SUPPLEMENTARY MATERIAL

Indigodole E from *Strobilanthes cusia* exhibits anti-IL-17A effect

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ABSTRACT

One new indazole alkaloid, indigodole E (**1**), was isolated from a traditional Chinese medicine Qing Dai prepared from the aerial parts of *Strobilanthes cusia*. The structure of **1** was elucidated by NMR, MS, UV, and IR spectra as well as optical rotation. Additionally, compound **1** could obviously inhibit not only IL-17A protein production at concentrations from 1.25 to 2.5 μg/mL, but also IL-17 gene expression at concentrations from 5.0 to 10.0 μg/mL without cytotoxicity toward Th17 and Jukat cells, respectively. Overall, indazole analogue **1** could be the anti-IL 17A contributor of Qing Dai in this investigation.

KEYWORDS: Qing Dai; *Strobilanthes cusia*; Acanthaceae; indigodole E; indazole alkaloid; IL-17A inhibition

Experimental in detail

1.1. Generals

NMR spectra were taken on Bruker Avance III 500 MHz. The chemical shift (δ) values are in ppm (part per million) with $CDCl_3$ as the solvent, and coupling constants (J) are in Hz. Low- and high-resolution ESIMS were measured on a Bruker Daltonics Esquire HCT ultra high capacity trap mass spectrometer, and an Orbitrap mass spectrometer (LTQ Orbitrap XL and Q Exactive Plus, Thermo Fisher Scientific), respectively. The optical rotation was recorded on a JASCO-P-2000 polarimeter (cell length 10 mm). UV and IR spectra were measured on a Perkin Elmer#Lambda265 and a Shimadzu model IR Prestige-21 Fourier- transform infrared spectrophotometer, respectively. TLC was performed on Kieselgel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) coated alumina plates, spots were viewed under ultraviolet light at 254 nm, and then stained by spraying with 5% H_2SO_4 in MeOH before heating on a hot plate. Silica gel (Silicycle 70–230 and 230–400 mesh, Sigma-Aldrich) and RP-18 (LiChroprep® 40–63 μm, Merck) were used for column chromatography.

1.2. T helper 17 (Th17) cell polarization and intracellular staining

Naïve CD4 T cells were isolated from spleens and peripheral lymph nodes harvested from BALB/c mice via EasySep™ Mouse T Cell Isolation Kit (STEMCELL Technologies Inc., Vancouver, Canada) according to the manufacturer's protocol as previously described (Ben-Shaanan et al., 2018). The kit is designed to isolate T cells from single-cell suspensions of splenocytes and lymphocytes by negative selection. Unwanted cells are targeted for removal with biotinylated antibodies directed against non-T cells and streptavidin-coated magnetic particles. Desired CD4+ T cells are isolated. 1×10^5 cells/well CD4+ T cells were cultured in 96 well plate coated with 5 μg/mL anti-CD3 Ab for 5 days in the following polarization condition: 1 μg/mL anti-CD28 Ab (Biolegend), 20 ng/mL IL-6 (PeroTech), 1.25 ng/mL TGF-β (PeroTech), 20 ng/mL IL-1β (PeroTech), 20 ng/mL IL-23 (R&D, USA), 20 μg/mL anti-IFN-γ Ab (Biolegend), and 20 μg/mL anti-IL-4 Ab (Biolegend). Culture medium used was IMDM (Gibco) supplemented with 1.0 mM sodium pyruvate (Gibco), 0.1 mM nonessential amino acids (Gibco), 100 IU/mL penicillin, 100 μg/mL streptomycin (Gibco), and 5% heat-inactivated FBS (Hyclone) (Harris et al., 2007; Yen et al., 2009).

For investigation of the level of IL-17A production after polarization, the polarized Th17 cells were treated with or without indicated compounds for 24 h. After incubation, cells were restimulated for 5 h in the presence of PMA (50 ng/mL), ionomycin (500 ng/mL), and GolgiStop (BD Biosciences). The IL-17A secreting cells were stained with anti-CD4 Ab (BD Biosciences) and anti-IL-17A Ab (BioLegend) and analyzed by a BD FACSVerse flow cytometer.

1.3. IL-17 luciferase reporter assay

The resultant IL-17 promoter-luciferase gene was cloned and constructed as described previously (Lee et al., 2019). Jurkat cells (4×10^6) were transfected with 10 μg IL-17 promoter-luciferase gene construct (pGL4-hIL-17prom) or control vector (pGL4.18) by electroporation according to the manufacturer's protocol (Neon transfection system, Invitrogen). Stable expression of IL-17 luciferase reporter from a clone of Jurkat cells were selected with G418 (800 μg/mL, GIBCO) for neomycin resistance. IL-17 luciferase reporter stably transfected cells (IL-17Luc cells) were seeded at the density of 1×10^5 cells/well in 96-well plate followed by treatment with PMA (50 ng/mL), ionomycin (500 ng/mL) and indicating compounds for 5 h. Assay medium was renewed and added a volume of Steady-Glo® Reagent equal to the volume of culture medium in the well for 30 min. Then the activity of luciferase in the transfected cells was measured with the BioTek Synergy microplate reader.

1.4. HPLC analysis of indigodole E

A SHIMADZU LC-20AT (Kyoto, Japan) equipped with a degasser, a binary pump, a DAD detector SPD–M20A, and a liquid handler SIL–20A autosampler was applied to analyze the chemical. The mobile phase consisted of water containing 0.1% formic acid (eluent A) and acetonitrile (eluent B). The gradient program was used as following: 10% to 76% B (22 min), 76% to 85% (10 min), 85% isocratic elution (5 min), 85% to 100% (5 min), 100% isocratic elution (8 min) with a HPLC column, COSMOSIL® 5C18–MS–II (5 μm, 4.6 × 250 mm I.D.) performed at room temperature. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The concentration of indigodole E was 0.1 mg/mL. UV/Vis spectra were recorded in the range of 200–800 nm.

Reference

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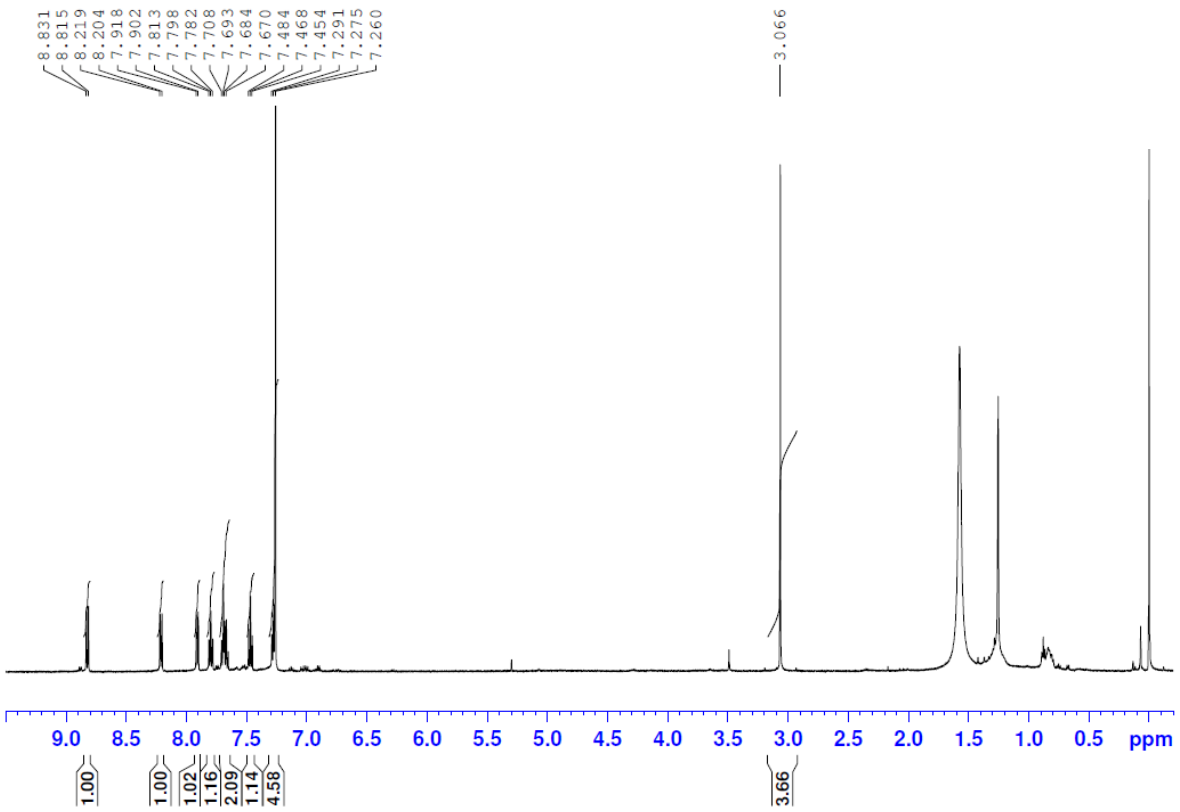

Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) of indigodole E.

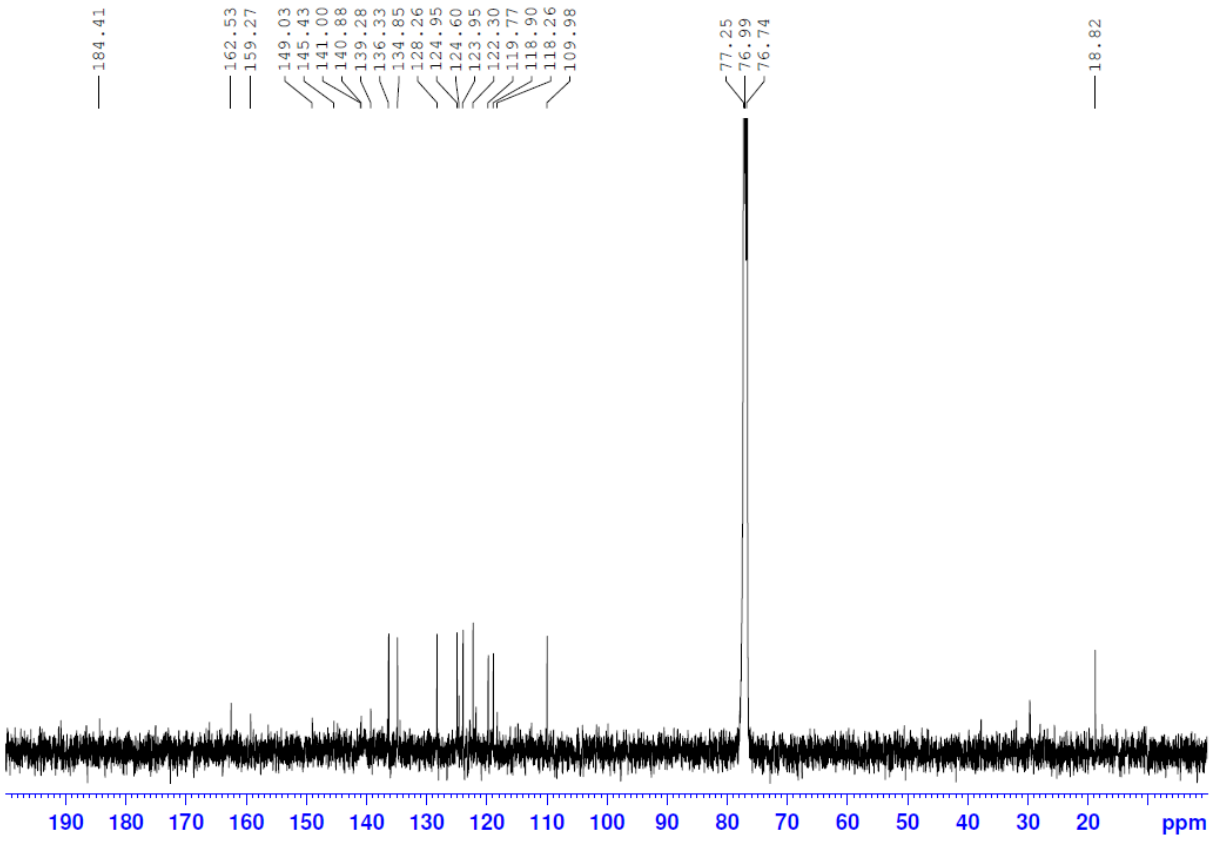

Figure S2. ^{13}C NMR (125 MHz, $CDCl_3$) of indigodole E.

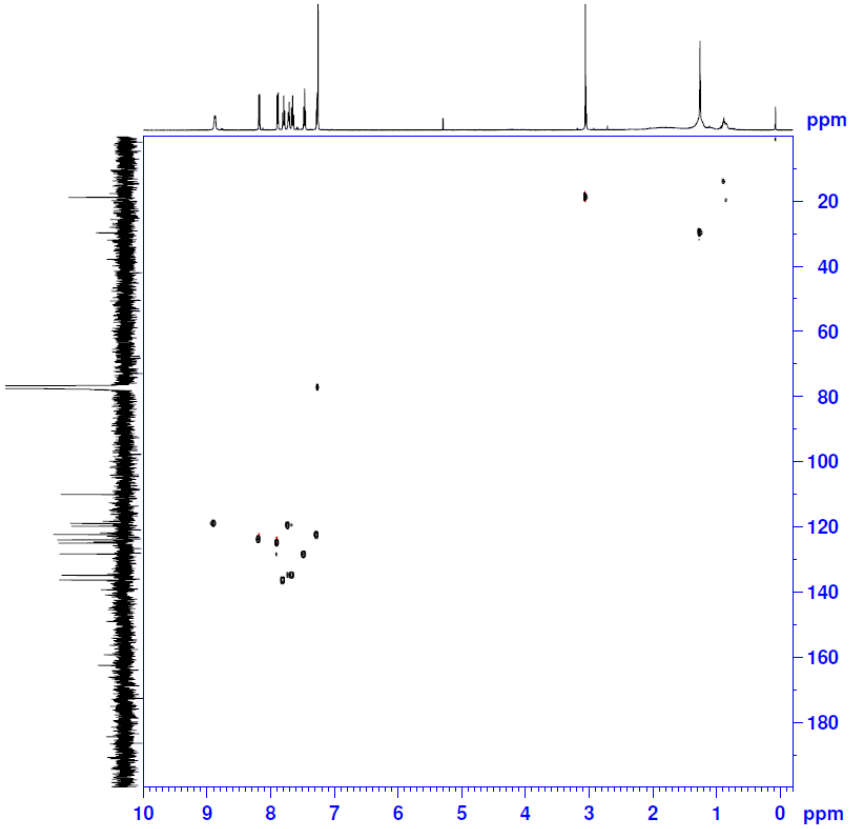

Figure S3. HSQC spectrum (500 MHz, $CDCl_3$) of indigodole E.

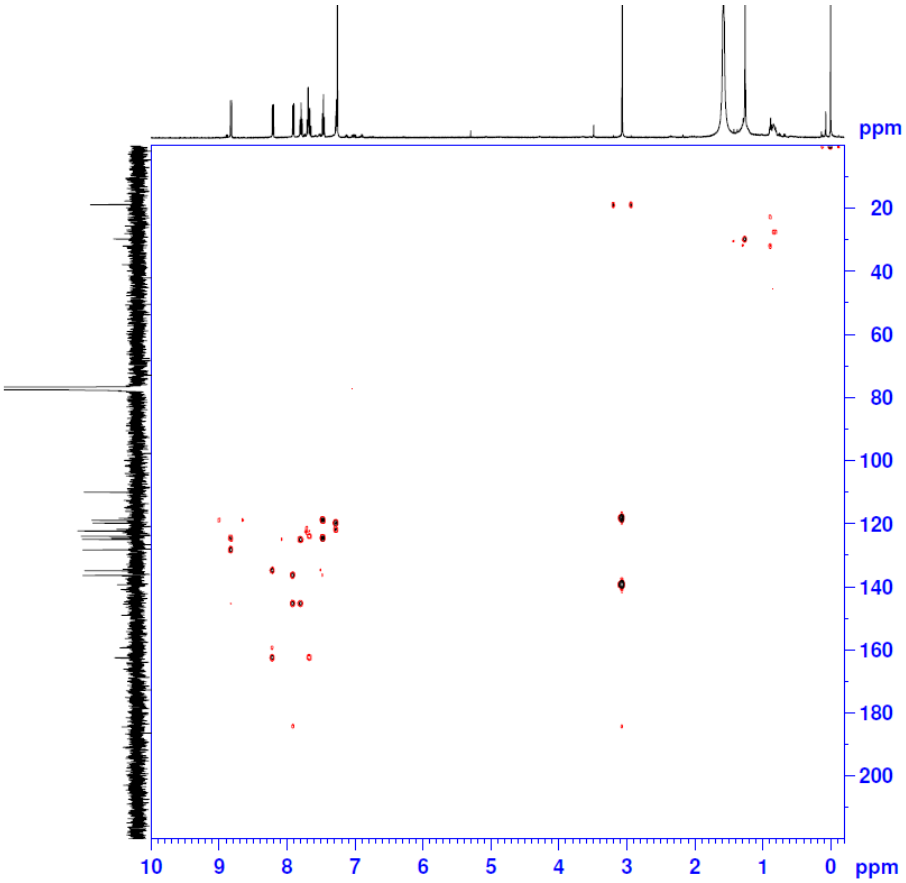

Figure S4. HMBC spectrum (500 MHz, $CDCl_3$) of indigodole E.

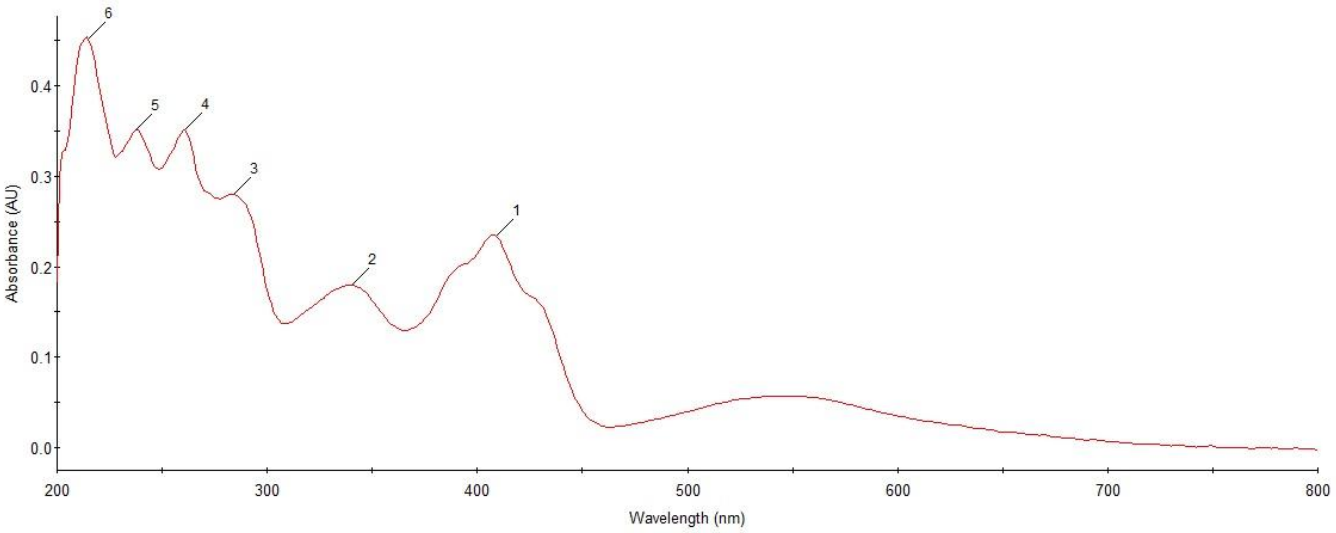

Figure S5. UV spectrum of indigodole E.

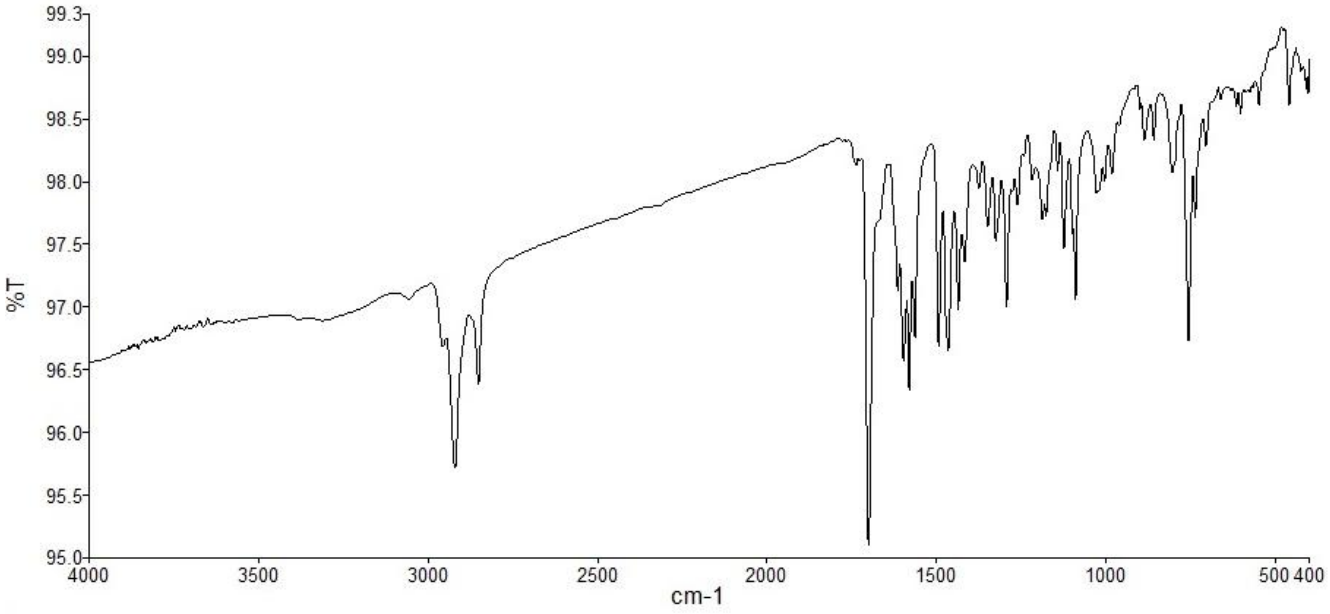

Figure S6. IR spectrum of indigodole E.

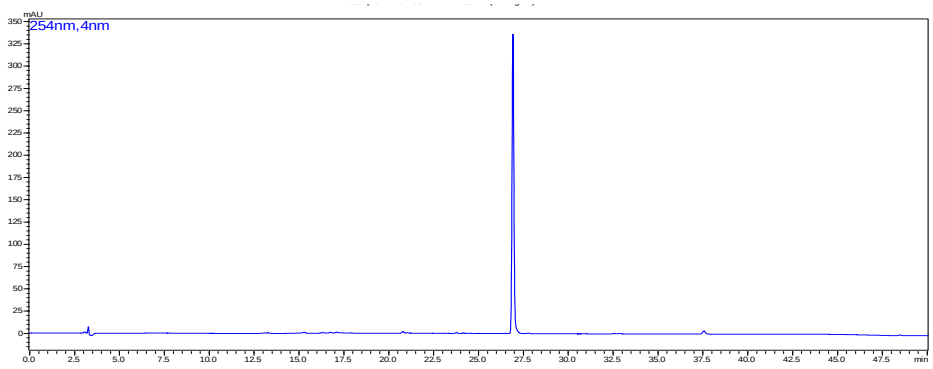

Figure S7. The HPLC retention time of indigodole E at 254 nm (Rt = 26.949 min).

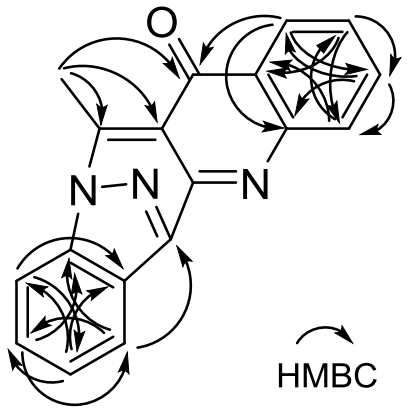

Figure S8. The key HMBC correlations of indigodole E.

Table S1 ^{1}H and ^{13}C NMR spectroscopic data (500 and 125 MHz, $CDCl_3$) for indigodole E.

	Indigodole E	
position	δ_H (J in Hz)	δ_C
1	7.89 (d, 7.5)	125.0
2	7.47 (t, 7.5)	128.5
3	7.80 (t, 7.5)	136.5
4	8.87 (d, 7.5)	119.0
4a		145.1
5a		140.9
6		158.7
6a		121.4
7	8.18 (d, 7.5)	123.9
8	7.28 (t, 7.5)	122.5
9	7.66 (t, 7.5)	134.9
10	7.73 (d, 7.5)	119.5
10a		161.0
11		139.9
11a		118.2
12		184.2
12a		124.5
13	3.05 (3H, s)	18.9

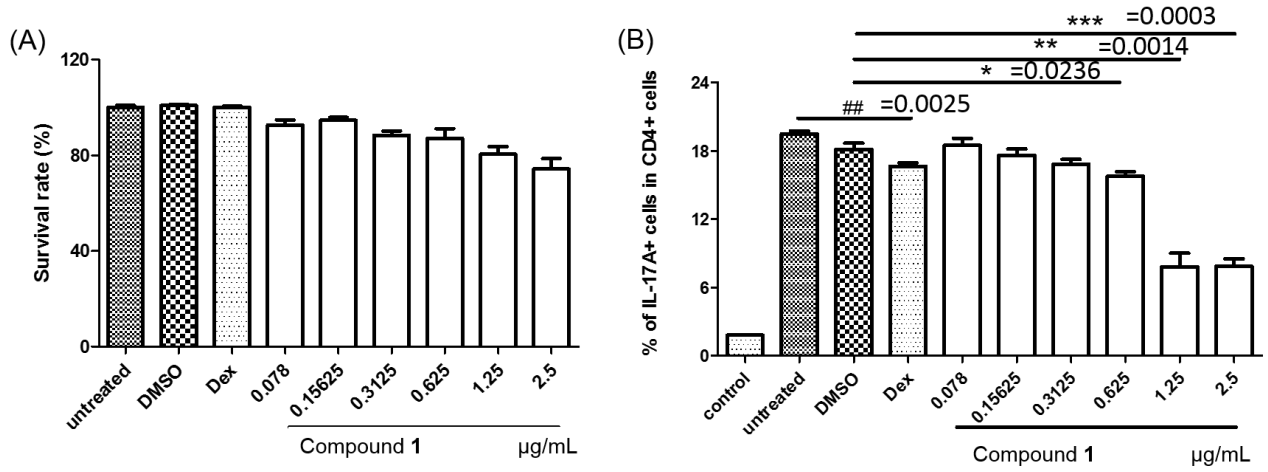

Figure S9. Cytotoxicity toward Th17 cells (A) and IL-17A protein expression (B) data of compound **1**. The polarized Th17 cells were treated with or without compound **1** for 24 h. After incubation, cells were restimulated with PMA (phorbol 12-myristate 13-acetate) and ionomycin for 5 h, and then cell viability was measured by staining with 1 μg/mL propidium iodide. The IL-17 secreting cells were stained with anti-CD4 Ab and anti-IL-17 Ab and then analyzed by flow cytometry. The data shown are representative of three independent experiments. ## *p* compared with the untreated group. **p*, ***p*, ****p* compared with the DMSO group. The concentration of dexamethasone (Dex) was 5 μg/mL.

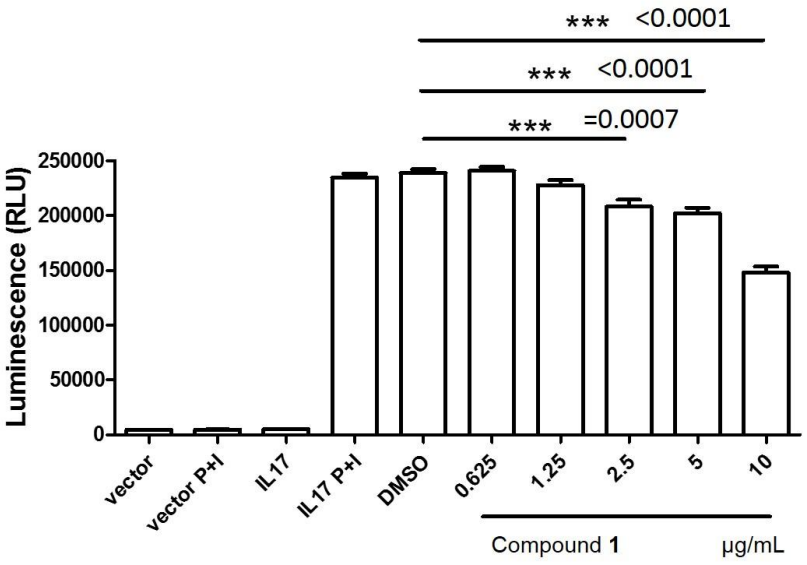

Figure S10. Compound **1** inhibited the IL-17 gene expression in the IL-17 luciferase reporter assay. IL-17Luc cells were stimulated with PMA and ionomycin, and co-treated with DMSO or compound **1** for 5 h. Then the activity of luciferase in the transfected cells was measured with the microplate reader. Vector means control vector (pGL4.18) transfected cells. IL 17 means IL-17 promoter-luciferase gene (pGL4-hIL-17prom) transfected cells. P+I means PMA and ionomycin. The data shown are representative of three independent experiments. ****p* compared with the DMSO group.