Lactones and Quinones from the Tubers of *Sinningia aggregata*

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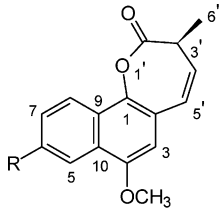
Three new aromatic ε-lactones, aggregatins A (**1**), B (**2**), and C (**3**), a new naphthoquinone derivative, aggregatin D (**4**), and three known anthraquinones, 2-methylanthraquinone, 7-methoxy-2-methylanthraquinone, and 7-hydroxy-2-methylanthraquinone, were isolated from the tubers of *Sinningia aggregata* (Gesneriaceae). Compounds **1** and **4** and the anthraquinones showed marginal antimicrobial activity.

Sinningia aggregata (Ker-Gawl.) Wiehler (Gesneriaceae) is an annual herb, with perennial tubers, found in Brazil (midwestern and southeastern regions) and Paraguay.[1] The composition of its essential oil has been reported,[2] but to date there have been no phytochemical investigations of this plant.

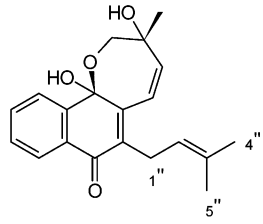
Successive chromatographic fractionation of extracts from the tubers of *S. aggregata* yielded four new compounds, aggregatins A (**1**), B (**2**), C (**3**), and D (**4**), and three known anthraquinones.



1 R = OH

2 R = H

3 R = OCH_3



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Compound **1** was isolated as a brown solid with a molecular formula of $C_{16}H_{14}O_4$, as determined from GC-HRMS and NMR data, which is consistent with 10 degrees of unsaturation. The ^1H NMR spectrum (Table 1) showed signals due to the presence of one secondary methyl group (δ_H 1.53), one methine proton (δ_H 2.80), one *O*-methyl group (δ_H 3.98), one hydroxy group (δ_H 5.40), two olefinic protons (δ_H 5.73 and 6.80) with coupling constants indicating a *cis* relation, and four aromatic protons (δ_H 6.56−8.22), one being isolated and the other three showing a typical pattern of coupling consistent with a 1,3,4-trisubstituted benzene moiety. The ^{13}C and DEPT 135 NMR spectra (Table 1) gave signals for 16 carbon atoms, corresponding to methyl and *O*-methyl groups (δ_C 14.4 and 55.7), seven methines, including an aliphatic methine (δ_C 38.5), an ester carbonyl (δ_C 171.1), and six aromatic quaternary carbons. Analysis of one-bond $^1H-^{13}C$ correlations from an HSQC experiment led to the identification of the tertiary carbons. The location of the double bond was established by long-range $^1H-^{13}C$ correlations from the HMBC experiment, mainly by the correlation of H-3 with C-5′, C-1, and C-10, while the correlations of H-6′ with C-2′ (carbonyl) and C-4′ as well as of H-8 with C-1 led to identification of an ε-lactone moiety. The position of the *O*-methyl group was determined by the long-range $^1H-^{13}C$ correlations of *O*-methyl protons with C-4 as well as by 1D NOE experiments. Selective irradiation of the resonance frequencies of H-3 (δ_H 6.56) and H-5 (δ_H 7.55) caused an NOE enhancement of the *O*-methyl protons. The location of the hydroxy group was established by the long-range $^1H-^{13}C$ correlations of H-8 with C-6 and C-10 and the correlations of the hydroxy hydrogen with C-6. The proposed structure was also supported by the fragment ions at *m*/*z* 242 ($[M]^{+\bullet}$ − CO) and 228 (base-peak) by HREIMS analysis. Accordingly, compound **1** was identified as 6-hydroxy-4-methoxy-3′-methylnaphtho[1,2-*b*]oxepin-2′(3′*H*)-one, named aggregatin A.

Compound **2** was isolated as a green solid with a molecular formula of $C_{16}H_{14}O_3$, as determined by HRESIMS and NMR data. The ^1H and ^{13}C NMR spectra (Table 1) were similar to those of compound **1**, suggesting the same skeleton. However, the ^1H NMR spectrum showed five aromatic protons, one being isolated and the other four showing the typical coupling pattern of a 1,2-disubstituted benzene unit. Analysis of one-bond and long-range $^1H-^{13}C$ correlations from the HSQC and HMBC experiments as well as 1D NOE and MS data supported the proposed structure for compound **2**, which was identified as 4-methoxy-3′-methylnaphtho[1,2-*b*]oxepin-2′(3′*H*)-one and named aggregatin B.

Compound **3** was isolated as a yellow solid with a molecular formula of $C_{17}H_{16}O_4$, as determined by HRESIMS and NMR data. The ^1H and ^{13}C NMR spectra (Table 1) were also similar to those of compound **1**, but with an *O*-methyl group replacing the hydroxy group. Therefore, compound **3** was identified as 4,6-dimethoxy-3′-methylnaphtho[1,2-*b*]oxepin-2′(3′*H*)-one and named aggregatin C.

The seven-membered ε-lactone ring in aggregatins A, B, and C can adopt two different conformations with the methyl group, either pseudoaxial or pseudoequatorial. Therefore, a conformational search was performed in order to find the one with minimal energy through structure geometry optimization and density functional theory.[3,4] The computational *ab initio* studies showed that the conformation with the methyl group pseudoequatorial is more stable (around 11 kJ mol^{-1}) than the pseudoaxial conformation. This conformation was supported by 1D NOE experiments. Selective irradiation of the resonance frequency of H-4′ caused an NOE enhancement only in the signal of hydrogens from the methyl group. Moreover, selective irradiation of the resonance frequency of H-3′ did not cause any NOE enhancement in the signal of H-4′, while the irradiation

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Table 1. NMR Data (400 MHz, $CDCl_3$) of Compounds **1**, **2**, and **3**

	1			**2**			**3**		
position	δ_C	δ_H mult. (*J* in Hz)	HMBC	δ_C	δ_H mult. (*J* in Hz)	HMBC	δ_C	δ_H mult. (*J* in Hz)	HMBC
1	139.6, qC			139.3, qC			139.6, qC		
2	120.6, qC			122.9, qC			120.6, qC		
3	103.4, CH	6.56 s	1, 4, 5, 5′, 10	102.6, CH	6.59 s	1, 4, 5, 5′, 10	103.4, CH	6.59 s	1, 4, 5′, 10
4	150.7, qC			151.7, qC			150.9, qC		
5	104.8, CH	7.55 d (2.5)	4, 6, 7, 9	121.9, CH	8.24 ddd (8.0, 1.7, 0.6)	4, 7, 9	100.7, CH	7.53 d (2.6)	4, 7, 9
6	154.4, qC			126.7, CH	7.57 ddd (8.0, 6.9, 1.3)	8, 10	158.6, qC		
7	118.7, CH	7.21 dd (9.1, 2.5)	5, 6, 9	127.5, CH	7.62 ddd (8.4, 6.9, 1.7)	5, 9	119.3, CH	7.24 dd (9.2, 2.6)	5, 9
8	124.7, CH	8.22 d (9.1)	1, 4, 5, 6, 10	122.4, CH	8.31 ddd (8.4, 1.3, 0.6)	1, 6, 10	124.3, CH	8.21 d (9.2)	1, 6, 10
9	122.1, qC			127.1, qC			122.1, qC		
10	127.5, qC			126.1, qC			127.4, qC		
2′	171.1, qC			171.2, qC			171.2, qC		
3′	38.5, CH	2.80 qdd (6.8, 5.0, 2.4)	2′, 4′, 5′	38.6, CH	2.80 qdd (6.8, 4.9, 2.3)	2′, 4′, 5′	38.6, CH	2.78 qdd (6.8, 5.0, 2.3)	2′, 4′, 5′
4′	130.1, CH	5.73 dd (9.5, 5.0)	1, 2, 2′, 3′, 5′, 6′	131.0, CH	5.78 dd (9.5, 4.9)	2, 2′, 3′, 6′	130.1, CH	5.72 dd (9.5, 5.0)	2, 2′, 3′, 6′
5′	129.1, CH	6.80 dd (9.5, 2.4)	1, 3, 3′, 4′	129.2, CH	6.83 dd (9.5, 2.3)	1, 3, 3′	129.1, CH	6.80 dd (9.5, 2.3)	1, 3, 3′
6′	14.4, CH_3	1.53 d (6,8)	2′, 3′, 4′	14.6, CH_3	1.54 d (6.8)	2′, 3′, 4′	14.6, CH_3	1.52 d (6.8)	2′, 3′, 4′
CH_3O-4	55.7	3.98 s	4	55.6, CH_3	4.02 s	4	55.8	4.01 s	4
HO-6		5.40 s							
CH_3O-6							55.5	3.96 s	6

of hydrogens from the methyl group caused an NOE intensification in the H-4′ signal (Figure 1).

After geometrical optimization, optical rotations were calculated for the *R* and *S* isomers of the more stable conformation (pseudoequatorial), employing the method proposed by Pedersen and Hansen.[5] Thus, it was predicted that the *S* isomers of aggregatins A, B, and C should be dextrorotatory. Since the signal of the experimental optical rotations was positive for compounds **1**, **2**, and **3**, their absolute configurations were assigned as 3′*S*.

Compound **4** was isolated as a yellow oil with a molecular formula of $C_{20}H_{22}O_4$, as determined by HRESIMS and NMR data, consistent with 10 degrees of unsaturation. The ^{1}H NMR spectrum (Table 2, $CDCl_3$) showed signals due to a spin system consisting of four aromatic protons (δ 7.52−8.10), in accordance with the pattern of a 1,2-disubstituted benzene, two olefinic protons (δ 6.43 and 6.73), two carbinolic protons (δ 4.00 and 3.83), a prenyl (δ 5.02, 3.37, 3.19, 1.77, and 1.66), and a methyl group (δ 1.70). The ^{13}C NMR spectrum (Table 2, $CDCl_3$) showed peaks for 20 carbon atoms. In particular, signals were observed for a carbonyl group (δ 183.9), two oxygenated aliphatic carbons (δ 74.8 and 80.6), and a dioxygenated carbon (δ 99.4). The carbon atom classification was determined by the DEPT 135 NMR spectrum as well as by one-bond ^{1}H−^{13}C correlations from the HSQC experiment. The positions of the carbonyl and prenyl groups were established by long-range ^{1}H−^{13}C correlations from an HMBC experiment, mainly by the correlations of H-5 and H-1″ with C-4. H-5′ exhibited cross-peaks with C-1, C-3, and C-3′, while H-4′ showed correlations with C-2, C-3′, and C-6′, determining the location of the heterocyclic ring. The overall analysis of the HMBC experiment confirmed compound **4** as 1,3′-dihydroxy-3′-methyl-3-prenyl-2′,3′-dihydronaphtho[1,2-*b*]oxepin-4(1*H*)-one, named aggregatin D.

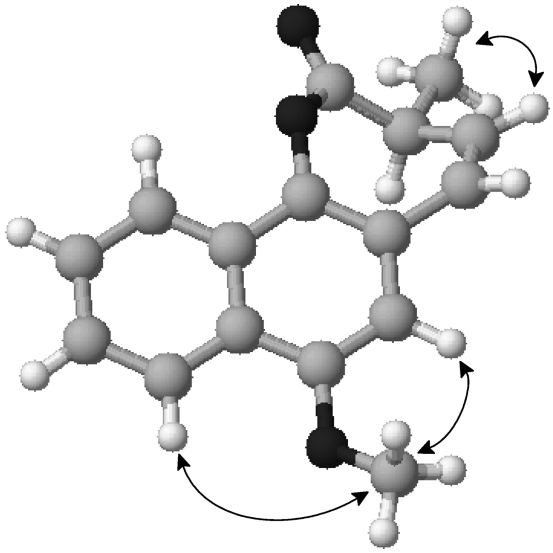

Figure 1. Conformation and key NOE 1D correlations for compounds **1**−**3**.

The relative configuration of compound **4** was deduced from NMR data, analysis of molecular models, and computational calculations. The hydroxy groups can be either *trans* or *cis* oriented. The hydroxy group at C-1 remains in the axial position for all isomers, while the hydroxy group at C-3′ can be pseudoaxial or pseudoequatorial. For the *trans* isomers, molecular models and computational *ab initio* studies showed that, due to steric hindrance, only one conformation is possible, with the methyl group pseudoequatorial and the hydroxy group pseudoaxial. However, the key NOE enhancements observed in the 1D NOE NMR experiments were incompatible with this possibility. On the other hand, *cis* isomers can adopt two main conformations, with the hydroxy group at C-3′ as pseudodiaxial or pseudoequatorial. In the first case intramolecular hydrogen bonding occurs, but no typical signal was observed in the ^{1}H NMR spectra acquired in both C_6D_6 and $CDCl_3$ (Table 2). In addition, the key NOE enhancements were incompatible with this conformer, but fully in accordance with the other. The selective irradiation of H-2′b (δ 3.84) showed a strong NOE intensification of the H-6′ signal and a weak intensification of the H-8 signal. Furthermore, the selective irradiation of H-2′a (δ 4.00) caused an NOE enhancement of H-4′ and only a very weak NOE intensification of H-6′ (Figure 2). These results support the *cis* isomers with the methyl group in a pseudoaxial position. In addition, the selective irradiation of H-2″ caused an NOE enhancement of the H-5′ and H-4″ signals, suggesting that the prenyl group is as indicated in Figure 2.

As for the ε-lactones, optical rotation calculation predicts that the 1*R*, 3′*S* isomer should be levorotatory. Therefore, since compound **4** showed a negative experimental optical rotation deviation, its absolute configuration was assigned as 1*R*, 3′*S*.

The known compounds isolated from *S. aggregata* were identified by comparison of their spectroscopic data with reported data, as 2-methylanthraquinone (tectoquinone),[6] 7-methoxy-2-methylanthraquinone,[7] and 7-hydroxy-2-methylanthraquinone.[8]

Compounds **1** and **4** and the three anthraquinones were evaluated for antimicrobial activity,[9] but were found to be only marginally

Table 2. NMR Data (400 MHz) of Compound **4**

	δ_C, mult.		δ_H mult. (*J* in Hz)		
position	$CDCl_3$	benzene-d_6	$CDCl_3$	benzene-d_6	HMBC
1	99.5, qC	100.0			
2	143.6, qC	143.9			
3	132.1, qC	132.4			
4	183.9, qC	183.6			
5	126.4, CH	126.7	8.10, ddd (7.8, 1.4, 0.5)	8.29, ddd (7.8, 1.4, 0.5)	4, 7, 9
6	129.8, CH	129.9	7.52, ddd (7.8, 7.4, 1.3)	7.04, ddd (7.8, 7.4, 1.3)	8, 10
7	132.8, CH	132.5	7.63, ddd (7.8, 7.4, 1.4)	7.20, ddd (7.8, 7.4, 1.4)	5, 9
8	125.9, CH	126.5	7.75, ddd (7.8, 1.3, 0.5)	7.76, ddd (7.8, 1.3, 0.5)	1, 6, 9, 10
9	137.8, qC	138.7			
10	131.7, qC	132.3			
2′	74.8, CH_2	74.9	4.00, d (6.4)	3.57, d (6.4)	1, 3′, 4′
			3.83, d (6.4)	3.44, d (6.4)	3′, 4′, 6′
3′	80.6, qC	80.4			
4′	139.7, CH	139.5	6.43, d (9.7)	5.81, d (9.7)	2, 3′, 6′
5′	123.9, CH	124.2	6.73, d (9.7)	6.52, d (9.7)	1, 3, 3′
6′	19.5, CH_3	19.2	1.70, s	1.18, s	2′, 3′, 4′
1″	23.7, CH_2	24.0	3.37, dd (14.4, 6.8)	3.26, dd (14.4, 6.7)	2, 4, 2″, 3″
			3.19, dd (14.4, 6.7)	3.46, dd (14.4, 7.2)	
2″	121.4, CH	122.4	5.02, ddqq (6.8, 6.7, 1.2, 0.9)	5.18, ddq (7.2, 6.7, 1.4)	3, 1″, 4″, 5″
3″	132.6, qC	132.4			
4″	25.7, CH_3	25.7	1.66, d (1.2)	1.52, d (1.4)	2″, 3″, 5″
5″	18.0, CH_3	18.1	1.77, d (0.9)	1.72, s	2″, 3″, 4″
OH			1.56, s	3.45, s	

active, with MIC values greater than 100 μg mL^{-1}, mainly for the *Candida dubliniensis* strains ATCC 777 and ATCC 778157.

Experimental Section

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ on a Rudolph Research polarimeter. The UV spectra were obtained in MeOH on a Shimadzu UV-2401PC spectrophotometer. The IR spectra were recorded in KBr pellets on a Biorad FTIR spectrophotometer. 1D and 2D NMR determinations were carried out in $CDCl_3$ at 295 K on a Bruker AVANCE 400 NMR spectrometer operating at 9.4 T, observing ^{1}H and ^{13}C at 400 and 100 MHz, respectively. The spectrometer was equipped with a 5 mm multinuclear direct detection probe with a *z*-gradient. All ^{1}H and ^{13}C NMR chemical shifts are given in ppm (δ) and were related to the TMS signal at 0.00 ppm as internal reference, with the coupling constants (*J*) in Hz. HR-MS spectra were obtained on an HP-5000 Shimadzu GC-HRMS or on a Micromass ESI-QqTof mass spectrometer. GC-MS analyses were performed using an HP5-MS column (30 m × 0.25 mm × 2.25 mm). Geometry optimization and density functional theory calculations on the electronic structure of the compounds employed the B3LYP hybrid functional, using the LANL2DZ basis set, as implemented in the Gaussian03 suite.[10] Silica gel (Merck, 230−400 mesh) was used for column chromatography, while silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) and preparative (1.0 mm) TLC. Compounds were visualized by exposure under $UV_{254/366}$ light and by spraying with 5% (v/v) H_2SO_4 in EtOH solution, followed by heating on a hot plate.

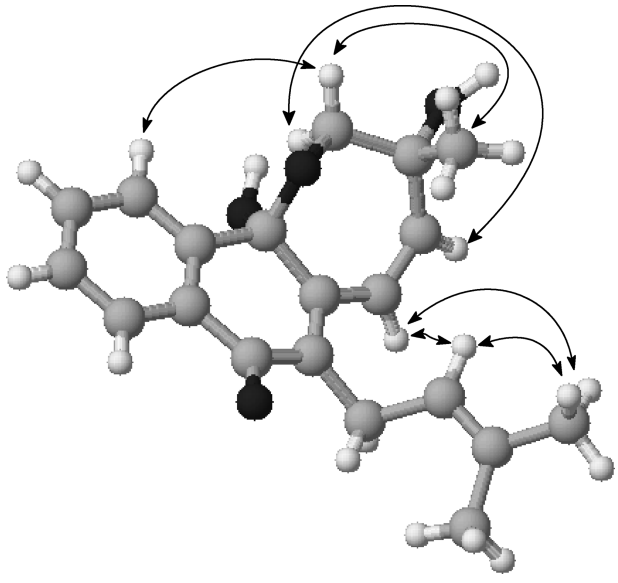

Figure 2. Conformation and key NOE 1D correlations for compound **4**.

Plant Material. Tubers of *S. aggregata* were harvested in two localities of Paraná State, Brazil. Material A was collected in Curitiba, in October 2004, and was identified by Dr. Armando C. Cervi, who deposited a voucher specimen (Cervi 3873) in the herbarium of the Universidade Federal do Paraná (UPCB). Material B was collected in Tibagi, in May 2007, and was identified by Clarice B. Poliquesi. A voucher specimen (#290738) was deposited in the herbarium of the Museu Botânico Municipal.

Extraction and Isolation. Dried and powdered tubers (A 18.3 g; B 349.2 g) were extracted, at room temperature successively, with hexane (mixture of isomers) and EtOAc. The hexane and EtOAc extracts of each collection were similar by CCDC and subsequently pooled, yielding the extracts EA (0.23 g, material A) and EB (1.59 g, material B). EA was subjected to silica gel column chromatography (CC), eluted by a gradient system with increasing concentrations of EtOAc in hexane, giving 50 fractions. Fractions 3−7 (14.0 mg) were purified by silica gel preparative TLC (PTLC), using hexane−EtOAc (9:1) as solvent, to give 7-methoxy-2-methylanthraquinone (4.5 mg). Fractions 20−36 (17.0 mg) were purified by PTLC using hexane−CH_2Cl_2−EtOAc (1:1:0.5) as solvent to give **1** (7.0 mg). Fraction 49 (37 mg) was purified by PTLC using hexane−CH_2Cl_2−acetone−MeOH (3:3:2:0.1) as solvent to give 7-hydroxy-2-methylanthraquinone (3.0 mg). EB was submitted to silica gel CC, eluted by a gradient system with increasing concentrations of acetone in hexane, to give seven fractions after TLC analysis. Fraction 2 (1.21 g) was subjected to further CC on silica gel, eluted again with increasing concentrations of acetone in hexane, to give 19 fractions after TLC analysis. Fraction VI yielded 2-methylanthraquinone (3.0 mg) after repeated silica gel PTLC using hexane−EtOAc (9:1) as solvent. Fraction VII (19.0 mg) was purified by silica gel PTLC, eluted with petroleum ether−acetone (5:0.2), yielding compound **2** (1.0 mg). Fraction VIII (26.0 mg) was submitted to the same procedure, yielding **2** (2.3 mg). Fraction X (112.0 mg) was purified by silica gel PTLC using hexane−$CHCl_3$−MeOH (5:5:0.1) as solvent to give compound **4** (50 mg). Fraction XIV (41.0 mg) was purified by repeated silica gel preparative TLC, eluted with hexane−CH_2Cl_2 (1:1), to give compound **3** (1.4 mg).

Aggregatin A (1): brown needles; mp 95 °C; $[\alpha]^{25}_D$ +37.6 (*c* 0.01, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 291 (3.8), 239 (3.9), 208 (4.6) nm; IR ν_{max} (KBr) 3410, 1717, 1620 cm^{-1}; ^{1}H and ^{13}C and NMR data, see

Table 1; GC-HRMS *m/z* 270.1131 (52) $[M]^{+\bullet}$ (calcd for $C_{16}H_{14}O_4$ 270.0892), 242.1176 (13), 228.0986 (13), 227.0950 (100), 212.0720 (7).

Aggregatin B (2): green powder; $[\alpha]^{25}_D$ +53.8 (*c* 0.065, $CHCl_3$); λ_{max} (MeOH) (log ε) 263 (3.8), 233 (3.7), 217 (3.7) nm; IR ν_{max} (KBr) 1729, 1597, 1275 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; GC-MS *m/z* 254 (24) $[M]^{+\bullet}$, 239 (5), 226 (17), 211 (100), 196 (16), 165 (14), 77 (14); HRESIMS *m/z* 255.1010 $[M + H]^+$ (calcd for $C_{16}H_{15}O_3$ 255.1021).

Aggregatin C (3): yellow powder; $[\alpha]^{25}_D$ +52.0 (*c* 0.05, $CHCl_3$); λ_{max} (MeOH) (log ε) 265 (3.7), 233 (3.6), 217 (3.6) nm; IR ν_{max} (KBr) 1729, 1275 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; GC-MS *m/z* 284 (28) $[M]^{+\bullet}$, 269 (5), 256 (10), 241 (100), 226 (14), 106 (14), 77 (17); HRESIMS *m/z* 285.1127 $[M + H]^+$ (calcd for $C_{17}H_{17}O_4$ 285.1127).

Aggregatin D (4): yellow oil; $[\alpha]^{25}_D$ −27.3 (*c* 0.05, $CHCl_3$); λ_{max} (MeOH) (log ε) 219 (3.7), 229 (3.7) nm; IR ν_{max} (KBr) 3416, 3070, 2932, 1715, 1649, 1600, 1309, 1070 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 349.1482 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_4Na$ 349.1416).

Antimicrobial Activity. Compounds **1** and **4** and the anthraquinones were evaluated for antimicrobial activity using the broth microdilution method, as previously described.[9] The following strains of microorganisms were utilized: Gram-positive bacteria (*Staphylococcus aureus* ATCC 14458, *Staplylococcus epidermidis* ATCC 12228), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 10799), and yeasts (*Candida tropicalis* ATCC 157, *Candida glabrata* ATCC 30070, *Candida dubliniensis* ATCC 777, and *C. dubliniensis* ATCC 778157).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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