Abstract: A phytochemical investigation on the roots of medicinal plant *Eurycoma longifolia* resulted in the isolation of 10 new highly oxygenated C$_{20}$ quassinoids longifolactones G-P (1–10), along with four known ones (11–14). Their chemical structures and absolute configurations were unambiguously elucidated on the basis of comprehensive spectroscopic analysis and X-ray crystallographic data. Notably, compound 1 is a rare pentacyclic C$_{20}$ quassinoid featuring a densely functionalized 2,5-dioxatricyclo[5.2.2.0$^{1}$4]$^{1}$undecane core. Compound 4 represents the first example of quassinoids containing a 14,15-epoxy functionality, and 7 features an unusual α-oriented hydroxyl group at C-14. All isolated compounds were evaluated for their anti-proliferation activities on human leukemia cells. Among the isolates, compounds 5, 12, 13, and 14 potently inhibited the in vitro proliferation of K562 and HL-60 cells with IC$_{50}$ values ranging from 2.90 to 8.20 μM.

Keywords: *Eurycoma longifolia*; Simaroubaceae; quassinoids; natural products; anti-proliferation activities

1. Introduction

Quassinoids are a class of highly oxygenated degraded triterpenoids mainly distributed in plant family Simaroubaceae [1]. Based on the number of carbon atoms involving the construction of their basic scaffolds, quassinoids are commonly categorized into six distinct groups: C$_{26}$, C$_{25}$, C$_{22}$, C$_{20}$, C$_{19}$, and C$_{18}$ types [2]. Quassinoids have been reported to display a wide range of biological activities, including antitumor, antimalarial, anti-inflammatory, antiviral, neuroprotective, and antifeedant activities [2,3]. Especially since the discovery of bruceantin, a C$_{20}$ quassinoid isolated from *Brueca antidysenteria* (Simaroubaceae) in the early 1970s that showed remarkable antileukemic activity, the antitumor activities of quassinoids have attracted extensive attention from both chemical and biological communities [4–7].

*Eurycoma longifolia* Jack (Simaroubaceae), commonly known as “Tongkat Ali”, is a flowering shrub plant that widely distributed in Southeast Asia [8]. The roots of *E. longifolia* were traditionally used by local people for the treatment of malaria, dysentery, glandular swelling, persistent fever, aches, and sexual insufficiency [8]. Besides, the antitumor activities of the crude extract of *E. longifolia* roots were reported in 2005 [9]. Previous phytochemical investigations on the roots of *E. longifolia* have afforded a wide variety of chemical components, including quassinoids, canthin-6-one alkaloids, β-carboline alkaloids,
tirucallane-type triterpenes, squalene derivatives, and biphenyl neolignans [10]. Among them, quassinoids are the most characteristic chemical constituents of this plant [11–13].

Previously, our group had reported the isolation and characterization of six novel quassinoids (longifolactones A-F) with unprecedented C\textsubscript{26} or C\textsubscript{20} scaffolds from the petroleum ether-soluble fraction of the ethanol extract of \textit{E. longifolia} roots [14]. Among them, longifolactone F is the first example of quassinoids containing an unprecedented densely functionalized 2,5-dioxatricyclo[5.2.2.0\textsubscript{4,8}]undecane ring system. In our continuing studies on searching structurally unique and biologically interesting metabolites from medicinal plants, the ethyl acetate-soluble fraction of the title plant was further investigated. As a result, longifolactones G–P (1–10), 10 new C\textsubscript{20} quassinoids, together with four known ones were isolated. Their structures and absolute configurations were unambiguously established by extensive spectroscopic data analysis and single-crystal X-ray diffraction experiment. Notably, compound 1 is the second member of the rare class of quassinoids featuring densely functionalized 2,5-dioxatricyclo[5.2.2.0\textsubscript{4,8}]undecane core. Besides, compound 4 represents the first example of quassinoids containing a 14,15-epoxy functionality, and compound 7 features an unusual 14α-OH substituent that makes 7 the second member of this rare class of quassinoids so far. Herein, we reported the isolation and structure elucidation of these new quassinoids. In addition, the in vitro anti-proliferation activities of all isolates on two human leukemia cell lines (K562 and HL-60 cells) were also described.

2. Results and Discussions

2.1. Quassinoids Isolated from \textit{E. longifolia}

The air-dried and powdered roots of \textit{E. longifolia} (10 kg) were extracted with 95% ethanol under room temperature for five times. The ethanol extract (270 g) was suspended in water and partitioned successively with petroleum ether, ethyl acetate, and \textit{n}-butanol. The ethyl acetate-soluble fraction was investigated in present study. By performing a series of chromatographic procedures on the aforementioned fraction, 10 new C\textsubscript{20} quassinoids (longifolactones G–P, 1–10), along with four known C\textsubscript{20} quassinoids, chaparrolide (11) [15], 15β-hydroxyklaineanone (12) [16], 14,15β-dihydroxyklaineanone (13) [17], and eurycomanone (14) [18], were isolated (Figure 1).

![Chemical structures of quassinoids isolated from the roots of \textit{E. longifolia}.](image)

Figure 1. Chemical structures of quassinoids isolated from the roots of \textit{E. longifolia}.

2.2. Structure Elucidation of the New Quassinoids

Compound 1 was obtained as colorless needles. Its molecular formula was determined as C\textsubscript{20}H\textsubscript{26}O\textsubscript{8} by the HR-ESI-MS ion peak at \textit{m/z} 395.1696 [\textit{M} + \textit{H}]\textsuperscript{+} (calcd for C\textsubscript{20}H\textsubscript{27}O\textsubscript{8}, 395.1700) and \textsuperscript{13}C NMR data. The UV spectrum of 1 displayed absorption maxima at
241 nm. Its IR spectrum revealed the characteristic absorptions for hydroxyl (3455 cm\(^{-1}\)) and carbonyl (1730 and 1667 cm\(^{-1}\)) functional groups. In the \(^1\)H and \(^{13}\)C NMR spectra of I, signals corresponding to two hydroxyl groups [\(\delta_H\) 7.05 (1H, s) and 6.89 (1H, d, \(J = 5.7\) Hz)], a ketone carbonyl (\(\delta_C\) 199.7), a ester carbonyl (\(\delta_C\) 171.5), a trisubstituted double bond [\(\delta_H\) 6.07 (1H, br s); \(\delta_C\) 163.3 and 125.6], a hemiketal carbon (\(\delta_C\) 111.0), an oxygenated quaternary carbon (\(\delta_C\) 82.9), four oxygenated methines [\(\delta_H\) 5.45 (1H, m), 5.13 (1H, s), 4.72 (1H, dd, \(J = 4.6, 1.8\) Hz), and 4.00 (1H, s); \(\delta_C\) 85.5, 83.5, 82.7, and 69.5], three methines, a methylene, two quaternary carbons, and four methyl groups [\(\delta_H\) 1.96 (3H, s), 1.70 (3H, s), 1.39 (3H, s), and 1.34 (3H, d, \(J = 7.2\) Hz); \(\delta_C\) 21.6, 13.0, 12.9, and 12.8] were observed, indicative of a \(C_{20}\) quassinoid skeleton for I. With the aid of 2D NMR spectroscopic data, all proton and carbon resonances of I were assigned (Tables S1 and S2).

The above NMR spectroscopic data of I were closely similar to those of longifolactone F [14], suggesting the structural similarity of these two compounds. Different from longifolactone F, the NMR signals corresponding to a methylene group (CH\(_2\)-3) and a methine group (CH-4) were replaced by resonances of a double bond [\(\delta_H\) 6.07 (1H, br s); \(\delta_C\) 163.3 and 125.6] in I. In the HMBC spectrum, key correlations between H\(_3\)-29 and C-3, H-5 and C-3, H\(_2\)-6 and C-4 were observed, suggesting the presence of an \(\alpha,\beta\)-unsaturated ketone motif in ring A of I, which was also confirmed by the characteristic chemical shift values of C-2-C-4 (\(\delta_C\) 199.7, 125.6, and 163.3). After a comprehensive interpretation of its \(^1\)H–\(^1\)H COSY and HMBC spectra, the gross structure of I was established as a \(C_{20}\) quassinoid with a rare 2,5-dioxatricyclo[5.2.2.0\(^{4,8}\)]undecane core (Figure 2).
Figure 3. Key NOESY correlations of compounds 1–10.

Figure 4. X-ray ORTEP drawings of compounds 1–10.

The molecular formula of 2 was deduced as C_{21}H_{30}O_{8} on the basis of its HR-ESI-MS data (m/z 433.1832 [M + Na]^+; calcd for C_{21}H_{30}O_{8}Na, 433.1833), and the $^{13}$C NMR data analysis. The $^1$H and $^{13}$C NMR spectroscopic data of 2 (Tables S1 and S2) highly resembled those of 6-dehydroxylongilactone [19], except for the presence of additional signals due to a hemiketal carbon (δC 102.5) and an oxygenated methyl group [δH 3.83 (3H, s); δC 52.7] in 2. Subsequently, detailed analysis of its $^1$H-$^1$H COSY and HMBC spectra allowed the establishment of a 6/6/6/5 ring system for 2 that was identical to 6-dehydroxylongilactone. Besides, the HMBC correlation between $^{1'}$-OCH$_3$ and C-16 indicated the presence of an extra methoxycarbonyl group in 2. Based on the molecular formula information, as well as the obvious down-field shift of C-15 (δC 102.5), the remaining methoxycarbonyl and hydroxyl groups were both assigned to attach to C-15, which was also confirmed by the HMBC correlation between H-14 and C-16 (Figure 2). Thus, the planar structure of 2 was established. Finally, the structure of 2 was fully resolved by an X-ray diffraction experiment.
With an excellent Flack parameter of 0.09 (8), the absolute configuration of 2 was assigned as 1S,5S,7R,8S,9R,10S,11R,12R,13S,14S,15S (Figure 4).

The molecular formula of 3 was determined to be C_{20}H_{26}O_{5} on the basis of its sodiated molecular ion peak at m/z 371.1831 [M + Na]^+ (calcd for C_{20}H_{26}O_{5}Na, 371.1829) and 13C NMR data. The 1H and 13C NMR spectral data of 3 were similar to those of the co-isolated known compound chaparrolide (11), which indicated that 3 was also a C_{20} quassinoid. Compared with those of 11, the 1H and 13C NMR spectra of 3 showed additional signals for a cis-disubstituted double bond [δH 4.31 (1H, d, J = 8.9 Hz) and 5.76 (1H, d, J = 9.6 Hz); δC 132.7 and 130.2] and an exo-olefin group [δH 5.00 (1H, s) and 4.84 (1H, s); δC 145.8 and 111.3], while the signals corresponding to a ketone carbonyl, a methylene group, a methine group, and a methyl group were absent. In the 1H–1H COSY spectrum of 3, the correlation between H-11 and H-12 indicated that the C-11 in 3 was an oxygenated-substituted methine instead of the ketone carbonyl in 11 (Figure 2). Moreover, the observed HMBC cross-peaks between H_{2}-29 and C-3/C-5, H-3 and C-5, H-3 and C-1 indicated the presence of two conjugated double bonds in ring A of 3 (Figure 2). Similarly, an X-ray diffraction experiment using Cu Kα radiation was performed, which led to the full assignment of planar structure and absolute configuration for 3 (1R,5S,7R,8S,9R,10S,11S,12R,13R,14S,15S, Figure 4).

The molecular formula of 4 was assigned as C_{20}H_{26}O_{7} based on its HR-ESI-MS data (m/z 401.1574 [M + Na]^+; calcd for C_{20}H_{26}O_{7}Na, 401.1571) and 13C NMR spectroscopic data, 18 mass units less than the co-isolated known C_{20} quassinoid, 14,15β-di-hydroxyklaineaneone (13). The NMR spectra of 4 showed characteristic signals similar to those of 13, except for the presence of one oxygen-bearing methine [δH 3.36 (1H, s); δC 52.9] and one oxygenated bearing quaternary carbon (δC 67.9). Considering the molecular formula information, the two hydroxyl groups at C-14 and C-15 in 13 were replaced by an epoxide ring in 4. Furthermore, the NOE correlation between H-15 and H-18 in the NOESY spectrum suggested that the epoxide ring had the β-orientation (Figure 3). Similar to 1–3, the structure with absolute configuration (1S,5S,7R,8S,9R,10S,11R,12R,13S,14R,15R) of 4 was definitively assigned by an X-ray diffraction experiment (Figure 4).

The molecular formula of 5 was determined as C_{20}H_{26}O_{7} by the HR-ESI-MS ion peak at m/z 401.1574 [M + Na]^+ (calcd for C_{20}H_{26}O_{7}Na, 401.1571) and 13C NMR data. The 1H and 13C NMR spectral data of 5 (Tables S1 and S2) were very similar to those of 11-dehydroklaineaneone [20]. The main differences were that the signals corresponding to a methylene group [δH 3.69 (1H, d, J = 19.4, 12.7 Hz) and 2.70 (1H, dd, J = 19.4, 6.6 Hz); δC 29.1] in the known compound were replaced by the signals due to an oxygenated methine [δH 5.42 (1H, d, J = 10.1 Hz); δC 67.3] in 5, suggesting the presence of an additional hydroxyl group at C-15 in 5. This assumption was further confirmed by the HMBC cross-peak between H-15 and C-16 (Figure 2). Subsequently, the planar structure and absolute configuration (1S,5S,7R,8S,9R,10S,11R,12R,13S,14R,15R) of 5 were completely deduced by a single-crystal X-ray diffraction experiment (Figure 4).

The HR-ESI-MS of 6 displayed a sodiated molecular ion peak at m/z 435.1626 [M + Na]^+, corresponding to a molecular formula of C_{20}H_{28}O_{9}. Comparison of the 1H and 13C NMR spectral data of 6 (Tables S1 and S3) with those of Δ^{4,5,14}-hydroxyklaurubol [21] revealed that they were closely similar, except for signals for the endo-olefin (δC 130.1 and 127.5) and a methyl [δH 1.75 (3H, s); δC 20.2] in Δ^{4,5,14}-hydroxyklaurubol were replaced by signals of an exo-olefin [δH 4.98 (1H, s) and 4.74 (1H, s); δC 147.4 and 110.1] and a methine [δH 2.74 (1H, overlapped); δC 42.5] in 6. In the HMBC spectrum, correlations between H_{2}-29 and C-3/C-5 indicated that the exo-olefin was located at C-4 (29) (Figure 2). Thus, the planar structure of 6 was established. Similarly, the relative stereostructure and absolute configuration (1S,2S,5S,7R,8R,9R,10S,11R,12R,13S,14R,15R) of 6 were established by a single-crystal X-ray diffraction experiment (Figure 4).

The molecular formula of 7 was deduced as C_{20}H_{28}O_{9} by its HR-ESI-MS data (m/z 435.1621 [M + Na]^+; calcd for C_{20}H_{28}O_{9}Na, 435.1626) and 13C NMR data. The NMR spectroscopic features of 7 were similar to those of 14-epi-13,21-dihydroeryucomanone [22], except for the presence of signals assigned to an oxygenated methine [δH 4.63 (1H, br s); δC 72.3]...
in 7, while the signal corresponding to a ketone carbonyl carbon was absent. The above data suggested that 7 was the C-2 hydroxylated derivative of 14-\(\text{e}^\text{pi}\)-13,21-dihydroeurycomanone. This assumption was confirmed by the spin system deduced from H-1 to H-3 in the \(\text{H}^1\)-\(\text{H}^\text{COSY}\) spectrum of 7 (Figure 2). Furthermore, the NOE correlation between H-2 and H3-19 in the NOESY spectrum suggested that the H-2 and H3-19 had the same orientation (Figure 3). Finally, with a Flack parameter of \(-0.12\) (9), the absolute structure of 7 (1\(S\),2\(S\),5\(S\),7\(R\),8\(R\),9\(R\),10\(S\),11\(R\),12\(R\),13\(S\),14\(S\),15\(R\)) was unambiguously established (Figure 4).

The HR-ESI-MS of compound 8 displayed a sodiated molecular ion peak at \(\text{m}/\text{z} 431.1311 [\text{M} + \text{Na}]^+\) (calcd for \(\text{C}_{20}\text{H}_{24}\text{O}_6\text{Na}, 431.1313\)), allowing the determination of a molecular formula of \(\text{C}_{20}\text{H}_{22}\text{O}_6\) that was identical to the known \(\text{C}_{20}\) quassinoid 13-\(\text{e}^\text{pi}\)-eurycomadilactone [21]. The \(\text{H}^1\) and \(\text{C}^{13}\) NMR spectral data of 8 (Tables S1 and S3) closely resembled those of 13-\(\text{e}^\text{pi}\)-eurycomadilactone, combined with its molecular formula information, suggesting that 8 was a stereoisomer of the known compound. Further analysis of the 2D NMR data of 8 confirmed that 8 had the same planar structure as 13-\(\text{e}^\text{pi}\)-eurycomadilactone. Different from 13-\(\text{e}^\text{pi}\)-eurycomadilactone, the NOESY spectrum of 8 showed the correlation between H-13 and H2-30, indicating the \(\alpha\)-orientation for the H3-18 in 8 (Figure 3). The structure with absolute configuration (1\(S\),5\(S\),7\(R\),8\(R\),9\(R\),10\(S\),11\(S\),13\(R\),14\(R\),15\(R\)) of 8 was finally determined on the basis of an X-ray crystallography study by using the anomalous dispersion of Cu K\(\alpha\) radiation (Figure 4).

Compound 9 was assigned to possess a molecular formula of \(\text{C}_{20}\text{H}_{26}\text{O}_6\) by the HR-ESI-MS ion peak at \(\text{m}/\text{z} 433.1472 [\text{M} + \text{Na}]^+\) (calcd for \(\text{C}_{20}\text{H}_{26}\text{O}_6\text{Na}, 433.1469\)) and 1D NMR spectral data analysis, which was two mass units more than that of 8. The \(\text{H}^1\) and \(\text{C}^{13}\) NMR spectra of 9 exhibited similar signals to those of 8 (Tables S1 and S3), except for the signal assigned to a ketone carbonyl (\(\delta \text{C} 197.0\), C-2 in 8) was replaced by the signals of an oxygenated methine (\(\delta \text{H} 4.55\) (1H, overlapped); \(\delta \text{C} 72.5\)) in 9. Thus, compound 9 was assumed to be a C-2 hydroxylated derivative of 8. This deduction was further verified by the spin system from H-1 to H-3 in the \(\text{H}^1\)-\(\text{H}^\text{COSY}\) spectrum of 9 (Figure 2). Furthermore, the \(\alpha\)-orientation of the 2-OH was determined on the basis of key NOE correlation between H-2 and H3-19 (Figure 3). A further crystallographic analysis led to the unambiguous establishment of the structure and absolute configuration (1\(S\),2\(S\),5\(S\),7\(R\),8\(R\),9\(R\),10\(S\),11\(S\),13\(R\),14\(R\),15\(R\)) of 9 (Figure 4).

The molecular formula of 10 was deduced to be identical to that of 9 on the basis of its HR-ESI-MS data (\(\text{m}/\text{z} 433.1470 [\text{M} + \text{Na}]^+\); calcd for \(\text{C}_{20}\text{H}_{26}\text{O}_6\text{Na}, 433.1469\)) and \(\text{C}^{13}\) NMR data. Comparison of the NMR data of 10 with those of 9 (Tables S1 and S3) indicated that 10 possessed the identical gross structure to 9. The main differences of the NMR spectral data between 10 and 9 were the obvious down-field shifts of C-5 (\(\Delta \delta +5.1\)) and C-6 (\(\Delta \delta +5.2\)) in 10, suggesting that 10 might be a C-5 epimer of 9. Further analysis of its 2D NMR spectroscopic data verified that 10 possessed the identical planar structure to 9. In the NOESY spectrum, NOE correlation between H-5 and H2-19 was observed, suggesting the \(\beta\)-orientation for H-5 in 10 (Figure 3). Similar to 1–9, the single-crystal X-ray diffraction study (Cu K\(\alpha\)) allowed the assignment of the complete stereochemistry of 10. As a result, the absolute configuration of 10 was definitively assigned to be 1\(S\),2\(S\),5\(S\),7\(R\),8\(R\),9\(R\),10\(S\),11\(S\),13\(R\),14\(R\),15\(R\)) (Figure 4).

2.3. Anti-proliferation Activities of Isolated Quassinoids

The isolated compounds were tested for their anti-proliferation activities on two human leukemia cell lines, K562 and HL-60. As shown in Table S5, compounds 5, 12, 13, and 14 exhibited potent inhibitory effects on the proliferation of both K562 and HL-60 cells with IC\(_{50}\) values ranging from 2.90 to 8.20 \(\mu\)M.

3. Materials and Methods

3.1. General Methods

Melting points were measured on an X-5 melting point instrument (Fukai, Beijing, China) without correction. Optical rotations were determined in MeOH on a P-1020 polarimeter (JASCO, Tokyo, Japan) with a 1 cm cell at room temperature. UV spectra
were acquired on a JASCO V-500 UV/vis spectrometer. IR spectra were obtained with a JASCO FT/IR-480 plus infrared spectrometer using KBr pellets. HR-ESI-MS data were collected using an Agilent 6210 TOF-MS spectrometer (Agilent Technologies, Santa Clara, CA, USA). Other experimental procedures were performed as described previously [14]. The human leukemia cell lines, HL-60 and K562, were purchased from the American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine.

3.2. Plant Material

The roots of *Eurycoma longifolia* were collected from Malacca, Malaysia, in June 2014 and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A voucher specimen (No. 20140501) was deposited in the Center for Bioactive Natural Molecules and Innovative Drugs Research, College of Pharmacy, Jinan University.

3.3. Extraction and Isolation

The air-dried and powdered roots of *E. longifolia* (10 kg) were extracted with 95% (v/v) EtOH five times at room temperature. The combined EtOH extract was concentrated under vacuum to yield a crude extract (270 g), which was suspended in water and then partitioned successively with petroleum ether, ethyl acetate, and *n*-BuOH.

The ethyl acetate-soluble fraction (95 g) was subjected to silica gel column chromatography using gradient mixture of CHCl₃-MeOH (100:0 → 0:100, v/v) as eluent to afford six subfractions (Fr.1–Fr.6). Fr.2 (30.5 g) was further separated on a silica gel column (petroleum ether-EtOAc, 100:0 → 100:0, v/v) to give six subfractions Fr.2A–Fr.2F. Fr.2B (1.2 g) was purified by a Sephadex LH-20 column (CHCl₃-MeOH, 1:1) followed by semipreparative HPLC (CH₃CN-H₂O, 35:65, v/v) to yield compounds 2 (5.0 mg) and 4 (8.0 mg). Then, Fr.2D (15.0 g) was separated over an ODS column (MeOH-H₂O, 20:80 → 100:0) to afford six subfractions (Fr.2D-1–Fr.2D-6). Fr.2D-2 (3.0 g) was subsequently purified by semipreparative HPLC (MeOH-H₂O, 30:70, v/v) to give compounds 1 (14.5 mg) and 11 (8.4 mg), and Fr.2D-4 (1.5 g) was also purified by preparative HPLC (MeOH-H₂O, 35:65, v/v) to afford compound 3 (6.0 mg). Fr.3 (5.0 g) was applied to a Sephadex LH-20 column (MeOH) and gave five subfractions Fr.3A–Fr.3E. Furthermore, Fr.3B was further subjected to preparative HPLC (MeOH-H₂O, 32:68, v/v) and gave compound 12 (45.0 mg).

Fr. 6 (20.0 g) was purified over an ODS column using MeOH-H₂O (20:80 → 100:0, v/v) as eluent to afford eight subfractions (Fr.6A–Fr.6H). Fr.6B (8.0 g) was subjected to a Sephadex LH-20 column (MeOH) to yield five subfractions (Fr.6B-1–Fr.6B-5). Fr.6B-2 (3.0 g) was purified by semipreparative HPLC (CH₃CN-H₂O, 18:82, v/v) to give compounds 5 (7.4 mg), 6 (10.0 mg), 7 (40.5 mg), and 13 (1.5 g), respectively. Fr.6B-4 (500 mg) was purified by preparative HPLC (CH₃CN-H₂O, 18:82, v/v) to give compounds 8 (25.3 mg), 9 (5.2 mg), and 10 (5.1 mg).

Fr.9 (3.5 g) was applied to a Sephadex LH-20 column (MeOH) to obtain four subfractions Fr.9A–Fr.9D. Then, Fr.9C (1.2 g) was further subjected to preparative HPLC separation (CH₃CN-H₂O, 12:88, v/v) to afford compound 14 (80 mg).

3.4. Compounds Characterization

**Longifolactone G** (1): colorless needles (MeOH); mp 290–291 °C; [α]D²⁵ +58.0 (c 0.55, MeOH); UV (CH₃CN) λmax (log ε): 241 (3.81) nm; IR (KBr) νmax 3455, 2957, 1730, 1667, 1432, 1379, 1348, 1229, 1198, 1115, 1017, 973, 878 cm⁻¹; 1H and 13C NMR spectral data, see Tables S1 and S2; HR-ESI-MS m/z 395.1696 [M + H]⁺ (calcd for C₂₀H₂₀O₈, 395.1700).

**Longifolactone H** (2): colorless needles (MeOH); mp 225–226 °C; [α]D²⁵ −6.2 (c 0.34, MeOH); UV (CH₃CN) λmax (log ε): 241 (4.01) nm; IR (KBr) νmax 3432, 2941, 1725, 1662, 1380, 1262, 1122, 998, 819, 564 cm⁻¹; 1H and 13C NMR spectral data, see Tables S1 and S2; HR-ESI-MS m/z 433.1832 [M + Na]⁺ (calcd for C₂₁H₃₀O₈Na, 433.1833).
Longifolactone I (3): colorless needles (MeOH); mp 178–179 °C; $[\alpha]^{25}_D +56.0$ (c 0.46, MeOH); UV (CH₃CN) λ_max (log ε): 230 (3.97) nm; IR (KBr) ν_max; 3468, 3346, 2953, 2904, 2577, 1727, 1496, 1411, 1316, 1226, 1128, 1057, 1015, 963, 813, 712, 638 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S2; HR-ESI-MS: m/z 371.1831 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 371.1829).

Longifolactone J (4): colorless needles (MeOH); mp 255–256 °C; $[\alpha]^{25}_D +18.2$ (c 0.29, MeOH); UV (CH₃CN) λ_max (log ε): 241 (3.75) nm; IR (KBr) ν_max; 3450, 2943, 1726, 1656, 1434, 1379, 1347, 1261, 1196, 1123, 1063, 998, 959, 589, 523 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S2; HR-ESI-MS m/z 401.1574 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 401.1571).

Longifolactone K (5): colorless needles (MeOH); mp 266–267 °C; $[\alpha]^{25}_D −7.2$ (c 0.31, MeOH); UV (CH₃CN) λ_max (log ε): 240 (4.01) nm; IR (KBr) ν_max; 3429, 2944, 1726, 1660, 1435, 1381, 1261, 1122, 1064, 997, 964, 902, 819, 698, 449 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S2; HR-ESI-MS m/z 401.1574 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 401.1571).

Longifolactone L (6): colorless needles (MeOH); mp 265–266 °C; $[\alpha]^{25}_D +22.2$ (c 0.92, MeOH); UV (CH₃CN) λ_max (log ε): 195 (3.80) nm; IR (KBr) ν_max; 3468, 3354, 2951, 2904, 2719, 1729, 1499, 1389, 1314, 1226, 1125, 1055, 964, 814 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S3; HR-ESI-MS m/z 435.1626 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 435.1626).

Longifolactone M (7): colorless needles (MeOH); mp 300–301 °C; $[\alpha]^{25}_D +10.1$ (c 0.70, MeOH); UV (CH₃CN) λ_max (log ε): 198 (3.60) nm; IR (KBr) ν_max; 3304, 2886, 1737, 1654, 1507, 1456, 1426, 1332, 1281, 1234, 1081, 993, 917 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S3; HR-ESI-MS m/z 434.1627 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 434.1607).

Longifolactone N (8): colorless needles (MeOH); mp 248–249 °C; $[\alpha]^{25}_D +44.6$ (c 0.67, MeOH); UV (CH₃CN) λ_max (log ε): 240 (3.46) nm; IR (KBr) ν_max; 3495, 3324, 1739, 1664, 1491, 1394, 1252, 1155, 1058, 1066, 973, 823 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S3; HR-ESI-MS m/z 434.1311 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 434.1313).

Longifolactone O (9): colorless needles (MeOH); mp 245–246 °C; $[\alpha]^{25}_D +4.2$ (c 3.07, MeOH); UV (CH₃CN) λ_max (log ε): 198 (3.63) nm; IR (KBr) ν_max; 3307, 2915, 1737, 1659, 1489, 1434, 1389, 1192, 1156, 1044, 974 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S3; HR-ESI-MS m/z 432.1472 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 433.1469).

Longifolactone P (10): colorless needles (MeOH); mp 265–266 °C; $[\alpha]^{25}_D −1.3$ (c 0.15, MeOH); UV (CH₃CN) λ_max (log ε): 196 (3.70) nm; IR (KBr) ν_max; 3491, 3305, 1739, 1661, 1389, 1250, 1154, 1108, 1064, 973, 835 cm⁻¹; ¹H and ¹³C NMR spectral data, see Tables S1 and S3; HR-ESI-MS m/z 433.1470 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 434.1469).

Chaparrilide (11): colorless needles (MeOH); mp 184–185 °C; $[\alpha]^{25}_D +15.6$ (c 0.11, MeOH); UV (CH₃CN) λ_max (log ε): 196 (3.94) nm; IR (KBr) ν_max; 3484, 3369, 2961, 1720, 1381, 1242, 1098, 1054, 982, 810 cm⁻¹; ¹H and ¹³C NMR spectral data, see Table S4 in Supplementary Materials; HR-ESI-MS m/z 389.1940 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 389.1935).

15β-Hydroxyklaineanone (12): colorless needles (MeOH); mp 223–224 °C; $[\alpha]^{25}_D +6.3$ (c 1.73, MeOH); UV (CH₃CN) λ_max (log ε): 242 (4.05) nm; IR (KBr) ν_max; 3460, 2950, 1733, 1671, 1436, 1378, 1259, 1124, 1068, 1000, 986, 920, 814, 697, 633 cm⁻¹; ¹H and ¹³C NMR spectral data, see Table S4 in Supplementary Materials; HR-ESI-MS m/z 402.1743 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 402.1727).

14,15β-Dihydroxyklaineanone (13): colorless needles (MeOH); mp 266–267 °C; $[\alpha]^{25}_D +54.3$ (c 0.51, MeOH); UV (CH₃CN) λ_max (log ε): 241 (4.40) nm; IR (KBr) ν_max; 3425, 2945, 1726, 1660, 1435, 1381, 1344, 1262, 1122, 1064, 998, 964, 902, 818, 698 cm⁻¹; ¹H and ¹³C NMR spectral data, see Table S4 in Supplementary Materials; HR-ESI-MS m/z 419.1674 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 419.1676).

Eurycomanone (14): colorless needles (MeOH); mp 285–286 °C; $[\alpha]^{25}_D +39.5$ (c 0.65, MeOH); UV (CH₃CN) λ_max (log ε): 241 (3.46) nm; IR (KBr) ν_max; 3402, 2981, 2880, 1736, 1676, 1622, 1504, 1435, 1312, 1231, 1121, 1056, 985, 826, 765 cm⁻¹; ¹H and ¹³C NMR spectral data, see Table S4 in Supplementary Materials; HR-ESI-MS m/z 431.1314 [M + Na]^+ (calcd for C₂₀H₂₅O₂Na, 431.1313).
3.5. X-ray Crystallographic Analyses

The crystal data of compounds 1-10 were collected using an Oxford-Diffraction SuperNova diffractometer (Agilent Technologies, Yarnton, UK) with Cu Kα radiation. The crystal structures were solved by direct methods using the SHELXS program (Sheldrick, 2019) [23], and refined by the SHELXL-2018 program (Sheldrick, 2019) [23] and full-matrix least-squares calculation. Crystal data of compounds 1-10 in standard CIF format were deposited with the Cambridge Crystallographic Data Centre (CCDC 2,105,724 for 1, CCDC 2,105,722 for 2, CCDC 2,105,723 for 3, CCDC 2,105,731 for 4, and CCDC 2,105,730 for 5, CCDC 2,105,729 for 6, CCDC 2,105,727 for 7, and CCDC 2,105,725 for 8, CCDC 2,105,726 for 9, and CCDC 2,105,728 for 10).

Crystal data for compound 1 (M = 394.41 g/mol): orthorhombic, space group P2₁2₁2₁, a = 7.17350(10) Å, b = 10.18810(10) Å, c = 24.5189(2) Å, β = 90°, V = 1791.95(3) Å³, Z = 4, T = 99.99(10) K, μ (Cu Kα) = 0.948 mm⁻¹, Dcalc = 1.462 g/cm³, 21,948 reflections measured (7.21° ≤ 2θ ≤ 147.045°), 3593 unique (Rint = 0.0301, Rsigma = 0.0140) which were used in all calculations. The final R₁ was 0.0306 (I > 2σ(I)), and wR₂ = 0.0964 (all data). Flack parameter = 0.06(4).

Crystal data for compound 2 (M = 410.45 g/mol): monoclinic, space group P2₁, a = 8.0642(2) Å, b = 10.6239(3) Å, c = 11.9568(3) Å, β = 90.421(2)°, V = 977.44(5) Å³, Z = 2, T = 100.00(10) K, μ (Cu Kα) = 0.888 mm⁻¹, Dcalc = 1.395 g/cm³, 9576 reflections measured (7.75° ≤ 2θ ≤ 146.844°), 3595 unique (Rint = 0.0302, Rsigma = 0.0220) which were used in all calculations. The final R₁ was 0.0353 (I > 2σ(I)), and wR₂ was 0.0966 (all data). Flack parameter = 0.09(8).

Crystal data for compound 3 (M = 364.44 g/mol): monoclinic, space group P2₁, a = 7.0067(2) Å, b = 13.4001(4) Å, c = 9.7581(3) Å, β = 90.421(2)°, V = 916.17(5) Å³, Z = 2, T = 293(2) K, μ (Cu Kα) = 0.795 mm⁻¹, Dcalc = 1.328 g/cm³, 8966 reflections measured (9.062° ≤ 2θ ≤ 147.176°), 3440 unique (Rint = 0.0639, Rsigma = 0.0435) which were used in all calculations. The final R₁ was 0.0695 (I > 2σ(I)), and wR₂ was 0.0908 (all data). Flack parameter = 0.09(9).

Crystal data for compound 4 (M = 378.41 g/mol): orthorhombic, space group P2₁2₁2₁, a = 9.63490(10) Å, b = 12.0407(2) Å, c = 15.6703(2) Å, β = 90°, V = 1817.93(4) Å³, Z = 4, T = 100.00(10) K, μ (Cu Kα) = 0.868 mm⁻¹, Dcalc = 1.383 g/cm³, 14,343 reflections measured (9.262° ≤ 2θ ≤ 146.826°), 3607 unique (Rint = 0.0285, Rsigma = 0.0201) which were used in all calculations. The final R₁ was 0.0305 (I > 2σ(I)), and wR₂ was 0.0746 (all data). Flack parameter = −0.05(6).

Crystal data for compound 5 (M = 457.51 g/mol): monoclinic, space group I2, a = 7.84600(10) Å, b = 12.85380(10) Å, c = 22.0124(2) Å, β = 95.8390(10)°, V = 2208.45(4) Å³, Z = 4, T = 100.00(10) K, μ (Cu Kα) = 0.828 mm⁻¹, Dcalc = 1.376 g/cm³, 20,973 reflections measured (7.976° ≤ 2θ ≤ 147.044°), 4407 unique (Rint = 0.0497, Rsigma = 0.0283) which were used in all calculations. The final R₁ was 0.0334 (I > 2σ(I)), and wR₂ was 0.0884 (all data). Flack parameter = 0.05(7).

Crystal data for compound 6 (M = 466.47 g/mol): orthorhombic, space group P2₁2₁2₁, a = 13.8037(6) Å, b = 12.1850(6) Å, c = 12.0498(5) Å, β = 90°, V = 2026.75(16) Å³, Z = 4, T = 113(20) K, μ (Cu Kα) = 1.079 mm⁻¹, Dcalc = 1.529 g/cm³, 7421 reflections measured (7.336° ≤ 2θ ≤ 147.508°), 3904 unique (Rint = 0.0401, Rsigma = 0.0517) which were used in all calculations. The final R₁ was 0.0503 (I > 2σ(I)), and wR₂ was 0.1464 (all data). Flack parameter = −0.04(9).

Crystal data for compound 7 (M = 430.44 g/mol): orthorhombic, space group P2₁2₁2₁, a = 6.94750(10) Å, b = 9.86740(10) Å, c = 28.8229(4) Å, β = 90°, V = 1975.92(4) Å³, Z = 4, T = 100.00(10) K, μ (Cu Kα) = 0.983 mm⁻¹, Dcalc = 1.447 g/cm³, 22,539 reflections measured (6.132° ≤ 2θ ≤ 147.044°), 3946 unique (Rint = 0.0695, Rsigma = 0.0368) which were used in all calculations. The final R₁ was 0.0372 (I > 2σ(I)), and wR₂ was 0.1016 (all data). Flack parameter = −0.12(9).

Crystal data for compound 8 (M = 480.45 g/mol): orthorhombic, space group P2₁2₁2₁, a = 7.02070(10) Å, b = 13.3311(2) Å, c = 22.4885(3) Å, β = 90°, V = 2104.78(5) Å³, Z = 4,
Author Contributions: W.-Q.Y. and W.T. performed the isolation, purification, structure determination and written the manuscript. X.-J.H. performed the structural identification of the compounds. Y.X. and C.-L.F. performed the extraction, isolation and structural identification of the compounds. J.-G.S. conducted the single crystal X-ray diffraction experiments. Y.-Y.L. worked in biological experiments. Y.W., Z.-L.W., and W.-C.Y. designed the whole experiments and revised the paper. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials: The following are available online, Detailed UV, IR, HR-ESI-MS, and NMR spectra of compounds 1–14, as well as crystallographic data of compounds 1-10 are available as Supplementary Materials.

Author Contributions: W.-Q.Y. and W.T. performed the isolation, purification, structure determination and written the manuscript. X.-J.H. performed the structural identification of the compounds. J.-G.S. conducted the single crystal X-ray diffraction experiments. Y.-Y.L. worked in biological experiments. Y.X. and C.-L.F. performed the extraction, isolation and structural identification of the compounds. Y.W., Z.-L.W., and W.-C.Y. designed the whole experiments and revised the paper. All authors have read and agreed to the published version of the manuscript.

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