SUPPLEMENTARY INFORMATION

Artemannuols A–C, three novel sesquiterpenoid-flavonol hybrids with antihepatoma activity from Artemisia annua

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General Experimental Instruments and Procedures
ECD Calculations
Antihepatoma assay

Figure S1. Key chemical shifts difference of 1-bisabolen-3-ols according to the references (J. Nat. Prod., 2014, 77, 1708–1717; J. Chem. Eco., 2014, 40, 1260–1268)

S1. 1H NMR (600 MHz, acetone-d$_6$) of compound 1
S2. 13C NMR (DEPT) (150 MHz, acetone-d$_6$) of compound 1
S3. 1H-1H COSY (600 MHz, acetone-d$_6$) of compound 1
S4. HSQC (600 MHz, acetone-d$_6$) of compound 1
S5. HMBC (600 MHz, acetone-d$_6$) of compound 1
S6. ROESY (600 MHz, acetone-d$_6$) of compound 1
S7. $[\alpha]_D$ spectrum of compound 1 in MeOH
S8. IR of compound 1
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S11. 1H NMR (600 MHz, acetone-d$_6$) of compound 2
S12. 13C NMR (DEPT) (150 MHz, acetone-d$_6$) of compound 2
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S14. HSQC (600 MHz, acetone-d$_6$) of compound 2
S15. HMBC (600 MHz, acetone-d$_6$) of compound 2
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S17. $[\alpha]_D$ spectrum of compound 2 in MeOH
S18. IR of compound 2
S19. ECD and UV of compound 2
S20. HRESIMS of compound 2
S21. 13C NMR (DEPT) (150 MHz, CD$_3$OD) of compound 3
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S30. HRESIMS of compound 3 .............................................................................................................26
General Experimental Instruments and Procedures

A Shimadzu LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan) was used to gain the HRESIMS. UV spectra were conducted on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan), and IR (KBr) spectra were obtained on a Bio-Rad FTS-135 spectrometer (Hercules, California, USA). 1D and 2D NMR spectra were conducted on Advance III-600 spectrometers (Bruker, Bremerhaven, Germany) with TMS as the internal standard. Optical rotations were determined on a JASCO P-1020 digital polarimeter (Horiba, Tokyo, Japan). Electronic circular dichroism (ECD) spectra were measured on an Applied Photophysics Circular Dichromatograph (Applied Photophysics, Britain). Thin-layer chromatography (TLC) analyses were performed on silica gel GF254 plates (Yantai Jiangyou Silicon Development Company, Yantai, China), and spots were monitored under UV light or by heating after sprayed with 10% H2SO4 in EtOH (v/v). Silica gel (200–300 mesh, Linyi Haixiang, Linyi, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with Agilent XDB-C18 (5 μm, 9.4 × 250 mm) columns.

ECD Calculations

The conformation search was performed by Spartan '14 software using molecular mechanics MMFF94x. The appropriate low-energy conformers were selected and optimized in the gas phase by semi-empirical method in Gaussian 09 program package, and were further optimized and analyzed for frequency using the density functional theory (DFT) at the B3LYP/6-31G(d,p) level, resulting in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). All the conformers were used for calculating electronic circular dichroism (ECD) by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d,p) level with the CPCM model in MeOH.1 The overall calculated ECD curves were generated by Boltzmann weighting of the selected low-energy conformers using SpecDis 1.62 with σ = ~0.3 eV.

Antihepatoma assay

The antihepatoma assay of the compounds 1–3 was tested by the MTT assay. Briefly, cells in a density of 3×10^4 cells/well were seeded into 96-well plates and incubated at 37 °C with 5% CO2 for 24 h. The culture medium was replaced with fresh medium containing different concentrations of compounds 1–3, and cells were incubated for additional 48 h. After removal of the medium, 100 μL of MTT reagent (1mg/mL) was added into each well, and the plates were kept in incubator for 4 h. After that, 100 μL of dimethyl sulfoxide (DMSO) was added into each well, and the plates were measured at 490 nm using microplate reader (BIO-RAD, USA). The inhibitory ratio was calculated as [(A490 control – A490 treated)/A490 control] ×100%. The antihepatoma assay of compounds was expressed as IC50 values calculated by GraphPad Prism 5 (GraphPad Software, California, USA).
Figure S1. Key chemical shifts differences of 1-bisabolen-3-ols according to the references (J. Nat. Prod., 2014, 77, 1708–1717; J. Chem. Eco., 2014, 40, 1260–1268)
S1. $^1$H NMR (600 MHz, acetone-$d_6$) of compound 1
S2. $^{13}$C NMR (DEPT) (150 MHz, acetone-$d_6$) of compound 1
S3. $^1$H-$^1$H COSY (600 MHz, acetone-d$_6$) of compound I

S4. HSQC (600 MHz, acetone-d$_6$) of compound I
S5. HMBC (600 MHz, acetone-d₆) of compound 1

S6. ROESY (600 MHz, acetone-d₆) of compound 1
S7. $[\alpha]_D$ spectrum of compound 1 in MeOH

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S8. IR of compound 1
S9. ECD and UV of compound 1
S10. HRESIMS of compound 1
S11. $^1$H NMR (600 MHz, acetone-$d_6$) of compound 2
S12. $^{13}$C NMR (DEPT) (150 MHz, acetone-d$_6$) of compound 2
S13. $^1$H-$^1$H COSY (600 MHz, acetone-d$_6$) of compound 2

S14. HSQC (600 MHz, acetone-d$_6$) of compound 2
S15. HMBC (600 MHz, acetone-d<sub>6</sub>) of compound 2

S16. ROESY (600 MHz, acetone-d<sub>6</sub>) of compound 2
S17. $[\alpha]_D$ spectrum of compound 2 in MeOH

S18. IR of compound 2
S19. ECD and UV of compound 2
S20. HRESIMS of compound 2

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**MSn Loge Mode:** AND  
**Max Results:** 500

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S22. $^{13}$C NMR (DEPT) (150 MHz, CD$_3$OD) of compound 3

S23. $^1$H-$^1$H COSY (600 MHz, CD$_3$OD) of compound 3
S24. HSQC (600 MHz, CD$_3$OD) of compound 3

S25. HMBC (600 MHz, CD$_3$OD) of compound 3
S26. ROESY (600 MHz, CD3OD) of compound 3

S27. [α]D spectrum of compound 3 in MeOH

**Rudolph Research Analytical**

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.
Measurement Date: Tuesday, 13-DEC-2022
Set Temperature: 20.0
Time Delay: Disabled
Delay between Measurement: Disabled

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S28. IR of compound 3
S29. ECD and UV of compound 3
S30. HRESIMS of compound 3

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**Error Margin (ppm): 50**

**DBE Ranges: -2.0 - 1000.0**

**Electron Ions:** both

**Apply N Rule:** yes

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MSn Iso RI (%): 75.00

MSn Logic Mode: AND

Max Results: 500

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**Event #: 1**

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**Measured region for 597.2687 m/z**

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**C33 H40 O10 [M+H]+: Predicted region for 597.2694 m/z**

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