New Lanostanoids from the Mushroom *Ganoderma lucidum*

Jiyuan Ma, Qing Ye, Yanjun Hua, Decheng Zhang, Raymond Cooper, Michael N. Chang, Joseph Y. Chang, and Hao H. Sun*

Pharmanex, Inc., 75 West Center Street, Provo, Utah 84601

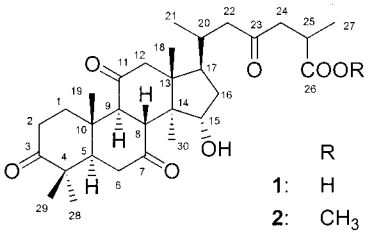
Received July 30, 2001

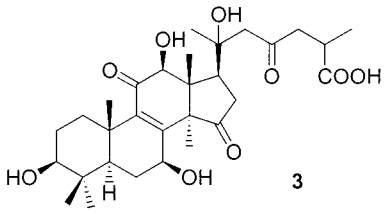
From a lipophilic extract of the fruiting body of *Ganoderma lucidum*, three new lanostanoids, 8*β*,9α-dihydroganoderic acid J (**1**), methyl 8*β*,9α-dihydroganoderate J (**2**), and 20-hydroxylganoderic acid G (**3**), along with 12 known lanostanoids and two ergostane sterols were isolated. The structures of **1**−**3** were determined by interpretation of their spectroscopic data.

Ganoderma lucidum (W. Curt.: Fr.) Karst. (Ganodermataceae), or "reishi" as it is most commonly known, is a wood rotting fungus generally found growing on trees and stumps. "Reishi" occurs in most parts of the world,[1] and the mushroom has been used in traditional Chinese medicine (TCM) for thousands of years as a herbal tonic that promotes longevity. The fruiting bodies of *G. lucidum* are distributed in Asia and are called "Ling Zhi," "Young Zhi", and "reishi" in China, Korea, and Japan, respectively. In the 16th century Chinese pharmacopoeia *Pen T'sao Kang Mu*, "Ling Zhi" was cited as being useful for enhancing "vital energy", increasing "intellectual capacity", preventing "forgetfulness", and producing "longevity".[2,3] Even today it is still prescribed by TCM doctors in the treatment of neurasthenia, debility from prolonged illness, insomnia, anorexia, dizziness, chronic hepatitis, hypercholesterolemia, coronary heart disease, hypertension, altitude sickness, fatigue, carcinoma, and bronchial cough.[3,4]

In our ongoing investigations of the use of TCM as dietary supplements we have evaluated the health benefits of *G. lucidum*, particularly in relation to its immune-modulating activity.[5,6] We found that a combined water and ethanol extract of *G. lucidum* induced the modulation of cytokine secretion[7] including IL-2, IL-4, and IFN-*γ* from normal human peripheral blood mononuclear leukocytes with IC_{50} = 3.74, 1.79, and 6.22 *μ*M, respectively. Other biological activities of *G. lucidum*, including anti-HIV,[8,9] antinociceptive,[10] inhibition of histamine release,[11] and inhibition of angiotensin converting enzyme,[12] have also been known. These distinct pharmacological activities prompted us to further investigate the constituents of *G. lucidum*.

We first examined an ethanolic extract of the dried, crushed powder of *G. lucidum*. Solvent partitioning and repeated chromatography followed by crystallization resulted in the isolation of 15 highly oxidized terpenoids belonging to the lanostanoid type.[13,14] These were three new lanostanoids, named 8*β*,9α-dihydroganoderic acid J (**1**), methyl 8*β*,9α-dihydroganoderate J (**2**), and 20-hydroxylganoderic acid G (**3**), and 12 known triterpenes including ganoderic acids A,[15,16] AM_1,[17] B,[15,16] C_2,[18] DM,[19] G,[20−22] H,[21] I,[18,20] methyl ganoderate I,[18,20] 12-deacetylganoderic acid H,[21] lucidone A,[23] and ganoderiol B.[12,24] Two ergostane sterols, ergosta-7,22-dien-3*β*-ol [25,26] and 5α,8α-epidioxyergosta-6,22-dien-3*β*-ol,[26] were also isolated. The structures of the known compounds were identified by comparison of their ^1H and ^{13}C NMR data with those reported in the literature. The elucidation of structures of the new compounds (**1**−**3**) is presented herein.





A molecular formula of $C_{30}H_{44}O_7$ was established for compound **1** from its HRFABMS. The ^1H NMR spectrum (Table 1) showed the presence of five tertiary methyl groups at *δ* 0.84 (s), 1.05 (s), 1.10 (s), 1.24 (s), and 1.48 (s) and two secondary methyl groups at *δ* 0.84 (d) and 1.17 (d). The ^{13}C NMR spectrum (Table 2) and DEPT measurement of **1** revealed the molecule contained 30 carbons including seven methyls, seven methylenes, seven methines, five carbonyls, and four quaternary carbons. Among the five carbonyl signals, one was a carboxylic acid at *δ* 179.6 and four were ketone carbonyls at *δ* 210.1, 211.2, 215.6, and 217.5. The existence of a hydroxyl methine was supported by the signals at *δ* 4.13 (1H, m) and 75.5. These NMR characteristics are very similar to those of ganoderic acid J.[27] However, the *trans*-annular conjugated moiety, 8-en-7,11-dione, between rings B and C, was not found in **1**. Instead, it was found to possess a 7,11-dione moiety. This was substantiated by the appearance of a pair of doublets at *δ* 3.11 (1H, d, *J* = 13.2 Hz, H-8) and 2.59 (1H, d, *J* = 13.2 Hz, H-9) in the ^1H NMR spectrum and two methine carbons at *δ* 55.0 (C-8) and 60.4 (C-9) in the ^{13}C NMR spectrum. Furthermore, no olefinic protons or carbons were found in the NMR spectra. The conjugated enone chromophore also did not appear in the UV spectrum. The HMBC NMR spectrum showed long-range carbon to hydrogen connectivities from C-3 to H-5, H_3-28, and H_3-29; from C-7 to H-5 and H-9; from C-11 to H-12, H-8, and H-9; from C-15 to H-8, H-17, and H_3-30; from C-23 to H-20 and H-25; and from C-26 to H-24 and H_3-27, establishing the positions of

* To whom correspondence should be addressed. Tel: (801) 345-6353. Fax: (801) 345-2299. E-mail: hsun@pharmanex.com.

10.1021/np010385e CCC: $22.00
Published on Web 12/27/2001

Table 1. ^{1}H NMR Data for Compounds **1**−**3**

proton	**1**[a]	**2**[b]	**3**[c]
1α	1.50 (1H, ddd, J = 13.3, 13.3, 5.2)	1.38 (1H, ddd, J = 14.0, 14.0, 5.1)	1.22 (1H, overlapping)
1β	2.98 (1H, ddd, J = 13.3, 4.5, 2.5)	3.04 (1H, ddd, J = 14.0, 4.5, 2.5)	2.81 (1H, ddd, J = 13.5, 3.2, 3.2)
2α	2.33 (1H, m)	2.36 (1H, m)	1.78 (1H, m)
2β	2.79 (1H, m)	2.74 (1H, m)	1.83 (1H, m)
3			3.35 (1H, dd, J = 11.6, 4.7)
5	1.81 (1H, dd, J = 14.6, 3.1)	1.67 (1H, brd, J = 12.2)	1.16 (1H, dd, J = 12.8, 1.5)
6α	2.36 (1H, dd, J = 15.7, 3.1)	2.41 (1H, m)	2.43 (1H, ddd, J = 12.1, 8.5, 1.5)
6β	2.71 (1H, dd, J = 15.7, 14.6)	2.55 (1H, dd, J = 13.5, 12.2)	1.80 (1H, m)
7			5.02 (1H, overlapping)
8	3.11 (1H, d, J = 13.2)	2.86 (1H, d, J = 12.1)	
9	2.59 (1H, d, J = 13.2)	2.36 (1H, overlapping)	
12α	2.82 (1H, d, J = 14.0)	2.68 (1H, d, J = 13.5)	4.70 (1H, s)
12β	2.32 (1H, d, J = 14.0)	2.40 (1H, overlapping)	
15	4.13 (1H, m)	4.07 (1H, brs)	
16α	1.90 (1H, m)	1.91 (1H, m)	2.90 (1H, m)
16β	1.90 (1H, m)	1.91 (1H, m)	2.68 (1H, dd, J = 19.6, 11.1)
17	1.90 (1H, m)	1.84 (1H, m)	3.06 (1H, m)
18	0.84 (3H, s)	0.83 (3H, s)	1.14 (3H, s)
19	1.48 (3H, s)	1.46 (3H, s)	1.50 (3H, s)
20	1.95 (1H, m)	1.97 (1H, m)	
21	0.84 (3H, d, J = 6.0)	0.84 (3H, d, J = 6.5)	1.45 (3H, s)
22a	2.47 (1H, dd, J = 16.6, 2.7)	2.41 (1H, m)	3.19 (1H, d, J = 14.2)
22b	2.30 (1H, dd, J = 16.6, 9.0)	2.23 (1H, dd, J = 16.6, 9.2)	2.87 (1H, d, J = 14.2)
24a	2.81 (1H, m)	2.82 (1H, dd, J = 16.0, 8.5)	3.08 (1H, dd, J = 17.4, 9.4)
24b	2.53 (1H, dd, J = 16.0, 3.0)	2.48 (1H, dd, J = 16.0, 5.0)	2.93 (1H, dd, J = 17.4, 4.4)
25	2.84 (1H, m)	2.94 (1H, m)	3.05 (1H, m)
27	1.17 (3H, d, J = 7.0)	1.17 (3H, d, J = 7.0)	1.36 (3H, d, J = 6.9)
28	1.05(3H, s)	1.07 (3H, s)	1.22 (3H, s)
29	1.10(3H, s)	1.09 (3H, s)	1.05 (3H, s)
30	1.24(3H, s)	1.22 (3H, s)	1.67 (3H, s)
OMe		3.67 (3H, s)	

[a] Measured in $CD_3OD/CDCl_3$. [b]Measured in $CDCl_3$. [c]Measured in CD_3OD.

Table 2. ^{13}C NMR Data of Compounds **1**−**3**[a]

^{13}C	**1** [b]	**2** [c]	**3**[d]
1	37.2 (t)	36.3 (t)	35.9 (t)
2	35.2 (t)	34.0 (t)	28.4 (t)
3	217.5 (s)	214.2 (s)	79.2 (d)
4	49.0 (s)	47.8 (s)	39.9 (s)
5	53.6 (d)	52.8 (d)	50.5 (d)
6	40.9 (t)	40.0 (t)	28.4 (t)
7	215.6 (s)	212.8 (s)	67.7 (d)
8	55.0 (d)	54.0 (d)	157.3 (s)
9	60.4 (d)	59.5 (d)	144.2 (s)
10	38.0 (s)	36.5 (s)	39.8 (s)
11	210.1 (s)	207.6 (s)	199.4 (s)
12	53.7 (t)	52.6 (t)	78.7 (d)
13	51.0 (s)	50.0 (s)	53.3 (s)
14	51.3 (s)	49.7 (s)	62.7 (s)
15	75.5 (d)	74.1 (d)	217.4 (s)
16	39.0 (t)	38.4 (t)	38.6 (t)
17	49.0 (d)	47.7 (d)	53.9 (d)
18	17.1 (q)	16.6 (q)	13.8 (q)
19	13.7 (q)	13.1 (q)	19.5 (q)
20	33.5 (d)	32.0 (d)	73.8 (s)
21	20.0 (q)	19.4 (q)	28.3 (q)
22	50.5 (t)	49.6 (t)	52.3 (t)
23	211.2 (s)	208.3 (s)	211.5 (s)
24	47.8 (t)	46.8 (t)	49.4 (t)
25	36.1 (d)	34.6 (d)	36.2 (d)
26	179.6 (s)	176.2 (s)	180.2 (s)
27	17.8 (q)	17.1 (q)	17.8 (q)
28	25.9 (q)	25.2 (q)	28.9 (q)
29	21.9 (q)	21.2 (q)	16.4 (q)
30	12.8 (q)	12.5 (q)	23.3 (q)
OMe		51.9 (q)	

[a] Multiplicity derived from DEPT measurements. [b] Measured in $CD_3OD/CDCl_3$. [c] Measured in $CDCl_3$. [d] Measured in CD_3OD.

the carbons containing functional groups, including one carboxyl (C-26), one hydroxyl (C-15), and four ketones (C-3, C-7, C-11, and C-23). The relative stereochemistry of H-8, H-9, and H-15 was defined by using NOESY experiments. NOEs were observed from H-9 to H-5, H-12α, and H_3-28; and from H-8 to H-6β, H-12β, H-15, H_3-18, and H_3-19. Accordingly, H-8 and H-15 were assigned as β and H-9 as α, leading to the structural determination of compound **1** as 8β,9α-dihydroganoderic acid J.

HRFABMS and ^{13}C NMR data established the elemental formula $C_{31}H_{46}O_7$ for compound **2**. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were closely related to those of **1.** Analysis of its 2D NMR data including COSY, HMQC, HMBC, and NOESY showed **2** to be the methyl ester of **1**. This was evident from the presence of a methyl signal at δ 3.67, a methoxyl carbon signal at δ 51.9, and a carboxylate signal at δ 176.2 (C-26). The methoxyl protons were found to have a cross-peak with C-26 in the HMBC spectrum, establishing compound **2** to be methyl 8β,9α-dihydroganoderate J.

Compound **3** was accorded the molecular formula $C_{30}H_{44}O_9$ as established by HRFABMS and ^{13}C NMR data. The ^{13}C NMR spectrum (Table 2) revealed the presence of seven methyls, six methylenes, six methines, one carboxyl, three ketone carbonyls, two olefinic carbons, and five quaternary carbons. The ^{1}H NMR spectrum showed six methyl singlets (δ 1.05, 1.14, 1.22, 1.45, 1.50, and 1.67), one methyl doublet (δ 1.36), and three hydroxyl methine signals (δ 3.35, 4.70, and 5.02). These NMR data closely resembled those of ganoderic acid G.[20−22] Examination of the ^{13}C NMR chemical shifts of C-1 to C-14, C-19, C-28, and C-29 clearly indicated that rings A, B, and C in **3** were the same as those in ganoderic acid G,[20−22] having the *trans*-annular conjugated moiety 7,11-dihydroxy-8-ene-11-one. The UV absorbance at 252 nm also supported an eneone moiety. Further comparison of their NMR spectra revealed that a methyl doublet at δ 1.13 (H_3-21) and a tertiary carbon at δ 29.2 (C-20) in ganoderic acid G had disappeared, while a methyl singlet at δ 1.45 and a quaternary carbon at δ 73.8 in **3** had appeared instead. In **3**, the multiplicity in each of the two adjacent methylene

protons (H_2-22) at δ 2.87 and 3.19 changed to a doublet (J = 14.2 Hz) from a doublet of doublets in ganoderic acid G. A hydroxyl group must then be attached to C-20 in **3**, yielding the same side chain, 2-methyl-4-oxo-6-hydroxylheptanoic acid, as in ganoderic acid I.[18,20] ^{13}C NMR chemical shifts of the D ring and the side chain in **3** were in agreement with those of ganoderic acid I. The ^{1}H−^{1}H and ^{1}H−^{13}C correlations obtained from the COSY, HMQC, HMBC, and NOESY data substantiated the positions of all oxygenated atoms and the relative stereochemistry of three hydroxyl methine protons, leading **3** to be proposed as 20-hydroxylganoderic acid G.

Experimental Section

General Experimental Procedures. Melting points were recorded on a Fisher-Johns melting point apparatus. Optical rotations were recorded on a JASCO ORD/UV5 spectropolarimeter. IR spectra were obtained on a Magna FTIR-750 spectrometer. UV spectra were recorded on a HP-8453 spectrophotometer. One- and two-dimensional NMR spectra were recorded on a Bruker AMX 400 spectrometer. Chemical shifts were reported as δ values in ppm relative to TMS. EIMS was performed on an Hewlett-Packard 5989A mass spectrometer. HRFABMS was measured on a tandem double-focusing Micromass 70-SE-4F spectrometer. HPLC was performed on a Waters 600 instrument equipped with a Waters 486 UV detector at 254 nm. Separations of triterpenoids and ergosterols were achieved on Nova-pak C_{18} and Dynamax-60A cyano columns using linear solvent gradient systems of MeOH−0.5% HCOOH (45:55 to 60:40) for the C_{18} column and CH_3CN−0.5% HCOOH (30:70 to 35:65) for the cyano column.

Fungal Material. The fruiting body of *G. lucidum* was purchased from Shanghai Company of Traditional Chinese Medicine, Shanghai, People's Republic of China, in July 1998. The mushroom was identified by Professor Guanyun Gu, School of Pharmacy, Shanghai Medical University, Shanghai, People's Republic of China. A voucher specimen (GL-9807) has been deposited at the Department of Pharmacognosy, Shanghai Medical University, Shanghai, People's Republic of China.

Extraction and Isolation. *G. lucidum* (2 kg) mushrooms were chipped and extracted with 95% EtOH under reflux at 80 °C three times. The combined ethanolic extracts were evaporated under reduced pressure. The residue (100 g) was suspended in H_2O and extracted with $CHCl_3$. The volume of the combined $CHCl_3$ extracts was reduced to one-third under vacuum. A saturated $NaHCO_3$ aqueous solution was then added. The $CHCl_3$ layer (fraction A, 40 g) was subjected to silica gel column chromatography for isolation of nonacidic triterpenoids and sterols. The water layer was extracted with $CHCl_3$ again after the pH value was adjusted to 2−3 with 6 N HCl solution. The combined $CHCl_3$ extracts were evaporated to dryness under reduced pressure, and the residue (fraction B, 20 g) was applied to column chromatography for the separation of acidic triterpenoids. By repeated column chromatography, HPLC separation, and crystallization, fraction B yielded **1** (10 mg), **2** (6 mg), **3** (8 mg), ganoderic acids A (40 mg), AM_1 (8 mg), B (30 mg), C_2 (10 mg), G (100 mg), H (50 mg), I (25 mg), methylganoderate I (5 mg), and 12-deacetylganoderic acid H (42 mg). In turn, fraction A afforded ganoderic acid DM (34 mg), lucidone A (5 mg), ganoderiol B (5 mg), ergosta-7,22-dien-3β-ol (15 mg), and 5α,8α-epidioxyergosta-6,22-dien-3β-ol (60 mg). The spectral data of new compounds (**1**−**3**) are listed herein.

8β,9α-Dihydroganoderic acid J (1): colorless prisms (MeOH−H_2O); mp 205−208 °C; $[\alpha]^{25}_D$ +24° (*c* 0.04, MeOH); IR (KBr) ν_{max} 3463, 3400, 1701 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; HMBC cross-peaks C-1/H-9; C-2/H-1; C-3/H-1, H-2, H_3-28, H_3-29; C-4/H-2, H-5, H-6, H_3-28, H_3-29; C-5/H-1, H-6, H-9, H_3-28, H_3-29; C-6/H-5; C-7/H-5, H-6, H-8, H-9; C-8/H-6, H-9, H-15, H_3-30; C-9/H-8, H-12; C-10/H-1, H-5, H-6, H_3-19; C-11/H-8, H-9, H-12; C-12/H_3-18; C-13/H-8, H-12, H_3-30; C-14/H-8; C-15/H-8, H-16, H_3-30; C-16/H-15, H-17; C-17/H-12, H_3-21, H-22; C-18/H-12, H-17; C-19/H-1, H-5, H-9; C-20/H-16, H-17, H_3-21, H-22; C-21/H-22; C-22/H_3-21; C-23/H-22, H-24; C-24/H-25, H_3-27; C-25/H-24, H_3-27; C-26/H-24, H-25, H_3-27; C-27/H-24; C-28/H-5, H_3-29; C-29/H-5, H_3-28; C-30/H-8, H-15; NOESY cross-peaks H-1β/H-19; H-5/H-1α, H-9, H_3-28; H-6α/H-5; H-8/H_3-18, H_3-19; H-9/H-1α; H-15/H-8, H_3-18; H_3-18/H-12β; H_3-19/H-1β, H-6β, H-8; H_3-28/H-1α, H-5, H-6α; H_3-29/H-6β, H_3-19; H_3-30/H-9, H-12α; EIMS *m*/*z* 517 [M + 1]$^+$ (4), 516 [M]$^+$ (2), 499 (3), 481 (4), 443 (4), 386 (59), 368 (56), 356 (31), 259 (9), 234 (26), 207 (13), 179 (24), 161 (15), 139 (57), 121-(51), 109 (37); HRFABMS *m*/*z* 517.3163 [M + 1]$^+$ (calcd for $C_{30}H_{45}O_7$, 517.3165).

Methyl 8β,9α-dihydroganoderate J (2): colorless prisms (MeOH−H_2O); mp 202−205 °C; $[\alpha]^{25}_D$ +52° (*c* 0.22, MeOH); IR (KBr) ν_{max} 3437, 1732, 1705 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; HMBC cross-peaks C-1/H-2, H-19; C-2/H-1; C-3/H-1, H-2, H_3-28, H_3-29; C-4/H_3-28, H_3-29; C-5/H-1, H_3-28, H_3-29; C8-/H-9, H_3-30; C-9/H-1, H-12, H_3-19; C-10/H-1, H-2, H_3-19; C-11/H-12; C-12/H_3-18; C-13/H-12, H_3-30; C-14/H-12; C-15/H_3-30; C-17/H-12, H_3-18, H_3-21; C-18/H-12; C-19/H-1; C-20/H_3-21; C-21/H-22; C-22/H_3-21; C-23/H-22, H-24; C-24/H-25, H_3-27; C-25/H-24, H_3-27; C-26/H-24, H-25, H_3-27, OMe; C-27/H-24, H-25; C-28/H_3-29; C-29/H_3-28; NOESY cross-peaks H-5/H-1α, H-9, H_3-28; H-9/H-1α; H-12α/H-30α; H_3-30/H-9; EIMS *m*/*z* 531 [M + 1]$^+$ (35), 513 (18), 495 (27), 481 (15), 463 (11), 386 (56), 368 (99), 356 (24), 234 (19), 179 (25), 144 (95); HRFABMS *m*/*z* 531.3322 [M + 1]$^+$ (calcd for $C_{31}H_{47}O_7$ 531.3322).

20-Hydroxylganoderic acid G (3): pale yellow needles (MeOH−H_2O); mp 175−177 °C; $[\alpha]^{25}_D$ +42° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (3.76) nm; IR (KBr) ν_{max} 3435, 1722, 1684, 1385 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; HMBC cross-peaks C-1/H-2, H_3-19; C-3/H_3-28, H_3-29; C-4/H-5, H-6, H_3-28, H_3-29; C-5/H-6, H_3-19, H_3-28, H_3-29; C-6/H-7; C-7/H-5, H-6; C-8/H-7, H_3-30; C-9/H-7, H_3-19; C-10/H-2, H_3-19; C-11/H-12; C-12/H_3-18; C-13/H-12, H-16, H-17, H_3-18, H_3-30; C-14/H_3-18, H_3-30; C-15/H-16, H_3-30; C-17/H-16, H_3-21; C-19/H-5; C-20/H-16, H-17, H_3-21, H-22; C-21/H-22; C-22/H_3-21; C-23/H-22, H-24; C-24/H-22, H_3-27; C-25/H-24, H_3-27; C-26/H-25, H_3-27; C-27/H-24; C-28/H-3, H_3-29; C-29/H-3, H-5, H_3-28; NOESY cross-peaks H-1β/H_3-19, H-3/H-1α, H-5, H-6α, H_3-28; H-6α/H-5, H_3-28; H-7/H-5, H-6α, H_3-30; H-12α/H-17, H_3-30; H-17/H-16α, H_3-30; H_3-18/H-16β; H_3-19/H-1β, H-16β, H_3-29; H_3-28/H-6α; H_3-29/H-6β, H_3-19; H_3-30/H-16α, H-17; EIMS *m*/*z* 512 [M − 2H_2O]$^+$ (7), 494 (12), 466 (10), 433 (4), 366 (5), 338 (5), 305 (27), 290 (9), 241 (8), 215 (9), 195(11); HRFABMS *m*/*z* 549.3066 [M + 1]$^+$ (calcd for $C_{30}H_{45}O_9$ 549.3064).

Immune Assays. The mediator release, IL-2, IL-4, and IFN-γ assays[7] were used to measure the IC_{50} values for the extract of *G. lucidum*. The extract was dissolved in a 0.4% DMSO solution, then incubated with normal human peripheral blood mononuclear leukocytes in a RPMI 1640 buffer containing 10% FBS, 50 unit/mL penicillin, and 50 μg/mL streptomycin at 37 °C for 16 h. Levels of IL-2, IL-4, and IFN-γ were measured using ELISA (performed at Panlabs).

Acknowledgment. We thank Dr. Steven L. Mullen, Mass Spectrometry Laboratory, University of Illinois at Urbana−Champaign for accurate mass measurements and MDS Panlabs Pharmacology Services for immune assays.

References and Notes

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NP010385E