubated 24 hr. After preincubation, cells were transfer to serum-free medium and treated with **1** or **4** (conc. 0,78, 1.56, 3.125 and 6.25 μM). After incubation for 24 hr, the activity was measured by WST-1 assay. As the results, **1** and **4** showed the 120% - 150% increase of cells in the concentration range tested in this study (Figure 4).

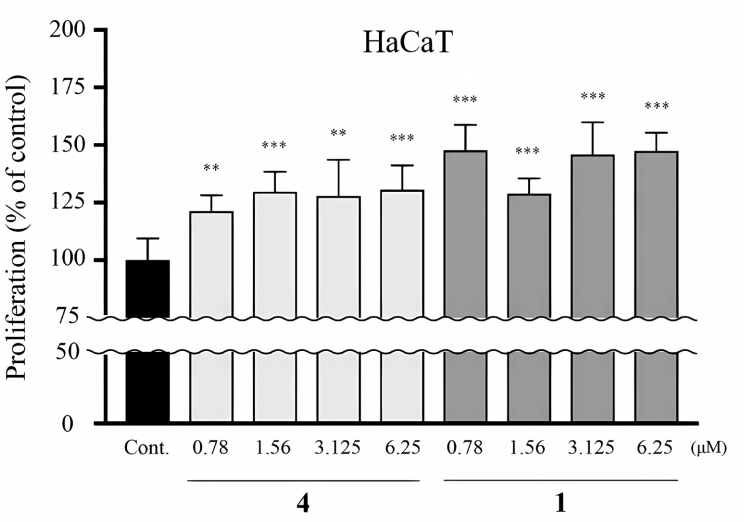

Figure 4. Cell proliferation effect of **1** and **4** against HaCaT cell line.

The cell proliferation effect of **1** and **4** isolated from *Phialocephala scopiformis* FC-1873 against HaCaT cell line were tested by WST-1 assay. HaCaT cell was treated with **1** and **4** (conc. 0.78, 1.56, 3.125 and 6.25 μM). The absorbance at 450 nm were measured after added WST-1 reagent. The Smirnov-Grubbs test was used to evaluate the outliers (a < 0.05). Data are mean ± SD (n = 3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by student's *t*-test).

4. Discussion

In the course of searching for new bioactive compounds from *Phialocephala scopiformis* FC-1873, new isocoumarin derivatives (+)-phaeosphaerin A (**1**), (+)-phaeosphaerin B (**2**) and (*S*)-6-demethylkigelin (**3**) and together with known isocoumarin derivatives lignicol (**4**) and 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**5**).

(+)-Phaeospharins A (**1**), B (**2**) isolated from *Phialocephala scopiformis* FC-1873 were enantiomer of phaeosphaerins A, B isolated from *Phaeosphaeriopsis* sp. WP-26, respectively, but these fungi don't produce enantiomer or racemic

compounds reported to be isolation from each fungus. Therefore, stereochemistry of these compounds may be control by biosynthetic enzyme as opposed to spontaneous reaction.

The cytotoxicity of (+)-phaeosphaerin A (**1**) and lignicol (**4**) against HL60, PANK-1, HepG2, HT-29 and T98 cell lines was investigated by WST-1 assay. In the results, **1** and **4** showed weak cytotoxicity against HL-60. Phaeosphaerins A-E don't show the cytotoxicity against BEL-7402, SGC-7901, K562, A549 and HL-60 [14], compounds of this family were expected to have no/weak cytotoxicity without regard for stereochemistry of C-3 or C-4 and existence of chlorine atom.

Variations in the intracellular concentrations of compounds **1** and **4** may underlie the differential cytotoxic effects observed across various cell lines. To date, no studies have elucidated the membrane permeation mechanisms of these compounds. Nonetheless, cellular uptake is likely governed by passive diffusion, active transport, or a combination of both. In the case of passive diffusion, physicochemical parameters such as molecular weight, logD, and pKa are critical determinants, and permeability can be approximated using Fick's law. In contrast, carrier-mediated transport would depend on the expression and activity of specific transporters associated with each compound, thereby influencing uptake kinetics. In cancer cells, efflux transporters such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are commonly overexpressed and play a pivotal role in drug resistance. Therefore, efflux via these transporters could represent an additional factor contributing to the observed cytotoxicity profiles. Notably, several 3,4-dihydroisocoumarin derivatives have been reported to function as both substrates and inhibitors of P-gp and BCRP [16]. Given the structural similarity of com-pounds **1** and **4** to these derivatives, it is plausible that they may also interact with these transporters, thereby modulating their intracellular bioavailability and contributing to the cell line-dependent differences in cytotoxic response.

Gene expression of P-gp and BCRP in these cancer cell lines was examined in the database of Cancer Cell Line Encyclopedia (CCLE) and HL-60 had very low expression of both genes compared to other cancer cell lines (**Supplementary Table**) [16]. These results suggest that the reason why **1** and **4** were cytotoxic only to HL60 was because the different gene expression profiles of P-gp and BCRP led to different intracellular drug concentrations.

The cell proliferative effects of **1** and **4** on human noncancer cells (HaCaT) were also examined. For the results, **1** and **4** showed 120% - 150% cell proliferation effect against HaCaT cell line at 0.78 - 6.25 μM by WST-1 assay. In previous literature, the ethanolic extract of rhizome of *Boesenbergia rotunda* was reported 120% - 150% cell proliferation effect against HaCaT cell line and to stimulate wound healing, and the mechanism was reported to activation of extracellular signal-regulated kinase (ERK) 1/2 and phosphatidylinositol 3-kinase (PI3K)/Akt pathway by phosphorylation [17]. In addition, tracheloside, lignan glycoside isolated from *Trachelospermum jasminoides*, showed cell proliferation effect against HaCaT cell line and the mechanism was reported to phosphorylation of ERK1/2

[18]. Also, it was reported that mitogen-activated protein kinase (MAPK), important for regulation of cell proliferation [19], was activated by isocoumarin derivative [20]. In this study, although the precise mechanisms underlying the proliferative effects of compounds **1** and **4** on HaCaT cells remain unclear, the observed responses suggest a possible involvement of the MAPK signaling pathway, particularly via phosphorylation of ERK, Jun-*N*-terminal kinase (JNK), and p38. However, this remains a hypothesis at present, as no direct experimental evidence has been provided. Further studies, including Western blot analysis for phosphorylated MAPK family members, are warranted to validate this possibility and are planned for future investigation.

5. Conclusion

In this study, the novel isocoumarin derivatives (+)-phaeosphaerin A (**1**), (+)-phaeosphaerin B (**2**) and (*S*)-6-demethylkigelin (**3**) were isolated from *Phialocephala scopiformis* FC-1873. Compounds 1 and 4 showed weak cytotoxicity only against cancer cell HL60 and strong cell proliferative activity against HaCaT cells. In the near future, elucidation of the mechanism of proliferative action of HCaT cells exhibited by **1** and **4** may be used for cosmetic and skin treatment applications.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Table

Gene / Cell line	ABCB1 (MDR1: P-gp)	ABCG2 (BCRP)
HL60	0.028569152	0.014355293
HEPG2	4.110196178	3.270528942
HT-29	0.137503524	4.114367025
PANC1	0.042644337	5.271649772
T98G	0.097610797	3.752748591