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Nitrogen-Containing Dihydro-β-agarofuran Derivatives from *Tripterygium wilfordii*

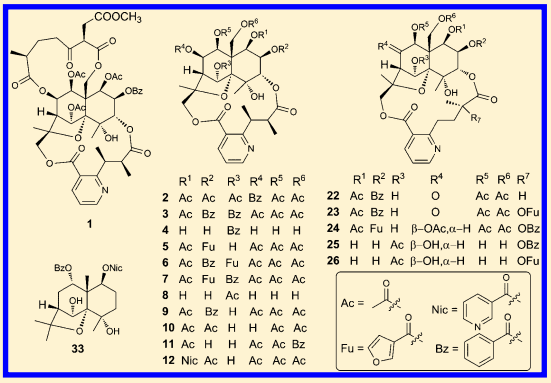
Yinggang Luo,*[,†] Xiang Pu,[†,‡] Guoyong Luo,[†,‡] Min Zhou,[†] Qi Ye,[†] Yan Liu,[†] Jian Gu,[†] Huayi Qi,[†] Guoyou Li,[†] and Guolin Zhang*[,†]

[†]Center for Natural Products Research, Chengdu Institute of Biology, Chinese Academy of Sciences, 9 Section 4, Renmin Road South, Chengdu 610041, People's Republic of China

[‡]University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, People's Republic of China

Supporting Information

ABSTRACT: Thunder god vine, the dried roots of *Tripterygium wilfordii*, is a widely used traditional Chinese medicine. More than 200 bioactive complex natural products have been isolated from this herb. Inspired by the diversity of chemical structures and bioactivities of the components of this herb, the investigation to mine new chemical entities as potential drug leads led to the identification of 36 nitrogen-containing compounds. Among them, 18 new dihydro-β-agarofuran alkaloids (tripterygiumines A–L (**1**–**12**), M–Q (**22**–**26**), and R (**33**)) were identified from the spectroscopic data and chemical degradation studies. Tripterygiumine Q (**26**) exhibited immunosuppressive activity against human peripheral mononuclear cells with an IC_{50} value of 8.67 μM and showed no cytotoxicity, even at 100 μM, indicating that **26** may represent a novel scaffold for the development of new immunosuppressants.



	R^1	R^2	R^3	R^4	R^5	R^6
2	Ac	Ac	Ac	Bz	Ac	Ac
3	Ac	Bz	Bz	Ac	Ac	Ac
4	H	H	Bz	H	H	H
5	Ac	Fu	H	Ac	Ac	Ac
6	Ac	Bz	Fu	Ac	Ac	Ac
7	Ac	Fu	Bz	Ac	Ac	Ac
8	H	H	Ac	H	H	H
9	Ac	Bz	H	Ac	Ac	Ac
10	Ac	Ac	H	H	Ac	Ac
11	Ac	H	H	Ac	Ac	Bz
12	Nic	Ac	H	Ac	Ac	Ac

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
22	Ac	Bz	H	O	Ac	Ac	H
23	Ac	Bz	H	O	Ac	Ac	OFu
24	Ac	Fu	H	β-OAc,α-H	Ac	Ac	OBz
25	H	H	Ac	β-OH,α-H	H	H	OBz
26	H	H	Ac	β-OH,α-H	H	H	OFu

The air-dried root of *Tripterygium wilfordii* (Celastraceae), known as thunder god vine, is a traditional Chinese medicine that has been widely used for more than 400 years to treat inflammation, cancers, and autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and lupus.[1] A number of sesqui-, di-, and triterpenoids, steroids, and sesquiterpenoid pyridine alkaloids exhibiting beneficial and/or adverse effects have been isolated from this plant.[2] The sesquiterpenoid pyridine alkaloids possess a characteristic macrocyclic dilactone skeleton consisting of a dicarboxylic acid moiety, 2-(carboxyalkyl)nicotinic acid, and a polyoxygenated dihydro-β-agarofuran sesquiterpenoid. The hydroxy groups of the latter are usually esterified by various organic acids including acetic, benzoic, furanoic, nicotinic, and cinnamic acids. The 2-(carboxyalkyl)nicotinic acid moiety originates from evoninic acid, wilfordic acid, hydroxywilfordic acid, or their congeners.[2]

Our previous phytochemical investigation of aqueous EtOH extracts of the dried roots of *T. wilfordii* yielded five new and 13 known alkaloids.[3] Some of these alkaloids exhibited moderate inhibition against herpes simplex virus type II.[3] As a continuation of the chemical studies of *Tripterygium* alkaloids to mine new chemical entities with drug potential, 36 sesquiterpenoid alkaloids, of which 18 are new nitrogen-containing derivatives, were identified. Herein, the isolation and structure elucidation of the 18 new alkaloids are reported. The immunosuppressive activities of these alkaloids were evaluated for their therapeutic potential.

RESULTS AND DISCUSSION

The $CHCl_3$-soluble extract of *T. wilfordii* was separated by repeated column chromatography on silica gel. Semipreparative HPLC of the subfractions afforded 36 compounds. The detailed extraction and isolation procedures of each compound are presented in the Supporting Information.

Compound **1** was isolated as a white powder. The molecular formula of $C_{50}H_{57}NO_{21}$ was determined by ^{13}C NMR (Table 1) and HRESIMS data [*m*/*z* 1030.3320 ([M + Na]$^+$)] and revealed 23 indices of hydrogen deficiency. The IR spectrum indicated the presence of hydroxy (3437 cm^{-1}), carbonyl (1740 cm^{-1}), and pyridine (1631 cm^{-1}) groups, and the UV spectrum suggested the presence of aromatic groups (244 and 262 nm). The NMR spectroscopic data (Table 1) indicated the presence of three acetyls, two oxygenated methylenes, three aliphatic methylenes, five aliphatic methines, six oxygenated methines, five methyls, one methoxy, one 2,3-disubstituted pyridine, and one benzoyl group. In addition to the resonances of the aforementioned groups, resonances for a ketocarbonyl, five ester carbonyls, three oxygenated quaternary carbons, and one aliphatic quaternary carbon were observed in the ^{13}C NMR spectrum of **1**. A polyoxygenated dihydro-β-agarofuran skeleton was established from the ^1H–^1H COSY cross signals for the H-1/H-2/H-3 and H-5/H-6/H-7/H-8 coupling systems and the following HMBC correlations: H-1/C-9; H-2/C-9; H-3/C-4,

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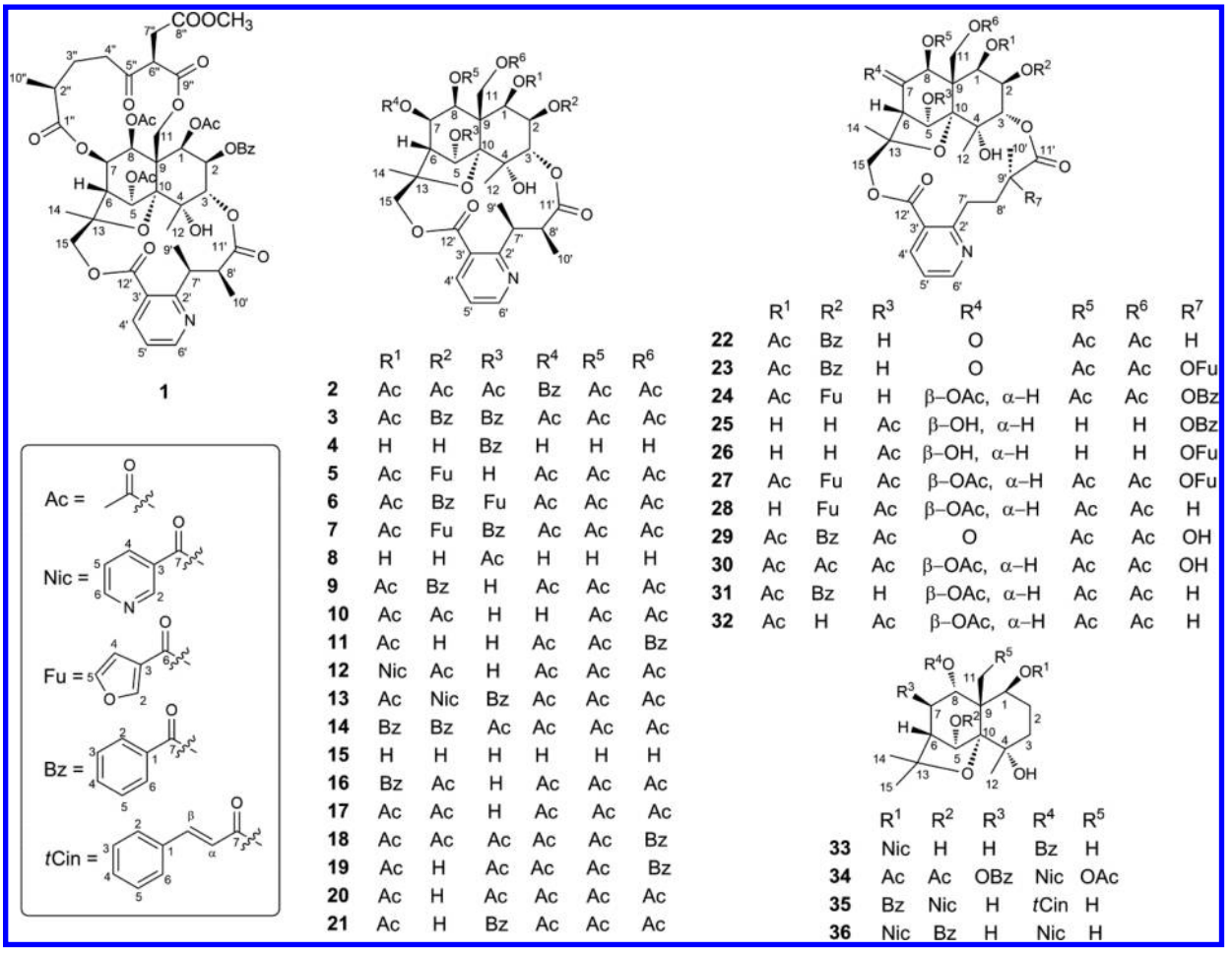

	R^1	R^2	R^3	R^4	R^5	R^6
2	Ac	Ac	Ac	Bz	Ac	Ac
3	Ac	Bz	Bz	Ac	Ac	Ac
4	H	H	Bz	H	H	H
5	Ac	Fu	H	Ac	Ac	Ac
6	Ac	Bz	Fu	Ac	Ac	Ac
7	Ac	Fu	Bz	Ac	Ac	Ac
8	H	H	Ac	H	H	H
9	Ac	Bz	H	Ac	Ac	Ac
10	Ac	Ac	H	H	Ac	Ac
11	Ac	H	H	Ac	Ac	Bz
12	Nic	Ac	H	Ac	Ac	Ac
13	Ac	Nic	Bz	Ac	Ac	Ac
14	Bz	Bz	Ac	Ac	Ac	Ac
15	H	H	H	H	H	H
16	Bz	Ac	H	Ac	Ac	Ac
17	Ac	Ac	H	Ac	Ac	Ac
18	Ac	Ac	Ac	Ac	Ac	Bz
19	Ac	H	Ac	Ac	Ac	Bz
20	Ac	H	Ac	Ac	Ac	Ac
21	Ac	H	Bz	Ac	Ac	Ac

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
22	Ac	Bz	H	O	Ac	Ac	H
23	Ac	Bz	H	O	Ac	Ac	OFu
24	Ac	Fu	H	β–OAc, α–H	Ac	Ac	OBz
25	H	H	Ac	β–OH, α–H	H	H	OBz
26	H	H	Ac	β–OH, α–H	H	H	OFu
27	Ac	Fu	Ac	β–OAc, α–H	Ac	Ac	OFu
28	H	Fu	Ac	β–OAc, α–H	Ac	Ac	H
29	Ac	Bz	Ac	O	Ac	Ac	OH
30	Ac	Ac	Ac	β–OAc, α–H	Ac	Ac	OH
31	Ac	Bz	H	β–OAc, α–H	Ac	Ac	H
32	Ac	H	Ac	β–OAc, α–H	Ac	Ac	H

	R^1	R^2	R^3	R^4	R^5
33	Nic	H	H	Bz	H
34	Ac	Ac	OBz	Nic	OAc
35	Bz	Nic	H	*t*Cin	H
36	Nic	Bz	H	Nic	H

C-10; H-5/C-7, C-10, C-13; H-6/C-7; H-7/C-9; H-8/C-1, C-9, C-11; H-11/C-8, C-9; H-12/C-3, C-4, C-10; H-14/C-6, C-13, C-15; and H-15/C-13, C-14 (Figure 1A). A 2-(3-carboxybutan-2-yl)nicotinic acid moiety was established based on the 2,3-disubstituted pyridine and the −CH(CH$_3$)−CH(CH$_3$)− moieties corresponding to the HMBC correlations of H-4′/C-2′, C-6′, C-12′; H-5′/C-3′; H-6′/C-4′; H-7′/C-2′, C-8′, C-9′, C-11′; H-8′/C-2′, C-11′; H-9′/C-2′, C-7′, C-8′; and H-10′/C-7′, C-8′, C-11′ (Figure 1A). A monoterpene tricarboxylic acid moiety (C-1″−C-10″) was established from the ^{1}H−^{1}H COSY cross signals for the H-10″/H-2″/H-3″/H-4″ coupling systems and the following HMBC correlations: H-10″/C-1″, C-3″; H-4″/C-2″, C-5″; H-6″/C-5″, C-7″, C-8″, C-9″; and H-7″/C-5″, C-6″, C-8″, C-9″ (Figure 1A). These data suggested that compound **1** was a dimacrocyclic sesquiterpenoid pyridine alkaloid. The HMBC cross signals of H-3/C-11′ and H-15/C-12′ confirmed the C-3−O−C-11′ and C-15−O−C-12′ linkages, respectively, for the first macrocyclic system between the polyoxygenated dihydro-*β*-agarofuran and the substituted nicotinic acid moieties (Figure 1A). The linkages of the second macrocyle were between the dihydro-*β*-agarofuran and the monoterpene tricarboxylic acid moiety at C-7−O−C-1″ and C-11−O−C-9″ and were established from the HMBC cross signals of H-7/C-1″ and H-11/C-9″, respectively (Figure 1A). The third carboxy group of the tricarboxylic acid moiety was a methyl ester, as confirmed by the HMBC correlation of OCH$_3$/C-8″ (Figure 1A). The 1-OAc, 5-OAc, 8-OAc, and 2-OBz substituents were determined from the corresponding HMBC correlations of H-1/C-2$_{1\text{-OAc}}$, H-5/C-2$_{5\text{-OAc}}$, H-8/C-2$_{8\text{-OAc}}$, and H-2/C-7$_{2\text{-OBz}}$ (Figure 1A). A free hydroxy group was assigned to C-4 by comparing the ^{13}C NMR chemical shift of C-4 (δ_C 70.50) with the reported value[3,4] and was confirmed by the HMBC cross signals of 4-OH/C-12 and H-12/C-3, C-4, C-10 (Figure 1A).

The relative configuration of the polyoxygenated dihydroagarofuran moiety of **1** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-11/H-12 (Figure 1B). Owing to the flexibility of the macrocyclic rings, the relative configurations of the substituted pyridine and the monoterpene tricarboxylic acid moieties could not be determined by NOESY experiments. However, *J*-based configuration analysis is a powerful tool for the stereochemical determination of macrocyclic structures.[5] Coupling constants were calculated using HETLOC[6] and HSQC-HECADE[7] techniques. The C-7′−C-10′ and C-2″−C-4″ moieties are characteristic of the 2,3-dimethyl- and 2-methylbutane systems, respectively. The $^3J_{H,H}$ and $^{2,3}J_{C,H}$ coupling constants of the C-7′−C-10′ moiety are summarized in Figure 1C. Comparison of the reported $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values for the 2,3-disubstituted and 2-substituted butane systems with methyl/hydroxy, alkyloxy, or acyloxy groups permitted the deduction of the relative configurations of the C-7′−C-10′ and C-2″−C-4″ moieties, which were consistent with those of triptonine A. The relative configuration of triptonine A had been previously established by X-ray crystallography analysis.[8a] Thus, compound **1** was identified as 2-de-*O*-acetyl-2-*O*-benzoyltriptonine A and was named tripterygiumine A. Besides triptonines A and B,[8] **1** is the third reported dimacrocyclic structure in which the

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Table 1. NMR Spectroscopic Data for Tripterygiumine A (1)[a]

position	δ_H (J in Hz)	δ_C, type	position	δ_H (J in Hz)	δ_C, type
1	5.68, d (4.6)	73.82, CH	10′	1.20, d (7.2)	9.69, CH_3
2	5.53, m	69.83, CH	11′		173.87, C
3	4.85, d (2.4)	75.78, CH	12′		168.53, C
4		70.50, C	1″		175.67, C
5	7.14, s	73.41, CH	2″	2.62, m	37.32, CH
6	2.51, d (3.6)	50.22, CH	3″	1.98, m	28.06, CH_2
				1.83, m	
7	5.52, m	69.18, CH	4″	3.47, m	42.12, CH_2
				2.97, m	
8	5.37, d (6.1)	70.86, CH	5″		204.19, C
9		52.01, C	6″	4.82, t (7.3)	51.92, CH
10		94.07, C	7″	3.07, d (7.4)	32.64, CH_2
11	5.67, d (13.6)	62.13, CH_2	8″		171.82, C
	4.36, d (13.6)				
12	1.63, s	22.71, CH_3	9″		167.84, C
13		84.50, C	10″	1.19, d (7.0)	18.00, CH_3
14	1.72, s	18.54, CH_3	4-OH	4.61, s	
15	6.04, d (11.7)	69.65, CH_2	8″-OMe	3.70, s	52.0, CH_3
	3.74, d (11.6)				
2′		165.43, C	1-OAc	1.79, s	20.34, CH_3; 168.94, C
3′		124.97, C	5-OAc	2.26, s	21.67, CH_3; 170.15, C
4′	8.09, dd (7.7, 1.4)	137.76,CH	8-OAc	1.92, s	20.36, CH_3; 168.66, C
5′	7.28, dd (7.7, 4.8)	121.09, CH	2-OBz-1		124.97, C
6′	8.70, dd (4.8, 1.5)	151.53, CH	2-OBz-2,6	8.12, d (7.5)	130.10, CH
7′	4.68, q (6.8)	36.41, CH	2-OBz-3,5	7.50, t (7.7)	128.70, CH
8′	2.58, q (7.4)	45.03, CH	2-OBz-4	7.62, t (7.5)	133.72, CH
9′	1.40, d (7.0)	11.74, CH_3	2-OBz-7		164.73, C

[a]Recorded in $CDCl_3$ at 600 MHz for ^1H and 150 MHz for ^{13}C NMR spectra. Chemical shifts and coupling constants (in parentheses) are given in ppm and Hz, respectively.

polyoxygenated sesquiterpene is esterified with a monoterpene carboxylic acid.

Tripterygiumine B (**2**) was shown to have the molecular formula $C_{43}H_{49}NO_{18}$ by ^{13}C NMR (Table 2) and HRESIMS data [m/z 890.2819 ([M + Na]$^+$)], revealing 20 indices of hydrogen deficiency. The IR, UV, and NMR spectroscopic data (Table 2) of **2** were similar to those of **1** and the alkaloids reported previously.[3] The same polyoxygenated dihydro-β-agarofuran and 2-(3-carboxybutan-2-yl)nicotinic acid moieties as those in **1** were established on the basis of spectroscopic data analysis (Figure 1D). The HMBC cross signals of H-3/C-11′ and H-15/C-12′ confirmed the C-3−O−C-11′ and C-15−O−C-12′ linkages between the dihydro-β-agarofuran and the substituted nicotinic acid moieties, respectively. A free hydroxy group was assigned to C-4 by comparison of the ^{13}C NMR chemical shift of C-4 (δ_C 70.68) with the reported value[3,4] and was confirmed by the HMBC cross signals of 4-OH/C-12 and H-12/C-3, C-4, C-10 (Figure 1D). The presence of 1-OAc, 2-OAc, 5-OAc, 8-OAc, and 7-OBz substituents was determined on the basis of the corresponding HMBC correlations of H-1/C-$2_{1\text{-OAc}}$, H-2/C-$2_{2\text{-OAc}}$, H-5/C-$2_{5\text{-OAc}}$, H-8/C-$2_{8\text{-OAc}}$, and H-7/C-$7_{7\text{-OBz}}$ (Figure 1D). The relative configuration of the polyoxygenated dihydroagarofuran moiety of **2** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-11/H-12 (Figure 1E). The relative configuration of the C-7′−C-10′ moiety was determined by J-based configuration analysis (Figure 1F).

Compound **3**, tripterygiumine C, was shown to have the molecular formula $C_{48}H_{51}NO_{18}$ by ^{13}C NMR (Table S2) and HRESIMS data [m/z 952.2998 ([M + Na]$^+$)], revealing 24 indices of hydrogen deficiency. The IR and UV data of **3** were similar to those of **2**. Comparison of the NMR spectroscopic data of **3** (Tables S1 and S2) with those of **2** suggested that two acetyl groups and one benzoyl group in **2** were replaced by two benzoyl groups and one acetyl group, respectively, in **3**. 2D NMR experiments established the planar structure of **3** (Figure S1A). The relative configuration of the polyoxygenated dihydroagarofuran moiety of **3** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-11/H-12 (Figure S1B). The relative configuration of the 2-(3-carboxybutan-2-yl)nicotinic acid was determined by comparison of the NMR data of **3** with those of **1** and **2** based on their similar biosynthetic origins.

Compound **4** (tripterygiumine D) was shown to have the molecular formula $C_{33}H_{39}NO_{13}$ by ^{13}C NMR (Table S2) and HRESIMS data [m/z 680.2293 ([M + Na]$^+$)]. The IR and UV data of **4** were similar to those of **2**. Comparison of the NMR spectroscopic data of **4** (Tables S1 and S2) with those of **2** and **3** revealed that only the 5-OH group was benzoylated in **4**. The planar structure and relative configuration of **4** were established by the methods similar to those used for **3** (Figure S1).

Alkaloid **5** (tripterygiumine E) was shown to have the molecular formula $C_{39}H_{45}NO_{18}$ by ^{13}C NMR (Table S2) and HRESIMS data [m/z 838.2553 ([M + Na]$^+$)]. The IR and UV data of **5** were similar to those of **2**. Comparison of the NMR spectroscopic data of **5** (Tables S1 and S2) with those of **2** suggested that the 5-OH group was free. The 2-OAc and 8-OBz groups in **2** were replaced with 2-OFu and 8-OAc groups, respectively, in **5**. The planar structure and relative config-

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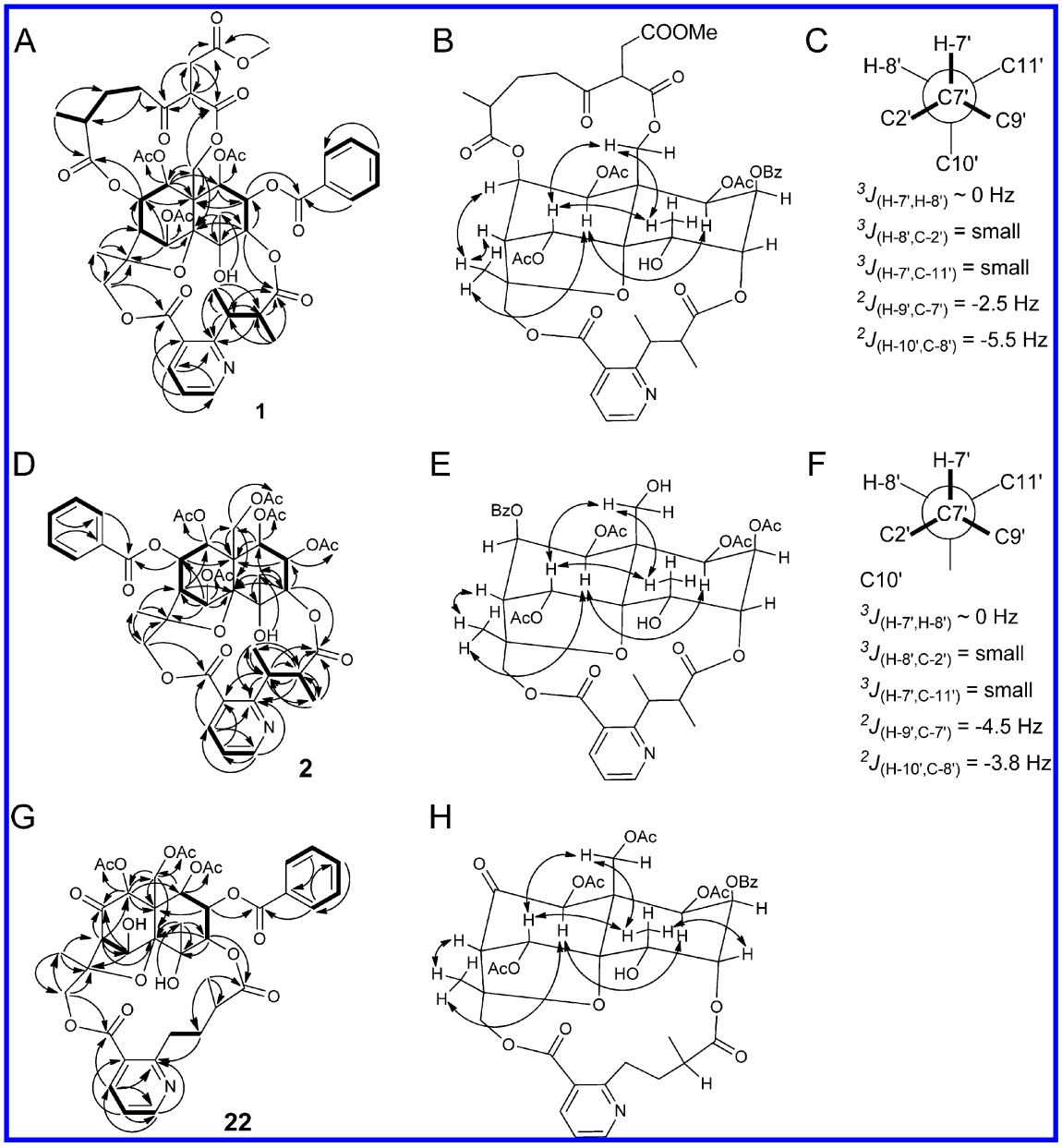

Figure 1. Key HMBC (curved arrows), ^{1}H−^{1}H COSY (bold bonds), and NOESY (curved double arrows) correlations and *J*-based configuration analysis (Newman projection) of alkaloids **1**, **2**, and **22**.

uration of **5** were established by the methods described above (Figure S1).

Alkaloid **6** (tripterygiumine F) was shown to have the molecular formula $C_{46}H_{49}NO_{19}$ by ^{13}C NMR (Table S2) and HRESIMS data [*m*/*z* 942.2797 ([M + Na]$^+$)]. The IR and UV data of **6** were similar to those of **3**. Comparison of the NMR spectroscopic data of **6** (Tables S1 and S2) with those of **3** suggested that the C-5 benzoyl group in **3** was replaced by a furanoyl group in **6**. The planar structure and relative configuration of **6** were established by the method described above (Figure S1).

Alkaloid **7** (tripterygiumine G) was shown to have the molecular formula $C_{46}H_{49}NO_{19}$ by ^{13}C NMR (Table S2) and HRESIMS data [*m*/*z* 942.2783 ([M + Na]$^+$)]. The spectroscopic data (Tables S1 and S2) suggested that **7** was isomeric with alkaloid **6**. The spectroscopic data revealed that alkaloid **7** possessed 2-OFu and 5-OBz substituents instead of the 2-OBz and 5-OFu groups in **6** (Figure S1).

Alkaloid **8** was shown to have the molecular formula $C_{28}H_{37}NO_{13}$ by ^{13}C NMR (Table S2) and HRESIMS data [*m*/*z* 618.2165 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **8** (Tables S1 and S2) with those of **4** suggested that the benzoyl group in **4** was replaced by an acetyl group in **8**. The planar structure and relative configuration of **8** were established by the methods described above (Figure S1). The structure of **8** was identical to the methanolysis product of cathedulin K2.[9a] However, only the ^{1}H NMR data in methanol-d_4 were reported for the methanolysis product of cathedulin K2. Herein, **8** is reported as a naturally occurring sesquiterpenoid pyridine alkaloid and was named tripterygiumine H.

Alkaloid **9** (tripterygiumine I) was shown to have the molecular formula $C_{41}H_{47}NO_{17}$ by ^{13}C NMR (Table S2) and HRESIMS data [*m*/*z* 848.2772 ([M + Na]$^+$)]. The IR and UV data of **9** were similar to those of **5**. Comparison of the NMR spectroscopic data of **9** (Tables S1 and S2) with those of **5**

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Table 2. NMR Spectroscopic Data for Tripterygiumine B (2)[a]

position	δ_H (J in Hz)	δ_C, type	position	δ_H (J in Hz)	δ_C, type
1	5.69, d (4.0)	73.22, CH	6′	8.70, dd (4.8, 1.7)	151.50, CH
2	5.52, m	69.28, CH	7′	4.66, q (6.9)	36.48, CH
3	4.86, d (2.3)	75.73, CH	8′	2.60, q (7.2)	44.92, CH
4		70.68, C	9′	1.39, d (7.0)	11.90, CH_3
5	7.03, s	73.74, CH	10′	1.22, d (7.1)	9.70, CH_3
6	2.36, d (3.9)	50.58, CH	11′		173.88, C
7	5.54, m	68.95, CH	12′		168.47, C
8	5.40, d (6.0)	70.46, CH	4-OH	4.53, s	
9		52.13, C	1-OAc	1.81, s	20.41, CH_3; 169.10, C
10		94.04, C	2-OAc	2.16, s	20.98, CH_3; 169.86, C
11	5.45, d (13.4)	60.47, CH_2	5-OAc	2.22, s	21.60, CH_3; 170.01, C
	4.39, d (13.4)				
12	1.66, s	23.26, CH_3	8-OAc	1.94, s	20.41, CH_3; 168.92, C
13		84.17, C	11-OAc	2.28, s	21.18, CH_3; 170.33, C
14	1.70, s	18.52, CH_3	7-OBz-1		128.90, C
15	5.97, d (11.6)	69.87, CH_2	7-OBz-2,6	8.10, d (7.3)	129.98, CH
	3.71, d (11.6)				
2′		165.36, C	7-OBz-3,5	7.51, t (7.7)	128.72, CH
3′		125.07, C	7-OBz-4	7.62, t (7.4)	133.65, CH
4′	8.07, dd (7.8, 1.7)	137.71, CH	7-OBz-7		164.81, C
5′	7.27, dd (7.8, 4.8)	121.06, CH			

[a]Recorded in $CDCl_3$ at 600 MHz for ^1H and 150 MHz for ^{13}C NMR spectra. Chemical shifts and coupling constants (in parentheses) are given in ppm and Hz, respectively.

Table 3. NMR Spectroscopic Data for Tripterygiumine M (22)[a]

position	δ_H (J in Hz)	δ_C, type	position	δ_H (J in Hz)	δ_C, type
1	5.89, d (3.4)	71.77, CH	5′	7.29, dd (7.9, 4.7)	121.26, CH
2	5.42, t (3.1)	69.85, CH	6′	8.77, dd (4.7, 1.7)	153.68, CH
3	5.21, d (3.0)	74.31, CH	7′	4.12, m	33.00, CH_2
				2.89, m	
4		71.96, C	8′	2.39, m	33.44, CH_2
				1.90, m	
5	5.37, brs	75.90, CH	9′	2.38, m	38.17, CH
6	3.18, brs	64.52, CH	10′	1.22, d (6.4)	18.92, CH_3
7		197.61, C	11′		174.99, C
8	5.63, s	78.70, CH	12′		167.14, C
9		52.5, C	4-OH	6.59, s	
10		94.10, C	5-OH	6.22, s	
11	4.91, d (12.8)	60.69, CH_2	1-OAc	1.90, s	20.11, CH_3; 169.30, C
	4.69, d (12.8)				
12	1.98, s	23.94, CH_3	8-OAc	1.99, s	20.48, CH_3; 169.64, C
13		86.37, C	11-OAc	1.99, s	20.54, CH_3; 169.33, C
14	1.63, s	19.33, CH_3	2-OBz-1		128.56, C
15	5.92, d (12.4)	71.33, CH_2	2-OBz-2,6	8.02, d (7.3)	129.73, CH
	3.78, d (12.4)				
2′		164.97, C	2-OBz-3,5	7.64, t (7.4)	128.94, CH
3′		123.63, C	2-OBz-4	7.52, t (7.8)	133.94, CH
4′	8.37, dd (7.9, 1.7)	138.65, CH	2-OBz-7		165.32, C

[a]Recorded in $CDCl_3$ at 600 MHz for ^1H and 150 MHz for ^{13}C NMR spectra. Chemical shifts and coupling constants (in parentheses) are given in ppm and Hz, respectively.

suggested that the furanoyl group in **5** was replaced by a benzoyl group in **9**. The planar structure and relative configuration of **9** were established using the methods described above (Figure S1).

Alkaloid **10** (tripterygiumine J) was shown to have the molecular formula $C_{34}H_{43}NO_{16}$ by ^{13}C NMR (Table S2) and HRESIMS data [m/z 744.2480 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **10** (Tables S1 and S2) with those of **9** suggested that the benzoyl group in **9** was replaced by an acetyl group in **10**, with free 5-OH and 7-OH groups. The planar structure and relative configuration of **10** were established by the methods described above (Figure S1).

Alkaloid **11** (tripterygiumine K) was shown to have the molecular formula $C_{39}H_{45}NO_{16}$ by ^{13}C NMR (Table S2) and HRESIMS data [m/z 806.2638 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **11** (Tables S1 and S2) with

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those of **9** suggested that the 11-OAc group was replaced by a benzoyl group in **11**, as well as free hydroxy groups at C-2 and C-5. The planar structure and relative configuration of **11** were established by the methods described above (Figure S1).

Alkaloid **12** (tripterygiumine L) was shown to have the molecular formula $C_{40}H_{46}N_2O_{17}$ by ^{13}C NMR (Table S2) and HRESIMS data [*m*/*z* 849.2725 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **12** (Tables S1 and S2) with those of **9** revealed that the 1-OAc and 2-OBz groups of **9** were replaced by nicotinoyl and acetyl groups, respectively, in **12**. The planar structure and relative configuration of **12** were established using the methods described above (Figure S1).

Tripterygiumines A−L (**1**−**12**) contain the same 2-(3-carboxybutan-2-yl)nicotinic acid moiety; the relative configuration of this moiety was determined from the NOESY data, *J*-based configuration analysis, and analysis of the biosynthetic pathway. Owing to the limited amounts of these new alkaloids, we performed only chemical degradation of alkaloid **4** (Supporting Information), using a reported procedure.[9] The 2-(3-carboxybutan-2-yl)nicotinic acid moiety of **4** was defined as (1′*S*,2′*S*)-evoninic acid. These results were consistent with the NMR spectroscopic data.

Compound **22** was shown to have the molecular formula $C_{39}H_{43}NO_{16}$ by ^{13}C NMR (Table 3) and HRESIMS data [*m*/*z* 804.2511 ([M + Na]$^+$)], revealing 19 indices of hydrogen deficiency. The IR, UV, and NMR spectroscopic data (Table 3) of **22** were similar to those of the alkaloids reported previously.[3] A polyoxygenated dihydroagarofuran-7-one skeleton and a 2-(3-carboxybutan-1-yl)nicotinic acid moiety were evident from the $^1H-^1H$ COSY, HSQC, and HMBC correlations (Figure 1G). The HMBC cross signals of H-3/C-11′ and H-15/C-12′ confirmed the C-3−O−C-11′ and C-15−O−C-12′ linkages between the dihydroagarofuran and the substituted nicotinic acid moieties, respectively (Figure 1G). The 1-OAc, 8-OAc, 11-OAc, and 2-OBz substituents were established from the corresponding HMBC correlations of H-1/C-$2_{1\text{-OAc}}$, H-8/C-$2_{8\text{-OAc}}$, H-11/C-$2_{11\text{-OAc}}$, and H-2/C-$7_{2\text{-OBz}}$ (Figure 1G). The presence of a free C-4 hydroxy group was determined by comparison of the ^{13}C NMR chemical shift of C-4 (δ_C 71.96) with reported chemical shifts[3,4] and confirmed by the HMBC cross signals of 4-OH/C-12 and H-12/C-3, C-4, C-10 (Figure 1G). A free C-5 hydroxy group was evident by comparison of the ^{13}C NMR chemical shift of C-5 (δ_C 75.90) with the reported values and the molecular composition of **22**.

The relative configuration of the polyoxygenated dihydroagarofuran moiety of **22** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-12/H-11, H-3 (Figure 1H). The relative configuration of the C-7′−C-10′ moiety was determined by comparing the chemical shifts and coupling constants of the H-7′/H-8′/H-9′/H-10′ spin system with those of ebenifolin W-I. The configuration of ebenifolin W-I had been previously determined by NMR spectroscopic analysis and confirmed by X-ray crystallography analysis.[10] Thus, compound **22** was named tripterygiumine M.

Alkaloid **23** (tripterygiumine N) was shown to have the molecular formula $C_{44}H_{45}NO_{19}$ by ^{13}C NMR (Table S4) and HRESIMS data [*m*/*z* 914.2503 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **23** (Tables S3 and S4) with those of **22** suggested the presence of an additional furanoyl group in **23**. The 2D NMR experiments established the planar structure of **23** (Figure S1A). The relative configuration of the polyoxygenated dihydroagarofuran moiety of **23** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-11/H-12 (Figure S1B). The relative configuration of the 2-(3-carboxybutan-1-yl)nicotinic acid moiety was established by comparing the NMR spectroscopic data of **23** with those of **22** and 7-*epi*-mekongensine, the configuration of which had been previously confirmed by X-ray crystallography analysis,[4c] and based on their similar biosynthetic origins.

Alkaloid **24** (tripterygiumine O) was shown to have the molecular formula $C_{46}H_{49}NO_{20}$ by ^{13}C NMR (Table S4) and HRESIMS data [*m*/*z* 958.2772 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **24** (Tables S3 and S4) with those of **23** suggested that the C-7 carbonyl group in **23** had been reduced and acetylated to a 7-OAc group. In addition, the 2-OBz and 9′-OFu substituents in **23** were replaced with 2-OFu and 9′-OBz groups in **24**. The planar structure and relative configuration of **24** were established using the methods described above (Figure S1).

Tripterygiumine P (**25**) was shown to have the molecular formula $C_{35}H_{41}NO_{15}$ by ^{13}C NMR (Table S4) and HRESIMS data [*m*/*z* 738.2348 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **25** (Tables S3 and S4) with those of **24** revealed that only the 5-OH and 9′-OH groups were esterified by acetyl and benzoyl groups, respectively, in **25**. The planar structure and relative configuration of **25** were established using the methods described above (Figure S1).

Compound **26**, tripterygiumine Q, was shown to have the molecular formula $C_{33}H_{39}NO_{16}$ by ^{13}C NMR (Table S4) and HRESIMS data [*m*/*z* 728.2185 ([M + Na]$^+$)]. Comparison of the NMR spectroscopic data of **26** (Tables S3 and S4) with those of **25** suggested that the benzoyl group in **25** was replaced by a furanoyl group in **26**. The planar structure and relative configuration of **26** were established using the methods described above (Figure S1).

Compound **33** was isolated as a white powder. A molecular formula of $C_{28}H_{33}NO_7$ was determined by ^{13}C NMR and HRESIMS data [*m*/*z* 496.2324 ([M + H]$^+$)] and revealed 13 indices of hydrogen deficiency. The IR spectrum showed absorption bands at 3437, 1723, and 1630 cm^{-1}, corresponding to hydroxy, carbonyl, and aromatic groups, respectively. The NMR spectroscopic data were similar to those for wilforcidine (**35**),[11] suggesting that **33** was a nitrogen-containing sesquiterpenoid ester derivative. The planar structure of **33** was determined from the $^1H-^1H$ COSY, HSQC, and HMBC correlations (Figure S1A). The relative configuration of **33** was established by the NOESY correlations of H-1/H-3 and H-5/H-7, H-11, H-12 (Figure S1B). Compound **33** was named tripterygiumine R.

By comparing the spectroscopic and physicochemical data with the reported data, the 18 known nitrogen-containing dihydroagarofuran derivatives were identified as hyponine D (**13**),[12] 2-*O*-benzoyl-2-deacetylmayteine (**14**),[4c] hexadesacetyleuomynine (**15**),[13] euojaponine A (**16**),[14] neoeuonymine (**17**),[13] hyponine C (**18**),[15] 7-(acetyloxy)-O^{11}-benzoyl-$O^{2,11}$-deacetyl-7-deoxoevonine (**19**),[16] 4-hydroxy-7-*epi*-chuchuhuanine E-V (**20**),[17] wilfornine F (**21**),[18] triptonine B (9′-*O*-(3-furoyl)wilfortrine, **27**),[19] 1-desacetylwilforgine (**28**),[4b] alatamine (**29**),[20] alatusinine (**30**),[21] wilforzine (**31**),[22] wilforjine (**32**),[23] 1β,5α,11-triacetoxy-7β-benzoyl-4α-hydroxy-8β-nicotinoyldihydroagarofuran (**34**),[24] wilforcidine (**35**),[11] and 5α-benzoyl-4α-hydroxy-1β,8α-dinicotinoyldihydroagarofuran (**36**).[24]

The immunosuppressive activities of compounds **1**−**36** were evaluated against human peripheral mononuclear cells, as

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previously described.[25] Tripterygiumine I (**9**), alatusinine (**30**), and wilforcidine (**35**) exhibited weak immunosuppressive activity and cytotoxicity (Table S5). Triptonine B (9′-*O*-(3-furoyl)wilfortrine, **27**) exhibited immunosuppressive activity with an IC_{50} value of 4.95 μM but also showed cytotoxicity with an IC_{50} value of 26.41 μM (Table S5). Tripterygiumine Q (**26**) exhibited immunosuppressive activity with an IC_{50} value of 8.67 μM, and no cytotoxicity was observed even at a dose of 100 μM, indicating that **26** is an ideal lead for the development of novel immunosuppressive drugs (Table S5). This work thus validates chemical mining as an efficient strategy for the discovery of new chemical entities with therapeutic potential.

EXPERIMENTAL SECTION

General Experimental Procedures and Plant Material. See ref 3.

Extraction and Isolation. See the Supporting Information.

*Tripterygiumine A (**1**):* white powder; $[\alpha]_D^{20}$ −20 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.23), 244 (3.59), and 262 (3.25) nm; IR (KBr) ν_{max} 3437, 1740, 1631, 1384, 1319, 1268, 1232, 1164, and 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 1030 [M + Na]$^+$; HRESIMS *m*/*z* 1030.3320 (calcd for $C_{50}H_{57}NO_{21}$+Na^+, 1030.3315), error −0.5 ppm.

*Tripterygiumine B (**2**):* white powder; $[\alpha]_D^{20}$ +6 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.27), 243 (3.59), and 262 (3.17) nm; IR (KBr) ν_{max} 3453, 1746, 1633, 1432, 1371, 1314, 1231, 1171, 1095, 1070, and 901 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 890 [M + Na]$^+$; HRESIMS *m*/*z* 890.2819 (calcd for $C_{43}H_{49}NO_{18}$+Na^+, 890.2842), error −2.6 ppm.

*Tripterygiumine C (**3**):* yellow powder; $[\alpha]_D^{20}$ +2 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.38), 244 (3.56), and 262 (3.05) nm; IR (KBr) ν_{max} 3438, 1745, 1632, 1452, 1433, 1372, 1314, 1253, 1170, 1116, 1071, 895, and 713 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 952 [M + Na]$^+$; HRESIMS *m*/*z* 952.2998 (calcd for $C_{48}H_{51}NO_{18}$+Na^+, 952.2998), error 0.0 ppm.

*Tripterygiumine D (**4**):* yellowish powder; $[\alpha]_D^{20}$ −36 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (4.25), 243 (4.19), and 262 (3.12) nm; IR (KBr) ν_{max} 3436, 1718, 1632, 1358, 1278, 1118, 1063, 895, 714, and 619 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 680 [M + Na]$^+$; HRESIMS *m*/*z* 680.2293 (calcd for $C_{33}H_{39}NO_{13}$+Na^+, 680.2314), error 3.0 ppm.

*Tripterygiumine E (**5**):* white powder; $[\alpha]_D^{20}$ +3 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (4.25), 243 (4.19), and 262 (3.12) nm; IR (KBr) ν_{max} 3436, 1748, 1633, 1497, 1456, 1433, 1373, 1307, 1253, 1230, 1160, 1116, 1079, 1060, 785, 751, and 605 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 838 [M + Na]$^+$; HRESIMS *m*/*z* 838.2553 (calcd for $C_{39}H_{45}NO_{18}$+Na^+, 838.2529), error −2.8 ppm.

*Tripterygiumine F (**6**):* white powder; $[\alpha]_D^{20}$ −4 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.31), 242 (4.12), and 262 (3.08) nm; IR (KBr) ν_{max} 3436, 1744, 1632, 1384, 1313, 1268, 1248, 1227, 1160, 1118, and 627 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 942 [M + Na]$^+$; HRESIMS *m*/*z* 942.2797 (calcd for $C_{46}H_{49}NO_{19}$+Na^+, 942.2791), error −0.7 ppm.

*Tripterygiumine G (**7**):* white powder; $[\alpha]_D^{20}$ +10 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.35), 244 (4.13), and 262 (3.02) nm; IR (KBr) ν_{max} 3437, 1744, 1631, 1384, 1305, 1254, 1228, 1117, 912, 714, and 618 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 942 [M + Na]$^+$; HRESIMS *m*/*z* 942.2783 (calcd for $C_{46}H_{49}NO_{19}$+Na^+, 942.2791), error 0.9 ppm.

*Tripterygiumine H (**8**):* white powder; $[\alpha]_D^{20}$ −24 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 242 (3.46), and 261 (2.96) nm; IR (KBr) ν_{max} 3437, 1722, 1632, 1384, 1319, 1279, 1200, 1120, 1060, 881, 786, and 577 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 618 [M + Na]$^+$; HRESIMS *m*/*z* 618.2165 (calcd for $C_{28}H_{37}NO_{13}$+Na^+, 618.2157), error −1.2 ppm.

*Tripterygiumine I (**9**):* white powder; $[\alpha]_D^{20}$ +20 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (3.91) and 246 (3.50) nm; IR (KBr) ν_{max} 3437, 1748, 1631, 1371, 1270, 1251, 1228, 1168, 1114, 1061, 713, 618, and 510 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 848 [M + Na]$^+$; HRESIMS *m*/*z* 848.2772 (calcd for $C_{41}H_{47}NO_{17}$+Na^+, 848.2736), error −4.3 ppm.

*Tripterygiumine J (**10**):* white powder; $[\alpha]_D^{20}$ −20 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (3.64) and 245 (3.51) nm; IR (KBr) ν_{max} 3422, 1748, 1434, 1371, 1235, 1171, 1116, 911, and 871 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 744 [M + Na]$^+$; HRESIMS *m*/*z* 744.2480 (calcd for $C_{34}H_{43}NO_{16}$+Na^+, 744.2474), error −0.8 ppm.

*Tripterygiumine K (**11**):* white powder; $[\alpha]_D^{20}$ +16 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.21), 243 (4.03), and 262 (3.03) nm; IR (KBr) ν_{max} 3436, 1744, 1632, 1373, 1274, 1251, 1200, 1117, 1072, and 715 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 806 [M + Na]$^+$; HRESIMS *m*/*z* 806.2638 (calcd for $C_{39}H_{45}NO_{16}$+Na^+, 806.2631), error −0.9 ppm.

*Tripterygiumine L (**12**):* white powder; $[\alpha]_D^{20}$ +9 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 201 (4.12) and 242 (3.88) nm; IR (KBr) ν_{max} 3437, 1745, 1631, 1456, 1433, 1372, 1276, 1252, 1229, 1170, 1114, 1060, 738, and 617 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2, respectively; ESIMS *m*/*z* 849 [M + Na]$^+$; HRESIMS *m*/*z* 849.2725 (calcd for $C_{40}H_{46}N_2O_{17}$+Na^+, 849.2689), error −4.2 ppm.

*Tripterygiumine M (**22**):* white powder; $[\alpha]_D^{20}$ +35 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.17) and 250 (3.50) nm; IR (KBr) ν_{max} 3436, 1739, 1632, 1451, 1384, 1264, 1241, 1153, 1131, 1092, 862, 712, and 624 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESIMS *m*/*z* 804 [M + Na]$^+$; HRESIMS *m*/*z* 804.2511 (calcd for $C_{39}H_{43}NO_{16}$+Na^+, 804.2474), error −4.6 ppm.

*Tripterygiumine N (**23**):* yellowish powder; $[\alpha]_D^{20}$ −29 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.37) and 252 (3.62) nm; IR (KBr) ν_{max} 3437, 1741, 1632, 1384, 1306, 1227, 1129, 713, and 618 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S3 and S4, respectively; ESIMS *m*/*z* 914 [M + Na]$^+$; HRESIMS *m*/*z* 914.2503 (calcd for $C_{44}H_{45}NO_{19}$+Na^+, 914.2478), error −2.7 ppm.

*Tripterygiumine O (**24**):* yellowish powder; $[\alpha]_D^{20}$ −41 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.18) and 252 (3.49) nm; IR (KBr) ν_{max} 3436, 1742, 1632, 1440, 1373, 1289, 1256, 1230, 1130, 1055, 1003, 715, and 604 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S3 and S4, respectively; ESIMS *m*/*z* 958 [M + Na]$^+$; HRESIMS *m*/*z* 958.2772 (calcd for $C_{46}H_{49}NO_{20}$+Na^+, 958.2740), error −3.4 ppm.

*Tripterygiumine P (**25**):* white powder; $[\alpha]_D^{20}$ −42 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 200 (4.28) and 244 (3.66) nm; IR (KBr) ν_{max} 3437, 1717, 1637, 1384, 1293, 1250, 1172, 1136, 1110, 1061, 987, 884, 761, 719, and 618 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S3 and S4, respectively; ESIMS *m*/*z* 738 [M + Na]$^+$; HRESIMS *m*/*z* 738.2348 (calcd for $C_{35}H_{41}NO_{15}$+Na^+, 738.2368), error 2.7 ppm.

*Tripterygiumine Q (**26**):* yellowish powder; $[\alpha]_D^{20}$ −15 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 250 (3.60) nm; IR (KBr) ν_{max} 3437, 1731, 1631, 1374, 1243, 1115, 720, and 618 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S3 and S4, respectively; ESIMS *m*/*z* 728 [M + Na]$^+$; HRESIMS *m*/*z* 728.2185 (calcd for $C_{33}H_{39}NO_{16}$+Na^+, 728.2161), error −3.3 ppm.

*Tripterygiumine R (**33**):* white powder; $[\alpha]_D^{20}$ +48 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (4.04) and 241 (3.57) nm; IR (KBr) ν_{max} 3437, 1723, 1630, 1384, 1290, 1119, and 618 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.63 (dd, *J* = 12.0, 4.2 Hz, H-1), 2.16 (m, H-2a), 1.61 (m, H-2b), 2.02 (m, H-3a), 1.76 (dt, *J* = 13.3, 3.2 Hz, H-3b), 4.50 (d, *J* = 5.5 Hz, H-5), 2.23 (brs, H-6), 2.33 (ddd, *J* = 11.1, 7.5, 3.6 Hz, H-7a), 2.16 (dd, *J* = 11.1, 3.0 Hz, H-7b), 5.09 (d, *J* = 7.3 Hz, H-8), 1.55 (s, H-14), 1.60 (s, H-15), 1.65 (s, H-12), 1.51 (s, H-11), 3.27 (s, OH-4), 5.12 (d, *J* = 5.5 Hz, OH-5), 8.64 (brs, H-1_{Nic}), 7.75 (d, *J* = 5.0 Hz, H-4_{Nic}), 7.19 (dd, *J* = 7.5, 5.0 Hz, H-5_{Nic}), 8.68 (brs, H-6_{Nic}), 7.76 (d, *J* = 7.4 Hz, H-2_{Bz}, 6_{Bz}), 7.32 (t, *J* = 7.7 Hz, H-3_{Nic}, 5_{Nic}), and 7.50 (t, *J* = 7.5 Hz, H-4_{Nic}); ^{13}C NMR ($CDCl_3$) δ 73.96 (C-1), 23.47 (C-2), 37.22 (C-3), 73.03 (C-4), 91.55 (C-10), 79.71 (C-5), 50.17 (C-6), 32.05 (C-7), 73.40 (C-8), 50.63 (C-9), 84.68 (C-13), 26.62 (C-14), 30.13 (C-15), 23.80 (C-12), 20.24 (C-11), 153.10 (C-2_{Nic}), 125.59 (C-3_{Nic}), 136.32 (C-4_{Nic}), 122.87 (C-5_{Nic}), 150.41 (C-6_{Nic}), 164.23 (C-7_{Nic}), 129.20 (C-1_{Bz}), 129.74 (C-2_{Bz}, 6_{Bz}), 128.10 (C-3_{Bz}, 5_{Bz}), 133.13 (C-

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4_{Bz}), and 165.17 (C-7_{Bz}); ESIMS m/z 496 $[M + H]^+$; HRESIMS m/z 496.2324 (calcd for $C_{28}H_{33}NO_7+H^+$, 496.2330), error 1.1 ppm.

Immunosuppressive Activity Assay. The immunosuppressive activity assays were performed according to a published procedure[25] with minor modifications. Briefly, human peripheral mononuclear cells (PBMCs) were isolated from healthy donors by density-gradient centrifugation with Lymphoprep and resuspended in complete medium. To evaluate the proliferative activity of the isolated compounds, PBMCs (10^6 cells/mL) were stimulated by plate-bound anti-CD3 (2 μg/mL) and soluble anti-CD28 (1 μg/mL) monoclonal antibodies and incubated for 96 h in the presence of varying concentrations of the isolated compound dissolved in DMSO (less than 0.025%) or vehicle alone. PBMC proliferation was analyzed by flow cytometry on an Accuri C6 system (Becton Dickinson). To evaluate the cytotoxicity of the isolated compounds, PBMCs (10^6 cells/mL) were incubated in the presence of different concentrations of compound or vehicle alone for 96 h. Cell survival was evaluated by flow cytometry on an Accuri C6 system.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Extraction and isolation, methanolysis/hydrolysis of tripterygiumine D (**4**), supplementary references, Figure S1, Tables S1−S5, and spectroscopic data, including ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, HMBC, NOESY, and HRESIMS data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(Y. Luo) Tel: +86-28-82890813. Fax: +86-28-82890288. E-mail: yinggluo@cib.ac.cn.

*(G. Zhang) Tel: +86-28-82890996. Fax: +86-28-82890288. E-mail: zhanggl@cib.ac.cn.

Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on June 25, 2014, with a structure graphic inadvertently omitted. The corrected version reposted on June 26, 2014.