Sorocenols G and H, Anti-MRSA Oxygen Heterocyclic Diels−Alder-Type Adducts from *Sorocea muriculata* Roots

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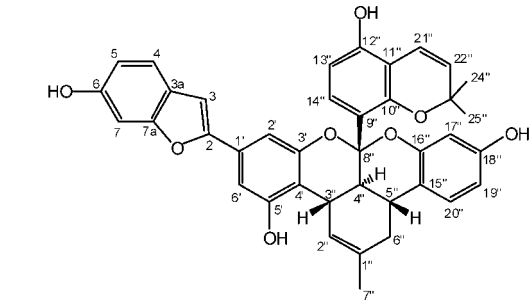
Bioassay-guided fractionation of a root extract of *Sorocea muriculata* led to the isolation and identification of two new oxygen heterocyclic Diels−Alder-type adducts, sorocenols G (**1**) and H (**2**), along with lupeol-3-(3′*R*-hydroxytetradecanoate) and oxyresveratrol. The structures of **1** and **2** were elucidated using 1D and 2D NMR spectroscopic and HRMS data and by comparison with reported values. The absolute configurations of **1** and **2** were established by analysis of their experimental and theoretically calculated CD spectra. Compounds **1** and **2** showed significant and selective activity against methicillin-resistant *Staphylococcus aureus* with IC_{50} values of 1.5 and 0.5 μM, respectively. Compound **2** also displayed antifungal activity against *Candida albicans, Cryptococcus neoformans*, and *Aspergillus fumigatus*, with IC_{50} values of 5.4, 5.4, and 10.0 μM, respectively.

The plant genus *Sorocea* includes 25 species, distributed mainly in the western Amazon region of South America. Various species in this genus have been used traditionally to treat inflammation and gastric ulcers.[1,2] Previous phytochemical investigations of *Sorocea bonplandii* Baillon (Moraceae) resulted in the isolation of six Diels−Alder-type adducts named sorocenols A−F, but no biological evaluation of these metabolites was reported.[3,4] We report herein the isolation and characterization of two new oxygen heterocyclic Diels−Alder-type adducts, sorocenols G (**1**) and H (**2**), from the EtOH extract of the roots of *S. muriculata* Miq. This extract showed an IC_{50} value of <8.0 μg/mL against MRSA and no discernible cytotoxicity against Vero cells (African green monkey kidney fibroblasts). On the basis of these biological screening results and the absence of published data on *S. muriculata,* this plant was selected for further chemical and biological evaluation.

Sorocenol G (**1**) was obtained as an amorphous, red solid, showing $[\alpha]_D^{27}$ +44.0 (*c* 0.20, MeOH) and UV absorption maxima at 315 and 282 nm. A molecular formula of $C_{39}H_{32}O_8$ was determined by HREIMS {*m*/*z* 629.2176 $[M + H]^+$ (calcd 629.2175)}, suggesting 24 degrees of unsaturation. Analysis of the ^{1}H and COSY NMR spectra showed two sets of ABX aromatic spin systems [δ 7.33 (d, J = 8.4 Hz, H-4), 6.83 (dd, J = 8.4, 2.4 Hz, H-5), 6.31 (d, J = 2.4 Hz, H-7), and 6.31 (d, J = 2.4 Hz, H-17″), 6.43 (dd, J = 8.4, 2.4 Hz, H-19″), 7.05 (d, J = 8.4 H-20″)], two *meta*-coupled resonances [δ 6.74 (d, J = 2.4 Hz, H-2″), 6.21 (d, J = 2.4 Hz, H-6″)], two *ortho*-coupled aromatic protons [δ 6.09 (d, J = 8.7 Hz, H-13″), 6.93 (d, J = 8.7 Hz, H-14″)], two coupled olefinic protons [δ 6.64 (d, J = 9.9 Hz, H-21″), 5.55 (d, J = 9.9 Hz, H-22″)], a sharp aromatic singlet [δ 6.88 (s, H-3)], and two tertiary methyl resonances [δ 1.31 (s, H_3-24″) and 1.30 (s, H_3-25″)]. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) also showed the presence of a methylcyclohexene moiety [δ_H 6.52 (d, J = 5.4 Hz, H-2″), 3.48 (dd, J = 12.0, 5.4 Hz, H-3″), 3.35 (t, J = 12.0 Hz, H-4″), 2.90 (dd, J = 12.0, 5.7 Hz, H-5″), 2.67 (dd, J = 17.4, 12.0, H_β-6″), 2.02 (dd, J = 17.4, 5.7 H_α-6″), 1.82 (3H, s, H-7″); δ_C 135.3 (C-1″), 122.3 (C-2″), 34.7 (C-3″), 37.6 (C-4″), 28.4 (C-5″), 37.1 (C-6″)]. The ^{13}C NMR, DEPT, and HMQC spectra of **1** revealed the presence of 39 carbon resonances including three methyls, one methylene, 17 methines, and 18 quaternary carbons



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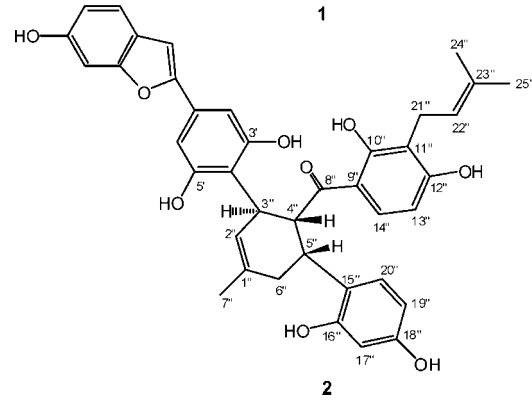

2

(eight oxyaryl, two oxygenated sp^3, and eight aryl sp^2 carbons). The UV and ^{1}H NMR spectroscopic data of **1** were similar to those reported for mulberrofuran K.[5,6] Analysis of both the ^{1}H NMR coupling constants and the ROESY spectrum allowed the determination of the relative configurations at C-3″, C-4″, and C-5. The large coupling constant between both H-3″/H-4″ (12.0 Hz) and H-4″/H-5″ (12.0 Hz) suggested that H-3″, H-4″, and H-5″ are *trans*-diaxially interrelated (Table 2). This was supported by ROESY correlations between H-3″ (δ 3.48), H-5″ (δ 2.90), and H_β-6″ (δ 2.67) and between H-4″ (δ 3.35) and H_α-6 (δ 2.02). The absolute configurations of the stereogenic centers C-3″, C-4″, C-5″, and C-8″ of **1** were established by theoretical calculations of its electronic circular dichroism (ECD) using the time-dependent density functional theory (TDDFT) method at the B3LYP/6-31G* level.[7−9] Since the relative configuration of C-8″ could not be determined by the ROESY experiment, there were four possible structures considered for compound **1**, with the absolute configuration (3″*R*,4″*R*,5″*S*,8″*R*), (3″*R*,4″*R*,5″*S*,8″*S*), (3″*S*,4″*S*,5″*R*,8″*R*), or

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **1** (CD_3OD) and **2** (acetone-d_6)[a]

	1		2	
position	δ_C	δ_H, mult., (J in Hz)	δ_C	δ_H, mult., (J in Hz)
2	156.6		154.5	
3	102.3	6.88 s	101.8	6.93 s
3a	124.7		122.2	
4	120.6	7.33 d (8.4)	119.2	7.20 d (8.2)
5	113.7	6.83 dd (8.4, 2.4)	113.4	6.67 d(8.2)
6	152.1		153.5	
7	104.0	6.31 d (2.4)	103.6	6.50 bs
7a	154.7		155.2	
1′	133.4		133.0	
2′	103.9	6.74 d (2.4)	103.0	6.77 d (2.0)
3′	152.9		158.9	
4′	109.8		112.3	
5′	160.2		158.9	
6′	103.8	6.21 d (2.4)	103.3	6.77 d (2.0)
1″	135.3		134.1	
2″	122.3	6.52 d (5.4)	122.1	5.72 d (6.5)
3″	34.7	3.48 dd (12.0, 5.4)	41.9	4.07 dd (14.0, 6.5)
4″	37.6	3.35 t (12.0)	48.8	4.75 dd (14.0, 5.8)
5″	28.4	2.90 dd (12.0, 5.7)	34.3	4.44 t (5.8)
6″	37.1	2.67 dd (17.4, 12.0) 2.02 dd (17.4, 5.7)	29.9	2.05 m, 2.52 m
7″	23.7	1.82 s	23.2	1.95 s
8″	102.6		207.3	
9″	117.8		113.6	
10″	153.1		163.5	
11″	111.9		114.9	
12″	154.8		161.9	
13″	107.9	6.09 d (8.7)	107.2	6.43 d (9.0)
14″	128.9	6.93 d (8.7)	130.7	8.25 d (9.0)
15″	118.3		122.0	
16″	153.4		155.9	
17″	104.0	6.31 d (2.4)	102.8	6.36 d (2.2)
18″	157.8		156.6	
19″	109.9	6.43 dd (8.4, 2.4)	106.9	6.26 dd (8.4, 2.0)
20″	127.8	7.05 d (8.4)	129.9	6.95 d (8.4)
21″	118.6	6.64 d (9.9)	21.5	3.20 d (6.8)
22″	128.9	5.55 d (9.9)	122.6	5.12 dd (7.2, 1.2)
23″	76.8		130.7	
24″	27.5	1.31	25.2	1.57 s
25″	27.6	1.30	17.2	1.68 s

[a] Assignments confirmed by DEPT-135, HMQC, COSY, ROESY, and HMBC NMR experiments.

Table 2. ^{1}H NMR Coupling Constants and Configurations of the Methylcyclohexene Rings of **1** and **2**.

	1		2	
	J value in Hz	configuration	J value in Hz	configuration
H-3″/H-4″	12.0	*trans*	14.0	*trans*
H-4″/H-5″	12.0	*trans*	6.5	*cis*
H-5″/H-6″$_\beta$	12.0	*trans*	m	
H-5″/H-6″$_\alpha$	5.7	*cis*	m	

(3″*S*,4″*S*,5″*R*,8″*S*). The results showed that the calculated ECD of the (3″*S*,4″*S*,5″*R*,8″*R*)-isomer strongly resembled the experimental spectrum (Figure 1). Accordingly, the calculated Cotton effects (CE) for the electronic transitions of the aromatic π-systems around 325 nm (positive), 280 nm (negative), and 225 nm (positive) corresponded to the experimental Cotton effects observed around 300, 260, and 235 nm, respectively. Thus, the structure of sorocenol G was established as **1**. It should be noted that the absolute configuration of a similar compound, mongolocin F,[10] with a 3″,4″-*cis*, 4″,5″-*trans* arrangement was tentatively assigned as 3″*S*, 4″*R*, 5″*S* based on a positive optical rotation and a positive Cotton effect (310 nm). The absolute configuration at C-8″ of mongolocin F could not be assigned.[10]

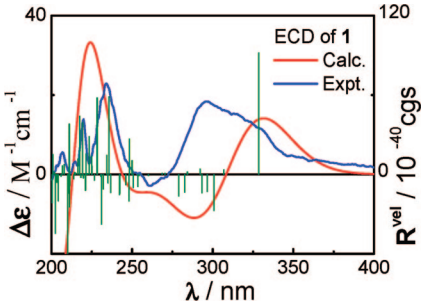

Figure 1. Experimental (blue) and calculated (red) CD spectra of **1**.

Sorocenol H (**2**) was isolated as an optically active {$[\alpha]_D^{27}$ +46.0 (*c* 0.20, MeOH)} red, amorphous solid. Its UV spectrum displayed absorption maxima at 316, 288, and 220 nm. The molecular formula of $C_{39}H_{36}O_9$ was deduced from its HREIMS {*m*/*z* 649.2467 [M + H]$^+$ (calcd 649.2437)} and ^{13}C, ^{1}H, and DEPT NMR data. The molecular formula indicated 22 degrees of unsaturation, and the IR spectrum exhibited hydroxy and conjugated carbonyl absorption bands at 3346 and 1635 cm^{-1}, respectively. Analysis of the ^{1}H and COSY NMR spectra of **2** (Table 1) suggested the presence of two sets of ABX aromatic spin systems (H-4, H-5, H-7 and H-17″, H-19″, H-20″), two *meta*-coupled protons (H-2′, H-6′), two *ortho*-coupled aromatic protons (H-13″, H-14″), and a hydrogen-bonded hydroxy proton (δ_H 12.87, OH-10″). The ^{13}C and DEPT NMR data displayed 39 carbon resonances, comprising three methyls, two methylenes, 16 methines, and 18 quaternary carbons [one carbonyl (δ_C 207.3, C-8″), nine oxyaryl, and eight aryl sp^2 carbons]. The physical data of **2** were in agreement with those reported for magnolicin F[10] except for the configuration at C-5″, which was determined by analysis of the coupling constants between H-3″/

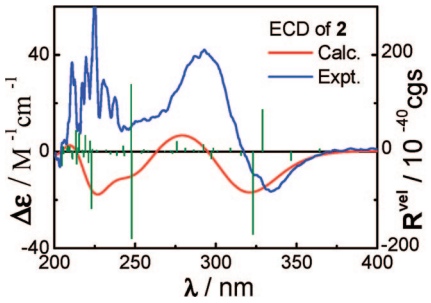

Figure 2. Experimental (blue) and calculated (red) CD spectra of **2**.

H-4″ and H-4″/H-5″ as well as ROESY correlations. The large coupling constants between H-3″/H-4″ (14.0 Hz) and the small coupling constant between H-4″/H-5″ (5.8 Hz) indicated the 3″,4″-*trans* and 4″,5″-*cis* relative configurations (Table 2). The ROESY correlation between H-4″ (δ 4.75) and H-5″ (δ 4.44) confirmed these orientations. In a similar manner, theoretical ECD calculations of **2** were performed to determine the absolute configuration at C-3″, C-4″, and C-5″. The calculated ECD spectrum of this structure with 3″*R*, 4″*R*, 5″*R* absolute configuration corresponded to the experimental ECD that showed typical Cotton effects for the electronic transition of the aromatic and carbonyl moieties around 334 nm (negative) and 293 nm (positive). However, the Cotton effects in the short-wavelength range 200−250 nm were not consistent with those in the calculated spectrum (Figure 2). This inconsistency may be due to minor conformational differences between the theoretically optimized geometry and the real solution conformers, particularly in view of the potential for rotation of the three large substituents at the stereogenic centers in solution.

The absolute configuration of the related compounds, cathayanons A and B, and sanggenon O[11] at the stereogenic centers of the cyclohexenyl ring was defined on the basis of positive or negative Cotton effects in the 280−320 nm region and subsequent application of the excitation chirality theory, with no indication being given as to which cyclohexenyl substituents were responsible for the excitation coupling. Our work has clearly demonstrated more complexity in the 280−350 nm regions of the CD spectra of compounds **1** and **2** than the single Cotton effects indicated in earlier work. We are confident that our assignments represent a more accurate picture of the complexities of assigning absolute configuration in this rather complex group of naturally occurring phenolic compounds.

The known compounds were identified as 3-*O*-(3′*R*-hydroxytetradecanoyl)lupeol and oxyresveratrol by comparing their NMR data with those reported.[12,13] This is the first report of the isolation of these metabolites from *S. muriculata*.

The isolated compounds were evaluated for in vitro antimicrobial activity against the fungi *Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus* and the bacteria methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*. Compounds **1** and **2** showed significant and selective activity against MRSA with IC_{50} values of 1.5 and 0.5 μM, respectively (Table 3). Compound **2** also displayed antifungal activity against the yeasts *C. neoformans* and *C. albicans*, with an IC_{50} of 5.4 μM each, and the filamentous fungus *A. fumigatus* (IC_{50} 10.0 μM) (Table 3). Compounds **1** and **2** showed no activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* or *M. intracellulare* at the highest test concentration used of 20 μg/mL (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Analytical Autopol IV automatic polarimeter model 589-546. UV spectra were recorded on a Varian Cary 50 Bio UV−visible spectrometer. IR spectra were recorded on a Bruker Tensor 27 instrument. CD spectra were measured on a JASCO J-715 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a 400 MHz Ultrashield NMR spectrometer Bruker Advance DRX with a 3 mm inverse probe using TMS as internal standard. The mass spectrometric analysis was performed on an Agilent Series 1100 SL mass spectrometer equipped with an ESI source. HPLC was carried out on a Waters Empower pro Delta 4000 system with a 2487 dual absorbance detector. The columns used were Phenomenex HyperClone 5 μm ODS C_{18} (250 × 10.0 mm) and analytical NovaPak C_{18}, 60 Å pore size silica gel (3.9 × 150 mm). Column chromatography was performed on silica gel (EM Science, 60 Å, 230−400 mesh ASTM, Bakerbond Polar Plus C_{18} bonded phase) and Sepahex LH-20. Preparative TLC was performed on Analtech Uniplates, having a silica gel GF preparative layer with UV 254, 20 × 20 cm, 1500 μm thick. Computational ECD calculations were performed at 298 K by the Gaussian 03 program package, and the details are described in a previous paper.[9]

Plant Material. *Sorocea muriculata* root material was collected and identified in June 1997 in Loreto Region, District of Punchata, Peru, by Dr. Manuel Rimachi. A voucher specimen (IBE11917) is housed in Dr. Sydney McDaniel's collection at 2020 Sessums Rd., Starkville, MS 39759.

Extraction and Isolation. Dried, milled plant material (166.45 g) was extracted by maceration and sonication at room temperature with 100% EtOH. The extract was evaporated in vacuo to yield 3.64 g of a dried residue. A portion (2.94 g) of this extract was subjected to separation using silica gel vacuum liquid chromatography (VLC) eluted with hexanes and gradient hexanes/EtOAc/MeOH to 30% MeOH. Ten fractions (A to J) were collected. Fraction B (432 mg) was further chromatographed on silica gel using EtOAc−hexanes (5:95) as eluent to yield lupeol-3-(3′*R*-hydroxytetradecanoate) (52.7 mg). Fractions F and G (1.90 g) showed strong antimicrobial activity and were passed over Sephadex LH-20 eluted with MeOH, followed by final purification by C_{18}-HPLC using gradient MeOH−H_2O (50:50) to 100% MeOH to yield **1** (2.9 mg), **2** (8.3 mg), and oxyresveratrol (16.7 mg).

Sorocenol G (1): amorphous, red solid; $[\alpha]^{25}_D$ +44.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 315 (4.32), 282 (4.14) nm; CD (*c* 0.10, MeOH) λ_{max} ($\Delta\varepsilon$) 300 (+18.6), 260 (−3.4), 235 (+2.4); IR (neat) ν_{max} 3404, 2930, 1568 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRESIMS *m/z* 629.2176 [M + H]$^+$ (calcd for $C_{39}H_{33}O_8$, 629.2175); R_f = 0.4 ($CHCl_3$−MeOH, 85:15).

Sorocenol H (2): amorphous, red solid; $[\alpha]^{25}_D$ +46.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 316 (4.80), 288 (4.62), 220 (4.78) nm; CD (*c* 0.10, MeOH) λ_{max} ($\Delta\varepsilon$) 334 (−16.2), 293 (+42.2); IR (neat) ν_{max} 3346, 2932, 1635, 1568 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRESIMS *m/z* 649.2467 [M + H]$^+$ (calcd for $C_{39}H_{37}O_9$, 649.2437); R_f = 0.6 ($CHCl_3$−MeOH, 85:15).

Antimicrobial Assays. All organisms were obtained from the American Type Culture Collection (Manassas, VA) and included the fungi *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC

Table 3. In Vitro Antimicrobial Activity of Compounds **1** and **2** (all values in μM)

	MRSA			*A. fumigatus*			*C. neoformans*			*C. albicans*		
sample	IC_{50}[a]	MIC[b]	MBC[c]	IC_{50}[a]	MIC[b]	MFC[c]	IC_{50}[a]	MIC[b]	MFC[c]	IC_{50}[a]	MIC[b]	MFC[c]
1	1.5	4.0	31.8	na[d]	na[d]	na[d]	na[d]	na[d]	na[d]	na[d]	na[d]	na[d]
2	0.5	1.9	30.8	10.0	15.4	30.8	5.4	7.7	7.7	5.4	15.4	30.8
amphotericin B	nt[e]	nt[e]	nt[e]	1.6	2.7	2.7	0.9	1.4	2.5	0.3	0.7	1.4
ciprofloxacin	0.3	0.8	na[d]	nt[e]	nt[e]	nt[e]	nt[e]	nt[e]	nt[e]	nt[e]	nt[e]	nt[e]

[a] IC_{50} = the test concentration that affords 50% growth. [b] MIC (minimum inhibitory concentration) = the lowest test concentration that allows no detectable growth. [c] MBC/MFC (minimum bactericidal/fungicidal concentration) = the lowest test concentration that kills the organism. [d] na = not active at the highest test concentration. [e] nt = not tested.

90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. All organisms were tested using modified versions of the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) methods. For all organisms excluding *M. intracellulare* and *A. fumigatus*, optical density was used to monitor growth.[14,15] Medium supplemented with 5% Alamar Blue (BioSource International, Camarillo, CA) was utilized for growth detection of *M. intracellulare*[16,17] and *A. fumigatus*.[18] Samples dissolved in DMSO were serially diluted in 20% DMSO−saline and transferred (10 μL) in duplicate to 96-well flat-bottomed microplates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth [RPMI 1640/2% dextrose/0.03% glutamine/MOPS at pH 6.0 (Cellgro) for *C. albicans*, Sabouraud dextrose for *C. neoformans*, cation-adjusted Mueller-Hinton (Difco) at pH 7.0 for MRSA, *E. coli*, and *P. aeruginosa*, 5% Alamar Blue in Middlebrook 7H9 broth with OADC enrichment, pH = 7.0 for *M. intracellulare*, and 5% Alamar Blue/RPMI 1640 broth (2% dextrose, 0.03% glutamine, buffered with 0.165 M MOPS at pH 7.0) for *A. fumigatus*, to afford an assay volume of 200 μL and final target inocula of *C. albicans* 1.0×10^4, *C. neoformans* 1.0×10^5, *M. intracellulare* 2.0×10^6, MRS, *E. coli*, *P. aeruginosa* 5.0×10^5 cfu/mL, and *A. fumigatus* 3.0×10^4 cfu/mL. Final sample test concentrations were 1/100 the DMSO stock concentration. Drug controls [ciprofloxacin (ICN Biomedicals, Columbus, OH) for bacteria and amphotericin B (ICN Biomedicals) for fungi] were included in each assay. All organisms were read at either 630 nm, using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Winooski, VT), or 544ex/590em (*M. intracellulare, A. fumigatus*), using a Polarstar Galaxy plate reader (BMG LabTechnologies, Offenburg, Germany) prior to and after incubation: *C. albicans*, MRSA, *E. coli*, and *P. aeruginosa* at 37 °C for 18−24 h, *C. neoformans* and *A. fumigatus* at 30 °C for 68−72 h, and *M. intracellulare* at 37 °C and 10% CO_2 for 68−72 h. The MIC is defined as the lowest test concentration that allows no detectable growth (for *M. intracellulare* and *A. fumigatus*, no color change from blue to pink). Minimum fungicidal or bactericidal concentrations were determined by removing 5 μL from each clear (or blue) well, transferring to agar, and incubating as previously mentioned. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar).

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Supporting Information Available: Computational ECD calculation data of **1** and **2**. This information is available free of charge via the Internet at http:/pubs.acs.org.

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