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•Research article•

# Three new carabrane sesquiterpenoid derivatives from the whole plant of *Carpesium abrotanoides* L.

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**[ABSTRACT]** Dicarabrols B and C (**1** and **2**), two new carabrane sesquiterpenoid dimers, along with one new carabrane sesquiterpenoid (**3**) were isolated from the whole plant of *Carpesium abrotanoides* L. Their full structures were established by extensive analysis of HR-ESI-MS and NMR spectroscopic data, and time-dependent density functional theory (TDDFT) electronic circular dichroism (ECD) calculations. Dicarabrol B possesses a novel $C_{30}$ skeleton featuring a methylene-tethered bridge between two sesquiterpene moieties, while dicarabrol C presents the unique linkage of a cyclopentane ring in the molecule. Dicarabrol C exhibited potent inhibitory effects on HL−60 cells with an $IC_{50}$ value of 3.7 μmol·$L^{-1}$.

**[KEY WORDS]** Sesquiterpenoid dimer; *Carpesium abrotanoides* L.; Carabrane sesquiterpenoid; Dicarabrol B; Dicarabrol C; 11*R*-hydroxycarabrol

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## Introduction

Sesquiterpenoid dimers (SDs), characterized with unusual carbon skeletons biosynthetically derived through coupling two sesquiterpenoid monomers, are in essence potential bioactive molecules. In recent years, SDs have gathered considerable research interest for their peculiar and diversified structures featured with complex linkage patterns and multiple stereocenters [1-4]. Nowadays, SDs are considered as useful scaffolds for the development of drugs as they exhibit more "biological friendly" and "drug-like" molecular effects than their monomeric precursors [5-8].

*Carpesium abrotanoides* L., belonging to the genus *Carpesium* (Asteraceae), is distributed all over China and Korea, and predominantly in the mountainous areas of southwest China [9]. As a traditional Chinese herbal medicine, the whole plant of *C. abrotanoides* is applied for the treatment of bruises, fever, bronchitis, ascariasis, insect stings, snake bites, enterobiasis, and ancylas-tomiasis [9, 10]. Moreover, its fruits, commonly known as "Nan-He-Shi" in Chinese, are famous insecticides used in northern China [9]. In previous chemical investigations of *C. abrotanoides*, a variety of sesquiterpenoid monomers and dimers were identified, which are believed to be the principal and characteristic constituents of this species [11-14]. In our previous studies, several SDs with novel structures were isolated, such as dicarabrones A and B, featuring a cyclopentane ring connecting two sesquiterpene units, which represent the first examples of dimeric carabrone sesquiterpenes [13], dicarabrol A and dicarabrone C are the first examples of carabranolide dimers linking through a spiro-tetrahydrofuran ring [14]; and dipulchellin A is the first dimer of a guaianolide unit and a carabranolide unit linking through a cyclopentane ring [14].

As a part of our continuing search for bioactive constituents with novel structures from natural resources, a detailed investigation of *C. abrotanoides* resulted in the isolation of two new sesquiterpene lactone dimers, dicarabrols B and C (**1**– **2**), along with one new carabrane sesquiterpenoid (**3**) (Fig. 1). Herein, the structural characterization, proposed biogenetic pathways, and biological evaluation of these compounds are presented.

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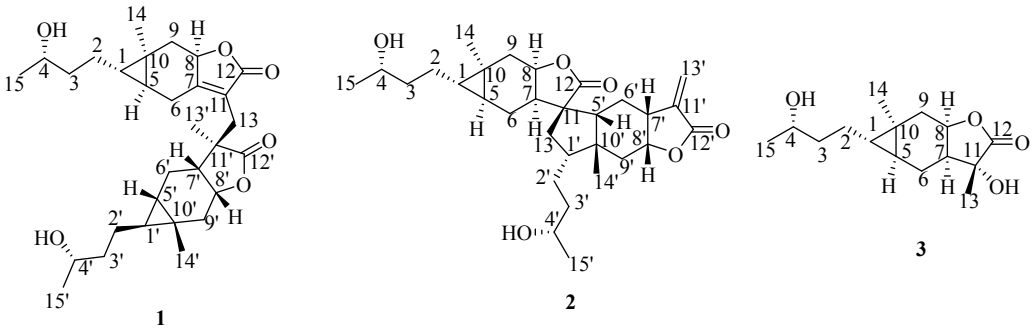

**Fig. 1 Chemical structures of compounds 1–3**

## Results and Discussion

Dicarabrol B (**1**) was obtained as yellow amorphous powder. The molecular formula of $C_{30}H_{44}O_6$ was deduced from its HR-ESI-MS wherein a quasi-molecular positive ion was measured at *m/z* 523.3034 $[M + Na]^+$ (Calcd for $C_{30}H_{44}O_6Na$, 523.3036), corresponding to an unsaturation equivalence of nine in the molecule. Two characteristic absorptions at 1753 and 3432 $cm^{-1}$ in the IR spectrum suggested the presence of carbonyl and hydroxyl groups. The $^{13}C$ NMR and DEPT spectra of **1**, combined with its HSQC experiment, revealed the existence of 30 carbon resonances ascribed to five methyls, nine methylenes, nine methines, and seven quaternary carbons. From these signals, two ester carboxyls ($\delta_C$ 174.5, 179.9), two doublet methyls ($\delta_C$ 23.7, 23.8), and four oxygenated methines ($\delta_C$ 67.9, 68.4, 77.2, 78.0) were clearly identified. Additionally, its $^1H$ NMR spectrum displayed four sets of methines at $\delta_H$ 0.48 (m), 0.61 (m), 0.54 (m) and 0.51 (m), indicating the presence of two cyclopropane moieties. The above-mentioned data unveiled two sets of similar NMR resonances, together with the MS data, suggesting that **1** might be a dimeric sesquiterpene.

A detailed examination of the $^1H-^1H$ COSY and HMBC data of **1** led to the construction of two carabranolide moieties, whose $^1H$ and $^{13}C$ NMR signals showed high similarity to those of carabrol [15]. When compared with the exocyclic double bond between C-11 and C-13 in carabrol, one conjugated double bond between C-7 and C-11 ($\delta_C$ 121.2, 167.1) in moiety A, as well as a quaternary carbon ($\delta_C$ 45.4) and a methyl ($\delta_H$ 1.15, s/$\delta_C$ 21.9) in the moiety B, were the major differences observed. The linkage between these two moieties was established by the HMBC experiment (Fig. 2). The HMBC correlations between $H_2$-13 ($\delta_H$ 2.49, 2.17) and C-7 ($\delta_C$ 167.1), C-12 ($\delta_C$ 174.5), C-7′ ($\delta_C$ 48.5), C-12′ ($\delta_C$ 179.9) and C-13′ ($\delta_C$ 21.9) were indicative of a new C–C bond formed between C-13 and C-11′. The planar structure of **1** was therefore proposed as a carabrane-type sesquiterpene dimer tethered by one bridge of methylene.

The relative configuration of **1** was inferred from the ROESY experiment. The cross-peaks of H-5/$H_3$-14, $H_3$-14/H-8, H-5′/$H_3$-14′, $H_3$-14′/H-8′, and H-7′/H-8′ were observed, suggesting *cis*-diaxial like structure of carabrol (Fig. 3). Additionally, the observed ROESY correlations of H-8′/$H_a$-13 and H-8′/$H_b$-13 implied the co-facial of H-8′ and $H_2$-13. In light of the biogenetic relationship between **1** and carabrol, the absolute configuration of **1** was proposed as 1*S*, 4*S*, 5*S*, 8*R*, 10*R*, 1′*S*, 4′*S*, 5′*S*, 7′*R*, 8′*R*, 10′*R*, 11′*R*.

To substantiate such elucidation, time-dependent density functional theory (TDDFT) ECD calculation was performed. The ECD spectrum of **1** was measured, indicating a strong negative Cotton effect at 245 nm (Fig. 4). To facilitate the conformational searching, a truncated structure **1a** with two ethyl rather than two 3-hydroxy-butanyl side chains were used for the computational calculation. Conformational search of **1a** was conducted using the Conflex in a 5.0 $kcal \cdot mol^{-1}$ energy window, yielding 12 conformers above 1.0% Boltzmann population. The conformers were re-optimized at the M06-2X/6-311G (d,p) with SMD solvent model for methanol. TDDFT ECD spectra were calculated at the level of M06-2X/def2TZVP with SMD solvent model for methanol. The calculated ECD spectrum showed a strong negative Cotton effect around 240 nm, very similar to that of

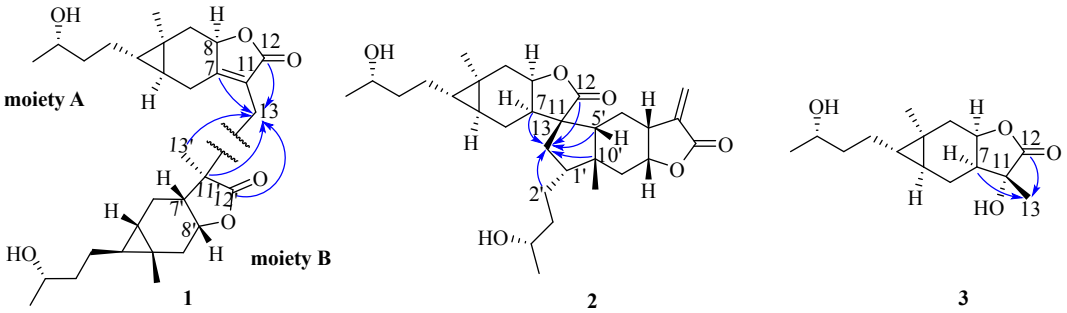


**Fig. 2 Key HMBC correlations (C → H) of 1–3**

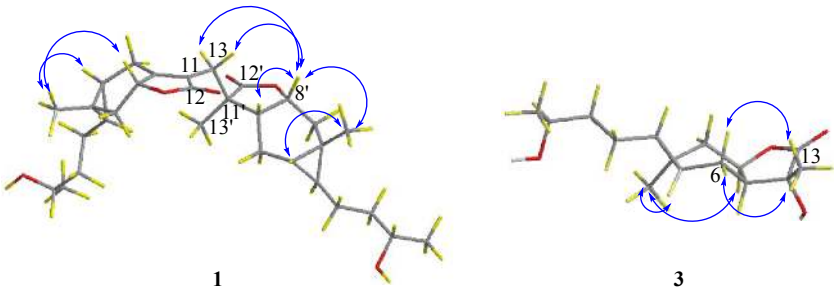


**Fig. 3 Key ROESY correlations of 1 and 3**

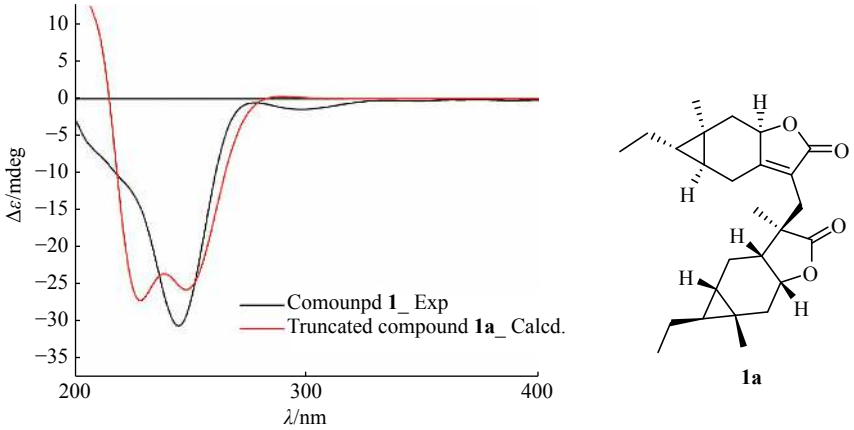


**Fig. 4 Experimental ECD spectra of compound 1 in MeOH compared with the Boltzmann-weighted M06-2X/def2TZVP SMD/MeOH ECD spectra of the truncated 1a computed for the M06-2X/6-311G(d, p) optimized conformers**

compound **1**. The matching between compound **1** and its truncated derivative **1a** suggested that the monomeric halves of **1** remained the same absolute configuration with carabrol. Consequently, the full structure of **1** was established, and named dicarabrol B.

Dicarabrol C (**2**) was obtained as yellow amorphous powder. Its molecular formula was established as the same as that of **1** by the HR-ESI-MS data wherein a sodiated ion $[M + Na]^+$ was measured at *m/z* 523.3023. The $^1$H NMR spectrum of **2** displayed characteristic signals including two sets of methines at $\delta_H$ 0.40 (m) and 0.28 (m), and two sets of oxygenated methines at $\delta_H$ 3.80 (overlapped) and 4.62 (dt, *J* = 7.2, 9.8 Hz), which also indicated a carabrol dimer. In-depth analysis further revealed that the NMR data of **2** were very close to those of dicarabrone B, a known compound characterized in our previous study [13]. Their differences were observed, in which two hydroxymethines were presented in **2** rather than two carbonyl groups in dicarabrone B. The $^1$H–$^1$H COSY and HMBC data further supported that **2** might be dimerized from two same halves of carabrol.

The key ROESY correlations revealed that most chiral carbons in **2** remained the same configurations as those in carabrol. However, due to the overlapping of the key ROESY correlations, the stereochemistry of the cyclopentane moiety including the absolute configurations of C-11, C-1′ and C-5′, remained unclear. Therefore, chemical conversion was conducted, where compound **2** was converted to dicarabrone B through oxidation using Dess-Martin periodinane as the catalyst (Fig. S1). Thus, the absolute configuration of **2** was deduced to be the same with that of dicarabrone B. In a similar way descried for compound **1**, the TD-DFT ECD spectrum of a truncated structure of **2a** was calculated at the level of M06-2X/def2TZVP//M06-2X/6-311G (d, p) with SMD solvent model for methanol. The results showed that the experimental and calculated spectra well matched, both exhibiting a positive Cotton effect around 210 nm (Fig. S27). Accordingly, the whole structure of **2** was finally determined, and named dicarabrol C.

Compound **3**, isolated as translucent colorless oil, with a molecular formula of $C_{15}H_{24}O_4$ as deduced from its $^{13}$C NMR and an HR-ESI-MS ion at *m/z* 291.1577 $[M + Na]^+$ (Calcd. 291.1572), corresponding to four indices of hydrogen deficiency. The NMR data demonstrated that these signals showed great similarities to those of the known compound carabrol except for the signals of an oxygen-bearing quaternary carbon ($\delta_C$ 75.0) and a methyl ($\delta_H$ 1.31, s, 3H; $\delta_C$ 20.9) taking place of the exocyclic double bond between C-11 and C-13 in carabrol. The deduction was further evidenced by the HMBC experiment, which showed correlations from $H_3$-13 to C-11 and C-12. This newly-presented methyl at C-11 was deduced to be of *β*-orientation based on the NOESY correlations of $H_3$-13/H-6*α* and $H_3$-13/H-6*β*. Taken the biogenetic relationship into consideration, the absolute configuration of **3** was proposed as 1*S*, 4*S*, 5*S*, 7*S*, 8*R*, 10*R*, 11*R*, and further

confirmed by comparison of its experimental and calculated ECD spectra (Fig. S28). The TD-DFT ECD spectrum of the truncated structure of **3a** was calculated, which was also at the level of M06-2X/def2TZVP//M06-2X/6-311G (d, p) with SMD solvent model for methanol. The good matching of the experimental and calculated ECD spectra supported the structural construction of **3**, which was named 11*R*-hydroxycarabrol.

Previous investigations indicated that sesquiterpenoid dimers are important sources for new anticancer agents [6, 7][10, 11][13, 14]. Therefore, the isolated compounds were evaluated for their cytotoxicity against human tumour cell lines A549 and HL-60 using MTT assay. Compound **2** exhibited selective cytotoxicity against HL-60 cells with an $IC_{50}$ value of 3.7 μmol·L$^{-1}$, without activity on A549 cells ($IC_{50}$ > 20 μmol·L$^{-1}$). However, neither compounds **1** nor **3** displayed cytotoxicity on the test cancer cell lines at the concentration of 20 μmol·L$^{-1}$. The results were consistent with the fact that the cytotoxic activities of sesquiterpene lactones were considered to be associated with introduction of an *α*-methylene-*γ*-lactone moiety into the molecules [16, 17]. In this study, only compound **2** possessed an *α*-methylene-*γ*-lactone moiety in the structure, and accordingly exhibited better cytotoxicity than the other two compounds.

Recently, more and more sesquiterpene oligomers have been identified from natural sources, especially the family Compositae. Tricarabrols A–C, for example, three sesquiterpene trimers were reported from *C. faberi* [18]. In the proposed biosynthetic pathway of tricarabrols A–B, compound **1** was identified as a key intermediate. Undoubtedly, the discovery of naturally occurring **1** from the plant provides evidence to support the proposed biogenetic pathway, and more sesquiterpene oligomers with **1** as a precursor is expected in future investigations.

## Experimental

### *General experimental procedures*

Optical rotation values were measured on a Perkin-Elmer 341 polarimeter (Perkin-Elmer, Boston, MA, USA). IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. NMR spectra were recorded on a Bruker Advance-500 spectrometer with TMS as internal standard (Bruker BioSpin AG, Fallanden, Switzerland). ESI-MS and HR-ESI-MS data were recorded on Waters 2695−3100 LC-MS and Waters Xevo TOF mass spectrometers. Analytical HPLC was performed on a Waters 2690 instrument with an Alltech ELSD 2000 detector. Preparative HPLC was performed on a Varian PrepStar system with an Alltech 3300 ELSD. Chromatographic separation was performed on a Waters Sunfire® RP $C_{18}$, 5 μm, 30 mm × 150 mm column and a Waters Sunfire® RP $C_{18}$, 5 μm, 19 mm × 150 mm column, using a gradient solvent system consisting of $H_2O$ and $CH_3CN$, with a flow rate of 25.0 and 10.0 mL·min$^{-1}$, respectively. Silica gel (Qingdao Marine Chemical Industrials, Qingdao, China) was used for flash chromatography. MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and Sephadex LH−20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). TLC was carried out on precoated silica gel $GF_{254}$ plates (Yantai Chemical Industrials, Yantai, China), and the TLC spots were observed at 254 nm and visualized with 5% $H_2SO_4$ in EtOH containing 10 mg·mL$^{-1}$ vanillin. All solvents were of analytical grade (Shanghai Chemical Plant, Shanghai, China).

### *Plant materials*

The whole plant of *C. abrotanoid* was collected in Henan Province, China, in 2012, and identified by Professor SHEN Jin-Gui from the Shanghai Institute of Materia Medica. A sample (20120901) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

### *Extraction and isolation*

The air-dried whole plant of *C. abrotanoides* (50.0 kg) was extracted with 95% ethanol (3 × 40 L, 7 days each) at room temperature. After evaporation of the solvent, the obtained residue was dissolved in water (10 L), and then extracted with dichloromethane ($CH_2Cl_2$) (3 × 10 L) and ethyl acetate (3 × 10 L), successively. The $CH_2Cl_2$-soluble partition (1.7 kg) was fractionated over MCI gel (EtOH/$H_2O$, from 50% to 95%) to return five fractions (A−E). Fractions B (305.0 g) and C (300.0 g) were further fractionated over MCI gel (EtOH/$H_2O$, from 30% to 70%) to afford subfractions B1−B7 and C1−C11, respectively. The above subfractions were analyzed by LC-MS method, and the subfractions B6 (5.6 g) and C9 (26.5 g), bearing two peaks of potential dimeric sesquiterpenoids with molecular weight of 500 were selected. Consequently, these two subfractions were subjected to column chromatography (CC) over silica gel eluted with $CH_2Cl_2$/MeOH (50 : 1 to 10 : 1) in a step manner to give subfractions B6A−B6G and C9A−C9E, respectively. Subsequently, subfractions C9D and B6E was subjected to CC over Sephadex LH-20 (MeOH) and then purified further by preparative HPLC ($CH_3CN$/$H_2O$, 30% to 60%, 90 min) to yield **1** (25.0 mg), and **2** (160.0 mg), respectively. Similarly, subfraction B3 (39.6 g) was subjected to CC over silica gel eluted with $CH_2Cl_2$/MeOH (50 : 1 to 10 : 1) to afford subfractions B3A−B3G. Subfraction B3E was selected for further purification by CC over Sephadex LH-20 (MeOH) and preparative HPLC ($CH_3CN$/$H_2O$, from 30% to 60%, 90 min), yielding **3** (50.0 mg).

Dicarabrol B (**1**): yellow amorphous powder; $[\alpha]_D^{20}$ +16 (*c* 0.1, $CH_3OH$); IR (KBr) $\nu_{max}$ 3432, 2965, 2926, 2865, 1753, 1458, 1383, 1102, 1016 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 1; HR-ESI-MS *m*/*z* 523.3034 $[M + Na]^+$ (Calcd. for $C_{30}H_{44}O_6Na$, 523.3036).

Dicarabrol C (**2**): yellow amorphous powder; $[\alpha]_D^{20}$ +69 (*c* 0.1, $CHCl_3$); IR (KBr) $\nu_{max}$ 3439, 2960, 2925, 2857, 1755, 1460, 1359, 1269, and 1163 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 1; HR-ESI-MS *m*/*z* 523.3023 $[M + Na]^+$ (Calcd. for $C_{30}H_{44}O_6Na$, 523.3036).

11*R*-hydroxycarabrol (**3**): translucent colorless oil;

$[\alpha]_D^{20}$ +46 (*c* 0.1, $CH_3OH$); IR (KBr) $\nu_{max}$ 3404, 3278, 2969, 2939, 2860, 1750, 1458, 1374, 1208, 1114, 979 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see Table 1; HR-ESI-MS *m/z* 291.1577 $[M + Na]^+$ (Calcd. for $C_{15}H_{24}O_4Na$, 291.1572).

*Conversion of dicarabrol C (**2**) to dicarabrone B*

A solution of **2** (2.0 mg, 4 μmol) in $CH_2Cl_2$ (2 mL) was treated with Dess-Martin periodinane (10.0 mg, 24 μmol) at room temperature for 12 h. After reaction, the mixture was evaporated to dryness. The residue was redissolved in MeOH, and the insoluble material was removed by centrifugation. The supernatant was subjected to column chromatography over Sephadex LH-20 (MeOH) to afford dicarabrone B (0.8 mg, 1.6 μmol, HPLC, $^1H$ NMR).

*Cytotoxicity bioassay*

The anti-proliferative activity of compounds **1**–**3** were evaluated against HL-60 and A-549 tumor cell lines using MTT [19] and sulforhodamine B [20] methods, respectively. Doxorubicin was used as the positive control. The anti-proliferative activities of the compounds were evaluated on the HL-60 cell line using MTT assay. The cells were plated in 96-well tissue plates at a density of $1 \times 10^4$ cells/well. Adherent cell lines were previously incubated for 24 h to ensure adhesion to the wells in an atmosphere of 5% $CO_2$. The compounds were applied at various concentrations, while control

**Table 1 $^1H$ and $^{13}C$ NMR Data for 1–3 (500, 125 MHz, TMS, δ ppm) in $CDCl_3$**

| No. | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| | $\delta_H$ (mult, *J* Hz) | $\delta_C$ | $\delta_H$ (mult, *J* Hz) | $\delta_C$ | $\delta_H$ (mult, *J* Hz) | $\delta_C$ |
| 1 | 0.48 m | 32.4 | 0.40 (ddd, 4.1, 6.9, 7.1) | 35.2 | 0.41 (ddd, 4.2, 6.9, 7.2) | 35.2 |
| 2 | 1.37 overlapped | 25.5 | 1.53 m | 25.6 | 1.35 m | 25.5 |
| 3 | 1.55 overlapped | 39.2 | 1.59 m | 39.4 | 1.52 m | 39.4 |
| 4 | 3.81 overlapped | 67.9 | 3.80 overlapped | 68.1 | 3.80 m | 68.1 |
| 5 | 0.61 m | 25.8 | 0.28 (ddd, 4.1, 7.8, 8.4) | 23.8 | 0.30 (ddd, 4.1, 7.8, 8.8) | 23.8 |
| 6*α*<br>6*β* | 2.82 m | 25.8 | 1.92 overlapped<br>0.75 (dt, 8.4, 13.6) | 25.6 | 2.04 m<br>0.63 (dt, 8.8, 13.7) | 24.2 |
| 7 | | 167.1 | 2.34 overlapped | 39.3 | 2.39 overlapped | 45.3 |
| 8 | 4.72 (dd, 7.5, 11.3) | 78.0 | 4.62 (dt, 7.2, 9.8) | 75.7 | 4.95 (dt, 7.4, 10.3) | 76.7 |
| 9*α*<br>9*β* | 2.54 (dd, 7.5, 13.4)<br>1.37 overlapped | 42.6 | 2.34 overlapped<br>0.99 (dd, 10.0, 14.4) | 37.8 | 2.39 overlapped<br>0.91 (dd, 10.1, 14.2) | 37.4 |
| 10 | | 18.3 | | 16.0 | | 16.1 |
| 11 | | 121.2 | | 53.6 | | 75.0 |
| 12 | | 174.5 | | 181.7 | | 178.7 |
| 13 | 2.49 (d, 13.8)<br>2.17 (d, 13.8) | 24.9 | 1.79 (dd, 5.6, 12.7)<br>1.36 overlapped | 36.3 | 1.31 s | 20.9 |
| 14 | 1.14 s | 21.3 | 1.05 s | 19.2 | 1.04 s | 18.9 |
| 15 | 1.19 (d, 6.0) | 23.7 | 1.20 (d, 6.2) | 23.8 | 1.18 (d, 6.2) | 23.7 |
| 1′ | 0.54 m | 29.1 | 2.51 overlapped | 47.5 | | |
| 2′ | 1.37 overlapped | 25.6 | 1.35 overlapped | 25.7 | | |
| 3′ | 1.55 overlapped | 39.2 | 1.45 m | 38.8 | | |
| 4′ | 3.81 overlapped | 68.4 | 3.80 overlapped | 68.5 | | |
| 5′ | 0.51 m | 25.8 | 1.99 (dd, 5.6, 13.0) | 51.7 | | |
| 6′ | 1.97 m | 21.4 | 1.6 m/H-6′α<br>1.36 overlapped/H-6′β | 31.0 | | |
| 7′ | 1.47 m | 48.5 | 3.02 m | 36.7 | | |
| 8′ | 4.12 (dt, 5.8, 11.2) | 77.2 | 4.74 (dt, 7.2, 9.5) | 75.6 | | |
| 9′*α*<br>9′*β* | 1.55 overlapped<br>2.30 (dd, 5.8, 12.9) | 39.6 | 1.35 overlapped<br>1.90 overlapped | 31.5 | | |
| 10′ | | 18.5 | | 42.7 | | |
| 11′ | | 45.4 | | 139.3 | | |
| 12′ | | 179.9 | | 170.2 | | |
| 13′ | 1.15 s | 21.9 | 6.30 (d, 2.1, H-13′a)<br>5.68 (d, 1.8, H-13′b) | 123.4 | | |
| 14′ | 1.11 s | 22.2 | 1.16 s | 28.4 | | |
| 15′ | 1.20 (d, 6.2) | 23.8 | 1.19 (d, 6.2) | 23.7 | | |

cells were treated with DMSO at the highest concentration used in the test wells (0.5%). Then, 1 h prior to the end of incubation, 20 mL of MTT (5 mg·mL$^{-1}$ in PBS, 5% MTT) were added to each well and further incubated at 37 °C for another 4 h. The supernatants were removed and 150 μL DMSO were afterwards added to each well in order to dissolve the formazan crystals. The mixture was oscillatored at room temperature for 10 min and its absorbance was measured at 490 nm (Genios, Tecan, Austria). The anti-proliferative activities of the compounds were evaluated on the A-549 cell line using the sulforhodamine B (SRB) assay. In the SRB assay, A-549 cells ($4 \times 10^3$ cell/well) were seeded into 96-well plates and grown for 24 h. The cells were then treated with increased concentrations of the compounds and grown for further 72 h. At the end of exposure, 100 μL of ice-cold 10% trichloroacetic acid (TCA) was added to each well, ketpt at 4 °C for 1 h, and washed five times with distilled water. The TCA-fixed cells were stained for 15 min with 100 μL of 4 mg·mL$^{-1}$ SRB in 1% HOAc. The plates were washed five times with 1% HOAc and air-dried overnight. After air-drying, protein-bound dye was dissolved in 150 μL of 10 mmol·L$^{-1}$ Tris base for 5 min and measured at 515 nm using multiwall spectrophotometer (SpectraMax, Molecular Devices, USA). The inhibitory rate was calculated as (1-A treated/A control) × 100%.

*Computational section*

TDDFT calculations were performed using the Gaussian 09 program [21]. Conformational search was carried out by the Conflex 8.0 software using the MMFF force field within an energy window of 5.0 kcal·mol$^{-1}$ [22]. The conformers with the Boltzmann population above 1.0% were re-optimized. TDDFT ECD calculations were run at the M06-2X/TZVP or M06-2X/def2TZVP level with the SMD solvent model for methanol. ECD spectra were generated using the SpecDis Version 1.71 with 0.4 sigma/gamma (eV) after UV correction [23, 24].

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