Abietane-type Diterpenoid Constituents from Tripterygium hypoglaucum with Cytotoxicity against Tumor Cells (Postprint)

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Abstract

To investigate the cytotoxic abietane-type diterpenoid constituents in Tripterygium hypoglaucum, this study employed chromatographic separation techniques including silica gel, microporous resin, and semi-preparative liquid chromatography to isolate and purify the ethyl acetate extract of Tripterygium hypoglaucum, identified their structures using NMR and MS, and evaluated the cytotoxicity of the compounds against SH-SY5Y human neuroblastoma cells, SW1990 human pancreatic cancer cells, and 4T1 mouse breast cancer cells by the CCK-8 assay. The results showed that: (1) Ten abietane-type diterpenoid compounds were isolated from Tripterygium hypoglaucum and identified as triptophenolide (1), isoneotriptophenolide (2), triptobenzene I (3), triptotin A (4), triptotin B (5), triptobenzene N (6), triptobenzene M (7), triptophenol F (8), triptobenzene A (9), and p-quinone 21 (10). Among them, compounds 4, 5, 6, and 7 were isolated from Tripterygium hypoglaucum for the first time. (2) Compound 1 exhibited cytotoxic effects on SH-SY5Y human neuroblastoma cells with an IC50 value of $(1.10\$\pm0.03)\mu mol\ L-$ $1; compounds 1, 7, and 8 showed cytotoxicity against SW1990 human pancreatic cancer cells with IC50 values of (0.$ $1, (9.26\pm1.39)\mu mol\ L-1, and (4.81\pm0.77)\mu mol\ L-1, respectively; compounds 7, 8, and 9 exhibited cytotoxicity ag$ $1, (0.79\pm0.05)\mu mol\ L-1, and (2.12\pm\$0.08)$ mol · L-1, respectively. These findings enrich the chemical constituents of Tripterygium hypoglaucum and provide a foundation for the study of its antitumor activity.

Full Text

Abietane Diterpenoids with Cytotoxic Activity from *Tripterygium hypoglaucum*

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Abstract: To investigate the cytotoxic abietane diterpenoids from *Tripterygium hypoglaucum*, the ethyl acetate extract was purified using chromatographic techniques including silica gel, MCI, and semi-preparative high-performance liquid chromatography (HPLC). Compound structures were identified by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), and cytotoxic activities were evaluated against SH-SY5Y human neuroblastoma cells, SW1990 human pancreatic cancer cells, and 4T1 mouse breast cancer cells using the CCK-8 assay. The results showed that: (1) Ten abietane diterpenoids were isolated and identified as triptophenolide (1), isoneotriptophenolide (2), triptobenzene I (3), triptotin A (4), triptotin B (5), triptobenzene N (6), triptobenzene M (7), wilforol F (8), triptobenzene A (9), and quinone 21 (10). Among these, compounds 4, 5, 6, and 7 were obtained from *T. hypoglaucum* for the first time. (2) Compound 1 exhibited cytotoxicity against SH-SY5Y cells with an IC_{50} value of $(1.10\$\pm0.03)\mu mol\cdot L\hat{}\{-1\}.Compounds1,7,and8showedcytotoxiceffectsonSW1990cellswithIC\{50\}\$$ *values of (0.47$*±0.02), (9.26±1.39), *and*(4.81±0.77)$\mu mol\cdot L\hat{}\{-1\}, respectively.Compounds7,8,and9demonstr$ values of $(3.98\$\pm0.73), (0.79\pm0.05), and(2.12\pm0.08)\mu mol\cdot L\hat{}\{-1\}\$$, respectively. These findings enrich the chemical profile of *T. hypoglaucum* and provide a foundation for further investigation of its antitumor potential.

Keywords: *Tripterygium hypoglaucum*; *Tripterygium*; chemical constituents; abietane diterpenoids; cytotoxicity

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Introduction

Tripterygium hypoglaucum, also known as "Kunming Mountain Begonia," is a member of the Celastraceae family and the genus *Tripterygium*. This plant is primarily distributed in southwestern China, as well as in Fujian, Zhejiang, and Hunan provinces (Pan et al., 2016). The dried roots and rhizomes of *T.*

hypoglaucum have been used traditionally in Chinese medicine for dispelling wind and dampness, promoting blood circulation and hemostasis, relaxing muscles and bones, and detoxification (Wang et al., 2023). Notably, the *Yunnan Anti-cancer Chinese Herbal Medicine* records its use in treating leukemia, osteosarcoma, lymphosarcoma, thyroid cancer, and lung cancer (Xie, 2016).

The characteristic chemical constituents of *T. hypoglaucum* are terpenoids, with triptolide—a diterpenoid containing a five-membered lactone ring—serving as a representative compound (Duan et al., 1997; Lin et al., 2015). The chemical composition and therapeutic indications of *T. hypoglaucum* are remarkably similar to those reported for the congeneric species *Tripterygium wilfordii* (Huang et al., 2010), both exhibiting anti-inflammatory and antitumor activities. The main constituents, including triptolide (Sun et al., 2017), celastrol (Yu et al., 2015), and triptonide, have been reported to possess broad-spectrum and highly potent antitumor activities through diverse mechanisms such as inhibiting tumor cell proliferation and inducing apoptosis. Miyata et al. (2005) demonstrated that phosphatidylinositol 3-kinase is a crucial molecular target for triptolide' s antitumor action, whereby reduced PI3K activity inhibits tumor cell proliferation. Triptonide effectively suppresses pancreatic cancer cell-mediated angiogenesis by decreasing promoter activity of VE-cadherin and chemokine ligand 2 genes, thereby inhibiting migration and invasion (Han et al., 2018). Additionally, triptonide inhibits prostate cancer cell invasion and migration by suppressing Caveolin-1 expression (Yuan et al., 2020). Celastrol also induces cell cycle arrest in breast cancer cells by reducing mRNA expression levels (Jang et al., 2011) and triggers apoptosis in myeloma U266 cells by inhibiting NF- B nuclear translocation and causing G1 phase arrest (Tozawa et al., 2011).

Our research group has previously investigated *T. hypoglaucum* and identified diverse chemical constituents with significant anti-inflammatory and antitumor activities (Chen et al., 2018; Chen et al., 2022). To further elucidate the chemical foundation and explore the bioactive constituents of this plant, we employed silica gel, MCI, and semi-preparative HPLC to fractionate the ethyl acetate extract of *T. hypoglaucum*. The isolated abietane diterpenoids were subsequently screened for cytotoxicity against SH-SY5Y human neuroblastoma cells, SW1990 human pancreatic cancer cells, and 4T1 mouse breast cancer cell lines. This study aimed to address two primary questions: (1) characterize the chemical constituents of *T. hypoglaucum*, and (2) evaluate the cytotoxic activity of the isolated compounds against selected tumor cell lines.

Materials and Methods

Plant Material

Dried roots and rhizomes of *Tripterygium hypoglaucum* were purchased from the Luosifen herbal medicine market in Kunming in November 2020. The material was authenticated by Professor Zhang Rongping of Yunnan University of Chi-

nese Medicine as *Tripterygium hypoglaucum* (Levl.) Hutch (voucher specimen No. 2019-0115) and stored at the School of Chinese Materia Medica & Yunnan Key Laboratory of Southern Medicine Utilization.

Instruments and Reagents

The following instruments were used: Bruker AV-600 MHz and AV-400 MHz NMR spectrometers (Bruker, Germany); Agilent 1290 UPLC/6545 Q-TOF mass spectrometer (Agilent Technologies); YMC-ODS column (5 m particle size, 10 mm diameter, 250 mm length, Beijing Saipuris Technology); LC-52 semi-preparative HPLC (Beijing Saipuris Technology); MCI CHP-20P gel (Mitsubishi Chemical, Japan); Sephadex LH-20 (Pharmacia); silica gel G for column chromatography (200–300 mesh, 60–80 mesh) and silica gel H for TLC (Qingdao Marine Chemical); HPLC-grade acetonitrile (Merck, Germany); HPLC-grade formic acid (Shanghai Aladdin); ultrapure water; analytical-grade petroleum ether, ethyl acetate, dichloromethane, and methanol (Yunnan Liyan Technology); low-temperature cooling circulation pump (Gongyi Yuhua Instrument); deuterated reagents (Beijing InnoChem); rotary evaporator (Tokyo Rikakikai); ELX-800 microplate reader (BioTek, USA); ZHJH-C1109C clean bench (Shanghai Zhicheng Analytical Instrument); HHB11360-S CO_2 incubator (Shanghai Yuejin Medical Equipment); CCK-8 kit (Shanghai Titan Technology); SH-SY5Y human neuroblastoma cells, SW1990 human pancreatic cancer cells, and 4T1 mouse breast cancer cells (Wuhan Procell Life Technology).

Extraction and Isolation

Dried *T. hypoglaucum* rhizomes (20 kg) were pulverized and soaked overnight in 60 L of 90% ethanol at room temperature, followed by reflux extraction for 2 hours. This process was repeated three times, and the combined extracts were concentrated to remove ethanol. The residue was dispersed in an equal volume of water and extracted three times with two volumes of ethyl acetate. The combined ethyl acetate extracts were concentrated to yield the ethyl acetate fraction. The ethyl acetate fraction (500.0 g) was subjected to silica gel column chromatography (5.0 kg, 23 cm × 75 cm) eluted with petroleum ether-ethyl acetate (98:2, 95:5, 90:10, 80:20, V/V) to obtain four fractions (Fr.1–Fr.4). LC-MS analysis revealed that Fr.2 contained primarily diterpenoids, which was selected for further investigation. Fraction Fr.2 was separated by MCI column chromatography using a methanol-water gradient (70:30$→$100:0, V/V) to yield subfractions Fr.2-1–Fr.2-4. Subfraction Fr.2-2 (25.0 g) was further purified by silica gel column chromatography with chloroform-acetone (90:10, 80:20, V/V), followed by RP-18 column chromatography (70% methanol-water, V/V) and semi-preparative HPLC (YMC-ODS column, 50% acetonitrile-water, V/V, λ = 220/254 nm) to afford compounds **7** (7.0 mg), **8** (20.0 mg), and **9** (15.0 mg). Subfraction Fr.2-3 (309.0 g) was separated by RP-18 column chromatography (methanol-water, 70:30$→$90:10, V/V) and semi-preparative HPLC (YMC-ODS column, 60%

acetonitrile-water, V/V, λ = 220/254 nm) to yield compound **5** (35.0 mg). Subfraction Fr.2-4 (40.0 g) was subjected to RP-18 column chromatography with a methanol-water gradient (50:50$→90 : 10, V/V), followedbyRP − 18columnchromatography(methanol − water, 60 : 30→$90:10, V/V), semi-preparative HPLC (YMC-ODS column, 40% acetonitrile-water, V/V, λ = 220/254 nm), and repeated recrystallization to obtain compounds **1** (30.0 mg), **2** (100.0 mg), **3** (70.0 mg), **4** (100.0 mg), **6** (10.0 mg), and **10** (100.0 mg).

Cytotoxicity Assay (CCK-8 Method)

SH-SY5Y human neuroblastoma cells, SW1990 human pancreatic cancer cells, and 4T1 mouse breast cancer cells were cultured in DMEM medium supplemented with penicillin (100 $U \cdot mL^{-1}$) and streptomycin (100 $g \cdot mL^{-1}$) at 37 °C in a 5% CO_2 incubator. Cells were passaged when confluence exceeded 90%, and those in logarithmic growth phase were used for experiments.

For the assay, 96-well plates were seeded with 100 L of cell suspension at a density of 5×10^3 cells per mL and incubated for 24 h to allow attachment. After removing the culture medium, test compounds dissolved in DMSO were added at concentrations of 100, 50, and 25 $mol \cdot L^{-1}$ (three replicates per concentration). The control group received medium containing 0.1% DMSO, while paclitaxel served as the positive control. Following 24 h incubation, 10% CCK-8 solution was added to each well and incubated for an additional 2 h. Absorbance (A) was measured at 450 nm using a microplate reader. Cell viability was calculated as: Viability (%) = (A_{sample} - A_{blank}) / (A_{vehicle} - A_{blank}) × 100. All experiments were performed in triplicate.

Results

Compound Structure Identification

Ten abietane diterpenoids were isolated from the ethyl acetate extract of *T. hypoglaucum* and identified as triptophenolide (**1**), isoneotriptophenolide (**2**), triptobenzene I (**3**), triptotin A (**4**), triptotin B (**5**), triptobenzene N (**6**), triptobenzene M (**7**), wilforol F (**8**), triptobenzene A (**9**), and quinone 21 (**10**). Their chemical structures are shown in Figure 1 [Figure 1: see original paper].

Compound 1 was obtained as a white powder with HR-ESI-MS m/z: 311.1672 $[M-H]^-$ and molecular formula $C_{20}H_{24}O_3$. $[\alpha]^{20}{}_{.0}D$ +35.00 (c = 0.29, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 6.43 (s, 1H, H-11), 4.99 (s, 1H, 12-OH), 4.84 (dd, J = 17.2, 1.4 Hz, 1H, H-19a), 4.74 (d, J = 17.2 Hz, 1H, H-19b), 3.68 (s, 3H, 14-OMe), 3.17–3.32 (m, 1H, H-15), 3.08 (dd, J = 17.6, 5.0 Hz, 1H, H-7a), 2.75–2.79 (m, 2H, H-7b/5), 2.40–2.41 (m, 2H, H-5/2a), 1.82–1.84 (m, 1H, H-2b), 1.71–1.82 (m, 1H, H-1a), 1.57–1.53 (m, 3H, H-1b/6), 1.20 (s, 3H, H-16), 1.19 (s, 3H, H-17), 1.16 (s, 3H, H-20); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 31.0 (t, C-1), 18.6 (t, C-2), 163.3 (s, C-3), 125.2 (s, C-4), 44.0 (d, C-5), 19.5 (t,

C-6), 25.5 (t, C-7), 129.3 (s, C-8), 149.2 (s, C-9), 37.3 (s, C-10), 111.9 (d, C-11), 139.9 (s, C-12), 130.8 (s, C-13), 150.8 (s, C-14), 26.1 (d, C-15), 23.8 (q, C-16), 23.7 (q, C-17), 174.5 (s, C-18), 70.6 (t, C-19), 17.3 (q, C-20). These data are consistent with literature reports (Wang et al., 2017) and identified the compound as triptophenolide.

Compound 2 was isolated as a white powder with HR-ESI-MS m/z: 343.1880 $[M+H]^+$ and molecular formula $C_{21}H_{26}O_4$. ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 6.43 (s, 1H, H-11), 4.99 (s, 1H, 12-OH), 4.84 (dd, J = 17.2, 1.4 Hz, 1H, H-19a), 4.74 (d, J = 17.2 Hz, 1H, H-19b), 3.68 (s, 3H, 14-OMe), 3.17–3.32 (m, 1H, H-15), 3.08 (dd, J = 17.8, 5.0 Hz, 1H, H-7a), 2.75–2.79 (m, 2H, H-7b/5), 2.40–2.41 (m, 2H, H-5/2a), 1.82–1.84 (m, 1H, H-2b), 1.71–1.82 (m, 1H, H-1a), 1.53–1.57 (m, 3H, H-1b/6), 1.20 (s, 3H, H-16), 1.19 (s, 3H, H-17), 1.16 (s, 3H, H-20); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 31.0 (t, C-1), 18.6 (t, C-2), 125.2 (s, C-3), 163.3 (s, C-4), 44.0 (d, C-5), 19.5 (t, C-6), 25.5 (t, C-7), 129.3 (s, C-8), 149.2 (s, C-9), 37.3 (s, C-10), 111.9 (d, C-11), 139.9 (s, C-12), 130.8 (s, C-13), 150.8 (s, C-14), 26.1 (d, C-15), 23.8 (q, C-16), 23.7 (q, C-17), 174.5 (s, C-18), 70.6 (t, C-19), 17.3 (q, C-20), 60.2 (q, OCH_3). These data matched literature values (Peng et al., 2008) and identified the compound as isoneotriptophenolide.

Compound 3 was obtained as a white powder with HR-ESI-MS m/z: 327.1553 $[M-H]^-$ and molecular formula $C_{20}H_{24}O_4$. ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 7.06 (d, J = 11.8 Hz, 1H, H-12), 6.93 (d, J = 11.8 Hz, 1H, H-11), 6.10 (s, 1H, H-19), 3.08–3.15 (m, 1H, H-15), 2.87–2.91 (m, 1H, H-7a), 2.79–2.85 (m, 1H, H-7b), 2.71 (d, J = 12.8 Hz, 1H, H-5), 2.49–2.52 (m, 2H, H-1a/2a), 2.32–2.40 (m, 2H, H-2b/6a), 1.90–1.93 (m, 1H, H-6b), 1.64–1.69 (m, 1H, H-1b), 1.27 (d, J = 6.8 Hz, 3H, H-17), 1.26 (d, J = 6.8 Hz, 3H, H-16), 1.03 (s, 3H, H-20); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 32.4 (t, C-1), 18.9 (t, C-2), 128.8 (s, C-3), 161.1 (s, C-4), 40.3 (d, C-5), 18.1 (t, C-6), 22.5 (t, C-7), 120.6 (s, C-8), 143.7 (s, C-9), 36.2 (s, C-10), 116.2 (d, C-11), 123.3 (d, C-12), 131.0 (s, C-13), 150.8 (s, C-14), 26.9 (d, C-15), 22.6 (q, C-16), 22.7 (q, C-17), 171.1 (s, C-18), 97.3 (d, C-19), 22.5 (q, C-20). These data corresponded with literature reports (Xu et al., 2011) and identified the compound as triptobenzene I.

Compound 4 was isolated as a light yellow powder with HR-ESI-MS m/z: 359.1453 $[M+H]^+$ and molecular formula $C_{20}H_{22}O_6$. $[\alpha]$D +68.66 (c = 0.067, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 6.27 (d, J = 0.8 Hz, 1H, H-12), 4.84 (dd, J = 17.6, 1.4 Hz, 1H, H-19a), 4.70 (dd, J = 17.6, 1.4 Hz, 1H, H-19b), 4.42 (dd, J = 11.4, 6.2 Hz, 1H, H-1), 2.96 (dd, J = 13.4, 6.6 Hz, 1H, H-15), 2.76 (dd, J = 19.0, 6.2 Hz, 2H, H-2a/7a), 2.56 (d, J = 12.8 Hz, 1H, H-5), 2.37 (ddd, J = 19.0, 10.8, 7.8 Hz, 1H, H-7b), 2.22–2.30 (m, 1H, H-2b), 1.93–2.02 (m, 1H, H-6a), 1.77–1.89 (m, 1H, H-6b), 1.31 (s, 3H, H-20), 1.10 (d, J = 6.8 Hz, 3H, H-16), 1.05 (d, J = 6.8 Hz, 3H, H-17); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 84.1 (d, C-1), 22.6 (t, C-2), 147.6 (s, C-3), 161.9 (s, C-4), 41.0 (d, C-5), 17.7 (t, C-6), 21.9 (t, C-7), 130.1 (s, C-8), 147.6 (s, C-9), 36.4 (s, C-10), 93.1 (s, C-11), 132.8 (d, C-12), 147.6 (s, C-13), 184.5 (s, C-14), 26.6 (d, C-15), 21.5 (q, C-16), 21.3 (q, C-17), 172.6 (s, C-18), 70.2 (t, C-19), 14.1 (s, C-20). These data aligned with

literature values (Guo et al., 1999) and identified the compound as triptotin A.

Compound 5 was obtained as a light yellow powder with HR-ESI-MS m/z: 363.1814 $[M+H]^+$ and molecular formula $C_{20}H_{22}O_6$. $[\alpha]^2._0D$ +91.75 (c = 0.166, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 6.22 (1H, s, H-12), 4.51–4.48 (1H, m, H-11), 3.78 (1H, d, J = 11.6 Hz, H-18a), 3.71 (1H, d, J = 11.6 Hz, H-18b), 2.92–2.97 (2H, m, H-2a/15), 2.76 (1H, dd, J = 18.4, 3.8 Hz, H-7a), 2.47–2.52 (1H, m, H-2b), 2.19–2.25 (1H, m, H-7b), 1.90–1.93 (1H, m, H-6a), 1.72 (2H, brs, H-5/6b), 1.37 (3H, s, H-20), 1.18 (3H, s, H-19), 1.07 (3H, d, J = 6.8 Hz, H-17), 1.03 (3H, d, J = 6.8 Hz, H-16); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 82.9 (d, C-1), 37.4 (t, C-2), 214.2 (s, C-3), 51.2 (s, C-4), 48.2 (d, C-5), 17.2 (t, C-6), 24.2 (t, C-7), 130.9 (s, C-8), 146.8 (s, C-9), 37.7 (s, C-10), 93.8 (s, C-11), 133.5 (d, C-12), 147.0 (s, C-13), 184.8 (s, C-14), 26.4 (d, C-15), 21.5 (q, C-16), 21.3 (q, C-17), 65.6 (t, C-18), 24.3 (q, C-19), 13.9 (q, C-20). These data matched literature values (Guo et al., 1999) and identified the compound as triptotin B.

Compound 6 was isolated as a white powder with HR-ESI-MS m/z: 315.1932 $[M+H]^+$ and molecular formula $C_{20}H_{26}O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 7.89 (d, J = 1.5 Hz, 1H, H-14), 7.43 (dd, J = 8.1, 1.8 Hz, 1H, H-12), 7.29 (d, J = 8.2 Hz, 1H, H-12), 4.02 (d, J = 11.2 Hz, 1H, H-18a), 3.69 (d, J = 11.2 Hz, 1H, H-18b), 2.89–2.97 (m, 1H, H-15), 2.79–2.85 (m, 2H, H-6), 2.74 (dd, J = 17.4, 3.6 Hz, 1H, H-2a), 2.60–2.70 (m, 2H, H-1a/2b), 2.49 (dd, J = 14.4, 3.2 Hz, 1H, H-1b), 2.08 (td, J = 12.6, 6.6 Hz, 1H, H-5), 1.41 (s, 3H, H-19), 1.25 (s, 3H, H-20), 1.23 (t, J = 6.6 Hz, 6H, H-16/17); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 36.2 (t, C-1), 35.4 (t, C-2), 215.2 (s, C-3), 52.1 (s, C-4), 49.9 (d, C-5), 36.2 (t, C-6), 197.9 (s, C-7), 130.2 (s, C-8), 150.9 (s, C-9), 37.2 (s, C-10), 124.3 (d, C-11), 132.9 (d, C-12), 147.5 (s, C-13), 125.3 (d, C-14), 33.6 (d, C-15), 23.7 (q, C-16), 23.7 (q, C-17), 65.7 (t, C-18), 21.4 (q, C-19), 23.5 (q, C-20). These data corresponded with literature reports (Duan et al., 1999) and identified the compound as triptobenzene N.

Compound 7 was obtained as a white powder with HR-ESI-MS m/z: 315.1962 $[M-H]^-$ and molecular formula $C_{20}H_{28}O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δH: 6.84 (s, 1H, H-14), 6.61 (s, 1H, H-11), 4.08 (d, J = 11.4 Hz, 1H, H-18a), 3.51 (d, J = 11.4 Hz, 1H, H-18b), 3.13 (dt, J = 13.6, 6.8 Hz, 1H, H-15), 2.86 (dd, J = 16.8, 5.4 Hz, 1H, H-7a), 2.61–2.70 (m, 2H, H-2), 2.42–2.61 (m, 1H, H-1a/7b), 2.09 (d, J = 13.0 Hz, 1H, H-5), 1.92–2.06 (m, 2H, H-1b/6a), 1.60–1.68 (dd, J = 12.8, 5.8 Hz, 1H, H-6b), 1.35 (s, 3H, H-19), 1.24 (s, 3H, H-20), 1.23 (t, J = 6.6 Hz, 6H, H-16/17); ^{13}C-NMR (150 MHz, $CDCl_3$) δC: 37.2 (t, C-1), 34.8 (t, C-2), 221.0 (s, C-3), 50.8 (s, C-4), 51.2 (d, C-5), 19.8 (t, C-6), 30.3 (t, C-7), 126.4 (s, C-8), 144.8 (s, C-9), 36.8 (s, C-10), 112.1 (d, C-11), 151.3 (s, C-12), 132.6 (s, C-13), 126.6 (d, C-14), 26.8 (d, C-15), 22.6 (q, C-16), 22.5 (q, C-17), 65.7 (t, C-18), 22.2 (q, C-19), 25.7 (q, C-20). These data matched literature values (Duan et al., 1997) and identified the compound as triptobenzene M.

Compound 8 was isolated as a white powder with HR-ESI-MS m/z: 345.2064 $[M-H]^-$ and molecular formula $C_{21}H_{30}O_4$. ^{1}H-NMR (400 MHz, $CDCl_3$) δH: 6.35 (s, 1H, H-11), 4.08 (d, J = 11.3 Hz, 1H, H-18a), 3.76 (s, 3H, 12-OMe),

3.54 (d, J = 11.3 Hz, 1H, H-18b), 3.41–3.50 (m, 1H, H-15), 2.79 (dt, J = 21.8, 10.9 Hz, 1H, H-7a), 2.57–2.74 (m, 2H, H-2), 2.39–2.55 (m, 2H, H-1a/7b), 2.01–2.14 (m, 2H, H-1b/6a), 1.92–2.00 (m, 1H, H-6b), 1.34 (s, 3H, H-19), 1.33 (s, 3H, H-20), 1.32 (overlap, 3H, H-16), 1.30 (overlap, 3H, H-17); ^{13}C-NMR (100 MHz, $CDCl_3$) δC: 37.3 (t, C-1), 35.0 (t, C-2), 220.2 (s, C-3), 51.12 (s, C-4), 50.9 (d, C-5), 19.1 (t, C-6), 24.4 (t, C-7), 114.0 (s, C-8), 145.3 (s, C-9), 37.1 (s, C-10), 100.8 (d, C-11), 156.9 (s, C-12), 119.5 (s, C-13), 151.7 (s, C-14), 24.4 (d, C-15), 20.9 (q, C-16), 20.8 (q, C-17), 65.6 (t, C-18), 22.1 (q, C-19), 25.3 (q, C-20), 55.7 (q, 12-OMe). These data corresponded with literature reports (Morota et al., 1995) and identified the compound as wilforol F.

Compound 9 was obtained as a white powder with HR-ESI-MS m/z: 317.2116 $[M+H]^+$ and molecular formula $C_{20}H_{28}O_3$. ^{1}H-NMR (400 MHz, $CDCl_3$) δH: 7.04 (d, J = 8.2 Hz, 1H, H-12), 6.84 (d, J = 8.3 Hz, 1H, H-11), 4.08 (d, J = 11.3 Hz, 1H, H-18a), 3.55 (d, J = 11.2 Hz, 1H, H-18b), 3.15 (dt, J = 13.6, 6.8 Hz, 1H, H-15), 2.92 (dd, J = 16.8, 5.4 Hz, 1H, H-7a), 2.74–2.78 (m, 1H, H-7b), 2.29–2.73 (m, 1H, H-2a), 2.55–2.61 (m, 1H, H-2b), 2.35–2.40 (m, 1H, H-1a), 2.13 (d, J = 13.0 Hz, 1H, H-5), 2.04–2.07 (m, 2H, H-1b), 1.83–1.86 (m, 1H, H-6a), 1.70 (dd, J = 12.8, 5.8 Hz, 1H, H-6b), 1.34 (s, 3H, H-19), 1.28 (s, 3H, H-20), 1.24 (t, J = 6.4 Hz, 6H, H-16/17); ^{13}C-NMR (100 MHz, $CDCl_3$) δC: 37.4 (t, C-1), 35.0 (t, C-2), 220.2 (s, C-3), 51.2 (s, C-4), 50.9 (d, C-5), 19.1 (t, C-6), 24.9 (t, C-7), 120.9 (s, C-8), 145.6 (s, C-9), 36.9 (s, C-10), 117.6 (d, C-11), 123.7 (d, C-12), 130.7 (s, C-13), 150.2 (s, C-14), 26.8 (d, C-15), 22.8 (q, C-16), 22.6 (q, C-17), 65.7 (t, C-18), 22.1 (q, C-19), 25.5 (q, C-20). These data matched literature values (Takaishi et al., 1997) and identified the compound as triptobenzene A.

Compound 10 was isolated as a yellow powder with HR-ESI-MS m/z: 325.1443 $[M-H]^-$ and molecular formula $C_{20}H_{22}O_4$. ^{1}H-NMR (400 MHz, $CDCl_3$) δH: 6.42 (s, 1H, H-12), 4.65–4.88 (m, 2H, H-19), 3.09–3.16 (m, 1H, H-1a), 2.95–3.06 (m, 1H, H-15), 2.77–2.84 (m, 1H, H-7a), 2.62 (d, J = 12.8 Hz, 1H, H-7b), 2.46–2.58 (m, 1H, H-1b), 2.37–2.45 (m, 3H, H-2a/6), 1.41–1.54 (m, 1H, H-5), 1.22–1.33 (m, 1H, H-2b), 1.16 (s, 3H, H-20), 1.13 (d, J = 6.8, 2.3 Hz, 6H, H-16/17); ^{13}C-NMR (100 MHz, $CDCl_3$) δC: 18.7 (t, C-1), 30.8 (t, C-2), 125.6 (s, C-3), 161.3 (s, C-4), 42.5 (d, C-5), 18.5 (t, C-6), 24.3 (t, C-7), 142.6 (s, C-8), 147.8 (s, C-9), 36.8 (s, C-10), 187.5 (s, C-11), 131.7 (d, C-12), 153.5 (s, C-13), 187.3 (s, C-14), 26.5 (d, C-15), 21.4 (q, C-16), 21.3 (q, C-17), 173.8 (s, C-18), 70.3 (t, C-19), 18.3 (q, C-20). These data corresponded with literature reports and identified the compound as quinone 21.

Cytotoxicity Screening Results

The cytotoxicity screening results for compounds **1–10** are summarized in Table 1 . Compound **1** exhibited potent cytotoxicity against SH-SY5Y cells with an IC_{50} value of $(1.10\$\pm0.03)\mu mol{\cdot}L\hat{}\{-1\}, significantlystrongerthanthepositivecontrolpaclitaxel[IC\{50\}\$ = (39.40\$\pm0.87)\mu mol\cdot L\hat{}\{-1\}]. Compounds**1**, **7**, **8**, **9**, and** 10 ** demonstratedcytotoxiceffectsagainstSW1990cellswithIC\{50\}\$ values of $(0.47\$\pm0.02), (9.26\pm1.39), (4.81\pm0.77), (39.74\pm2.46), and (75.38\pm6.07)\mu mol\cdot$

$L\hat{}\{-1\}, respectively. Notably, compounds * *1 * *, * * 7 * *, and * *8 * *showed significantly greater potency than paclitaxel [IC\{50\}\$ = (92.16\$\pm$5.53)\mu mol\cdot L\hat{}\{-1\}] against SW1990 cells. Compounds * *7 * *, * * 8 * *, and * *9 * *displayed cytotoxicity against 4T1 cells with IC\{50\}\$ values of (3.98\$\pm$0.73), (0.79\pm0.05), and (2.12\pm0.08)\mu mol\cdot L\hat{}\{-1\}\$, respectively.

Discussion

This study isolated ten abietane diterpenoids from *T. hypoglaucum*, among which compounds **4**, **5**, **6**, and **7** were reported from this species for the first time. Previous studies have shown that compound **1** exerts anti-asthmatic effects by inhibiting NF- B and reducing EMT-related transcription factors (Cen and Weng, 2024) and exhibits cytotoxicity against HepG2 human liver cancer cells. Compounds **4** and **5** have demonstrated cytotoxic activity against U251 glioma cells (Gao et al., 2016). Although *T. hypoglaucum* contains numerous diterpenoids, antitumor studies on these individual constituents remain limited.

Our cytotoxicity screening revealed that triptophenolide (**1**), triptobenzene M (**7**), wilforol F (**8**), and triptobenzene A (**9**) exhibited strong cytotoxic effects against all three tumor cell lines. Notably, compounds **1**, **7**, **8**, **9**, and **10** showed significantly greater potency than paclitaxel against SW1990 pancreatic cancer cells. While compounds **7** and **10** displayed weaker or no cytotoxicity against other tumor cell lines, suggesting selective activity. Compound **1** demonstrated excellent cytotoxicity against SH-SY5Y neuroblastoma cells, and compound **8** showed notable activity against 4T1 breast cancer cells. Particularly, triptobenzene A (**9**) exhibited strong cytotoxicity across all three cell lines, highlighting the differential tumor-specific cytotoxic profiles among these compounds.

Triptolide, a major diterpenoid from *Tripterygium* species, is well-known for its potent antitumor activity. Our findings demonstrate that other diterpenoids from *T. hypoglaucum* also possess significant cytotoxic properties, albeit with varying selectivity toward different cell lines. This suggests that expanding the panel of tumor cell lines in future studies would be valuable. The present investigation enriches the chemical and biological understanding of abietane diterpenoids from *T. hypoglaucum*. Given the chemical complexity of this plant, the observed antitumor effects may result from synergistic interactions among multiple constituents. Therefore, further research will focus on identifying additional bioactive compounds and elucidating their antitumor mechanisms.

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Note: Figure translations are in progress. See original paper for figures.