The Journal of Antibiotics (2019) 72:15–21
https://doi.org/10.1038/s41429-018-0106-3

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

ARTICLE

Cyathane diterpenoids and drimane sesquiterpenoids with neurotrophic activity from cultures of the fungus *Cyathus africanus*

Rong-Wei Kou[1] · Shuang-Tian Du[2] · Yi-Xiao Li[1] · Xi-Tao Yan[1] · Qiang Zhang[1] · Chen-Yu Cao[1] · Xia Yin[1] · Jin-Ming Gao[1]

Received: 7 May 2018 / Revised: 11 September 2018 / Accepted: 21 September 2018 / Published online: 16 October 2018

Abstract

Five terpenoids, including two new cyathane diterpenoids neocyathin S (**1**) and neocyathin T (**2**), together with three drimane sesquiterpenoids, one known 3β,6β-dihydroxycinnamolide (**3**), two new ones 3β,6α-dihydroxycinnamolide (**4**) and 2-keto-3β,6β-dihydroxycinnamolide (**5**), were isolated from the cultures of the basidiomycete *Cyathus africanus*. Their structures were established based on extensive spectroscopic methods including 2D NMR (HSQC, ^{1}H–^{1}H-COSY, HMBC, ROESY) and HRESIMS experiments. The absolute configurations of two pairs of epimers, **1** and **2** as well as **3** and **4**, were determined by ECD quantum chemical calculation. All the five compounds enhanced nerve growth factor (NGF)-mediated neurite outgrowth using rat pheochromocytoma (PC12) cells at concentration 10 μM.

Introduction

Cyathus, well-known as the "bird's nest fungi", belongs to the fungus in the Nidulariaceae. The characteristic metabolites from genus *Cyathus* were related to the cyathane-type diterpenoids with a contiguous 5/6/7 tricyclic skeleton. Most of these diterpenoids have been proven to display a wide range of fascinating biological properties including anti-microbial, anti-inflammatory, anti-proliferative activities and most importantly stimulating the synthesis of neurotrophic factors (NGF) [1–4]. As a part of our research for therapeutic agents for neurogenerative disorders, we have studied several *Cyathus*, *Sarcodon*, and *Hericium* species, focusing on the chemical diversity of the cyathane diterpenoids [5–9] and the signaling pathways of their neurotrophic activity [9, 10], our current investigation on the solid cultures of *C. africanus* 5.1117 led to the isolation of two new cythane-type diterpenoids, Neocyathin S and T. In addition, unexpectedly, it is the first time that three drimane-type sesquiterpenoids were obtained from genus *Cyathus*, including one known 3β,6β-dihydroxycinnamolide (**3**) [11] and two new congeners, 3β,6α-dihydroxycinnamolide (**4**) and 2-keto-3β,6β-dihydroxycinnamolide (**5**) (Fig. 1).

Results and discussion

Compound **1** was isolated as colorless oil, with the molecular formula of $C_{21}H_{34}O_6$ based on the HRESIMS at m/z 381.2301 $[M–H]^-$, indicating four degrees of unsaturation. Its ^{13}C NMR data (Table 1) revealed 21 carbon resonances, corresponding to five methyls (including one methoxy), five methylenes (including one oxygenated), five methines (including three oxygenated) and six quaternary carbons (including one oxygenated, two olefinic, and one ketal carbon). The above data coupled with the ^{1}H NMR (Table 1) indicated that compound **1** was a cyathane-type diterpenoid substituted by a methoxy, which was further identified as analog of neocyathin P by a literature survey

These authors contributed equally: Rong-Wei Kou, Shuang-Tian Du.

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0106-3) contains supplementary material, which is available to authorized users.

✉ Xia Yin
yinxiabb@outlook.com

✉ Jin-Ming Gao
jinminggao@nwsuaf.edu.cn

[1] Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, 3 Taicheng Road, Yangling, Shaanxi 712100, China

[2] College of Life Sciences, Northwest A&F University, 3 Taicheng Road, Yangling, Shaanxi 712100, China

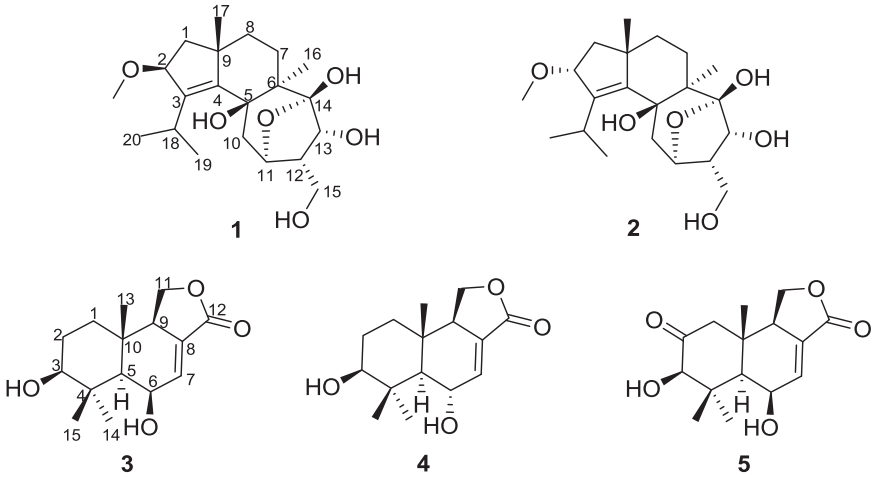

Fig. 1 Structures of compounds **1–5**

Table 1 ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **1–2** (CD_3OD)

Entry	1		2	
	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)	δ_C mult.
1	1.82, d(13.3); 1.45, dd(13.3, 6.4)	47.9, CH_2	2.14, m; 1.35, overlap	51.0, CH_2
2	4.12, d(6.4)	85.8, CH	4.45, dd, (7.2)	87.6, CH
3		141.9, qC		142.6, qC
4		144.9, qC		142.4, qC
5		80.5, qC		80.5, qC
6		47.2, qC		46.3, qC
7	2.07, m; 1.33, overlap	25.6, CH_2	2.09, m; 1.35, overlap	25.8, CH_2
8	1.58, m; 1.52, dd(13.5, 4.4)	37.9, CH_2	1.64, m; 1.56, m	37.6, CH_2
9		48.4, qC		46.9, qC
10	2.29, m	31.3, CH_2	2.40, m; 2.24, m	31.0, CH_2
11	4.41, ddd(8.7, 2.5, 2.5)	70.4, CH	4.40, ddd, (8.5, 2.2, 2.2)	70.5, CH
12	2.48, m	47.0, CH	2.46, m	46.8, CH
13	4.14, br. s	75.9, CH	4.11, br. s	75.9, CH
14		107.3, qC		107.1, qC
15	3.74, dd(10.7, 2.2); 3.64, dd (10.7, 1.1)	61.4, CH_2	3.72, dd(10.8,1.5); 3.66, brd (10.8)	61.4, CH_3
16	1.05, s	16.8, CH_3	1.16, s	16.7, CH_3
17	1.32, s	31.4, CH_3	1.17, s	30.3, CH_3
18	3.02, m	27.3, CH	3.04, m	27.5, CH
19	1.07, d(6.9)	23.3, CH_3	1.11, d (6.7)	23.6, CH_3
20	1.04, d(6.9)	22.4, CH_3	1.06, d (6.7)	19.7, CH_3
OCH_3	3.26, s	56.4, CH_3	3.24, s	56.0, CH_3

[6]. Detailed analysis of the HMBC and ^{1}H–^{1}H COSY spectrum revealed that compound **1** shared the same planar structure with neocyathin Q, which were supported by the ^{1}H–^{1}H COSY correlations of H-1/H-2, H-10/H-11/H-12/H-13/H-15 and H18/19/20, and the HMBC correlations from H-17 to C-1, C-4, C-8, C-9, H-16 to C-5, C-6, C-7, C-14, H-15 to C-12, and H-10 to C-5, C-6 (Fig. 2). Hence, compound **1** was supposed to be an isomer of neocyathin P. In the ROESY spectrum, cross peaks between H-1$_\beta$ (δH 1.84) and Me-17 (δH 1.33)/-OMe (δH 3.26) indicated that the methoxy should be β-orientated. The chemical shift of C-12 (δC 47.0) indicated that C-12 was α-orientated comparing with the literatures. According to the literatures and our previous results, α-oriented C-12 had δC12 in the range of 48.9–50.3 ppm, while β-oriented C-12 had δC12 around 56 ppm [12–14]. The result was also supported by the cross peaks between H-11 (δH 4.41) and H-12 (δH 2.48) in the ROESY spectrum. Finally, the structure of compound **1** was determined as shown, and the absolute configuration was further confirmed by ECD calculation (Fig. 3).

Compound **2** was isolated as colorless oil. It had the same molecular of $C_{21}H_{34}O_6$ as compound **1** on the base of HRESIMS at m/z 381.2296 $[M–H]^-$. The ^{1}H and ^{13}C NMR spectral data (Table 1) revealed that compound **2** was an epimer of **1**, and the methoxyl at C-2 in **2** was α-oriented. This conclusion was verified by HMBC correlations from H-17 to C-1, C-4, C-8, C-9, H-16 to C-5, C-6, C-7, C-14, H-15 to C-12, H-13 to C-11, C-14 and H-10 to C-5, C-6 and ^{1}H-^{1}H COSY correlations between H-1/H-2, H-18/H-19, H-18/H-20, H-11/H-12/H-13, and H-12/H-15 (Fig. 2). The configuration of the methoxyl was supported by the NOESY correlations of Me-17 (δH 1.17)/H-2 (δH 4.45), H-17/H-7$_\beta$ (δH 2.09)/H-13 (δH 4.11), and Me-16/H-10α. Detailed analysis of the 2D NMR indicated that other parts of compound **2** were the same with those of compound **1**. Its structure was finally confirmed by ECD (Fig. 3), and named neocyathin T.

Compound **4**, colorless oil, had the molecular formula of $C_{15}H_{22}O_4$ base on the HRESIMS at m/z 289.1411 $[M + Na]^+$. ^{1}H and ^{13}C NMR spectra (Table 2) in

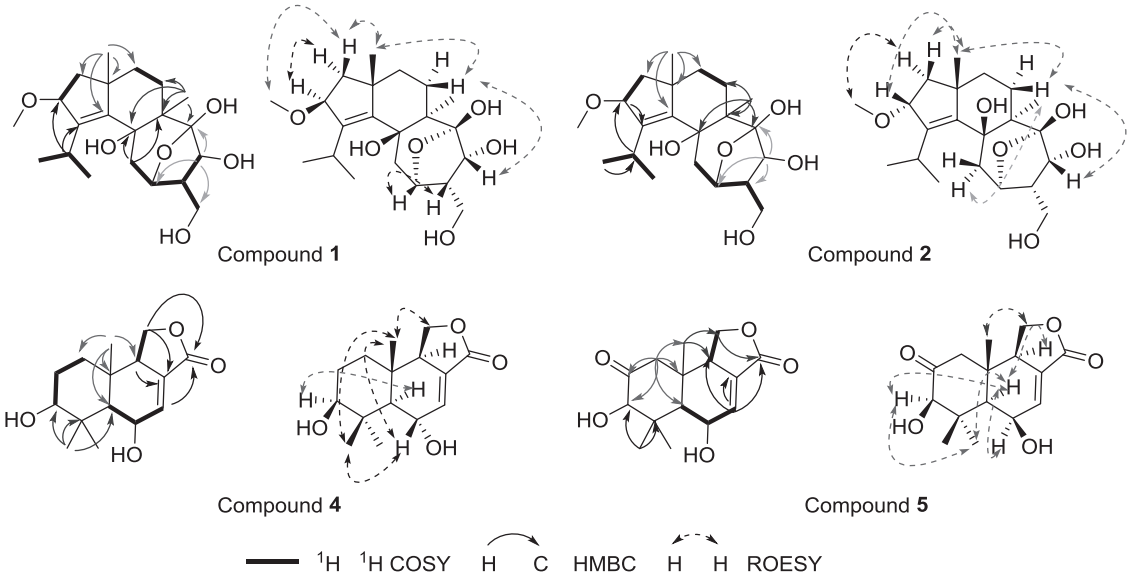

Fig. 2 Key COSY, HMBC, ROESY correlations of compounds **1, 2, 4**, and **5**

combination with HSQC spectrum demonstrated 15 carbons, which were classified as three methyls (three singlet in ^{1}H NMR), three methylene's (one oxygenated), five methines (two oxygenated, one olefinic) and four quaternary carbons (one olefinic and one lactone). The NMR data together with the molecular formula indicated that **4** was a drimane-type sesquiterpenoid and an epimer of **3** [8]. This conclusion was supported by the ^{1}H-^{1}H COSY correlations of H-1/H-2/H-3, H-5/H-6/H-7, and H-9/H-11 and the HMBC correlations of Me-13 to C-1, C-5, C-9 and C-10, H-15 to C-4, C-3 and C-5, and H-11 to C-9, C-1, C-8 and C-12 (Fig. 2). The NOESY correlations of Me-13 (δH 0.87)/ Me-15 (δH 1.02), Me-13/ H-6 (δH 4.52) indicated that the configuration of HO- at C-6 in **4** should be *α*-oriented instead of *β*-orientated in **3**. In-depth analysis of 2D NMR suggested that the other parts were the same to those of 3*β*,6*β*-dihydroxycinnamolide (**3**). Finally, the ECD confirmed the difference between **4** and **3**, and **4** was identified as 3*β*,6*α*-dihydroxycinnamolide (Fig. 3).

Compound **5** was isolated as colorless oil. Its molecular formula was determined to be $C_{15}H_{20}O_5$ base on the HRESIMS at m/z 279.1237 $[M–H]^-$, 14 units higher than compounds **3** and **4**. Preliminary analysis of 1D NMR suggested that one methylene was oxygenated into a ketone in compound **5** comparing with that of **3**. Careful analysis of HMBC spectrum indicated that C-2 should be the ketone supported by the correlations of H-1 to C-2, C-3, C-10, and C-13. The correlations of H-5/H-6/H-7 and H-9/H-11 in the COSY spectrum and the correlations from H-13 to C-9, C-10 and C-11, H-7 to C-8, C-9 and C-12, and H-11 to C-12 suggested that the other parts of compound **5** remained the same as those of **3**. The configuration of HO-3, HO-6, and C-11 was determined to be *β*, *β*, and *β* according to the correlations of Me-13 (δH 1.05)/H-11 (δH 4.16), and H-5 (δH 1.95)/H-3 (δH 4.08), H-5/H-6 (δH 4.79), H-5/H-9 in NOESY spectrum (Fig. 2). Therefore, compound **5** was identified as 2-keto-3*β*,6*β*-dihydroxycinnamolide.

All five compounds were tested for their enhancement of NGF-induced neurite outgrowth in PC12 cells. They all enhanced neurite formation induced by NGF at concentration 10 μM (Fig. 4).

In summary, two new cyathane-type diterpenoids, neocyathins S and T (**1**, **2**), and three drimane-type sesquiterpenoids (**3–5**) were isolated from cultures of a fungal strain of *Cyathus afticanus*. Besides, it is the first report that drimane sesquiterpenoids have been obtained from genus *Cyathus*. These metabolites were found to exert neurite outgrowth-promoting activity in NGF-mediated PC12 cells. Basing on our previous research, normally, cyathane diterpenoids combined with NGF showed significant increase in neurite-bearing cells compared to NGF-treated PC-12 cells, and few of them was active without NGF. In this study, the NGF-mediated neurite outgrowth activities of compounds **1** and **2** are much weaker than their analogs and it might be the result of the substituted hydroxyl at C-5. Further research is needed to explore why the cyathane diterpenoids and drimane sesquiterpenoids with different scaffolds show similar phenotypes.

Experimental section

General experimental procedures

Optical rotations (OR) were recorded on an Autopol III automatic polarimeter (Rudolph Research Analytical). UV

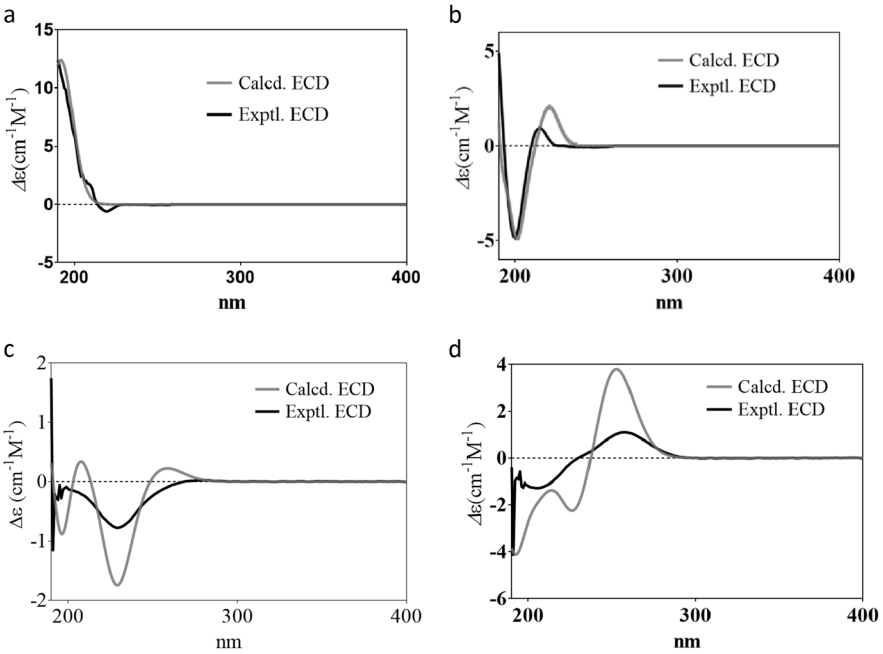

Fig. 3 Calculated and experimental ECD spectra of compounds **1** (**a**), **2** (**b**), **3** (**c**), and **4** (**d**)

and the IR spectra were obtained on a Thermo Scientific Evolution-300 UV–visible spectrophotometer and a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance III 500 spectrometers with tetramethylsilane (TMS) as an internal standard at room temperature. High-resolution (HR) ESIMS were recorded on an AB Sciex Triple TOF 4600 system. ECD spectra were obtained on a Chirascan spectrometer. Silica gel (300–400 mesh, Qingdao Marine Chemical Ltd., People's Republic of China) and RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd., Japan) were used for column chromatography (CC). Semi-preparative HPLC was performed on a Waters 1525 liquid chromatography system equipped with a Hypersil BDS C-18 column (4.6 mm × 250 mm, and 10.0 mm × 250 mm). Fractions were monitored by TLC. Spots were visualized by heating silica gel plates immersed in 10% H_2SO_4 in ethanol. Nutrient mixture F-12 (Ham) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Horse serum (HS) was purchased from Gibco (Life Technologies Corporation, USA). FBS was purchased from Hyclone (Thermo Scientific, China). The growth substrates poly-L-lysine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Nerve growth factor (NGF) was purchased from WuHan Hitech Biological Pharma Co., LTD, China.

Fungal material

The fungus of *Cyathus africanus* was purchased from China General Microbiological Culture Collection Center (CGMCC) with the accession number 5.1117. A voucher specimen of *C. africanus* was deposited in the College of Chemistry & Pharmacy, Northwest A&F University (No. Ca 5.1117).

Extraction and isolation

The *C. africanus* was cultured on the stilling medium of rice for 2 months, and then extracted with methanol. The solutions were filtered and evaporated under reduced pressure, and diluted with water to 5 L. The filtrate was extracted three times with EtOAc. Finally, the organic extract was evaporated to dryness to obtain the crude extract (5.63 g).

The crude extract was subjected to silica gel CC with a $CHCl_3$/MeOH gradient solvent system (v/v, 100:1–1:1) to obtain five fractions (A–E). Fraction B (956.1 mg) was subjected to a RP-18 silica gel with stepwise gradient of MeOH/H_2O (v/v 50:50–100:0) to afford eight subfractions (B1–B8). B2 (177.9 mg) was separated on Sephadex LH-20 (MeOH) then subjected on prep-HPLC (MeOH–H_2O 20%) to get compound **3** (16 min, 5.2 mg) and **4** (20 min, 3.7 mg). B6 (142.3 mg) was subjected to silica gel CC with $CHCl_3$–MeOH solvent system (v/v, 60:1), followed by prep-HPLC (MeOH–H_2O 65%) to get compound **1** (34 min, 4.4 mg) and **2** (23 min, 8.1 mg). Compound **5** (3.0 mg) was obtained from subfraction C (415.7 mg) by using Sephadex LH-20 (MeOH) then on prep-HPLC (MeOH–H_2O 20%, 18 min).

Neocyathin S (1)

Colorless oil, $[\alpha]_D^{25} + 20.37$ (*c*0.545, MeOH); IR 3394, 2950, 2837, 2523, 1651, 1457, 1409, 1021 cm^{-1},; UV

Table 2 ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **3–5** (CD_3OD)

Entry	3		4		5	
	δ_H (*J* in Hz)	δ_C mult.	δ_H (*J* in Hz)	δ_C mult.	δ_H (*J* in Hz)	δ_C mult.
1	1.65, m; 1.38, m	39.9, CH_2	1.50, m; 1.44, m	37.9, CH_2	2.31, brs	53.0, CH_2
2	1.73, m; 1.59, m	27.8, CH_2	1.65, m	27.8, CH_2		210.6, qC
3	3.18, dd (11.6, 3.8)	79.9, CH	3.23, dd (11.0, 5.0)	79.4, CH	4.08, s	84.0, CH
4		40.8, qC		40.4, qC		45.9, qC
5	1.17, overlap	55.5, CH	1.44, overlap	57.7, CH	1.95, d (4.8)	54.2, CH
6	4.69, m	66.2, CH	4.52, m	68.6, CH	4.79, overlap	66.2, CH
7	6.71, dd (3.6, 3.6)	138.1, CH	6.64, dd (3.5, 3.5)	137.9, CH	6.77, dd (3.6, 3.6)	138.3, CH
8		128.5, qC		129.6, qC		128.3, qC
9	2.75, m	52.8 CH	2.96, m	50.9, CH	3.14, m	52.8, CH
10		34.9, qC		41.0, qC		40.5, qC
11	4.48, dd (9.2, 9.2) 4.15, dd (9.0, 9.0)	69.1, CH_2	4.43, dd 4.09, dd	68.8, CH_2	4.50, dd (9.3, 9.3) 4.16, dd (9.1, 9.1)	68.6, CH_2
12		172.6, qC		172.2, qC		171.9, qC
13	1.021, s	15.4, CH_3	0.87, s	14.7, CH_3	1.05, s	16.5, CH_3
14	1.28, s	27.4, CH_3	1.25, s	30.9, CH_3	1.35, s	26.7, CH
15	1.19, s	17.7, CH_3	1.02, s	16.3, CH_3	1.15, s	18.6, qC

(MeOH) λ_{max} (log ε) 213 (2.73); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOD-*d*$_4$), see Table 1; negative ion HRESIMS *m/z* 381.2301 [M–H]−(calcd for $C_{21}H_{33}O_6^-$, 381.2300).

Neocyathin T (2)

Colorless oil, $[\alpha]_D^{25}$ −21.15 (*c*0.260, MeOH); IR 3394, 2950, 2837, 1651, 1456, 1410, 1021 cm^{-1},; UV (MeOH) λ_{max} (log ε) 213 (3.11); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOD-*d*$_4$), see Table 1; negative ion HRESIMS *m/z* 381.2296 [M–H]$^-$(calcd for $C_{21}H_{33}O_6^-$, 381.2300).

3*β*,6*β*-dihydroxycinnamolide (3)

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOD-*d*$_4$), see Table 2.

3*β*,6*α*-dihydroxycinnamolide (4)

$[\alpha]_D^{25}$ + 40 (*c*0.400, MeOH); IR 3450, 2969, 1639, 1108, 1049 cm^{-1},; UV (MeOH) λ_{max} (log ε) 230 (3.19); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOD-*d*$_4$), see Table 2; positive ion HRESIMS *m/z* 289.1411 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na^+$, 289.1400).

2-keto-3*β*,6*β*-dihydroxycinnamolide (5)

$[\alpha]_D^{25}$ −64.7 (*c*0.420, MeOH); IR 3394, 2950, 2837, 1651, 1457, 1411, 1021 cm^{-1},; UV (MeOH) λ_{max} (log ε) 230 (3.25); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOD-*d*$_4$), see Table 2; negative ion HRESIMS *m/z* 279.1237 $[M–H]^-$ (calcd for $C_{15}H_{19}O_5^-$, 279.1200).

ECD calculation

A preliminary conformational search was performed in Conflex6.7 using MMFF94s forcefield [15]. Conformers were saved and further optimized using the density functional theory (DFT) method and CPCM solvent model at B3LYP/6-31 + G(d,p) level in Gaussian 09 software package [16]. Frequency was calculated at the same level of theory to check optimized results. The stable conformers with populations greater than 1% and without imaginary frequencies were submitted to ECD calculation by the TDDFT using cam-B3LYP/TZVP method associated with CPCM solvent model in MeCN. The excitation energies (E), oscillator strength (f), rotatory strength in velocity form (Rvel), and rotatory strength in length form (Rlen) of the lowest 32 excited states were calculated. ECD spectra of different conformers were summated in SpecDis according to their Boltzmann-calculated distributions [17].

Bioassay methods

Cell culture

The rat adrenal pheochromocytoma cell line, PC-12, were obtained from China Center for Type Culture Collection (CCTCC). PC-12 cells were maintained in nutrient mixture F-12 (Ham) medium supplemented with 10% inactivated HS, 5% inactivated fetal bovine serum (FBS), 100 U/mL penicillin G, 100 µg/mL streptomycin, and 2.5 g/L sodium

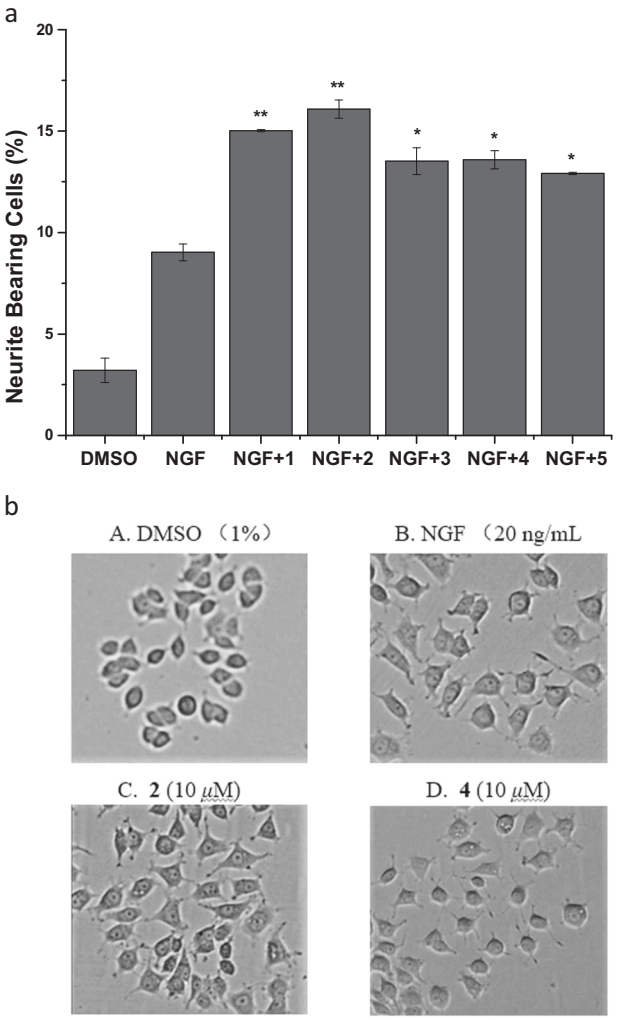

Fig. 4 **a** Effect of compounds **1**–**5** on neurite outgrowth in PC12 cells. (NGF 20 ng/mL as positive control, $^{**}p < 0.01$, $^{*}p < 0.05$); **b** Effect of compounds **2** and **4** as examples on neurite outgrowth in PC12 cells treated with 1% DMSO, NGF (20 ng), NGF(20 ng) + **2** (10 μM), and NGF(20 ng) + **4** (10 μM)

bicarbonate at 37 °C in humidified air containing 5% CO_2 [18].

Analysis of neurite outgrowth of PC-12 cells

Morphological analysis and quantification of neurite-bearing cells were carried out using phase-contrast microscope as described previously. PC-12 cells were seeded on poly-L-lysine-coated 24-well plates at a density of 2×10^4 cells/mL in normal serum medium for 24 h. The F-12 medium containing low serum (1% HS and 0.5% FBS) was replaced prior to exposure to vehicle (0.1% DMSO) or indicated reagents. The cells were treated with tested compounds at various concentrations of 10 μM with 20 ng/mL of NGF. Cells without treatment served as a negative control. Cells treated with 20 ng/mL of NGF served as a positive control. One concentration experiment was repeated in three wells. After an additional 48 h of incubation, neurite outgrowth of PC-12 cells was observed under an inverted microscope using phase-contrast objectives and photographed by the digital camera. Five images were selected randomly under a microscope for each well. At least 100 cells in each of five randomly separated fields were scored and the proportion of cells with neuritis greater than or equal to the length of one cell body were positive for neurite outgrowth and expressed as a percentage of the total cell number in five fields. Experiments were repeated at least three times and data are as mean ± SD [19].

Acknowledgements This work was financially supported by National Natural Science Foundation of China (81502938, 21572182, 21503168), and the Fundamental Research Funds for the Central Universities (No. 2452016094).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Tang HY, Yin X, Zhang CC, Jia Q, Gao JM. Structure diversity, synthesis, and biological activity of cyathane diterpenoids in higher fungi. Curr Med Chem. 2015;22:2375–91.
2. Kita T, Takaya Y, Tomihisa YO. Scabronines B, C, D, E and F, novel diterpenoids showing stimulating activity of the nerve growth factor synthesis, from the mushroom Sarcodon scabrosus. Tetrahedron. 1998;54:11886–7.
3. Hitro M, Morimura K, Shibata H. Anti-inflammatory compounds from the bitter mushroom, Sarcodon scabrosus. Biosci Biotechnol Biochem. 2002;68:1362–5.
4. Obara Y, Nakahata N, Kita T, Takaya Y, Kobayashi H, Hosoi S, et al. Stimulation of neurotrophic factor secretion from 1321N1human astrocytoma cells by novel diterpenoids, scabronines A and G. Eur J Pharmacol. 1999;370:79–84.
5. Bai R, Zhang CC, Yin X, Wei J, Gao JM, Striatoids A-F. cyathane diterpenoids with neurotrophic activity from cultures of the fungus Cyathus striatus. J Nat Prod. 2015;78:783–8.
6. Wei J, Cheng YY, Guo WH, Wang DC, Zhang Q, Li D, et al. Molecular diversity and potential anti-neuroinflammatory activities of cyathane diterpenoids from the basidiomycete *Cyathus africanus*. Sci Rep. 2017;7:8883.
7. Wei J, Guo WH, Cao CY, Kou RW, Xu YZ, Gorecki M, et al. Polyoxygenated cyathane diterpenoids from the mushroom *Cyathus africanus*, and their neurotrophic and anti-neuroinflammatory activities. Sci Rep. 2018;8:2175.
8. Shi XW, Liu L, Gao JM, Zhang AL. Cyathane diterpenes from Chinese mushroom *Sarcodon scabrosus* and their neurite outgrowth-promoting activity. Eur J Med Chem. 2011;46:3112.
9. Zhang C-C, Cao C-Y, Kubo M, Harada K, Yan X-T, Fukuyama Y, et al. Chemical constituents from *Hericium erinaceus* promote neuronal survival and potentiate neurite outgrowth via the TrkA/Erk1/2 pathway. Int J Mol Sci. 2017;18:1659.
10. Cao CY, Zhang CC, Shi XW, Li D, Cao W, Yin X, et al., Sarcodonin G derivatives exhibit distinctive effects on neurite outgrowth by modulating NGF signaling in PC12 cells. ACS Chem. Neurosci. https://doi.org/10.1021/acschemneuro.7b00488.

11. Guo HY, Lu TD, Li ZH, Wang G, Liu JK. A new drimane-type sesquiterpenoid from fermentation broth of Fomitiporia punicata. Nat Prod Res Dev. 2014;26:6–10.
12. Shiono Y, Hiramatsu F, Murayama T, Koseki T, Funakoshi T. Two cyathane-type diterpenoids from the liquid culture of Strobilurus tenacellu. Chem Biodivers. 2008;5:1811–6.
13. Xu Z, Yan S, Bi K, Han J, Chen Y, Wu Z, et al. Anti-inflammatory and cytotoxic cyathane diterpenoids from the medicinal fungus Cyathus hookeri Berk. Fitoterapia. 2013;86:159–62.
14. Wang B, Han J, Xu W, Chen Y, Liu H. Production of bioactive cyathane diterpenes by a bird's nest fungus Cyathus gansuensis growing on cooked rice. Food Chem. 2014;152:169–76.
15. Conflex 6.7, Conflex Corp: Tokyo Yokohama, Japan, 2010.
16. Frisc MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, et al. Gaussian 09 revision D.01; Gaussian Inc.: Wallingford CT, 2013.
17. Bruhn T, Schaumloeffel A, Hemberger Y, Bringmann G. SpecDis: quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality. 2013;25:243–9.
18. Lin CW, Wu MJ, Liu IYC, Su JD, Yen JH. Neurotrophic and cytoprotective action of luteolin in PC12 cells through ERL-dependent induction of Nrf2-deiven HO-1 expression. J Agric Food Chem. 2010;58:4477–86.
19. Suzuki-Mizushima Y, Gohda E, Okamura T, Kanasaki K, Yamamoto I. Enhancement of NGF- and cholera toxin-induced neurite outgrouth by butyrate in PC12 cells. Brain Res. 2002;951:209–17.