Butyrolactones from the Fungus *Aspergillus terreus* BCC 4651

Rachada Haritakun, Pranee Rachtawee, Rungtiwa Chanthaket, Nattawut Boonyuen, and Masahiko Isaka*

National Center for Genetic Engineering and Biotechnology; 113 Thailand Science Park, Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand.

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Two new butenolides, butyrolactones VI (1) and VII (2), were isolated together with six known compounds, butyrolactones I (3), II (4), IV (5), and V (6), aspernolide B (7), and bisdethiodi(methylthio)acetylaranotin (8) from the fungus *Aspergillus terreus* BCC 4651. Compound 8, exhibiting a minimum inhibitory concentration (MIC) value of 1.56 μg/ml against *Mycobacterium tuberculosis* H37Ra, proved to be the antimycobacterial principle from the culture of this fungus. On the other hand, butyrolactone V (6) showed antiplasmodial activity against *Plasmodium falciparum* K1 with an IC_{50} of 7.9 μg/ml.

Key words *Aspergillus terreus*; butyrolactone; antimalarial activity; antitubercular activity

As part of our research program on the utilization of bioresouces in Thailand, we have been screening fungal extracts for *in vitro* assays inclusive of antimycobacterial and antiplasmodial activities. An extract from *Aspergillus terreus* BCC 4651 exhibited moderate activity against *Mycobacterium tuberculosis* H37Ra (minimum inhibitory concentration (MIC) 25 μg/ml) and the ^{1}H-NMR spectrum of the extract showed a unique profile, therefore, we selected the strain for large scale fermentation and chemical investigation. This study led to the isolation of two new butenolides, butyrolactones VI (**1**) and VII (**2**), together with the known compounds, butylolactone I (**3**),[1] butyrolactone II (**4**),[2] butyrolactone IV (**5**),[3] butyrolactone V (**6**),[4] and aspernolide B (**7**).[5] A known diketopiperazine, bisdethiodi(methylthio)-acetylaranotin (**8**),[6—8] was also isolated as an antimycobacterial constituent of the culture extract. We report herein the structure elucidation of the new compounds and biological activities of isolated compounds.

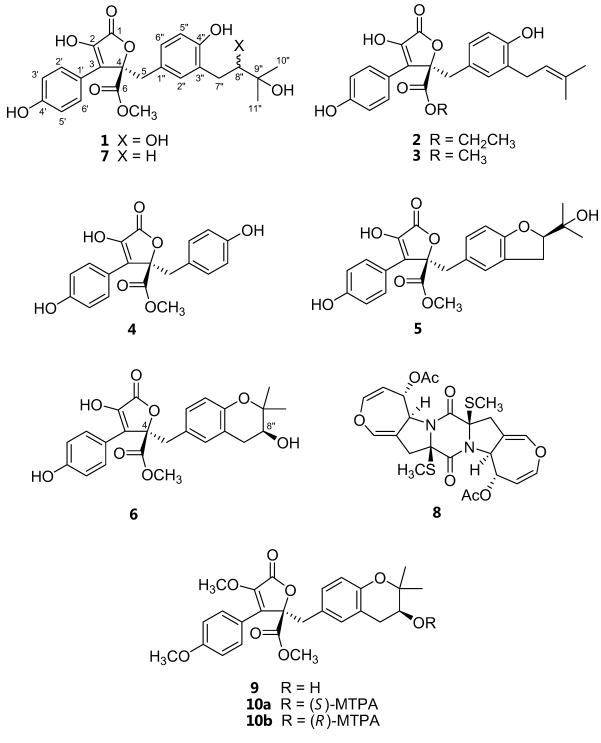

Chart 1

Results and Discussion

Initial study of the cultures grown in a common liquid medium, Czapek–Dox broth, under shaking resulted in the isolation of the known compounds **3**—**5**, **7**, and **8**. Further studies revealed that butyrolactone derivatives were efficiently produced when the fungus BCC 4651 was fermented in yeast extract sucrose medium (YES; a sucrose-rich liquid medium) under static conditions. The culture provided with two new compounds **1** and **2** along with **3**, **5**, **6** and terrein, which is one of the commonly occurring *Aspergillus* toxins.[9] These compounds were isolated by column chromatography (Sephadex LH-20, silica gel, and reversed-phase HPLC), wherein fractions were analyzed by TLC and ^{1}H-NMR. Compounds **3**—**5**, and **7** were identified on the basis of the MS and NMR data as butyrolactone I,[1,3] butyrolactone II,[2,3] butyrolactone IV,[3] and aspernolide B,[5] respectively. All these butenolides were previously isolated from *A. terreus*.[2,3,5,10,11] The positive optical rotations of **3** ($[\alpha]_D^{25}$ +63, c=0.25, EtOH), **5** ($[\alpha]_D^{26}$ +55, c=0.10, MeOH), and **7** ($[\alpha]_D^{25}$ +77, c=0.104, MeOH) were consistent with the literature data, which confirmed the 4*R*-configuration. Butyrolactone V (**6**) was recently isolated from *Aspergillus* sp. F1.[4] However, the original report didn't refer to the absolute configuration of the secondary alcohol (C-8″), and the reported specific rotation ($[\alpha]_D^{20}$ +24.8, c=15.7, MeOH)[4] was considerably different from that of our sample ($[\alpha]_D^{26}$ +79, c=0.095, MeOH). Therefore, we determined the absolute configuration (C-8″) of **6**, from *A. terreus* BCC 4651, by application of the modified Mosher's method.[12] Methylation of **6** (MeI, K_2CO_3, *N*,*N*-dimethylformamide (DMF)) gave the dimethylated derivative **9**, which was subsequently converted to the (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester derivatives **10a** and **10b**, respectively. The $\Delta\delta$-values (δ_S–δ_R) of the Mosher esters unambiguously indicated the 8″*S*-configuration (Fig. 1).

Butyrolactone VI (**1**) was isolated as a colorless solid. The molecular formula was determined to be $C_{24}H_{26}O_9$ by high resolution-electrospray ionization-mass spectra (HR-ESI-MS). The IR spectrum showed broad and intense absorption bands at ν_{max} 3442 (broad) and 1739 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were similar to those of other butyrolactone

* To whom correspondence should be addressed. e-mail: isaka@biotec.or.th

derivatives. The differences were observed at the prenyl-derived side chain (C-7″—C-11″). The side chain was composed of an oxygenated quaternary carbon resonating at δ_C 71.8, an oxygenated methine at δ_C 80.1 (δ_H 3.48, dd, J=9.6, 2.0 Hz), a methylene at δ_C 33.6 (δ_H 2.65, dd, J=14.2, 2.0 Hz, and 2.58, dd, J=14.2, 9.6 Hz), and two methyl groups at δ_C 24.8 (δ_H 1.19, 3H, s) and δ_C 24.2 (δ_H 1.18, 3H, s). The connectivity of these side chain carbons was deduced by the heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2). HMBC correlations from H_2-7″ to C-2″, C-3″, and C-4″, and from H-2″ to C-7″ indicated the connection of C-7″ to C-3″. The structure of all other part of the molecule was identical to butyrolactone I (**3**), which was also confirmed on the basis of HMBC correlations (Fig. 2). Although the resonances of hydroxyl protons (8″-OH and 9″-OH) were not observed, the carbon chemical shifts and the molecular formula (HR-MS) of this molecule required the diol structure of C-8″ and C-9″. Thus, butyrolactone VI (**1**) is a new analogue wherein the double bond of the prenyl group in butyrolactone I (**3**) is dihydroxylated. The ^{13}C-NMR spectrum of **1** suggested the presence of a minor isomer, whose carbon resonances were mostly superimposed but some were separate from the major isomer with *ca.* 5 : 1 peak intensity ratio: C-5 (δ_C 38.4/38.3), C-5″ (115.5/115.6), C-7″ (33.6/33.7), C-8″ (80.1/80.5), C-10″ (24.8/25.0), and C-11″ (24.2/24.1). These data indicated that the isolated compound (**1**) was a mixture of C-8″ epimers. For the assignment of the C-8″ absolute configuration of the major isomer by the modified Mosher's method, the same derivatization procedure as **6** was examined. However, treatment of **1** with MeI/K_2CO_3 in DMF gave unexpectedly a complex mixture of products.

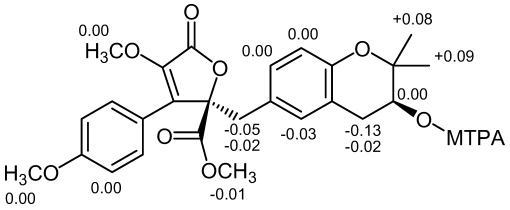

Fig. 1. $\Delta\delta$-Values (δ_S–δ_R) of the Mosher Esters **10a** and **10b**

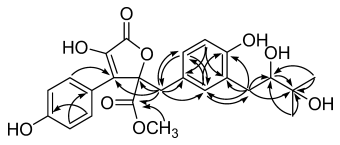

Fig. 2. Selected HMBC Correlations for **1**

The molecular formula of butyrolactone VII (**2**) was determined to be $C_{25}H_{26}O_7$ (HR-ESI-MS). The ^{1}H- and ^{13}C-NMR spectroscopic data were very similar to those of **3** except for the replacement of the methoxycarbonyl group of **3** with an ethoxycarbonyl. The ethyl group resonated at δ_C 62.2 (δ_H 4.26, 2H, q, J=7.1 Hz) and 13.3 (δ_H 1.20, 3H, t, J=7.1 Hz). The HMBC correlations from the ethoxyl methylene protons (δ_H 4.26) and the diastereotopic methylene protrons (H_2-5) at δ_H 3.46 and 3.45 to the ester carbonyl carbon at δ_C 169.5 (C-6) confirmed the location of the ethoxycarbonyl group. Therefore, **2** was assigned as the ethyl ester variant of butyrolactone I (**3**).

The isolated compounds were tested for antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra, antiplasmodial activity against the malarial parasite *Plasmodium falciparum* K1, and cytotoxicity to three human cancer cell-lines, KB (oral epidermoid carcinoma), MCF-7 (breast cancer) and NCI-H187 (small-cell lung cancer), and non-cancerous Vero cells (African green monkey kidney fibroblasts) (Table 1). Compound **2** was not tested for these assays due to the sample limitation. Butyrolactones were weakly active or inactive against *M. tuberculosis*, whereas diketopiperazine **8** exhibited a significant growth inhibitory effect (MIC 1.56 μg/ml). On the other hand, butyrolactone V (**6**) showed moderate antimalarial activity (IC_{50} 7.9 μg/ml). Butyrolactone derivatives **1** and **3**—**7** exhibited very weak or no cytotoxicity. Butyrolactones I (**3**), II (**4**), III, and IV (**5**) are known to exhibit weak cytotoxicity to human cancer cell-lines, while they were inactive against several Gram-positive and Gram-negative bacteria.[3] Butyrolactone I (**3**) is also known to be a specific inhibitor of eukalyotic cyclin-dependent kinases.[13—15]

Experimental

General Experimental Procedures Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on an Analytikjena SPEKOL 1200 spectrophotometer. Fourier transform-infrared (FT-IR) spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker AV500D and DRX400 spectrometers. ESI-time-of-flight (TOF) mass spectra were measured with a Bruker micrOTOF mass spectrometer.

Fungal Material The fungus used in this study was isolated from a tree hole in Nam Nao National Park, Phetchabun Province, Thailand, by Dr. Wipapat Kladwang. This fungus was deposited in the BIOTEC Culture Col-

Table 1. Antimycobacterial, Antiplasmodial, and Cytotoxic Activities of Compounds **1** and **3**—**8**

Compound	*M. tuberculosis* (MIC, μg/ml)	*P. falciparum* (IC_{50}, μg/ml)	Cytotoxicity (IC_{50}, μg/ml)			
			KB	MCF-7	NCI-H187	Vero
1	>50	>10	22	>50	>50	>50
3	>50	>10	>50	>50	>50	>50
4	>50	>10	27	>50	>50	>50
5	50	>10	>50	>50	>50	>50
6	>50	7.9	>50	>50	49	>50
7	25	>10	>50	>50	>50	>50
8	1.56	>10	>50	>50	14	>50
Isoniazid[a]	0.050	—[e]	—[d]	—[d]	—[d]	—[d]
Dihydroartemisinin[b]	—[d]	0.0011	—[d]	—[d]	—[d]	—[d]
Ellipticine[c]	—[d]	—[d]	0.45	0.57	0.18	0.63

a) Standard antitubercular compound. *b*) Standard antimalarial compound. *c*) Reference compound for the cytotoxicity assay. *d*) Not tested.

lection (BCC) as BCC 4651 on September 3, 1998. It was identified as *Aspergillus terreus* on the basis of the sequence data of 18S rDNA and internal transcribed spacer (ITS) genes by one of the authors (N.B.).

Fermentation in Yeast Extract Sucrose (YES) Medium, Extraction, and Isolation *Aspergillus terreus* BCC 4651 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 4×250 ml Erlenmeyer flasks containing 25 ml of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 6 d on a rotary shaker (200 rpm), each primary culture was transferred into a 1 l Erlenmeyer flask containing 250 ml of the same liquid medium (PDB), and incubated at 25 °C for 6 d on a rotary shaker (200 rpm). These secondary cultures were transferred into 40×1 l Erlenmeyer flasks containing 250 ml of yeast extract sucrose medium (YES; yeast extract 20.0 g, sucrose 150.0 g, per liter), and final fermentation was carried out at 25 °C for 20 d under static conditions. The cultures were filtered to separate broth (filtrate) and mycelia (residual cakes). The filtrate was extracted with EtOAc (4×10 l) to give a brown gum (9.03 g, extract A). The wet mycelia were macerated in MeOH (2×3 l, rt, 3 d). The combined MeOH solution was partially concentrated by evaporation, diluted with H_2O (100 ml), and defatted with hexane (300 ml). The residual solution was partially concentrated by evaporation, and extracted with EtOAc (3×1.5 l) and concentrated under reduced pressure to obtain a brown gum (5.60 g, extract B). Extracts A and B were separately subjected to chromatographic fractionation. Extract A was fractionated by column chromatography (CC) on Sephadex LH-20 (4.6×60 cm, MeOH) to obtain 8 pooled fractions A-1—A-8. Fraction A-4 (765 mg) was subjected to CC on silica gel (MeOH/$CHCl_3$, step gradient elution from 0 : 100 to 100 : 0) followed by preparative HPLC using a reverse phase column (Phenomenex Luna 10u C18(2) 100A, 21.2 mm×250 mm; MeOH/H_2O=35 : 65, flow rate 8 ml/min) to furnish **6** (7.8 mg). Fraction A-5 (4.03 g) was repeatedly fractionated by CC on silica gel (MeOH/$CHCl_3$) to afford **3** (5.7 mg). Fraction A-6 (3.39 g) mostly composed of terrein. Extract B was fractionated by CC on Sephadex LH-20 (4.6×60 cm, MeOH) to obtain 9 pooled fractions B-1—B-9. Fraction B-3 (830 mg) was subjected to CC on silica gel (3.0×20 cm, MeOH/CH_2Cl_2, step gradient elution from 5 : 95 to 100 : 0) and preparative HPLC (MeOH/H_2O=40 : 60) to furnish **5** (5.7 mg), **2** (3.0 mg), and **3** (70.8 mg). Fraction B-4 (1.77 g) was separated by CC on silica gel (MeOH/CH_2Cl_2, step gradient elution) and preparative HPLC (MeOH/H_2O=40 : 60) to obtain **5** (422 mg), **6** (62.8 mg), and ergosterol (28.6 mg). Fractions B-5 (652 mg) and B-6 (736 mg) were combined and subjected to CC on silica gel (MeOH/CH_2Cl_2, step gradient elution) to afford **1** (161 mg).

Fermentation in Czapek–Dox Medium, Extraction, and Isolation The seed cultures of BCC 4651 in PDB were prepared in the same manner as described above. The final fermentation was performed using 20×1000 ml Erlenmeyer flasks each containing 250 ml of Czapek–Dox broth (sucrose 30.0 g, $NaNO_3$ 3.0 g, K_2HPO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.1 g, per liter), and incubated for 20 d on rotary shakers (200 rpm). The cultures broth extract (1.70 g) was subjected to chromatographic fractionations (Sephadex LH-20, silica gel, and reverse phase HPLC) to furnish **3** (6.0 mg), **4** (4.8 mg), **5** (5.4 mg), **7** (12.1 mg), and **8** (47.1 mg).

Butyrolactone VI (**1**): Colorless solid, mp 136—137 °C; $[\alpha]_D^{27}$ +71 (c=0.105, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.83), 304 (4.10) nm; IR (neat) ν_{max} 3442, 1739, 1664, 1610, 1518, 1385, 1261, 1181, 1066, 1038, 839, 757 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 7.63 (2H, d, J=8.8 Hz, H-2′, H-6′), 6.98 (2H, d, J=8.8 Hz, H-3′, H-5′), 6.61 (1H, d, J=2.1 Hz, H-2″), 6.56 (2H, m, H-5″, H-6″), 3.79 (3H, s, CO_2CH_3), 3.48 (1H, dd, J=9.6, 2.0 Hz, H-8″), 3.46 (1H, d, J=14.4 Hz, Ha-5), 3.45 (1H, d, J=14.4 Hz, Hb-5), 2.65 (1H, dd, J=14.2, 2.0 Hz, Ha-7″), 2.58 (1H, dd, J=14.2, 9.6 Hz, Hb-7″), 1.19 (3H, s, H-10″), 1.18 (3H, s, H-11″); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 170.0 (C, C-6), 168.0 (C, C-1), 158.0 (C, C-4′), 155.1 (C, C-4″), 138.4 (C, C-2), 133.2 (CH, C-2″), 129.5 (CH, C-6″), 129.3 (CH, C-2′, C-6′), 127.4 (C, C-3), 126.3 (C, C-3″), 124.3 (C, C-1″), 122.0 (C, C-1′), 115.7 (CH, C-3′, C-5′), 115.5 (CH, C-5″), 85.2 (C, C-4), 80.1 (CH, C-8″), 71.8 (C, C-9″), 52.8 (CH_3, CO_2CH_3), 38.4 (CH_2, C-5), 33.6 (CH_2, C-7″), 24.8 (CH_3, C-10″), 24.2 (CH_3, C-11″); HR-MS (ESI-TOF) m/z: 481.1482 $[M+Na]^+$ (Calcd for $C_{24}H_{26}O_9Na$, 481.1469).

Butyrolactone VII (**2**): Colorless solid, mp 124—125 °C; $[\alpha]_D^{27}$ +54 (c=0.08, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.08), 303 (4.17) nm; IR (neat) ν_{max} 3444, 1738, 1665, 1610, 1257 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 9.27 (1H, br s, 2-OH), 9.10 (1H, br s, 4′-OH), 8.27 (1H, br s, 4″-OH), 7.64 (2H, d, J=8.9 Hz, H-2′, H-6′), 6.97 (2H, d, J=8.9 Hz, H-3′, H-5′), 6.59 (1H, d, J=8.1 Hz, H-5″), 6.55 (1H, dd, J=8.1, 2.1 Hz, H-6″), 6.51 (1H, d, J=2.1 Hz, H-2″), 5.11 (1H, m, H-8″), 4.26 (2H, q, J=7.1 Hz, $CO_2CH_2CH_3$), 3.46 (1H, d, J=14.7 Hz, Ha-5), 3.45 (1H, d, J=14.7 Hz, Hb-5), 2.12 (2H, br d, J=7.2 Hz, H-7″), 1.65 (3H, br s, H-11″), 1.59 (3H, br s, H-10″), 1.20 (3H, t, J=7.1 Hz, $CO_2CH_2CH_3$); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 169.5 (C, C-6), 167.9 (C, C-1), 158.0 (C, C-4′), 153.9 (C, C-4″), 138.2 (C, C-2), 131.5 (C, C-9″), 131.4 (CH, C-2″), 129.3 (CH, C-2′, C-6′), 128,6 (CH, C-6″), 127.5 (C, C-3), 127.0 (C, C-3″), 124.1 (C, C-1″), 122.6 (CH, C-8″), 121.9 (C, C-1′), 115.7 (CH, C-3′, C-5′), 114.1 (CH, C-5″), 85.2 (C, C-4), 62.2 (CH_2, $CO_2CH_2CH_3$), 38.4 (CH_2, C-5), 27.7 (CH_2, C-7″), 25.0 (CH_3, C-11″), 16.9 (CH_3, C-10″), 13.3 (CH_3, $CO_2CH_2CH_3$); HR-MS (ESI-TOF) m/z: 461.1577 $[M+Na]^+$ (Calcd for $C_{25}H_{26}O_7Na$, 461.1571).

Butyrolactone V (**6**): Colorless solid, mp 139—140 °C; $[\alpha]_D^{26}$ +79 (c=0.095, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.99), 306 (4.15) nm; IR (KBr) ν_{max} 3406, 1741, 1668, 1610, 1263 cm^{-1}; ^{1}H-NMR (500 MHz, acetone-d_6) δ: 7.62 (2H, d, J=8.8 Hz, H-2′, H-6′), 6.98 (2H, d, J=8.8 Hz, H-3′, H-5′), 6.60 (1H, dd, J=8.3, 2.0 Hz, H-6″), 6.49 (1H, d, J=8.3 Hz, H-6″), 6.48 (1H, m, H-2″), 3.79 (3H, s, CO_2CH_3), 3.69 (1H, dd, J=8.4, 5.5 Hz, H-8″), 3.48 (1H, d, J=14.4 Hz, Ha-5), 3.46 (1H, d, J=14.4 Hz, Hb-5), 2.74 (1H, dd, J=16.5, 5.5 Hz, Ha-7″), 2.52 (1H, dd, J=16.5, 8.4 Hz, Hb-7″), 1.29 (3H, s, H-10″), 1.14 (3H, s, H-11″); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 169.9 (C, C-6), 167.8 (C, C-1), 158.0 (C, C-4′), 152.3 (C, C-4″), 138.3 (C, C-2), 131.7 (CH, C-2″), 129.3 (CH, C-2′, C-6′), 129.2 (CH, C-6″), 127.5 (C, C-3), 124.7 (C, C-1″), 121.9 (C, C-1′), 119.7 (C, C-3″), 116.0 (CH, C-5″), 115.7 (CH, C-3′, C-5′), 85.1 (C, C-4), 76.9 (C, C-9″), 69.0 (CH, C-8″), 52.8 (CH_3, CO_2CH_3), 38.4 (CH_2, C-5), 31.2 (CH_2, C-7″), 25.3 (CH_3, C-10″), 19.3 (CH_3, C-11″); HR-MS (ESI-TOF) m/z: 441.1535 $[M+H]^+$ (Calcd for $C_{24}H_{25}O_8$, 441.1544), 463.1351 $[M+Na]^+$ (Calcd for $C_{24}H_{24}O_8Na$, 463.1363).

Synthesis of the Mosher Esters 10a and 10b To a solution of **6** (5.2 mg) in DMF (0.3 ml) were added MeI (30 μl) and K_2CO_3 (25 mg), and the mixture was stirred at room temperature for 15 h. The mixture was diluted with EtOAc, washed three times with H_2O, and the organic layer was concentrated under reduced pressure to obtain a dimethylated product **9** (3.7 mg): ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.60 (2H, d, J=8.9 Hz, H-2′, H-6′), 7.08 (2H, d, J=8.9 Hz, H-3′, H-5′), 6.57 (1H, dd, J=8.3, 1.6 Hz, H-6″), 6.51 (1H, d, J=8.3 Hz, H-5″), 6.50 (1H, br s, H-2″), 4.19 (1H, br s, OH), 3.89 (3H, s, 4′-OCH$_3$), 3.83 (1H, m, H-8″), 3.81 (3H, s, CO_2CH_3), 3.72 (3H, s, 2-OCH$_3$), 3.47 (1H, d, J=14.6 Hz, Ha-5), 3.39 (1H, d, J=14.6 Hz, Hb-5), 2.78 (1H, dd, J=16.6, 5.6 Hz, Ha-7″), 2.54 (1H, dd, J=16.6, 8.3 Hz, Hb-7″), 1.29 (3H, s, H-10″), 1.14 (3H, s, H-11″); HR-MS (ESI-TOF) m/z: 491.1670 $[M+Na]^+$ (Calcd for $C_{26}H_{28}O_8Na$, 491.1676). A portion (1.5 mg) of this methylated product was treated with (−)-(*R*)-MTPACl (20 ml) in pyridine (0.2 ml) at room temperature for 15 h. The mixture was diluted with EtOAc and washed with H_2O, and 1 M $NaHCO_3$, and the organic layer was concentrated under reduced pressure to furnish the (*S*)-MTPA ester derivative **10a**. Similarly, (*R*)-MTPA ester **10b** was prepared from **9** and (+)-(*S*)-MTPACl.

(*S*)-MTPA Ester **10a**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60—7.30 (7H, m, H-2′, H-5′, and phenyl of MTPA), 7.35—7.28 (3H, m, phenyl of MTPA), 6.98 (2H, d, J=8.9 Hz, H-3′, H-5′), 6.58 (2H, m, H-5″, H-6″), 6.51 (1H, br s, H-2″), 5.08 (1H, dd, J=7.0, 5.3 Hz, H-8″), 3.87 (3H, s, 4′-OCH$_3$), 3.79 (3H, s, CO_2CH_3), 3.78 (3H, s, 2-OCH$_3$), 3.47 (1H, d, J=14.7 Hz, Ha-5), 3.44 (3H, br s, –OCH$_3$ of MTPA), 3.35 (1H, d, J=14.7 Hz, Hb-5), 3.02 (1H, dd, J=16.8, 5.3 Hz, Ha-7″), 2.65 (1H, dd, J=16.8, 7.0 Hz, Hb-7″), 1.30 (3H, s, H-10″), 1.20 (3H, s, H-11″); HR-MS (ESI-TOF) m/z: 707.2079 $[M+Na]^+$ (Calcd for $C_{36}H_{37}F_3O_{10}Na$, 707.2075).

(*R*)-MTPA Ester **10b**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60—7.30 (7H, m, H-2′, H-5′, and phenyl of MTPA), 7.35—7.28 (3H, m, phenyl of MTPA), 6.98 (2H, d, J=8.9 Hz, H-3′, H-5′), 6.58 (2H, m, H-5″, H-6″), 6.54 (1H, br s, H-2″), 5.08 (1H, m, H-8″), 3.87 (3H, s, 4′-OCH$_3$), 3.80 (3H, s, CO_2CH_3), 3.78 (3H, s, 2-OCH$_3$), 3.52 (1H, d, J=14.8 Hz, Ha-5), 3.44 (3H, br s, –OCH$_3$ of MTPA), 3.37 (1H, d, J=14.8 Hz, Hb-5), 3.04 (1H, dd, J=17.0, 5.2 Hz, Ha-7″), 2.78 (1H, dd, J=17.0, 6.2 Hz, Hb-7″), 1.22 (3H, s, H-10″), 1.11 (3H, s, H-11″); HR-MS (ESI-TOF) m/z: 707.2079 $[M+Na]^+$ (Calcd for $C_{36}H_{37}F_3O_{10}Na$, 707.2075).

Biological Assays Growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra and cytotoxicity to Vero cells were performed using the green fluorescent protein microplate assay (GFPMA).[16] Assay for activity against *Plasmodium falciparum* K1 (multi-drug resistant strain) was performed using the microculture radioisotope technique.[17] Cytotoxic activity against KB, MCF-7, and NCI-H187 cell-lines was evaluated using the resazurin microplate assay.[18]

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References

1) Kiriyama N., Nitta K., Sakaguchi Y., Taguchi Y., Yamamoto Y., *Chem. Pharm. Bull.*, **25**, 2593—2601 (1977).
2) Nitta K., Fujita N., Yoshimura T., Arai K., Yamamoto Y., *Chem. Pharm. Bull.*, **31**, 1528—1533 (1983).
3) Rao K. V., Sadhukhan A. K., Veerender M., Ravikumar V., Mohan E. V., Dhanvantri S. D., Sitaramkumar M., Babu J. M., Vyas K., Reddy G. O., *Chem. Pharm. Bull.*, **48**, 559—562 (2000).
4) Lin T., Lu C., Shen Y., *Nat. Prod. Res.*, **23**, 77—85 (2009).
5) Parvatkar R. R., D'Souza C., Tripathi A., Naik C. G., *Phytochemistry*, **70**, 128—132 (2009).
6) Nagarajan R., Huckstep L. L., Lively D. H., DeLong D. C., Marsh M. M., Neuss N., *J. Am. Chem. Soc.*, **90**, 2980—2982 (1968).
7) Moncrief J. W., *J. Am. Chem. Soc.*, **90**, 6517—6518 (1968).
8) Kamata S., Sakai H., Hirota A., *Agric. Biol. Chem.*, **47**, 2637—2638 (1983).
9) Cole R. J., Cox R. H., "Handbook of Toxic Fungal Metabolites," Chap. 18, Academic Press, New York, 1981, pp. 753—822.
10) Cazar M. E., Schmeda-Hirschmann G., Atsudillo L., *World J. Microbiol. Biotechnol.*, **21**, 1067—1075 (2005).
11) Niu X., Dahse H.-M., Menzel K.-D., Lozach O., Walther G., Meijer L., Grabley S., Sattler I., *J. Nat. Prod.*, **71**, 689—692 (2008).
12) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4096 (1991).
13) Kitagawa M., Okabe T., Ogino H., Matsumoto H., Suzuki-Takahashi I., Kokubo T., Higashi H., Saitoh S., Taya Y., Yasuda H., Ohba Y., Nishimura S., Tanaka N., Okuyama A., *Oncogene*, **8**, 2425—2432 (1993).
14) Nishio K., Ishida T., Arioka H., Kurokawa H., Fukuoka K., Nomoto T., Fukumoto H., Yokote H., Saijo N., *Anticancer Res.*, **16**, 3387—3395 (1996).
15) Schimmel T. G., Coffman A. D., Parsons J., *Appl. Environ. Microbiol.*, **64**, 3707—3712 (1998).
16) Changsen C., Franzblau S. G., Palittapongarnpim P., *Antimicrob. Agents Chemother.*, **47**, 3682—3687 (2003).
17) Desjardins R. E., Canfield C. J., Haynes J. D., Chulay J. D., *Antimicrob. Agents Chemother.*, **16**, 710—718 (1979).
18) O'Brien J., Wilson I., Orton T., Pognan F., *Eur. J. Biochem.*, **267**, 5421—5426 (2000).