DNA Polymerase β Inhibitors from *Tetracera Boiviniana*

Ji Ma, Shelley R. Starck, and Sidney M. Hecht*

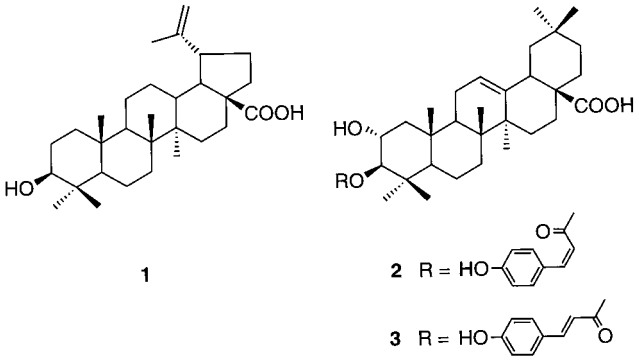
Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901

Received July 2, 1999

Bioassay-guided fractionation of an active methyl ethyl ketone extract of *Tetracera boiviniana,* using a sensitive assay to monitor DNA polymerase β inhibition, resulted in the isolation of three known triterpenoids, betulinic acid (**1**), 3-*cis*-*p*-coumaroyl maslinic acid (**2**), and 3-*trans*-*p*-coumaroyl maslinic acid (**3**). Compounds **1**−**3** inhibited DNA polymerase β with IC_{50} values of 14, 15, and 4.2 μM in the presence of bovine serum albumin (BSA) and 6.5, 7.5, and 2.0 μM in the absence of BSA, respectively. Further, compounds **1**−**3** potentiated the effects of bleomycin in cultured P-388D_1 cells.

DNA damage induced by a myriad of exogenous factors such as sunlight, ionizing radiation, and chemicals is repaired constantly by sophisticated DNA repair systems, such as the base excision repair system and nucleotide excision repair system.[1] Therefore, it is not surprising that human tumor cells utilize the same repair pathways to resist the effects of chemotherapy.[2] DNA polymerase β, one of the key enzymes involved in base excision repair, is believed to play an important role in repairing various forms of chemotherapeutically relevant DNA damage caused by antitumor agents such as the bleomycins,[3,4] monofunctional DNA alkylating agents,[5] cisplatin,[6] and neocarzinostatin.[4] Despite the recent identification of some naturally occurring inhibitors of DNA polymerase β,[7−10] it is still quite important to identify additional nontoxic natural products that can block the function of DNA polymerase β selectively and which may, consequently, be able to potentiate the activity of DNA-damaging antitumor agents.

Tetracera boiviniana Baill. (Dilleniaceae) belongs to a family of common shrubs indigenous to the Savanna of the Shimba Hills just southwest of Mombassa, Kenya. To date, no chemical constituents have been identified from this species, though some triterpenoids[11] and flavonoids[12] have been isolated from several plants of the genus *Tetracera*. In screening crude plant extracts for their ability to inhibit DNA polymerase β, the methyl ethyl ketone (MEK) extract of *T. boiviniana* exhibited potent inhibition (98% inhibition at 100 μg/mL). The crude extract still possessed significant activity (78% inhibition at 100 μg/mL) after being passed through a polyamide 6S column to remove polyphenols, such as tannins. Accordingly, the crude extract of *T. boiviniana* was chosen for bioassay-guided fractionation, using an assay sensitive to DNA polymerase β inhibition. Bioassay-guided fractionation of the MEK extract of *T. boiviniana* resulted in the isolation of three known compounds (**1**−**3**) that belong to two different structural classes of triterpenoids. We describe herein the isolation of these three triterpenoids and their potency of inhibition of DNA polymerase β, as well as the potentiation of bleomycin cytotoxicity by **1**−**3** in cultured mammalian cells.



1

Results and Discussion

The twigs and stem bark of *T. boiviniana* were soaked successively with hexane, MEK, methanol, and water. The MEK extract exhibited strong DNA polymerase β inhibitory activity. The active MEK extract was first passed through a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. The 4:1 MeOH−CH_2Cl_2 fraction, which exhibited significant inhibition of DNA polymerase β (80% at 100 μg/mL) was subjected to fractionation on a C_{18} column, using MeOH−H_2O mixtures for elution. The 9:1 MeOH−H_2O fraction possessed the most potent inhibitory activity and was fractionated further on a C_8 open column; two active fractions (17:3 and 9:1 MeOH−H_2O) were obtained, exhibiting 86% and 88% inhibition of polymerase β function, respectively, at 50 μg/mL. Further isolation and purification of these two active fractions by HPLC using C_{18} columns afforded three pure active principles (**1**−**3**).

Compounds **1**−**3** were all obtained as amorphous white powders. Comparison of ^1H and ^{13}C NMR data of **1** with the literature[13,14] led to the identification of **1** as betulinic acid, a species widely distributed in the plant kingdom. Analysis of the ^1H NMR and ^{13}C NMR spectra of **2** and **3** with published data allowed the identification of the basic skeleton as maslinic acid.[15,16] The cis and trans coumaric substituents of these two compounds were also identified by comparison of their NMR data with the literature.[17] Subsequently, **2** and **3** were determined as 3-*cis*-*p*-coumaroyl maslinic acid and 3-*trans*-*p*-coumaroyl maslinic acid.[18,19] During the isolation and purification of **2** and **3**, it was found that **2** underwent equilibration within the olefin

* To whom correspondence should be addressed at Department of Chemistry. Tel.: (804)924-3906. Fax: (804)924−7856. E-mail: sidhecht@virginia.edu.

10.1021/np990326p CCC: $18.00
Published on Web 11/10/1999

Table 1. IC_{50} Values for Compounds **1**−**3** from *Tetracera Boiviniana*

compound	IC_{50} (μM)	
	BSA[a]	no BSA[a]
1	14	6.5
2	15	7.5
3	4.2	2.0

[a] BSA, bovine serum albumin, present at 0.1 mg/mL.

moiety to give an olefin ratio of 75% cis to 25% *trans* in methanol during the first 2 days at room temperature, and finally reached an equilibrium ratio of 75% trans to 25% cis within about one week.[19] Likewise, **3** reached the same equilibrium ratio of 75% trans to 25% cis when dissolved in methanol under the same conditions.

As shown in Table 1, compounds **1**−**3** inhibited DNA polymerase β with reasonable potency both in the presence and absence of bovine serum albumin (BSA). The moderate influence of BSA on the inhibitory effect of the three compounds (Table 1) suggests that the binding of compounds **1**−**3** to DNA polymerase β occurs selectively,[20] consistent with the utility of these compounds in vivo.

More recently, betulinic acid (**1**) has been reported to possess antitumor activity toward cultured human melanoma cells in both in vitro and in vivo models.[21] Further, the ability of betulinic acid to induce apoptosis in melanoma[22] and other cell types[23] and the favorable therapeutic index suggests that betulinic acid may be regarded as an attractive and promising candidate for antitumor therapy. Other biological activities reported for betulinic acid include antiinflammatory activity,[24] inhibition of phorbol ester-induced epidermal ornithine decarboxylase accumulation in the mouse-ear model with subsequent inhibition of the carcinogenic response in the two-stage mouse-skin model,[25] anti-HIV activity in H9 lymphocytes,[26] and allelopathic activity.[27] This is the first time that betulinic acid (**1**) has been reported to inhibit DNA polymerase β. Further, the isolation of betulinic acid (**1**) from *T. boiviniana* provides an additional type of triterpenoid capable of inhibiting DNA polymerase β.[7e,f,8]

The geometry of the double bond of the coumaroyloxy substituent appears to be important for the inhibition of DNA polymerase β, as demonstrated by the greater potency of **3** both in the presence and absence of BSA (Table 1). It is important to note that the IC_{50} values given for **2** and **3** in Table 1 are actually for mixtures. Compound **2** was studied as a 75:25 mixture of cis and trans isomers, while **3** was a 75:25 trans-cis mixture. Thus the pure trans isomer would presumably be substantially more potent than the pure cis isomer, although it is conceivable that the isomers could act synergistically.

Bleomycin, which is used therapeutically as blenoxane (a mixture consisting predominantly of bleomycins A_2 and B_2), is known to mediate both single and double-strand DNA breaks, and to induce the formation of alkali-labile lesions.[28,29] Because DNA polymerase β has been implicated in the repair of single-strand breaks induced by bleomycin and other DNA-damaging antitumor agents,[3,4] it was logical to think that the inhibitors reported here (**1**−**3**) might exert these effects in cell culture and potentiate the effects of bleomycin. Compound **1** was not cytotoxic to cultured P-388D_1 cells when tested alone at a 10-μM concentration. However, when tested concurrently with an otherwise nontoxic (75 nM) concentration of bleomycin, there was a 30% decrease in the number of viable cells (Figure 1, Table 2). Compounds **2** and **3** did not exhibit cytotoxicity when tested alone at a 10-μM concentration,

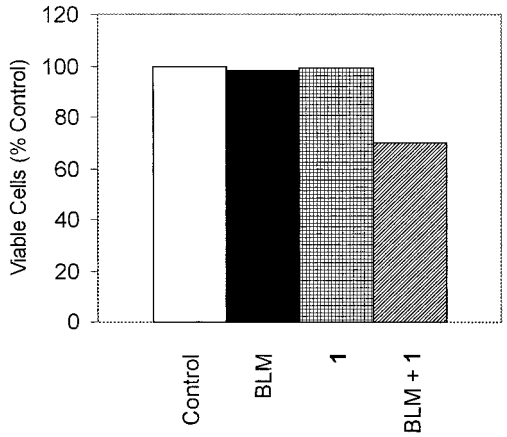


Figure 1. Potentiation of bleomycin cytotoxicity in P-388D_1 cells by **1**. Cells were treated as described for 6 h. Viability was assessed by trypan blue exclusion. White bar, control − no treatment; black bar, 75 nM bleomycin; squares, 10 μM **1**; diagonal lines, 75 nM bleomycin + 10 μM **1**.

Table 2. Potentiation of Bleomycin[a,b] Cytotoxicity in P-388D_1 Cells by Compounds **1**−**3**[c] from *Tetracera Boiviniana*

compound	viable cells (% of control)	
	compound alone	bleomycin + compound
1	99	70
2	100	77
3	100	82

[a] Bleomycin used at 75 nM concentration. [b] Bleomycin alone, 98% viable cells (% of control). [c] Compounds **1**−**3** were each utilized at a 10-μM concentration.

but showed analogous reductions in the numbers of viable cells when 75 nM bleomycin was added simultaneously (Table 2). The results reported herein and the recent finding that the sensitivity of CHO cells to several DNA damaging agents is diminished as a result of overexpression of DNA polymerase β[30] support the idea that DNA polymerase β inhibition may be a valid strategy for potentiating the effects of DNA-damaging agents employed for antitumor therapy.

Experimental Section

General Experimental Procedures. Polyamide 6S (a product of Riedel−de Haen, Germany) was purchased from Crescent Chemical Co. Silica RP C_{18} and RP C_8 (40 μm) chromatographic supports were obtained from J. T. Baker. Alltech Econosil C_{18} (250 × 10 mm, 10 μm) and Alltech Alltima C_{18} columns (250 × 4.6 mm, 5 μm) were used for reversed-phase HPLC. ^1H and ^{13}C NMR were performed on a Varian Unity Inova 300 NMR spectrometer. Low-resolution chemical ionization mass spectra (LRCIMS) were recorded on a Finnigan MAT 4600 mass spectrometer. Compounds **1**−**3** were dissolved in 1:1 DMSO−methanol for the DNA polymerase β inhibition assay and in 100% DMSO for the cell culture studies; the final DMSO concentration was 0.25% in the cell culture medium. The recombinant rat liver DNA polymerase β preparation employed was a gift from Xiangyang Wang and Hongge Wang, prepared as described previously.[31−34] Antibiotic antimycotic solution; Hank's balanced salt solution; Dulbecco's modified Eagle's medium containing 4500 mg glucose/L; unlabeled dNTPs; and calf thymus DNA were purchased from Sigma Chemicals; calf thymus DNA was activated by the method described previously.[35] [^3H]dTTP was purchased from ICN Pharmaceuticals. DEAE-cellulose paper (DE-81) was from Whatman. Cell line P-388D_1 (a

mouse lymphoid neoplasm) was purchased from American Type Culture Collection. Trypan blue dye and donor horse serum were from Gibco BRL. Blenoxane, the clinically used mixture of bleomycins consisting predominantly of bleomycin A_2 and bleomycin B_2, was a gift from Bristol Myers Squibb Pharmaceuticals.

Plant Material. The twigs and stem bark of *T. boiviniana* Baill. were collected in Kenya in October 1980. Voucher specimen SS-1601 is preserved at the United States National Arboretum Herbarium (NA), Washington, DC.

Extraction and Isolation. The dried plant material was soaked successively with hexane, MEK, methanol, and water. The MEK extract exhibited strong DNA polymerase β inhibition (98% inhibition at 100 μg/mL). Because the crude extract still possessed significant activity (78% inhibition at 100 μg/mL) after passage through a polyamide 6S column, it was chosen for further bioassay-guided fractionation. In a typical experiment, 166 mg of the crude MEK extract *of T. boiviniana* was first passed through a polyamide 6S column (15 g), eluting successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. The 4:1 MeOH−CH_2Cl_2 fraction (107 mg) strongly inhibited DNA polymerase β (80% inhibition) at 100 μg/mL and was subjected to further fractionation employing a C_{18} column (15 g) and (1:1 → 1:0) MeOH−H_2O mixtures for elution. The 9:1 MeOH−H_2O fraction (67 mg total) possessed the strongest inhibitory activity toward DNA polymerase β (81% inhibition at 50 μg/mL). By further fractionation using a 15-g C_8 open column and elution with (1:1 → 1:0) MeOH−H_2O mixtures, two strongly active fractions (17:3, 40 mg; 9:1, 18 mg) were obtained (86% and 88% inhibition at 50 μg/mL, respectively). The 9:1 MeOH−H_2O fraction was applied to a 10-μm C_{18} reversed-phase HPLC column (250 × 10 mm), which was washed with 9:1 CH_3CN−H_2O over a period of 30 min at a flow rate of 3.0 mL/min (UV monitoring at 215 nm); this afforded one active fraction (96% inhibition at 50 μg/mL). The further purification of this active fraction was performed on a 5-μm C_{18} reversed-phase HPLC column (250 × 4.6 mm) using a linear gradient of 4:1 → 19:1 CH_3CN−H_2O over a period of 30 min to afford pure compound **1** (2.8 mg). The fractionation and purification of the second active fraction from the C_8 column (17:3 MeOH−H_2O) was performed as described above for **1**, and led to the isolation of two pure compounds, **2** (0.9 mg) and **3** (2.6 mg).

Betulinic acid (1): white amorphous powder; positive CIMS *m/z* 457 (20), 439 (30), 273 (25), 263 (55), 137 (100), 91 (65); ^{1}H and ^{13}C NMR data consistent with the literature.[13,14]

3-*trans*-*p*-Coumaroyl maslinic acid (2): white amorphous powder; negative CIMS *m/z* 618 (6.5); positive CIMS *m/z* 619 (13), 437 (24), 409 (25), 243 (48), 153 (28), 141 (28), 127 (34), 121 (47), 107 (80), 91 (83), 69 (66), 51 (100); ^{1}H and ^{13}C NMR data consistent with the literature.[18,19]

3-*trans*-*p*-Coumaroyl maslinic acid (3): white amorphous powder; negative CIMS *m/z* 618 (39); ^{1}H and ^{13}C NMR data consistent with the literature.[18,19]

DNA Polymerase β Inhibition Assay. To 50 μL of 62.5 mM 2-amino-2-methyl-1,3-propanediol buffer, pH 8.6, containing 10 mM $MgCl_2$, 1 mM DTT, 100 μg/mL BSA, 6.25 μM dNTPs including [^{3}H]dTTP (0.04 Ci/mmol), and 0.25 mg/mL of activated calf thymus DNA was added 6 μL of a solution containing the test compound and 4 μL of the recombinant rat DNA polymerase β preparation (6.9 units, 4.8×10^4 units/mg). After incubation at 37 °C for 60 min, the radiolabeled DNA product was collected on DEAE-cellulose paper (DE-81), dried, and rinsed successively with 0.4 M K_2HPO_4, pH 9.4, and 95% ethanol for radioactivity determination.

Bleomycin Cytotoxicity Assay in Cell Culture. P-388D_1 cells were maintained as suspension cultures in 90% (v/v) Dulbecco's modified Eagle's medium containing 4500 mg glucose/L; 10% (v/v) donor horse serum; and 100 IU penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B per mL at 37 °C in a 5% CO_2 in air atmosphere. To 12.5-mL tissue culture flasks were added 5 mL of cell suspension containing ca. 5×10^5 cells/mL; this was incubated for 1 h to stabilize the cells. Assays were carried out in 6-mL reaction cultures containing the desired amounts of test compound and blenoxane dissolved in media. Cultures were incubated at 37 °C in a 5% CO_2 in air atmosphere for 6 h followed by cell viability determination using trypan blue dye.

Acknowledgment. We wish to thank Xiangyang Wang and Hongge Wang for preparation of the rat DNA polymerase β employed in this work. This work was supported by Research Grant CA50771 from the National Cancer Institute.

Supporting Information Available: Supporting Information Available: Table showing DNA polymerase β inhibition data from the bioassay-guided fractionation of a methyl ethyl ketone crude extract of *Tetracera boiviniana* (percent inhibition of polymerase β). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*, ASM Press: New York, 1995. (b) Wood, R. D. *Annu. Rev. Biochem.* **1996**, *65*, 135−167. (c) Dianov, G. L.; Prasad, R.; Wilson, S. H.; Bohr, V. A. *J. Biol. Chem.* **1999**, *274*, 13741−13743.
(2) (a) Carmichael, J.; Hickson, I. D. *Int. J. Radiation Oncology Biol. Phys.* **1991**, *20*, 197−202. (b) Chaney, S. G.; Sancar, A. *J. Natl. Cancer Inst.* **1996**, *88*, 1346−1360.
(3) (a) Seki, S.; Oda, T. *Carcinogenesis* **1986**, *7*, 77−82. (b) Seki, S.; Oda, T. *Carcinogenesis* **1988**, *9*, 2239−2244. (c) DiGiuseppe, J. A.; Dresler, S. L. *Biochemistry* **1989**, *28*, 9515−9520. (d) Park, I.-S.; Koh, H. Y.; Park, J. K.; Park, S. D. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 1226−1233. (e) Zhang, B.; Seki, S.; Ikeda, S. *Int. J. Biochem.* **1991**, *23*, 703−711.
(4) Miller, M. R.; Chinault, D. N. *J. Biol. Chem.* **1982**, *257*, 10204−10209.
(5) (a) Sobol, R. W.; Horton, J. K.; Kühn, R.; Gu, H.; Singhal, R. K.; Prasad, R.; Rajewsky, K.; Wilson, S. H. *Nature* **1996**, *379*, 183−186. (b) Narayan, S.; He, F.; Wilson, S. H. *J. Biol. Chem.* **1996**, *271*, 18508−18513. (c) Ogawa, A.; Murate, T.; Izuta, S.; Takemura, M.; Furuta, K.; Kobayashi, J.; Kamikawa, T.; Nimura, Y.; Yoshida, S. *Int. J. Cancer* **1998**, *76*, 512−518.
(6) (a) Ali-Osman, F.; Berger, M. S.; Rairkar, A.; Stein, D. E. *J. Cell. Biochem.* **1994**, *54*, 11−19. (b) Hoffmann, J.-S.; Pillaire, M.-J.; Maga, G.; Podust, V.; Hübscher, U.; Villani, G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5356−5360. (c) Horton, J. K.; Srivastava, D. K.; Zmudzka, B. Z.; Wilson, S. H. *Nucleic Acids Res.* **1995**, *23*, 3810−3815.
(7) (a) Chen, J.; Zhang, Y.-H.; Wang, L.-K.; Sucheck, S. J.; Snow, A. M.; Hecht, S. M. *J. Chem. Soc., Chem. Commun.* **1998**, 2769−2770. (b) Deng, J.-Z.; Starck, S. R.; Hecht, S. M. *J. Nat. Prod.* **1999**, *62*, 477−480. (c) Deng, J.-Z.; Sun, D.-A.; Starck, S. R.; Hecht, S. M.; Cerny, R. L.; Engen, J. R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1147−1149. (d) Deng, J.-Z.; Starck, S. R.; Hecht, S. M.; Ijames, C. F.; Hemling, M. E. *J. Nat. Prod.* **1999**, *62*, 1000−1002. (e) Sun, D.-A.; Deng, J.-Z.; Starck, S. R.; Hecht, S. M. *J. Am. Chem. Soc.* **1999**, *121*, 6120−6124. (f) Sun, D.-A.; Starck, S. R.; Locke, E. P.; Hecht, S. M. *J. Nat. Prod.* **1999**, *62*, 1110−1113.
(8) (a) Tanaka, N.; Kitamura, A.; Mizushina, Y.; Sugawara, F.; Sakaguchi, K. *J. Nat. Prod.* **1998**, *61*, 193−197. (b) Mizushina, Y.; Tanaka, N.; Kitamura, A.; Tamai, K.; Ikeda, M.; Takemura, M.; Sugawara, F.; Arai, T.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biochem. J.* **1998**, *330*, 1325−1332. (c) Sun, H.-D.; Qiu, S.-X.; Lin, L.-Z.; Wang, Z.-Y.; Lin, Z.-W.; Pengsuparp, T.; Pezzuto, J. M.; Fong, H. H. S.; Cordell, G. A.; Farnsworth, N. R. *J. Nat. Prod.* **1996**, *59*, 525−527.
(9) Ono, K.; Nakane, H.; Fukushima, M. *Eur. J. Biochem.* **1988**, *172*, 349−353.
(10) (a) Mizushina, Y.; Yoshida, S.; Matsukage, A.; Sakaguchi, K. *Biochim. Biophys. Acta* **1997**, *1336*, 509−521. (b) Ishiyama, H.; Ishibashi, M.; Ogawa, A.; Yoshida, S.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3831−3836.
(11) Dan, S.; Dan, S. S. *J. Indian Chem. Soc.* **1980**, *57*, 760.
(12) Harrison, L. J.; Sia, G.-L.; Sim, K.-Y. *Planta Med.* **1994**, *60*, 493−494.
(13) Reher, G.; Budesinsky, M. *Phytochemistry* **1992**, *31*, 3909−3914.
(14) Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O. *Chem. Pharm. Bull.* **1980**, *28*, 1006−1008.
(15) Kojima, H.; Ogura, H. *Phytochemistry* **1989**, *28*, 1703−1710.

(16) Yaguchi, Y.; Sakurai, N.; Nagai, M.; Inoue, T. *Chem. Pharm. Bull.* **1988**, *36*, 1419−1424.
(17) Numata, A.; Yang, P.; Takahashi, C.; Fujiki, R.; Nabae, M.; Fujita, E. *Chem. Pharm. Bull.* **1989**, *37*, 648−651.
(18) Yagi, A.; Okamura, N.; Haraguchi, Y.; Noda, K.; Nishioka, I. *Chem. Pharm. Bull.* **1978**, *26*, 3075−3079.
(19) Häberlein, H.; Tschiersch, K.-P. *Phytochemistry* **1994**, *35*, 765−768.
(20) Ohta, K.; Mizushina, Y.; Hirata, N.; Takemura, M.; Sugawara, F.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biol. Pharm. Bull.* **1999**, *22*, 111−116.
(21) Kim, D. S. H. L.; Pezzuto, J. M.; Pisha, E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1707−1712.
(22) Pisha, E.; Chai, H.; Lee, I.-S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Das Gupta, T. K.; Pezzuto, J. M. *Nat. Med.* **1995**, *1*, 1046−1051.
(23) (a) Fulda, S.; Friesen, C.; Los, M.; Scaffidi, C.; Mier, W.; Benedict, M.; Nuñez, G.; Krammer, P. H.; Peter, M. E.; Debatin, K. M. *Cancer Res.* **1997**, *57*, 4956−4964. (b) Noda, Y.; Kaiya, T.; Kohda, K.; Kawazoe, Y. *Chem. Pharm. Bull.* **1997**, *45*, 1665−1670. (c) Hashimoto, F.; Kashiwada, Y.; Cosentino, L. M.; Chen, C.-H.; Garrett, P. E.; Lee, K.-H. *Bioorg. Med. Chem.* **1997**, *5*, 2133−2143.
(24) (a) Recio, M. D. C.; Giner, R. M.; Máñez, S.; Gueho, J.; Julien, H. R.; Hostettmann, K.; Ríos, J. L. *Planta Med.* **1995**, *61*, 9−12. (b) Mukherjee, P. K.; Saha, K.; Das, J.; Pal, M.; Saha, B. P. *Planta Med.* **1997**, *63*, 367−369.
(25) Yasukawa, K.; Takido, M.; Matsumoto, T.; Takeuchi, M.; Nakagawa, S. *Oncology* **1991**, *48*, 72−76.
(26) Kashiwada, Y., Hashimoto, F.; Cosentino, L. M.; Chen, C.-H.; Garrett, P. E.; Lee, K.-H. *J. Med. Chem.* **1996**, *39*, 1016−1017.
(27) Macías, F. A.; Simonet, A. M.; Esteban, M. D. *Phytochemistry* **1994**, *36*, 1369−1379.
(28) (a) Suzuki, H.; Nagai, K.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot.* **1969**, *22*, 446−448. (b) Terasima, T.; Yasukawa, M.; Umezawa, H. *Gann.* **1970**, *61*, 513−516. (c) Terasima, T.; Takabe, Y.; Katsumata, T.; Watanabe, M.; Umezawa, H. *J. Natl. Cancer Inst.* **1972**, *49*, 1093−1100. (d) Iqbal, Z. M.; Kohn, K. W.; Ewig, R. A. G.; Fornace, A. J., Jr. *Cancer Res.* **1976**, *36*, 3834−3838. (e) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608−3612. (f) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310−4318. (g) Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544−10546. (h) Berry, D. E.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 3207−3214.
(29) (a) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107−1136. (b) Natrajan, A.; Hecht, S. M. In *Molecular Aspects of Anticancer Drug−DNA Interactions* Neidle, S., Waring, M., Eds. Macmillan: London, 1994; pp 197−242. (c) Kane, S. A.; Hecht, S. M. *Prog. Nucleic Acids Res. Mol. Biol.* **1994**, *49*, 313−352.
(30) Canitrot, Y.; Cazaux, C.; Fréchet, M.; Bouayadi, K.; Lesca, C.; Salles, B.; Hoffmann, J.-S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12586−12590.
(31) Zmudzka, B. Z.; SenGupta, D.; Matsukage, A.; Cobianchi, F.; Kumar, P.; Wilson, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5106−5110.
(32) Matsukage, A.; Nishikawa, K.; Ooi, T.; Seto, Y.; Yamaguchi, M. *J. Biol. Chem.* **1987**, *262*, 8960−8962.
(33) Abbotts, J.; SenGupta, D. N.; Zmudzka, B.; Widen, S. G.; Notario, V.; Wilson, S. H. *Biochemistry* **1988**, *22*, 901−909.
(34) Date, T.; Yamaguchi, M.; Hirose, F.; Nishimoto, Y.; Tanihara, K.; Matsukage, A. *Biochemistry* **1988**, *27*, 2983−2990.
(35) Wang, T. S.-F.; Korn, D. *Biochemistry* **1980**, *19*, 1782−1790.

NP990326P