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Biotransformation and tumor multidrug resistance reversal potency of polyoxygenated taxadienes

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Abstract

The biotransformation of four taxadienes, 2α,5α-diacetoxy-14β-hydroxy-10β-methoxytaxa-4(20),11(12)-diene (**1**), 10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**), 2α,5α,10β-triacetoxytaxa-4(20),11(12)-dien-13-one (**3**), and 2α,5α-diacetoxy-10β-methoxytaxa-4(20),11(12)-dien-13-one (**4**) were individually investigated by the cultured cells of *Ginkgo biloba*. Six new metabolites together with four known metabolites were obtained from their biotransformations. Most compounds were evaluated for the MDR reversal activity against taxol-resistant human non-small cell lung cancer (NSCLC)-lung adenocarcinoma cell line, A549/taxol. Two compounds, **5** and **6**, exhibited significant MDR reversal activity when co-administered with taxol at 5 μM. The result showed that the methoxyl group at C-10 and hydroxyl group at C-14 may be potential pharmacophores with taxadiene MDR reversal agents.

Keywords: Taxadienes; *Ginkgo biloba*; Biotransformation; Tumor MDR reversal agents

1. Introduction

For decades, taxol (paclitaxel) has been a highly successful anticancer drug since it was initially approved for the treatment of breast and ovarian cancers, and then potently expanded to the treatment of lung, gastrointestinal and other cancers. However, its poor water solubility and the development of resistance have increasingly limited the clinical efficacy for some kinds of cancers. The multidrug resistance (MDR), a cellular resistance to structurally and functionally unrelated chemotherapeutic drugs, has become the major obstacles for successful chemotherapy of taxol in recent years. By now, one of the mechanisms of MDR is associated with overexpression of P-glycoprotein (P-gp) in tumor cells [1,2]. As a 170-kDa plasma membrane glycoprotein, P-gp acts as an energy-dependent drug efflux pump. The overexpression of P-gp results in reduced drug accumulation in tumor cells and subsequently increases the possibility of chemotherapeutic failure. Although some chemical compounds (verapamil, quinidine, cyclosporine A, etc.) have been reported to reverse MDR *in vivo*, these MDR reversing agents are still failed to be developed for further clinical trails due to their unacceptable toxicities [3]. Therefore restoring taxol sensitivity at a maximum to resistant cancers has fuelled the search for novel MDR reversing agents with strong potency and minimal side effects.

In the previous study, we have reported that sinenxan A and some derivatives increased intercellular accumulation of taxol in MDR cells *in vitro* [4]. In order to search for potent MDR-reversing agents from taxane diterpenoids, we targeted to synthesize some new compounds from the biotransformation of polyoxygenated taxadienes by plant cultured cells. In this study, four derivatives of 10-methoxyl or 13-oxo taxadienes, 2α,5α-diacetoxy-14β-hydroxy-10β-methoxytaxa-4(20),11(12)-diene (**1**), 10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**), 2α,5α,10β-triacetoxytaxa-4(20),11(12)-dien-13-one (**3**), and 2α,5α-diacetoxy-10β-methoxytaxa-4(20),11(12)-

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dien-13-one (**4**) were selected as substrates to carry out the transformation by employing the cultured cells of *Ginkgo biloba*, and the MDR reversal activities of ten compounds, against a taxol-resistant human non-small cell lung cancer (NSCLC)-lung adenocarcinoma cell line, A549/taxol, were also evaluated.

2. Experimental

2.1. General methods

Melting points were determined on an XT-4 micro point apparatus and are uncorrected. Specific rotations were measured with PerkinElmer 241 polarimeter. IR spectra were recorded on Nicolet 5700 spectrophotometer (FT-IR Microscope Transmission). NMR spectra were recorded in $CDCl_3$ with an INOVA 500 NMR spectrometer, using visual resonances (^{1}H δ 7.27, ^{13}C δ 77.0) for internal reference. Mass spectra and accurate mass measurements were recorded on an AutoSpec Ultima-TOF spectrometer. Silica gel 200–300 mesh (Qingdao Haiyang Chemical Co., Qingdao, PR China) were used for column chromatography. Semi-preparative HPLC was carried out using a Shimadzu system with a pump (model LC-6AD), detector (RID-10A), and YMC C_{18} column (250 mm × 10 mm, 5 μm).

2.2. Substrates

2α,5α-Diacetoxy-14β-hydroxy-10β-methoxytaxa-4(20), 11(12)-diene (**1**), 10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**), 2α,5α,10β-triacetoxytaxa-4(20), 11(12)-dien-13-one (**3**), and 2α,5α-diacetoxy-10β-methoxytaxa-4(20),11(12)-dien-13-one (**4**) were synthesized and biotransformed from sinenxan A [4,5].

2.3. Organism, media and cultivation condition

The cultured cells of *Ginkgo biloba* were prepared as described previously [6]. And then the suspension cells of *Ginkgo biloba* were cultured in 500 mL conical flasks, each containing 150 mL liquid MS media supplemented with 0.5 mg/L of naphthalene acetic acid, 0.5 mg/L of 6-benzylaminopurine and 0.2 mg/L of 2,4-dichlorophenoxy acetic acid. The media solution was adjusted to pH 5.8 before sterilization at 121 °C for 20 min. The cultivation procedure was carried out with the inoculum size (5 g/L of cell cultures) and subcultured with shaking on a shaker at 110 rpm [7].

2.4. Biotransformation procedure and purification of metabolites

The substrates, respectively, previously dissolved as a 5% solution in DMF, and then were uniformly distributed at a final concentration of 0.1 mg/mL among the flasks followed by incubation for additional 7 days. Cultures were filtered, and the filtrate was extracted with EtOAC. The extracts were evaporated under reduced pressure, and the crude extracts were fractionated by silica gel column chromatography. The fractions obtained from the biotransformation of **1** (400 mg) was further chromatographed on C_{18} Si gel column. Elution with MeOH–H_2O led to the isolation of substrate **1** (46.6 mg), and the metabolites **5** (33.6 mg), **6** (11.3 mg), **7** (4.0 mg) and **8** (2.7 mg). The extract obtained from the biotransformation of **2** (305 mg) was repeatedly subjected to C_{18} Si gel column with MeOH-H_2O to achieve the substrate **2** (195.5 mg), and the metabolite **9** (4.5 mg). The further purification of the fractions from the biotransformation of **3** (100 mg) was developed on C_{18} Si gel column, and eluted with MeOH–H_2O to afford the metabolites **10** (9.1 mg), **11** (15.5 mg), **12** (2.3 mg) and **13** (3.8 mg). And the extract residue obtained from the biotransformation of substrate **4** (23 mg) was applied on C_{18} Si gel column with elution of MeOH–H_2O, to yield the purified compound **14** (4.6 mg).

2.5. Analysis

2.5.1. 2α,5α-Diacetoxy-9α,14β-dihydroxy-10β-methoxytaxa-4(20),11(12)-diene (*5*)

White amorphous powder, mp 199–201 °C, $[\alpha]_D^{20}$ +48.8° (*c* 0.205, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3465, 1738, 1642, 1437, 1372, 1235, 1201, 1083, 1020, 911, 881; EI-MS *m/z*: 390 [M–CH_3COOH], 375 [M–CH_3COOH–CH_3]$^+$, 330 [M–2 × CH_3COOH], 315 [M–2 × CH_3COOH–CH_3]$^+$, 165; HRFABMS *m/z*: 473.2517 [M+Na]$^+$ (calcd for $C_{25}H_{38}O_7Na$: 473.2515); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.40 (1H, dd, *J* = 1.5, 5.0 Hz, H-2), 5.28 (1H, s, H_a-20), 5.25 (1H, br s, H-5), 4.94 (1H, s, H_b-20), 4.34 (1H, d, *J* = 9.5 Hz, H-10), 4.08 (1H, m, H-14), 4.05 (1H, d, *J* = 9.5 Hz, H-9), 3.32 (3H, s, 10-OCH_3), 2.85 (1H, *J* = 5.0 Hz, H-3), 2.65 (1H, dd, *J* = 9.0, 18.5 Hz, H_β-13), 2.55 (1H, dd, *J* = 4.5, 18.5 Hz, H_α-13), 2.13 (3H, s, 2-$OCOCH_3$), 2.06 (3H, s, 5-$OCOCH_3$), 2.03 (3H, s, H-18), 1.84 (1H, dd, *J* = 2.5, 14.0 Hz, H_α-7), 1.80 (1H, m, H-6), 1.71 (1H, br s, H-1), 1.68 (1H, m, H-6), 1.54 (3H, s, H-16), 1.46 (1H, dt, *J* = 4.0, 14.0, H_β-7), 1.22 (3H, s, H-17), 1.02 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

2.5.2. 2α-Acetoxy-5α,14β-dihydroxy-10β-methoxytaxa-4(20),11(12)-diene (*6*)

White amorphous powder, mp 106–108 °C, $[\alpha]_D^{20}$ +20.0° (*c* 0.05, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3431, 2927, 1730, 1445, 1375, 1242, 1087, 1025, 914; EI-MS *m/z*: 392 [M]$^+$, 374 [M–H_2O]$^+$, 359 [M–H_2O–CH_3], 332 [M–CH_3COOH]$^+$; HRFABMS *m/z*: 415.2477 [M+Na]$^+$ (calcd for $C_{23}H_{36}O_5Na$: 415.2460); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.40 (1H, dd, *J* = 1.6, 6.0 Hz, H-2), 5.09 (1H, s, H_a-20), 4.79 (1H, s, H_b-20), 4.64 (1H, dd, *J* = 12.0, 5.2 Hz, H-10), 4.18 (1H, br s, H-5), 4.10 (1H, dd, *J* = 9.2, 5.0 Hz, H-14), 3.27 (3H, s, 10-OCH_3), 3.16 (1H, d, *J* = 6.0 Hz, H-3), 2.67 (1H, dd, *J* = 9.2, 18.8 Hz, H_β-13), 2.46 (1H, dd, *J* = 5.0, 18.8 Hz, H_α-13), 2.24 (1H, dd, *J* = 12.0, 15.0, H_β-9), 2.07 (3H, s, 2-$OCOCH_3$), 2.00 (3H, s, H-18), 1.72 (1H, br s, H-1), 1.62 (3H, s, H-16), 1.60 (1H, m, H_α-9), 1.22 (3H, s, H-17), 0.79 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

Table 1
^{13}C NMR spectroscopic data for compounds **1**, **5**, **6**, **9**, **12**, **13** and **14** (in $CDCl_3$, 125 MHz)

C	1	5	6	9	12	13	14
1	63.7	63.5	63.5	58.7	48.8	48.6	48.6
2	71.4	70.8	71.7	70.6	77.2	69.9	69.9
3	42.2	44.0	40.2	44.0	67.4	38.6	42.8
4	143.0	142.5	148.2	141.9	143.2	59.8	142.3
5	78.8	79.3	76.7	78.9	76.7	69.9	76.4
6	28.9	28.6	31.2	25.7	25.6	24.3	28.7
7	33.8	26.0	33.1	28.6	38.1	32.9	26.4
8	39.5	44.0	39.9	44.0	43.3	38.4	44.1
9	45.2	77.1	45.2	77.3	45.7	43.3	78.4
10	75.9	81.3	76.1	81.3	77.9	70.8	82.8
11	137.0	137.4	136.3	136.8	62.9	154.1	154.1
12	135.2	135.0	136.1	135.0	52.6	136.8	138.0
13	42.5	42.6	42.4	39.1	215.1	198.9	199.1
14	67.8	67.7	68.0	70.2	38.6	36.3	37.9
15	37.8	37.7	37.8	37.3	42.6	37.0	37.1
16	25.2	26.0	25.2	26.1	26.7	24.7	25.0
17	31.2	31.1	31.4	31.4	28.1	36.2	36.1
18	21.1	21.3	21.0	21.9	15.4	13.7	14.0
19	22.4	17.3	22.2	17.4	28.5	22.9	17.4
20	116.6	117.4	113.3	117.6	128.6	50.2	116.9
OCH_3	55.2	55.4	55.1	55.5			56.5
2-$OCOCH_3$	169.7	169.7	169.6	169.9	169.7	169.6[a]	170.2[a]
	22.0	22.0	21.6	21.4	21.4	21.2	21.4
5-$OCOCH_3$	169.6	169.5		169.8	169.7	168.9[a]	169.6[a]
	21.6	21.6		21.2	21.4	21.2	21.4
14-$OCOCH_3$				170.0			
				21.5			
10-$OCOCH_3$					170.2	170.0	
					21.2	21.4	

[a] Data may be interchanged.

*2.5.3. 9α-Hydroxy-10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**9**)*

Colorless solid, HRFABMS *m/z*: 515.2595 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_8Na$: 515.2620); mp 152–154 °C, $[\alpha]_D^{20}$ +36.7° (*c* 0.21, Acetone); IR (KBr) ν_{max} (cm^{-1}): 3483, 3020, 2937, 2854, 1732, 1450, 1369, 1097, 1034, 928, 885; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 5.35 (1H, dd, *J* = 2.0, 6.4 Hz, H-2), 5.30 (1H, br s, H-5), 5.20 (1H, s, H_a-20), 4.97 (1H, dd, *J* = 4.8, 9.2 Hz, H-14), 4.91 (1H, s, H_b-20), 4.35 (1H, d, *J* = 9.2 Hz, H-10), 4.04 (1H, d, *J* = 9.2 Hz, H-9), 3.30 (3H, s, 10-OCH_3), 2.91 (1H, d, *J* = 6.4 Hz, H-3), 2.85 (1H, dd, *J* = 8.8, 18.8 Hz, H_β-13), 2.46 (1H, dd, *J* = 4.4, 18.8 Hz, H_α-13), 2.17 (3H, s, H-18), 2.04 (2 × 3H, s, 14, 2-$OCOCH_3$), 2.02 (3H, s, 5-$OCOCH_3$), 1.88 (1H, s, H-1), 1.82 (1H, m, H-6), 1.70 (1H, m, H_α-7), 1.56 (3H, s, H-16), 1.46 (1H, m, H_β-7), 1.18 (3H, s, H-17), 1.03 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

*2.5.4. 2α,5α,10β-Triacetoxy-3,11-cyclotaxa-4(20)-en-13-one (**12**)*

Colorless solid, mp 128–130 °C; $[\alpha]_D^{20}$ +33.3° (*c* 0.03, MeOH); IR (KBr) ν_{max} (cm^{-1}): 2931, 1732, 1670, 1461, 1372, 1228, 1055, 894; FABMS *m/z*: 483 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 6.10 (1H, d, *J* = 5.5 Hz, H-2), 5.75 (1H, s, H_a-20), 5.58 (1H, s, H_b-20), 5.54 (1H, t, *J* = 8.5 Hz, H-5), 5.28 (1H, dd, *J* = 8.5, 10.5 Hz, H-10), 3.40 (1H, q, *J* = 7.0 Hz, H-12), 2.55 (1H, d, *J* = 20.0 Hz, H_a-14), 2.48 (1H, dd, *J* = 20.0, 7.0 Hz, H_b-14), 2.23 (1H, dd, *J* = 8.5, 14.0 Hz, H_α-9), 2.16 (1H, m, H-1), 2.14 (1H, m, H_a-6), 2.08 (3H, s, 10-$OCOCH_3$), 2.06 (2 × 3H, s, 2, 5-$OCOCH_3$), 2.03 (1H, m, H_β-9), 1.61 (3H, s, H-17), 1.53 (1H, m, H_b-6), 1.51 (2H, m, H-7), 1.36 (3H, s, H-19), 1.28 (3H, d, *J* = 7.0 Hz, H-18), 1.22 (3H, s, H-16); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

*2.5.5. 2α,5α,10β-Triacetoxy-4β,20-epoxytaxa-11(12)-en-13-one (**13**)*

Colorless solid, mp 66–68 °C, $[\alpha]_D^{20}$ +200.0° (*c* 0.025, MeOH); IR (KBr) ν_{max} (cm^{-1}): 2926, 1736, 1670, 1372, 1227, 1018, 960, 876; FABMS *m/z*: 499 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 6.06 (1H, dd, *J* = 6.0, 12.0 Hz, H-10), 5.48 (1H, d, *J* = 3.0 Hz, H-2), 4.19 (1H, br s, H-5), 3.45 (1H, d, *J* = 5 Hz, H_a-20), 2.89 (1H, dd, *J* = 6.5, 19.5 Hz, H_a-14), 2.84 (1H, d, *J* = 4.0 Hz, H-3), 2.49 (1H, dd, *J* = 12, 15 Hz, H_β-9), 2.36 (1H, d, *J* = 19.5 Hz, H_b-14), 2.28 (1H, *J* = 5 Hz, H_b-20), 2.20 (3H, s, 10-$OCOCH_3$), 2.10 (3H, s, 2-$OCOCH_3$), 2.04 (3H, s, 5-$OCOCH_3$), 2.01 (1H, br s, H-1), 2.00 (3H, s, H-18), 1.67 (1H, dd, *J* = 5.5, 15 Hz, H_α-9), 1.66 (3H, s, H-17), 1.15 (3H, s, H-16), 1.13 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

*2.5.6. 2α,5α-Diacetoxy-9α-hydroxy-10β-methoxytaxa-4(20),11(12)-dien-13-one (**14**)*

Colorless solid, mp 155–157 °C, $[\alpha]_D^{20}$ +80.0° (*c* 0.125, MeOH), IR (KBr) ν_{max} (cm^{-1}): 3468, 2933, 1731, 1661, 1370, 1237, 1094, 1030, 920, 870; FABMS *m/z*: 471 $[M+Na]^+$,

HRFABMS *m/z*: 471.2381 $[M+Na]^+$ (calcd for $C_{25}H_{36}O_7Na$: 471.2359); ^1H NMR (500 MHz, $CDCl_3$) δ: 5.48 (1H, d, J = 5.0 Hz, H-2), 5.30 (1H, s, H_a-20), 5.24 (1H, br s, H-5), 4.87 (1H, s, H_b-20), 4.47 (1H, d, J = 9.5 Hz, H-10), 4.15 (1H, d, J = 9.5 Hz, H-9), 3.40 (3H, s, 10-OCH_3), 3.18 (1H, d, J = 6.0 Hz, H-3), 2.98 (1H, s, 9-OH), 2.80 (1H, dd, J = 6.5, 20.0 Hz, H_a-14), 2.32 (1H, d, J = 20.0 Hz, H_b-14), 2.14 (3H, s, 2-$OCOCH_3$), 2.12 (1H, br s, H-1), 2.06 (3H, s, 5-$OCOCH_3$), 1.98 (3H, s, H-18), 1.89 (1H, br d, J = 13 Hz, H_a-7), 1.80 (1H, br d, J = 14.5 Hz, H_a-6), 1.71 (1H, br t, J = 14.5 Hz, H_b-6), 1.62 (3H, s, H-17), 1.46 (1H, dt, J = 5, 13 Hz, H_b-7), 1.20 (3H, s, H-16), 1.09 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

2.6. Evaluation of MDR reversal activity for compounds **2–6**, and **10–14** in vitro

2.6.1. Cells and culture

Human non-small cell lung cancer (NSCLC)-lung adenocarcinoma cells A549, and its drug-resistant subclone, A549/taxol, were maintained in Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences. The MDR tumor cells was established by culturing the cells with gradually increasing concentrations of taxol and identified with molecular techniques. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 incubator. Cells were subcultured for two generations every week by digesting with mixture of 0.25% trypsin and 0.01% EDTA solution.

2.6.2. Cytotoxicity assay

The procedures were performed as described in reference [4]. The fold-reversal of MDR was calculated by dividing the IC_{50} values in the absence of MDR reversal agents by those in the presence of the MDR reversal agents.

3. Results and discussion

3.1. Biotransformation and structural elucidation of new products

The substrates **1–4** were added into the cultured medium of *Ginkgo biloba* for biotransformation, respectively. Subsequently, the corresponding culture medium was extracted by ethyl acetate and the solvents were evaporated under the reduced pressure. The residues were fractionated by normal column chromatography, and further purified by HPLC chromatography. In this report, six new metabolites (**5**, **6**, **9**, **12–14**), together with four known compounds (**7**, **8**, **10**, **11**) were obtained from the biotransformation by *Ginkgo biloba* (Scheme 1).

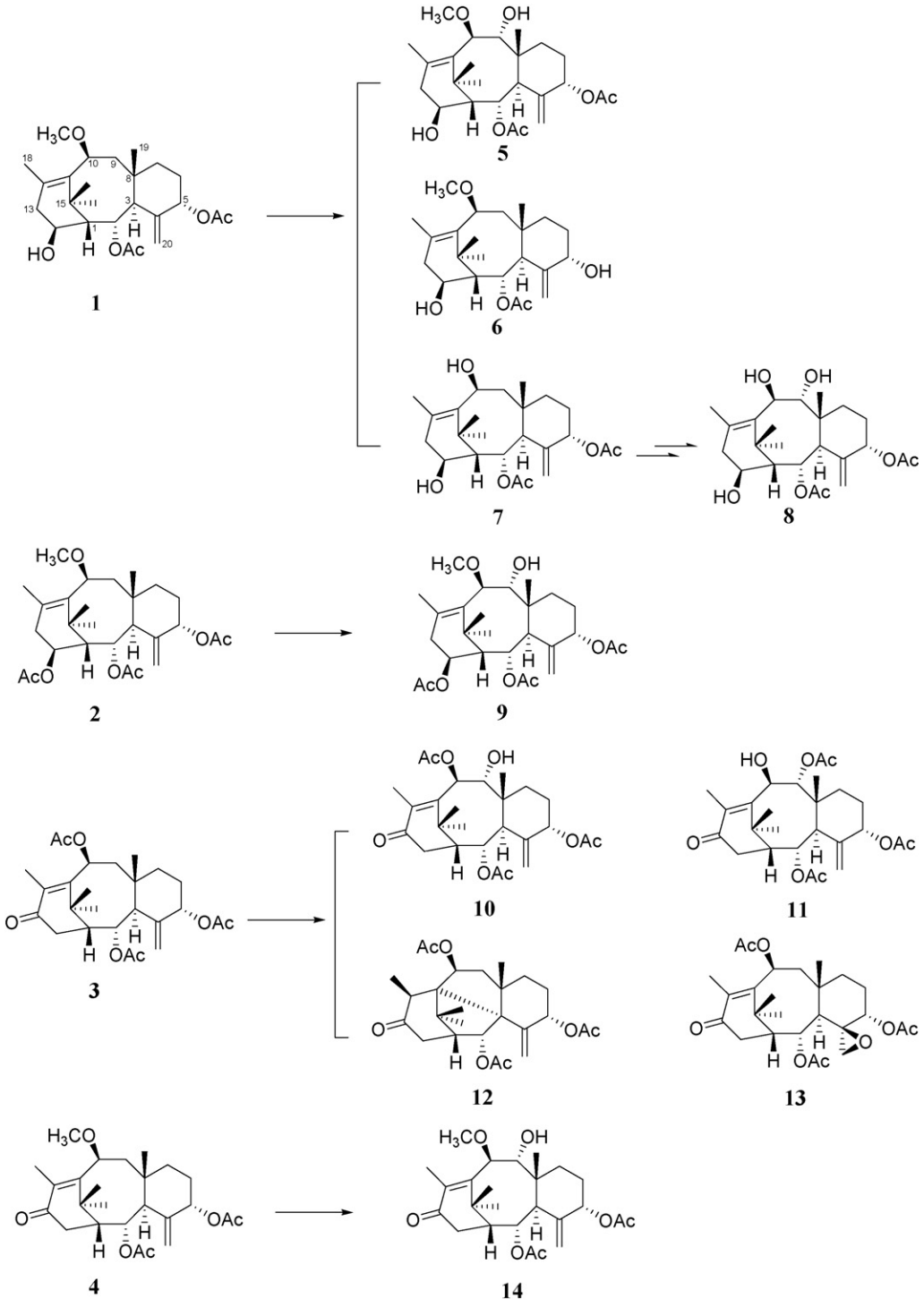
Biotransformation of 2α,5α-diacetoxy-14β-hydroxy-10β-methoxytaxa-4(20),11(12)-diene (**1**) with cultured cells of *Ginkgo* led to the isolation of four metabolites (**5–8**). Among these metabolites, the spectroscopic data of two metabolites were compatible with those of the known 2α,5α-diacetoxy-10β,14β-dihydroxytaxa-4(20),11(12)-diene (**7**) and 2α,5α-diacetoxy-9α,10β,14β-trihydroxytaxa-4(20),11(12)-diene (**8**), respectively [7].

The metabolite **5** had a molecular formula established as $C_{25}H_{38}O_7$ from its HRFABMS, and the strong peak at *m/z* 390 observed in EIMS which is formed by loss of acetic acid. The ^1H NMR spectrum of compound **5** exhibited a typical AB system as a pair of doublets at 4.05 (H-9) and 4.34 (H-10), with a coupling constant of 9.5 Hz, which appeared to limit the attachment of the hydroxyl group to C-9. Moreover, comparison of the ^1H NMR spectrum of **1** and **5** showed that the singlet of C-19 methyl group in **5** had undergone diamagnetic shifts of 0.2 ppm, thus clearly pointing to C-9 as the locus of the hydroxyl group. That the introduced hydroxyl group was at C-9 was also confirmed by the analysis of the ^{13}C NMR spectrum. In the ^{13}C NMR of compound **1**, the C-2, C-5, C-10, and C-14 are found near 70 ppm. As the ^{13}C NMR spectrum of **5** exhibited five carbon signals near 70 ppm, it was clear that C-9 was oxygenated, and the signals of C-8 and C-10 had undergone the expected downfield shifts of 4.5 and 5.4 ppm, respectively. As regards the stereochemistry of **5**, the magnitude of the coupling constants involving H-9 ($J_{9,10}$ = 9.5 Hz) made it obvious that the introduced hydroxyl group was at α-orientation. Thus, compound **5** was concluded to be 2α,5α-diacetoxy-9α,14β-dihydroxy-10β-methoxytaxa-4(20),11(12)-diene.

As stated above, the α-hydroxylation on C-9 was the characteristic biotransformation reaction of polyoxygenated taxadienes by cultured cells of *Ginkgo*. The biotransformation of 10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**) and 2α,5α-diacetoxy-10β-methoxytaxa-4(20),11(12)-dien-13-one (**4**), both of which have a methoxyl group on C-10 in the skeletons, are naturally occurring into the corresponding C-9 α-hydroxylated derivatives, metabolites **9** and **14**. Hence the structures of the metabolites **9** and **14** were elucidated obviously, similar to the analysis of compound **5** detailed above, as 9α-hydroxy-10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**9**) and 2α,5α-diacetoxy-9α-hydroxy-10β-methoxytaxa-4(20),11(12)-dien-13-one (**14**) from their MS and NMR data, respectively.

Then again, the HRFABMS spectrum of metabolite **6** suggested a molecular formula of $C_{23}H_{36}O_5$. The ^{13}C NMR spectrum of **6**, in comparison with that of substrate **1** (Table 1), showed the presence of signals for a sole acetoxy group (δ 169.6 and 21.6). Moreover the downfield shift by 4.8 ppm corresponding to C-4 observed in the ^{13}C NMR spectrum (Table 1) suggested that the transformation was located on its neighbour position C-5. Since the ^1H NMR spectrum also exhibited, near 2.0 ppm, only two methyl singlets at 2.00 (18-CH_3) and 2.07 (2-$OCOCH_3$), it was clear that the acetoxy group at C-5 was lost and substituted with hydroxyl group, perhaps resulting from the preferential hydrolysis of acetoxy group at C-5 adjacent to the terminal olefinic bond. Therefore, the metabolite **6** was 2α-acetoxy-5α,14β-dihydroxy-10β-methoxytaxa-4(20),11(12)-diene.

The compound **3** was obtained by the allylic oxidation of 2α,5α,10β-triacetoxytaxa-4(20),11(12)-diene, and the compound **4** was one of the by-products isolated from the procedure [5]. Both of the substrates **3** and **4** available for further bio-



Scheme 1. Metabolism of compounds **1**, **2**, **3** and **4** by cultured cells of *Ginkgo biloba*.

transformation had a carbonyl group at C-13 conjugated to the $\Delta^{11(12)}$-double bond, and the major difference of two compounds was the diversity of oxygen-bearing functional group at C-10. The biotransformation of the substrate **3** afforded four metabolites **10–13**, in which the major reaction of the plant cell was also the hydroxylation on the substrate to produce the known compounds 9α-hydroxy-2α,5α,10β-triacetoxytaxa-4(20),11(12)-dien-13-one (**10**) and 10β-hydroxy-2α,5α,9α-triacetoxytaxa-4(20),11(12)-dien-13-one (**11**) identical with the literature data [8]. However, the new products **12** and **13** were minor compounds obtained from the biotransformation. The molecular formula of **12** was the same as the substrate **3** as $C_{26}H_{36}O_7$, which was deduced from its FABMS (*m*/*z* 483 $[M+Na]^+$). The ^{1}H NMR of **12** exhibited the usual signals for a taxane skeleton: four methyl groups at δ 1.61, 1.36, 1.28, and 1.22, three acetyl methyl groups at δ 2.08, 2.06, and 2.06, and

an exocyclic methylene group at δ 5.75 and 5.58. However, one of the methyl groups in **12** was resonated as a doublet signal of a secondary methyl group at δ 1.28 (d, $J = 7.0$ Hz), not as all four tertiary methyl groups in regular taxanes. Additionally, the ^{1}H NMR spectrum of **12** showed a quartet signal at δ 3.40 (1H, q, $J = 7.0$ Hz) coupling with the methyl group at δ 1.28 in the ^{1}H-^{1}H COSY spectrum, accompanied by the disappearance of signal of H-3α that usually appears at δ 3.2–3.6 in regular taxanes. These characteristic signals were assigned as Me-18 and H-12, respectively [9]. Interestingly, the ^{13}C NMR spectrum of **12** (Table 1) showed the signals of sole exocyclic double bond at δ 143.2 and 128.6, which suggested the absence of the $\Delta^{11(12)}$ double bond normally existed in most taxanes. This observation was also confirmed by the downfield shift of signal at δ 215.1 corresponding to a ketone carbonyl group at C-13. Based on these data, compound **12** was characterized as a 3,11-cyclotaxane. The HMQC and HMBC spectra of compound **12** further supported the structure drawn in Scheme 1. In addition, the NOESY 1D spectra showed that correlation between the signals of H-12 (δ 3.40) and H-10α (δ 5.28) was compatible only with β-stereochemistry for the methyl attached at C-12. Hence, the compound **12** was assigned as 2α,5α,10β-triacetoxy-3,11-cyclotaxa-4(20)-en-13-one.

The metabolite **13** was obtained as an epoxide product from the biotransformation of the substrate **3**. The FABMS of **13** was in accordance with the formula $C_{26}H_{36}O_8$, indicating that a new oxygen was introduced in the molecule of **3**. The ^{1}H NMR spectrum of compound **13** showed the absence of two signals corresponding to exocyclic methylene protons, whereas the presence of an AB system corresponding to two germinal protons at δ 3.45 and 2.28. The signal of proton H-3 was also shifted upfield of δ 2.84 due to the disappearance of exocyclic double bond, which indicated that the substrate was oxidized into a C-4(20) epoxide. And the large chemical shift difference (Δδ 1.17) between two germinal epoxide protons H-20a and H-20b revealed that the 4(20)-expoxide group was in β-orientation [10,11], which was further supported by the carbon resonances at δ 59.8 and 50.2 assigned to C-4 and C-20 in the ^{13}C NMR spectrum (Table 1). Therefore, the structure of **13** was then established as 2α,5α,10β-triacetoxy-4β,20-epoxytaxa-11(12)-en-13-one.

*3.2. The MDR reversal potency of compounds **2–6**, and **10–14** against taxol-resistant A549 tumor cells in vitro*

The drug-resistant A549/taxol tumor cell line was established by culturing the cells with gradually increasing concentrations of taxol. In this text, the potency of ten compounds **2–6**, and **10–14** to reverse the drug-resistant A549/taxol were examined at 2.5 and 5 μM, respectively, and compared with that of verapamil at 20 μM. The effects of the test compounds against A549/taxol cells are summarized in Table 2. Among the test compounds, compounds **4**, **10**, and **14** showed weak or no reversal activity, however, the other seven compounds showed strong or medium activity. Interestingly, **5**, and **6** were much more active than **2** although their structures varied only by the degree of oxygenation at the C-5, C-9, and C-14, which indicated that hydroxyl groups at C-5, C-9, and C-14 afforded to increase reversal activity. However, unlike these three compounds, compounds **4**, and **14**, which substituted with carbonyl group at C-13 and without oxygenated group at C-14, exhibited much less active than **2**, **5**, and **6**. Compared with the substrates **3**, the metabolites **11**, **12**, and **13** afforded differential improvement against A549/taxol. The result suggested that the carbonyl group on C-13 may be not suitable for the development on the MDR-reversal agents.

Table 2
Reversal effect of compounds on A549/taxol cells *in vitro*

Compounds	Concentration (μM)	A549/taxol	
		IC_{50} (mol/L)	Fold-reversal
Taxol		5.02×10^{-8}	–
2	2.5	9.46×10^{-11}	530.66
	5	2.79×10^{-9}	17.99
3	2.5	1.24×10^{-9}	40.48
	5	6.82×10^{-12}	–
4	2.5	4.61×10^{-8}	1.09
	5	1.30×10^{-7}	0.39
5	2.5	9.30×10^{-10}	53.98
	5	5.24×10^{-11}	958.02
6	2.5	1.35×10^{-10}	371.85
	5	5.09×10^{-11}	986.25
10	2.5	2.78×10^{-8}	1.81
	5	–	–
11	2.5	5.41×10^{-11}	927.91
	5	8.81×10^{-9}	5.70
12	2.5	8.47×10^{-9}	5.93
	5	7.93×10^{-10}	63.30
13	2.5	2.44×10^{-10}	205.74
	5	5.73×10^{-9}	8.76
14	2.5	1.01×10^{-8}	4.97
	5	4.18×10^{-8}	1.20
Verapamil	20	7.34×10^{-9}	6.84

4. Conclusions

As a good biomimic methodology, biotransformation using plant cultured cells of exogenous substrates has been broadly developed to overcome much disadvantages companied with the organic synthesis of complicated and expensive natural products [12]. In conclusion, taxadienes, one kind of special diterpenoids with 6/8/6 carbon skeleton, can be regio- and stereoselective hydroxylated, hydrolyzed to yield a series of structure-diversity polyoxygenated taxadienes by *Ginkgo biloba*. Again, the bioassay of these compounds showed that some agents exhibited significant reversal activity against A549/taxol. Importantly, the compounds **5**, and **6**, which greatly exceeded verapamil in MDR-reversing activity, are potent for further research to be good MDR-reversal agents. Despite the close similarity in structure among the taxadiene compounds, a structure-activity relationship can be also hinted that the methoxyl group at C-10 and the hydroxyl group at C-14 are possible to be effective pharmacophores in MDR-reversal activity. In addition, the hydroxyl groups at C-5 and C-10 are useful to enhance MDR reversal activity of polyoxygenated taxadienes. Further investigation on

the design, synthesis and mechanistic study of those active taxadienes may be needed.

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