# Antimalarial (+)-*trans*-Hexahydrodibenzopyran Derivatives from *Machaerium multiflorum*

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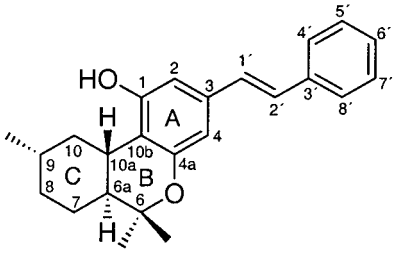
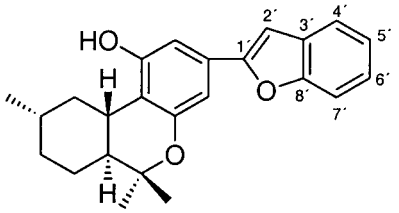
Bioassay-guided fractionation of *Machaerium multiflorum* yielded the hitherto unreported (+)-*trans*-hexahydrodibenzopyrans machaeriol A (**1**) and machaeriol B (**2**), as well as the known guaiane sesquiterpene (−)-kessane. Structure elucidation was based on $^1$H and $^{13}$C NMR data, mainly 2D NMR $^1$H−$^1$H COSY, $^1$H−$^{13}$C HMQC, $^1$H−$^{13}$C HMBC, and $^1$H−$^1$H NOESY experiments. This is the first report of the hexahydrodibenzopyrans from a higher plant other than the genus *Cannabis*. The cannabimimetic activity was thus evaluated by radioligand binding assay for cannabinoid receptor CB1, which indicated, notably, that both **1** and **2** were inactive. In addition, the cross reactivity of **1** and **2** toward antibodies designed for urinary metabolites of cannabinoids was evaluated with the EMIT and On Line cannabinoids assays. Both compounds showed no response at 100 000 ng/mL in both assays. Machaeriol B (**2**) demonstrated in vitro antimalarial activity ($IC_{50}$ = 120 ng/mL) against *Plasmodium falciparum* W-2 clone.

*Machaerium* is a genus of scandent to upright shrubs or lianas and small to medium sized, rarely large trees found throughout the tropical part of Mexico and as far as South America.[1,2] Chemical investigations of several species from Brazil, Peru, and Venezuela have revealed the presence of triterpenes,[3] benzoquinones,[4] and flavonoids,[5−7] including isoflavonoids, neoflavonoids, isoflavans, and lectins from *M. vestitum*, *M. villosum*, and *M. biovulatuml*. Some of these isoflavonoids and lectins exhibited anti-HIV and anti-inflamatory activities.[7,8] In addition, antigiardial isoflavonoids from *M. aristulatum*[9] and biologically active procyanidins from *M. floribundum* have been reported.[10]

*M. multiflorum* Spruce (Fabaceae), a native Amazonian liane found in Loreto, Peru, has not previously been the subject of phytochemical analysis. An ethanolic extract of the stem bark showed sufficient antimalarial and antibacterial activities to warrant bioassay-guided fractionation. This led to the isolation of two new hexahydrodibenzopyrans (HHDBP), named machaeriol A (**1**) and machaeriol B (**2**), as well as the known guaiane sesquiterpene (−)-kessane.[11] To our knowledge, this is the first report of HHDBPs **1** and **2** from a natural source.




**1**




**2**

## Results and Discussion

Re-extraction of the active dried EtOH extract of *M. multiflorum* with *n*-hexane, followed by $CH_2Cl_2$, resulted in localization of the antimalarial[12] and antibacterial activities in the *n*-hexane fraction. Centrifugal preparative TLC, followed by reversed-phase HPLC (see Experimental Section) of the *n*-hexane fraction, resulted in the isolation of two HHDBPs (**1** and **2**), as well as (−)-kessane,[11] a sesquiterpene oxide previously isolated from *Valeriana officinalis*.[13]

The molecular formula $C_{24}H_{28}O_2$ for **1** was established by positive-ion ESI-HRMS. The UV spectrum demonstrated the conjugated system for a stilbene chromophore, and the IR bands showed a hydroxyl group and aromatic ring(s) ($\nu_{max}$ 3550, 1630, 1515 cm$^{-1}$). The NMR spectral data of **1** (Table 1) were in agreement with hexahydrocannabinol,[14−17] except for the presence of a styrene moiety at C-3, instead of the $C_5H_{11}$ substituent. The $^1$H NMR spectrum demonstrated the presence of three methyls ($\delta$ 0.95, d, $J$ = 6.6 Hz; $\delta$ 1.08, s and 1.40, s) and two aromatic protons ($\delta$ 6.39, d and 6.61, d; each $J$ = 1.3 Hz), while the $^{13}$C NMR revealed five singlets ($\delta_C$ 77.8, 113.5, 137.2, 155.6, 155.8), five doublets ($\delta_C$ 33.3, 36.1, 49.5, 106.1, 108.9), and three triplets ($\delta_C$ 28.5, 39.4, 35.9), consistent with an HHDBP base skeleton.[15,16] The gradient $^1$H−$^1$H DQF-COSY spectra established the partial monoterpene unit for the HHDBP nucleus and a *trans*-coupled AB system ($\delta$ 6.88, d and 6.92, d; each $J$ = 16.3 Hz) of a styrene moiety. The gradient HMBC experiment established the assignments of the C-6 and C-9 methyl groups by $^3J$ -correlations between $\delta_{C-6a}$ 49.5, $\delta$ 1.08 (C-6-Me), and $\delta$ 1.40 (C-6-Me); $\delta_{C-10}$ 39.4, $\delta_{C-8}$ 35.9, and $\delta$ 0.95 (C-9-Me). The oxygenated carbons C-1 and -4a were placed by the $^3J$ -correlations between $\delta$ 2.50 (H-10a), $\delta_{C-4a}$ 153.8, and $\delta_{C-1}$ 155.1; the latter showed $^2J$ -correlation with $\delta$ 6.39 (H-2). Finally, placement of the C-3

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**Table 1.** $^1$H and $^{13}$C NMR Data for Compounds **1** and **2**

| | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| proton/carbon | $^1$H | $^{13}$C | HMBC | $^1$H | $^{13}$C | HMBC |
| 1 | | 155.8 s[b] | H-2<br>H-10a | | 156.0 s | H-2′<br>H-10a |
| 2 | 6.39 d (1.3)[a] | 106.1 d | H-1′<br>H-4 | 6.62 d (1.5) | 104.5 s | H-1′<br>H-4 |
| 3 | | 137.2 s | H-2′ | | 130.0 s | H-2′ |
| 4 | 6.61 d (1.3) | 108.9 d | H-1′<br>H-2 | 6.95 d (1.5) | 107.5 s | H-1′<br>H-2 |
| 4a | | 155.6 s | H-10a | | 156.0 s | H-10a |
| 6 | | 77.8 s | – | | 78.0 s | – |
| 6a | 1.49 ddd<br>(2.1,11.1,11.4) | 49.5 d | H-6-Me<br>H-10,<br>H-8 | 1.51 ddd<br>(1.75, 9.6, 11.3) | 49.5 d | H-6-Me<br>H-10,<br>H-8 |
| 7 | 1.85 m; 1.12 m | 28.5 t | | 1.85 m; 1.13 m | 28.5 t | |
| 8 | 1.85 m; 1.12 m | 35.9 t | | 1.85 m; 1.13 m | 35.9 t | |
| 9 | 1.64 m | 33.3 d | H-7<br>H-10a | 1.63 m | 33.3 d | H-7<br>H-10a |
| 10 | 3.07 d br (12.7)<br>0.79 dd br (12.7, 13.4) | 39.4 t | H-6a<br>C-9-Me | 3.10 d br (12.8)<br>0.80 dd br (12.8, 13.7) | 39.2 t | H-6a<br>C-9-Me |
| 10a | 2.50 ddd (2.4, 11.1,13.4) | 36.1 d | H-9 | 2.53 ddd (2.5,11.3, 13.7) | 36.2 d | H-9 |
| 10b | | 113.5 s | H-2, H-4<br>H-6a | | 114.5 s | H-2, H-4<br>H-6a |
| 6α-Me | 1.08 s | 19.5 q | H-6-Me<br>H-6a | 1.07 s | 19.5 q | H-6-Me<br>H-6a |
| 6*β*-Me | 1.40 s | 28.1 q | H-6-Me<br>H-6a | 1.41 s | 28.1 q | H-6-Me<br>H-6a |
| 9α-Me | 0.95 d (6.6) | 23.0 q | H-8<br>H-10 | 0.95 d (6.6) | 23.0 q | H-8<br>H-10 |
| 1′ | 6.88 d (16.3) | 128.9 d | H-2, H-4<br>H-2′ | | 155.8 s | H-2, H-4<br>H-2′ |
| 2′ | 6.92 d (16.3) | 128.6 d | H-1′, H-4′, H-8′ | 6.78 s | 101.6 d | H-4′ |
| 3′ | | 137.8 s | H-5′<br>H-7′ | | 129.6 s | H-5′<br>H-7′ |
| 4′ | 7.43 d br (7.4) | 126.9 d | H-2′ H-6′, H-8′ | 7.50 d br (7.6) | 121.3 d | H-2′<br>H-6′ |
| 5′ | 7.32 t (7.4, 7.7) | 129.1 d | H-7′ | 7.22 t br (7.6, 7.3) | 123.3 d | H-7′ |
| 6′ | 7.23 t (7.7, 7.7) | 127.9 d | H-4′<br>H-8′ | 7.24 ddd (1.0, 7.3, 7.9) | 124.5 d | H-4′ |
| 7′ | 7.32 t (7.4, 7.7) | 129.1 d | H-5′ | 7.44 d br (7.9) | 114.5 d | H-5′ |
| 8′ | 7.43 d br (7.4) | 126.9 d | H-4′<br>H-6′ | | 155.2 s | H-4′<br>H-6′ |
| OH | 5.24 br s | | | 5.30 br s | | |

[a] Coupling constants (*J* values in Hz) are in parentheses. [b] Multiplicities of carbon signals were determined by DEPT (135°) experiments.

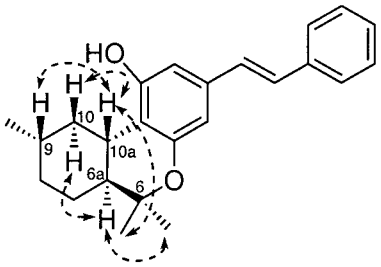


**Figure 1.** Key 2D NMR $^1$H−$^1$H NOESY correlations of compound **1**.

styrene substituent was established by cross-peaks between $\delta_{C-3}$ 137.2 and $\delta$ 6.92 (H-2′), and $\delta_{C-3'}$ 137.8 and $\delta$ 6.88 (H-1′).

The stereochemical assignments of carbons C-6a, C-9, and C-10a were resolved using optical rotation and NOESY experiments (Figure 1). A close comparison of the NMR spectral data and $[\alpha]_D$ values of **1** ($[\alpha]_D$ +115.4°) with enantiomeric HHDBPs,[17−20] (+)-hexahydrocannabinol ($[\alpha]_D$ +79.5°) and (−)-hexahydrocannabinol ($[\alpha]_D$ −73.2°), as well as their derivatives, indicates that **1** is an analogue of the (+)-enantiomer, i.e., possessing absolute configuration 6a*S*, 9*S*, 10a*S*. On the basis of this assumption the spatial orientation of the relevant protons and methyl groups was confirmed by NOESY correlations as follows. The NOESY showed correlations between H-10a ($\delta$ 2.50), H-10*β* ($\delta$ 3.07), and C-6*β*-Me ($\delta$ 1.40), as well as between $\delta$ 2.50 (H-10a) and 1.64 (H-9), indicating that they are *cis* to each other and *β*-oriented. As a result, the C-9-axial methyl group was placed at the α-face of the molecule. On the other hand, the NOESY data also showed a *cis* (α-face) relationship between H-10α ($\delta$ 0.79) and H-6a ($\delta$ 1.49), thereby confirming the α-orientation of H-6a; hence, compound **1** has a *trans*-fused B/C ring junction. This was further evident from the $^1$H NMR, which showed large *trans* coupling ($J_{6a,10a}$ = 11.1 Hz) between H-6a and -10a.[21] Molecular modeling simulation of **1** indicates that the dihedral angle ($\phi$) between the H-6aα-axial and H-10a*β*-axial protons is 169.9°. The dihedral angles between the H-10a*β*-axial and H-10*β*-equatorial, and H-10a*β*-axial and H-10α-axial, protons are 62.2° and 179.7°, respectively. This is consistent with the observed $J^{H_{6a}-H_{10a}}$ of 11.1 Hz, $J^{H_{10a}-H_{10\beta}}$ of 2.4 Hz, and $J^{H_{10a}-H_{10\alpha}}$ of 13.4 Hz. On the basis of the foregoing data, the stereochemistry was assigned as shown in Figure 1.

The $^1$H and $^{13}$C NMR spectra of **2** ($C_{24}H_{26}O_3$) were generally similar to those observed for **1** (Table 1), except for the differences associated with the presence of a benzo[*b*]furan substituent at C-3, instead of the C-3-styrene moiety in **1**. The $^1$H NMR spectrum revealed four aromatic protons ($\delta$ 7.50, 7.22, 7.24, and 7.44; H-4′−H-7′) for an ABCD system, as well as a one proton singlet at $\delta$ 6.78

**Table 2.** Antimalarial Activity of Compounds **1** and **2**

| | *P. falciparum* (W-2 clone)[a] | | *P. falciparum* (D-6 clone)[b] | |
|---|---|---|---|---|
| compound | $IC_{50}$ (ng/mL) | SI[c] | $IC_{50}$ (ng/mL) | SI[c] |
| **1** | 600 | >7.9 | 1500 | >3.2 |
| **2** | 120 | >40 | 720 | >6.6 |
| chloroquine | 140 | >34 | 19 | >251 |
| artemisinin | 4.4 | >1082 | 5.0 | >952 |

[a] Chloroquine-resistant clone. [b] Chloroquine-sensitive clone. [c] Selectivity index = $IC_{50}$ (Vero cells)/$IC_{50}$ *P. falciparum*.

**Table 3.** Antimicrobial Activity of Compounds **1** and **2**

| | $IC_{50}$ ($\mu$g/mL)[a] | | |
|---|---|---|---|
| compound | *C. neoformans* | *S. aureus* | MR *S. aureus* |
| **1** | 50 | 15 | 10 |
| **2** | *b* | 5.0 | 4.5 |
| amphotericin B | 0.375 | *b* | *b* |
| tetracycline | NT | 0.15 | 0.15 |

[a] $IC_{50}$ values after 48 h of incubation at 37 °C. [b] Inactive.

(H-2′), suggesting the presence of a C-1′-substituted benzo[*b*]furan moiety. Furthermore, a close comparison with the $^{13}C$ NMR data of benzo[*b*]furan[22] led to the conclusion that indeed **2** was the benzo[*b*]furan derivative of (+)-HHDBP. The structure and sterochemistry of **2** was confirmed by optical rotation and 2D NMR studies just as for **1**. The optical rotation ($[\alpha]_D$ +113.7°) indicates 6a*S*, 9*S*, 10a*S* stereochemistry. The linkage between C-1′ of the benzo[*b*]-furan side chain and C-3 of HHDBP was established by $^3J$-HMBC, which revealed correlations between $\delta_{C-3}$ 130.0 and H-2′, and $\delta_{C-1'}$ 155.8, $\delta$ 6.62 (H-2), and $\delta$ 6.95 (H-4). In addition, HMBC displayed cross-peaks between $\delta_{C-3'}$ 129.6, H-5′ and H-7′; and $\delta_{C-8'}$ 155.2, H-4′ and H-6′, confirming the assignments of the ABCD protons of the benzo[*b*]furan side chain. A NOESY experiment on **2** showed *cis* ($\beta$-face) correlation between H-9 ($\delta$ 1.63), H-10a ($\delta$ 2.53), and C-6-Me ($\delta$ 1.41), as well as cross-peaks between $\delta$ 1.51 (H-6a) and $\delta$ 0.80 (H-10$\alpha$), as observed in **1**. These correlations indicated that H-6a was $\alpha$-oriented and *trans* to the $\beta$-oriented H-10a, while C-9-Me was $\alpha$-axial. Other NOESY correlations observed for the monoterpene unit of **2** were also consistent with **1**. A similar molecular modeling simulation study of **2** ($^{H_{6a}-H_{10a}}\phi$ = 169.7°; $^{H_{10a}-H_{10\beta}}\phi = 62.6°$; $^{H_{10a}-H_{10\alpha}}\phi = 179.4°$) was in agreement with **1**, suggesting that the H-6a and H-10a protons were $\alpha$- and $\beta$-axially disposed, respectively.

Machaeriol B (**2**) showed inhibition of the growth of chloroquine-resistant *P. falciparum* clone W-2 ($IC_{50}$ = 120 ng/mL; SI = >40), while **1** was found to be weakly active.[23,24] When tested for antibacterial activity (Table 3) against *Staphylococcus aureus* and methicillin-resistant *S. aureus* using a modified microplate assay,[25] compound **2** showed inhibitory activities against both the organisms with $IC_{50}$ values of 5 and 4.5 $\mu$g/mL, respectively. Compounds **1** and **2** were evaluated against human recombinant HEK-293 cells (cannabinoid receptor CB1), using a radioligand binding assay.[26] They displayed insignificant activity against the CB1 receptor at concentrations of 1 and 0.1$\mu$M with an affinity range of 7% and 10% for **1** and −3% and 3% for **2**, respectively. The $IC_{50}$ value of reference cannabinoid was found to be 0.029 $\mu$M [$K_I$ = 0.023 $\mu$M; $n_H$ = 0.8]. The synthetic (−)-*trans* HHDBP-enantiomer, *cis*-hept-1-ene analogue of 9$\beta$-hydroxyhexahydrocannabinoid, previously exhibited strong affinities toward CB1-enriched rat brain microsome preparations ($K_I$ = 0.89 nM).[27] Furthermore, the (9*S*)-epimer of hexahydrocannabinol was found to be 20 times less psychoactive than its corresponding (9*R*)-epimer.[28]

The cross reactivity of **1** and **2** against antibodies designed for immunoassays to detect the presence of cannabinoids in biological fluids (mainly urine specimens) was investigated. No response was elicited by 100 000 ng/mL of either **1** or **2** in urine for the EMIT cannabinoids assay calibrated at 2 ng/mL cutoff or the On Line cannabinoids assay calibrated at 50 ng/mL. This is not at all surprising since these compounds have a stereochemistry exact opposite of the cannabinoids.

This appears to be the first report of the HHDBPs machaeriol A (**1**) and machaeriol B (**2**) from a natural source. Various HHDBPs had previously been synthesized, including (+)-hexahydrocannabinol and (−)-hexahydrocannabinol,[17−20] but to our knowledge the only known hexahydrocannabinoid as a natural product was cannabiripsol, isolated from a South African *Cannabis* variant.[29] Natural cannabinoids are restricted to *Cannabis sativa* and its variants in higher plants,[30,31] and a bibenzyl analogue of $\Delta^9$-THC, perrottetinen, had previously been reported from liverwort *Radula perrottetii*.[32] It is intriguing to note that the antimalarial activity of Peruvian *M. multiflorum* is contributed by two nonpsychotropic HHDBP derivatives **1** and **2**. Recently, antimalarial stilbenes from *Artocarpus integer* exhibited in vitro activities against multi-drug-resistant K1 strain.[33]

## Experimental Section

**General Experimental Procedures.** UV spectra were obtained in MeOH, using a Hewlett-Packard 8452A spectrophotometer, and IR spectra were taken as KBr disks on an Ati Mattson (Genesis Series) FTIR spectrophotometer. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz ($^1H$) and 125 MHz ($^{13}C$) in $CDCl_3$, using TMS as internal standard. Multiplicity determinations (DEPT) and 2D NMR spectra (gradient DQF-COSY, HMQC, gradient HMBC, and NOESY) were run using a standard Bruker pulse program. HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with electro-spray ionization (ESI). Optical rotation measurements were taken on a JASCO DIP-370 digital polarimeter in MeOH at ambient temperature. Centrifugal preparative TLC (CPTLC, using Chromatotron, Harrison Research Inc. Model 8924): 1 or 2 mm Si gel GF Chromatotron rotors (Analtech, Inc.), solvent $CHCl_3$−*n*-hexane (9:1), using an $N_2$ flow rate of 4 mL min$^{-1}$. HPLC: Waters LC module I plus, using semipreparative C-18 column. TLC was carried out on Si gel F254 with solvent system $CH_2Cl_2$−*n*-hexane (8:2). The isolated compounds were visualized by observing plates under UV-254 nm, followed by spraying with anisaldehyde−$H_2SO_4$ reagent. Molecular modeling was done using CS Chem3D Pro Version 5.0 MM2 molecular dynamics minimization followed by MM2 steric minimization. The software was obtained from CambridgeSoft Corporation, 100 Cambridge Park Drive, Cambridge, MA 02140-2312.

**Plant Material.** The stem bark of *M. multiflorum* was collected in November 1997 from open sandy forest near Loreto (Maynas), Peru, and was identified by Dr. Sidney T. McDaniel. A voucher specimen (IBE 12161) has been deposited at the Herbarium of the University of Mississippi.

**Extraction and Bioassay.** The powdered stem bark of *M. multiflorum* (0.5 kg) was extracted by percolation with 95% EtOH (3 × 2 L), and the combined extracts were evaporated under reduced pressure and then freeze-dried (yield 17.7 g). A portion of the dried EtOH extract (15 g) was percolated with *n*-hexane, followed by $CH_2Cl_2$, and finally the residual extract was washed with MeOH (each 200 mL × 3). The *n*-hexane, $CH_2Cl_2$, and MeOH fractions were separately filtered and dried, which afforded 3.8, 8.9, and 4.5 g, respectively. Antimalarial and antibacterial screenings (vide infra) of these

fractions showed that the main activity resided in an *n*-hexane-soluble fraction ($IC_{50}$ = 400 ng/mL [SI = >119] against *P. falciparum* D-6 clone; $IC_{50}$ <20 $\mu$g/mL against *S. aureus* and methicillin-resistant *S. aureus*).

**Isolation of Compounds.** The *n*-hexane fraction (3.5 g) was subjected to CPTLC (4 mm silica gel rotor), using *n*-hexane−$CHCl_3$ (2:1; 500 mL) and then with *n*-hexane−$CHCl_3$ (1:1) as eluant, to afford brown resinous mixture (mixture A; 512 mg). Mixture A was further separated by another CPTLC (2 mm silica gel rotors), using *n*-hexane−$CH_2Cl_2$ (9:1) as eluant, to give (−)-kessane as a pale yellow oil (36 mg; $[\alpha]_D$ −1.7°; lit.[11] $[\alpha]_D$ −6.1°], followed by a mixture of **1** and **2** (300 mg). A portion of the mixture (50 mg) was separated by semipreparative RP-HPLC (column: ODS prodigy 10$\mu$, 250 × 10 mm; detector: UV-254 nm), using 95% MeCN−$H_2O$ as solvent, which afforded **1** [16 mg; $R_f$ 0.73, silica gel, solvent: *n*-hexane−$CH_2Cl_2$ (8:2)], followed by **2** (16 mg; $R_f$ 0.70). The spectral data of (−)-kessane were in agreement with those reported in the literature.[11]

**Machaeriol A (6a$\alpha$,7,8,9$\beta$,10,10a$\beta$-Hexahydro-6,6,9-trimethyl-3-phenylethylene-6*H*-dibenzo[*b*,*d*]pyran-1-ol) (1):** amorphous solid; $[\alpha]_D$ +115.4° (*c* 0.39, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 212 (4.45), 314 (4.52) nm; IR (KBr) $\nu_{max}$ 3550 (OH), 2980−2820, 1630, 1515, 1480, 1440, 1330, 1300, 1285, 1265, 1100, 740 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; HRMS *m*/*z* 349.2129 $[MH]^+$ (calcd for $C_{24}H_{29}O_2$, 349.2162).

**Machaeriol B (6a$\alpha$,7,8,9$\beta$,10,10a$\beta$-Hexahydro-6,6,9-trimethyl-3-benzo[*b*]furan-1′-yl-6*H*-dibenzo[*b*,*d*]pyran-1-ol) (2):** amorphous solid; $[\alpha]_D$ +113.7° (*c* 0.53, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 214 (4.4), 246 (4.41), 306 (4.56) nm; IR (KBr) $\nu_{max}$ 3527 (OH), 2921, 2865, 1621, 1563, 1453, 1421, 1248, 1141, 1038, 950, 884, 799, 749 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; HRMS *m*/*z* 363.1936 $[MH]^+$ (calcd for $C_{24}H_{27}O_3$, 363.1955).

**Antimalarial/Parasite LDH Assay.** The in vitro antimalarial assay procedure[12] utilized at the NCNPR, University of Mississippi, is an adaptation of the parasite lactate dehydrogenase (pLDH) assay developed by Makler et al.,[23,24] using a 96-well microplate assay protocol with two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The primary screening involves determination of pLDH inhibition (percentage) of each sample tested at 15.9 and 1.59 $\mu$g/mL for extracts and pure compounds, respectively. The $IC_{50}$ values are determined only for the samples that inhibit parasite growth by >50% for one of the clones. The antimalarial agents chloroquine and artemisinin are used as positive controls, with DMSO as the negative (vehicle) control.

**Antimicrobial Assay.** The preliminary antimicrobial activities of the crude extracts/fractions and the $IC_{50}$ values of compounds **1**−**3** were determined by using a modified 96-well microplate assay protocol.[25] The test organisms used were ATCC strains of *Cryptococcus neoformans* (#90113), *Staphylococcus aureus* (# 6535), and methicillin-resistant *S. aureus* (#33591). Amphotericin B and tetracycline were used as positive controls, with DMSO as the negative control.

**Pharmacological Method for CB1 Activity.** The cannabimimetic activty was evaluated at MDS Panlabs, Taipei, Taiwan, using radioligand binding assay for cannabinoid receptor CB1 as described by Felder et al.[26] Human recombinant HEK-293 cell was used as the source material to assess the affinity of compounds **1** and **2** for the CB1 binding site. For primary assays, only the lowest concentration (1 and 0.1 $\mu$M) with a significant response (>50% of maximum stimulation or inhibition) was judged by the assay's criteria, where both **1** and **2** exhibited no significant response. The reference cannabinoid WIN-55,212-2 (Panlab standard) was used as positive control, while DMSO was used as the placebo.

**Cross Reactivity against Cannabinoid Antibodies.** Methanolic solutions of **1** and **2** were prepared at 2 mg/mL. Urine specimens (blank) were spiked at 100 000 ng/mL of either **1** or **2** (50 $\mu$L of the stock solution/mL of urine). The spiked urine samples were tested in two immunoassays, namely, EMIT cannabinoids 20 (Dade Behring, Cupertino, CA) and Abuscreen On Line cannabinoids (THC) assay (Roche Diagnostic Corporation, Indianapolis, IN). Since no positive response was achieved at the 100 000 ng/mL concentration of either compound, no further investigation was carried out.

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