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Inhibitory activities against topoisomerase I and II by isoaurostatin derivatives and their structure–activity relationships

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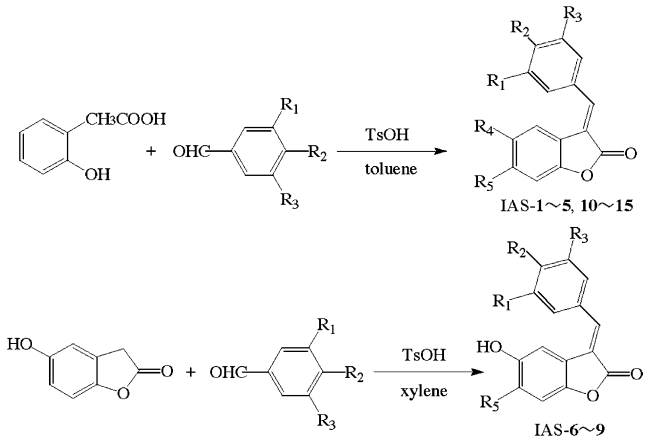
Abstract—Isoaurostatin A (IAS-A) isolated from *Thermomonospora alba* showed weak inhibition against topoisomerase (topo) I (IC_{50} = 307 μM). To get more strong inhibition, derivatives of IAS-A were prepared and their structure–activity relationships against topo I and II were investigated. The addition of hydroxyl group on aromatic rings increased the activities, 3-(3,4,5-trihydroxybenzylidene)-5-hydroxy-3*H*-benzofuran-2-one (IAS-**9**) showed strong inhibition (IC_{50} = 3 μM) against topo I. And also, the increasing of hydroxyl group increased growth inhibition against a variety of cancer cells, and IAS-**9** showed most potent inhibition. Unlike camptothecin and etoposide, IAS-**9** neither stabilized DNA–topo cleavable complex nor intercalated into DNA, and it inhibited topo I and II noncompetitively. The inhibitory activities also increased by opening of lactone ring in the molecule of IAS-**9**.

1. Introduction

DNA topoisomerases (topo) are a family of enzymes that are essential for a variety of DNA associated processes such as replication, recombination and transcription.[1] Topo are principal intercellular targets for a number of clinically important anticancer drugs.[2–7] Topo inhibitors like camptothecin,[8] etoposide[9] and doxorubicin[10] bind to the cleavable complex formed between topo and DNA, and keep it from going back to the original DNA. This action is associated with severe side effects as well as other anticancer drugs targeted at DNA.[11,12] Now drugs directly inhibiting topo are urgently being requested. We have previously reported isoaurostatin A (IAS-A) isolated from *Thermomonospora alba* directly inhibited topo I.[13] In this short communication, we tried to prepare the modified compounds in order to enhance topo inhibition, and discussed their structure–activity relationships.

2. Chemistry

Synthetic methods for the preparation of IAS-A derivatives (IAS-**1**–**15**) are summarized in Scheme 1. IAS-**1**–**5** and **10**–**15** are prepared by reaction of 2-hydroxyphenylacetic acid with various benzaldehyde in the presence of *p*-toluene sulfonic acid. IAS-**6**–**9** are prepared by



Scheme 1.

Keywords: Isoaurostatin; Topoisomerase; Anticancer; Structure–activity relationship.
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reaction of 5-hydroxybenzofurane-2(3*H*)-one with various benzaldehyde in existence of *p*-toluene sulfonic acid.

3. Inhibitory activity

Inhibition against relaxation activity of topo was measured by detecting the conversion of supercoiled pBR322 DNA to its relaxed form.[14,15] As shown in Table 1, IAS-**9**, which have four hydroxyl groups showed most potent topo I inhibitory activity with IC_{50} 3 μM, one hundred times stronger than IAS-A. Trihydroxyl derivatives (IAS-**5**, -**8**) indicated IC_{50} 4 and 6 μM, respectively. Dihydroxyl and monohydroxyl derivatives indicated weak inhibition. The benzoyl derivatives without hydroxyl group showed no activities. From these results, it is suggested that hydroxyl group is important for inhibitory activity. Additionally, the hydroxyl group on benzene ring is more related to inhibitory activity than hydroxyl group on benzofluoro ring.

4. Growth inhibition of various human cancer cells

Growth inhibition of IAS-A derivatives were investigated on a human cancer cell line panel (HCC panel) of Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, consisting of 39 cell lines as shown in Table 2.[16,17] As well as topo inhibition, growth inhibition increased with the increase of hydroxyl group. Especially, IAS-**9** showed potent growth inhibition against lung cancer cell. It is also similar to topo inhibition that hydroxyl group on benzene ring is more deeply related to the cell growth inhibition than hydroxyl group on benzofluoro ring.

5. Inhibitory property

Inhibitory properties of IAS-**9** were examined as the following four methods.

Table 1. Inhibition of topoisomerase I and II by IAS derivatives

IAS	R_1	R_2	R_3	R_4	R_5	Inhibition (IC_{50}, μM)	
						Topo I	Topo II
1	H	H	H	H	H	>1000	>400
2	OH	H	H	H	H	262	>400
3	H	OH	H	H	H	250	327
4	OH	OH	H	H	H	160	272
5	OH	OH	OH	H	H	4	63
6	H	H	H	OH	H	960	>400
7	H	OH	H	OH	H	555	>400
8	OH	OH	H	OH	H	6	240
9	OH	OH	OH	OH	H	3	120
10	H	OCH_3	H	H	H	>1000	>400
11	OH	OCH_3	H	H	H	800	>400
12	OCH_3	OH	H	H	H	630	351
13	H	NO_2	H	H	H	120	>400
14	H	F	H	H	H	392	>400
15	H	CH_2CO_2Et	H	H	H	>1000	>400
IAS-A	H	OH	H	H	OH	307	>400

Table 2. Growth inhibition of IAS-**4**, -**5**, -**8** and -**9** against various cancer cells

Cancer (cell)	Growth inhibition (GI_{50}, μM)			
	IAS-**4**	IAS-**5**	IAS-**8**	IAS-**9**
Breast				
HBC4	>100	45	24	75
HBC-5	>100	23	21	19
BSY-1	70	15	30	16
MCF-7	>100	26	29	20
MDA-MB-231	>100	16	34	18
Brain				
U251	>100	32	40	31
SF-268	68	66	28	30
SF-295	>100	20	27	20
SF-539	48	22	22	20
SNB-75	74	44	14	23
SNB-78	>100	35	21	21
Colon				
HCC2998	82	33	19	23
KM-12	>100	21	49	17
HT-29	>100	28	95	29
HCT-15	>100	61	25	67
HCT-116	>100	22	21	21
Lung				
NCI-H23	>100	41	70	14
NCI-H226	>100	9	46	9
NCI-H522	21	11	22	6
NCI-H460	>100	20	56	33
A549	>100	51	63	99
DMS273	68	19	22	19
DMS114	24	14	18	16
Melanoma				
LOX-IMVI	>100	21	66	21
Ovary				
OVCAR-3	80	23	29	12
OVCAR-4	>100	17	56	15
OVCAR-5	>100	20	>100	17
OVCAR-8	>100	19	62	23
SK-OV-3	>100	31	>100	38
Renal				
RXF-631L	>100	55	66	74
ACHN	59	>100	52	85
Stomach				
St-4	>100	19	>100	17
MKN1	99	17	20	25
MKN7	>100	17	38	27
MKN28	>100	40	55	29
MKN45	49	23	19	20
MKN74	>100	20	34	19
Prostate				
DU-145	>100	35	>100	29
PC-3	78	19	29	23

5.1. Inhibitory manner of IAS-9 against topo I and II

The type of inhibition was determined by Lineweaver–Burk plots[18] of substrate concentrations against the rate of relaxation of supercoiled pBR322 DNA by topo I and II in the presence or absence of IAS-**9**. IAS-**9** inhibited the relaxation activities of topo I and II noncompetitively with respect to pBR322 DNA exhibiting K_i values of 3.6 μM and 34.3 μM, respectively. The Michaelis con-

stants (K_m values) of topo I and II were 3.6 nM and 8.3 nM, respectively. In view of inhibitory potency (K_i/K_m) against DNA relaxation by topo I and II, IAS-**9** was 8-fold potent against topo I than topo II. From these results, IAS-**9** was considered to bind with a different site from the binding site of the substrate DNA in the enzyme molecule.

5.2. Stabilization of topo-cleavable complex by IAS-9

Topo inhibitors of the cleavable complex-forming type such as camptothecin and etoposide stabilize the cleavable complex (topo–DNA reaction intermediate) and inhibit the DNA rejoining reaction of topo, which is the inhibitory mechanism of the inhibitors, therefore the inhibitors induce nicked DNA in the cleavage assay.[8,19] To determine whether IAS-**9** is an inhibitor of the cleavable complex-forming type or not, cleavage assays were carried out. Camptothecin used as the controls of cleavable complex-forming inhibitors against topo I induced nicked DNA with increasing concentrations. Unlike camptothecin, IAS-**9** could not induce the nicked DNA even at 100 μM. This results suggests that IAS-**9** is an inhibitor of the cleavable-nonforming type. IAS-**9** may directly acts on topo I molecule in earlier step than the formation of the topo–DNA complex and inhibits the DNA breaking and rejoining reactions by the enzyme.

5.3. DNA interaction by IAS-9

Some topo inhibitors such as doxorubicin and amsacrine are DNA intercalators. To determine whether IAS-**9** has the ability to intercalate into DNA strands, CD (circular dichroism) spectral change of DNA by addition of IAS-**9** was measured, because the spectrum is sensitive to the conformation changes of DNA by intercalators.[20,21] The spectrum of DNA changed greatly with increasing concentrations of doxorubicin used as control of intercalator. On the other hand, the spectral changes by IAS-**9** did not occur, therefore, it is clear that IAS-**9** has no ability to intercalate into DNA. Thus, IAS-**9** is different from inhibitors causing DNA damage such as cleavable complex-forming inhibitors and DNA intercalators.

5.4. Effect of IAS-9 on the growth and cell cycle of HeLa cells

The cell growth inhibition of IAS-**9** was determined in HeLa cells by Alamar Blue assay.[22] The values of cell growth inhibition (GI_{50}) of IAS-**9**, camptothecin and etoposide were 24, 0.6 and 40 μM, respectively. Camptothecin and etoposide arrest the cell cycle progression. The cell cycle progression was analyzed with a flow cytometer (Becton Dickinson FACS Calibur) using the ModFit LT, which is a software to determine the percentage of cells in G0/G1, S and G2/M phases as shown in Figure 1.[23] HeLa cells were arrested at S phase and G2/M phase when cultured with 0.1 μM camptothecin and 2 μM etoposide, respectively. On the other hand, IAS-**9** showed weak arrest against the cell cycle. When 100 μM of IAS-**9** was added, it arrested G2/M phase from late S. The results suggest that the cytotoxicity of IAS-**9** is different from that of camptothecin and etoposide.

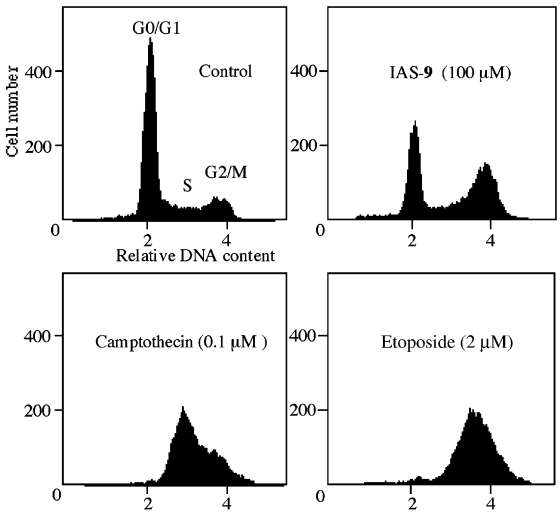

Figure 1. Effects of IAN-**9**, camptothecin and etoposide on cell cycle of HeLa cells.

6. Effect of pH on the structure and inhibitory activity of IAS-9

It has been reported that camptothecin is hydrolyzed in the blood, and converted to the carboxylate form (open ring). The carboxylate form is inactive with respect to topo inhibition.[24,25] IAS-**9** also has a lactone ring. Therefore, it was investigated whether carboxylate form of IAS-**9** inhibits topo I or not. The lactone and carboxylate forms of IAS-**9** were detected by HPLC. As shown in Figure 2, the lactone form decreases the rise of pH and the carboxylate form increases above pH 7. The inhibitory activity of IAS-**9** went up based on increasing of carboxylate form. The result suggest that IAS-**9** is activated by open ring contrary to camptothecin.

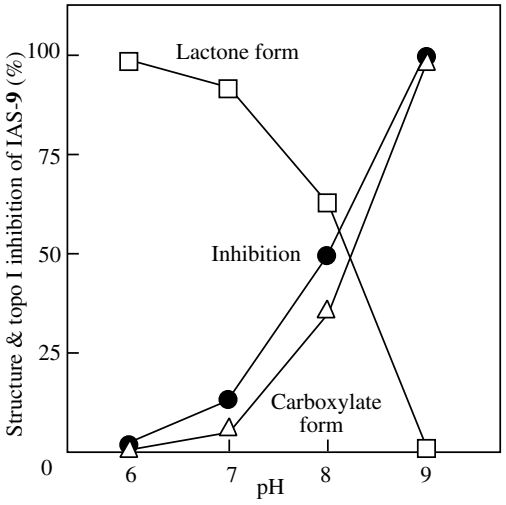

Figure 2. Effects of pH on the structure and topo I inhibition of IAS-**9**.

7. Materials and general experimental procedures

2-Hydroxyphenylacetic acid, 5-hydroxybenzofurane-2(3*H*)-one, and 3,4,5-tri-hydroxybenzaldehyde were obtained from Aldrich. Other aldehydes were purchased from Tokyo Kasei and Wako.

7.1. Preparation of IAS-1–5 and 10–15

A solution of 2-hydroxyphenylacetic acid (0.76 g, 5 mmol) and benzaldehyde (5 mmol) in toluene (15 mL) was refluxed for 3–4 h in the presence of a catalytic amount of *p*-toluenesulfonic acid. In the case of IAS-**1**–**4**, **10** and **12**–**15** toluene was removed under reduced pressure and the residue was recrystallized from toluene to give yellow powder in yields of 6.2–82.5%. The residue of IAS-**5** and -**11** was purified by column chromatography on silica gel with an eluent of CH_2Cl_2/MeOH (19:1) to give the product as a yellow powder in yields of 4.0% and 23.0%, respectively.

7.2. Preparation of IAS-6–9

A solution of 5-hydroxybenzofurane-2(3*H*)-one (0.30 g, 2 mmol) and benzaldehyde (2 mmol) in xylene (10 mL) was heated at 140 °C for 3–4 h in the presence of a catalytic amount of *p*-toluenesulfonic acid. After removal of xylene under reduced pressure, the residue was treated on silica gel column chromatography with an eluent of CH_2Cl_2/MeOH (19:1) to afford the product as a yellow powder in yields of 34.0–53.0%.

Structures of all compounds were confirmed on the basis of spectral data and elemental analyses.

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