Arch. Pharm. Res. Vol.19, No.1, pp. 6-11, 1996

Cytotoxicity of Trichothecenes to Human Solid Tumor Cells *in Vitro*

Sang Un Choi[1], Eun Jung Choi[1], Kwang Hee Kim[1], Nam Young Kim[1], Byoung-Mog Kwon[2], Sung Uk Kim[2], Song Hae Bok[2], So Young Lee[2] and Chong Ock Lee[1]

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

(Received September 4, 1995)

The trichothecenes are sesquiterpenoid mycotoxins characterized by the 12,13-epoxytrichothec-9-ene ring system. We have tested cytotoxicity of several naturally-occurring or synthesized trichothecenes against human solid tumor cell lines. Among them, trichothecin (I) and 4-β-Acetoxy-12,13-epoxytrichothec-9-ene (trichodermin, II) exhibited highly cytotoxic activities. 4-β-Hydroxy-12,13-epoxytrichothec-9-ene (trichodermol, III) and 4-β-Methoxy-12,13-epoxytrichothec-9-ene (IV) had mild cytotoxicities. But 12,13-Epoxytrichothec-9-ene-4-one (V) and 4-α-Hydroxy-12,13-epoxytrichothec-9-ene(VI) had no cytotoxicities up to 10 μg/ml. And in the tested cell lines, HCT15 colon cancer cell line was the most sensitive to all tested trichothecenes.

Key Words : Trichothecenes, *Gliocladium, Trichothecium,* Cytotoxicity, Human tumor cell line

INTRODUCTION

Trichothecene mycotoxins are toxic secondary metabolites produced by various species of fungi such as Fusarium, *Trichoderma, Trichothecium, Myrothecium, Stachybotrys, Cepholosparium* and *Verticimonosporium* (Anderson *et al.*, 1989; Schoental *et al.*, 1985; Ueno, 1983). Structually, they are sesquiterpenoids usually containing a relatively unreactive epoxide moiety, and their biological activities depend mainly on the number, position and nature of the ester substituents, and the epoxide group on the nucleus (Anderson *et al.*, 1989; Bamburg, 1983; Mekhancha-Dahel *et al.*, 1991). These molecules have strong biological activities such as immunosuppressive properties (Mekhancha-Dahel *et al.*, 1991), and they also strongly inhibit protein and DNA synthesis (Mekhancha-Dahel *et al.*, 1991; Melmed *et al.*, 1985; Rosenstein and Lafarge-Frayssinet, 1983; Ueno *et al.*, 1973). Furthermore, their biological activities are very dependent on cell types, and especially, the digestive tract and lymphoid cells are very sensitive. The fact that the lymphoid organs are one of their preferential targets implies a serious immunosuppressive activity of these compounds (Forsell *et al.*, 1985; Pestka and Bondy, 1990; Tomer *et al.*, 1998; Visconti *et al.*, 1991). That is the reason why the trichothecenes could not be used for antitumor agents even though these compounds have strong cytotoxicities against tumor cells (Mekhancha-Dahel *et al.*, 1991). Therefore, the study of structure-activity relationships of trichothecenes is very important with regard to the possibility as a novel anticancer drug and the etiology of mycotoxicoses occurring in animals and human (Adler *et al.*, 1984; Anderson *et al.*, 1989; De Simone *et al.*, 1986; Porcher *et al.*, 1988; Porcher *et al.*, 1987). Actually, trichothecenes are still on the consideration or under investigations to be developed as an antitumor agent with all their implication of numerous human and animal diseases (Robbana-Barnat *et al.*, 1989; Schoental *et al.*, 1985; Ueno, 1986). And the structure-activity relationship studies of these analogues have also been reported from many laboratories (Anderson *et al.*, 1989; Chanh and Jewetson, 1991; Kaneto *et al.*, 1982; Mekhancha-Dahel *et al.*, 1991; Ramu *et al.*, 1989).

In this paper, we have reported additional structure-cytotoxic activity studies of naturally occurring trichothecenes from *Gliocladium virence* (sp. F1127) or *Trichothecium roseum* (sp. F1064) and several synthetic analogues against human solid tumor cell lines *in vitro*. And we also discussed the relationship

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

between the stereochemistry of C4 position and biological activities.

MATERIALS AND METHODS

General procedure

FT-IR spectra were recorded on a Bio-Rad Digible Division FTS-80 spectrophotometer, and the high-resolusion MS spectrum was measured on a VZ ZAB-7070 by the University of California at Riverside Mass Spectrometry Facility using CI or EI modes. NMR spectra were recorded in $CDCl_3$ on a Brucker AM 500 or 300 MHz.

Isolation of trichothecene analogoues (I, II, III)

The culture broth was filtered and separated into the mycelial cake and the culture filtrate. The filtrate was extracted with chloroform, and the extract was concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with a linear gradient 10 to 60% ethyl acetate/hexane. The crude products were purified further by pre-TLC with ethyl acetate/hexane (6 : 4). Physicochemical properties were summarized by Cole and Cox (1981).

4-β-Methoxy-12,13-epoxytrichothec-9-ene (IV)

4-β-Hydroxy-12,13-epoxytrichothec-9-ene (trichodermol, 60 mg, III) was treated with methyl iodide (excess amount) and sodium hydride (suspended in oil) in THF at 0°C for 1 hour. The reaction mixture was extracted with ethyl acetate, and the organic layer was dried with $MgSO_4$ and concentrated, and the residue was chromatographed to give 30 mg of IV as an oil. $[\alpha]_D$: -49.4 (c. 0.42), IR (film): 2970, 1060, 960 cm^{-1}, HRMS: [M+] 264.1714, calcd. 264.1725, $C_{16}H_{24}O_3$, ^{1}H-NMR: δ 5.35 (dd, 1H, J=6.8, 1.3 Hz), 3.86 (dd, 1H, J=7.0, 4.0 Hz), 3.75 (d, 1H, J=15.3 Hz), 3.48 (d, 1H, δ J=5.4 Hz), 3.31 (s, 3H), 3.08 (d, 1H, 4.1 Hz), 2.72 (d, 1H, J=4.1 Hz), 2.33 (dd, 1H, J=14.9, 7.1 Hz), 1.98 (m, 4H), 1.65 (s, 3H), 1.33 (m, 1H), 0.85 (s,3H), 0.72 (s,3H). ^{13}C-NMR: δ 140.03, 118.67, 82.03, 79.19, 70.37, 65.22, 57.02, 49.17, 47.65, 39.95, 36.32, 27.97, 24.13, 23.17, 15.98, 5.88.

12,13-Epoxytrichothec-9-ene-4-one (V)

To oxidation of 4-β-Hydroxy-12,13-epoxytrichothec-9-ene, 55 mg of III was dissolved in 30 ml of methylene chloride, and the solution was treated with an excess amount of MnO_2 at room temperature for 2 hours. The reaction solution was separated via filtration and the filtrate was concentrated. 12,13-Epoxytrichothec-9-ene-4-one (20 mg, V) was obtained after columm chromatography of the reaction mixture. $[\alpha]_D$: -29.8 (c. 0.45), IR (film): 2974, 1739, 1172, 1060, 960 cm^{-1}, HRMS: [M+] 248.1501, calcd. 248.1495, $C_{15}H_{20}O_3$, ^{1}H-NMR: δ 5.36 (dd, 1H, J=6.8, 1.3 Hz), 4.06 (d, 1H, J=5.2 Hz), 3.61 (d, 1H, J=5.4 Hz), 3.21 (d, 1H, δ J=4.1 Hz), 2.82 (d, 1H, J=4.1 Hz), 2..05 (m, 2H), 1.68 (s, 3H), 1.33 (m, 1H), 0.75 (s,3H), 0.74 (s, 3H). ^{13}C-NMR: δ 214.68, 140.31, 118.07, 75.81, 70.97, 65.02, 54.97, 49.77, 42.34, 41.66, 27.53, 23.45, 23.29, 15.19, 5.22.

4-α-Hydroxy-12,13-epoxytrichothec-9-ene (VI)

To a solution of 12,13-Epoxytrichothec-9-ene-4-one (50 mg, V) in THF (10 ml) containing about 0.5 ml of methanol was added sodium borohydride (5 mg) and the reactant was stirred at room temperature for 30 minutes. The mixture was partitioned between 100 ml of ethyl acetate and water. The organic layer was dried with $MgSO_4$ and concentrated, and the residue was chromatographed to give 35 mg of VI. $[\alpha]_D$: +65.7 (c. 0.52), IR (film): 2970, 1106, 1060, 960 cm^{-1}, HRMS: [M+] 250.1582, calcd. 250.1573, $C_{15}H_{22}O_3$, ^{1}H-NMR: δ 5.35 (dd, 1H, J=6.8, 1.3 Hz), 4.27 (dd, 1H, J=10.5, 5.2 Hz), 4.21 (d, 1H, J=6.1 Hz), 3.66 (d, 1H, δ J=5.4 Hz), 3.05 (d, 1H, 4.1 Hz), 2.77 (d, 1H, J=4.1 Hz), 2.53 (ddd, 1H, J=14.9, 10.5, 5.4 Hz), 1.98 (m, 4H), 1.65 (s, 3H), 1.33 (m, 1H), 0.85 (s, 3H), 0.72 (s, 3H). ^{13}C-NMR: δ 139.72, 119.45, 80.28, 78.02, 70.19, 66.15, 49.06, 47.05, 40.37, 39.42, 27.42, 25.43, 23.05, 15.72, 9.87.

Cells

Human tumor cells used in the experiment were A 549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF498 (central nerve system) and HCT15 (colon). Stock cell cultures were grown in T-25 (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 up the NCI (USA)'s protocol based on the Sulforhodamine B (SRB) method as described previously (Ryu *et al.*, 1992; Skehan *et al.*, 1990). Briefly, tumor cells were inoculated over a series of standard 96-well flat bottom microtiter plates on day 0. These cells were then preincubated on the microtiter plate for 24 hours.

The compounds were added to the wells in six 2-fold dilutions starting from the highest concentrations. The actual assay involved an incubation of the compounds for 48 hours with the cancer cells. At the termination of the incubation, 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.

The data were transferred and transformed into a 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

Chemistry

Compounds II (trichodermin, 4-β-Acetoxy-12,13-epoxytrichothec-9-ene) and III (trichodermol, 4-β-Hydroxy-12,13-epoxytrichothec-9-ene) were isolated from the fermentation broth of *Gliocladium virence* (sp. F1127), and compound I (trichothecin) was isolated from the cultures of *Trichothecium roseum* strain F1064 in liquid media. Analogues IV-V modified at C4 were prepared by the reactions shown in Fig. 1. New compounds IV and V were characterized by NMR experiments and mass spectrometry. NMR assignments were based on already reported data. The stereochemistry of protons at C4 was determined by NOE experiments. Trichodermol (III) was isolated from the fermentation broth or prepared by hydrolysis of trichodermin (I). Oxidation of C4 hydroxy group in III gave trichodermone (V), and reduction of V with sodium borohydride gave exclusively compound VI, 4-α-epimer of III. New trichothecene analogue IV was synthesized by methylation of C4 hydroxy group in III with methyl iodide and sodium hydride.

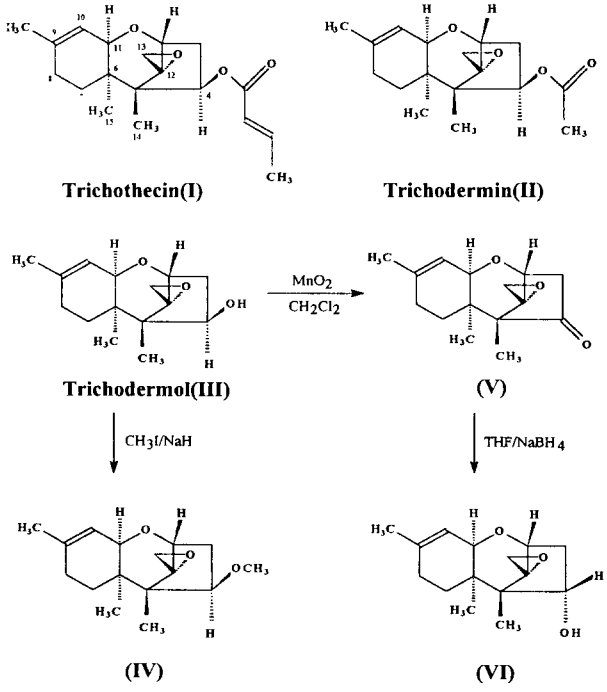

Fig. 1. Structures of some naturally occuring (I, II, III) and synthetic (IV, V, VI) trichothecenes.

Biological activities

We have tested cytotoxicity *in vitro* of several trichothecenes (compounds I-VI), which had the same structure except the moiety of C4 side chain. Trichothecin (I) and trichodermin (II), both compounds including the ester group in the C4 side chain, were highly cytotoxic ones among the tested trichothecenes. These two compounds revealed roughly the same cytotoxicity, which was 0.03~0.09 μg/ml of ED_{50} values (the concentration that caused 50% inhibition of cell growth) against different cancer cell lines, and these cytotoxicities were comparable with that of doxorubicin. Trichodermol (III) and 4-β-Methoxy-12,13-epoxytrichothec-9-ene (IV) were relatively less cytotoxic than the two ester compounds I and II. However they also had considerable cytotoxicities in comparison with cisplatin against colon cancer cell line HCT15, especially. Compound III was generally more cytotoxic than IV even though both of them showed about 5~10 times less biological activities to the human tumor cell lines than doxorubicin. The most interesting point was that both compounds V and VI showed no cytotoxicity up to 10 μg/ml in all the tested cell lines. The difference between compounds III and VI is only the stereochemistry of C4 position. Compound III has a β-hydroxyl group at C4 while the stereochemistry at C4 of compound VI is α-hydroxy, which means that compound VI is an epimer of 4-β-Hydroxy-12,13-epoxytrichethec-9-ene (III). The ED_{50} values of two standard drugs (cisplatin and doxorubicin) and tested trichothecenes (I~VI) were summarized in Table I, and the cytotoxicities of tested trichothecenes at each concentration were shown in Fig. 2. These results suggest that the moiety of C4 side chain of trichothecene was an important factor to its cytotoxicity, and these results were in agreement with that of the previous reports. In the experiment of cytotoxicity tests of T-2

Table I. Cytotoxicity of some trichothecences against human humor cell lines *in vitro*

COMPOUNDS	ED_{50} (μg/ml)[a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
CISPLATIN	1.24±0.11[b]	0.83±0.27	0.79±0.18	0.88±0.26	2.13±0.35
DOXORUBICIN	0.09±0.02	0.03±0.01	0.05±0.01	0.07±0.01	0.83±0.17
I	0.08±0.05	0.09±0.03[c]	0.07±0.04	0.06±0.04	0.04±0.02[d]
II	0.06±0.04[c]	0.05±0.03	0.07±0.03	0.07±0.04	0.02±0.02[d]
III	0.48±0.21[d]	0.63±0.16[d]	0.53±.019[d]	0.58±0.27[d]	0.23±0.11[d]
IV	0.95±0.27[d]	1.01±0.32[d]	0.83±0.25[d]	1.34±0.39[d]	0.66±0.30[c]
V	>10.0[d]	>10.0[d]	>10.0[d]	>10.0[d]	>10.0[d]
VI	>10.0[d]	>10.0[d]	>10.0[d]	>10.0[d]	>10.0[d]

[a]ED_{50} value of the compound against each cancer cell line, which is defined as the concentration that caused 50% inhibition of cell growth

[b]Data are Mean±S.E. of at least three distinct experiments.

[c]P<0.01, [d]P<0.001 compared to Doxorubicin by t-test

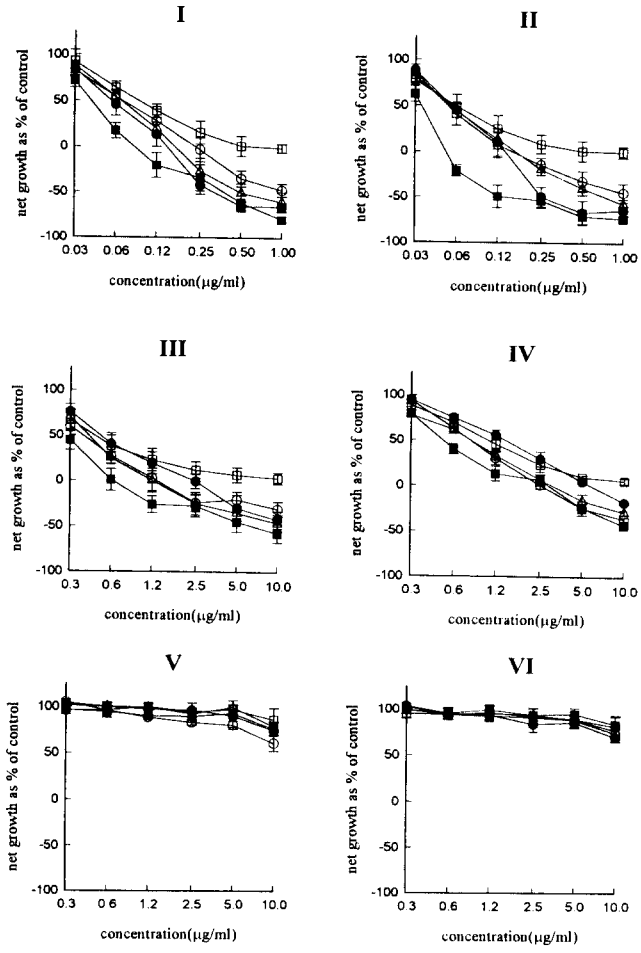

Fig. 2. Cytotoxicities of some trichothecenes against human tumor cell lines in vitro. Cells were incubated with each trichothecene for 48 hours and then compared its cytotoxicity to the cells which were incubated without drug. Each point represents the mean±S.E. of three experiments. Key: A 549(○), SK-OV-3(□), SK-MEL-2(△), XF498(●), HCT15(■)

toxin, one of the most potent naturally-occurring cytotoxic trichothecene, and its derivatives, Anderson *et al.* (1989) had reported that the cytotoxicity of trichothecenes was particularly susceptible to changes at C3, C4, C9 and C10 but was relatively unaffected by changes at C8. It was reported in another experiment in which had tested the analogues of anguidin, one of cytotoxic trichothecenes produced by Fusarium aguiseti, that the changes of C4 side chain had affected its cytotoxicity (Kaneto *et al.*, 1982). Therefore, our data showed similar results to those of other groups using the compounds which we had tested in these experiments. So far the importance of the stereochemistry at C4 of trichothecences which showed a dramatical change in the cytotoxicity against tumor cell lines was not evaluated extensively.

It was generelly accepted that cytotoxic compounds enter the cell for conducting its biological action *in vitro* and they penetrated the cell membrane by simple passive diffusion through solubilization in the lipid matrix of the membrane (John *et al.*, 1979). Therefore, the lipophilic properties of analogues were considered as the most important factor in determining its biological activity. In the previous report which investigated the influence of the 3-substituent on the cytotoxicity of the 6-aziridinylpyrrolo[1,2-α] benzimidazole quinones, the 6-acetamidopyrrolo[1,2-α]benzimidazole quinones and the 6-acetamidopyrrolo[1,2-α]benzimidazole iminoquinones, it was shown that increasing lipophilicity of the 3-substituent increased the cytotoxicity against melanoma cell lines (Schulz *et al.*, 1995). Manfredini *et al* (1994) also reported that the highest cytotostatic activity was associated with the highest global lipophilicity in the geiparvarin analogues against lymphoid tumor cell lines. However, we could not detect any correlation between the cytotoxicity and lipophilicity of trichothecenes in our experiment (We have compared the relative lipophilicity of tested trichothecenes by thin-layer chromatography: data not shown). Actually, there was a reverse correlation between the cytotoxicity and lipophilicity of compounds III (C4-OH) and IV (C4-OCH_3) because compound III was more active than IV against all of the human tumor cell

lines which were examined in this study. And also similar results were reported by Ramu *et al.* (1989), which said that the trichothecene analogues did not give the correlation between their hydrophobicity and anti-cancer activity against adriamycin-sensitive and adriamycin-resistant P388 mouse leukemia cell line. So it may be possible to suppose that trichothecene enter the cell via other mechanism instead of simple diffusion which was already reported.

So far cytotoxicities of trichithecene derivatives against cancer cells were observed by many laboratories, and they also reported the biological activities of these compounds at the level of macromolecule synthesis. However, these reports were mainly about lymphoid cells because many trichothecenes were preferentially active on lymphoid cells(Anderson *et al.*, 1989; Goetsch *et al.*, 1990; Mekhancha-Dahel *et al.*, 1991; Porcher *et al.*, 1988; Ramu *et al.*, 1989; Visconti *et al.*, 1991), and the data about solid tumor cells were relatively few. Therefore, we observed the cytotoxicity of trichothecenes against human solid tumor cell lines in this study. Among the tested cell lines, colon cancer cell line HCT15 was most sensitive to all of the compounds I~VI. It was very interesting because HCT15 cell line was most resistent to cisplatin and doxorubicin which are currently widely used in cancer therapy (Table I). And HCT15 was also relatively resistent to carboplatin, taxol and etoposide but was sensitive to vinblastin and podophyllotoxin among the tested cell lines (data not shown).

It is difficult to make generalized conclusion about the structure-activity relationship with these limited sizes of cell panels and analogues. However, it is the very interesting point that the biological activities of these compounds depend on the stereochemistry of C 4. In fact the relationship between cytotoxicities and the epoxide ring has already been reported from many research groups, but few reports that studied the relationship between the stereochemistry at C4 and biological activities of trichothecenes were found in the literature. In this study, we found that the trichothecene derivatives exhibited relatively higher cytotoxicity against HCT15 colon cancer cells than ones against other cells.

REFERENCES CITED

Adler, S. S., Lowenbraun, S., Birch, B., Jarrel, R. and Garraud, J., Anguidine, a broad phase II study of the Southeastern Cancer Study Group, *Cancer Treat. Rep.*, 68, 423-425 (1984).

Anderson, D. W., Black, R. M., Lee, C. G., Pottage, C., Richard, R. L., Sandford, M. S. and Webber, T. D., Structure-activity studies of trichothecenes: cytotoxicity of analogues and reaction products derived from T-2 toxin and neosolaniol. *J. Med. Chem.*, 32, 555-562 (1989).

Bamburg, J. R., Biological and biochemical actions of trichothecenes mycotoxins, In Hahn, F. E. (ed.), *Progress in molecular and subcellular biology* 8, Springer Verlag, Berlin, 1983, pp41-110.

Chanh, T. C. and Jewetson, J. F., Structure/function studies of T-2 mycotoxin with a monoclonal antibody. *Immunopharmacology*, 21, 83-89 (1991).

Cole, R. J. and Cox, R. H. (ed), *Handbook of Toxic Fungal Metabolite*. Academic Press, N.Y. 1981.

De Simone, P. A., Grew, F. A., Lessner, H. F. and Bartolucci, A., Phase II evaluation of anguidine (NSC 141537) in 5-day courses in colorectal adenocarcinoma. A Southeastern Cancer Study Group Trial, *Am. J. Clin. Oncol.*, 9, 187-188 (1986).

Forsell, J. H., Kateley, J. R., Yoshizawa, T. and Pestka, J. J., Inhibition of mitogen-induced blastogenesis in human lymphocytes by T-2 toxin and its metabolites. *Appl. Environ. Microbial.*, 43, 1523-1526 (1985).

Goetsch, L., Thomasset, N., Vila, J., Philip, I. and Dore J. F., Selective effect of trichotechlone on hemopoietic tumor cells. *Anticancer Res.*, 10, 1013-1018 (1990).

John, W. K., Joanne S., Robert K. G. and Alan C. S., Approaches to the acquisition of new anticancer drugs: Analog development. *Methods in Cancer Res.*, 16, 127-162 (1979).

Kaneto, T., Schmitz, H., Essery, J. M., Rose, W., Howell, H. G., O'Herron, F. A., Nachfolger, S., Huftalen, J., Bradner, W. T., Partyka, R. A., Doyle, T. W., Davies, J. and Cundliffe, E., Structural modifications of angudine and antitumor activities of its analogues. *J. Med. Chem.*, 27, 579-589 (1982).

Manfredini, P. G., Baraldi, R., Bazzanini, M., Guarneri, D., Simoni, J., Balzarini and E. De Clercq, Geiparvarin analogues. 4. synthesis and cytotoxic activity of geiparvarin analogues bearing a carbamate moiety or frrocoumarin fragment on the alkenyl side chain. *J. Med. Chem.*, 37, 2401-2405 (1994).

Mekhancha-Dahel, C., Lafarge-Frayssinet, C., Venuat, A. M., Rosenfeld, C. and Frayssinet, C., Trichothecenes as antitumoral substances: inhibiting effect on the growth human lymphocytes, lymphoma and leukaemia cells. *J. Cell. Pharmacol.*, 2, 343-350 (1991).

Melmed, R. N., Ishai-Michaeli, R. and Yagen, B., Differential inhibition by T-2 toxin of total proteins, DNA and isoprenoid synthesis in the culture macrophage cell line J774. *Biochem. Pharmacol.*, 34, 2809-2812 (1985).

Pestka, J. J. and Bondy, G. S., Alteration of immune function following dietary mycotoxin exposure. *Can. J. Physiol. Pharmacol.*, 68, 1009-1016 (1990).

Porcher, J. M., Lafarge-Frayssinet, C. and Frayssinet, C., Determination of cytotoxic trichothecenes in corn by cell culture toxicity assay. *J. Assoc. Off. Anal. Chem.*, 70, 844-849 (1987).

Porcher, J. M., Dahel, C., Fafarge-Frayssinet, C., Chu, F. S. and Frayssinet, C., Uptake and metabolism of T-2 toxin in relation to its cytotoxicity in lymphoid cells. *Food Chem. Toxicol.*, 26, 587-593 (1988).

Ramu, A., Yagen, B. and Ramu, N., The cytotoxicity of T-2 toxin and related 12,13-epoxytrichothecenes to adriamycin-sensitive and -resistant P388 leukemia cells. *Cancer Chemother. Pharmacol.*, 24, 264-267 (1985).

Robbana-Barnat, S., Lafarge-Frayssinet, C. and Frayssinet, C., Use of cell cultures for predicting the biological effects of mycotoxins. *Cell Biol. Toxicol.*, 5, 217-226 (1989).

Rosenstein, Y. and Lafarge-Frayssinet, C., Inhibitory effect of Fusarium T-2 toxin on lymphoid DNA and protein synthesis. *Toxicol. Appl. Pharmacol.*, 70, 283-288 (1983).

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).

Schoental, R., Trichothecenes, zerealenone and other carcinogenic metabolites in *Fusarium* and related microfungi, Academic Press, Florida, 1985.

Schu!z, W. G., Islam, I. and Skibo, E. B., Pyrrolo[1,2-α]benzimidazole-based quinones and iminoquinones. The role of the 3-substituent of cytotoxicity. *J. Med. Chem.*, 38, 109-118 (1995).

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

Tomer,R. S., Blakley, B. R. and De Coteau, W. E., In vitro effects of T-2 toxin on the mitogen responsiveness and antibody producing ability of human lymphocytes. *Toxicol. Lett.*, 40, 109-117 (1988).

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N. and Shimada, N., Inhibition of protein synthesis in animal cells. *J. Biochem.*, 74, 285-296 (1973).

Ueno, Y., Trichothecenes: chemical, biological and toxicological aspects, Elsevier, Amsterdam, 1983.

Ueno, Y., The toxicology of mycotoxins, CRC Press Inc., Florida, 1986.

Visconti, A., Minervini. F., Lucivero, G. and GAmbastesa, V., Cytotoxic and immunotoxic effects of Fusarium mycotoxins using a rapid colorimetric bioassay. *Mycophathologia*, 113, 181-186 (1991).