SAINTOPIN, A NEW ANTITUMOR ANTIBIOTIC WITH TOPOISOMERASE II DEPENDENT DNA CLEAVAGE ACTIVITY, FROM *PAECILOMYCES*

Sir:

DNA topoisomerases I and II are enzymes that alter DNA conformation through a concerted breaking and rejoining of DNA strands[1]. Recently topoisomerase II has been identified as the primary cellular target for a number of clinically important antitumor agents with diverse and unrelated chemical structures[2,3]. These antitumor drugs, termed "topoisomerase II poisons" have the common property of stabilizing the DNA-topoisomerase II complex which upon exposure to denaturing agents results in the induction of DNA cleavage[2,3]. Several lines of evidence indicate that the ability to induce topoisomerase II dependent DNA cleavage (TDC) is responsible for the antitumor activity of these drugs[2~4].

In order to identify a specific new topoisomerase II poison, we have screened cultures of actinomycetes and fungi for their ability to induce TDC *in vitro*. We found that flavonoids such as genistein and orobol are potent inducer of TDC[5], and have now isolated a novel compound with TDC activity, saintopin, from the culture broth of *Paecilomyces* sp.

The producing organism was isolated from a soil collected at a vineyard in Yamanashi Prefecture, Japan and was assigned to the genus, *Paecilomyces*. Fermentation was carried out at 25°C for 5 days under aeration and agitation in 30-liter jar fermenter containing 18 liters of a culture medium consisting of sucrose 5%, dry yeast 3%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05%, pH 7.0. Saintopin accumulated in mycelium but not in extracellular medium and so was extracted with MeOH from the mycelium after the filtration of culture broth. The extract was diluted with an equal volume of deionized water and then applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The column was washed with deionized water-MeOH (5:5) and eluted with MeOH. The active eluate was concentrated and applied to a column of Diaion HP-20SS. The column was washed with deionized water-MeOH (5:5) and the saintopin containing fraction was eluted with deionized water-MeOH (2:8). The eluate was concentrated and oily materials were removed by extraction with *n*-hexane at pH 7.0. The active constituent was extracted with EtOAc at pH 2.5 and then the organic layer was concentrated to dryness. Further purification was effected by two stages of silica gel chromatography using *n*-hexane-EtOAc-MeOH-AcOH (10:5:0.5:0.1) and CH_2Cl_2-MeOH (8:2). The active fractions were combined, concentrated and precipitated by the addition of deionized water at 4°C to yield 30 mg of saintopin.

The physico-chemical properties of saintopin are summarized in Table 1. Saintopin was obtained as a reddish purple powder and was readily soluble in DMSO but insoluble in H_2O and *n*-hexane. The molecular formula of saintopin was determined as $C_{18}H_{10}O_7$ by HREI-MS. The UV spectrum and the IR spectrum suggest the presence of a polyaromatic quinone ring structure. The structure of saintopin (Fig. 1) was assigned by ^1H and ^{13}C NMR spectroscopic studies which will be reported elsewhere[6].

The TDC activity of saintopin was studied *in vitro* using purified calf thymus topoisomerase II and plasmid DNA. Fig. 2 shows a photograph of agarose gel electrophoresis comparing the TDC activity of saintopin with other well known topoisomerase II poisons, *m*-AMSA and VP-16. At saintopin concentrations greater than 2.5 μM, the linear full

Table 1. Physico-chemical properties of saintopin.

Appearance	Reddish purple powder
Molecular formula	$C_{18}H_{10}O_7$
HREI-MS	Calcd: 338.0425
	Found: 338.0422
UV λ_{max} nm (ε)	249 (27,000), 277 (46,000), 313 (15,000), 354 (11,000), 529 (17,000)
IR (KBr) cm^{-1}	3400, 3250, 2930, 1615, 1600, 1580, 1400, 1370, 1330, 1275, 1160
Rf value[a]	0.50
Solubility:	
Easily soluble	DMSO
Soluble	MeOH, EtOH, acetone
Insoluble	*n*-Hexane, water

[a] Silica gel TLC (Merck 5715), *n*-hexane-EtOAc-MeOH-AcOH (5:5:0.5:0.1)

Fig. 1. Structure of saintopin.

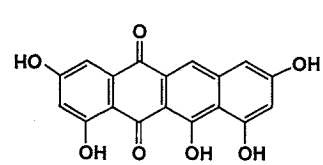
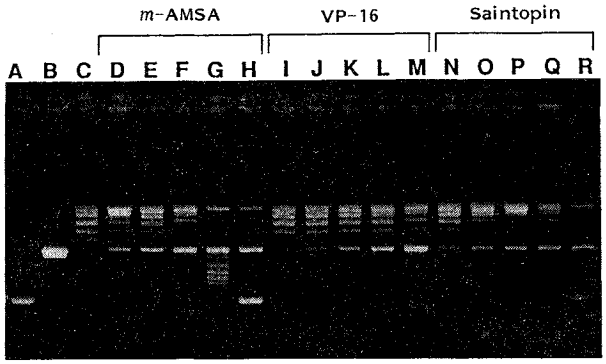


Fig. 2. Topoisomerase II dependent DNA cleavage activity of saintopin, *m*-AMSA and VP-16.



Supercoiled pBR322 DNA was incubated with calf thymus DNA topoisomerase II and drugs at 37°C for 60 minutes. Reactions were terminated by the addition of 2 μl of a solution containing 5% sodium dodecyl sulfate and 2.5 mg/ml proteinase K. Following an additional incubation for 60 minutes at 37°C, the samples were electrophoresed through an 1.2% agarose gel. (Lane A) supercoiled DNA (no enzyme, no drug); (lane B) linear DNA; (lane C) no drug; (lanes D~H) *m*-AMSA; (lanes I~M) VP-16; (lanes N~R) saintopin. Drug concentrations were 0.5 μM (lanes D, I and N), 2.5 μM (lanes E, J and O), 12.5 μM (lanes F, K and P), 50 μM (lanes G, L and Q) and 250 μM (lanes H, M and R).

Table 2. Antimicrobial spectrum of saintopin.

Organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	1.30
Enterococcus faecium ATCC 10541	0.65
Bacillus subtilis No. 10107	20.8
Klebsiella pneumoniae ATCC 10031	>100
Escherichia coli ATCC 26	>100
Pseudomonas aeruginosa Bin H No. 1	>100
Salmonella typhi ATCC 9992	>100
Proteus vulgaris ATCC 6897	>100
Shigella sonnei ATCC 9290	>100
Candida albicans ATCC 10231	>100

length DNA appeared as a result of DNA double strand cleavage. In the absence of topoisomerase II, saintopin did not induce any changes on the supercoiled structure of pBR322 DNA (data not shown), indicating that saintopin is a new compound with TDC activity.

Saintopin exhibits a weak antimicrobial activity against Gram-positive bacteria but not against both Gram-negative bacteria and fungi (Table 2). Saintopin shows cytotoxic activity against a human tumor cell line, HeLa S3 (IC_{50} 0.35 μg/ml) *in vitro*, and furthermore shows antitumor activity against murine leukemia P388 (ip) *in vivo*, exhibiting a statistically significant increase in life span (ILS 30%) at a dose of 25 mg/kg (ip).

Saintopin is the first example of a new topoisomerase II targeting drug which has been found from microbial cultures using mammalian topoisomerase II in mechanistically oriented screening. Detailed studies on the mechanism of action and antitumor activity of saintopin are in progress.

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