Cardenolides from *Antiaris toxicaria* as Potent Selective Nur77 Modulators

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Toxicarioside D (1), a new cardenolide, along with 10 other known ones, was isolated from the stem of *Antiaris toxicaria* LESCH. by bioassay-guided fractionation. Their structures were determined on the basis of spectroscopic analysis. All the reported compounds effectively inhibited the growth of various cancer cell lines at nanomolar concentrations. Inhibition of cancer cell growth was accompanied with induction of the expression of Nur77, a potent apoptotic member of the steroid/thyroid hormone receptor superfamily.

Key words *Antiaris toxicaria*; cardenolides; Nur77; anticancer

Nur77 (also known as TR3 or NGFI-B) is perhaps the most potent apoptotic member of the nuclear receptor superfamily.[1,2] The death effect of Nur77 in cancer cells was originally observed in cancer cells treated with the retinoid-related molecule AHPN.[3] Rapid induction of Nur77 has since been observed in cancer cells after stimulation of apoptosis by a variety of apoptosis-inducing agents, including calcium ionophores, etoposide (VP-16),[4,5] phorbol ester,[4—7] synthetic chenodeoxycholic acid derivatives,[8] di-*n*-butyltin dichloride,[9] histone deacetylase inhibitors,[10] cadmium,[11] and 1,1-bis(3′-indolyl)-1-(*p*-substituted phenyl)methanes.[12]

Antiaris toxicaria LESCH. (Moraceae) is known as "upas tree" and widespread over tropic rain forest of Southeast Asia. Previous investigations concentrated on the latex of *A. toxicaria* and led to the isolation of cardiac glycosides.[13—16] In addition to the effect of the cardenolides on the activity of the ubiquitous cell surface enzyme Na^+/K^+-ATPase, recent studies have demonstrated that cardiac glycosides could inhibit the growth of cancer cells[17,18] and induce their apoptosis *via* activation of caspase-3, induction of cytochrome C release from mitochondria, and generation of reactive oxygen species.[19]

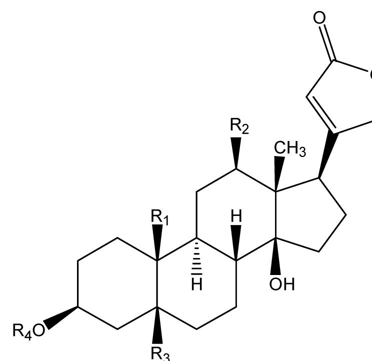
In our present work searching for components with anticancer activity, 11 cardenolides (Fig. 1) were isolated from the stem of *A. toxicaria* by cytotoxicity assay-guided fractionation. All these compounds showed potent growth-inhibitory activity in a variety of cancer cell lines. Moreover, we found that several cardenolides induced the expression of Nur77 protein in cancer cells.

Results and Discussion

Toxicarioside D (**1**), a new cardenolide, was isolated from the bioassay-guided chromatographic fractionation of the 60% ethanol extract of the stem of *A. toxicaria*, of which the structure was elucidated by comprehensive analysis of 1D and 2D NMR spectra.

Compound **1** was obtained as white powder. The molecular formula was established as $C_{30}H_{44}O_{10}$ by HR-ESI-MS. In the ^{1}H- and ^{13}C-NMR spectra (Table 1), the characteristic signals of a lactone (δ_C 174.4), an olefin (δ_H 6.21, δ_C 117.7, 175.6), an oxygenated methylene (δ_H 5.27, 5.01, δ_C 73.6), and an anomeric sugar signal (δ_H 4.80, δ_C 100.8) indicated that **1** was an α,β-unsaturated γ-lactone cardenolide with a sugar residue, which was consistent with UV and IR absorptions at 217 nm and 3383, 1744, and 1716 cm^{-1}. The signals in the ^{1}H-NMR spectrum at δ 10.41 (1H, s, H-19) and 1.00 (3H, s, H-18) suggested a cardenolide skeleton with 19-CHO and 18-CH_3. The aglycone of **1** was established on the basis of its key HMBC and ROESY correlations (Fig. 2), and the ^{1}H- and ^{13}C-NMR spectra were similar to those reported for 3-*O*-substituted strophanthidins.[13,20] Thus the aglycone of **1** was identified as strophanthidin. In addition, the doublet at δ 1.57 (3H, d, J=5.7 Hz, H-6′) indicated a C-6′-deoxy hexose residue.

According to the HMBC correlations of H-3/C-1′ and H-1′/C-3, the sugar moiety was connected to C-3. The large coupling constant of anomeric proton H-1′ (1H, d, J=8.0 Hz) indicated a β-configuration of the hexose moiety. In the HMBC spectrum, H-2′ correlated with the carbon signal of



1 R_1 = CHO, R_2 = H, R_3 = OH, R_4 = 6-deoxy-2-O-methyl-β-glucopyranosyl
2 R_1 = CHO, R_2 = H, R_3 = OH, R_4 = H
3 R_1 = CH_2OH, R_2 = H, R_3 = OH, R_4 = H
4 R_1 = CHO, R_2 = H, R_3 = H, R_4 = α-L-rhamnopyranosyl
5 R_1 = CHO, R_2 = H, R_3 = OH, R_4 = β-D-javopyranosyl
6 R_1 = CHO,R_2 = H, R_3 = OH, R_4 = 6-deoxy-β-D-gulopyranosyl
7 R_1 = CHO, R_2 = H, R_3 = OH, R_4 = 6-deoxy-β-D-allopyranosyl
8 R_1 = CH_2OH, R_2 = H, R_3 = H, R_4 = α-L-rhamnopyranosyl
9 R_1 = CH_2OH, R_2 = H, R_3 = OH, R_4 = 6-deoxy-β-D-allopyranosyl
10 R_1 = CHO, R_2 = OH, R_3 = OH, R_4 = 6-deoxy-β-D-allopyranosyl
11 R_1 = CHO, R_2 = OH, R_3 = OH, R_4 = β-D-javopyranosyl

Fig. 1. The Structures of Compounds **1**—**11**

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Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) Data of Compound **1** (Pyridine-d_5, J in Hz)

Atom	δ(H)	δ(C)	Atom	δ(H)	δ(C)
1	2.62 (1H, m), 1.91 (1H, m)	18.6	16	2.04 (1H, m)	27.2
2	2.19 (1H, m), 1.72 (1H, m)	25.7	17	2.77 (1H, m)	51.1
3	4.45 (1H, br s)	73.9	18	1.00 (3H, s)	15.9
4	2.31 (1H, m), 1.85 (1H, m)	37.4	19	10.41 (1H, s)	208.4
5		73.6	20		175.6
6	2.21 (1H, m), 1.86 (1H, m)	35.2	21	5.27 (1H, d, 17.6) 5.01 (1H, dd, 18, 1.2)	73.6
7	2.45 (1H, m), 1.44(1H, m)	24.9	22	6.21 (1H, s)	117.7
8	2.28 (1H, m)	41.8	23		174.4
9	1.35 (1H, m)	39.5	1′	4.80 (1H, d, 7.6)	100.8
10		55.4	2′	3.39 (1H, dd, 8.8, 8.0)	85.0
11	1.57 (1H, m), 1.34 (1H, m)	22.6	3′	4.01 (1H, dd, 8.0, 8.4)	77.7
12	1.77 (1H, m), 1.35 (1H, m)	39.5	4′	3.62 (1H, m)	76.7
13		49.8	5′	3.66 (1H, m)	73.0
14		84.4	6′	1.57 (3H, d, 5.2)	18.4
15	2.05 (1H, m), 1.85 (1H, m)	32.1	2-*O*-Methyl ether	3.82 (3H, s)	60.8

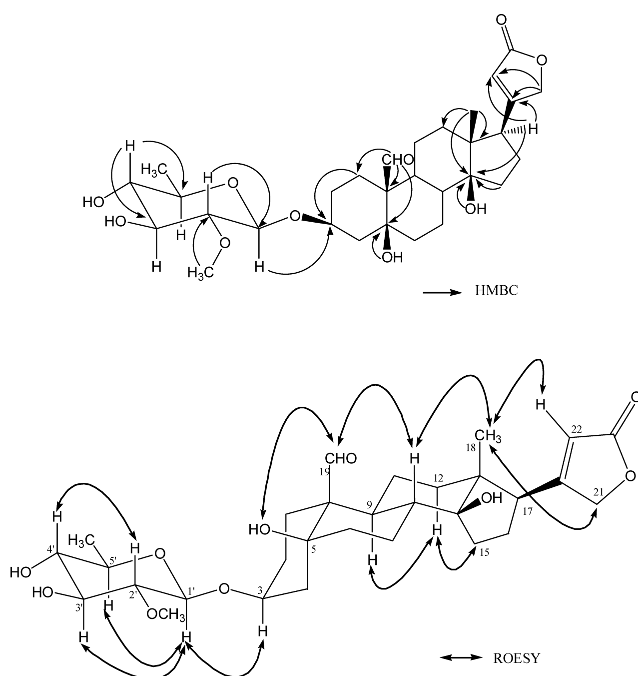

Fig. 2. The Key HMBC and ROESY Correlations of Compound **1**

the methoxy group, indicating that the *O*-methyl ether attachment point was at C-2′ of the pyranose, which was also supported by the relatively low field shift observed for C-2′ (δ 85.0) in the ^{13}C-NMR spectrum of **1**. According to the vicinal coupling constants for the sugar proton signals and the ^{13}C-NMR shifts, the sugar moiety was determined as 6-deoxy-2-*O*-methyl-glucopyranose, which agreed with the literature report.[16] Thus compound **1** was elucidated as strophanthidin-3-*O*-β-(6-deoxy-2-*O*-methyl)-glucopyranoside.

In addition, on the basis of the spectroscopic features and direct comparison with the respective literature data, the structures of the known compounds were determined as strophanthidine (**2**),[21] strophanthidol (**3**),[22] malayoside (**4**),[23] strophanthojavoside (**5**),[13] deglucocheirotoxin (**6**),[20] strophalloside (**7**),[20] cannogenol-3-*O*-α-L-rhamnoside (**8**),[20] strophanolloside (**9**),[24] antiarigenin-3-*O*-β-D-6-deoxy-alloside (**10**),[25] and antiarojavoside (**11**).[16]

The growth inhibitory activity of all the compounds was examined in a panel of cancer cell lines, including NIH-H460, A549, and Calu-6 lung cancer, LNCaP prostate cancer, MCF-7 breast cancer, SW480 colon cancer, and HeLa ovarian cancer cell lines. The results demonstrated that these compounds exhibited significant inhibitory activities against the proliferation of these human cancer cell lines, with IC_{50} in nanomolar range (Table 2). The research on antitumor activity of these compounds provided more data on structure–activity relationships (SAR) of cardenolides. Compounds **1**, **5**, **6**, **7**, and **9** were more active than their corresponding aglycones: **2** and **3**, respectively. Thus 3-glycosidation seems crucial for high cell-growth inhibition, which is consistent with literature data.[26] Cytotoxic activities also could be remarkably altered after 12β-hydroxylation of aglycones (compounds **5**/**11** and **7**/**10**), but the influence of the cytotoxic activity of 5β-hydroxylation was complex. Additionally, activity against cancer cell-growth increased after hydrogenation at 19-CHO of aglycones (compounds **3**/**8** and **7**/**9**) and the activity decreased after 2-*O*-methylation of sugar moieties (compounds **5**/**7** and **10**/**11**). After all, aglycone glycosidation and 12β-hydroxylation seem necessary in terms of high cancer cell-growth inhibition. However, more experiments are needed to clarify this point.

The growth inhibitory effect of cardenolides might be attributed to their induction of apoptosis.[27,28] Cardenolides promote apoptosis of cancer cells by inducing release of cytochrome C from mitochondria.[27] To explore the potential molecules involved in mediating the apoptotic action of cardenolides, their effect on expression of a number of protein factors was examined. Because NIH-H460 is sensitive cell line to cardenolides compared with the other cell lines tested, NIH-H460 cells were used in our subsequent experiments. The most potent compounds **1**, **4**, **6**, **7**, and **8** were tested for their regulation of Nur77 expression at mRNA and protein levels. As shown in Fig. 3A, **4**, **6**, and **7** potently induced expression of Nur77 protein in NIH-H460 lung cancer cells. The inducing effect was observed when cells were treated with nanomolar concentrations of the compounds for as short as 6 h. Consistently, we found that the Nur77 transcripts were strongly upregulated by **4** and **6** in the same cells within nanomolar concentrations. At higher concentrations (10 μM),

Table 2. IC_{50} Values for the Inhibition of Human Cancer Cell Lines of Compounds **1**—**11** (Mean±S.D., $n=3$)

Drug	IC_{50}±S.D. (nM)						
	H460	LNCaP	MCF-7	SW-480	Hela	A549	Calu-6
1	30.3±4.4	115.5±23.3	48.5±0.9	103.0±1.4	99.7±9.8	67.1±45.8	619.0±195.0
2	>5000	>5000	>5000	>5000	>5000	>5000	>5000
3	>5000	>5000	>5000	>5000	>5000	>5000	>5000
4	14.6±2.3	52.4±3.6	28.2±1.9	61.6±8.5	37.0±4.7	24.8±13.5	345.5±10.6
5	21.5±2.9	89.0±2.9	34.1±0.4	76.3±2.3	67.9±15.0	48.3±8.3	331.0±81.3
6	15.0±1.8	47.6±5.2	23.4±3.3	64.0±0.6	44.9±1.9	25.8±8.0	207.0±90.4
7	19.6±4.7	69.7±29.3	41.0±1.8	77.6±76.1	59.1±8.0	32.2±15.9	221.3±13.6
8	10.8±5.4	42.8±17.3	37.0±2.2	65.8±46.5	27.7±10.1	17.6±5.8	163.3±47.7
9	5.8±0.4	21.9±2.0	15.2±1.5	43.6±34.7	11.5±6.7	8.3±3.9	67.5±9.6
10	215.0±60.8	688.5±275	437.0±140	990.5±41.7	493.0±95.1	450.7±24.4	2893±536
11	81.6±16.1	478.5±235.5	123.0±7.1	239.5±70	259.7±75.6	238.0±31.1	>500

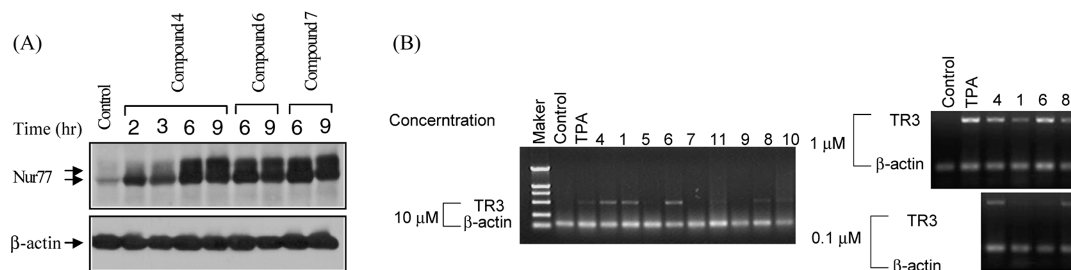

Fig. 3. Induction of Nur77 Expression by Cardenolides

(A) NIH-H460 lung cancer cells were treated with compounds **4** (10 nM), **6** (20 nM), or **7** (20 nM) for the indicated time. Cell lysates were prepared and analyzed for expression of Nur77 protein by Western blotting using anti-Nur77 antibody. Expression of β-actin was used for loading control. (B) NIH-H460 lung cancer cells were treated with vehicle, TPA (100 ng/ml), or various cardenolides (10 μM, 1 μM, 0.1 μM) for 3 h. Nur77 mRNA expression was examined by RT-PCR method.

Nur77 mRNA could also be elevated by **1** and **8** (Fig. 3B). Compound **7** appeared to stabilize Nur77 protein rather than to regulate Nur77 expression at the mRNA level. As a positive control, upregulation of Nur77 mRNA expression could also be seen in NIH-H460 cells treated with the phorbol ester 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) at 100 ng/ml with extensively expressed Nur77 mRNA. Since Nur77 induction plays a critical role in mediating the death effect of a variety of apoptotic stimuli,[1,2] our results suggest that induction of Nur77 may be a mechanism by which cardenolides exert their antitumor effects, and more investigations needed to be conducted to ascertain.

Experimental

General Optical rotations were measured by a JASCO P-1020 digital polarimeter. UV spectra were determined by JASCO V-550 UV/vis spectrophotometer in MeOH. IR spectra were acquired by JASCO FT/IR-480 plus spectrophotometer. ESI-mass spectra were taken on a Finigan LCQ Advantage MAX mass spectrometer. HR-mass spectra were recorded on a Micromass Q-TOF mass spectrometer. 1D and 2D NMR spectra were measured on a Bruker AV-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Preparative high-performance liquid chromatography (HPLC) was run on a Varian chromatograph equipped with a Prostar 215 pump and a Prostar325 UV–vis detector with a C_{18} reversed-phase column (Cosmosil, 20×250 mm, 5 μm). Analytical HPLC were run on a Dionex chromatograph equipped with a P680 pump and a PDA-100 photodiode array detector with a C_{18} reversed-phase column (Cosmosil, 4.6×250 mm, 5 μm). Column chromatography was performed on silica gel (200—300 mesh, Qingdao Haiyang Chemical Group Corp., Qingdao, China), Sephadex LH-20 (Pharmacia) and ODS (60—80 mesh, Merck).

Plant Materials The stem of *A. toxicaria* was collected from Yunnan province of P.R. China in August 2005 and identified by Prof. Hao Zhang, West China School of Pharmacy, Sichuan University. A voucher specimen (YANTO2005-08) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University.

Extraction and Isolation Air-dried plant material (6 kg) was minced finely then refluxed with 60% ethanol (50 l×2 h) three times. The combined extract was concentrated under reduced pressure to afford a brown residue (300 g). The residue was suspended in water then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction exhibited significant cytotoxicity activity (IC_{50}<0.625 μg/ml) against human lung carcinoma cells (NIH-H460), while the EtOAc-soluble fraction showed only weak activity. Then the *n*-BuOH extract (35 g) was subjected to open silica gel column (ϕ5.0×60 cm) and eluted with $CHCl_3$/MeOH/H_2O gradiently (19 : 1 : 0.05, 9 : 1 : 0.1, 8.5 : 1.5 : 0.15, 8 : 2 : 0.2, 7 : 3 : 0.3). Fraction A ($CHCl_3$/MeOH/H_2O 19 : 1 : 0.05 elution, 1.7 g) was chromatographed on Sephadex LH-20 (ϕ2.0×65 cm) and eluted with MeOH/H_2O (1 : 1) to afford two subfractions. Subfraction A1 (650 mg) was applied to preparative RP-HPLC using eluted with MeOH/H_2O (45 : 55) as mobile phase to afford compounds **2** (2 mg) and **3** (3 mg). Fraction B ($CHCl_3$/MeOH/H_2O 9 : 1 : 0.1 elution, 2.9 g) was chromatographed over ODS (ϕ3.0×40 cm) using solvent of MeOH/H_2O (40 : 60). Subfraction B2 (930 mg) was subjected to preparative RP-HPLC and eluted with MeOH/H_2O (35 : 65) to obtain compounds **4** (15 mg), **5** (12 mg), **6** (11 mg), and **7** (17 mg). Fraction C ($CHCl_3$/MeOH/H_2O 8.5 : 1.5 : 0.15 elution, 3.7 g) was subjected to ODS column (ϕ3.0×40 cm) by eluting with MeOH/H_2O (40 : 60) to give two subfractions. Both were applied to RP-HPLC: subfraction C1 (750 mg) was eluted by MeOH/H_2O (33 : 67) to afford compounds **8** (15 mg) and **1** (18 mg) and C2 (990 mg) was eluted by MeOH/H_2O (35 : 65) to obtain compounds **9** (12 mg), **10** (14 mg), and **11** (10 mg).

Toxicarioside D (**1**): White powder; $[\alpha]_D^{25}$ +37.0° (c=0.2, MeOH); UV λ_{max} (MeOH) nm (log ε): 217 (4.27); IR (KBr) cm^{-1}: 3383, 2935, 2380, 1744, 1716, 1065; ^{1}H-NMR (CD_3OD, 400 MHz) δ_H and ^{13}C-NMR (CD_3OD, 100 MHz) δ_C were given in Table 1. ESI-MS m/z: 587 $[M+Na]^+$, 563 $[M-H]^-$; HR-ESI-MS m/z: 587.2817 $[M+Na]^+$ (Calcd for $C_{30}H_{44}O_{10}Na$, 587.2832).

Cytotoxicity Assay Human lung carcinoma cells (NIH-H460, A549, Calu-6), human prostate carcinoma cells (LNCaP), human breast adenocarcinoma cells (MCF-7), human colon adenocarcinoma cells (SW480), and human cervical carcinoma cells (HeLa) were perchased from the American Type Culture Collection. All the cells were maintained in RPMI 1640 medium containing 10% FBS and 1% L-glutamine, 100 units/ml penicillin

and 100 μg/ml streptomycin. By the standard trypsinization method, the cells were transferred into 96-well plates (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator for 24 h. The medium was removed and replaced by the growth medium containing various concentrations of each sample. The cells were incubated for a further 72 h, then cell proliferation was evaluated with an MTT assay procedure as previously described.[29,30] Twenty milliliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (4.14 mg/ml) was added to each well and incubated at 37 °C for 4 h. The formed formazan crystals were dissolved in 100 ml DMSO for 10 min with shaking. The UV/vis absorbance at 570 nm was measured by microplate reader. Cytotoxicity was expressed as the concentration of the extract required to inhibit cellular growth 50% (IC_{50})±standard deviation (S.D.).

Western Blotting Analysis for Nur77 Expression Nur77 expression was determined by Western blotting using polyclonal anti-Nur77 antibody as previously described.[3,4] Briefly, cell lysates were boiled in SDS sample buffer, resolved by SDS-polyacrylamide gel electrophoresis (12.5% polyacrylamide), and transferred to nitrocellulose. After transfer, the membranes were blocked in 5% milk in TBST (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing the anti-Nur77 antibody. The membranes were then washed three times with TBST, and incubated at room temperature for 1 h in TBST containing horseradish peroxide-linked anti-immunoglobulin. After 3 washes in TBST, immunoreactive products were detected by chemiluminescence with an enhanced chemiluminescence system (ECL, Amersham).

RT-PCR Analysis NIH-H460 lung cancer cells seeded in six-well plate (5×10^5 cells/well) were allowed to adhere overnight and were starved for 12 h before treatment. After treatment with the compounds at desired concentrations, total RNA was isolated with Trizol LS (Invitrogen) and first-strand synthesis was performed with SuperScript II (Invitrogen) according to the manufacturer's instructions. Two microliters of cDNA was used for PCR reactions in Eppendorf AG 22331 Hamburg (Eppendorf, U.S.A.) with primers specific to Nur77 (forward primer: 5′-TCA TGG ACG GCT ACA CAG-3′; reverse primer: 5′-GTA GGC ATG GAA TAG CTc-3′) and β-actin (forward primer: 5′-CTG GAG AAG AGC TAC GAG-3′; reverse primer: 5′-TGA TGG AGT TGA AGG TAG-3′). Cycling conditions were 30 s denaturation at 94 °C, 30 s annealing at 55 °C, and 30 s elongation at 72 °C for 35 cycles. In all, 5 μl of PCR reactions were electrophoresed on 2% agarose gels containing 0.1 mg/ml ethidium bromide in Tris-Borate-EDTA. Gel images were captured with a Gel logic 200 system (Kodak, U.S.A.).

Statistical Analysis Statistical significance of differences between groups was analyzed by Student's *t*-test. Values of $p<0.05$ were considered significant.

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References

1) Moll U. M., Marchenko N., Zhang X. K., *Oncogene*, **25**, 4725—4743 (2006).
2) Zhang X. K., *Expert Opin. Ther. Targets*, **11**, 69—79 (2007).
3) Li Y., Lin B., Agadir A., Liu R., Dawson M. I., Reed J. C., Fontana J. A., Bost F., Hobbs P. D., Zheng Y., Chen G. Q., Shroot B., Mercola D., Zhang X. K., *Mol. Cell. Biol.*, **18**, 4719—4731 (1998).
4) Li H., Kolluri S. K., Gu J., Dawson M. I., Cao X., Hobbs P. D., Lin B., Chen G., Lu J., Lin F., Xie Z., Fontana J. A., Reed J. C., Zhang X. K., *Science*, **289**, 1159—1164 (2000).
5) Liu S., *World J. Gastroenterol.*, **8**, 446—450 (2002).
6) Cao X., Liu W., Lin F., Li H., Kolluri S. K., Lin B., Han Y. H., Dawson M. I., Zhang X. K., *Mol. Cell. Biol.*, **24**, 9705—9725 (2004).
7) Lin B., Kolluri S., Lin F., Liu W., Han Y. H., Cao X., Dawson M. I., Reed J. C., Zhang X. K., *Cell*, **116**, 527—540 (2004).
8) Jeong J. H., Park J. S., Moon B., Kim M. C., Kim J. K., Lee S., Suh H., Kim N. D., Kim J. M., Park Y. C., Yoo Y. H., *Ann. N.Y. Acad. Sci.*, **1010**, 171—177 (2003).
9) Gennari A., Bleumink R., Viviani B., Galli C. L., Marinovich M., Pieters R., Corsini E., *Toxicol. Appl. Pharmacol.*, **181**, 27—31 (2002).
10) Chinnaiyan P., Varambally S., Tomlins S. A., Ray S., Huang S., Chinnaiyan A. M., Harari P. M., *Int. J. Cancer*, **118**, 1041—1050 (2005).
11) Shin H. J., Lee B. H., Yeo M. G., Oh S. H., Park J. D., Park K. K., Chung J. H., Moon C., Lee M. O., *Carcinogenesis*, **25**, 1467—1475 (2004).
12) Chintharlapalli S., Burghardt R., Papineni S., Ramaiah S., Yoon K., Safe S., *J. Biol. Chem.*, **280**, 24903—24914 (2005).
13) Muehlradt P., Weiss E. K., Reichstein T., *Helv. Chim. Acta*, **47**, 2164—2186 (1964).
14) Wehrli W., *Helv. Chim. Acta*, **45**, 1206—1211 (1962).
15) Carter C. A., Forney R. W., Gray E. A., Gehring A. M., Schneider T. L., Young D. B., Lovett C. M., Scott L., Messer A. C., Richardson D. P., *Tetrahedron*, **53**, 13557—13566 (1997).
16) Carter C. A., Gray E. A., Schneider T. L., Lovett C. M., Scott L., Messer A. C., Richardson D. P., *Tetrahedron*, **53**, 16959—16968 (1997).
17) Schoner W., Scheiner-Bobis G., *Am. J. Physiol.*, **293**, C509—C536 (2007).
18) Nesher M., Shpolansky U., Rosen H., Lichtstein D., *Life Sci.*, **80**, 2093—2107 (2007).
19) Huang Y. T., Chueh S. C., Teng C. M., *Biochem. Pharmacol.*, **67**, 727—733 (2004).
20) Schenk B., Junior P., Wichtl M., *Planta Med.*, **40**, 1—11 (1980).
21) Kocovsky P., Stieborova I., *Tetrahedron Lett.*, **30**, 4295—4298 (1989).
22) Sharipov A. K., Gorovits M. B., Makarichev G. K., Yagudaev M. R., Abubakirov N., *Chem. Nat. Compd.*, **5**, 270—273 (1969).
23) Abe F., Yamauchi T., Wan A., *Phytochemistry*, **31**, 3189—3193 (1992).
24) Kubelka W., Kopp B., Jentzsch K., *Pharm. Acta Helv.*, **50**, 353—359 (1975).
25) Muehlradt P., Weiss E., Reichstein T., *Justus Liebigs Ann. Chem.*, **685**, 253—261 (1965).
26) Ueda J., Tezuka Y., Banskota A. H., Le Tran Q., Tran Q. K., Saiki I., Kadota S., *Biol. Pharm. Bull.*, **26**, 1431—1435 (2003).
27) McConkey D. J., Lin Y., Nutt L. K., Ozel H. Z., Newman R. A., *Cancer Res.*, **60**, 3807—3812 (2000).
28) Frese S., Frese-Schaper M., Andres A. C., Miescher D., Zumkehr B., Schmid R. A., *Cancer Res.*, **66**, 5867—5874 (2006).
29) Mizutani Y., Bonavida B., Koishihara Y., Akamatsu K., Ohsugi Y., Yoshida O., *Cancer Res.*, **55**, 590—596 (1995).
30) Carmichel J., DeGraff W. G., Gazdar A. F., Minna J. D., Mitchell J. B., *Cancer Res.*, **47**, 936—942 (1987).