Arch. Pharm. Res. Vol.19, No.4, pp. 286-291, 1996

Cytotoxicity of a Novel Biphenolic Compound, Bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl)methane against Human Tumor Cells *In vitro*

Sang Un Choi[1], Kwang Hee Kim[1], Nam Young Kim[1], Eun Jung Choi[1], Chong Ock Lee[1], Kwang Hee Son[2], Sung Uk Kim[2], Song Hae Bok[2] and Young Kook Kim[2]

[1]Pharmaceutical Screening Team, Korea Research Institute of Chemical Technology, P.O.Box 107, Daedeog-Danji, Yusung, Taejeon 305-606, Korea and [2]Bioproducts Research Group, Korea Research Institute of Bioscience & Technology, KIST, P.O.Box 115, Yusung, Taejeon, 305-600, Korea

(Received March 18, 1996)

Phenolic compounds are prevalent as toxins or environmental pollutants, but they are also widely used as drugs for various purpose including anticancer agent. A novel biphenolic compound, bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl)methane (GERI-BP002-A) was isolated from the fermentation broth of *Aspergillus fumigatus* F93 previously, and it has revealed cytotoxicity against human solid tumor cells. Its effective doses that cause 50% inhibition of cell growth *in vitro* against non-small cell lung cancer cell A549, ovarian cancer cell SK-OV-3, skin cancer cell SK-MEL-2 and central nerve system cancer cell XF498 were 8.24, 10.60, 8.83, 9.85 μg/ml respectively. GERI-BP002-A has also revealed cytotoxicity against P-glycoprotein-expressed human colon cancer cell HCT15 and its multidrug-resistant subline HCT15/CL02, and its cytotoxicity was not affected by P-glycoprotein. We have also tested cytotoxicities of structurally related compounds of GERI-BP002-A such as diphenylmethane, 1,1-bis(3,4-dimethylphenyl)ethane, 2,2-diphenylpropane, 2-benzylpyridine, 3-benzylpyridine, 4,4'-di-*tert*-butylphenyl, bibenzyl, 2,2'-dimethylbibenzyl, *cis*-stilbene, *trans*-stilbene, 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide, sulfadiazine and sulfisomidine for studying of structure and activity relationship, and from these data we could suppose that hydroxyl group of GERI-BP002-A conducted important role in its cytotoxicity.

Key words : Bis(2-hydroxy-3-tert-butyl-5-methylphenyl)methane, Human tumor cell, Cytotoxicity, Multidrug-resistance

INTRODUCTION

There are many agents which were developed as anticancer drugs so far, and these chemotherapeutic agents have proven to be effective in the cure of some human cancers (Boven *et al.*, 1988; Brown *et al.*, 1991). In spite of these facts, cancer is still a major cause of human death in almost nations, and very extensive studies on searching for new anticancer drugs have been conducted in many laboratories worldwidely (Boven *et al.*, 1988; Brown *et al.*, 1991; Douros and Stuffness, 1979; Weiss *et al.*, 1988; Slavik, 1978). And we have also been investigating for the searching of new antineoplastic compounds from resources of varieties such as medicinal plants, fungi and bacteria based on the activity-guided fractionation of the crude materials (Choi *et al.*, 1996; Lee *et al.*, 1995; Ryu *et al.*, 1994; Ryu *et al.*, 1992).

Cancer cells developed resistance to anticancer drugs in many ways, and the emergence of insentive cells is a major problem encountered in cancer chemotherapy. Despite the high prevalence of the drug resistance, the mechanisms underlying resistance are poorly understood. Recently, several mechanisms of drug resistance have been suggested, and the discovery that colon cancer cells have high levels of P-glycoprotein (PGP) led to the attractive hypothesis that enhanced drug efflux could explain in part the refractoriness to therapy seen in this malignancy (Fojo *et al.*, 1987). PGP is 170-kilodalton transmembrane protein, and functions as an energy-dependent drug efflux pump that reduces intracellular drug accumulation, thereby causing resistance to many structually different drugs (Endicott and Ling, 1989; Kartner *et al.*, 1983; Park *et al.*,

Correspondence to: Sang Un Choi, Pharmaceutical Screening Team, Korea Research Institute of Chemical Technology, P.O.Box 107, Daedeog-Danji, Yusung, Taejeon 305-606, Korea

1994). And recently, a direct relationship between the PGP expression and chemosensitivity has been reported in several human cancers (Chen *et al.*, 1994; Goldstein *et al.*, 1989; Park *et al.*, 1994).

We have already reported the isolation of a novel biphenolic compound, Bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl)methane (GERI-BP002-A), from the fermentation broth of *Aspergillus fumigatus* F93 (Kim *et al.*, in press). In this paper, we report the cytotoxic activity of GERI-BP002-A against some human solid tumor cell lines *in vitro*. We have also studied its cytotoxicity against naturally PGP-expressed human colon cancer cell line, HCT15 and against HCT15/CL 02 multidrug resistant subline derived from parental HCT15 cells.

MATERIALS AND METHODS

Chemicals

GERI-BP002-A was isolated from the fermentation broth of *Aspergillus fumigatus* F93 and the structure was determined to be bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl)methane on the basis of several methods including ^{1}H-NMR and ^{13}C-NMR in Korea Research Institute of Bioscience and Technology. The details of isolation and determination of structure were previously described (Kim *et al.*, in press), and the structure of GERI-BP002-A is shown in Fig. 1. The standard anticancer agents such as doxorubicin, cisplatin and tamoxifen were purchased from Sigma Chemical Co. (St Louis, MO). Verapamil, a reversal agent of multidrug-resistance, and analogues of GERI-BP002-A such as diphenylmethane, 1,1-bis(3,4-dimethylphenyl)ethane, 2,2-diphenylpropane, 2-benzylpyridine, 3-benzylpyridine, 4,4'-di-*tert*-butylphenyl, bibenzyl, 2,2'-dimethylbibenzyl, cis-stilbene, trans-stilbene, 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide, sulfadiazine and sulfisomidine were also purchased from Sigma Chemical Co. And the cell growth medium, RPMI-1640 and fetal bovine serum were purchased from Gibco Ltd (Grand Island, N.Y.).

Cells

Human tumor cells used in the experiment, that is non-small cell lung cancer cell line A549, ovarian cancer cell line SK-OV-3, malignant melanoma cell line SK-MEL-2, central nerve system cancer cell line XF498 and colorectral adenocarcinoma cell line HCT 15 were originally provided by National Cancer Institute (NCI) in U.S.A. and maintained in Korea Research Institute of Chemical Technology (KRICT). The multidrug resistant HCT15/CL02 cells were established from parental HCT15 cells by stepwise and continuous doxorubicin exposure in KRICT (Choi *et al.*, submitted). Stock cell cultures were grown in T-25

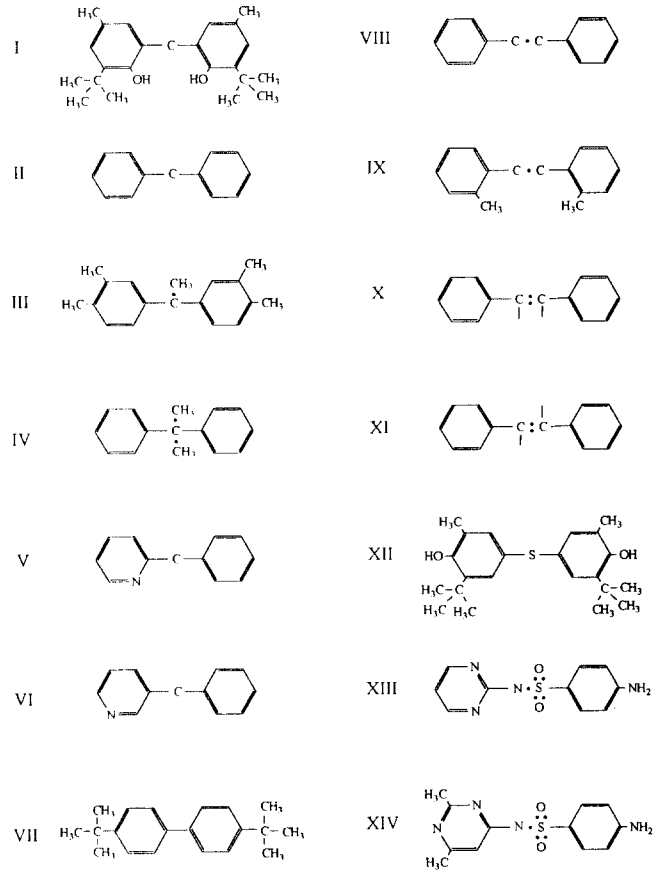

Fig. 1. Structure of GERI-BP002-A (I) and its structurally related compounds, diphenylmethane (II), 1,1-bis(3,4-dimetylphenyl) ethane (III), 2,2-diphenylpropane (IV), 2-benzylpyridine (V), 3-benzylpyridine (VI), 4,4'-di-*tert*-butylphenyl (VII), bibenzyl (VIII), 2,2'-dimethylbibenzyl (IX), cis-stilbene (X), *trans*-stilbene (XI), 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide (XII), sulfadiazin (XIII) and sulfisomidine (XIV)

(Falcon) flasks containing 10 ml of RPMI 1640 medium with glutamine, sodium bicarbonate, gentamycin, amphotericin and 5% fetal bovine serum. The cells were dissociated with 0.25% trypsin and 3 mM 1,2-cyclohexanediaminetetraacetic acid (CDTA) in PBS in case of transferring or dispensing before experiment. The cells were maintained in the incubator at 37°C in a humidified atmosphere of 5% CO_2 in air continuously except when adding drugs.

Cytotoxicity assay *in vitro*

All experimental procedures were followed the NCI (U.S.A.)'s protocol (Skehan *et al.*, 1990) based on the Sulforhodamine B (SRB) method as described previously (Ryu *et al.*, 1992). Briefly, tumor cells were inoculated over a series of standard 96-well flat bottom microtiter plates on day 0. These cells were then preincubated for attachment on the microtiter plate for 24 hours. The compounds were added to the wells in serial dilutions starting from the highest con-

centrations. At the termination of incubation with each drug for 48 hours in cases of A549, SK-OV-3, SK-MEL-2 and XF498 cells or 72 hours in cases of HCT15 and HCT15/CL02, the culture medium in each well was removed and the cells were fixed with cold 10% trichloroacetic acid (TCA). The microplates were washed and dried after incubation at 4°C for 1 hour with TCA. And then, 0.4% SRB solution was added and incubated for 30 minutes at room temperature. The cells were washed again, and the bound stain was solubilized with 10 mM unbuffered Tris base solution (pH 10.5) and the absorbances were measured spectrophotometrically at 520 nm and 690 nm in a microtiter plate reader. The absorbance measured at 690 nm was substracted from the absorbance at 520 nm so as to eliminate the effects of non-specific absorbance.

For the study of effects of verapamil on the cytotoxicities of anticancer drugs, attached cells were incubated with serial dilutions of drugs in the presence or absence of 4 μg/ml verapamil. After 72 hours of continuous drug-expose incubation, the survival fractions were measured by the same method to the previous test.

The data were transferred and transformed into Lotus-123 format and survival fractions were calculated by comparing the drug treated with controls. All data represented the average values for a minimum of three wells.

RESULTS AND DISCUSSION

Phenolic compounds are widely distributed in nature and they have very diverse activities in both beneficial and harzardous effects (Nohl *et al.*, 1986; Stich, 1991). And some of them have been shown to be cytotoxic and/or genotoxic in a variety of biological systems (O'Brine, 1991; Li and Trush, 1994; Ryu *et al* 1994). GERI-BP002-A is a novel biphenolic compound, and we have already reported its inhibitory activity of acyl-CoA:cholesterol acyltransferase which is a responsible enzyme for catalyzing the intracellular esterification of cholesterol and acyl CoA (Kim *et al.*, in press). And in this cytotoxicity study, GERI-BP002-A has revealed cytotoxicity against all the tested human solid tumor cells, and its cytotoxicities of each cell line were not shown any statistically significant difference (Fig. 2, Fig. 3). We have also tested the cytotoxicities of some other compounds which have similar structure with GERI-BP 002-A for studying of the structure and activity relationship. Among the tested compounds, 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide has revealed considerable cytotoxicities against all the tested cancer cell lines. But we have not been able to detect any cytotoxicity of other tested compounds such as di-

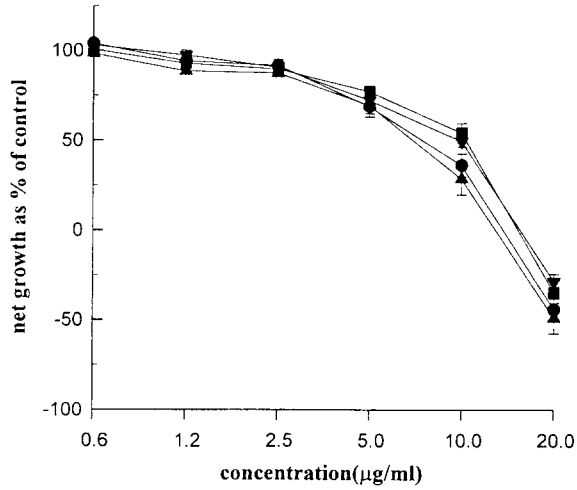

Fig. 2. Cytotoxicities of GERI-BP002-A against human solid tumor cells such as non-small cell lung cancer cell line A549 (●), ovarian cancer cell line SK-OV-3 (■), malignant melanoma cell line SK-MEL-2 (▲) and central nerve system cancer cell line XF498 (▼). Each point represents mean±S.E.M. of three distinct experiments.

phenylmethane, 2,2-diphenylpropane, bibenzyl, 2,2'-dimethylbibenzyl and 4,4'-di-*tert*-butylphenyl, 2-benzylpyridine and 3-benzylpyridine. We have also tested the cytotoxicities of *cis*-stilbene, *trans*-stilbene, sulfadiazine and sulfisomidine, and these compounds have not shown any cytotoxicity up to 50 μg/ml. However, in previous report, we have already reported that some naturally occurring hydroxystilbenes such as 3,5-dihydroxy-4'-methoxystilbene, 3,3',5-trihydroxy-4'-methoxystilbene and 3,4',5-trihydroxystilbene have cytotoxic activities against same human tumor cell lines used in this study *in vitro* (Ryu *et al.*, 1994). And in another previous study on the cytotoxic effects of biphenyl and its hydroxylated derivatives, Nakagawa *et al.* (1993) have reported that o-phenylphenol, m-phenylphenol, p-phenylphenol and phenyl-hydroquinone were more cytotoxic to freshly isolated rat hepatocytes than biphenyl and 2-biphenylglycidyl ether, and they have concluded that the addition of a hydroxyl group to the aromatic ring of biphenyl enhanced biphenyl-induced cytotoxicity and that the mitochondria were a common target of the o-phenylphenol isomers and other biphenyl derivatives. It is generally appreciated that the toxicity associated with some phenolic compounds, if not all, is mediated by their further oxidative activation (Li and Trush, 1994; Nakagawa *et al.*, 1993; Thompson *et al.*, 1989), and in this study it is possible to suppose that hydroxyl group of GERI-BP 002-A have conducted key role in its cytotoxicity. The effective dose that cause 50% inhibition of cell growth (ED_{50}) of each tested compound was summarized in Table I.

Frequently, normal tissues such as adrenal gland,

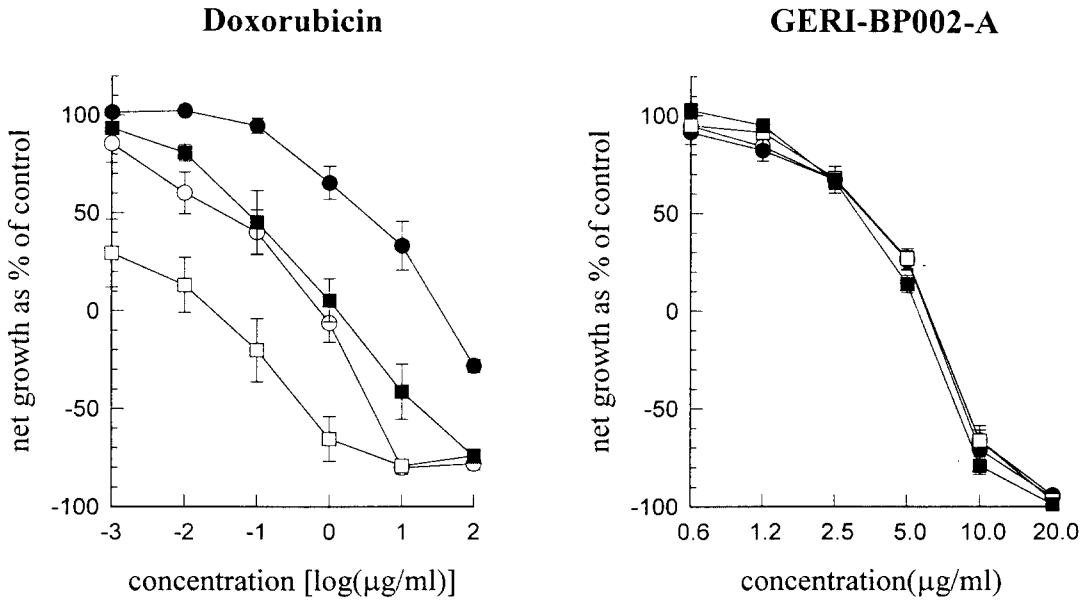

Fig. 3. Cytotoxicities of doxorubicin and GERI-BP002-A against human colorectal adenocarcinoma cell line HCT15 and its multidrug-resistant subline HCT15/CL02 cells, and the effects of verapamil on the cytotoxicity of GERI-BP002-A. Each point represents mean±S.E.M. of at least three distinct experiments. Key: HCT15 cells in the absence of verapamil (■), HCT15 cells in the presence of verapamil 4.0 μg/ml (□), HCT15/CL02 cells in the absence of verapamil (●), HCT15/CL02 cells in the presence of verapamil 4.0 μg/ml (○).

Table I. Cytotoxicities of GERI-BP002-A and its structurally related compounds *in vitro*

Compound	ED_{50} (μg/ml)[a]			
	A549	SK-OV-3	SK-MEL-2	XF498
Doxorubicin	0.09±0.02b	0.05±0.02	0.04±0.01	0.07±0.00
Cisplatin	1.24±0.11	0.83±0.07	0.79±0.08	0.88±0.06
Tamoxifen	4.45±0.04	4.27±0.05	3.98±0.16	4.08±0.07
GERI-BP002-A	8.24±1.58	10.60±0.97	8.83±1.83	9.85±1.04
Diphenylmethane	>50.0	>50.0	>50.0	>50.0
1,1-Bis(3,4-dimethylphenyl)ehane	>50.0	>50.0	>50.0	>50.0
2,2-Diphenylpropane	>50.0	>50.0	>50.0	>50.0
2-Benzylpyridine	>50.0	>50.0	>50.0	>50.0
3-Benzylpyridine	>50.0	>50.0	>50.0	>50.0
4,4'-Di-tert-butylphenyl	>20.0	>20.0	>20.0	>20.0
Bibenzyl	>50.0	>50.0	>50.0	>50.0
2,2'-Dimethylbibenzyl	>50.0	>50.0	>50.0	>50.0
cis-Stilbene	>50.0	>50.0	>50.0	>50.0
trans-Stilbene	>50.0	>50.0	>50.0	>50.0
3-tert-Butyl-4-hydroxy-5-methylphenylsulfide	9.37±3.34	11.85±2.19	8.97±3.16	10.24±1.88
Sulfadiazin	>50.0	>50.0	>50.0	>50.0
Sulfisomidine	>50.0	>50.0	>50.0	>50.0

[a]Effective dose that cause 50 % inhibition of cell growth
[b]Data represent mean±S.E.M. of at least three distinct experiments.

kidney, liver, colon, rectum and brain have highly expressed PGP (Bello-Reuss and Ernest, 1994; Goldstein *et al.*, 1989; Henson *et al.*, 1992; Park *et al.*, 1994). And the pharmacological properties of PGP, together with its distribution in normal tissues have led to the hypothesis that P-glycoprotein plays a role in detoxification mechanisms of the body by transferring various harmful substances from the inner compartment of the body to the external compartment (Gheuens *et al.*, 1993). In fact, the intrinsic or acquired multidrug-resistant human cancers have been reported in many cases from the cells that are constantly exposed to naturally occurring toxins, and so the colon carcinoma represents a complex model in which to study multidrug resistance (Lai *et al.*, 1991a; Lai *et al.*, 1991b). Since GERI-BP002-A is a cytotoxic compound derived from natural products, we have also tested its cytotoxicities against parental HCT15

and multidrug-resistanct HCT15/CL02 cells. The HCT 15 cells were established from a colorectal adenocarcinoma after surgical resection before chemotherapy treatment (Tompkins *et al.*, 1974), and it was reported that these HCT15 cells expressed PGP on its plasma membrane (Iwahashi *et al.*, 1991; Mickley *et al.*, 1989). And we have already reported the isolation of multidrug-resistant subline HCT15/CL02 from parental HCT15 cells, and that its multidrug-resistance may be attributed by complex mechanisms including PGP. We have also reported that these HCT15/CL02 cells have resistance to several standard anticancer drugs such as doxorubicin, actinomycin D, etoposide and vinblastin in the previous report. But HCT15/CL02 cells have shown no resistance to GERI-BP002-A in comparison with parental HCT15 cells. And verapamil, a reversal agent of multidrug resistance by PGP, has not been able to enhance the cytotoxicity of GERI-BP002-A against both HCT15 and HCT15/CL02 cells (Fig. 3). From these results, we have concluded that the cytotoxicity of GERI-BP002-A was not affected by PGP.

In previous report, we have reported that GERI-BP 002-A had no antimicrobial activity against *Streptococccus pyogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella oxytoca, Klebsiella aerogeres* and *Enterobacter cloacae* at concentration up to 200 μg/ml *in vitro*. And in spite of its cytotoxicity against human tumor cells shown in this study, it was also previously reported that GERI-BP002-A has no acute toxic effect on the ICR mice at 500 mg/kg (Kim *et al.*, in press). Therefore, it is possible for GERI-BP002-A to become a leading compound for new anticancer agent which have the overcoming properties of drug resistance.

REFERENCES CITED

Bello-Reuss, E. and Ernest, S., Expression and function of P-glycoprotein in human mesangial cells. *Am. J. Physiol.*, 267, c1351-c1358 (1994).

Boven, E., Winograd, B., Fodstad, O., Lobbezoo, M. W. and Pinedo, H. M., Preclinical phase II studies in human tumor lines: a european multicenter study. *Eur. J. Cancer Clin. Oncol.*, 24, 567-573 (1988).

Brown, T. D., Burris, H. A., Havlin, K. A., O'Rourke, T. J., Rodriguez, G. I., Wall, J. G. and Weiss, G. R., New anticancer agents. *Cancer Chemother. Biol. Response Modif.*, 12, 111-146 (1991).

Choi, S. U., Choi, E. J., Kim, K. H., Kim, N. Y., Kwon, B. M., Kim, S. U., Bok, S. H. and Lee, J. O., Cytotoxicity of trichothecenes to human solid tumor cells *in vitro*. *Arch. Pharm. Res.*, 19, 6-11 (1996).

Douros, J., and Suffness, M., New natural products of interest under development at the National Cancer Institute. *Cancer Chemother. Pharmacol.*, 1, 91-100 (1978).

Endicott, J. A. and Ling, V., The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu. Rev. Biochem.*, 58, 137-171 (1989).

Fojo, A. T., Ueda, K., Slaman, D. J., Poplack, D. G., Gottesman, M. M. and Pastan, I., Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci.*, 84, 265-269 (1987).

Gheuens, E., van der Heyden, S., Elst, H., Eggermont, A., Oosterom, A. V. and De Bruijn E., Multidrug resistance in rat colon carcinoma cell lines CC531, $CC531^{mdr+}$ and $CC531^{rev}$. *Jpn. J. Cancer Res.*, 84, 1201-1208 (1993).

Goldstein, L. J., Galski, H., Fojo, A., Willingham, M., Lai, S-L., Gazdar, A., Cossman, J., Gottesman, M. M. and Pastan, I., Expression of a multidrug resistance gene in brain cancer. *J. Natl. Cancer Inst.*, 81, 116-124 (1989).

Henson, J. W., Cordon-Cardo, C. and Posner, J. B., P-glycoprotein expression in brain tumors. J. Neurooncol., 14, 37-43 (1992).

Iwahashi, T., Okochi, E., Ono, K., Sugawara, I., Tsuruo, T. and Mori, S., Establishment of multidrug resistant human coloredtal carcinoma HCT15 cell lines and their properties. *Anticancer Res.*, 11, 1309-1312 (1991).

Kartner, N., Riordan, J. R. and Ling, V., Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science*, 221, 1285-1288 (1983).

Kim, Y. K., Lee, H. W., Son, K. H., Kwon, B. M., Jeong, T. S., Lee, D. H., Shin, J., Seo, Y., Kim, S. U. and Bok, S. H., GERI-BP002-A, novel inhibitor of acyl-CoA:cholesterol acyltransferase produced by *Aspergillus fumigatus* F93. J. Antibiotics (in press).

Lai, G. M., Chen, Y. N., Mickley, L. A., Fojo, A. T. and Bates, S. E., P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma. *Int. J. Cancer*, 49, 696-703 (1991a).

Lai, G. M., Moscow, J. A., Alvarez, M. G., Fojo, A. T. and Bates, S. E., Contribution of glutathione and glutathione-dependent enzymes in the reversal of adriamycin resistance in colon carcinoma cell lines. *Int. J. Cancer*, 49, 688-695 (1991b).

Lee, S. H., Ryu, S. Y., Choi, S. U., Lee, C. O., No, Z., Kim, S. K. and Ahn J. W., Hydrolysable tannins and related compound having cytotoxic activity from the fruits of *Terminalia chebula*. *Arch. Pharm. Res.*, 18, 118-120 (1995).

Li Y. and Trush, M. A., Reactive oxygen-dependent DNA damage resulting from the oxidation of phenolic compounds by a copper-redox cycle mechanism. *Cancer Res.*, 54, 1895s-1898s (1994).

Mickley, L. A., Bates, S. E., Richert, N. D., Currier, S., Tanaka, S., Foss, F., Rosen, N. and Fojo, A. T., Modulation of the expression of a multidrug resistance gene (*mdr*-1/Pglycoprotein) by differentiating agents. *J. Biol. Chem.*, 264, 18031-18040 (1989).

Nakagawa Y., Tayama, S., Moore, G. and Moldeus, P., Cytotoxic effects of biphenyl and hydroxybiphenyls on isolated rat hepatocytes. *Biochem. Pharmacol.*, 45, 1959-1965 (1993).

Nohl, H., Jordan, W. and Youngman, R. J., Quinones in biology: functions in electron transfer and oxygen activation. *Adv. Free Radical Biol. Med.*, 2, 211-279 (1986).

O'Brien, P. J., Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.*, 80, 1-41 (1991).

Park, J. G., Lee, S. K., Hong, I. G., Kim, H. S., Lim, K. H., Choe, K. J., Kim, W. H., Kim, Y. I., Tsuruo, T. and Gottesman, M. M., MDR1 gene expression: its effects on drug resistance to doxorubicin in human hepatocellular carcinoma cell lines. *J. Natl. Cancer Inst.*, 86, 700-705 (1994).

Ryu, S. Y., Choi, S. U., Lee, C. O., Lee, S. H., Ahn, J. W. and Zee, O. P., Antitumor activity of some phenolic components in plant. *Arch. Pharm. Res.*, 17, 42-44 (1994).

Ryu, S. Y., Choi, S. U., Lee, C. O. and Zee, O. P., Anticancer activity of *Psoralea corylifolia*. *Arch. Pharm. Res.*, 15, 356-359 (1992).

Skehan, P., Streng, R., SCudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M.R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1112 (1990).

Slavik, M., New anticancer antibiotics under development and clinical evaluation in the National Cancer Institute. *Recent Result Cancer Res.*, 63, 282-287 (1978).

Stich, H. F., The beneficial and harzardous effects of simple phenolic compounds. *Mut. Res.*, 259, 307-324 (1991).

Tompkins, W. A. F., Watrach, A. M., Schmale, J. D., Schultz, R. M. and Harris, J. A., Cultural and antigenic properties of newly established cell strains derived from adenocarcinomas of the human colon and rectum. *J. Natl. Cancer Inst.*, 52, 1101-1110 (1974).

Thompson, D. C., Cha, Y-N. and Trush M. A., The peroxidase-dependent activation of butylated hydroxyanisole and butylated hydroxytoluene (BHT) to reactive intermediates. *J. Biol. Chem.*, 264, 3957-3965 (1989).

Weiss, G. R., Arteaga, G. L., Brown, T. D., Craig, J. B., Harman, G. S., Havlin, K. A., Koeller, J. M., Kuhn, J. G. and Von Hoff, D. D., New anticancer agents. *Cancer Chemother. Biol. Response Modif.*, 10, 85-116 (1988).