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Novel prenylated and geranylated aromatic compounds isolated from *Polysphondylium* cellular slime molds

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ABSTRACT

We have studied the diversity of secondary metabolites of cellular slime molds to utilize them as new biological resources for natural product chemistry. From the methanol extract of fruiting bodies of *Polysphondylium tenuissimum*, we obtained five prenylated and geranylated aromatic compounds, Pt-1–5 (**1**–**5**). An additional aromatic compound, Ppc-1 (**6**), was isolated from *Polysphondylium pseudo-candidum*. The structures of these compounds were determined by spectral analysis, and synthetic routes to **4**, **5**, and **6** were developed. Compound **5** showed the glucose consumption-promotive activity on 3T3-L1 cells.

1. Introduction

The cellular slime mold *Dictyostelium discoideum* is thought to be an excellent model organism for the study of cell and developmental biology because of its simple pattern of development.[1] Vegetative cells of *D. discoideum* grow as single amoebae by eating bacteria. When starved, they initiate a developmental program of morphogenesis and gather to form a slug-shaped multicellular aggregate. This aggregate then differentiates into two distinct cell types, prespore, and prestalk cells, which are precursors of spores and stalk cells, respectively. At the end of its development, the aggregate forms a fruiting body consisting of spores and a multicellular stalk.

Several small molecules including DIFs (Differentiation-Inducing Factors),[2] discadenine,[3] and cAMP[4] have been reported as development-regulating substances of cellular slime molds. DIFs and their derivatives also exhibit many biological effects in mammalian cells, such as suppression of cell growth,[5–7] induction/promotion of cell differentiation,[5a,d] promotion of glucose consumption[7,8] and regulation of IL-2 production.[9] A chlorine-substituted dibenzofuran derivative AB0022A[10] and a resorcinol derivative MPBD[11] were also isolated from cellular slime molds, although no other reports have, to date, described the secondary metabolites of cellular slime molds.

We have focused on the utility of cellular slime molds as a resource for novel drug development, and have studied the diversity of secondary metabolites of cellular slime molds.[12] Cellular slime molds are classified into three genera, *Dictyostelium*, *Polysphondylium*, and *Acytostelium*, according to their morphology.[13] We have recently isolated α-pyronoids,[12a,f] amino sugar derivatives,[12c,d] and aromatics[12b,e] with unique structures from several species of *Dictyostelium*. This paper reports the isolation, structure elucidation and synthesis of new prenylated and geranylated aromatic compounds Pt-1–5 (**1**–**5**) and Ppc-1 (**6**) from *Polysphondylium* cellular slime molds (Fig. 1). The antiproliferative and glucose consumption–promotive activities of these compounds in mammalian cells are also described.

2. Results and discussion

2.1. Isolation and structure elucidation

Fruiting bodies (dry weight 73.6 g) of the cellular slime mold, *Polysphondylium tenuissimum*, were cultured on plates and extracted three times with methanol at room temperature to yield an extract (18.2 g) that was subsequently partitioned between ethyl acetate and water. The ethyl acetate solubles (5.49 g) were separated by repeated column chromatography over SiO_2 and ODS to

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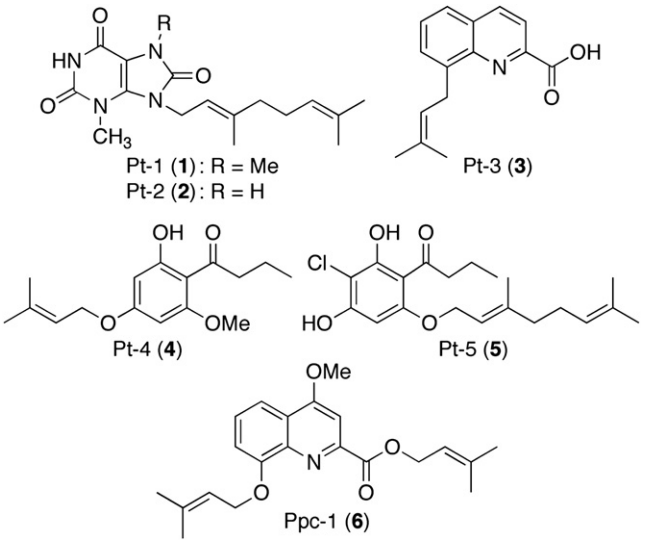


Figure 1. Structures of Pt-1–5 (**1**–**5**) and Ppc-1 (**6**).

yield Pt-1 (**1**) (1.8 mg), Pt-2 (**2**) (9.7 mg), Pt-3 (**3**) (0.8 mg), Pt-4 (**4**) (2.6 mg), and Pt-5 (**5**) (4.7 mg). In the same manner, Ppc-1 (**6**) (1.8 mg) was obtained from the fruiting bodies (dry weight 57.3 g) of *Polysphondylium pseudo-candidum*.

HREIMS (*m*/*z* 332.1835 $[M]^+$), ^1H and ^{13}C NMR spectra indicated that the molecular formula of **1** was $C_{17}H_{24}N_4O_3$. The ^{13}C NMR spectrum of **1** showed the presence of seven quaternary sp^2 carbons, two tertiary sp^2 carbons, three methylene carbons and five methyl carbons (Table 1). ^1H–^1H COSY revealed that C-1′–C-2′ and C-4′–C-5′–C-6′ were connected. The correlations of H_3-9′–C-2′, C-3′, and C-4′; and H_3-10′–C-6′, C-7′, and C-8′ in the HMBC spectrum indicated a geranyl group. The molecular formula of the structure with neither the geranyl group ($C_{10}H_{17}$) nor the two methyl groups was $C_5HN_4O_3$, suggesting the presence of a trisubstituted uric acid moiety. This was supported by the observation that the chemical shifts of the five remaining sp^2 carbons (δ 152.6 (C-6), 151.8 (C-8), 149.7 (C-2), 137.1 (C-4), and 99.7 (C-5)) were consistent with those of uric acid[14] (δ 153.4, 152.6, 150.1, 136.6, and 97.1). The correlations between the three sets of peaks: H_3-11 with C-2 and C-4; H_3-12 with C-5 and C-8; and H_2-1′ with C-4 and C-8, in the HMBC spectrum, indicated that two methyl groups (C-11 and C-12) and a geranyl group were attached to N-3, N-7, and N-9, yielding the complete structure of **1** (Fig. 2).

Table 1
^{13}C and ^1H NMR spectral data of Pt-1 (**1**), Pt-2 (**2**) and **7**

	Pt-1 (**1**)[a]		Pt-2 (**2**)[b]		**7**[a]
	^{13}C	^1H	^{13}C	^1H	^{13}C
2	149.7		151.5		150.8
4	137.1		137.6		135.4
5	99.7		99.8		99.5
6	152.6		154.4		153.5
8	151.8		153.2		151.9
10					28.2
11	30.5	3.61 (3H, s)	30.0	3.68 (3H, s)	30.5
12	29.3	3.56 (3H, s)			29.3
1′	41.8	4.66 (2H, d, *J*=6.0 Hz)	41.2	4.82 (2H, d, *J*=5.7 Hz)	41.8
2′	119.4	5.08–5.12 (1H, m)	121.4	5.36–5.40 (1H, m)	119.6
3′	140.7		139.5		140.5
4′	39.2	2.03 (2H, t, *J*=7.2 Hz)	39.4	2.05 (2H, t, *J*=7.0 Hz)	39.2
5′	26.1	2.07 (2H, q, *J*=7.2 Hz)	26.4	2.11 (2H, q, *J*=7.0 Hz)	26.1
6′	123.3	4.98–5.03 (1H, m)	124.3	5.07–5.12 (1H, m)	123.3
7′	132.3		131.8		132.3
8′	25.7	1.64 (3H, d, *J*=1.0 Hz)	25.7	1.63 (3H, d, *J*=0.8 Hz)	25.7
9′	16.7	1.72 (3H, d, *J*=1.0 Hz)	16.4	1.71 (3H, d, *J*=1.0 Hz)	16.7
10′	17.7	1.56 (3H, s)	17.7	1.52 (3H, s)	17.7
1-NH		8.08 (1H, br s)		13.40 (1H, br s)[c]	
9-NH				13.29 (1H, br s)[c]	

[a] ^1H (600 MHz) and 150 MHz for ^{13}C in $CDCl_3$.
[b] ^1H (600 MHz) and 150 MHz for ^{13}C in pyridine-d_5.
[c] These signals were indistinguishable.

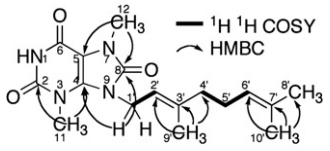


Figure 2. Structural elucidation of Pt-1 (**1**).

HREIMS of **2** (*m*/*z* 318.1688 $[M]^+$) indicated a molecular formula, $C_{16}H_{22}N_4O_3$, which differed from that of **1** by a methylene unit. The ^1H and ^{13}C NMR spectra of **2** were nearly identical to those of **1** (Table 1), suggesting that the methyl group and geranyl group were attached to the uric acid moiety in the structure of **2**. In the HMBC spectrum of **2**, the two sets of correlation peaks: H_3-11 to the signals at δ_c 137.6 (C-4) and 151.5; and H_2-1′ to the signals at δ_c 137.6 (C-4) and 153.2, indicated that these groups were bonded to N-3 and N-9 (Fig. 3). However, the positions of each substituent could not be determined because the chemical shifts of the signals at δ_c 151.5 and 153.2 were too close to assign them to C-2 and C-8, respectively. To resolve this issue, compound **2** was methylated. The HMBC spectrum of the product **7** showed two sets of correlation peaks: between H_3-12 and the signals at δ_c 99.5 and 151.9; and between H_2-1′ and the signals at δ_c 135.4 and 151.9. Because the signals at δ_c 99.5 and 135.4 could be readily assigned to C-5 and C-4, respectively, the position of the geranyl group was assigned to N-9.

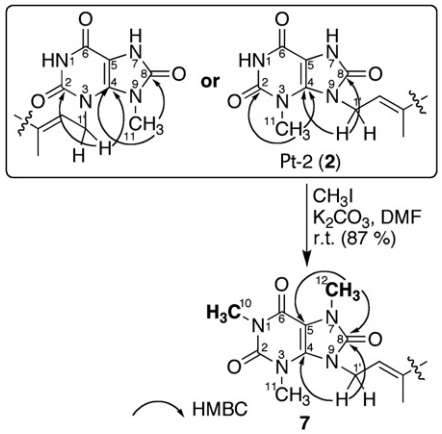


Figure 3. Structural elucidation of Pt-2 (**2**).

Compound **3** showed a molecular ion peak at *m*/*z* 241.1091 in HREIMS, suggesting that its molecular formula was $C_{15}H_{15}NO_2$. The ^{13}C NMR spectrum of **3** showed the presence of one carbonyl, five sp^2 quaternary, six sp^2 tertiary, one methylene, and two methyl carbons (Table 2). The ^1H NMR spectrum showed the presence of a 1,2,3-trisubstituted benzene ring (δ 7.78 (1H, dd, *J*=8.1, 1.2 Hz, H-5), 7.62 (1H, dd, *J*=8.1, 7.1 Hz, H-6), and 7.65 (1H, dd, *J*=7.1, 1.2 Hz, H-7)). Other low field-shifted proton signals at δ 8.26 (1H, d, *J*=8.3 Hz, H-3) and 8.39 (1H, d, *J*=8.3 Hz, H-4) implied the presence of a nitrogen-containing heterocycle, and the HMBC correlations shown in Figure 4 gave 2,8-disubstituted quinoline moiety. Moreover, the presence of a prenyl group at C-8 was revealed by the correlation of H_2-1′–H-2′ in the ^1H–^1H COSY and the correlations of H_3-5′–C-2′, C-3′, and C-4′; and H_2-1′–C-7, C-8, and C-8a in the HMBC spectrum. Therefore, the remaining moiety, CHO_2, was assigned as a carboxyl

group, and the structure of **3** was determined to be 8-prenylquinoline-2-carboxylic acid. This structure was supported by the fact that ^{13}C NMR signals of the quinoline ring in **3** corresponded with those from 8-ethylquinoline-2-carboxylic acid.[15]

Table 2
^{13}C and ^{1}H NMR spectral data of Pt-3 (**3**)[a]

	^{13}C	^{1}H
2	144.7	
3	118.8	8.26 (1H, d, J=8.3 Hz)
4	139.4	8.39 (1H, d, J=8.3 Hz)
4a	130.3	
5	126.0	7.78 (1H, dd, J=8.1, 1.2 Hz)
6	129.2	7.62 (1H, dd, J=8.1, 7.1 Hz)
7	130.2	7.65 (1H, dd, J=7.1, 1.2 Hz)
8	140.5	
8a	144.4	
9	164.3	
1′	30.1	3.97 (2H, d, J=7.2 Hz)
2′	122.2	5.38–5.44 (1H, m)
3′	133.4	
4′	25.7	1.76 (3H, br s)
5′	17.9	1.80 (3H, br s)

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.

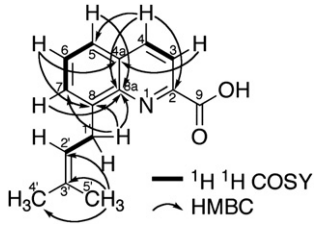


Figure 4. Structural elucidation of Pt-3 (**3**).

The molecular formula for **4**, $C_{16}H_{22}O_4$, was proposed based on the observation of a molecular ion peak at m/z 278.1498 in HREIMS. The ^{13}C NMR spectrum of **4** showed the presence of a keto carbonyl, five sp^2 quaternary, three sp^2 tertiary, one oxymethylene, one methoxyl, two methylene, and three methyl carbons (Table 3). The ^{1}H NMR spectrum revealed the presence of a 1,2,3,5-tetrasubstituted benzene ring (δ 6.05 (1H, d, J=2.4 Hz) and 5.92 (1H, d, J=2.4 Hz)). The ^{1}H NMR signal at δ 14.09 (1H, s) was assigned to a phenolic proton hydrogen-bonded to a carbonyl group. A butanoyl group was suggested by the correlation of H_2-2′–H_2-3′–H_3-4′ in the ^{1}H–^{1}H COSY and the correlations of H_2-2′–C-1′ in the HMBC spectrum. A prenyl group was also deduced by the correlation of H_2-1″–H-2″ in the ^{1}H–^{1}H COSY and the correlations of H_3-5″–C-2″, C-3″, and C-4″ in the HMBC spectrum. The hydrogen bond between a phenolic proton and a carbonyl group indicated that the butanoyl group was located on *ortho* position of the phenolic hydroxyl group. Thus, the HMBC correlations of a phenolic proton to C-1, C-2, and C-3 revealed that the hydroxyl group and the butanoyl group were attached to C-2 and C-1, respectively. The HMBC correlations of H-3, H-5, and H_2-1″–C-4 showed that the prenyloxy group was bonded to C-4. Finally, the remaining methoxyl group was attached to C-6, and the structure of **4** was elucidated (Fig. 5).

Table 3
^{13}C and ^{1}H NMR spectral data of Pt-4 (**4**) and Pt-5 (**5**)[a]

	Pt-4 (**4**)		Pt-5 (**5**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	105.7		106.3	
2	167.6		162.0	
3	94.2	6.05 (1H, d, J=2.4 Hz)	99.9	
4	165.0		157.5	
5	91.3	5.92 (1H, d, J=2.4 Hz)	91.0	6.13 (1H, s)
6	162.7		160.9	
1′	205.8		206.3	
2′	46.1	2.94 (2H, t, J=7.4 Hz)	46.4	2.99 (2H, t, J=7.4 Hz)
3′	18.1	1.67 (2H, sextet, J=7.4 Hz)	18.3	1.68 (2H, sextet, J=7.4 Hz)
4′	14.0	0.96 (3H, t, J=7.4 Hz)	13.9	0.97 (3H, t, J=7.4 Hz)
1″	65.0	4.50 (2H, d, J=6.8 Hz)	65.9	4.58 (2H, d, J=6.7 Hz)
2″	118.7	5.42–5.48 (1H, m)	117.9	5.48–5.51 (1H, m)
3″	139.3		142.7	
4″	25.8	1.78 (3H, br s)	39.5	2.08–2.16 (2H, m)
5″	18.2	1.72 (3H, br s)	26.2	2.08–2.16 (2H, m)
6″			123.5	5.07–5.11 (1H, m)
7″			132.1	
8″			25.6	1.68 (3H, br s)
9″			16.7	1.75 (3H, br s)
10″			17.7	1.61 (3H, br s)
2-OH		14.09 (1H, s)		14.83 (1H, s)
4-OH				6.14 (1H, br s)
6-OCH_3	55.5	3.82 (3H, s)		

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.

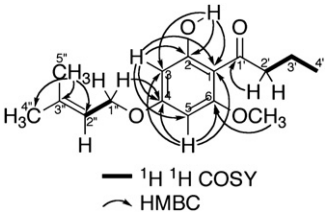


Figure 5. Structural elucidation of Pt-4 (**4**).

EIMS of **5** showed two peaks, m/z 366 and 368, for molecular ion peaks in a ratio of 3:1, suggesting that compound **5** contained one chlorine atom. HREIMS (m/z 366.1597 $[M]^+$), ^{1}H and ^{13}C NMR spectra indicated the molecular formula of **5** as $C_{20}H_{27}ClO_4$. The ^{13}C NMR spectrum of **5** showed the presence of a keto carbonyl, seven sp^2 quaternary, three sp^2 tertiary, one oxymethylene, four methylene, and four methyl carbons (Table 3). The presence of a geranyloxy group and a butanoyl group in **5** was deduced by the ^{1}H–^{1}H COSY and HMBC spectra as well as in compound **1** and **4**, respectively. The ^{1}H NMR signal at δ 14.83 (1H, s) was assigned to a phenolic proton hydrogen-bonded to a carbonyl group. Five quaternary sp^2 carbons (δ_C 162.0, 160.9, 157.5, 106.3, 99.9) and a tertiary sp^2 carbon (δ_C 91.0 and δ_H 6.13) revealed a benzene ring substituted by two phenolic hydroxy groups, a geranyloxy group, a butanoyl group and a chlorine atom. The HMBC correlation peaks for H-2–OH (δ_H 14.83 (1H, s)) to C-1, C-2, and C-3 showed that the butanoyl group and the chlorine atom were connected to either of C-1 (δ_C 106.3) and C-3 (δ_C 99.9). The HMBC correlation peaks for H-5 to C-1, C-3, C-4, and C-6 showed that the geranyloxy group and the hydroxy group were connected to either of C-4 (δ_C 157.5) and C-6 (δ_C 160.9). Thus, there are two possible structures of **5** (Fig. 6). To resolve this problem, the methylation and catalytic hydrogenation

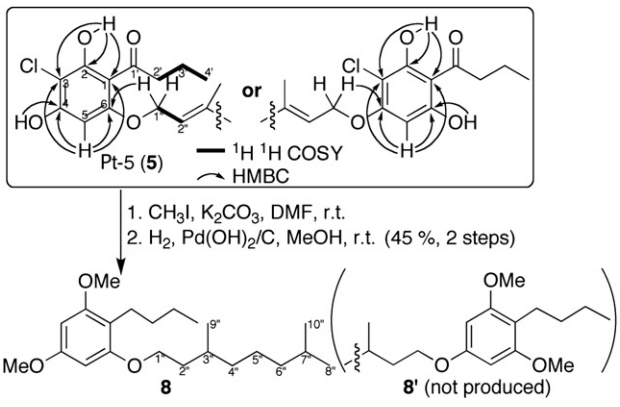


Figure 6. Structural elucidation of Pt-5 (**5**).

of **5** were carried out. The ^{1}H NMR spectrum of the resulting compound showed two aromatic and two methoxyl proton signals, suggesting that the product was the non-symmetrical compound **8**, instead of the symmetric **8′**. Therefore, the geranyloxy group in **5** was shown to be located on C-6.

Compound **6** showed a molecular ion peak at *m*/*z* 355.1754 in HREIMS, suggesting that its molecular formula was $C_{21}H_{25}NO_4$. The ^{13}C NMR spectrum of **6** showed the presence of one carboxyl, seven sp^2 quaternary, six sp^2 tertiary, two oxymethylene, one methoxyl, and four methyl carbons (Table 4). The ^{1}H–^{1}H COSY, and HMBC spectra showed the presence of two prenyl groups in **6** as well as in compound **4**. The ^{1}H NMR spectrum showed the presence of a 1,2,3-trisubstituted benzene ring (*δ* 7.76 (1H, dd, *J*=8.1, 1.1 Hz, H-5), 7.48 (1H, t, *J*=8.1 Hz, H-6) and 7.08 (1H, dd, *J*=8.1, 1.1 Hz, H-7)). Other low field-shifted proton signal at *δ* 7.60 (1H, s, H-3) implied the presence of a nitrogen-containing heterocycle, and the HMBC correlation peaks for H-3 to C-2, C-4, C-4a, and C-9; H-5 to C-4 and C-8a; H-6 to C-4a and C-8; and H-7 to C-8 and C-8a depicted in Figure 7 gave 2,4,8-trisubstituted quinoline moiety. Finally, the correlation peaks for a methoxyl proton to C-4; H$_2$-1′ to C-9; and H$_2$-1″ to C-8 in HMBC spectrum revealed the methoxyl group, the two prenyloxy groups were attached to C-8, C-4, and C-9, respectively, the structure of **6** was elucidated (Fig. 7).

Table 4
^{13}C and ^{1}H NMR spectral data of Ppc-1 (**6**).[a]

	^{13}C	^{1}H
2	148.2	
3	100.7	7.60 (1H, s)
4	163.2	
4a	123.5	
5	113.1	7.76 (1H, dd, *J*=8.1, 1.1 Hz)
6	127.9	7.48 (1H, t, *J*=8.1 Hz)
7	110.0	7.08 (1H, dd, *J*=8.1, 1.1 Hz)
8	155.3	
8a	140.7	
9	166.0	
1′	63.2	4.95 (2H, d, *J*=7.3 Hz)
2′	118.8	5.55–5.61 (1H, m)
3′	138.8	
4′	25.8	1.78 (3H, br s)
5′	18.4	1.79 (3H, br s)
1″	66.2	4.81 (2H, d, *J*=6.4 Hz)
2″	120.1	5.67–5.73 (1H, m)
3″	136.7	
4″	25.8	1.80 (3H, br s)
5″	18.2	1.81 (3H, br s)
4-OCH$_3$	56.1	4.11 (3H, s)

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$.

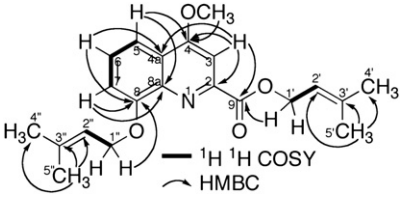
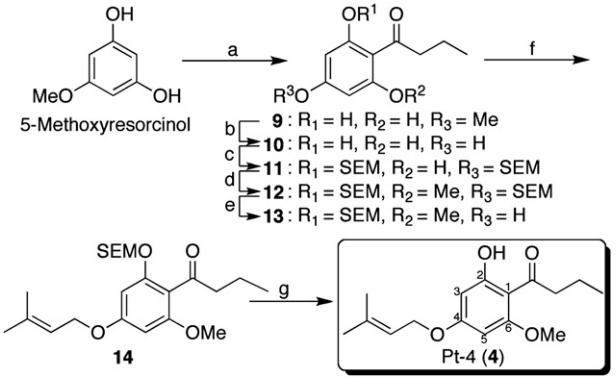


Figure 7. Structural elucidation of Ppc-1 (**6**).

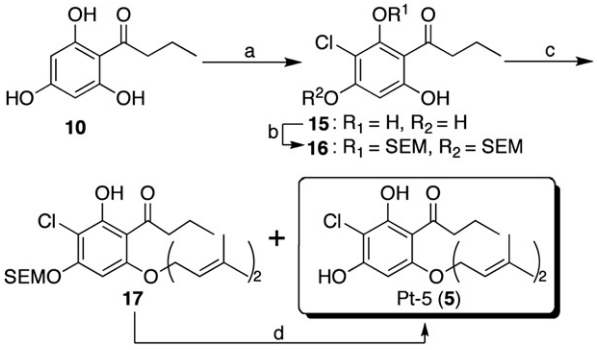
2.2. Syntheses of 4–6

Compounds **4**–**6** were synthesized to confirm the structures and obtain a sufficient quantity of sample for biological assays. The synthesis of **4** was shown in Scheme 1. Friedel–Crafts acylation of commercially available 5-methoxyresorcinol with butanoyl chloride gave compound **9**.[6] After demethylation of the 4-methoxyl group, both of 2- and 4-hydroxyl groups were protected by SEM groups, and the 6-hydroxyl group was methylated to produce **12**. The TBAF–HMPA system[16] removed only one SEM group to afford 4-hydroxyl compound **13** and the 2-hydroxyl isomer. The phenolic hydroxyl group of **13** was prenylated to give **14**. Finally, removal of the SEM group on C-2 by using the TBAF–HMPA system again allowed us to synthesize **4**.



Scheme 1. Synthesis of Pt-4 (**4**). Reagents and conditions: (a) butanoyl chloride, $AlCl_3$, CH_2Cl_2, 0 °C (81%); (b) BBr_3, CH_2Cl_2, −78 °C→rt (95%); (c) SEMCl, DIPEA, CH_2Cl_2, rt (86%); (d) MeI, K_2CO_3, acetone, rt (90%); (e) TBAF, HMPA–THF (8:1), 40 °C (23%); (f) 1-bromo-3-methyl-2-butene, K_2CO_3, DMF, rt (60%); (g) TBAF, HMPA–THF (8:1), 40 °C (45%).

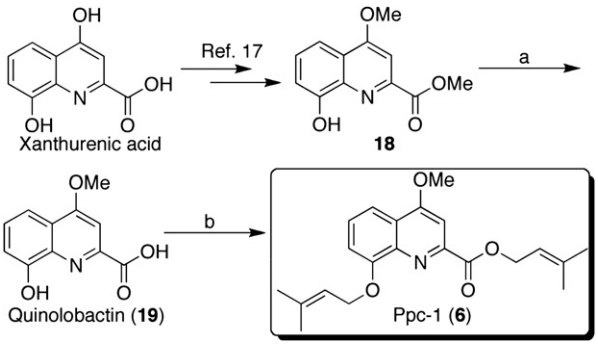
In the course of synthesis of **5** (Scheme 2), C-3 in compound **10** was selectively chlorinated by using 1 equiv of sulfuryl chloride to give **15**. The two phenolic hydroxyl groups on C-2 and C-4 were subsequently protected by SEM groups to produce **16**. During geranylation of **16**, the SEM groups were simultaneously eliminated to afford compounds **17** and Pt-5 (**5**). By using TBAF–HMPA system,



Scheme 2. Synthesis of Pt-5 (**5**). Reagents and conditions: (a) SO_2Cl_2, $CHCl_3$–EtOH (100:1), rt (95%); (b) SEMCl, DIPEA, CH_2Cl_2, rt (53%); (c) geranyl bromide, K_2CO_3, DMF, rt (25% (for **17**) and 19% (for **5**)); (d) TBAF, HMPA, 50 °C (66% borsm).

compound **17** was also converted into **5**.

Synthesis of **6** proceeded via the synthetic method described for quinolobactin (**19**),[17] a siderophore from *Pseudomonas fluorescens*



Scheme 3. Synthesis of Ppc-1 (**6**). Reagents and conditions: (a) 5% NaOHaq, MeOH, reflux; (b) 1-bromo-3-methyl-2-butene, K_2CO_3, DMF, rt (87% for two steps).

(Scheme 3). Hydrolysis of **18**, prepared from xanthurenic acid, gave quinolobactin (**19**), which was prenylated to produce **6**.

2.3. Biological evaluation

The antiproliferative activities of **1**–**6** were evaluated on K562 human leukemia, Hela cervical carcinoma, and 3T3-L1 mouse embryonic fibroblast cells (Fig. 8A). Ppc-1 (**6**) showed about 50% inhibition at 15 μM in all cell lines. Pt-1 (**1**) and Pt-5 (**5**) decreased the cell number into less than 30% of control on K562 cells. The

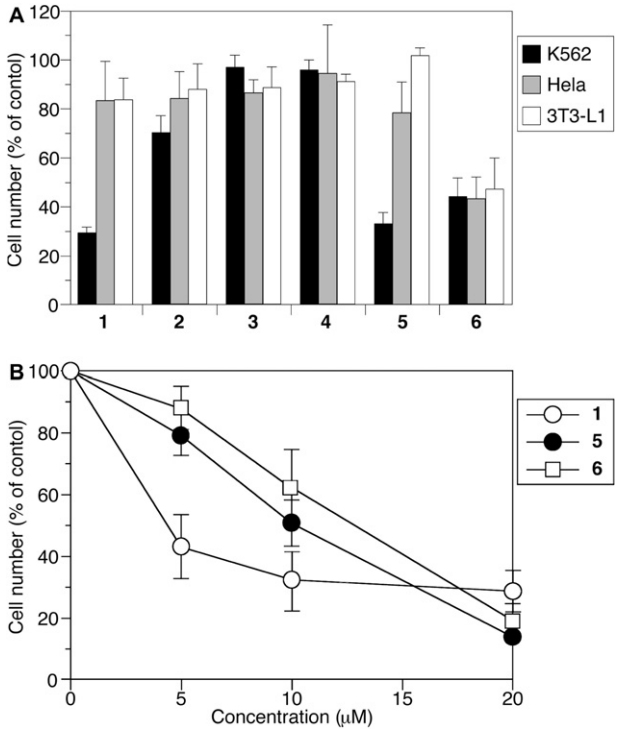
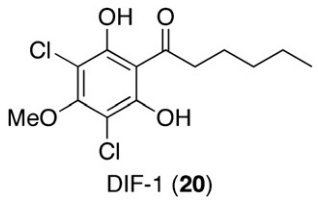


Figure 8. A. Antiproliferative activities of 15 μM of **1**–**6** on K562, Hela and 3T3-L1 cells. B. Detailed analysis of antiproliferative activities of **1**, **5**, and **6** on K562 cells. Cells were incubated in vitro with the indicated concentrations of compounds for several days, and the relative cell number was assessed. The mean values and SD (bars) of the triplicate (n=3) are presented.

concentration response curves for K562 cells exposed to **1**, **5** and **6** gave the EC_{50} values of 4.4 μM, 10 μM, and 13 μM, respectively (Fig. 8B).

Compound **5** is a chlorine-containing acylphloroglucinol derivative, and is related to DIF-1 (**20**) (Fig. 9),[2] which induces differentiation in *Dictyostelium* cellular slime molds. As mentioned, **20** promoted glucose consumption in 3T3-L1 cells.[8] Therefore, we examined the activities of **1**–**6** toward glucose consumption. As shown in Figure 10, only **5** showed activity at 20 μM, similar to DIF-1 (**20**).



DIF-1 (**20**)

Figure 9. Structure of DIF-1 (**20**).

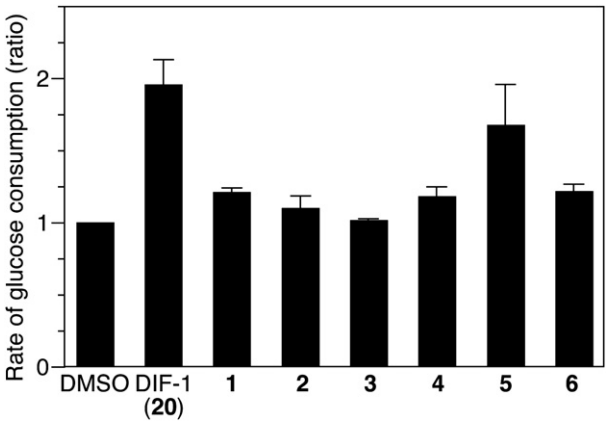


Figure 10. Effects of **1**–**6** on glucose consumption in 3T3-L1 cells. Confluent 3T3-L1 cells were incubated for several hours in DMEM-MG containing 0.2% DMSO or 20 μM of compounds. The glucose concentration was measured, and the rate of glucose consumption was calculated. The mean values and SD (bars) of the triplicate (n=3) are presented.

3. Conclusions

The prenylated and geranylated compounds, such as Pt-1–5 (**1**-**5**) and Ppc-1 (**6**) have been scarcely reported, although their structural skeletons are composed of familiar aromatic rings. Isolation of this type of compounds shows that cellular slime molds are promising resources for natural product chemistry. Compounds **4** and **5**, which bear butanoyl groups, may be biosynthesized via an analog of DIF-1 (**20**), which contains a hexanoyl group. The difference in length of the acyl groups may be a result of the chemotaxonomic differences between the genera *Dictyostelium* and *Polysphondylium*.

4. Experimental section

4.1. General methods

Analytical TLC was performed on silica gel 60 F_{254}. Column chromatography was carried out on silica gel 60 (70–230 mesh). ^{1}H NMR spectra were recorded on at 600 MHz, 500 MHz or 400 MHz. ^{13}C NMR spectra were recorded on at 150 MHz, 125 MHz or 100 MHz. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and residual solvent signals (δ_C 77.0, 29.8, and 123.5 for CDCl3, acetone-d_6 and pyridine-d_5, respectively) as internal standards.

4.2. Organism and culture conditions

The cellular slime molds, *P. tenuissimum* and *P. pseudo-candidum*, were kindly supplied by Kyorin Pharmaceutical Co. Ltd., Tokyo, Japan and Dr. Hagiwara, National Science Museum, Tokyo, Japan. Spores were cultured at 22 °C with *Escherichia coli* Br on A-medium consisting of 0.5% glucose, 0.5% polypeptone, 0.05% yeast extract, 0.225% KH_2PO_4, 0.137% $Na_2HPO_4{\cdot}12H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$, and 1.5% agar. When fruiting bodies had formed after four days, they were harvested for extraction.

4.3. Isolation of Pt-1–5 (1–5)

The cultured fruiting bodies (dry weight 73.6 g) of *P. tenuissimum* were extracted three times with MeOH at room temperature to give an extract (18.2 g), which was then partitioned with EtOAc and H_2O to yield EtOAc solubles (5.49 g). The EtOAc solubles were chromatographed over SiO_2 and the column eluted with hexane–EtOAc and EtOAc–MeOH solutions with increasing polarity to afford hexane–EtOAc (19:1) eluent (fraction A, 718 mg), hexane–EtOAc (4:1)

eluent (fraction B, 450 mg), EtOAc eluent (fraction C, 36 mg) and EtOAc–MeOH (1:1) eluent (fraction D, 510 mg). Fraction A was chromatographed over ODS using H_2O–acetonitrile (3:7) to give Pt-4 (**4**) (2.6 mg). Fraction B was separated by ODS column (elutant: H_2O–acetonitrile (1:1)) and silica gel column (elutant: hexane–$CHCl_3$ (1:1)) to give Pt-5 (**5**) (4.7 mg). Fraction C was chromatographed over silica gel using $CHCl_3$–MeOH (99:1) and then ODS using H_2O–MeOH (1:4) to afford Pt-2 (**2**) (9.7 mg). Fraction D was further separated by silica gel column using $CHCl_3$–MeOH solvent system to give $CHCl_3$–MeOH (99:1) elutant (fraction D1, 25 mg) and $CHCl_3$-MeOH (49:1) elutant (fraction D2, 8 mg). Fraction D1 was purified by ODS column using H_2O–acetonitrile (1:2) to give Pt-3 (**3**) (0.8 mg). Fraction D2 was also purified by ODS column using H_2O–MeOH (1:1) to give Pt-1 (**1**) (1.8 mg).

Data for **1** ((*E*)-9-(3,7-dimethylocta-2,6-dienyl)-3,7-dimethyl-1*H*-purine-2,6,8(3*H*,7*H*,9*H*)-trione). Colorless amorphous solid; UV (EtOH) λ_{max}, nm (log ε) 204 (4.34), 236 (3.83), 295 (4.01); IR (KBr) ν_{max} (cm^{-1}) 1693; ^1H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 332 $[M]^+$, 196 (100%), 153, 139, 81, 69; HREIMS *m*/*z* 332.1835 $[M]^+$ (332.1848 calcd for $C_{17}H_{24}N_4O_3$).

Data for **2** ((*E*)-9-(3,7-dimethylocta-2,6-dienyl)-3-methyl-1*H*-purine-2,6,8(3*H*,7*H*,9*H*)-trione). Colorless amorphous solid; UV (EtOH) λ_{max}, nm (log ε) 204 (4.31), 234 (3.81), 294 (3.99); IR (KBr) ν_{max} (cm^{-1}) 1700; ^1H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 318 $[M]^+$, 182 (100%), 137, 81, 69; HREIMS *m*/*z* 318.1688 $[M]^+$ (318.1692 calcd for $C_{16}H_{22}N_4O_3$).

Data for **3** (8-(3-methylbut-2-enyl)quinoline-2-carboxylic acid). Brownish amorphous solid; UV (EtOH) λ_{max}, nm (log ε) 206 (4.09), 239 (4.06), 314 (3.29); IR (KBr) ν_{max} (cm^{-1}) 2924, 2852, 1723; ^1H NMR and ^{13}C NMR data are shown in Table 2; EIMS *m*/*z* 241 $[M]^+$, 198 (100%), 180, 154; HREIMS *m*/*z* 241.1091 $[M]^+$ (241.1103 calcd for $C_{15}H_{15}NO_2$).

Data for **4** (1-(2-hydroxy-6-methoxy-4-(3-methylbut-2-enyloxy)phenyl)butan-1-one). Yellowish amorphous solid; UV (EtOH) λ_{max}, nm (log ε) 206 (4.26), 225 (sh, 4.11), 288 (4.19); IR (KBr) ν_{max} (cm^{-1}) 2959, 2932, 2872, 1620, 1594; ^1H NMR and ^{13}C NMR data are shown in Table 3; EIMS *m*/*z* 278 $[M]^+$, 210, 167 (100%), 69; HREIMS *m*/*z* 278.1498 $[M]^+$ (278.1518 calcd for $C_{16}H_{22}O_4$).

Data for **5** ((*E*)-1-(3-chloro-6-(3,7-dimethylocta-2,6-dienyloxy)-2,4-dihydroxyphenyl)butan-1-one). Yellowish amorphous solid; UV (EtOH) λ_{max}, nm (log ε) 205 (4.36), 229 (sh, 4.01), 293 (3.97), 329 (3.91); IR (KBr) ν_{max} (cm^{-1}) 2964, 2924, 2852, 1613, 1556; ^1H NMR and ^{13}C NMR data are shown in Table 3; EIMS *m*/*z* 368$[M+2]^+$, 366 $[M]^+$, 232, 230, 189, 187, 137, 93, 81, 69 (100%); HREIMS *m*/*z* 366.1597 $[M]^+$ (366.1598 calcd for $C_{20}H_{27}{}^{35}ClO_4$).

4.4. Isolation of Ppc-1 (6)

In a similar manner to the isolation of **1**–**5**, the cultured fruiting bodies of *P. pseudo-candidum* (dry weight 85.1 g) yielded a MeOH extract (16.9 g), which was then partitioned with EtOAc and H_2O to yield EtOAc solubles (3.60 g). The EtOAc solubles were chromatographed over SiO_2, and the column eluted with hexane–EtOAc with increasing polarity. Hexane–EtOAc elutant (55 mg) was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (φ 20.0 mm×500 mm); elutant, $CHCl_3$) to give Ppc-1 (**6**) (1.8 mg). Data for **6** (3-methylbut-2-enyl 4-methoxy-8-(3-methylbut-2-enyloxy) quinoline-2-carboxylate): colorless oil; UV (EtOH) λ_{max}, nm (log ε) 204 (4.33), 212 (sh, 4.32), 248 (4.36), 301 (3.43), 343 (3.45); IR (KBr) ν_{max} (cm^{-1}) 2925, 2855, 1715; ^1H NMR and ^{13}C NMR data are shown in Table 4; EIMS *m*/*z* 355 $[M]^+$, 286, 219 (100%), 201, 173; HREIMS *m*/*z* 355.1754 $[M]^+$ (355.1784 calcd for $C_{21}H_{25}NO_4$).

4.5. Conversion of Pt-2 (2) into 7

To a solution of **2** (2.0 mg, 6.3 μmol) in DMF (1.0 mL) were added methyl iodide (10 μL, 161 μmol) and potassium carbonate (6.3 mg, 46 μmol). After being stirred for 3 h at room temperature, the reaction mixture was poured into 0.3 M HCl, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel and eluted by $CHCl_3$–MeOH (99:1) to give **7** (1.9 mg, 5.5 μmol, 87%).

Data for **7** ((*E*)-9-(3,7-dimethylocta-2,6-dienyl)-1,3,7-trimethyl-1*H*-purine-2,6,8(3*H*,7*H*,9*H*)-trione). Colorless amorphous solid; ^1H NMR (600 MHz, $CDCl_3$) δ 5.08–5.12 (1H, m), 4.97–5.02 (1H, m), 4.65 (2H, d, *J*=6.0 Hz), 3.61 (3H, s), 3.56 (3H, s), 3.36 (3H, s), 2.07 (2H, q, *J*=7.2 Hz), 2.03 (2H, t, *J*=7.2 Hz), 1.72 (3H, d, *J*=1.0 Hz), 1.64 (3H, d, *J*=1.0 Hz), 1.56 (3H, s); ^{13}C NMR datum is shown in Table 1; EIMS *m*/*z* 346 $[M]^+$, 210 (100%), 153, 69; HREIMS *m*/*z* 346.2012 $[M]^+$ (346.2005 calcd for $C_{18}H_{26}N_4O_3$).

4.6. Conversion of Pt-5 (5) into 8

To a solution of **5** (2.1 mg, 5.7 μmol) in DMF (1.0 mL) were added methyl iodide (10 μL, 161 μmol) and potassium carbonate (6.1 mg, 44 μmol). After being stirred for 1 h at room temperature, the reaction mixture was poured into 0.3 M HCl, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue and 20% $Pd(OH)_2$ on carbon (5.5 mg) in MeOH (1.0 mL) were stirred at room temperature for 24 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was chromatographed over silica gel and eluted by hexane–EtOAc (49:1) to give **8** (1.0 mg, 2.9 μmol, 50% (two steps)). Data for **8** (2-butyl-1-(3,7-dimethyloctyloxy)-3,5-dimethoxybenzene): colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.09–6.14 (2H, m), 3.92–3.98 (2H, m), 3.79 (3H, s), 3.78 (3H, s), 2.55 (2H, t, *J*=7.5 Hz), 1.77–1.84 (1H, m), 1.68–1.73 (1H, m), 1.50–1.57 (2H, m), 1.40–1.45 (2H, m), 1.28–1.35 (6H, m), 1.13–1.18 (2H, m), 0.90–0.94 (6H, m), 0.86–0.88 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 158.8, 158.3, 112.4, 91.4, 90.5, 66.4, 55.7, 55.3, 39.3, 37.3, 36.5, 31.9, 29.8, 28.0, 24.8, 22.8, 22.7, 22.6, 22.4, 19.6, 14.1; EIMS *m*/*z* 350 $[M]^+$, 307 (100%), 169, 167; HREIMS *m*/*z* 350.2812 $[M]^+$ (350.2821 calcd for $C_{22}H_{38}O_3$).

4.7. 1-[2-Hydroxy-4,6-bis[(2-trimethylsilylethoxy)methoxy] phenyl]butan-1-one (11)

To a solution of **9**[6] (793 mg, 3.77 mmol) in dichloromethane (20 mL) was added 1.0 M boron tribromide solution in dichloromethane (20 mL) at −78 °C. After being stirred for 2 h at room temperature, the reaction mixture was poured into saturated sodium bicarbonate solution and extracted with EtOAc three times. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (2:1) to give 1 -(2,4,6-Trihydroxyphenyl)butan-1-one (**10**) (701 mg, 3.57 mmol, 95%).

To a solution of **10** (205 mg, 1.05 mmol) in dichloromethane (4.0 mL) were added 2-(trimethylsilyl)ethoxymethyl chloride (0.4 mL, 2.26 mmol) and DIPEA (0.4 mL, 2.30 mmol). After being stirred for 2.5 h at room temperature, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give **11** (411 mg, 0.90 mmol, 86%). Data for **11**: yellowish oil; ^1H NMR (400 MHz, $CDCl_3$) δ 13.79 (1H, s), 6.26 (1H, d, *J*=2.4 Hz), 6.25 (1H, d, *J*=2.4 Hz), 5.28 (2H, s), 5.20 (2H, s), 3.71–3.80 (4H, m), 2.99 (2H, t, *J*=7.4 Hz), 1.70 (2H, sextet, *J*=7.4 Hz), 0.93–1.01 (7H, m), 0.01 (9H, s), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.9, 166.8, 163.4, 160.4, 106.7, 97.2, 94.1, 92.9, 92.5, 67.1, 66.7, 46.2, 18.2, 18.01, 18.00, 14.0, −1.44 (3C), −1.45 (3C); EIMS *m*/*z* 456 $[M]^+$, 398, 355,

340, 325, 297, 281, 103, 73 (100%); HREIMS m/z 456.2361 $[M]^+$ (456.2363 calcd for $C_{22}H_{40}O_6Si_2$).

4.8. 1-[2-Methoxy-4,6-bis[(2-trimethylsilylethoxy)methoxy] phenyl]butan-1-one (12)

To a solution of **11** (188 mg, 0.41 mmol) in acetone–DMF (1:1) (4.0 mL) were added methyl iodide (75 μL, 1.20 mmol) and potassium carbonate (249 mg, 2.30 mmol). After being stirred for 6 h at room temperature, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (39:1) to give **12** (473 mg, 0.37 mmol, 90%). Data for **12**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (1H, d, J=1.9 Hz), 6.30 (1H, d, J=1.9 Hz),, 5.20 (2H, s), 5.16 (2H, s), 3.69–3.77 (4H, m), 3.76 (3H, s), 2.71 (2H, t, J=7.4 Hz), 1.68 (2H, sextet, J=7.4 Hz), 0.92–0.98 (7H, m), 0.01 (9H, s), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.5, 159.7, 157.6, 155.4, 115.5, 95.9, 93.6, 93.0, 92.9, 66.38, 66.33, 55.7, 46.9, 18.0, 17.9, 17.3, 13.7, −1.44 (3C), −1.48 (3C); EIMS m/z 470 $[M]^+$, 397, 369, 354, 339, 311 (100%), 295, 103, 73; HREIMS m/z 470.2519 $[M]^+$ (470.2520 calcd for $C_{23}H_{42}O_6Si_2$).

4.9. 1-[4-Hydroxy-2-methoxy-6-[(2-trimethylsilylethoxy) methoxy]phenyl]butan-1-one (13)

To a solution of **12** (160 mg, 0.34 mmol) in HMPA (8.0 mL) was added 1.0 M TBAF in THF (1.0 mL). After being stirred for 4 h at 50 °C, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (17:3) and hexane–EtOAc (4:1) to give 2-hydroxyl isomer (42.1 mg, 0.124 mmol, 36%) and **13** (26.4 mg, 0.076 mmol, 23%), respectively. Data for **13**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (1H, d, J=2.0 Hz), 6.07 (1H, d, J=2.0 Hz), 5.66 (1H, br s), 5.13 (2H, s), 3.69–3.73 (2H, m), 3.71 (3H, s), 2.71 (2H, t, J=7.3 Hz), 1.69 (2H, sextet, J=7.3 Hz), 0.92–0.96 (5H, m), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.1, 158.23, 158.16, 155.8, 114.2, 95.0, 92.95, 92.94, 66.5, 55.7, 47.0, 18.0, 17.5, 13.8, −1.47 (3C); EIMS m/z 340 $[M]^+$, 311, 282, 267, 239 (100%), 210, 167, 73; HREIMS m/z 340.1714 $[M]^+$ (340.1706 calcd for $C_{17}H_{28}O_5Si$).

4.10. 1-[2-Methoxy-4-(3-methylbut-2-enyloxy)-6-[(2-trimethylsilylethoxy)methoxy]phenyl] butan-1-one (14)

To a solution of **13** (14.9 mg, 44 μmol) in DMF (1.0 mL) were added 1-bromo-3-methyl-2-butene (20 μL, 0.17 mmol) and potassium carbonate (18.1 mg, 0.13 mmol). After being stirred for 3 h at room temperature, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give **14** (10.8 mg, 26 μmol, 60%). Data for **14**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (1H, d, J=2.0 Hz), 6.15 (1H, d, J=2.0 Hz), 5.44–5.48 (1H, m), 5.16 (2H, s), 4.49 (2H, d, J=6.9 Hz), 3.74 (3H, s), 3.70–3.73 (2H, m), 2.71 (2H, t, J=7.4 Hz), 1.79 (3H, d, J=0.9 Hz), 1.75 (3H, d, J=1.0 Hz), 1.67 (2H, sextet, J=7.4 Hz), 0.91–0.96 (5H, m), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.6, 161.1, 157.8, 155.7, 138.6, 119.2, 114.7, 93.9, 93.2, 92.9, 66.4, 64.9, 55.7, 47.0, 25.8, 18.2, 18.0, 17.4, 13.8, −1.4 (3C); EIMS m/z 408 $[M]^+$, 378, 350, 335, 307, 267, 239 (100%), 223, 167, 73; HREIMS m/z 408.2346 $[M]^+$ (408.2332 calcd for $C_{22}H_{36}O_5Si$).

4.11. Synthesis of Pt-4 (4)

To a solution of **14** (10.8 mg, 26 μmol) in HMPA (1.0 mL) was added 1.0 M TBAF in THF (50 μL). After being stirred for 4 h at room temperature, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give Pt-4 (**4**) (3.3 mg, 12 μmol, 45%). All spectral data of the synthetic product were identical with those of the natural product.

4.12. 1-(3-Chloro-2,4,6-trihydroxyphenyl)butan-1-one (15)

To a solution of **10** (106 mg, 0.54 mmol) in $CHCl_3$ (5.0 mL) were added ethanol (50 μL) and sulfuryl chloride (72.5 mg, 0.54 mmol) at 0 °C. After being stirred for 30 min, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by $CHCl_3$–MeOH (19:1) to give **15** (119 mg, 0.51 mmol, 95%). Data for **15**: yellowish amorphous solid; ^{1}H NMR (400 MHz, acetone-d_6) δ 6.13 (1H, s), 3.06 (2H, t, J=7.3 Hz), 1.65 (2H, sextet, J=7.3 Hz), 0.94 (3H, t, J=7.3 Hz); ^{13}C NMR (100 MHz, acetone-d_6) δ 206.8, 161.8, 161.7, 160.2, 105.5, 100.1, 95.7, 46.4, 18.6, 14.1; EIMS m/z 232 $[M+2]^+$, 230 $[M]^+$, 189, 187 (100%); HREIMS m/z 230.0335 $[M]^+$ (230.0246 calcd for $C_{10}H_{11}{}^{35}ClO_4$).

4.13. 1-[3-Chloro-2-hydroxy-4,6-bis[(2-trimethylsilylethoxy) methoxy]phenyl]butan-1-one (16)

To a solution of **15** (80.3 mg, 0.35 mmol) in dichloromethane (2.0 mL) were added 2-(trimethylsilyl)ethoxymethyl chloride (0.13 mL, 0.73 mmol) and DIPEA (0.13 mL, 0.75 mmol). After being stirred for 30 min at room temperature, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (99:1) and hexane–EtOAc (49:1) to give **16** (91.0 mg, 0.19 mmol, 53%) and tri-SEM compound (62.9 mg, 0.10 mmol, 29%), respectively. Data for **16**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.99 (1H, s), 6.59 (1H, s), 5.30 (2H, s), 5.20 (2H, s), 3.75–3.81 (4H, m), 3.14 (2H, t, J=7.3 Hz), 1.70 (2H, sextet, J=7.3 Hz), 0.92–0.98 (7H, m), 0.01 (9H, s), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 163.2, 158.7, 155.7, 111.8, 108.5, 100.3, 99.0, 93.4, 68.9, 67.2, 45.2, 18.2, 17.98, 17.97, 13.8, −1.4 (3C), −1.5 (3C); EIMS m/z 492 $[M+2]^+$, 490 $[M]^+$, 361, 359 (100%), 333, 331, 103, 73; HREIMS m/z 490.2012 $[M]^+$ (490.2012 calcd for $C_{22}H_{39}{}^{35}ClO_6Si_2$).

4.14. (*E*)-1-[3-Chloro-6-(3,7-dimethylocta-2,6-dienyloxy)-2-hydroxy-4-[(2-trimethylsilylethoxy)-methoxy]phenyl]butan-1-one (17) and Pt-5 (5)

To a solution of **16** (70.3 mg, 0.14 mmol) in DMF (1.0 mL) were added geranyl bromide (30 μL, 0.14 mmol) and potassium carbonate (39.8 mg, 0.29 mmol). After being stirred for 1.5 h at room temperature, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (99:1) and hexane–EtOAc (19:1) to give **17** (17.5 mg, 35 μmol, 25%) and Pt-5 (**5**) (10.1 mg, 28 μmol, 19%), respectively. Data for **17**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.51 (1H, s), 6.33 (1H, s), 5.46–5.50 (1H, m), 5.33 (2H, s), 5.05–5.08 (1H, m), 4.58 (2H, d, J=6.8 Hz), 3.78–3.82 (2H, m), 2.98 (2H, t, J=7.5 Hz), 2.07–2.15 (4H, m), 1.75 (3H, s), 1.62–1.70 (5H, m), 1.59 (3H, s), 0.93–0.97 (5H, m), 0.00 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 161.8, 160.5, 158.9, 142.7, 132.0, 123.5, 118.0, 107.0,

103.5, 93.2, 90.7, 67.1, 65.8, 46.6, 39.5, 26.3, 25.6, 18.3, 18.1, 17.7, 16.7, 13.9, −1.4 (3C); EIMS *m*/*z* 498 $[M+2]^+$, 496 $[M]^+$, 362, 360, 304, 302 (100%), 261, 259, 137, 81, 73; HREIMS *m*/*z* 496.2397 $[M]^+$ (496.2412 calcd for $C_{26}H_{41}{}^{35}ClO_5Si$). All spectral data of synthetic Pt-5 (**5**) were identical with those of the natural product.

4.15. Conversion of 17 into Pt-5 (5)

To a solution of **17** (10.0 mg, 20 μmol) in HMPA (1.0 mL) was added 1.0 M TBAF in THF (30 μL). After being stirred for 4 h at 50 °C, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane–EtOAc (19:1) and hexane–EtOAc (9:1) to give recovered **17** (1.8 mg, 4 μmol, 18%) and **5** (4.0 mg, 11 μmol, 54%), respectively.

4.16. Synthesis of Ppc-1 (6)

To a solution of **18**[17] (11.6 mg, 50 μmol) in MeOH–water (9:1) (2.0 mL) was added sodium hydroxide (50 mg). After being refluxed for 30 min, the reaction mixture was acidified by 3 M HCl, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated to give crude quinolobactin (**19**). This crude was dissolved in DMF (1.0 mL), and 1-bromo-3-methyl-2-butene (20 μL, 0.17 mmol) and potassium carbonate (30.1 mg, 0.22 mmol) were added. After being stirred for 14 h, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue chromatographed over silica gel eluted by hexane–EtOAc (19:1) to give Ppc-1 (**6**) (15.6 mg, 44 μmol, 87% (two steps)). All spectral data of the synthetic product were identical with those of the natural product.

4.17. Assay for cell growth in K562, HeLa, and 3T3-L1 cells

K562 cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with RPMI growth medium (RPMI1640 medium with 10% [v/v] fetal bovine serum, 25 μg/ml penicillin, and 50 μg/ml streptomycin). HeLa and 3T3-L1 cells were maintained at 37 °C (5% CO_2) in DMEM-HG (Dulbecco's modified Eagle's medium containing a high concentration (4,500 mg/l) of glucose supplemented with 25 μg/ml penicillin, 50 μg/ml streptomycin and 10% [v/v] fetal bovine serum). K562 cells (2×10^4 cells/well), HeLa and 3T3-L1 cells ($3–10\times10^3$ cells/well) were allowed to grow for 3 days in 12-well plates; each well contained 1 ml of RPMI (for K562 cells) or DMEM-HG (for HeLa and 3T3-L1 cells) with DMSO or sample compounds. The relative cell number was assessed using Alamar blue (cell number indicator) as described previously.[6,8b]

4.18. Assay for glucose consumption in confluent 3T3-L1 cells

3T3-L1 cells were transferred into 12-well plates, each containing 1 ml of DMEM-HG, and incubated for 5–6 days until they reached the confluent state with exchanging DMEM-HG every 2–3 days. The cells were then pre-incubated for 2–3 days with 1 ml of DMEM-MG [DMEM containing a medium concentration (2,000 mg/l) of glucose supplemented with the antibiotics, 10% [v/v] fetal bovine serum, and 10 mM HEPES–NaOH (pH 7.4)] with exchanging the media every day. The confluent cells thus obtained were treated for the appropriate number of hours with 1 ml of DMEM-MG containing DMSO (vehicle) or sample compounds. Aliquots of the incubation media were then assayed for their glucose concentration using a blood glucose test meter and appropriate sensor chips, and the rate of glucose consumption was calculated as previously described.[8]

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Supplementary data

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