New Water-Soluble Duocarmycin Derivatives: Synthesis and Antitumor Activity of A-Ring Pyrrole Compounds Bearing β-Heteroarylacryloyl Groups

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A series of A-ring pyrrole compounds of duocarmycin bearing 4′-methoxy-β-heteroarylacryloyl 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 4′-methoxy-β-heteroarylacrylates displayed in vitro anticellular activity equivalent to that of 4′-methoxycinnamates. Among the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxy-β-heteroarylacrylates, compound **15b** having a (4-methoxy-3,5-pyrimidinyl)acryloyl as segment-B (Seg-B) showed remarkably potent in vivo antitumor activity and low peripheral blood toxicity compared with the A-ring pyrrole derivatives having the trimethoxyindole skeleton in Seg-B, which were equal to 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxycinnamates. Moreover, these 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxy-β-heteroarylacrylates had high aqueous solubility.

Introduction

Duocarmycins (A, **1a**; SA, **1b**; B1, **1e**; B2, **1c**; C1, **1f**; C2, **1d**) are novel antitumor antibiotics isolated from the culture broth of *Steptomyces* sp. (Chart 1).[1−3] Since duocarmycins B1 (**1e**), B2 (**1c**), C1 (**1f**), and C2 (**1d**) readily yield duocarmycin A (**1a**)[1a−c] in aqueous solution, duocarmycin A is thought to be an active form among these antibiotics. Duocarmycins A and SA (**1b**) have a unique cyclopropane ring responsible for the sequence-selective alkylation of double-stranded DNA mediating N3 adenine covalent adduct formation.[4] This mechanism is similar to that of CC-1065 (**2**) which has been reported to show a high cytotoxicity.[5,6] KW-2189 (**3b**),[7] selected as the best compound in analogues of A-ring pyrrole derivatives of duocarmycin B2, showed good stability in the culture medium and aqueous solubility greater than 10 mg/mL.[8,9] It also showed strong activities against murine ascitic and human solid tumors.[7b] KW-2189 (**3b**) was designed as a prodrug which requires enzymatic hydrolysis followed by regeneration of DU-86 (**3a**) as an active metabolite.[10] KW-2189 (**3b**) is currently under phase II clinical evalution.

The segment-A (Seg-A) containing a spirocyclopropylhexadienone moiety is necessary for the formation of covalent binding with DNA. Our previous results indicate that the Seg-A structure will influence the electrophilicity of cyclopropane.[7c] On the other hand, the segment-B (Seg-B) of duocarmycin has been considered to play an important role for noncovalent binding to the minor groove of DNA.[11] We have previously synthesized a series of duocarmycin analogues bearing a simplified DNA noncovalent binding segment such as acetyl, indol-2-ylcarbonyl, benzofuran-2-ylcarbonyl, cinnamoyl, and phenoxyacetyl groups to increase antitumor activity and decrease toxicity. Among them, the cinnamoyl and phenoxyacetyl derivatives were more potent and had lower toxicity than benzofuran-2-ylcarbonyl or indol-2-ylcarbonyl derivatives including the trimethoxyindole derivative, the natural type of Seg-B.[12] In previous papers,[13] we have reported the structure−activity relationship of 4′-substituted-*trans*-cinnamoyl derivatives. Among derivatives modified at Seg-B of KW-2189 (**3b**), compound **4b** was found to exhibit sufficient in vivo activity against sarcoma 180 with low peripheral blood toxicity.[12] The reduction of toxicity of compound **4b**, of which Seg-B is 4-methoxycinnamoyl, is considered to result from the decrease of noncovalent binding to DNA and the increase of reversibility of covalent bonding to DNA.[7c,14] In the same manner, it is shown that the derivatives modified at the noncovalent binding moiety of CC-1065 have excellent broad-spectrum in vivo antitumor activity without causing delayed deaths.[15]

However, the aqueous solubility of compound **4b** is below 0.1 mg/mL. Due to its poor solubility, compound **4b** is difficult to use in clinical studies. We have previously synthesized a variety of analogues by modifying the substituent on the aromatic ring of the 4′-methoxycinnamoyl group.[3d,16] The 8-*O*-(*N*,*N*-dialkylcarbamoyl)cinnamates having an amino group at the 3′-position were found to possess adequate water solubility in excess of 10 mg/mL.[13]

Recently, it has been reported that several efforts are being made to modify DNA sequence selectivity of the distamycin molecule, one of the DNA minor groove binders. Structural modifications have been based on replacement of *N*-methylpyrrole by an *N*-methylimidazole to accommodate hydrogen bonding to the 2-amino group of guanine. The result was that imidazole-containing analogues of distamycin changed AT sequence specificity to GC in the DNA minor groove.[17]

It is expected that our approach to synthesize new Seg-B analogues having β-heteroarylacryloyl groups may enhance potency and decrease toxicity by noncovalent binding site changes attributable to an interaction between nitrogen on the heteroaryl and DNA. Moreover, it is expected that these new analogues could

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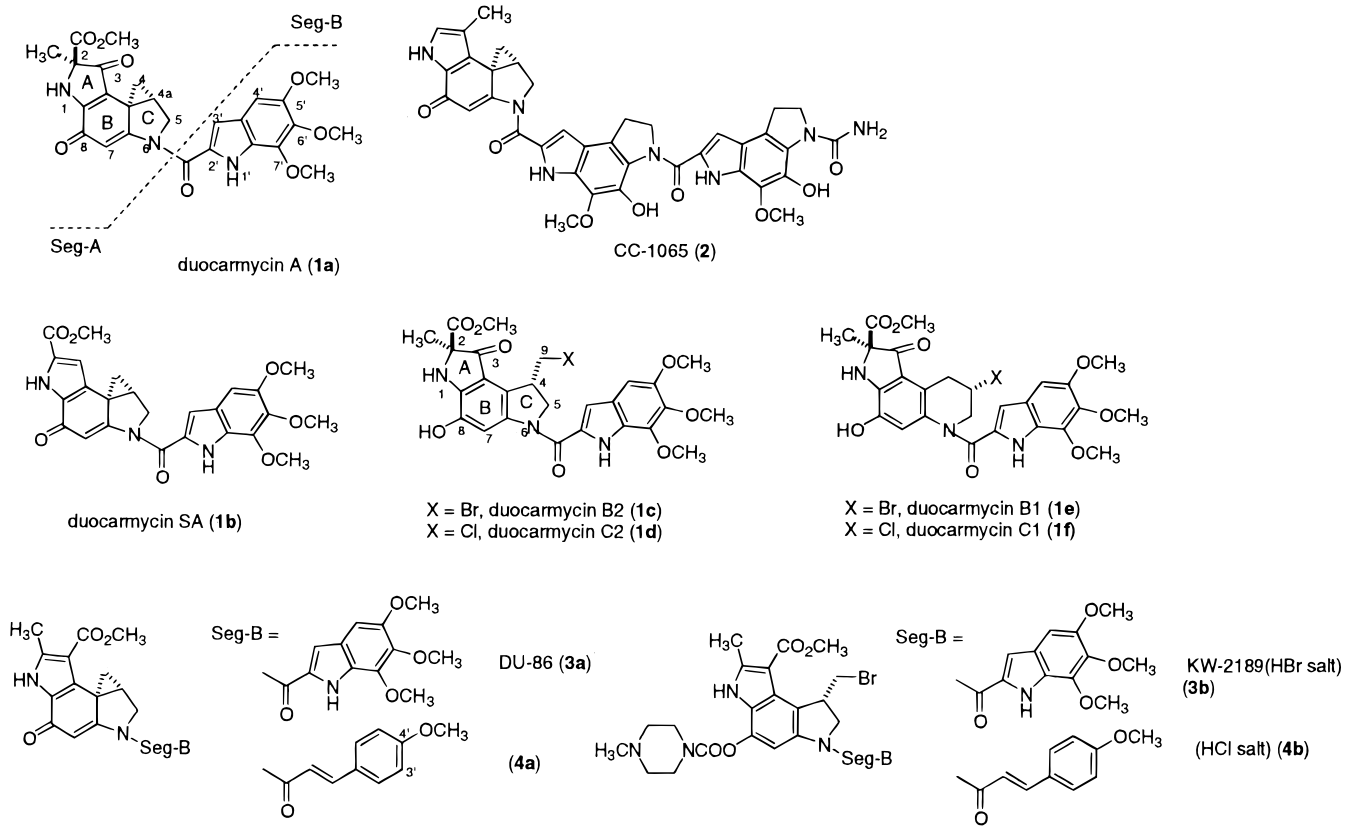
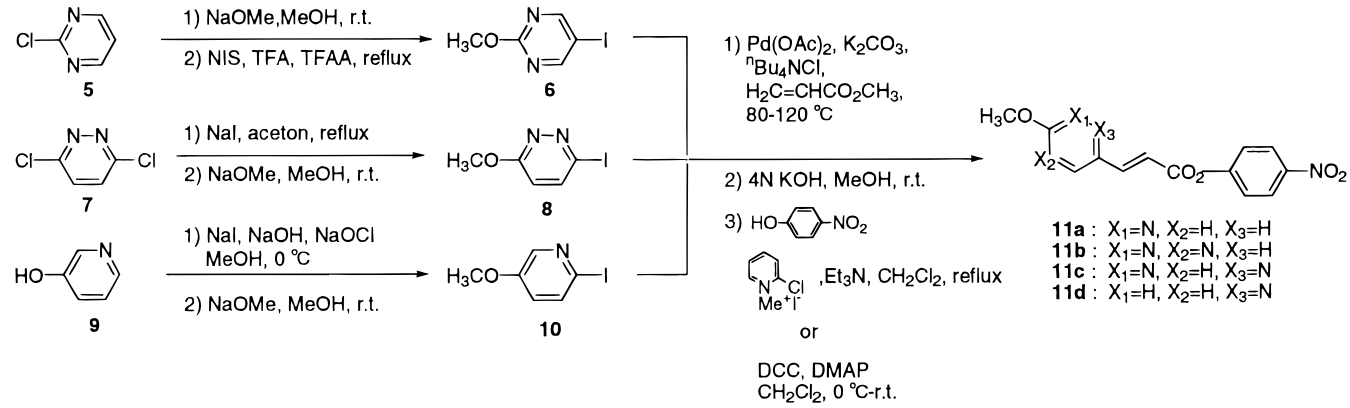
Chart 1. Structures of Duocarmycins, CC-1065, and Duocarmycin Derivatives



Scheme 1



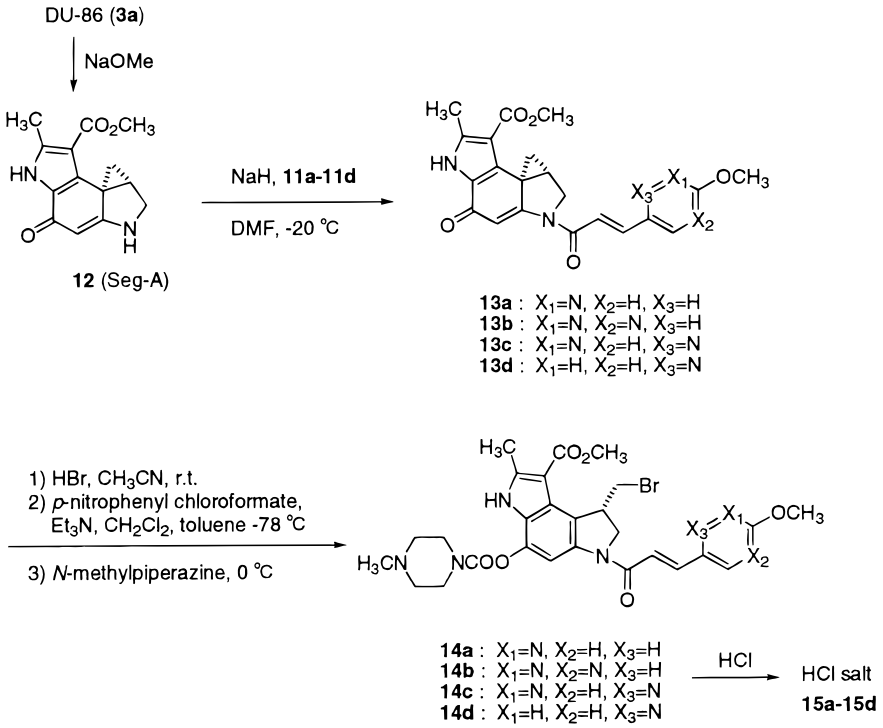
improve aqueous solubility. In this paper, we report our investigation regarding the synthesis, anticellular and antitumor activities, hematotoxicity, and structure−activity relationships (SAR) of these derivatives.

Chemistry

Initially, the various *β*-heteroarylacrylic acids were synthesized according to Scheme 1. The synthetic intermediate for **11a** was (*E*)-3-(6-methoxypyridinyl)-acrylic acid, which was prepared by Nishikawa's methods.[18] The synthetic intermediates (**6**, **8**, **10**) of the other *β*-heteroarylacrylic acids were synthesized from starting materials **5**, **7**, and **9**. The coupling reaction of compounds **6**, **8**, and **10** and methyl acrylate was carried out by Pd−Heck reaction.[19] We tried Pd−Heck reaction under various conditions to find that the reaction would successfully proceed in the presence of a catalytic amount of palladium acetate under phase-transfer conditions using potassium carbonate as a base and tetrabutylammonium chloride as the phase-transfer agent.[20] The *β*-heteroarylacrylic methyl esters were treated with 4 N KOH to afford *β*-heteroarylacrylic acid derivatives, which were converted to *p*-nitrophenyl esters **11a**−**11d** by the reaction with *p*-nitrophenol using 1-methyl-2-chloropyridinium salt (Mukaiyama's method)[21] or condensing agent (DCC). We obtained better results by Mukaiyama's methods.

The *β*-heteroarylacrylates of duocarmycin (**13a**−**13d**, **14a**−**14d**, **15a**−**15d**) were prepared as follows (Scheme 2). The 2-methyl-3-methoxycarbonyl A-ring pyrrole compound of duocarmycin (DU-86, **3a**) was prepared by employing the Wagner−Meerwein type rearrangement of the 8-*O*-protected-3-hydroxyduocarmycin B2 followed by deprotection of the protecting group under basic conditions.[13,22] Compound **3a** was treated with NaOMe in MeOH to afford Seg-A (**12**). The reaction of **12** and a

Scheme 2



suitable acylating agent, *p*-nitrophenyl esters **11a**−**11d**, was carried out with base (NaH) in DMF to give **13a**−**13d**, respectively.[12,13] Compounds **13a**−**13d** were treated with 48% HBr followed by addition of *p*-nitrophenyl chloroformate in the presence of triethylamine in CH_2Cl_2−toluene at −78 °C to give carbonates as an intermediate. By treating carbonates as an intermediate with *N*-methylpiperazine, the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives **14a**−**14d** were obtained. When CH_2Cl_2 was used as the solvent, the reaction yield was low due to the heterogeneous system. We tried various solvent systems. The result was that compounds **14a**−**14d** were obtained in high yield by using CH_2Cl_2−toluene as solvent, which was a homogeneous system.

Formation of salts of **14a**−**14d** was examined for aqueous solubility. Treatment of **14b** with 48% HBr resulted in a degradation product, 4′-hydroxy-3′,5′-pyrimidinyl acrylate, which was formed by demethylation on the pyrimidine ring of Seg-B and detected with ^{1}H NMR and FAB-MS. It was considered that the 4′-hydroxy-3′,5′-pyrimidinyl acrylate was obtained by H_2O in 48% HBr. Treatment of **14a**−**14d** with 6.86 N HCl in EtOH provided the salts **15a**−**15d** without decomposition. The aqueous solubility of the salts **15a**−**15d** was found to be more than 20 mg/mL.

Results and Discussion

The antitumor activity of some representative derivatives was evaluated primarily by assays of the inhibition of HeLa S_3 cell growth (in vitro) and antitumor activity against murine sarcoma 180 (in vivo). As shown in Table 1, the efficacy in vivo is expressed as T/C, where T and C represent means of tumor volume in treated and control mice, respectively. Compound **4b** showed 100 times weaker anticellular activity compared with compound **4a** (72-h exposure). It appears that compound **4a** is obtained as an active metabolite by enzymatic hydrolysis of compound **4b**, which is the same action as that of DU-86 (**3a**), an active metabolite of KW-2189 (**3b**) (data not shown).[7d,10] The same tendency was shown between **13a**−**13d** and **15a**−**15d.** Compounds **13a**−**13c** exhibited strong anticellular activity with IC_{50} values below 1.0 nM at 72-h exposure, which was almost equivalent to that of compound **4a**. Compound **13d**, in which the position of nitrogen in the *β*-heteroarylacryloyl of Seg-B was different from that in **13a**, showed anticellular activity inferior to that of **13a**. This result supported the results we have shown previously that the 3′-position of the 4′-methoxycinnamates did not seriously influence the association between DNA and

Table 1. Anticellular Activity, Antitumor Activity, Hematotoxicity, and Water Solubility of Duocarmycin Derivatives

	HeLa S_3 IC_{50} (nM)[a]		sarcoma 180 (sc-iv)[b]		hematotoxicity		water
			dose		WBC[d]	PL[e]	solubility
no.	1 h	72 h	(mg/kg)	T/C[c]	(%)	(%)	(mg/mL)
13a	3.3	0.42	0.25	0.60	50	84	
15a	900	110	1	0.36	38	92	>20
13b	2.5	0.90	2	0.45	28	86	
15b	1300	170	2	0.26	26	69	>20
13c	0.5	0.28					
15c	1100	90	1	0.31	51	115 (2)[f]	>20
13d	9.7	2.9					
15d	1300	150	4	0.5	38	105	>20
4a	2.9−7.0	0.26−0.94	0.83	0.34	50	63	
4b	1800	37	4	0.2	25	44[g]	<0.1

[a] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%. [b] Mice (5 mice/group) were implanted subcutaneously (sc) with tumor cells, and the drug was dosed (mg/mg) intravenously (iv). [c] T and C are the values of the mean of tumor volume of treated and control mice, respectively. [d] Number of peripheral platelets of normal mice on day 7 (percent of control). [e] Number of white blood cells of tumor-bearing mice on day 4 (percent of control). [f] Mortality (5 mice/group). [g] Dose of 5.33 mg/kg.

drug.[13] The biological activity indicated that the heteroaryl substituents did not influence improvement of potency.

Peripheral blood toxicity (reduction of the number of peripheral blood platelets) of 4′-methoxy-β-heteroarylacrylates was lower than that of the derivatives bearing Seg-B of natural type and was equal to that of 4′-methoxycinnamates. Among new Seg-B derivatives, compound **15b** showed antitumor activity against murine solid tumor with T/C values less than 0.3, which is comparable to that of compound **4b** [**15b**: T/C = 0.26; KW-2189: T/C = 0.15 (0.63 mg/kg dose)].[7] Indeed, compound **15b** exhibited sufficient efficacy with low peripheral blood toxicity.[12,13] The other compounds showed a tendency to decrease antitumor activity in vivo. Compound **15b** was evaluated for in vivo antitumor activity against nude mice bearing human xenograft St-4 (poorly differentiated stomach adenocarcinoma). Compound **15b** showed excellent activity in vivo with T/C values of 0.28 (12 mg/kg dose). Its potency against St-4 human stomach tumor xenograft was nearly comparable to the potency of our clinical candidate KW-2189 (**3b**) [T/C = 0.12 (0.63 mg/kg dose)].[7]

Compound **4b** exhibits poor solubility below 0.1 mg/mL in water or phosphate buffer (pH 7). However, the 8-*O*-[(*N*-methylpiperazinyl)carbonyl] derivatives of 4′-methoxy-β-heteroarylacrylates (**15a**−**15d**) were found to possess adequate water solubility in excess of 20 mg/mL. Compounds **15a**−**15d** were over 200-fold more soluble in aqueous solution than compound **4b**.

Conclusions

A series of A-ring pyrrole compounds of duocarmycin bearing 4′-methoxy-β-heteroarylacryloyl groups were prepared and evaluated for their anticellular activity against HeLa S_3 cells and for antitumor activity against sarcoma 180 murine solid tumor and St-4 human stomach tumor xenograft. Some showed potent antitumor activity nearly comparable to that of our clinical candidate KW-2189. Moreover, these compounds had lower peripheral blood toxicity than the derivatives bearing a trimethoxyindole skeleton in Seg-B and higher aqueous solubility than 4′-methoxycinnamates.

Experimental Section

All melting points were measured on a Yanagimoto micro-melting point 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 and are reported in δ units. Mass spectra were measured with JEOL JMS-DX303, JMS-SX102, and SHIMAZU QP-1000 spectrometers. Elemental analyses were performed with a Perkin-Elmer 2400 C, N, 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}. Usual workup refers to washing of organic layers with brine, drying over anhydrous Na_2SO_4, and evaporating off the solvents under reduced pressure.

2-Methoxy-5-iodopyrimidine (6). To a solution of 2-chloropyrimidine (**5**) (970 mg, 8.47 mmol) in methanol (7 mL) was added 28 wt % NaOMe in methanol (3.40 g, 10.9 mmol), and the mixture was stirred at room temperature for 30 min. Then water was added, and the resulting mixture was extracted with $CHCl_3$ and worked up as usual. To a solution of the residue in TFA (14 mL) and trifluoroacetic anhydride (3 mL) was added *N*-iodosuccinimide (3.96 g, 16.9 mmol), and the mixture was stirred at reflux (80 °C) for 11 h. Then water was added, and the resulting mixture was extracted with $CHCl_3$, washed with aqueous $NaHCO_3$ and 5% $Na_2S_2O_3$, and worked up as usual. The residue was purified by column chromatography (hexane−AcOEt, 3:1) to give 523 mg (26%) of 2-methoxy-5-iodopyrimidine (**6**): ^{1}H NMR (90 MHz, $CDCl_3$) 8.64 (2 H, s), 3.99 (3 H, s).

3-Methoxy-6-iodopyridazine (8). To a solution of 3,6-dichloropyridazine (**7**) (2.00 g, 13.4 mmol) in acetone (60 mL) was added NaI (20.1 g, 268 mmol), and the mixture was heated under reflux (70 °C) for 2 h. Water was added, and the resulting mixture was extracted with AcOEt. Usual workup gave 2.73 g (61%) of 3,6-diiodopyridazine. To a solution of 3,6-diiodopyridazine (2.73 g, 8.23 mmol) in methanol (80 mL) was added 28 wt % NaOMe in methanol (3.17 g, 16.5 mmol), and the mixture was stirred at room temperature for 13 h. Then water was added; the whole was extracted with AcOEt. Usual workup and purification by column chromatography (hexane−AcOEt, 8:1) gave 1.661 g (86%) of 3-methoxy-6-iodopyridazine (**8**): ^{1}H NMR (90 MHz, $CDCl_3$) 7.64 (1 H, d, J = 9.1 Hz), 8.31 (1 H, d, J = 9.1 Hz), 4.09 (3 H, s).

3-Methoxy-6-iodopyridine (10). To a methanol (80 mL) solution of 3-hydroxypyridine (**9**) (3.00 g, 31.5 mmol), NaI (4.72 g, 31.5 mmol), and NaOH (1.26 g, 31.5 mmol) was added dropwise 4% NaOCl in water (58.5 g) at 0 °C over 100 min. Then the mixture was stirred at 0 °C for 3 h; 5% $Na_2S_2O_3$ (10 mL), 0.1 N HCl was added, and the whole was extracted with AcOEt. Usual workup and purification by column chromatography (hexane−AcOEt, 4:1) afforded 2.38 g (34%) of 3-hydroxy-6-iodopyridine. To a solution of 60% NaH (647 mg, 16.2 mmol) in DMF (20 mL) was added a DMF solution (20 mL) of 3-hydroxy-6-iodopyridine (2.38 g, 10.8 mmol), and the mixture was stirred under Ar atomosphere at room temperature. After 2 h, NaI (2.14 g, 15.1 mmol) was added and stirred for 1 h; 0.01 M phosphoric buffer (pH 7) was added; the whole was extracted AcOEt and then worked up as usual. The residue was purified by column chromatography (hexane−AcOEt, 5:1−4:1) to give 2.57 g (100%) of 3-methoxy-6-iodopyridine (**10**): ^{1}H NMR (90 MHz, $CDCl_3$) 8.03 (1 H, m), 7.22−7.30 (2 H, m), 3.96 (3 H, s).

(*E*)-*p*-Nitrophenyl 3-(2-Methoxy-5-pyridinyl)acrylate (11a). To a solution of (*E*)-3-(2-methoxy-5-pyridinyl)acrylic acid[16] (182 mg, 1.02 mmol) in CH_2Cl_2 (15 mL) were added *p*-nitrophenol (241 mg, 1.73 mmol), trimethylamine (0.483 mL, 3.47 mmol), and 2-chloro-1-methylpyridinium iodide (443 mg, 1.73 mmol), and the mixture was heated under reflux (50 °C) for 2 h. The reaction mixture was added to aqueous $NaHCO_3$ and extracted with $CHCl_3$. Usual workup and purification by column chromatography ($CHCl_3$−MeOH, 100:1) gave 220 mg (72%) of **11a**: ^{1}H NMR (270 MHz, $CDCl_3$) 8.35 (1 H, d, J = 2.3 Hz), 8.31 (2 H, d, J = 8.9 Hz), 7.86 (1 H, d, J = 16.2 Hz), 7.85 (1 H, dd, J = 8.6, 2.3 Hz), 7.37 (2 H, d, J = 8.9 Hz), 6.82 (1 H, d, J = 8.6 Hz), 6.52 (1 H, d, J = 16.2 Hz), 4.00 (3 H, s).

(*E*)-*p*-Nitrophenyl 3-(2-Methoxy-5-pyrimidinyl)acrylate (11b). To a solution of 2-methoxy-5-iodopyrimidine (**6**) (1.74 g, 7.36 mmol) in DMF (36 mL) were added $Pd(OAc)_2$ (99 mg), K_2CO_3 (2.54 g, 18.4 mmol), ^{n}Bu_4NCl (2.05 g, 7.36 mmol), and methyl acrylate (3.17 g, 36.8 mmol), and the mixture was heated at 80 °C under Ar atmosphere for 1 h. After the mixture was cooled, water was added; the whole was extracted with AcOEt. Usual workup and purification by column chromatography (hexane−AcOEt, 2:1−1:1) gave 1.26 g (88%) of (*E*)-methyl 3-(2-methoxy-5-pyrimidinyl)acrylate. To a solution of (*E*)-methyl 3-(2-methoxy-5-pyrimidinyl)acrylate (1.26 g, 6.49 mmol) in methanol (40 mL) was added 4 N KOH (3.25 mL), and the mixture was heated at 50 °C for 2 h. After the mixture was cooled, 0.5 N HCl was added; the whole was extracted with AcOEt. Usual workup afforded 1.12 g (96%) of (*E*)-3-(2-methoxy-5-pyrimidinyl)acrylic acid. The synthesis of compound **11b** was performed according to the same procedure as for **11a**. **11b**: yield 90%; ^{1}H NMR (270 MHz, $CDCl_3$) 8.69 (2 H, s), 8.25 (2 H, d, J = 9.4 Hz), 7.74 (1 H, d, J = 16.3 Hz), 7.31 (2 H, d, J = 8.9 Hz), 6.58 (1 H, d, J = 15.8 Hz), 4.03 (3 H, s).

(*E*)-*p*-Nitrophenyl 3-(3-Methoxy-6-pyridazinyl)acrylate (11c). To a solution of 3-methoxy-6-iodopyridazine (**8**) (1.66 g, 7.04 mmol) in DMF (34 mL) were added $Pd(OAc)_2$ (99 mg), K_2CO_3 (1.06 g, 7.67 mmol), $^nBu_4NCl$ (1.96 g, 7.05 mmol), and methyl acrylate (12.1 g, 141 mmol), and the mixture was heated at 80 °C under Ar atmosphere for 4.5 h. After the mixture had cooled, water was added; the whole was extracted with AcOEt, $CHCl_3$. Usual workup and purification by column chromatography (hexane−AcOEt, 3:1−2:1) gave 828 mg (60%) of (*E*)-methyl 3-(3-methoxy-6-pyridazinyl)acrylate. To a solution of (*E*)-methyl 3-(3-methoxy-6-pyridazinyl)acrylate (818 mg, 4.21 mmol) in methanol (25 mL) was added 4 N KOH (4.22 mL), and the mixture was heated at 50 °C for 8 h. After the mixture was cooled, 0.5 N HCl was added; the whole was extracted with AcOEt, $CHCl_3$. Usual workup afforded 668 mg (88%) of (*E*)-3-(3-methoxy-6-pyridazinyl)acrylic acid. The synthesis of compound **11c** was performed according to the same procedure as for **11a**. **11c**: yield 60%; ^{1}H NMR (270 MHz, $CDCl_3$) 8.30−8.33 (2 H, m), 8.04 (1 H, d, J = 16.2 Hz), 7.62 (1 H, d, J = 9.2 Hz), 7.38−7.41 (2 H, m), 7.05 (1 H, d, J = 8.9 Hz), 7.01 (1 H, d, J = 16.2 Hz), 4.22 (3 H, s).

(*E*)-*p*-Nitrophenyl 3-(3-Methoxy-6-pyridinyl)acrylate (11d). To a solution of 3-methoxy-6-iodopyridine (**10**) (500 mg, 2.13 mmol) in DMF (6 mL) were added $Pd(OAc)_2$ (29 mg), K_2CO_3 (736 mg, 5.33 mmol), $^nBu_4NCl$ (592 mg, 2.13 mmol), and methyl acrylate (917 mg, 10.7 mmol), and the mixture was heated in a sealed tube at 120 °C for 12 h. After the mixture was cooled, 0.01 M phosphoric buffer (pH 7) was added; the whole was extracted with AcOEt. Usual workup and purification by column chromatography (hexane−AcOEt, 4:1−3:1) afforded 168 mg (41%) of (*E*)-methyl 3-(3-methoxy-6-pyridinyl)acrylate. To a solution of (*E*)-methyl 3-(3-methoxy-6-pyridinyl)acrylate (429 mg, 2.22 mmol) in methanol (12 mL) was added 4 N KOH (1.11 mL), and the mixture was heated at room temperature for 20 h. After the mixture was cooled, 1 N HCl was added; the whole was extracted with AcOEt, $CHCl_3$. Usual workup afforded 277 mg (70%) of (*E*)-3-(3-methoxy-6-pyridinyl)acrylic acid. To a solution of (*E*)-3-(3-methoxy-6-pyridinyl)acrylic acid (268 mg, 1.50 mmol) in CH_2Cl_2 (28 mL) was added DCC (620 mg, 3.00 mmol), and the mixture was stirred at 0 °C for 45 min. Then, *p*-nitrophenol (418 mg, 3.00 mmol) and DMAP (367 mg, 3.00 mmol) were added to the reaction mixture, and the mixture was stirred at room temperature for 2 h 10 min; 1 N HCl was added; the whole was extracted with $CHCl_3$, washed with aqueous $NaHCO_3$, and worked up as usual. The whole was added to AcOEt and was obtained as a crystalline material. Recrystallization from EtOH gave 136 mg (30%) of **11d**: ^{1}H NMR (270 MHz, $CDCl_3$) 8.31 (1 H, d, J = 15.2 Hz), 8.27−8.33 (3 H, m), 7.36−7.40 (2 H, m), 7.32 (1 H, d, J = 4.3 Hz), 7.29 (1 H, d, J = 1.7 Hz), 7.20 (1 H, d, J = 15.8 Hz), 3.94 (3 H, s).

Methyl (7b*R*,8a*S*)-2-[2-(4-Methoxy-3-pyridinyl)ethen-1-ylcarbonyl]-6-methyl-4(5*H*)-oxo-1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-7-carboxylate (13a). To a solution of 60% NaH (4.7 mg, 0.12 mmol) in DMF (0.38 mL) was added a DMF solution (0.5 mL) of **12** (Seg-A) (25.0 mg, 0.0970 mmol), and the mixture was stirred under Ar atomosphere at −20 °C for 2 h 20 min. Then a solution of **11a** (32.1 mg, 0.107 mmol) in DMF (0.5 mL) was added and stirred for 1 h 20 min; 0.01 M phosphoric buffer (pH 7) was added; the whole was extracted with AcOEt, and then worked up as usual. The residue was purified by column chromatography ($CHCl_3$−MeOH, 100:1−70:1) to give 31.8 mg (78%) of **13a**: mp 245−250 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 11.52 (1 H, br), 8.32 (1 H, d, J = 2.0 Hz), 7.81 (1 H, dd, J = 2.3, 8.6 Hz), 7.77 (1 H, d, J = 15.2 Hz), 6.77 (1 H, d, J = 15.5 Hz), 6.77 (1 H, d, J = 8.9 Hz), 6.74 (1 H, br), 4.24 (1 H, d, J = 10.9 Hz), 4.16 (1 H, dd, J = 10.9, 4.6 Hz), 3.98 (3 H, s), 3.81 (3 H, s), 3.55−3.61 (1 H, m), 2.62 (3 H, s), 2.40 (1 H, dd, J = 7.4, 3.5 Hz), 1.31 (1 H, dd, J = 4.6, 3.6 Hz); IR (KBr, cm^{-1}) 1701, 1668, 1614, 1601, 1495, 1462, 1389, 1292, 1244, 1219, 1111; FAB-MS m/z 420 (M + H)$^+$; FAB-HRMS calcd for $C_{23}H_{22}N_3O_5$ (M + H)$^+$ m/z 420.1559, found 420.1552. Anal. ($C_{23}H_{21}N_3O_5$·1.5H_2O) H, N; C: calcd, 61.88; found, 62.60.

The synthesis of compounds **13b**−**13d** was performed according to the same procedure as for **13a**.

Methyl (7b*R*,8a*S*)-2-[2-(4-methoxy-3,5-pyrimidinyl)-ethen-1-ylcarbonyl]-6-methyl-4(5*H*)-oxo-1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-7-carboxylate (13b): yield 74%; mp 280−290 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 12.39 (1 H, brs), 9.04 (2 H, s), 7.65 (1 H, d, J = 15.8 Hz), 7.21 (1 H, d, J = 15.8 Hz), 6.91 (1 H, br), 4.38 (1 H, d, J = 10.9 Hz), 4.21 (1 H, dd, J = 10.9, 4.6 Hz), 3.97 (3 H, s), 3.74 (3 H,s), 3.46−3.52 (1 H, m), 2.47 (3 H, s), 2.11 (1 H, dd, J = 7.6, 3.0 Hz), 1.32 (1 H, dd, J = 4.0, 4.0 Hz); IR (KBr, cm^{-1}) 1701, 1676, 1624, 1618, 1595, 1477, 1400, 1340, 1250, 1111; FAB-MS m/z 421 (M + H)$^+$; FAB-HRMS calcd for $C_{22}H_{21}N_4O_5$ (M + H)$^+$ m/z 421.1500, found 420.1512. Anal. ($C_{22}H_{20}N_4O_5$·0.3H_2O) C, H, N.

Methyl (7b*R*,8a*S*)-2-[2-(4-methoxy-2,3-pyridazinyl)-ethen-1-ylcarbonyl]-6-methyl-4(5*H*)-oxo-1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-7-carboxylate (13c): yield 73%; mp 210−215 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 11.48 (1 H, brs), 7.84 (1 H, d, J = 15.2 Hz), 7.52 (1 H, d, J = 9.2 Hz), 7.44 (1 H, d, J = 15.2 Hz), 7.00 (1 H, d, J = 9.2 Hz), 6.93 (1 H, br), 4.29 (1 H, d, J = 10.9 Hz), 4.20 (1 H, dd, J = 10.9, 4.6 Hz), 4.19 (3 H, s), 3.81 (3 H, s), 3.54−3.66 (1 H, m), 2.62 (3 H, s), 2.39 (1 H, dd, J = 7.6, 3.6 Hz), 1.31 (1 H, dd, J = 5.0, 3.6 Hz); IR (KBr, cm^{-1}) 1701, 1610, 1411, 1396, 1294, 1248, 1217, 1109, 1072; FAB-MS m/z 421(M + H)$^+$; FAB-HRMS calcd for $C_{22}H_{21}N_4O_5$ (M + H)$^+$ m/z 421.1500, found 421.1512. Anal. ($C_{22}H_{20}N_4O_5$·1.5 H_2O) C, H; N: calcd, 12.52; found, 12.11.

Methyl (7b*R*,8a*S*)-2-[2-(4-methoxy-2-pyridinyl)ethen-1-ylcarbonyl]-6-methyl-4(5*H*)-oxo-1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-7-carboxylate (13d): yield 83%; mp 235−240 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 11.80 (1 H, br), 8.23 (1 H, d, J = 15.2 Hz), 8.22 (1 H, dd, J = 4.1, 1.5 Hz), 7.46 (1 H, d, J = 15.2 Hz), 7.21−7.30 (2 H, m), 6.92 (1 H, br), 4.31 (1 H, d, J = 10.9 Hz), 4.20 (1 H, dd, J = 10.6, 4.6 Hz), 3.90 (3 H,s), 3.81 (3 H, s), 3.55−3.62 (1 H, m), 2.62 (3 H, s), 2.36 (1 H, dd, J = 7.6, 3.3 Hz), 1.29 (1 H, dd, J = 4.6, 3.6 Hz); IR (KBr, cm^{-1}) 1701, 1672, 1618, 1578, 1450, 1390, 1296, 1252, 1217, 1113; FAB-MS m/z 420 (M + H)$^+$; FAB-HRMS calcd for $C_{23}H_{22}N_3O_5$ (M + H)$^+$ m/z 420.1559, found 420.1561. Anal. ($C_{22}H_{21}N_3O_5$·1.0H_2O) C, H; N: calcd, 9.61; found, 9.08.

Methyl (1*S*)-1-(Bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[2-(4-methoxy-3-pyridinyl)ethen-1-ylcarbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (14a). To a solution of **13a** (21.2 mg, 0.0510 mmol) in CH_3CN (1.27 mL) was added 48% HBr (0.012 mL), and the mixture was stirred at room temperature for 30 min. Then the mixture was concentrated under reduced pressure. *p*-Nitrophenyl chloroformate (29.6 mg, 0.158 mmol) and triethylamine (0.0210 mL, 0.153 mmol) were added to the residue in dry CH_2Cl_2 (1.07 mL) and toluene (0.42 mL) under cooling at −78 °C. The mixture was stirred at −78 °C for 30 min. Then *N*-methylpiperazine (0.0196 mL, 0.179 mmol) was added, and stirring was continued at 0 to −78 °C for 20 min. The mixture was extracted with $CHCl_3$, washed with aqueous $NaHCO_3$, and worked up as usual. The residue was purified by PTLC ($CHCl_3$−MeOH, 9:1) to give 25 mg (78%) of **14a**: mp 150−155 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 9.20 (1 H, brs), 8.34 (1 H, d, J = 2.3 Hz), 8.21 (1 H, brs), 7.89 (1 H, dd, J = 8.6, 2.3 Hz), 7.79 (1 H, d, J = 15.2 Hz), 6.82 (1 H, d, J = 15.2 Hz), 6.81 (1 H, d, J = 8.6 Hz), 4.49−4.60 (1 H, m), 4.45 (1 H, d, J = 10.2 Hz), 4.30 (1 H, dd, J = 9.6, 8.9 Hz), 3.98 (3 H, s), 3.95 (3 H, s), 3.79 (1 H, dd, J = 9.6 Hz), 3.76 (2 H, br), 3.63 (2 H, br), 3.21 (1 H, dd, J = 10.2, 9.9 Hz), 2.53 (3 H, s), 2.49 (4 H, br), 2.36 (3 H, s); IR (KBr, cm^{-1}) 1701, 1697, 1649, 1495, 1435, 1410, 1381, 1290, 1217, 1153, 1095; FAB-MS m/z 628, 626 (M + H)$^+$; FAB-HRMS calcd for $C_{29}H_{33}{}^{79}BrN_5O_6$ (M + H)$^+$ m/z 626.1614, found 626.1642.

The synthesis of compounds **14b**−**14d** was performed according to the same procedure as for **14a**.

Methyl (1*S*)-1-(bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[2-(4-methoxy-3,5-pyrimidinyl)-ethen-1-ylcarbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (14b): yield 65%; mp 160−165 °C; ^{1}H NMR

(270 MHz, $CDCl_3$) 9.01 (1 H, s), 8.75 (2 H, s), 8.21 (1 H, s), 7.71 (1 H, d, J = 15.5 Hz), 6.93 (1 H, d, J = 15.5 Hz), 4.53−4.63 (1 H, m), 4.45 (1 H, d, J = 10.2 Hz), 4.31 (1 H, dd, J = 9.6, 9.6 Hz), 4.07 (3 H, s), 3.95 (3 H, s), 3.80 (1 H, dd, J = 7.3, 2.6 Hz), 3.74 (2 H, br), 3.64 (2 H, br), 3.23 (1 H, dd, J = 10.2, 9.9 Hz), 2.61 (3 H, s), 2.50 (4 H, brs), 2.37 (3 H, s); IR (KBr, cm^{-1}) 1714, 1701, 1653, 1473, 1435, 1412, 1338, 1219, 1153, 1095; FAB-MS m/z 629, 627 $(M + H)^+$; FAB-HRMS calcd for $C_{28}H_{32}{}^{79}BrN_6O_6$ $(M + H)^+$ m/z 627.1567, found 627.1538.

Methyl (1*S*)-1-(bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[2-(4-methoxy-2,3-pyridazinyl)-ethen-1-ylcarbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (14c): yield 70%; mp 165−170 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 9.15 (1 H, brs), 8.24 (1 H, s), 7.84 (1 H, d, J = 15.2 Hz), 7.57 (1 H, d, J = 15.2 Hz), 7.54 (1 H, d, J = 8.9 Hz), 7.01 (1 H, d, J = 8.9 Hz), 4.53−4.60 (1 H, m), 4.52 (1 H, d, J = 9.9 Hz), 4.33 (1 H, dd, J = 9.6, 9.6 Hz), 4.20 (3 H, s), 3.95 (3 H, s), 3.79 (1 H, dd, J = 10.2, 2.6 Hz), 3.75 (2 H, br), 3.61 (2 H, br), 3.21 (1 H, dd, J = 10.2, 9.9 Hz), 2.57 (3 H, s), 2.49 (4 H, br), 2.36 (3 H, s); IR (KBr, cm^{-1}) 1714, 1705, 1699, 1653, 1466, 1412, 1294, 1217, 1153, 1093, 1005; FAB-MS m/z 629, 627 $(M + H)^+$; FAB-HRMS calcd for $C_{28}H_{32}{}^{79}BrN_6O_6$ $(M + H)^+$ m/z 627.1567, found 627.1539.

Methyl (1*S*)-1-(bromomethyl)-7-methyl-5-[(4-methylpiperazinyl)carbonyloxy]-3-[2-(4-methoxy-2-pyridinyl)ethen-1-ylcarbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-8-carboxylate (14d): yield 58%; mp 230−235 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 9.47 (1 H, brs), 8.28 (1 H, d, J = 15.2 Hz), 8.27 (1 H, dd, J = 4.0, 1.3 Hz), 7.55 (1 H, d, J = 14.9 Hz), 7.21−7.30 (2 H, m), 4.54 (1 H, br), 4.52 (1 H, d, J = 9.6 Hz), 4.33 (1 H, dd, J = 9.9, 9.2 Hz), 3.93 (3 H, s), 3.89 (3 H, s), 3.76 (1 H, dd, J = 9.9, 2.6 Hz), 3.74 (2 H, br), 3.60 (2 H, br), 3.21 (1 H, dd, J = 9.9, 9.9 Hz), 2.51 (3 H, s), 2.46 (4 H, br), 2.34 (3 H, s); IR (KBr, cm^{-1}) 1722, 1701, 1697, 1433, 1408, 1292, 1259, 1217, 1153, 1093; FAB-MS m/z 628, 626 $(M + H)^+$; FAB-HRMS calcd for $C_{29}H_{33}{}^{79}BrN_5O_6$ $(M + H)^+$ m/z 626.1614, found 626.1609.

14a Hydrochloride (15a). A solution of **14a** (20.9 mg, 0.0334 mmol) in ethanol (0.91 mL) and methanol (0.46 mL) was treated with anhydrous 6.86 N HCl in ethanol (0.0146 mL) at room temperature for 4 h. The mixture was concentrated under reduced pressure to give 22.1 mg of **15a**: mp 235−240 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 12.12 (1 H, s), 10.70 (1 H, br), 8.50 (1 H, d, J = 2.3 Hz), 7.35 (1 H, dd, J = 8.6, 2.3 Hz), 8.10 (1 H, br), 7.61 (1 H, d, J = 15.5 Hz), 7.19 (1 H, d, J = 15.2 Hz), 6.92 (1 H, d, J = 8.9 Hz), 4.36−4.51 (4 H, m), 4.12−4.18 (2 H, br), 3.91 (3 H, s), 3.85 (3 H, s), 3.79 (1 H, brd, J = 8.3 Hz), 2.85 (3 H, s), 2.68 (3 H, s); IR (KBr, cm^{-1}) 1714, 1695, 1657, 1651, 1435, 1414, 1219, 1173, 1095. Anal. ($C_{29}H_{32}BrN_5O_6$·2.0HCl·2.5H_2O) C, H, N.

The synthesis of compounds **15b**−**15d** was performed according to the same procedure as for **15a**.

14b Hydrochloride (15b): mp 250−260 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 12.06 (1 H, s), 10.44 (1 H, br), 9.08 (2 H, s), 8.11 (1 H, s), 7.59 (1 H, d, J = 15.8 Hz), 7.36 (1 H, d, J = 15.8 Hz), 4.40−4.49 (3 H, m), 4.10−4.24 (1 H, br), 3.98 (3 H, s), 3.85 (3 H, s), 3.76−3.84 (1 H, m), 2.86 (3 H, s), 2.68 (3 H, s); IR (KBr, cm^{-1}) 1705, 1701, 1659, 1477, 1433, 1412, 1336, 1215, 1186, 1095. Anal. ($C_{28}H_{31}BrN_6O_6$·2.0HCl·0.7H_2O) C, H; N: calcd, 11.79; found, 10.68.

14c Hydrochloride (15c): mp 145−150 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 12.12 (1 H, s), 10.54 (1 H, br), 8.31 (1 H, d, J = 8.9 Hz), 7.74 (1 H, d, J = 15.2 Hz), 7.54 (1 H, d, J = 15.2 Hz), 7.34 (1 H, d, J = 8.6 Hz), 4.34−4.60 (4 H, m), 4.02−4.24 (1 H, br), 4.09 (3 H, s), 3.85 (3 H, s), 3.76−3.82 (1 H, m), 2.85 (3 H, br), 2.69 (3 H, s); IR (KBr, cm^{-1}) 1716, 1705, 1699, 1417, 1435, 1414, 1252, 1219, 1093. Anal. ($C_{28}H_{31}BrN_6O_6$·2.0HCl·5.5H_2O) C, H, N.

14d Hydrochloride (15d): mp 230−235 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 12.15 (1 H, s), 10.75 (1 H, br), 8.28 (1 H, d, J = 3.6 Hz), 8.11 (1 H, s), 7.96 (1 H, d, J = 15.2 Hz), 7.62 (1 H, d, J = 8.6 Hz), 7.54 (1 H, d, J = 15.5 Hz), 7.45−7.50 (1 H, m), 4.30−4.58 (4 H, m), 4.08−4.24 (1 H, br), 3.94 (3 H, s), 3.85 (3 H, s), 3.81 (1 H, br), 2.85 (3 H, br), 2.69 (3 H, s); IR (KBr, cm^{-1}) 1722, 1699, 1655, 1614, 1437, 1416, 1255, 1219. Anal. ($C_{29}H_{32}BrN_5O_6$·2.0 HCl·3.5H_2O) C, H; N: calcd, 9.18; found, 8.41.

Biological Studies. Human uterine cervix carcinoma HeLa S_3 cells were obtained from 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 compond 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 by 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 (sc) at the axillary region of mice. Drugs were administered intravenously (iv) beginning 1 day after tumor inoculation. Antitumor efficacy is expressed as T/C, where T and C are the values of mean tumor volume of 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.[23]

The criteria for effectiveness against murine solid tumors were 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 is 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 were T/C values with 50% and less, and statistical significance was determined by the Mann−Whitney U test ($p < 0.01$, one-sided).[24]

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 days, 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 percentage of the absolute value of the treated group versus that of control (percent of control).

Effect on PB white blood cell counts: Drug were administered intravenously (iv) beginning 1 day after tumor inoculation. After 4 days, 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 percentage of the absolute value of the treated group versus that of control (percent of control).

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Supporting Information Available: HPLC analytical data of compounds **13a**, **13c**, **13d**, **15b**, and **15d**. This ma-

terial is available free of charge via the Internet at http://pubs.acs.org.

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