ELSEVIER

Biochemical Pharmacology 64 (2002) 473–480

Biochemical Pharmacology

A novel stereo-selective sulfonylurea, 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one, has antitumor efficacy in *in vitro* and *in vivo* tumor models

Chang Woo Lee[a,1], Dong Ho Hong[a,1], Sang Bae Han[a], Sang-Hun Jung[b], Hyung Chin Kim[a], Robert L. Fine[c], Sang-Han Lee[a,1], Hwan Mook Kim[a,*]

[a]*Biopotency Evaluation Laboratory, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon 305-333, South Korea*
[b]*College of Pharmacy, Chungnam National University, Taejon 305-618, South Korea*
[c]*Division of Medical Oncology, Columbia University College of Physicians & Surgeons, New York, NY 10032, USA*

Received 13 July 2001; accepted 2 January 2002

Abstract

The antitumor activities of novel 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-ones were studied to determine the potential of these compounds as antitumor candidates. The agents studied were: DW2143 (1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one), a racemic mixture, and DW2282 [(4*S*)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one], an *S*-isomer. DW2143 and DW2282 suppressed the *in vitro* growth of tumor cells at lower concentrations than doxorubicin, but tumor specificity was not observed between the compounds. These compounds when administered orally were not active in syngeneic models of murine Colon 26 adenocarcinoma and L1210 leukemia. However, DW2143 suppressed the growth of SW620 (human colon cancer) and NCI-H23 (human lung cancer) cells in nude mice, inhibiting tumor growth by 87 and 67%, respectively. DW2282 was a more potent inhibitor of SW620 tumor cell growth in nude mice and was also lower in toxicity than DW2143. Moreover, DW2282 did not produce a series of toxic symptoms caused by the aniline metabolites of sulfonylureas, including hypoglycemia. These results suggest that DW2282, an *S*-isomer, could be a novel antitumor candidate with higher specificity and lower toxicity than other orally active sulfonylureas.

Keywords: Stereo-selective; Antitumor agents; *In vitro* and *in vivo* tumor models; Sulfonylureas

1. Introduction

Multiple derivatives of orally active diarylsulfonylureas have been synthesized and examined for their antitumor activities in syngeneic rodent and human tumor xenograft models [1]. These disulfonylureas showed a broad spectrum of antitumor activity against several solid tumor models including MDR cancer cell lines [2]. Some compounds, such as LY186641, LY295501, and sulofenur, have been studied in clinical trials [3]. However, the unpredicted onset of dose-limiting toxicities, such as methemoglobulinemia and hemolytic anemia, associated with its aniline metabolites, complicated the clinical study of some of these sulfonylureas, e.g. LY186641 [3]. To further explore other pharmacologically potent agents containing a sulfonylurea backbone, we and other groups synthesized and evaluated a series of novel 4-phenyl-1(*N*)-arylsulfonylimidazolidinones [4]. These compounds showed poor bioavailability and weak potency in animals. To improve bioavailability, we substituted the aryl group for a benzoylindoline group, and synthesized a novel 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one as a racemic mixture (DW2143) and as an *S*-isomer (DW2282) (Fig. 1). Stereo-specific modification of the structure increased the oral bioavailability up to 40% in mice (data not shown).

* Corresponding author. Tel.: +82-42-860-4660; fax: +82-42-860-4605.
E-mail address: hwanmook@mail.kribb.re.kr (H.M. Kim).
[1] Authors contributed equally to this work.
Abbreviations: TGI, tumor growth inhibition; ILS, increase of life span; GI, growth inhibition.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01105-X

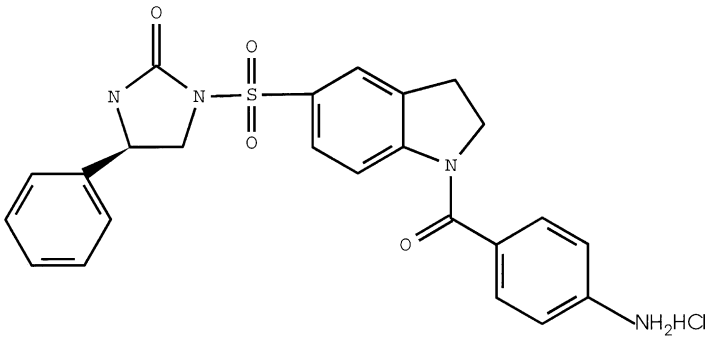

Fig. 1. Chemical structure of sulfonylureas. DW2143: 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one. DW2282: (4*S*)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one.

In the present paper, the antitumor effects of DW2143 and DW2282 were examined by determining their *in vitro* cytotoxicity and their *in vivo* inhibition of tumor growth in animal models. DW2143 and DW2282 suppressed the *in vitro* growth of various human cancer cells at lower concentrations than doxorubicin. Tumor growth was also suppressed by these compounds in human SW620 (colon) and NCI-H23 (lung) transplanted nude mice models.

2. Materials and methods

2.1. Materials

The structural formula of DW2822 [(4*S*)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one·hydrochloride] and DW2143 (its racemic mixture) is presented in Fig. 1. DW2143 and DW2282 were solubilized, and diluted in DMSO [for the *in vitro* cytotoxicity assay; final concentration was 0.1% (v/v) in the medium] and 1,2-propanediol [for the *in vivo* xenograft model; solubilizing solvent]. Doxorubicin was solubilized in 0.85% NaCl.

2.2. Mice and tumors

Specific pathogen-free female BALB/c-*nu/nu* mice (nude mice), used for all the human tumor xenograft experiments, were obtained from Charles River Japan, Inc. The mice used for all experiments had an average weight of 18–25 g and were 6–8 weeks old. Transplantable murine tumors were maintained in the mouse of origin and transferred to the appropriate F_1 hybrid for chemotherapy studies (DBA/2 and BALB/c × DBA/2 F_1 for L1210 murine leukemia and Colon 26 murine adenocarcinoma). These animals were obtained from Charles River Laboratories. The mice were housed in a pathogen-free barrier facility where ambient light was controlled automatically to produce 12-hr light and dark cycles. The L1210 leukemia (murine tumor; i.p. injection) and the Colon 26 adenocarcinoma (s.c. injection) were provided by the Cell Bank Facility, Korea Research Institute of Biotechnology (KRIBB). The human tumor cell lines NCI-H23 (lung carcinoma) and SW620 (colon adenocarcinoma) also were obtained from KRIBB. These human cell lines were grafted into nude mice (at concentrations of 6×10^6 cells/mouse, and 2×10^6 cells/mouse, respectively).

2.3. Cell lines

Twenty-seven human tumor cell lines, obtained from the National Cancer Institute (NCI), NIH, USA, were used: 4 lung (A549, NCI-H26, NCI-H23, and NCI-H522), 3 renal (UO-31, ACHN, and Caki-1), 6 colon (COLO205, HCT15, HT29, HCT116, KM12, and SW620), 3 gliomas (SF539, SNB19, and SNB75), 4 melanoma (UACC62, LOX-IMVI, M14, and SK-MEL-2), 3 breast (MCF-7, MCF-7/ADR, and MDA-MB-231), 2 leukemia (K562 and MOLT-4F), 1 ovary (SK-OV-3), and 1 prostate (PC-3) cell line(s). The cells were routinely maintained in a humidified CO_2 incubator (95% air and 5% CO_2) at 37° with RPMI 1640 containing 10% fetal bovine serum (FBS; Gibco BRL). All the cell lines were kept under liquid nitrogen until used and were passaged *in vitro* to maintain exponential tumor growth.

2.4. In vitro cytotoxicity assay

A sulforhodamine B (SRB) assay was performed as described previously [5]. In brief, cells were split into 96-well plates. After incubation, anchorage-dependent cells were directly fixed by the slow addition of 50 μL of 50% trichloroacetic acid (TCA) solution per well. Anchorage-independent cells were fixed by pre-centrifugation (150 *g*, 1 min at 20°) and the drop-wise addition of 50% TCA. Fixation proceeded for 1 hr at 4°. After fixation, plates were washed five times with tap water, and air-dried. One hundred microliters of SRB solution (0.4% in 1% acetic acid) was added to each well of the 96-well microplates. Staining was done at room temperature for 30 min. Residual dye was washed out with 1% acetic acid and air-dried. To each well, 100 μL of Tris solution (10 mM, pH 10.5) was added. Optical density (O.D.) was measured in a microtiter plate reader at 540 nm. Each drug concentration was tested in triplicate at least three times.

2.5. Evaluation of activity in murine tumor models

To evaluate the sensitivity of transplantable murine tumors to DW2143 and DW2282, DBA/2 mice were inoculated i.p. with L1210 leukemia cells (2×10^5/mouse). BALB/c × DBA/2F$_1$ (hereafter called CD2F$_1$) mice were implanted s.c. with Colon 26 adenocarcinoma cells (5×10^5/mouse). Mice were randomized into groups of six animals each for anti-cancer studies for leukemia and colon cancer. Colon 26 adenocarcinoma cells (5×10^5/mouse) were inoculated s.c. on day 0, and treatment was started when the tumor reached between 50 and 100 mm^3. Drugs were administered p.o. on days 6, 8, 10, and 12 after Colon 26 tumor transplantation. L1210 leukemia cells (2×10^5/mouse) were inoculated i.p. on day 0, and drugs were administered p.o. on day 1, 3, 5, 7, and 9. All experiments and antitumor testing were conducted according to NCI protocols [6]. For ascitic tumors, the percentage of ILS (increase in life span) was calculated as:

$$\text{ILS}\,(\%) = \left(\frac{\text{T}}{\text{C}} - 1\right) \times 100$$

where T and C indicate the survival days of treated and control mice, respectively. The criteria for effectiveness was denoted as an ILS value of 25% or more; statistical significance was determined by the log-rank test ($P < 0.05$).

For murine solid tumors, the percentage of T/C was calculated as:

$$\frac{\text{T}}{\text{C}}\,(\%) = \left(\frac{\text{mean}\,(V_{\text{treated}}/V_{\text{o}})}{\text{mean}\,(V_{\text{control}}/V_{\text{o}})}\right) \times 100$$

The relative tumor volume was expressed as the V/V_{o} index, where V is the tumor volume on the last day of measurement, and V_{o} is the initial volume of the same tumor on the day the treatment was started. In the case of the murine solid tumors, the relative tumor volume was expressed as the $\text{T}_{\text{RV}}/\text{C}_{\text{RV}}$ index.

$$\frac{\text{T}_{\text{RV}}}{\text{C}_{\text{RV}}}\,(\%) = \left(\frac{\text{Relative tumor volume of treated group}}{\text{Relative tumor volume of control group}}\right) \times 100$$

where RV is the mean tumor volume at day 18 versus the mean tumor volume at day 6.

The criteria for effectiveness against murine solid tumors were denoted as a T/C value of 42% or less, and statistical significance was determined by Student's *t*-test.

2.6. Evaluation of activity in human tumor models

To evaluate the sensitivity of human tumor xenografts to DW2143 and doxorubicin, NCI-H23 cells (6×10^6/mouse) and SW620 cells (2×10^6/mouse) were injected s.c. into the right flank of the nude mice. DW2143 was administered p.o. on days 1, 3, 5, 7, 9, and 11 after tumor cell transplantation. Doxorubicin was administered i.p. every day as 1 mg/kg on days 1–11, 2 mg/kg on days 12–14, and 3 mg/kg on days 15–18. Tumor volume was measured every 11–19 days. The compounds did not produce skin ulcers.

To evaluate the effects of DW2282 and doxorubicin, SW620 cells (2×10^6/mouse) were injected s.c. at day 0 into the right flank of the nude mice. Drug treatment was initiated on day 1 after tumor cell transplantation. Tumor volumes were measured on days 9, 10, 12, 14, 16, 17, and 18. DW2282 was administered by the p.o. route on days 1, 3, 5, 7, 9, and 11, and doxorubicin was administered by the i.p. route on days 1, 3, 5, 7, and 9. In the other model (one model was the initiation model, the other was the regression model), treatment was started when the tumor reached a volume of approximately 50–100 mm^3 (day 0). DW2282 was administered p.o. on days 0, 2, 4, 6, 8, and 10, and doxorubicin was administered by the i.p. route on days 0, 2, 4, and 6. Tumor volume was measured on days 0, 1, 3, 5, 7, 9, and 15.

Tumor volume was estimated using two-dimensional caliper measurements and the formula for an ellipsoid [7]:

$$\text{Tumor volume} = \frac{\text{L} \times \text{W}^2}{2}$$

where L is the major axis and W is the width of the tumor.

To examine whether or not the compounds inhibited tumor growth, mice were weighed and euthanized at day 15 or day 18 after the initiation of chemotherapy. On the final day, tumors were excised and weighed. Mean tumor weights were determined, and used to calculate the TGI expressed as a percentage:

$$\%\,\text{TGI} = 1 - \left(\frac{\text{mean final tumor weight}_{\text{treated}}}{\text{mean final tumor weight}_{\text{control}}}\right) \times 100$$

Moderate activity was defined as a TGI of 58–89%, and >90% TGI as significant activity [8]. Tumor growth inhibition was analyzed for statistical significance using Student's *t*-test.

3. Results

3.1. Inhibitory effects of DW2143 and DW2282 on in vitro growth of tumor cells

Table 1 shows the cytotoxic potential (GI_{50}) of DW2143 and DW2282 against 27 human tumor cell lines tested, i.e. 4 lung, 3 renal, 6 colon, 3 brain, 4 skin, 3 breast, 2 leukemia, 1 ovarian, and 1 prostate cancer cell line(s). DW2143 inhibited the *in vitro* growth of human cancer cells at low concentrations. Among them, KM12 (a colon cancer cell line) was the most sensitive and SNB75 (a brain cancer cell line) was the most resistant to DW2143. However, organ specificity was not observed. The mean GI_{50} was calculated to be 0.133 μg/mL. Similar to DW2143, DW2282 also exhibited *in vitro* growth inhibition, also with no organ

Table 1
Cytotoxicities of DW2l43, DW2282. and doxorubicin against a panel of human tumor cell lines

Cell type	Cell line	GI_{50} (μg/mL)			
		DW2143	Doxorubicin	DW2282	Doxorubicin
Lung	A549	0.12	0.44	0.13	0.22
	NCI-H26	0.13	0.36	0.56	0.70
	NCI-H23	0.08	0.17	0.04	0.16
	NCI-H522	0.09	0.36	0.04	0.27
Renal	UO-31	0.37	0.20	0.22	0.88
	ACHN	0.09	0.31	0.01	0.18
	Caki-1	0.83	0.49	0.46	0.15
Colon	COLO205	0.09	0.19	0.04	0.45
	HCT15	0.14	0.84	0.09	2.35
	HT29	0.11	0.34	0.06	0.49
	HCT116	0.10	0.17	0.05	0.26
	KM12	<0.03	0.30	0.02	0.30
	SW620	0.08	0.14	0.04	0.10
Gliomas	SF539	0.16	0.07	0.05	0.37
	SNB19	0.17	0.14	0.06	0.13
	SNB75	>3.00	0.16	>1.00	0.08
Melanoma	UACC62	0.04	0.11	0.05	0.15
	LOX-IMV1	0.13	0.25	0.07	0.13
	M14	0.09	0.06	0.05	0.08
	SK-MEL-2	0.11	0.41	0.03	0.19
Breast	MCF-7	0.06	0.15	0.03	0.13
	MCF-7/ADR	0.04	0.97	<0.01	0.06
	MDA-MB-231	0.11	0.27	0.07	0.33
Leukemia	K562	0.11	0.42	0.03	0.28
	MOLT-4F	0.11	0.14	0.09	0.21
Ovary	SK-OV-3	0.09	0.13	0.02	0.13
Prostate	PC-3	0.05	0.47	0.06	0.39

The present data are from one of two independent experiments. Growth inhibition of 50% (GI_{50}) is calculated from $100 \times [(T - T_0)/(C - T_0)] = 50$, which is the drug concentration causing a 50% reduction in the net protein increase in control cells during the drug concentration (T: treatment group; T_0: time zero group; and C: control group).

specificity. DW2282 showed a higher potency than DW2143 on an equimolar basis in *in vitro* cytotoxicity. The mean GI_{50} of DW2282 was 0.095 μg/mL. The activity of both compounds was slightly higher than that of doxorubicin, which was utilized as a positive control. Among the tested human cancer cell lines, we selected NCI-H23 and SW620 as cell lines to be transplanted into the nude mice.

3.2. Antitumor activities in the murine tumor models

The *in vivo* dose of DW2143 was selected from prior acute toxicity studies. A single dose of 80 and 100 mg/kg to female Balb/c mice produced a 33% death rate, but 65 mg/kg did not cause death even after 5 injections (once/2 days). In the case of DW2282, treatment at 50 mg/kg for 5 days (once/day) did not cause death or loss of body weight. Therefore, we chose to use compound doses of 50–65 mg/kg in the following animal studies. The compounds were first tested in murine solid and ascitic tumor models. Table 2 shows the results of a murine solid tumor model. Against Colon 26 adenocarcinoma cells, DW2143 and DW2282 produced 70.65 and 80.7% T_{RV}/C_{RV} (the ratio of tumor volume), respectively. Because these values were higher than 42% of T/C, we decided that both compounds did not elicit any moderate or significant activity in this model. In the murine ascitic model with L1210 leukemia

Table 2
Antitumor activity of DW2143 and DW2282 against murine Colon 26 adenocarcinoma

Drug	Dose (mg/kg)	Relative tumor volume (RV)[a]	T_{RV}/C_{RV} (%)[b]
Vehicle only		12.54	
DW2143	50	8.86	70.6[c]
DW2282	50	10.12	80.70

Colon 26 cells (5×10^5/mouse) were inoculated s.c. on day 0. When estimated tumor volume reached about 50 mm^3, mice were treated p.o. with DW2143 and with DW2282 on days 6, 8, 10, and 12 after tumor transplantation.

[a] RV = mean tumor volume at day 18/mean tumor volume at day 6.

[b] T_{RV}/C_{RV} = relative tumor volume of the treated group/relative tumor volume of the control group.

[c] T/C ≤ 42% represented moderate activity, and T/C ≤ 10% represented significant activity.

Table 3
Antitumor activity of DW2143 and DW2282 against murine ascitic L1210 leukemia

Drug	Dose (mg/kg)	Mean survival (days)[a]	ILS (%)[b]
Vehicle only		8.5 ± 0.3	
DW2143	50	10.2 ± 0.3	19.7[c]
DW2282	50	10.2 ± 0.2	19.7

L1210 cells (2×10^5/mouse) were inoculated i.p. on day 0. Drugs were administered p.o. on days 1, 3, 5, 7, and 9.

[a] Values are mean survival days ± SD, N = 7.

[b] Maximum increase in life span, [(treated group/control group) − 1] ×100.

[c] The criterion for effectiveness was an ILS value of 25% or more.

cells, oral treatment with both compounds increased the mean survival time, and the ILS was 19.7% in both cases (Table 3). However, these activities against mouse leukemia were also judged not to be promising, because the ILS values were lower than 25%. DW2282 was also examined in the B16 melanoma model; the activity was found to be similar to that in the L1210 study (data not shown). These results indicate that the murine tumor models may not be the most appropriate model to test for cytotoxicity-mediated inhibition of tumor growth by these diarylsulfonylureas (Tables 2 and 3).

3.3. Antitumor activities in human tumor xenograft models

To evaluate the antitumor activity of DW2143 against human tumor xenografts, two human tumor cell lines were transplanted into nude mice. After s.c. implantation of NCI-H23 lung cancer or SW620 colon cancer cells into the right flank of the nude mice, DW2143 (65 mg/kg/day) was administered via the oral route on days 1, 3, 5, 7, 9, and 11. The treatment induced a loss of body weight (about 20% of that of the untreated group) in the tested animals, but the body weight recovered and reached control levels shortly after the end of drug treatment (Fig. 2C and D). The formation and growth of solid tumors were greatly inhibited (Fig. 2A and B). DW2143 induced significant inhibition of NCI-H23 tumor growth by day 11 ($P < 0.01$), whereas doxorubicin did not affect tumor growth until day 16. At day 19 after the initiation of chemotherapy, tumors were excised and their weights were measured. In NCI-H23 lung cancer and SW620 colon cancer, DW2143 produced 67 and 87% TGI, while doxorubicin produced 39 and 49% TGI, respectively (Table 4). Because the treatment dose, route of administration, and treatment schedule of DW2143 were different from that of doxorubicin, a direct comparison of growth-inhibiting potential is not possible.

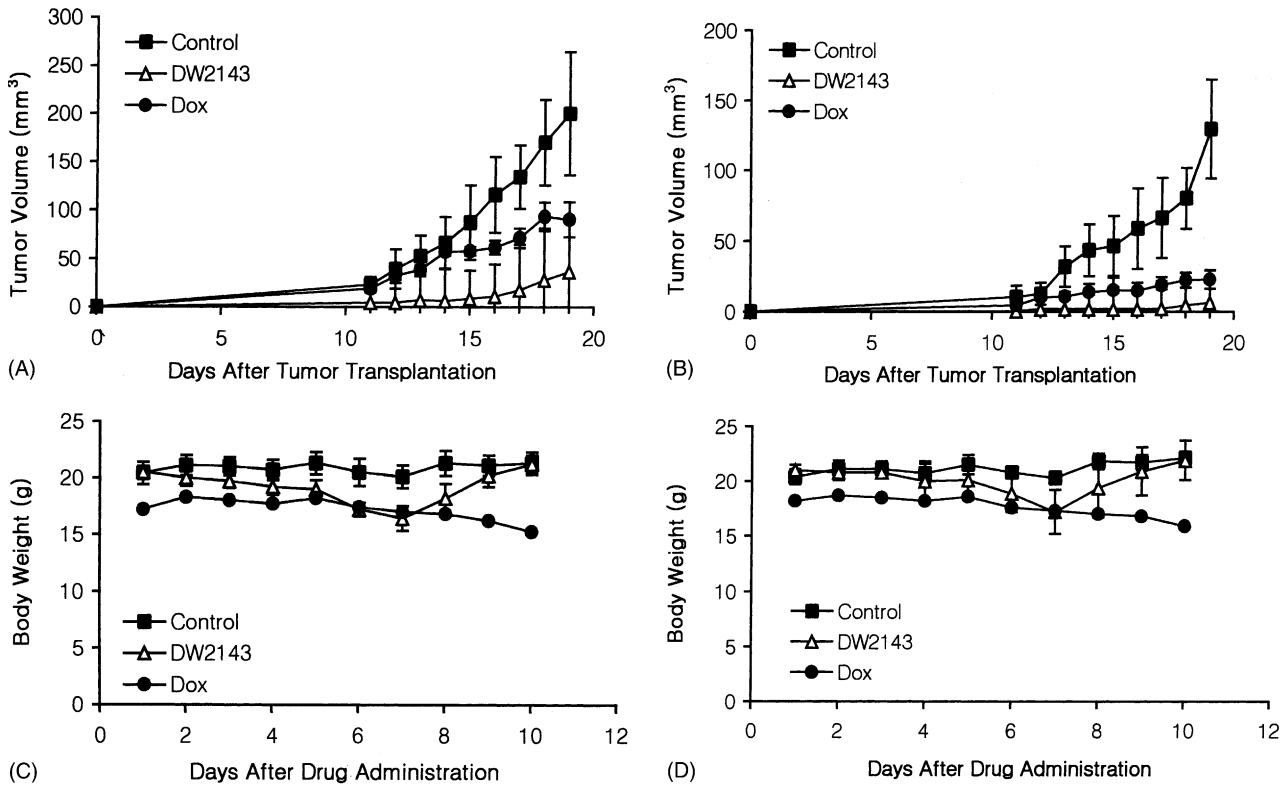

Fig. 2. Growth curve of human xenografts treated with DW2143 and doxorubicin. Human NCI-H23 lung (A) and SW620 colon (B) cancer cells were implanted s.c. on day 0. Body weight changes of mice transplanted with NCI-H23 (C) and SW620 (D) cells are also shown. Body weight was serially measured during the experimental period at every 11–19 days. DW2143 was administered p.o. on days 1, 3, 5, 7, 9, and 11 after tumor transplantation into nude mice. Tumor volume was measured every 11–19 days. Vehicle (propylene glycol, ■); DW2143 (65 mg/kg, △); doxorubicin (1, 2, and 3 mg/kg, ●). Values denote mean tumor volume ± SD, N = 9 (A and B), and mean body weight ± SD, N = 9 (C and D).

Table 4
Antitumor activity of DW2143 and doxorubicin against human tumor xenografts

Tumor[a]	DW2143				Doxorubicin			
	Schedule (days)	Dose (mg/kg)	TGI (%)[b]	Body weight (g)[c]	Schedule (days)	Dose (mg/kg)	TGI (%)	Body weight (g)
NCI-H23	1, 3, 5, 7, 9, 11	65	67[d]	0.6	1–11 12–14 15–18	1 2 3	39	−2.6
SW620	1, 3, 5, 7, 9, 11	65	87[d]	1	1–11 12–14 15–18	1 2 3	49	−2.6

At day 0, tumors were injected s.c. into the right flank of nude mice. DW2143 was administered p.o. on days 1, 3, 5, 7, 9, and 11 after tumor cell transplantation, and doxorubicin was administered i.p. every day as 1 mg/kg on days 1–11, 2 mg/kg on days 12–14, and 3 mg/kg on days 15–18. Mice were weighed and euthanized at day 19, and tumors were excised and weighed.

[a] NCI-H23 (6×10^6 cells/mouse) and SW620 (2×10^6 cells/mouse) were implanted s.c. on day 0.

[b] %TGI = 1 − (mean final tumor weight of the treated group/mean final tumor weight of the control group) × 100.

[c] Difference in body weight (g) between days 1 and 19.

[d] Moderate activity and significant activity were defined as TGI of 58–89 and ≥90%, respectively.

The antitumor activity of DW2282 against NCI-H23 lung cancer and SW620 colon cancer cells was studied using regression models (Fig. 3). NCI-H23 cells were injected s.c. into the right flank of the nude mice, and drug treatment was started when the tumor reached a volume of between 50 and 100 mm^3. DW2282 did not cause a loss of body weight during the experimental period (Fig. 3C). Tumor growth was arrested by DW2282 in a dose-dependent manner, and statistical significance was recorded at 30 and 65 mg/kg from the beginning of the measurement of tumor size (Fig. 3A). At day 19 after tumor cell transplantation, tumors were excised and

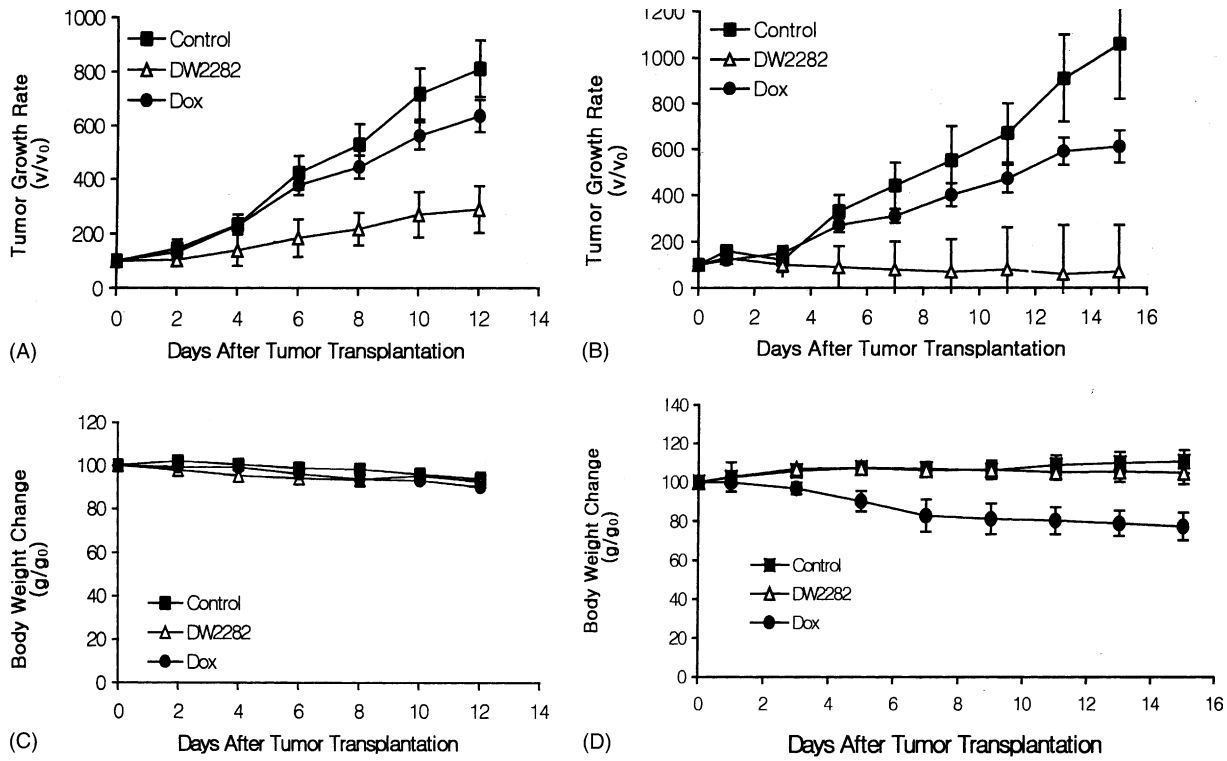

Fig. 3. Efficacy of DW2282 against human tumor xenografts. On day 0, tumor cells were injected s.c. into the right flank of nude mice. On day 19 after the initiation of chemotherapy, mice were weighed and euthanized, and tumors were excised and weighed. Human tumor xenografts were treated with DW2282 and doxorubicin. Human NCI-H23 lung (A) and SW620 colon (B) cancer cells were implanted s.c. on day 0. Body weight changes of mice transplanted with NCI-H23 (C) and SW620 (D) cells were measured at every 11–19 days. Body weight was serially measured during the experimental period. DW2282 was administered p.o. on days 1, 3, 5, 7, 9, and 11 after tumor transplantation into nude mice. Tumor volume was measured every 11–19 days. Vehicle (propylene glycol, ■); DW2282 (65 mg/kg, △); doxorubicin (1, 2, and 3 mg/kg, ●). Values denote mean tumor growth rate ± SD, N = 9 (A and B), and mean body weight change ± SD, N = 9 (C and D).

Table 5
Antitumor activity of DW2282 and doxorubicin in the NCI-H23 and SW620 xenograft line

Tumor[a]	DW2143				Doxorubicin			
	Schedule (days)	Dose (mg/kg)	TGI[b] (%)	Body weight (g)	Schedule (days)	Dose (mg/kg)	TGI (%)	Body weight (g)
NCI-H23[c]	1, 3, 5, 7, 9, 11	65	97[d]	2.4[e]	1, 3, 5, 7, 9	3	73	−4.9[e]
	1, 3, 5, 7, 9, 11	30	58[d]	1.3[e]				
SW620[f]	0, 2, 4, 6, 8, 10	65	87[d]	1[g]	0, 2, 4, 6	3	17	−4.4[g]
	0, 2, 4, 6, 8, 10	30	31[d]	1.2[g]		2		

[a] SW620 cells (2×10^6/mouse) and NCI-H23 cells (6×10^6/mouse) were implanted s.c. on day 0.
[b] %TGI = 1 − (mean final tumor weight, treated group/mean final tumor weight, control group) × 100.
[c] Nude mice were grafted s.c. with the tumor, and treatment was started when the tumor reached 50–100 mm^3 (day 0). DW2282 was administered by the p.o. route at days 1, 3, 5, 7, 9, and 11. Doxorubicin was administered by the i.p. route at days 1, 3, 5, 7, and 9. Mice were weighed and euthanized at day 18, and tumors were excised and weighed.
[d] Moderate activity and significant activity were defined as TGI of 58–89 and ≥90%, respectively.
[e] Difference in body weight (g) between days 1 and 18.
[f] Difference in body weight (g) between days 1 and 15.
[g] Nude mice were grafted s.c. with the tumor, and treatment was started when the tumor reached 50–100 mm^3 (day 0). DW2282 was administered by the p.o. route at days 0, 2, 4, 6, 8, and 10. Doxorubicin was administered by the i.p. route at days 0, 2, 4, and 6. Mice were weighed and euthanized at day 15, and tumors were excised and weighed.

weighed (Table 5). In 30 and 65 mg/kg/day dose groups, DW2282 produced 58 and 97% TGI, respectively, in NCI-H23 cells. Of special note, when administered at 65 mg/kg/day, complete tumor growth inhibition occurred in 5 of 7 animals. Tumor growth inhibition by doxorubicin was 73%. In SW620 cells, treatment began when the tumor size reached approximately 50–100 mm^3. As shown in Fig. 3D, body weight was not affected by DW2282, but doxorubicin-treated animals lost body weight starting early after treatment. DW2282 at 30 mg/kg inhibited tumor growth, but this dose did not reduce the tumor size. At 65 mg/kg of DW2282, regression was observed from day 3 after initiation of chemotherapy (Fig. 3B). Tumor weights on day 15, taken from animals treated with 30 and 65 mg/kg of DW2282, were 31 and 87% that of the control group, respectively (Table 5).

4. Discussion

The racemic mixture (DW2143) and *S*-isomer (DW2282) of a novel 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one were examined for their pharmacological antitumor activities. DW2282 showed higher potency as an antitumor drug than did DW2143. By removing the *R*-isomer from the racemic mixture, the GI_{50}, as measured by the *in vitro* cytotoxicity assay, was lowered from 0.133–0.095 mg/mL. The results indicate that the *S*-isomer of the racemic mixture is more active than the *R*-isomer in inhibiting the *in vitro* growth of cancer cells, but not by very much as both were effective. In nude mice experiments, stable body weights were maintained in animals treated with DW2282. During the treatment period, DW2143 caused a loss of body weight (about 20% of that of the untreated group) that was a marker of its *in vivo* toxicity. Even though the same dose and schedule were applied, DW2282 did not cause a change in body weight. From these results, it can be assumed that the *R*-isomer, but not the *S*-isomer is responsible for the *in vivo* toxicity of DW2143. The efficacy of DW2282 in the inhibition of tumor formation and growth was also superior to that of DW2143. In initiation models of human tumor xenografts, the activity of DW2282 was 97% TGI and that of DW2143 was 87% TGI. Seventy-one percent of cancer cell-inoculated animals did not develop any palpable tumor mass after six injections of DW2282. Most cytotoxic antitumor drugs are known to cause toxicity to both tumors and host. This nonselective action of the drug limits the therapeutic dose and causes a deterioration in the quality of the life of the patient. Tumor selectivity is another beneficial aspect of DW2282.

It has been reported that DW2143 and DW2282 may not cause methemoglobinemia and hemolytic anemia, toxicities associated with aniline metabolites of sulfonylureas [9]. Hemolysis did not occur until 48 hr after direct exposure of human blood to DW2282 (0.4–10 mg/mL). Injection of DW2282 (50–150 mg/kg) in mice also did not elevate the level of methemoglobulin in serum [9]. The hypogylcemic action of sulfonylureas was not observed in DW2282-treated animals (data not shown). These results showed that the metabolic fate of DW2282 was different from that of other sulfonylureas, and the problem of toxicity-related *in vivo* metabolism was not observed. It has been reported that DW2282 induces apoptosis in A549 cells and fragmented DNA was detected in K562 cells, demonstrating that apoptosis is one of the mechanisms by which DW2282 inhibits the proliferation of A549 and K562 cells [10]. Another antitumor diarylsulfonylurea (DSU) showed cytotoxicity in GC3/c1 human colon adenocarcinoma cells, causing the formation of nucleosomal DNA ladders [11,12]. Along with other sulfonylureas, DW2282 could be administered orally, and oral treatment

was effective in both tumor initiation and regression models. The possibility of treating cancer patients with an oral-dosing formulation is another benefit for DW2282, although the precise mode of action of DW2282 remains to be elucidated. An effective change of dosing schedule might make it possible to maximize the therapeutic outcome in diverse patient cases. DW2282 was applied only to colon cancer models for pre-clinical evaluation. However, it showed potential as a therapeutic drug candidate due to the low toxicity during repeated administration.

In conclusion, DW2143, the racemic mixture, and DW2282, the *S*-isomer, of a novel 1-[1-(4-aminobenzoyl)-2,3-dihydro-1*H*-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one were examined for antitumor activities. The results suggest that the *S*-isomer is the most active form for inhibition of *in vitro* growth of cancer cells. Using the same dose and schedule in a human tumor xenograft nude mouse model, DW2143 caused a loss of body weight but DW2282 did not. The efficacy of DW2282 for inhibition of tumor formation and growth was also superior to that of DW2143. In initiation models of human tumor xenografts, the tumor growth inhibitory activity of DW2282 and DW2143 was 97 and 87%, respectively. In conclusion, our data suggest that DW2282 is a novel sulfonylurea with activity *in vivo* per the oral route and possibly low toxicity. DW2282 could be a novel antitumor candidate with high specificity and with low toxicity compared to other orally active sulfonylureas.

References

[1] Houghton PJ, Houghton JA. Antitumor diarylsulfonylureas: novel agents with unfulfilled promise. Invest New Drugs 1996;14:271–80.

[2] Sosinski J, Thakar JH, Germain GS, Dias P, Harwood FC, Kuttesch JF, Houghton PJ. Cross-resistance to antitumor diarylsulfonylureas and collateral sensitivity to mitochondrial toxins in a human cell line selected for resistance to the antitumor agent *N*-(5-indanylsulfonyl)-*N*′(-(4-chlorophenyl)urea. Mol Pharmacol 1994;45:962–70.

[3] Mahjoubi M, Kattan J, Bonnay M, Schmitt H, Droz JP. Phase II trial of LY 186641 in advanced renal cancer. Invest New Drugs 1993;11:323–8.

[4] Jung SH, Kwak SJ, Kim ND, Lee SU, Lee CO. Stereochemical requirement at 4-position of 4-phenyl-1-arylsulfonylimidazolidinones for their cytotoxicities. Arch Pharm Res 2000;23:35–41.

[5] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107–12.

[6] Louie KG, Hamilton TC, Shoemaker RH, Young RC, Ozols RF. Evaluation of *in vitro* drug screening leads using experimental models of human ovarian cancer. Invest New Drugs 1992;10:73–8.

[7] Dixon JM, Renshaw L, Bellamy C, Stuart M, Hoctin-Boes G, Miller WR. The effects of neoadjuvant anastrozole (Arimidex) on tumor volume in postmenopausal women with breast cancer: a randomized, double-blind, single-center study. Clin Cancer Res 2000;6:2229–35.

[8] Maswadeh H, Demetzos C, Dimas K, Hatziantoniou S, Georgopoulos A, Rallis M, Dallas P, Papaioannou G. Accumulation of vinblastine into transfersomes and liposomes in response to a transmembrane ammonium sulfate gradient and their cytotoxic/cytostatic activity in vitro. Anticancer Res 2001;21:2577–83.

[9] Goldin A, Venditti JM, Macdonald JS, Muggia FM, Henney JE, DeVita Jr VT. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. Eur J Cancer 1981;17:129–42.

[10] Moon EY, Hwang HS, Choi CH, Jung SH, Yoon SJ. Effect of DW2282 on the induction of methemoglobinemia, hypoglycemia or WBC count and hematological changes. Arch Pharm Res 1999;22:565–70.

[11] Hwang HS, Moon EY, Seong SK, Choi CH, Chung YH, Jung SH, Lee DK, Yoon SJ. Characterization of the anticancer activity of DW2282, a new anticancer agent. Anticancer Res 1999;19:5087–93.

[12] Sosinski J, Thakar JH, Germain GS, Harwood FC, Houghton PJ. Proliferation-dependent and -independent cytotoxicity by antitumor diarylsulfonylureas. Indication of multiple mechanisms of drug action. Biochem Pharmacol 1993;45:2135–42.