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A Furantaxane with an Unusual 6/8/6/5 Ring System and Potent Tumor MDR Reversal Activity Obtained via Microbial Transformation

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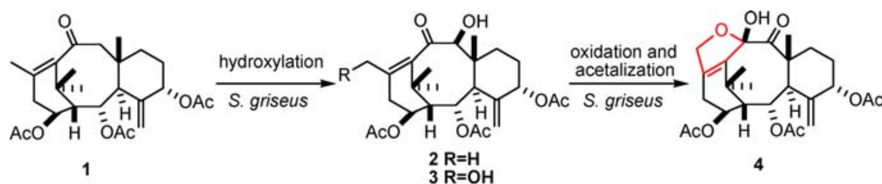
ABSTRACT



A furantaxane (4) with an unusual 6/8/6/5 ring system and two hydroxylated products (2, 3) were isolated following the biotransformation of a taxane (1) by *Streptomyces griseus*. The structures of the isolates were elucidated by spectroscopic analysis. The absolute configuration of 4, which exhibited potent reversal activity in the A549/taxol MDR tumor cell line, was unambiguously deduced by single-crystal X-ray diffraction.

The clinical treatment of cancer with chemotherapeutic drugs is frequently hindered by either intrinsic or acquired resistance of the tumor cells. When tumor cells acquire resistance against a single chemotherapeutic drug, they often show cross resistance to a variety of antitumor drugs, a state termed multidrug 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 develop by several mechanisms, a common cause is believed to be overexpression of an energy-dependent drug efflux pump, P-glycoprotein (P-gp). P-gp lowers the intracellular concentration of cytotoxic agents by pumping them outside of the tumor cells.[3] Although several chemical compounds, including verapamil, quinidine, and cyclosporin A have been reported to reverse MDR *in vivo*, these agents were not developed further due to their unacceptable toxicity profiles.[4] Identifying novel MDR reversal agents that can act as competitive inhibitors to the binding of antitumor drugs to P-gp would be an effective strategy to overcome this clinical problem.[5]

In a previous investigation, we obtained in high yield the natural taxane sinenxan A, a type of 4(20),11(12)-taxadiene with a C-14 oxygenated substituent (Figure 1), along with several analogs, from cell cultures of *Taxus chinensis*. These compounds possessed potent reversal activity against several MDR tumor cells[6] (see Supporting Information). In an effort to identify more potent derivatives, these compounds were subjected to structural modification by chemical and/or enzymatic methods and the derivatives were evaluated for pharmacological activity.[7]

(1) Hosoyama, H.; Shigemori, H.; Tomida, A.; Tsuruo, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 389–394.
(2) Longley, D. B.; Johnston, P. G. *J. Pathol.* **2005**, *205*, 275–292.
(3) Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.* **1993**, *62*, 385–427.
(4) Tan, B.; Piwnica-Worms, D.; Ratner, L. *Curr. Opin. Oncol.* **2000**, *12*, 45–48.
(5) (a) Riordan, J. R.; Ling, V. *Pharmacol. Therapeut.* **1985**, *28*, 51–75. (b) Tsuruo, T.; Saito, H. I.; Kawabata, H.; Ohhara, T.; Hamada, H.; Utakoji, T. *Jpn. J. Cancer Res.* **1986**, *77*, 682–692. (c) Naito, M.; Hamada, H.; Tsuruo, T. *J. Biol. Chem.* **1988**, *263*, 11887–11891.
(6) Cheng K. D., Chen W. M., Zhu W. H., Fang Q. C. WO 9,406,740, 1994.
(7) (a) Zhao, X.; Gu, J.; Yin, D.; Chen, X. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4767–4770. (b) Dai, J.; Qu, R.; Zou, J.-H.; Chen, X. *Tetrahedron* **2008**, *64*, 8102–8116. (c) Dai, J. *Curr. Top. Med. Chem.* **2009**, *9*, 1625–1635. (d) Huang, M.; Zhao, X.; Zhang, M.; Gu, J.; Chen, X.; Yin, D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5418–5420. (e) Zhang, Y.; Li, H.; Wang, H.; Su, F.; Qu, R.; Yin, D.; Dai, J.; Li, Y.; Chen, X. *Cancer Chemother. Pharmacol.* **2010**, *66*, 999–1003.

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One derivative of sinenxan A, 10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**1**, Figure 1), which was obtained by chemical synthesis (Scheme S1) and possessed a C-10 carbonyl group in place of an acetoxy group, was found to have highly potent MDR reversing activity in the A549/taxol MDR cell line.

To further investigate the structure−activity relationships of these taxanes and to obtain more potent derivatives, structural modification of **1** by biological and/or chemical transformations was performed. Herein, we report three new products (**2**−**4**) derived from the microbial transformation of **1** by *Streptomyces griseus* CACC200300. Of them, **4** is the first taxane reported having an unusual 6/8/6/5 ring system including a 10α,18-epoxy moiety (a furan ring) in the skeleton. The absolute structure was determined by HR-MS, 1D-NMR, 2D-NMR, NOE, and single crystal X-ray diffraction analysis. The bioassay results showed that **4** exhibited more potent reversing activity toward MDR tumor cells A549/taxol than **1**.

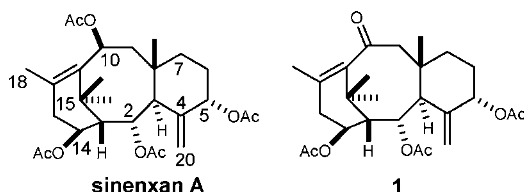


Figure 1. Structures of sinenxan A and **1**.

Fourteen species of filamentous fungi distributed in 10 genera (*Absidia*, *Alternaria*, *Aspergillus*, *Botrytis*, *Cunninghamella*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Gibberella*) and four species of actinomyces belonging to the *Nocardia* and *streptomyces* genera were employed as biocatalysts for screening transformations of **1**. Based on the TLC and HPLC analyses, the strain *S. griseus* CACC200300 was selected for preparative scale biotransformation due to the diversity and relatively high yields of products. After the standard two-stage fermentation protocol,[8] three new products (**2**−**4**, Scheme 1) were obtained by a combination of open silica gel column chromatography and semipreparative HPLC. On the basis of IR, HR-MS, 1D-NMR, 2D-NMR, and/or single crystal X-ray diffraction analysis, their structures were established as 9β-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**, ∼1.5%), 9β,18-dihydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**3**, ∼0.5%), and 10α,18-epoxy-10β-hydroxy-9-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**4**, ∼8.5%). These products evidence several enzymatic reactions including hydroxylation, oxidation, and intramolecular acetalization.

Compound **2** was obtained as a white amorphous powder.[9] Its molecular formula, $C_{26}H_{36}O_8$, was established by positive HR-ESI-MS, which showed a quasimolecular ion peak at *m*/*z* 499.2310 [M+Na]$^+$ (calcd for $C_{26}H_{36}O_8Na$, 499.2308). Its molecular weight was 16 amu more than that of **1**, suggesting the introduction of one hydroxyl group, which was further supported by an IR absorption at 3447 cm^{-1}. The ^{1}H NMR spectroscopic data of **2** were similar to those of **1** except for the disappearance of the methylene signals corresponding to H-9 (δ_H2.34, d, 15.2 Hz; δ_H2.84, d, 15.2 Hz; Table 1) and the appearance of an oxygen-bearing methine signal at δ_H5.36 (br s). The ^{13}C NMR spectrum showed an oxygentaed carbon resonance at δ_C77.1 (d) in place of the **1**'s C-9 signal at δ_C58.1 (t). These data indicate the introduction of a hydroxyl group at the C-9 position. This result was further supported by the shift of H-3 of **1** at δ_H3.04 (d, 6.4 Hz) downfield to δ_H3.58 (d, 7.5 Hz) in **2** and the shift of C-8/C-10 of **1** at δ_C40.9 (s)/204.1 (s) downfield to δ_C 55.7 (s)/214.6 (s) in **2**. The β-configuration of the 9-OH group was determined by the NOE difference spectrum, in which the integration value of H-9 was enhanced when H-3 was irradiated, whereas the enhancement of H-9 was not observed when H_3-19 was irradiated. Therefore, the structure of **2** was determined as 9β-hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene. This is the first report of a stereoselective hydroxylation of a taxane at the 9β position by biotransformation.

The positive HR-ESI-MS spectrum of **3** displayed a quasimolecular ion peak at *m*/*z* 515.2262 [M+Na]$^+$ (calcd for $C_{26}H_{36}O_9Na$, 515.2257),[10] consistent with the molecular formula $C_{26}H_{36}O_9$ and an MW 16 amu more than that of **2**, indicating the presence of an additional hydroxyl group in the molecule. The ^{1}H NMR spectrum of **3** was very similar to that of **2**, except that the signal of the H_3-18 at δ_H2.01 (3H, s) in **2** was absent, while an additional coupling of the oxygenated methylene proton signals at δ_H4.60 (d, 12.6 Hz) and 4.42 (d, 12.6 Hz) was observed, suggesting hydroxylation at C-18. This was further supported by the observation that the signal of C-18 of **3** shifted downfield to δ_C63.4 (t) from δ_C21.3 (q) in **2**. Thus, **3** was identified as 9β,18-dihydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene, which might be biosynthesized from **2** by a subsequent 18-hydroxylation.

Compound **4** was obtained as colorless crystals from MeOH after purification by semipreparative HPLC.[11] Its molecular formula was determined to be $C_{26}H_{34}O_9$ with 10 degrees of unsaturation by HR-ESI-MS, in which an ion peak [M+Na]$^+$ at *m*/*z* 513.2118 (calcd for $C_{26}H_{34}O_9Na$, 513.2101) was observed. The ^{1}H NMR spectrum of **4** was

(8) Betts, R. E.; Walters, D. E.; Rosazza, J. P. *J. Med. Chem.* **1974**, *17*, 599–602.

(9) 9β-Hydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**2**): White amorphous powder; $[\alpha]^{20}_D$ −89.6 (*c* 0.115, CH_3OH); IR (ν_{max}): 3447, 3001, 2931, 1739, 1693, 1435, 1383, 1370, 1253, 1026, 1014, 973, 932 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS (positive) *m*/*z* [M+Na]$^+$ 499.2310 (calcd 499.2308 for $C_{26}H_{36}O_8Na$).

(10) 9β,18-Dihydroxy-10-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12) -diene (**3**): White powder; mp 147−148 °C, $[\alpha]^{20}_D$ −61.7 (*c* 0.06, CH_3OH); IR (ν_{max}): 3427, 2936, 1734, 1715, 1433, 1372, 1261, 1231, 1015, 988, 958 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS (positive) *m*/*z* [M+Na]$^+$ 515.2262 (calcd 515.2257 for $C_{26}H_{36}O_9Na$).

(11) 10α,18-Epoxy-10β-hydroxy-9-oxo-2α,5α,14β-triacetoxytaxa-4(20),11(12)-diene (**4**): Colorless crystal; mp 170−172 °C, $[\alpha]^{20}_D$ −90.8 (*c* 0.26, CH_3OH); IR (ν_{max}): 3432, 2986, 2943, 2859, 1730, 1693, 1439, 1382, 1247, 1189, 1051, 1034, 1018, 957, 919, 898 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS (positive) *m*/*z* [M+Na]$^+$ 513.2118 (calcd 513.2101 for $C_{26}H_{34}O_9Na$).

Table 1. ^{1}H NMR and ^{13}C NMR Data for Compounds **1**–**4** in $CDCl_3$[a]

	1		**2**		**3**		**4**	
no.	δ_C	δ_H[b]	δ_C	δ_H[b]	δ_C	δ_H[b, c]	δ_C	δ_H[b, d]
1	57.4 d	2.04 (br s)	58.2 d	1.97 (br s)	58.3 d	2.16 (br s)	58.6 d	2.10 (d, 3.2)
2	70.3 d	5.44 (d, 6.4)	69.9 d	5.23 (dd, 7.5, 3.5)	70.5 d	5.24 (dd, 7.5, 3.0)	70.2 d	5.30 (dd, 8.0, 3.2)
3	43.9 d	3.04 (d, 6.4)	45.0 d	3.58 (d, 7.5)	45.1 d	3.56 (d, 7.5)	44.2 d	3.27 (d, 8.0)
4	144.1 s		141.0 s		140.8 s		141.6 s	
5	76.0 d	5.31 (br s)	73.5 d	5.37 (br s)	72.5 d	5.35 (br s)	76.7 d	5.33 (br s)
6	30.5 t	1.63 (m, 2H)	29.9 t	1.89 (m, 2H)	29.8 t	1.96 (m, 2H)	28.8 t	1.89 (m, 2H)
7α	36.3 t	1.25 (m)	34.3 t	1.23 (m)	38.0 t	1.23 (m)	35.3 t	1.34 (m)
7β		1.74 (m)		1.89 (m)		1.96 (m)		2.38 (m),
8	40.9 s		55.7 s		55.6 s		55.1 s	
9α	58.1 t	2.34 (d, 15.2)	77.1 d	5.36 (br s)	76.9 d	4.15 (s)	209.7 s	
9β		2.84 (d, 15.2)						
10	204.1 s		214.6 s		213.9 s		107.6 s	
11	142.4 s		135.1 s		138.3 s		139.9 s	
12	135.4 s		134.9 s		138.2 s		138.8 s	
13α	38.6 t	2.45 (dd, 18.8, 4.8)	38.3 t	2.54 (dd, 18.5, 5.0)	34.3 t	2.52 (dd, 18.9, 5.1)	29.5 t	2.45 (dd, 17.6, 4.8)
13β		2.93 (dd, 18.8, 9.2)		2.85 (dd, 18.5, 9.0)		3.13 (dd, 18.9, 9.0)		2.94 (dd, 17.6, 9.2)
14	70.1 d	5.17 (dd, 9.2, 4.8)	70.7 d	5.13 (dd, 9.0, 5.0)	69.9 d	5.17 (dd, 9.0, 5.1)	69.9 d	5.37 (dd, 9.2, 4.8)
15	35.6 s		35.5 s		34.4 s		33.5 s	
16	26.2 q	1.46 (s)	24.4 q	1.29 (s)	24.4 q	1.30 (s)	23.2 q	1.08 (s)
17	28.6 q	1.18 (s)	28.7 q	1.21 (s)	28.3 q	1.22 (s)	28.3 q	1.24 (s)
18	21.6 q	1.91 (s)	21.3 q	2.01 (s)	63.4 t	4.60 (d, 12.6)	74.7 t	5.06 (d, 13.2)
						4.42 (d, 12.6)		4.63 (d, 13.2)
19	22.1 q	0.96 (s)	15.4 q	1.09 (s)	15.6 q	1.13 (s)	17.8 q	1.39 (s)
20a	115.8 t	5.28 (br s)	117.9 t	5.35 (br s)	118.1 t	5.37 (br s)	116.8 t	5.35 (br s)
20b		4.87 (br s)		4.80 (br s)		4.77 (br s)		4.69 (br s)
OAc	21.6 q	2.16 (s)	21.7 q	2.19 (s)	21.5 q	2.19 (s)	21.5 q	2.14 (s)
(CH_3)	21.4 q	2.06 (s)	21.0 q	2.12 (s)	21.3 q	2.02 (s)	21.3 q	2.05 (s)
	21.3 q	2.04 (s)	21.0 q	2.02 (s)	21.3 q	2.00 (s)	21.3 q	2.03 (s)
OAc	170.2 s		169.9 s		169.8 s		170.0 s	
(CO)	169.9 s		169.7 s		169.7 s		169.8 s	
	169.6 s		169.5 s		169.6 s		169.3 s	

[a] ^{1}H NMR data were recorded at 400 MHz for **1** and **4**, at 500 MHz for **2** and at 300 MHz for **3**, respectively. ^{13}C NMR data were recorded at 100 MHz for **1** and **4** and at 125 MHz for **2** and **3**. 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 hydroxy protons of **3**: δ_H 5.47 (s, *OH*-9). [d] Data for hydroxy protons of **4**: δ_H 5.57 (s, *OH*-10).

very similar to that of **3** except for the absence of H_1-9 (δ_H4.15, s). The ^{13}C NMR spectrum of **4** was also very similar to that of **3** except for the disappearance of C-9 (δ_C76.9, d) and the appearance of a quaternary carbon resonance at δ_C107.6(s). The long-range heteronuclear correlations of 10-OH/C-10 and C-12, as well as H_2-18/C-11 and C-12, were observed in the HMBC spectrum. Based upon the HR-MS and NMR data, the presence of a furan hemiacetal moiety composed of C_{12}–C_{18}–O–C_{10}–C_{11} in **4** was deduced. To prove the above assignment and to determine the absolute configuration of **4**, a single crystal X-ray diffraction pattern was obtained by anomalous scattering of Cu Kα radiation. An ORTEP drawing with the atom-numbering scheme indicated is shown in Figure 2 and demonstrates a configuration of 1*S*,2*S*,3*R*,5*S*,8*R*,10*S*,14*S* for **4**. The structure of **4** was determined as 10α,18-epoxy-10β-hydroxy-9-oxo-2α,5α,14β-triacetoxy-taxa-4(20),11(12)-diene.

To the best of our knowledge, this is the first report of a 10α,18-epoxy taxane (a furantaxane). Based on a comparison of the chemical structures of substrate (**1**) and the products (**2**–**4**), a plausible bioconversion route is proposed (Scheme 1). Stereoselective hydroxylation at the C-9β position of **1** yields **2**, and **3** is obtained *via* further hydroxylation at the C-18 position. The furantaxane **4** could be formed from **3a**, which should be formed from **3** by oxidation of the 9-hydroxy to a 9-keto followed by an intramolecular acetalization. Considering that acetalization could occur under acidic conditions, the pH kinetics during the whole incubation was investigated, and the results displayed that the lowest pH value was *ca.* 6.3 during the whole process (Figure S3). Thus, to verify whether this acetalization step was enzymatic or chemical under acidic conditions, the incubations of **1** and **2** with

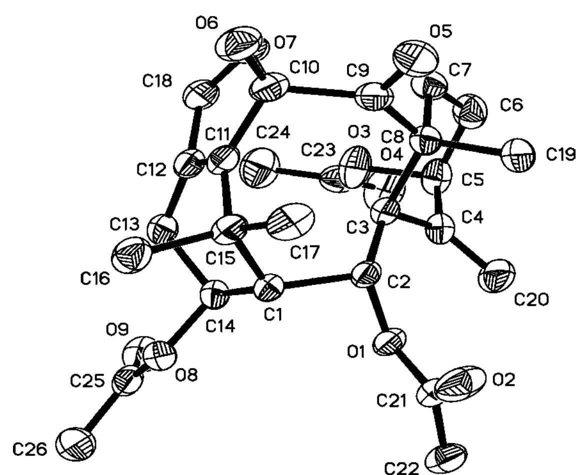
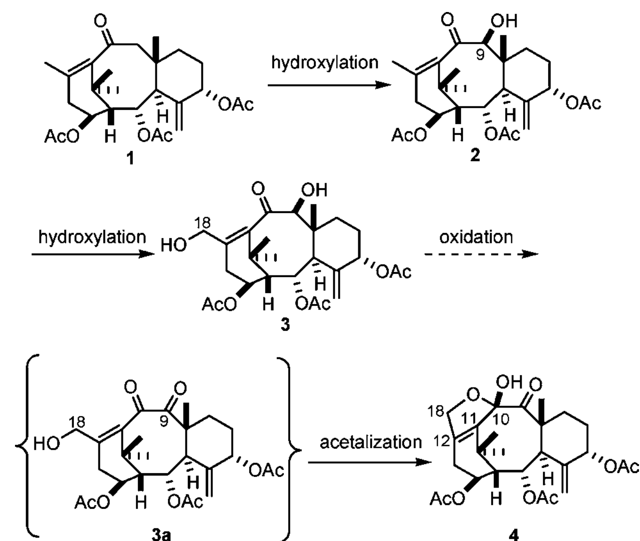


Figure 2. ORTEP diagram of **4**.

resting cells and/or crude enzymes in the phosphate buffer solutions (PBS, 50 mM), in which the pH was designated as 6.3 and 7.0 (a neutral and normal pH value for P450 enzymes), were performed, respectively. The production of **4** was detected by HPLC analysis, while it was not detected in the controls (incubations of **1** and **2** in PBS buffer without resting cells or crude enzymes). These results solidly indicated that **4** was an enzymatic product (Figures S4–S6).

Scheme 1. Plausible Bioconversion Route from **1** to **2**–**4**



Taxanes **1**–**4**, coadministered with paclitaxel, were tested at 10 μM for reversal activity toward the MDR tumor cell line of taxol-resistant A549 having a P-gp overexpressing phenotype (Table 2). **4** displayed significant reversal activity with a reversal fold (RF) of 4.1, which is two times higher than that of **1**. The RF of verapamil, the positive control, was 4.4 at 10 μM. **2** and **3** exhibited comparable reversal activity to **1**. All the compounds showed low cytotoxicity against five human tumor cell lines, including HCT-8, Bel-7402, BGC-823, A549, and A2780 ($IC_{50} > 10^{-5}$ M, Table S4). Low cytotoxicity is a good property for a reversing agent.[12]

(12) (a) Kobayashi, J.; Hosoyama, H.; Wang, X.; Shigemori, H.; Sudo, Y.; Tsuruo, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1555–1558. (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*, 2218–2228.

Table 2. Reversal Activities of **1**–**4** against MDR A549/Taxol Cells

samples (10 μM)	IC50 of paclitaxel (nM)[a]	RF (A549/T)[b]
paclitaxel	26	–
paclitaxel + **1**	14.4	1.8
paclitaxel + **2**	13.0	2.0
paclitaxel + **3**	11.8	2.2
paclitaxel + **4**	6.3	4.1
paclitaxel + Verapamil	5.9	4.4

[a] A549/taxol, an MDR subline of human lung adenocarcinoma cell line A549. [b] RF (reversal fold) = IC_{50} (antitumor agent)/IC_{50} (reversal agent + antitumor agent).

In summary, this communication reports successful structural diversification by microbial transformation of a 4(20),11(12)-taxadiene (**1**). The biotransformations observed included hydroxylations, oxidation of a hydroxy group to a ketone, and an intramolecular acetalization. The transformations provided three new compounds, **2**–**4**. The stereoselective hydroxylation at C-9β and intramolecular acetalization are the first reported for biotransformations of taxanes. **4** is an unusual 6/8/6/5 taxane bearing an additional furan ring comprising C_{12}–C_{18}–O–C_{10}–C_{11}. **4** exhibited significant reversal activity, comparable with verapamil, toward MDR A549/taxol cells when coadministered with paclitaxel. These results suggest that **4** is a good lead as an MDR reversal agent and warrants further study. Also, bioconversion is a versatile means for obtaining diversity from structural leads and can play an important role in the discovery of drug leads/candidates.

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Supporting Information Available. Experimental procedure, selected NMR (1D-NMR, 2D-NMR, and NOE), HR-ESI-MS, and IR spectra of compounds **1**–**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.The authors declare no competing financial interest.