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Short Communication

In vitro Cytotoxicity of Sambutoxin

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***In vitro* cytotoxicity of sambutoxin was measured by using various human and murine tumor cells and IC_{50} values of sambutoxin ranged from 46.2 to 1,425.6 ng/ml.**

Key words : *cytotoxicity, sambutoxin,* Fusarium sambucinum.

In the course of our screening of toxic metabolites from *Fusarium* species, a new toxin, sambutoxin (Fig. 1) was purified from wheat cultures of *F. sambucinum* PZF-4 isolate.[1] The chemical structure of sambutoxin has been reported previously.[2] The toxin has caused some toxic effects in rats including body weight loss, hemorrhages in tissues, and death. The toxin was also toxic to chick embryos and its LD_{50} was 29.6 μg/egg. A potent cytotoxicity against various human and murine tumor cell lines, has been found through our study of the biological activities of sambutoxin.

In vitro cytotoxicity assay was carried out against 3 human leukemia cell lines (MOLT-4, CCRF-CEM, and K 562), 1 human lymphoma cell line (Daudi), 2 mouse leukemia cell lines (P388 and L1210), and 3 human carcinoma cell lines (KATO III, A549, and COLO320DM) by MTT (3-[4,5-dimethyl-2-yl]-2,5-diphenyl tetrazolium bromide) microculture tetrazolium assay.[3]

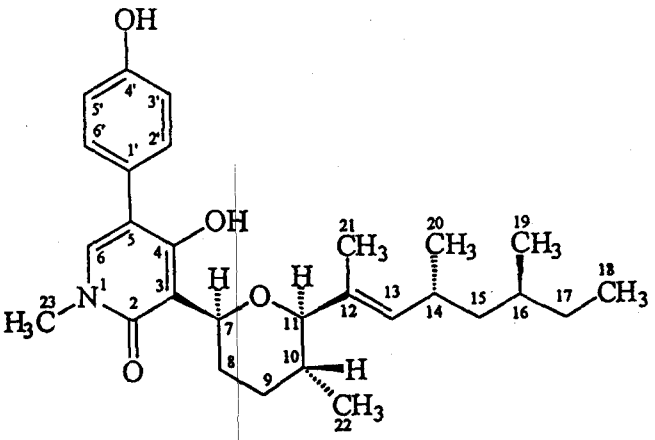

Fig. 1. Chemical structure of sambutoxin.

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Sambutoxin was first dissolved in 50% ethanol (1 mg/ml) and the solution was serially diluted with RPMI-1640 supplemented with 10% fetal bovine serum (FBS), penicillin-G (100 units/ml), and streptomycin (100 μg/ml), (RPMI-FBS). Doxorubicin was used as a positive control. A 0.1-ml of each tumor cell suspension (5×10^4 cells/ml) in RPMI-FBS and 0.1 ml of the test sample solutions in RFMI-FBS were prepared in 96-well flat-bottomed microplates and incubated at 37°C for 72 hr with 5% CO_2 under highly humid condition. Further a 4 hr-incubation was carried out for the incorporation of MTT into the cells.

IC_{50} values of sambutoxin and doxorubicin for 9 tumor cell lines are summarized in Table 1. Both compounds showed the cytotoxic effect in a dose-dependent manner within the concentration ranges tested in the individual experiment. Sambutoxin showed the potent cytotoxic effect against MOLT-4, CCRF-CEM, K562, P388, L1210, KATO III, and A549 cell lines with IC_{50} values below 200 ng/ml. Both Daudi and COLO320DM cell lines were relatively resistant to sambutoxin with IC_{50} values of 604.7 and 1425.6 ng/ml, respectively. Doxorubicin used as a positive control showed a higher cytotoxicity against haematopoetic cell lines than carcinoma cell lines irrespective of its origin; the IC_{50} values for haematopoetic cell lines were below 50 ng/ml, whereas those for carcinoma cells were above 200 ng/ml. Haematopoletic cell lines are generally more sensitive to topoisomerase II inhibitors namely doxorubicin than are the carcinoma cell lines.[4] However, sambutoxin did not fit into this trend; Daudi lymphoma cell line was much more resistant to sambutoxin than A549 and KATO III carcinoma cell lines and the IC_{50} values of sambutoxin for the two carcinoma cell

Table 1. *In vitro* cytotoxicities of sambutoxin and doxorubicin against various tumor cell lines.

Cell line	IC_{50} (ng/ml)[a] of	
	Sambutoxin	Doxorubicin
MOLT-4	120.1	26.8
CCRF-CEM	59.7	49.9
K562	56.0	12.4
KATO III	94.9	256.8
A549	174.3	209.9
COLO320DM	1,425.6	496.6
Daudi	604.7	11.5
P388	76.4	7.7
L1210	46.2	7.7

[a]Concentration causing 50% inhibition of cell growth

lines were similar to those of the haematopoetic cell lines except Daudi cell line. This result suggests that sambutoxin may have a different cytotoxic mechanism for tumor cells from the topoisomerase II inhibitors including doxorubicin and daunorubicin. The potent cytotoxicity of sambutoxin against various tumor cells *in vitro* warrants further studies on the mechanism of antitumor activity and the cytotoxicity against tumor cells *in vivo*. In addition, no antibiotic, phytotoxic, or mutagenic activities was shown by sambutoxin.

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