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Studies on Duocarmycin SA and its Derivatives

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Abstract—New duocarmycin SA derivatives have been 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 results suggested that the *N,N*-dialkylcarbamoyl derivatives bearing the *p*-methoxy cinnamoyl group, which was prepared from duocarmycin SA, showed good in vivo antitumor activities superior to native duocarmycin SA.

Introduction

Duocarmycin SA (**1**) is an exceptionally potent antitumor antibiotic, isolated in trace quantities from a culture broth of the *Streptomyces* species.[1] This structure is related to other duocarmycins and pyrindamycins (Fig. 1).[2] Among these antibiotics, **1** and duocarmycin A (**2a**) especially resemble CC-1065 (**2b**) in that they have a unique cyclopropane ring responsible for the sequence-selective and reversible alkylation of duplex DNA.[3,4] Duocarmycin SA is the most stable compound among the duocarmycins, and exhibits high in vitro anticellular activities relative to other structurally related compounds.[5] Based on its unique structure and mode of action, the total synthesis of **1** and structurally related compounds have been performed by some groups.[6] However, according to our preliminarily biological and physiological tests, duocarmycin SA (**1**) exhibited the in vivo antitumor activity only at very narrow range of doses against murine sarcoma 180, and showed very poor water solubility. Therefore, these results dissuaded us from further developing **1** as a novel antitumor drug.

We had already been synthesizing analogues of duocarmycin B2 (**2c**) in order to enhance and broaden the spectrum of the antitumor activity, and to improve the solubility.[7] Recently, KW-2189 (**3a**), a novel derivative of duocarmycin B2 (**2c**), was synthesized and the compound demonstrated excellent in vivo antitumor activity with aqueous solubility greater than 10 mg/mL.[8] KW-2189 is currently under phase I clinical trial. With the goal of finding new candidates having greater activity or less toxicity than those of conventional duocarmycins or their derivatives, we have designed the duocarmycin SA derivatives modified at the segment-A and the segment-B based on our SAR information.

In this paper, we first report our investigation into the synthesis, anticellular and antitumor activities of duocarmycin SA derivatives. Furthermore, we will describe the relationship between the aqueous stability of duocarmycin SA and the related compounds, and their biological properties.

Chemistry

We had already reported that the introduction of a *N,N*-dialkylcarbamoyl moiety at the 8-*O*-phenolic hydroxy group of duocarmycins showed excellent in vivo antitumor activity, superior to that of the parent compounds.[7] Based on this strategy, a series of the 8-*O*-*N,N*-dialkylcarbamoyl derivatives of duocarmycin SA were prepared and evaluated, as shown in Scheme 1.

Initially, the 2-methyl-3-methoxycarbonyl-A-ring pyrrole-duocarmycin (DU-86, **3b**) was prepared by employing the Wagner–Meerwein type rearrangement of the 8-*O*-protected-3-hydroxy-duocarmycin B2 followed by deprotection of the protecting group under basic conditions.[9] Treatment of **3b** with HBr or HCl exclusively afforded their adducts, carrying a bromomethyl or a chloromethyl group in the C-ring part.[10] When duocarmycin SA was used for this reaction, their adducts carrying a bromomethyl or a chloromethyl group were obtained exclusively. Addition of 4-nitrophenyl chloroformate in the presence of triethylamine in methylene chloride at −78 °C gave a carbonate as an intermediate, which, without isolation, furnished the 8-*O*-*N,N*-dialkylcarbamoyl derivatives (**4a, b** and **5a, b**) in good yields upon treatment with dimethylamine solution or 1-methylpiperazine.

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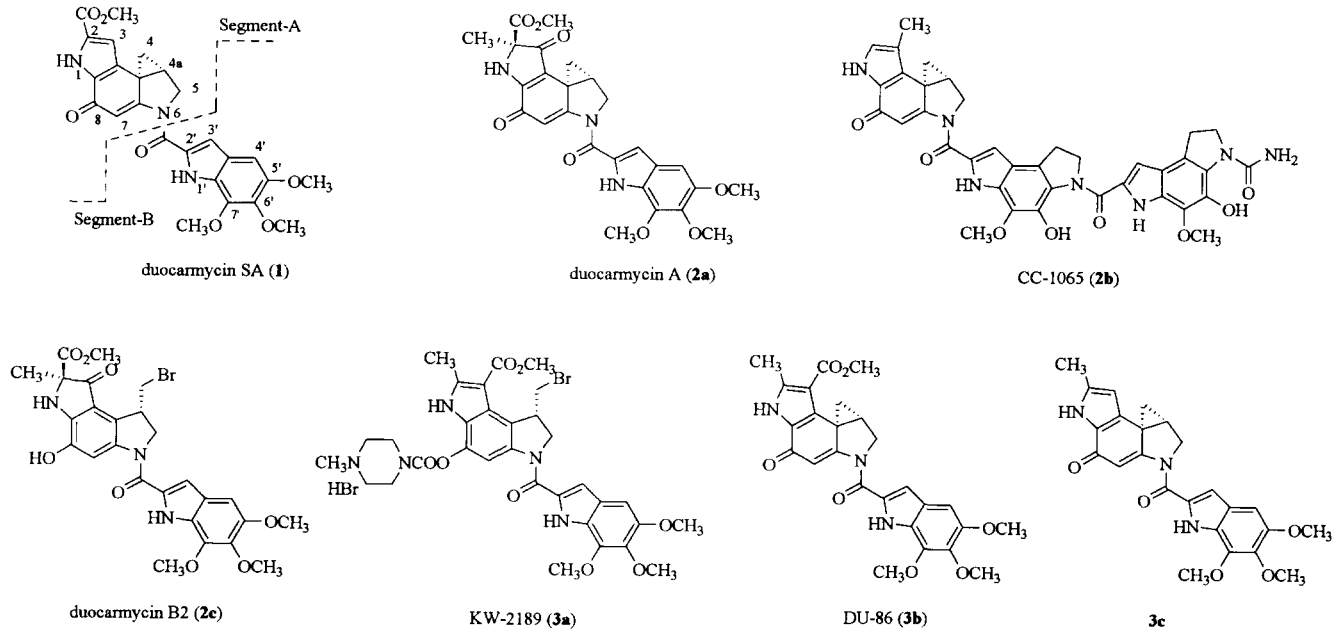
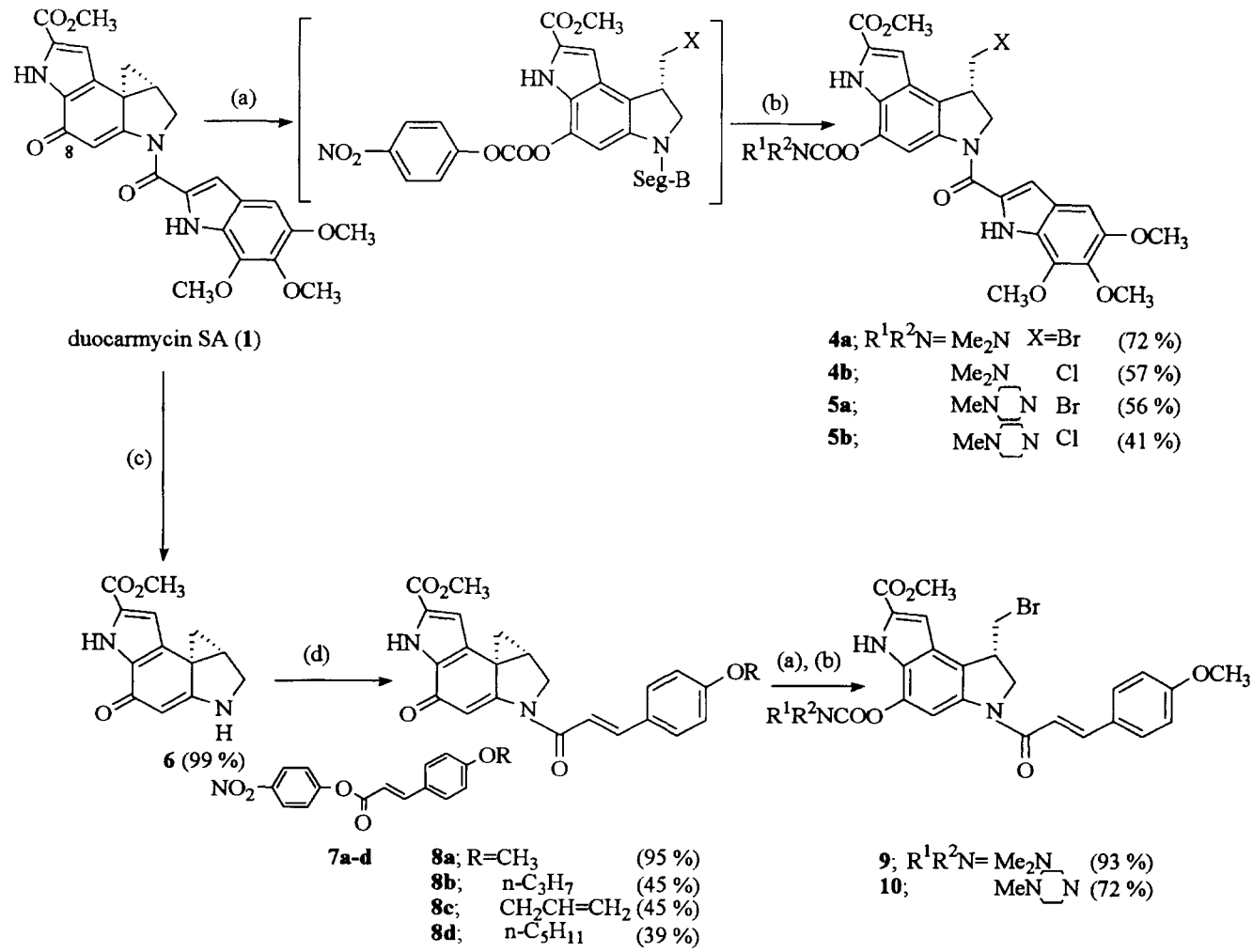


Figure 1. Structures of duocarmycins, CC-1065 and duocarmycin derivatives.



Scheme 1. (a) HBr or HCl, CH_3CN, rt, 1 h, then 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, −78 °C, 0.5 h; (b) Me_2NH or 1-methylpiperazine CH_2Cl_2, 0 °C, 1 h; (c) NaOMe, MeOH–CH_3CN, rt, 1 h; (d) NaH, DMF, 0 °C, 2 h, then **7a–d**, −50 °C, 2 h.

On the other hand, we have synthesized a series of duocarmycin analogues bearing the simplified moieties such as a cinnamoyl group at the segment-B.[11] The 8-*O*-*N*,*N*-dialkylcarbamoyl derivatives introduced the cinnamoyl moiety exhibited sufficient in vivo activity against sarcoma 180 in the wide range of doses without detectable toxic effect. Accordingly, duocarmycin SA analogues bearing the cinnamoyl moiety were also synthesized by the same method. Duocarmycin SA was treated with NaOMe in MeOH to afford compound **6** and methyl 5,6,7-trimethoxyindole-2-carboxylate quantitatively. The compound **6** thus obtained was treated with *p*-alkoxy-*trans*-cinnamic acid *p*-nitrophenyl esters (**7a–d**) in the presence of NaH to yield the corresponding *p*-substituted cinnamates **8a-d**. *p*-Alkoxy-*trans*-cinnamic acid *p*-nitrophenyl esters were synthesized from the corresponding *p*-substituted cinnamic acid and *p*-nitrophenol by the Mukaiyama reagent.[12] The cinnamate (**8a**) was converted to the 8-*O*-*N*,*N*-dialkylcarbamoyl derivatives (**9** and **10**) by the aforementioned method.

Results and Discussion

Duocarmycin SA (**1**) and its derivatives were studied in vitro for their anticellular activity against HeLa S_3 cells (Tables 1 and 2). As can be seen in the tables, the in vitro anticellular activity of the 4′-methoxy cinnamoyl duocarmycin SA (**8a**) was almost equivalent to that of duocarmycin SA (**1**), the IC_{50} value at 72 h exposure was 0.0016 nM. In addition, there is a marked tendency for the 4′-alkoxy cinnamoyl analogues of duocarmycin SA to decrease the anticellular potency with increasing size of the alkoxy moiety (**8a–d**).[13]

On the other hand, the 8-*O*-*N*,*N*-dialkylcarbamoyl derivatives (**4a, b**, **5a, b**, **9**, **10**) showed decreased in vitro anticellular activity. The IC_{50} values at 72 h exposure were approximately four orders of magnitude inferior to **1** or **8a**. These 8-*O*-*N*,*N*-dialkylcarbamoyl derivatives do not directly interact with DNA. They need to be activated by enzyme, especially carboxyesterase, to exhibit the biological activities, as previously described.[7]

The in vivo activity of the 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. The results at maximum tolerated doses (MTD) are indicated in Tables 1 and 2. Tumor volume was calculated according to the method described previously.[7,8] Compounds **1** and **8a**, which have electrophilic cyclopropane rings responsible for DNA alkylation, showed sufficient efficacy with *T*/*C* values of 0.21 and 0.068, respectively. At a half dose of MTD, however, they exhibited no in vivo antitumor activities. Therefore, their effective ranges of doses were extremely narrow. Also their water solubilities were very poor. In view of

Table 1. Anticellular activity and antitumor activity of duocarmycin SA derivatives

Compound	HeLa S_3 IC_{50} (nM)[a]	S-180 (sc-iv)[b]	
	72 h	Dose (mg/kg)	T/C
4a	0.44	0.5	0.22
4b	0.78		
5a	0.62	0.5	0.31
5b	1.5	0.5	0.28
8a	0.0016	1.0	0.068
8b	5.3		
8c	4.2		
8d	38		
9	47	8.0	0.20
10	6.9	8.0	0.19

[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 cell, and the drug was dosed (mg/kg) intravenously (iv).

Table 2. Anticellular activity, antitumor activity and stability tests of duocarmycin SA and its related compounds

Compound	Stability ($T_{1/2}$)[a] in aqueous solution (pH 7)	HeLa S_3 IC_{50} (nM)[b] 72 h	S-180 (sc-iv)[c] Dose (mg/kg)	T/C
1	330	0.00069	0.10	0.21
2a	<1	0.0058	0.075	0.26
3b	130	0.0052	0.13	0.18
3c	320	0.0004	0.063	0.40

[a]A half-life at 35 °C. Drug concentratin was 0.02 mg/mL. See the experimental section.
[b]Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.
[c]Mice (five mice/group) were implanted subcutaneously (sc) with tumor cell, and the drug was dosed (mg/kg) intravenously (iv).

this information, we stopped developing these compounds as new drug candidates.

The 8-*O*-*N*,*N*-dialkylcarbamoyl analogues (**4a** and **5a**, **b**), which were enzymatically converted to duocarmycin SA (**1**) as an active metabolite, exhibited marginal in vivo antitumor activities. Their biological activities are not better than that of KW-2189 (**3a**), a novel derivative of duocarmycin B2. In contrast, the compounds (**9**, **10**) bearing a cinnamoyl moiety showed good in vivo antitumor activities with *T*/*C* values below 0.2. Moreover, as anticipated, they were significantly effective in the wide range from MTD to a half dose of it without showing detectable toxic effects. Consequently, compound **10** has been selected as one of the candidates for further development.

In general, the hydrolysis of the 8-*O*-*N*,*N*-dialkylcarbamoyl derivatives modified at a phenolic hydroxyl group is catalyzed by carboxyesterase, and the polymorphism of enzymes or the difference of enzyme activities may cause the interpatient variation in efficacy and toxicity.[7,8,14] Thus, we have to focus special attention on the interpatient variation. Further studies on antitumor spectra and toxicity of these derivatives (**9**, **10**) are in progress.

Herein, the stability of duocarmycin SA and the related compounds was measured in 0.01 M phosphate buffer (pH 7) containing 20% CH_3CN by HPLC analysis. As shown in Table 2, duocarmycin SA (**1**) and compound **3c**, which was derived by chemical modification from duocarmycin B2 (**2c**),[8] exhibited good stability with their half-lives of almost 330 h under these conditions. In contrast, compound **3b**, an active metabolite of KW-2189 (**3a**), was not as stable as **1** or **3c**, being gradually hydrolyzed to the corresponding diol, which was produced by a nucleophilic addition of H_2O to the cyclopropane ring.[10,15] Duocarmycin A (**2a**) was extremely unstable ($T_{1/2}$ <1 h), and decomposed to inactive forms.[7] While the correlation is limited to the comparisons made on the three A-ring pyrrole compounds except duocarmycin A (**2a**), the solvolytically more stable compounds tended to be more potent in anticellular activity (in vitro) against HeLa S_3 cells, as reported by Boger et al.[16] The electron-withdrawing group at the C-3 position may especially contribute to a decrease of the aqueous stability. However, the compounds having a cyclopropane ring exhibited marginal or weak in vivo antitumor activities against murine sarcoma 180 at a very narrow range of doses, as mentioned above.[17] In addition, in other mice or human tumor models, they did not show statistically significant in vivo antitumor activities. In order to exhibit any critical in vivo antitumor activities, it is considered that the conversion of the cyclopropane-containing agents to seco-compounds (HBr or HCl adducts, etc.) and the strategy of prodrug will be necessary. These findings may prove useful in preparing the next generation of active duocarmycin derivatives.

Experimental

All mps were measured on a Yanagimoto micromelting point apparatus and are uncorrected. IR were recorded on a JASCO IR-810. ^{1}H NMR 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 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.

8-*O*-*N*,*N*-Dimethylcarbamoyl-9-bromo-duocarmycin SA (4a). Hydrobromic acid (48%, 0.5 mL) was added to a solution of **1** (20 mg, 0.042 mmol) in CH_3CN (1.5 mL), and the mixture was stirred for 1 h at rt. It 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 (16.9 mg, 0.084 mmol) and triethylamine (0.012 mL, 0.087 mmol) were added to a stirred solution of the residue in dry methylene chloride (0.5 mL) at −78 °C. Then, the resulting mixture was stirred at the same temperature for 0.5 h. An aqueous solution of 50% dimethylamine (0.038 mL, 0.422 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 0.01 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 19 mg (72 %) of **4a** as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (1 H, br s), 9.09 (1 H, br s), 8.26 (1 H, s), 7.17 (1 H, d, J = 2.1 Hz), 6.96 (1 H, d, J = 2.2 Hz), 6.89 (1 H, s), 4.74 (1 H, dd, J = 10.7, 9.5 Hz), 4.60 (1 H, dd, J = 10.7, 4.0 Hz), 4.19 (1 H, m), 4.08 (3 H, s), 3.96 (3 H, s), 3.95 (3 H, s), 3.93 (1 H, dd, J = 10.2, 3.8 Hz), 3.92 (3 H, s), 3.47 (1 H, dd, J = 10.2, 10.2 Hz), 3.21 (3 H, s), 3.08 (3 H, s). IR (KBr) 1938, 1718, 1617, 1522, 1490, 1437, 1411, 1387, 1311, 1213, 1165, 1111, 1049, 998 cm^{-1}. EIMS *m*/*z* 630 628 $(M)^+$, 548, 397 395, 315, 234. Anal. calcd for $C_{28}H_{29}BrN_4O_8 \cdot 1.5\ H_2O$: C, 51.23; H, 4.91; N, 8.53. Found: C, 51.31; H, 4.87; N, 8.61.

8-*O*-*N*,*N*-Dimethylcarbamoyl-9-chloro-duocarmycin SA (4b). The procedure was the same as that employed for the preparation of **4a** except for the use of 6 N HCl. The crude product was purified by silica-gel chromatography to afford 14 mg (57%) of **4b** as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (1 H, br s), 9.09 (1 H, br s), 8.27 (1 H, s), 7.17 (1 H, d, J = 2.0 Hz), 6.96 (1 H, d, J = 2.3 Hz), 6.88 (1 H, s), 4.73 (1 H, dd, J = 10.7, 10.7 Hz), 4.64 (1 H, dd, J = 10.7, 3.9 Hz), 4.13 (1 H, m), 4.09 (3 H, s), 4.04 (1 H, dd, J = 11.1, 3.6 Hz), 3.96 (3 H,

s), 3.95 (3 H, s), 3.92 (3 H, s), 3.61 (1 H, dd, J = 11.1, 10.0 Hz), 3.21 (3 H, s), 3.08 (3 H, s). IR (KBr) 2938, 1705, 1616, 1521, 1489, 1436, 1410, 1386, 1310, 1212, 1160, 1110, 1049, 997 cm^{-1}. EIMS m/z 584 $(M)^+$, 351, 234. Anal. calcd for $C_{28}H_{29}ClN_4O_8 \cdot 2.0\ H_2O$: C, 54.15; H, 5.36; N, 9.02. Found: C, 54.33; H, 5.65; N, 9.46.

8-*O*-(4-Methyl-1-piperaziny carbonyl)-9-bromo-duocarmycin SA hydrochloride (5a). The procedure was the same as that employed for the preparation of **4a** except for the use of 4-methylpiperazine. The crude product was purified by silica-gel chromatography to afford the free base 16 mg (56%) of **5a**. ^1H NMR (400 MHz, DMSO-d_6) δ 11.27 (1 H, d, J = 1.7 Hz), 10.51 (1 H, br s), 8.02 (1 H, s), 7.44 (1 H, d, J = 1.4 Hz), 7.01 (1 H, d, J = 1.7 Hz), 6.96 (1 H, s), 4.74 (1 H, dd, J = 10.1, 9.7 Hz), 4.42 (2 H, br s), 4.37 (1 H, dd, J = 10.1, 4.5 Hz), 4.26 (1 H, m), 4.15 (2 H, br s), 4.04 (1 H, dd, J = 10.3, 10.3 Hz), 3.97 (1 H, dd, J = 10.3, 10.3 Hz), 3.94 (3 H, s), 3.91 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.51 (4 H, br s), 2.85 (3 H, s). IR (KBr) 2938, 1706, 1616, 1521, 1489, 1430, 1310, 1251, 1214, 1166, 1109 cm^{-1}. EIMS m/z 685 683$(M)^+$, 234. Anal. calcd for $C_{31}H_{34}BrN_5O_8 \cdot 1.5\ H_2O$: C, 52.33; H, 5.24; N, 9.84. Found: C, 52.36; H, 5.36; N, 10.32.

8-*O*-(4-Methyl-1-piperaziny carbonyl)-9-chloro-duocarmycin SA hydrochloride (5b). The procedure was the same as that employed for the preparation of **5a** except for the use of 6 N HCl. The whole was evaporated in vacuo to give 11.4 mg (41%) of **5b** as a white powder. ^1H NMR (400 MHz, DMSO-d_6) δ 11.27 (1 H, d, J = 1.8 Hz), 10.50 (1 H, br s), 8.02 (1 H, s), 7.43 (1 H, d, J = 2.1 Hz), 7.01 (1 H, d, J = 1.8 Hz), 6.96 (1 H, s), 4.74 (1 H, dd, J = 11.1, 11.1 Hz), 4.42 (2 H, br s), 4.40 (1 H, dd, J = 11.1, 4.5 Hz), 4.20 (1 H, m), 4.15 (2 H, br s), 4.13 (1 H, dd, J = 11.1, 3.4 Hz), 4.06 (1 H, dd, J = 11.1, 6.5 Hz), 3.94 (3 H, s), 3.91 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.51 (4 H, br s), 2.86 (3 H, s). IR (KBr) 2940, 1701, 1611, 1559, 1539, 1521, 1490, 1457, 1313, 1216, 1171 cm^{-1}. EIMS m/z 639 $(M)^+$, 603, 406, 370, 234. Anal. calcd for $C_{31}H_{34}ClN_5O_8 \cdot 1.0\ H_2O$: C, 56.58; H, 5.51; N, 10.64. Found: C, 56.38; H, 5.66; N, 10.46.

Methyl-1,2,8,8a-tetrahydrocycloprop[1,2-*c*]pyrrolo-[3,2-*e*]-indol-4(*5H*)-one-6-carboxylate (6). Sodium methoxide (25 wt% solution in methanol; 0.04 mL, 0.21 mmol) was added to a solution of duocarmycin SA (**1**; 20 mg, 0.042 mmol) in MeOH (2 mL) and CH_3CN (1 mL), and the mixture was stirred at rt for 1 h. Then, 0.2 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, 25:1) to give 10 mg (99%) of **6** as a light-tan powder. ^1H NMR (400 MHz, $CDCl_3$ + DMSO-d_6) δ 11.78 (1 H, br s), 7.34 (1 H, br s), 6.54 (1 H, d, J = 2.2 Hz), 5.38 (1 H, s), 3.79 (3 H, s), 3.71 (1 H, dd, J = 11.0, 5.4 Hz), 3.53 (1 H, dd, J = 11.0, 11.0 Hz), 2.83 (1 H, m), 1.52 (1 H, dd, J = 7.8, 2.5 Hz), 1.16 (1 H, dd, J = 4.5, 3.7 Hz). IR (KBr) 1701, 1599, 1541, 1521, 1419, 1302, 1251, 1240, 1000 cm^{-1}. SIMS m/z 245 $(M + H)^+$. Anal. calcd for $C_{13}H_{12}N_2O_3 \cdot 1.0\ CH_3OH$: C, 60.86; H, 5.84; N, 10.14. Found: C, 61.54; H, 5.48; N, 10.35.

4′-Methoxy cinnamoyl duocarmycin SA (8a). NaH (60%; 9 mg, 0.23 mmol) was added to a solution of **6** (33 mg, 0.14 mmol) in DMF (0.5 mL) at argon atmosphere, and the mixture was stirred for 2 h at 0 °C. p-Nitrophenyl ester of 4-methoxycinnamic acid (68 mg, 0.28 mmol) dissolved in DMF (2.5 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 54 mg (95%) of **8a** as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (1 H, br s), 7.78 (1 H, d, J = 15.5 Hz), 7.52 (2 H, d, J = 8.7 Hz), 7.26 (1 H, s), 6.92 (2 H, d, J = 8.7 Hz), 6.74 (1 H, d, J = 15.5 Hz), 6.59 (1 H, s), 4.22 (1 H, dd, J = 11.4, 11.4 Hz), 4.16 (1 H, dd, J = 11.4, 4.8 Hz), 3.91 (3 H, s), 3.86 (3 H, s), 2.70 (1 H, m), 1.75 (1 H, dd, J = 7.6, 4.8 Hz), 1.51 (1 H, dd, J = 4.8, 4.8 Hz). IR (KBr) 1718, 1669, 1601, 1511, 1390, 1282, 1232, 1173 cm^{-1}. SIMS m/z 405 $(M + H)^+$. Anal. calcd for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.33; H, 5.10; N, 6.89.

4′-Propoxy cinnamoyl duocarmycin SA (8b). The procedure was the same as that employed for the preparation of **8a** except for the use of p-nitrophenyl ester of 4-propoxycinnamic acid. The crude product was purified by silica-gel chromatography to afford 8 mg (45%) of **8b** as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 9.87 (1 H, br s), 7.77 (1 H, d, J = 15.5 Hz), 7.31 (2 H, d, J = 7.9 Hz), 7.13 (2 H, d, J = 7.6 Hz), 7.06 (1 H, br s), 6.94 (1 H, dd, J = 7.8, 2.3 Hz), 6.84 (1 H, d, J = 15.5 Hz), 6.59 (1 H, d, J = 2.3 Hz), 4.23 (1 H, dd, J = 11.3, 11.3 Hz), 4.17 (1 H, dd, J = 11.3, 4.8 Hz), 3.95 (2 H, dt, J=7.5, 1.7 Hz), 3.91 (3 H, s), 2.72 (1 H, m), 1.82 (2 H, m), 1.76 (1 H, dd, J = 7.5, 4.7 Hz), 1.51 (1 H, dd, J = 4.7, 4.7 Hz), 1.05 (3 H, t, 7.4 Hz). IR (KBr) 1713, 1672, 1616, 1389, 1233 cm^{-1}. SIMS m/z 433 $(M + H)^+$. Anal. calcd for $C_{25}H_{24}N_2O_5 \cdot 1.0\ H_2O$: C, 66.66; H, 5.82; N, 6.22. Found: C, 67.22; H, 5.89; N, 6.49.

4′-Propenyloxy cinnamoyl duocarmycin SA (8c). The procedure was the same as that employed for the preparation of **8a** except for the use of p-nitrophenyl ester of 4-propenyloxycinnamic acid. The crude product was purified by silica-gel chromatography to afford 8 mg (45%) of **8c** as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (1 H, br s), 7.77 (1 H, d, J = 15.4 Hz), 7.31 (2H, t, J = 7.9 Hz), 7.16 (2H, d, J = 7.6 Hz), 7.08 (1 H, br s), 6.97 (1 H, dd, J = 8.0, 2.1 Hz), 6.83 (1 H, d, J = 15.4 Hz), 6.59 (1 H, d, J = 2.2 Hz), 6.05 (1 H, m), 5.43 (1 H, dd, J = 17.3, 1.4 Hz), 5.31 (1 H, dd, J = 10.5, 1.3 Hz), 4.57 (2 H, d, J = 5.1 Hz), 4.23 (1 H, dd, J = 11.2, 11.2 Hz), 4.17 (1 H, dd, J = 11.2, 4.6 Hz), 3.91 (3 H, s), 2.72 (1 H, m), 1.77 (1 H, dd, J=7.5, 4.7 Hz), 1.52 (1 H,

dd, J = 4.7, 4.7 Hz). IR (KBr) 1696, 1610, 1516, 1387, 1244 cm^{-1}. SIMS m/z 431 $(M + H)^+$. Anal. calcd for $C_{25}H_{22}N_2O_5 \cdot 1.0\ H_2O$: C, 66.95; H, 5.39; N, 6.25. Found: C, 66.95; H, 5.36; N, 6.13.

4′-Pentyloxy cinnamoyl duocarmycin SA (8d). The procedure was the same as that employed for the preparation of **8a** except for the use of *p*-nitrophenyl ester of 4-penthyloxycinnamic acid. The crude product was purified by silica-gel chromatography to afford 15 mg (39%) of **8d** as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 9.69 (1 H, br s), 7.77 (1 H, d, J = 15.4 Hz), 7.30 (2 H, t, J = 7.1 Hz), 7.14 (2 H, d, J = 7.8 Hz), 7.05 (1 H, br s), 6.95 (1 H, dd, J = 8.3, 2.0 Hz), 6.83 (1 H, d, J = 15.4 Hz), 6.59 (1 H, d, J = 2.5 Hz), 4.23 (1 H, dd, J = 11.2, 11.2 Hz), 4.17 (1 H, dd, J = 11.2, 4.6 Hz), 3.98 (2 H, t, J = 6,5 Hz), 3.91 (3 H, s), 2.71 (1 H, m), 1.78 (3 H, m), 1.50 (3 H, m), 1.42 (2 H, m), 0.94 (3 H, t, J = 7.1 Hz). IR (KBr) 1700, 1654, 1611, 1507, 1457, 1383, 1250 cm^{-1}. SIMS m/z 461 $(M + H)^+$. Anal. calcd for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.41; H, 5.98; N, 6.12.

4′-Methoxy cinnamoyl 8-*O*-(*N*,*N*-dimethylcarbamoyl) duocarmycin SA (9). Forty-eight percent hydrobromic acid (0.5 mL) was added to a solution of **8a** (10 mg, 0.025 mmol) in CH_3CN (1 mL), and the mixture was stirred for 1 h at rt. 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 (13 mg, 0.065 mmol) and triethylamine (0.009 mL, 0.065 mmol) were added to a stirred solution of the residue in dry methylene chloride (1 mL) at −78 °C. Then, the resulting mixture was stirred at the same temperature for 0.5 h. An aqueous solution of 50% dimethylamine (0.012 mL, 0.13 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 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 (100:1) to give 13 mg (93%) of **9**. ^1H NMR (400 MHz, $CDCl_3$) δ 9.05 (1 H, br s), 8.34 (1 H, s), 7.79 (1 H, d, J=15.2 Hz), 7.56 (2 H, d, J = 8.7 Hz), 6.93 (2 H, d, J = 8.7 Hz), 7.14 (1 H, d, J = 2.0 Hz), 6.74 (1 H, d, J = 15.2 Hz), 4.48 (1 H, dd, J = 10.7, 6.3 Hz), 4.35 (1 H, dd, J = 10.7, 4.1 Hz), 4.14 (1 H, m), 3.95 (3 H, s), 3.90 (1 H, dd, J = 10.4, 2.8 Hz), 3.86 (3 H, s), 3.45 (1 H, dd, J = 10.4, 10.4 Hz), 3.19 (3 H, s), 3.07 (3 H, s). IR (KBr) 1718, 1648, 1601, 1511, 1438, 1406, 1306, 1250, 1163 cm^{-1}. SIMS m/z 558 556 $(M + H)^+$. Anal. calcd for $C_{26}H_{26}BrN_3O_6 \cdot 0.5\ H_2O$: C, 55.23; H, 4.81; N, 7.43. Found: C, 55.22; H, 4.99; N, 7.46.

4′-Methoxy-cinnamoyl-8-*O*-(*N*-methylpiperaziny carbonyl) duocarmycin SA (10). The procedure was the same as that employed for the preparation of **9** except for the use of 4-methylpiperazine. The crude product was purified by silica-gel chromatography to afford 9.6 mg (72%) of **10** as a white crystalline. ^1H NMR (400 MHz, $CDCl_3$) δ 9.29 (1 H, br s), 8.33 (1 H, s), 7.78 (1 H, d, J = 15.2 Hz), 7.56 (2 H, d, J = 8.8 Hz), 7.12 (1 H, d, J = 2.0 Hz), 6.93 (2 H, d, J = 8.8 Hz), 6.74 (1 H, d, J = 15.3 Hz), 4.49 (1 H, dd, J = 10.7, 10.3 Hz), 4.34 (1 H, dd, J = 10.7, 4.2 Hz), 4.14 (1H, m), 3.95 (3 H, s), 3.94 (2 H, br s), 3.89 (1 H, dd, J = 10.2, 3.4 Hz), 3.86 (3 H, s), 3.80 (2 H, br s), 3.46 (1 H, dd, J = 10.2, 10.2 Hz), 2.78 (4 H, br s), 2.56 (3 H, s). IR (KBr) 2344, 1715, 1648, 1603, 1512, 1424, 1406, 1307, 1252, 1174, 1100 cm^{-1}. SIMS m/z 613 611 $(M + H)^+$. Anal. calcd for $C_{29}H_{31}BrN_4O_6 \cdot 1.0\ H_2O$: C, 55.33; H, 5.28; N, 8.90. Found: C, 55.66; H, 5.00; N, 9.13.

Stability of drug in aqueous solution

The stability of duocarmycin SA (**1**) and its related compounds under aqueous buffer condition was examined by chromatography on a UNISIL pack 5C18 reversed-phase HPLC column (GL Science, Co., Ltd, Tokyo, Japan). The compound (1 mg) was dissolved in CH_3CN (10 mL). This solution (2 mL) was diluted with aqueous solution (8 mL). Aqueous solution was composed of 0.01 M phosphate buffer (pH 7). The resulting solution was incubated at 35 °C. Samples were removed at intervals and injected directly into the HPLC injection port. The compound was eluted with 0.05 M phosphate buffer (pH 5.9)–CH_3CN (30:70) and detected by measuring absorbance at 330 nm.

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, Michigan, U.S.A.) 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 was 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 one 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.[18]

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 ($P < 0.05$).[19]

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References

1. (a) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037. (b) Yasuzawa, T.; Saitoh, Y.; Ichimura, M.; Takahashi, I.; Sano, H. *J. Antibiot.* **1991**, *44*, 445.

2. (a) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915. (b) Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H. *Chem. Pharm. Bull.* **1988**, *36*, 3728. (c) Ichimura, M.; Muroi, K.; Asano, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1285. (d) Ogawa, T.; Ichimura, M.; Katsumata, S.; Morimoto, M.; Takahashi, K. *J. Antibiot.* **1989**, *42*, 1299. (e) Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045. (f) Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H.; Saitoh, Y. *Chem. Pharm. Bull.* **1995**, *43*, 378. (g) Ohba, K.; Watabe, H.; Sasaki T.; Takeuchi, Y.; Kodama, Y.; Nakazawa, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S. *J. Antibiot.* **1988**, *41*, 1515. (h) Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inoue, S.; Kondo, S. *J. Antibiot.* **1989**, *42*, 1713.

3. (a) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. *J. Antibiot.* **1978**, *31*, 1211. (b) Martin, D. G.; Chidester, C. G.; Duchamp, D. J.; Mizsak S. A. *J. Antibiot.* **1980**, *33*, 902. (c) Reynolds, V. L.; McGovren, J. P.; Hurley, L. H. *J. Antibiot.* **1986**, *39*, 319. (d) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843. (e) Raynolds, V. L.; Molineaux, I. J.; Kaplan, D. J.; Swensen, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228. (f) Tang, M. S.; Lee, C. S.; Doisy, R.; Ross, L.; Needham-Van Devanter, D. R.; Hurley, L. H. *Biochemistry* **1988**, *27*, 893.

4. (a) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Org. Chem.* **1990**, *55*, 4499. (b) Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197. (c) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961. (d) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645. (e) Sugiyama, H.; Ohmori, K.; Chan, K. L.; Hosoda, M.; Asai, A.; Saito, H.; Saito, I. *Tetrahedron Lett.* **1993**, *34*, 2179. (f) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.

5. Ichimura, M.; Ogawa, T.; Takahashi, K.; Mihara, A.; Takahashi, I.; Nakano, H. *Oncol. Res.* **1993**, *5*, 165.

6. (a) Boger, D. L.; Machiya, K. *J. Am. Chem. Soc.* **1992**, *114*, 10056. (b) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.* **1993**, *115*, 9025. (c) Muratake, H.; Matsumura, N.; Natsume, M. *Chem. Pharm. Bull.* **1995**, *43*, 1064. (d) Boger, D. L.; Yun, W.; Han, N. *Bioorg. Med. Chem.* **1995**, *3*, 1429. (e) Muratake, H.; Abe, I.; Natsume, M. *Chem. Pharm. Bull.* **1996**, *44*, 67. (f) Boger, D. L.; Goldberg, J.; McKie, J. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1955. (g) Boger, D. L.; Bollinger, B.; Johnson, D. S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2207.

7. (a) Gomi, K.; Kobayashi, E.; Miyoshi, K.; Ashizawa, T.; Okamoto, A.; Ogawa, T.; Katsumata, S.; Mihara, A.; Okabe, M.; Hirata, T. *Jpn. J. Cancer Res.* **1992**, *83*, 113. (b) Nagamura, S.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1995**, *43*, 1530.

8. (a) Nagamura, S.; Asai, A.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, 1723. (b) Kobayashi, E.; Okamoto, A.; Asada, M.; Okabe, M.; Nagamura, S.; Asai, A.; Saito, H.; Gomi, K.; Hirata, T. *Cancer Res.* **1994**, *54*, 2404. (c) Asai, A.; Nagamura, S.; Saito, H. *J. Am. Chem. Soc.* **1994**, *116*, 4171.

9. (a) Nagamura, S.; Kanda, Y.; Asai, A.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, 933. (b) Berner, D.; Cox, D. P.; Dahn, H. *J. Am. Chem. Soc.* **1982**, *104*, 2631.

10. Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem.* **1996**, *4*, 1379.

11. Asai, A.; Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1215.

12. Mukaiyama, T.; Usui, M.; Shimada, E.; Saigo, K. *Chem. Lett.* **1975**, 1045.

13. (a) The biological activities of other functional moieties except alkoxy groups were examined, but we could not find the good functional group superior to the alkoxy group (*J. Med. Chem. 1997 40*, in press). (b) The reduced intracellular permeability by charged molecules in this series has been previously discussed. Boger, D. L.; Yun, W.; Han, N.; Johnson, D. S. *Bioorg. Med. Chem.* **1995**, *3*, 611.

14. (a) Hansen, J.; Mork, N.; Bundgaard, H. *Int. J. Pharm.* **1992**, *81*, 253. (b) Heymann, E. *Metabolic Basis of Detoxification*; Jakoby, W. B.; Bend, J. R.; Caldwell, J., Eds.; Academic Press: New York, 1982. (c) Ohe, Y.; Sasaki, Y.; Shinkai, T.; Eguchi, K.; Tamura, T.; Kojima, A.; Kunikane, H.; Okamoto, H.; Karato, A.; Ohmatsu, H.; Kanzawa, F.; Saijo, N. *J. Natl. Cancer Inst.* **1992**, *84*, 972.

15. (a) Ogasawara, H.; Nishio, K.; Takeda, Y.; Ohmori, T.; Kubota, N.; Funayama, Y.; Ohira, T.; Kuraishi, Y.; Isogai, Y.; Saijo, N. *Jpn. J. Cancer Res.* **1994**, *85*, 418. (b) Okamoto, A.; Asai, A.; Saito, H.; Okabe, M.; Gomi, K. *Jpn. J. Cancer Res.* **1994**, *85*, 1304. (c) Ogasawara, H.; Nishio, K.; Kanzawa, F.; Lee, Y.-S.; Funayama, Y.; Ohira, T.; Kuraishi, Y.; Isogai, Y.; Saijo, N. *Jpn. J. Cancer Res.* **1995**, *86*, 124.

16. (a) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 5523. (b) Boger, D. L. *Acc. Chem. Res.* **1995**, *28*, 20. (c) Boger, D. L.; Mesini, P. *J. Am. Chem. Soc.* **1995**, *117*, 11647. (c) Boger, D. L.; McKie, J. A.; Han, N.; Tarby, C. M.; Riggs, H. W.; Kitos, P. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 659. (d) Boger, D. L.; Han, N.; Tarby, C. M.; Boyce, C. W.; Cai, H.; Jin, Q.; Kitos, P. A. *J. Org. Chem.* **1996**, *61*, 4894.

17. Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2147.

18. Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.

19. Inaba, M.; Kobayashi, T.; Tashiro, T.; Sakurai, Y.; Maruo, K.; Ohnishi, Y.; Ueyama, Y.; Nomura, T. *Cancer* **1989**, *64*, 1577.

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