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Synthesis of Natural Acylphloroglucinol-Based Antifungal Compounds against *Cryptococcus* Species

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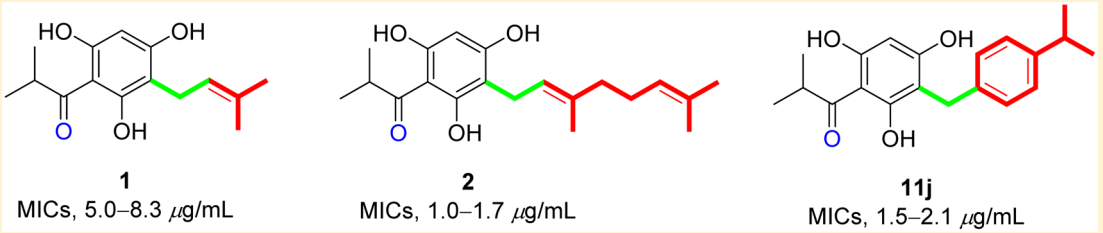
Supporting Information



ABSTRACT: Thirty-three natural-product-based acylphloroglucinol derivatives were synthesized to identify antifungal compounds against *Cryptococcus* spp. that cause the life-threatening disseminated cryptococcosis. In vitro antifungal testing showed that 17 compounds were active against *C. neoformans* ATCC 90113, *C. neoformans* H99, and *C. gattii* ATCC 32609, with minimum inhibitory concentrations (MICs) in the range 1.0−16.7 μg/mL. Analysis of the structure and antifungal activity of these compounds indicated that the 2,4-diacyl- and 2-acyl-4-alkylphloroglucinols were more active than *O*-alkylacylphloroglucinols. The most promising compound found was 2-methyl-1-(2,4,6-trihydroxy-3-(4-isopropylbenzyl)phenyl)-propan-1-one (**11j**), which exhibited potent antifungal activity (MICs, 1.5−2.1 μg/mL) and low cytotoxicity against the mammalian Vero and LLC-PK1 cell lines (IC_{50} values >50 μg/mL). This compound may serve as a template for further synthesis of new analogues with improved antifungal activity. The findings of the present work may contribute to future antifungal discovery toward pharmaceutical development of new treatments for cryptococcosis.

Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* in immunocompromised patients with AIDS, cancer, and organ transplants or by *Cryptococcus gattii* in immunocompetent hosts. A significant feature of this infection is that the fungal cells can pass through the blood−brain barrier, invading the central nervous system, leading to life-threatening meningitis and meningoencephalitis.[1,2] Cryptococcosis is the most prevalent opportunistic invasive mycosis, affecting greater than 1 million people per year, with a mortality rate of 20−70%.[1] There are few treatment options for this disease due to a limited number of effective antifungal drugs.[2] Therefore, there is a need to discover new lead compounds for drug development in this therapeutic area.

Naturally occurring acylphloroglucinols constitute a prominent class of structurally diverse compounds that have been found in plants, marine organisms, and microbes.[3] While a large number of compounds within this structural class have shown antimicrobial,[3−5] anti-HIV,[6] antidepressant,[7] antiproliferative,[8] antiprotozoal,[9] antiangiogenic,[10] anti-inflammatory,[11] antioxidant,[12] and catalytic activities,[13] only a few compounds were reported to be active against *Cryptococcus* spp.[14−16] A synthetic formylated acylphloroglucinol, 2,4,6-trihydroxy-3-(3-hydroxy-2-methylacryloyl)benzaldehyde, has shown good activity against *C. neoformans* with an IC_{50} value of 2.5 μg/mL. However, this compound may not be chemically stable due to the presence of a formyl group on the aromatic ring and the enol functionality on the side chain.[17] During our recent work on the synthesis of the plant-derived antibacterial acylphloroglucinol psorothatin C,[18] the intermediate 2-isobutyryl-4-isoprenylphloroglucinol (**1**), which is also a natural product isolated from the plant *Helichrysum kraussii*,[19] was found to be active against *C. neoformans* with an MIC value of 5.0 μg/mL. The potent activity of this prenylated acylphlorogluconol prompted the design and synthesis of additional analogues, with the aim of identifying structurally simple and chemically stable antifungal compounds against *Cryptococcus* spp.

RESULTS AND DISCUSSION

The general design for the synthesis of target compounds was based on the analysis of structure and activity of compound **1**

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and other naturally occurring acylphloroglucinols shown in Figure 1. Since limited information was available on antifungal

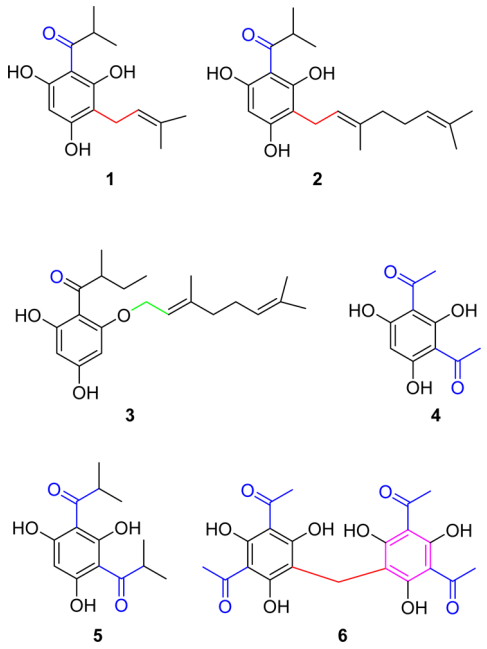

Figure 1. Structures of antimicrobial acylphloroglucinols.

activity of this class of compounds against *Cryptococcus* spp., the structures of those active against other fungal pathogens or bacteria were considered as synthetic templates, with assumptions that compounds with similar core structures would have antifungal activity or exhibit both antifungal and antibacterial activities. In fact, compound **1** and its synthetic analogues with different acyl substituents have demonstrated antifungal and antibacterial activities.[20] The structurally similar geranylated acylphloroglucinols **2** and **3** isolated from *Hypericum punctatum*[21] and *Hypericum olympicum*,[22] respectively, showed potent antibacterial activity, but their antifungal activity has not been explored. 2,4-Diacetylphloroglucinol (**4**), isolated from the red marine alga *Corpus spongiosum*, and its synthetic analogue 2,4-diisobutyrylphloroglucinol (**5**), which showed antibacterial activity,[23,24] may possess antifungal activity. Finally, the microbial metabolite bis(2,4-diacetylphloroglucyl)-methane (**6**) isolated from *Pseudomonas aurantiaca*[25] was also considered as a template because it represents a large number of structurally similar dimers or trimers formed through a methylene linker within this class.[3,7] Mimicking the aforementioned templates, three chemotypes of compounds, 2,4-diacylphloroglucinols (**5**, **9**, and **10a**−**10e**), 2-acyl-4-alkylphloroglucinols (**1**, **2**, **11a**−**11l**), and *O*-alkylacylphloroglucinols (**12a**−**12i**), were synthesized.

The synthetic approach for preparation of these compounds is shown in Scheme 1, which is an adaptation from synthetic methodologies available in the literature for preparation of alkylated acylphloroglucinols.[26−28] Thus, Friedel−Crafts acylation of phloroglucinol (**7**) with acyl chloride (RCOCl) and $AlCl_3$ in nitrobenzene afforded the monoacylated products **8a**−**8c** and the diacylated products **5** and **9**. Further acylation of **8a**−**8b** with a different acylating reagent gave the diacylated products **10a**−**10e**, while treatment of **8a**−**8c** with alkyl halide yielded the C-alkylated products **1**, **2** and **11a**−**11l** and the O-alkylated products **12a**−**12i**.

The rationale for the design and synthesis of these particular compounds is further explained below. First, 2,4-diacylphloroglucinols **5**, **9**, **10a**, and **10b** and 2-acyl-4-alkylphloroglucinols **1**, **11a**, and **11b** with different acyl groups were synthesized and evaluated for in vitro antifungal activity against *C. neoformans* ATCC 90113. It was observed that compounds with an isobutyryl group (**1**, **5**, and **10a**) and those with a corresponding butyryl substituent (**9**, **10b**, and **11a**) showed similar antifungal activity, while compound **11b**, with a bulky pivaloyl group, was inactive. Thus, the isobutyryl group that is commonly present in natural acylpholoroglucinols[3] was considered as an appropriate acyl group substituted on the phloroglucinol ring of new analogues to be synthesized. Then, using isobutyrylated phloroglucinol **8a** as the key intermediate, the 2,4-diacylphloroglucinols **10c**−**10e** were prepared to examine the effects of aromatic acyl substitutions on antifungal activity, while the 2-acyl-4-alkylphloroglucinols **11c**−**11l** and the *O*-alkylacylphloroglucinols **12a**−**12i** with varying alkyl chains were synthesized to identify meaningful structure−activity relationships (SAR).

All 33 synthetic compounds were tested for in vitro antifungal activity against *C. neoformans* ATCC 90113. Compounds exhibiting greater than 50% growth inhibition (IC_{50}) at a concentration of 20 μg/mL were considered to be active, which were further tested against this strain as well as *C. neoformans* H99 and *C. gattii* ATCC 32609 to obtain MIC values. The results showed that 17 active compounds had overall MIC values in the range 1.0−16.7 μg/mL (Table 1). For comparison, the antifungal drugs fluconazole and amphotericin B gave MIC values in the range 6.3−25.0 and 0.3−0.4 μg/mL, respectively, against the three fungal strains. Cytotoxicity testing against the mammalian Vero and LLC-PK1 cell lines showed that all these antifungal compounds had IC_{50} values of greater than 10 μg/mL (Table 1), indicating they were moderately or marginally cytotoxic.

Analysis of the potency of individual antifungal compounds revealed SAR information. Within the chemotype of 2,4-diacylphloroglucinols, the aliphatically acylated compounds **5** and **9** showed potent activity with MIC values in the range 1.5−3.3 μg/mL. Replacement of one aliphatic acyl group with an aromatic acyl group in the molecule decreased antifungal activity by 3−4-fold, e.g., **5** versus **10a**. Additional substitutions on the aromatic ring with either an electron-withdrawing or an electron-donating group such as in **10c** and **10d** altered antifungal activity slightly when compared with **10a**. However, compound **10e**, with a bulky tri-OMe-substituted aromatic acyl group, was inactive at 20 μg/mL. This suggested that the antifungal activity of this chemotype of compounds is associated with acyl groups, and aliphatic acyl substitutions appear to improve potency.

For 2-acyl-4-alkylphloroglucinols, a short alkyl chain that contains a double bond, a triple bond, a cyano group, or an ester functionality disabled antifungal activity as in the cases of compounds **11c**−**11f**, which were inactive at 20 μg/mL. Compound **2**, with a C_{10} geranyl group, was more potent than **1**, with a C_5 isoprenyl group (Table 1). Compound **11l**, with an extended C_{15} farnesyl group, showed decreased activity by 3−7-fold when compared with **2**. Although **2** and **1** are potent antifungal compounds against *Cryptococcus* spp., their chemical instability is a disadvantage, as they can undergo readily intramolecular cyclization to form corresponding chromane derivatives.[29] Such cyclized products have been isolated and demonstrated to be inactive at 20 μg/mL in this study (data

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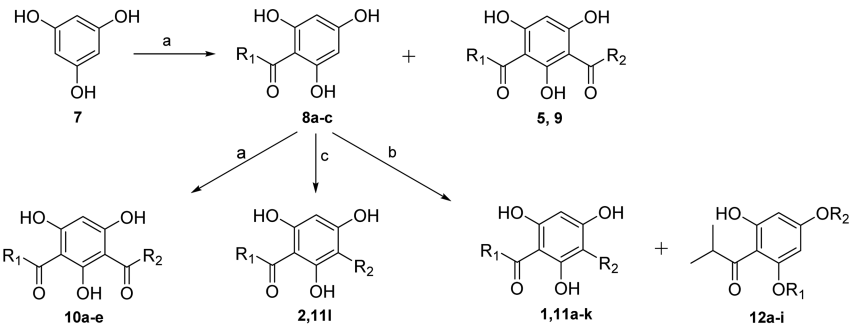
Scheme 1. Synthesis of Acylphloroglucinols[a]



	R1	R2		R1	R2		R1	R2
8a		-	11a			12a	H	
8b		-	11b			12b		H
8c		-	11c			12c		
5			11d			12d	H	
9			11e		N	12e		H
10a			11f			12f		H
10b			11g			12g	H	
10c		F	11h			12h		H
10d		OCH3	11i			12i	H	
10e			11j			2		
1			11k		NO2	11l		

[a]Reagents and conditions: (a) RCOCl, $AlCl_3$, $PhNO_2$, 65 °C, 21 h. (b) RX, DBU, THF, rt, 40 h. (c) RBr, K_2CO_3, acetone, 65 °C, 6 h.

not shown). Compounds **11g**–**11k** with varying aromatic-containing alkyl chains showed strong antifungal activity (Table 1), which is of interest, as these compounds are chemically stable compared to compounds **1** and **2** and structurally simple compared to template **6**. It appears that different substituents and substitution patterns on the aromatic ring in **11g**–**11k** influence antifungal activity. Compound **11j** demonstrated the best activity profiles due to its strong antifungal activity against the three fungal pathogens, with MIC values in the range 1.5–2.1 μg/mL, as well as low cytotoxicity against the two mammalian cell lines, Vero and LLC-PK1, with IC_{50} values greater than 50 μg/mL. It was also interesting to note that there was a 2–4-fold difference in antifungal activity between **11j** and **11k**, with a *para*-isopropyl group and a *para*-nitro group on the aromatic ring, respectively, indicating that further modifications of the aromatic-containing alkyl chain may afford compounds with improved antifungal activity.

Within the chemotype of *O*-alkylacylphloroglucinols, only **12f** and **12h** showed antifungal activity against the three *Cryptococcus* strains, with MIC values in the range 5.0–8.3 μg/mL. Structural comparison of the active compound **12h** and the inactive compound **12g** with the same (2-chloro-6-fluorophenyl)methyl group substituted at the *ortho* and *para* positions of the phloroglucinol ring, respectively, indicated that different substitution patterns are associated with antifungal activity of this chemotype of compounds. Considering the low

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Table 1. In Vitro Antifungal Activity and Cytotoxicity of Synthetic Acylphloroglucinols[a]

	antifungal activity (MIC, μg/mL)[b]			cytotoxicity (IC_{50}, μg/mL)[c]		SI[d]	
	C. neoformans ATCC 90113	*C. neoformans* H99	*C. gattii* ATCC 32609	Vero[e]	LLC-PK1[f]	Vero IC_{50}/ fungal MIC[g]	LLC-PK1 IC_{50}/fungal MIC[g]
1	5.0	8.3	5.0	18.0	31.0	3.6	6.2
2	1.7	1.7	1.0	22.5	24.5	13.2	14.4
5	2.5	3.3	2.1	11.5	17.5	4.6	7.0
9	2.1	2.5	1.5	14.5	24.5	6.9	11.7
10a	10.0	10.0	8.3	23	42.5	2.3	4.3
10b	6.7	6.7	5.0	14.3	27	2.1	4.0
10c	8.3	11.7	6.7	13.5	27.5	1.6	3.3
10d	13.3	10.0	8.3	10.5	24.8	0.8	1.9
11a	3.3	8.3	5.0	16.3	28.0	4.9	8.5
11g	6.7	16.7	10.0	28	28.5	4.2	4.3
11h	1.0	4.2	2.1	27.5	29.5	27.5	29.5
11i	1.7	6.7	4.2	28	26.3	16.5	15.5
11j	1.5	2.1	2.1	>50	>50	>33.3	>33.3
11k	6.7	10.0	5.0	18.0	28.0	2.7	4.2
11l	7.5	5.0	7.5	25.0	27.0	3.3	3.6
12f	8.3	5.0	5.0	32.5	34.5	3.9	4.2
12h	5.0	8.3	5.0	>50	>50	>10	>10
amphotericin B	0.4	0.3	0.3	–[h]	–	–	–
fluconazole	6.3	12.5	25.0	–	–	–	–
doxorubicin	–	–	–	>10	1.3	–	–

[a]Both antifungal activity and cytotoxicity data are expressed as mean values of three experimental data. [b]MIC: minimum inhibitory concentration (the lowest concentration that allows no detectable growth). The highest test concentration for synthetic acylphloroglucinols, amphotericin B, and fluconazole were 20, 5, and 50 μg/mL, respectively. [c]IC_{50}: concentration responsible for 50% growth inhibition of mammalian cells. The highest test concentration for synthetic acylphloroglucinols and the positive control doxorubicin was 50 and 10 μg/mL, respectively. [d]Selectivity index. [e]African green monkey kidney fibroblasts. [f]Pig kidney epithelial cells. [g]Calculated by using the *C. neoformans* ATCC 90113 strain. [h]Not tested.

cytotoxicity of **12h** (IC_{50} values of >50 μg/mL) against the two mammalian cell lines tested, this antifungal compound may warrant further studies.

To assess whether these antifungal compounds possess antibacterial activity, **5**, **11j**, and **12h**, representing the aforementioned three chemotypes of acylphloroglucinols, were tested using a published protocol[18] for in vitro activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Mycobacterium intracellulare*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The three compounds were active only against *S. aureus* (data shown in the Supporting Information). While compound **5** showed the most potent activity, with an MIC value of 1.3 μg/mL, which is consistent with the data reported previously in the literature,[24] **11j** was the least antibacterial compound, with an MIC value of 10.0 μg/mL. Taking into account its antifungal potency, selectivity over mammalian cell lines (selectivity indices >33.3, Table 1) and bacteria, and chemical stability, **11j** is considered to be the most promising compound and may serve as a template for further synthesis of new analogues with improved antifungal properties.

In conclusion, the present work with a focus on natural-products-based chemical synthesis has led to the discovery of several potent antifungal acylphloroglucinols against *Cryptococcus* spp. and a promising antifungal template. These findings may contribute to future antifungal discovery toward pharmaceutical development of new treatments for cryptococcosis.

EXPERIMENTAL SECTION

General Experimental Procedures. The NMR spectra using standard pulse programs were recorded at room temperature on a Bruker Avance DPX-400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz. The chemical shift (δ, ppm) values were calibrated using the residual NMR solvent. High-resolution TOFMS were measured on an Agilent series 1100 SL spectrometer equipped with an ESI source. TLC was performed on silica gel aluminum sheets (silica gel 60 F254, Merck, Darmstadt, Germany) and reversed-phase silica gel glass plates (RP-18 F254S, Merck, Darmstadt, Germany) and visualized by UV 254 nm and spraying 10% H_2SO_4, followed by heating. Flash column chromatography was done on normal-phase silica gel (230 × 400 mesh, J. T. Baker, Center Valley, PA, USA) and reversed-phase silica gel (RP-18, 40 μm, J. T. Baker). Reactants and reagents including anhydrous phloroglucinol, isobutyryl chloride, trimethylacetyl chloride, butanoyl chloride, 3,3-dimethylallyl bromide, 3-methyl-2-butenal, aluminum chloride ($AlCl_3$), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), benzoyl chloride, 4-nitrobenzyl bromide, 3-bromo-1-propene, bromoacetonitrile, 3-bromo-1-propyne, ethyl bromoacetate, butyryl chloride, 4-fluorobenzoyl chloride, 4-methoxybenzoyl chloride, 3,4,5-trimethoxybenzoyl chloride, geranyl bromide, farnesyl bromide, *n-tert*-butyl-2-chloroacetamide, nitrobenzene, tetrahydrofuran, and sodium sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA) in appropriate grades and were used without further purification. 2,6-Dichlorobenzyl chloride, 2-chloro-6-fluorobenzyl chloride, and 4-isopropylbenzyl chloride were purchased from Otava, Ltd. (Vaughan, Ontario, Canada). The yield of each synthetic product after column chromatography is reported. The ^{1}H and ^{13}C NMR assignments for all synthetic products and key intermediates with appropriate numbering systems are shown in the Supporting Information.

General Procedure for the Preparation of the 2-Acylphloroglucinols 8a–8c and 2,4-Diacylphloroglucinols 5 and 9. To a solution of anhydrous phloroglucinol (7, 79.4 mmol) in nitrobenzene (80 mL) was added $AlCl_3$ (317 mmol). The reaction mixture was

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stirred at room temperature under nitrogen for 30 min. Isobutyryl chloride or *n*-butyryl chloride (87.2 mmol) was added dropwise, and the reaction mixture was stirred at 65 °C for 21 h for production of **8a** and **5** or **8b** and **9**, respectively.[27] Acylation using trimethylacetyl chloride occurred rapidly, and the reaction mixture was stirred at room temperature for 20 min to afford the monoacylated product **8c**. After addition of ice−water (40 mL), the reaction mixture was extracted with EtOAc (100 mL × 3). The combined EtOAc layers were treated with 2 M NaOH (160 mL × 2). The aqueous layers were neutralized with 2 M HCl and extracted with EtOAc (100 mL × 3). The combined EtOAc layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford a residue, which was chromatographed on silica gel using $CHCl_3$−MeOH (19:1 for **5** and **8b**, 9:1 for **8a**, and 10:0 for **9**) or $CHCl_3$−acetone (9:1 for **8c**) to give the respective purified compounds. Identification of compounds **8a**,[27] **8b**,[30] **8c**,[31] **5**,[24] and **9**[24] was made by comparison of their NMR spectroscopic and high-resolution ESIMS data (see Supporting Information) with those reported in the literature.

General Procedure for the Preparation of the 2,4-Diacylphloroglucinols 10a−10e. Compound **8a** or **8b** was acylated with an aromatic acyl chloride using the same reaction conditions described above for preparation of the starting material. Similar workup and purification procedures of the reaction mixture afforded the respective purified compounds.

*1-(3-Benzoyl-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**10a**):* 20% yield, yellow powder; ^{1}H NMR ($CDCl_3$) δ 7.92 (2H, d, J = 8.0 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.24 (2H, t, J = 8.0 Hz), 5.76 (1H, s), 3.76 (1H, m), 1.01 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.5, 199.5, 172.3 (2C), 170.4, 140.6, 134.0, 130.4, 128.7, 103.9, 103.8, 96.3, 39.6, 19.3 (2C); HRESIMS m/z 299.0930 $[M - H]^-$ (calcd for $C_{17}H_{15}O_5^-$, 299.0925).

*1-(3-Benzoyl-2,4,6-trihydroxyphenyl)butan-1-one (**10b**):* 29% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.52 (2H, d, J = 8.0 Hz), 7.39 (1H, t, J = 8.0 Hz), 7.29 (2H, t, J = 8.0 Hz), 5.83 (1H, s), 2.96 (1H, t, J = 7.8 Hz), 1.59 (2H, m), 0.90 (3H, t, J = 7.5 Hz); ^{13}C NMR (MeOH-d_4) δ 207.6, 200.4, 169.9, 169.5, 167.5, 142.1, 132.5, 129.3 (2C), 128.8 (2C), 105.5, 104.9, 95.9, 48.7, 18.9, 14.4; HRESIMS m/z 299.0939 $[M - H]^-$ (calcd for $C_{17}H_{15}O_5^-$, 299.0925).

*1-(3-(4-Fluorobenzoyl)-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**10c**):* 38% yield, yellow powder; ^{1}H NMR ($CDCl_3$) δ 7.61 (2H, t, J = 8.0 Hz), 7.05 (2H, t, J = 8.0 Hz), 5.86 (1H, s), 3.91 (1H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 212.2/211.5, 198.8/198.1, 172.1/170.3, 166.4/163.9, 166.2/163.7,136.9/136.5, 133.0/132.9, 131.00/131.96/130.91/130.87 (2C), 115.9/115.7/115.4/115.3/115.2/115.1 (2C), 103.8/103.7, 102.6, 96.0, 39.6, 19.3 (2C); HRESIMS m/z 317.0812 $[M - H]^-$ (calcd for $C_{17}H_{14}O_5F^-$, 317.0831).

*2-Methyl-1-(2,4,6-trihydroxy-3-(4-methoxybenzoyl)phenyl)-propan-1-one (**10d**):* 11% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.65 (2H, d, J = 8.0 Hz), 6.87 (2H, d, J = 8.0 Hz), 5.92 (1H, s), 3.93 (1H, m), 3.78 (3H, s), 1.10 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.0, 197.8, 167.9, 167.1, 165.6, 164.8, 133.3, 132.5 (2C), 114.2 (2C), 107.0, 104.1, 95.7, 55.9, 40.2, 19.5 (2C); HRESIMS m/z 329.1043 $[M - H]^-$ (calcd for $C_{18}H_{17}O_6^-$, 329.1031).

*2-Methyl-1-(2,4,6-trihydroxy-3-(3,4,5-trimethoxybenzoyl)phenyl)-propan-1-one (**10e**):* 7% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.29 (2H, s), 5.81 (1H, s), 3.93 (1H, m, J = 7.8 Hz), 3.87 (6H, s), 3.86 (3H, s), 1.14 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.4, 205.4, 172.8, 170.5, 170.1, 149.0 (2C), 142.0, 108.2, 105.3, 104.1 (2C), 96.0, 58.3, 56.9 (2C), 40.5, 19.7 (2C); HRESIMS m/z 389.1272 $[M - H]^-$ (calcd for $C_{20}H_{21}O_8^-$, 389.1242).

General Procedure for the Preparation of the 2-Acyl-4-alkylphloroglucinols 1 and 11a−11k and the *O*-Alkyl-acylphloroglucinols 12a−12i. A solution of **8a** or **8b** or **8c** (2.8 mmol), alkyl halide (3 mmol), and DBU (3.1 mmol) in dry tetrahydrofuran (THF) (12.5 mL) was stirred at room temperature under nitrogen for 48 h. The reaction was quenched with 2 M HCl (12.5 mL), and the solution was extracted with EtOAc (12.5 mL × 3). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. Removal of the solvent afforded a residue, which was chromatographed on silica gel using hexanes−EtOAc (19:1) or $CHCl_3$−acetone (10:0−9:1) to afford purified compounds. Note that some reactions afforded only 2-acyl-4-alkylphloroglucinols (**1**, **11a**−**11e**, **11g**, and **11k**) or *O*-alkyl-acyl-phloroglucinols (**12d**/**12f**), while others produced both chemotypes of compounds, including **11f**/**12a**/**12b**/**12c**, **11h**/**12f**, **11i**/**12g**/**12h**, and **11j**/**12i**. The known compounds **1**[16] and **11a**[32] were identified by comparison of their NMR and HRESIMS spectroscopic data (see Supporting Information) with those reported in the literature. The new compounds were characterized by spectroscopic methods as follows (the detailed ^{1}H and ^{13}C NMR assignments are shown in the Supporting Information).

*2,2-Dimethyl-1-(2,4,6-trihydroxy-3-(3-methylbut-2-en-1-yl)-phenyl)propan-1-one (**11b**):* 14% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 5.91 (1H, s), 5.11 (1H, t, J = 7.0 Hz), 3.17 (2H, d, J = 6.5 Hz), 1.68 (3H, s), 1.60 (3H, s), 1.21 (9H, s); ^{13}C NMR (MeOH-d_4) δ 217.9, 159.2, 156.5, 155.1, 132.0, 124.4, 110.8, 108.6, 96.1, 46.2, 27.8 (3C), 26.0, 22.7, 18.0; HRESIMS m/z 277.1480 $[M - H]^-$ (calcd for $C_{16}H_{21}O_4^-$, 277.1445).

*1-(3-Allyl-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**11c**):* 10% yield, red powder; ^{1}H NMR (MeOH-d_4) δ 5.87 (1H, s), 5.81 (1H, m), 4.89 (1H, br d, J = 17.2 Hz), 4.80 (1H, br d, J = 9.8 Hz), 3.96 (1H, m), 3.19 (2H, d, J = 6.5 Hz), 1.08 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 165.4, 163.7, 161.5, 138.0, 114.1, 106.3, 104.4, 95.0, 40.0, 27.4, 19.9 (2C); HRESIMS m/z 235.0976 $[M - H]^-$ (calcd for $C_{13}H_{15}O_4^-$, 235.0976).

*2-Methyl-1-(2,4,6-trihydroxy-3-(prop-2-yn-1-yl)phenyl)propan-1-one (**11d**):* 28% yield, red powder; ^{1}H NMR (MeOH-d_4) δ 5.87 (1H, s), 3.95 (1H, m), 3.30 (2H, s), 1.96 (1H, t, J = 2.6 Hz, H-13), 1.08 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 165.3, 163.2, 161.9, 104.4, 103.7, 95.0, 84.2, 67.0, 40.0, 19.8 (2C), 12.3; HRESIMS m/z 233.0806 $[M - H]^-$ (calcd for $C_{13}H_{13}O_4^-$, 233.0819).

*2-(2,4,6-Trihydroxy-3-isobutyrylphenyl)acetonitrile (**11e**):* 21% yield, red powder; ^{1}H NMR (MeOH-d_4) δ 5.93 (1H, s), 3.90 (1H, m), 3.51 (2H, s), 1.08 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 165.7, 163.4, 163.0, 120.0, 104.3, 97.4, 94.9, 40.1, 19.8 (2C), 11.2; HRESIMS m/z 234.0765 $[M - H]^-$ (calcd for $C_{12}H_{12}O_4N^-$, 234.0772).

*Ethyl 2-(2,4,6-trihydroxy-3-isobutyrylphenyl)acetate (**11f**).* 9% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 5.78 (1H, s), 4.42 (1H, s), 4.09 (1H, q, J = 7.8 Hz), 3.66 (2H, m), 1.12 (3H, t, J = 7.5 Hz), 0.95 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 175.6, 165.4, 163.5, 162.0, 104.6, 101.1, 95.9, 62.0, 39.8, 28.8, 19.6 (2C), 14.4; HRESIMS m/z 281.1066 $[M - H]^-$ (calcd for $C_{14}H_{17}O_6^-$, 281.1031).

*1-(3-Benzyl-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**11g**):* 57% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.19 (2H, d, J = 7.4 Hz), 7.11 (2H, t, J = 7.4 Hz), 7.00 (1H, t, J = 7.2 Hz), 5.90 (1H, s), 3.96 (1H, m), 3.80 (2H, s), 1.09 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 165.7, 163.8, 161.6, 143.5, 129.6 (2C), 128.9 (2C), 126.3, 108.1, 104.6, 95.1, 40.1, 28.9, 19.9 (2C); HRESIMS m/z 285.1149 $[M - H]^-$ (calcd for $C_{17}H_{17}O_4^-$, 285.1132).

*1-(3-(2,6-Dichlorobenzyl)-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**11h**):* 16% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.16 (2H, d, J = 8.0 Hz), 6.98 (1H, t, J = 8.0 Hz), 5.82 (1H, s), 4.09 (2H, s), 3.95 (1H, m), 1.07 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 166.2, 164.1, 161.6, 139.1, 137.5 (2C), 129.0 (2C), 128.1, 105.3, 104.5, 95.1, 40.0, 26.3, 19.9 (2C); HRESIMS m/z 353.0380 $[M - H]^-$ (calcd for $C_{17}H_{15}O_4Cl_2^-$, 353.0353).

*1-(3-(2-Chloro-6-fluorobenzyl)-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**11i**):* 14% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.02 (2H, m), 6.82 (1H, t, J = 8.0 Hz), 5.84 (1H, s), 3.97 (1H, m), 3.94 (2H, s, H-11), 1.07 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.0, 166.2, 164.1, 163.1, 162.4, 161.7, 136.9/136.8, 129.1/129.0, 128.3/128.2, 126.1/126.0, 114.7/114.5, 105.6, 104.5, 95.1, 40.0, 21.3, 20.0 (2C); HRESIMS m/z 337.0674 $[M - H]^-$ (calcd for $C_{17}H_{15}O_4ClF^-$, 337.0648).

*2-Methyl-1-(2,4,6-trihydroxy-3-(4-isopropylbenzyl)phenyl)-propan-1-one (**11j**):* 15% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.14 (2H, d, J = 8.0 Hz), 7.02 (1H, d, J = 8.0 Hz), 5.93 (1H, s), 4.00

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(1H, m), 3.79 (2H, s), 2.80 (1H, m), 1.20 (6H, d, J = 7.0 Hz), 1.12 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.8, 165.6, 163.7, 161.4, 146.8, 140.7, 129.4 (2C), 126.7 (2C), 108.2, 104.5, 95.0, 39.9, 35.0, 28.3, 24.6 (2C), 19.8 (2C); HRESIMS m/z 327.1642 $[M - H]^-$ (calcd for $C_{20}H_{23}O_4^-$, 327.1602).

*2-Methyl-1-(2,4,6-trihydroxy-3-(4-nitrobenzyl)phenyl)propan-1-one (**11k**):* 25% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.92 (2H, d, J = 8.8 Hz), 7.36 (2H, d, J = 8.7 Hz), 5.92 (1H, s), 3.92 (1H, m, J = 7.8 Hz), 3.85 (2H, s), 1.07 (6H, d, J = 6.7 Hz); ^{13}C NMR (MeOH-d_4) δ 211.8, 165.0, 163.7, 162.0, 151.9, 147.1, 130.5 (2C), 124.1 (2C), 106.5, 104.5, 95.0, 40.1, 29.0, 19.9 (2C); HRESIMS m/z 330.0992 $[M - H]^-$ (calcd for $C_{17}H_{16}O_6N^-$, 330.0983).

*Ethyl 2-(3,5-dihydroxy-4-isobutyrylphenoxy)acetate (**12a**):* 16% yield, yellow powder; ^{1}H NMR ($CDCl_3$) δ 5.96 (2H, s), 4.59 (2H, s), 4.27 (2H, q, J = 7.5 Hz), 3.85 (1H, m), 1.30 (3H, t, J = 7.5 Hz), 1.12 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 211.1, 169.5, 163.9, 163.2 (2C), 105.0, 94.9 (2C), 64.9, 62.4, 39.5, 19.3 (2C), 14.3; HRESIMS m/z 281.1046 $[M - H]^-$ (calcd for $C_{14}H_{17}O_6^-$, 281.1031).

*Ethyl 2-(3,5-dihydroxy-2-isobutyrylphenoxy)acetate (**12b**):* 16% yield, yellow powder; ^{1}H NMR ($CDCl_3$) δ 5.99 (1H, s), 5.74 (1H, s), 4.60 (2H, s), 4.24 (2H, q, J = 7.5 Hz), 3.94 (1H, m), 1.27 (3H, t, J = 7.5 Hz), 1.12 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 211.1, 168.5, 167.5, 163.2, 160.9, 104.9, 97.7, 91.9, 65.7, 62.2, 39.6, 19.4 (2C), 14.2; HRESIMS m/z 281.1056 $[M - H]^-$ (calcd for $C_{14}H_{17}O_6^-$, 281.1031).

*Diethyl 2,2′-((5-hydroxy-4-isobutyryl-1,3-phenylene)bis(oxy))-diacetate (**12c**):* 14% yield, yellow powder; ^{1}H NMR ($CDCl_3$) δ 5.89 (1H, s), 5.80 (1H, s), 4.55 (2H, s), 4.50 (2H, s), 4.16 (4H, q, J = 7.5 Hz), 3.90 (1H, m), 1.20 (6H, t, J = 7.5 Hz), 1.06 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 210.3, 167.7, 167.6, 167.2, 163.1, 160.0, 105.3, 94.8, 91.7, 65.3, 64.7, 61.3 (2C), 39.3, 19.0 (2C), 13.9 (2C); HRESIMS m/z 367.1371 $[M - H]^-$ (calcd for $C_{18}H_{23}O_8^-$, 367.1398).

*N-(tert-Butyl)-2-(3,5-dihydroxy-4-isobutyrylphenoxy)acetamide (**12d**):* 27% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 5.98 (2H, s), 4.63 (2H, s), 3.99 (1H, m), 1.37 (9H, s), 1.14 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.3, 171.5, 170.3, 165.6, 164.8, 106.3, 95.2 (2C), 67.7, 51.9, 40.4, 29.2 (3C), 19.7 (2C); HRESIMS m/z 308.1573 $[M - H]^-$ (calcd for $C_{16}H_{22}O_5N^-$, 308.1503).

*N-(tert-Butyl)-2-(3,5-dihydroxy-2-isobutyrylphenoxy)acetamide (**12e**):* 14% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 5.93 (1H, s), 5.81 (1H, s), 4.69 (2H, s), 3.95 (1H, m), 1.34 (9H, s), 1.11 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.4, 171.1, 165.7, 162.2, 106.0, 98.2, 93.3, 68.2, 52.0, 40.8, 29.2 (3C), 19.9 (2C); HRESIMS m/z 308.1508 $[M - H]^-$ (calcd for $C_{16}H_{22}O_5N^-$, 308.1503).

*1-(2-((2,6-Dichlorobenzyl)oxy)-4,6-dihydroxyphenyl)-2-methylpropan-1-one (**12f**):* 7% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.47 (2H, d, J = 8.0 Hz), 7.39 (1H, t, J = 8.0 Hz), 6.16 (1H, s), 5.96 (1H, s), 5.34 (2H, s), 3.49 (1H, m), 0.85 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.4, 168.4, 166.3, 163.5, 138.2 (2C), 132.8, 132.5, 129.9 (2C), 105.7, 97.6, 92.9, 66.7, 40.4, 19.7 (2C); HRESIMS m/z 353.0365 $[M - H]^-$ (calcd for $C_{17}H_{15}O_4Cl_2^-$, 353.0353).

*1-(4-((2-Chloro-6-fluorobenzyl)oxy)-2,6-dihydroxyphenyl)-2-methylpropan-1-one (**12g**):* 6% yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.40 (1H, q, J = 8.0 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.15 (1H, t, J = 8.0 Hz), 6.03 (2H, s), 5.17 (2H, s), 3.99 (1H, m), 1.15 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.2, 166.2, 165.7 (2C), 164.7, 162.3, 137.7, 132.7, 126.9, 123.4/123.3, 115.7/115.5, 105.9, 95.2 (2C), 62.2, 40.3, 19.7 (2C); HRESIMS m/z 337.0655 $[M - H]^-$ (calcd for $C_{17}H_{15}O_4ClF^-$, 337.0648).

*1-(2-((2-Chloro-6-fluorobenzyl)oxy)-4,6-dihydroxyphenyl)-2-methylpropan-1-one (**12h**):* 12%, yield, yellow powder; ^{1}H NMR (MeOH-d_4) δ 7.41 (1H, q, J = 8.0 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.15 (1H, t, J = 8.0 Hz), 6.15 (1H, s), 5.96 (1H, s), 5.21 (2H, s), 3.50 (1H, m), 0.87 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.4, 168.3, 166.2, 163.4, 164.7/162.2, 137.6/137.5, 132.9/132.8, 126.9/126.8, 123.0/122.9, 115.7/115.5, 105.7, 97.7, 93.0, 62.7, 40.4, 19.7 (2C); HRESIMS m/z 337.0674 $[M - H]^-$ (calcd for $C_{17}H_{15}O_4ClF^-$, 337.0648).

*1-(2,6-Dihydroxy-4-((4-isopropylbenzyl)oxy)phenyl)-2-methylpropan-1-one (**12i**):* 9% yield, yellow solid; ^{1}H NMR (MeOH-d_4) δ 7.30 (2H, d, J = 8.0 Hz), 7.22 (2H, d, J = 8.0 Hz), 5.98 (2H, s), 5.00 (2H, s), 3.97 (1H, m), 2.90 (1H, m), 1.23 (6H, d, J = 7.0 Hz), 1.12 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 212.1, 166.4, 165.6 (2C), 150.2, 135.4, 128.9 (2C), 127.7 (2C), 105.6, 95.4 (2C), 71.1, 40.2, 35.3, 24.5 (2C), 19.7 (2C); HRESIMS m/z 327.1603 $[M - H]^-$ (calcd for $C_{20}H_{23}O_4$, 327.1602).

Synthesis of the 2-Acyl-4-alkylphloroglucinols 2 and 11l. A solution of **8a** (2.6 mmol), geranyl bromide or farnesyl bromide (1.2 mmol), and anhydrous K_2CO_3 (5.4 mmol) in acetone (10 mL) was stirred at 65 °C under nitrogen for 6 h. After removal of the solvent and addition of a 2 M HCl solution (20 mL), the reaction mixture was extracted with EtOAc (20 mL × 3). The combined EtOAc layers were washed with brine and dried over Na_2SO_4. Removal of the solvent afforded a crude product, which was purified by column chromatography on silica gel using $CHCl_3$–acetone (19:1). The known compound **2** was identified by comparison of its NMR spectroscopic data (see Supporting Information) with those reported in the literature.[33]

*2-Methyl-1-(2,4,6-trihydroxy-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)phenyl)propan-1-one (**11l**):* 25% yield, yellow solid; ^{1}H NMR (MeOH-d_4) δ 5.86 (1H, s), 5.14 (1H, t, J = 6.7 Hz), 4.99 (2H, br t, J = 6.0 Hz), 3.95 (1H, m), 3.15 (2H, d, J = 7.0 Hz), 2.10–1.75 (8H, m), 1.69 (3H, s), 1.59 (3H, s), 1.51 (3H, s), 1.49 (3H, s), 1.08 (6H, d, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 211.9, 165.4, 163.4, 161.0, 135.8, 134.7, 131.9,125.5 (2C), 124.8, 108.4, 104.7, 95.2, 40.93, 40.87, 40.0, 27.8, 27.6, 26.1, 22.3, 20.0 (2C), 17.9, 17.9, 16.4, 16.3; HRESIMS m/z 399.2527 $[M - H]^-$ (calcd for $C_{25}H_{35}O_4^-$, 399.2541).

In Vitro Antifungal Assay. A modified version of the CLSI (formerly NCCLS) method was used for susceptibility testing. The fungal strains *C. neoformans* ATCC 90113 and *C. gattii* ATCC 32609 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). *C. neoformans* H99 was obtained from the Department of Molecular Genetics and Microbiology at Duke University School of Medicine. Amphotericin B (ICN Biomedicals, Aurora, OH, USA) and fluconazole (ICN Biomedicals) were used as positive controls. The detailed procedure has been described previously.[34,35]

In Vitro Cytotoxicity Assay. The two mammalian cell lines Vero (African green monkey kidney fibroblast) and LLC-PK1 (pig kidney epithelial) were obtained from ATCC. Cytotoxicity was determined by the neutral red method.[36] The detailed assay procedure has been described previously.[34,37]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00224.

In vitro antibacterial data of compounds **5**, **11j**, and **12h** and original NMR and HRESIMS spectra of synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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