Isariotins E and F, Spirocyclic and Bicyclic Hemiacetals from the Entomopathogenic Fungus *Isaria tenuipes* BCC 12625

Taridaporn Bunyapaiboonsri,* Seangaroon Yoiprommarat, Kamolphan Intereya, Pranee Rachtawee, Nigel L. Hywel-Jones, and Masahiko Isaka

National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phahonyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

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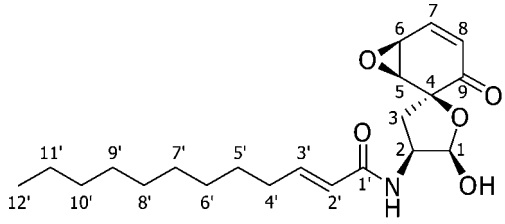
New spirocyclic and bicyclic hemiacetals, isariotins E (**1**) and F (**2**), together with TK-57-164A (**3**) were isolated from the entomopathogenic fungus *Isaria tenuipes* BCC 12625. The absolute configuration of **3** was addressed by application of the modified Mosher's method. Isariotin F (**2**) exhibited activity against the malaria parasite *Plasmodium falciparum* K1 with an IC_{50} value of 5.1 μM and cytotoxic activities against cancer cell lines (KB, BC, and NCI-H187) and nonmalignant (Vero) cells with respective IC_{50} values of 15.8, 2.4, 1.6, and 2.9 μM.

Isaria tenuipes (also known as *Paecilomyces tenuipes* or *Isaria japonica*)[1,2] is a parasitic fungus infecting pupae or larvae of lepidopteran insects.[2,3] Its cultivated fruiting bodies are known to contain a variety of secondary metabolites including trichothecanes (tenuipesine A[4] and paecilomycines A−C[5]), spirocyclic trichothecanes (spirotenuipesines A and B),[6] and a pseudodipeptide (hanasanagin).[7] As part of our continuing research on bioactive secondary metabolites of entomopathogenic fungi,[8] liquid fermentations of two *Isaria tenuipes* strains, BCC 1614 and BCC 7831, were reported to produce beauvericins[9] and isariotins A−D,[10] respectively. Investigation of *I. tenuipes* BCC 12625 led to the isolation and structure elucidation of new spirocyclic and bicyclic hemiacetals, isariotins E (**1**) and F (**2**), and TK-57-164A (**3**).[11]



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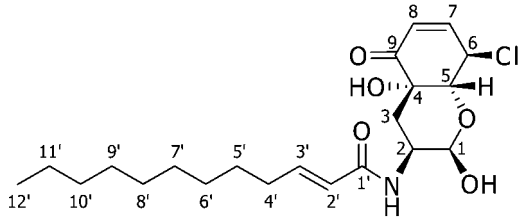

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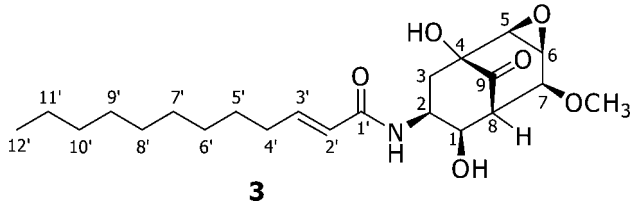

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The crude extract from mycelia of *I. tenuipes* BCC 12625 contained beauvericin[12] as a major metabolite. Compounds **1**−**3** were obtained as minor constituents after purification by Sephadex LH-20 and silica gel column chromatography.

Isariotin E (**1**) had the molecular formula $C_{21}H_{31}NO_5$ as established by HRESIMS. The IR spectrum showed absorption bands at 3345 (OH), 1685 (conjugated ketone), and 1668 (amide carbonyl) cm^{-1}. Analysis of ^{13}C NMR and DEPT spectra indicated the presence of a ketone carbonyl (δ_C 194.7), an amide carbonyl (δ_C 165.0), four olefinic methines (δ_C 145.0, 143.6, 131.0, and 124.0), a hemiacetal methine (δ_C 96.6), an oxygenated quaternary carbon (δ_C 83.9), three methines (δ_C 58.7, 50.5, and 47.5), nine methylenes, and a methyl group. $^1H-^1H$ COSY spectroscopic data revealed the connection of C-5−C-6−C-7−C-8. The conjugation of the *cis*-olefin (δ_H 7.29, 1H, dd, J = 9.9, 3.9 Hz, H-7; δ_H 6.08, 1H, dd, J = 9.9, 1.7 Hz, H-8) with a carbonyl (δ_C 194.7, C-9) was determined on the basis of ^1H and ^{13}C NMR data (Table 1) and a HMBC correlation from H-7 to C-9. The presence of a *cis*-epoxide was suggested by the observation of corresponding resonances at δ_H 3.71 (1H, d, J = 3.9 Hz)/δ_C 58.7 for CH-5 and δ_H 3.65 (1H, dt, J = 1.7, 3.9 Hz)/δ_C 47.5 for CH-6. HMBC correlations from H-5 to C-9 and from H-8 to an oxygenated quaternary carbon at δ_C 83.9 (C-4) required the epoxycyclohexenone substructure. The tetrahydrofuran ring was determined on the basis of $^1H-^1H$ COSY correlations from H-2 to H-1, H-3, and amide proton and from H-1 to hydroxy proton (1-O*H*), together with HMBC correlations from H-3b to C-4 and from H-3a to C-1. The spirocyclic structure of **1** was confirmed by the observed HMBC correlations from H-3b to C-5 and C-9. The carboxyamide side chain from C-4′ to C-12′ was determined on the basis of $^1H-^1H$ COSY and HMBC spectroscopic data. The *trans*-olefinic protons (δ_H 6.75, 1H, dt, J = 15.2, 7.0 Hz, H-3′; δ_H 6.06, 1H, dt, J = 15.2, 1.4 Hz, H-2′) showed HMBC correlations to the amide carbonyl carbon C-1′ (δ_C 165.0) and C-4′. The relative configuration of **1** was deduced from NOESY spectroscopic data and ^1H NMR coupling constants. The large coupling constant of 11.6 Hz between H-2 and H-3b and the strong NOESY cross-peak between H-2 and H-3a indicated an antiperiplanar relationship between H-2 and H-3b. NOESY correlations from H-3b to H-5 and amide proton (N*H*) and from H-2 to H-1 established the relative configuration of **1** (Figure 1).

Isariotin F (**2**) was isolated as a white solid. The molecular formula, $C_{21}H_{32}ClNO_5$, was established on the basis of HRESIMS. The relative intensity of molecular ion peaks showed the presence of a chlorine atom in the molecule. Inspection of ^1H NMR, ^{13}C NMR (Table 1), and DEPT spectra revealed the presence of a chlorinated methine (δ_H 4.92/δ_C 56.2, CH-6) and an oxygenated methine (δ_H 4.18/δ_C 74.9, CH-5) instead of two epoxide methines as in compound **1**. Data from the $^1H-^1H$ COSY spectrum indicated

* To whom correspondence should be addressed. Tel: +66-25646700, ext. 3553. Fax: +66-25646707. E-mail: taridaporn@biotec.or.th.

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** in Acetone-d_6

	1		**2**	
position	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.
1	5.53, dd (4.3, 3.8)	96.6, CH	5.20, dd (4.3, 3.8)	90.7, CH
2	4.48, m	50.5, CH	4.52, m	44.9, CH
3	2.16, dd (12.3, 7.7), H-3a; 2.08, dd (12.3, 11.6), H-3b	35.5, CH_2	2.01, dd (13.3, 4.5), H-3α; 1.87, t (13.3), H-3β	29.6, CH_2
4		83.9, qC		72.9, qC
5	3.71, d (3.9)	58.7, CH	4.18, br d (8.7)	74.9, CH
6	3.65, dt (1.7, 3.9)	47.5, CH	4.92, dt (8.7, 2.2)	56.2, CH
7	7.29, dd (9.9, 3.9)	145.0, CH	6.90, dd (10.3, 2.2)	146.8, CH
8	6.08, dd (9.9, 1.7)	131.0, CH	5.99, dd (10.3, 2.2)	126.6, CH
9		194.7, qC		195.4, qC
1′		165.0, qC		164.8, qC
2′	6.06, dt (15.2, 1.4)	124.0, CH	6.04, dt (15.2, 1.4)	124.3, CH
3′	6.75, dt (15.2, 7.0)	143.6, CH	6.76, dt (15.2, 7.0)	143.4, CH
4′	2.16, dq (1.4, 7.0)	31.6, CH_2[a]	2.16, dq (1.4, 7.0)	31.6, CH_2[c]
5′	1.44, quint (7.0)	28.2, CH_2	1.44, m	28.2, CH_2
6′−9′	1.37−1.23, m	29.3, CH_2[b]	1.35−1.22, m	29.4, CH_2[d]
		29.2, CH_2[b]		29.3, CH_2[d]
		29.1, CH_2[b]		29.1, CH_2[d]
		29.0, CH_2[b]		29.0, CH_2[d]
10′	1.37−1.23, m	31.7, CH_2[a]	1.35−1.22, m	31.7, CH_2[c]
11′	1.37−1.23, m	22.4, CH_2	1.35−1.22, m	22.4, CH_2
12′	0.87, t (7.0)	13.4, CH_3	0.87, t (7.0)	13.4, CH_3
1-O*H*	6.16, br d (3.8)		5.99, dd (4.3, 1.2)	
4-O*H*			5.17, d (1.1)	
N*H*	6.93, br d (8.4)		6.90, br d (8.2)	

[a−d] Assignment of carbons of the same index can be interchanged.

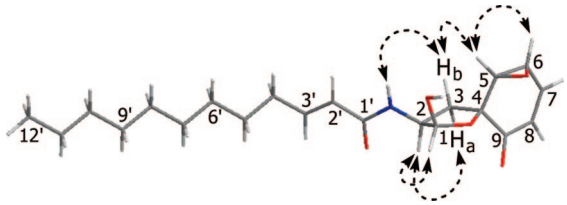


Figure 1. Selected NOESY correlations for **1**.

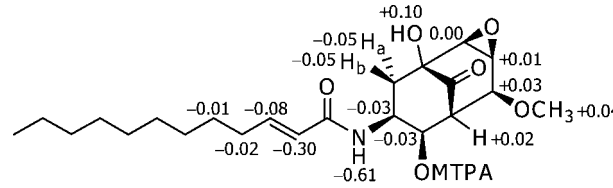


Figure 2. $\Delta\delta$ values ($\delta_S - \delta_R$) of the MTPA esters **3a** and **3b**.

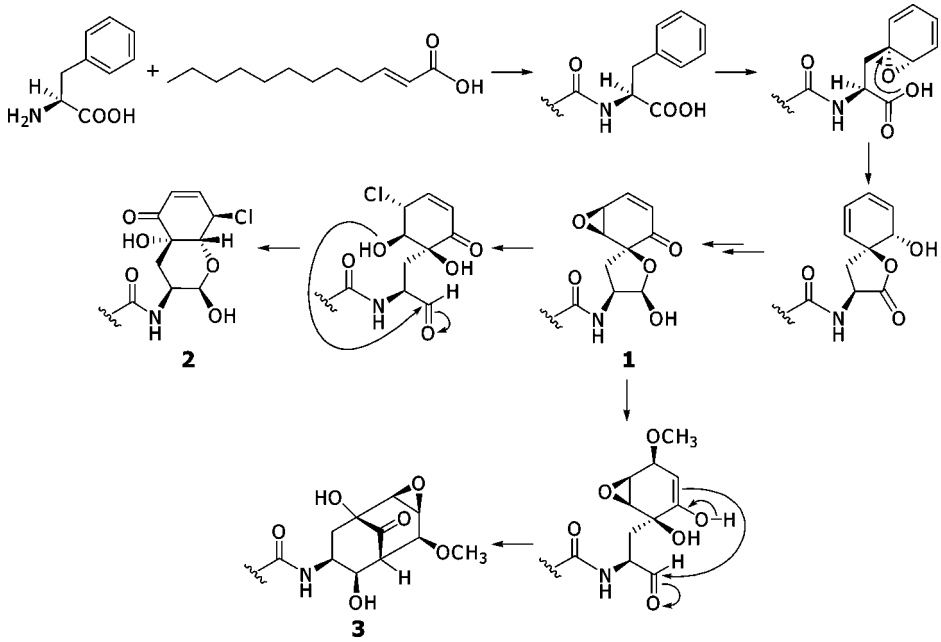
the connections of C-1−C-2−C-3 and C-5−C-6−C-7−C-8. Key HMBC correlations observed from H-1 to C-5; H-3α to C-4 and C-5; 4-O*H* to C-4; H-5 to C-4; H-7 to C-5 and C-9; and H-8 to C-4 and C-6 demonstrated the planar structure of the bicyclic core. The relative configuration of **2** was deduced from NOESY spectroscopic data and $^{1}H-^{1}H$ J values. A NOESY cross-peak observed between H-3β and H-5 and the large coupling constant, $J_{2,3\beta}$ of 13.3 Hz, indicated a chair conformation of the tetrahydropyran ring and axial orientations of H-2, H-3β, and H-5, while the $^{1}H-^{1}H$ J value, $J_{1,2}$ of 3.8 Hz, suggested an equatorial position of H-1. The large coupling constant (8.7 Hz) between H-5 and H-6 proposed a pseudo *trans*-diaxial relationship of both protons, *trans*-ring junction, and α-position of 4-OH.

Compound **3** was isolated as a white solid with the molecular formula $C_{22}H_{35}NO_6$ (HRMS) and assigned as TK-57-164A (an antibacterial substance isolated from *Isaria* sp. TK-57)[11] by comparison of the ^{1}H and ^{13}C NMR data in $CDCl_3$ and specific rotation (**3**, $[\alpha]^{26}_D$ −23.5; TK-57-164A,[11] $[\alpha]^{25}_D$ −21.3). The relative configuration of **3**, which has not been reported, was established on the basis of $^{1}H-^{1}H$ J values, NOESY spectroscopic data, NOEDIFF experiments, and chemical shift comparison to isariotins A−D.[10] The NOESY cross-peak between H-5 and H-6 and a coupling constant of 3.7 Hz indicated the *cis*-epoxide ring. The large coupling constant (12.7 Hz) suggested a *trans*-diaxial orientation of H-2 and H-3b. A *W*-type long-range coupling (J = 1.8 Hz) was observed between two equatorial protons, H-1 and H-3a. Irradiation of H-7 in a NOEDIFF experiment resulted in signal enhancement of H-1, while irradiation of H-3a gave signal enhancement of H-5. From these data along with the consistency of UV, IR, and NMR data with isariotins A−D, the relative configuration of **3** was concluded to be the same as that of isariotins A−D. The absolute configuration of **3** was determined by application of the modified Mosher's method.[13] (*R*)-MTPA and (*S*)-MTPA esters were prepared by treatment of **3** with (*S*)-(+)- and (*R*)-(−)-MTPA chlorides, respectively. The differences of proton chemical shifts ($\Delta\delta$ values, $\delta_S - \delta_R$) for the MTPA esters (Figure 2) indicated the 1*R*-configuration; hence the absolute configuration of **3** was deduced as depicted.

Compounds **1**−**3** possess chemical skeletons similar to the gymnastatins/dankastatins. However, the carboxyamide side chain and chloro substituent on the cyclic core of **1**−**3** were different. Gymnastatins and dankastatins are secondary metabolites isolated from the sponge-derived fungus *Gymnascella dankaliensis* OUPS-N134 and were reported to exhibit significant growth inhibition against murine P388 lymphocytic leukemia cells.[14−17] Numata and co-workers proposed biosynthetic pathways for gymnastatins starting with condensation of 4,6*R*-dimethyldodeca-2*E*,4*E*-dienoic acid and L-tyrosine.[16] Biosynthetic routes to compounds **1**−**3** may resemble those for gymnastatins (as shown in Scheme 1), which require the condensation of *trans*-2-dodecenoic acid and L-phenylalanine. Compound **1** was suggested to be a possible biosynthetic intermediate for **2** and **3**. Based on this hypothesis, the absolute configurations of **1** and **2** were assumed to correlate with their co-metabolite **3** as depicted.

Compounds **2** and **3** were screened for antimalarial, antitubercular, antifungal, and anticancer activities. Compound **2** displayed antimalarial activity against *Plasmodium falciparum* K1 with an IC_{50} value of 5.1 μM, antitubercular activity against *Mycobacterium tuberculosis* H37Ra with an MIC value of 60.4 μM, and antifungal activity against *Candida albicans* with an IC_{50} value of 13.9 ± 2.1 μM. It also showed cytotoxic activities against three cancer cell

Scheme 1. Proposed Biosynthetic Pathways for **1**−**3**



lines (KB, BC, and NCI-H187) and nonmalignant (Vero) cells with respective IC_{50} values of 15.8 ± 0.6, 2.4 ± 0.2, 1.6 ± 0.1, and 2.9 ± 0.5 μM. Compound **3** was inactive in these assays. The biological activities of **1** were not evaluated due to sample limitation.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV and IR spectra were taken on a GBC Cintra 404 UV−visible spectrophotometer and a Bruker VECTOR 22 spectrometer, respectively. NMR spectra were recorded on a Bruker DRX400 and a Bruker AV500D spectrometer. ESI-TOF mass spectra were obtained on Micromass LCT and Bruker micrOTOF spectrometers.

Fungal Material. The fungus *Isaria tenuipes* was collected on Lepidoptera larva from Huai Kha Khaeng Wildlife Sanctuary, Uthai Thani Province, western Thailand, and was deposited at the BIOTEC Culture Collection as BCC 12625 on October 28, 2002.

Fermentation, Extraction, and Isolation. The fungus BCC 12625 was cultured in 15 L (60 × 250 mL) of minimum salt medium (MM; glucose 20.0 g, NH_4NO_3 3.0 g, KH_2PO_4 0.5 g, NaH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2$ 0.5 g, and yeast extract 1.0 g, per 1 L of distilled water) on rotary shakers (200 rpm) for 19 days at 25 °C. The mycelium was harvested by filtration and then macerated in MeOH (1.5 L, rt, 2 days) and filtered. The filtrate was added to H_2O (150 mL) and defatted with hexane (1 L). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (1 L) and washed with H_2O (300 mL). The organic layer was concentrated under reduced pressure to leave a brown gum (7.00 g). The crude mycelial extract was passed through a Sephadex LH-20 column (elution with MeOH) to provide 10 fractions (1−10). Fraction 3 (2.86 g) yielded beauvericin.[12] Fraction 6 (1.90 g) was subjected to silica gel column chromatography (acetone/CH_2Cl_2, step gradient elution from 0:100 to 30:70) to provide nine subfractions (6-1−6-9). Subfraction 6-6 (190.7 mg) was triturated in diethyl ether (5.0 mL) and then filtered to leave a white solid (**3**, 30.0 mg). Fraction 7 (1.16 g) was purified by Sephadex LH-20 column (elution with MeOH) and silica gel column chromatography (acetone/CH_2Cl_2, step gradient elution from 0:100 to 30:70) to yield **1** (1.6 mg). Fraction 8 (246 mg) was purified by silica gel column chromatography (acetone/CH_2Cl_2, step gradient elution from 0:100 to 30:70) to provide 12 subfractions (8-1−8-12). Subfraction 8-6 (86.3 mg) was repeatedly purified by silica gel column chromatography (EtOAc/hexane, 80:20) to provide 10 subfractions (8-6A−8-6J). Subfraction 8-6B (47.6 mg) was triturated in diethyl ether (5 mL) and then filtered to give a white powder (**2**, 10.4 mg).

Isariotin E (1): yellow powder; mp 123−125 °C; $[\alpha]^{27}_D$ −14 (*c* 0.07, acetone); UV (MeCN) λ_{max} (log ε) 211 (4.33) nm; IR (KBr) ν_{max} 3345, 2923, 1685, 1668, 1628, 1537, 1037, 983, 847 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in acetone-d_6, see Table 1; HRMS (ESI-TOF) *m*/*z* 378.2277 $[M + H]^+$ (calcd for $C_{21}H_{32}NO_5$, 378.2280).

Isariotin F (2): white powder; mp 116−119 °C; $[\alpha]^{25}_D$ −108 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.25) nm; IR (KBr) ν_{max} 3425, 2924, 1693, 1670, 1545, 1035 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in acetone-d_6, see Table 1; HRMS (ESI-TOF) *m*/*z* 414.2032 $[M + H]^+$ (calcd for $C_{21}H_{33}{}^{35}ClNO_5$, 414.2047).

TK-57-164A (3): white solid; mp 159−161 °C; $[\alpha]^{26}_D$ −23.5 (*c* 0.05, $CHCl_3$), (lit.,[11] $[\alpha]^{25}_D$ −21.3, *c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (4.36) nm; IR (KBr) ν_{max} 3402, 3289, 2925, 1741, 1685, 1651, 1531, 1101, 974 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 7.00 (1H, br d, J = 8.1 Hz, N*H*), 6.77 (1H, dt, J = 15.3, 7.0 Hz, H-3′), 6.02 (1H, dt, J = 15.3, 1.5 Hz, H-2′), 4.74 (1H, m, H-2), 4.61 (1H, d, J = 3.6 Hz, 1-O*H*), 4.49 (1H, s, 4-O*H*), 4.37 (1H, m, H-1), 3.87 (1H, dd, J = 7.5, 1.2 Hz, H-7), 3.52 (1H, dd, J = 3.7, 1.2 Hz, H-5), 3.47 (3H, s, 7-OC*H*$_3$), 3.45 (1H, dd, J = 3.7, 1.5 Hz, H-6), 3.09 (1H, ddd, J = 7.5, 3.4, 1.5 Hz, H-8), 2.29 (1H, ddd, J = 12.7, 5.0, 1.8 Hz, H-3a), 2.15 (2H, dq, J = 1.5, 7.0 Hz, H-4′), 2.00 (1H, t, J = 12.7 Hz, H-3b), 1.43 (2H, m, H-5′), 1.35−1.22 (12H, m, H-6′−H-11′), 0.86 (3H, t, J = 6.9 Hz, H-12′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 207.1 (C, C-9), 164.5 (C, C-1′), 143.3 (CH, C-3′), 124.3 (CH, C-2′), 77.5 (CH, C-7), 75.7 (C, C-4), 71.3 (CH, C-1), 63.9 (CH, C-5), 57.5 (CH$_3$, 7-OC*H*$_3$), 55.4 (CH, C-8 or C-6), 55.1 (CH, C-6 or C-8), 46.1 (CH, C-2), 38.0 (CH$_2$, C-3), 31.7 (CH$_2$, C-10′ or C-4′), 31.6 (CH$_2$, C-4′ or C-10′), 2 × 29.3, 29.1, 29.0 (CH$_2$, C-6′−C-9′), 28.3 (CH$_2$, C-5′), 22.4 (CH$_2$, C-11′), 13.4 (CH$_3$, C-12′); ^1H and ^{13}C NMR data in $CDCl_3$ were identical to those reported in the literature;[11] HRMS (ESI-TOF) *m*/*z* 410.2540 $[M + H]^+$ (calcd for $C_{22}H_{36}NO_6$, 410.2543).

Preparation of MTPA Ester Derivatives of 3. To a solution of **3** (4.2 mg) in pyridine (300 μL) was added (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (20 μL). The reaction mixture was stirred at room temperature overnight and then dried under vacuum. The residue was dissolved in EtOAc (5 mL) and washed with aqueous $NaHCO_3$ (1 M, 5 mL) and H_2O (2 × 5 mL). The organic layer was concentrated *in vacuo* and then purified by HPLC on a reversed-phase column (LiChroCART C_{18}, 10 μm, 10 × 250 mm; CH_3CN/H_2O, 80:20; flow rate 4 mL/min) to yield (*S*)-MTPA ester **3a** (1.2 mg). Preparation of (*R*)-MTPA ester **3b** (1.7 mg) was performed in the same manner by treatment of **3** (4.4 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. (*S*)-MTPA ester **3a**: ^1H NMR (acetone-d_6, 400 MHz) δ 7.53−7.42 (5H, m, phenyl of MTPA), 6.75 (1H, dt, J = 15.3, 6.8 Hz, H-3′), 6.50 (1H, br d, J = 6.1 Hz, N*H*), 5.92 (1H, m, H-1), 5.69 (1H, dt, J = 15.3, 1.2 Hz, H-2′), 4.92 (1H, br s, 4-O*H*), 4.85 (1H, m, H-2), 4.09 (1H, dd, J = 7.3, 1.0 Hz, H-7), 3.63

(3H, s, OCH_3 of MTPA), 3.62 (1H, dd, J = 3.7, 1.0 Hz, H-5), 3.52 (1H, dd, J = 3.7, 1.3 Hz, H-6), 3.50 (3H, s, 7-OCH_3), 3.38 (1H, ddd, J = 7.3, 2.8, 1.3 Hz, H-8), 2.41 (1H, ddd, J = 13.2, 5.2, 2.1 Hz, H-3a), 2.18 (2H, dq, J = 1.2, 6.8 Hz, H-4′), 1.98 (1H, t, J = 13.2 Hz, H-3b), 1.45 (2H, quint, J = 6.8 Hz, H-5′), 1.35−1.23 (12H, m, H-6′−H-11′), 0.88 (3H, t, J = 7.0 Hz, H-12′); HRMS (ESI-TOF) m/z 626.2943 [M + H]$^+$ (calcd for $C_{32}H_{43}F_3NO_8$, 626.2941). (*R*)-MTPA ester **3b**: ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.51−7.40 (5H, m, phenyl of MTPA), 7.11 (1H, br d, J = 6.2 Hz, N*H*), 6.83 (1H, dt, J = 15.4, 7.0 Hz, H-3′), 5.99 (1H, br d, J = 15.4 Hz, H-2′), 5.95 (1H, m, H-1), 4.88 (1H, m, H-2), 4.82 (1H, br s, 4-O*H*), 4.06 (1H, dd, J = 7.4, 0.9 Hz, H-7), 3.63 (3H, s, OCH_3 of MTPA), 3.62 (1H, m, H-5), 3.51 (1H, dd, J = 3.6, 1.1 Hz, H-6), 3.46 (3H, s, 7-OCH_3), 3.36 (1H, dd, J = 7.4, 2.2 Hz, H-8), 2.46 (1H, ddd, J = 13.3, 5.1, 1.8 Hz, H-3a), 2.20 (2H, dq, J = 1.0, 7.0 Hz, H-4′), 2.03 (1H, t, J = 13.3 Hz, H-3b), 1.46 (2H, quint, J = 7.0 Hz, H-5′), 1.35−1.23 (12H, m, H-6′−H-11′), 0.87 (3H, t, J = 7.0 Hz, H-12′); HRMS (ESI-TOF) m/z 626.2938 [M + H]$^+$ (calcd for $C_{32}H_{43}F_3NO_8$, 626.2941).

Biological Assays. Growth inhibition against *Mycobacterium tuberculosis* H37Ra was evaluated using the microplate Alamar Blue assay (MABA).[18] The MIC value (at which ≥90% of growth was inhibited) of a standard antitubercular drug, isoniazid, was 0.37 μM. A microculture radioisotope technique was used to determine the antimalarial activity against *Plasmodium falciparum* K1.[19] Average IC_{50} value (n = 2) of a standard antimalarial compound, dihydroartemisinin, was 0.004 μM. Antifungal activity against *Candida albicans* and cytotoxicity assays against oral human epidermoid carcinoma (KB) cells, human breast cancer (BC) cells, human small-cell lung cancer (NCI-H187) cells, and African green monkey kidney fibroblast (Vero) cells were determined by using a colorimetric method.[20,21] The IC_{50} values are given as the mean ± SD (n = 3) with R^2 values greater than 0.9. An antifungal agent, amphotericin B, displayed antifungal activity with an IC_{50} value of 0.030 ± 0.003 μM. Ellipticine was used as positive controls for cytotoxicity assays against KB, BC, NCI-H187, and Vero cell lines with respective IC_{50} values of 1.38 ± 0.54, 1.20 ± 0.56, 4.40 ± 0.30, and 1.69 ± 0.24 μM.

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