Synthesis and Antitumor Activity of Duocarmycin Derivatives: A-Ring Pyrrole Compounds Bearing Cinnamoyl Groups

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A series of *N*-cinnamates of the A-ring pyrrole compound of duocarmycin 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. The 4′-methoxy- and 4′-BocNH-cinnamates exhibited strong *in vitro* anticellular activity among the synthesized compounds. The ortho substitution of the 4′-methoxycinnamate did not affect the anticellular activity and contributed to an enhancement of water solubility. Most of the 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives of the 4′-methoxycinnamates displayed remarkably superior *in vivo* antitumor activity to duocarmycin A or B2. Moreover, it is noteworthy that these 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives exhibited significant antitumor activity at wider range of doses as compared with the A-ring pyrrole derivatives having the trimethoxyindole skeleton in segment B.

Introduction

Duocarmycins (A, **1a**; SA, **1c**; B2, **1d**; C2, **1e**; B1, **1f**; C1, **1g**) are novel antitumor antibiotics isolated from the culture broth of *Streptomyces* sp. (Figure 1).[1] Duocarmycin A (**1a**),[1a−c] which is considered as an active form among these duocarmycins, possesses a unique cyclopropane ring with the ability to alkylate DNA. Duocarmycin A (**1a**) has been reported to show its cytotoxicity through a sequence-selective minor groove alkylation of double-stranded DNA resulting in N3 adenine covalent adduct formation[2] as in the case of the antitumor antibiotic CC-1065 (**1b**).[3,4] Duocarmycins are known to exhibit potent growth-inhibitory activity against human uterine cervix carcinoma HeLa S_3 *in vitro* and modest broad antitumor spectrum against murine transplantable solid tumors.[5,6] However, their marginal activity against human solid tumors, their poor stability, and their insolubility in water dissuaded us from considering them for further evaluation. We were interested in synthesizing analogs in order to enhance and broaden their spectrum of antitumor activity and to improve their stability and solubility.[7,8] One such analog, KW-2189 (**2b**), was prepared by the modification of the segment A in duocarmycin B2 (**1d**), demonstrating excellent *in vivo* antitumor activity, good stability in the culture medium, and aqueous solubility greater than 10 mg/mL.[9] It was designed as a prodrug which requires enzymatic hydrolysis followed by regeneration of DU-86 (**2a**) as an active metabolite.[10] KW-2189 (**2b**) is currently under going phase I clinical evaluation.

On the other hand, segment B of duocarmycins has been considered to play an important role in the noncovalent binding to DNA.[11] Moreover, the modification of the noncovalent binding segments may contribute to a decrease in toxicity. These strategies overcame the delayed irreversible toxicity of CC-1065, and several analogs of CC-1065 were taken into clinical trials.[12] Therefore, we have synthesized a series of duocarmycin analogs bearing the simplified moieties such as acetyl, indol-2-ylcarbonyl, benzofuran-2-ylcarbonyl, cinnamoyl, and phenoxyacetyl group. These compounds exhibited varied *in vitro* anticellular activities. In order to enhance *in vivo* antitumor activity, the compounds that showed potent *in vitro* anticellular activity were converted to the 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives, in view of our acquired information.[7] Among them, the 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives introduced into the cinnamoyl derivatives exhibited sufficient *in vivo* activity against sarcoma 180 over a wide range of doses without detectable toxic effects.[13] With the goal of finding new candidates having greater activity or less toxicity than that of conventional duocarmycins or their derivatives, a series of the *N*-cinnamates of A-ring pyrrole compound of duocarmycin were synthesized and evaluated for their anticellular and antitumor activities. The 4′-methoxy- and 4′-BocNH-cinnamates seemed to be especially suitable for evaluation of *in vitro* activity, and the 4′-methoxycinnamate minimized the decreased *in vitro* activity caused by the substitution at the 3′-position.[6d,14] In this paper, we report our investigation into the synthesis, anticellular and antitumor activities, and structure−activity relationships of these derivatives.

Chemistry

Initially, the 2-methyl-3-methoxycarbonyl A-ring pyrrole compound of duocarmycin (DU-86, **2a**) 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.[15] Compound **2a** was treated with NaOMe in MeOH to afford compound **3** and methyltrimethoxyindole-2-carboxylate quantitatively, as shown in Scheme 1. The obtained compound **3** was allowed to react with 4′-substituted-*trans*-cinnamic acid

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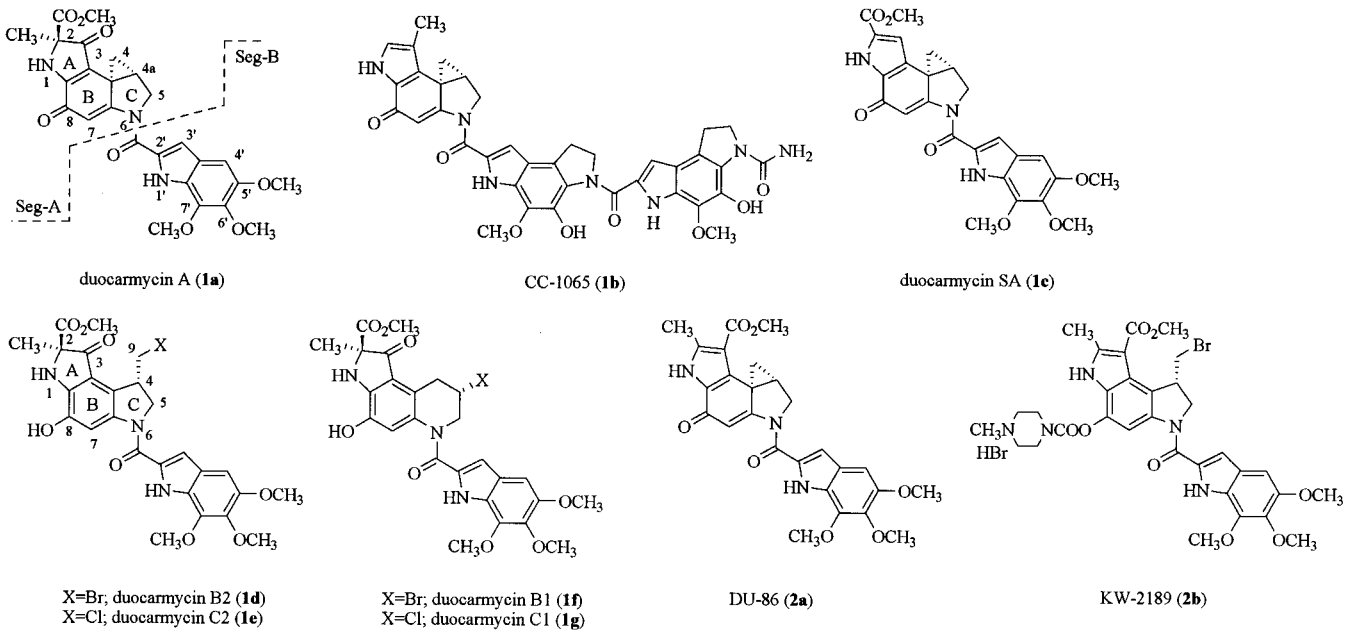
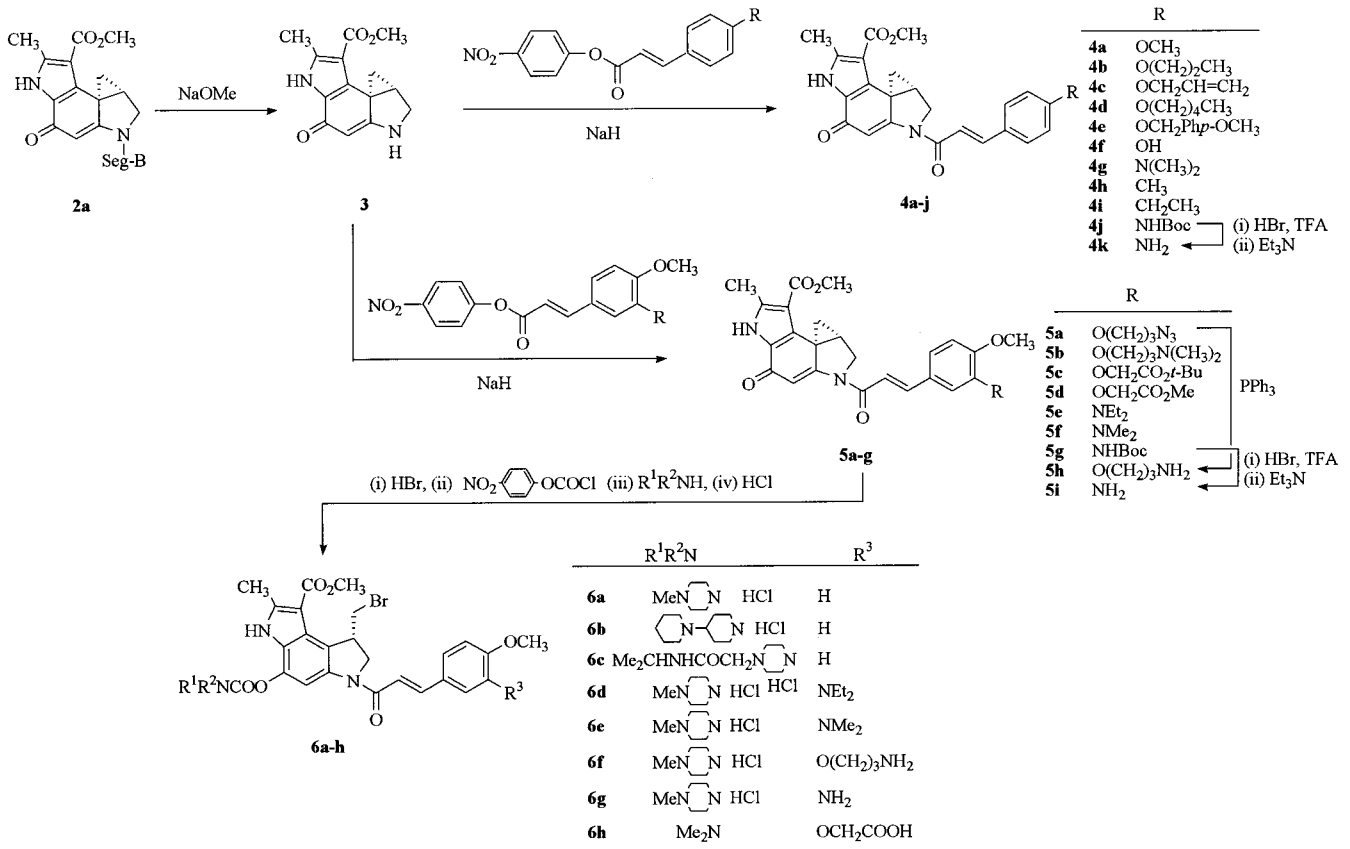


Figure 1. Structures of duocarmycins, CC-1065, and duocarmycin derivatives.

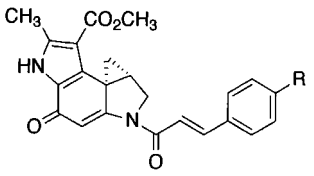
Scheme 1



p-nitrophenyl esters in the presence of NaH to yield the corresponding 4′-substituted cinnamates **4a**−**j** in reasonable yields, as described previously.[13] The 4′-substituted-*trans*-cinnamic acids were prepared from the available 4′-substituted benzaldehyde and malonic acid in pyridine. Their *p*-nitrophenyl esters were then synthesized from the corresponding 4′-substituted cinnamic acid and *p*-nitrophenol using the Mukaiyama reagent in good yields.[16] Compound **4k** was prepared in 47% yield by the reaction of **4j** with 48% HBr and TFA in CH_3CN followed by the treatment of triethylamine. The 4′-methoxy-3′-substituted-cinnamates **5a**−**g** were also prepared by the same procedure as that of **4a**−**j**. Conversion of **5a** to **5h** was achieved in acceptable yield by the method of PPh_3 reduction. Compound **5i** was synthesized by the same method as that of **4k**.

In order to enhance *in vivo* antitumor activities of these cinnamates (**4**, **5**), the 8-*O*-(*N*,*N*-dialkylcarbamoyl) series were prepared as described in preceding papers.[7,9] The cinnamates were treated with HBr, followed by conversion to the *p*-nitrophenyl carbonate by the reaction with *p*-nitrophenyl chloroformate in the presence

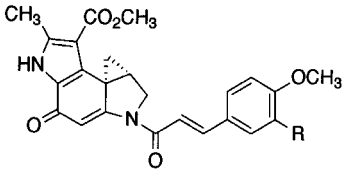
Table 1. Anticellular Activity of 4′-Substituted-cinnamates of Duocarmycin



no.	R	HeLa S_3 IC_{50} (nM)[a] 1 h	72 h
4a	OCH_3	2.9−7.0	0.26−0.94
4b	$O(CH_2)_2CH_3$	51	8.3
4c	$OCH_2CH{=}CH_2$	39	7.5
4d	$O(CH_2)_4CH_3$	>100	50
4e	OCH_2Ph-*p*-OMe	13	0.81
4f	OH	19	3.5
4g	$N(CH_3)_2$	12	1.6
4h	CH_3	33	2.3
4i	CH_2CH_3	51	3.8
4j	NHBoc	4.0	0.56
4k	NH_2	32	2.6
2a		0.39−0.50	0.15−0.16

[a] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.

Table 2. Anticellular Activity of 4′-Methoxy-3′-substituted-cinnamates of Duocarmycin



no.	R	HeLa S_3 IC_{50} (nM)[a] 1 h	72 h
5a	$O(CH_2)_3N_3$	2.0	0.74
5b	$O(CH_2)_3NMe_2$	3.0	1.6
5c	OCH_2CO_2-*t*-Bu	7.4	2.3
5d	$OCH_2CO_2CH_3$	1.3	0.86
5e	NEt_2	9.5	2.3
5f	NMe_2	2.5	0.92
5h	$O(CH_2)_3NH_2$	5.9	1.4
5i	NH_2	2.1	0.53
4a	H	2.9−7.0	0.26−0.94

[a] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.

of triethylamine. The carbonates were allowed to react with the corresponding secondary amines to produce the 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives (**6a**−**h**) in reasonable yields.

Results and Discussion

Tables 1 and 2 show the *in vitro* anticellular activity of those cinnamates against HeLa S_3 cells. As can be seen, the anticellular activity of these cinnamates depends on the substituent at the 4′-position. The methoxy (**4a**) and the BocNH (**4j**) moiety as a substituent impact increased *in vitro* activity in this series. They exhibited strong anticellular activities with the IC_{50} values below 1.0 nM at 72 h exposure. Among the 4′-alkoxycinnamates, the anticellular potency decreased with increasing size of the alkoxy moiety (**4a**−**d**). Many compounds having polar functional groups (e.g., OH, NH_2, $N(CH_3)_2$) at the 4′-position did not show sufficient activity superior to that of **4a** or **4j**. Noteworthy, the 4′-methyl and -ethyl compounds (**4h** and **4i**) have similar bulkiness to that of the 4′-methoxycinnamate (**4a**); however, they exhibited about 10 times decreased anticellular activity compared to **4a**.

Table 3. Anticellular Activity and Antitumor Activity of Cinnamates of Duocarmycin

	HeLa S_3 IC_{50} (nM)[a] 1 h	72 h	sarcoma 180 (sc−iv)[b] mg/kg	T/C[c]
6a	1800	37	4	0.2
6b	1000	224	4	0.17
6c	1200	61	8	0.12
6d	2100	280	8	0.2
6e	430	52	2	0.13
6f	290	30	0.13	0.77
6g	290	30	8	0.39
6h	10000	>1000	4	0.72

[a] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%. [b] Mice (five 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 mean tumor volume of treated and control mice, respectively.

On the other hand, compounds **5a**−**i**, bearing an ortho substituent, showed anticellular activity similar to that of **4a**. It is suggested, therefore, that the 3′-substituent of the 4′-methoxycinnamates did not seriously influence the association between DNA and drug.

The *in vivo* activity of selected compounds was evaluated against sarcoma 180 murine solid tumor. The *in vivo* efficacy was expressed as T/C, which is defined as treated versus control value of tumor volume. Tumor volume was calculated according to the method described previously.[7,9] As shown in Table 3, all of the 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives of the 4′-methoxycinnamates showed 10^2 −10^3 times inferior anticellular activity to that of the corresponding cyclopropane derivatives. However, compounds **6a**−**e** exhibited potent antitumor activity *in vivo*. In contrast, compounds **6f**−**h** having hydrophilic moieties at the 3′-position were somewhat less active than **6a**−**e**, suggesting that a charged group in these analogs may reduce cell permeability and therefore antitumor activity.[17] Moreover, compound **6a**−**e** also showed efficient *in vivo* antitumor activity even at the second highest doses (a half of maximally tolerated dose). Indeed, the cinnamoyl derivatives generally exhibited sufficient efficacy over a wide range of doses without detectable toxic effect.[13] At the same time, the *in vivo* antitumor activities of some representative cyclopropane compounds (**4a**−**c**) were also evaluated, but they did not demonstrate significant antitumor activity (T/C = 0.38−0.47), and the effective range of doses was narrow, similar to that of duocarmycin A or SA or DU-86 which is an active metabolite of KW-2189 (data not shown).[9,18]

Compound **6a** exhibits poor solubility, below 0.1 mg/mL in water or phosphate buffer (pH 7). In contrast, the 8-*O*-(*N*,*N*-dialkylcarbamoyl)cinnamates having an amino group at the 3′-position (**6d**−**g**) were found to possess adequate water solubility in excess of 10 mg/mL. Several analogs demonstrating both potent antitumor activity against sarcoma 180, and adequate water solubility were evaluated *in vivo* for efficacy in nude mice bearing human xenograft St-4 (poorly differentiated stomach adenocarcinoma). Compound **6a** and **6d** showed excellent activity *in vivo* with T/C values of 0.15 (12 mg/kg dose) and 0.29 (18 mg/kg dose), respectively. They caused significant tumor size regression with less toxicity as judged by body weight loss. These activities against St-4 human stomach tumor xenograft were

nearly comparable to our clinical candidate KW-2189.[9] Consequently, compound **6d** is one of the candidates for the next generation of duocarmycin derivatives. Further studies on antitumor spectra and toxicity of these derivatives are in progress.[19]

Conclusions

A series of 8-*O*-(*N*,*N*-dialkylcarbamoyl) derivatives of the 4′-methoxycinnamates were prepared, and some of them were effective against sarcoma 180 murine solid tumor and St-4 human stomach tumor xenograft, nearly comparable to our clinical candidate KW-2189, a novel derivative of duocarmycin B2. Moreover, the effective range of doses was significantly wider than the A-ring pyrrole derivatives, bearing a trimethoxyindole skeleton in segment B. It was shown that the methoxy group at the 4′-position of these cinnamates plays a significant role for the biological activity, and the substituent at the 3′-position contributes to an enhancement of water solubility.

Experimental Section

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO IR-810. ^{1}H spectra were measured on a Varian EM-390, a JEOL JNM-GX270, and a Bruker AM-400 spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. Elemental analyses were performed with a Perkin-Elmer 2400 C, H, N analyzer. Mass spectra were measured with a Hitachi B-80 and a Shimadzu QP-1000 spectrometer. For column chromatography, silica gel (SiO_2, Wako C-200) was used. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck). All organic solvent extracts were dried over anhydrous sodium sulfate prior to concentration *in vacuo*.

Methyl 6-Methyl-1,2,8,8a-tetrahydrocycloprop[1,2-*c*]-pyrrolo[3,2-*e*]indol-4(5*H*)-one-7-carboxylate (3). Sodium methoxide (25 wt % solution in methanol; 0.38 mL, 1.76 mmol) was added to a solution of DU-86 (**2a**; 285 mg, 0.58 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature for 3 h. Then, 0.01 M phosphate buffer (pH 7) was added to the reaction mixture, and the mixture was extracted with $CHCl_3$. The combined extracts were washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography ($CHCl_3$−MeOH, 20:1) to give 113 mg (76%) of **3** as a light-tan powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (1 H, br s), 7.81 (1 H, br s), 6.92 (1 H, br s), 5.34 (1 H, s), 4.15 (1 H, dd, J = 5.4, 5.4 Hz), 3.72 (3 H, s), 3.67 (1 H, dd, J = 10.6, 5.4 Hz), 3.41 (1 H, m), 2.65 (3 H, s), 1.98 (1 H, dd, J = 7.7, 2.8 Hz), 0.99 (1 H, dd, J = 4.5, 2.8 Hz); IR (KBr) 1682, 1607, 1573, 1458, 1305, 1229, 1108, 1083 cm^{-1}; SIMS m/z 259 (M + H)$^+$. Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

General Synthetic Method for Type 4 Compounds (4a−j). 4′-Methoxycinnamoyl A-Ring Pyrrole Duocarmycin 4a. NaH (60%; 23mg, 0.57 mmol) was added to a solution of **3** (96 mg, 0.37 mmol) in DMF (2 mL) at argon atmosphere, and the mixture was stirred for 3 h at −50 °C. The *p*-nitrophenyl ester of 4-methoxycinnamic acid (166 mg, 0.56 mmol) dissolved in DMF (2 mL) was added to a stirred solution at −50 °C. Then, the resulting mixture was stirred at the same temperature for 1 h. The mixture was diluted with AcOEt, and the combine was washed with 0.01 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (50:1) to give 132 mg (85%) of **4a** as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.14 (1 H, br s), 7.79 (1 H, d, J = 15.4 Hz), 7.52 (2 H, d, J = 8.8 Hz), 7.26 (1 H, s), 6.91 (2 H, d, J = 8.8 Hz), 6.75 (1 H, d, J = 15.4 Hz), 4.24 (1 H, dd, J = 10.9, 10.9 Hz), 4.15 (1 H, dd, J = 10.9, 4.8 Hz), 3.85 (3 H, s), 3.82 (3 H, s), 3.56 (1 H, m), 2.62 (3 H, s), 2.39 (1 H, dd, J = 7.6, 3.5 Hz), 1.31 (1 H, dd, J = 4.9, 3.5 Hz); IR (KBr) 1702, 1601, 1512, 1292, 1225, 1173, 1110, 1072 cm^{-1}; EIMS m/z 418 (M)$^+$. Anal. ($C_{24}H_{22}N_2O_5\cdot0.5H_2O$) C, H, N.

4′-Propoxycinnamoyl A-ring pyrrole duocarmycin 4b: yield 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (1 H, br s), 7.77 (1 H, d, J = 15.7 Hz), 7.32−6.93 (4 H, m), 6.84 (1 H, d, J = 15.7 Hz), 6.67 (1 H, br s), 4.24 (1 H, dd, J = 11.0, 11.0 Hz), 4.15 (1 H, dd, J = 11.0, 4.7 Hz), 3.94 (2 H, t, J = 6.3 Hz), 3.82 (3 H, s), 3.55 (1 H, m), 2.59 (3 H, s), 2.39 (1 H, dd, J = 7.5, 3.7 Hz), 1.82 (2 H, m), 1.31 (1 H, dd, J = 4.9, 3.7 Hz), 1.05 (3 H, t, J = 7.4 Hz); IR (KBr) 1697, 1654, 1596, 1437, 1388, 1292, 1246, 1213, 1110 cm^{-1}; SIMS m/z 447 (M + H)$^+$, 259. Anal. ($C_{26}H_{26}N_2O_5\cdot0.5H_2O$) C, H, N.

4′-(Propenyloxy)cinnamoyl A-ring pyrrole duocarmycin 4c: yield 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (1 H, br s), 7.77 (1 H, d, J = 15.6 Hz), 7.33−6.95 (4 H, m), 6.84 (1 H, d, J = 15.6 Hz), 6.77 (1 H, br s), 6.07 (1 H, m), 5.43 (1 H, dd, J = 17.4, 1.8 Hz), 5.31 (1 H, dd, J = 10.5, 1.2 Hz), 4.57 (2 H, dt, J = 5.1, 1.5 Hz), 4.25 (1 H, dd, J = 11.0, 10.9 Hz), 4.15 (1 H, dd, J = 11.0, 4.6 Hz), 3.82 (3 H, s), 3.57 (1 H, m), 2.60 (3 H, s), 2.40 (1 H, dd, J = 7.6, 3.7 Hz), 1.32 (1 H, dd, J = 4.7, 3.7 Hz); IR (KBr) 1701, 1603, 1486, 1445, 1388, 1292, 1246, 1215, 1109 cm^{-1}; SIMS m/z 445 (M + H)$^+$, 259. Anal. ($C_{26}H_{24}N_2O_5\cdot0.5H_2O$) C, H, N.

4′-(Pentyloxy)cinnamoyl A-ring pyrrole duocarmycin 4d: yield 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (1 H, br s), 7.78 (1 H, d, J = 15.4 Hz), 7.52−6.93 (4 H, m), 6.85 (1 H, d, J = 15.4 Hz), 6.67 (1 H, br s), 4.24 (1 H, dd, J = 11.0, 11.0 Hz), 4.15 (1 H, dd, J = 11.0, 4.6 Hz), 3.99 (2 H, t, J = 6.6 Hz), 3.82 (3 H, s), 3.55 (1 H, m), 2.61 (3 H, s), 2.39 (1 H, dd, J = 7.6, 3.7 Hz), 1.81 (2 H, m), 1.44 (4 H, m), 1.31 (1 H, dd, J = 4.9, 3.7 Hz), 0.94 (3 H, t, J = 7.0 Hz); IR (KBr) 1701, 1628, 1599, 1457, 1389, 1255, 1216, 1109 cm^{-1}; SIMS m/z 475 (M + H)$^+$, 259. Anal. ($C_{28}H_{30}N_2O_5\cdot1.5\ H_2O$) C, H, N.

4′-[(Methoxyphenyl)methyl]cinnamoyl A-ring pyrrole duocarmycin 4e: yield 66%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.78 (1 H, br s), 7.77 (1 H, d, J = 15.4 Hz), 7.52 (2 H, d, J = 8.9 Hz), 7.36 (2 H, d, J = 8.4 Hz), 6.97 (2 H, d, J = 8.9 Hz), 6.92 (2 H, d, J = 8.4 Hz), 6.75 (1 H, d, J = 15.4 Hz), 6.62 (1 H, br s), 5.03 (2 H, s), 4.22 (1 H, dd, J = 10.9, 10.9 Hz), 4.14 (1 H, dd, J = 10.9, 4.5 Hz), 3.82 (6 H, s), 3.55 (1 H, m), 2.61 (3 H, s), 2.39 (1 H, dd, J = 7.4, 3.4 Hz), 1.31 (1 H, dd, J = 4.5, 4.5 Hz); IR (KBr) 1701, 1512, 1390, 1242, 1225, 1171 cm^{-1}; SIMS m/z 525 (M + H)$^+$. Anal. ($C_{31}H_{28}N_2O_6\cdot0.5H_2O$) C, H, N.

4′-Hydroxylcinnamoyl A-ring pyrrole duocarmycin 4f: yield 23%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.40 (1 H, br s), 10.05 (1 H, s), 7.62 (1 H, d, J = 14.9 Hz), 7.60 (2 H, d, J = 8.4 Hz), 6.87 (1 H, d, J = 14.9 Hz), 6.83 (2 H, d, J = 8.4 Hz), 6.77 (1 H, br s), 4.35 (1 H, dd, J = 11.4, 11.4 Hz), 4.21 (1 H, dd, J = 11.2, 4.9 Hz), 3.76 (3 H, s), 3.47 (1 H, m), 2.49 (3 H, s), 2.11 (1 H, dd, J = 7.5, 3.5 Hz), 1.32 (1 H, dd, J = 4.5, 3.5 Hz); IR (KBr) 1701, 1603, 1489, 1395, 1240, 1169, 1088 cm^{-1}; SIMS m/z 405 (M + H)$^+$. Anal. ($C_{23}H_{20}N_2O_5\cdot3.5H_2O$) C, H, N.

4′-(*N*,*N*-Dimethylamino)cinnamoyl A-ring pyrrole duocarmycin 4g: yield 64%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.73 (1 H, br s), 7.77 (1 H, d, J = 15.2 Hz), 7.46 (2 H, d, J = 9.0 Hz), 6.59 (2 H, d, J = 9.0 Hz), 6.64 (1 H, d, J = 15.2 Hz), 6.63 (1 H, br s), 4.21 (1 H, dd, J = 10.9, 10.9 Hz), 4.14 (1 H, dd, J = 10.9, 4.6 Hz), 3.82 (3 H, s), 3.53 (1 H, m), 3.04 (6 H, s), 2.61 (3 H, s), 2.36 (1 H, dd, J = 7.3, 4.3 Hz), 1.30 (1 H, dd, J = 4.9, 4.3 Hz); IR (KBr) 1701, 1593, 1525, 1389, 1360, 1242, 1217, 1169 cm^{-1}; SIMS m/z 432 (M + H)$^+$. Anal. ($C_{25}H_{25}N_3O_4\cdot0.5H_2O$) C, H, N.

4′-[(*tert*-Butoxycarbonyl)amino]cinnamoyl A-ring pyrrole duocarmycin 4h: yield 65%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 11.32 (1 H, br s), 7.76 (1 H, d, J = 15.5 Hz), 7.51 (2 H, d, J = 8.9 Hz), 7.41 (2 H, d, J = 8.9 Hz), 6.80 (1 H, d, J = 15.5 Hz), 6.62 (1 H, br s), 4.23 (1 H, dd, J = 11.2, 11.2 Hz), 4.12 (1 H, dd, J = 11.2, 7.3 Hz), 3.82 (3 H, s), 3.59 (1 H, m), 2.62 (3 H, s), 2.40 (1 H, dd, J = 7.6, 4.3 Hz), 2.05 (1 H, s), 1.54 (9 H, s), 1.30 (1 H, dd, J = 4.3, 4.0 Hz); IR (KBr) 1707, 1620, 1587, 1525, 1520, 1394, 1294, 1240, 1159 cm^{-1}; SIMS m/z 504 (M + H)$^+$. Anal. ($C_{28}H_{29}N_3O_6\cdot0.5H_2O$) C, H, N.

4′-Methylcinnamoyl A-ring pyrrole duocarmycin 4i: yield 83%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.49 (1 H, br s), 7.79

(1 H, d, J = 15.5 Hz), 7.46 (2 H, d, J = 8.2 Hz), 7.20 (2 H, d, J = 8.2 Hz), 6.83 (1 H, d, J = 15.5 Hz), 6.67 (1 H, br s), 4.23 (1 H, dd, J = 10.9, 10.9 Hz), 4.13 (1 H, m), 3.82 (3 H, s), 3.56 (1 H, m), 2.61 (3 H, s), 2.39 (3 H, s), 2.39 (1 H, m), 1.30 (1 H, dd, J = 7.6, 3.6 Hz); IR (KBr) 1701, 1606, 1487, 1294, 1242, 1215, 1109 cm^{-1}; SIMS m/z 403 (M + H)$^+$. Anal. ($C_{24}H_{22}N_2O_4 \cdot 0.2H_2O$) C, H, N.

4′-Ethylcinnamoyl A-ring pyrrole duocarmycin 4j: yield 62%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.39 (1 H, br s), 7.79 (1 H, d, J = 15.6 Hz), 7.49 (2 H, d, J = 8.1 Hz), 7.23 (2 H, d, J = 8.1 Hz), 6.84 (1 H, d, J = 15.6 Hz), 6.65 (1 H, br s), 4.23 (1 H, dd, J = 11.2, 11.2 Hz), 4.14 (1 H, dd, J = 11.2, 4.6 Hz), 3.82 (3 H, s), 3.54 (1 H, m), 2.68 (2 H, q, J = 7.6 Hz), 2.60 (3 H, s), 2.39 (1 H, dd, J = 7.6, 3.7 Hz), 1.31 (1 H, dd, J = 5.3, 3.7 Hz), 1.26 (3 H, t, J = 7.6 Hz); IR (KBr) 1701, 1618, 1609, 1458, 1390, 1244, 1109 cm^{-1}; SIMS m/z 417 (M + H)$^+$. Anal. ($C_{25}H_{24}N_2O_4 \cdot 1.5H_2O$) C, H, N.

4′-Aminocinnamoyl A-Ring Pyrrole Duocarmycin 4k. HBr (1 N, 0.5 mL) and TFA (0.2 mL) were added to a solution of **4h** (8 mg, 0.016 mmol) in CH_3CN (5 mL), and the reaction mixture was stirred for 4 h at room temperature. Then, the mixture was concentrated *in vacuo*. The residue was dissolved in CH_3CN (1 mL), H_2O (0.2 mL), and Et_3N (0.2 mL), and the mixture was stirred for 24 h. Then, 0.2 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (10:1) to give 3 mg (47%) of **4k** as a white powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.28 (1 H, br s), 7.74 (1 H, d, J = 15.5 Hz), 7.39 (2 H, d, J = 8.6 Hz), 6.68 (1 H, d, J = 15.5 Hz), 6.65 (2 H, d, J = 8.6 Hz), 6.62 (1 H, br s), 4.21 (1 H, dd, J = 11.2, 11.2 Hz), 4.11 (1 H, dd, J = 11.2, 6.3 Hz), 3.82 (3 H, s), 3.56 (1 H, m), 2.60 (3 H, s), 2.36 (1 H, dd, J = 7.6, 3.4 Hz), 1.32 (1 H, dd, J = 4.5, 3.4 Hz); IR (KBr) 1697, 1595, 1518, 1443, 1392, 1242, 1219, 1174 cm^{-1}; SIMS m/z 404 (M + H)$^+$. Anal. ($C_{23}H_{21}N_3O_4 \cdot 1.0H_2O$) C, H; N: calcd, 9.97; found, 10.43.

General Synthetic Method for Type 5 Compounds (5a−g). 3′-(Azidopropoxy)-4′-methoxycinnamoyl A-Ring Pyrrole Duocarmycin 5a. NaH (60%; 12 mg, 0.3 mmol) was added to a solution of **3** (60 mg, 0.23 mmol) in DMF (0.6 mL) at argon atmosphere, and the mixture was stirred for 2 h at −50 °C. The *p*-nitrophenyl ester of 3-(3-azidopropoxy)-4-methoxycinnamic acid (124 mg, 0.31 mmol) dissolved in DMF (1.5 mL) was added to a stirred solution at −50 °C. Then, the resulting mixture was stirred at the same temperature for 2 h. The mixture was diluted with AcOEt, and the combine was washed with 0.2 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (50:1) to give 77 mg (65%) of **5a** as a white powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.81 (1 H, br s), 7.68 (1 H, d, J = 15.5 Hz), 7.11 (1 H, dd, J = 8.3, 2.0 Hz), 7.01 (1 H, d, J = 2.0 Hz), 6.81 (1 H, d, J = 8.2 Hz), 6.66 (1 H, d, J = 15.5 Hz), 6.56 (1 H, br s), 4.15 (1 H, dd, J = 11.2, 11.2 Hz), 4.07 (2 H, t, J = 3.6 Hz), 4.06 (1 H, m), 3.84 (3 H, s), 3.76 (3 H, s), 3.50 (2 H, t, J = 6.6 Hz), 3.46 (1 H, m), 2.52 (3 H, s), 2.31 (1 H, dd, J = 7.3, 3.4 Hz), 2.05 (2 H, m), 1.25 (1 H, dd, J = 5.3, 3.4 Hz); IR (KBr) 2098, 1697, 1622, 1608, 1516, 1392, 1263, 1217 cm^{-1}; SIMS m/z 518 (M + H)$^+$. Anal. ($C_{27}H_{27}N_5O_6 \cdot 1.5H_2O$) C, H, N.

3′-[(*N*,*N*-Dimethyamino)propoxy]-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5b: yield 52%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.38 (1 H, br s), 7.59 (1 H, d, J = 15.6 Hz), 7.39 (1 H, br s), 7.28 (1 H, br d, J = 8.5 Hz), 7.00 (1 H, d, J = 8.6 Hz), 6.94 (1 H, d, J = 15.6 Hz), 6.90 (1 H, br s), 4.34 (1 H, br d, J = 10.8 Hz), 4.27 (1 H, m), 4.06 (2 H, t, J = 6.2 Hz), 3.81 (3 H, s), 3.76 (1 H, m), 3.73 (3 H, s), 3.12 (2 H, m), 2.59 (3 H, s), 2.23 (1 H, m), 2.16 (2 H, m), 1.46 (1 H, m), 1.23 (6 H, s); IR (KBr) 1684, 1601, 1443, 1437, 1385, 1263 cm^{-1}; FABMS m/z 520 (M + H)$^+$. Anal. ($C_{29}H_{33}N_3O_6 \cdot 1.5H_2O$) C, H, N.

3′-[[(*tert*-Butoxycarbonyl)methyl]oxy]-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5c: yield 52%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.61 (1 H, br s), 7.65 (1 H, d, J = 15.5 Hz), 7.15 (1 H, dd, J = 8.2, 1.6 Hz), 6.92 (1 H, d, J = 2.0 Hz), 6.83 (1 H, d, J = 8.5 Hz), 6.60 (1 H, d, J = 15.5 Hz), 6.58 (1 H, br s), 4.52 (2 H, s), 4.14 (1 H, dd, J = 10.9, 10.9 Hz), 4.09 (1 H, m), 3.86 (3 H, s), 3.75 (3 H, s), 3.49 (1 H, m), 2.54 (3 H, s), 2.31 (1 H, dd, J = 7.6, 3.4 Hz), 1.55 (9 H, s), 1.25 (1 H, dd, J = 4.6, 3.4 Hz); IR (KBr) 1751, 1701, 1616, 1512, 1458, 1392, 1294, 1142 cm^{-1}; SIMS m/z 549 (M + H)$^+$. Anal. ($C_{30}H_{32}N_2O_8 \cdot 0.5H_2O$) C, H, N.

3′-[[(Methoxycarbonyl)methyl]oxy]-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5d: yield 88%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.69 (1 H, br s), 7.65 (1 H, d, J = 15.5 Hz), 7.15 (1 H, dd, J = 8.2, 1.7 Hz), 6.96 (1 H, d, J = 2.0 Hz), 6.83 (1 H, d, J = 8.6 Hz), 6.63 (1 H, d, J = 15.5 Hz), 6.60 (1 H, br s), 4.66 (2 H, s), 4.14 (1 H, dd, J = 10.9, 10.9 Hz), 4.07 (1 H, m), 3.86 (3 H, s), 3.76 (3 H, s), 3.74 (3 H, s), 3.48 (1 H, m), 2.52 (3 H, s), 2.31 (1 H, dd, J = 7.6, 3.4 Hz), 1.25 (1 H, dd, J = 5.0, 3.4 Hz); IR (KBr) 1699, 1653, 1616, 1516, 1458, 1396, 1219 cm^{-1}; SIMS m/z 507 (M + H)$^+$. Anal. ($C_{27}H_{26}N_2O_8$) C, H, N.

3′-(Diethylamino)-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5e: yield 87%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.39 (1 H, br s), 7.76 (1 H, d, J = 15.5 Hz), 7.25 (1 H, dd, J = 8.6, 1.9 Hz), 7.11 (1 H, d, J = 2.0 Hz), 6.86 (1 H, d, J = 8.6 Hz), 6.70 (1 H, d, J = 15.5 Hz), 6.67 (1 H, br s), 4.22 (1 H, dd, J = 10.9, 10.9 Hz), 4.15 (1 H, dd, J = 10.9, 4.6 Hz), 3.90 (3 H, s), 3.82 (3 H, s), 3.54 (1 H, m), 3.19 (4 H, q, J = 7.3 Hz), 2.60 (3 H, s), 2.36 (1 H, dd, J = 7.6, 3.6 Hz), 1.32 (1 H, dd, J = 5.3, 3.6 Hz), 1.05 (6 H, t, J = 7.0 Hz); IR (KBr) 1701, 1616, 1508, 1389, 1255, 1109 cm^{-1}; FABMS m/z 490 (M + H)$^+$. Anal. ($C_{28}H_{31}N_3O_5 \cdot 1.8H_2O$) C, H, N.

3′-(Dimethylamino)-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5f: yield 64%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.73 (1 H, br s), 7.77 (1 H, d, J = 15.5 Hz), 7.22 (1 H, dd, J = 8.6, 2.0 Hz), 7.11 (1 H, d, J = 2.1 Hz), 6.87 (1 H, d, J = 8.5 Hz), 6.73 (1 H, d, J = 15.5 Hz), 4.23 (1 H, dd, J = 11.0, 10.9 Hz), 4.15 (1 H, dd, J = 11.0, 4.6 Hz), 3.94 (3 H, s), 3.82 (3 H, s), 3.55 (1 H, m), 2.82 (6 H, s), 2.61 (3 H, s), 2.11 (1 H, dd, J = 7.6, 3.3 Hz), 1.32 (1 H, dd, J = 4.9, 3.3 Hz); IR (KBr) 1705, 1614, 1576, 1387, 1240, 1219, 1109 cm^{-1}; FABMS m/z 462 (M + H)$^+$. Anal. ($C_{26}H_{27}N_3O_5 \cdot 1.7H_2O$) C, H, N.

3′-[(*tert*-Butoxycarbonyl)amino]-4′-methoxycinnamoyl A-ring pyrrole duocarmycin 5g: yield 97%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 10.76 (1 H, br s), 8.37 (1 H, br s), 7.78 (1 H, d, J = 15.5 Hz), 7.17 (1 H, dd, J = 8.6, 2.3 Hz), 7.11 (1 H, br s), 6.84 (1 H, d, J = 8.6 Hz), 6.79 (1 H, br s), 6.74 (1 H, d, J = 15.5 Hz), 4.24 (1 H, dd, J = 10.5, 10.5 Hz), 4.16 (1 H, dd, J = 10.5, 3.9 Hz), 3.92 (3 H, s), 3.82 (3 H, s), 3.54 (1 H, m), 2.60 (3 H, s), 2.36 (1 H, dd, J = 7.5, 3.3 Hz), 1.54 (9 H, s), 1.32 (1 H, dd, J = 4.0, 3.3 Hz); IR (KBr) 1705, 1614, 1531, 1390, 1261, 1219, 1157 cm^{-1}; FABMS m/z 534 (M + H)$^+$. Anal. ($C_{29}H_{31}N_3O_7$) C, H; N: calcd, 7.88; found, 8.30.

3′-(Aminopropoxy)-4′-methoxycinnamoyl A-Ring Pyrrole Duocarmycin (5h). A solution of **5a** (15 mg, 0.029 mmol) in dry THF (1.5 mL) was stirred at room temperature. PPh_3 (23 mg, 0.088 mmol) was added, and the mixture was stirred for 24 h. Then, aqueous $NaHCO_3$ was added to the resulting mixture, and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH−Et_3N (200:10:1) to give 4 mg (28%) of **5h** as a yellowish powder: ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.75 (1 H, d, J = 15.2 Hz), 7.54 (1 H, br s), 7.49 (1 H, br d, J = 8.6 Hz), 7.18 (1 H, d, J = 8.6 Hz), 7.09 (1 H, d, J = 15.2 Hz), 7.03 (1 H, br s), 4.15 (1 H, br d, J = 11.2 Hz), 4.39 (1 H, m), 4.27 (2 H, t, J = 3.6 Hz), 3.96 (3 H, s), 3.87 (3 H, s), 3.61 (1 H, m), 3.12 (2 H, t, J = 7.2 Hz), 2.61 (3 H, s), 2.23 (1 H, m), 2.16 (2 H, m), 1.46 (1 H, m); IR (KBr) 1647, 1610, 1512, 1458, 1394, 1385, 1294, 1219 cm^{-1}; SIMS m/z 492 (M + H)$^+$. Anal. ($C_{27}H_{29}N_3O_6$) C, H, N.

3′-Amino-4′-methoxycinnamoyl A-Ring Pyrrole Duocarmycin 5i. The procedure was the same as that employed for the preparation of **4k**. **5g** (30 mg, 0.056 mmol) was subjected to the reaction to afford 12 mg (49%) of **5i** as a light-tan powder: ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.40 (1 H, br s), 7.53 (1 H, d, J = 15.5 Hz), 7.03 (1 H, d, J = 1.7 Hz), 6.96 (1 H, dd, J = 8.3, 1.8 Hz), 6.88 (1 H, d, J = 8.3 Hz), 6.83 (1 H, br s), 6.76 (1 H, d, J = 15.5 Hz), 4.88 (2 H, s), 4.30 (1 H, dd, J

= 10.5, 10.5 Hz), 4.22 (1 H, dd, J = 10.5, 4.9 Hz), 3.85 (3 H, s), 3.76 (3 H, s), 3.48 (1 H, m), 2.50 (3 H, s), 2.11 (1 H, dd, J = 7.3, 4.6 Hz), 1.35 (1 H, dd, J = 4.6, 3.3 Hz); IR (KBr) 1703, 1612, 1514, 1446, 1390, 1271, 1217, 1111 cm^{-1}; FABMS m/z 434 $(M + H)^+$. Anal. ($C_{24}H_{23}N_3O_5 \cdot 0.5H_2O$) C, H, N.

General Synthetic Method for Type 6 Compounds (6a−e). 4′-Methoxycinnamoyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride 6a. Hydrobromic acid (48%, 2.5 mL) was added to a solution of **4a** (50 mg, 0.12 mmol) in CH_3CN (5 mL), and the mixture was stirred for 1 h at room temperature. The resulting mixture was poured into 1 N HBr, and the combine was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. *p*-Nitrophenyl chloroformate (61 mg, 0.30 mmol) and triethylamine (0.042 mL, 0.30 mmol) were added to a stirred solution of the residue in dry methylene chloride (5 mL) at −78 °C. Then, *N*-methylpiperazine (0.040 mL, 0.36 mmol) was added to the solution, and the mixture was stirred at 0 °C for 0.5 h. The mixture was diluted with $CHCl_3$ and washed with 0.2 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (20:1) to give 67 mg (89%) of the free base of **6a**. A solution of the free base (45 mg, 0.072 mmol) in ethanol (2 mL) and methanol (4 mL) was treated with anhydrous 5.8 N HCl in EtOH (0.025 mL) at room temperature for 1 h. The resulting mixture was evaporated *in vacuo* to give 47 mg (99%) of **6a** as a white crystalline compound: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (1 H, br s), 10.57 (1 H, br s), 8.10 (1 H, s), 7.74 (2 H, d, J = 8.7 Hz), 7.58 (1 H, d, J = 15.3 Hz), 7.06 (1 H, d, J = 15.3 Hz), 7.00 (2 H, d, J = 8.7 Hz), 4.50 (1 H, m), 4.42 (3 H, br s), 4.17 (1 H, br s), 3.85 (3 H, s), 3.82 (3 H, s), 3.79 (1 H, br s), 3.58 (3 H, br s), 3.50 (4 H, br s), 2.86 (3 H, s), 2.68 (3 H, s). IR (KBr) 1705, 1648, 1599, 1511, 1405, 1218, 1173, 1095, 1023 cm^{-1}; SIMS (the free base) m/z 627 625 $(M + H)^+$. Anal. ($C_{30}H_{33}BrN_4O_6 \cdot 1.0HCl \cdot 3.0H_2O$) C, H, N.

4′-Methoxycinnamoyl 8-*O*-[(4-piperidinopiperidinyl)-carbonyl] A-ring pyrrole duocarmycin B2 hydrochloride 6b: yield 87%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.02 (1 H, s), 9.96 (1 H, br s), 8.05 (1 H, s), 7.74 (2 H, d, J = 8.9 Hz), 7.57 (1 H, d, J = 15.4 Hz), 7.05 (1 H, d, J = 15.4 Hz), 6.99 (2 H, d, J = 8.9 Hz), 4.46 (2 H, m), 4.41 (2 H, br s), 4.19 (1 H, br d, J = 13.3 Hz), 3.84 (3 H, s), 3.80 (3 H, s), 3.77 (1 H, br s), 3.47 (5 H, br s), 3.13 (1 H, br d, J = 12.9 Hz), 2.95 (2 H, br s), 2.66 (3 H, s), 2.15 (2 H, br s), 1.82 (6 H, br s), 1.70 (2 H, br s); IR (KBr) 1688, 1646, 1598, 1514, 1407, 1252, 1213, 1093, 1023 cm^{-1}; FABMS (the free base) m/z 695 693 $(M + H)^+$. Anal. ($C_{35}H_{41}BrN_4O_6 \cdot 1.0HCl \cdot 3.5H_2O$) C, H, N.

4′-Methoxycinnamoyl 8-*O*-[[[[(*N*-isopropylamino)carbonyl]methyl]piperazinyl]carbonyl] A-ring pyrrole duocarmycin B2 hydrochloride 6c: yield 70%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.21 (1 H, s), 10.39 (1 H, br s), 8.60 (1 H, br s), 8.09 (1 H, s), 7.75 (2 H, d, J = 8.4 Hz), 7.57 (1 H, d, J = 14.3 Hz), 7.06 (1 H, d, J = 14.3 Hz), 6.99 (1 H, d, J = 8.4 Hz), 4.41 (3 H, m), 4.12 (1 H, m), 3.91 (4 H, m), 3.84 (3 H, s), 3.81 (3 H, s), 3.77 (1 H, m), 3.69 (6 H, m), 2.68 (3 H, s), 1.22 (1 H, m), 1.11 (6 H, d, J = 6.4 Hz); IR (KBr) 1678, 1643, 1599, 1515, 1409, 1251, 1212, 1095, 1032 cm^{-1}; FABMS (the free base) m/z 712 710 $(M + H)^+$. Anal. ($C_{34}H_{40}BrN_5O_7 \cdot 1.0HCl \cdot 3.0H_2O$) C, H, N.

3′-(Diethylamino)-4′-methoxycinnamoyl 8-*O*-[(*N*-methylpiperazinyl]carbonyl] A-ring pyrrole duocarmycin B2 hydrochloride 6d: yield 50%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.19 (1 H, br s), 10.88 (1 H, br s), 8.10 (2 H, br s), 7.60 (1 H, br d, J = 8.6 Hz), 7.40 (1 H, br s), 7.24 (1 H, br s), 7.00 (1 H, br s), 4.58 (3 H, m), 3.85 (6 H, s), 3.81−3.26 (2 H, m), 3.21 (4 H, q, J = 7.2 Hz), 2.88 (4 H, br s), 2.69 (3 H, s), 2.51 (4 H, br s), 2.37 (3 H, s), 1.07 (6 H, t, J = 7.0 Hz); IR (KBr) 1714, 1645, 1435, 1417, 1410, 1255, 1219 cm^{-1}; FABMS (the free base) m/z 698 696 $(M + H)^+$. Anal. ($C_{34}H_{42}BrN_5O_6 \cdot 2.0HCl \cdot 3.0H_2O$) C, H, N.

3′-(Dimethylamino)-4′-methoxycinnamoyl 8-*O*-[(*N*-methylpiperazinyl)carbonyl] A-ring pyrrole duocarmycin B2 hydrochloride 6e: yield 55%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.09 (1 H, br s), 10.55 (1 H, br s), 8.10 (1 H, br s),7.58 (1 H, d, J = 15.2 Hz), 7.46 (2 H, br s), 7.04 (1 H, d, J = 15.2 Hz), 7.03 (1 H, br s), 4.49 (4 H, m), 4.18 (1 H, m), 3.86 (3 H, s), 3.85 (3 H, s), 3.79 (1 H, br d, J = 9.9 Hz), 3.48 (3 H, br s), 2.85 (10 H, br s), 2.68 (3 H, s), 2.50 (3 H, s); IR (KBr) 1716, 1697, 1647, 1510, 1434, 1414, 1246, 1217, 1095 cm^{-1}; FABMS (the free base) m/z 670 668 $(M + H)^+$. Anal. ($C_{32}H_{38}BrN_5O_6 \cdot 1.0HCl \cdot 1.0H_2O$) C, H, N.

3′-(Aminopropoxy)-4′-methoxycinnamoyl 8-*O*-[(*N*-methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride 6f. Hydrobromic acid (48%, 1 mL) was added to a solution of **5a** (50 mg, 0.096 mmol) in CH_3CN (5 mL), and the mixture was stirred for 1 h at room temperature. The resulting mixture was poured into 1 N HBr, and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. *p*-Nitrophenyl chloroformate (58 mg, 0.29 mmol) and triethylamine (0.04 mL, 0.29 mmol) were added to a stirred solution of the residue in dry methylene chloride (5 mL) at −78 °C. Then, the resulting mixture was stirred at the same temperature for 0.5 h. *N*-Methylpiperazine (0.038 mL, 0.34 mmol) was added to the solution, and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with $CHCl_3$, and the combine was washed with 0.2 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (20:1) to give 51 mg of the 8-*O* derivative. A solution of the 8-*O* derivative (35 mg, 0.049 mmol) with 10% Pd/$BaSO_4$ (22 mg) in acetone (1 mL) and CH_3OH (5 mL) was stirred under 1 atm of H_2 at room temperature for 4 h. The reaction mixture was filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$−CH_3OH−NH_4OH (10:1:1) as an eluent to afford 12 mg of the free base of **6f**. The obtained free base was employed in the same procedure as that for the preparation of HCl salt to give 10 mg of **6f** in 41% yield from **5a**: ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.18 (1 H, br s), 10.93 (1 H, br s), 8.09 (1 H, s), 7.89 (2 H, br s), 7.56 (1 H, d, J = 15.3 Hz), 7.43 (1 H, br s), 7.33 (1 H, d, J = 8.2 Hz), 7.06 (1 H, d, J = 15.3 Hz), 7.03 (1 H, d, J = 8.3 Hz), 4.50 (1 H, m), 4.40 (2 H, m), 4.15 (2 H, t, J = 5.6 Hz), 3.85 (3 H, s), 3.83 (3 H, s), 3.46 (6 H, br s), 3.26 (4 H, br s), 2.99 (2 H, m), 2.84 (3 H, s), 2.68 (3 H, s), 2.05 (2 H, m); IR (KBr) 1716, 1647, 1509, 1437, 1408, 1263, 1140 cm^{-1}; FABMS m/z 700 698 $(M + H)^+$. Anal. ($C_{33}H_{40}BrN_5O_7 \cdot 2.0HCl \cdot 1.0H_2O$) C, H, N.

3′-Amino-4′-methoxycinnamoyl 8-*O*-[(*N*-Methylpiperazinyl)carbonyl] A-Ring Pyrrole Duocarmycin B2 Hydrochloride 6g. The procedure was the same as that employed for the preparation of **5i**. **5g** (29 mg, 0.054 mmol) was subjected to the reaction to afford 16 mg of **6g** in 44% yield from **5g**: ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.09 (1 H, br s), 10.64 (1 H, br s), 8.10 (1 H, br s), 7.49 (1 H, d, J = 15.2 Hz), 7.22 (1 H, br s), 7.16 (1 H, d, J = 8.6 Hz), 6.95 (1 H, d, J = 2.9 Hz), 6.90 (1 H, d, J = 15.2 Hz), 4.42 (2 H, m), 4.19 (1 H, m), 3.86 (3 H, s), 3.85 (3 H, s), 3.79 (1 H, br s), 3.49 (9 H, br s), 2.89 (3 H, s), 2.68 (3 H, s); IR (KBr) 1699, 1645, 1514, 1439, 1412, 1281, 1219 cm^{-1}; FABMS m/z 642 640 $(M + H)^+$. Anal. ($C_{30}H_{34}BrN_5O_6 \cdot 2.0HCl \cdot 3.5H_2O$) C, H, N.

3′-[(Carboxymethyl)oxy]-4′-methoxycinnamoyl 8-*O*-(Dimethylcarbamoyl) A-Ring Pyrrole Duocarmycin B2 (6h). Hydrobromic acid (48%, 0.16 mL) was added to a solution of **5c** (99 mg, 0.18 mmol) in CH_3CN (5 mL), and the mixture was stirred for 1 h at room temperature. The resulting mixture was poured into 1 N HBr, and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. *p*-Nitrophenyl chloroformate (109 mg, 0.54 mmol) and triethylamine (0.076 mL, 0.54 mmol) were added to a stirred solution of the residue in dry methylene chloride (5 mL) at −78 °C. Then, the resulting mixture was stirred at the same temperature for 0.5 h. Dimethylamine (50%, 0.162 mL, 1.8 mmol) was added to the solution, and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with $CHCl_3$, and the combine was washed with 0.2 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (20:1) to give 44 mg of the 8-*O*

derivative. A solution of the 8-*O* derivative (30 mg, 0.043 mmol) in CH_2Cl_2 (1 mL) and TFA (0.051 mL) was stirred at 80 °C for 24 h. The mixture was diluted with $CHCl_3$, and the combine was washed with 1 N HBr and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3-CH_3OH-CH_3COOH$ (100:10:1) as an eluent to afford 23 mg of **6h** in 29% yield from **5c**: ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.01 (1 H, br s), 8.02 (1 H, br s), 7.51 (1 H, d, J = 15.1 Hz), 7.26 (1 H, br d, J = 8.5 Hz), 7.17 (1 H, br s), 6.97 (1 H, d, J = 15.1 Hz), 6.96 (1 H, d, J = 8.5 Hz), 4.50 (1 H, m), 4.38 (2 H, m), 4.30 (2 H, m), 3.84 (3 H, s), 3.81 (3 H, s), 3.78 (2 H, br s), 3.15 (3 H, s), 2.96 (3 H, s), 2.65 (3 H, s); IR (KBr) 1701, 1585, 1437, 1416, 1317, 1267, 1169 cm^{-1}; FABMS m/z 645 643 $(M + H)^+$. Anal. ($C_{29}H_{29}BrN_3O_9$) C, N; H: calcd, 4.53; found, 4.12.

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 compound for 1 h, washed with Dulbecco's phosphate-buffered saline [Ca^{2+}-, 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 (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 was 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.[20]

The criteria for effectiveness against murine solid tumors were the percentage T/C value with 42% and less, and statistical significance 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 at the day of evaluation and V_0 is the tumor volume at the day of initial drug treatment. The criteria for effectiveness were T/C value with 50% and less and statistical significance determined by the Mann−Whitney U test ($p < 0.01$, one-sided).[21]

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