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A Neoprzewaquinone Analogue from *Salvia miltiorrhiza* Bunge

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Abstract: (3*R*,3′*R*)-2,2′,3,3′-tetrahydroneoprzewaquinone A (**1**), a previously undescribed neoprzewaquinone analogue, was isolated from the root of *Salvia miltiorrhiza* Bunge. Its absolute configuration was elucidated by comprehensive analyses of spectra including NMR and MS combined with ECD calculations. MTT assay indicated that **1** can inhibit the proliferation of MV-4-11, TMD-8, MOLM-13, and H460 cell lines with IC_{50} values of 2.21 μM, 2.48 μM, 3.39 μM, and 2.02 μM respectively.

Keywords: *Salvia miltiorrhiza* Bunge; (3*R*,3′*R*)-2,2′,3,3′-tetrahydroneoprzewaquinone A; cytotoxic activity.

1. Introduction

The dry root and rhizome of *Salvia miltiorrhiza* Bunge (Labiatae), also known as red sage or danshen, is a popular traditional Chinese medicine (TCM), which is used for cardiovascular disease, liver cirrhosis, nephrotic syndrome, and pneumonia [1-3]. The compounds tanshinone, propanoic acid, salvianolic acid, flavonoids, and polysaccharides are mainly accountable for the therapeutic effects [4-5]. For example, tanshinone I showed obvious anti-inflammatory effects [6], and tanshinone IIA can resist atherosclerosis [7] and anti-tumor [8-11].

Many undescribed compounds have been found of *S. miltiorrhiza* in recent years, such as neo-przewaquinone A, 3-hydroxy-2-(2'-formyloxy-1'-methylethyl)-8-methyl-1,4-phenanthrenedione, and

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(8'*R*)-isosalvianolic acid C methyl ester [12,13], etc. As a part of our work to search for more bio-active compounds with novel structure from TCM, *S. miltiorrhiza* was selected. After a systematic phytochemical investigation, a previously undescribed compound tetrahydroneoprzewaquinone A (**1**) has been found (see Figure 1). Herein, we reported the isolation, structural elucidation, and cytotoxicity.

2. Materials and Methods

2.1. Instruments and Materials

The semi-preparative HPLC (Waters, USA) was conducted by using an Ultimate XB-C_{18} column (4.6×250 mm, 5 μm) (Welch Technology Co., Ltd, China). The 1D and 2D NMR data were obtained using a Bruker Bruker-Ascend-600-MHz spectrometer (Bruker Corporation, Billerica, MA, USA). HR-ESI-MS was measured on a Q Exactive UHMR Hybrid Quadrupole-Orbitrap 16 mass spectrometer (Thermo Fisher Scientific, MA, USA). Different types of cancer cells were cultured in an MCO-15AC CO_2 incubator (Sanyo Semiconductor, Japan), MCV-13161FT clean bench (Sanyo Semiconductor, Japan). The MKG9823 inverted microscope (Carl Zeiss AG, German) and Cell counting plate (Shanghai Qiujing Biochemical Reagent Instrument Co., Ltd, China) were used to observe and count the cells, and the OD value was measured with a MK 3 automatic microplate reader (Thermo Fisher, USA). HepG-2 (Human liver cancer cell), HeLa (Human cervical cancer cell), H460 (Human large cell lung cancer cell), TMD-8 (Human diffuse large B lymphoma cells), MOLM-13 (Human acute myeloid leukemia cell), and MV-4-11 (Human myeloid monocytic leukemia cells) were purchased from the American type culture collection.

2.2. Separation and Purification

After crushing and blending the dried *S. miltiorrhiza*, the powder was extracted 3 times with 80~90% ethanol under reflux for 1 hour each time (the weight of ethanol is 8~10 times of medicinal powder) [14, 15]. Then recovered the solvent to no alcohol smell and the concentrated extract was obtained. Adding 6-10 times of water to disperse evenly, the obtained aqueous dispersion was separated by using AB-8 macroporous adsorption resin column chromatography (methanol/water, *v/v* = 80:20 as the mobile phase) [16]. Collect the chromatographic solution containing diterpene quinones, and then concentrate it until no alcohol smell to obtain a concentrated solution by reduced pressure. Filter the concentrated solution, and then the filtrate was prepared and separated by C_{18} reverse phase chromatography packing (acetonitrile/water, *v/v* = 62:38 as the mobile phase, detection wavelength 270nm).

2.3. Cytotoxicity Test

The inhibitory effects of compound **1** on MV-4-11, TMD-8, MOLM-13, H460, HeLa, and HepG-2 cells were determined by the MTT method with the positive control Cisplatin [17-21]. The cells in the logarithmic growth phase were added to the complete medium to make a cell suspension with a concentration of 2×10^4 cells/mL, and 100μL complete medium was added per well containing 0.63, 1.25, 2.50, 5.00, 10.00, and 20.00 μg/mL of compound **1**. Set the cells containing 0.1% DMSO complete medium as the solvent control group, and 3 multiple wells for each drug concentration. After 72h incubate them at 37℃ and 5% CO_2, 20 μL of 5mg/mL MTT solution was added to each well. After incubating for another 2～4h, then add 80 μL of 20% SDS to each well and incubate overnight. On the next day, using a microplate reader to determine the absorbance at 570nm (optical density, OD). Calculate the cell inhibition rate according to the following formula: cell inhibition rate = (control group OD value-experimental group OD value)/control group OD value×100%. The biostatistical software Graphpad Prism was used to fit the growth inhibition curves of the drugs on different cells, and the half-maximal inhibitory concentration (IC_{50}) value was calculated.

2.4. Spectroscopic Data

Tetrahydroneoprzewaquinone A (**1**): Red solid powder; $[\alpha]_D^{20}$ = -56.25 (c 0.02, MeOH); UV (MeOH): λmax = 219 (4.64) and 276 (4.78) nm; IR (KBr) ν_{max}: 3017, 2966, 2879, 1747, 1689, 1617, 1555, 1480, 1372, 1299, 1027 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR (600/150 MHz, CD_3OD) see Table 1; HR-ESI-MS calcd. for $C_{36}H_{32}O_6Na$ $[M+Na]^+$ *m/z*: 583.2092, found *m/z*: 583.2090.

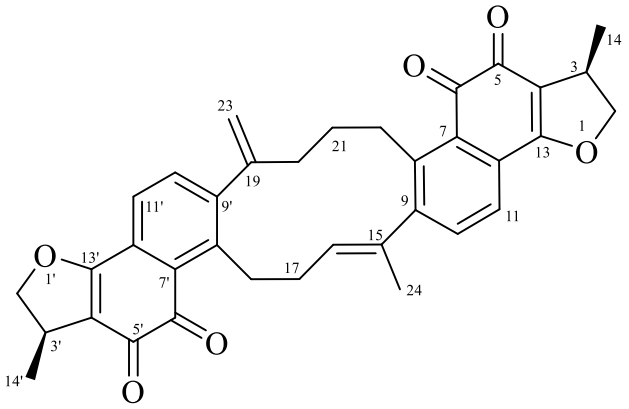

Figure 1. Chemical structure of compound **1**

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of compound **1** (δ in ppm, *J* in Hz) in CD_3OD

Position	δ_H	δ_C	Position	δ_H	δ_C
2/2′	4.95 (2H, td, 9.5, 3.5) 4.42 (2H, m)	81.8	18	3.31 (2H, dd, 8.1, 2.3)	24.4
3/3′	3.55 (2H, m)	34.4	19		143.1
4/4′		118.4	20	2.50 (2H, m)	31.7
5		175.9	21	1.85 (2H, quin, 6.4)	23.1
6		184.3	22	3.23 (2H, t, 6.4)	29.0
7		128.2	23	5.59 (1H, s) 5.11 (1H, s)	110.7
8		143.4	24	2.05 (3H, s)	18.5
9		140.8	5′		175.4
10	7.50 (1H, d, 8.4)	127.5	6′		183.6
11	7.55 (1H, d, 7.9)	123.4	7′		127.4
12		126.0	8′		143.3
13		171.9	9′		140.5
14/14′	1.32 (6H, d, 6.9)	17.4	10′	7.96 (1H, d, 8.1)	130.0
15		131.2	11′	7.52 (1H, d, 8.1)	122.6
16	6.12 (1H, s)	129.3	12′		127.7
17	2.22 (2H, m)	22.0	13′		171.6

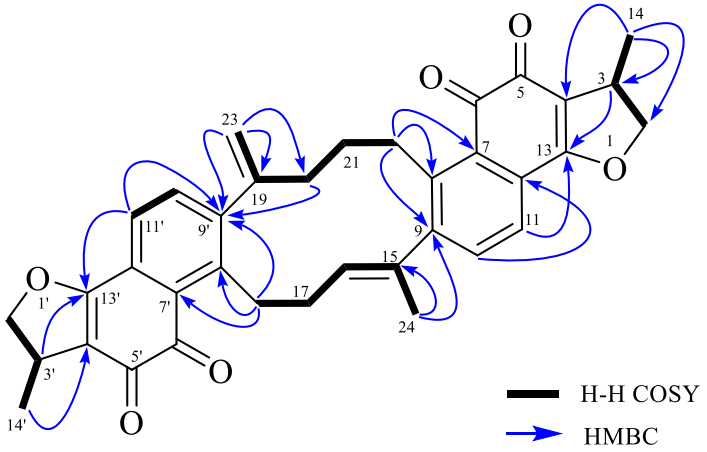

Figure 2. Key HMBC (arrows) and ^{1}H-^{1}H COSY (bold) correlations of **1**

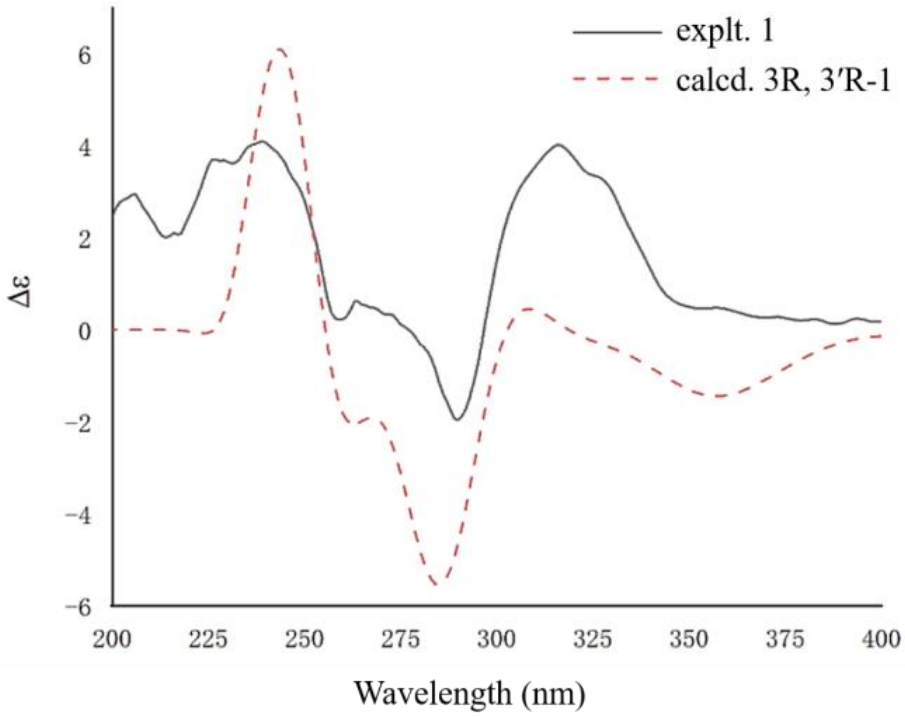

Figure 3. Calculated ECD curve and experimental ECD spectrum of **1**

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a red solid powder. Its molecular formula was assigned as HR-ESI-MS by the pseudo-molecular ion peak at *m/z* 583.2090 $[M+Na]^+$ (calculated for 583.2092, $C_{36}H_{32}O_6Na^+$). The ^{1}H-NMR spectrum indicated the presence of three methyl protons at δ 1.32 (6H, d, J = 6.9 Hz, H-14/H14′) and δ 2.05 (3H, s, H-24), seven methylene protons at δ 4.95 (2H, td, J = 9.5, 3.5 Hz, H-2/H-2′), 4.42 (2H, ddd, J = 8.7, 6.2, 2.3 Hz, H-2/H-2′), 3.31 (2H, dd, J = 8.1, 2.3 Hz, H-18), 3.23 (2H, t, J = 6.4 Hz, H-22), 2.50 (2H, m, H-20), 2.22 (2H, m, H-17), 1.85 (2H, quin, J = 6.4 Hz, H-21), and seven double bond proton at δ 7.96 (1H, d, J = 8.1 Hz, H-10′), 7.55 (1H, d, J = 7.9 Hz, H-11), 7.52 (1H, s, H-

11′), 7.50 (1H, d, *J* = 8.4 Hz, H-10), 6.12 (1H, m, H-16), 5.59 (1H, s, H-23), 5.11 (1H, s, H-23). The ^{13}C-NMR spectrum of **1** indicated the presence of four carbonyl carbon signals at δ_C 184.3 (C-6), 183.6 (C-6′), 175.9 (C-5), 175.4 (C-5′), and twenty olefinic and aromatic carbon signals at δ_C 171.9 (C-13), 171.6 (C-13′), 143.4 (C-8), 143.3 (C-8′), 143.1 (C-19), 140.8 (C-9), 140.5 (C-9′), 131.2 (C-15), 130.0 (C-10′), 129.3 (C-16), 128.2 (C-7), 127.7 (C-12′), 127.5 (C-10), 127.4 (C-7′), 126.0 (C-12), 123.4 (C-11), 122.6 (C-11′), 118.4 (C-4′), 110.7 (C-23). (see Table 1)

The ^{1}H-NMR spectra of **1** are similar to neo-przewaquinone A [12, 22], expecting the absence of two olenfic protons, and the presence of two more methylene and two methine signals. The HMBC correlations from H-14/14′ to C-2/2′, C-3/C-3′, C-4/4′ indicates methylene and methane replayed the olenfic carbons at C2/2′ and C3/3′. The ^{1}H-^{1}H COSY correlations of H-18/H-17/H-16/H-15/H-24, of H-10′/H-11′, and H-22/H-21/H-20 as well as the HMBC correlations of H-24/C-16, C-15, C-9, of H-22/ C-20, C-19, C-9′, of H-20 /C-9′ and H-22/C-9, C-8, C-7 generated the planar structure of **1** as shown in Figure 2. The weak NOESY correlations of H-24/H-17 indicated the E configuration of the double bond between C-15 and C-16. The absolute configuration of the chiral carbons at the positions of C-3 and C-3′ were deduced as *R* by subsequent ECD calculations [23] shown in Figure 3. Thus, compound **1** was finally elucidated as (3*R*,3′*R*)-2,2′,3,3′-tetrahydroneoprzewaquinone A.

3.2. Cell Viability Assay

MTT test indicated that compound **1** showed obvious inhibitory activity against MV4-11, TMD-8, MOLM-13, and H460 cells with IC_{50} values of 2.21 μM, 2.48 μM, 3.39 μM, and 2.02 μM, respectively (The IC_{50} value of each cell line is shown in Table 2 and Figure 4). The compound **1** is sensitive to MV4-11, TMD-8, MOLM-13, and H460 cells, but insensitive to HeLa and HepG-2 cells, which may have a certain potential targeting effect and worth further study.

Table 2. The results of the measurement of IC_{50} of Compound **1** and Cisplatin on different cells

Cell line	Cell type	Compd. **1** IC_{50} (μM)	Cisplatin IC_{50} (μM)
MV-4-11	Human acute myeloid leukemia cells	2.21	8.23
TMD-8	Human diffuse large B lymphoma cells	2.48	ND
Molm-13	Human acute myeloid leukemia cells	3.39	17.27
H460	Human lung cancer cell	2.02	9.36
HeLa	Human cervical cancer cells	>35.70	18.43
HepG-2	Human hepatoma cells	>35.70	5.78

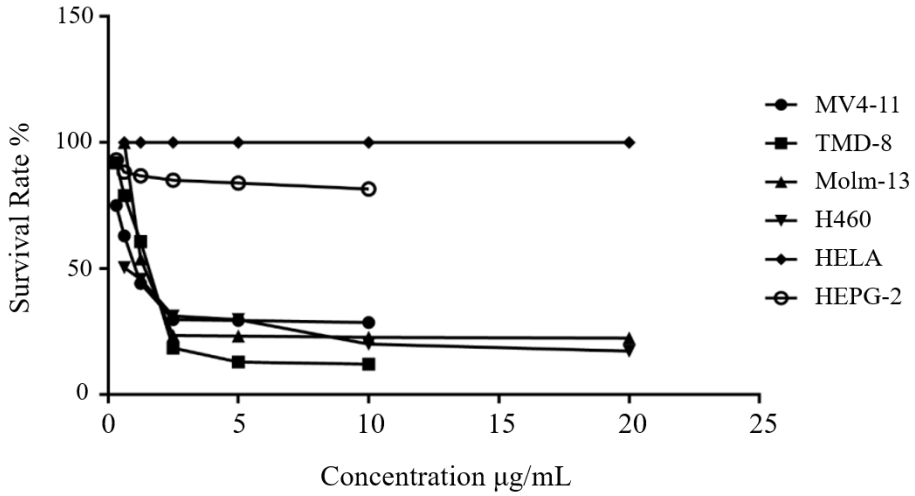

Figure 4. Growth inhibition curves of **1** on MV4-11, TMD-8, MOLM-13, H460, HepG-2, and HeLa cells (72 hours)

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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