Microbial Transformation of Cephalomannine by *Luteibacter* sp.

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Luteibacter sp., a new bacterium isolated from the soil around a *Taxus cuspidata* Sieb. et Zucc plant, was studied for its capability to metabolize cephalomannine (**1**). After preparative fermentation, eight metabolites were obtained and characterized as baccatin III (**2**), baccatin V (**3**), 10-deacetylbaccatin III (**4**), 10-deacetyl-10-oxobaccatin V (**5**), 7-epicephalomannine (**6**), 10-deacetylcephalomannine (**7**), 10-deacetyl-7-epicephalomannine (**8**), and 3′-*N*-debenzoyl-3′-*N*-(2-methylbutyryl)-7-epitaxol (**9**). Among these metabolites, **9** is a new compound. Epimerization of the 7β-OH group and hydrolysis of the C-13 side-chain were the two major reactions in this bioprocess. However, the biotransformation of 7β-D-xylosyl-10-deacetyltaxol (**10**) with the same strain yielded a C-13 side-chain eliminated product without epimerization at C-7 (**11**). Metabolites **5**–**9** and **11**, together with **1** and paclitaxel, were evaluated for their inhibitory activities against five human cancer cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780). All these compounds showed less potent activities than paclitaxel, which is currently used in clinical chemotherapy.

Microbial transformation is an interesting alternative approach to chemical synthesis to produce new compounds and useful intermediates for the semisynthesis of bioactive natural products due to its high regio- and stereoselectivity.[1] When compared to chemical synthesis, biotransformation is environmentally friendly and is usually performed under mild conditions. Because of these advantages, microbial transformation has become an important tool for the structural modification of natural products and chemical synthesis.

Taxoids are a family of diterpenes containing a tricyclic taxane core. The best known taxoid is paclitaxel (Taxol, Figure 1), which is an effective drug for the treatment of a variety of human cancers. So far, over 350 naturally occurring taxoids have been reported.[2] However, few investigations have been conducted on their biotransformations.[3–7] Cephalomannine (**1**, Figure 1) is one of the most stubborn congeners of paclitaxel, and their structures differ only in the amide side-chain at C-3′.[8] Thus, separation of these two compounds at a late stage of the purification of paclitaxel from plant extracts is not trivial, and the resulting cephalomannine is generally considered as a byproduct. Development of an improved method for modifying cephalomannine to other intermediates for the semisynthesis of paclitaxel or other bioactive taxoids is considered to be of great importance. In this context, we have been carrying out a systematic investigation of microbial transformation of cephalomannine. Here, we report the biotransformation of cephalomannine by a newly discovered bacterium derived from soil and cytotoxicities of the biotransformed metabolites in human cancer cell lines.

Results and Discussion

A total of 197 microbial strains were screened for their capabilities to metabolize cephalomannine. Of the five strains that could metabolize cephalomannine, *Luteibacter* sp. was selected for scale-up transformation. After incubation for six days, eight products were obtained by a combination of Si gel column chromatography and semipreparative HPLC. On the basis of the analyses of the IR, ^{1}H NMR, ^{13} C NMR, and MS data, these metabolites (Figure 2) were identified as baccatin III (**2**),[9] baccatin V (**3**),[10] 10-deacetylbaccatin III (**4**),[11] 10-deacetyl-10-oxobaccatin V (**5**),[12] 7-epicephalomannine (**6**),[13] 10-deacetylcephalomannine (**7**),[14] 10-deacetyl-7-epicephalomannine (**8**),[14] and 3′-*N*-debenzoyl-3′-*N*-(2-methylbutyryl)-7-epitaxol (**9**), a new compound.

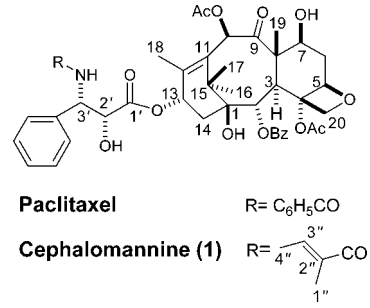

Figure 1. Structures of paclitaxel and cephalomannine (**1**).

Compound **9** was isolated as a white powder. The MS spectrum showed two quasi-molecular ion peaks at *m*/*z* 834 and 856 corresponding to $[M + H]^+$ and $[M + Na]^+$, respectively, indicating a molecular formula of $C_{45}H_{55}NO_{14}$. The major differences between the ^{1}H NMR spectra of **9** and **1** were the resonances of H-7 and H-20. In the ^{1}H NMR spectrum of **1**, these resonances occur as a multiplet at δ 4.39 (H-7) and a pair of doublets at δ 4.29 and 4.21 (H-20a and H-20b), respectively. In the ^{1}H NMR spectrum of **9**, however, H-7 gave a broad singlet at δ 3.71 and both H-20 protons resonated at δ 4.38. Other differences included the chemical shift of H-10 and the coupling constants of H-5. These changes strongly suggested that the 7-OH group occupied an α- rather than a β-orientation.[8] The ^{1}H NMR spectrum of **9** was very similar to that of **6**, except that the H-3″ vinyl proton at δ 6.42 disappeared. The H-1″ methyl protons at δ 1.78 (singlet) and H-4″ methyl protons at δ 1.70 (doublet) in **6** shifted upfield to δ 1.11 (doublet) and 0.83 (triplet) in **9**, respectively. In the ^{13}C NMR spectrum of **9**, two aliphatic carbons resonated at δ 43.2 (d, C-2″) and 27.1 (t, C-3″), in contrast to two olefinic carbons at δ 138.1 (s, C-2″) and 131.9 (d, C-3″) in **6**. These data suggested that the *N*-tigloyl group in **6** was reduced to an *N*-(2-methylbutyryl) in **9**. The structure of **9** was thus identified as 3′-*N*-debenzoyl-3′-*N*-(2-methylbutyryl)-7-epitaxol.

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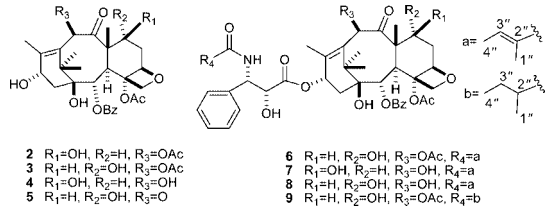

Figure 2. Structures of metabolites of cephalomannine (**1**) by *Luteibacter* sp.

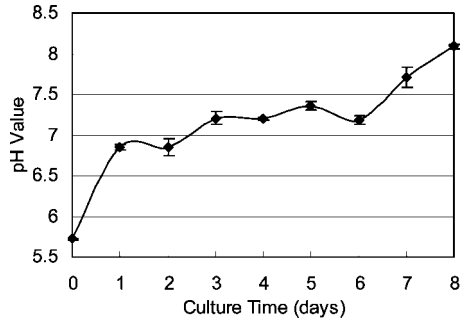

Figure 3. pH value variation of cell cultures of *Luteibacter* sp.

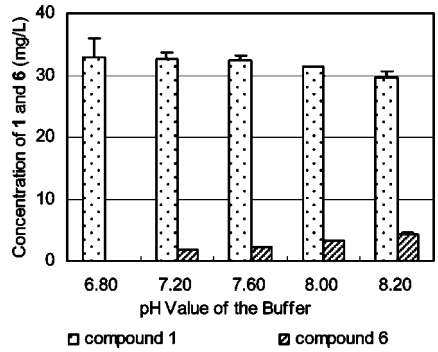

Figure 4. Effects of the pH value on the epimerization of **1**.

The major reactions of this biotransformation were the epimerization of the 7β-OH group and the hydrolysis of the C-13 side-chain. The minor reactions included hydrolysis of the C-10 acetoxy group, reduction of the *N*-tigloyl group, and oxidation of the 10-OH group following deacetylation.

It had been reported that partial epimerization of the 7β-OH group in 10-deacetyltaxol and cephalomannine could happen under neutral and mild basic conditions without enzymatic catalysis.[14,15] This raised the question whether the epimerization resulted from chemical transformation under basic conditions or enzymatic transformation. To address this question, we examined the change in pH value during the fermentation cycle. The results (Figure 3) showed that the pH values changed significantly over the biotransformation duration. The pH increased sharply from 5.72 to 6.85 on the first day, was maintained at 6.85 to 7.19 for the next five days, and then increased to 8.09 on the eighth day. According to these data, we can see that the pH values of the cultural system remained in the neutral or mild basic ranges throughout the growth period.

On the basis of the above pH observations, the incubation of **1** in PBS (phosphate buffer solution) with five different pH values for six days was examined. The results (Figure 4) showed that (1) epimerization of the 7β-OH group occurred when the pH was above 7.20, but not when the pH was below 6.80; and (2) the yield of the 7α-OH epimer increased with the pH value of the PBS, and the maximum yield was 4.3 mg/L (12.6%) when the pH value was 8.20.

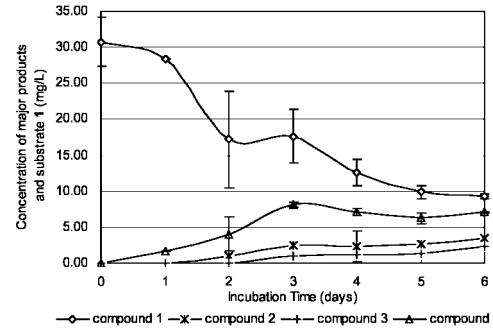

Figure 5. Time course of biotransformation of **1** to **2**, **3**, and **6** by *Luteibacter* sp.

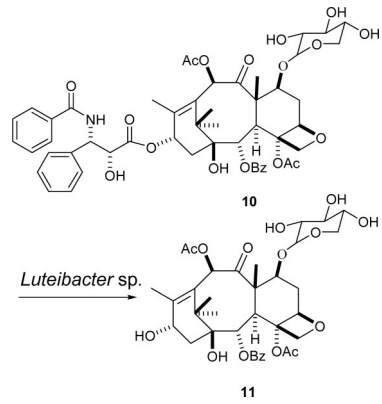

Figure 6. Biotransformation of 7β-D-xylosyl-10-deacetyltaxol (**10**) by *Luteibacter* sp.

Finally, we investigated the biotransformation kinetics of **1** and the three major metabolites **2**, **3**, and **6**. The results (Figure 5) showed that (1) during the period of incubation, the concentration of **1** decreased gradually and the final value was 9.24 mg/L; (2) the yield of metabolite **6** reached a maximum of 8.14 mg/L on day 3 and decreased slightly thereafter; (3) the yields of metabolites **2** and **3** increased throughout the period and reached maximum values of 3.50 and 2.40 mg/L, respectively; and (4) the total yield of metabolites without the C-13 side-chain (metabolites **2** and **3**) was 5.90 mg/L (ca. 20%), and the yield of the 7α-OH epimers (metabolites **3** and **6**) was 9.50 mg/L (ca. 31%).

The pH value of the culture was 8.09 after fermentation for 8 days (in this experiment, the substrate was added on day 3), and almost 31% of **1** was epimerized not including the two trace metabolites (**8** and **9**). However, only 12.6% of the substrate was epimerized in PBS (pH 8.2) after six days of incubation. On the basis of these data, the epimerization was due to the combined effects of enzymatic transformation and chemical transformation under basic conditions.

It has been suggested that the 7α-OH epimers were more stable than their 7β-epimers because of the existence of intramolecular hydrogen bonding of the 7α-OH group to the acyl oxygen atom of the 4α-*O*-acetyl group. However, the fact that the 7β-acyltaxol derivatives failed to be epimerized may be because of the loss of such intramolecular hydrogen bonding.[14–16] This was also confirmed by our test in which 7β-D-xylosyl-10-deacetyltaxol (**10**) was used as the substrate and was bioconverted to 7β-D-xylosylbaccatin III (**11**) as the only product in 16.6% yield under the same conditions (Figure 6). This outcome suggested that the xylose moiety at C-7 destroys the intramolecular hydrogen bonding and the action of epimerase. This finding may be useful for the development of an efficient method for site-specifically hydrolyzing the C-13 side-chain of taxoids such as cephalomannine and 10-deacetylcephalomannine and for the production of the important intermediates baccatin III and 10-deacetylbaccatin III by this

Table 1. Cytotoxicity of Metabolites **5–9** and **11** against Human Cancer Cells

	IC_{50} (μM)				
compound	HCT-8	Bel-7402	BGC-823	A549	A2780
1	0.31	0.92	0.1	0.1	0.1
5	<10	<10	1.38	<10	4.89
6	0.23	0.69	0.09	0.09	0.07
7	3.29	2.69	0.1	<10	0.09
8	2.23	3.53	0.79	3.24	0.35
9	1.57	3.30	0.53	2.24	0.65
11	<10	<10	<10	<10	<10
paclitaxel	0.037	0.1	0.007	0.019	0.0063

bacterium for the semisynthesis of paclitaxel or other second-generation taxoid agents. Thus, it is necessary that the 7β-OH group is protected before enzymatic hydrolysis of the C-13 side-chain, or the pH value is strictly controlled at pH <6.8.

Metabolites **5–9** and **11**, of which cytotoxicities have not been reported previously, together with **1** and the positive control paclitaxel, were tested for their cytotoxicity against five human cancer cell lines. The results are given in Table 1. As listed, these compounds showed less potent activities than paclitaxel. Some preliminary conclusions on the structure–activity relationships of this type of compounds may be drawn: (1) the C-13 side-chain is necessary for the activity (comparing **5** and **11** with the other compounds); (2) deacetylation of the C-10 *O*-acetyl group leads to loss of activity (comparing **7** and **8** with **6** and **9**); (3) replacement of the *N*-tigloyl group by the *N*-(2-methylbutyryl) group results in poor activity (comparing **6** with **9**); (4) the orientation of the 7-OH group has little effect on the activity (comparing **7** with **6**, **8**, and **9**); and (5) the presence of the 7-*O*-D-xylosyl moiety causes substantial loss of activity (comparing **11** with **1** and **6–9**).

Experimental Section

General Experimental Procedures. Optical rotations in $CHCl_3$ were carried out on a Perkin-Elmer model-341 digital polarimeter. IR spectra were obtained on a Nicolet 5700 FT-IR microscope spectrometer. The ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-400 and Bruker ARX-500 spectrometers using $CDCl_3$ as solvent and internal reference. ESIMS spectra were obtained using a VG ZabSpec mass spectrometer. Analytical HPLC was carried out on an Agillent 1100 with a BDS HYDERSIL column (C_{18}, 5 μm, 250 mm × 4.6 mm i.d., flow rate 1 mL/min), the UV detector was set at 230 nm, and the column was operated at 30 °C. Semipreparative normal-phase HPLC was performed on a Shimadzu LC-6AD instrument with an Appolo Si gel column (5 μm, 250 mm × 10 mm i.d., flow rate 4 mL/min) and a Shimadzu RID-10A detector. Semipreparative reversed-phase HPLC was performed on the same instrument with a YMC-Pack ODS-A (5 μm, 250 mm × 10 mm i.d., flow rate 2 mL/min). Si gel (200–300 mesh) was used for flash column chromatography. Analytical TLC was carried out on Si gel GF254 plates (Qingdao Oceanic Chemicals, China), and the visualization of TLC plates was performed by spraying with 5% H_2SO_4 in EtOH followed by heating at 105 °C.

Microorganism Isolation and Identification. The strain used in this biotransformation was isolated from soil in the Botanical Garden of Beijing, China. A 1.0 g sample of soil was added to 99 mL of sterilized water and shaken at 120 rpm at 25 °C in the dark for 2 h. The diluted supernatant was placed onto potato dextrose/agar medium (PDA medium, potato 200 g/L, dextrose 20 g/L, agar 20 g/L). After cultivation for several days, a single colony was transferred to an additional PDA plate, and this process was repeated two to three times. A total of 197 strains were isolated and kept on agar slants at 4 °C. The strain *Luteibacter* sp. was identified by CICC (China Center of Industrial Culture collection) using 16S rRNA sequence analysis and deposited there (CICC200641).

Substrates. Cephalomannine (**1**) and 7β-D-xylosyl-10-deacetyltaxol (**10**) were kind gifts from Guilin Hui'ang Biochemistry Medicine Industry Co., Ltd., China. The structures were characterized on the basis of ^1H NMR and ^{13}C NMR spectra, and the purities were determined to be >95% by HPLC analyses.

Screening Procedure. The strains were cultivated at 24 °C in 250 mL Erlenmeyer flasks containing 50 mL of liquid medium (PDA medium without agar). A 2 mg sample of **1** was dissolved in 0.3 mL of DMF and added to 2-day-old cultures. The cultures with substrate were incubated for another 6 days at 24 °C. The broth was extracted with EtOAc (1 × 100 mL) and analyzed by TLC and/or HPLC.

Preparative Biotransformation of 1. A two-stage fermentation procedure was used. A 1 mL sample of 2-day-old seed culture was added to one flask (350 mL of medium per 1000 mL flask), and 1.01 g of **1** was dissolved in 25 mL of DMF and distributed among 45 flasks after cultivation for 2 days. After incubation for additional 6 days, the cultures were pooled and filtered. The filtrate was saturated with NaCl and extracted with EtOAc (4 × 15 L), and the dried cell mass was extracted with EtOAc (4 × 100 mL) by sonication. The extracts were combined and concentrated under vacuum at 40 °C to afford 3.81 g of yellowish residue and subjected to Si gel column chromatography eluted with a gradient of CH_2Cl_2/MeOH to afford eight fractions. Fraction 3 was further subjected to semipreparative normal-phase HPLC with *n*-hexane−EtOAc (60/40, v/v) to give metabolite **9** (4.7 mg, ~0.5%) and metabolite **6** (129.3 mg, ~12.8%). Fraction 4 was purified with *n*-hexane−EtOAc (52/48, v/v) to afford **2** (66.1 mg, ~9.3%), **3** (62.1 mg, ~8.8%), and **5** (2.0 mg, ~0.3%). Fraction 6 was purified with *n*-hexane−EtOAc (47/53, v/v) to give **8** (8.0 mg, ~0.8%). Fraction 8 was purified with *n*-hexane−EtOAc (30/70, v/v) to give **4** (9 mg, ~1.4%) and **7** (5.8 mg, ~0.6%).

3′-*N*-Debenzoyl-3′-*N*-(2-methylbutyryl)-7-epitaxol (9): white power; $[\alpha]^{20}_D$ –45.8 (*c* 0.059, $CHCl_3$); IR ν_{max} 3433, 2965, 1732, 1713, 1639 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (2 H, d, J = 7.6 Hz, *o*-H of OBz), 7.62 (1H, brt, J = 7.2 Hz, *p*-H of OBz), 7.54 (2 H, brt, J = 7.2 Hz, *m*-H of OBz), 7.39 (4 H, brd, J = 4.0 Hz, *o*- and *m*-H of 3′-Ph), 7.34 (1 H, m, *p*-H of 3′-Ph), 6.81 (1 H, s, H-10), 6.23 (1 H, overlapped, NH), 6.23 (1 H, overlapped, H-13), 5.82 (1 H, d, J = 7.6 Hz, H-2), 5.61 (1 H, dd, J = 6.8, 2.0 Hz, H-3′), 4.91 (1 H, dd, J = 9.6, 4.0 Hz, H-5), 4.71 (1 H, d, J = 2.4 Hz, H-2′), 4.38 (2 H, s, H-20), 3.93 (1 H, d, J = 7.6 Hz, H-3), 3.71 (1 H, brs, H-7), 2.47 (3 H, s, 4-OCO *CH*$_3$), 2.35 (2 H, m, H-14), 2.33 (2 H, m, H-6), 2.21 (3 H, s, 10-OCO *CH*$_3$), 2.18 (1 H, m, H-2″), 1.82 (3 H, s, H-18), 1.68 (3 H, s, H-19), 1.59 (1 H, m, H-3″a), 1.38 (1 H, m, H-3″b), 1.23 (3 H, s, H-16), 1.17 (3 H, s, H-17), 1.11 (3 H, d, J = 6.8 Hz, H-1″), 0.83 (3 H, t, J = 7.6 Hz, H-4″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.3 (C-9), 176.3 (NH-CO),172.8 (C-1′), 172.2 (4-*C*OCH$_3$), 169.4 (10-*C*OCH$_3$), 167.1 (C=O of OBz), 139.7 (C-12), 138.1 (quaternary C of 3′-Ph), 133.7 (*p*-C of OBz), 133.4 (C-11), 130.2 (*o*-C of OBz), 129.6 (quaternary C of OBz), 129.0 (*p*-C of 3′-Ph), 128.7 (*m*-C of 3′-Ph), 128.2 (*m*-C of OBz), 126.8 (*o*-C of 3′-Ph), 82.7 (C-5), 82.1 (C-4), 79.0 (C-1), 78.1 (C-10), 77.6 (C-20), 75.7 (C-2), 75.3 (C-7), 73.0 (C-2′), 72.4 (C-13), 57.5 (C-8), 54.1 (C-3′), 43.2 (C-2″), 42.7 (C-15), 40.3 (C-3), 36.0 (C-6), 35.3 (C-14), 27.1 (C-3″), 25.9 (C-17), 22.3 (4-CO *CH*$_3$), 21.4 (C-16), 20.9 (10-CO *CH*$_3$), 17.5 (C-1″), 16.2 (C-19), 14.7 (C-18), 11.8 (C-4″); TOF-MS *m*/*z* 856 $[M + Na]^+$, 834 $[M + H]^+$, 816 $[M + H - H_2O]^+$, 774 $[M + H - CH_3COOH]^+$.

Biotransformation Kinetics of 1 to 2, 3, and 6. A 2 mg sample of **1** was added to each flask with 50 mL of medium. At intervals of 24 h, one flask was sampled and extracted with EtOAc (3 × 50 mL). The pooled extract was concentrated under reduced pressure and the resulting residue diluted to 10 mL with MeOH prior to being analyzed by HPLC. Analysis was achieved with a two-pump gradient program for pump A (solution A: MeCN) and pump B (solution B: H_2O) as follows: 40% solution A maintained for 9 min, then ramped to 46% solution A until 27 min; held at 46% solution A for 10 min, then reset to 40% solution A until 55 min for equilibrating the column and stabilizing the baseline before the next injection.

pH Value Kinetics of *Luterbacter* sp. Cell Growth Period. A 1 mL sample of 2-day-old seed cultures was added to each of 9 flasks with 50 mL of liquid medium. The flasks were rotated at 120 rpm at 25 °C. Every 24 h, the pH value of one flask was measured.

Incubation of 1 in Gradient PBS. A 2 mg sample of **1** in 0.3 mL of DMF was added to each flask with 50 mL of 0.1 M PBS. The pH value of the buffer solution was 6.80, 7.20, 7.60, 8.00, or 8.20. The flasks were rotated for 6 days at 25 °C in the dark, and then the mixture was extracted with EtOAc (3 × 50 mL). The pooled extract was concentrated under vacuum before HPLC analysis.

Biotransformation of 10. The procedures were carried out as described above, except that 54.6 mg of **10** was used; finally 1.58 g of an oil-like extract was recovered. The extract was fractionated by Si gel column chromatography with gradient elution with CH_2Cl_2−MeOH to give two fractions. With further purification of fraction 1 (20.6 mg),

semipreparative reversed-phase HPLC was performed (MeCN−H_2O, 4/6, v/v) to afford **11** (6.5 mg, ~16.6%, t_R = 17.5 min). Compound **10** (26.3 mg, ~48.2%) was recovered from fraction 2 (47.7 mg) by further semipreparative HPLC.

Cytotoxicity Bioassays of Metabolites. The HCT-8 human colorectal adenocarcinoma cell line, the Bel-7402 human liver cancer cell line, and the BGC-823 human gastric cancer cell line were purchased from the Institute of Cell Biology (Shanghai, China); the A549 human lung carcinoma cell line and the A2780 human ovarian cancer cell line were obtained from ATCC. All five tumor cell lines were maintained in RRMI1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere. The tumor cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After incubation for 96 h, cell viability was determined by measuring the metabolic conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into purple formazan crystals by active cells. The MTT assay results were read using an MK 3 well scan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested at five concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M) and were dissolved in 100% DMSO with a final concentration of DMSO of 0.1% (v/v) in each well. Each concentration of the compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

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References and Notes

(1) Ishihara, K.; Hamada, H.; Hirata, T.; Nakajima, N. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 145–170.
(2) Baloglu, E.; Kingston, D. G. I. *J. Nat. Prod.* **1999**, *62*, 1448–1472.
(3) Dai, J.; Yang, L.; Sakai, J.; Ando, M. *Tetrahedron* **2005**, *61*, 5507–5517.
(4) Zhang, J.; Zhang, L.; Wang, X.; Qiu, D.; Sun, D.; Gu, J.; Fang, Q. *J. Nat. Prod.* **1998**, *61*, 497–500.
(5) Arnone, A.; Bava, A.; Alemani, S.; Nasini, G.; Bombardelli, E.; Fontana, G. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 95–98.
(6) Hanson, R. L.; Wasylyk, J. M.; Nanduri, V. B.; Cazzulino, D. L.; Patel, R. N.; Szarka, L. J. *J. Biol. Chem.* **1994**, *35*, 22145–22149.
(7) Patel, R. N. *Annu. Rev. Microbiol.* **1998**, *98*, 361–395.
(8) Chmurny, G. N.; Hilton, B. D.; Brobst, S.; Look, S. A.; Witherup, K. M.; Beutler, J. A. *J. Nat. Prod.* **1992**, *55*, 414–423.
(9) Sénilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potie, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131–137.
(10) Della Casa de Marcano, D. P.; Halsall, T. G.; Castellano, E.; Hodder, O. J. R. *J. Chem. Soc., Chem. Commun.* **1970**, 1382–1383.
(11) Zhang, Z.; Jia, Z. *Phytochemistry* **1990**, *29*, 3673–3675.
(12) Fuji, K.; Tanaka, K.; Li, B.; Shingu, T.; Sun, H.; Taga, T. *J. Nat. Prod.* **1993**, *56*, 1520–1531.
(13) De Bellis, P.; Lovati, M.; Pace, R.; Peterlongo, F.; Zini, G. F. *Fitoterapia* **1995**, *66*, 521–524.
(14) McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R., Jr *J. Nat. Prod.* **1981**, *44*, 312–319.
(15) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Araold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469–1474.
(16) Chen, W. M.; Zhang, P. L.; Zhou, J. Y. *Acta Pharm. Sin.* **1994**, *29*, 207–214.

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