Synthesis and Antitumor Activity of Duocarmycin Derivatives: A-Ring Pyrrole Compounds Bearing 5-Membered Heteroarylacryloyl Groups

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A series of A-ring pyrrole compounds of duocarmycin bearing 5-membered heteroarylacryloyl groups (thienylacryloyl and pyrrolylacryloyl) and heteroarylcarbonyl groups were synthesized and evaluated for *in vitro* anticellular activity against HeLa S_3 cells and *in vivo* antitumor activity against murine sarcoma 180 in mice. Most of the thienylacrylates displayed *in vitro* anticellular activity equivalent to 4′-methoxycinnamates. Among the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of methoxy-thienylacrylates, compound 11b, having 4′-methoxy-2′-thienylacryloyl as segment-B (Seg-B), showed remarkably potent antitumor activity and low peripheral blood toxicity *in vivo*, which were equal to those of 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxycinnamates, compared with the A-ring pyrrole derivatives having the trimethoxyindole skeleton in Seg-B. On the other hand, the 2′-pyrrolylacrylates having a double bond as spacer showed 10^2- to 10^3-fold stronger anticellular activity than 2′-pyrrolecarboxylates (IC_{50}<0.3 nM, 72 h-exposure). The 8-*O*-acetate and 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 2′-pyrrolylacrylates exhibited an antitumor effect at a lower dose compared with the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxycinnamate (1j). Moreover, it was expected that the antitumor activity would be increased by the strength of the extra hydrogen bond formed between the nitrogen of the pyrrole amido group and DNA, owing to the increase of the number of *N*-methyl-2′-pyrrolecarboxamide units. However, 2′-pyrrolylacrylates having three *N*-methyl-2′-pyrrolecarboxamide units showed nearly equal antitumor activity to 2′-pyrrolylacrylates having only one *N*-methyl-2′-pyrrolecarboxamide unit.

Key words duocarmycin; heteroarylacryloyl derivative; antitumor activity; DNA minor groove; KW-2189

Duocarmycins (A, **1a**; SA, **1b**; B1, **1e**; B2, **1c**; C1, **1f**; C2, **1d**) are novel antitumor antibiotics isolated from the culture broth of *Streptomyces* sp. (Fig. 1).[1—3] Since duocarmycins **1e**, **1c**, **1f**, and **1d** readily yield **1a**[1a—c] in aqueous solution, **1a** is considered to be an active form among these duocarmycins. Duocarmycins **1a** and **1b** have a unique cyclopropane ring responsible for the sequence-selective and reversible alkylation of double-stranded DNA mediating N3 adenine covalent adduct formation.[4] This mechanism is similar to that of CC-1065 (**2a**) which has been reported to show high cytotoxicity (Fig. 2).[5—6] KW-2189 (**1i**),[7] which was selected as the best compound of the analogs of A-ring pyrrole derivatives of duocarmycin B2, showed good stability in the culture medium, and the aqueous solubility was greater than 10 mg/ml.[8,9] KW-2189 (**1i**) is currently in phase II clinical trials. Segment-A (Seg-A) has an electrophilic cyclopropane ring necessary for the formation of covalent bonding with DNA.[7c] On the other hand, segment-B (Seg-B) has been considered to play important roles in noncovalent binding to the minor groove of DNA.[10] In previous papers,[11,12] we have reported on 4′-substituted-*trans*-cinnamoyl derivatives. The 4′-methoxy- and 4′-(*tert*-butoxycarbonyl)amino (BocNH)- cinnamates exhibited strong *in vitro* anticellular activity. A series of 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives of the 4′-methoxycinnamates exhibited significant antitumor activity with low peripheral blood toxicity compared with the A-ring pyrrole derivatives having the trimethoxyindole skeleton in Seg-B. Moreover, the 8-*O*-(*N*,*N*-dialkylcarbamoyl) cinnamates having an amino group at the 3′-position, and 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 6-membered *N*-heteroarylacrylates[12b] were found to possess adequate water solubility in excess of 10 mg/ml.

Some minor groove alkylators were found to be very potent cytotoxic agents.[13] In contrast, noncovalent minor groove binders exhibit only marginal antitumor activity. The representatives of these reagents are distamycin (**2b**) and netropsin (**2c**). Distamycin (**2a**) and netropsin (**2c**) bind to the minor groove, predominantly recognizing A·T-rich sequences.[13] Distamycin derivatives which bind to DNA by covalent interaction have been developed.[13b] Among the alkylating derivatives of distamycin, tallimusine (FCE 24517, **2d**) has been identified and is being tested in early clinical studies.[14]

Recently, Fregeau, Lown and co-workers have reported the structural features of the covalent bonding of a novel cyclopropylpyrroloindole (CPI)-lexitropsin conjugate (**2e**) to a model duplex DNA, which was examined by high field ^{1}H-NMR analysis and restrained molecular dynamics calculations.[15] The CPI-lexitropsin conjugate, which was designed for enhanced DNA binding compared with natural (+)-CC-1065, exhibits an exceptional cytotoxic potency against KB human nasopharangeal tumor cells *in vitro* of IC_{50}=0.76 fg/l.[15a] The racemic CPI-lexitropsin conjugate reacted readily with the duplex oligonucleotide $d(CGCAATTGCG)_2$ to form a single covalent adduct as the major product.

Our goal is to design an A-ring pyrrole of duocarmycin derivatives having greater activity and/or less toxicity than that of 4′-methoxycinnamates which exhibited the best results in our previous studies. For this purpose, our approach is to synthesize new compounds which have 5-membered heteroarylacryloyl groups (thienylacryloyl or pyrrolylacryloyl) as Seg-B. We are interested in beneficial effects on the binding potency of the conjugated molecule to DNA by changing the 6-membered ring to a 5-membered ring. More-

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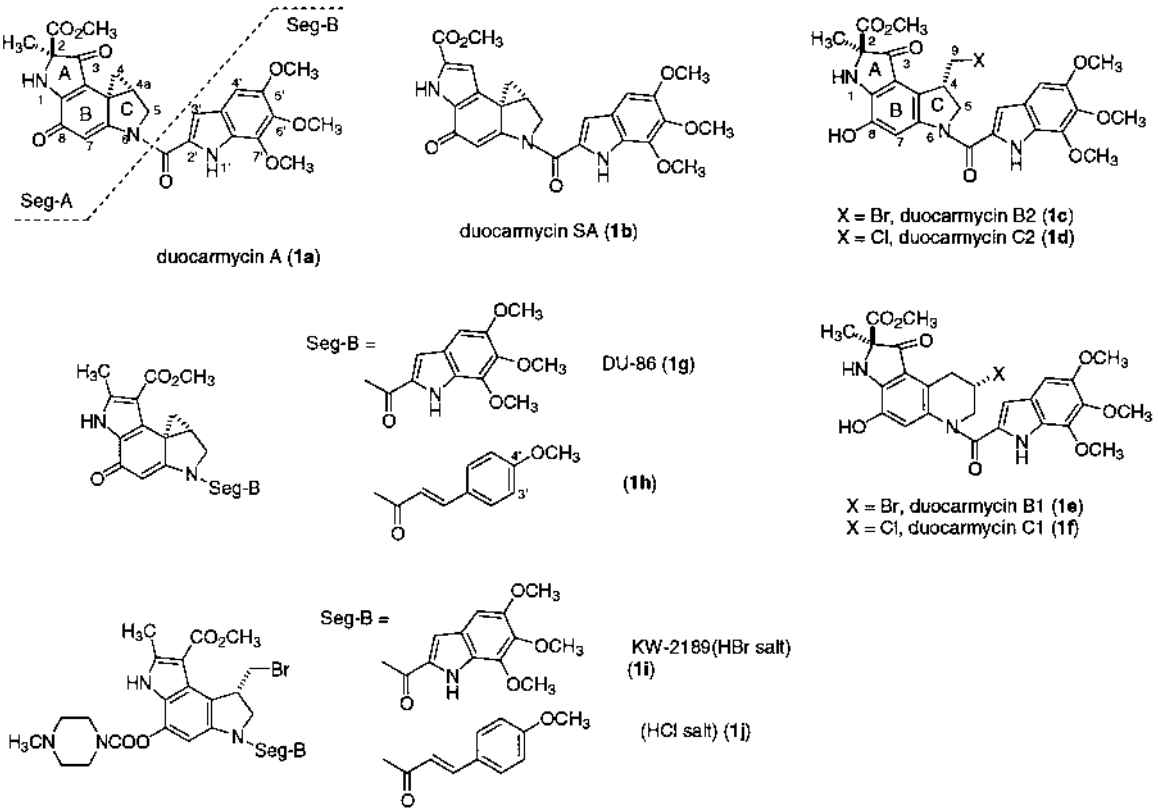


Fig. 1. Structures of Duocarmycins and Duocarmycin Derivatives

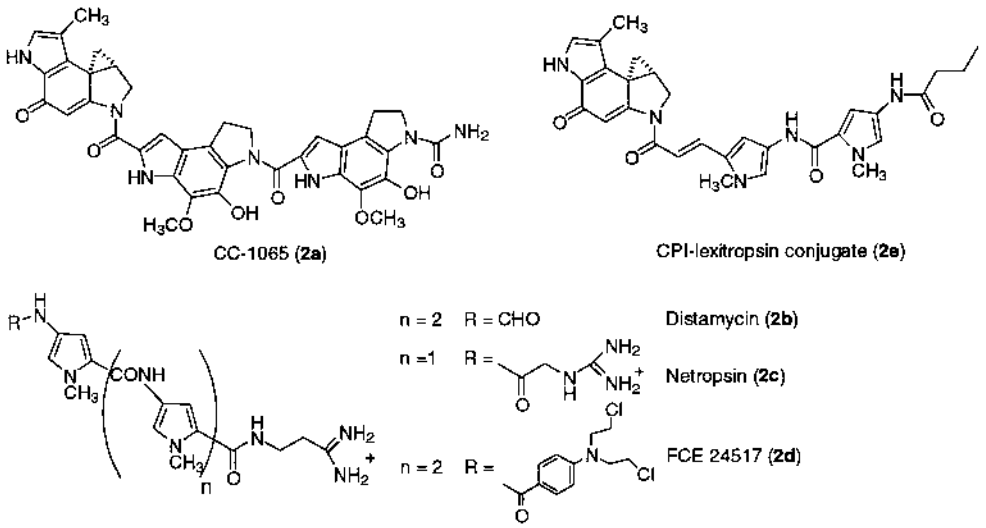
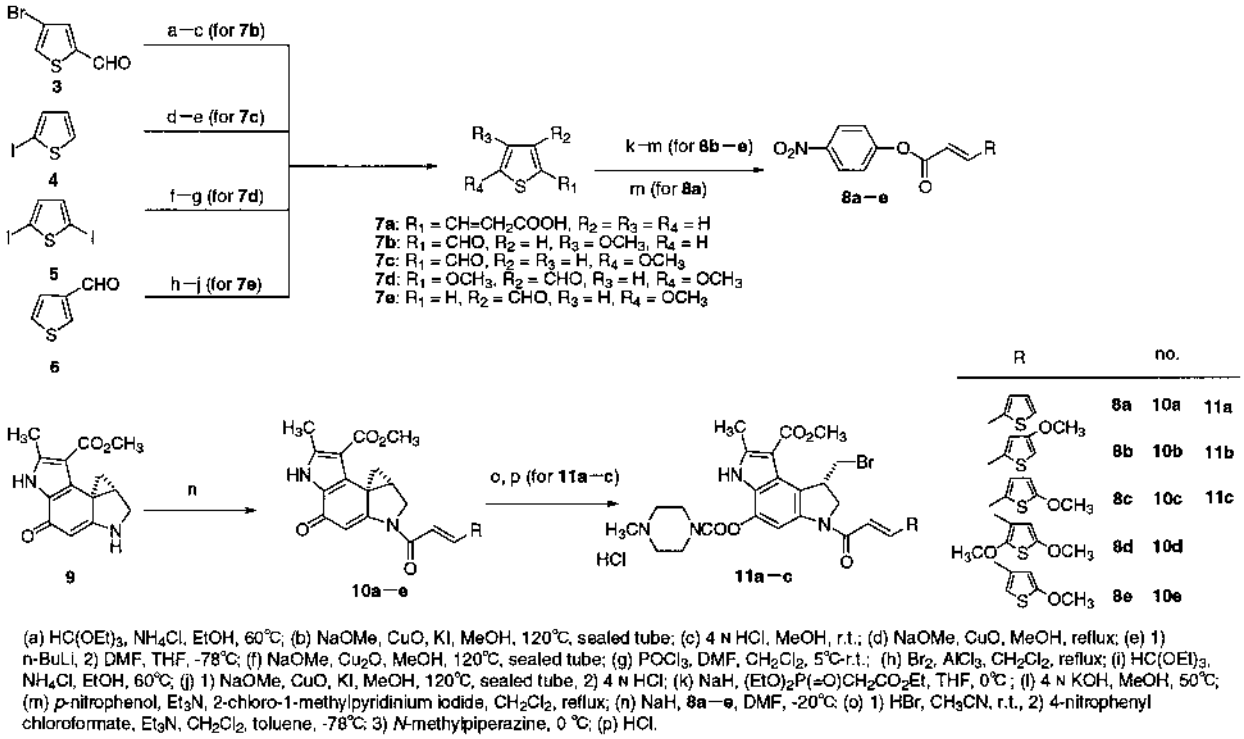


Fig. 2. Structures of CC-1065, Distamycin, Netropsin, FCE24517 and CPI-Lexitropsin Conjugate

over, we synthesized derivatives without the double bond as a spacer in Seg-B in order to examine whether the double bond as a spacer increases anticellular and antitumor activity by enhancing noncovalent binding to the minor groove of DNA. In this paper, we report our investigation regarding the synthesis, anticellular and antitumor activities, hematotoxicity, and structure-activity relationships (SAR) of these derivatives.

Chemistry For the formation of a thienylacryloyl moiety as Seg-B parts, we first synthesized the *p*-nitrophenyl esters of methoxy-substituted or non-substituted thienylacrylic acid, as shown in Chart 1. The methoxy-thiophene aldehydes (**7b**,[16a] **7c**,[16b] **7d**,[16c] **7e**[16d,e]) were prepared from starting materials (**3**—**6**). Their methoxy-thienylacrylic acid ethyl esters[17] were synthesized from the methoxy-thiophene aldehydes and triethyl phosphonoacetate by a Horner–Emmons reaction.[18] The obtained methoxy-thienylacrylic acid ethyl esters were treated with 4 N KOH to yield the corresponding methoxy-thienylacrylic acid. Their *p*-nitrophenyl esters (**8a**—**e**) were then prepared from the corresponding methoxy-thienylacrylic acid and *p*-nitrophenol using the Mukaiyama reagent[19] in good yields. The 2-methyl-3-methoxycarbonyl A-ring pyrrole compound of duocarmycin (DU-86, **1g**) was prepared by employing Wagner–Meerwein



(a) $HC(OEt)_3$, NH_4Cl, EtOH, 60°C; (b) NaOMe, CuO, KI, MeOH, 120°C, sealed tube; (c) 4 N HCl, MeOH, r.t.; (d) NaOMe, CuO, MeOH, reflux; (e) 1) n-BuLi, 2) DMF, THF, -78°C; (f) NaOMe, Cu_2O, MeOH, 120°C, sealed tube; (g) $POCl_3$, DMF, CH_2Cl_2, 5°C-r.t.; (h) Br_2, $AlCl_3$, CH_2Cl_2, reflux; (i) $HC(OEt)_3$, NH_4Cl, EtOH, 60°C; (j) 1) NaOMe, CuO, KI, MeOH, 120°C, sealed tube, 2) 4 N HCl; (k) NaH, $(EtO)_2P(=O)CH_2CO_2Et$, THF, 0°C; (l) 4 N KOH, MeOH, 50°C; (m) *p*-nitrophenol, Et_3N, 2-chloro-1-methylpyridinium iodide, CH_2Cl_2, reflux; (n) NaH, **8a—e**, DMF, -20°C; (o) 1) HBr, CH_3CN, r.t., 2) 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, toluene, -78°C; 3) *N*-methylpiperazine, 0 °C; (p) HCl.

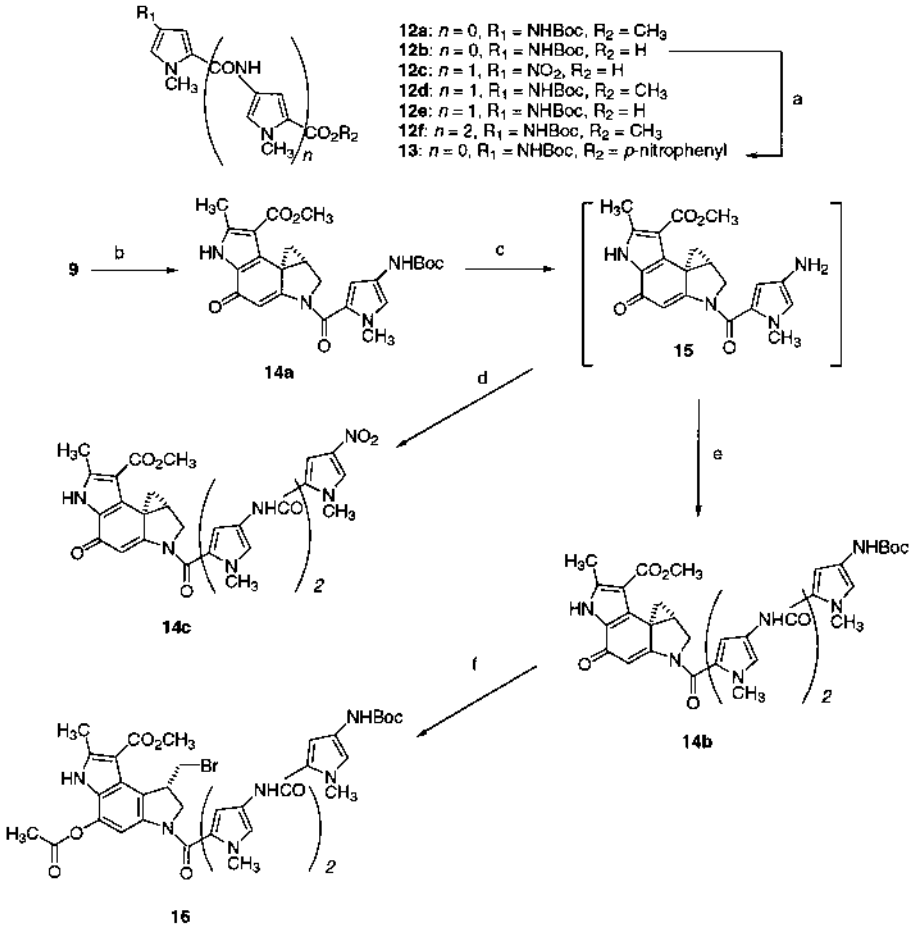
Chart 1

type rearrangement of the 8-*O*-protected-3-hydroxyduocarmycin B2, followed by deprotection of the protecting group under basic conditions.[12,20] The treatment of **1g** with NaOMe in MeOH provided Seg-A (**9**). The obtained compound **9** was allowed to react with *p*-nitrophenyl thienylacrylates in the presence of NaH to yield the corresponding thienylacrylates (**10a**—**e**) in reasonable yields.[11,12]

The 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives were synthesized from these thienylacrylates (**10a**—**e**) as described in preceding papers,[12] in order to increase *in vivo* antitumor activities and their stability under various conditions. The thienylacrylates were converted to *p*-nitrophenyl carbonate by treatment with HBr, followed by the reaction with *p*-nitrophenyl chloroformate in the presence of triethylamine. The carbonates were allowed to react with the *N*-methylpiperazine to produce 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives. The obtained 8-*O*-[(*N*-methylpiperazinyl)-carbonyl] derivatives were converted to hydrochloride salts (**11a**—**c**) by the treatment with HCl in MeOH and EtOH. However, the aqueous solubility of these salts (**11a**—**c**) was found to be below 0.1 mg/ml. Though we tried the same procedure with 3′-thienylacrylates (**10d**—**e**), we were unable to obtain the corresponding 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives because of decomposition in the reaction system.

We studied those derivatives with a pyrrole skeleton in Seg-B as the other 5-membered heterocycle. Compounds **12a**—**f** were prepared according to the method reported previously.[21] The synthesis of compound **14a** was performed according to the same procedures used for **10a**. Formation of the pyrrolecarboxylate analog with three *N*-methyl-2-pyrrolecarboxyamide units, like the distamycin molecule, was examined by coupling compound **14a** and 2-pyrrolecarboxylic acids (**12c**, **e**). Compound **14a** was deprotected with HBr in MeOH and $ClCH_2CH_2Cl$ at 50 °C and treated with saturated $NaHCO_3$ to give 4′-amino-2′-pyrrolecarboxylate (**15**) as the intermediate. **15** was condensed with 2-pyrrolecarboxylic acid (**12c** or **12e**) by using diethyl cyanophosphonate (DECP) to give Seg-B analogs with three *N*-methyl-2-pyrrolecarboxyamide units (**14b**, **c**). The 8-*O*-acetate (**16**) was prepared by the reaction of **14b** with HBr in CH_3CN and MeOH, followed by the addition of acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP).

The preparation of 2′-pyrrolylacrylates having a double bond as a spacer is outlined in Chart 3. The methyl *N*-methyl-2-pyrrolecarboxylates (**12a**, **12d**, **12f**) were reduced with $LiAlH_4$, followed by oxidation with MnO_2 to give the corresponding *N*-methyl-2-pyrrolecarboxyaldehydes. 2-Pyrrolylacrylic acid (**17a**—**c**) and *p*-nitrophenyl *N*-methyl-2-pyrrolylacrylic (**18**) were prepared by the same method as that for the thienylacrylic acids and their *p*-nitrophenyl ester (see Chart 1). Although we attempted condensation with the 4′-amino-pyrrole compound, shown in Chart 2, it did not succeed. Compounds **19b** and **19c** were prepared by the reaction of **9** with 6.86 N HCl in AcOEt, followed by the addition of **17b** or **17c** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) as a condensing agent.[22] Compounds **19b** and **19c** were then treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give cyclopropane compounds **20b** and **20c**, respectively. To synthesize the 8-*O*-acetate (**21a**) or 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives (**22**), preparation of the C8 phenolic hydroxyl compound with a bromomethyl group in the C-ring part was attempted by the reaction of **20a** with HBr at room temperature as in previous method;[12,23a] however, compound **20a** was decomposed. When the reaction temperature was lowered to −20 °C, compounds **21a** and **22** were obtained in high yield without decomposition. The *O*-acetates (**21b**—**c**) were also



(a) *p*-nitrophenol, Et_3N, 2-chloro-1-methylpyridinium iodide, CH_2Cl_2, reflux; (b) NaH, **13**, DMF, -20°C; (c) 1) HBr, MeOH, $ClCH_2CH_2Cl$, 50°C, 2) sat. $NaHCO_3$; (d) **12c**, DECP, Et_3N, THF, 0°C-r.t.; (e) **12e**, DECP, Et_3N, THF, 0°C-r.t.; (f) 1) HBr, MeOH, CH_3CN, r.t., 2) Ac_2O, DMAP, CH_2Cl_2, 0°C.

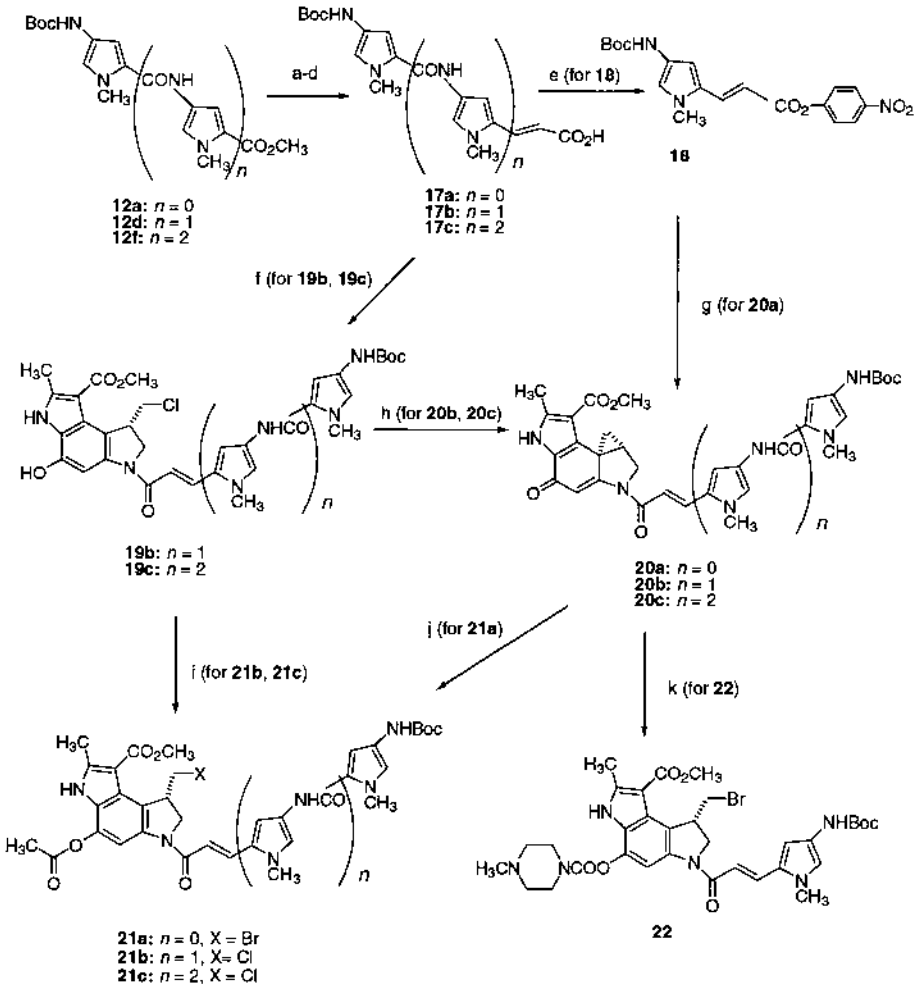
Chart 2

prepared by the same procedure as that for **16**.

Results and Discussion

The anticellular activity of thienylacrylates against HeLa S_3 cells was evaluated *in vitro*. The thienylacrylates (**10a**, **b**, **10d**, **e**) exhibited potent anticellular activity with IC_{50} values below 3.0 nM at 72 h exposure, which were almost equivalent to compound **1h**. The anticellular activity of all the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of the thienylacrylates was about 1/100 of that of the corresponding cyclopropane derivatives. This result showed that the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives were not converted to cyclopropane derivatives as active metabolites in the culture medium.[7d,23] The antitumor activity of the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives (**11a**—**c**) against sarcoma 180 murine solid tumor was evaluated *in vivo*. The *in vivo* efficacy was expressed as T/C, which is defined as the treated *versus* control value of the tumor volume. Tumor volume was calculated according to the method described previously.[7,8] Compounds **11a**—**c** exhibited potent antitumor activity *in vivo* (T/C; 0.09—0.35). Compound **11b**, with 4′-methoxy-2′-thienylacryloyl as Seg-B, showed more effective antitumor activity than compound **11a** with 2′-thienylacryloyl without a methoxy moiety. This result was supported by our observation[12a] that 4′-methoxycinnamates exhibited the strongest anticellular activity among cinnamates. Moreover, compound **11c**, in which the position of the methoxy group on the thiophene ring of Seg-B was different from **11b**, showed antitumor activity inferior to **11b**.[24]

On the other hand, the 2′-pyrrolylacrylates having a double bond as a spacer showed 10^2—10^3 times stronger anticellular activity than 2′-pyrrolecarboxylates (**14a** *vs*. **20a**, **14b** *vs*. **20c**). All of the 2′-pyrrolylacrylates with a cyclopropane ring showed anticellular activity superior to 4′-methoxycinnamate (**1h**) (IC_{50}<0.3 nM, 72 h-exposure). The 4′-BocNH-2′-pyrrolecarboxylate (**14b**) showed stronger anticellular activity than the 4′-nitro-2′-pyrrolecarboxylate (**14c**). This result was parallel to our expectation based on the strong anticellular activity of 4′-BocNH-cinnamates in 4′-substituted-cinnamates. The anticellular potency of 2′-pyrrolecarboxylates (**14a** *vs*. **14b**) increased by the increase in the number of *N*-methyl pyrrolecarboxyamide units. However, the same tendency was not found in 2′-pyrrolylacrylates (**20a** *vs*. **20b** *vs*. **20c**). The conversion of the 8-*O*-acetates to cyclopropane compounds in the culture medium resulted in strong anticellular activity below 0.3 nM, which was different from 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives (**21a** *vs*. **22**). The 8-*O*-acetate and 8-*O*-[(*N*-methylpiperazinyl)carbonyl] 2′-pyrrolylacrylates (**21a**, **22**) exhibited a significant antitumor effect on sarcoma 180 murine solid tumor at a lower



(a) $LiAlH_4$, THF; (b) MnO_2, THF, 50°C; (c) NaH, $(EtO)_2P(=O)CH_2CO_2Et$, THF, 0°C; (d) 4 N KOH, EtOH, 50°C; (e) *p*-nitrophenol, Et_3N, 2-chloro-1-methylpyridinium iodide, CH_2Cl_2, reflux; (f) 1) **9**, HCl, AcOEt, r.t., 2) **17b** or **17c** EDCI, DMF, r.t.; (g) NaH, **9**, DMF, -2 °C; (h) DBU, CH_3CN, Py, r.t.; (i) Ac_2O, DMAP, CH_2Cl_2, 0°C; (j) 1) HBr, CH_2Cl_2, -20°C, 2) Ac_2O, DMAP, CH_2Cl_2, -20°C; (k) 1) HBr , CH_2Cl_2, -20°C, 2) *p*-nitrophenyl chloroformate, Et_3N, 3) *N*-methylpiperazine, CH_2Cl_2, -78–0°C.

Chart 3

dose compared with compound **1j**. In contrast, the 8-*O*-acetate of 2′-pyrrolecarboxylate (**16**) exhibited no antitumor effect.

The peripheral blood toxicity (reduction of the number of peripheral blood platelets) of compound **11b**, demonstrating potent antitumor activity against sarcoma 180, was lower than that of the derivatives bearing Seg-B of a natural type, and was equal to that of 4′-methoxycinnamates. On the other hand, 2′-pyrrolylacrylates showed a tendency to increase the number of peripheral blood platelets, although the reason is unclear.

We also established a simple method of investigating by HPLC whether the drug directly interacted with calf thymus DNA.[7c,8] As shown in Fig. 3, compound **20c** disappeared faster in the presence of calf thymus DNA than in its absence, while the rate of disappearance of compound **14b** was less influenced by DNA. Accordingly, compound **14b** had little direct interaction with calf thymus DNA, and thus showed weak anticellular activity. The double bond as a spacer is very important for strong anticellular and antitumor activity. 4′-Methoxycinnamate (**1h**) was examined by the same method. Compound **20c** was similar to compound **1h**. These results indicated the same tendency of anticellular activity of **20c** and **1h**.

Conclusions

A series of A-ring pyrrole duocarmycins bearing 5-membered heteroarylacryloyl groups were prepared and evaluted for their anticellular activity against HeLa S_3 cells, as well as antitumor activity against sarcoma 180 murine solid tumor. The 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives bearing 4′-methoxy-2′-thienylacryloyl as Seg-B (**11b**) showed remarkably potent antitumor activity and low peripheral blood toxicity *in vivo* compared with derivatives with the trimethoxyindole skeleton in Seg-B (**1i**). The 2′-pyrrolylacrylates with double bonds as spacers showed strong anticellular and antitumor activity superior to the 2′-pyrrolecarboxylates. The 8-*O*-acetate and the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 2′-pyrrolylacrylates exhibited an antitumor effect at a lower dose compared with the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxycinnamates. The double bond as a spacer would be very important for the strong association between the DNA minor groove and drugs.

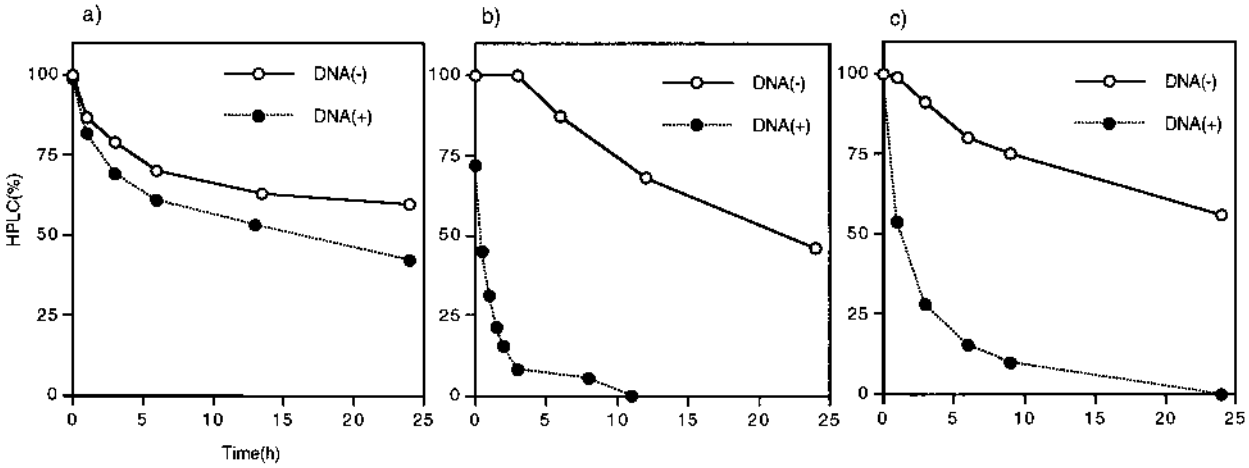


Fig. 3. Interaction of **14b**, **20c** and **1h** with Calf Thymus DNA

a) Time course of disappearance of compound **14b** in the presence of DNA (●) and in the absence of DNA (○). The reactions were performed in 0.01 M phosphate buffer (pH 7) containing 10% DMF at 35 °C. *b*) Interaction of compound **20c** (●) and its control (○) with DNA. *c*) Interaction of compound **1h** (●) and its control (○) with DNA.

Table 1. Anticellular Activity, Antitumor Activity, and Hematotoxicity of 5-Membered Heteroarylacrylates and Heteroarylcarboxylates

No.	HeLa S_3[a] IC_{50} (nM)		Sarcoma 180 (s.c.-i.v.)[b]		Hematotoxicity	
	1 h	72 h	Dose (mg/kg)	T/C[c]	WBC[d] (%)	PL[e] (%)
10a	12	2.9	NT[f]		NT	NT
10b	29	1.9	NT		NT	NT
10d	7.7	1.2	NT		NT	NT
10e	1.5	0.67	NT		NT	NT
11a	1100	230	8	0.35	19	90
11b	1400	200	8	0.09	19	94
11c	970	95	8	0.33	13	22
14a	1200	140	NT		NT	NT
14b	98	17	NT		NT	NT
14c	1300	87	NT		NT	NT
16	330	18	8	1.20	84	114
19b	0.98	0.14	NT		NT	NT
20a	0.92	0.21	NT		NT	NT
20b	1.0	0.28	0.5	0.36	49	119
20c	0.79	0.27	0.25	0.20	73	137
21a	1.2	0.15	0.5	0.36	63	110
21b	1.0	0.24	1	0.17	44	95
21c	4.0	0.2	0.25	0.36	93	NT
22	410	35	1	0.22	57	113
1g	0.045	0.0052	0.25	0.21	22	38
1h	2.9—7.0	0.26—0.94	0.83	0.34	50	63
1i	53	1.6	0.63	0.15	24	10
1j	1800	37	4	0.20	25	44[g]

a) Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%. *b*) Mice (5 mice/group) were implanted subcutaneously (s.c.) with tumor cells, and the drug was dosed (mg/mg) intravenously (i.v.). *c*) T and C are the values of the mean of tumor volume of treated and control mice, respectively. *d*) Number of white blood cells of tumor-bearing mice on day 4 (percent of control). *e*) Number of peripheral platelets of normal mice on day 7 (percent of control). *f*) Not tested. *g*) Dose of 5.33 mg/kg.

Experimental

All melting points were measured on a Yanagimoto micromelting points apparatus without correction. Infrared spectra (IR) were recorded on a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were measured on JEOL JNM-EX270 and Hitachi R-90H spectrometers. Mass spectra were measured with JEOL JMS-DX303, JMS-SX102, and Shimadzu QP-1000 spectrometers. Elemental analyses were performed with a Perkin-Elmer 2400 C, H, N analyzer. For column chromatography, silica gel (SiO_2, Merck Kieselgel 60 F_{254}) was used. Preparative TLC (PTLC) was carried out on glass plates coated with Merck Kieselgel 60 F_{254s}. The usual work-up refers to washing the organic layers with brine, drying them over anhydrous Na_2SO_4, and evaporating off the solvents under reduced pressure.

4-Methoxy-2-thiophenecarboxaldehyde (7b)[16a] To a solution of 4-bromo-2-thiophenecarboxaldehyde (**3**) (2.00 g, 10.5 mmol) in EtOH (12 ml) were added NH_4Cl (180 mg) and $HC(OEt)_3$ (2.61 ml, 15.7 mmol), and the mixture was stirred at 60 °C for 2 h 10 min. The reaction mixture was poured into H_2O, and thus combination was extracted with AcOEt. The usual work-up afforded 2.98 g (100%) of 2-bromo-4-thiophenecarboxaldehyde diethyl acetal. To a solution of 2-bromo-4-thiophenecarboxaldehyde diethyl acetal in MeOH (14 ml) were added CuO (150 mg), KI (21 mg) and 28% NaOMe in MeOH (7.27 g, 37.7 mmol), and the mixture was heated in a sealed tube at 120 °C for 11 h. The reaction mixture was filtered through the Celite. The filtrate was poured into 0.5 N HCl, and new mixture was extracted with AcOEt, then worked up as usual. The residue was purified by column chromatography (hexane–AcOEt, 10 : 1) to give 544 mg (67%) of 2-methoxy-4-thiophenecarboxaldehyde diethyl acetal. To a solution of 2-methoxy-4-thio-

phenecarboxaldehyde diethyl acetal (496 mg, 2.29 mmol) in MeOH (10 ml) was added 4 M HCl, and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into 0.5 N HCl, and the new mixture was extracted with AcOEt. The usual work-up afforded 335 mg (100%) of 2-methoxy-4-thiophenecarboxaldehyde (**7b**): ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 9.81 (1H, s), 7.39 (1H, br s), 6.74 (1H, br s), 3.85 (3H, s).

5-Methoxy-3-thiophenecarboxaldehyde (7e)[16d,e] To a solution of 3-thiophenecarboxaldehyde (**6**) (1.00 g, 8.92 mmol) in CH_2Cl_2 (30 ml) was added $AlCl_3$ (2.97 g, 22.3 mmol) at room temperature for 10 min. Then, a solution of bromine (0.426 ml) in CH_2Cl_2 (1 ml) was added to the mixture. After stirring at reflux for 1 h and 40 min, the reaction mixture was poured into H_2O, and the new mixture was extracted with $CHCl_3$. The organic layer was washed with 5% $Na_2S_2O_3$, and worked up as usual. The residue was purified by column chromatography (hexane–AcOEt, 30 : 1) to give 1.03 g (60%) of 5-bromo-3-thiophenecarboxaldehyde.[16d] To a solution of 5-bromo-3-thiophenecarboxaldehyde (1.01 g, 5.27 mmol) in EtOH (8 ml) were added NH_4Cl (40 mg) and $HC(OEt)_3$ (1.31 ml, 7.91 mmol), and the mixture was stirred at 60 °C for 50 min. The reaction mixture was poured into H_2O, and the new mixture was extracted with AcOEt. The usual work-up afforded 1.363 g (98%) of 5-bromo-2-thiophenecarboxaldehyde diethyl acetal. To a solution of 5-bromo-3-thiophenecarboxaldehyde diethyl acetal (200 mg, 0.754 mmol) in MeOH (1.5 ml) were added CuO (30 mg), KI (6.3 mg) and 28% NaOMe in MeOH (727 mg, 3.77 mmol), and the mixture was heated in a sealed tube at 120 °C for 8 h and 10 min. After the addition of CuO (15 mg) and 28% NaOMe in MeOH (436 mg), the mixture was further heated in a sealed tube at 120 °C for 22 h. The mixture was filtered through the Celite. Then, the filtrate was poured into 4 N HCl. After being stirred at room temperature for 20 min, the mixture was extracted with AcOEt, and worked up as usual. The residue was purified by column chromatography (hexane–AcOEt, 20 : 1—10 : 1) to give 67 mg (63%) of 5-methoxy-3-thiophenecarboxaldehyde (**7e**):[16e] ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 9.66 (1H, s), 7.39 (1H, d, J=1.5 Hz), 6.58 (1H, d, J=1.5 Hz), 3.91 (3H, s).

(*E*)-*p*-Nitrophenyl 3-(2-Thienyl)acrylate (8a) To a solution of 3-(2-thienyl)acrylic acid (**7a**, Aldrich) (500 mg, 3.24 mmol) in CH_2Cl_2 (20 ml) were added *p*-nitrophenol (766 mg, 5.51 mmol), triethylamine (1.54 ml, 11.0 mmol) and 2-chloro-1-methylpyridinium iodide (1.41 g, 5.51 mmol), and the mixture was heated under reflux for 2 h 30 min. The reaction mixture was poured into aqueous $NaHCO_3$, and the new mixture was extracted with $CHCl_3$, and worked up as usual. The residue was purified by column chromatography ($CHCl_3$) to give 842 mg (94%) of (**8a**): ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.30 (2H, d, J=9.2 Hz), 8.01 (1H, d, J=15.8 Hz), 7.48 (1H, d, J=5.0 Hz), 7.37 (1H, d, J=4.6 Hz), 7.36 (2H, d, J=9.2 Hz), 7.12 (1H, dd, J=5.0, 3.6 Hz), 6.41 (1H, d, J=15.8 Hz); IR (KBr) 1734, 1626, 1520, 1348, 1200, 1134, 966 cm^{-1}; FAB-MS m/z 276 $(M+H)^+$. *Anal*. Calcd for $C_{13}H_9NO_4S$: C, 56.72; H, 3.30; N, 5.09. Found: C, 56.99; H, 3.40; N, 5.11.

(*E*)-*p*-Nitrophenyl 3-(4-Methoxy-2-thienyl)acrylate (8b) To a solution of 60% NaH (112 mg, 2.81 mmol) in tetrahydrofuran (THF) (2 ml) was added a solution of triethyl phosphonoacetate (630 mg, 2.81 mmol) in THF (1 ml), and the mixture was stirred under Ar atmosphere at 0 °C. After 5 min, a solution of 4-methoxy-2-thiophenecarboxaldehyde (**7b**) (337 mg, 2.37 mmol) in THF (2 ml) was added, and the mixture was stirred at the same temperature for 1 h. The reaction was quenched by the addition of ice, and the resulting mixture was poured into 0.5 N HCl. The whole was extracted with AcOEt, and worked up as usual. The residue was purified by column chromatography (hexane–AcOEt, 15 : 1) to give 424 mg (84%) of ethyl 3-(4-methoxy-2-thienyl)acrylate.[17a] To a solution of ethyl 3-(4-methoxy-2-thienyl)acrylate (414 mg, 1.95 mmol) in MeOH (9 ml) was added 4 N KOH (1.46 ml, 5.85 mmol), and the mixture was stirred at 50 °C for 1 h. The reaction mixture was poured into 0.5 N HCl, and the new mixture was extracted with AcOEt. The usual work-up afforded 361 mg (100%) of 3-(4-methoxy-2-thienyl)acrylic acid.

The synthesis of compound **8b** was performed according to the same procedure as that for **8a**: Yield 91%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.29 (2H, d, J=8.9 Hz), 7.86 (1H, d, J=15.5 Hz), 7.35 (2H, d, J=8.9 Hz), 7.02 (1H, s), 6.43 (1H, s), 6.38 (1H, d, J=15.8 Hz), 3.83 (3H, s); IR (KBr) 1738, 1622, 1524, 1352, 1284, 1217, 1128, 1034, 964, 870 cm^{-1}; EI-MS m/z 305 (M^+). *Anal*. Calcd for $C_{14}H_{11}NO_5S$: C, 55.08; H, 3.63; N, 4.59. Found: C, 55.07; H, 3.75; N, 4.59.

The synthesis of compounds **8c**—**e** was performed according to the same procedure as that for **8b**.

(*E*)-*p*-Nitrophenyl 3-(5-Methoxy-2-thienyl)acrylate (**8c**): Yield 58%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.28 (2H, d, J=9.2 Hz), 7.85 (1H, d, J=15.5 Hz), 7.34 (2H, d, J=9.2 Hz), 7.06 (1H, d, J=4.3 Hz), 6.22 (1H, d, J=4.3 Hz), 6.08 (1H, d, J=15.2 Hz), 3.97 (3H, s); IR (KBr) 1722, 1612, 1520, 1483, 1348, 1221, 1132, 1051 cm^{-1}; FAB-MS m/z 306 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{11}NO_5S \cdot 0.3H_2O$: C, 54.12; H, 3.76; N, 4.51. Found: C, 54.15; H, 3.67; N, 4.43.

(*E*)-*p*-Nitrophenyl 3-(2,5-Dimethoxy-3-thienyl)acrylate (**8d**): Yield 73%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.28 (2H, d, J=9.2 Hz), 7.86 (1H, d, J=15.8 Hz), 7.35 (2H, d, J=9.2 Hz), 6.13 (1H, d, J=15.8 Hz), 6.02 (1H, s), 3.95 (3H, s), 3.87 (3H, s); IR (KBr) 1736, 1614, 1520, 1308, 1221, 1144, 999 cm^{-1}; FAB-MS m/z 336 $(M+H)^+$. *Anal*. Calcd for $C_{15}H_{13}NO_6S$: C, 53.73; H, 3.91; N, 4.18. Found: C, 53.92; H, 4.10; N, 4.17.

(*E*)-*p*-Nitrophenyl 3-(5-Methoxy-3-thienyl)acrylate (**8e**): Yield 64%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.29 (2H, d, J=9.2 Hz), 7.69 (1H, d, J=15.8 Hz), 7.35 (2H, d, J=9.2 Hz), 6.91 (1H, d, J=1.6 Hz), 6.42 (1H, d, J=1.3 Hz), 6.28 (1H, d, J=15.8 Hz), 3.94 (3H, s); IR (KBr) 1632, 1593, 1514, 1352, 1282, 1219, 1134, 974, 858 cm^{-1}; FAB-MS m/z 306 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{11}NO_5S$: C, 55.08; H, 3.63; N, 4.59. Found: C, 55.36; H, 3.77; N, 4.57.

2′-Thienylacryloyl A-Ring Pyrrole Duocarmycin (10a) To a solution of 60% NaH (4.7 mg, 0.12 mmol) in *N*,*N*-dimethylformamide (DMF) (0.3 ml) was added a DMF solution (0.4 ml) of **9** (Seg-A) (25 mg, 0.097 mmol), and the mixture was stirred under Ar atmosphere at −20 °C for 2 h 30 min. A solution of **8a** (30.7 mg, 0.112 mmol) in DMF (0.4 ml) was added, and the mixture was stirred at −20 °C for 1 h 40 min. Phosphoric buffer (0.01 M, pH 7) was added, and the whole was extracted with AcOEt, and worked up as usual. The residue was purified by column chromatography ($CHCl_3$–MeOH, 100 : 1) to give 28 mg (73%) of **10a**: mp 135—140 °C; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 11.91 (1H, br s), 7.93 (1H, d, J=14.9 Hz), 7.40 (1H, d, J=5.0 Hz), 7.31 (1H, d, J=3.3 Hz), 7.07 (1H, dd, J=5.0, 3.9 Hz), 6.79 (1H, br), 6.63 (1H, d, J=15.2 Hz), 4.23 (1H, d, J=10.9 Hz), 4.16 (1H, dd, J=10.9, 4.6 Hz), 3.81 (3H, s), 3.54—3.63 (1H, m), 2.63 (3H, s), 2.38 (1H, dd, J=7.3, 3.0 Hz), 1.30 (1H, dd, J=4.3, 3.6 Hz); IR (KBr) 1701, 1670, 1606, 1487, 1390, 1294, 1248, 1217, 1109, 1072 cm^{-1}; FAB-MS m/z 395 $(M+H)^+$; FAB-HR-MS Calcd for $C_{21}H_{19}N_2O_4S$ $(M+H)^+$ m/z 395.1066, Found 395.1045. *Anal*. Calcd for $C_{21}H_{18}N_2O_4S \cdot 0.3\,CHCl_3$: C, 59.46; H, 4.29; N, 6.51. Found: C, 59.82; H, 4.45; N, 6.37.

The synthesis of compounds **10b**—**e** was performed according to the same procedure as that for **10a**.

4′-Methoxy-2′-thienylacryloyl A-Ring Pyrrole Duocarmycin (**10b**): Yield 73%; mp 190—195 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.64 (1H, d, J=15.2 Hz), 6.87 (1H, s), 6.62 (1H, br), 6.49 (1H, d, J=15.2 Hz), 6.29 (1H, s), 4.11 (1H, d, J=10.9 Hz), 4.04 (1H, dd, J=10.6, 4.3 Hz), 3.70 (6H, s), 3.46—3.49 (1H, m), 2.27 (1H, dd, J=7.3, 3.3 Hz), 1.20 (1H, dd, J=4.3, 4.0 Hz); IR (KBr) 1701, 1606, 1549, 1450, 1387, 1360, 1277, 1217, 1109, 1070 cm^{-1}; FAB-MS m/z 425 $(M+H)^+$; FAB-HR-MS Calcd for $C_{22}H_{21}N_2O_5S$ $(M+H)^+$ m/z 425.1171, Found 425.1150. *Anal.* Calcd for $C_{22}H_{20}N_2O_5S \cdot 0.8\,H_2O$: C, 60.21; H, 4.96; N, 6.38. Found: C, 60.21; H, 4.68; N, 6.30.

3′-Methoxy-2′-thienylacryloyl A-Ring Pyrrole Duocarmycin (**10c**): Yield 63%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 11.69 (1H, br), 7.79 (1H, d, J=14.8 Hz), 7.00 (1H, d, J=4.0 Hz), 6.79 (1H, br), 6.32 (1H, d, J=14.8 Hz), 6.18 (1H, d, J=4.0 Hz), 4.19 (1H, d, J=10.6 Hz), 4.13 (1H, dd, J=10.9, 4.3 Hz), 3.94 (3H, s), 3.80 (3H, s), 3.53—3.58 (1H, m), 2.63 (3H, s), 2.36 (1H, dd, J=7.4, 3.1 Hz), 1.28 (1H, dd, J=4.6, 3.6 Hz); IR (KBr) 1701, 1601, 1541, 1483, 1450, 1389, 1279, 1279, 1215, 1174, 1109 cm^{-1}; FAB-MS m/z 425 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{20}N_2O_5S \cdot 1.2\,H_2O \cdot 0.3\,CHCl_3$: C, 55.62; H, 4.69; N, 5.82. Found: C, 55.65; H, 4.54; N, 5.63.

2′,4′-Dimethoxy-3′-thienylacryloyl A-Ring Pyrrole Duocarmycin (**10d**): Yield 67%; mp 150—155 °C; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 12.04 (1H, br s), 7.75 (1H, d, J=15.2 Hz), 6.77 (1H, br s), 6.42 (1H, d, J=15.2 Hz), 5.96 (1H, s), 4.19 (1H, d, J=10.9 Hz), 4.12 (1H, dd, J=10.9, 4.0 Hz), 3.90 (3H, s), 3.82 (3H, s), 3.78 (3H, s), 3.52—3.58 (1H, m), 2.61 (3H, s), 2.34 (1H, dd, J=7.3, 3.0 Hz), 1.27 (1H, dd, J=4.0, 4.0 Hz); IR (KBr) 1701, 1603, 1452, 1389, 1292, 1248, 1215, 1174, 1109, 991 cm^{-1}; FAB-MS m/z 455 $(M+H)^+$; FAB-HR-MS Calcd for $C_{23}H_{23}N_2O_6S$ $(M+H)^+$ m/z 455.1277, Found 455.1275. *Anal*. Calcd for $C_{23}H_{22}N_2O_6S \cdot 1.0\,H_2O$: C, 58.46; H, 5.12; N, 5.93. Found: C, 58.24; H, 4.84; N, 5.78.

4′-Methoxy-3′-thienylacryloyl A-Ring Pyrrole Duocarmycin (**10e**): Yield 62%; mp 155—160 °C; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.52 (1H, d, J=15.2 Hz), 6.81 (1H, d, J=1.7 Hz), 6.60 (1H, br), 6.46 (1H, d, J=15.2 Hz), 6.33 (1H, d, J=1.7 Hz), 4.15 (1H, d, J=10.9 Hz), 4.07 (1H, dd, J=10.9, 4.6 Hz), 3.85 (3H, s), 3.75 (3H, s), 3.47—3.54 (1H, m), 2.50 (3H, s), 2.32 (1H, dd, J=7.6, 3.6 Hz), 1.24 (1H, dd, J=5.0, 3.3 Hz); IR (KBr) 1701, 1668, 1620, 1601, 1485, 1454, 1392, 1292, 1219, 1109, 1072 cm^{-1}; FAB-MS m/z 425 $(M+H)^+$; FAB-HR-MS Calcd for $C_{22}H_{21}N_2O_5S$ $(M+H)^+$ m/z 425.1171, Found 425.1158. *Anal*. Calcd for $C_{22}H_{20}N_2O_5S \cdot 0.8\,H_2O$: C, 60.21; H, 4.96;

N, 6.38. Found: C, 60.25; H, 4.76; N, 5.76.

2′-Thienylacryloyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride (11a) To a solution of **10a** (26.6 mg, 0.0674 mmol) in CH_3CN (1.32 ml) was added 48% HBr (0.015 ml), and the mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. *p*-Nitrophenyl chloroformate (40.8 mg, 0.202 mmol) and triethylamine (0.038 ml, 0.270 mmol) were added to a residue in dry CH_2Cl_2 (1.32 ml) and toluene (0.53 ml) at −78 °C. Then, the mixture was stirred at −78 °C for 65 min. *N*-methypiperazine (0.0262 ml, 0.236 mmol) was added to the solution, and the mixture was stirred at 0 °C for 1 h 25 min. The mixture was diluted with $CHCl_3$, and the combine was washed with aqueous $NaHCO_3$, and worked up as usual. The residue was purified by PTLC ($CHCl_3$–MeOH, 9 : 1) to give 31.6 mg (78%) of the free base of **11a**: ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.45 (1H, s), 8.19 (1H, br s), 7.94 (1H, d, J=14.9 Hz), 7.38 (1H, d, J=5.0 Hz), 7.30 (1H, d, J=3.3 Hz), 7.07 (1H, dd, J=5.9, 3.6 Hz), 6.72 (1H, d, J=14.9 Hz), 4.46—4.58 (1H, m), 4.42 (1H, d, J=10.6 Hz), 4.29 (1H, dd, J=9.6, 8.9 Hz), 3.94 (3H, s), 3.79 (1H, dd, J=9.9, 2.7 Hz), 3.73 (2H, br), 3.63 (2H, br), 3.20 (1H, dd, J=10.2, 9.9 Hz), 2.47 (4H, br), 2.43 (3H, s), 2.34 (3H, s); IR (KBr) 1697, 1645, 1435, 1421, 1406, 1292, 1217, 1151, 1093, 1005 cm^{-1}; FAB-MS m/z 603, 601 $(M+H)^+$; FAB-HR-MS Calcd for $C_{27}H_{30}{}^{79}BrN_4O_5S$ $(M+H)^+$ m/z 601.1120, Found 601.1104.

A solution of the free base of **11a** (19.7 mg, 0.0328 mmol) in EtOH (0.98 ml) and MeOH (0.49 ml) was treated with anhydrous 6.86 N HCl in EtOH (0.0096 ml) at room temperature for 2 h 30 min. The mixture was concentrated under reduced pressure to give 19.8 mg of **11a**: mp 265—270 °C (dec.); ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 12.08 (1H, s), 10.49 (1H, br), 8.09 (1H, br s), 7.77 (1H, d, J=15.2 Hz), 7.71 (1H, d, J=5.0 Hz), 7.58 (1H, d, J=2.6 Hz), 7.17 (1 H, dd, J=4.3, 4.3 Hz), 6.86 (1 H, d, J=14.9 Hz), 4.31—4.51 (3H, m), 4.20—4.30 (2H, br), 3.84 (3H, s), 3.80 (1H, dd, J=9.2, 2.0 Hz), 3.40—3.56 (7H, m), 2.85 (3H, s), 2.68 (3H, s); IR (KBr) 1716, 1695, 1647, 1435, 1408, 1340, 1250, 1221, 1171, 1093 cm^{-1}. *Anal*. Calcd for $C_{27}H_{29}BrN_4O_5S\cdot HCl\cdot H_2O$: C, 49.44; H, 4.92; N, 8.54. Found: C, 49.50; H, 4.69; N, 8.28.

The synthesis of compounds **11b** and **11c** was performed according to the same procedure as that for **11a**.

4′-Methoxy-2′-thienylacryloyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride (**11b**): The free base of **11b**: Yield 89%; mp 265—270 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.51 (1H, s), 8.17 (1H, br s), 7.79 (1H, d, J=15.2 Hz), 6.95 (1H, s), 6.69 (1H, d, J=14.9 Hz), 6.33 (1H, s), 4.51 (1H, br), 4.40 (1H, d, J=10.2 Hz), 4.27 (1H, dd, J=9.9, 8.6 Hz), 3.93 (3H, s), 3.81 (3H, s), 3.78 (1H, br d, J=10.1 Hz), 3.72 (2H, br), 3.62 (2H, br), 3.19 (1H, dd, J=10.2, 9.9 Hz), 2.48 (4H, br), 2.41 (3H, s), 2.33 (3H, s); IR (KBr) 1699, 1645, 1551, 1433, 1404, 1383, 1348, 1292, 1215, 1151, 1093 cm^{-1}; FAB-MS m/z 633, 631 $(M+H)^+$; FAB-HR-MS Calcd for $C_{28}H_{32}{}^{79}BrN_4O_6S$ $(M+H)^+$ m/z 631.1226, Found 631.1204.

11b: mp 240—250 °C (dec.); ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 12.09 (1H, s), 10.53 (1H, br), 8.08 (1H, br s), 7.61 (1H, d, J=14.9 Hz), 7.32 (1H, s), 6.87 (1H, d, J=14.9 Hz), 6.78 (1H, s), 4.28—4.56 (3H, m), 4.05—4.26 (2H, br), 3.84 (3H, s), 3.81—3.82 (1H, br), 3.77 (3H, s), 3.41—3.60 (7H, br), 2.85 (3H, s), 2.68 (3H, s); IR (KBr) 1716, 1697, 1647, 1551, 1435, 1406, 1221, 1157, 1093 cm^{-1}. *Anal*. Calcd for $C_{28}H_{31}BrN_4O_6S\cdot HCl\cdot 1.5\,H_2O$: C, 48.39; H, 5.08; N, 8.06. Found: C, 48.59; H, 4.84; N, 7.93.

3′-Methoxy-2′-thienylacryloyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride (**11c**): The free base of **11c**: Yield 73%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.23 (1H, br s), 8.19 (1H, br s), 7.80 (1H, d, J=14.8 Hz), 6.97 (1H, d, J=4.0 Hz), 6.40 (1H, d, J=14.8 Hz), 6.17 (1H, d, J=4.0 Hz), 4.53 (1H, br t, J=9.2 Hz), 4.39 (1H, d, J=10.2 Hz), 4.26 (1H, dd, J=9.9, 8.9 Hz), 3.95 (3H, s), 3.94 (3H, s), 3.79 (1H, dd, J=9.9, 2.3 Hz), 3.73 (2H, br), 3.62 (2H, br), 3.20 (1H, dd, J=10.2, 9.9 Hz), 2.51 (3H, s), 2.48 (4H, br), 2.35 (3H, s); IR (KBr) 1726, 1697, 1643, 1485, 1404, 1290, 1215, 1151, 1098 cm^{-1}; FAB-MS m/z 633, 631$(M+H)^+$; FAB-HR-MS Calcd for $C_{28}H_{32}{}^{79}BrN_4O_6S$ $(M+H)^+$ m/z 631.1226, Found 631.1216.

11c: mp 240—245 °C (dec.); ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 12.06 (1H, br s), 10.53 (1H, br), 8.07 (1H, br s), 7.64 (1H, d, J=15.2 Hz), 7.25 (1H, d, J=4.0 Hz), 6.50 (1H, d, J=14.9 Hz), 6.38 (1H, d, J=3.6 Hz), 4.10—4.52 (5H, m), 3.94 (3H, s), 3.84 (3H, s), 3.78—3.81 (1H, m), 2.86 (3H, s), 2.67 (3H, s); IR (KBr) 1716, 1697, 1637, 1541, 1437, 1417, 1406, 1250, 1219, 1095 cm^{-1}. *Anal*. Calcd for $C_{28}H_{31}BrN_4O_6S\cdot HCl\cdot 2.0\,H_2O$: C, 47.77; H, 5.15; N, 7.95. Found: C, 47.67; H, 4.42; N, 7.56.

p-Nitrophenyl 1-Methyl-4-[(*tert*-butoxycarbonyl)amino]-2-pyrrolecarboxylate (**13**): The synthesis of compound **13** was performed according to the same procedure as that for **8a**. Yield 91%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.29 (2H, d, J=8.6 Hz), 7.35 (2H, d, J=8.6 Hz), 7.21 (1H, br s), 6.91 (1H, d, J=1.6 Hz), 6.30 (1H, br s), 3.91 (3H, s), 1.51 (9H, s); IR (KBr) 3244, 1738, 1680, 1525, 1348, 1209, 1161, 1038 cm^{-1}; FAB-MS m/z 362 $(M+H)^+$. *Anal*. Calcd for $C_{17}H_{19}N_3O_6$: C, 56.51; H, 5.30; N, 11.63. Found: C, 56.73; H, 5.55; N, 11.68.

1′-Methyl-4′-[(*tert*-butoxycarbonyl)amino]-2′-pyrrolecarboxy A-Ring Pyrrole Duocarmycin (**14a**): The synthesis of compound **14a** was performed according to the same procedure as that for **10a**. Yield 62%; mp 205—215 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.03 (1H, s), 6.70 (1H, br), 6.40 (2H, s), 4.21 (1H, dd, J=10.9, 4.6 Hz), 4.08 (1H, d, J=10.9 Hz), 3.77 (3H, s), 3.76 (3H, s), 3.45—3.51 (1H, m), 2.54 (3H, s), 2.35 (1H, dd, J=7.6, 3.3 Hz), 1.45 (9H, s), 1.34 (1H, dd, J=5.0, 3.6 Hz); IR (KBr) 1705, 1701, 1653, 1608, 1587, 1450, 1381, 1296, 1269, 1215, 1159, 1107 cm^{-1}; FAB-MS m/z 481 $(M+H)^+$; FAB-HR-MS Calcd for $C_{25}H_{29}N_4O_6$ $(M+H)^+$ m/z 481.2087, Found 481.2115. *Anal*. Calcd for $C_{25}H_{28}N_4O_6\cdot 1.0\,H_2O\cdot 0.3\,CHCl_3$: C, 58.62; H, 5.89; N, 10.81. Found: C, 58.24; H, 5.62; N, 10.52.

1′-Methyl-4′-[1″-methyl-4″-[1‴-methyl-4‴-[(*tert*-butoxycarbonyl)-amino]-2‴-pyrrolecarboxyamido]-2″-pyrrolecarboxamido]-2′-pyrrolecarboxy A-Ring Pyrrole Duocarmycin (14b) Hydrobromic acid in MeOH (5%, 875 mg, 0.541 mmol) was added to a solution of **14a** (26.0 mg, 0.0541 mmol) in $ClCH_2CH_2Cl$ (2 ml), and the mixture was stirred at 50 °C for 4 h. Saturated $NaHCO_3$ was added, and the whole was extracted with $CHCl_3$. The usual work-up afforded 21.6 mg of **15**. Compound **12e** was added to a solution of **15** (21.6 mg, 0.0541 mmol), DECP (0.0123 ml, 0.0812 mmol) and Et_3N (0.0226 ml, 0.0162 mmol) in THF (1.3 ml), and the mixture was stirred at room temperature at 0 °C for 16 h. Phosphoric buffer (0.01 M, pH 7) was added, the whole was extracted with AcOEt, then worked up as usual. The residue was purified by PTLC ($CHCl_3$–MeOH, 12 : 1) to give 17.5 mg (45%) of **14b:** mp 235—240 °C; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.81 (1H, br s), 8.61 (1H, br s), 7.30 (1H, s), 7.15 (1H, br s), 7.05 (1H, br s), 6.75 (1H, br s), 6.69 (1H, br s), 6.58 (1H, s), 6.55 (1H, br s), 6.36 (1H, s), 4.20 (1H, dd, J=11.1, 4.5 Hz), 4.06 (1H, d, J=10.6 Hz), 3.82 (3H, s), 3.80 (3H, s), 3.75 (3H, s), 3.74 (3H, s), 3.42—3.50 (1H, m), 2.49 (3H, s), 2.32 (1H, dd, J=7.8, 3.5 Hz), 1.43 (9H, s), 1.32 (1H, dd, J=4.9, 4.0 Hz); IR (KBr) 1701, 1653, 1635, 1616, 1583, 1458, 1398, 1381, 1263, 1161 cm^{-1}; FAB-MS m/z 725 $(M+H)^+$; FAB-HR-MS Calcd for $C_{37}H_{41}N_8O_8$ $(M+H)^+$ m/z 725.3047, Found 725.3064.

1′-Methyl-4′-[1″-methyl-4″-(1‴-methyl-4‴-nitro-2‴-pyrrolecarboxyamido)-2″-pyrrolecarboxamido]-2′-pyrrolecarboxy A-Ring Pyrrole Duocarmycin (**14c**): The synthesis of compound **14c** was performed according to the same procedure as that for **14b**: Yield 36%; mp 230—240 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.55 (1H, d, J=2.0 Hz), 7.31 (2H, br), 7.24—7.26 (2H, br), 7.19 (1H, s), 6.70 (1H, s), 6.57 (1H, d, J=2.0 Hz), 6.29 (1H, s), 4.21 (1H, dd, J=11.2, 4.6 Hz), 4.07 (1H, d, J=11.2 Hz), 3.97 (3H, s), 3.87 (3H, s), 3.77 (3H, s), 3.76 (3H, s), 3.49—3.53 (1H, m), 2.51 (3H, s), 2.38 (1H, dd, J=7.3, 3.3 Hz), 1.38 (1H, dd, J=4.6, 4.0 Hz); IR (KBr) 1701, 1684, 1653, 1647, 1578, 1560, 1437, 1381, 1309, 1257, 1109 cm^{-1}; FAB-MS m/z; 655 $(M+H)^+$; FAB-HR-MS Calcd for $C_{32}H_{31}N_8O_8$ $(M+H)^+$ m/z 655.2265, Found 655.2269. *Anal*. Calcd for $C_{32}H_{30}N_8O_8\cdot 0.2\,H_2O$: C, 52.07; H, 4.15; N, 15.18. Found: C, 52.50; H, 4.55; N, 14.11.

1′-Methyl-4′-[1″-methyl-4″-[1‴-methyl-4‴-[(*tert*-butoxycarbonyl)amino]-2‴-pyrrolecarboxyamido]-2″-pyrrolecarboxamido]-2′-pyrrolecarboxy 8-*O*-Acetate A-Ring Pyrrole Duocarmycin B2 (16) Hydrobromic acid in MeOH (5%, 117 mg, 0.0723 mmol) was added to a solution of **14b** (17.5 mg, 0.0241 mmol) in CH_3CN (1.19 ml), and the mixture was stirred at room temperature for 60 min. Then, the reaction mixture was concentrated under reduced pressure. To a solution of the residue in dry CH_2Cl_2 (1.19 ml) were added Ac_2O (0.0070 ml, 0.075 mmol) and DMAP (9.41 mg, 0.0771 mmol), and the mixture was stirred at 0 °C for 2 h and 10 min. Phosphoric buffer (0.01 M, pH 7) was added, then the whole was extracted with $CHCl_3$ and worked up as usual. The residue was purified by PTLC ($CHCl_3$–MeOH, 12 : 1) to give 15.1 mg (74%) of **16**: mp 220—230 °C; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.82 (1H, br s), 8.27 (1H, br s), 7.74 (1H, br s), 7.26 (1H, s), 7.08 (1H, s), 7.08 (1H, d, J=1.7 Hz), 6.70 (1H, s), 6.63 (1H, s), 6.56 (1H, br s), 4.31 (1H, d, J=10.9 Hz), 4.12—4.22 (1H, m), 4.05 (1H, dd, J=9.6, 9.2 Hz), 3.84 (3H, s), 3.83 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 3.63 (1H, dd, J=9.6, 2.0 Hz), 3.22 (1H, dd, J=9.2, 9.2 Hz), 2.43 (3H, s), 2.32 (3H, s), 1.45 (9H, s); IR (KBr) 1697, 1641, 1587, 1541, 1489, 1443, 1404, 1367, 1252, 1205, 1190, 1107 cm^{-1}; FAB-MS m/z 849, 847 $(M+H)^+$; FAB-HR-MS Calcd for $C_{39}H_{44}{}^{79}BrN_8O_9$ $(M+H)^+$ m/z 847.2415, Found 847.2443. *Anal*. Calcd for $C_{39}H_{43}BrN_8O_9\cdot 0.7\,CHCl_3$: C, 51.20; H, 4.73; N, 12.03. Found: C, 51.32; H, 4.89; N, 11.73.

3-[1-Methyl-4-[(*tert*-butoxycarbonyl)amino]-2-pyrrolyl]acrylic Acid

(17a) To a solution of **12a** (50.0 mg, 0.197 mmol) in THF (3 ml) was added a solution of $LiAlH_4$ (22.4 mg, 0.591 mmol) in THF (1.5 ml), and the mixture was stirred at room temperature for 2 h and 10 min. The reaction mixture was quenched by the addition of H_2O, and the whole was extracted with AcOEt. The usual work-up afforded 35.3 mg (79%) of 1-methyl-2-hydroxymethyl-4-[*N*-(*tert*-butyloxycarbonyl)amino]pyrrole. MnO_2 (969 mg, 11.1 mmol) was added to a solution of 1-methyl-2-hydroxymethyl-4-[*N*-(*tert*-butyloxycarbonyl)amino]pyrrole (126 mg, 0.557 mmol) in Et_2O (7 ml), and the mixture was stirred at room temperature for 1 h and 30 min. The whole was filtered through Celite. Then, the filtrate was concentrated under reduced pressure to give 98.4 mg (79%) of 1-methyl-4-[*N*-(*tert*-butyloxycarbonyl)amino]pyrrole-2-carboxyaldehyde. The synthesis of compound **17a** was performed according to the same procedure as that for **8b**: Yield 72%; ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 7.61 (1H, d, J=15.4 Hz), 7.03 (1H, d, J=0.7 Hz), 6.42 (1H, d, J=1.3 Hz), 6.35 (1H, br), 6.05 (1H, d, J=15.4 Hz), 3.66 (3H, s), 1.50 (9H, s).

The synthesis of compounds **17b** and **17c** was performed according to the same procedure as that for **17a**.

3-[1-Methyl-4-[1-methyl-4-[(*tert*-butoxycarbonyl)amino]-2-pyrrolecarboxyamido]-2-pyrrolyl]acrylic Acid (**17b**): Yield 34%; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.51 (1H, d, J=15.5 Hz), 7.30 (1H, d, J=1.7 Hz), 7.29 (1H, br s), 7.28 (1H, br s), 6.76 (1H, br s), 6.58 (1H, br s), 6.52 (1H, d, J=1.7 Hz), 6.02 (1H, d, J=15.8 Hz), 3.83 (3H, s), 3.62 (3H, s), 1.44 (9H, s); FAB-MS m/z 389 $(M+H)^+$.

3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[(*tert*-butoxycarbonyl)amino]-2-pyrrolecarboxyamido]-2-pyrrolecarboxyamido]-2-pyrrolyl]acrylic Acid (**17c**): Yield 38%; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.79 (1H, br), 8.69 (1H, br), 7.58 (1H, d, J=15.5 Hz), 7.38 (1H, d, J=1.3 Hz), 7.14 (1H, d, J=1.7 Hz), 7.14 (1H, br), 6.82 (1H, d, J=2.0 Hz), 6.81 (1H, br s), 6.66 (1H, br s), 6.61 (1H, d, J=1.3 Hz), 6.09 (1H, d, J=15.5 Hz), 3.93 (3H, s), 3.90 (3H, s), 3.69 (3H, s), 1.51 (9H, s); IR (KBr) 1691, 1651, 1587, 1437, 1400, 1255, 1163 cm^{-1}; FAB-MS m/z 511 $(M+H)^+$. *Anal*. Calcd for $C_{25}H_{30}N_6O_6\cdot 1.8\,H_2O$: C, 55.30; H, 6.24; N, 15.48. Found: C, 55.43; H, 6.25; N, 14.94.

(*E*)-*p*-Nitrophenyl 1-Methyl-4-[(*tert*-butoxycarbonyl)amino]-2-pyrrolylacrylate (**18**): The synthesis of compound **18** was performed according to the same procedure as that for **8a**: Yield 47%; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.28 (2H, d, J=8.9 Hz), 7.71 (1H, d, J=15.5 Hz), 7.34 (2H, d, J=8.9 Hz), 7.12 (1H, br s), 6.51 (1H, d, J=1.7 Hz), 6.28 (1H, br s), 6.21 (1H, d, J=15.2 Hz), 3.70 (3H, s), 1.51 (9H, s); IR (KBr) 3390, 1697, 1593, 1524, 1338, 1290, 1194, 1159 cm^{-1}; FAB-MS m/z 388 $(M+H)^+$. *Anal*. Calcd for $C_{19}H_{21}N_3O_6\cdot 1.0\,H_2O$: C, 56.29; H, 5.72; N, 10.36. Found: C, 56.07; H, 5.77; N, 10.56.

1′-Methyl-4′-[1″-methyl-4″-[(*tert*-butoxycarbonyl)amino]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl 8-Hydroxy A-Ring Pyrrole Duocarmycin C2 (19b) 6.86 N HCl in EtOH (0.226 ml, 1.55 mmol) was added to a solution of **9** (40.0 mg, 0.155 mmol) in AcOEt (2.2 ml), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure. To a solution of the residue in DMF (2 ml) was added **17b** (72.2 mg, 0.186 mmol) and EDCI (88.9 mg, 0.465 mmol), and the mixture was stirred at room temperature for 17 h and 30 min. Phosphoric buffer (0.01 M, pH 7) was added, then the whole was extracted with AcOEt and worked up as usual. The residue was purified by PTLC ($CHCl_3$–MeOH, 10 : 1) to give 32 mg (31%) of **19b**: mp 180—190 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.74 (1H, br s), 7.69 (1H, s), 7.58 (1H, d, J=14.9 Hz), 7.31 (1H, br s), 7.18 (1H, s), 6.75 (1H, s), 6.67 (1H, s), 6.55 (1H, s), 6.52 (1H, d, J=15.5 Hz), 4.30 (1H, d, J=15.5 Hz), 4.24—4.33 (1H, br), 4.14 (1H, dd, J=10.2, 8.9 Hz), 3.83 (3H, s), 3.78—3.83 (1H, m), 3.80 (3H, s), 3.63 (3H, s), 3.19 (1H, dd, J=10.6, 10.2 Hz), 2.59 (3H, s), 1.41 (9H, s); IR (KBr) 1657, 1635, 1585, 1489, 1446, 1404, 1367, 1296, 1248, 1161, 1093 cm^{-1}; FAB-MS m/z 667, 665 $(M+H)^+$; FAB-HR-MS Calcd for $C_{33}H_{38}{}^{35}ClN_6O_7$ $(M+H)^+$ m/z 665.2491, Found 665.2482. *Anal*. Calcd for $C_{33}H_{37}ClN_6O_7\cdot 1.0\,H_2O\cdot 0.3\,CHCl_3$: C, 55.63; H, 5.51; N, 11.69. Found: C, 55.52; H, 5.38; N, 11.06.

1′-Methyl-4′-[1″-methyl-4″-[1‴-methyl-4‴-[(*tert*-butoxycarbonyl)amino]-2‴-pyrrolecarboxyamido]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl 8-Hydroxy A-Ring Pyrrole Duocarmycin C2 (**19c**): The synthesis of compound **19c** was performed according to the same procedure as that for **19b**: Yield 28%; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.50 (1H, s), 8.47 (1H, s), 7.96 (1H, s), 7.55 (1H, d, J=15.2 Hz), 7.14 (1H, s), 7.08 (1H, s), 7.04 (1H, s), 6.76 (1H, s), 6.72 (1H, s), 6.62 (1H, s), 6.56 (1H, s), 6.42 (1H, d, J=14.8 Hz), 4.22—4.32 (1H, m), 4.24 (1H, d, J=10.9 Hz), 4.11—4.15 (1H, m), 3.86 (3H, s), 3.83 (6H, br s), 3.73—3.80 (1H, br), 3.18 (1H, dd, J=10.2, 10.2 Hz), 2.60 (3H, s) 1.46 (9H, s); FAB-MS m/z 789, 787 $(M+H)^+$.

1′-Methyl-4′-[(*tert*-butoxycarbonyl)amino]-2′-pyrrolylacryloyl A-Ring Pyrrole Duocarmycin (**20a**): The synthesis of compound **20a** was performed according to the same procedure as that for **10a**: Yield 59%; mp 180—190 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 11.38 (1H, br s), 7.68 (1H, d, J=15.2 Hz), 7.01 (1H, br s), 6.79 (1H, br), 6.50 (1H, s), 6.48 (1H, d, J=14.8 Hz), 6.34 (1H, br s), 4.19 (1H, d, J=10.9 Hz), 4.13 (1H, dd, J=10.9, 4.3 Hz), 3.81 (3H, s), 3.67 (3H, s), 3.55—3.57 (1H, m), 2.61 (3H, s), 2.35 (1H, dd, J=7.4, 3.5 Hz), 1.50 (9H, s), 1.28 (1H, dd, J=4.9, 3.6 Hz); IR (KBr) 1701, 1684, 1653, 1616, 1608, 1576, 1387, 1290, 1244, 1219, 1163 cm^{-1}; FAB-MS m/z 507 $(M+H)^+$; FAB-HR-MS Calcd for $C_{27}H_{31}N_4O_6$ $(M+H)^+$ m/z 507.2244, Found 507.2256. *Anal*. Calcd for $C_{27}H_{30}N_4O_6\cdot 0.4\,CHCl_3$: C, 59.37; H, 5.53; N, 10.11. Found: C, 59.32; H, 5.84; N, 10.25.

1′-Methyl-4′-[1″-methyl-4″-[(*tert*-butoxycarbonyl)amino]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl A-Ring Pyrrole Duocarmycin (20b) A solution of **19b** (12.6 mg, 0.0194 mmol) in CH_3CN (1.1 ml) and pyridine (0.5 ml) was treated with DBU (0.0145 ml, 0.0970 mmol), and the mixture was stirred at room temperature for 2 h and 40 min. Phosphoric buffer (0.01 M, pH 7) was added, then the whole was extracted with $CHCl_3$ and worked up as usual. The residue was purified by PTLC ($CHCl_3$–MeOH, 10 : 1) to give 8.4 mg (69%) of **20b**: mp 200—220 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.87 (1H, s), 7.61 (1H, d, J=15.2 Hz), 7.34 (1H, s), 6.78 (1H, s), 6.64 (2H, br s), 6.58—6.62 (1H, br), 6.54 (1H, s), 6.43 (1H, d, J=15.2 Hz), 4.13 (1H, d, J=10.9 Hz), 4.05 (1H, dd, J=10.9, 4.6 Hz), 3.79 (3H, s), 3.73 (3H, s), 3.61 (3H, s), 3.46—3.52 (1H, m), 2.48 (3H, s), 2.29 (1H, dd, J=7.4, 3.5 Hz), 1.41 (9H, s), 1.22 (1H, dd, J=4.3, 4.0 Hz); IR (KBr) 1705, 1651, 1605, 1564, 1452, 1387, 1288, 1244, 1217, 1165 cm^{-1}; FAB-MS m/z 629 $(M+H)^+$; FAB-HR-MS Calcd for $C_{33}H_{37}N_6O_7$ $(M+H)^+$ m/z 629.2723, Found 629.2700. *Anal*. Calcd for $C_{33}H_{36}N_6O_7\cdot 0.6\,CHCl_3$: C, 57.63; H, 5.27; N, 12.00. Found: C, 57.98; H, 5.59; N, 11.63.

1′-Methyl-4′-[1″-methyl-4″-[1‴-methyl-4‴-[(*tert*-butoxycarbonyl)amino]-2‴-pyrrolecarboxyamido]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl A-Ring Pyrrole Duocarmycin (**20c**): The synthesis of compound **20c** was performed according to the same procedure as that for **20b**: Yield 62%; mp 220—230 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.91 (1H, s), 8.73 (1H, s), 7.60 (1H, d, J=14.9 Hz), 7.27 (1H, s), 7.26 (1H, s), 7.11 (1H, s), 6.76 (1H, s), 6.74 (1H, d, J=1.7 Hz), 6.68 (1H, br), 6.65 (1H, s), 6.58 (1H, s), 6.40 (1H, d, J=14.9 Hz), 4.12 (1H, d, J=10.6 Hz), 4.06 (1H, dd, J=11.1, 4.5 Hz), 3.83 (3H, s), 3.80 (3H, s), 3.74 (3H, s), 3.59 (3H, s), 3.47—3.54 (1H, m), 2.48 (3H, s), 2.29 (1H, dd, J=7.4, 3.5 Hz), 1.42 (9H, s), 1.22 (1H, dd, J=4.6, 3.6 Hz); IR (KBr) 1701, 1653, 1605, 1560, 1489, 1458, 1389, 1286, 1244, 1167 cm^{-1}; FAB-MS m/z 751 $(M+H)^+$; FAB-HR-MS Calcd for $C_{39}H_{43}N_8O_8$ $(M+H)^+$ m/z 751.3204, Found 751.3208. *Anal*. Calcd for $C_{39}H_{42}N_8O_8\cdot 1.0\,H_2O\cdot 0.6\,CHCl_3$: C, 56.59; H, 5.35; N, 13.33. Found: C, 56.93; H, 5.49; N, 12.78.

1′-Methyl-4′-[(*tert*-butoxycarbonyl)amino]-2′-pyrrolylacryloyl 8-Acetate A-Ring Pyrrole Duocarmycin B2 (21a) Hydrobromic acid in MeOH (5%, 63.8 mg, 0.0394 mmol) was added to a solution of **20a** (10 mg, 0.0197 mmol) in CH_2Cl_2 (0.6 ml), and the mixture was stirred at −20 °C for 20 min. Then, Ac_2O (0.0056 ml, 0.059 mmol) and DMAP (7.2 mg, 0.059 mmol) were added to the reaction mixture. After stirring at −20 °C for 45 min, phosphoric buffer (0.01 M, pH 7) was added, the whole was extracted with $CHCl_3$, and worked up as usual. The reside was purified by PTLC ($CHCl_3$–MeOH, 25 : 1) to give 10.3 mg (83%) of **21a**: mp 170—175 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.76 (1H, br s), 8.26 (1H, s), 7.22 (1H, d, J=15.9 Hz), 6.63 (1H, s), 6.43 (1H, d, J=1.7 Hz), 6.28 (1H, d, J=14.8 Hz), 6.18 (1H, s), 4.43—4.53 (1H, m), 4.23—4.29 (2H, m), 3.96 (3H, s), 3.76 (1H, dd, J=9.6, 2.6 Hz), 3.44 (3H, s), 3.38 (1H, dd, J=10.2, 10.1 Hz), 2.51 (3H, s), 2.38 (3H, s), 1.54 (9H, s); IR (KBr) 1716, 1697, 1686, 1576, 1414, 1400, 1367, 1200, 1188, 1159 cm^{-1}; FAB-MS m/z 631, 629 $(M+H)^+$; FAB-HR-MS Calcd for $C_{29}H_{34}{}^{79}BrN_4O_7$ $(M+H)^+$ m/z 629.1611, Found 629.1628.

1′-Methyl-4′-[1″-methyl-4″-[(*tert*-butoxycarbonyl)amino]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl 8-Acetate A-Ring Pyrrole Duocarmycin C2 (21b) Ac_2O (0.0085 ml, 0.090 mmol) and DMAP (11 mg, 0.090 mmol) were added to a solution of **19b** (20.0 mg, 0.0301 mmol) in CH_2Cl_2 (2.2 ml), and the mixture was stirred at 0 °C for 65 min. Phosphoric buffer (0.01 M, pH 7) was added, then the whole was extracted with $CHCl_3$ and worked up as usual. The reside was purified by PTLC ($CHCl_3$–MeOH, 12 : 1) to give 18.4 mg (86%) of **21b**: mp 210—230 °C (dec.); ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 8.62 (1H, s), 8.07 (1H, br s), 7.56 (1H, d, J=14.9 Hz), 7.22 (1H, br s), 7.14 (1H, s), 6.75 (1H, s), 6.62 (1H, s), 6.53 (1H, s), 6.47 (1H, d, J=15.2 Hz), 4.32—4.43 (1H, m), 4.32 (1H, d, J=9.9 Hz), 4.18 (1H, dd, J=8.9, 8.9 Hz), 3.86 (3H, s), 3.81 (3H, s), 3.80—3.84 (1H, m), 3.59 (3H, s), 3.23 (1H, dd, J=10.9, 10.2 Hz), 2.56 (3H, s), 2.30 (3H, s), 1.43 (9H ,s); IR (KBr) 1697, 1637, 1576, 1444, 1402, 1367, 1348, 1205, 1163, 1090 cm^{-1}; FAB-MS m/z 709, 707 $(M+H)^+$; FAB-HR-MS

Calcd for $C_{35}H_{40}{}^{35}ClN_6O_8$ $(M+H)^+$ *m/z* 707.2596, Found 707.2623. *Anal*. Calcd for $C_{35}H_{39}ClN_6O_8 \cdot 0.6\,CHCl_3$: C, 54.90; H, 5.12; N, 10.79. Found: C, 55.29; H, 5.31; N, 10.54.

1′-Methyl-4′-[1″-methyl-4″-[1‴-methyl-4‴-[(*tert*-butoxycarbonyl)amino]-2‴-pyrrolecarboxyamido]-2″-pyrrolecarboxyamido]-2′-pyrrolylacryloyl 8-Acetate A-Ring Pyrrole Duocarmycin C2 (**21c**): The synthesis of compound **21c** was performed according to the same procedure as that for **21b**: Yield 74%; mp 220—230 °C; ^{1}H-NMR (270 MHz, $CDCl_3+CD_3OD$) δ: 8.63 (1H, s), 8.41 (1H, s), 8.13 (1H, s), 7.52 (1H, d, J=14.8 Hz), 7.12 (1H, s), 7.06 (1H, s), 7.05 (1H, s), 6.68 (1H, s), 6.66 (1H, s), 6.56 (1H, s), 6.49 (1H, s), 6.43 (1H, d, J=15.2 Hz), 4.28—4.40 (1H, m), 4.28 (1H, d, J=11.6 Hz), 3.86 (3H, s), 3.82 (3H, s), 3.79 (3H, s), 3.75 (1H, br), 3.48 (3H, s), 3.17 (1H, dd, J=10.2, 10.2 Hz), 2.49 (3H, s), 2.34 (3H, s), 1.46 (9H, s); IR (KBr) 1695, 1645, 1585, 1446, 1402, 1367, 1250, 1205, 1163, 1092 cm^{-1}; FAB-MS *m/z* 829 $(M+H)^+$; FAB-HR-MS Calcd for $C_{41}H_{46}{}^{35}ClN_8O_9$ $(M+H)^+$ *m/z* 829.3076, Found 829.3065. *Anal*. Calcd for $C_{41}H_{45}ClN_8O_9 \cdot 0.5\,H_2O \cdot 0.5\,CHCl_3$: C, 55.51; H, 5.22; N, 12.48. Found: C, 55.87; H, 5.38; N, 11.97.

1′-Methyl-4′-[(*tert*-butoxycarbonyl)amino]-2′-pyrrolylacryloyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 (22) Hydrobromic acid in MeOH (5%, 160 mg, 0.0988 mmol) was added to a solution of **20a** (25.0 mg, 0.0494 mmol) in CH_2Cl_2 (1.25 ml), and the mixture was stirred at −20 °C for 20 min. The reaction mixture was treated with *p*-nitrophenyl chloroformate (29.9 mg, 0.148 mmol) and Et_3N (0.0207 ml, 0.148 mmol), then the mixture was stirred at −78 °C for 20 min. Next, *N*-methypiperazine (0.0192 ml, 0.173 mmol) was added, and the mixture was stirred at 0 °C for 30 min. The mixture was diluted with $CHCl_3$, and washed with aqueous $NaHCO_3$. After the usual work-up, the residue was purified by PTLC ($CHCl_3$–MeOH, 12 : 1) to give 25.8 mg (73%) of **22**: mp 175—180 °C; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.52 (1H, s), 8.20 (1H, s), 7.61 (1H, d, J=14.9 Hz), 6.88 (1H, s), 6.55 (1H, s), 6.52 (1H, d, J=14.9 Hz), 6.35 (1H, s), 4.45—4.56 (1H, m), 4.38 (1H, d, J=10.2 Hz), 4.25 (1H, dd, J=10.2, 8.9 Hz), 3.94 (3H, s), 3.77 (1H, dd, J=9.9, 2.3 Hz), 3.60 (3H, s), 3.57—3.75 (4H, br), 3.22 (1H, dd, J=10.2, 9.9 Hz), 2.48 (7H, br s), 2.33 (3H, s), 1.51 (9H, s); IR (KBr) 1701, 1576, 1446, 1412, 1402, 1385, 1292, 1238, 1217, 1157 cm^{-1}; FAB-MS *m/z* 715, 713 $(M+H)^+$; FAB-HR-MS Calcd for $C_{33}H_{42}{}^{79}BrN_6O_7$ $(M+H)^+$ *m/z* 713.2298, Found 713.2319. *Anal*. Calcd for $C_{33}H_{41}BrN_6O_7 \cdot 2\,H_2O$: C, 52.87; H, 6.05; N, 11.21. Found: C, 52.90; H, 5.74; N, 11.02.

Interaction of 14b, 20c and 1h with Calf Thymus DNA The interaction of the duocarmycin derivatives with calf thymus DNA were examined by chromatography on a UNISIL pack 5C18 reversed-phase HPLC column (GL Science Co., Ltd., Tokyo, Japan). Calf thymus DNA from Sigma (16.1 mg) was dissolved in 0.01 M phosphate buffer (pH 7, 50 ml). The test compounds were dissolved in DMF to give 6.10×10^{-4} M drug concentration. To this drug solution (9 ml) was added the buffer solution (1 ml) containing calf thymus DNA (or no DNA as the control), and the mixture was kept at 35 °C. Samples were collected at intervals and injected directly into the HPLC injection port. The compound was eluted with 0.05 M phosphate buffer (pH 5.9)–acetonitrile (30 : 70) and detected by measuring the absorbance at 330 nm.

Biological Studies Human uterine cervix carcinoma HeLa S_3 cells were obtained from the American Type Culture Collection through Dainippon Pharmaceutical Co. (Osaka, Japan). The cells (2×10^4/well) were precultured in the culture medium in 24-well multidishes (Nunc, Roskilde, Denmark) for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. For the pulse exposure experiment, cells were treated with each compound for 1 h, washed with Dulbecco's phosphate-buffered saline [Ca^{2+}- and Mg^{2+}-free, PBS(−)], and further incubated in fresh medium for 71 h. For the continuous exposure experiment, cells were treated with each compound for 72 h. Then, cells were treated with PBS(−) containing 0.05% trypsin (Difco Laboratories, Detroit, MI) and 0.02% EDTA (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan) and counted using a Microcell Counter (CC-180A, Toa Medical Electronics Co., Ltd., Kobe, Japan). The IC_{50} values (drug concentration required for 50% inhibition of the cell growth) were determined.

Sarcoma 180 cells were kindly supplied by the National Cancer Center (Tokyo, Japan). Sarcoma 180 cells were passaged and used for the experiment in adult male ddY mice. Murine solid tumor was inoculated subcutaneously (s.c.) at the axillary region of mice. Drugs were administered intravenously (i.v.) beginning 1 d after tumor inoculation. The antitumor efficacy is expressed as T/C, where T and C are the values of mean tumor volume of the treated and control mice. The length and width of the tumors were measured, and tumor volume was calculated as

$$\text{tumor volume (mm}^3\text{)} = \text{length (mm)} \times [\text{width (mm)}]^2/2$$

according to the method of the National Cancer Institute.[25]

The criteria for effectiveness against murine solid tumors was the percentage T/C values with 42% and less, and statistical significance was determined by the Mann-Whitney *U* test ($p<0.05$). Drug efficacy against human xenografts was expressed as the percentage of mean V/V_0 value against that of the control group, where V is the tumor volume on the day of evaluation and V_0 is the tumor volume on the day of initial drug treatment. The criteria for effectiveness was T/C values of 50% and less, and statistical significance was determined by the Mann–Whitney *U* test ($p<0.01$, one-sided).[26]

Hematotoxicity (Effect of Compounds on Peripheral Blood (PB) Platelet Counts and White Blood Cell Counts) Effect on PB Platelet Counts: Each drug was dissolved with saline and was administered into the tail vein of normal male ddY mice (mean weight 20±1 g). After 7 d, peripheral blood was obtained from the orbital vein to measure the platelet counts using a microcell counter (CC-180A, Toa Medical Electronics Co., Ltd., Kobe, Japan). Results are presented as the percentage of the absolute value of the treated group *versus* that of the control (percent of control).

Effect on PB White Blood Cell Counts: Drugs were administered intravenously (i.v.) beginning 1 d after tumor inoculation. After 4 d, peripheral blood was obtained from the orbital vein of tumor-bearing mice to measure the white blood cell counts using a microcell counter (CC-180A, Toa Medical Electronics Co., Ltd., Kobe, Japan). Results are presented as a percentage of the absolute value of the treated group *versus* that of the control (percent of control).

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