JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Aflaquinolones A–G: Secondary Metabolites from Marine and Fungicolous Isolates of *Aspergillus* spp.

Scott A. Neff,[†] Sang Un Lee,[‡] Yukihiro Asami,[§] Jong Seog Ahn,[§] Hyuncheol Oh,[‡] Jonas Baltrusaitis,[†] James B. Gloer,*[,†] and Donald T. Wicklow[⊥]

[†]Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States

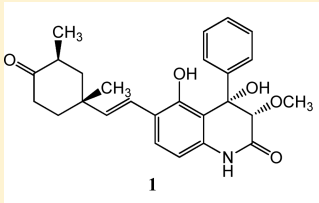
[‡]College of Medical and Life Sciences, Silla University, Busan 617-736, Korea

[§]Korea Research Institute of Bioscience and Biotechnology (KRIBB), 52 Eoun-dong, Yuseong-gu, Daejeon 305-333, Republic of Korea

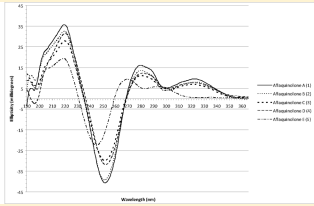
[⊥]Bacterial Foodborne Pathogens and Mycology Research Unit, Agricultural Research Service, National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604, United States

Supporting Information

ABSTRACT: Seven new compounds (aflaquinolones A–G; **1**–**7**) containing dihydroquinolin-2-one and terpenoid units have been isolated from two different fungal sources. Two of these metabolites (**1** and **2**) were obtained from a Hawaiian fungicolous isolate of *Aspergillus* sp. (section *Flavipedes*; MYC-2048 = NRRL 58570), while the others were obtained from a marine *Aspergillus* isolate (SF-5044) collected in Korea. The structures of these compounds were determined mainly by analysis of NMR and MS data. Relative and absolute configurations were assigned on the basis of NOESY data and ^{1}H NMR *J*-values, comparison of calculated and experimental ECD spectra, and analysis of a Mosher's ester derivative of **2**. Several known compounds, including alantrypinone, aspochalasins I and J, methyl 3,4,5-trimethoxy-2((2-((3-pyridinylcarbonyl)amino)benzoyl)-amino)benzoate, and *trans*-dehydrocurvularin were also encountered in the extract of the Hawaiian isolate.



1



Our long-term studies of fungi from a variety of ecological groups have resulted in the discovery of many new bioactive natural products.[1−3] Fungicolous and mycoparasitic isolates from Hawaii have been a productive source of such compounds over the past few years,[4−6] as have marine fungi.[7] Chemical investigations of two isolates of *Aspergillus* sp. (Trichocomaceae) obtained from these two different habitats carried out independently in our two laboratories led to the isolation of seven new dihydroquinolin-2-one-containing natural products that we named aflaquinolones A–G (**1**–**7**). These compounds are members of a known general class of fungal metabolites that includes aspoquinolones and penigequinolones.[8−10] Details of the isolation and structure elucidation of these compounds are presented here.

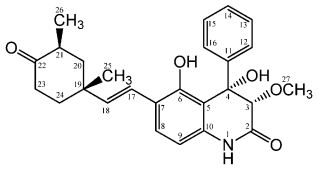
RESULTS AND DISCUSSION

A culture of *Aspergillus* sp. (MYC-2048 = NRRL 58570) was obtained from a basidioma of *Rigidoporus microsporus* found on a dead hardwood branch in a Hawaiian alien wet forest. The fungus was cultured by solid-substrate fermentation on rice, and the EtOAc extract of the resulting fermentation mixture showed antifungal activity against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) as well as the ability to reduce the growth rate of *Spodoptera frugiperda* (fall armyworm) in a dietary assay. The extract was therefore subjected to chemical investigation, leading to the isolation of compounds **1** and **2**, as well as the known metabolites alantrypinone,[11] aspochalasins I and J,[12] methyl 3,4,5-trimethoxy-2((2-((3-pyridinylcarbonyl)amino)benzoyl)amino)benzoate,[13] and *trans*-dehydrocurvularin.[14] The known compounds were identified by analysis of MS and NMR spectroscopic data in comparison with literature values.[11−14]

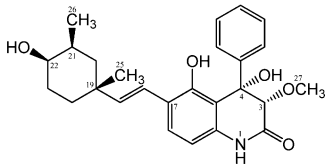
Aflaquinolone A (**1**) was found to have the molecular formula $C_{26}H_{29}NO_5$ (13 unsaturations) on the basis of HRESITOFMS and NMR data. The ^{1}H NMR spectrum of **1** (Table 1) exhibited signals for a phenyl group, an isolated *trans* olefin unit, a pair of *ortho*-coupled aromatic protons indicative of a 1,2,3,4-tetrasubstituted benzene ring, an isolated amide NH, and three methyl groups, including one methoxy group. There were also numerous signals for diastereotopic methylene protons. ^{13}C NMR data (Table 1) revealed the presence of two carbonyl carbons (one ketone and one amide), 14 aromatic/olefinic carbons (corresponding to the two aromatic rings and the *trans* olefin), and two quaternary sp^3 carbons. Chemical shift data indicated that one of the aromatic ring carbons is oxygenated (δ_C 155.0). The remaining eight carbon signals were located in the aliphatic region of the spectrum. These units account for 11 degrees of unsaturation, requiring two additional rings to be present.

Special Issue: Special Issue in Honor of Gordon M. Cragg

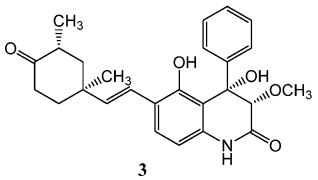
Received: December 9, 2011
Published: February 1, 2012



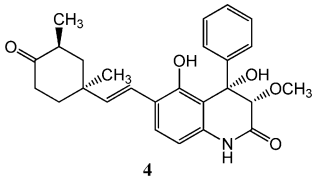
1



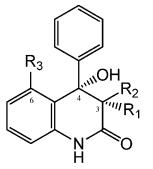
2



3



4



5 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$
6 $R_1 = OH$, $R_2 = H$, $R_3 = H$
7 $R_1 = H$, $R_2 = OH$, $R_3 = H$

The structure of the dihydroquinolone portion of **1** was established by analysis of 2D NMR data and through comparison to a known series of compounds that includes the aspoquinolines and penigequinolones.[7−9] HMBC correlations from the H-12/H-16 signal for the phenyl group to C-4; from the isolated oxymethine H-3 to C-2, C-4, C-5, and C-11; and from the amide NH to C-5 and C-10 were consistent with the corresponding features of the structure of aspoquinolone C, albeit without the *para*-methoxy group on the aromatic ring substituent.[8]

The remaining portion of the structure was significantly different from those of the aforementioned compounds. HMBC correlations of H_3-25 to C-20 and C-24 and correlations of H_3-26 to C-20 and C-22, with the last remaining methylene unit (C-23) bridging C-22 and C-24, complete a cyclohexane ring, accounting for the remaining unit of unsaturation. This ring was connected to the *trans* olefin unit at C-18 on the basis of correlations of H-17 and H-18 to C-19 and of H_3-25 to C-18. Finally, the two main structural units of **1** were linked on the basis of HMBC correlations of both *trans* olefinic protons to C-7 to complete the gross structure as shown. Additional HMBC data were fully consistent with this conclusion. The differences in structures relative to those of the aspoquinolones led to the proposal of a distinct name for **1** (aflaquinolone A). However, the numbering system shown is consistent with that of the aspoquinolones.[7]

Analysis of NOESY data and ^{1}H NMR *J*-values enabled assignment of the relative configuration of each half of **1**. The dihydroquinolone unit of **1** exhibited NMR shifts and *J*-values virtually identical to those of the corresponding unit in the aspoquinolones. In addition, the appearance of the H-3 signal as a doublet long-range-coupled to the NH (1.5 Hz) matched a characteristic signal and *J*-value described for these compounds in the literature.[8] Observation of such a *J*-value would be consistent with the near coplanarity of the H-3 and NH bonds reflected in the energy-minimized molecular model of **1**, which approximates the kind of arrangement that sometimes results in a long-range "*w*-coupling". NOESY results for this structural unit were also analogous to those previously reported. Key NOESY data included correlations of H-3 with signals for both the phenyl group and the 4-OH (requiring H-3 to be pseudoequatorial), as well as a correlation from the methoxy group (C-27) to the 4-OH, all of which are consistent with literature observations for molecules having the relative configuration shown for **1** at C-3 and C-4.[10] The relative configuration of the left-hand portion of **1** was assigned on the basis of NMR *J*-values and NOESY data (Table 1 and Figure 1). H-21 was assigned an axial (or pseudoaxial) orientation by virtue of a large *trans*-diaxial coupling (J = 13 Hz) with H_{ax}-20. NOESY correlations of H-21 with both H_{ax}-23 and olefinic proton H-17 supported this assignment and placed H-21 and the *trans* olefin unit on the same face of the cyclohexanone ring. Further NOESY correlations shown in Figure 1 were consistent with these conclusions. On the basis of these data, the cyclohexanone unit of **1** was assigned the relative configuration shown. However, no NOESY correlations were observed that enabled relative stereochemical correlation of the cyclohexanone and dihydroquinolone portions of the molecule.

ECD (electronic circular dichroism) data were collected for **1** and matched closely with the spectrum of the literature compound peniprequinolone,[10] a member of this class having a

Table 1. NMR Spectroscopic Data for Aflaquinolones A (1) and B (2) in $CDCl_3$[a]

	1		2	
position	δ_C	δ_H, mult (J in Hz)	δ_C	δ_H, mult (J in Hz)
1-NH		7.52, br s		7.44, br s
2	165.1		165.2	
3	84.0	3.67, d (1.5)	84.1	3.65, d (1.5)
4	79.0		78.9	
5	110.9		110.8	
6	155.0		154.7	
7	122.3		121.2	
8	127.2	7.40, d (8.3)	127.1	7.35, d (8.3)
9	106.9	6.35, d (8.3)	106.8	6.32, d (8.3)
10	134.3		133.9	
11	137.2		137.8	
12/16	128.9	7.26−7.33, m	128.9	7.25−7.32, m
13/15	126.2	7.26−7.33, m	126.3	7.25−7.32, m
14	129.2	7.30, m	129.3	7.25−7.32, m
17	122.5	6.67, d (17)	123.0	6.58, d (17)
18	135.9	6.26, d (17)	137.4	6.06, d (17)
19	37.3		30.7	
20_{eq}	47.5	2.09, m	40.0	1.32−1.45, m
20_{ax}		1.45, t (13)		1.32−1.45, m
21	41.3	2.53, dpent (13, 6.0)	37.1	1.55−1.73, m
22[b]	213.7		70.1	3.72, br q (2.7)
23_{ax}	38.6	2.47, ddd (14, 14, 5.8)	32.4[c]	1.55−1.73, m
23_{eq}		2.22, ddd (14, 4.5, 2.5)		1.55−1.73, m
24_{eq}	38.4	2.13, m	31.7[c]	1.55−1.73, m
24_{ax}		1.71, td (14, 4.5)		1.55−1.73, m
25	30.4	1.09, s	30.1	1.01, s
26	14.4	0.96, d (6.4)	18.3	0.91, d (6.9)
27	58.9	3.60, s	58.9	3.59, s
4-OH		4.60, s		4.58, s
6-OH		9.08, s		9.02, s

[a]Data were collected at 400 MHz (^{1}H) or 100 MHz (^{13}C). [b]A 22-OH ^{1}H NMR signal for **2** was not observed, presumably due to signal broadness. [c]These assignments may be interchanged.

dx.doi.org/10.1021/np200958r | *J. Nat. Prod.* 2012, 75, 464−472

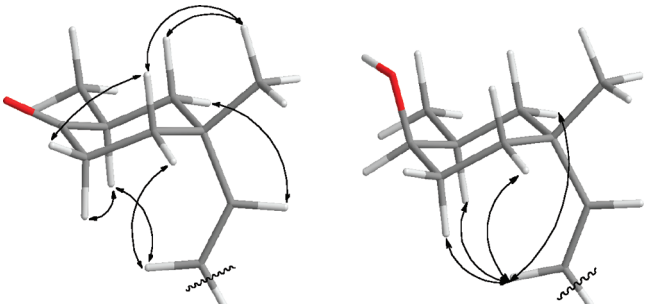


Figure 1. Key NOESY correlations for the terpene-derived portions of aflaquinolones A (**1**) and B (**2**).

simple prenyl substituent at C-7 and a *para*-methoxyphenyl group in place of the phenyl group of **1**. A simpler, co-occurring metabolite in the same report lacking the prenyl group also afforded an ECD curve of similar shape.[10] However, these data were provided to help characterize the compounds and were not used to propose the absolute configuration. The similarity in ECD data among these compounds suggested that the shape of the ECD curve is dictated largely by the configuration of the dihydroquinoline unit and that these three compounds all share the same absolute configuration in that portion of the molecule. However, the literature does not offer definitive assignment of absolute configuration for a member of this class, and the structure does not lend itself well to stereochemical analysis by standard empirical methods. Ultimately, TDDFT computational methods proved to be helpful in making a stereochemical assignment.

After geometry optimization of each possible isomer of **1** to obtain minimum energy conformers, TDDFT-calculated, smoothed ECD spectra were generated for each and compared with the experimental data. Comparison of the experimental and calculated spectra for **1** showed excellent agreement for the 3*S*, 4*S*-absolute configuration at C-3 and C-4 in **1** (Figure 2), regardless of the choice of configuration for the cyclohexanone portion of the molecule. Both the calculated and experimental data spectra showed a pair of positive Cotton effects (CE) above 275 nm, a negative CE near 250−260 nm, and a positive CE below 220 nm. These close similarities enabled assignment of the absolute configuration for the dihydroquinoline unit of **1** as shown. However, those of the remote terpenoid-derived portion could not be assigned by these methods. This issue is addressed further below.

Compound **2** was assigned the molecular formula $C_{26}H_{31}NO_5$ (12 unsaturations) on the basis of HRESITOFMS and NMR data. The structure of **2** was nearly identical to that of **1**. The main difference was evident in the absence of a ketone signal in the ^{13}C NMR spectrum, the appearance of ^{1}H and ^{13}C NMR signals for an oxymethine unit (CH-22), and associated changes in shifts and multiplicities of nearby diastereotopic protons (Table 1). These observations indicated that **2** differs from **1** by reduction of the C-22 ketone unit to a secondary alcohol moiety. Some of the key ^{1}H NMR *J*-values were difficult to measure due to overlap in the upfield region, but a spectrum in acetone-d_6 afforded better resolution of these signals. The resulting data indicated that the axial proton at C-20 showed a large *trans*-diaxial-type coupling (12.5 Hz) to H-21, indicating that H-21 must be axially oriented, and placing the C-21 methyl group in an equatorial position. The oxymethine signal (H-22) shows only small couplings, with a large *trans*-diaxial coupling clearly absent, thereby placing H-22 in an

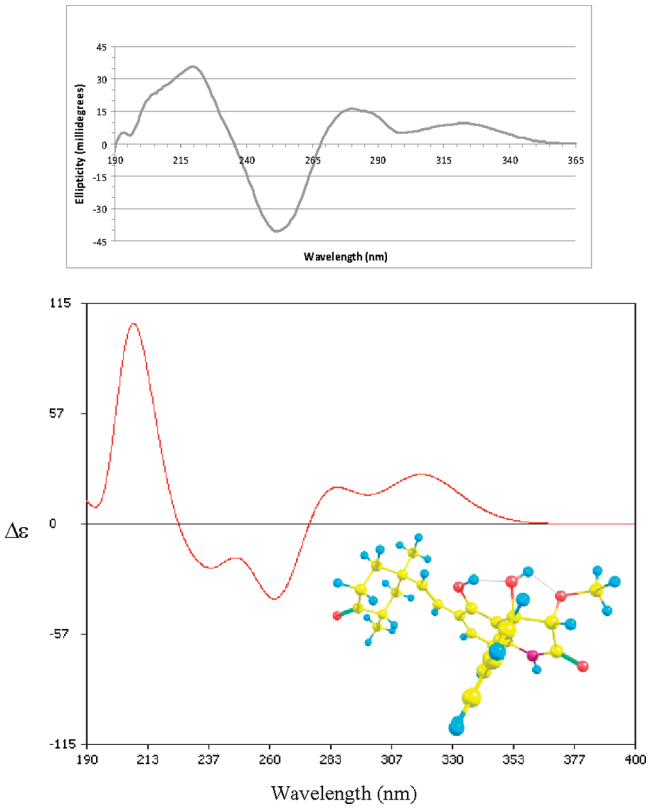


Figure 2. Experimental ECD spectrum (top) and TDDFT-calculated ECD spectrum with energy-minimized model (bottom) for aflaquinolone A (**1**).

equatorial position and indicating that the new C-22 hydroxy group adopts an axial orientation. A NOESY correlation between the axial H-21 and H-17 of the disubstituted olefin unit required these two units to be on the same face of the molecule, thereby setting the relative configuration at C-19. Other NOESY data (Figure 1) were consistent with these relative stereochemical assignments. The data also showed correlations for the dihydroquinolone moiety that matched those observed for **1**, enabling assignment of the analogous relative configuration at C-3 and C-4.

The ECD spectrum collected for **2** was virtually identical to that of **1**, enabling assignment of the analogous 3*S*, 4*S*-absolute configuration to the dihydroquinolinone portion of the molecule. Unlike compound **1**, the presence of the secondary alcohol moiety on the terpenoid-derived unit of **2** suggested that the absolute configuration of this portion of the molecule could be assigned through the use of Mosher's method.[15] Treatment of **2** with *R*-(−)-MTPA-Cl or *S*-(+)-MTPA-Cl in pyridine-d_6 and $CDCl_3$ afforded the *S*-MTPA ester (**2a**) or *R*-MTPA ester (**2b**), respectively. Formation of the esters was confirmed by the significant downfield shift of the signal for H-22 in each case and the appearance of the expected new aromatic and methoxy signals in the ^{1}H NMR spectra. The analysis was complicated somewhat by the formation of minor products arising from a second acylation at the phenolic OH group. However, assignment of ^{1}H NMR signals for relevant portions of **2a** and **2b** was accomplished by comparison with the data for **2** and verified by $^{1}H-^{1}H$ decoupling experiments. The resulting $\Delta\delta$ values observed for key signals of **2a** and **2b** (Figure 3) were consistent with the *S*-configuration at C-22, leading to assignment of the overall absolute configuration

dx.doi.org/10.1021/np200958r | *J. Nat. Prod.* 2012, 75, 464−472

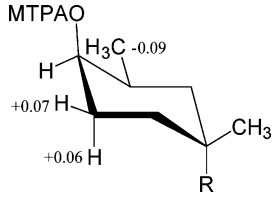


Figure 3. Observed chemical shift differences ($\Delta\delta = \delta_S - \delta_R$, ppm; 400 MHz) for the *R*- and *S*-MTPA esters of aflaquinolone B (**2**).

shown for **2**. The absolute configuration for the cyclohexanone unit of **1** was then proposed as shown by analogy to **2**. The name aflaquinolone B is proposed for compound **2**.

In an effort to chemically correlate **1** and **2** to further support the above conclusions, attempts were made to oxidize **2** to **1** under mild conditions using TPAP/NMO. Unfortunately, the approaches employed led to decomposition and did not succeed in providing detectable product. More extensive efforts toward this end were hampered by sample limitations. However, treatment of **1** with $NaBH_4$ afforded a mixture of alcohol products, one of which gave NMR signals and chromatographic properties identical to those of **2**.

In the course of this work, it came to our attention that colleagues at another institution (coauthors on this manuscript) had independently encountered and identified members of the same class of compounds from a marine isolate of *Aspergillus* sp. (SF-5044), including one metabolite that initially appeared to be identical to **1**. Because of this overlap, a decision was made to pool our efforts and compile a joint report. Earlier studies of this marine isolate led to isolation of an unrelated set of compounds.[7]

The first of these compounds, aflaquinolone C (**3**), was assigned the same molecular formula as **1** on the basis of HRESIMS data and was initially thought to be identical to **1**, as the structure and relative configuration of the two stereochemically insulated portions of the molecule were found to be identical to those of **1** by independent analysis of COSY, HMBC, and NOESY data, as well as ^{1}H NMR *J*-values. Moreover, the NMR shifts for the two compounds (as measured for both samples in acetone-d_6) were virtually identical by direct comparison. However, despite the nearly identical ECD spectra obtained for the two samples, the specific rotation values recorded under matching conditions were of somewhat different magnitudes and, more importantly, of opposite sign. These observations led to the hypothesis that **1** and **3** have the same dihydroquinolinone unit absolute configuration and the same relative configuration in the cyclohexanone ring, but have opposite absolute configurations at both stereocenters in the latter unit. This would make the two compounds diastereomers of one another, but because the two stereogenic features in the structures are well insulated from one another, they might be expected to possess virtually identical NMR data. Their specific rotations might well differ, even though their ECD curves might match. Indeed, a mixed sample of **1** and **3** gave a single ^{1}H NMR spectrum, as well as a single peak by HPLC analysis on three different stationary phases. While an inability to resolve the two stereoisomers was somewhat unexpected, the reproducible difference in the sign (and magnitude) of specific rotation for **1** and **3**, together with the accompanying virtually identical ECD spectra, was consistent with this hypothesis. It has been noted that diastereomers having stereochemical differences in regions remote from one another can give identical NMR spectra.[16]

Isolation of reduced analogue **2** from the same source as **1** and the assignment of its absolute configuration by Mosher's method as noted above led us to propose that **1** has the absolute configuration analogous to that of **2**, thereby enabling the proposal of the opposite cyclohexanone unit absolute configuration for **3**, as shown.

The ^{1}H NMR spectrum of aflaquinolone D (**4**), another isomer of **1** and **3** as established by HRESIMS data, was almost identical to those of **1** and **3** except for some chemical shift variations of signals corresponding to the C-17 to C-26 portion of the molecule (Table 1). Therefore, aflaquinolone D was presumed to be another stereoisomer of compounds **1** and **3**. Detailed analysis of ^{1}H NMR and COSY data supported the conclusion that **4** has the same planar structure as **1** and **3**, and the NOESY correlation of H-3 with the aromatic protons of the phenyl group, along with ECD analysis, indicated that the quinolin-2-one unit in **4** has the same absolute configuration as in **1**−**3**. However, comparisons of the NOESY data and *J*-values of **4** with those of **1** and **3** revealed a difference in relative configuration in the cyclohexanone unit A *trans*-diaxial *J*-value (13 Hz) for H-21 and H_{ax}-20 again indicated an axial orientation for H-21 and an equatorial orientation for CH_3-26. However, in this instance, the H-21 signal showed a NOESY correlation with H_3-25, rather than H-17 as in **1**−**3**, placing CH_3-25 in an axial orientation *cis* to H-21 and *trans* to CH_3-26. Correlations of H_{ax}-20 with H-18 and H_3-26 were consistent with the location of all of these protons on the same face of the cyclohexanone ring. Therefore, aflaquinolone D (**4**) was identified as a diastereomer of **1** and **3** possessing an inverted configuration at one of the centers in the cyclohexanone ring. The structure shown for **4** displays an inverted orientation at C-21 (α to the ketone carbonyl) relative to that of **3**, but the other possible diastereomer (i.e., with inversion at C-19 instead) could not be ruled out. Unlike the case for **1** and **3**, an isomer with the same relative configuration in the cyclohexanone unit that would have enabled an analogous comparison was not obtained from the fungicolous fungal culture, and sample limitations precluded further study of the issue for this compound.

The molecular formula of aflaquinolone E (**5**) was established as $C_{16}H_{15}NO_4$ on the basis of HRESIMS analysis and NMR data, making it significantly smaller than **1**−**4**. Analysis of the ^{1}H and ^{13}C NMR data for **5** and comparison with those of **1**−**4** suggested that **5** retains the phenylquinolin-2-one skeleton, but lacks the terpenoid side-chain appended to the aromatic ring in **1**−**4**. The planar structure of **5** was readily determined by independent analysis of 1D- and 2D-NMR data (Table 2). NOESY correlation of H-3 with the aromatic protons of the phenyl group again indicated that **5** possesses a relative configuration analogous to those found in the quinolin-2-one moieties of **1**−**4**, and the ECD spectrum was again very similar to those of **1**−**4**, aside from a modest blue shift in wavelength values, indicating analogous absolute configurations as well. The observed shift to lower wavelength in the ECD curve of **5** might be expected given the change in the chromophore associated with the absence of the olefin unit, but the overall shape was relatively unaffected. In fact, the similarity in ECD spectral shape among all five metabolites **1**−**5** (Figure 4) strongly supports the conclusion that the quinolin-2-one moiety is dominant in dictating the shape of the ECD curve in this class of compounds, regardless of the presence or absence of side-chains like those found in **1**−**4** and any stereocenters that might be associated with them.

Table 2. NMR Spectroscopic Data for Aflaquinolones E–G (5–7) in CD_3OD[a]

	5		6		7	
position	δ_C	δ_H, mult (J in Hz)	δ_C	δ_H, mult (J in Hz)	δ_C	δ_H, mult (J in Hz)
2	169.1		172.7		172.6	
3	86.3	3.64, s	75.8	4.76, s	76.6	4.58, s
4	79.9		78.7		78.2	
5	113.0		130.1		132.4	
6	159.1		130.1	6.71, dd (7.8, 2.1)	127.6	7.53, dd (7.7, 1.2)
7	108.1	6.45, d (8.0)	124.0	6.90, t (7.8)	124.8	7.11, t (7.7)
8	131.0	7.14, t (8.0)	130.6	7.25, dt (7.8, 1.4)	130.1	7.29, dt (7.7, 1.2)
9	113.2	6.53, d (8.0)	117.0	6.95, d (7.8)	116.8	6.95, d (7.7)
10	138.1		138.4		136.7	
11	140.9		143.2		141.5	
12/16	127.6	7.24–7.29, m	128.3	7.51, d (7.5)	128.6	7.34, d (8.0)
13/15	129.6	7.26–7.31, m	129.0	7.39, d (7.5)	128.7	7.19–7.25, m
14	129.8	7.26–7.31, m	128.4	7.32, t (7.5)	128.7	7.19–7.25, m
17	59.2	3.52, s				

[a]Data were collected at 400 MHz (^1H) or 100 MHz (^{13}C).

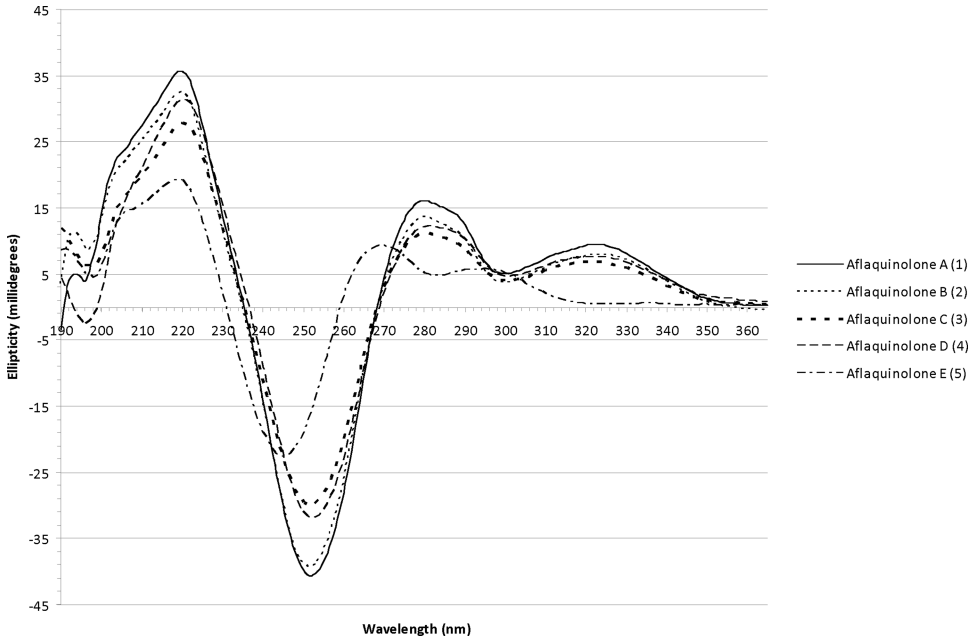


Figure 4. ECD spectra of aflaquinolones A–E (1–5).

HRESIMS data established the molecular formula $C_{15}H_{13}NO_3$ for both aflaquinolones F (6) and G (7). ^1H and ^{13}C NMR data of 6 and 7 showed almost identical patterns with some minor chemical shift variations. Comparison of these data with the ^1H and ^{13}C NMR data for 5 indicated replacement of the phenolic OH group with an additional aromatic proton and also revealed the absence of the methoxy group (Table 2). These observations led to the proposal that 6 and 7 are diastereomers of one another, each having a 3,4-dihydroxy-4-phenyl-3,4-dihydro-2(1*H*)-quinolinone structure. Detailed analysis of 2D-NMR data confirmed this proposal. The relative configurations of 6 and 7 were determined by analysis of NOESY data. NOESY correlation of H-3 with aromatic protons of the phenyl group in 6 indicated a *cis* arrangement of the two hydroxy groups in 6, thus leading to assignment of the same relative configuration found in the quinolin-2-one moieties of 1–5. In order for 7 to be a diastereomer of 6, a *trans* orientation of the two hydroxy groups is required, and this assignment is consistent with the absence of a NOESY correlation between H-3 and the protons of the phenyl group in the data for 7. A literature search revealed that similar spectroscopic patterns were observed for the two diastereomers of 3,4-dihydroxy-4-(4′-methoxyphenyl)-3,4-dihydro-2(1*H*)-quinolinone.[17]

Comparison of the ECD spectra of 6 and 7 with that of 5, the closest relative among the other metabolites encountered, showed some differences in both cases. However, as might be expected given the relative configuration assignments for 6 and 7, the data for 6 more closely resembled those of 5, as both compounds show a positive CE at approximately 270 nm, a negative CE near 250 nm, and a strongly positive CE below 230 nm. By contrast, the ECD data for 7 included a negative CE at 286 nm not present in any of the spectra for compounds 1–6, although other ECD features retained some resemblance with those of 1–6. Thus, while the absolute configuration of 6 was assigned to match those of 1–5, that of 7 (the only metabolite encountered in this study with a *trans* orientation of

dx.doi.org/10.1021/np200958r | J. Nat. Prod. 2012, 75, 464–472

the oxygen substituents at C-3 and C-4) could not be assigned with confidence by comparison of the experimental ECD curves. In an effort to resolve this ambiguity, we again employed computational methods to calculate ECD spectra for the two possible enantiomers of **7**. Although the calculated absorption wavelengths (Figure 5) did not match with the

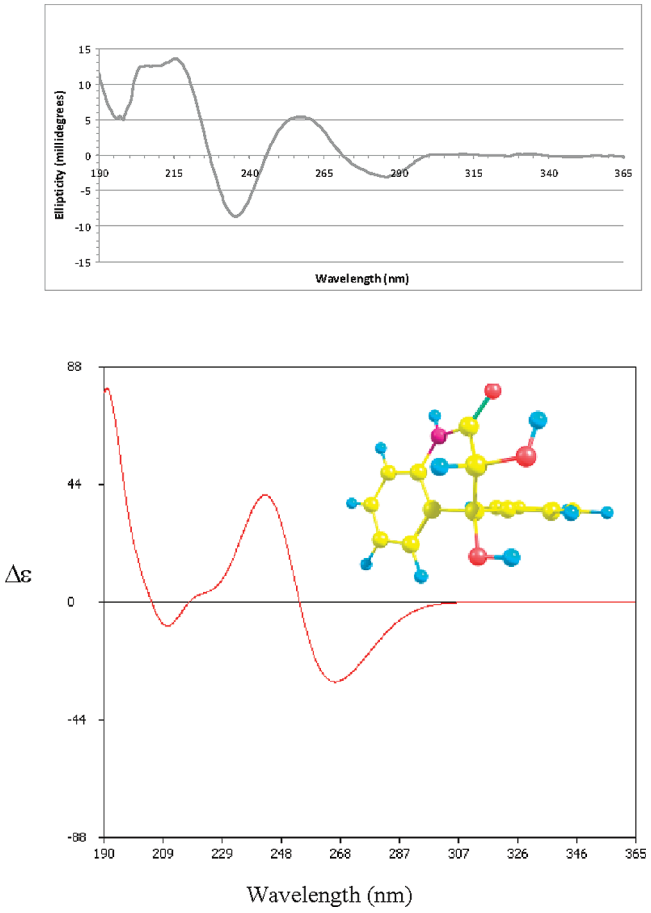


Figure 5. Experimental ECD spectrum (top) and TDDFT-calculated ECD spectrum (bottom) for aflaquinolone G (**7**).

experimental peak positions as well as in the cases above, the sequence and signs of the key higher wavelength peaks calculated for structure **7** (the C-3 epimer of **6**) showed a much better match with the experimental data than the inverted spectrum calculated for its enantiomer, resulting in assignment of the 3*R*,4*S*-configuration shown for **7**.

Biogenetically, compounds **1**–**4** appear likely to be derived from one unit each of anthranilic acid and phenylalanine, along with two isoprene units via processes analogous to those proposed for the aspoquinolones and the penigequinolones,[8−10] although these precedents would presumably incorporate tyrosine rather than phenylalanine and also have some significant differences in the arrangement/cyclization of the 10-carbon unit appended to the aromatic ring of the quinolinone unit. Compounds **5**–**7** lack the prenyl units, but would otherwise be similarly constructed. In view of the results described here, it is likely that ECD could be readily applied to assignment of the absolute configuration of the dihydroquinolone portions of other members of this class. In some cases, comparison with data provided in the literature now enable such an assignment.[10] However, establishment of the configurations of any centers that are remote from this unit remains a complicated issue and would still require independent analysis on a case-by-case basis.

The fungicolous *Aspergillus* isolate from Hawaii (MYC-2048 = NRRL 58570) was initially suggested to be *Aspergillus flavipes* Section *Flavipedes* on the basis of morphological characteristics. However, DNA sequence information and subsequent BLAST queries of GenBank produced a 100% match with an undescribed species of *Aspergillus* sp. NRRL 32683 (= MYC-1580 = NRRL 58569), another isolate from our own collection that was shown to be most closely related to *Aspergillus aureofulgens* Section *Flavipedes* (NRRL 6326).[18] The NRRL 6326 isolate was originally described from truffle soil in France. Its occurrence in truffle soil is intriguing because this environment is also suggestive of a possible fungicolous origin.

Neither compound **1** nor **2** showed bioactivity that would account for the initial activity seen in the extract of the Hawaiian isolate. However, numerous known compounds were also encountered in the extract, including alantrypinone,[11] aspochalasins I and J,[12] methyl 3,4,5-trimethoxy-2((2-((3-pyridinylcarbonyl)amino)benzoyl)amino)benzoate,[13] and *trans*-dehydrocurvularin.[14] The abundance of these compounds in the extract, particularly the aspochalasin and curvularin analogues, which are known to display various bioactivities, likely explains the effects originally observed. All of the aflaquinolones except for **4** were evaluated for growth inhibitory activity against chronic myelogenous leukemia cells (K562), murine melanoma cells (B16F10), human acute promyelocytic leukemia cells (HL-60), human breast cancer adenocarcinoma cells (MDA-MB-231), and hepatocellular carcinoma cells (Hep3B) using *in vitro* cell viability assays. While some of the compounds showed effects at high concentrations, all were effectively inactive, with IC_{50} values > 80 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Rudolph automatic polarimeter, model APIII. UV data were recorded with a Varian Cary III UV−visible spectrophotometer, and ECD data were collected using an Olis Cary-17 instrument (0.1 cm cell). ^{1}H NMR, ^{13}C NMR, and NOESY spectra were recorded using Bruker AVANCE-400, DRX-400, or AVANCE-600 or JEOL JNM ECP-400 spectrometers. Chemical shift values were referenced to residual solvent signals for $CDCl_3$ (δ_H/δ_C, 7.24/77.0), acetone-d_6 (δ_H/δ_C, 2.05/29.8), or CD_3OD (δ_H/δ_C, 3.31/49.0). HMQC and HMBC data were recorded using the Bruker AVANCE-600 instrument. All ^{13}C NMR multiplicities were determined by analysis of DEPT and/or HMQC data and are consistent with the position assignments. HRESITOFMS data were obtained using a Waters Q-ToF Premier mass spectrometer (or a similar instrument at Korea University) or a hybrid ion-trap time-of flight mass spectrometer (Shimadzu) at KBSI. HPLC separations involving **1** and **2** were performed using a Beckman System Gold instrument with a model 166P variable-wavelength UV detector connected to a 127P solvent module. The reversed-phase HPLC method used to isolate metabolites from the MYC-2048 cultures employed a program of 25% MeCN/H_2O to 100% MeCN over 25 min and 100% MeCN for 5 min using an Alltech HyperPrep 8 μm particle size C_{18} column (10 × 250 mm) at a flow rate of 2 mL/min with UV detection at 244 nm. Other HPLC separations were performed on an Agilent prep-C_{18} column (21.2 × 150 mm; 5 μm; 5 mL/min; UV detection at 210 nm). Flash column chromatography was carried out using YMC octadecyl-functionalized silica gel (C_{18}).

Fungal Material. MYC-2048 was originally obtained by D.T.W. from a basidioma of *Rigidoporus microsporus* found on a dead hardwood branch in an alien wet forest near milepost 7 on Scenic Route 19, Onomea Bay, Hawaii Co., Hawaii, in November 2002. This isolate was initially identified as *Aspergillus flavipes* (Section *Flavipedes*) on the basis

dx.doi.org/10.1021/np200958r | *J. Nat. Prod.* 2012, 75, 464−472

of *in vitro* colony growth and micromorphology. A subculture was deposited in the culture collection at the USDA National Center for Agricultural Utilization Research and assigned the accession number NRRL 58570. The culture was subjected to partial sequence analysis of the internal transcribed spacer region (ITS) and domains D1 and D2 of the nuclear large subunit (28S) rDNA gene using ITS5 and NL4 as polymerase chain reaction and sequencing primers.[19,20] A nucleotide-to-nucleotide BLAST query of the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) recovered EF669595 *Aspergillus* sp. NRRL 32683 (= MYC-1580; NRRL 58569)[17] as the closest match to the ITS rDNA of NRRL 58570 (100%). This undescribed species of *Aspergillus* (Section *Flavipedes*) was also initially identified as *A. flavipes* and also came from our Hawaiian collection (isolated by D.T.W. from a basidioma of *Earliella scabrosa* found on a dead hardwood branch, alien wet forest, Hilo Zoo, Hawaii Co., HI). It was shown to be a sibling species to the clade containing *Aspergillus aureofulgens* NRRL 6326, which, in turn, was originally described from a single known isolate obtained from truffle soil in France.[18] These results suggest that NRRL 58570 and NRRL 32683 represent separate Hawaiian isolates of the same undescribed species of *Aspergillus* (Section *Flavipedes*). Because the matching isolate has not been taxonomically described, we characterize the Hawaiian isolate (NRRL 58570) at present only as "*Aspergillus* sp."

Aspergillus sp. SF-5044 (deposited at the College of Medical and Life Sciences fungal strain repository, Silla University) was isolated from an intertidal sediment sample collected from Dadaepo Beach, Busan, Korea, in April 2006 using procedures that have been described.[15] Analysis of 28S rRNA sequences (Genbank accession number FJ935999) and a subsequent GenBank search indicated *Aspergillus protuberus* (FJ176897) and *Aspergillus asperescens* (EF652495) as the closest matches, showing sequence identities of 99.64% and 98.22%, respectively. Therefore, the marine-derived fungal strain SF-5044 was also identified as an *Aspergillus* sp. As was the case for the Hawaiian isolate, it could not be assigned to species, but the two isolates do not represent the same species.

Aspergillus sp. MYC-2048 was grown on 100 g of autoclaved rice for 30 days at 25 °C. The EtOAc extract (802 mg) of the resulting fermentation mixture showed antifungal activity against *A. flavus* and *F. verticillioides*. The extract also caused significant reduction in the growth rate of fall armyworm. *Aspergillus* sp. SF-5044 was cultured on 110 Petri plates (90 mm), each containing 20 mL of PDA with 3% NaCl. Plates were individually inoculated with 2 mL seed cultures of the fungal strain and incubated at 25 °C for a period of 10 days. Extraction of the combined agar media with EtOAc (2 L) provided an organic phase, which was then concentrated *in vacuo* to yield 2.0 g of an extract.

Isolation. The extract from *Aspergillus* sp. MYC-2048 (= NRRL 58570) was partitioned between MeCN and hexanes (~8 mL of each). The resulting MeCN fraction (478 mg) was then chromatographed on a silica gel column (Scientific Adsorbent, Inc.; 63−200 μm) using a hexanes/EtOAc/MeOH step gradient (hexanes, hexanes/EtOAc, EtOAc, EtOAc/MeOH, MeOH, ratios used: hexanes, 3:1, 1:1, 1:3; pure EtOAc, 99:1, 49:1, 19:1 9:1; and pure MeOH) to give fifteen 100 mL fractions. Fraction 5 (34 mg), eluted with 1:1 hexanes/EtOAc, was further separated by reversed-phase HPLC to afford *trans*-dehydrocurvularin[14] (15 mg, t_R 19.0 min) and aflaquinolone A (**1**; 4 mg, t_R 20.4 min). A sample of compound **2** was also obtained from this HPLC step (1 mg, t_R 17.0 min). Fraction 6 (14 mg), eluted with 1:1 hexanes/EtOAc, was further separated by HPLC under the same conditions to afford aflaquinolone B (**2**; 4 mg, t_R 17.8 min), aspochalasin J[12] (3 mg, t_R 24.0 min), and an additional sample of compound **1** (2 mg). Fraction 8 (19 mg), eluted with 1:3 hexanes/EtOAc, was similarly separated by HPLC to afford alantripinone[11] (1 mg, t_R 14.1 min) and methyl 3,4,5-trimethoxy-2((2-((3-pyridinylcarbonyl)amino)benzoyl)amino)-benzoate[13] (2 mg, t_R 22.2 min). Fraction 10 (17 mg), eluted with 100% EtOAc, contained only aspochalasin I.[12]

The EtOAc extract from *Aspergillus* sp. SF-5044 was subjected to C_{18} flash column chromatography (5 × 26 cm), eluting with a stepwise gradient of 20%, 40%, 60%, 80%, and 100% (v/v) MeOH in H_2O (500 mL each). The fraction eluted at 60% MeOH was resubjected to C_{18} flash column chromatography (4.5 × 12 cm), eluting with a stepwise gradient of 20% to 70% MeOH in H_2O (250 mL each, 10% increment from 20%, to 50%, followed by two additional 50% MeOH fractions, 60% MeOH, and 70% MeOH). The fraction eluted with the first 50% MeOH in H_2O elution solvent (29.9 mg) was further purified by semipreparative reversed-phase HPLC eluting with a gradient from 30% to 60% MeOH in H_2O (0.1% formic acid) over 40 min to yield **4** (3.5 mg, t_R = 30.9 min). The fraction eluted with the second 50% MeOH elution solvent (35 mg) was purified by semipreparative reversed-phase HPLC eluting with a gradient from 45% to 65% MeOH in H_2O (0.1% formic acid) over 65 min to yield compounds **3** (3.4 mg, t_R = 27.5 min) and **5** (16 mg, t_R = 25.1 min). The fraction from C_{18} flash column chromatography of the extract that eluted at 80% MeOH (261 mg) was subjected to silica flash column chromatography (3.5 × 10 cm), eluting with a stepwise gradient of 0% to 20% (v/v) MeOH in CH_2Cl_2 (200 mL each, 1% increment for each fraction). The fraction eluted with 1% MeOH (30 mg) was further purified by semipreparative reversed-phase HPLC eluting with 75% MeOH in H_2O (0.1% formic acid) to yield **6** (3.0 mg, t_R = 19.5 min). Compound **7** was purified by re-HPLC of the fraction collected between 1 and 10 min in the above HPLC procedure using a gradient from 50% to 100% MeOH in H_2O (0.1% formic acid) over 50 min (1.5 mg, t_R = 33.5 min).

*Aflaquinolone A (**1**):* pale orange, amorphous solid; $[\alpha]^{21}_D$ +14 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.3), 233sh (4.2), 278 (4.1), 324 (4.1) nm; CD (43 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+36), 252 (−41), 280 (+16), 286 (+15), and 323 (+9.5) nm; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data, NH → C-2, 3, 5, 9, 10; H-3 → C-2, 4, 5, 11; H-8 → C-5, 6, 7, 10, 17; H-9 → C-4, 5, 6, 7, 10; H-12/16 → C-11, 12/16, 13/15, 14; H-13/15 → C-11, 12/16, 13/15, 14; H-14 → C-11, 12/16, 13/15; H-17 → C-6, 7, 8, 18, 19, 25; H-18 → C-7, 19, 20, 25; H_{ax}-20 → C-18, 19, 21, 22, 25, 26; H_{eq}-20 → C-19, 21, 22, 24; H-21 → C-20, 22, 26; H_{ax}-23 → C-22, 24; H_{eq}-23 → C-19, 21, 22; H_{ax}-24 → C-19, 23, 25; H_{eq}-24 → C-19, 21, 22; H_3-25 → C-18, 20, 23, 24; H_3-26 → C-20, 21, 22; H_3-27 → C-3; key NOESY data (400 MHz; $CDCl_3$) 4-OH ↔ H_3-27; 4-OH ↔ H-3; H-3 ↔ H-12/16; H_{ax}-20 ↔ H_3-25; H_{eq}-20 ↔ H-18; H-21 ↔ H-17; H_{ax}-24 ↔ H_{eq}-23; H_3-25; H_{eq}-24 ↔ H-17; HRESITOFMS *m/z* 458.1940 $[M + Na]^+$ (calcd for $C_{26}H_{29}NO_5Na$, 458.1943).

*Aflaquinolone B (**2**):* pale yellow, amorphous solid; $[\alpha]^{22}_D$ +20 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.3), 233sh (4.1), 278 (4.0), 288sh (3.9), 324 (4.0) nm; CD (54 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+32), 252 (−39), 280 (+14), 286 (+12), and 323 (+7.9) nm; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.31 (br s, NH), 9.52 (br s, OH-6), 7.43 (d, *J* = 8.3 Hz, H-8), 7.37−7.34 (m, 5H, H-12 through H-16), 6.62 (d, *J* = 17 Hz, H-17), 6.56 (d, *J* = 8.3 Hz, H-9), 6.30 (br s, OH-4), 6.17 (d, *J* = 17 Hz, H-18), 3.66 (m, H-3), 3.66 (m, H-22), 3.51 (s, H_3-27), 3.23 (d, *J* = 4.2 Hz, OH-22), 1.71 (m, H_{ax}-24), 1.67 (m, H-21), 1.64 (m, H_{ax}-23), 1.62 (m, H_{eq}-23), 1.49 (t, *J* = 13 Hz, H_{ax}-20), 1.48 (br dd, *J* = 13, 2.7 Hz, H_{eq}-24), 1.36 (dt, *J* = 13, 2.7 Hz, H_{eq}-20), 1.00 (s, H_3-25), 0.91 (d, *J* = 6.8 Hz, H_3-26); key NOESY data (400 MHz; acetone-d_6) H-3 ↔ H-12/16, H-17 ↔ H_{eq}-20; H-17 ↔ H-21, H-17 ↔ H_{ax}-23; H-17 ↔ H_{eq}-24; additional NOESY data (400 MHz; $CDCl_3$) 4-OH ↔ H_3-27; 4-OH ↔ H-3; HRESITOFMS obsd *m/z* 460.2089 $[M + Na]^+$ (calcd for $C_{26}H_{31}NO_5Na$, 460.2091).

*Aflaquinolone C (**3**):* pale yellow solid; $[\alpha]^{25}_D$ −33 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.2), 212sh (4.1), 235sh (4.0), 278 (3.8), 324 (3.8) nm; CD (70 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+28), 252 (−30), 281 (+11), 286 (+10), and 322 (+6.8) nm; ^{1}H and ^{13}C NMR data in $CDCl_3$ matched those of **1** (Table 1); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.4 (C-2), 85.8 (C-3), 80.1 (C-4, 112.2 (C-5), 156.1 (C-6), 122.0 (C-7), 127.9 (C-8), 107.8 (C-9), 137.2 (C-10), 140.3 (C-11), 129.6 (C-12/16), 127.5 (C-13/15), 129.8 (C-14), 123.6 (C-17), 135.9 (C-18), 37.9 (C-19), 48.3 (C-20), 41.7 (C-21), 212.1 (C-22), 39.1 (C-23), 39.3 (C-24), 30.8 (C-25), 14.9 (C-26), 58.9 (C-27); HMBC data, H-3 → C-2, 4, 5, 6, 11, 27; H-8 → C-5, 6, 10, 11; H-9 → C-4, 5, 6, 7, 10; H-12/16 → C-4; H-17 → C-6, 8, 19, 25; H-18 → C-7, 19, 20, 24, 25; H_2-20 → C-18, 19, 21, 25, 26; H-21 → C-20, 22, 26; H_2-23 → C-22, 24; H_2-24 → C-18, 19, 25;

H_3-25 → C-18, 19, 20, 24; H_3-26 → C-20, 21, 22; H_3-27 → C-3; NOESY data H-3 ↔ H-12/16; H-17 ↔ H-23_{ax}, H-21; H-23_{ax} ↔ H-24_{ax}, H_3-25, H_3-26; HRESIMS m/z 434.1954 $[M - H]^-$ (calcd for $C_{26}H_{28}NO_5$, 434.1967).

*Aflaquinolone D (**4**):* pale yellow solid; $[\alpha]^{25}_D$ −10 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.3), 212sh (4.3), 235sh (4.2), 278 (4.0), 323 (4.0) nm; CD (47 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+31), 252 (−32), 282 (+12), 286 (+12), and 324 (+7.6) nm; ^{1}H NMR (400 MHz, acetone-d_6) δ 9.40 (br s, NH), 7.42 (d, J = 8.4 Hz, H-8), 7.38−7.31 (m, 5H), 6.64 (d, J = 17 Hz, H-17), 6.58 (d, J = 8.4 Hz, H-9), 6.24 (d, J = 17 Hz, H-18), 3.66 (s, H-3), 3.51 (s, H_3-27), 2.69 (dq, J = 13, 6.6 Hz, H-21), 2.62 (dt, J = 14, 7.3 Hz, H_{ax}-23), 2.17 (m, H_{eq}-23), 1.89 (m, H_{eq}-20), 1.86 (m, H_a-24), 1.81 (m, H_b-24), 1.54 (t, J = 13 Hz, H_{ax}-20), 1.43 (s, H_3-25), 0.94 (d, J = 6.6 Hz, H_3-26); NOESY data, H-3 ↔ H-12/16; H-17 ↔ H-20_{ax}; H-20_{ax} ↔ H_3-26; H-21 ↔ H_3-25; H-23_{ax} ↔ H_3-25; HRESIMS m/z 434.1954 $[M - H]^-$ (calcd for $C_{26}H_{28}NO_5$, 434.1967).

*Aflaquinolone E (**5**):* white solid; $[\alpha]^{25}_D$ −41 (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.2), 252 (3.5), 295 (3.5) nm; CD (62 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 218 (+19), 245 (−23), 270 (+9.2), and 295 (+5.7) nm; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data, H-3 → C-2, 4, 5, 6, 11, 27; H-7 → C-5, 6; H-8 → C-5, 6, 7, 10; H-9 → C-5, 6, 7, 10; H-12/16 → C-4, 11, 14; H_3-17 → C-3; HRESIMS m/z 308.0893 $[M + Na]^+$ (calcd for $C_{16}H_{15}NO_4Na$, 308.0899).

*Aflaquinolone F (**6**):* white solid; $[\alpha]^{25}_D$ +10 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.2), 252 (3.6), 295 (3.5) nm; CD (56 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 200 (−9.5), 223 (+13), 252 (−3.2), 269 (+2.1), and 290 (+0.8) nm; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data, H-3 → C-2, 4, 5, 11; H-6 → C-4, 8, 10; H-7 → C-6, 9; H-8 → C-6, 10; H-9 → C-5, 7; H-12/16 → C-4; H-13/15 → C-11, 12/16; H-14 → C-12/16; HRESIMS m/z 256.0975 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_3$, 256.0974).

*Aflaquinolone G (**7**):* white solid; $[\alpha]^{25}_D$ −6 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.2), 253 (3.7), 285sh (3.3) nm; CD (57 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 206 (+13), 216 (+14), 235 (−8.6), 257 (+5.4), and 286 (−3.1) nm; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data, H-3 → C-2, 4, 11; H-6 → C-4, 8, 10; H-7 → C-5, 6, 9, 10; H-8 → C-6, 9, 10; H-9 → C-5, 7; H-12/16 → C-4, 13/15; HRESIMS m/z 278.0788 $[M + Na]^+$ (calcd for $C_{15}H_{13}NO_3Na$, 278.0793).

Preparation of Aflaquinolone B Mosher's Esters. A solution of aflaquinolone B (**2**; 0.2 mg) in $CDCl_3$ (100 μL) was treated with dry pyridine-d_6 (10 μL) and *R*-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (*R*-MTPA-Cl, 5 μL). The mixture was allowed to react at room temperature for 24 h in a Teflon-lined screw-cap vial, resulting in formation of the *S*-MTPA ester **2a**. Additional $CDCl_3$ was then added directly to the vial, and the resulting solution was placed in an NMR tube for analysis. Analogous treatment of an additional 0.2 mg portion of **2** using *S*-MTPA-Cl afforded the *R*-MTPA ester **2b**. *S*-MTPA ester **2a**: key ^{1}H NMR data (400 MHz, $CDCl_3$) δ 0.736 (d, J = 6.9 Hz, H_3-26), 0.947 (s, H_3-25), 1.29 (m, H_{ax}-23), 1.71 (m, H_{eq}-23). *R*-MTPA ester **2b**: key ^{1}H NMR data (400 MHz, $CDCl_3$) δ 0.822 (d, J = 6.9 Hz H_3-26), 0.891 (s, H_3-25), 1.23 (m, H_{ax}-23), 1.64 (m, H_{eq}-23). Precise δ values for other potentially relevant signals (e.g., H-21) could not be established due to signal overlap.

Sodium Borohydride Reduction of Aflaquinolone A. A 0.7 mg sample of aflaquinolone A (**1**) was transferred to a 0.5 mL Reacti-vial. To this vial were added 0.75 mg of $NaBH_4$ and 75 μL of MeOH, and a small triangular stir bar was placed into the vial. The vial was then sealed under Ar, and the course of the reaction was monitored by TLC (9:1 Et_2O/MeOH). After 1 h, all of the starting material had reacted. The solvent was then evaporated with air flow and worked up using aqueous NH_4Cl and ether. The ether layer was collected and evaporated to dryness. ^{1}H NMR analysis of the ether-soluble material indicated the presence of a mixture of products, which were separated by reversed-phase HPLC (C_{18}, column: 4.6 × 250 mm, 1.0 mL/min, 50−100% MeCN in H_2O over 25 min), resulting in the collection of two fractions (t_R = 11.2 and 12.8 min). ^{1}H NMR analysis of the first fraction to elute exhibited signals matching those of aflaquinolone B (**2**), indicating that reduction of **1** to **2** had occurred. HPLC co-injection with a sample of **2** supported this conclusion.

Energy Minimization and ECD Calculations. All geometry optimizations were performed in the gas phase using resolution-of-identity (RI) approximation and a BP functional,[21,22] combined with the SV(P) basis set[23] and the corresponding auxiliary basis set.[24,25] No symmetry constraints were used during the optimization. ECD spectra were calculated for geometries obtained from the RI-BP/SV(P) calculations. Time-dependent density functional (TDDFT) calculations were used with B3LYP functional[26,27] using the RIJCOSX approximation[28] and TZVP basis set[23] with the corresponding auxiliary basis set[24,25] in the gas phase and in MeOH solution (COSMO solvation model).[29]

A total of 80 excited states were calculated, and only singlet excited states were considered. All of the quantum chemical calculations were performed with ORCA version 2.7.[30] ECD spectra were obtained using SpecDis version 1.50 software.[31] A broadening factor of 0.24 was used in an effort to match the resolution level of the experimental data as closely as possible.

Cell Proliferation Assay. K562 (3×10^4 cells per well), B16F10 (3×10^3 cells per well), HL-60 (1×10^5 cells per well), MDA-MB-231 (5×10^3 cells per well), and Hep3B (5×10^3 cells per well) cells were seeded on 96-well microplates. Cell proliferation assays were carried out using the enhanced cell viability assay kit EZ-CyTox (Daeil Lab Service Co., Ltd.) protocol. DMSO solutions of the test compounds were used, and cells were treated for 48 h. The absorbance (450 nm) of each well was measured using a Power WaveX 340 (Bio-Tek Instruments).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 319-335-1361. Fax: 319-335-1270. E-mail: james-gloer@uiowa.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Research in the University of Iowa lab was supported by grants from the National Science Foundation (CHE-0718315 and CHE-11011847 to J.B.G. and AGS 0927944 to J.B.). Assistance from the staff of the University of Iowa NMR and Mass Spectrometry Facilities is gratefully acknowledged. Valuable assistance from an anonymous reviewer in recognizing a significant error in the original manuscript is appreciated. Acquisition of the HRESITOFMS and 600 MHz NMR instruments was made possible by grants from the National Institutes of Health (S10 RR023384 and S10 RR025500, respectively). The USDA is an equal opportunity provider and employer. Research carried out at Silla University and KRIBB was supported by the grants from the Global R&D Center (GRDC) Program funded by the Korean Ministry of Education, Science & Technology (MEST) and from the Marine Biotechnology Program funded by the Ministry of Land, Transport, and Maritime Affairs of the Korean Government.

■ DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

REFERENCES

(1) Gloer, J. B. Applications of Fungal Ecology in the Search for New Bioactive Natural Products. In *The Mycota*, Vol. *IV*, 2nd ed.; Kubicek, C. P.; Druzhinina, I. S., Eds.; Springer-Verlag: New York, 2007; pp 257−283.
(2) Schmidt, L. E.; Deyrup, S. T.; Baltrusaitis, J.; Swenson, D. C.; Wicklow, D. T.; Gloer, J. B. *J. Nat. Prod.* **2010**, *73*, 404−408.
(3) Wicklow, D. T.; Jordan, A. M.; Gloer, J. B. *Mycol. Res.* **2009**, *113*, 1433−1442.
(4) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3573−3577.
(5) Sy, A. A.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2008**, *71*, 415−419.
(6) Shim, S. H.; Swenson, D. C.; Gloer, J. B.; Dowd, P. F.; Wicklow, D. T. *Org. Lett.* **2006**, *8*, 1255−1228.
(7) Lee, S. U.; Asami, Y.; Lee, D.; Jang, J.-H.; Ahn, J.-S.; Oh, H. *J. Nat. Prod.* **2011**, *74*, 1284−1287.
(8) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2006**, *4*, 3517−3520.
(9) Kimura, Y.; Kusano, M.; Koshino, H.; Uzawa, J.; Fujioka, S.; Tani, K. *Tetrahedron Lett.* **1996**, *37*, 4961−4964.
(10) Kusano, M.; Koshino, H.; Uzawa, J.; Fujioka, S.; Kawano, T.; Kimura, Y. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2559−2568.
(11) Larsen, T. O.; Frydenvang, K.; Frisvad, J. C.; Christophersen, C. *J. Nat. Prod.* **1998**, *61*, 1154−1157.
(12) Zhou, G. X.; Wijeratne, E. M. K.; Bigelow, D.; Pierson, L. S. III; VanEtten, H. D.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2004**, *67*, 328−332.
(13) Arai, K.; Shimizu, S.; Yamamoto, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1005−1012.
(14) Munro, H. D.; Musgrave, O. C.; Templeton, R. *J. Chem. Soc. C* **1967**, *10*, 947−948.
(15) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451−2457.
(16) Bajpai, R.; Curran, D. P. *J. Am. Chem. Soc.* **2011**, *133*, 20435−20443.
(17) He, J.; Lion, U.; Sattler, I.; Gollmick, F. A.; Grabley, S.; Cai, J.; Meiners, M.; Schünke, H.; Schamann, K.; Dechert, U.; Krohn, M. *J. Nat. Prod.* **2005**, *68*, 1397−1399.
(18) Peterson, S. W. *Mycologia* **2008**, *100*, 205−226.
(19) O'Donnell, K. *Sydowia* **1996**, *48*, 57−70.
(20) White, T. J.; Bruns, T.; Lee, S.; Taylor, J. W. In *PCR Protocols: a Guide to Methods and Applications*; Innis, M. A.; Gelfand, D. H.; Sninsky, J. J.; White, T. J., Eds.; Academic Press: New York, 1990; pp 315−322.
(21) Perdew, J. P. *Phys. Rev. B* **1986**, *33*, 8822−8824.
(22) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098−3100.
(23) Schaefer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys.* **1992**, *97*, 2571−2577.
(24) Eichkorn, K.; Weigend, F.; Treutler, O.; Ahlrichs, R. *Theor. Chim. Acta* **1997**, *97*, 119−124.
(25) Eichkorn, K.; Treutler, O.; Öhm, H.; Häser, M.; Ahlrichs, R. *Chem. Phys. Lett.* **1995**, *240*, 283−289.
(26) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(27) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789.
(28) Neese, F.; Wennmohs, F.; Hansen, A.; Becker, U. *Chem. Phys.* **2009**, *356*, 98−109.
(29) Sinnecker, S.; Rajendran, A.; Klamt, A.; Diedenhofen, M.; Neese, F. *J. Phys. Chem. A* **2006**, *110*, 2235−2245.
(30) Neese, F. *ORCA*, Version 2.7.0; Universität Bonn: Bonn, Germany, 2010.
(31) Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *SpecDis*, Version 1.50; Universität Würzburg: Würzburg, Germany, 2010.