Ten new aurovertins from cultures of the basidiomycete *Albatrellus confluens*

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**Abstract:** Aurovertins J–S (1–10), together with four known metabolites, aurovertins B, C, E, and I (11–14), were isolated from cultures of the basidiomycete *Albatrellus confluens*. The structures of compounds 1–10 were elucidated on the basis of extensive spectroscopic analysis. All compounds were evaluated for their cytotoxic activities on five tumor cell lines.

**Keywords:** *Albatrellus confluens*, aurovertins, cytotoxic activities

**Introduction**

The first four aurovertin-type compounds, aurovertins A–D, were isolated as toxic substances from fermentation broth of the fungus *Calcarisporium arbuscula*\(^1\). Aurovertins are a class of compounds with the basic structure of 2,6-dioxabicyclo[3.2.1]octane ring system and a conjugated \(\alpha\)-pyrone moiety originated from polyketide pathway. They have attracted considerable attention of chemists because of their activities as potent inhibitors of ATP synthesis and antitumor agents\(^2–7\). There have been nine aurovertins (A–I) structurally elucidated to date\(^1,8–10\), while the relative and absolute configurations of aurovertin B were determined by spectroscopic analysis and total synthesis, respectively\(^11,12\). The absolute configuration of aurovertin F was determined by analysis of the CD spectrum recently\(^9\). It is deduced that aurovertin-type of compounds obtained in literature probably share the same absolute configuration on biosynthetic grounds\(^10,13\). During our continuing investigation of bioactive microbial secondary metabolites from the fungus\(^8,14–23\), ten new aurovertin derivatives, named as aurovertins J–S (1–10), along with four previously reported aurovertins B, C, E and I (11–14), were isolated from cultures of the basidiomycete *Albatrellus confluens* (Alb. et Schw.: Fr.) Kolt. et Pouz. All compounds were evaluated for their cytotoxicities against five human cancer cell lines. This paper deals with the isolation, structural elucidation, and bioactivities of these isolates.

**Results and Discussion**

A total of 14 aurovertins including 10 previously-unreported compounds were isolated from cultures of the basidiomycete *Albatrellus confluens*. Comparison with all the reported nine aurovertin-type metabolites to date, it should be noticed that the methyl (C-22) in the moiety of \(\alpha\)-pyrone in aurovertins was missing in aurovertin J (1) and aurovertin K (2), the moiety of...
alpha-pyrones was degraded in aurovertin L (3) and the polynene side chain was partly oxidized in aurovertins J–S (5–10). In addition, compounds 7 and 11 exhibited certain cytotoxicities.

Aurovertin J (1) was obtained as yellow syrup. The molecular formula of 1 was determined to be C_{17}H_{24}O_{5} on the basis of HREIMS m/z 446.1934 (calcld for 446.1941), corresponding to ten degrees of unsaturation. The IR spectrum showed absorption bands for hydroxy group (3441 cm\(^{-1}\)) and double bonds (1632 cm\(^{-1}\)). The \(^{1}H\) and \(^{13}C\) NMR spectra (Tables 1 and 3) of 1 were similar to those of aurovertin B (11)\(^{3}\) except for the loss of the methyl group at C-16. The upfield shift of C-16 (δc 101.0, d) clearly indicated that 1 was a demethylated analogue of 11, which was supported by the observed HMBC correlations from δc 5.84 (1H, d, J = 1.9 Hz, H-16) to δc 122.0 (d, C-14), 158.6 (s, C-15), 171.0 (s, C-17), and 88.7 (d, C-18). In the light of the evidences mentioned above and the key \(^{1}H\)-\(^{1}H\) COSY and HMBC correlations (Figure 2), the planar structure of 1 was therefore elucidated as shown in Figure 1, named aurovertin J. The key ROESY correlations of H-2 with H-8, H-3 with Me-20, and H-5 with H-7 were observed in the ROEY spectrum of compound 1 (Figure 2), which established the relative stereochemistry of 1 as that of 11. Because the NMR data and other physico-chemical properties of 1 are very similar to those of 11, the absolute configuration and geometrical isomerism of both compounds are suggested to be the same. Therefore, the structure of aurovertin J (1) was established, as shown.

Aurovertin K (2) was inferred to possess the molecular formula C_{17}H_{24}O_{5} on the basis of its HREIMS at m/z 404.1832 [M\(^{+}\)]. Preliminary analysis of the NMR data also indicated that 2 possessed a similar skeleton to that of aurovertin I (14).\(^{10}\) The same demethylation at C-16 (δc 101.0) was concluded by the observed HMBC correlations from H-16 to C-14, C-15, C-17, and C-18 as the same to those of 1. In addition, a methyl carbon at δc 26.5 (q) was assigned to be placed at C-8 according to the HMBC correlations from δc 1.26 (3H, s, H-8) to δc 79.6 (d, C-7), 78.2 (s, C-8), and 147.1 (d, C-9). The ROEY correlations of Me-2 with Me-8, H-3 with Me-20, and H-5 with H-7 indicated the same configuration to that of 1. Thus, compound 2 (aurovertin K) was established, as shown.

Aurovertin L (3) exhibited an ion peak at m/z 324.1564 [M\(^{+}\)] in the HREIMs, in agreement with the molecular formula C_{17}H_{24}O_{5}. Preliminary analysis of 1D NMR data suggested that compound 3 was still an aurovertin derivative, as indicated by the characteristic carbon signals for C-1 to C-8, as well as the olefinic carbons for C-9 to C-12 (Tables 1 and 3). However, compound 3 might be identified as a degraded product of 11, in which the double bond between C-13 and C-14 was cleaved, then an aldehyde group was formed at C-13 (δc 194.0, d), as supported by the HMBC correlation from δc 9.58 (1H, d, J = 8.0 Hz, H-13) to δc 151.3 (d, C-11) and 132.1 (d, C-12). Further analysis of other spectroscopic data (HSQC, HMBC, \(^{1}H\)-\(^{1}H\) COSY, ROESY) established the structure of compound 3 (aurovertin L) as shown in Figure 1.

**Table 1. \(^{1}H\) NMR spectroscopic data of aurovertins J–N (1–5) in CDCl\(_{3}\)**

| no. | 1\(^{a}\) | 2\(^{a}\) | 3\(^{a}\) | 4\(^{a}\) | 5\(^{a}\) |
|-----|----------|----------|----------|----------|----------|
| 1   | 1.09 (3H, t, 7.5) | 1.09 (3H, t, 7.5) | 1.08 (3H, t, 7.6) | 1.00 (3H, t, 7.5) |
| 2   | 1.70 (2H, m) | 1.65 (2H, br. s) | 1.68 (2H, m) | 1.61 (2H, m) |
| 3   | 3.93 (1H, dd, 8.8, 4.3) | 3.95 (1H, dd, 9.0, 4.6) | 3.91 (1H, dd, 8.8, 4.3) | 3.91 (1H, dd, 8.7, 4.6) |
| 4   | 4.80 (1H, s) | 4.80 (1H, s) | 4.80 (1H, s) | 3.42 (1H, s) |
| 5   | 3.28 (1H, d, 8.3) | 3.30 (1H, d, 8.5) | 3.28 (1H, br. s) | 3.12 (1H, d, 8.1) |
| 6   | 4.14 (1H, dd, 8.3, 6.5) | 4.20 (1H, dd, 8.5, 5.2) | 4.14 (1H, dd, 7.1, 6.5) | 3.83 (1H, m) |
| 7   | 5.94 (1H, dd, 14.5, 6.3) | 5.98 (1H, dd, 15.2) | 6.37 (1H, dd, 15.3, 5.2) | 5.92 (1H, dd, 14.3, 6.5) |
| 8   | 6.45 (1H, dd, 14.5, 10.6) | 6.34 (1H, dd, 15.2, 10.9) | 6.63 (1H, dd, 15.3, 11.0) | 6.45 (1H, overlapped) |
| 9   | 6.50 (1H, dd, 14.5, 10.6) | 6.44 (1H, dd, 15.2, 10.9) | 7.12 (1H, dd, 15.3, 11.0) | 6.46 (1H, overlapped) |
| 10  | 6.33 (1H, dd, 14.5, 11.3) | 6.29 (1H, dd, 14.5, 10.9) | 6.18 (1H, dd, 15.3, 8.0) | 6.35 (1H, overlapped) |
| 11  | 7.16 (1H, dd, 15.2, 11.3) | 7.15 (1H, dd, 15.2, 10.9) | 9.58 (1H, d, 8.0) | 7.16 (1H, dd, 14.7, 11.3) |
| 12  | 6.04 (1H, dd, 15.2) | 6.04 (1H, dd, 15.2) | 6.33 (1H, d, 14.7) | 6.34 (1H, d, 15.2) |
| 13  | 5.84 (1H, d, 1.9) | 5.84 (1H, d, 1.9) | 5.48 (1H, s) | 5.50 (1H, s) |
| 14  | 5.45 (1H, d, 1.9) | 5.46 (1H, d, 1.9) | 5.48 (1H, s) | 5.50 (1H, s) |
| 15  | 1.91 (3H, s) | 1.33 (3H, s) | 1.21 (3H, s) | 1.17 (3H, s) |
| 16  | 1.27 (3H, s) | 1.36 (3H, s) | 1.27 (3H, s) | 1.24 (3H, s) |
| 17  | 1.95 (3H, s) | 1.95 (3H, s) | 1.35 (3H, s) | 1.35 (3H, s) |
| 18  | 1.26 (3H, s) | 2.17 (3H, s) | 2.17 (3H, s) | 2.43 (2H, m) |
| 19  | 3.80 (3H, s) | 3.81 (3H, s) | 3.82 (3H, s) | 3.82 (3H, s) |

\(^{a}\)Measured at 600 MHz; \(^{b}\)Measured at 500 MHz; \(^{c}\)Measured at 400 MHz.
The HREIMS indicated the molecular formula of aurovertin M (4) to be C_{29}H_{30}O_{10} by the ion peak at m/z 474.2264 [M^+]. The NMR data of 4 resembled those of 11 except that the acetoxy group at C-5 was replaced by a propionyloxy group, which was supported by the ^1H-^1H COSY correlation between δ_H 2.43 (2H, m, H-2') and 1.19 (3H, t, J = 7.6 Hz, Me-3'), as well as the HMBC correlation from Me-3' to δ_C 27.5 (t, C-2') and 173.3 (s, C-1'). Accordingly, compound 4 was identified as aurovertin M.

Aurovertin N (5) was isolated as yellow syrup. The molecular formula C_{29}H_{30}O_{10} was determined by HREIMS at m/z 436.2097 [M^+]. The 1D and 2D NMR data suggested that the structure of 5 was related to that of aurovertin E (13), except that the double bond between C-9 and C-10 in 14 was opened to form a hydroxymethine group at C-10 (δ_C 69.8), as implied by the HMBC correlations from δ_H 4.53 (1H, br, s, H-10) to δ_C 75.9 (d, C-8), 39.6 (t, C-9), and 141.5 (d, C-11). Detailed analysis of other spectroscopic data suggested that the other parts of 5 were the same to those of 13. Thus, compound 5 was established as aurovertin N.

The same to 5, all the spectral data suggested that compound 6 also showed similar patterns to those of aurovertin B (11), except that the double bond between C-9 and C-10 was opened to form a hydroxymethine group at C-10 (δ_C 69.8), as implied by the HMBC correlations from δ_H 4.53 (1H, br, s, H-10) to δ_C 75.9 (d, C-8), 39.6 (t, C-9), and 141.5 (d, C-11). Detailed analysis of other spectroscopic data suggested that the other parts of 6 were the same to those of 11. Thus, compound 6 was established as aurovertin O.

Aurovertin P (7) had the molecular formula C_{29}H_{30}O_{10} as established by the HREIMS at m/z 494.2157 [M^+]. All the data suggested that the structure of 7 was closely related to that of 11. A significant change was that C-9 and C-12 in 7 were two oxygennated methines at δ_C 72.3 (C-9) and 71.8 (C-12) instead of two olefinic carbons in 11, which was supported by the HMBC correlations from δ_H 4.33 (1H, m, H-9) to δ_C 79.3 (d, C-8) and 130.0 (d, C-10), and from δ_H 4.84 (1H, m, H-12) to δ_C 131.7 (d, C-11) and 138.6 (d, C-13), as well as the 1H-1H COSY correlations from H-8 to H-13. Further analysis of other 2D NMR data suggested that the other parts were the same to those of 11. Therefore, compound 7 was established as aurovertin P, as shown.

The NMR data of aurovertin Q (8) were closely related to those of 7, except for the existence of a methoxy group at C-12 (δ_C 81.0) in 8 rather than the hydroxy group at C-12 in 7, as established by the HMBC correlation of δ_H 3.34 (3H, s, OMe) to δ_C 81.0 (d, C-12). Thus, compound 8 was elucidated as aurovertin Q, as shown.

Aurovertin R (9) possessed a molecular formula C_{30}H_{32}O_{10} according to the ion peak at m/z 494.2144 [M^+] in the HREIMS. The NMR data suggested that the structure of 9 was related to that of 6, except for one more hydroxy group placed at C-9, as indicated by the HMBC correlation from δ_H 3.79 (1H, m, H-9) to δ_C 77.0 (d, C-8) and 71.3 (d, C-10), as well as the 1H-1H COSY correlation from H-8 to H-10. Thus, compound 9 was determined as aurovertin R, as shown.

The NMR data suggested that aurovertin S (10) had the related structure to that of 9 except for the methoxy group at C-10 in 10 rather than the hydroxy group in 9, as indicated by the signal at δ_H 3.34 (3H, s, OMe), as well as the HMBC correlation from δ_H 3.34 (3H, s, OMe) to δ_C 83.1 (d, C-10). Detailed analysis of other 2D NMR data suggested that the other parts of 10 were the same to those of 9. Compound 10 was, therefore, determined as aurovertin S, as shown.

The structures of the known compounds 11–14 isolated were identified as aurovertin B, aurovertin C, aurovertin E, and aurovertin L, respectively, by comparison of their spectroscopic data with literature values. All aurovertins

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Table 2. ^1H NMR spectroscopic data of aurovertins O–S (6–10) in CDCl₃

| no. | δ_H (ppm) | δ_C (ppm) |
|-----|-----------|-----------|
| 6   | 1.03 (3H, t, 7.5) | 1.03 (3H, t, 7.5) |
| 2   | 1.03 (3H, t, 7.5) | 1.03 (3H, t, 7.5) |
| 3   | 3.89 (1H, m) | 3.88 (1H, dd, 9.0, 4.2) |
| 4   | 4.77 (1H, s) | 4.69 (1H, s) |
| 5   | 3.24 (1H, d, 8.3) | 3.53 (1H, d, 7.9) |
| 25  | 3.82 (1H, m) | 3.70 (1H, dd, 7.9, 4.3) |
| 9   | 2.02 (1H, m), 1.93 (1H, m) | 4.34 (1H, overlapped) |
| 10  | 4.53 (1H, br, s) | 5.87 (1H, overlapped) |
| 11  | 6.06 (1H, dd, 15.2, 4.6) | 5.69 (1H, dd, 15.5, 7.2) |
| 12  | 6.50 (1H, dd, 15.2, 11.3) | 4.32 (1H, overlapped) |
| 13  | 7.18 (1H, dd, 15.2, 11.3) | 6.62 (1H, dd, 15.5, 6.4) |
| 14  | 6.36 (1H, dd, 15.2) | 5.50 (1H, s) |
| 15  | 5.51 (1H, s) | 5.50 (1H, s) |
| 20  | 1.15 (3H, s) | 1.14 (3H, s) |
| 21  | 1.25 (3H, s) | 1.27 (3H, s) |
| 22  | 1.95 (3H, s) | 1.95 (3H, s) |
| 23  | 2.15 (3H, s) | 2.14 (3H, s) |

*Measured at 600 MHz; *Measured at 500 MHz; *Measured at 400 MHz.
Table 3. 13C NMR spectroscopic data of aurovertins J–S (1–10) in CDCl3

| no. | 1° | 2° | 3° | 4° | 5° | 6° | 7° | 8° | 9° | 10° |
|-----|----|----|----|----|----|----|----|----|----|-----|
| 1   | 11.8 q | 11.7 q | 11.7 q | 11.9 q | 11.8 q | 11.7 q | 11.7 q | 11.8 q | 11.9 q |
| 2   | 20.1 t | 13.2 q | 20.1 t | 20.1 t | 20.1 t | 20.1 t | 20.1 t | 20.2 t | 20.1 t | 20.3 t |
| 3   | 85.5 d | 80.3 d | 85.5 d | 84.6 d | 85.3 d | 85.2 d | 85.2 d | 85.4 d | 85.3 d |
| 4   | 82.7 s | 83.0 s | 82.9 s | 83.9 s | 82.8 s | 82.5 s | 82.6 s | 82.8 s | 82.6 s |
| 5   | 80.4 d | 75.4 d | 80.2 d | 80.3 d | 80.3 d | 80.4 d | 80.3 d | 80.5 d | 80.2 d |
| 6   | 83.4 s | 82.6 s | 83.4 s | 84.1 s | 83.3 s | 83.5 s | 83.3 s | 83.3 s | 83.5 s |
| 7   | 76.3 d | 79.6 d | 76.2 d | 76.2 d | 76.3 d | 76.1 d | 75.4 d | 72.6 d | 71.9 d |
| 8   | 77.9 d | 78.2 s | 77.2 d | 77.8 d | 75.9 d | 79.3 d | 79.6 d | 77.0 d | 76.2 d |
| 9   | 134.5 d | 147.1 d | 142.1 d | 134.2 d | 39.6 t | 39.6 t | 72.5 d | 72.8 d | 75.6 d | 71.9 d |
| 10  | 131.5 d | 128.0 d | 128.4 d | 131.6 d | 69.8 d | 69.8 d | 130.0 d | 131.9 d | 71.3 d | 83.1 d |
| 11  | 137.6 d | 137.7 d | 151.3 d | 137.0 d | 141.5 d | 141.5 d | 131.7 d | 130.3 d | 138.3 d | 135.2 d |
| 12  | 131.5 d | 130.9 d | 132.1 d | 132.0 d | 128.5 d | 128.7 d | 71.8 d | 81.0 d | 130.5 d | 133.9 d |
| 13  | 135.9 d | 135.8 d | 194.0 d | 135.6 d | 135.3 d | 135.1 d | 138.6 d | 136.8 d | 135.1 d | 134.3 d |
| 14  | 122.0 d | 121.9 d | 119.4 d | 119.4 d | 119.3 d | 117.4 d | 118.6 d | 119.8 d | 120.3 d |
| 15  | 158.6 s | 158.7 s | 154.2 s | 154.3 s | 154.2 s | 153.7 s | 153.4 s | 154.2 s | 152.8 s |
| 16  | 101.0 d | 101.0 d | 108.0 s | 108.0 s | 108.0 s | 108.2 s | 108.3 s | 108.2 s | 108.6 s |
| 17  | 171.0 s | 171.1 s | 170.6 s | 170.8 s | 170.7 s | 170.9 s | 170.7 s | 170.7 s | 170.5 s |
| 18  | 88.7 d | 88.7 d | 88.7 d | 88.7 d | 88.7 d | 88.7 d | 89.1 d | 88.9 d | 89.2 d |
| 19  | 164.1 s | 164.0 s | 163.7 s | 163.9 s | 163.8 s | 164.1 s | 163.7 s | 163.7 s | 163.6 s |
| 20  | 16.4 q | 17.3 q | 16.3 q | 16.4 q | 16.5 q | 16.5 q | 16.4 q | 16.4 q | 16.5 q |
| 21  | 15.0 q | 18.6 q | 15.0 q | 15.0 q | 14.6 q | 15.0 q | 15.0 q | 14.9 q | 15.1 q |
| 22  | 8.8 q | 8.8 q | 8.8 q | 8.8 q | 8.7 q | 8.9 q | 8.8 q | 8.9 q | 8.9 q |

Me-8  26.5 q
1°  169.9 s  169.9 s  173.3 s  169.9 s  170.0 s  170.0 s  169.8 s  169.7 s
2°  20.8 q  20.8 q  27.5 t  20.8 q  20.7 q  20.8 q  20.7 q  20.7 q
3°  9.2 q
OMe-10  57.0 q
OMe-12  56.2 q
OMe-17  56.9 q  55.9 q  56.1 q  56.2 q  56.2 q  56.2 q  56.3 q  56.2 q  56.2 q

*Measured at 150 MHz; †Measured at 125 MHz; ‡Measured at 100 MHz.

obtained from cultures of the basidiomycete A. confluentus were yellow syrup, and could not obtain as crystals in diverse solution systems. In order to elucidate the absolute configuration of the side chain in compounds 5–10, modified Mosher reaction25 of compound 5 and acylation of compound 7 with 4-bromobenzoxy chloride26 for crystals were all in failure. Thus, the stereocountries of compounds 5–10 were not determined wholly.

All compounds were evaluated for their cytotoxicities against five human cancer cell lines. The results showed that compounds 7 and 11 exhibited moderate cytotoxicities compared with those of cisplatin (Table 4), while the other compounds were inactive (IC50 > 40 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco-P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instruments of Avance III 600 or Bruker DRX-500 or Bruker AV 400. ESIMS and HREIMS were measured on Bruker HCT/Esquire and VG Autospec-3000 mass spectrometer respectively. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 x 150 mm) column. Preparative MPLC was performed on a Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605 and manager C-615. Silica gel (200–300 mesh and 80–100 mesh, Qingdao Marine Chemical Inc., China), RP-18 gel (40–75 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots visualized by heating silica gel plates immersed in vanillin-H2SO4 in EtOH.

Fungal Material and Cultivation Conditions. The fungus A. confluentus was collected from Ailao Mountain of Yunnan Province, China, in July 2003, and identified by Prof. Mu Zang, Kunming Institute of Botany. The voucher specimen
Aurovertin M (4): yellow syrup; \([\alpha]_D^{15} = 38.5 (c 0.40, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 359 (3.5), 271 (3.6), 227 (3.4), 199 (3.4) \text{ nm; IR (KBr) } v_{\text{max}} 3441, 1630, 1110 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 600 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 150 \text{ MHz data, see Tables 1 and 3; ESIMS (positive) } m/z 497 [M + Na]; \text{HREIMS m/z } 474.2264 \text{ (calcd for C}_{20}H_{32}O_{10}, 474.2254).}

Aurovertin N (5): yellow syrup; \([\alpha]_D^{15} = 10.6 (c 0.31, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 337 (3.2), 246 (3.4), 229 (3.4), 207 (3.3) \text{ nm; IR (KBr) } v_{\text{max}} 3442, 1632 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 600 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz data, see Tables 1 and 3; ESIMS (positive) } m/z 459 [M + Na]; \text{HREIMS m/z } 436.2097 \text{ (calcd for C}_{19}H_{28}O_{10}, 436.2097).}

Aurovertin O (6): yellow syrup; \([\alpha]_D^{15} = 13.9 (c 0.30, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 320 (2.9), 226 (3.2) \text{ nm; IR (KBr) } v_{\text{max}} 3442, 1631 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 600 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 150 \text{ MHz data, see Tables 2 and 3; ESIMS (positive) } m/z 501 [M + Na]; \text{HREIMS m/z } 478.2190 \text{ (calcd for C}_{20}H_{32}O_{10}, 478.2203).}

Aurovertin P (7): yellow syrup; \([\alpha]_D^{15} = 10.3 (c 0.20, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 319 (3.2), 225 (3.7) \text{ nm; IR (KBr) } v_{\text{max}} 3443, 1638, 1251, 1037 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 400 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz data, see Tables 2 and 3; ESIMS (positive) } m/z 517 [M + Na]; \text{HREIMS m/z } 494.2157 \text{ (calcd for C}_{20}H_{32}O_{10}, 494.2152).}

Aurovertin Q (8): yellow syrup; \([\alpha]_D^{15} = 13.4 (c 0.30, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 333 (3.2), 245 (3.4), 227 (3.5), 197 (3.3) \text{ nm; IR (KBr) } v_{\text{max}} 3440, 1631 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 400 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz data, see Tables 2 and 3; ESIMS (positive) } m/z 531 [M + Na]; \text{HREIMS m/z } 508.2305 \text{ (calcd for C}_{20}H_{32}O_{10}, 508.2308).}

Aurovertin R (9): yellow syrup; \([\alpha]_D^{15} = 4.2 (c 0.30, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 340 (3.3), 247 (3.5), 212 (3.5) \text{ nm; IR (KBr) } v_{\text{max}} 3449, 1639, 1038 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 500 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 125 \text{ MHz data, see Tables 2 and 3; ESIMS (positive) } m/z 517 [M + Na]; \text{HREIMS m/z } 494.2144 \text{ (calcd for C}_{20}H_{32}O_{10}, 494.2152).}

Aurovertin S (10): yellow syrup; \([\alpha]_D^{15} = 70.0 (c 0.20, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 338 (3.2), 248 (3.4), 197 (3.2) \text{ nm; IR (KBr) } v_{\text{max}} 3443, 1632 \text{ cm}^{-1}; ^1H \text{ NMR (CDCl}_3, 600 \text{ MHz) and } ^{13}C \text{ NMR (CDCl}_3, 150 \text{ MHz data, see Tables 2 and 3; ESIMS (positive) } m/z 531 [M + Na]; \text{HREIMS m/z } 508.2326 \text{ (calcd for C}_{20}H_{32}O_{10}, 508.2308).}

Cytotoxic Assay. All compounds were evaluated for their cytotoxicity against five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human
myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The cytotoxicity assay was performed according to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method in 96-well microplates. Briefly, 100 µL of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1 × 10⁵ cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 µM in triplicates for 48 h, and all tests were done in twice with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC₅₀ values were calculated by Reed and Muench’s method.²⁷

Electronic Supplementary Material
Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-012-0088-y and is accessible for authorized users.

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