ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Meyeroguilline E, a New Isoindolinone Alkaloid from the Poisonous Mushroom *Chlorophyllum molybdites*, and Identification of Compounds with Multidrug Resistance (MDR) Reversal Activities

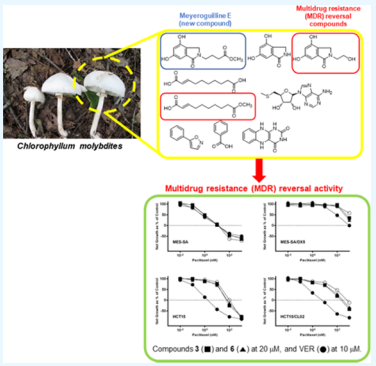
Bum Soo Lee, Rhim Ryoo, Jin Song Park, Sang Un Choi, Se Yun Jeong, Yoon-Joo Ko, Jung Kyu Kim, Jin-Chul Kim,* and Ki Hyun Kim*

Cite This: *ACS Omega* 2022, 7, 39456−39462

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Three isoindolinone alkaloids (**1**−**3**), including one new isoindolinone-type alkaloid, meyeroguilline E (**1**), and six other known compounds (**4**−**9**) were isolated from the poisonous mushroom *Chlorophyllum molybdites* (Agaricaceae). The structure of the new compound was determined using extensive spectroscopic analyses via one-dimensional (1D) and two-dimensional (2D) NMR data interpretation and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). To the best of our knowledge, compound **1** is the first example of a natural isoindolinone with a butanoic acid moiety, and this study is the first to detect the other known compounds (**2**−**9**) in *C. molybdites*. The isolated compounds (**1**−**9**) were examined for their multidrug resistance (MDR) reversal activity against MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 human cancer cells. Based on the results, 20 μM of compounds **3** and **6** slightly potentiated paclitaxel (TAX)-induced cytotoxicity in MES-SA/DX5, HCT15, and HCT15/CL02 cells; however, the compounds had no effect on the cytotoxicity against MES-SA and nonMDR cells.



1. INTRODUCTION

Chlorophyllum molybdites (Meyer. ex Fr.) Mass. (Agaricaceae), a false parasol or green-spored parasol, is a widespread poisonous mushroom that usually grows on lawns and parks in tropical and subtropical regions.[1] This poisonous mushroom is also distributed in a restricted area throughout Korea and Japan.[1] Owing to a mushroom poisoning accident, *C. molybdites* has been reported to be a gastrointestinal irritant for over a century in many countries, including North America.[2] Further, there are increasing reports of *C. molybdites* poisoning worldwide each year.[3,4] The representative toxin of *C. molybdites*, which causes serious gastrointestinal discomfort that can last 1−6 h after ingestion, is a toxic protein called molybdophylysin.[5] Previous chemical investigation of this mushroom revealed the presence of two pyrrolidine alkaloids, lepiothins A and B,[6] α-methylglyceric acid,[7] fatty acid derivatives,[8] simple alkaloids,[8] and steroid derivatives.[9] In particular, some steroids isolated from *C. molybdites* were reported to exert cytotoxicity against human gastric carcinoma Kato III cells, with IC_{50} values ranging from 2.69 to 14.27 μg/mL.[9]

As part of the continuing research to isolate bioactive novel natural products and elucidate their structures from diverse natural resources,[10−16] we investigated potentially bioactive metabolites from poisonous mushrooms.[17,18] In the present study, the bioactive compounds from a methanol (MeOH) extract of the fruiting bodies of *C. molybdites* were assessed using liquid chromatography−mass spectrometry (LC/MS)-guided analysis. The MeOH extract of *C. molybdites* fruiting bodies was subjected to solvent partitioning and intensive chemical analysis using repeated column chromatography and semipreparative HPLC. This separation process led to the isolation of three isoindolinone alkaloids (**1**−**3**) and six other known compounds (**4**−**9**). The structure of the new compound (**1**) was established via detailed one-dimensional (1D) and two-dimensional (2D) NMR and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analyses. Herein, the isolation and structural determination of compounds **1**−**9** have been described, and their multidrug resistance (MDR) reversal activity has been determined.

2. RESULTS AND DISCUSSION

2.1. Isolation and Structural Elucidation of Compounds 1−9. The MeOH extract of the fruiting bodies of *C. molybdites* was subjected to solvent partitioning using four organic solvents, namely, hexane, CH_2Cl_2, EtOAc, and BuOH. The four main fractions derived from solvent partitioning, i.e., hexane-, CH_2Cl_2-, EtOAc-, and BuOH-soluble fractions, were

Received: September 23, 2022
Accepted: October 13, 2022
Published: October 24, 2022

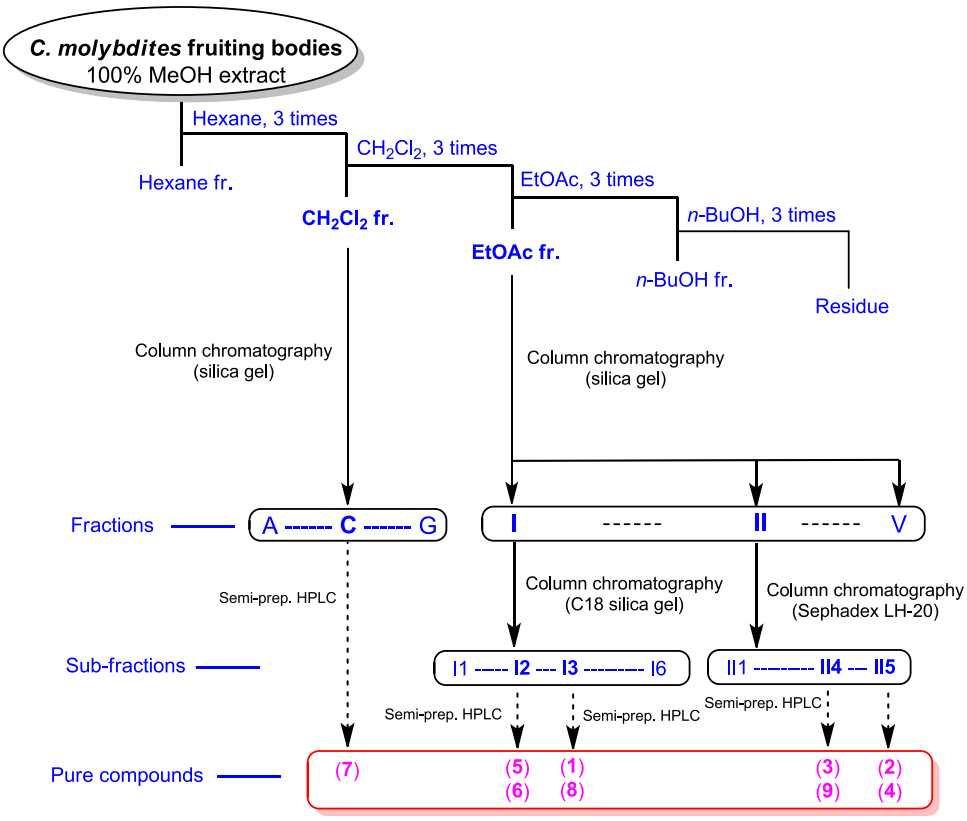
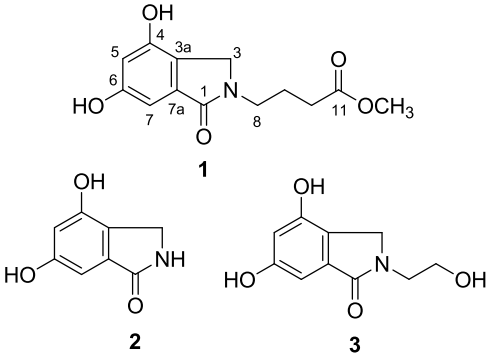


Figure 1. Separation scheme for compounds **1**–**9**.

subjected to LC/MS analysis, which revealed that the CH_2Cl_2- and EtOAc-soluble fractions contained metabolites of interest, including *N*-containing compounds. Accordingly, the CH_2Cl_2- and EtOAc-soluble fractions were subjected to intensive chemical analysis by repeated column chromatography and semipreparative HPLC under the guidance of LC/MS analysis (Figure 1). This analysis led to the isolation of three isoindolinone alkaloids (**1**–**3**) (Figure 2), including one new isoindolinone-type alkaloid, meyeroguilline E (**1**), and six other known compounds (**4**–**9**).



1 **2** **3**

Figure 2. Structures of the isolated isoindolinone alkaloids (**1**–**3**).

Meyeroguilline E (**1**) was isolated as a white powder. Further, the HR-ESI-MS data of **1** revealed quasimolecular ion peaks of $[M + Na]^+$ at *m*/*z* 288.0845 (calculated for $C_{13}H_{15}NO_5Na$, 288.0848) and $[2M + Na]^+$ at *m*/*z* 553.1794 (calculated for $C_{26}H_{30}N_2O_{10}Na$, 553.1798) in the positive-ion mode (Figure S1), which aligns with the molecular formula $C_{13}H_{15}NO_5$. Of note, this molecular formula was corroborated by the NMR spectroscopic data (Table 1). The IR spectrum of

Table 1. ^{1}H (850 MHz) and ^{13}C (212.5 MHz) NMR Data of Meyeroguilline E (1) in CD_3OD[a]

	1	
position	δ_H	δ_C
1		169.6 C
3	4.30, s	47.2 CH_2
3a		118.9 C
4		153.1 C
5	6.47, d (2.0)	105.4 CH
6		159.1 C
7	6.67, d (2.0)	99.9 CH
7a		133.0 C
8	3.63, t (7.0)	41.3 CH_2
9	1.99, m	23.1 CH_2
10	2.38, t (7.0)	30.4 CH_2
11		173.7 C
OCH_3	3.58, s	47.8 CH_3

[a]Coupling constants (Hz) are presented in parentheses, and the ^{13}C NMR data were assigned based on the HSQC and HMBC experiments.

1 exhibited absorption bands for the hydroxyl (3440 cm^{-1}) and carbonyl (1712 and 1620 cm^{-1}) groups, and its ^{1}H NMR spectrum (Table 1 and Figure S2) revealed the presence of resonances for two meta-coupled aromatic protons at δ_H 6.67 (1H, d, J = 2.0 Hz) and 6.47 (1H, d, J = 2.0 Hz); four methylenes at δ_H 4.30 (2H, s), 3.63 (2H, t, J = 7.0 Hz), 2.38 (2H, t, J = 7.0 Hz), and 1.99 (2H, m); and one methoxy group

https://doi.org/10.1021/acsomega.2c06155
ACS Omega 2022, 7, 39456−39462

at δ_H 3.58 (3H, s). The ^{13}C NMR data (Table 1) revealed 13 carbon resonances comprising six nonprotonated carbons [δ_C 173.7, 169.6, 159.1, 153.1, 133.0, and 118.9], two protonated aromatic carbons [δ_C 105.4 and 99.9], four methylene carbons [δ_C 47.2, 41.3, 30.4, and 23.1], and one methyl carbon [δ_C 47.8]. The above NMR data suggest that compound **1** was similar to meyeroguilline D (**3**),[19] except for the presence of an additional methylene group and one methoxy group in **1**. Accordingly, compound **1** could be an analogue belonging to the isoindolinone class. Detailed analysis of the $^1H-^1H$ COSY and heteronuclear single quantum coherence (HSQC) data (Figures S3 and S4) indicated the presence of one spin fragment of H_2-8/H_2-9/H_2-10 (Figure 3). The heteronuclear

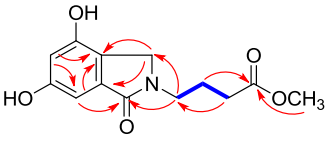


Figure 3. Key $^1H-^1H$ COSY (bold blue line) and HMBC (red arrows) correlations of compound **1**.

multiple bond correlation (HMBC) correlations from H_2-9 to C-11 and OCH_3 to C-11 confirmed the methoxyl group at C-11, indicating the presence of butanoic acid methyl ester as a side chain (Figure S5). The HMBC correlations from H-5 to C-3a and C-7; from H-7 to C-1, C-3a, and C-5; and from H_2-3 to C-3a and C-7a constructed an isoindolinone moiety, and the linkage of the butanoic acid methyl ester to N-2 of the isoindolinone core was established by the HMBC correlation from H_2-8 to C-3 and C-1 (Figure 3). Hence, the chemical structure of **1** was elucidated, as shown in Figure 2, and named meyeroguilline E. To the best of our knowledge, compound **1** is the first example of a natural isoindolinone with a butanoic acid moiety.

The known compounds were identified as 4,6-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one (**2**),[20] meyeroguillines D (**3**),[19] 5′-methylthioadenosine (**4**),[21] (*E*)-2-decenedioic acid (**5**),[22] pantheric acid C (**6**),[23] 5-phenylisoxazole (**7**),[24] benzoic acid (**8**),[25] and 5,10-dihydrobenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione (**9**)[26] (Figure 4) based on a comparison of their NMR spectroscopic data with reported values and the results of LC/MS analysis. To the best of our knowledge, all known compounds (**2**–**9**) were isolated for the first time from *C. molybdites*. Among the substances isolated from *C. molybdites*, 5′-methylthioadenosine (**4**) is called 5′-methylthioadenosine

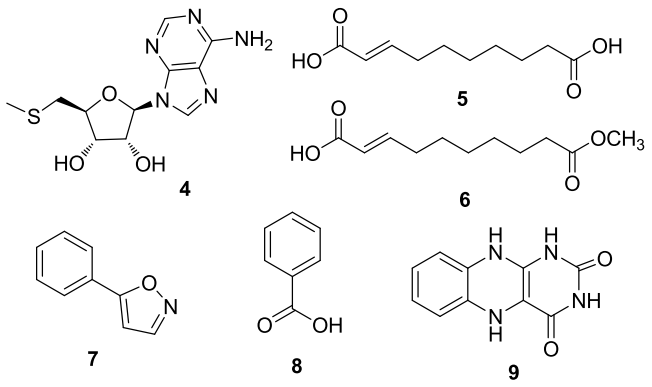


Figure 4. Structures of the known compounds (**4**–**9**).

(MTA) and is the *S*-methyl derivative of adenosine. MTA is an intermediate in the methionine salvage pathway, also called the 5′-methylthioadenosine (MTA) cycle, which recycles the sulfur of MTA, a byproduct of the biosynthesis of polyamine and the plant hormone, ethylene.[27] As the source of the sulfur atom in MTA, methionine (MET) was first identified in 1923, and the methyl group of MTA is derived from *S*-adenosylmethionine, which is synthesized from MET and adenosine triphosphate (ATP) through the catalysis of MET adenosyltransferases.[28]

2.2. Evaluation of the Multidrug Resistance (MDR) Reversal Activity of the Isolated Compounds. According to our previous study,[29] isoindolinone alkaloids isolated from the mushroom *Hericium erinaceum* showed cytotoxic activity against the human cancer cell lines, A549, SK-OV-3, SK-MEL-2, and HCT-15. Based on these results, the cytotoxic activity of the isoindolinone alkaloids and the other known compounds identified in *C. molybdites* was investigated. All compounds tested in this study had little effect on the growth of A549, SK-OV-3, SK-MEL-2, and HCT-15 human cancer cells up to 30 μM (data not shown).

The MDR reversal activities of these compounds were tested in the human colorectal cancer cell line (HCT15), doxorubicin-resistant HCT15/CL02 cells, uterine carcinoma cell line (MES-SA), and doxorubicin-resistant MES-SA/DX5 cells. The cytotoxicity of paclitaxel (TAX) against the MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 human cancer cells increased in a concentration-dependent manner. The effects of the compounds tested in this study and verapamil (VER) on the cytotoxicity of TAX in these cells are summarized in Table 2. The EC_{50} of TAX was 0.81, 1251.14, 25.64, and 91.73 nM against MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 cells, respectively (Table 2). These data indicate that the MES-SA/DX5 and HCT15/CL02 cells were more resistant to TAX than parental MES-SA and HCT15 cells by approximately 1544.6- and 3.6-fold, respectively. The isolated compounds did not inhibit cell growth at the experimental concentration of 20 μM (data not shown). However, 20 μM of compounds **3** and **6** slightly potentiated TAX-induced cytotoxicity in MES-SA/DX5, HCT15, and HCT15/CL02 cells (Figure 5). When 100 nM

Table 2. Cytotoxicity of Paclitaxel (TAX) against MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 Human Cancer Cells in the Absence or Presence of Compounds 1–9 (20 μM) and Verapamil (VER) (10 μM)

	cytotoxicity of paclitaxel (EC_{50}[a])			
compounds cotreated	MES-SA	MES-SA/DX5	HCT15	HCT15/CL02
none	0.81	1251.14	25.64	91.73
1	0.93	1009.24	27.18	84.52
2	0.86	1204.27	24.73	93.44
3	1.07	643.92	13.49	22.71
4	0.79	1012.80	23.18	94.75
5	0.76	1013.39	30.27	108.76
6	0.92	834.65	21.07	24.78
7	0.90	988.46	26.14	81.45
8	1.04	1067.45	20.84	62.91
9	0.95	1087.96	23.48	64.35
VER	0.81	96.43	0.28	0.75

[a]EC_{50}: effective concentration that induces 50% cell growth inhibition.

https://doi.org/10.1021/acsomega.2c06155
ACS Omega 2022, 7, 39456−39462

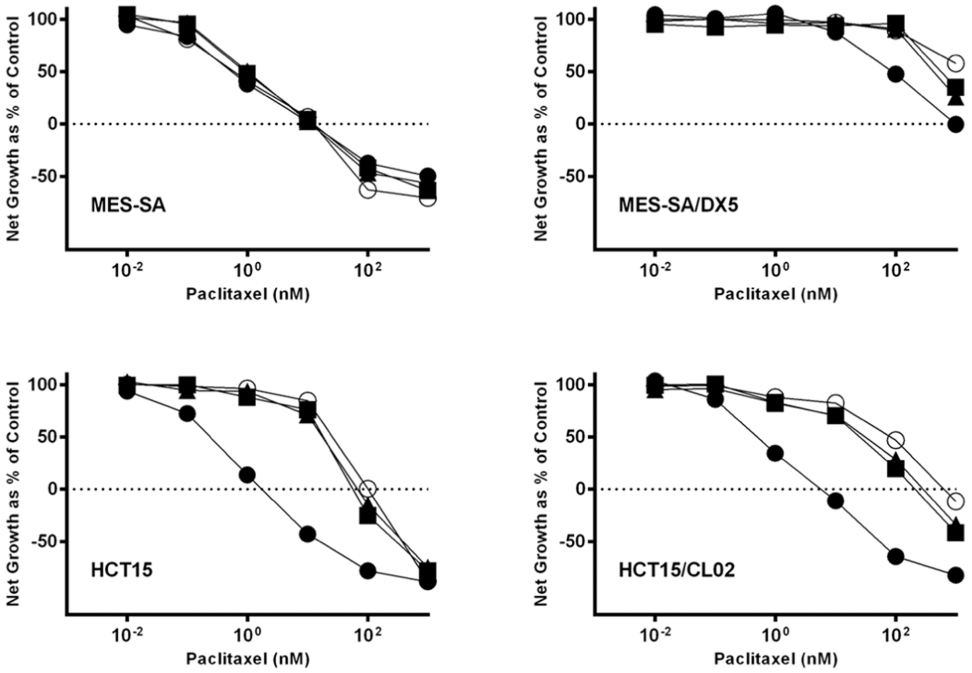


Figure 5. Effects of compounds **3** and **6** and VER on the cytotoxicity of TAX against MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 human cancer cells *in vitro*. The cells were cultured with serial dilutions of TAX in the absence (○) or presence of 20 μM of compounds **3** (■) and **6** (▲) and 10 μM of VER (●). The cell survival fractions were assessed after continuous drug exposure for 3 days using the SRB assay.

of TAX was employed, the survival fraction against HCT15 cells was shifted by compounds **3** and **6** from 0.43 to −14.59 and −25.01, respectively. For HCT15/CL02 cells, compounds **3** and **6** enhanced the survival fraction from 46.94 to 28.40 and 19.65 at 100 nM TAX, and −11.66 to −33.93 and −41.46 at 1000 nM TAX, respectively. The two active compounds, **3** and **6**, also shifted the survival fraction of TAX against MES-SA/CX5 cells from 57.78 to 25.76 and 35.01, respectively, at 1000 nM TAX. In the presence of 10 μM VER, the survival fraction of TAX was potentiated in MES-S/DX5, HCT15, and HCT15/CL02 cells (Figure 5). However, all compounds (20 μM) or VER (10 μM) had no effect on the cytotoxicity against MES-SA and nonMDR cells.

3. CONCLUSIONS

In this study, a chemical investigation of the MeOH extract from the fruiting bodies of the poisonous mushroom *C. molybdites* resulted in the isolation and identification of a new isoindolinone-type alkaloid, meyeroguilline E (**1**), and eight known compounds (**2**−**9**). In the present study, the new compound (**1**) was reported to be the first example of a natural isoindolinone with a butanoic acid moiety, and the other known compounds **2**−**9** were isolated from *C. molybdites* for the first time. Among the isolated compounds, 20 μM of compounds **3** and **6** slightly potentiated the paclitaxel (TAX)-induced cytotoxicity to MES-SA/DX5, HCT15, and HCT15/CL02 cells. Further, compounds **3** and **6** enhanced the survival fraction at 1000 nM of TAX from −11.66 to −33.93 and −41.46, respectively, in HCT15/CL02 cells.

4. EXPERIMENTAL SECTION

4.1. General Experimental Procedure. Optical rotation was measured using a Jasco P-2000 polarimeter (Jasco, Easton, MD). The infrared (IR) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany), while the ultraviolet (UV) spectra were acquired on an Agilent 8453 UV−visible spectrophotometer (Agilent Technologies, Santa Clara, CA). The nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AVANCE III HD 850 NMR spectrometer with a 5 mm TCI CryoProbe operating at 850 MHz (proton, ^{1}H) and 212.5 MHz (^{13}C, Bruker, Karlsruhe, Germany), with chemical shifts given in ppm (δ) for the ^{1}H and ^{13}C NMR analyses. Semipreparative HPLC was performed using a Shimadzu Prominence HPLC System, with SPD-20A/20AV Series Prominence HPLC UV−vis detectors (Shimadzu, Tokyo, Japan) and a Phenomenex Luna C18 column (250 × 10 mm, 5 μm; flow rate: 2 mL/min; Phenomenex, Torrance, CA). Liquid chromatography−mass spectrometry (LC/MS) analysis was performed using an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C18 100 Å column (100 × 2.1 mm, 5 μm; flow rate: 0.3 mL/min; Phenomenex, Torrance, CA). All high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data were obtained using an Agilent 6545 Q-TOF LC/MS spectrometer (Agilent Technologies, Santa Clara, CA). Silica gel 60 (230−400 mesh; Merck, Darmstadt, Germany) and RP-C_{18} silica gel (230−400 mesh; Merck) were used for column chromatography. Sephadex LH-20 (Pharmacia, Uppsala, Sweden) was employed as the packing material for molecular sieve column chromatography. Merck precoated silica gel F_{254} plates and RP-C_{18} F_{254s} plates were used for thin-layer chromatography. After thin-layer chromatography, spots were detected under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

4.2. Fungal Material. Fruiting bodies of *Chlorophyllum neomastoideum* were collected from a mixed forest in Pocheon, GyeongGi-do, Korea, in August 2020. The samples were identified by one of the authors (R. Ryoo) via DNA analysis; fungal-specific PCR primers, ITS1 and ITS4, were used to

https://doi.org/10.1021/acsomega.2c06155
ACS Omega 2022, 7, 39456−39462

amplify the internal transcribed spacer (ITS) region. The DNA sequence of this material was analyzed according to a modified method.[30] The homology of the ITS sequence was matched with that of *C. neomastoideum* (syn. *Macrolepiota neomastoidea*) with the highest score in the NCBI GenBank database. A voucher specimen (SKKU 2020-08-CN) was deposited at the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

4.3. Extraction and Isolation. Dried *C. molybdite* fruiting bodies (1.7 kg) were extracted with 100% methanol (MeOH, 3.0 L × 3) and filtered. The resulting extract was vacuum-concentrated using a rotary evaporator to obtain the MeOH extract (449.3 g). The concentrated MeOH extract was suspended in distilled water (700 mL) and solvent-partitioned three times each with hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) to obtain four fractions: hexane-soluble (24.2 g), CH_2Cl_2-soluble (3.7 g), EtOAc-soluble (1.0 g), and *n*-BuOH-soluble (15.4 g). Based on LC-MS analysis of each fraction derived from solvent partitioning, the CH_2Cl_2- and EtOAc-soluble fractions were identified to harbor compounds of interest, including *N*-containing compounds since we found that HRESIMS of peak in the CH_2Cl_2 fraction showed an *m*/*z* 146.0610 $[M + H]^+$ (calculated for C_9H_8NO, 146.0606) in the positive-ion mode, and HRESIMS of peaks in the EtOAc fraction revealed *m*/*z* 288.0845 $[M + Na]^+$ (calculated for $C_{13}H_{15}NO_5Na$, 288.0848), *m*/*z* 188.0328 $[M + Na]^+$ (calculated for $C_8H_7NO_3Na$, 188.0324), and *m*/*z* 232.0583 $[M + Na]^+$ (calculated for $C_{10}H_{11}NO_4Na$, 232.0586) in the positive-ion mode. The CH_2Cl_2-soluble fraction (3.7 g) was separated using silica gel open column chromatography using a gradient solvent system of CH_2Cl_2/MeOH (80:1, CH_2Cl_2/MeOH to 100% MeOH) to yield seven fractions (A−G). Fraction C (37.1 mg) was purified using semipreparative HPLC (20% MeOH) on a Phenomenex Luna phenyl-hexyl column to obtain compound **7** (t_R = 32.0 min, 0.6 mg). The EtOAc-soluble fraction (1.0 g) was separated using silica gel open column chromatography using a gradient solvent system of CH_2Cl_2/MeOH (30:1, CH_2Cl_2/MeOH to 100% MeOH) to yield five fractions (I−V). Fraction I (190.0 mg) was separated by reversed phase silica gel open column chromatography using 100% MeOH to yield six subfractions (I1−I6). Subfraction I2 (98.1 mg) was purified by semipreparative HPLC (55% MeOH) on a Phenomenex Luna phenyl-hexyl column to obtain compounds **5** (t_R = 17.0 min, 4.0 mg) and **6** (t_R = 23.5 min, 0.5 mg). Subfraction I3 (30.0 mg) was purified by semipreparative HPLC (45% MeOH) on a Phenomenex Luna C18 column to obtain compounds **1** (t_R = 26.5 min, 1.0 mg) and **8** (t_R = 38.0 min, 1.0 mg). Fraction II (124.3 mg) was separated by Sephadex LH-20 open column chromatography using 100% MeOH to yield five subfractions (II1−II5). Subfraction II4 (45.9 mg) was purified by semipreparative HPLC (20% MeOH) on a Phenomenex Luna C18 column to obtain compounds **3** (t_R = 15.0 min, 1.0 mg) and **9** (t_R = 19.0 min, 0.8 mg). Subfraction II5 (19.6 mg) was purified by semipreparative HPLC (18% MeOH) on a Phenomenex Luna C18 column to obtain compounds **2** (t_R = 17.0 min, 1.0 mg) and **4** (t_R = 25.5 min, 1.0 mg).

*4.3.1. Meyeroguilline E (**1**).* White powder; UV (MeOH) λ_{max} (log ε) 215 (4.18), 252 (2.00), 305 (1.76) nm; IR (KBr): ν_{max} = 3440, 2928, 1712, 1620, 1353, 1155 cm^{-1}; ^1H (850 MHz) and ^{13}C (212.5 MHz) NMR data, Table 1; HRESIMS (positive-ion mode) *m*/*z* 288.0848 $[M + Na]^+$ (calculated for $C_{13}H_{15}NO_5Na$, 288.0845), and *m*/*z* 553.1798 $[2M + Na]^+$ (calculated for $2[C_{13}H_{15}NO_5]^+Na$, 553.1794).

4.4. Cancer Cells. The human colorectal cancer cell line HCT15, uterine carcinoma cell line MES-SA, and its doxorubicin-resistant subline MES-SA/DX5 were purchased from American Type Culture Collection (Rockville, Maryland). HCT15/CL02 cells were established from HCT15 cells at the Korea Research Institute of Chemical Technology.[31] Cell cultures were conducted in Falcon T-25 (Becton Dickinson, Lincoln Park, NJ) flasks containing 10 mL of RPMI-1640 medium with glutamine, sodium bicarbonate, gentamycin, amphotericin, and 5% fetal bovine serum (FBS). The cells were dissociated using 0.25% trypsin and 3 mM CDTA solution for passaging. The cells were maintained in an incubator at 37 °C in a humidified atmosphere of 5% CO_2.

4.5. MDR Reversal Assay. The ability of the samples to potentiate the cytotoxicity of paclitaxel (TAX) was evaluated in the MES-SA, MES-SA/DX5, HCT15, and HCT15/CL02 cell lines as previously described.[32] The cells were then inoculated over a series of standard 96-well flat-bottom microplates and preincubated for 24 h to allow attachment to the microtiter plate. The attached cells were incubated with serial dilutions of TAX in the absence or presence of each sample or verapamil. After continuous exposure to the compounds for 72 h, the culture medium was removed from each well and the cells were fixed with 10% cold TCA at 4 °C for 1 h. After washing with distilled water, the cells were stained with 0.4% SRB solution and incubated for 30 min at room temperature. The cells were washed again and solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 and 690 nm using a microtiter plate reader (Molecular Devices, Sunnyvale, CA). To eliminate the effects of nonspecific absorbance, the absorbance at 690 nm was subtracted from that at 520 nm. The cell survival fractions were calculated via three basic measurements: time zero (Tz) at the beginning of drug incubation, cell control (CC) at the end of incubation without drug, and drug treatment (DT) at the end of the drug incubation period. CC is the fraction of cells incubated with the MDR reversal sample alone. If DT ≥ Tz, the net perfect cell growth inhibition was calculated as (DT − Tz)/(CC − Tz) × 100. If DT < Tz, the net percentage of cell-killing activity was calculated as (DT − Tz)/Tz × 100. All data represent the average values of three wells in each experiment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06155.

HRESIMS, 1D and 2D NMR spectra of compound **1** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Jin-Chul Kim − *KIST Gangneung Institute of Natural Products, Natural Product Research Center, Gangneung 25451, Republic of Korea*; Email: jckim@kist.re.kr

Ki Hyun Kim − *School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Republic of Korea*; orcid.org/0000-0002-5285-9138; Phone: +82-31-290-7700; Email: khkim83@skku.edu; Fax: +82-31-290-7730

https://doi.org/10.1021/acsomega.2c06155
ACS Omega 2022, 7, 39456−39462

Authors

Bum Soo Lee − *School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Republic of Korea*

Rhim Ryoo − *Special Forest Products Division, Forest Bioresources Department, National Institute of Forest Science, Suwon 16631, Republic of Korea*

Jin Song Park − *Korea Research Institute of Chemical Technology, Deajeon 34114, Republic of Korea*

Sang Un Choi − *Korea Research Institute of Chemical Technology, Deajeon 34114, Republic of Korea*

Se Yun Jeong − *School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Republic of Korea*

Yoon-Joo Ko − *Laboratory of Nuclear Magnetic Resonance, National Center for Inter-University Research Facilities (NCIRF), Seoul National University, Seoul 08826, Republic of Korea*

Jung Kyu Kim − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea;* orcid.org/0000-0002-8218-0062

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c06155

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (2019R1A5A2027340 and 2021R1A2C2007937) and a Korea Institute of Science and Technology intramural research grant (2E31881).

■ REFERENCES

(1) Imazeki, R.; Hongo, T. *Coloured Illustrations of Mushrooms of Japan*; Hoikusya: Osaka, 1987; Vol. *1*.

(2) Stenklyft, P. H.; Lynn Augenstein, W. *Chlorophyllum molybdites*-severe mushroom poisoning in a child. *J. Toxicol.: Clin. Toxicol.* **1990**, *28*, 159−168.

(3) Yokoyama, K.; Gonmori, K. Increase of poisoning by tropical mushrooms in Japan in recent years. *Jpn. J. Toxicol. Sci.* **2009**, *22*, 240−248.

(4) Whitaker, G.; Box, J. *Chlorophyllum molybdites* mushroom poisoning: a case report and review of the syndrome. *J. Med. Soc.* **1985**, *82*, 220−222.

(5) Yamada, M.; Tokumitsu, N.; Saikawa, Y.; Nakata, M.; Asano, J.; Miyairi, K.; Okuno, T.; Konno, K.; Hashimoto, K. Molybdophyllysin, a toxic metalloendopeptidase from the tropical toadstool, *Chlorophyllum molybdites*. *Bioorg. Med. Chem.* **2012**, *20*, 6583−6588.

(6) Ohta, T.; Inoue, H.; Kusano, G.; Oshima, Y. Lepiotins A and B, new alkaloids from the mushrooms, *Macrolepiota neomastoidea* and *Chlorophyllum molybdites*. *Heterocycles* **1998**, *47*, 883−891.

(7) Shore, F. L. Chemical Constituents of *Chlorophyllum molybdites* and the Absolute Configuration of α-Methylglyceric Acid. Dissertation Abstracts International. B, The Sciences and Engineering, University Microfilms, 1972.

(8) Su, Z.; Wang, P.; Yuan, W.; Li, S. Chemical constituents from the fruit body of *Chlorophyllum molybdites*. *Nat. Prod. Commun.* **2013**, *8*, 1227−1228.

(9) Yoshikawa, K.; Ikuta, M.; Arihara, S.; Matsumura, E.; Katayama, S. Two new steroidal derivatives from the fruit body of *Chlorophyllum molybdites*. *Chem. Pharm. Bull.* **2001**, *49*, 1030−1032.

(10) Lee, S.; Lee, D.; Ryoo, R.; Kim, J.-C.; Park, H. B.; Kang, K. S.; Kim, K. H. Calvatianone, a sterol possessing a 6/5/6/5-fused ring system with a contracted tetrahydrofuran B-ring, from the fruiting bodies of *Calvatia nipponica*. *J. Nat. Prod.* **2020**, *83*, 2737−2742.

(11) Lee, S. R.; Kang, H.; Yoo, M. J.; Yu, J. S.; Lee, S.; Yi, S. A.; Beemelmanns, C.; Lee, J.; Kim, K. H. Anti-adipogenic pregnane steroid from a Hydractinia-associated fungus, *Cladosporium sphaerospermum* SW67. *Nat. Prod. Sci.* **2020**, *26*, 230−235.

(12) Lee, S.; Ryoo, R.; Choi, J. H.; Kim, J.-H.; Kim, S.-H.; Kim, K. H. Trichothecene and tremulane sesquiterpenes from a hallucinogenic mushroom *Gymnopilus junonius* and their cytotoxicity. *Arch. Pharm. Res.* **2020**, *43*, 214−223.

(13) Lee, K. H.; Kim, J. K.; Yu, J. S.; Jeong, S. Y.; Choi, J. H.; Kim, J.-C.; Ko, Y.-J.; Kim, S.-H.; Kim, K. H. Ginkwanghols A and B, osteogenic coumaric acid-aliphatic alcohol hybrids from the leaves of *Ginkgo biloba*. *Arch. Pharm. Res.* **2021**, *44*, 514−524.

(14) Ha, J. W.; Kim, J.; Kim, H.; Jang, W.; Kim, K. H. Mushrooms: An important source of natural bioactive compounds. *Nat. Prod. Sci.* **2020**, *26*, 118−131.

(15) Yu, J. S.; Li, C.; Kwon, M.; Oh, T.; Lee, T. H.; Kim, D. H.; Ahn, J. S.; Ko, S.-K.; Kim, C. S.; Cao, S.; Kim, K. H. Herqueilenone A, a unique rearranged benzoquinone-chromanone from the Hawaiian volcanic soil-associated fungal strain *Penicillium herquei* FT729. *Bioorg. Chem.* **2020**, *105*, No. 104397.

(16) Lim, S. H.; Yu, J. S.; Lee, H. S.; Choi, C.-I.; Kim, K. H. Antidiabetic flavonoids from fruits of *Morus alba* promoting insulin-stimulated glucose uptake via Akt and AMP-activated protein kinase activation in 3T3-L1 adipocytes. *Pharmaceutics* **2021**, *13*, 526−539.

(17) Lee, S.; Kim, C. S.; Yu, J. S.; Kang, H.; Yoo, M. J.; Youn, U. J.; Ryoo, R.; Bae, H. Y.; Kim, K. H. Ergopyrone, a Styrylpyrone-Fused Steroid with a Hexacyclic 6/5/6/6/6/5 Skeleton from a Mushroom *Gymnopilus orientispectabilis*. *Org. Lett.* **2021**, *23*, 3315−3319.

(18) Lee, S.; Yu, J. S.; Lee, S. R.; Kim, K. H. Non-peptide secondary metabolites from poisonous mushrooms: overview of chemistry, bioactivity, and biosynthesis. *Nat. Prod. Rep.* **2022**, *39*, 512−559.

(19) Cui, H.; Yu, J.; Chen, S.; Ding, M.; Huang, X.; Yuan, J.; She, Z. Alkaloids from the mangrove endophytic fungus *Diaporthe phaseolorum* SKS019. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 803−807.

(20) Lü, W.-W.; Gao, Y. J.; Su, M. Z.; Luo, Z.; Zhang, W.; Shi, G. B.; Zhao, Q. C. Isoindolones from *Lasiosphaera fenzlii* Reich. and their bioactivities. *Helv. Chim. Acta* **2013**, *96*, 109−113.

(21) Granato, A. C.; Berlinck, R. G.; Magalhães, A.; Schefer, A. B.; Ferreira, A. G.; Sanctis, B.; Freitas, J. C. d.; Hajdu, E.; Migotto, A. E. Natural products from the marine sponges *Aaptos* sp. and *Hymeniacidon* aff. *heliophila*, and from the *nudibranch Doris* aff. *verrucosa*. *Quim. Nova* **2000**, *23*, 594−599.

(22) Noda, N.; Umebayashi, K.; Nakatani, T.; Miyahara, K.; Ishiyama, K. Isolation and characterization of some hydroxy fatty and phosphoric acid esters of 10-hydroxy-2-decenoic acid from the royal jelly of honeybees (*Apis mellifera*). *Lipids* **2005**, *40*, 833−838.

(23) Lee, S. R.; Yi, S. A.; Nam, K. H.; Ryoo, R.; Lee, J.; Kim, K. H. Pantheric acids a−c from a poisonous mushroom, *Amanita pantherina*, promote lipid accumulation in adipocytes. *J. Nat. Prod.* **2019**, *82*, 3489−3493.

(24) Rosa, F. A.; Machado, P.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. Reaction of β-dimethylaminovinyl ketones with hydroxylamine: A simple and useful method for synthesis of 3-and 5-substituted isoxazoles. *J. Heterocycl. Chem.* **2008**, *45*, 879−885.

(25) Emmler, T.; Gieschler, S.; Limbach, H.; Buntkowsky, G. A simple method for the characterization of OHO-hydrogen bonds by ^{1}H-solid state NMR spectroscopy. *J. Mol. Struct.* **2004**, *700*, 29−38.

(26) El Azab, I. H.; Elkanzi, N. A.; Gobouri, A. A. Design and Synthesis of Some New Quinoxaline-Based Heterocycles. *J. Heterocycl. Chem.* **2018**, *55*, 65−76.

(27) Albers, E. Metabolic characteristics and importance of the universal methionine salvage pathway recycling methionine from 5′-methylthioadenosine. *IUBMB Life* **2009**, *61*, 1132−1142.

(28) Li, Y.; Wang, Y.; Wu, P. 5′-Methylthioadenosine and cancer: old molecules, new understanding. *J. Cancer* **2019**, *10*, 927−936.

(29) Kim, K. H.; Noh, H. J.; Choi, S. U.; Lee, K. R. Isohericenone, a new cytotoxic isoindolinone alkaloid from *Hericium erinaceum*. *J. Antibiot.* **2012**, *65*, 575−577.

(30) Lee, S. B.; Taylor, J. W. Isolation of DNA from Fungal Mycelia and Single Spores. In *PCR Protocols, A Guide to Methods and Applications*; Academic Press, 1990; pp 282−287.
(31) Choi, S. U.; Kim, N. Y.; Choi, E. J.; Kim, K. H.; Lee, C. O. Establishment of doxorubicin-resistant subline derived from HCT15 human colorectal cancer cells. *Arch. Pharm. Res.* **1996**, *19*, 342−347.
(32) Choi, S. U.; Lee, C. O.; Kim, K. H.; Choi, E. J.; Park, S. H.; Shin, H. S.; Yoo, S. E.; Jung, N. P.; Lee, B. H. Reversal of multidrug resistance by novel verapamil analogs in cancer cells. *Anticancer Drugs* **1998**, *9*, 157−165.

https://doi.org/10.1021/acsomega.2c06155
ACS Omega 2022, 7, 39456−39462