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Sinulariaone A: a novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species†

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Chemical composition screening of an octocoral identified as *Sinularia* species led to the isolation of a novel diterpenoid, sinulariaone A (**1**), featuring a 13-membered carbocyclic skeleton. The structure of **1** was established by spectroscopic elucidation, computed calculation, and X-ray diffraction analysis. Moreover, a single-crystal X-ray diffraction analysis of chlorofurancembranoid B (**2**), obtained in our previous study from the same octocoral species, was reported for the first time to demonstrate the absolute configuration. Diterpenoid **1** showed cytotoxicity towards human promyelocytic leukemia HL-60 cells, with an IC_{50} value of 38.01 μM.

1 Introduction

Octocorals of the genus *Sinularia* (phylum Cnidaria, subphylum Anthozoa, class Octocorallia, order Malacalcyonacea, family Sinulariidae)[1] are one of the most common marine invertebrates natively distributed throughout tropical and subtropical regions of the Indo-Pacific Ocean. Despite their ecological importance, the secondary metabolites, in particular terpenoid derivatives from these organisms were proven to have potential for biomedical uses.[2–4] In this research, we completed the preparation, structural identification, and cytotoxicity assessment of sinulariaone A (**1**), a diterpenoid featuring with a rare 13-membered carbocyclic skeleton and chlorofurancembranoid B (**2**)[5] (Fig. 1), from an octocoral identified as *Sinularia* sp., collected from the waters of Taiwan, an area with high biodiversity at the intersection of the Kuroshio current, South China Sea surface current, and Mainland Coastal current.

2 Results and discussion

Sinulariaone A (**1**) was obtained as colorless prisms with the molecular formula determined to be $C_{20}H_{32}O_2$ by (+)-HRESIMS at *m*/*z* 327.22928 (calcd for $C_{20}H_{32}O_2$ + Na, 327.22945), corresponding to five double-bond equivalents (DBEs). The IR spectrum of **1** showed a strong absorption at ν_{max} 1716 cm^{-1}, consistent with a ketone moiety in the structure. The ^{13}C spectrum (Table 1), in combination with the DEPT and HSQC spectrum, showed signals of 20 carbons, including a ketonic carbonyl (δ_C 210.0, C-17), four olefinic carbons (δ_C 149.8, C-1;

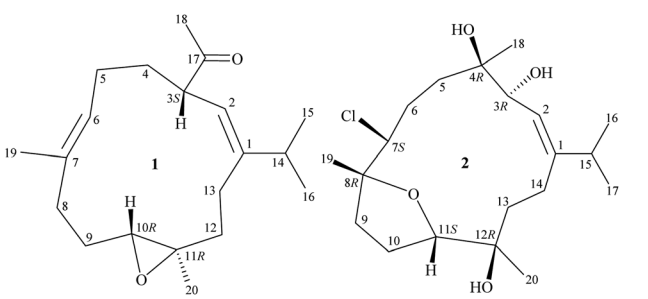

Fig. 1 Structures of sinulariaone A (**1**) and chlorofurancembranoid B (**2**).

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† Electronic supplementary information (ESI) available: HRESI-MS, 1D and 2D NMR spectra of **1**; experimental and calculated SOR values of **1**; X-ray crystallo-graphic data of **1** and **2**. CCDC 2226689 and 2208811. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d3ra01589k

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Table 1 ^{1}H and ^{13}C NMR spectroscopic data of sinulariaone A (**1**)

Position	δ_H[a] (J in Hz)	δ_C[b], Mult.[c]
1		149.8, C
2	5.06 d (10.0)	121.6, CH
3	3.16 ddd (10.0, 10.0, 2.0)	49.9, CH
4	2.23 m	31.5, CH_2
4′	1.04 m	
5	1.97–2.05 m[d]	25.6, CH_2
6	5.13 dd (6.8, 6.8)	127.4, CH
7		134.8, C
8	2.36 ddd (12.4, 5.2, 4.4)	36.7, CH_2
8′	2.18 dd (12.4, 4.0)	
9	2.24 ddd (13.2, 5.2, 4.0)[e,h]	24.4, CH_2
9′	1.37 dddd (13.2, 10.0, 4.4, 4.0)	
10	2.74 dd (10.0, 4.0)	62.6, CH
11		61.3, C
12α	2.26 m[e]	40.7, CH_2
β	1.07 m	
13	1.97–2.05 m[d]	25.2, CH_2
14	2.25 m[e]	35.4, CH
15	0.99 d (6.8)[f]	21.9, CH_3[g]
16	0.99 d (6.8)[f]	21.9, CH_3[g]
17		210.0, C
18	2.06 s	29.4, CH_3
19	1.62 br s	14.5, CH_3
20	1.30 s	16.0, CH_3

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [c] Multiplicity deduced by DEPT and HSQC spectrum and indicated by usual symbols. [d] Signals overlapped. [e] Signals overlapped. [f] Signals overlapped. [g] Signals overlapped. [h] The coupling constants for H-9 were assigned by its geminal coupling with H-9′ and vicinal couplings with H-8 and H-10, respectively.

121.6, CH-2; 127.4, CH-6; 134.8, C-7), and two oxygenated carbons (δ_C 62.6, CH-10; 61.3, C-11), as well as five methyls, six aliphatic sp^3 methylenes, and two aliphatic sp^3 methines.

Analysis of ^{1}H (Table 1), ^{13}C, and HSQC spectra illustrated that **1** contained two trisubstituted carbon–carbon double bonds (δ_H 5.13, 1H, dd, J = 6.8, 6.8 Hz/δ_C 127.4, CH-6; δ_C 134.8, C-7; δ_H 5.06, 1H, d, J = 10.0 Hz/δ_C 121.6, CH-2; δ_C 149.8, C-1) and an acetyl group (δ_H 2.06, 3H, s/δ_C 29.4, CH_3-18; δ_C 210.0, C-17). The ^{3}J-proton-proton coupling information in the COSY spectrum led to the assignment of four continuous spin systems from H-2/H-3/H_2-4/H_2-5/H-6, H_2-8/H_2-9/H-10, H_2-12/H_2-13, and H-14/H_3-15 (H_3-16) (Fig. 2). The HMBC spectrum showed ^{2}J- and ^{3}J-heteronuclear correlations from neighbor protons to the non-protonated carbons such as H-3, H_2-12, H_2-13, H-14, H_3-15, H_3-16/C-1; H_2-5, H_2-8, H_2-9, H_3-19/C-7; H_2-12, H_2-13, H_3-20/C-11; and H-2, H-3, H_2-4, H_3-18/C-17 (Fig. 2), confirming the presence of central 13-membered carbon macrocyclic ring system.[6–10] The HMBC correlations from H_3-20/C-10, C-11, and C-12 indicated that Me-20 was placed at C-11. The presence of a vinyl methyl (Me-19) at C-7 was substantiated by the HMBC correlations from H-6, H-8′ (δ_H 2.18)/C-19 and H_3-19/C-6, C-7, C-8, and further confirmed by a long-range allylic coupling between H-6/H_3-19 (Fig. 2). The presence of an isopropyl group at C-1 was substantiated by the HMBC correlations from H-14, H_3-15, H_3-16 to C-1. The above analysis enabled the establishment of the carbon skeleton of **1**. A trisubstituted epoxide

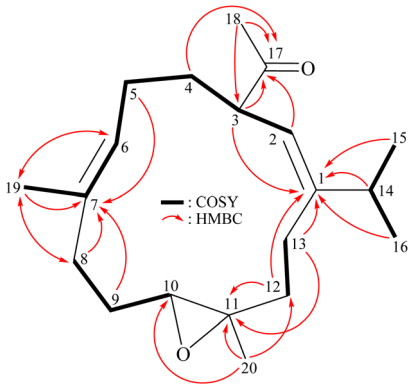


Fig. 2 Key COSY and HMBC correlations of **1**.

containing a methyl substituent in **1** was established from the signals of an oxygenated quaternary carbon at δ_C 61.3 (C-11) and an oxymethine (δ_H 2.74, 1H, dd, J = 10.0, 4.0 Hz/δ_C 62.6, CH-10), and from the proton signal of a methyl at δ_H 1.30 (3H, s, H_3-20). An acetyl group at C-3 was confirmed by the HMBC correlations from the methine proton at δ_H 3.16 (H-3) to the ketonic carbonyl at δ_C 210.0 (C-17); the other HMBC correlations from the methyl protons resonating at δ_H 2.06 (H_3-18) to C-17 ketonic carbonyl (δ_C 210.0) and C-3 methine (δ_C 49.9), further supporting that this group was positioned at C-3.

The relative stereochemistry of **1** was determined based on correlations obtained from NOESY experiments. In the NOESY spectrum (Fig. 3), H-10 exhibited cross-peaks with H-3 and one of the diastereotopic methylene protons at C-12 (δ_H 1.07, H-12β); and H-12β was correlated with H-3 but not with H_3-20, which illustrated the β-orientations of H-3 and H-10, and the α-orientation of Me-20. The Z-form of Δ^1 and Δ^6 was confirmed by

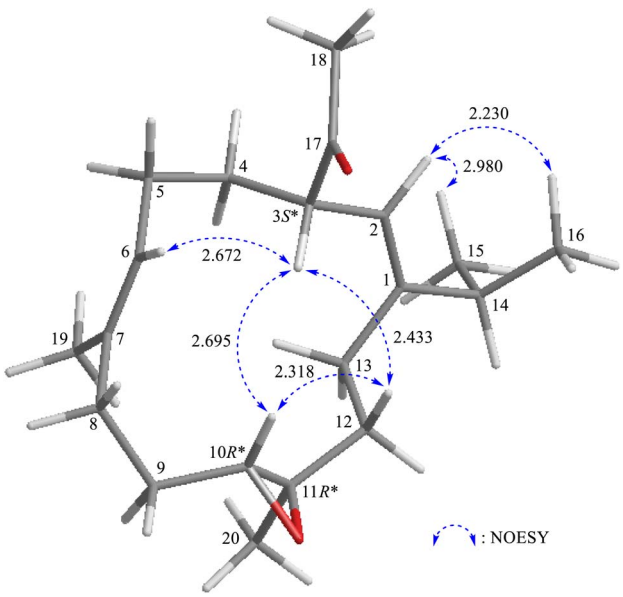
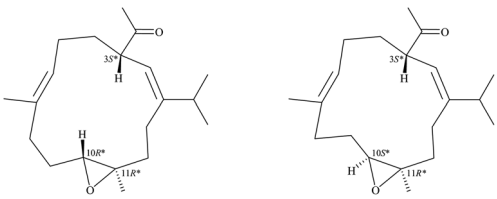
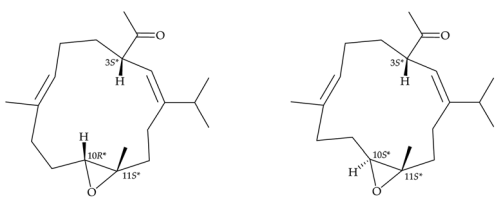


Fig. 3 Stereo-view of **1** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

NOESY correlations between H-2 (olefin proton)/H_3-15 (H_3-16); and H-6 (olefin proton)/H-3, respectively, and there were no NOESY correlations were found between H-6/H_3-19 (vinyl methyl) and H-2/H_2-13.

After the program of the above analysis, the gross structure of **1** displayed four possible relative configurations, including **1**-3*S**, 10*R**, 11*R**; **1**-3*S**, 10*S**, 11*R**; **1**-3*S**, 10*R**, 11*S**; and **1**-3*S**, 10*S**,11*S**. The four possible relative configurations were inputted into Spartan'16 and optimized at the MMFF94 level.[11–13] The predicted distance of key NOESY of possible configurations is shown in Table 2. The **1**-3*S**, 10*R**, 11*R** displayed the best result matching the experimental key NOESY correlations, and the calculated single optical rotation (SOR) value of **1**-3*S*, 10*R*, 11*R* and **1**-3*R*, 10*S*, 11*S* were +226 and −226, respectively. Comparing the calculated with the experimental SOR value of **1** (+236), the absolute configuration of **1** could be assigned as 3*S*, 10*R*, 11*R*.

Table 2 The predicted distance (Å) of key NOESY of optimized top 3 possible relative configurations of **1**

	3*S**, 10*R**, 11*R**	3*S**, 10*S**, 11*R**
Structures		
	Top 1	
H-3/H-6	2.775	2.688
H-3/H-10	2.404	4.931
H-3/H-12	2.468	2.479
	Top 2	
H-3/H-6	2.742	2.740
H-3/H-10	2.430	4.857
H-3/H-12	2.565	2.308
	Top 3	
H-3/H-6	2.636	2.517
H-3/H-10	2.409	5.071
H-3/H-12	2.411	2.479
	3*S**, 10*R**, 11*S**	3*S**, 10*S**, 11*S**
Structures		
	Top 1	
H-3/H-6	2.597	3.197
H-3/H-10	3.235	3.896
H-3/H-12	4.123	2.346
	Top 2	
H-3/H-6	2.636	3.199
H-3/H-10	3.138	3.839
H-3/H-12	4.161	3.829
	Top 3	
H-3/H-6	2.604	3.269
H-3/H-10	3.237	3.935
H-3/H-12	4.229	3.220

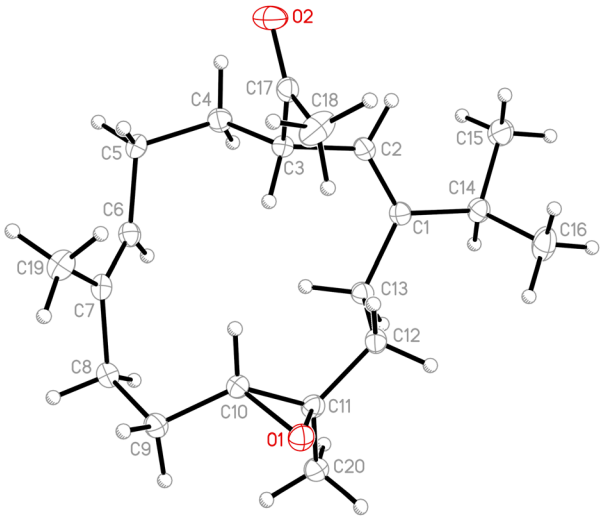


Fig. 4 The computer-generated ORTEP diagram of **1**.

Due to the conformational mobility of the macrocycle, the stereochemistry of the stereogenic centers C-3, C-10, and C-11 of **1** would be further determined from an X-ray diffraction analysis. Regarding validation of the structure of **1**, a single-crystal X-ray diffraction analysis was employed. The structure of **1** was fully established by X-ray crystallography, as observed by Cu Kα radiation (λ = 1.54178 Å) and the Flack parameter x = 0.0(3).[14,15] The X-ray structure (Fig. 4) demonstrates the location of an acetyl group at C-3 and an epoxy group between C-10/11 in the 13-membered macrocycle ring. Based on the X-ray diffraction analysis, the stereogenic centers in **1** were assigned as 3*S*, 10*R*, 11*R*. From the above findings, the structure, including the absolute configuration, of **1** was therefore elucidated unambiguously.

Chlorofurancembranoid B (**2**), a cytotoxic cembranoid toward human promyelocytic leukemia HL-60 cells, was reported in our previous publication, and its stereochemistry was established by combination of a NOESY experiment.[5] Thus, in order to determine the absolute configuration. This compound has been crystallized, and the diffraction experiment was carried out with a diffractometer equipped with molybdenum radiation (Mo Kα, λ = 0.71073 Å) source. The ORTEP diagram (Fig. 5) showed the absolute configuration for all stereogenic centers were assigned as 3*R*, 4*R*, 7*S*, 8*R*, 11*S*, 12*R*.

The cytotoxicity of **1** against cancer cells HL-60 and HepG2 (human hepatoma cell line) were investigated. The assay used in this study was performed as described in previous

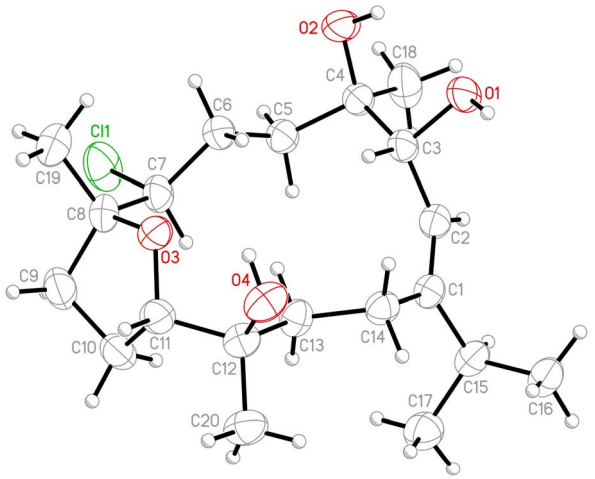


Fig. 5 The computer-generated ORTEP diagram of **2**.

Table 3 Effects of compound **1** on cell viability in HL-60 and HepG2 tumor cells

	HL-60		HepG2	
Compound	Cell viability[a] (%)	IC_{50}[b] (μM)	Cell viability[a] (%)	IC_{50}[b] (μM)
1	42.42 ± 0.70***	38.01 ± 1.21	71.76 ± 2.90	> 50
DMSO	100.00 ± 2.16		100.00 ± 0.72	

[a] Cell viability at 50 μM for 48 h. Results are expressed as mean ± SEM (n = 3). ***p < 0.001 compared with DMSO alone. [b] Concentration necessary for 50% inhibition (IC_{50}).

publications.[16,17] The results are shown in Table 3. According to the outcomes of cytotoxic assays, diterpenoid **1** showed cytotoxicity towards human promyelocytic leukemia HL-60 cells, with an IC_{50} value of 38.01 μM.

3 Conclusions

In this study, the chemical composition of an octocoral identified as *Sinularia* sp. was screened, resulted in the isolation of a novel diterpenoid, sinulariaone A (**1**). It is to note that diterpenoid **1**, involving an uncommon 13-membered carbocyclic carbon system, which was suggested biosynthesized from the common 14-membered carbocyclic cembrane analogues by ring contraction,[6,7] however, to be one of a kind, this is the first time to obtain a 13-membered carbocyclic cembranolide analogue featuring with an acetyl group at C-3. The structure of **1**, including the absolute configuration, was determined by spectroscopic methods and further confirmed by a single-crystal X-ray diffraction analysis and this compound showed cytotoxicity toward the HL-60 tumor cells. In addition, the absolute configuration of a known cytotoxic cembranoid, chlorofurancembranoid B (**2**), was determined using a single-crystal X-ray diffraction analysis with the molybdenum radiation source, with the material obtained in previous study.[5]

4 Experimental

4.1 General experimental procedures

Optical rotation values were measured using a JASCO P-1010 digital polarimeter. IR spectra were obtained with a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. NMR spectra were recorded on a 400 MHz Jeol ECZ NMR spectrometer using the residual $CHCl_3$ (δ_H 7.26 ppm) and $CDCl_3$ signals (δ_C 77.0 ppm) as internal standards for ^1H and ^{13}C NMR, respectively; coupling constants (J) are presented in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solariX FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck). TLC was performed on plates precoated with silica gel 60 F_{254} (Merck) and RP-18W/UV_{254} (0.15 mm-thick, Macherey-Nagel), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots.

4.2 Animal material

Specimens of *Sinularia* sp. were collected on Turtle Island, Yilan County, Taiwan. The samples were stored in a freezer at −20 °C until extraction. A voucher specimen was deposited in the National Museum of Marine Biology & Aquarium, Taiwan (NMMBA-TW–SC–2018-0619). Identification of this organism was performed by comparison with previous descriptions.[1,18]

4.3 Extraction and isolation

Freeze-dried and sliced bodies (wet/dry weight = 510/172 g) of the coral specimens were extracted with a mixture of MeOH/CH_2Cl_2 (1 : 1) to give 17.8 g of crude extract, which was partitioned between EtOAc and H_2O. The EtOAc extract (6.8 g) was subjected to silica gel column chromatography (Si C. C.) and eluted with gradients of *n*-hexane/EtOAc (100% *n*-hexane–100% EtOAc, stepwise) to furnish 14 sub-fractions A–N. Fraction D was chromatographed by Si C. C. and eluted with a mixture of CH_2Cl_2/EtOAc (20 : 1) to obtain 18 sub-fractions D1–D18. Fraction D13 was further separated by Si C. C. and eluted with CH_2Cl_2 to afford **1** (3.5 mg).

4.4 Structural characterization of undescribed compound

4.4.1 Sinulariaone A (1). Colorless prisms (MeOH); mp 102–104 °C; [α] + 236 (c 0.05, $CHCl_3$); IR (KBr) ν_{max} 1716 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data (see Table 1); ESIMS: m/z 327 $[M + Na]^+$; HRESIMS m/z 327.22928 (calcd for $C_{20}H_{32}O_2$ + Na, 327.22945).

4.5 Single-crystal X-ray crystallography of sinulariaone A (1)

Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.600 × 0.484 × 0.138 mm^3) was identified as being of the orthorhombic system, space group $P2_12_12_1$ (#19), with a = 9.0173(3) Å, b = 10.8659(3) Å, c = 18.6542(6) Å, V = 1827.76(10) $Å^3$, Z = 4, D_{calcd} = 1.106 Mg m^{-3} and λ (Cu Kα) = 1.54178 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a θ_{max} of 69.999°. All measurement data of 18 147 reflections were collected, of which

3472 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[19,20] The refined structural model converged to a final $R_1 = 0.0601$; $wR_2 = 0.1637$ for 3095 observed reflections [$I > 2\sigma(I)$] and 199 variable parameters; and the absolute configuration was established from the Flack parameter $x = 0.0(3)$.[14,15] Crystallographic data for the structure of sinulariaone A (**1**) were submitted to the Cambridge Crystallographic Data Center (CCDC) with supplementary publication number CCDC 2226689 (data can be obtained from the CCDC website at https://www.ccdc.cam.ac.uk/conts/retrieving.html).

4.6 Single-crystal X-ray crystallography of chlorofurancembranoid B (2)

Suitable colorless prisms of **2** were obtained from a solution of MeOH. The crystal ($0.388 \times 0.145 \times 0.028$ mm^3) was identified as being of the triclinic system, space group $P1$ (#1), with $a = 10.1046(4)$ Å, $b = 10.4180(3)$ Å, $c = 10.9661(4)$ Å, $V = 1066.93(7)$ Å^3, $Z = 2$, $D_{calcd} = 1.167$ Mg m^{-3} and λ (Mo Kα) = 0.71073 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a θ_{max} of 29.998°. All measurement data of 38 323 reflections were collected, of which 12 414 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[19,20] The refined structural model converged to a final $R_1 = 0.0561$; $wR_2 = 0.1188$ for 8865 observed reflections [$I > 2\sigma(I)$] and 468 variable parameters; and the absolute configuration was established from the Flack parameter $x = -0.01(3)$.[14,15] Crystallographic data for the structure of chlorofurancembranoid B (**2**) were deposited with the Cambridge Crystallographic Data Center (CCDC) as supplementary publication number CCDC 2208811 (data can be obtained from the CCDC website at https://www.ccdc.cam.ac.uk/conts/retrieving.html).

4.7 *In silico* calculations

The conformational search and calculated SOR results were carried out with the same method published as ref. 11–13. The brief procedure was described as follows. First, we optimized the minimized energy of the structure in the MM2 level and outputted an xyz file. Then, we submitted the file into spartan'16 software (Wavefunction Inc.; Irvine, CA, USA) at MMFF94 to generate conformational search results. The output data were imported into the Gaussian 09 software (Gaussian Inc.; Wallingford, CT, USA) and optimized using the time-dependent density functional theory (TDDFT) methodology at the B3LYP/6-31G* level in the gas phase and the B3LYP/6-31(d) levels in the solvent phase for SOR calculation, and the GIAO-DFT at the PCM/mpw1pw91/6-311 + g(d,p) level in the solvent phase for GIAO-NMR DP4+ analysis. The results were averaged by the proportion of each conformer.

4.8 *In vitro* cytotoxic assay

The cytotoxicity assay used in this study was performed as described in previous publications.[16,17]

Author contributions

Hsuan-Jung Tseng: investigation, analysis of results. Liang-Mou Kuo: investigation, analysis of results. Yu-Chi Tsai: investigation, software, modelling and simulation. Hao-Chun Hu: software, modelling and simulation. Po-Jen Chen: data curation, methodology. Su-Ying Chien: formal analysis, X-ray analysis. Jyh-Horng Sheu: conceptualization, supervision, visualization. Ping-Jyun Sung: analysis of results, conceptualization, visualization, supervision, writing-original draft, writing-reviewing and editing.

Conflicts of interest

There are no conflicts to declare.

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