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New Furanoid Diterpenes from *Caesalpinia pulcherrima*

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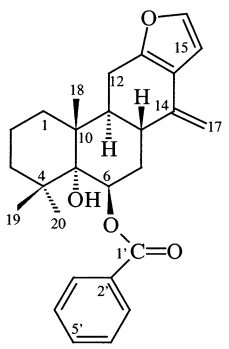
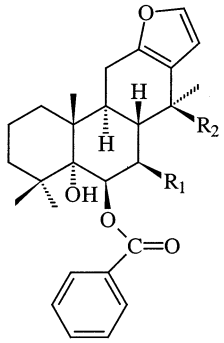
Four new cassane-type furanoditerpenoids (**1**−**4**) were isolated from the air-dried leaves of *Caesalpinia pulcherrima*. Their structures were elucidated by spectral data interpretation. The exocyclic methylene compound **1** readily isomerized and oxidized to the benzofuran **4**. Benzyl 2,6-dimethoxybenzoate (**5**) was also identified in this study. Antimicrobial tests on **1**−**5** indicated that they were active against several bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis*) and fungi (*C. albicans* and *T. mentagrophytes*).

Caesalpinia pulcherrima (L.) Swartz. (Leguminosae) is a tropical shrub grown as an ornamental plant in the Philippines. Decoctions of the leaves, bark, and roots are used to treat liver disorders and ulcers of the mouth and throat, reduce fevers, cause abortions, and alleviate fungal infections.[1] The fruit is used to check bleeding and prevent diarrhea and dysentery,[1] while the flowers are utilized to combat oxidative stresses.[2] A number of studies have been reported on the chemical constituents of *C. pulcherrima*. Diterpene benzoates that were found to be active against DNA repair-deficient yeast mutant were isolated from the roots of the plant.[3] A cassane-type diterpene ester, pulcherralpin, isolated from the stems had potential fertility-regulating and antitumor activities.[4] The furanoditerpenoids voucapen-5α-ol,6*β*-cinnamoyl-7*β*-hydroxyvouacapen-5-ol and 8,9,11,14-didehydrovouacapen-5α-ol were also reported from the roots of *C. pulcherrima*.[5] 2,6-Dimethoxybenzoquinone and 4′-methoxyisoliquritrigen were both reported to have significant cytotoxic activity in the KB test system.[6] Other studies have reported the isolation of pulcherrimin, 6-methoxypulcherrimin, bonducellin, and 8-methoxybonducellin from the stem,[6] while the seeds afforded galactomannan.[7] Myricetin glycoside and 5,7,3′,4′,5′-pentahydroxyflavanol were isolated from the leaves,[8] while the flowers afforded *β*-sitosterol, gallic acid, lupeol, lupeol acetate, myricetin, quercetin, and rutin.[9] The stem bark afforded *β*-sitosterol, ellagitannins, leucodelphinidin, quercimeritrin, and sebacic acid.[10,11]

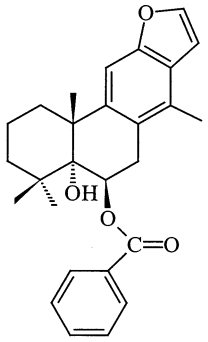
We now report the isolation, structure elucidation, and antimicrobial test results of four new diterpene metabolites (**1**−**4**) and the known metabolite **5** from the dichloromethane extract of the air-dried leaves of *C. pulcherrima*.



1



2 R_1 = H, R_2 = OH
3 R_1 OH, R_2 = H



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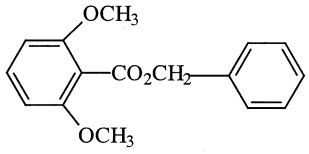

5

Results and Discussion

Silica gel chromatography of the initial crude extract, using increasing proportions of acetone in dichloromethane (10% increments), afforded four new cassane diterpenoids (**1**−**4**). Their structures were elucidated by extensive 1D and 2D NMR spectroscopy and mass spectrometry as follows. Compound **1** gave a ^{13}C NMR spectrum (Table 1) with 27 carbon signals. From the ^{1}H NMR spectrum (Table 1), seven of the ^{13}C NMR signals were due to a benzoate ester, indicating **1** was a diterpenoid. The ^{1}H NMR spectrum also indicated three methyl groups (*δ* 1.04, 1.24, 1.60), an exocyclic methylene (*δ* 4.83, 5.07), and a 2,3-disubstituted furan (*δ* 6.43, 7.23), which were confirmed by a *J* modulated spin−echo spectrum for X-nuclei coupled to ^{1}H (J_{MOD} ^{13}C NMR) and HMQC 2D NMR spectroscopy. The J_{MOD} spectrum also indicated additional oxygenated quaternary and methine carbons (*δ* 76.1 and 72.3, respectively) plus two quaternary, two methine, and five methylene carbons. Assuming an alcohol functionality, the proposed molecular formula for **1** was $C_{27}H_{32}O_4$, and this was confirmed by HREIMS.

The COSY 2D NMR spectrum of **1** showed correlations for five spin systems, H_2-1/H_2-2/H_2-3, H-6/H_2-7/H-8/H-9/H_2-11, and H-15/H-16 (Figure 1), plus the exocyclic methylene protons and a monosubstituted aromatic ring of the benzoate ester. Protons attached to carbon were assigned (Table 1) from HMQC 2D NMR spectral data, and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Figure 1. The quaternary oxygenated carbon was located at C-5 by long-range correlations to three methyl group protons

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Table 1. NMR Spectral Data for Compounds **1**−**3** ($CDCl_3$)

	1		**2**		**3**	
position	δ_C	δ_H mult. (*J* Hz)	δ_C	δ_H mult. (*J* Hz)	δ_C	δ_H mult. (*J* Hz)
1	34.5	1.52	34.8	1.46	35.1	1.49
		1.60		1.58		1.54
2	18.2	1.54	18.2	1.55	18.1	1.56
		1.79		1.75		1.70
3	38.0	1.16	38.1	1.17	37.8	1.18
		1.75		1.72		1.67
4	38.9		39.0		39.3	
5	76.1	1.62 s (OH)	76.3	1.66 s (OH)	77.9	1.75 s (OH)
6	72.3	5.59 t (3.1)	72.3	5.61 t (3.0)	74.0	5.81 d (4.1)
7	31.6	2.24 (2H)	27.4	2.05	69.3	4.41 dd (4.1 11.0)
				2.26		1.57 s (OH)
8	31.6	2.57	40.3	1.96 dt (4.4, 12.3)	38.1	2.02
9	44.1	2.40	41.2	2.44	37.2	2.43
10	41.3		41.5		41.0	
11	22.3	2.65 (2H)	21.7	2.56 (2H)	21.8	2.57 (2H)
12	152.2		149.4		149.2	
13	142.0		124.9		122.0	
14	118.7		71.6	1.55 s (OH)	27.3	3.04
15	106.3	6.43 d (1.9)	107.3	6.39 d (1.9)	109.7	6.20 d (1.9)
16	141.3	7.23 d (1.9)	141.3	7.24 d (1.9)	140.5	7.24 d (1.9)
17	104.2	4.83 br d (1.8)	25.3	1.29 s (Me)	17.1	1.09 d (6.8) (Me)
		5.07 d (2.2)				
18	17.1	1.60 s (Me)	17.2	1.57 s (Me)	17.6	1.54 s (Me)
19	27.6	1.24 s (Me)	25.9	1.24 s (Me)	25.5	1.18 s (Me)
20	25.9	1.04 s (Me)	27.6	1.05 s (Me)	27.3	1.12 s (Me)
1′	165.7		165.7		167.2	
2′	130.4		130.5		130.0	
3′/7′	129.7	8.06	129.7	8.04	129.9	8.05
4′/6′	128.6	7.46	128.5	7.44	128.6	7.45
5′	133.1	7.57	133.4	7.56	133.2	7.57

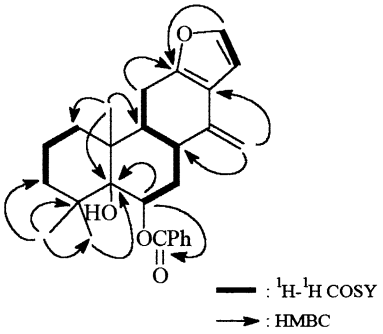

Figure 1. ^{1}H−^{1}H COSY and key ^{1}H−^{13}C long-range correlations for **1**.

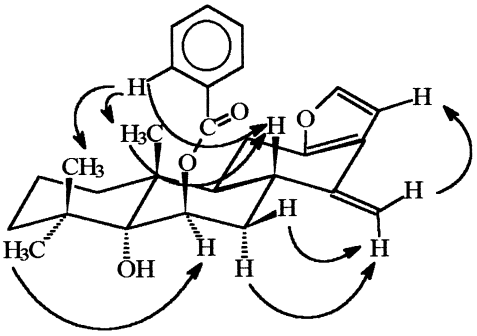

Figure 2. Key NOESY correlations for **1**.

and the H-6 proton, the ester group at C-6 by long-range correlations from H-6 to C-1′, and the exocyclic methylene group at C-14 from the correlations of the vinyl protons to C-8, C-13. All other long-range HMBC correlations supported the proposed structure for **1**.

The stereochemistry of **1** was determined by an analysis of coupling constants and NOESY 2D NMR data (Figure 2). The ^{1}H NMR signal for H-6 was a triplet with a small coupling constant (J = 3.1 Hz) to both protons on C-7, suggesting a conformation in which H-6 was equatorial or pseudoequatorial (though care was required since the H_2-7 protons had almost identical chemical shifts). Therefore, the benzoate group was axial. This was supported by the NOESY correlation of the *ortho*-benzoate ester protons to both the C-18 and C-19 methyl protons; this also placed the C-5 hydroxyl on the opposite face of the molecule and axial. The H-6 signal also showed a NOESY correlation to the C-20 methyl protons, indicating both were equatorial, and was further supported by a NOESY correlation of the C-5 hydroxyl proton to H-6 and the C-20 methyl protons. Both the H-8 and H-9 signals, although not completely first-order, had half-height widths of >25 Hz, indicating both had two large couplings ($J \approx$ 10, 12 Hz for H-9), consistent with a *trans*-diaxial orientation. NOESY correlations of H-8 to the C-18 methyl and the *ortho*-benzoate ester protons located H-8 on the same face as the benzoate group. The upfield exocyclic methylene proton (δ 4.83) showed a NOE to the H-7 protons, while the downfield proton (δ 5.07) showed a NOE to H-15. Other NOESY correlations observed were consistent with the stereochemistry proposed for **1**. Thus, compound **1** was established as (4aα,5β,6aβ,
11aα,11bβ)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,11b-trimethyl-7-methylenephenanthro[3,2-*b*]furan-4a,5-diol-5-benzoate. The trivial name isovouacapenol A is proposed.

The ^{1}H NMR data of **2** were similar to those of **1**, except for the absence of the exocyclic methylene protons and the appearance of a fourth singlet methyl resonance. The J_{MOD} ^{13}C NMR spectrum also revealed a second quaternary oxygenated carbon in **2**. Thus, **2** appeared to be the 5,14-diol that might result from double-bond hydration of **1**, and this was confirmed by HMBC correlations from the new quaternary C-14 (δ 71.6) to H-8 and H-15 and the HREIMS measurement of a molecular ion for $C_{27}H_{34}O_5$. The stereo-

Table 2. NMR Spectral Data for Compound **4** ($CDCl_3$)

position	δ_C	δ_H mult. (*J* Hz)
1	35.8	2.08
		2.21
2	19.0	1.72
		1.95
3	38.2	1.17
		1.98
4	38.6	
5	75.8	1.74 s (OH)
6	70.6	5.89 dd (0.8, 7.3)
7	32.4	2.89 d (18.7)
		3.58 dd (18.7, 7.3)
8	123.6	
9	143.4	
10	43.6	
11	105.0	7.43 s
12	154.1	
13	126.1	
14	128.5	
15	105.8	6.73 dd (0.9, 2.2)
16	144.6	7.55 d (2.2)
17	16.0	2.35 s (Me)
18	28.8	1.83 s (Me)
19	26.0	1.30 s (Me)
20	27.6	1.12 s (Me)
1′	166.1	
2′	130.4	
3′/7′	129.8	8.03
4′/6′	128.5	7.44
5′	133.0	7.55

chemistry of **2**, as determined by NOESY and coupling constant analysis, was identical with **1** at common stereocenters, and the methyl group at C-14 was placed on the opposite face of the molecule to the benzoate ester group by NOESY correlations from the methyl protons to H-7_{axial} and H-9. All remaining 2D NMR data were consistent with the proposed structure. Thus, compound **2** was established as (4aα,5*β*,6a*β*,7*β*,11aα,11b*β*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,7,11b-tetramethylphenanthro[3,2-*b*]-furan-4a,5,7-triol-5-benzoate. The trivial name isovouacapenol B is proposed.

Compound **3** was isomeric to **2**, having the same molecular formula on the basis of HREIMS. The J_{MOD} ^{13}C NMR spectrum of **3** indicated replacement of a methylene and a quaternary oxygenated carbon of **2** by two methine carbons (one oxygenated) in **3**. The ^1H NMR spectrum showed doublet resonances for H-6 and H-17 methyl protons (J = 4.1 Hz, 6.8 Hz, respectively) and a new doublet of doublets resonance (δ 4.41, J = 4.1, 11.0 Hz) for a single, axial H-7 proton. The relative stereochemistry of **3** was the same as **1** for the stereocenters in common, including the NOE from the benzoate protons *ortho* to H-8. A NOESY correlation from H-8 to H-14 determined that both protons were on the same face, further supported by NOESY correlations between the C-17 protons and H-7, which placed both on the opposite face of **3**. Thus, compound **3** was established as (4aα,5*β*,6a*β*,7α,11aα,11b*β*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,7,11b-tetramethylphenanthro[3,2-*b*]-furan-4a,5,7-triol-5-benzoate. The trivial name isovouacapenol C is proposed.

The benzofuran diterpenoid **4** was first observed as the major product of decomposition of an NMR sample of **1** in $CDCl_3$ and subsequently identified in the *C. pulcherrima* extract. Reisolation of **1** from another batch of freshly extracted samples afforded **1**−**4**. The ^1H NMR spectrum of **4** (Table 2) showed the loss of exocyclic methylene and other aliphatic protons of **1** and the appearance of a new singlet resonances for methyl (δ 2.35) and aromatic (δ 7.43) protons, with deshielded resonances observed for the H-7 protons (δ 2.89, 3.58). The J_{MOD} ^{13}C NMR spectrum of **4** (Table 2) showed four new aromatic carbons (one protonated) and one new methyl carbon in place of the exocyclic methylene and C-8, C-9 methine carbons of **1**. Compound **4** was thus identified as arising from double-bond isomerization and dehydrogenation of **1**. The molecular formula for **4** was confirmed by HREIMS for $C_{27}H_{30}O_4$. NOESY and coupling constant analysis showed that the relative stereochemistry of remaining stereocenters in **4** was unchanged from **1**. Thus, compound **4** was established as (4aα,5*β*,11b*β*)-1,2,3,4,4a,5,6,11b-octahydro-4,4,7,11b-tetramethylphenanthro[3,2-*b*]furan-4a,5-diol-5-benzoate. The trivial name isovouacapenol D is proposed.

The known compound benzyl 2,6-dimethoxybenzoate (**5**) was identified by comparison with literature data.[15] Ester **5** has not been previously reported from *C. pulcherrima*.

Since *C. pulcherrima* has been used ethnomedically to heal various infections,[1] **1**−**5** were tested for their antimicrobial potential. The results obtained (Table 3) indicated that **1** and **3** have moderate activity against *S. aureus*, while **2**, **4**, and **5** have low activity against this bacterium. Compounds **1**−**3** exhibited higher potencies against *B. subtilis* than **4** and **5**, although they had much lower activities when compared to the standard antibiotic used. All the compounds tested had low activity against *E. coli* and *P. aeruginosa*. Furthermore, except for **4**, all those tested had moderate activity against *C. albicans*. Compound **5** was the most active among the tested compounds against *T. mentagrophytes*, but lower than the standard antifungal agent, chlortrimazole. Compounds **1**−**5** were all inactive against *A. niger*.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Fourier transform IR spectrometer and UV spectra on a HP 8452A diode array spectrometer. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer in $CDCl_3$ (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). The high- and low-resolution EIMS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70−230 mesh). TLC was performed with plastic-backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin−H_2SO_4 and warming.

Plant Material. The plant sample was collected near Manila in July 2001. It was identified at the Philippine National Museum as *Caesalpinia pulcherrima* (L.) Swartz., and a voucher specimen (# 052) is maintained at the Chemistry Department of De La Salle University.

Extraction and Isolation. The air-dried leaves (2.3 kg) of *C. pulcherrima* were ground in a blender, then extracted with CH_2Cl_2 (10 L) for 2 days. The mixture was filtered and the filtrate concentrated in vacuo to afford a crude extract (150.5 g). This extract was dissolved in EtOH (2.5 L), then placed in an ice bath. To the solution was added 4% aqueous $Pb(OAc)_2$ (1.25 L) to precipitate the more polar components.[12] The mixture was then filtered and the filtrate concentrated in vacuo until a mixture of water and an oily residue remained. The concentrate was extracted with $CHCl_3$, and the extract was dried with anhydrous Na_2SO_4, then filtered. The filtrate was concentrated in vacuo to afford the treated extract (23 g), which was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increments) as eluents. The 10%−20% acetone in dichloromethane fractions were rechromatographed (6×) in 5%−10% ethyl acetate in petroleum ether to afford **1** (20 mg) and **4** (2 mg). The 30%−40% acetone in dichloromethane fractions were rechromatographed (8×) in 15%−20% ethyl acetate in petroleum ether to afford **2** (10 mg) and **3** (5 mg). The dichloro-

Table 3. Antimicrobial Test Results on **1**−**5** at 30 μg

	S. aureus		*E. coli*		*P. aeruginosa*		*B. subtilis*		*C. albicans*		*A. niger*		*T. mentagrophytes*	
sample	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]	C.Z.[a]	A.I.[b]
1	16	0.6	12	0.2	12	0.2	15	0.5	14	0.4		0	12	0.2
2	14	0.4	12	0.2	14	0.4	14	0.4	14	0.4	12	0.2	13	0.3
3	16	0.6	12	0.2	12	0.2	15	0.5	13	0.3		0	13	0.3
4	12	0.2	12	0.2	12	0.2	12	0.2	12	0.2		0	12	0.2
5	12	0.2	12	0.2	13	0.3	13	0.3	14	0.4		0	14	0.4
standard antibiotic	28 chloramphenicol	1.8	24 chloramphenicol	1.4	12 chloramphenicol	0.2	35 chloramphenicol	2.5	20 chlortrimazole	1.0	13 cycloheximide	0.3	21 chlortrimazole	1.1

[a] C.Z., clearing zone in mm; average of three trials. [b] A.I., antimicrobial index

methane fraction was rechromatographed (8×) in 15% ethyl acetate in petroleum ether to afford **5** (5 mg).

Isovouacapenol A [(4aα,5*β*,6a*β*,11aα,11b*β*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,11b-trimethyl-7-methylenephenanthro[3,2-*b*]furan-4a,5-diol-5-benzoate] (1): colorless crystals (mp 163−165 °C, petroleum ether); $[\alpha]^{20}_D$ −25.5° (*c* 0.0092, $CDCl_3$); IR (neat) ν_{max} 3408 (br, OH), 1713 (ester), 1274, 1176, 1111, 1070, 1023 (C−O), 665, 711 cm^{-1}; UV (MeOH) λ_{max} 273 (ϵ 3850) nm; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 420 [M^+] (34), 280 (30), 265 (43), 145 (16), 105 (100); HREIMS *m*/*z* 420.2300 [M^+] ($C_{27}H_{32}O_4$ requires 420.2301].

Isovouacapenol B [(4aα,5*β*,6a*β*,7*β*,11aα,11b*β*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,7,11b-tetramethylphenanthro[3,2-*b*]furan-4a,5,7-triol-5-benzoate] (2): colorless crystals (mp 108−110 °C, petroleum ether); $[\alpha]^{20}_D$ +12.6° (*c* 0.0082, $CDCl_3$); IR (neat) ν_{max} 3476 (br, OH), 1702 (ester), 1276, 1113, 1176, 1070, 1034, 1026 (C−O), 665, 711 cm^{-1}; UV (MeOH) λ_{max} 273.5 (ϵ 2930) nm; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 438 [M^+] (10), 420 (21), 280 (38), 265 (59), 145 (21), 105 (100); HREIMS *m*/*z* 438.2397 [M^+] ($C_{27}H_{34}O_5$ requires 438.2406).

Isovouacapenol C [(4aα,5*β*,6a*β*,7α,11aα,11b*β*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,4,7,11b-tetramethylphenanthro[3,2-*b*]furan-4a,5,7-triol-5-benzoate] (3): colorless crystals (mp 116−118 °C, petroleum ether); $[\alpha]^{20}_D$ −18.4° (*c* 0.0044, $CDCl_3$); IR (neat) ν_{max} 3482 (br, OH), 1709 (ester), 1276, 1176, 1120, 1071, 1026 (C−O), 665, 715 cm^{-1}; UV (MeOH) λ_{max} 280 (ϵ 3080) nm; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 438 [M^+] (77), 283 (5), 232 (8), 140 (11), 108 (27), 105 (100); HREIMS *m*/*z* 438.2397 [M^+] ($C_{27}H_{34}O_5$ requires 438.2406).

Isovouacapenol D [(4aα,5*β*,11b*β*)-1,2,3,4,4a,5,6,11b-octahydro-4,4,7,11b-tetramethyl phenanthro[3,2-*b*]furan-4a,5-diol-5-benzoate] (4): colorless crystals (mp 211−213 °C, petroleum ether); $[\alpha]^{20}_D$ −71.6° (*c* 0.0031, $CDCl_3$); IR (neat) ν_{max} 3561 (br, OH), 1712 (ester), 1273, 1172, 1107, 1071, 1024 (C−O), 665, 711 cm^{-1}; UV (MeOH) λ_{max} 280 (ϵ 2910) and 289 (ϵ 2610) nm; ^1H NMR and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 418 [M^+] (15), 296 (100), 281 (51), 253 (26), 231 (23), 211 (18), 105 (40); HREIMS *m*/*z* 418.2214 [M^+] ($C_{27}H_{30}O_4$ requires 418.2144).

Antimicrobial Testing. The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These were *Pseudomonas aeruginosa* UPCC 1244, *Bacillus subtilis* UPCC 1149, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, *Candida albicans* UPCC 2168, *Trichophyton mentagrophytes* UPCC 4193, and *Aspergillus niger* UPCC 3701.

A microbial suspension containing approximately 6×10^8 cells/mL was prepared from each test organism for 24 h cultures of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, and *C. albicans* and from 5-day-old cultures of *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water. One-tenth (0.1) mL of the bacteria, yeast, and molds was transferred into a medium containing prepoured nutrient agar (NA, DISCO Laboratories, Detroit, MI), glucose yeast peptone agar (GYP),[13] and potato dextrose agar (PDA, DISCO Laboratories), respectively. About 5 mL of corresponding medium, autoclaved and cooled to about 45 °C, was poured into the 90 mm Petri dish. The plate was swirled to distribute the microbial cells evenly on the plate, and the agar overlay was allowed to solidify. Three 10 mm wells were cut from equidistant points of the seeded agar plates using a sterile cork borer. Seventy (70) μg of samples dissolved in 95% EtOH was transferred in each well. For the standard agent, 30 μg were used.

The NA-, GYP-, and PDA-based cultures were incubated at 30 ± 1 °C for 24, 48, and 72 h, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone in millimeters. The average diameter of the clear zones was used to calculate an antimicrobial index.[14]

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Supporting Information Available: Tables of complete HMBC and NOESY 2D NMR data of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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