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Total synthesis, structural revision and biological evaluation of γ -elemene-type sesquiterpenes†

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Total synthesis and absolute configuration confirmation of γ -elemene-type sesquiterpenes, which possess vast potential for biological activities, was investigated based on a convergent synthetic strategy. A key intermediate with all functional groups of this family of natural products was accessed by an intermolecular aldol reaction and then an acetylation of a known ketone (**12**) derived from commercially available verbenone. The versatile intermediate can be easily transformed into structurally different γ -elemene-type sesquiterpenes based on control of base-promoted cyclization manipulation in different solvents. The utility of this robust approach is illustrated by the first syntheses of elema-1,3,7(11),8-tetraen-8,12-lactam (**4'**) and 8 β -methoxy-isogermafurenolide (**6a**), as well as the syntheses of elem-1,3,7,8-tetraen-8,12-olide (**3**) and hydroxyisogermafurenolide (**5**) in only 6 or 7 steps. In addition, the structure of the reported 5 β H-elem-1,3,7,8-tetraen-8,12-olide (**1**) was revised as elem-1,3,7,8-tetraen-8,12-olide (**3**) by comparison of their identified datum, and the absolute configuration of elema-1,3,7(11),8-tetraen-8,12-lactam was confirmed as **4'**. Furthermore, the inhibitory effect of all synthesized natural compounds and their natural analogues on cancer cell proliferation was evaluated. Among them compounds **3**, **4** and **4'** were found to possess potent inhibitory activity against Kasumi-1 and Pfeiffer. Meanwhile, preliminary structure–activity relationships for these compounds are discussed.

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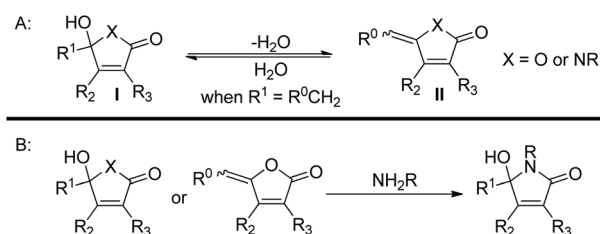
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Introduction

Heterocycles are a significantly important class of compounds, which are extensively present in various biologically active molecules.¹ Specifically, not only are the heterocyclic motifs of γ -hydroxybutenolides, γ -ylidenebutenolides, 5-hydroxy-1H-pyrrol-2(5H)-ones and 5-ylidenepyrrol-2(5H)-ones widely found as an essential unit in a vast variety of bioactive natural products and other substances of biomedical significance,^{1c,2,3} but they are also important synthons in organic synthesis⁴ as well. Structurally (Scheme 1A), the structure **I**, sometimes with different substituent groups in R¹, R² and R³, could be assigned to the fundamental skeleton of γ -hydroxybutenolides and 5-hydroxy-1H-pyrrol-2(5H)-ones, and the structure **II** for γ -ylidenebutenolides and 5-ylidenepyrrol-2(5H)-ones. Chemically, structure **II** can be regarded as a dehydrated

counterpart of structure **I** (Scheme 1A), which was generally regarded as a biogenetic process,^{5a} and 5-hydroxy-1H-pyrrol-2(5H)-ones could be obtained by ammonolysis of γ -hydroxybutenolides or γ -ylidenebutenolides in some transformations (Scheme 1B).⁵ Owing to their biological properties and unique structures, there are numerous synthetic approaches for the synthesis of these important scaffolds.^{5,6} And natural products containing these structural units have also attracted growing interest in the chemical and biological communities.^{5c,6g,7}

Elemenes are a group of natural products of closely related structure found in a variety of plants and possess various



Scheme 1 The structure of γ -hydroxybutenolides, γ -ylidenebutenolides, 5-hydroxy-1H-pyrrol-2(5H)-ones and 5-ylidenepyrrol-2(5H)-ones, and their chemical relations.

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activities. Among them, there are some γ -elemene-type sesquiterpenes bearing the abovementioned scaffolds (Fig. 1). In 2015, three elemene-type sesquiterpenes (**1**, **5** and **9**) were isolated from the essential oil of *Curcuma wenyujin* by Chen *et al.*⁸ Biological investigation for inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) shows that they exhibited weak inhibitory activities against NO production (IC_{50} 73.18 ± 3.81 , 59.97 ± 3.96 and 30.62 ± 2.63 μ M, respectively).^{8a} Originally, compound **1** was named 5 β H-elem-1,3,7,8-tetraen-8,12-olide and a structure was proposed for it as (5*R*,6*S*)-3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-5,6-dihydrobenzofuran-2(4*H*)-one (**1**, Fig. 1) by Chen and co-workers,^{8a} but the spectral data of compound **3** synthesized by us were found to be in good accordance with those of the isolated compound. So we revised the structure of this compound as **3**. Interestingly and logically, the stereochemistry of γ -elemene lactone (**2**) isolated from *Commiphora molmol* resin by Ayyad *et al.* in 2015, which displayed remarkable cytotoxic activity against NIH3T3 cells (IC_{50} 10 ± 0.02 μ M), should be structure **1** deduced by us.⁹ Compound **5** was also isolated from *Curcuma xanthorrhiza* by Fan *et al.*^{10a} in 2015 as well as from *Curcuma wenyujin* DREG by Yin *et al.* in 2016,^{10b} and showed moderate antibacterial activity in pathogenic bacteria.^{10b} Compound **9** was found in other plants as well, such as *Lindera strychnifolia* or *Neolitsea hiranensis*.¹¹ Specifically, elema-1,3,7(11),8-tetraen-8,12-lactam (**4**), the absolute configuration of which was ultimately confirmed as **4'** by us, together with hydroxyisogermafurenolide (**5**) and isogermafurenolide (**8**) were isolated from the rhizomes of *Curcuma wenyujin* in 2013, and compounds **4** and **5** showed stronger activities than compound **8** for the inhibition of LPS-induced nitric oxide production (IC_{50} 9.4 ± 1.6 , 17.5 ± 2.4 , >100 μ M, respectively).¹² 8 β H-Elema-1,3,7(11)-trien-8,12-lactam (**7**), a

γ -elemene-type sesquiterpene similar to compound **8** in structure, along with 8 β -methoxy-isogermafurenolide (**6a**) were isolated from the rhizomes of *Curcuma phaeocaulis* by Qiu *et al.* in 2015,¹³ all showing a weak inhibitory effect against LPS-induced nitric oxide production.

As early as 1988, Bohlmann had paid attention to the synthesis of various cytotoxic elemanolides found in the plant family of Compositae.¹⁴ The divinylcyclohexanone prepared from Hagemann's ester had been utilized as a key intermediate for a synthetic approach, which leads to the synthesis of compounds **5**, **8** and **9** in racemic form.¹⁴ Beyond that, there is some work focusing on the study of chemical transformation between this group natural products.^{11a,15} On the other hand, although some synthetic studies and several bioevaluations on the inhibition of LPS-induced nitric oxide production or cytotoxic activity of this group of natural products have been studied, the asymmetric total synthesis and bioevaluations on the anti-tumor effect of this group of natural products have not been reported yet. Moreover, a synthetic strategy suitable for these natural compounds from a common synthetic intermediate has not been reported yet. Herein, we describe the total synthesis and absolute configuration confirmation of **4** and its natural analogs (**3**, **5** and **6a**), as well as the *in vitro* anti-tumor evaluations of all synthesized natural products on the inhibitory effect towards the proliferation of leukemia and lymphoma cells.

Results and discussion

Our proposal for the synthesis of these γ -elemene-type sesquiterpenes is shown in Scheme 2. Retrosynthetically, compound

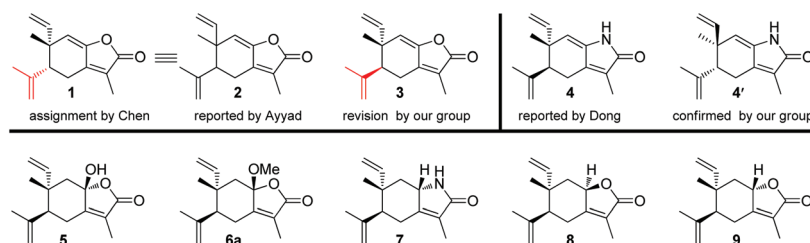
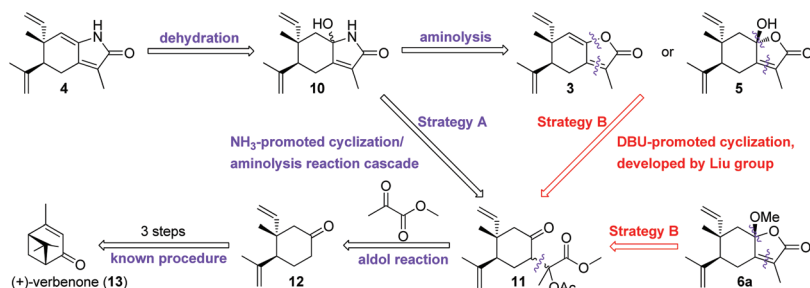


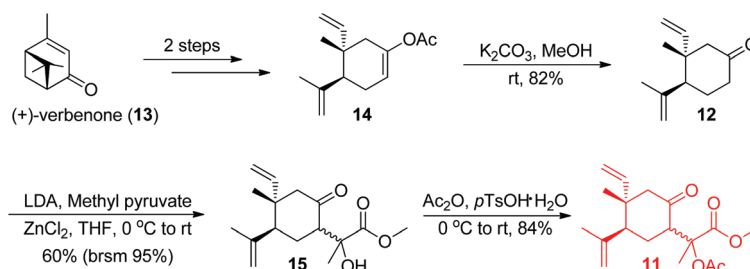
Fig. 1 Examples of γ -elemene-type sesquiterpenes.



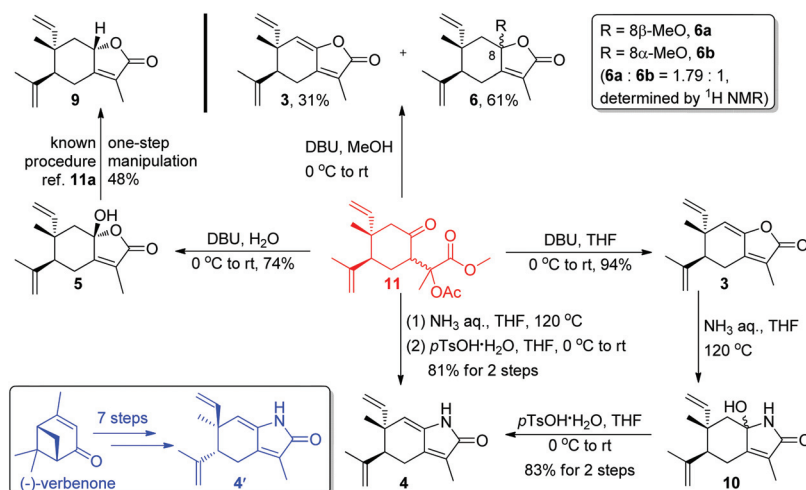
Scheme 2 Retrosynthetic analysis.

With the key intermediate **11** in hand, we firstly aimed towards the syntheses of **3** (Scheme 4) which can lead to the total synthesis of natural product **4** (strategy B, Scheme 2). The

cyclized methodology to furnish γ -alkylidenebutenolide promoted by DBU reveals a very powerful application in the total synthesis of lindenane sesquiterpenoids,^{7e,g,16} converting **11** to **3** in 94% yield. Interestingly, comparing the spectral data (¹H NMR and ¹³C NMR) of synthetic **3** with isolated natural products **1** and **2**, we found that it was in good accordance with natural product **1** reported by Chen *et al.* So the structural revision of this natural product was confirmed as **3** (see the ESI† for details), and the stereochemistry of γ -elemene lactone (**2**) should be structure **1**, whose relative configuration wasn't provided by Ayyad *et al.* originally.⁹ To achieve elema-1,3,7(11),8-tetraen-8,12-lactam (**4**), **3** was first converted into **10** through an aminolysis reaction by ammonium hydroxide in a sealed tube. Subsequently, **10** was treated with *p*TsOH-H₂O in THF, affording the natural product elema-1,3,7(11),8-tetraen-8,12-lactam (**4**) in 83% yield in 2 steps. The spectral data (¹H NMR, ¹³C NMR and HRMS) of synthetic **4** are in good agreement with those of the natural product, but the inconsistency of optical rotation exists between our synthesized sample and the natural product. The optical rotation for the natural product is positive, [α]_D²⁰ +39.0 (*c* 0.01, MeOH), whereas the optical rotation for synthetic **4** is negative, [α]_D²⁶ -72.7 (*c* 1.28, MeOH), which is similar to that of its synthetic analog **3**, [α]_D²⁶ -65.2 (*c* 2.46, CHCl₃). It is worth noting that the optical rotation of **12** is in good agreement with that of the literature (see the ESI†



Scheme 3 Construction of the key intermediate **11**.



Scheme 4 Syntheses of natural products **3**, **4'**, **5** and **6a** from **11**.

for details).¹⁷ These facts implied that the absolute configuration of the natural product **4** should be that of its enantiomer. In general, we completed the syntheses of **3** and **4** in 6 and 8 longest linear steps, respectively, from the commercially available (+)-verbenone (**13**).

Having successfully prepared **3** and **4**, our attention turned towards the synthesis of the natural analogs **5** and **6a** based on a diversity-oriented approach (Scheme 4). Compound **11** was subjected to DBU/H₂O affording a single isomer **5** in 74% yield, whose characterization data were in good accord with those reported except for the optical rotation (see the Experimental section for details). Meanwhile, the formal synthesis of isogermafurenolide (**9**) can be accomplished *via* one-step manipulation according to known chemistry.^{11a} 8β-Methoxy-isogermafurenolides **6a** and **6b** were acquired as an inseparable diastereomeric mixture in a ratio of 1.79:1 with 61% yield utilizing MeOH as a solvent, along with compound **3** in 31% yield. This inseparable diastereomixture can be further purified by preparative HPLC to give separable **6a** and **6b** (see the Experimental section for details), and their structures were confirmed by the NMR spectra of ¹H, ¹³C, NOESY, DEPT, HSQC and HMBC (see the ESI† for details). Pleasingly, the identified characterization data of **6a** were in full agreement with those reported as a racemate.¹³ Inspired and encouraged by this base-promoted cyclization methodology, we are impelled to try to synthesise 5-hydroxy-1*H*-pyrrol-2(5*H*)-one **10** with ammonium hydroxide as a promoter from intermediate **11**. Satisfactorily, **11** was subjected to the mixed solution of ammonium hydroxide (25% in H₂O) in THF with a volume ratio of 1:3 at 120 °C in a sealed tube, immediately affording compound **3**. Prolonging the reaction by 28 hours, the reaction afforded compound **10**. Dehydration of **10** gave compound **4** in 81% yield in two steps. Finally, elema-1,3,7(11),8-tetraen-8,12-lactam (**4**) can be prepared in only 7 steps from the commercially available (+)-verbenone (**13**). Meanwhile, the NH₃-promoted cyclization/aminolysis reaction

cascade, developed by us, can complement the established methodology of 5-hydroxy-1*H*-pyrrol-2(5*H*)-one construction. In consideration of the completely opposite optical rotation data between synthetic compound **4** and the natural one, we were on a journey toward the absolute configuration confirmation of this natural product. Our confirmed synthetic tour (Scheme 4, shown in blue) started with commercially available (–)-verbenone to afford compound **4'** in 7 steps by the use of exactly the same procedure as **4**, and pleasingly, the optical rotation of **4'** {[α]_D²² +54.3 (c 0.64, MeOH)} is similar to that of the natural product reported by Dong, [α]_D²⁰ +39.0 (c 0.01, MeOH).¹² Meanwhile, other characterized data of **4'** were in full agreement with those reported, securing the absolute configuration of the natural product as **4'**.

Successfully achieving these four natural products (**3**, **4'**, **5** and **6a**) and their natural analogs (**4** and **6b**), we turned our attention to evaluate their bioactivity assay. To detect whether these compounds were sufficient to inhibit cancer cell proliferation, we screened one leukemia (Kasumi-1) and two lymphoma (Pfeiffer and Karpas-422) cell lines using the CellTiter-Glo assay and EED226 was selected as a positive control.¹⁸ The IC₅₀ values (Fig. 2 and Table 1) demonstrate that compounds **3**, **4** and **4'** were potent in inhibiting cell proliferation in a

Table 1 Anti-proliferative effect of compounds in Kasumi-1, Pfeiffer and Karpas-422 cells. The unit of IC₅₀ is μM

Compounds	Kasumi-1 (IC ₅₀)	Pfeiffer	Karpas-422
3	41.35	41.99	64.49
4	21.10	24.87	69.18
4'	26.41	25.18	>100
5	>100	>100	>100
6	45.18	67.13	>100
6a	56.70	67.27	>100
6b	74.89	97.10	>100
EED226	—	0.1186	0.1155

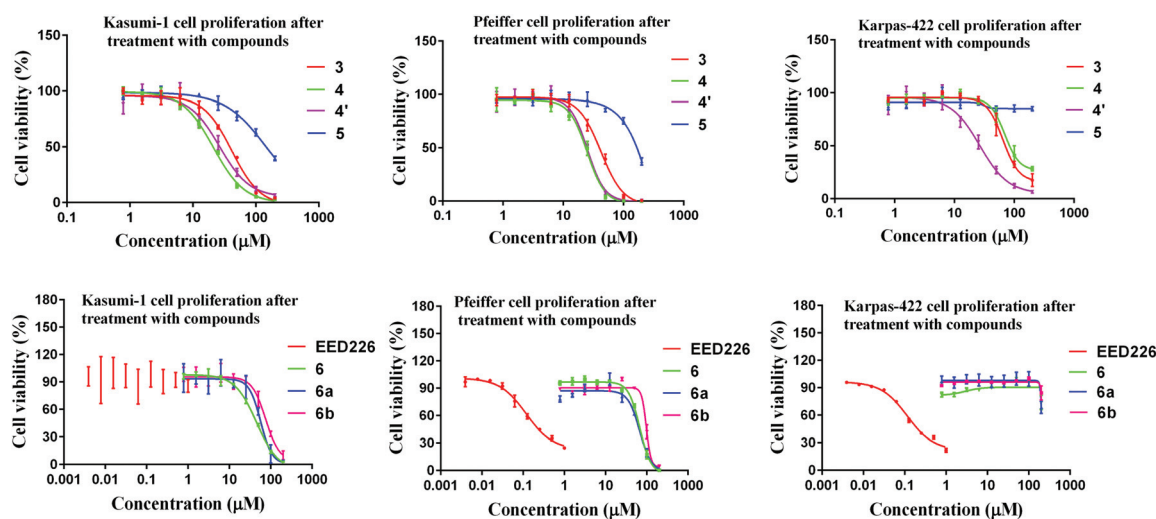


Fig. 2 Dose response curves for the synthesized compounds against three cancer cell lines with EED226 as a positive control.

dose-dependent manner and notably showed high potency toward Kasumi-1 and Pfeiffer (Table 1), whereas **4** and its enantiomer **4'** showed no significant difference. Compounds **4** and **4'** showed stronger inhibitory activity regarding cancer cell proliferation than compounds **3**, **5**, **6a** and **6b**, suggesting that the inhibitory activities of **4** and **4'** might benefit from the nitrogen atom. It is worth noting that the double bond of C8=C9 might also be a vital factor for activity against cancer cell proliferation according to the significant difference of effects between compounds **3**, **5**, **6a** and **6b**.

Conclusion

In summary, we have completed the total synthesis and absolute configuration confirmation of four γ -elemene-type sesquiterpenes in 6 or 7 steps from commercially available compounds (+)-verbenone or (–)-verbenone. The good match of the characterization data between synthetic **3** and the reported natural product **1** prompted us to revise its molecular structure as **3**, which also confirmed the relative configuration of natural product **2** as **1**. The mismatch of the optical rotation data between synthetic **4** and the natural product prompted us to revise its absolute configuration as **4'** and accomplish the total synthesis of **4'** in 7 steps, which confirmed the absolute configuration of this natural product. This convergent synthesis outlines the preparation of the key intermediate **11**, with further efficient and rapid cyclization to yield the natural products. It is anticipated that the described general synthesis strategy can be useful to prepare related classes of compounds. Inhibitory activity on cancer cell proliferation of these synthetic natural products and their natural analogues was also evaluated, and among them compounds **3**, **4** and **4'** were found to possess potent inhibitory activity against Kasumi-1 and Pfeiffer.

Experimental details

Materials and methods

All reactions were performed in flame-dried round-bottom flasks fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred *via* a syringe or cannula. Diisopropylamine (DIPA) was distilled from calcium hydride under argon; tetrahydrofuran (THF) was distilled from sodium-benzophenone under argon; and all other reagents were used directly from the supplier without further purification unless otherwise noted. Flash chromatography was performed using silica gel (200–300 mesh). Reactions were monitored using thin-layer chromatography (TLC). Visualization was achieved under a UV lamp (254 nm and 365 nm) and by developing the plates with *p*-anisaldehyde. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance III-HD 600 MHz NMR spectrometer with TMS as the internal standard and were calibrated using residual undeuterated solvent as an

internal reference (CDCl_3 : ^1H NMR = 7.26, ^{13}C NMR = 77.16; CD_3OD : ^1H NMR = 3.31 and 4.87, ^{13}C NMR = 49.00). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. Coupling constants (*J*) are reported in hertz (Hz). Optical rotations were measured in the sodium D line with a 100 mm path length cell, and are reported as follows: $[\alpha]_D^{25}$, concentration (g per 100 mL), and solvent. High-resolution mass spectra (HRMS) were recorded on a FTMS-7 spectrometer and are reported as *m/z* (relative intensity). Accurate masses are reported for the molecular ion $[\text{M} + \text{Na}]^+$, $[\text{M} + \text{H}]^+$, or $[\text{M} - \text{H}]^-$. Infrared (IR) spectra were recorded on a NEXUS 670 FT-IR Fourier transform infrared spectrophotometer and are reported in wavenumbers (cm^{-1}).

Synthesis

Compound 12. To a methanol (80.0 mL) solution of compound **14** (3.80 g, 17.2 mmol), which can be prepared according to the known procedure in 2 steps,¹⁶ was added K_2CO_3 (720 mg, 5.21 mmol) at 0 °C. Then the reaction was allowed to warm to rt and stirred overnight, before it was quenched with HCl (6 M in H_2O , 1.70 mL) at 0 °C. Then the mixture was concentrated to remove MeOH and diluted by H_2O (30.0 mL). The aqueous layer was extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified using flash column chromatography on silica gel (50 : 1 petroleum ether–EtOAc) to provide compound **12** (a known compound,^{17,19} 2.52 g, 82%,) as a yellow oil. $[\alpha]_D^{26} -25.0$ (*c* 6.88, CHCl_3), {Lit.^{17a} $[\alpha]_D^{20} -26.2$ (*c* 1.05, CHCl_3); {Lit.^{17b} $[\alpha]_D^{20} -29.2$ (*c* 1.44, CHCl_3); IR (thin film): 2961, 2948, 1717, 1638, 1383, 1339, 912, 905, 469, 459 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 5.84 (dd, *J* = 17.4, 10.2 Hz, 1H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.92 (s, 1H), 4.91 (d, *J* = 17.4 Hz, 1H), 4.69 (s, 1H), 2.48 (d, *J* = 13.2 Hz, 1H), 2.43–2.35 (m, 3H), 2.10 (dd, *J* = 13.8, 1.8 Hz, 1H), 2.05–1.98 (m, 1H), 1.95–1.90 (m, 1H), 1.77 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 210.8, 147.1, 145.8, 113.5, 111.20, 53.8, 51.8, 44.2, 41.3, 28.1, 25.1, 17.9; HRMS (ESI): *m/z* calcd for $\text{C}_{12}\text{H}_{19}\text{O}$ $[\text{M} + \text{H}]^+$ 179.1430; found, 179.1430. The enantiomer of compound **12** can be synthesized from commercially available (–)-verbenone in the same operation, and the optical rotation for this enantiomer is $[\alpha]_D^{22} +19.4$ (*c* 0.55, CHCl_3).

Compound 15. To a solution of LDA, prepared from DIPA (2.86 g, 3.96 mL, 28.3 mmol) and *n*BuLi (2.5 M in hexane, 11.3 mL, 28.3 mmol) in THF (60.0 mL) was added **12** (2.52 g, 14.1 mmol) in THF (20.0 mL) at 0 °C and the resulting mixture was stirred for 30 min at the same temperature. A mixture of methyl pyruvate (2.89 g, 2.56 mL, 28.3 mmol) and ZnCl_2 (3.86 g, 28.3 mmol) in THF (40.0 mL) was added to the above solution at 0 °C. After stirring for another 2 h, saturated NH_4Cl solution (30.0 mL) was added to this reaction. The resulting mixture was concentrated to remove THF and saturated Rochelle salt (30.0 mL) was added. Then the aqueous layer was extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated under

reduced pressure. The residue was purified using flash column chromatography on silica gel (10 : 1 petroleum ether–EtOAc) to recycle compound **12** (0.93 g, 37%) and furnish compound **15** (2.39 g, 60%; 95% brsm) as an inseparable diastereomixture and a yellow oil.

Compound 11. *p*TsOH·H₂O (2.42 g, 12.7 mmol) was added to a solution of **15** (2.39 g, 8.52 mmol) in Ac₂O (21.7 g, 20.0 mL, 0.213 mol) at 0 °C. Then the reaction was allowed to warm to rt and stirred overnight, before it was added to a solution of NaHCO₃ (35.8 g, 0.426 mol) in H₂O (200 mL) at 0 °C and stirred until bubbling ceased. The mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified using flash column chromatography on silica gel (10 : 1 petroleum ether–EtOAc) to furnish **11** (2.75 g, 84%) as an inseparable diastereomixture and a colorless oil. Compound **11** was not identified using ¹H and ¹³C NMR and was directly used for the following reaction.

Compound 3. Product **11** (0.344 g, 1.07 mmol) was dissolved in THF (15.0 mL) and DBU (0.812 g, 0.800 mL, 5.33 mmol) was slowly added at 0 °C. The resulting mixture was allowed to warm to rt and stirred overnight, before it was concentrated under reduced pressure to remove THF. Then the residue was directly purified using flash column chromatography on silica gel (10 : 1 petroleum ether–EtOAc) to provide compound **3** (0.232 g, 94%)^{8a} as a white solid. [α]_D²⁶ –65.2. (*c* 2.46, CHCl₃) or [α]_D²² –55.5 (*c* 2.46, MeOH) {Lit.^{8a} [α]_D²⁰ –12.7 (*c* 0.021, MeOH)}; IR (thin film): 3082, 2967, 2925, 1771, 1670, 1651, 1378, 1318, 1293, 1221, 1062, 918, 870, 758, 580, 553 cm^{–1}; ¹H NMR (600 MHz, CDCl₃) δ 5.84 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.47 (s, 1H), 5.07 (d, *J* = 10.8 Hz, 1H), 5.01 (d, *J* = 17.4 Hz, 1H), 4.92–4.92 (m, 1H), 4.78 (s, 1H), 2.74 (ddd, *J* = 16.8, 4.8, 1.2 Hz, 1H), 2.63 (ddd, *J* = 17.3, 8.4, 1.2 Hz, 1H), 2.47 (dd, *J* = 8.4, 4.8 Hz, 1H), 1.89 (s, 3H), 1.70 (s, 3H), 1.15 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 171.4, 149.0, 148.0, 146.0, 145.3, 120.5, 114.9, 114.5, 113.6, 50.9, 42.0, 25.6, 23.9, 22.2, 8.6; HRMS (ESI): *m/z* calcd for C₁₅H₁₈NaO₂ [M + Na]⁺ 253.1199; found, 253.1195.

Method A for compound 4. A solution of compound **3** (229.1 mg, 0.99 mmol) and ammonium hydroxide (25% in H₂O, 5.00 mL) in THF (15.0 mL) was heated to 120 °C in a sealed-tube for 24 h. After concentration under reduced pressure, crude product **10** was obtained and directly used for the next step. To a solution of the above crude product **10** in THF (20.0 mL) was added *p*TsOH·H₂O (94.2 mg, 0.495 mmol) at 0 °C. The resulting mixture was allowed to warm to rt and stirred overnight. The mixture was quenched with saturated aqueous NaHCO₃ solution (30.0 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified using flash column chromatography on silica gel (petroleum ether/EtOAc 4 : 1) to provide compound **4** (0.188 g, 83% for 2 steps) as a white solid.

Method B for compound 4. A solution of compound **11** (0.216 g, 0.67 mmol) and ammonium hydroxide (25% in H₂O,

5.00 mL) in THF (15.0 mL) was heated to 120 °C in a sealed-tube for 28 h. After concentration under reduced pressure, crude product **10** was obtained and directly used for the next step. To a solution of the above crude product **10** in THF (20.0 mL) was added *p*TsOH·H₂O (63.7 mg, 0.335 mmol) at 0 °C. The resulting mixture was allowed to warm to rt and stirred overnight. The mixture was quenched with saturated aqueous NaHCO₃ solution (30.0 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified using flash column chromatography on silica gel (petroleum ether/EtOAc 4 : 1) to provide compound **4** (0.124 g, 81% for 2 steps) as a white solid. [α]_D²⁶ –72.7 (*c* 1.28, MeOH), {Lit.¹² [α]_D²⁰ +39.0 (*c* 0.01, MeOH)}; IR (thin film): 3373, 3172, 1746, 1695, 1412, 1261, 1021, 912, 800, 737 cm^{–1}; ¹H NMR (600 MHz, CDCl₃) δ 7.68 (s, 1H), 5.84 (dd, *J* = 17.4, 10.2 Hz, 1H), 5.29 (s, 1H), 5.04 (dd, *J* = 10.2, 0.8 Hz, 1H), 4.99 (dd, *J* = 17.4, 0.8 Hz, 1H), 4.89 (s, 1H), 4.76 (s, 1H), 2.69 (ddd, *J* = 16.8, 4.8, 1.0 Hz, 1H), 2.58 (ddd, *J* = 17.4, 10.2, 1.2 Hz, 1H), 2.46 (dd, *J* = 8.4, 5.4 Hz, 1H), 1.86 (s, 3H), 1.69 (s, 3H), 1.12 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 173.2, 146.7, 146.1, 141.3, 136.6, 125.0, 115.9, 114.3, 113.0, 51.4, 42.0, 25.3, 24.0, 22.0, 8.3; HRMS (ESI): *m/z* calcd for C₁₅H₁₉NaO [M + Na]⁺ 252.1359 found 252.1361. Compound **4'** can be synthesized from commercially available (–)-verbenone in the same experimental procedure as compound **4**, and the optical rotation for this enantiomer is [α]_D²² +54.3 (*c* 0.64, MeOH).

Compound 5. Product **11** (0.126 g, 0.391 mmol) was dissolved in H₂O (10.0 mL) and DBU (0.297 g, 0.292 mL, 1.95 mmol) was slowly added at 0 °C. The resulting mixture was allowed to warm to rt and stirred overnight, before it was diluted by further H₂O (20.0 mL). Then the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified using flash column chromatography on silica gel (petroleum ether/EtOAc 5 : 1) to provide compound **5** (71.8 mg, 74%) as a white solid in a single product. The spectra of ¹H NMR and ¹³C NMR of compound **5** are well in accord with the natural product hydroxy-isogermafurenolide.^{11a,14} [α]_D²⁶ +125.6 (*c* 1.00, CDCl₃) or [α]_D²² +58.3 (*c* 2.67, dioxane) {Lit.^{11a} [α]_D²² +4.7 (*c* 0.996, dioxane)}; IR (thin film): 3342, 1748, 1689, 1643, 1435, 1380, 1325, 1239, 1143, 1128, 1084, 1008, 899, 913, 765, 726 cm^{–1}; ¹H NMR (600 MHz, CDCl₃) δ 5.70 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.00 (d, *J* = 10.8 Hz, 1H), 4.99 (s, 1H), 4.97 (d, *J* = 16.8 Hz, 1H), 4.74 (s, 1H), 2.86 (br s, 1H, –OH), 2.72 (t, *J* = 13.8 Hz, 1H), 2.56 (dd, *J* = 13.8, 3.6 Hz, 1H), 2.14 (d, *J* = 14.4 Hz, 1H), 2.05 (dd, *J* = 13.2, 3.0 Hz, 1H), 1.83 (s, 3H), 1.77 (s, 3H), 1.76 (d, *J* = 12.6 Hz, 1H), 1.27 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 172.0, 160.2, 147.6, 144.9, 122.2, 114.3, 112.1, 102.9, 54.4, 49.5, 40.8, 27.2, 24.5, 17.9, 8.4; HRMS (ESI): *m/z* calcd for C₁₅H₁₉O₃ [M – H][–] 247.1340; found, 247.1336.

Compounds 6a and 6b. Product **11** (0.328 g, 1.02 mmol) was dissolved in MeOH (15.0 mL) and DBU (0.776 g, 0.762 mL, 5.10 mmol) was slowly added at 0 °C. The resulting mixture

was allowed to warm to rt and stirred overnight, before it was concentrated under reduced pressure to remove MeOH. Then the residue was directly purified using flash column chromatography on silica gel (14 : 1 petroleum ether–EtOAc) to provide compound **3** (68.1 mg, 31%) as a white solid and compound **6** (0.163 mg, 61%) as a white solid. Compound **6** contains **6a** and **6b** as an inseparable diastereomixture in a ratio of 1.79 : 1 determined using ^1H NMR. An aliquot of this diastereomeric mixture **6** was further separated using preparative HPLC (YMC-Triart C18, hexane/*i*-PrOH = 80/20, flow rate = 3.0 mL min $^{-1}$, column temperature = 20 °C, λ = 254 nm) to give **6a** and **6b** (retention time: tr_1 = 14.5 min for **6b**, tr_2 = 16.1 min for **6a**). The ^1H NMR and ^{13}C NMR spectra of **6a** are identical to those of natural β -methoxy-isogermafurenolide,¹³ and the structures of **6a** and **6b** were also confirmed by NMR spectra of NOESY, DEPT, HSQC and HMBC (see the ESI† for details).

6a (β -methoxy-isogermafurenolide): $[\alpha]_{\text{D}}^{26}$ +96.3 (*c* 1.22, CDCl_3) (natural **6a** was reported as a racemate); ^1H NMR (600 MHz, CD_3OD) δ 5.76 (dd, *J* = 17.4, 10.8 Hz, 1H), 4.98 (s, 1H), 4.96 (dd, *J* = 11.4, 0.6 Hz, 1H), 4.95 (dd, *J* = 17.6, 1.2 Hz, 1H), 4.77 (s, 1H), 3.15 (s, 3H), 2.63 (dd, *J* = 4.2, 13.8 Hz, 1H), 2.59 (dt, *J* = 12.6, 1.2 Hz, 1H), 2.14 (dd, *J* = 12.6, 3.6 Hz, 1H), 2.10 (d, *J* = 13.8 Hz, 1H), 1.85 (d, *J* = 1.2 Hz, 3H), 1.77 (s, 3H), 1.67 (d, *J* = 13.8 Hz, 1H), 1.21 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.6, 160.7, 149.3, 146.4, 125.2, 114.5, 112.2, 107.5, 55.2, 50.6, 49.6, 41.8, 28.3, 24.9, 18.0, 8.1; HRMS (ESI): *m/z* calcd for $\text{C}_{16}\text{H}_{23}\text{O}_3$ [*M* + *H*] $^+$ 263.1642; found, 263.1641.

6b (α -methoxy-isogermafurenolide): $[\alpha]_{\text{D}}^{26}$ –149.4 (*c* 0.46, CDCl_3); ^1H NMR (600 MHz, CD_3OD) δ 6.22 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.08 (d, *J* = 17.4 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.86 (s, 1H), 4.68 (s, 1H), 3.07 (s, 3H), 2.77 (t, *J* = 4.8 Hz, 1H), 2.69–2.68 (m, 2H), 2.26 (d, *J* = 14.4 Hz, 1H), 1.89 (d, *J* = 14.4 Hz, 1H), 1.83 (s, 3H), 1.75 (t, *J* = 0.6 Hz, 3H), 1.00 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.4, 159.4, 148.7, 148.3, 127.0, 113.9, 110.2, 107.7, 50.4, 49.9, 46.6, 40.8, 28.0, 26.3, 25.5, 8.4; HRMS (ESI): *m/z* calcd for $\text{C}_{16}\text{H}_{23}\text{O}_3$ [*M* + *H*] $^+$ 263.1642; found, 263.1640.

Inhibitory effect on the proliferation of leukemia (Kasumi-1) and lymphoma cells (Pfeiffer and Karpas-422). These three human cancer cells were cultured in RPMI1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin under 5% CO_2 at 37 °C. After confirmation that cells were in linear log-phase growth, these cells were plated in 24-well plates in a volume of 100 μL and treated with compounds in corresponding concentration (DMSO as control) for 4 days. Cell viability tests were carried out using a Cell Titer-Glo luminescence assay. Briefly, 100 μL of reconstituted Cell Titer-Glo reagent was added to each well. Then the plates were shaken for 2 minutes for cell lysis and incubated at room temperature for 10 minutes. Then 100 μL of the mixture was transferred to a white 96-well luminometer plate and luminescence was measured *via* an Envision luminometer (PerkinElmer). All treatments were determined in triplicate, and data were normalized for control cells. GraphPad Prism 6.0 to was used fit the IC_{50} values.

Conflicts of interest

There are no conflicts to declare.

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