Tetrahedron 68 (2012) 9539–9549

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Microbial transformations of taxadienes and the multi-drug resistant tumor reversal activities of the metabolites

Xiao Liu, Ridao Chen, Dan Xie, Mei Mei, Jianhua Zou, Xiaoguang Chen, Jungui Dai *

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing 100050, PR China

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form 18 September 2012
Accepted 21 September 2012
Available online 26 September 2012

Keywords:
Taxadiene
Microbial transformation
Tumor MDR reversal activity

ABSTRACT

Microbial transformation of two taxadienes (**1**, **2**) was individually investigated with two filamentous fungi, *Cunninghamella echinulata* CGMCC 3.3400 and *Aspergillus niger* CGMCC 3.1858, and two actinomycete stains, *Streptomyces griseus* CACC 200300 and *Nocardia purpurea* CGMCC 4.1182. A total of 21 products were obtained, 13 of which were new compounds. The reactions that occurred exhibited diversity, including selective hydroxylation, epoxidation, oxidation, demethylation, acetylation, deacetylation, and O-alkylation, and we have proposed plausible bioconversion routes. The results of a pharmacological evaluation showed that compound **15** displayed significant reversal of activity toward multi-drug resistant (MDR) tumor cell line A549/taxol when co-administered with paclitaxel at 10 μM. This investigation provided a useful approach to prepare new active taxane derivatives that were difficult to access by chemical means.

1. Introduction

Clinical treatment of cancer with chemotherapeutic drugs is frequently hindered by either an intrinsic or acquired resistance of the tumor cells. In both cases, tumors can be refractory to a variety of antineoplastic drugs that have different structures and mechanisms of action. This process is termed multi-drug resistance (MDR).[1] According to statistical data, the failure of treatment in over 90% of patients with metastatic cancer is caused by MDR.[2] Although MDR can be developed by several different mechanisms, a common cause is believed to be overexpression of an Mr 170,000 plasma membrane glycoprotein (P-gp), which acts as an energy-dependent drug efflux pump to lower the intracellular concentration of cytotoxic agents by pumping them outside of the tumor cells.[3] A broad range of chemical compounds, including verapamil, quinidine, and cyclosporine A, have been reported to reverse MDR in vivo. These reversing agents competitively inhibit the binding of antitumor drugs to P-gp in MDR tumor cells and increase the intracellular accumulation of antitumor drugs to sufficient amounts to be cytotoxic to the MDR tumor cells.[4] However, these agents have not been developed for further clinical trials due to their unacceptable toxicities.[5] Therefore, identifying novel MDR reversal agents is an effective strategy to overcome this clinical problem.

In our previous investigation, we found that our patented natural taxanes, sinenxan A and other 4(20),11(12)-taxadienes with C-14 oxygenated substituents (Fig. 1) from cell cultures of *Taxus chinensis*,[6] possessed potent reversal activity against several MDR tumor cells. In an effort to find more potent derivatives, a systematic structural diversification and modification of these compounds was performed by chemical and/or enzymatic approaches.[7] Two derivatives, 10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**1**, Fig. 1) and 5α-hydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**2**, Fig. 1) were obtained from sinenxan A by chemical synthesis (see Experimental section 4.2). The change of an acetoxy group at C-10 to a carbonyl group (**1**) or a methoxy group (**2**) led to a remarkably potent MDR reversing activity toward the A549/taxol MDR cell line. Therefore, in an attempt to obtain more potent derivatives and further investigate the structure–activity relationship of this type of compound with different substituents at C-10 position, the structural modification of **1** and **2** by biological transformation was performed. Twenty-one derivatives were obtained via bioconversion of **1** and **2** through diverse reactions with two species of filamentous fungi, *Cunninghamella echinulata* CGMCC 3.3400 and *Aspergillus niger* CGMCC

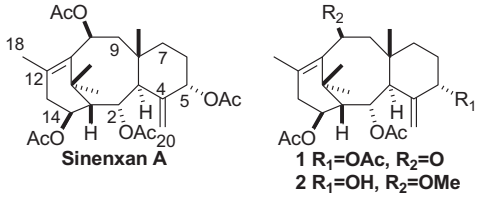

Fig. 1. The structures of sinenxan A, substrates **1** and **2**.

* Corresponding author. Tel.: +86 10 63165195; fax: +86 10 63017757; e-mail address: jgdai@imm.ac.cn (J. Dai).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.09.091

3.1858, and two actinomycete stains, *Streptomyces griseus* CACC 200300 and *Nocardia purpurea* CGMCC 4.1182. Thirteen of the products are new compounds, of which the structures were elucidated via extensive spectroscopic data analysis. Furthermore, the bioassays showed that product **15** exhibited more active reversal efficiency than verapamil toward taxol-resistant A549 tumor cells.

2. Results and discussion

In this investigation, 14 species of filamentous fungi distributed in 10 genera (*Absidia*, *Alternaria*, *Aspergillus*, *Botrytis*, *Cunninghamella*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Gibberella*) and 4 species of actinomycetes (*Nocardia* and *Streptomyces* genera) were employed as biocatalysts for the biotransformation of **1** and **2**. After analysis by TLC and HPLC–UV, four strains, *C. echinulata* CGMCC 3.3400, *A. niger* CGMCC 3.1858, *S. griseus* CACC 200300, and *N. purpurea* CGMCC 4.1182, were selected for the further scale-up biotransformations.

2.1. Biotransformations of 1

*2.1.1. Biotransformation of **1** by C. echinulata CGMCC 3.3400.* Following a standard two-stage fermentation protocol,[8] four new metabolites (**3**–**6**, Fig. 2) were obtained after incubation of **1** with cultures of *C. echinulata* for 7 days. The compounds were purified by silica gel chromatography and semi-preparative HPLC. The compounds were identified as 5α-hydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**3**, ~52.1%), 5α,6α-dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**4**, ~1.8%), 5α-hydroxy-10-oxo-2α,6α,14β-triacetoxytaxa-4(20),11(12)-diene (**5**, ~2.2%), and 7β-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**6**, ~6.6%). A plausible biotransformation pathway for all four metabolites (Fig. 2) was proposed starting from the initial structure **1**.

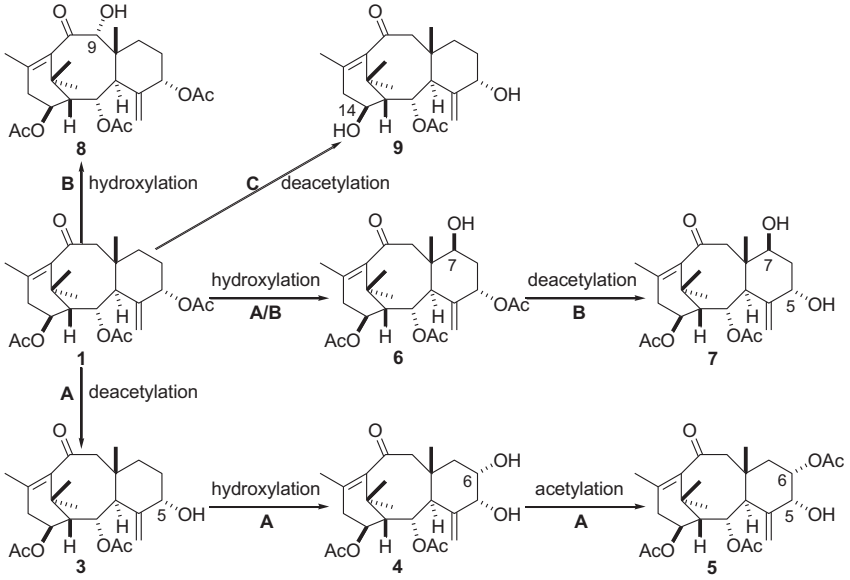

Fig. 2. The plausible bioconversion route for metabolites from **1** (A: by *Cunninghamella echinulata* CGMCC 3.3400; B: by *Streptomyces griseus* CACC 200300; C: by *Nocardia purpurea* CGMCC 4.1182).

Compound **3** was a white amorphous powder. Its molecular formula, $C_{24}H_{34}O_6$, was established by positive HR-ESI-MS; the spectrum contained a quasi-molecular ion peak at *m*/*z* 441.2237 $[M+Na]^+$ (calcd 441.2253 for $C_{24}H_{34}O_6Na$). The ^1H NMR spectroscopic data of **3** were similar to those of **1**, except that the signal of H-5 (δ_H 5.31, br s) shifted upfield to δ_H 4.20 (br s). Moreover, in the ^{13}C NMR spectrum of **3**, the carbon resonance of C-5 shifted upfield to δ_C 74.8 (d) from δ_C 76.0 (d) in **1**. These observations suggest the presence of a hydroxy group rather than an acetoxy group at C-5. This conclusion was further confirmed by the loss of the acetyl group signals at [δ_H 2.16 (s, $-COCH_3$), δ_C 169.6 (s, $-COCH_3$)] and the observation of IR absorption at 3478 cm^{-1} for a hydroxyl group. Thus, **3** was elucidated as 5α-hydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene, which is a selectively deacetylated product of **1**.

Compound **4** was obtained as a white amorphous powder. The positive HR-ESI-MS spectrum of **4** showed a quasi-molecular ion peak at *m*/*z* 457.2215 $[M+Na]^+$ (calcd 457.2203 for $C_{24}H_{34}O_7Na$), which is consistent with the molecular formula $C_{24}H_{34}O_7$. The molecular weight of compound **4** was 16 amu higher than that of **3**, which suggested the introduction of one hydroxyl group. The ^1H NMR spectroscopic data of **4** were similar to those of **3**, except that the signals of H_2-6 δ_H 1.63 (m) disappeared, and an additional oxymethine signal at δ_H 3.67 (m, 1H) was observed. These data suggested that the hydroxyl group may be introduced at the C-6 position. This deduction was further supported by the ^{13}C NMR spectrum of **4**, which had an oxygenated carbon resonance at δ_C 70.3 (d) in place of the C-6 signal at δ_C 31.0 (t) in **3**. The stereochemistry of the introduced hydroxyl group was determined by NOE difference spectra to be in the α-configuration; the integration values of H-5, H-7β, and H-19 were enhanced when H-6 was irradiated. Therefore, the structure of **4** was determined to be 5α,6α-dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene, a hydroxylated derivative of **3**.

Compound **5** was obtained as a white amorphous powder. The molecular formula of $C_{26}H_{37}O_8$ was established by positive HR-ESI-MS for the observation of a quasi-molecular ion peak at *m*/*z* 477.2460 $[M+H]^+$ (calcd 477.2489 for $C_{26}H_{37}O_8$). The ^1H NMR spectrum of **5** was similar to that of **4**, except that the signal of H-6 at δ_H 3.67 (m) was down-shifted to δ_H 4.78 (m). Moreover, the carbon resonance of C-6 in the ^{13}C NMR spectrum of **5** shifted downfield to δ_C 72.4 (d) from δ_C 70.3 (d) in **4**, suggesting the presence of an acetoxy group rather than a hydroxy group at C-6. This conclusion was further confirmed by the appearance of additional signals for an acetoxy group at [δ_H 2.05 (s, $-COCH_3$); δ_C 169.8 (s, $-COCH_3$) and 21.2 (q, $-COCH_3$)] in the spectrum of **5**. The stereochemistry of 6-OAc was determined to be in the α-configuration by an NOE difference spectral experiment, in which the integration values of H-5, H-7β, and H-19 were enhanced when H-

6 was irradiated. Therefore, compound **5** was elucidated as 5α-hydroxy-10-oxo-2α,6α,14β-triacetoxytaxa-4(20),11(12)-diene, which may be converted from **4** via subsequently enzymatic acetylation.

The positive HR-ESI-MS spectrum of **6** had a quasi-molecular ion peak at *m/z* 477.2515 $[M+H]^+$ (calcd 477.2489 for $C_{26}H_{37}O_8$), which is consistent with the molecular formula of $C_{26}H_{36}O_8$. Its molecular weight was 16 amu more than that of **1**, suggesting the introduction of a hydroxy group, which was further supported by an IR absorption band at 3427 cm^{-1}. The ^1H NMR spectroscopic data of **6** were similar to those of **1**, except that the signals of H_2-7 [δ_H 1.74 (m, 1H), 1.25 (m, 1H)] had disappeared, while a new oxymethine proton signal at δ_H 3.54 (dd, 11.5, 5.5) was observed. These data suggest that the OH group may be introduced at C-7 position. This hypothesis was further supported by the observation that the chemical shift of C-7 of **6** was shifted downfield to δ_C 70.9 (d) from δ_C 36.3 (t) in **1**. The stereochemistry of 7-OH was determined to be in the β-configuration by the NOE experiment, in which the integration value of H-3 was enhanced when H-7 was irradiated. Therefore, the structure of **6** was determined to be 7β-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene, which is a hydroxylated derivative of **1**.

*2.1.2. Biotransformation of **1** by S. griseus CACC 200300.* After incubation of **1** with cell cultures of *S. griseus* for 7 days, three new metabolites (**6**–**8**) were obtained by a combination of silica gel chromatography and semi-preparative HPLC (Fig. 2). Their structures were identified as 7β-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**6**, ~29.0%), 5α,7β-dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**7**, ~0.8%), and 9α-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**8**, ~0.8%). Compound **6** was also metabolite of **1** by *C. echinulata*. The hypothetical biotransformation route is illustrated in Fig. 2.

The molecular formula of **7** ($C_{24}H_{34}O_7$) was established by positive HR-ESI-MS (*m/z* 435.2388 $[M+H]^+$, calcd 435.2384 for $C_{24}H_{35}O_7$), which is 42 amu lower than that of **6**. The ^1H NMR spectroscopic data of **7** were similar to those of **6**, except that the signal of H-5 (δ_H 5.30, t) was shifted upfield to δ_H 4.25 (t). Moreover, in the ^{13}C NMR spectrum of **7**, the carbon resonance of C-5 was shifted upfield to δ_C 74.9 (d) from δ_C 75.7 (d) in **6**. The above observations suggest the presence of a hydroxy group instead of an acetoxy group at C-5. This conclusion was further confirmed by the absence of acetoxy signals [δ_H 2.16 (s, $-COCH_3$); δ_C 169.4 (s, $-COCH_3$), 21.5 (q, $-COCH_3$)] in the spectra of **6**. Accordingly, it was clear that deacetylation occurred at the C-5 position of **6** to form **7**, and the structure of **7** was determined to be 5α,7β-dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene.

The molecular formula of **8**, $C_{26}H_{36}O_8$, was established by positive HR-ESI-MS (*m/z* 499.2332 $[M+Na]^+$, calcd 499.2308 for $C_{26}H_{36}O_8Na$) to be 16 amu higher than that of **1**, suggesting the introduction of one hydroxy group. These data are supported by an IR absorption band at 3449 cm^{-1}. The ^1H NMR data of **8** were similar to those of **1**, except for the disappearance of the methylene signals corresponding to H-9 (δ_H 2.34, d, 15.2; δ_H 2.84, d, 15.2) and the appearance of an oxygen-bearing methine signal at δ_H 4.48 (s). The ^{13}C NMR spectrum had an oxygenated carbon resonance at δ_C 81.0 (d) in place of the C-9 signal at δ_C 58.1 (t) in **1**. These observations indicate that a hydroxy group was introduced at the C-9 position. This deduction was further supported by a downfield chemical shift of the H_3-19 signal of 1 at δ_H 0.96 (s) to δ_H 1.10 (s) in **8**, and the chemical shift of the C-8/C-10 signal of **1** at δ_C 40.9 (s)/204.1 (s) to δ_C 47.1 (s)/210.0 (s) in **8**. The α-configuration of the 9-OH group was determined by an NOE difference spectrum experiment, in which the integration values of H-2 and H_3-19 were enhanced when H-9 was irradiated. The structure of **8** was determined to be 9α-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene.

*2.1.3. Biotransformation of **1** by N. purpurea CGMCC 4.1182.* Compound **1** was efficiently bioconverted to one product (**9**, Fig. 2) by *N. purpurea* after 7 days of incubation. The structure of this new compound was determined to be 5α,14β-dihydroxy-10-oxo-2α-acetoxytaxa-4(20),11(12)-diene (**9**, in ca. 81.4% yield), which was formed through deacetylation of **1** at the C-5 and C-14 positions.

Compound **9** was obtained as a white amorphous powder. Its molecular formula, $C_{22}H_{32}O_5$, was established by positive HR-ESI-MS (*m/z* 377.2333 $[M+H]^+$, calcd 377.2329 for $C_{22}H_{33}O_5$). The ^1H NMR data of **9** were very similar to those of **1**, except that the chemical shifts of H-5 δ_H 5.31 (br s) and H-14 δ_H 5.17 (dd, 9.2, 4.8) of **1** shifted upfield to δ_H 4.20 (br s) and δ_H 4.28 (dd, 9.0, 4.5), respectively. Moreover, in the ^{13}C NMR spectrum of **9**, the carbon resonances of C-5 and C-14 shifted upfield to δ_C 74.7 (d) and δ_C 67.9 (d) from δ_C 76.0 (d) and δ_C 70.1 (d) in **1**, respectively. These chemical shifts indicated the presence of hydroxy groups rather than acetoxy groups at positions C-5 and C-14. This conclusion was further confirmed by the loss of two acetyl signals at [δ_H 2.16 (s, $-COCH_3$), δ_C 169.6 (s, $-COCH_3$)] and [δ_H 2.04 (s, $-COCH_3$), δ_C 170.2 (s, $-COCH_3$)] and an IR absorption band at 3478 cm^{-1}. Therefore, deacetylation occurred at both the C-5 and C-14 positions of **1** to form **9**, and the structure of **9** was determined to be 5α,14β-dihydroxy-10-oxo-2α-acetoxytaxa-4(20),11(12)-diene.

2.2. Biotransformations of 2

*2.2.1. Biotransformation of **2** by C. echinulata CGMCC 3.3400.* Compound **2** was efficiently converted to eight products (**10**–**14**, **16**–**18**, Fig. 3) after incubation with 2-day-old cultures of *C. echinulata* for 7 days. The compounds were identified as 5α,9α-dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**10**, ~0.7%), 5α,9α,10β-trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**11**, ~3.1%), 5α,10β-dihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene[9] (**12**, ~56.8%), 10β-hydroxy-2α,5α14β-triacetoxytaxa-4(20),11(12)-diene[9] (**13**, ~0.7%), 2α,10β-dihydroxy-5α,14β-diacetoxytaxa-4(20),11(12)-diene[7b] (**14**, ~1.9%), 5α,18-dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**16**, ~0.8%), 5α,10β,18-trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**17**, ~1.3%), and 5α-hydroxy-2α,10β,14β-triacetoxytaxa-4(20),11(12)-diene[9] (**18**, ~1.0%). Among these products, **10**, **11**, **16**, and **17** were new compounds. Based upon the comparison of the structures of the substrate and metabolites, a plausible biotransformation pathway was proposed in Fig. 3.

The positive HR-ESI-MS spectrum of **10** displayed a quasi-molecular ion peak at *m/z* 473.2517 $[M+Na]^+$, which was consistent with the molecular formula $C_{25}H_{38}O_7$ (calcd 473.2516 for $C_{25}H_{38}O_7Na$). Its molecular weight was 16 amu higher than that of **2**, suggesting the introduction of one hydroxyl group. The ^1H NMR data of **10** were similar to those of **2**, except for the disappearance of the methylene signals that corresponded to H-9 (δ_H 2.23, dd, 14.7, 11.7; δ_H 1.60, m) and the appearance of an oxygen-bearing methine signal at δ_H 4.02 (d, 9.2). The ^{13}C NMR spectrum had a signal for an oxygenated carbon resonance at δ_C 76.9 (d) in place of C-9 signal at δ_C 45.1 (t) for compound **2**. These observations indicate the introduction of a hydroxy group at the C-9 position. This deduction was further supported by the downfield shift of the H_3-19 signal of 2 at δ_H 0.80 (s) to δ_H 0.98 (s) in **10** and the shift of the C-8/C-10 signal of **2** at δ_C 39.6 (s)/76.1 (d) to δ_C 44.2 (s)/81.4 (d) in **10**. The α-configuration of the 9-OH group was determined by an NOE difference spectrum, in which the integration values of H-2 and H_3-19 were enhanced when H-9 was irradiated. Therefore, the structure of **10** was determined to be 5α,9α-dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene.

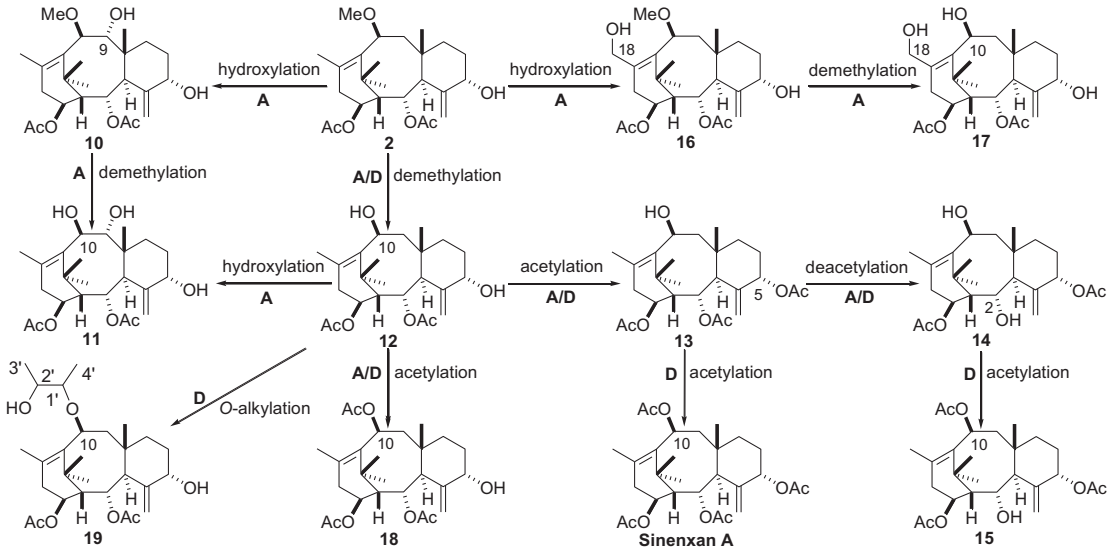

Fig. 3. The plausible bioconversion route for metabolites from **2** (A: by *Cunninghamella echinulata* CGMCC 3.3400; D: by *Aspergillus niger* CGMCC 3.1858).

The molecular formula of **11**, $C_{24}H_{36}O_7$, was established by positive HR-ESI-MS ($[M+Na]^+$ 459.2317 (calcd 459.2359 for $C_{24}H_{36}O_7Na$)). The ^1H NMR spectrum of **11** was very similar to that of **10**, except that the signal for H-10 shifted downfield from δ_H 4.39 (d, 9.2) for **10** to δ_H 4.84 (d, 9.0) for **11**. Moreover, in the ^{13}C NMR spectrum of **11**, the carbon resonance of C-10 was shifted upfield to δ_C 72.2 (d) from δ_C 81.4 (d) for **10**, which suggested the presence of a hydroxy group rather than a methoxy group at C-10. This conclusion was further confirmed by the disappearance of methoxy group signals [δ_H 3.33 (s, $-OCH_3$), δ_C 58.9 (q, $-OCH_3$)]. Thus, **11** was elucidated as 5α,9α,10β-trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene.

The positive HR-ESI-MS spectrum of **16** displayed a quasi-molecular ion peak at *m*/*z* 473.2482 $[M+Na]^+$ (calcd 473.2516 for $C_{25}H_{38}O_7Na$) that was consistent with the molecular formula $C_{25}H_{38}O_7$. The 16 amu increase of molecular mass indicated the presence of an additional hydroxy group in the molecule. The ^1H NMR spectrum of **16** was very similar to that of **2**, except that the signal of H_3-18 at δ_H 1.99 (3H, s) in **2** was absent, while an additional coupling of the oxygenated methylene proton signals at δ_H 4.71 (d, 12.6 Hz) and 4.00 (d, 12.6 Hz) was observed, suggesting hydroxylation at C-18. In addition, the signal of C-18 of **16** shifted downfield to δ_C 77.2 (t) from δ_C 24.9 (q) in **2**. Thus, **16** was identified as 5α,18-dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene, which may be biosynthesized from **2** by hydroxylation at C-18.

Compound **17** was obtained as a white amorphous powder. Its molecular formula, $C_{24}H_{36}O_7$, was established by positive HR-ESI-MS, which showed a quasi-molecular ion peak at *m*/*z* 459.2320 $[M+Na]^+$ (calcd 459.2359 for $C_{24}H_{36}O_7Na$). The ^1H NMR spectroscopic data of **17** were similar to those of **16**, except that the signal of H-10 shifted downfield from δ_H 4.72 (dd, 11.4, 5.4) for **16** to δ_H 5.22 (dd, 11.4, 5.4) for **17**. Moreover, in the ^{13}C NMR spectrum of **17**, the carbon resonance of C-10 shifted upfield to δ_C 70.6 (d) from δ_C 75.3 (d) for **16**, which suggested the presence of a hydroxy group rather than a methoxy group at C-10. This conclusion was further confirmed by the loss of the methoxy group signal at [δ_H 3.25 (s, $-OCH_3$), δ_C 59.3 (q, $-OCH_3$)] in the spectrum of **16**. Thus, **17** was identified as 5α,10β,18-trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene, which might be biosynthesized from **16** by a subsequent demethylation at C-10 position.

*2.2.2. Biotransformation of **2** by A. niger CGMCC 3.1858.* After incubation of **2** with cell cultures of the fungus *A. niger* for 7 days, seven metabolites (**12**–**15**, **18**, **19** and sinenxan A) were obtained by a combination of open silica gel chromatography and semi-preparative HPLC purification (Fig. 3). The compounds were identified as 5α,10β-dihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**12**, ~54.5%), 10β-hydroxy-2α,5α14β-triacetoxytaxa-4(20),11(12)-diene (**13**, ~0.8%), 2α,10β-dihydroxy-5α,14β-diacetoxytaxa-4(20),11(12)-diene (**14**, ~3.8%), 2α-hydroxy-5α,10β,14β-triacetoxytaxa-4(20),11(12)-diene (**15**, ~1.4%), 5α-hydroxy-2α,10β,14β-triacetoxytaxa-4(20),11(12)-diene (**18**, ~7.2%), 5α-hydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene-10β-*O*-(butan-2-ol)-ether (**19**, ~1.8%), and 2α,5α,10β,14β-tetraacetoxytaxa-4(20),11(12)-diene (sinenxan A, ~3.13%). Compounds **12**–**14**, and **18** were also metabolites of **2** by *C. echinulata*. Compound **15** was previously reported, and the spectroscopic data were in agreement with the reference.[10] Compound **19** was new compound. Based upon a comparison of the structures of the substrate and metabolites, a plausible biotransformation pathway was proposed (Fig. 3).

Compound **19** was obtained as a white amorphous powder. Its molecular formula, $C_{28}H_{44}O_7$, was established by positive HR-ESI-MS, which showed a quasi-molecular ion peak at *m*/*z* 515.2947 $[M+Na]^+$ (calcd 515.2985 for $C_{28}H_{44}O_7Na$). The ^1H NMR spectrum was very similar to that of **12**, except that a series of additional protons [δ_H 3.42 (dq, 3.0, 6.3, H_1-1′), δ_H 3.90 (dq, 3.0, 6.3, H_1-2′), δ_H 1.12 (d, 6.3, H_3-3′), δ_H 1.10 (d, 6.3, H_3-4′)] were observed. Signals for carbons connected to the new proton signals were also observed in the ^{13}C NMR spectrum. Thus, a butan-2,3-ol moiety was deduced to be attached at the C-10 position through an ether bond. According to the above evidence, **19** was identified as 5α-hydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene-10β-*O*-(butan-2-ol)-ether.

*2.2.3. Biotransformation of **2** by N. purpurea CGMCC 4.1182.* After incubation with cell cultures of *N. purpurea* for 7 days, four metabolites (**13**, **20**–**22**) were obtained from **2** by a combination of open silica gel chromatography and semi-preparative HPLC purification (Fig. 4). The compounds were identified as 10β-hydroxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**13**, ~30.5%), 10β-methoxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**20**, ~5.5%), 10β-methoxy-2α,5α,14β-trihydroxytaxa-4(20),11(12)-diene (**21**, ~22.6%), and 5α,10β,14β-trihydroxy-2α-acetoxytaxa-4(20),11(12)-diene (**22**, 7.46%). Of these compounds, **13**, **20**, and **22** were known, and the spectroscopic data were in good agreement with the reported data.[7b,12] Compound **13** was also metabolite of **2** by *C.*

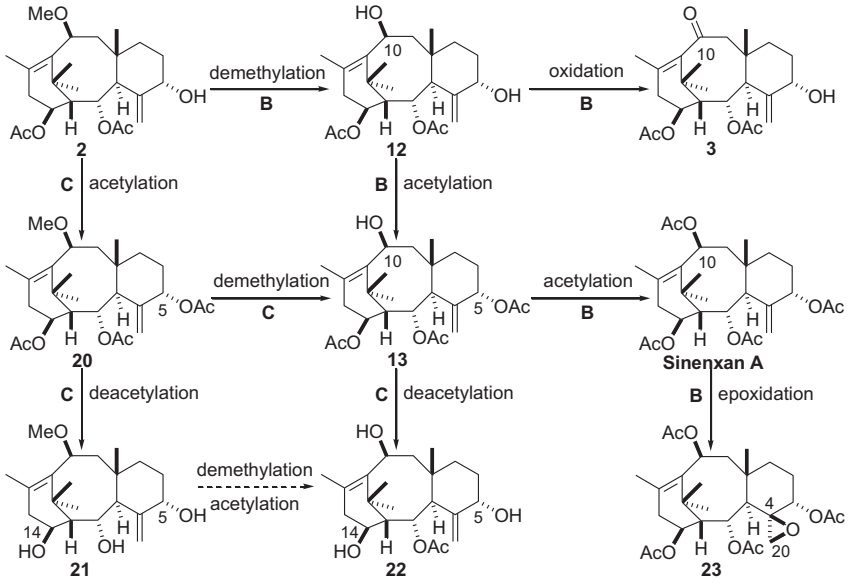

Fig. 4. The plausible bioconversion route for metabolites from **2** (B: by *Streptomyces griseus* CACC 200300; C: by *Nocardia purpurea* CGMCC 4.1182).

echinulata, and **21** was a new compound. Based upon the comparison of the structures of the substrate and metabolites, a plausible biotransformation pathway was proposed (Fig. 4).

The positive HR-ESI-MS spectrum of **21** displayed a quasi-molecular ion peak at *m*/*z* 351.2537 $[M+H]^+$ (calcd, 351.2536 for $C_{21}H_{35}O_4$), which was consistent with the molecular formula $C_{21}H_{34}O_4$. The ^{1}H NMR spectroscopic data of **21** were very similar to those of **2**, except that the signals for H-2 at δ_H 5.33 (dd, 6.0, 1.8) and H-14 at δ_H 5.04 (dd, 9.3, 4.5) shifted upfield to δ_H 4.10 (br d, 6.0) and δ_H 4.01 (dd, 9.0, 4.5), respectively. Moreover, in the ^{13}C NMR spectrum of **21**, the carbon resonance of C-2 shifted upfield to δ_C 69.7 (d) from δ_C 71.1 (d) for **2**, and the carbon resonance of C-14 shifted upfield to δ_C 67.7 (d) from δ_C 70.8 (d) for **2**, which suggested the presence of hydroxy groups rather than acetoxy groups at C-2 and C-14. This conclusion was further confirmed by the absence of acetoxy signals [δ_H 2.03, 2.04 (s, $-COCH_3$), δ_C 169.9, 170.3 (s, $-COCH_3$)] that were present in the spectrum of **2**. Therefore, **21** was identified as 10β-methoxy-2α,5α,14β-trihydroxytaxa-4(20),11(12)-diene, which might be biosynthesized from **2** by deacetylation at both the C-2 and C-14 positions.

*2.2.4. Biotransformation of **2** by S. griseus CACC 200300.* After 7 days of incubation of **2** with *S. griseus* and separation by silica gel chromatography and semi-preparative HPLC, five metabolites were obtained (**3**, **12**, **13**, **23**, and sinenxan A, Fig. 4). The spectroscopic data of these four metabolites were in accordance with those of known compounds, 5α,10β-dihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**12**, ~4.5%), 10β-hydroxy-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**13**, ~11.3%), 2α,5α,10β,14β-tetraacetoxytaxa-4β,20-epoxy-11(12)-ene (**23**, ~1.4%), and 2α,5α,10β,14β-tetraacetoxytaxa-4(20),11(12)-diene (sinenxan A, 28.7%).[7b,9a,11] Compounds **12**, **13**, and sinenxan A were also metabolites of **2** by *A. niger*. The new compound, 5α-hydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**3**, ~6.9%), was formed through oxidation of **2** at the C-10 position and was also metabolite of **1** by *C. echinulata*. Based upon the comparison of the structures of the substrate and metabolites, a plausible biotransformation pathway was proposed (Fig. 4).

2.3. The MDR reversal potency of compounds 1–23 against taxol-resistant A549 tumor cells in vitro

The multi-drug resistant A549/taxol tumor cell line was established by culturing the cells with gradually increasing concentrations of paclitaxel. For these experiments, the potency of compounds **1**–**23** in the reversal of the drug resistance of A549/taxol was examined at 10 μM. Verapamil was used as the positive control. The effects of the tested compounds are summarized in Table 5. Several of the

Table 1
^{1}H NMR spectral data of compounds **1** and **3**–**9** in $CDCl_3$[a]

No.	1[b]	3	4	5	6	7	8	9
1	2.04 (br s)	2.00 (d, 2.0)	2.01 (d, 2.0)	2.01 (br s)	2.07 (br s)	2.01 (br s)	2.10 (br s)	1.90 (d, 2.1)
2	5.44 (d, 6.4)	5.43 (dd, 6.0, 2.0)	5.44 (dd, 6.5, 2.0)	5.45 (dd, 6.5, 2.5)	5.49 (dd, 6.5, 2.5)	5.10 (dd, 6.0, 2.0)	5.53 (dd, 6.4, 2.0)	5.49 (dd, 6.0, 2.1)
3	3.04 (d, 6.4)	3.29 (d, 6.0)	3.24 (d, 6.5)	3.31 (d, 6.5)	2.87 (d, 6.5)	3.14 (d, 6.0)	2.86 (d, 6.4)	3.23 (d, 6.0)
5	5.31 (br s)	4.20 (br s)	4.17 (d, 4.0)	4.28 (d, 3.5)	5.30 (t)	4.25 (t)	5.32 (br s)	4.20 (br s)
6	1.63 (m, 2H)	1.63 (m, 2H)	3.67 (m)	4.78 (m)	1.71 (m, 2H)	2.01 (m, 2H)	1.75 (m, 2H)	1.71 (m, 2H)
7α	1.25 (m)	1.16 (m)	1.25 (m)	1.25 (m)	3.54 (dd, 11.5, 5.5)	3.65 (dd, 11.0, 5.0)	1.29 (m)	1.18 (m)
7β	1.74 (m)	1.65 (m)	1.80 (m)	1.70 (m)			1.70 (m)	1.74 (m)
9α	2.34 (d, 15.2)	2.85 (d, 15.6)	2.85 (d, 16.0)	2.93 (d, 15.5)	2.85 (d, 16.5)	2.79 (d, 16.5)		2.85 (d, 15.6)
9β	2.84 (d, 15.2)	2.28 (d, 15.6)	2.28 (d, 16.0)	2.25 (d, 15.5)	2.02 (m)	2.02 (m)	4.48 (s)	2.28 (d, 15.6)
13α	2.45 (dd, 18.8, 4.8)	2.40 (dd, 19.2, 4.4)	2.40 (dd, 19.0, 4.5)	2.95 (dd, 19.0, 9.0)	2.91 (dd, 19.0, 9.0)	2.89 (dd, 19.0, 9.5)	2.98 (dd, 19.2, 8.8)	2.78 (dd, 19.0, 9.0)
13β	2.93 (dd, 18.8, 9.2)	2.90 (dd, 19.2, 8.8)	2.91 (dd, 19.0, 9.0)	2.44 (dd, 19.0, 4.0)	2.47 (dd, 19.0, 4.5)	2.42 (dd, 19.0, 4.5)	2.52 (dd, 19.2, 4.4)	2.48 (dd, 19.0, 4.5)
14	5.17 (dd, 9.2, 4.8)	5.20 (dd, 8.8, 4.4)	5.17 (dd, 9.0, 4.5)	5.20 (dd, 9.0, 4.0)	5.17 (dd, 9.0, 4.5)	5.15 (dd, 9.5, 4.5)	5.09 (dd, 8.8, 4.4)	4.28 (dd, 9.0, 4.5)
16	1.46 (s)	1.46 (s)	1.46 (s)	1.46 (s)	1.46 (s)	1.52 (s)	1.48 (s)	1.45 (s)

(*continued on next page*)

Table 1 (*continued*)

No.	**1**[b]	**3**	**4**	**5**	**6**	**7**	**8**	**9**
17	1.18 (s)	1.16 (s)	1.18 (s)	1.18 (s)	1.16 (s)	1.17 (s)	1.21 (s)	1.21 (s)
18	1.91 (s)	1.92 (s)	1.91 (s)	1.91 (s)	1.91 (s)	1.92 (s)	1.97 (s)	1.91 (s)
19	0.96 (s)	0.90 (s)	0.96 (s)	0.96 (s)	0.90 (s)	0.80 (s)	1.10 (s)	0.90 (s)
20a	5.28 (br s)	5.16 (br s)	5.28 (br s)	5.33 (br s)	5.28 (br s)	5.18 (br s)	5.36 (br s)	5.16 (br s)
20b	4.87 (br s)	4.80 (br s)	4.87 (br s)	4.96 (br s)	4.80 (br s)	4.88 (br s)	4.93 (br s)	4.78 (br s)
−OAc	2.16 (s), 2.06 (s), 2.04 (s)	2.06 (s), 2.03 (s)	2.16 (s), 2.06 (s)	2.09 (s), 2.06 (s), 2.05 (s)	2.16 (s), 2.06 (s), 2.03 (s)	2.08 (s), 2.04 (s)	2.17 (s), 2.09 (s), 2.04 (s)	2.09 (s)

[a] ^{1}H NMR data were recorded at 300 MHz for **9**, at 400 MHz for **1**, **3**, and **8**, and at 500 MHz for **4**–**7**, respectively. Peaks were assigned by analyses of the 1D- and 2D-NMR spectra.
[b] Multiplicities and coupling constants (*J*) in hertz are in parentheses.

Table 2
^{13}C NMR data for compounds **1** and **3**–**9** in $CDCl_3$[a]

No.	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	57.4 d	57.4 d	56.9 d	56.6 d	51.4 d	51.0 d	57.1 d	57.9 d
2	70.3 d	70.5 d	69.6 d	70.0 d	70.2 d	70.0 d	69.7 d	71.0 d
3	43.9 d	41.4 d	39.4 d	39.9 d	42.0 d	39.5 d	43.9 d	41.3 d
4	144.1 s	147.3 s	144.0 d	144.0 s	144.8 s	145.7 s	148.0 s	143.8 s
5	76.0 d	74.8 d	74.8 d	76.2 d	75.7 d	74.9 d	78.5 d	74.7 d
6	30.5 t	31.0 t	70.3 d	72.4 d	35.6 t	35.5 t	28.5 t	30.4 t
7	36.3 t	30.7 t	31.0 t	31.1 t	70.9 d	70.2 d	26.9 t	35.5 t
8	40.9 s	41.5 s	43.9 s	39.6 s	46.0 s	46.5 s	47.1 s	41.9 s
9	58.1 t	57.8 t	57.1 t	57.0 t	57.5 t	57.5 t	81.0 d	61.6 t
10	204.1 s	204.1 s	202.9 s	202.6 s	203.6 s	203.4 s	210.0 s	204.4 s
11	142.4 s	143.7 s	140.0 s	142.6 s	140.8 s	144.4 s	141.2 s	147.7 s
12	135.4 s	136.5 s	136.8 s	137.2 s	136.4 s	137.5 s	140.0 s	137.2 s
13	38.6 t	38.5 t	38.6 t	39.1 t	38.9 t	39.0 t	39.5 t	41.3 t
14	70.1 d	70.4 d	70.1 d	70.2 d	69.6 d	69.9 d	70.3 d	67.9 d
15	35.6 s	35.5 s	35.3 s	38.6 s	36.8 s	38.9 s	35.5 s	35.9 t
16	26.2 q	26.2 q	26.2 q	26.3 q	26.5 q	26.5 q	26.6 q	26.4 q
17	28.6 q	30.7 q	29.4 q	31.1 q	30.7 q	31.0 q	31.2 q	31.4 q
18	21.6 q	21.4 q	21.4 q	21.5 q	21.5 q	21.4 q	21.8 q	21.7 q
19	22.1 q	21.7 q	24.5 q	22.3 q	21.6 q	15.5 q	17.1 q	21.6 q
20	115.8 t	113.2 t	116.8 t	117.9 t	117.1 t	114.5 t	118.1 t	112.9 t
−OAc	21.3, 21.4, 21.6 q; 169.6, 169.9, 170.2 s	21.3, 21.4 q; 2×170.1 s	21.3, 21.4 q; 2×170.1 s	21.1, 21.2, 21.4 q; 169.8, 2×170.1 s	21.3, 21.4, 21.5 q; 169.4, 169.9, 170.1 s	21.3, 21.4 q; 169.9, 170.0 s	3×21.3 q; 3×170.0 s	21.5 q; 169.8 s

[a] ^{13}C NMR data were recorded at 100 MHz for **1**, **8**, and **9**, at 125 MHz for **3**–**6**, and at 150 MHz for **7**, respectively. Peaks were assigned by analyses of the 1D- and 2D-NMR spectra.

Table 3
^{1}H NMR spectral data of compounds **2**, **10**, **11**, **16**, **17**, **19**, and **21** in $CDCl_3$[a]

No.	**2**[b]	**10**	**11**	**16**	**17**	**19**[c]	**21**
1	1.83 (br s)	1.81 (br s)	1.81 (br s)	1.90 (d, 2.1)	1.89 (br s)	1.82 (br s)	1.86 (d, br s)
2	5.33 (dd, 6.0, 1.8)	5.34 (d, 6.0)	5.35 (d, 6.0)	5.37 (dd, 6.3, 2.1)	5.37 (dd, 6.0, 2.0)	5.34 (d, 6.0)	4.10 (d, 6.0)
3	3.21 (d, 6.0)	3.21 (d, 6.0)	3.22 (d, 6.0)	3.14 (d, 6.3)	3.13 (d, 6.0)	3.19 (d, 6.0)	3.07 (d, 6.0)
5	4.18 (br s)	4.20 (br s)	4.19 (br s)	4.18 (br s)	4.15 (br s)	4.18 (br s)	4.19 (br s)
6	1.71 (m, 2H)	1.71 (m, 2H)	1.75 (m, 2H)	1.75 (m, 2H)	1.70 (m, 2H)	1.73 (m, 2H)	1.71 (m, 2H)
7α	1.26 (m)	1.26 (m)	1.23 (m)	1.01 (m)	1.09 (m)	1.25 (m)	1.07 (m)
7β	1.70 (m)	1.98 (m)	1.65 (m)	2.17 (m)	2.13 (m)	2.01 (m)	2.14 (m)
9α	2.23 (dd, 14.7, 11.7)	4.02 (d, 9.2)	4.05 (d, 9.0)	2.28 (dd, 15.0, 11.4)	2.24 (m)	2.10 (dd, 15.0, 12.0)	2.18 (dd, 15.0, 11.4)
9β	1.60 (m)			1.61 (m)	1.61 (dd, 15.0, 5.4)	1.59 (dd, 15.0, 4.5)	1.59 (dd, 15.0, 4.8)
10	4.65 (dd, 11.7, 5.4)	4.39 (d, 9.2)	4.84 (d, 9.0)	4.72 (dd, 11.4, 5.4)	5.22 (dd, 11.4, 5.4)	4.82 (dd, 12.0, 4.5)	4.62 (dd, 11.4, 4.8)
13α	2.77 (dd, 19.2, 9.3)	2.80 (dd, 19.6, 9.2)	2.78 (dd, 18.9, 9.0)	3.36 (dd, 19.2, 9.0)	3.28 (dd, 19.2, 9.3)	2.76 (dd, 18.9, 9.0)	2.66 (dd, 17.4, 9.0)
13β	2.37 (dd, 19.2, 4.5)	2.39 (dd, 19.6, 4.4)	2.36 (dd, 18.9, 4.8)	2.27 (dd, 19.2, 4.5)	2.37 (m)	2.34 (dd, 18.9, 4.5)	2.48 (dd, 17.4, 4.5)
14	5.04 (dd, 9.3, 4.5)	5.01 (dd, 9.2, 4.4)	5.01 (dd, 9.0, 4.8)	5.09 (dd, 9.0, 4.5)	5.07 (dd, 9.3, 4.8)	5.03 (dd, 9.0, 4.5)	4.01 (dd, 9.0, 4.5)
16	1.62 (s)	1.26 (s)	1.64 (s)	1.63 (s)	1.75 (s)	1.71 (s)	1.54 (s)
17	1.17 (s)	1.17 (s)	1.18 (s)	1.19 (s)	1.20 (s)	1.10 (s)	1.24 (s)
18	1.99 (s)	1.56 (s)	1.99 (s)	4.71 (d, 12.6), 4.00 (d, 12.6)	4.61 (d, 12.6), 4.04 (d, 12.6)	1.98 (s)	1.99 (s)
19	0.80 (s)	0.98 (s)	1.00 (s)	0.82 (s)	0.82 (s)	0.79 (s)	0.83 (s)

Table 3 (*continued*)

No.	**2**[b]	**10**	**11**	**16**	**17**	**19**[c]	**21**
20a	5.09 (br s)	5.13 (br s)	5.13 (br s)	5.12 (br s)	5.11 (br s)	5.09 (br s)	5.18 (br s)
20b	4.78 (br s)	4.84 (br s)	4.82 (br s)	4.78 (br s)	4.77 (br s)	4.77 (br s)	
$-OCH_3$	3.28 (s)	3.33 (s)		3.25 (s)			3.27 (s)
−OAc	2.03, 2.04 (s)	2.03, 2.04 (s)	2.03, 2.05 (s)	2.04, 2.05 (s)	2.03, 2.04 (s)	2.02, 2.03 (s)	

[a] ^{1}H NMR data were recorded at 300 MHz for **2**, **11, 16, 17 19** and **21,** and at 400 MHz for **10**, respectively. Peaks were assigned by analyses of the 1D- and 2D-NMR spectra.
[b] Multiplicities and coupling constants (*J*) in Hz are in parentheses.
[c] Data for protons of **19**: δ_H 3.42 (dq, 3.0, 6.3, H-1′), δ_H 3.90 (dq, 3.0, 6.3, H-2′), δ_H 1.12 (d, 6.3, H-3′), δ_H 1.10 (d, 6.3, H-4′).

Table 4
^{13}C NMR data for compounds **2**, **10**, **11**, **16**, **17**, **19**, and **21** in $CDCl_3$[a]

No.	**2**	**10**	**11**	**16**	**17**	**19**[b]	**21**
1	55.2 d	55.4 d	59.0 d	55.5 d	59.3 d	59.1 d	55.1 d
2	71.1 d	70.7 d	70.7 d	70.4 d	66.6 d	71.0 d	69.7 d
3	40.1 d	41.9 d	42.0 d	39.5 d	39.6 d	40.0 d	41.9 d
4	136.2 s	147.4 s	147.5 s	146.7 s	146.8 s	147.8 s	149.8 s
5	76.4 d	77.1 d	72.2 d	70.9 d	70.9 d	76.4 d	76.1 d
6	30.8 t	30.5 t	30.4 t	30.6 t	30.7 t	30.8 t	31.6 t
7	33.2 t	31.5 t	32.0 t	32.3 t	32.4 t	31.7 t	33.5 t
8	39.6 s	44.2 s	44.7 s	37.9 s	37.9 s	39.9 s	40.2 s
9	45.1 t	76.9 d	78.6 d	44.5 t	46.2 t	45.7 t	45.4 t
10	76.1 d	81.4 d	72.2 d	75.3d	70.6 d	72.6 d	76.0 d
11	147.9 s	138.0 s	135.6 s	141.0 s	142.5 s	136.9 s	135.9 s
12	135.6 s	134.1 s	135.6 s	138.6 s	136.2 s	134.4 s	135.7 s
13	39.9 t	39.8 t	39.4 t	44.3 t	40.0 t	39.4 t	42.3 t
14	70.8 d	70.5 d	70.5 d	61.5 d	61.7 d	70.8 d	67.7 d
15	37.4 s	37.4 s	37.5 s	34.1 s	34.1 s	37.4 s	37.6 s
16	22.3 q	21.1 q	21.2 q	25.2 q	25.5 q	25.1 q	22.6 q
17	31.6 q	25.3 q	25.2 q	31.2 q	31.7 q	33.3 q	31.8 q
18	24.9 q	25.7 q	26.0 q	77.2 t	76.4 t	20.9 q	25.5 q
19	20.9 q	17.2 q	17.4 q	22.5 q	22.4 q	22.3 q	21.1 q
20	113.4 t	114.1 t	114.1 t	113.8 t	113.7 t	113.5 t	113.8 t
$-OCH_3$	59.1 q	58.9 q		59.3 q			66.4 q
−OAc	2×21.5 q; 169.9 s, 170.3 s	2×21.5 q; 169.9 s, 170.3 s	2×21.5 q; 169.9 s, 170.3 s	2×21.5 q, 2×170.1 s	2×21.5 q; 169.9 s, 170.1 s	2×21.5 q; 2×169.9 s	

[a] ^{13}C NMR data were recorded at 125 MHz for **2**, and at 100 MHz for **10**, **11**, **16**, **17**, **19**, and **21**, respectively. Peaks were assigned by analyses of the 1D- and 2D-NMR spectra.
[b] Data for carbon of **19**: δ_C (75.7 d, C-1′), δ_C (67.8 d, C-2′), δ_C (17.6 q, C-3′), δ_C (14.2 q, C-4′).

Table 5
Reversal activities of **1**–**23** against MDR A549/taxol cells[a]

Samples	IC_{50} of paclitaxel[b] (nM)	RF[c] (A549/T)	Samples	IC_{50} of paclitaxel[b] (nM)	RF[c] (A549/T)
Paclitaxel (blank control)	26.0	—	12	23.6	1.1
Verapamil (positive control)	5.9	4.4	13	13.0	2.0
1	14.4	1.8	14	17.3	1.5
2	13.0	2.0	15	2.5	10.6
3	16.4	1.6	16	17.3	1.5
4	26.0	1.0	17	15.3	1.7
5	19.4	1.3	18	13.7	1.9
6	36.2	0.7	19	13.0	2.0
7	9.3	2.8	20	11.3	2.3
8	14.4	1.8	21	12.4	2.1
9	13.7	1.9	22	11.8	2.2
10	32.5	0.8	23	6.0	4.3
11	17.3	1.5			

[a] All the samples at 10 μM were co-administered with paclitaxel, and paclitaxel concentrations were designated to 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M. Paclitaxel without reversal agent was used as blank control, and 10 μM verapamil with paclitaxel was used as positive control.
[b] A549/taxol, an MDR subline of human lung adenocarcinoma cell line A549.
[c] RF (reversal fold)=IC_{50} (antitumor agent)/IC_{50} (reversal agent+antitumor agent).

metabolites exhibited higher reversal activities than the substrates **1** and **2**. Compound **15** had approximately two-fold higher activity than verapamil and five times higher activity than substrate **2**. Compound **23** had comparable activity to verapamil and two times higher activity than **2**. All of the metabolites exhibited weak or no cytotoxicity, which is a good property for a reversing agent.[13] Thus, the results showed that compounds **15** and **23** might be promising lead compounds for reversal agents against A549/taxol tumor MDR cells.

3. Conclusion

In summary, we report the successful structural diversification of two 4(20),11(12)-taxadienes (**1** and **2**) by microbial transformation. Seven transformation systems were established in two fungal and two actinomycete stains. In total, 21 derivatives were obtained, 13 of which were new compounds. Plausible bioconversion routes were predicted, and the reactions exhibited diversity, including selective

hydroxylation, epoxidation, oxidation, demethylation, acetylation, deacetylation, and O-alkylation. Most of these transformations are not easily accessible by a chemical approach. Moreover, bioassays showed that product **15** exhibited more active reversal efficiency than its parent substrate and the positive control (verapamil) toward taxol-resistant A549 tumor cells. These results suggest that biotransformation is a powerful approach to the structural diversification of bioactive natural products, perhaps even providing bioactive derivatives with increased potency.

4. Experimental section

4.1. General experimental procedures

Optical rotations in CH_3OH were recorded on a Perkin–Elmer Model-343 digital polarimeter. IR spectra were obtained on a Thermo Nicolet 5700 FT-IR microscope spectrometer. HR-ESI-MS were measured on a Q-trap ESI mass spectrometer (Applied Biosystems/MDS Sciex, Carlsbad, CA, USA). NMR spectra were recorded on Mercury-300, Mercury-400, and Bruker-500 spectrometers using the solvent ($CDCl_3$, ^1H δ 7.26, ^{13}C δ 77.0) as an internal reference. The chemical shifts (δ) are given in parts per million (ppm), and the coupling constants (J) are given in hertz (Hz). Analytical HPLC was performed on an Agilent series 1200 HPLC instrument equipped with a quaternary pump, a diode-array detector (DAD), an autosampler, a column compartment and a GraceSmart C_{18} column (250 mm×4.6 mm, i.d., 5 μm). The mobile phase consisted of ultrapure water and acetonitrile (CH_3CN) with a two-pump gradient program at the flow rate of 1.0 mL/min at 30 °C. Semi-preparative HPLC was performed on a Shiseido HPLC instrument equipped with a YRU-883A RI/UV detector and a Grace Allsphere silica column (250 mm×10 mm, i.d., 5 μm) by elution with mixtures of *n*-hexane and acetone or a Grace Adsorbosphere C_{18} column (250 mm×10 mm, i.d., 5 μm) by eluting with the mixtures of CH_3OH and H_2O. Silica gel (200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, PR China) and Sephadex LH-20 gel (Pharmacia Fine Chemical Co., Ltd., Sweden) were used for column chromatography (CC). Analytical TLC was performed using pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, PR China). The visualization of TLC plates was performed by illuminating at UV 254 nm, followed by spraying with 5% H_2SO_4 in EtOH and heating at 105 °C. All solvents used for CC were of analytical grade (Beijing Chemical Works., Beijing, PR China), and the solvents used for HPLC were HPLC grade (Mallinckrodt Baker Co. Ltd., Phillipsburg, USA).

4.2. Substrate preparation

Sinenxan A [2α,5α,10β,14β-tetraacetoxytaxa-4(20),11(12)-diene] was isolated from callus cultures (Ts-19 strain) of *T. chinensis*.[6]

*4.2.1. Preparation of 10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**1**).* 10-Deacetyl sinenxan A was prepared as reported by Yin.[14] To a solution of 675 mg (1.46 mmol) of 10-deacetyl sinenxan A in 15 mL of CH_2Cl_2 was added 855.8 mg (7.30 mmol) of *N*-methylmorpholine *N*-oxide (NMO). After the mixture was stirred for 10 min, 50.0 mg (0.15 mmol) of tetrapropylammonium perruthenate (TPAP) was added, and the mixture was allowed to stir for 10 h at 26 °C. The reaction mixture was filtered and then concentrated in vacuo. The residue was diluted with 20 mL of ddH_2O before extraction with EtOAc (60 mL×3). The pooled EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo at 40 °C to afford 1.5 g of residue, which was subjected to silica gel CC. A petroleum ether/acetone mixture (12:1, v/v) was used as the eluent to give **1** (600 mg, 89.3%). The same procedure was repeated additional three times, and totally obtain ca. 2.4 g of **1**.

*4.2.1.1. 10-Oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**1**).* White powder; $[\alpha]_D^{20}$ +3.6 (*c* 0.11, CH_3OH); IR (ν_{max}): 3001, 2955, 2936, 1738, 1679, 1436, 1372, 1241, 1103, 1030, 978, 928 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m*/*z*: $[M+Na]^+$ 483.2314 (calcd 483.2359 for $C_{26}H_{36}O_7Na$).

*4.2.2. Preparation of 5α-hydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**2**).* To a solution of sinenxan A (5.0 g, 10 mmol) in methanol (150 mL) was added 10 M aqueous KOH (14 mL). The reaction mixture was stirred at −10 °C for 20 min. The reaction was quenched with 1 M aqueous HCl, and the resulting mixture was concentrated and extracted with EtOAc (300 mL×3). The organic layer was washed with saturated aqueous $NaHCO_3$, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography to give 2α,5α,10β,14β-tetrahydroxytaxa-4(20),11-diene (2.85 g, 85%). Ac_2O (3 ml, 30 mmol) was added to a stirred pyridine (50 mL) solution of the compound 2α,5α,10β,14β-tetrahydroxytaxa-4(20),11-diene (2.85 g, 8.5 mmol). After the mixture was stirred at room temperature for 16 h, methanol (5 mL) was added to quench the reaction. The resulting mixture was neutralized with 1 M aqueous HCl and extracted with EtOAc (200 mL×3). The organic layer was successively washed with 1 M aqueous HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL) before it was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography (petroleum ether/EtOAc=5:1, v/v as elution) to afford **2** (1.1 g, in a total yield of 30%) as a white powder. The same procedure was repeated an additional time, and totally obtained ca. 2.2 g of **2**.

*4.2.2.1. 5α-Hydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**2**).* White powder; $[\alpha]_D^{20}$ +34.4 (*c* 0.125, CH_3OH); IR (ν_{max}): 3432, 3020, 2924, 2816, 1733, 1639, 1434, 1369, 1249, 1090, 1019 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m*/*z*: $[M+Na]^+$ 457.2566 (calcd 457.2568 for $C_{25}H_{38}O_6Na$).

4.3. Screening experiment

Fourteen species of filamentous fungi that were distributed in 10 genera (*Absidia*, *Alternaria*, Aspergillus, *Botrytis*, *Cunninghamella*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Gibberella*) and four species of actinomycetes (*Nocardia* and *Streptomyces* genera) were employed as biocatalysts for the biotransformations. Analytical scale fermentation was performed using 250 mL flasks containing 70 mL of culture medium. After 2 days of cultivation, 2 mg of the substrate was added. The culture controls consisted of fermentation blanks, in which the organisms were grown under identical conditions without addition of the substrate. After one week of incubation, the cultures were filtered, and the filtrate was extracted with EtOAc. The solvent was removed under vacuum, and the resulting residue was dissolved in CH_3OH before analysis via TLC and HPLC.

4.4. Organisms, media, and cultivation conditions

Two fungal (*C. echinulata* CGMCC 3.3400, *A. niger* CGMCC 3.1858) and two actinomycete stains (*S. griseus* CACC 200300, *N. purpurea* CGMCC 4.1182), which were purchased from the China General Microorganism Collection Center (Beijing, for code CGMCC) and the China Center for Antibiotics Culture Collection (Beijing, for code CACC), were screened for preparative scale biotransformations. The two fungal strains *C. echinulata* CGMCC

3.3400 and *A. niger* CGMCC 3.1858 were kept on PDA medium, containing 200 g of potato, 20 g of dextrose, 3.0 g of KH_2PO_4, 0.75 g of $MgSO_4$, and micro amounts of VB_1 in 1 L of distilled water. The culture medium for the actinomycete strains *S. griseus* CACC 200300 and *N. purpurea* CGMCC 4.1182 contained 20 g of dextrose, 5 g of yeast extract, 5 g of soy peptone, 5 g of NaCl, and 1.0 g of $K_2HPO_4 \cdot 3H_2O$ in 1 L of distilled water. The pH of the media was adjusted to 6.0/7.0 before autoclaving at 121 °C for 25 min. Microbial cultures were grown according to the standard two-stage fermentation protocol. The seed cultures were prepared in 250 mL flasks with 70 mL of liquid medium and incubated for 2 days. The larger biotransformation cultures (1000 mL flask containing 200 mL of medium) were inoculated with 2 mL of the seed culture and cultivated on a rotary shaker at 200 rpm at 28±1 °C in the dark.

4.5. Biotransformations of 1

*4.5.1. Biotransformation of **1** by C. echinulata CGMCC 3.3400.* After 2 days of cultivation, compound **1** was added to the cultures for a final concentration of 60 mg/L (stock solution of 300 mg of **1** in 12.5 mL ethanol). The cultures were pooled and filtered after 7 days of incubation. The supernatant was saturated with NaCl and extracted with EtOAc (7 L×3). The pooled EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure at 40 °C. The dried mycelia were extracted three times in an ultrasonic bath with 300 mL EtOAc, and the resulting extract was concentrated under vacuum at 40 °C. The two extracts were combined to afford 2.1 g of residue, which was subjected to silica gel CC. The fractions were further separated by a combination of normal phase semi-preparative HPLC and reverse-phase semi-preparative HPLC to afford **3** (142.1 mg, ca. 52.1%; t_R 26.05 min; mobile phase: *n*-hexane/EtOAc=3:1, v/v at 4 mL/min), **4** (5.0 mg, ca. 1.8%; t_R 44.85 min; mobile phase: CH_3OH/H_2O=65:35, v/v at 3.5 mL/min), **5** (6.8 mg, ca. 2.2%; t_R 13.83 min; mobile phase: CH_3OH/H_2O=70:30, v/v at 3.5 mL/min), and **6** (20.6 mg, ca. 6.6%; t_R 21.40 min; mobile phase: *n*-hexane/acetone=5:1, v/v at 4 mL/min). There was 12.0 mg (3.0%) of recovered substrate (**1**). All of the products were new compounds. Their physical and chemical data are as follows:

*4.5.1.1. 5α-Hydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**3**).* White powder; $[\alpha]_D^{20}$ +16.0 (*c* 0.225, CH_3OH); IR (ν_{max}): 3478, 3012, 2963, 2914, 1717, 1677, 1437, 1375, 1258, 1124, 1063, 1032 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 441.2237 (calcd 441.2253 for $C_{24}H_{34}O_6Na$).

*4.5.1.2. 5α,6α-Dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**4**).* White powder; $[\alpha]_D^{20}$ +34.3 (*c* 0.07, CH_3OH); IR (ν_{max}): 3436, 2928, 2858, 1807, 1736, 1679, 1434, 1373, 1247, 1180, 1033 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 457.2215 (calcd 457.2203 for $C_{24}H_{34}O_7Na$).

*4.5.1.3. 5α-Hydroxy-10-oxo-2α,6α,14β-triacetoxytaxa-4(20),11(12)-diene (**5**).* White powder; $[\alpha]_D^{20}$ −7.3 (*c* 0.165, CH_3OH); IR (ν_{max}): 3448, 3012, 2923, 2854, 1746, 1710, 1681, 1445, 1378, 1274, 1245, 1175, 1097, 1035 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+H]^+$ 477.2460 (calcd 477.2489 for $C_{26}H_{37}O_8$).

*4.5.1.4. 7β-Hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**6**).* White powder; $[\alpha]_D^{20}$ +20.6 (*c* 0.16, CH_3OH); IR (ν_{max}): 3427, 3019, 2941, 2936, 1738, 1691, 1436, 1374, 1236, 1092, 1019 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+H]^+$ 477.2515 (calcd 477.2489 for $C_{26}H_{37}O_8$).

*4.5.2. Biotransformation of **1** by S. griseus CACC 200300.* Four hundred milligrams of **1** in 18 mL of ethanol was evenly distributed among 36 flasks at a final concentration of 56 mg/L after 2 days of cultivation. The cultures were pooled and centrifuged after incubation for an additional 7 days. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The pooled EtOAc extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure at 40 °C. The dried cell mass was extracted three times in an ultrasonic bath with 300 mL EtOAc, and the resulting extract was concentrated under vacuum at 40 °C. The two extracts were combined to afford 1.5 g of residue, which was subjected to silica gel CC. The fractions were further separated by a combination of normal phase semi-preparative HPLC and reverse-phase semi-preparative HPLC to afford **6** (120.0 mg, ca. 29.0%), **7** (3.1 mg, ca. 0.8%; t_R 19.80 min; mobile phase: *n*-hexane/acetone=2:1, v/v at 4 mL/min), and **8** (3.4 mg, ca. 0.8%; t_R 21.87 min; mobile phase: *n*-hexane/acetone=7:1, v/v at 4 mL/min). There was 20.0 mg (5.0%) of recovered substrate (**1**). Compound **6** was also a metabolite of **1** by *C. echinulata*.

*4.5.2.1. 5α,7β-Dihydroxy-10-oxo-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**7**).* White powder; $[\alpha]_D^{20}$ +6.0 (*c* 0.05, CH_3OH); IR (ν_{max}): 3439, 2932, 2897, 1733, 1668, 1440, 1373, 1249, 1100, 1042 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+H]^+$ 435.2388 (calcd 435.2384 for $C_{24}H_{35}O_7$).

*4.5.2.2. 9α-Hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**8**).* White powder; $[\alpha]_D^{20}$ −33.3 (*c* 0.045, CH_3OH); IR (ν_{max}): 3449, 2949, 2854, 1737, 1675, 1435, 1372, 1252, 1104, 1028 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 499.2332 (calcd 499.2308 for $C_{26}H_{36}O_8Na$).

*4.5.3. Biotransformation of **1** by N. purpurea CGMCC 4.1182.* Four hundred milligrams of **1** in 18 mL ethanol was evenly distributed among 35 flasks at a final concentration of 57 mg/L after 2 days of cultivation. The cultures were pooled and centrifuged after an additional 7 days of incubation. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The dried mycelia were extracted three times in an ultrasonic bath with 300 mL of EtOAc. All the extracts were pooled, dried over anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to afford 2.2 g of residue, which was subjected to silica gel CC. A petroleum ether/acetone gradient (100:0 to 0:100, v/v) was used as the eluent to give 19 fractions based on the TLC analysis. Fraction 10 (210.2 mg) and fraction 11 (98.6 mg) were further separated via normal phase semi-preparative HPLC using a mobile phase of *n*-hexane/EtOAc (1:1, v/v) at 4 mL/min to yield **9** (266.1 mg, ca. 81.4%; t_R 16.34 min). There was 6.0 mg (1.5%) of recovered substrate (**1**).

*4.5.3.1. 5α,14β-Dihydroxy-10-oxo-2α-acetoxytaxa-4(20),11(12)-diene (**9**).* White powder; $[\alpha]_D^{20}$ +0.45 (*c* 0.22, CH_3OH); IR (ν_{max}): 3420, 2994, 2924, 2860, 1734, 1663, 1616, 1436, 1377, 1236, 1084, 1016 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESI-MS (positive) *m/z*: $[M+H]^+$ 377.2333 (calcd 377.2329 for $C_{22}H_{33}O_5$).

4.6. Biotransformations of 2

*4.6.1. Biotransformation of **2** by C. echinulata CGMCC 3.3400.* Four hundred milligrams of **2** in 18 mL acetone was evenly distributed among 36 flasks at a final concentration of 56 mg/L after 2 days of cultivation. The cultures were pooled and centrifuged after 7 days of additional incubation. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The dried mycelia were extracted three times in an ultrasonic bath with 300 mL EtOAc. All the extracts were pooled, dried over anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to afford 3.1 g of residue, which was subjected to silica gel CC. The fractions were further separated by a combination of normal phase semi-preparative HPLC and

reverse-phase semi-preparative HPLC to afford **10** (3.1 mg, ca. 0.7%; t_R 16.32 min; mobile phase: *n*-hexane/acetone=8:1, v/v at 4 mL/min), **11** (12.6 mg, ca. 3.1%; t_R 25.81 min; mobile phase: *n*-hexane/acetone=5:1, v/v at 4 mL/min), **12** (220.0 mg, ca. 56.8%; t_R 25.86 min; mobile phase: CH_3OH/H_2O=55:45, v/v at 3.5 mL/min), **13** (3.0 mg, ca. 0.7%; t_R 16.79 min; mobile phase: *n*-hexane/acetone=8:1, v/v at 4 mL/min), **14** (7.2 mg, ca. 1.9%; t_R 28.21 min; mobile phase: *n*-hexane/acetone=6:1, v/v at 4 mL/min), **16** (3.4 mg, ca. 0.8%; t_R 30.77 min; mobile phase: *n*-hexane/acetone=5:1, v/v at 4 mL/min), **17** (5.3 mg, ca. 1.3%; t_R 48.56 min; mobile phase: CH_3OH/H_2O=47:53, v/v at 2.5 mL/min), and **18** (4.1 mg, ca. 1.0%; t_R 21.61 min; mobile phase: *n*-hexane/acetone=7:1, v/v at 4 mL/min). There was 16.0 mg (4.0%) of the recovered substrate (**2**). Among these products, **10**, **11**, **16**, and **17** were new compounds, and their physical and chemical data are detailed as follows:

*4.6.1.1. 5α,9α-Dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**10**).* White powder; $[\alpha]_D^{20}$ +25.1 (*c* 0.215, CH_3OH); IR (ν_{max}): 3524, 3014, 2962, 2922, 1734, 1711, 1640, 1450, 1369, 1265, 1106, 1018 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 473.2517 (calcd 473.2516 for $C_{25}H_{38}O_7Na$).

*4.6.1.2. 5α,9α,10β-Trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**11**).* White powder; $[\alpha]_D^{20}$ +42.0 (*c* 0.2, CH_3OH); IR (ν_{max}): 3451, 2982, 2936, 1733, 1641, 1436, 1372, 1238, 1107, 1026 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 459.2317 (calcd 459.2359 for $C_{24}H_{36}O_7Na$).

*4.6.1.3. 5α,18-Dihydroxy-10β-methoxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**16**).* White powder; $[\alpha]_D^{20}$ +5.0 (*c* 0.08, CH_3OH); IR (ν_{max}): 3420, 3021, 2984, 2927, 1733, 1644, 1435, 1371, 1250, 1091, 1020 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 473.2482 (calcd 473.2516 for $C_{25}H_{38}O_7Na$).

*4.6.1.4. 5α,10β,18-Trihydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene (**17**).* White powder; $[\alpha]_D^{20}$ +5.0 (*c* 0.08, CH_3OH); IR (ν_{max}): 3412, 3020, 2985, 2926, 1733, 1647, 1434, 1372, 1252, 1102, 1028 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 459.2320 (calcd 459.2359 for $C_{24}H_{36}O_7Na$).

*4.6.2. Biotransformation of **2** by A. niger CGMCC 3.1858.* Five hundred milligrams of **2** in 20 mL acetone was evenly distributed among 40 flasks at a final concentration of 62.5 mg/L after 2 days of cultivation. The cultures were pooled and filtered after 7 days of additional incubation. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The dried mycelia were extracted three times in an ultrasonic bath with 300 mL EtOAc. All the extracts were pooled, dried over anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to afford 3.6 g of residue, which was subjected to silica gel CC. The fractions were further separated by a combination of normal phase semi-preparative HPLC and reverse-phase semi-preparative HPLC to give **12** (263.5 mg, ca. 54.5%), **13** (4.2 mg, ca. 0.8%), **14** (18.5 mg, ca. 3.8%), **15** (7.2 mg, ca. 1.4%; t_R 11.58 min; mobile phase: CH_3OH/H_2O=72:28, v/v at 3.5 mL/min), **18** (38.3 mg, ca. 7.2%), **19** (10.1 mg, ca. 1.8%; t_R 11.58 min; mobile phase: CH_3OH/H_2O=72:28, v/v at 3.5 mL/min), and sinenxan A (18.2 mg, ca. 3.13%; t_R 24.21 min; mobile phase: *n*-hexane/acetone=18:1, v/v at 4 mL/min). There was 10.0 mg (2.0%) of recovered substrate (**1**). Compounds **12**–**14** and **18** were also metabolites of **2** by *C. echinulata*. Compound **19** is new compound, and its physical and chemical data are shown as follows:

*4.6.2.1. 5α-Hydroxy-2α,14β-diacetoxytaxa-4(20),11(12)-diene-10β-O-(butan-2-ol)-ether (**19**).* White powder; $[\alpha]_D^{20}$ +17.3 (*c* 0.075, CH_3OH); IR (ν_{max}): 3451, 2980, 2928, 1734, 1643, 1446, 1372, 1250, 1103, 1026 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+Na]^+$ 515.2947 (calcd 515.2985 for $C_{28}H_{44}O_7Na$).

*4.6.3. Biotransformation of **2** by N. purpurea CGMCC 4.1182.* Four hundred milligrams of **2** in 18 mL ethanol was evenly distributed among 35 flasks at a final concentration of 57 mg/L after 2 days of cultivation. The cultures were pooled and centrifuged after 7 days of additional incubation. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The dried mycelia were extracted three times in an ultrasonic bath with 300 mL of EtOAc. All the extracts were pooled, dried over anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to afford 2.4 g of residue, which was subjected to silica gel CC. The fractions were further separated by a combination of normal phase semi-preparative HPLC and reverse-phase semi-preparative HPLC to afford **13** (130.0 mg, ca. 30.5%), **20** (24.0 mg, ca. 5.5%; t_R 14.98 min; mobile phase: *n*-hexane/acetone=15:1, v/v at 4 mL/min), **21** (73.0 mg, ca. 22.6%; t_R 18.60 min; mobile phase: *n*-hexane/acetone=5:1, v/v at 4 mL/min), and **22** (26.0 mg, ca. 7.46%; t_R 21.00 min; mobile phase: *n*-hexane/acetone=5:1, v/v at 4 mL/min). There was 15.0 mg (3.8%) of recovered substrate (**2**). Compound **13** was also a metabolite of **2** by *C. echinulata*. Compound **21** is a new compound, and its physical and chemical data are shown as follows:

*4.6.3.1. 10β-Methoxy-2α,5α,14β-trihydroxytaxa-4(20),11(12)-diene (**21**).* White solid; $[\alpha]_D^{20}$ +55.0 (*c* 0.12, CH_3OH); IR (ν_{max}): 3391, 3014, 2979, 2921, 1635, 1445, 1381, 1309, 1199, 1072, 1011 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; HR-ESI-MS (positive) *m/z*: $[M+H]^+$ 351.2537 (calcd 351.2536 for $C_{21}H_{35}O_4$).

*4.6.4. Biotransformation of **2** by S. griseus CACC 200300.* Three hundred milligrams of **2** in 12.5 mL acetone was evenly distributed among 25 flasks at a final concentration of 60 mg/L after 2 days of cultivation. The cultures were pooled and centrifuged after 7 days of additional incubation. The supernatant was saturated with NaCl and extracted with EtOAc (10 L×3). The pooled EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated in reduced pressure at 40 °C. The dried mycelia were extracted three times in an ultrasonic bath with 300 mL of EtOAc, and the resulting extract was concentrated under vacuum at 40 °C. The two extracts were combined to afford 2.2 g of residue, which was subjected to silica gel CC. The resulting fractions were further separated by a combination of normal phase semi-preparative HPLC and reverse-phase semi-preparative HPLC to afford **3** (20.0 mg, ca. 6.9%), **12** (13.0 mg, ca. 4.5%), **13** (36.0 mg, ca. 11.3%), sinenxan A (100.0 mg, ca. 28.7%), and **23** (5.0 mg, ca. 1.4%; t_R 22.31 min; mobile phase: CH_3OH/H_2O=70:30, v/v at 3.5 mL/min). There was 8.0 mg (2.7%) of recovered substrate (2). Compound **3** was also metabolite of **1** by *C. echinulata*, and **12**, **13**, and sinenxan A were also metabolites of **2** by *A. niger*.

4.7. Evaluation of MDR reversal activities for metabolites in vitro

The human non-small cell lung adenocarcinoma cell line A549 was maintained in the Department of Pharmacology, at the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. The drug resistant subline of A549/taxol was established by culturing the cells with gradually increasing concentrations of paclitaxel, and it was characterized as a phenotype with P-gp overexpression.[7a] The MDR tumor cells were incubated in the medium RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 atmosphere. Cells were subcultured twice every week by digesting with mixture of 0.025% trypsin and 0.01% EDTA solution. The

procedures were performed as described previously.[15] Reversing agents (10 μM) combined with paclitaxel (1×10^{-9}, 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M) were dissolved in 100 μL of DMSO, and a final DMSO concentration of 0.1% was used in each well. Each concentration was tested in three parallels and was administered to A549/taxol cells to detect the reversal activities of these agents. The IC_{50} values were defined as the paclitaxel concentration that resulted in a 50% reduction in the number of cells compared with the control cells after 4 days of incubation. These values were determined directly from the semi-logarithmic dose–response curves. Reversal fold (RF) was the ratio of the IC_{50} value of resistant cells only in the presence of paclitaxel to the IC_{50} value of resistant cells in the presence of both paclitaxel and reversal agents.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81102469), the Science & Technology Project of Guangdong Province (2011A080403020), the Program for New Century Excellent Talents in University (NCET-06-0155), and the National Science & Technology Major Project 'Key New Drug Creation and Manufacturing', China (2009ZX09103-033).

Supplementary data

Selected NMR, MS, and IR spectra of substrates (**1**–**11**) and new compounds (**3**–**11**, **16**, **17**, **19**, and **21**). Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.09.091.

References and notes

1. Hosoyama, H.; Shigemori, H.; Tomida, H.; Tsuruo, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 389.
2. Longley, D. B.; Johnston, P. G. *J. Pathol.* **2005**, *205*, 275.
3. Gottesman, M. M.; Pastan, I. *Ann. Rev. Biochem.* **1993**, *62*, 385.
4. (a) Riordan, J. R.; Ling, V. *Pharmacol. Ther.* **1985**, *28*, 51; (b) Tsuruo, T.; Saito, H. I.; Kawabata, H.; Ohhara, T.; Hamada, H.; Utakoji, T. *Jpn. J. Cancer Res.* **1986**, *77*, 682; (c) Naito, M.; Hamada, H.; Tsuruo, T. *J. Biol. Chem.* **1988**, *263*, 11887.
5. Tan, B.; Piwnica-Worms, D.; Ratner, L. *Curr. Opin. Oncol.* **2000**, *12*, 450.
6. (a) Cheng, K. D.; Chen, W. M.; Zhu, W. H.; Fang, Q. C. WO 9,406,740, 1994. (b) Wu, Y. Q.; Zhu, W. H.; Lu, J.; Hu, Q.; Li, X. L. *Chin. Pharm.. J.* **1998**, *33*, 15.
7. (a) Zhao, X.; Gu, J.; Yin, D.; Chen, X. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4767; (b) Dai, J.; Qu, R.; Zou, J.; Chen, X. *Tetrahedron* **2008**, *64*, 8102; (c) Dai, J. *Curr. Top. Med. Chem.* **2009**, *9*, 1625; (d) Huang, M.; Zhao, X.; Zhang, M.; Gu, J.; Chen, X.; Yin, D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5418; (e) Zhang, Y.; Li, H.; Wang, H.; Su, F.; Qu, R.; Yin, D.; Dai, J.; Li, Y.; Chen, X. *Cancer Chemother. Pharmacol.* **2010**, *66*, 851.
8. Betts, R. E.; Walters, D. E.; Rosazza, J. P. *J. Med. Chem.* **1974**, *17*, 599.
9. (a) Xu, H.; Cheng, K.; Fang, W.; Zhu, P.; Meng, C. *Chin. Chem. Lett.* **1997**, *8*, 1055; (b) Dai, J.; Cui, Y.; Zhu, W.; Guo, H.; Ye, M.; Hu, Q.; Zhang, D.; Zheng, J.; Guo, D. *Planta Med.* **2002**, *68*, 1113.
10. Bai, J.; Ito, N.; Sakai, J.; Kitabatake, M.; Fujisawa, H.; Bai, L.; Dai, J.; Zhang, S.; Hirose, K.; Tomida, A.; Tsuruo, T.; Ando, M. *J. Nat. Prod.* **2005**, *68*, 497.
11. Yang, L.; Qu, R.; Dai, J.; Chen, X. *J. Mol. Catal. B: Enzym.* **2007**, *46*, 8.
12. (a) Zou, J.-H.; Du, H.; Zhang, Y.; Dai, J.; Yin, D.; Chen, X. *J. Mol. Catal. B: Enzym.* **2008**, *55*, 12; (b) Dai, J.; Ye, M.; Guo, H.; Zhu, W.; Zhang, D.; Hu, Q.; Zheng, J.; Guo, D. *Bioorg. Chem.* **2003**, *31*, 345; (c) Hu, S.; Tian, X.; Zhu, W.; Fang, Q. *J. Biocatal. Biotransform.* **1997**, *14*, 241.
13. (a) Kobayashi, J.; Hosoyama, H.; Wang, X.; Shigemori, H.; Sudo, Y.; Tsuruo, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1555; (b) Ojima, I.; Borella, C. P.; Wu, X.; Bounaud, P. Y.; Oderda, C. F.; Sturm, M.; Miller, M.; Chakravarty, S.; Chen, J.; Huang, Q.; Pera, P.; Brooks, T. A.; Baer, M. R.; Bernacki, R. J. *J. Med. Chem.* **2005**, *48*, 2218.
14. Yin, D.; Liu, R.; Wang, D.; Guo, J.; Liang, X.; Sekiguchi, Y.; Kameo, K. *J. Chin. Pharm. Sci.* **1999**, *8*, 191.
15. Green, L. M.; Reade, J. L.; Ware, C. F. *J. Immunol. Methods* **1984**, *70*, 257.