JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

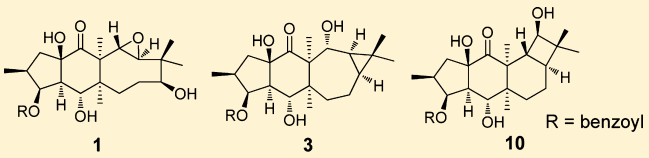
Diterpenoids with Diverse Skeletons from the Roots of *Euphorbia micractina*

Ye Tian,‡ Wendong Xu,‡ Chenggen Zhu, Sheng Lin, Ying Guo, and Jiangong Shi*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Supporting Information

ABSTRACT: Twelve new minor diterpenoids, possessing 5/6/8 (**1** and **2**), 5/6/7/3 (**3**–**9**), and 5/6/6/4 (**10**–**12**) fused-ring skeletons, as determined by spectroscopic analysis, were isolated from an ethanol extract of the roots of *Euphorbia micractina*, together with 25 known compounds. The structures of the diterpene skeletons are rare and have been found only in compounds isolated from *Euphorbia micractina* and *Euphorbia villosa*. On the basis of the octant rule for cyclohexanones, the absolute configurations of **1**–**12**, as well as of the known euphactins A–D and euphoractins A–D (**13**–**15**), could be assigned by circular dichroism spectroscopy. In addition, the co-occurring jolkinol B (**16**) was chemically transformed to euphoractin E (**17**), supporting the absolute configuration assignment and the biogenetic relationship between the different types of diterpenes. Compound **9** showed activity against HIV-1 replication in vitro, with an IC_{50} value of 8.8 ± 0.6 μM.



In mainland China, several species of the genus *Euphorbia* (Euphorbiaceae) have long been used for the treatment of various diseases such as ascites, cancer, edema, and warts.[1] Phytochemical and biological investigations have revealed that plants of this genus produce a diverse range of metabolites with interesting chemical structures and significant bioactivity. In particular, diterpenoids such as ingenanes, jatrophanes, lathyranes, myrsinols, and tiglianes, which display a variety of parent skeletons, exert a range of biological effects such as anti-inflammatory, antimicrobial, antiproliferation, cytotoxic, and modulation of multidrug resistance activities.[2] As a result, these compounds have attracted considerable interest.[3−9] Ingenol mebutate has recently been approved in the United States, countries of the European Union, Australia, and Brazil for the treatment of actinic keratosis, a precursor to a form of squamous-cell carcinoma.[10]

Euphorbia micractina Boiss. is distributed widely at high altitudes (2700–5000 m) in western mainland China, and its roots are used in Chinese folk medicine for the treatment of tumors and warts.[11] As part of a program to access the chemical diversity of Chinese traditional medicines and study their biological effects, our group has investigated *E. micractina* roots. In previous work, nine new minor triterpenoids and 17 new lathyrane diterpenoids, together with 20 known compounds, were characterized in several fractions obtained from a root EtOH extract.[12,13] Some of these compounds showed activity against HIV-1 replication and phenylephrine-induced vaso-constriction, as well as cytotoxicity against A2780 ovarian cells.[12,13] Herein, the investigation has been conducted of the remaining fractions of the same extract, leading to the isolation of 12 new minor diterpenes (**1**–**12**), along with 25 previously known compounds. Compounds **1** and **2** are found to have a 5/6/8 fused-ring skeleton, identical to that of two pairs of C-2 epimeric analogues (i.e., euphactins A–D) previously isolated from this plant.[14] Compounds **3**–**9** and compounds **10**–**12** were found to contain a 5/6/7/3 fused-ring core and a 5/6/6/4 fused-ring system, respectively. Such diterpene skeletons have been found previously in euphoractins A–E isolated from *E. micractina*[15,16] and *E. villosa* W. ex K.[17] An interesting, conserved structural feature is the C-2 configuration in compounds **1**–**12** and that of the biogenetically related lathyrane derivatives[2,13] present in the roots of *E. micractina*, which is opposite that of analogues isolated from *E. villosa*.[17] It should be noted that the same C-2 epimers (euphactins A–D and euphoractins A–E) have been characterized previously in whole plants (including roots, stems, and leaves) of *E. micractina*.[14−16,18]

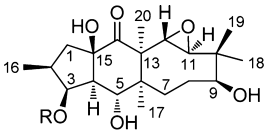
RESULTS AND DISCUSSION

Compound **1** showed IR absorptions characteristic for hydroxy group (3582, 3500, and 3377 cm^{-1}), carbonyl (1707 and 1687 cm^{-1}), and aromatic ring (1603 and 1497 cm^{-1}) functionalities. HRESIMS at m/z 495.2361 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_7Na^+$, 495.2353) indicated the molecular formula to be $C_{27}H_{36}O_7$, which was supported by the NMR data (Tables 1 and 3). The ^1H NMR spectrum of **1** displayed resonances that could be attributed to a single benzoyl moiety at δ_H 8.15 (2H, d, J = 7.0 Hz, H-2′ and H-6′), 7.61 (1H, t, J = 7.0 Hz, H-4′), and 7.50 (2H, t, J = 7.0 Hz, H-3′ and H-5′); five oxymethines at δ_H 5.76 (t, J = 5.0 Hz, H-3), 4.96 (dd, J = 11.5 and 5.0 Hz, H-5), 4.29 (d, J = 2.5 Hz, H-12), 3.47 (dd, J = 9.5 and 4.5 Hz, H-9), and 2.64 (d, J = 2.5 Hz, H-11); four tertiary methyl groups at δ_H 1.11 (H_3-18), 0.73 (6H, H_3-17 and H_3-20), and 0.55 (H_3-19); and a secondary methyl group at δ_H 1.15 (d, J = 7.5 Hz,

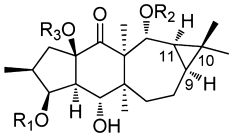
Received: January 12, 2013

ACS Publications © XXXX American Chemical Society and American Society of Pharmacognosy

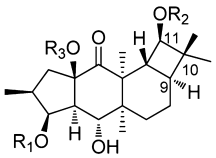
dx.doi.org/10.1021/np400029d | J. Nat. Prod. XXXX, XXX, XXX–XXX



1 R = benzoyl
2 R = cinnamoyl



3 R_1 = benzoyl, R_2 = R_3 = H
4 R_1 = cinnamoyl, R_2 = R_3 = H
5 R_1 = H, R_2 = Me, R_3 = benzoyl
6 R_1 = benzoyl, R_2 = Et, R_3 = H
7 R_1 = cinnamoyl, R_2 = Me, R_3 = H
8 R_1 = cinnamoyl, R_2 = Et, R_3 = H
9 R_1 = cinnamoyl, R_2,R_3 = isopropylidene
14 R_1 = R_3 = H, R_2 = cinnamoyl



10 R_1 = benzoyl, R_2 = R_3 = H
11 R_1 = cinnamoyl, R_2 = R_3 = H
12 R_1 = H, R_2 = Me, R_3 = cinnamoyl
13 R_1 = R_2 = H, R_3 = cinnamoyl
15 R_1 = R_2 = H, R_3 = benzoyl

H_3-16). In addition, resonances were present resulting from three exchangeable hydroxy protons at δ_H 4.82 (s, OH-15), 4.11 (d, J = 5.0 Hz, OH-5), and 3.61 (d, J = 5.0 Hz, OH-9), as well as partially overlapping resonances between δ_H 1.20 and 2.60 that could be ascribed to aliphatic methylene and methine units. Besides the resonances of the benzoyl moiety, the ^{13}C NMR and DEPT spectra showed 20 carbon resonances corresponding to the above-described functional units, as well as to five quaternary carbons (one carbonyl at δ_C 208.6 and an oxygen-bearing carbon at δ_C 86.0). Together, these spectroscopic data indicate that **1** is a diterpene alcohol benzoate, for which the structure was further deduced by 2D-NMR spectroscopic analysis.

The proton and protonated carbon resonances in the NMR spectra of **1** were assigned by HSQC experiments. In the $^1H-^1H$ COSY spectrum of **1**, the H_2-1/H-2/H-3/H-4/H-5/OH-5, H-2/H_3-16, H_2-7/H_2-8/H-9/OH-9, and H-11/H-12 cross-peaks indicated the presence of fragments with vicinal couplings. The HMBC spectrum showed two- and three-bond correlations between H_2-1/C-2, C-3, C-4, C-15, and C-16; H-3/C-1, C-15, and C-7′; H_3-16/C-1, C-2, and C-3; and OH-15/C-4 and C-14, which, in combination with the chemical shifts of these proton and carbon resonances, demonstrated the presence of a five-membered ring in **1** with a secondary methyl, a benzoyloxy functionality, and an OH group at C-2, C-3, and C-15, respectively. HMBC correlations between H-4/C-5 and C-6; H-5/C-3, C-4, C-6, and C-17; H_3-17/C-5, C-6, and C-13; H_3-20/C-6, C-13, and C-14; OH-5/C-4, C-5, and C-6; and OH-15/C-14, together with the corresponding shifts, revealed a cyclohexanone ring fused to positions C-4 and C-15 on the five-membered ring, which was substituted with an OH group at C-5 and two tertiary methyl groups at C-6 and C-13. In addition, HMBC correlations between H-9 and H-11/C-18 and C-19; H-11/C-12 and C-13; H-12/C-13 and C-20; H_3-17/C-7; H_3-18 and H_3-19/C-9, C-10, and C-11; H_3-20/C-12; and OH-9/C-9 and C-10 demonstrated the presence of an eight-membered ring that was fused to the six-membered ring at C-6 and C-13, which was substituted at C-9 by an OH group and two remaining tertiary methyl groups at C-10. The presence of an epoxy group between C-11 and C-12 was deduced from the molecular composition and the chemical shifts of H-11, H-12, C-11, and C-12. Therefore, compound **1** was determined to be an analogue of euphactins A–D. The structure of euphactin A was determined by X-ray crystallographic analysis.[14]

In the NOE difference spectrum of **1**, irradiation of H-5 enhanced the intensity of H-12 and OH-15, while H-12 was enhanced upon irradiation of H_3-19, indicating these protons to be in a cofacial position. In addition, irradiation of H-3 gave an enhancement of H-2, H-4, and OH-5; irradiation of H-4 enhanced H-2, H-3, and H_3-17; and irradiation of H-9 enhanced H-11 and H_3-18, revealing that these protons are cofacial as well. The circular dichroism (CD) spectrum of **1** displayed a positive Cotton effect at 314 nm ($\Delta\varepsilon$ = +1.54), corresponding to the n–π^* transition of the cyclohexanone chromophore. From the octant rule for cyclohexanones[19] and the aforementioned NOE enhancements, the absolute configuration of **1** could therefore be assigned. Thus, the structure of compound **1** was determined as shown. This compound has been designated as euphactin E, following the convention used for euphactins A–D.[14]

Compound **2**, $C_{29}H_{38}O_7$ (HRESIMS), exhibited similar IR and NMR spectra to **1**. Comparison of their NMR spectra demonstrated that **2** differs from **1** in that the benzoyl unit is substituted by a *trans* cinnamoyl moiety. This was confirmed by 2D-NMR, NOE difference, and CD data analysis. In the HMBC spectrum of **2**, the H-3/C-9′ correlation confirmed that the cinnamoyloxy group is located at C-3. The structure of compound **2** (euphactin F) was therefore designated as shown.

Compound **3** gave the molecular formula $C_{27}H_{36}O_6$, while the NMR data indicated it to be similar to the co-occurring euphoractin B (**14**),[15,16] possessing a 5/6/7/3 fused-ring skeleton, which is found also in a derivative isolated from *E. villosa*.[17] Comparison of the spectroscopic data indicated the replacement of the C-3 OH and C-12 *trans* cinnamoyloxy groups in **14** by a C-12 OH and a C-3 benzoyloxy group, respectively, in **3**. This was confirmed by 2D-NMR experiments. Thus, the $^1H-^1H$ gCOSY correlations between H_2-7/H_2-8/H-9/H-11/H-12 and HMBC correlations between H_3-17/C-5, C-6, C-7, and C-13; H_3-18 and H_3-19/C-9, C-10, and C-11; and H_3-20/C-6, C-12, C-13, and C-14, in combination with the observed shifts, demonstrated the presence of a 7/3 fused-ring moiety in **3** with an OH group at C-12. HMBC correlations between H-3, H-2′, and H-6′/C-7′ were used to verify the location of the benzoyl unit at C-3. NOE difference experiments showed that **3** has the same relative configuration as **14**. Specifically, irradiation of H-3 enhanced H-2 and H-4, while irradiation of H_3-17 gave enhancements of H-4 and H_3-20, and irradiation of H_3-20 in turn enhanced H-11 and H_3-17. Furthermore, H-9 and H-11 were enhanced upon irradiation of H_3-18. These data revealed that the protons enhanced are cofacial and located on the same side of the ring system. As H-12 was enhanced by irradiation of H-5 and irradiation of H_3-19 gave an enhancement of H-12, these protons were situated cofacially on opposing sides of the ring system. The CD spectra

dx.doi.org/10.1021/np400029d | J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 1. ^{1}H NMR Spectroscopic Data (δ) for Compounds 1−6 in Me_2CO-d_6[a]

position	1	2	3	4	5	6
1a	2.59 t (13.5)	2.61 t (13.5)	2.54 t (13.5)	2.56 t (13.5)	2.53 dd (15.0, 2.4)	2.57 t (13.5)
1b	1.64 dd (13.5, 3.5)	1.55 brd (13.5)	1.42 dd (13.5, 3.0)	1.40 dd (13.5, 3.0)	2.46 dd (15.0, 11.4)	1.47 dd (13.5, 2.5)
2	2.65 m	2.60 m	2.62 m	2.56 m	2.39 m	2.61 m
3	5.76 t (5.0)	5.61 t (ca. 4.0)	5.71 dd (6.0, 5.5)	5.55 dd (5.5, 4.5)	4.55 dt (6.6, 5.4)	5.71 t (6.0)
4	2.20 dd (11.5, 5.0)	2.17 dd (11.0, 4.0)	2.05 dd (11.0, 6.0)	2.02 dd (11.0, 5.5)	2.02 dd (11.4, 5.4)	2.07 dd (11.5, 6.0)
5	4.96 dd (11.5, 5.0)	4.92 dd (11.0, 4.5)	4.70 dd (11.0, 5.0)	4.64 dd (11.0, 5.0)	4.93 dd (11.4, 5.4)	4.62 dd (11.5, 5.0)
7a	1.86 ddd (15.5, 12.5, 2.0)	1.87 t (15.5)	1.96 m	2.00 m	2.07 m	1.97 dd (14.0, 7.5)
7b	1.32 m	1.32 dd (15.5, 4.0)	1.34 m	1.33 m	1.38 m	1.36 t (14.0)
8a	2.01 m	2.04 m	1.56 m	1.59 m	1.64 dt (14.4, 7.2)	1.58 m
8b	1.60 t (12.5)	1.57 t (13.5)	1.32 m	1.37 m	1.34 m	1.28 m
9	3.47 dd (9.5, 4.5)	3.49 dd (10.0, 5.5)	0.74 m	0.73 m	0.76 m	0.77 m
11	2.64 d (2.5)	2.63 d (1.5)	0.68 t (10.0)	0.69 t (10.0)	0.27 t (9.0)	0.44 t (9.5)
12	4.29 d (2.5)	4.26 d (1.5)	4.63 dd (10.0, 5.0)	4.58 dd (10.0, 5.0)	4.09 d (9.0)	4.52 d (9.5)
16	1.15 d (7.5)	1.13 d (7.5)	1.10 d (7.0)	1.11 d (7.0)	0.89 d (7.2)	1.08 d (7.0)
17	0.73 s	0.73 s	0.65 s	0.64 s	0.66 s	0.64 s
18	1.11 s	1.13 s	1.01 s	1.00 s	0.91 s	1.03 s
19	0.55 s	0.56 s	1.11 s	1.10 s	0.73 s	1.08 s
20	0.73 s	0.73 s	1.19 s	1.17 s	1.15 s	1.19 s
2′	8.15 d (7.0)	7.66 brd (7.0)	8.16 d (7.5)	7.66 brd (7.0)	8.22 d (7.2)	8.15 d (7.5)
3′	7.50 t (7.0)	7.43 brt (7.0)	7.49 t (7.5)	7.44 brt (7.0)	7.48 t (7.2)	7.49 t (7.5)
4′	7.61 t (7.0)	7.43 brt (7.0)	7.63 t (7.5)	7.44 brt (7.0)	7.63 t (7.2)	7.63 t (7.5)
5′	7.50 t (7.0	7.43 brt (7.0)	7.49 t (7.5)	7.44 brt (7.0)	7.48 t (7.2)	7.49 t (7.5)
6′	8.15 d (7.0)	7.66 brd (7.0)	8.16 d (7.5)	7.66 brd (7.0)	8.22 d (7.2)	8.15 d (7.5)
7′		7.79 d (16.5)		7.79 d (16.0)		
8′		6.71 d (16.5)		6.65 d (16.0)		
OH-3/5	/4.11 d (5.0)	/4.11 d (4.5)	/4.10 d (5.0)	/4.09 d (5.0)	3.54 d (5.4)/3.88 d (5.4)	/4.08 d (5.0)
OH-9/11	3.61 d (4.5)/	3.60 d (5.5)/				
OH-12/15	/4.82 s	/4.55 s	4.20 d (5.0)/4.65 s	3.94 d (5.0)/4.53 s		/3.77 s

[a]Data (δ) were measured in acetone-d_6 for **1**−**4** and **6** at 500 MHz; for **5** at 600 MHz. Coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. Data for OMe in **5**: δ 2.51 (3H, s); for OEt in **6**: δ 3.50 (1H, dq, J = 15.0 and 7.5 Hz), 3.22 (1H, dq, J = 15.0 and 7.5 Hz), and 1.03 (3H, t, J = 7.5 Hz).

of **3** and **14** showed positive Cotton effects at 308 nm ($\Delta\varepsilon$ = +11.47) and 313 nm ($\Delta\varepsilon$ = +9.38), respectively, corresponding to the n−π* transition of the cyclohexanone chromophore. On the basis of the octant rule for cyclohexanones,[19] the absolute configuration of **3** (euphoractin F) and **14** could be assigned as shown.

Comparison of the spectroscopic parameters of compound **4** with those of **3** (Tables 1 and 3; see also the Experimental Section) demonstrated the substitution of the benzoyl group of **3** by a *trans* cinnamoyl group in **4**. The structure of compound **4** (euphoractin G) was supported by 2D-NMR, NOE difference, and CD spectra.

The spectroscopic data of compound **5** (Tables 1 and 3; see also the Experimental Section) indicated it to be an analogue of **3** with the molecular formula $C_{28}H_{38}O_6$. Comparison of their NMR spectra revealed that a hydroxy group in **3** was replaced with a methoxy group (δ_H 2.51 and δ_C 54.3) in **5**. In addition, as compared to those of **3**, the NMR resonances of H-2, H-3, H-11, H-12, and H_3-19 and C-1, C-3, C-11, and C-14 in **5** were shielded by $\Delta\delta_H$ −0.23, −1.16, −0.41, −0.54, and −0.38 and $\Delta\delta_C$ −5.6, −4.2, −2.8, and −5.0 ppm, respectively, whereas the resonances of H-1b, H-5, C-12, and C-15 were deshielded by $\Delta\delta_H$ +1.04 and +0.23 and $\Delta\delta_C$ +6.6 and +7.1 ppm, respectively. Together with the coupling patterns of the two hydroxy protons, these shifts suggested that the OCH_3 and benzoyloxy groups are located at C-12 and C-15, respectively, while the two hydroxy groups are located at C-3 and C-5. This assumption was confirmed by 2D-NMR experiments. In particular, HMBC correlations between OH-3/C-3 and C-4, OH-5/C-4 and C-5, and OCH_3/C-12 were used to assign the location of the substituents. NOE difference and CD data confirmed that **5** displays the same configuration as **3**. The structure of compound **5** (euphoractin H) was therefore determined as shown.

The spectroscopic characterization of compound **6** ($C_{29}H_{40}O_6$) indicated it to be a further analogue of **3**, with the NMR data indicating the presence of an OEt group, which was attached to a chiral carbon [δ_H 3.50 (1H, dq, J = 15.0 and 7.5 Hz), 3.22 (1H, dq, J = 15.0 and 7.5 Hz), and 1.03 (3H, t, J = 7.5 Hz, H_3-2″)]. In addition, the H-11, H-12, and C-11 resonances in **6** were shielded by $\Delta\delta_H$ −0.24 and −0.11 and $\Delta\delta_C$ −2.1 ppm, respectively, as compared to the same resonances in **3**, whereas the C-12 resonance was deshielded by $\Delta\delta_C$ +6.3 ppm. The H-12 resonance appeared as a single doublet in **6**, while a double doublet was present in **3**. This demonstrated that **6** is the 12-OEt analogue of **3**, which was confirmed from the 2D-NMR, NOE difference spectra, and CD data. Compound **6** (euphoractin I) was designated as shown.

The spectroscopic features of compound **7** were found to be similar to those of **4**, and the HRESIMS indicated that **7** exhibited the molecular formula $C_{30}H_{40}O_6$ and thus possesses an additional CH_2 unit. The NMR data showed the substitution of the OH-12 group of **4** by a methoxy group in **7** (euphoractin J).

Compound **8** differed from **7** in that the OCH_3 group was replaced with an OEt group, as indicated by HRESIMS and by

dx.doi.org/10.1021/np400029d | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. ^{1}H NMR Spectroscopic Data (δ) for Compounds 7−12 in Me_2CO-d_6[a]

position	7	8	9	10	11	12
1a	2.50 t (ca. 13.5)	2.62 t (13.5)	2.59 t (13.5)	2.48 dd (14.0, 11.0)	2.48 m	2.44 dd (15.0,10.8)
1b	1.42 (13.5, 2.5)	1.41 dd (13.0, 3.0)	1.65 brd (13.5)	1.50 m	1.50 m	2.20 m
2	2.50 m	2.57 m	2.57 m	2.62 m	2.50 m	2.37 m
3	5.55 t (5.0)	5.56 t (5.0)	5.51 t (5.0)	5.74 t (6.0)	5.62 t (5.0)	4.45 ddd (7.8, 6.6, 5.4)
4	1.99 (11.0, 5.0)	2.05 dd (11.0, 5.0)	1.96 dd (11.0, 5.0)	2.11 m	2.07 m	1.99 dd (11.4, 5.4)
5	4.57 dd (11.0, 4.5)	4.57 dd (11.0, 4.5)	4.73 dd (11.0, 5.0)	4.87 dd (11.0, 5.0)	4.81 dd (11.5, 5.5)	5.01 dd (12.0, 5.4)
7a	1.99 m	2.00 m	1.93 m	2.06 m	2.05 m	2.22 m
7b	1.34 m	1.39 m	1.31 m	1.18 m	1.18 m	1.17 ddd (13.8,13.2,4.2)
8a	1.59 m	1.59 m	1.67 m	1.50 m	1.52 m	1.56 ddt (12.0, 12.0, 3.0)
8b	1.28 m	1.30 m	1.44 m	1.37 m	1.38 m	1.39 ddt (12.0, 3.0, 3.0)
9	0.78 m	0.75 m	0.68 m	1.11 m	1.16 m	1.11 dt (12.0, 3.6)
11	0.38 t (9.5)	0.43 t (9.5)	0.75 t (9.5)	3.41 m	3.41 m	2.89 d (8.4)
12	4.40 d (9.5)	4.47 d (9.5)	4.58 d (9.5)	3.45 m	3.41 m	2.92 dd (18.0, 8.4)
16	1.10 d (7.0)	1.09 d (7.0)	1.08 d (7.0)	1.11 d (7.5)	1.11 d (7.0)	1.00 d (7.2)
17	0.63 s	0.64 s	0.63 s	0.71 s	0.71 s	0.68 s
18	1.05 s	1.03 s	1.19 s	1.00 s	1.00 s	1.02 s
19	1.09 s	1.08 s	1.01 s	0.94 s	0.92 s	0.83 s
20	1.16 s	1.18 s	1.21 s	1.05 s	1.05 s	1.09 s
2′	7.65 brd (7.0)	7.65 brd (7.0)	7.74 brd (7.0)	8.18 d (7.5)	7.68 brd (7.0)	7.63 brd (7.8)
3′	7.44 brt (7.0)	7.45 brt (7.0)	7.44 brt (7.0)	7.51 t (7.5)	7.46 brt (7.0)	7.41 brt (7.8)
4′	7.44 brt (7.0)	7.45 brt (7.0)	7.44 brt (7.0)	7.64 t (7.5)	7.46 brt (7.0)	7.41 brt (7.8)
5′	7.44 brt (7.0)	7.45 brt (7.0)	7.44 brt (7.0)	7.51 t (7.5)	7.46 brt (7.0)	7.41 brt (7.8)
6′	7.65 brd (7.0)	7.65 brd (7.0)	7.74 brd (7.0)	8.18 d (7.5)	7.68 brd (7.0)	7.63 brd (7.8)
7′	7.79 d (16.0)	7.79 d (16.0)	7.82 d (16.0)		7.82 d (16.5)	7.72 d (16.2)
8′	6.63 d (16.0)	6.63 d (16.0)	6.55 d (16.0)		6.60 d (16.5)	6.62 d (16.2)
OH-3/5	/4.06 d (4.5)	/4.06 d (4.5)	/4.17 d (5.0)	/4.20 d (5.0)	/4.20 d (5.5)	3.31 d (5.4)/3.88 d (5.4)
OH-9/11				/3.69 d (5.0)	/3.41 m	
OH-12/15	/3.90 s	/3.69 s		/3.90 s	/3.90 s	

[a]Data (δ) were measured in acetone-d_6 for **8**−**11** at 500 MHz and for **12** at 600 MHz. Coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. Data for OMe in **7**: δ 3.09 (3H, s); for OEt in **8**: δ 3.46 (1H, dq, J = 15.0 and 7.5 Hz), 3.20 (1H, dq, J = 15.0 and 7.5 Hz), and 1.00 (3H, t, J = 7.5 Hz); for isopropylidene unit in **9**: δ 1.46 (3H, s) and 1.29 (3H, s); and for OMe in **12**: δ 2.98 (3H, s).

2D-NMR, NOE difference, and CD experiments. Thus, the structure of compound **8** (euphoractin K) was determined as shown.

Its NMR data indicated compound **9** to possess an isopropylidene unit [δ_H 1.46 (3H, s) and 1.29 (3H, s) and δ_C 104.2, 27.0, and 29.1], in place of the OH-12 and OH-15 groups in the close analogue **4**. As compared with the chemical shifts found in **4**, the C-1, C-9, and C-12 resonances in **9** were shielded by more than $\Delta\delta_C$ −2.3 ppm, whereas the C-4 and C-15 resonances were deshielded by more than $\Delta\delta_C$ +2.5 ppm. This suggested that **9** is the 12,15-acetonide of **4**, which was verified by various 2D-NMR experiments, especially by the correlation of H-12 with the quaternary carbon of the isopropylidene unit in the HMBC spectrum of **9**. Compound **9** (euphoractin L) was determined as shown, with the configuration confirmed by NOE difference spectra and from its CD data.

The spectroscopic characterization of compound **10** (Tables 2 and 3; see also the Experimental Section) indicated it to be an isomer of the co-occurring euphoractin C (**15**),[15,16] exhibiting the same 5/6/6/4 fused-ring skeleton as that of euphoractins A (**13**) and D isolated from *E. micractina*[15,16] and that of two analogues isolated from *E. villosa*.[17] The carbon skeleton and relative configuration of euphoractin A (**13**) have been determined by X-ray crystallographic analysis.[15] Comparison of the NMR spectra of **10** and **15** demonstrated, however, that the OH-3 and C-15 benzoyloxy groups in **15** are replaced with C-3 benzoyloxy and C-15 OH groups in **10**. This conclusion was confirmed from the 2D-NMR data, which showed COSY correlations between H_2-7/H_2-8/H-9/H-12/H-11 and HMBC correlations between H_3-18 and H_3-19/C-9, C-10, and C-11; H_3-20/C-12; and OH-11/C-11. These observations, in combination with the chemical shifts, suggested that the 6/4 fused-ring moiety in **10** is substituted with a hydroxy group at C-11. On the basis of the coupling constants and CD data, the configuration of **10** was assigned as being identical to those of **13** and **15**. Thus, the CD spectra of **10**, **13**, and **15** showed positive Cotton effects at 315 ($\Delta\varepsilon$ = +14.32), 317 ($\Delta\varepsilon$ = +2.49), and 313 nm ($\Delta\varepsilon$ = +10.42), respectively, corresponding to the n−π* transition of the cyclohexanone chromophore and allowing the assignment of these compounds. Compound **10** (euphoractin M) was thus assigned as shown.

The spectroscopic data for compound **11** indicated this compound to be an analogue of **10**. Comparison of the 2D-NMR and HRESIMS data (Tables 2 and 3; see also the Experimental Section) demonstrated that **11** differed from **10** in that the benzoyl unit is replaced with a *trans* cinnamoyl unit. Compound **11** (euphoractin N) was assigned the structure shown.

Compound **12**, with a molecular formula of $C_{30}H_{40}O_6$ (HRESIMS), was found to be similar structurally to **11** and differed in that an OCH_3 group replaced the C-11 OH group in **11**. In addition, H-3 and H-11 and C-3 in **12** were shielded by $\Delta\delta_C$ −1.17 and −0.52 and $\Delta\delta_C$ −3.8 ppm, respectively,

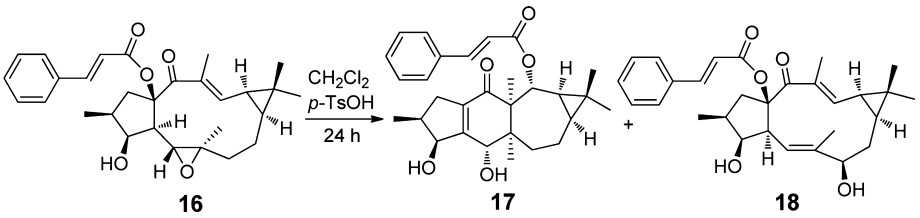
dx.doi.org/10.1021/np400029d | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{13}C NMR Spectroscopic Data (δ) of Compounds 1−12[a]

position	1	2	3	4	5	6	7	8	9	10	11	12
1	42.5	42.3	43.1	42.8	37.5	42.8	41.8	42.7	40.5	43.3	43.2	38.4
2	36.4	36.2	36.2	36.1	36.0	36.2	35.2	36.1	36.3	36.1	36.1	36.0
3	78.6	78.5	78.2	78.3	74.0	78.2	77.4	78.4	77.3	78.3	78.5	74.7
4	54.9	54.6	56.2	56.1	57.0	56.1	55.2	56.1	58.6	55.6	55.6	56.7
5	64.5	64.3	63.7	63.6	63.6	63.8	62.9	63.8	63.5	64.1	64.1	63.4
6	50.1	50.0	47.2	47.2	47.5	47.2	46.3	47.2	47.8	47.6	47.6	48.3
7	32.6	32.4	34.5	34.6	34.7	34.3	33.5	34.4	33.8	32.7	32.9	33.3
8	30.2	29.2	19.8	19.9	19.6	19.8	19.0	19.9	20.6	23.4	23.6	22.3
9	80.4	80.2	27.7	27.6	27.1	26.7	25.6	26.8	25.4	38.5	38.7	39.7
10	39.1	39.0	20.8	20.7	20.9	20.9	20.0	21.0	21.7	43.7	43.7	42.5
11	59.8	59.5	30.5	30.5	27.7	28.4	27.2	28.5	29.2	74.4	74.6	83.9
12	57.4	57.1	70.9	70.7	77.5	77.2	77.3	77.2	68.1	49.2	49.6	48.0
13	57.1	57.1	63.8	64.0	64.5	63.4	62.4	63.4	64.2	54.9	55.0	56.7
14	208.6	207.9	208.4	208.0	203.4	207.6	206.7	207.0	206.9	209.8	209.2	205.1
15	86.0	86.1	86.5	86.7	93.6	86.9	86.1	87.3	90.5	86.0	86.4	93.3
16	16.8	16.4	16.9	16.7	16.5	16.9	16.0	16.7	16.7	16.9	16.8	16.6
17	19.1	18.9	19.2	19.3	20.0	19.5	18.7	19.5	18.7	18.0	18.2	18.1
18	26.1	25.9	28.8	28.8	27.7	28.2	27.3	28.2	28.8	28.1	28.2	29.1
19	12.1	12.1	15.1	15.4	16.5	16.3	15.8	16.6	17.0	15.7	16.0	15.8
20	10.6	10.5	12.5	12.6	13.1	12.8	11.9	12.8	12.8	13.4	13.6	14.0
1′	131.8	135.3	131.9	135.5	132.9	131.8	134.6	135.4	135.5	131.9	135.6	136.1
2′	130.5	128.9	130.5	129.0	130.9	130.5	128.6	129.0	128.8	130.6	129.1	128.7
3′	129.3	129.7	129.2	129.8	129.2	129.2	129.0	129.8	129.9	129.3	130.0	129.7
4′	133.8	131.1	133.7	131.2	133.5	133.7	130.3	131.2	131.2	133.8	131.1	130.7
5′	129.3	129.7	129.2	129.8	129.2	129.2	129.0	129.8	129.9	129.3	120.0	129.7
6′	130.5	128.9	130.5	129.0	130.9	130.5	128.6	129.0	128.8	130.6	129.1	128.7
7′	167.4	145.6	167.3	145.6	164.4	167.2	144.7	145.6	145.0	167.3	145.7	144.0
8′		119.2		119.4			118.5	119.3	119.5		119.5	121.7
9′		167.8		167.9			167.0	167.8	166.8		166.8	165.0

[a]Data (δ) were measured in acetone-d_6 for **1**−**4**, **6**−**8**, and **10** at 125 MHz and for **5**, **9**, **11**, and **12** at 150 MHz. The assignments are based on DEPT, $^1H-^1H$ COSY, HSQC, and HMBC experiments. Data for OMe in **5**: δ 54.3; for OEt in both **6** and **8**: δ 62.1 and 16.0; for OMe in **7**: δ 53.6; for isopropylidene unit in **9**: δ 104.2, 29.1, and 27.0; and for OMe in **12**: δ 57.5.

Scheme 1. Chemical Transformation from 16 to 17 and 18



whereas C-11 and C-15 were deshielded by $\Delta\delta_C$ +9.3 and +6.9 ppm. This revealed that the OCH_3 and cinnamoyl units are located at C-11 and C-15 in **12**, which was confirmed by 2D-NMR data, where an HMBC correlation of OCH_3/C-11 verified the location of the OCH_3 group at C-11 (Supporting Information). Thus, the structure of compound **12** (euphoractin O) was assigned as shown.

Lathyrane diterpenes with a 5/11/3-membered ring system occur in *Euphorbia* species and are regarded as biosynthetic precursors of several polycyclic derivatives.[2] The 5/6/8, 5/6/7/3, and 5/6/6/4 fused-ring skeletons found in compounds **1**−**15** might be biosynthesized from their lathyrane analogues by transannular cyclization, followed by simultaneous or sequential opening and expansion of the cyclopropane ring.[17] A similar rearrangement was observed in the acid-catalyzed transformation of lathyrane analogues.[20−22] This prompted an examination of the acid-catalyzed transformation of jolkinol B (**16**), which was also isolated from *E. micractina*.[12] Treatment of **16** with *p*-toluenesulfonic acid in CH_2Cl_2 produced two compounds (Scheme 1), which were characterized spectroscopically as euphoractin E (**17**)[16] and 15-cinnamoyloxylathr-5*E*,12*E*-dien-3,7-diol-14-one (**18**). Interestingly, this indicated that 1,4-migration of the cinnamoyloxy group and concomitant double-bond formation occurred during the transannular cyclization of **16** to **17**. The formation of **18** from **16** is expected to result from ring-opening of the epoxide and subsequent allylic rearrangement. This supports the biogenetic relationship between lathyranes and euphactins and euphoractins isolated from *E. micractina*[12−16,18] and also confirmed the absolute configuration of **1**−**15**, as the absolute configuration of lathyrane diterpenes was previously determined by X-ray crystallographic analysis of 3,15-diacetoxy-5,6-epoxylathyr-12-en-14-one.[13]

dx.doi.org/10.1021/np400029d | J. Nat. Prod. XXXX, XXX, XXX−XXX

Compounds **5**−**9** (containing methoxy, ethoxy, and isopropylidene units) could well be artifacts produced from the present extraction and isolation procedure, which employed MeOH, EtOH, and acetone, even though it should be noted that *Euphorbia* diterpenes with the same or similar units have been reported.[2,17] Since etherification and acetonation did not occur when the corresponding diterpene alcohols (**3**, **4**, and **13**) were refluxed for 48 h in these solvents (with or without silica gel, which was the absorbent used in the isolation procedure), it was concluded that compounds **5**−**9** are true natural products.

The known compounds were identified by comparison of spectroscopic data with data reported in the literature. These compounds were euphoractins A−C (**13**−**15**),[15,16] helioscopinolide D,[23] stigamast-5-ene-3β,7α-diol, stigamast-5-ene-3β,7β-diol, stigmast-4-en-6β-ol-3-one, stigmast-3,6-dione,[24] stigmast-5-en-3β-ol-7-one,[24,25] (24*R*)-stigmast-1,4-dien-3-one,[26] loliolide,[27] (+)-dehydrovomifoliol,[28] 5α,6α-epoxy-3β-hydroxymegastigm-7-en-9-one,[29] 6-hydroxy-5,7-dimethoxycoumarin,[30] esculetin,[31] quercetin,[32] *m*-hydroxyphenylethyl alcohol, (*E*)-4-hydroxy-3-methoxycinnamic acid,[33] 3,4-dihydroxybenzoic acid,[34] vanillic acid,[35] *p*-hydroxybenzoic acid,[36] ethyl gallate,[37] methyl gallate,[38] ethyl 3,4-dihydroxybenzoate,[34] and 3,3′,4′-tri-*O*-methylellagic acid.[39]

In the in vitro bioassays performed in this study, compound **9** showed activity against HIV-1 replication[40] with an IC_{50} value 8.8 ± 0.6 μM (the positive control efavirenz gave an IC_{50} value of 0.06 ± 0.02 μM). All other compounds isolated in this experiment were inactive at concentrations of 10 μM. Although the compounds were also assessed for cytotoxicity against several human cancer cell lines[41] and inhibitory activity against protein tyrosine phosphatase 1B (PTP1B),[42] they were found to be inactive at a concentration of 10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 343 polarimeter. UV spectra were measured on a Cary 300 spectrometer. CD spectra were recorded on a JASCO-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR instrument. 1D- and 2D-NMR spectra were recorded at 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C on an INOVA 500 MHz or SYS 600 MHz spectrometer, respectively, in acetone-d_6. Solvent peaks were used as a reference. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical, Inc. Qingdao, People's Republic of China) or Sephadex LH-20 (Amersham Biosciences, Inc.). Preparative thin-layer chromatography was performed using high-performance silica gel preparative TLC plates ($HSGF_{254}$, glass precoated, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, People's Republic of China). High-performance liquid chromatography (HPLC) was performed on a Waters 600, using a Waters 2487 dual λ absorbance detector and a Prevail (250 × 10 mm i.d.) C_{18} column (5 μm). TLC was carried out on precoated glass silica gel GF_{254} plates. Spots were directly visualized under UV light or by spraying with 5% H_2SO_4 in 95% EtOH, followed by heating. Unless otherwise noted, all chemicals were commercially available and used without further purification.

Plant Material. See ref 12.

Extraction and Isolation. For extraction and preliminary fractionation of the EtOH extract of *E. micractina*, see refs 12 and 13. Fraction A4 (6.5 g) was separated by CC over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), to give fraction A4-1−A4-3. Fraction A4-2 (1.6 g) was separated over silica gel, eluting with 1:24 acetone in petroleum ether, to afford fractions A4-2-1−A4-2-4. Fraction A4-2-4 (62 mg) was separated by reversed-phase (RP) semipreparative HPLC, using MeOH−H_2O (70:30) as the mobile phase, to yield **6** (1.7 mg) and **8** (1.5 mg). Fraction A8 (1.5 g) was subjected to flash chromatography over RP silica gel, eluting with a gradient of EtOH (0 → 95%) in H_2O, to afford fraction A8-1−A8-8. Fraction A8-4 (173 mg) was subjected to preparative TLC (30% acetone in petroleum ether) to give a mixture (54 mg), which was further separated by RP HPLC, using MeOH−H_2O (75:25) as the mobile phase, to obtain **7** (2.2 mg), **9** (1.1 mg), and **11** (1.5 mg). Fraction A9-6 (0.5 g) was fractionated by flash chromatography over RP silica gel, eluting with a gradient of EtOH (0 → 95%) in H_2O, to afford fractions A9-6-1−A9-6-6. Fraction A9-6-3 (100 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), and then isolated by RP HPLC, using MeOH−H_2O (80:20) as the mobile phase, to yield **3** (1.2 mg) and **10** (1.1 mg). Fraction A10-6 (1.2 g) was fractionated by flash chromatography over RP silica gel, eluting with a gradient of EtOH (0 → 95%) in H_2O, to afford fraction A10-6-1−A10-6-5. Fraction A10-6-3 (109 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), and then purified by RP HPLC, using MeOH−H_2O (85:15) as the mobile phase, to yield **4** (8.5 mg). Fraction A13-5-6 (360 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), followed by separation by RP HPLC, using MeOH−H_2O (70:30) as a mobile phase, to yield **5** (1.8 mg), **12** (4.2 mg), **13** (7.8 mg), **14** (5.2 mg), and **15** (11.5 mg).

Fraction A14 (10.5 g) was separated by normal-phase silica gel CC, eluting with a gradient of acetone (10 → 70%) in petroleum ether, to give fractions A14-1−A14-8. Subsequent fractionation of fraction A14-5 (1.1 g) by flash chromatography over RP silica gel, eluting with a gradient of EtOH (0 → 95%) in H_2O, afforded fractions A14-5-1−A14-5-5. Fraction A14-5-4 (107 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), and then purified by RP HPLC, using MeOH−H_2O (60:40) as the mobile phase, to yield **1** (2.5 mg) and **2** (2.0 mg).

*Euphactin E (**1**):* white, amorphous powder; $[\alpha]^{20}_D$ −10.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.99), 273 (1.43) nm; CD (MeOH) 314 ($\Delta\varepsilon$ +1.54) nm; IR ν_{max} 3377, 2971, 2882, 1707, 1687, 1603, 1497, 1452, 1387, 1288, 1136, 1051, 1014, 964, 918, 713 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; ESIMS *m*/*z* 495 [M + Na]$^+$; HRESIMS *m*/*z* 495.2361 [M + Na]$^+$ (calcd for 495.2353).

*Euphactin F (**2**):* white, amorphous powder; $[\alpha]^{20}_D$ +14.6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.03), 277 (4.15) nm; CD (MeOH) 230 ($\Delta\varepsilon$ −0.44), 267 ($\Delta\varepsilon$ +1.24), 304 ($\Delta\varepsilon$ +2.48) nm; IR ν_{max} 3457, 2969, 2876, 1708, 1635, 1578, 1497, 1451, 1384, 1313, 1283, 1205, 1138, 1057, 914, 769 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; ESIMS *m*/*z* 521 [M + Na]$^+$; HRESIMS *m*/*z* 521.2528 [M + Na]$^+$ (calcd for 521.2510).

*Euphoractin F (**3**):* white, amorphous powder; $[\alpha]^{20}_D$ +15.6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.20), 266 (1.33) nm; CD (MeOH) 199 ($\Delta\varepsilon$ +3.41), 213 ($\Delta\varepsilon$ −9.49), 233 ($\Delta\varepsilon$ +1.22), 250 ($\Delta\varepsilon$ −0.32), 308 ($\Delta\varepsilon$ +11.47) nm; IR ν_{max} 3464, 2925, 2872, 1698, 1601, 1453, 1379, 1280, 1122, 1026, 999, 712 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; (+)-ESIMS *m*/*z* 479 [M + Na]$^+$, 935 [2M + Na]$^+$; HRESIMS *m*/*z* 479.2435 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_6Na$, 479.2410).

*Euphoractin G (**4**):* white, amorphous powder; $[\alpha]^{20}_D$ +38.4 (*c* 0.85, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.96), 277 (4.17) nm; CD (MeOH) 196 ($\Delta\varepsilon$ −5.06), 305 ($\Delta\varepsilon$ +6.23) nm; IR ν_{max} 3474, 2937, 2872, 1687, 1633, 1578, 1496, 1451, 1380, 1279, 1202, 1133, 1055, 998, 768 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; (+)-ESIMS *m*/*z* 505 [M + Na]$^+$, 987 [2M + Na]$^+$; HRESIMS *m*/*z* 505.2579 [M + Na]$^+$ (calcd for $C_{29}H_{38}O_6Na$, 505.2566).

*Euphoractin H (**5**):* white, amorphous powder; $[\alpha]^{20}_D$ −0.3 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.10), 273 (1.66) nm; CD (MeOH) 206 ($\Delta\varepsilon$ −2.21), 232 ($\Delta\varepsilon$ +0.82), 253 ($\Delta\varepsilon$ −0.26), 299 ($\Delta\varepsilon$

dx.doi.org/10.1021/np400029d | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

+0.48) nm; IR ν_{max} 3528, 2929, 2831, 1716, 1602, 1453, 1378, 1283, 1115, 1081, 1037, 995, 948, 903, 713 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; (+)-ESIMS m/z 493 $[M + Na]^+$, 509 $[M + K]^+$, 963 $[2M + Na]^+$; HRESIMS m/z 493.2590 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_6Na$, 493.2561).

*Euphoractin I (**6**):* white, amorphous powder; $[\alpha]^{20}_D$ +6.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.20), 275 (1.53) nm; CD (MeOH) 212 ($\Delta\varepsilon$ −1.69), 305 ($\Delta\varepsilon$ +2.71) nm; IR ν_{max} 3510, 2974, 2946, 2868, 1714, 1701, 1603, 1584, 1490, 1454, 1379, 1285, 1226, 1182, 1137, 1089, 1071, 1988, 708 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; ESIMS m/z 507 $[M + Na]^+$; HRESIMS m/z 507.2736 $[M + Na]^+$ (calcd for 507.2717).

*Euphoractin J (**7**):* colorless oil; $[\alpha]^{20}_D$ +41.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.96), 277 (4.20 nm); CD (MeOH) 203 ($\Delta\varepsilon$ −3.19), 304 ($\Delta\varepsilon$ +4.45) nm; IR ν_{max} 3474, 2936, 2873, 1711, 1635, 1578, 1497, 1451, 1379, 1313, 1282, 1205, 1136, 1109, 1084, 989, 769 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; ESIMS m/z 519 $[M + Na]^+$; HRESIMS m/z 519.2729 $[M + Na]^+$ (calcd for 519.2717).

*Euphoractin K (**8**):* white, amorphous powder; $[\alpha]^{20}_D$ −4.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.90), 278 (3.98) nm; CD (MeOH) 209 ($\Delta\varepsilon$ −3.38), 303 ($\Delta\varepsilon$ +4.89) nm; IR ν_{max} 3474, 2973, 2931, 2869, 1713, 1691, 1635, 1578, 1497, 1450, 1379, 1312, 1282, 1204, 1187, 1140, 1074, 1058, 1001, 768 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 3; ESIMS m/z 533 $[M + Na]^+$; HRESIMS m/z 533.2886 $[M + Na]^+$ (calcd for 533.2874).

*Euphoractin L (**9**):* white, amorphous powder; $[\alpha]^{20}_D$ +10.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.09), 277 (4.19) nm; CD (MeOH) 222 ($\Delta\varepsilon$ +0.80), 261 ($\Delta\varepsilon$ −0.78), 301 ($\Delta\varepsilon$ +4.70) nm; IR ν_{max} 3526, 2948, 2871, 1710, 1631, 1497, 1451, 1380, 1278, 1248, 1221, 1147, 1056, 1007, 989, 770 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; ESIMS m/z 545 $[M + Na]^+$; HRESIMS m/z 545.2881 $[M + Na]^+$ (calcd for 545.2874).

*Euphoractin M (**10**):* white, amorphous powder; $[\alpha]^{20}_D$ +7.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.14), 269 (1.33) nm; CD (MeOH) 193 ($\Delta\varepsilon$ +2.02), 214 ($\Delta\varepsilon$ −4.61), 232 ($\Delta\varepsilon$ +1.27), 315 ($\Delta\varepsilon$ +14.32) nm; IR ν_{max} 3402, 2927, 2863, 1693, 1600, 1453, 1379, 1282, 1125, 1045, 969, 712 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; (+)-ESIMS m/z 479 $[M + Na]^+$, 935 $[2M + Na]^+$; HRESIMS m/z 479.2396 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_6Na$, 479.2410).

*Euphoractin N (**11**):* white, amorphous powder; $[\alpha]^{20}_D$ +2.4 (*c* 0.11, MeOH); ^1H NMR (acetone-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; ESIMS m/z 505 $[M + Na]^+$; HRESIMS m/z 505.2567 $[M + Na]^+$ (calcd for 505.2561).

*Euphoractin O (**12**):* white, amorphous powder; $[\alpha]^{20}_D$ −10.1 (*c* 0.18,MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.91), 278 (4.10) nm; CD (MeOH) 237 ($\Delta\varepsilon$ +1.91), 257 ($\Delta\varepsilon$ +1.22), 282 ($\Delta\varepsilon$ +3.86), 308 ($\Delta\varepsilon$ −1.89), 331 ($\Delta\varepsilon$ +0.45) nm; IR ν_{max} 3449, 2930, 2871, 1708, 1641, 1578, 1497, 1451, 1381, 1339, 1204, 1186, 1135, 1094, 1067, 1035, 988, 765 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) data, see Table 2; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 3; (+)-ESIMS m/z 497 $[M + H]^+$, 519 $[M + Na]^+$, 535 $[M + K]^+$; HRESIMS m/z 519.2733 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_6Na$, 519.2723).

Chemical Transformation of Jolkinol B (16). Jolkinol B (**16**, 5.2 mg) was dissolved in CH_2Cl_2 (1.5 mL), and *p*-toluenesulfonic acid (5.0 mg) was added, after which the solution was stirred at room temperature for 24 h. The reaction solution was partitioned by addition of aqueous $NaHCO_3$ (1 M, 3 mL) and CH_2Cl_2 (2 × 3 mL). The CH_2Cl_2 phase was evaporated to a volume of 0.2 mL and separated by preparative TLC using petroleum ether−EtOAc (3:1) to yield **17** (1.2 mg) and **18** (1.6 mg). The spectroscopic data of **17** were identical to those of euphoractin E.[16] Compound **18** was identified as 15β-cinnamoyloxylathr-5*E*,12*E*-diene-3β,7β-diol-14-one from the following data: white, amorphous solid; CD (MeOH) 220 ($\Delta\varepsilon$ +0.02), 267 ($\Delta\varepsilon$ −0.07) nm; ^1H NMR (acetone-d_6, 500 MHz) δ 3.40 (1H, dd, *J* = 13.0 and 7.5 Hz, H-1a), 1.62 (1H, dd, *J* = 13.0 and 13.0 Hz, H-1b), 1.96 (1H, m, H-2), 3.90 (1H, m, H-3), 2.43 (1H, dd, *J* = 11.0 and 3.5 Hz, H-4), 6.36 (1H, d, *J* = 11.0 Hz, H-5), 4.01 (1H, dt, *J* = 11.0 and 3.5 Hz, H-7), 2.31 (1H, dt, *J* = 14.0 and 3.5 Hz, H-8a), 1.60 (1H, m, H-8b), 1.14 (1H, m, H-9), 1.43 (1H, dd, *J* = 11.5 and 8.0 Hz, H-11), 6.77 (1H, d, *J* = 11.5 Hz, H-12), 1.05 (3H, d, *J* = 6.5 Hz, H_3-16), 1.49 (3H, s, H_3-17), 1.09 (3H, s, H_3-18), 0.83 (3H, s, H_3-19), 1.77 (3H, s, H_3-20), 7.67 (2H, m, H-2′ and H-6′), 7.43 (3H, m, H-3′, H-4′, and H-5′), 7.68 (1H, d, *J* = 16.5 Hz, H-7′), 6.58 (1H, d, *J* = 16.5 Hz, H-8′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 44.6 (C-1), 40.3 (C-2), 79.3 (C-3), 53.4 (C-4), 121.0 (C-5), 146.0 (C-6), 75.5 (C-7), 38.0 (C-8), 29.8 (C-9), 25.1 (C-10), 31.6 (C-11), 146.5 (C-12), 132.5 (C-13), 195.3 (C-14), 95.6 (C-15), 14.0 (C-16), 19.0 (C-17), 29.1 (C-18), 16.6 (C-19), 12.5 (C-20), 131.3 (C-1′), 129.1 (C-2′), 129.8 (C-3′), 131.3 (C-4′), 129.8 (C-5′), 129.1 (C-6′), 146.0 (C-7′), 119.3 (C-8′), 165.9 (C-7′); (+)-ESIMS m/z 465 $[M + H]^+$, 487 $[M + Na]^+$, 503 $[M + K]^+$, 951 $[2M + Na]^+$.

Anti-HIV Activity Assay. See ref 40.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 41.

PTP1B Inhibition Assay. See ref 42.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of IR, MS, 1D- and/or 2D-NMR, and CD spectra for compounds **1**−**12**, **17**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(J. Shi) Tel: 86-10-83154789. Fax: 86-10-63017757. E-mail: shijg@imm.ac.cn.

Author Contributions

‡Y. Tian and W. Xu contributed equally to this study.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the National Natural Sciences Foundation of China (NNSFC; grant nos. 21172266, 30825044, and 20932007), the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, grant no. IRT1007), and the National Science and Technology Project of China (no. 2012ZX09301002-002) is acknowledged.

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