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Synthesis and Antitumor Activity of Duocarmycin Derivatives: A-Ring Pyrrole Analogues of Duocarmycin B2

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Abstract—A series of the eight-substituted A-ring pyrrole derivatives of duocarmycin B2 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. In addition, the stability of the analogues in aqueous solution was examined. The 8-H and the 8-CN compounds which cannot structurally release the cyclopropane compound (DU-86), exhibited extremely diminished anticellular activity compared with duocarmycin A (**1a**) or DU-86. The ethers and the sulfonates which were not converted to DU-86 under usual conditions (35 °C, pH 7), showed almost equal in vivo activities to that of **1a**. However, their optimal doses were significantly higher than that for **1a**. Most of the A-ring pyrrole analogues which can be chemically or enzymatically converted to DU-86, displayed remarkably superior in vivo antitumor activity to **1a**. These results suggest that the A-ring pyrrole analogues need to chemically or enzymatically release DU-86 as an active metabolite to exhibit potent in vivo antitumor activity. Copyright © 1996 Elsevier Science Ltd

Introduction

Duocarmycin (DUM)s (A; **1a**, SA; **1c**, B2; **1d**, C2; **1e**, B1; **1f**, C1; **1g**) are novel antitumor antibiotics isolated from the culture broth of *Streptomyces* sp (Fig. 1).[1] DUMA (**1a**),[1a–c] which is considered as an active form among these DUMs, possesses a unique cyclopropane ring with ability to alkylate DNA. DUMA 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] DUMs 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] However, their marginal activity against human solid tumors, their poor stability, and their insolubility in water dissuaded us from further evaluation. We were interested in synthesizing the analogues in order to enhance and broaden their spectrum of antitumor activity and to improve their stability and solubility.[6] KW-2189 (**2b**), a novel derivative of duocarmycin B2 (**1d**), demonstrated excellent in vivo antitumor activity, good stability in the culture medium, and aqueous solubility greater than 10 mg/mL.[7] It was designed as a prodrug which requires enzymatic hydrolysis followed by regeneration of DU-86 (**2a**) as an active metabolite. KW-2189 (**2b**) is currently under phase I clinical trial. We unexpectedly discovered that KW-2189 itself alkylates calf thymus DNA without release of **2a** in a buffer solution (pH 7.0) at 35 °C.[7a,8] Consequently, KW-2189 has two pathways for DNA alkylation by an active metabolite (**2a**) and by KW-2189 itself (Fig. 2). It is important to study whether DNA alkylation of KW-2189 itself is responsible for its antitumor activity, not only for the next development of duocarmycin derivatives, but also for prediction of the clinical effect of KW-2189. Therefore, we prepared several C8-substituted A-ring pyrrole derivatives of DUMB2 (**1b**) and evaluated their biological activity.

In this paper, we describe the synthesis of A-ring pyrrole derivatives modified at the C_8 phenolic hydroxyl group to ether, sulfonate, ester, carbonate, carbamate, cyano, and hydro compounds, and the evaluation of their anticellular and antitumor activity. In addition, we examined the stability and solubility of their analogues under aqueous conditions.

Chemistry

Initially, the 2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMA (DU-86, **2a**) was prepared by employing the Wagner–Meerwein type rearrangement of the 8-*O*-protected-3-hydroxy-DUMB2 followed by deprotection of the protecting group under basic conditions.[7a,9,10] Treatment of **2a** with HBr or HCl exclusively afforded their adducts, carrying a bromomethyl or a chloromethyl group in the C-ring part.[7a] When $HClO_4$ having no nucleophilicity was used for this reaction, the 9-hydroxy compound (**3**) was exclusively obtained in 78% yield as a versatile advanced intermediate (see Scheme 1).[11,12]

In the 8-*O*-ether series **3** was reacted with alkyl halides in the presence of potassium carbonate to yield the corresponding 8-*O*-benzyl (**4a**), 8-*O*-methyl (**4b**), and

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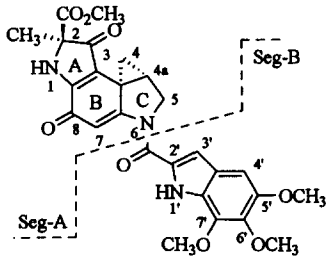
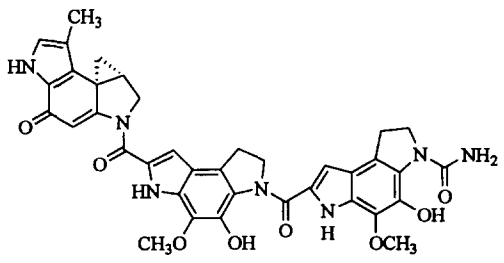
8-*O*-*N*,*N*-dimethylaminoethyl ethers (**4c**). Bromination of these compounds (**4a–c**) produced the 8-*O*-ether 9-bromo A-ring pyrrole analogues (**5a–c**). The sulfonates (**6a** and **b**) were prepared in good yields by the reaction of **2a** with 48% HBr in CH_3CN, followed by addition of methanesulfonyl chloride or trifluoromethanesulfonic anhydride in the presence of triethylamine in CH_2Cl_2 at −78 °C. To obtain the analogues which cannot be structurally converted to **2a**, conversions of the 8-*O*-trifluoromethanesulfonate (**6b**) to the 8-H (**7a**) or the 8-CN (**7b**) compound were achieved in acceptable yields by the method of palladium(0)-catalyzed reduction or cyanation.[13] The 9-hydroxy compounds (**9a** and **b**) of **7a** and **b** were independently prepared from **3** as authentic standards, since it was important to establish that **7a** and **b** are exclusively hydrolyzed not to **2a**, but to the corresponding 9-hydroxy analogues in aqueous solutions. Thus, treatment of **3** with trifluoromethanesulfonic anhydride gave the 8-*O*-formyl compound (**8a**) in *N*,*N*-dimethylformamide (DMF) quantitatively. Compound **3** was next treated with *N*-phenyltrifluoromethanesulfonimide as a mild triflating reagent[14] to produce the 8-*O*-triflate (**8b**) in 96% yield, which was then converted to the 8-H (**9a**) or the 8-CN (**9b**) compound by the same method as for **7a** or **b**.

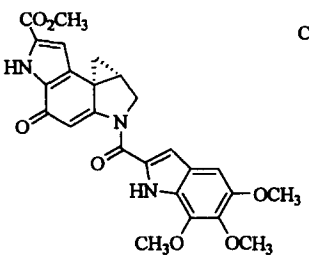
The 8-*O*-acetate (**10a**) and the 8-*O*-carbonates (**11a** and **b**) were prepared by the reaction of **2a** with 48% HBr in CH_3CN followed by the addition of acetic anhydride, methyl, and phenyl chloroformate in the presence of triethylamine, respectively (see Scheme 2). To improve the solubility of the ester, we prepared the 4-(4-methyl-1-piperazinylcarbony)butyric acid ester with a hydrophilic moiety by the reaction with the corresponding carboxylic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP).[6,15] The obtained ester was converted



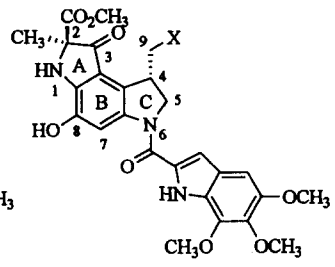
duocarmycin A (**1a**)



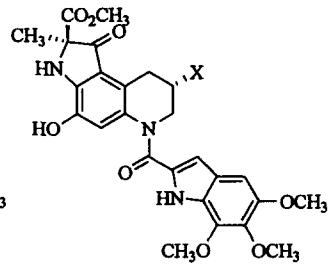
CC-1065 (**1b**)



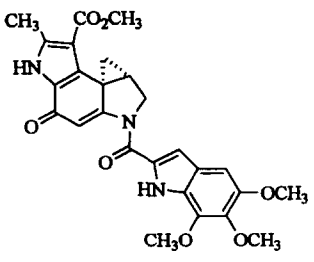
duocarmycin SA (**1c**)



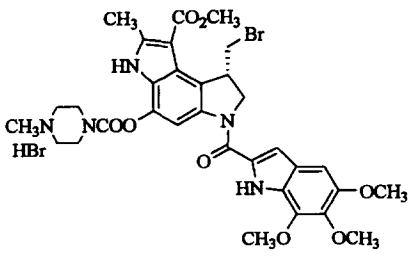
X=Br; duocarmycin B2 (**1d**)
X=Cl; duocarmycin C2 (**1e**)



X=Br; duocarmycin B1 (**1f**)
X=Cl; duocarmycin C1 (**1g**)



DU-86 (**2a**)



KW-2189 (**2b**)

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

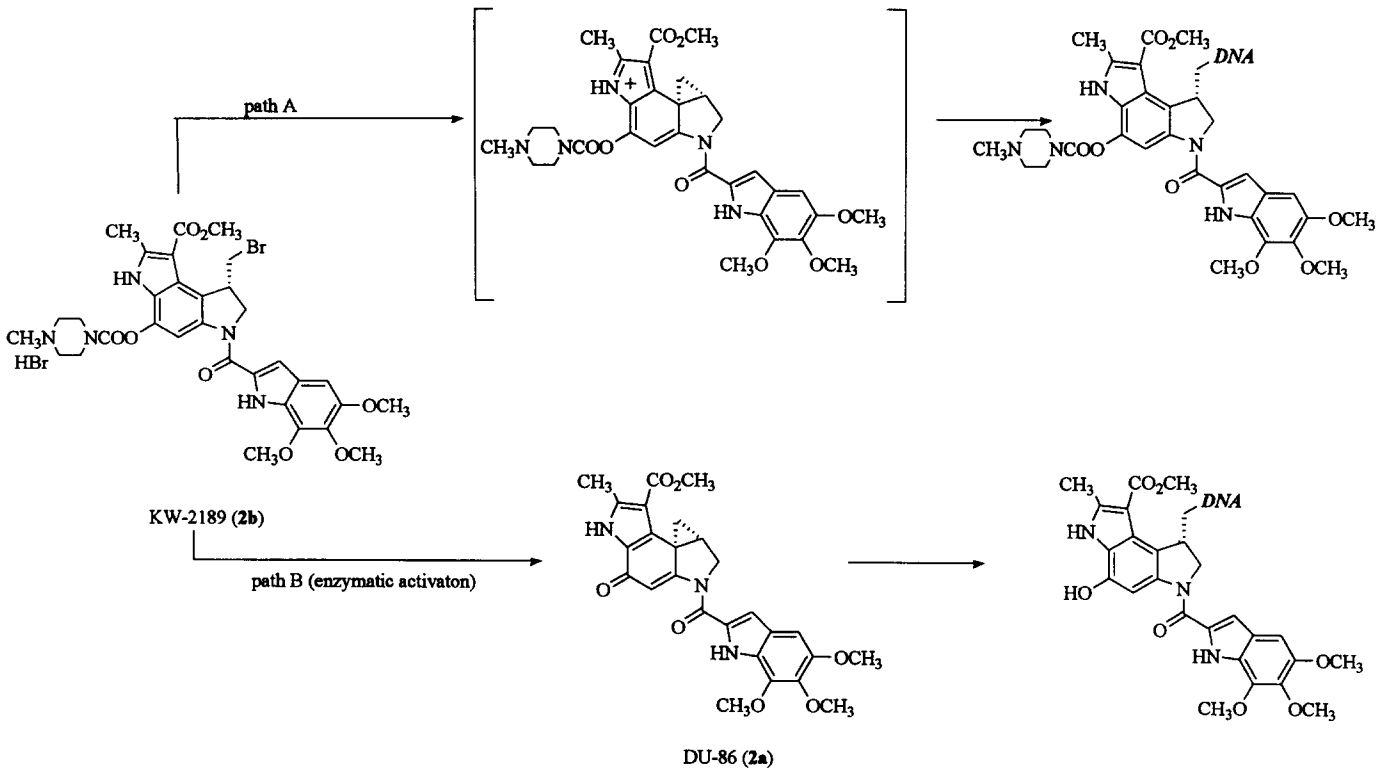


Figure 2. Mechanism of alkylation for DNA of **2b** (KW-2189).

to the hydrochloride salt (**10b**) upon treatment with HCl in ethanol. The solubility of this salt (**10b**) in water was found to be 10 mg/mL.

The preparation of the alkyl and arylcarbamoyl analogues is outlined in Scheme 2. We have already reported that the *N*-monomethylcarbamoyl or *N*-monophenylcarbamoyl derivatives of **1d** are readily hydrolyzed to **1a** in aqueous solutions, while the *N,N*-dialkylcarbamoyl derivatives gave no hydrolyzed products under the same conditions.[6] Thus, we have attempted to prepare the more chemically stable *N*-monoalkyl or *N*-monoarylcarbamoyl derivatives, or the more easily hydrolyzed *N,N*-dialkylcarbamoyl derivatives under aqueous conditions.[16] Compound **2a** was converted to the 4-nitrophenyl carbonate by the reaction with 4-nitrophenyl chloroformate in the presence of triethylamine, which was treated with the corresponding primary or secondary amines to produce the *N*-monoalkyl or *N,N*-dialkylcarbamoyl derivatives (**12a**, **c** and **13a–c**) in reasonable yields. The *N*-monoarylcarbamoyl derivatives (**12d** and **e**) were obtained by the reaction with the corresponding phenyl isocyanates. Deprotection of the benzyl group of **12a** using palladium on carbon afforded the glycinylcarbamoyl analogue (**12b**) in 89% yield. The *N*-methyl A-ring pyrrole derivatives at the N1 position were prepared to investigate the substituent effect upon hydrolysis under aqueous conditions. Compound **2a** was reacted with slightly excess iodomethane to produce the *N*-methyl compound (**14**) of **2a** followed by the above-mentioned methods to furnish the *N*-methyl dialkylcarbamoyl derivative (**15**).

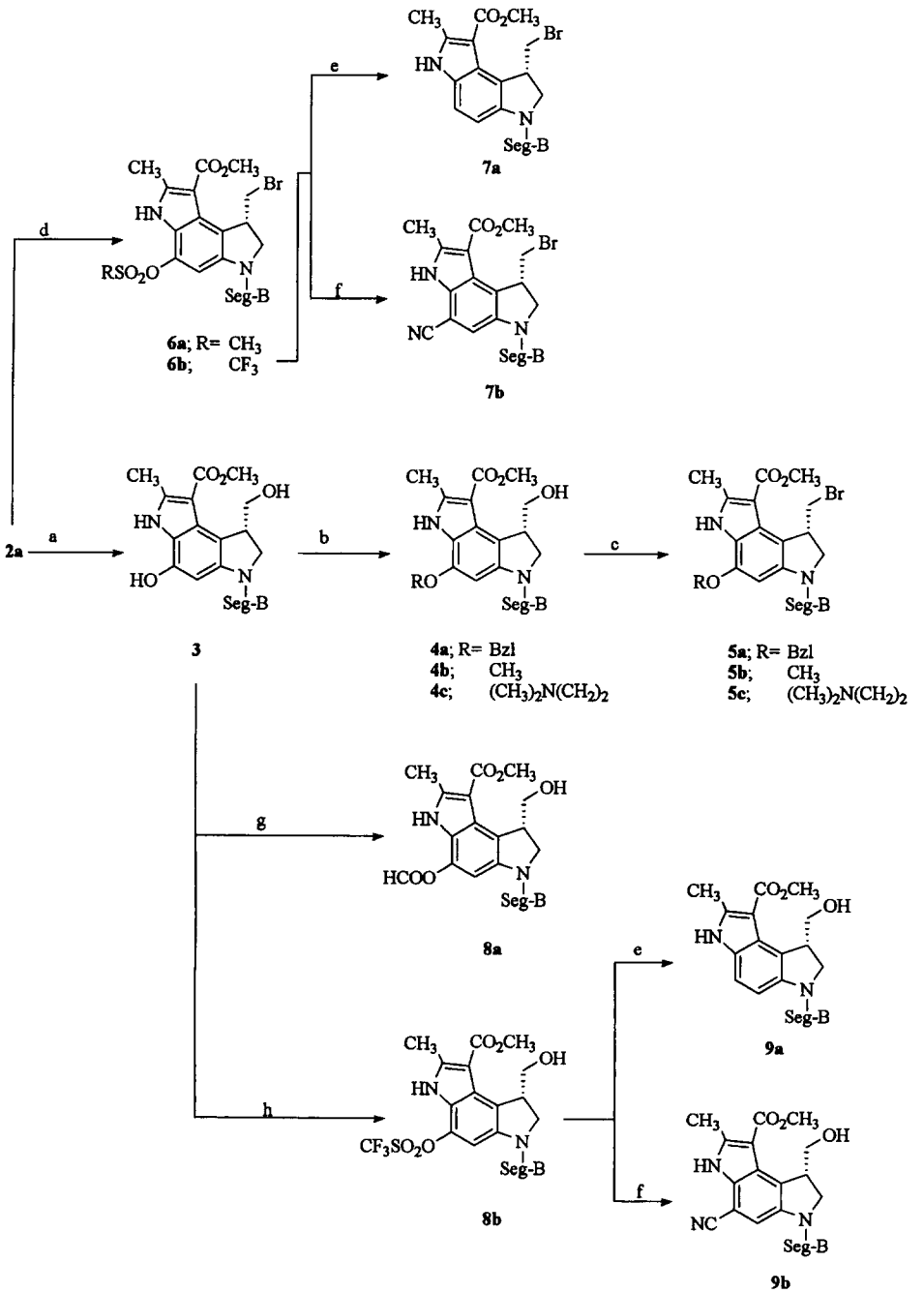
Results and Discussion

The stability of all compounds was measured in 0.01 M phosphate buffer (pH 7) containing 20% CH_3CN by HPLC analysis. The ethers (**5a–c**), sulfonates (**6a** and **6b**), 8-H (**7a**), 8-CN (**7b**), and *N,N*-dialkylcarbamoyls (**2b**, **13a–c** and **15**) decomposed to the single products under this condition, and they were confirmed to be the corresponding 9-OH derivatives from their authentic samples (Table 1). The order of increasing stability was 8-CN > sulfonates > 8-H = *N,N*-dialkylcarbamoyls > ethers. The 8-*O*-ether series (**5a–c**) were rather unstable in aqueous solution ($t_{1/2}$ = < 1 h), being readily hydrolyzed to **4a–c**. In contrast, compound **7b** was the most stable derivative among the A-ring pyrrole analogues synthesized ($t_{1/2}$ = 200 h). Interestingly, the stability of the *N,N*-dialkylcarbamoyl analogues was similar to that of the 8-H compound (**7a**). These results suggest that the electronic factor at the C8 position play an important role in the stability. Thus, the electron-withdrawing groups in the C8-substituents are useful for evaluation of their stability.

The esters (**10a** and **b**), carbonates (**11a** and **b**), *N*-monoalkylcarbamoyls (**12a–c**), and *N*-monoarylcarbamoyls (**12d** and **e**) were predominantly hydrolyzed to **2a** under the same conditions. The order of increasing stability was the carbonates > the esters > the *N*-monoalkylcarbamoyls > the *N*-monoarylcarbamoyls. These results are consistent with the fact that the *N*-monoalkylcarbamoyl and *N*-monoarylcarbamoyl derivatives of **1d** are very unstable ($T_{1/2}$ = < 1 h), and

they are readily converted to **1a**.[6] The *N*-methyl compounds demonstrated increased stability greater than the N—H compounds (**14** versus **2a** and **15** versus **2b**).

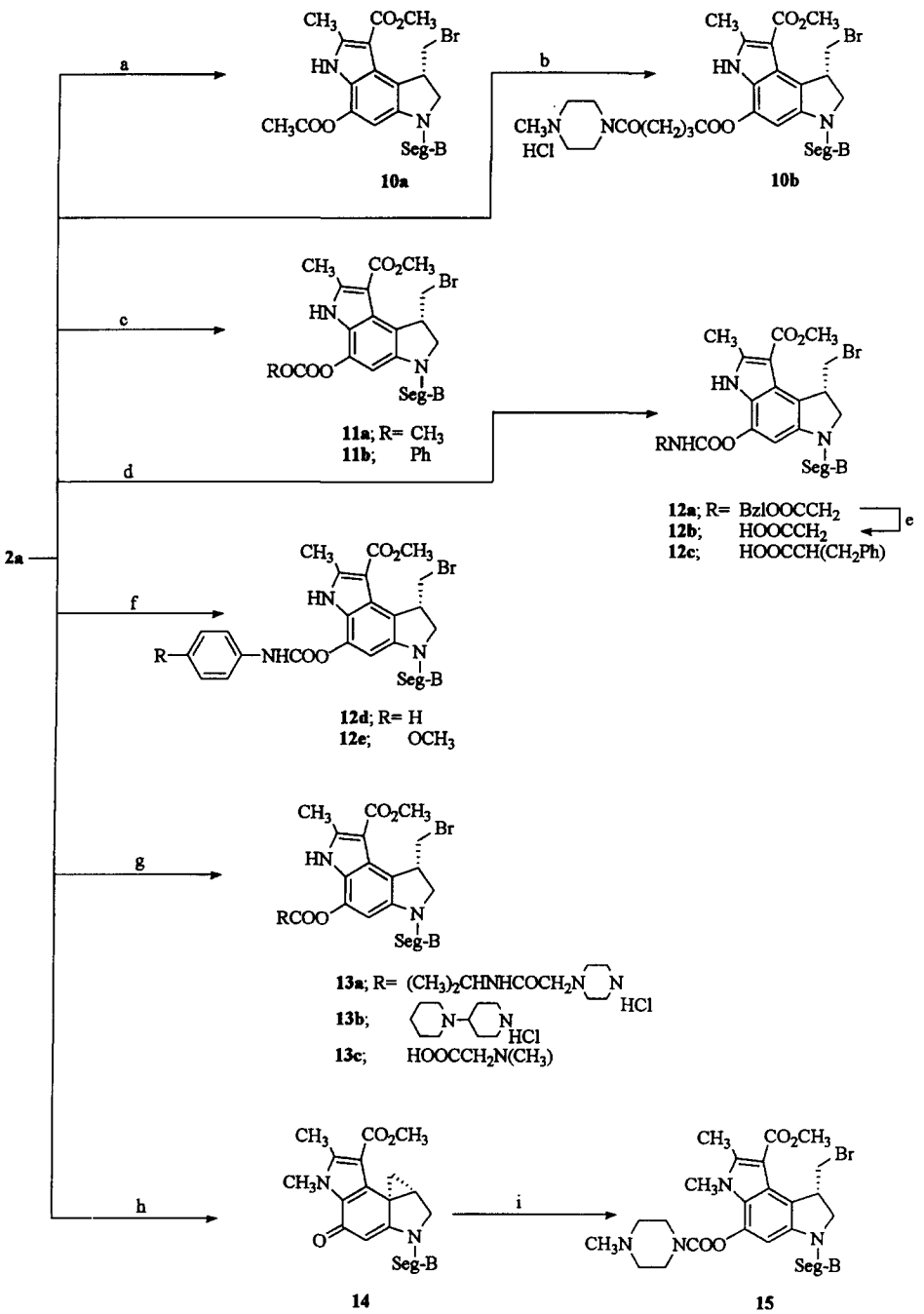
All of the C8 substituted A-ring pyrrole derivatives were studied in vitro for their anticellular activity against HeLa S_3 cells (Table 1). In order to obtain more meaningful comparisons of relative activities, **1a** and **2a** were tested at the same time. Compounds **7a** and **b** which cannot structurally release **2a**, showed extremely decreased anticellular activity. The IC_{50} values at 72 h exposure were approximately five orders of magnitude inferior to **2a**, and they were 100 times less potent than **2b** (KW-2189). The ethers (**5a–c**), sulfonates (**6a** and **6b**), and *N,N*-dialkylcarbamoyl analogues (**13a–c** and **2b**) exhibited weak anticellular activities with the IC_{50} values in the range 1.5–50 nM at 72 h exposure. In contrast, the esters (**10a** and **b**), carbonates (**11a** and **10b**), *N*-monoalkylcarbamoyls (**12a–c**), and *N*-monoarylcarbamoyl (**12d** and **e**) analogues, which can be chemically converted to **2a** in aqueous solutions, demonstrated strong anticellular activities below 0.5 nM. Moreover, the effect of meth-



Scheme 1. (a) 70% $HClO_4$, DMF; (b) BzlBr or CH_3I or *N,N*-dimethylaminoethyl chloride, K_2CO_3, DMF; (c) MsCl, pyridine, then LiBr, DMF; or CBr_4, PPh_3, CH_2Cl_2; (d) HBr, CH_3CN, then CH_3SO_2Cl or $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2; (e) $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)ferrocene, Et_3N, HCOOH, DMF; (f) *n*-Bu_3SnCN, $(PPh_3)_4Pd$, CH_2Cl_2; (g) $(CF_3SO_2)_2O$, Et_3N, DMF; (h) $(CF_3SO_2)_2NPh$, Et_3N, DMF.

ylation at the N1 position seemed not to contribute to an increase of in vitro anticellular activity (**14** versus **2a** and **15** versus **2b**).

The in vivo activity of selected compounds was evaluated against sarcoma 180 murine solid tumor. The in vivo efficacy was expressed as *T/C*, where *T* and *C* represent mean tumor volume of treated and control mice, respectively. Most compounds listed in Table 1 were more effective than **1a** in suppressing tumor volume. Among these compounds, the ethers (**5a** and **b**) and the sulfonates (**6a** and **b**), which were not converted to **2a** under usual conditions (35 °C, pH 7), showed almost equal activities to that of **1a** or **2a**. However, their optimal doses were significantly higher than that for **1a** or **2a**. There is a tendency for compounds having a carboxylic acid in the C8 substituent to show in vivo antitumor activity at high doses (**12b** and **c**) or decreased activity (**13c**). In general, the hydrolysis of the *N,N*-dialkylcarbamoyl



Scheme 2. (a) HBr, CH_3CN, then Ac_2O, 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; (b) HBr, CH_3CN, then 4-(4-methyl-1-piperazinycarbonyl)butyric acid, DMAP, dicyclohexylcarbidiimide (DCC), CH_2Cl_2, then HCl, EtOH; (c) HBr, CH_3CN, then CH_3OCOCl or PhOCOCl, Et_3N, CH_2Cl_2; (d) HBr, CH_3CN, then 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, then glycine benzylester TsOH or phenyalanine; (e) H_2, 10% Pd/C; (f) HBr, CH_3CN, then phenyl isocyanate or 4-methoxyphenyl isocyanate; (g) HBr, CH_3CN, then 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, then *N*-isopropyl-1-piperazineacetamide or 4-piperidinopiperidine or sarcosine, then HCl, EtOH; (h) CH_3I, K_2CO_3, DMF; (i) HBr, CH_3CN, then 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, then *N*-methylpiperazine.

derivatives modified at a phenolic hydroxyl group is catalysed by carboxyesterase, and the polymorphism of enzymes may cause the interpatient variation in efficacy and toxicity.[16c,d] Thus, we researched various carbamoyl moieties having little or no interpatient variation. It has been reported that carbamoyl moieties of compounds **13a** and **b** are more easily hydrolyzed by carboxyesterase among *N,N*-dialkylcarbamoyl moieties.[16d] However, in our assay systems, these carbamoyl derivatives (**13a** and **b**) demonstrated almost equal in vitro and in vivo biological activities to **2b**.

Among these A-ring pyrrole derivatives, the carbonates (**11a** and **b**), having suitable chemical stability, were selected for further evaluation against St-4 human stomach tumor xenograft. As the result, they showed significant antitumor activity below *T/C* values of 0.2. Further studies on antitumor spectra and toxicity of these derivatives are in progress.[17]

These findings suggest that A-ring pyrrole analogues need to release an active compound **2a** chemically or enzymatically to exhibit any significant in vivo antitumor activity. It seems that DU-86 (**2a**) as a metabolite of A-ring pyrrole analogues is responsible for the in vivo antitumor activity of KW-2189 (**2b**). KW-2189 itself seems not to be dominant in its antitumor potency.[18]

Experimental

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra 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 ppm downfield from tetramethylsilane. Elemental analyses were performed with a Perkin–Elmer 2400 C, H, N analyser. MS 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.

2-Methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (3). A solution of DU-86 (**2a**; 490 mg, 1.0 mmol) in DMF (53 mL) was cooled to 0 °C, and 70% perchloric acid (11 mL) and water (21 mL) were added. The mixture was stirred at room temperature for 3 h. Then, 0.2 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (30:1) as an eluent to give 396 mg

Table 1. Anticellular activity, antitumor activity and stability tests of duocarmycin A-ring pyrrole analogues

Compound	Stability $t_{1/2}$ (h)[a] in aq soln	HeLa S_3 1 h	IC_{50} (nM)[b] 72 h	S-180 (sc–iv)[c] mg/kg	*T/C*[d]
5a	1	300	5.1	4.0	0.27
5b	<1	29	6.3	8.0	0.27
5c	<1	100	20	N.T.[e]	
6a	84	26	4.0	16.0	0.2
6b	67	26	1.5	16.0	0.2
7a	21	>1000	330	N.T.	
7b	200	>1000	530	N.T.	
10a	22	0.95	0.13	0.5	0.18
10b	21	3.9	0.5	0.5	0.31
11a	20	0.53	0.082	0.5	0.10
11b	87	0.55	0.051	0.5	0.09
12a	1	0.59	0.12	2.0	0.21
12b	3	0.69	0.23	4.0	0.10
12c	3	1.2	0.17	4.0	0.07
12d	<1	<0.24	<0.24	0.5	0.087
12e	<1	0.59	0.066	0.5	0.16
13a	16	550	50	1.0	0.10
13b	18	330	29	1.0	0.14
13c	11	100	4.6	8.0	0.54
14	150	1.4	0.069	1.0	0.22
15	24	820	330	N.T.	
1a	1	0.0055	0.0058	0.0075	0.26
2a	130	0.045	0.0052	0.25	0.21
2b	16	53	1.6	0.5	0.14

[a] A half-life at 35 °C. Drug concentration was 0.02 mg/mL. See 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) intraveneously (iv).
[d] *T* and *C* are the values of mean tumor volume of treated and control mice, respectively.
[e] Not tested.

(78%) of **3** as a light-tan powder, mp 230–235 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.36 (1 H, br s), 9.52 (1 H, s), 7.82 (1 H, br s), 6.97 (1 H, d, J=2.2 Hz), 6.87 (1 H, s), 4.67 (1 H, br d, J=9.8 Hz), 4.47 (1 H, dd, J=5.3, 5.3 Hz), 4.39 (1 H, dd, J=10.5, 8.4 Hz), 4.18 (1 H, m), 4.05 (3 H, s), 3.89 (3 H, s), 3.88 (3 H, s), 3.86 (3 H, s), 3.73 (1 H, dd, J=10.5, 5.0 Hz), 2.66 (3 H, s). IR (KBr): 1595, 1490, 1442, 1320, 1223, 1161 cm^{-1}. SIMS: m/z 510 (M+ H)$^+$, 234. Anal. ($C_{26}H_{27}N_3O_8 \cdot 1.0\ H_2O$) C, H, N.

8-*O*-Benzyl-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (4a). Benzyl bromide (22 mL, 0.19 mmol) and potassium carbonate (20 mg, 0.14 mmol) were added to a solution of **3** (50 mg, 0.098 mmol) in anhyd DMF (3 mL), and the mixture was stirred at room temperature for 48 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (100:1) as an eluent to give 40 mg (68%) of **4a** as a white powder, mp 130–135 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (1 H, br s), 8.63 (1 H, br s), 8.18 (1 H, s), 7.50–7.37 (5 H, m), 6.99 (1 H, d, J=2.3 Hz), 6.86 (1 H, s), 5.24 (2 H, d, J=11.0 Hz), 4.66 (1 H, dd, J=10.1, 1.2 Hz), 4.52 (1 H, dd, J=10.1, 8.5 Hz), 4.38 (1 H, m), 4.07 (3 H, s), 3.94 (3 H, s), 3.91 (3 H, s), 3.90 (3 H, s), 3.85 (1 H, dd, J=10.5, 4.7 Hz), 3.58 (1 H, dd, J=10.5, 6.5 Hz), 2.68 (3 H, s). IR (KBr): 1671, 1636, 1597, 1492, 1443, 1417, 1313, 1219, 1113 cm^{-1}. EIMS: m/z 599 (M)$^+$, 234. Anal. ($C_{33}H_{33}N_3O_8 \cdot 1.0\ H_2O$) C, H, N.

8-*O*-Methyl-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (4b). The procedure was the same as that employed for the preparation of **4a**. Iodomethane (19 mL, 0.31 mmol), potassium carbonate (41 mg, 0.30 mmol) and **3** (100 mg, 0.195 mmol) were subjected to the reaction to afford 52 mg (51%) of **4b** as a white powder, mp 135–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (1 H, br s), 8.26 (1 H, br s), 8.05 (1 H, s), 6.99 (1 H, d, J=2.3 Hz), 6.86 (1 H, s), 4.66 (1 H, dd, J=10.2, 1.2 Hz), 4.52 (1 H, dd, J=10.2, 10.2 Hz), 4.37 (1 H, m), 4.07 (3 H, s), 4.00 (3 H, s), 3.94 (3 H, s), 3.91 (3 H, s), 3.90 (3 H, s), 3.85 (1 H, dd, J=10.5, 4.7 Hz), 3.59 (1 H, dd, J=10.5, 7.4 Hz), 2.69 (3 H, s). IR (KBr) 1670, 1634, 1521, 1446, 1411, 1313, 1221, 1113 cm^{-1}. SIMS: m/z 524 (M + H)$^+$, 234. Anal. ($C_{27}H_{29}N_3O_8 \cdot 0.5\ CH_3OH$) C, H; N: calcd, 7.79; found, 8.22.

8-*O*-Dimethylaminoethyl-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (4c). 2-Dimethylaminoethyl chloride hydrochloride (75 mg, 0.52 mmol), potassium iodide (18 mg, 0.11 mmol) and potassium carbonate (108 mg, 0.78 mmol) were added to a solution of **3** (130 mg, 0.26 mmol) in anhyd DMF (3 mL), and the mixture was stirred at room temperature for 72 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (10:1) as an eluent to give 22 mg (15%) of **4c** as a pale-yellow powder, mp 135–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.00 (1 H, br s), 9.54 (1 H, br s), 7.19 (1 H, s), 6.91 (1 H, d, J=2.2 Hz), 6.78 (1 H, s), 4.67 (1 H, br d, J=9.3 Hz), 4.52 (1 H, dd, J=9.7, 9.7 Hz), 4.45 (1 H, m), 4.26 (1 H, dd, J=10.5, 7.1 Hz), 4.22 (2 H, t, J=6.1 Hz), 4.07 (3 H, s), 3.94 (3 H, s), 3.89 (3 H, s), 3.86 (3 H, s), 3.69 (1 H, dd, J=10.5, 6.1 Hz), 3.42 (1 H, br s), 3.21 (2 H, br s), 2.80 (6 H, br s), 2.76 (3 H, s). IR (KBr) 1685, 1636, 1522, 1446, 1418, 1223, 1113, 1086 cm^{-1}. SIMS: m/z 581 (M + H)$^+$, 234. Anal. ($C_{30}H_{36}N_4O_8$) C, H, N.

8-*O*-Benzyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (5a). Methanesulfonyl chloride (6.5 mL, 0.084 mmol) was added to a solution of **4a** (17 mg, 0.028 mmol) in pyridine (1 mL), and the mixture was stirred at room temperature for 2 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was dissolved in DMF (1 mL). Lithium bromide (7.3 mg, 0.084 mmol) was added to this solution, and the mixture was stirred at 80 °C for 2 h. Phosphate buffer 0.01 M (pH 7) was poured into the resulting mixture and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using *n*-hexane:EtOAc (2:1) as an eluent to give 12 mg (64%) of **5a** as a white powder, mp 130–135 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.38 (1 H, br s), 8.62 (1 H, br s), 8.16 (1 H, s), 7.51–7.36 (5 H, m), 7.01 (1 H, d, J=2.3 Hz), 6.90 (1 H, s), 5.27 (1 H, d, J=11.0 Hz), 5.22 (1 H, d, J=11.0 Hz), 4.75 (1 H, br d, J=8.9 Hz), 4.56 (2 H, m), 4.08 (3 H, s), 3.97 (3 H, s), 3.95 (3 H, s), 3.93 (3 H, s), 3.82 (1 H, dd, J=8.1, 2.1 Hz), 3.22 (1 H, dd, J=8.1, 8.1 Hz), 2.72 (3 H, s). IR (KBr): 1697, 1605, 1525, 1494, 1415, 1214, 1112, 1088 cm^{-1}. SIMS: m/z 664 662 (M + H)$^+$, 430 428, 234. Anal. ($C_{33}H_{32}BrN_3O_7 \cdot 1.5\ H_2O$) C, H, N.

8-*O*-Methyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (5b). The procedure was the same as that employed for the preparation of **5a**, except for the use of **4b** (52 mg, 0.089 mmol). The crude product was purified by silica gel chromatography to afford 48 mg (92%) of **5b** as a white powder, mp 140–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.38 (1 H, br s), 8.59 (1 H, br s), 8.02 (1 H, s), 7.01 (1 H, d, J=2.3 Hz), 6.90 (1 H, s), 4.74 (1 H, br d, J=8.9 Hz), 4.54 (2 H, m), 4.08 (3 H, s), 4.01 (3 H, s), 3.97 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.82 (1 H, dd, J=9.9, 3.9 Hz), 3.21 (1 H, dd, J=9.9, 9.9 Hz), 2.73 (3 H, s). IR (KBr) 1697, 1584, 1492, 1411, 1312, 1215, 1112 cm^{-1}. SIMS m/z 588 586 (M + H)$^+$, 354 352, 234. Anal. ($C_{27}H_{28}BrN_3O_7 \cdot 1.0\ CH_3OH$) C, H, N.

8-*O*-Dimethylaminoethyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (5c). Triphenylphosphine (4

mg, 0.015 mmol) and carbon tetrabromide (5 mg, 0.015 mmol) were added to a stirred solution of **4c** (5 mg, 0,009 mmol) in anhyd CH_2Cl_2 (0.25 mL), and the mixture was stirred at room temperature for 2 h. Phosphate buffer 0.01 M (pH 7) was added to the resulting mixture, and the whole was extracted with $CHCl_3$. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (50:1) as an eluent to give 3.8 mg (66%) of **5c** as a light-tan powder, mp 98–103 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 12.20 (1 H, br s), 11.33 (1 H, br s), 7.82 (1 H, s), 6.99 (1 H, d, J=1.7 Hz), 6.97 (1 H, s), 4.60 (1 H, dd, J=9.5, 9.5 Hz), 4.41 (1 H, br d, J=10.5 Hz), 4.34 (2 H, br s), 4.11 (1 H, m), 3.94 (3 H, s), 3.83 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.79 (1 H, dd, J=10.3, 2.7 Hz), 3.51 (1 H, dd, J=10.3, 6.5 Hz), 3.40 (2 H, br s), 2.66 (3 H, s), 2.51 (6 H, br s). IR (KBr): 2358, 1686, 1525, 1490, 1313, 1224, 1112, 1090 cm^{-1}. SIMS: *m/z* 645 643 $(M + H)^+$, 234. Anal. ($C_{30}H_{35}BrN_4O_7 \cdot 1.0\ CH_3OH \cdot 0.5\ H_2O$) C, H, N.

8-*O*-Methanesulfonyl-2-methyl-3-methoxycarbonyl A-ring pyrrole-DUMB2 (6a). Hydrobromic acid 48% (1.5 mL) was added to a solution of DU-86 (**2a**; 22 mg, 0.044 mmol) in dry THF (1.5 mL), and the mixture was stirred at room temperature for 1 h. The resulting mixture was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The combined organic extracts were washed with brine and concentrated in vacuo. Methanesulfonyl chloride (16 mL, 0.21 mmol) and triethylamine (29 mL, 0.21 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (2 mL) at −78 °C. Then, the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (100:1) as an eluent to give 25 mg (87%) of **6a** as a light-tan powder, mp 132–137 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.36 (1 H, br s), 9.00 (1 H, br s), 8.31 (1 H, s), 7.01 (1 H, d, J=2.2 Hz), 6.90 (1 H, s), 4.77 (1 H, dd, J=10.5, 1.0 Hz), 4.68 (1 H, m), 4.57 (1 H, dd, J=10.5, 9.6 Hz), 4.08 (3 H, s), 3.98 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.81 (1 H, dd, J=10.1, 2.5 Hz), 3.33 (3 H, s), 3.26 (1 H, dd, J=10.1, 10.1 Hz), 2.75 (3 H, s). IR (KBr): 1698, 1522, 1410, 1364, 1217, 1177, 1106 cm^{-1}. SIMS: *m/z* 652 650 $(M + H)^+$, 418 416, 234. Anal. ($C_{27}H_{28}BrN_3O_9S \cdot 2.5\ H_2O$) C, H, N.

8-*O*-Trifluoromethanesulfonyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (6b). The procedure was the same as that employed for the preparation of **6a**. Trifluoromethanesulfonyl anhydride (75 mL, 0.45 mmol), triethylamine (63 mL, 0.45 mmol) and **2a** (74 mg, 0.15 mmol) were subjected to the reaction to afford 77 mg (73%) of **6b** as a light-tan powder, mp 108–112 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.39 (1 H, s), 8.74 (1 H, s), 8.45 (1 H, s), 7.00 (1 H, d, J=2.3 Hz), 6.89 (1 H, s), 4.78 (1 H, dd, J=10.5, 1.3 Hz), 4.69 (1 H, m), 4.58 (1 H, dd, J=10.5, 9.2 Hz), 4.08 (3 H, s), 3.99 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.79 (1 H, dd, J=10.1, 2.4 Hz), 3.26 (1 H, dd, J=10.1, 10.1 Hz), 2.78 (3 H, s). IR (KBr): 1697, 1611, 1522, 1412, 1311, 1213, 1137, 1114 cm^{-1}. SIMS: *m/z* 706 704 $(M + H)^+$, 234. Anal. ($C_{27}H_{25}BrF_3N_3O_9S \cdot 1.0\ CH_3OH \cdot 0.5\ H_2O$) C, H; N: calcd, 5.64; found, 5.23.

8-Dehydroxy-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (7a). A N_2-flushed flask was charged with **6b** (31 mg, 0.044 mmol), palladium acetate (12 mg, 0.046 mmol), DPPF [1,1′-bis(diphenyphosphino)ferrocene, 25 mg, 0.045 mmol], 98% formic acid (5 mL, 0.133 mmol) and anhydrous DMF (1 mL). The mixture was heated at 50 °C and was allowed to cool to ambient temperature. Then, the mixture was diluted with 0.01 M phosphate buffer (pH 7), and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using *n*-hexane:EtOAc (3:1) as an eluent to give 23 mg (94%) of **7a** as a white powder, mp 133–138 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.37 (1 H, s), 8.48 (1 H, s), 8.32 (1 H, d, J=8.3 Hz), 7.29 (1 H, d, J=8.3 Hz), 7.00 (1 H, d, J=2.1 Hz), 6.90 (1 H, s), 4.75 (1 H, br d, J=10.5 Hz), 4.65 (1 H, m), 4.54 (1 H, dd, J=9.0, 9.0 Hz), 4.09 (3 H, s), 3.98 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.84 (1 H, dd, J=10.0, 2.2 Hz), 3.25 (1 H, dd, J=10.0, 10.0 Hz), 2.75 (3 H, s). IR (KBr): 1685, 1601, 1457, 1312, 1263, 1197, 1091 cm^{-1}. SIMS: *m/z* 558 556 $(M + H)^+$, 234. Anal. ($C_{26}H_{26}BrN_3O_6 \cdot 2.5\ H_2O$) C, H, N.

8-Dehydroxy-8-cyano-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (7b). An argon-flushed round-bottomed flask was charged with tributyltin cyanide (27 mg, 0.085 mmol), tetrakis(triphenylphosphine)palladium (50 mg, 0.043 mmol) and anhyd CH_2Cl_2 (2 mL). The mixture was heated at reflux for 0.5 h. A solution of **6b** (30 mg, 0.043 mmol) in CH_2Cl_2 (1.5 mL) was added to the mixture, and the reaction mixture was heated at reflux for 7 h. Then, the mixture was diluted with 0.01 M phosphate buffer (pH 7), and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using *n*-hexane:EtOAc (1:1) as an eluent to give 6 mg (24%) of **7b** as a pale yellow powder, mp 180–190 °C dec. ^1H NMR (400 MHz, $CDCl_3$): δ 9.36 (1 H, s), 9.04 (1 H, s), 8.70 (1 H, s), 7.00 (1 H, d, J=2.3 Hz), 6.89 (1 H, s), 4.79 (2 H, m), 4.56 (1 H, dd, J=10.2, 10.2 Hz), 4.11 (3 H, s), 3.99 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.79 (1 H, dd, J=10.0, 3.0 Hz), 3.29 (1 H, dd, J=10.0, 10.0 Hz), 2.77 (3 H, s). IR (KBr): 2226, 1698, 1611, 1490, 1458, 1395, 1310, 1220, 1105 cm^{-1}. SIMS: *m/z* 583 581 $(M + H)^+$, 234. Anal. ($C_{27}H_{25}BrN_4O_6 \cdot 2.0\ H_2O$) C, H, N.

8-*O*-Formyl-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (8a). The procedure was the same as that employed for the preparation of **6b**. Trifluoromethanesulfonyl anhydride (12 mL, 0.071 mmol) and triethylamine (5 mL, 0.036 mmol) and **3** (10 mg, 0.02

mmol) were subjected to the reaction to afford 6 mg (56%) of **8a** as a pale yellow powder, mp 135–140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.84 (1 H, s), 11.23 (1 H, br s), 10.06 (1 H, s), 8.34 (1 H, s), 8.12 (1 H, s), 6.96 (1 H, d, J=2.0 Hz), 6.94 (1 H, s), 4.53 (1 H, dd, J=10.1, 9.6 Hz), 4.30 (1 H, br d, J=10.9 Hz), 4.26 (1 H, m), 3.93 (3 H, s), 3.91 (1 H, m), 3.81 (3 H, s), 3.80 (3 H, s), 3.76 (3 H, s), 3.35 (1 H, m), 2.61 (3 H, s). IR (KBr): 1722, 1697, 1589, 1464, 1444, 1319, 1219, 1111 cm^{-1}. SIMS: *m/z* 538 (M + H)$^+$, 234. Anal. ($C_{27}H_{27}N_3O_9 \cdot 0.5\ CH_3OH$) C, H, N.

8-*O*-Trifluoromethanesulfonyl-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (8b). *N*-Phenyltrifluoromethanesulfonimide (42 mg, 0.12 mmol) and triethylamine (17 mL, 0.12 mmol) were added to a solution of **3** (20 mg, 0.04 mmol) in anhyd DMF (3 mL), and the mixture was stirred at 50 °C for 6 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (100:1) as an eluent to give 24 mg (96%) of **4a** as a white powder, mp 132–140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (1 H, br s), 11.42 (1 H, br s), 8.36 (1 H, br s), 7.07 (1 H, d, J=2.0Hz), 6.96 (1 H, s), 4.58 (2 H, br d, J=4.8 Hz), 4.22 (1 H, m), 3.92 (3 H, s), 3.84 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.61 (1 H, dd, J=10.0, 3.6 Hz), 3.19 (1 H, dd, J=10.0, 8.6 Hz), 2.68 (3 H, s). IR (KBr): 1653, 1491, 1419, 1315, 1214, 1117 cm^{-1}. SIMS: *m/z* 642 (M + H)$^+$, 234. Anal. ($C_{27}H_{26}F_3N_3O_{10}S$) C, H, N.

8-Dehydroxy-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (9a). The procedure was the same as that employed for the preparation of **7a**, except for the use of **8b** (17 mg, 0.027 mmol). The crude product was purified by silica gel chromatography to afford 13 mg (98%) of **9a** as a white powder, mp 145–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.38 (1 H, br s), 8.46 (1 H, br s), 8.32 (1 H, d, J=8.8 Hz), 7.23 (1 H, d, J=8.8 Hz), 6.99 (1 H, d, J=2.2 Hz), 6.86 (1 H, s), 4.67 (1 H, br d, J=9.1 Hz), 4.49 (2 H, m), 4.08 (3 H, s), 3.94 (3 H, s), 3.92 (3 H, s), 3.91 (3 H, s), 3.87 (1 H, dd, J=10.6, 4.9 Hz), 3.61 (1 H, dd, J=10.6, 7.3 Hz), 2.70 (3 H, s). IR (KBr): 1735, 1577, 1437, 1312, 1200, 1094 cm^{-1}. SIMS: *m/z* 494 (M + H)$^+$, 234. Anal. ($C_{26}H_{27}N_3O_7$) C, H, N.

8-Dehydroxy-8-cyano-2-methyl-3-methoxycarbonyl-9-hydroxy-A-ring pyrrole-DUM (9b). The procedure was the same as that employed for the preparation of **7b**, except for the use of **8b** (25 mg, 0.039 mmol). The crude product was purified by silica gel chromatography to afford 18 mg (89%) of **9b** as a white powder, mp 130–140 °C dec. ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD): δ 8.49 (1 H, s), 6.94 (1 H, s), 6.77 (1 H, s), 4.66 (1 H, br d, J=8.7 Hz), 4.39 (2 H, m), 3.99 (3 H, s), 3.84 (3 H, s), 3.83 (3 H, s), 3.81 (3 H, s), 3.75 (1 H, dd, J=10.6, 4.6 Hz), 3.31 (1 H, dd, J=10.6, 7.8 Hz), 2.64 (3 H, s). IR (KBr): 2225, 1700, 1612, 1488, 1408, 1311, 1221 cm^{-1}. SIMS: *m/z* 519 (M + H)$^+$, 234. Anal. ($C_{27}H_{26}N_4O_7$) C, H, N.

8-*O*-Acetyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (10a). Hydrobromic acid 48% (2 mL) was added to a solution of DU-86 (**2a**; 29 mg, 0.059 mmol) in CH_3CN (4 mL) and the mixture was stirred at room temperature for 1 h. The resulting mixture was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The combined organic extracts were washed with brine and concentrated in vacuo. Acetic anhydride (20 mL, 0.21 mmol) and DMAP (25 mg, 0.20 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (4 mL) at 0 °C. Then, the resulting mixture was stirred at 0 °C for 2 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (50:1) as an eluent to give 23 mg (65%) of **10a** as a white powder, mp 140–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.35 (1 H, br s), 8.45 (1 H, s), 8.20 (1 H, s), 6.99 (1 H, d, J=2.2 Hz), 6.89 (1 H, s), 4.75 (1 H, br d, J=9.8 Hz), 4.62 (1 H, m), 4.55 (1 H, br d, J=9.8 Hz), 4.08 (3 H, s), 3.96 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.81 (1 H, dd, J=10.0, 2.4 Hz), 3.24 (1 H, dd, J=10.0, 10.0 Hz), 2.72 (3 H, s), 2.41 (3 H, s). IR (KBr): 1686, 1654, 1559, 1507, 1457, 1314, 1189 cm^{-1}. SIMS: *m/z* 616 614 (M + H)$^+$, 234. Anal. ($C_{28}H_{28}BrN_3O_8 \cdot 0.5\ H_2O$) C, H, N.

8-*O*-[4-(4-Methyl-1-piperzinylcarbonyl)butyryl]-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 Hydrochloride (10b). Hydrobromic acid 48% (1 mL) was added to a solution of DU-86 (**2a**; 36 mg, 0.073 mmol) in CH_3CN (3 mL) and the mixture was stirred at room temperature for 1 h. The resulting mixture was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The combined organic extracts washed with brine and concentrated in vacuo. DCC (30 mg, 0.15 mmol), DMAP (18 mg, 0.15 mmol) and 4-(4-methyl-1-piperazinyl-carbonyl)butyric acid (32 mg, 0.15 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (2.5 mL) at 0 °C. Then, the resulting mixture was stirred at room temperature for 24 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (50:1) as an eluent to give free base (44 mg) of **10b**. The free base was dissolved with EtOH (1 mL), and the resulting mixture was treated with anhyd 5.8 N HCl in EtOH (17 mL) at room temperature for 1 h. The whole was evaporated in vacuo to give 47 mg (77%) of **10b** as a pale yellow crystalline compound, mp 125–130 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (1 H, br s), 11.32 (1 H, br s), 10.26 (1 H, br s), 7.89 (1 H, s), 7.00 (1 H, d, J=2.2 Hz), 6.97 (1 H, s), 4.65 (1 H, dd, J=10.0, 9.0 Hz), 4.47 (1 H, m), 4.43 (1 H, br d, J=9.9 Hz), 3.94 (3 H, s), 3.85 (3 H, s), 3.83 (3 H, s), 3.80 (3 H, s), 3.59 (3 H, s), 3.60 (2 H, br s), 3.41 (2 H, m), 2.80 (2 H, br s), 2.79 (2 H, t, J=7.3 Hz), 2.68 (3 H, s), 2.51 (4 H, br s), 2.36 (2 H, t, J=7.3 Hz), 1.92 (2 H,

m). IR (KBr): 1735, 1693, 1617, 1413, 1315, 1218, 1109 cm^{-1}. SIMS: *m/z* 770 768 (M + H)$^+$, 234. Anal. ($C_{36}H_{42}BrN_5O_9 \cdot 1.0\ HCl \cdot 1.0\ H_2O$) C, H, N.

8-*O*-Methoxycarbonyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (11a). Hydrobromic acid 48% (2 mL) was added to a solution of DU-86 (**2a**; 29 mg, 0.059 mmol) in CH_3CN (4 mL), and the mixture was stirred at room temperature for 1 h. The resulting mixture was poured into 1 N HBr and the combine was extracted with $CHCl_3$. The combined organic extracts were washed with brine and concentrated in vacuo. Methyl chloroformate (14 mL, 0.18 mmol) and triethylamine (25 mL, 0.18 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (2.5 mL) at −78 °C. Then, the resulting mixture was stirred at 0 °C for 2 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using *n*-hexane:EtOAc (2:1) as an eluent to give 32 mg (87%) of **11a** as a white powder, mp 122–127 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.39 (1 H, s), 8.74 (1 H, s), 8.33 (1 H, s), 7.00 (1 H, d, J=2.0 Hz), 6.90 (1 H, s), 4.75 (1 H, br d, J=9.4 Hz), 4.58 (2 H, m), 4.08 (3 H, s), 3.97 (3 H, s), 3.96 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.72 (1 H, m), 3.24 (1 H, dd, J=9.9, 9.4 Hz), 2.72 (3 H, s). IR (KBr) 1768, 1696, 1617, 1495, 1442, 1315, 1263, 1219, 1111 cm^{-1}. FABMS: *m/z* 632 630 (M + H)$^+$, 234. Anal. ($C_{28}H_{28}BrN_3O_9 \cdot 1.5\ CH_3OH \cdot 0.5\ H_2O$) C, H, N.

8-*O*-Phenoxycarbonyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (11b). The procedure was the same as that employed for the preparation of **11a**. Phenyl chloroformate (27 mL, 0.22 mmol), triethylamine (30 mL, 0.22 mmol) and **2a** (34 mg, 0.069 mmol) were subjected to the reaction to afford 40 mg (82%) of **11b** as a light-tan powder, mp 115–120 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.39 (1 H, s), 8.84 (1 H, s), 8.45 (1 H, s), 7.46–7.29 (5 H, m), 7.00 (1 H, d, J=1.9 Hz), 6.90 (1 H, s), 4.76 (1 H, br d, J=9.4 Hz), 4.59 (2 H, m), 4.08 (3 H, s), 3.98 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.72 (1 H, m), 3.24 (1 H, dd, J=9.9, 9.4 Hz), 2.75 (3 H, s). IR (KBr): 1780, 1614, 1493, 1464, 1414, 1313, 1221, 1188, 1111 cm^{-1}. FABMS: *m/z* 694 692 (M + H)$^+$, 234. Anal. ($C_{33}H_{30}BrN_3O_9 \cdot 1.0\ CH_3OH$) C, H, N.

8-*O*-(*N*-Benzyloxycarbonylmethylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (12a). Hydrobromic acid 48% (0.5 mL) was added to a solution of DU-86 (**2a**; 15 mg, 0.03 mmol) in CH_3CN (1 mL), and the mixture was stirred at room temperature for 2 h. The resulting mixture was poured into 1 N HBr and the combine was extracted with $CHCl_3$. The combined organic extracts were washed with brine and concentrated in vacuo. *p*-Nitrophenyl chloroformate (19 mg, 0.09 mmol) and triethylamine (13 mL, 0.09 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (1 mL) at −78 °C. Then, the resulting mixture was stirred at −78 °C for 0.5 h. Glycine benzyl ester tosyl acid (36 mg, 0.11 mmol) and triethylamine (15 mL, 0.11 mmol) were added to the solution, and the mixture was stirred at 0 °C for 2 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (50:1) as an eluent to give 15 mg (65%) of **12a** as a white powder, mp 168–173 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.34 (1 H, br s), 9.31 (1 H, s), 8.10 (1 H, br s), 7.28–7.18 (5 H, m), 6.91 (1 H, d, J=2.0 Hz), 6.81 (1 H, s), 5.82 (1 H, t, J=5.6 Hz), 5.09 (2 H, br s), 4.65 (1 H, br d, J=10.0 Hz), 4.47 (2 H, m), 4.00 (3 H, s), 3.95 (3 H, s), 3.86 (3 H, s), 3.84 (3 H, s), 3.71 (1 H, br d, J=7.2 Hz), 3.14 (1 H, dd, J=9.9, 9.6 Hz), 2.95 (2 H, br s), 2.57 (3 H, s). IR (KBr): 1741, 1583, 1495, 1456, 1414, 1290, 1213, 1190 cm^{-1}. FABMS: *m/z* 765 763 (M + H)$^+$, 234. Anal. ($C_{36}H_{35}BrN_4O_{10}$) C, H, N.

8-*O*-(*N*-Glycinylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (12b). A solution of **12a** (15 mg, 0.03 mmol) with 10% Pd/C (4 mg) in EtOH (0.5 mL), CH_3OH (0.1 mL) and 1 N HBr (0.1 mL) was stirred under 1 atm of H_2 at room temperature for 2 h. The reaction mixture was filtered, and concentrated in vacuo. The residue was diluted with $CHCl_3$, and washed with water and brine. Then, the organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH:CH_3COOH (100:10:2) as an eluent to afford 12 mg (89%) of **12b** as a pale yellow powder, mp 210–215 °C dec. ^{1}H NMR (270 MHz, acetone-d_6 + TFA-*d*): δ 8.04 (1 H, s), 6.98 (1 H, s), 6.87 (1 H, s), 5.40 (2 H, br s), 4.54 (3 H, m), 3.91 (3 H, s), 3.78 (3 H, s), 3.77 (1 H, m), 3.75 (3 H, s), 3.74 (3 H, s), 3.74 (1 H, m), 2.57 (3 H, s). IR (KBr): 1697, 1601, 1444, 1416, 1219, 1109, 1088 cm^{-1}. FABMS: *m/z* 675 673 (M + H)$^+$, 234. Anal. ($C_{29}H_{29}BrN_4O_{10} \cdot 1.0\ CH_3OH \cdot 0.5\ H_2O$) C, H, N.

8-*O*-(*N*-Phenylalaninylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (12c). The procedure was the same as that employed for the preparation of **12a**. Phenylalanine (18 mg, 0.11 mmol), triethylamine (13 mL, 0.09 mmol) and **2a** (15 mg, 0.03 mmol) were subjected to the reaction to afford 8 mg (35%) of **12c** as a light-tan powder, mp 120–125 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ 12.35 (1 H, br s), 11.38 (1 H, br s), 7.81 (1 H, s), 7.27–7.16 (5 H, m), 6.95 (1 H, d, J=2.2 Hz), 6.93 (1 H, s), 4.58 (1 H, m), 4.41 (3 H, m), 4.15 (1 H, m), 3.88 (3 H, s), 3.79 (3 H, s), 3.77 (3 H, s), 3.74 (3 H, s), 3.54 (3 H, m), 2.98 (1 H, m), 2.62 (3 H, s). IR (KBr): 1699, 1525, 1416, 1313, 1217, 1111 cm^{-1}. FABMS: *m/z* 765 763 (M + H)$^+$, 234. Anal. ($C_{36}H_{35}BrN_4O_{10} \cdot 3.5\ H_2O$) C, H, N.

8-*O*-(*N*-Phenylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (12d). Hydrobromic acid 48% (1.6 mL) was added to a solution of DU-86 (**2a**; 36 mg, 0.073 mmol) in CH_3CN (3.3 mL), and the mixture was stirred at room temperature for 1 h. The resulting mixture was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The combined organic extracts

were washed with brine, and concentrated in vacuo. Phenyl isocyanate (51 mL, 0.39 mmol) and triethylamine (54 mL, 0.39 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (3.2 mL) at 0 °C. Then, the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$, and the combined organic extracts were washed with 0.2 M acetate buffer (pH 4) and brine, and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:acetone (100:1) as an eluent to give 38 mg (74%) of **12d** as a white powder, mp 157–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.34 (1 H, br s), 8.94 (1 H, br s), 8.26 (1 H, s), 7.43–7.29 (5 H, m), 7.11 (1 H, t, J=6.6 Hz), 6.99 (1 H, d, J=2.3 Hz), 6.89 (1 H, s), 4.73 (1 H, br d, J=9.9 Hz), 4.61 (1 H, m), 4.52 (1 H, br d, J=9.5 Hz), 4.08 (3 H, s), 3.96 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.79 (1 H, br d, J=9.4 Hz), 3.24 (1 H, dd, J=9.9, 9.9 Hz), 2.64 (3 H, s). IR (KBr): 1699, 1603, 1541, 1493, 1446, 1317, 1215, 1194 cm^{-1}. SIMS: m/z 693 691 (M + H)$^+$, 234. Anal. ($C_{33}H_{31}BrN_4O_8 \cdot 0.5\ H_2O$) C, H, N.

8-*O*-(*N*-4-Methoxyphenylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (12e). The procedure was the same as that employed for the preparation of **12d**. DU-86 (**2a**; 28 mg, 0.058 mmol) and 4-methoxyphenyl isocyanate (23 mL, 0.18 mmol) were subjected to the reaction to afford 40 mg (96%) of **12e** as a white powder, mp 155–160 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.41 (1 H, br s), 9.23 (1 H, br s), 8.26 (1 H, s), 7.49 (1 H, br s), 7.18–6.77 (5 H, m), 7.00 (1 H, d, J=2.3 Hz), 6.90 (1 H, s), 4.70 (1 H, br d, J=9.9 Hz), 4.53 (2 H, m), 4.06 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.79 (3 H, s), 3.76 (3 H, s), 3.76 (1 H, m), 3.21 (1 H, dd, J=9.5, 9.3 Hz), 2.58 (3 H, s). IR (KBr): 1736, 1697, 1606, 1545, 1512, 1416, 1313, 1217, 1111 cm^{-1}. FABMS: m/z 723 721 (M + H)$^+$, 234. Anal. ($C_{34}H_{33}BrN_4O_9 \cdot 0.5\ H_2O$) C, H, N.

8-*O*-(*N*-Isopropylaminocarbonylmethylpiperazinylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 hydrochloride (13a). Hydrobromic acid 48% (1 mL) was added to a solution of DU-86 (**2a**; 36 mg, 0.073 mmol) in CH_3CN (3 mL), and the mixture was stirred at room temperature for 2 h. The resulting mixture was poured into 1 N HBr and the combine was extracted with $CHCl_3$. The combined organic extracts were washed with brine, and concentrated in vacuo. *p*-Nitrophenyl chloroformate (44 mg, 0.22 mmol) and triethylamine (31 mL, 0.22 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (2.5 mL) at −78 °C. Then, the resulting mixture was stirred at −78 °C for 0.5 h. *N*-Isopropyl-1-piperazineacetamide (48 mg, 0.26 mmol) was added to the solution, and the mixture was stirred at 0 °C for 2 h. The mixture was diluted with $CHCl_3$, and washed with satd $NaHCO_3$ and brine. The organic extracts were concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (50:1) as an eluent to give free base (47 mg) of **13a**. The free base (38 mg) was dissolved with CH_3OH:EtOH (1:1, 2 mL), and the resulting mixture was treated with anhyd 5.8 N HCl in EtOH (14 mL) at room temperature for 1 h. The whole was evaporated in vacuo to give 39 mg (79%) of **13a** as a white crystalline compound, mp 185–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (1 H, s), 11.29 (1 H, br s), 10.33 (1 H, br s), 8.53 (1 H, br s), 7.93 (1 H, s), 7.00 (1 H, d, J=2.1 Hz), 6.97 (1 H, s), 4.64 (1 H, dd, J=10.4, 10.4 Hz), 4.47 (1 H, m), 4.42 (1 H, br d, J=10.8 Hz), 4.08 (2 H, br s), 3.95 (3 H, s), 3.85 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.65 (9 H, m), 3.01 (1 H, m), 2.58 (3 H, s), 1.60 (1 H, m), 1.11 (6 H, d, J=6.6 Hz). IR (KBr): 1678, 1412, 1313, 1216, 1109, 1088 cm^{-1}). SIMS: m/z 785 783 (M + H)$^+$, 234. Anal. ($C_{36}H_{43}BrN_6O_9 \cdot 1.0HCl \cdot 1.5\ CH_3OH \cdot 1.5\ H_2O$) C, H, N.

8-*O*-(*N*-4-Piperidinopiperidinylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 hydrochloride (13b). The procedure was the same as that employed for the preparation of **13a**. DU-86 (**2a**; 36 mg, 0.073 mmol) and 4-piperidinopiperidine (43 mg, 0.26 mmol) were subjected to the reaction to afford 48 mg (81%) of **13b** as a white crystalline powder, mp 205–210 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (1 H, br s), 11.30 (1 H, br s), 9.77 (1 H, br s), 7.88 (1 H, s), 7.00 (1 H, d, J=2.2 Hz), 6.97 (1 H, s), 4.64 (1 H, dd, J=10.5, 8.3 Hz), 4.43 (2 H, m), 4.21 (1 H, m), 3.94 (3 H, s), 3.85 (3 H, s), 3.82 (3 H, s), 3.80 (3 H, s), 3.42 (2 H, m), 3.15 (2 H, br s), 2.99 (2 H, br s), 2.68 (3 H, s), 2.50 (5 H, m), 1.84–2.17 (10 H, m). IR (KBr): 1698, 1411, 1313, 1217, 1108 cm^{-1}. SIMS: m/z 768 766 (M + H)$^+$, 234. Anal. ($C_{37}H_{44}BrN_5O_8 \cdot 1.0\ HCl \cdot 3.5\ H_2O$) C, H, N.

8-*O*-(*N*-Methylglycinylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (13c). The procedure was the same as that employed for the preparation of **13a**. DU-86 (**2a**; 22 mg, 0.044 mmol) and Sarcosine (28 mg, 0.32 mmol) were subjected to the reaction to afford 19 mg (66%) of **13c** as a pale yellow powder, mp 115–120 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ 13.48 (1 H, br s), 11.54 (1 H, s), 7.96 (1 H, s), 7.14 (1 H, br s), 7.11 (1 H, s), 4.74 (1 H, br d, J=10.1 Hz), 4.58 (2 H, m), 4.07 (3 H, s), 3.97 (3 H, s), 3.95 (3 H, s), 3.93 (3 H, s), 3.90 (1 H, m), 3.54 (1 H, dd, J=8.9, 5.3 Hz), 3.26 (3 H, s), 3.00 (2 H, s), 2.64 (3 H, s). IR (KBr): 1701, 1610, 1458, 1417, 1398, 1315, 1215, 1111 cm^{-1}. FABMS: m/z 688 686 (M + H)$^+$, 234. Anal. ($C_{30}H_{30}BrN_4O_{10} \cdot 0.5\ H_2O$) C, H, N.

1-*N*-Methyl-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMA (14). Iodomethane (19 mL, 0.31 mmol) and potassium carbonate (35 mg, 0.25 mmol) were added to a solution of **2a** (105 mg, 0.21 mmol) in anhydrous DMF (2 mL), and the mixture was stirred at room temperature for 2 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine, and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$:CH_3OH (100:1) as an eluent to give 52 mg (49%) of **14** as a white powder, mp 205–210 °C dec. ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.25 (1 H, br s), 6.98 (1 H, s), 6.93 (1 H, d, J=2.3 Hz), 6.80 (1

H, s), 4.38 (2 H, br d, $J=2.3$ Hz), 4.07 (3 H, s), 4.07 (3 H, s), 3.93 (3 H, s), 3.89 (3 H, s), 3.83 (3 H, s), 3.64 (1 H, m), 2.55 (3 H, s), 2.18 (1 H, dd, $J=7.6$, 3.8 Hz), 1.25(1 H, dd, $J=4.7$, 3.8 Hz). IR (KBr): 1699, 1639, 1610, 1458, 1400, 1304, 1292, 1265, 1105 cm^{-1}. FABMS: *m/z* 506 $(M + H)^+$, 234. Anal. ($C_{27}H_{27}N_3O_7 \cdot 0.5\ H_2O$) C, H, N.

1-*N*-Methyl-8-*O*-(4-methyl-1-piperazinylcarbamoyl)-2-methyl-3-methoxycarbonyl-A-ring pyrrole-DUMB2 (15). The procedure was the same as that employed for the preparation of **13a**. 4-Methylpiperazine (12 mL, 0.087 mmol) and **14** (14 mg, 0.027 mmol) were subjected to the reaction to afford 10 mg (52%) of **15** as a white powder, mp 120–125 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.35 (1 H, br s), 8.11 (1 H, s), 6.99 (1 H, d, $J=2.3$ Hz), 6.90 (1 H, s), 4.72 (1 H, dd, $J=10.4$, 1.2 Hz), 4.66 (1 H, m), 4.55 (1 H, dd, $J=10.4$, 10.4 Hz), 4.17 (2 H, br s), 4.08 (5 H, br s), 3.98 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.79 (3 H, s), 3.75 (1 H, dd, $J=10.3$, 2.0 Hz), 3.22 (1 H, dd, $J=10.3$, 10.1 Hz), 2.96 (4 H, br s), 2.79 (3 H, s), 2.72 (3 H, s). IR (KBr): 1718, 1625, 1405, 1307, 1231, 1115 cm^{-1}. SIMS: *m/z* 714 712 $(M + H)^+$, 234. Anal. ($C_{33}H_{38}BrN_5O_8 \cdot 1.5\ H_2O$) C, H, N.

Stability of drug in aqueous solution

The stability of the DUMB2 derivatives under aqueous conditions 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 Sir3 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 and St-4 (poorly differentiated stomach adenocarcinoma) 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. Human xenograft was passaged and used in adult male BALB/c-*nu/nu* mice. Murine solid tumor was inoculated subcutaneously (sc) at the axillary region of mice. Human xenograft was inoculated sc in the flank of nude 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:

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

according to the method of the National Cancer Institute.[19]

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 the *T/C* value with 50% and less and statistical significance determined by the Mann–Whitney *U* test ($P<0.01$, one-sided).[20]

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References and Notes

1. (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.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037; (f) Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045; (g) Yasuzawa, T.; Saitoh, Y.; Ichimura, M.; Takahashi, I.; Sano, H. *J. Antibiot.* **1991**, *44*, 445; (h) Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H.; Saitoh, Y. *Chem. Pharm. Bull.* **1995**, *43*, 378.

2. (a) Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197; (b) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Org. Chem.* **1990**, *55*, 4499; (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.

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.

4. Its analogues are promising candidates in the clinical development. (a) Li, L. H.; Kelly, R. C.; Warpehoski, M. A.; McGovren, J. P.; Gebhard, I.; Dekoning, T. F. *Invest. New Drugs* **1991**, *9*, 137; (b) Li, L. H.; Dekoning, T. F.; Kelly, R. C.; Krueger, W. C.; McGovren, J. P.; Pudbury, G. E.; Petzold, G. L.; Wallace, T. L.; Ouding, R. J.; Prairie, M. D.; Gebhard, I. *Cancer Res.* **1992**, *52*, 4904.

5. 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.

6. Nagamura, S.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1995**, *43*, 1530.

7. (a) Nagamura, S.; Asai, A.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, printed. (b) Kobayashi, E.; Okamoto, A.; Asada, M.; Okabe, M.; Nagamura, S.; Asai, A.; Saito, H.; Gomi, K.; Hirata, T. *Cancer Res.* **1994**, *54*, 2404.

8. Asai, A.; Nagamura, S.; Saito, H. *J. Am. Chem. Soc.* **1994**, *116*, 4171.

9. Nagamura, S.; Kanda, Y.; Asai, A.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *44*, 933.

10. (a) Berner, D.; Cox, D. P.; Dahn, H. *J. Am. Chem. Soc.* **1982**, *104*, 2631; (b) Abe, Y.; Suehiro, T. *Chem. Lett.* **1982**, 337.

11. In the case of reaction with duocarmycin A and $HClO_4$ under the same conditions, a 1:1 mixture of two 9-hydroxy compounds which had a five- and six-membered ring C was obtained. Unpublished data.

12. The anticellular activity of the 9-hydroxy compound (**3**) against HeLa S_3 cells was >1000 nM at 72 h exposure, and compound **3** did not exhibit other biological activities.

13. (a) Cacchi, S.; Ciattini, P. G.; Moreera, E.; Orter, G. *Tetrahedron Lett.* **1986**, *27*, 5541; (b) Mitchell, T. N. *Synthesis* **1992**, 803; (c) Scott, W. J.; Stille, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 3033.

14. Mc Murry, J. E.; Scott, W. J. *Tetrahedron Lett.* **1983**, *24*, 979.

15. Zitko, B. A.; Howes, J. F.; Razdan, R. K.; Dalzell, B. C.; Dalzell, H. C.; Sheehan, J. C.; Pars, H. G. *Science* **1972**, *177*, 442.

16. (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: 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; (d) Tsuji, T.; Kaneda, D.; Kado, K.; Yokokura, T.; Yoshimoto, T.; Tsuru, D. *Phamacobio-Dyn.* **1991**, *14*, 341.

17. (a) Asai, A.; Nagamura, S.; Saito, H.; Takahashi, I.; Nakano, H. *Nucl. Acids Res.* **1994**, *22*, 83; (b) McGovren, J. P.; Clarke, G. L.; Pratt, E. A.; Dekoning, T. F. *J. Antibiot.* **1984**, *37*, 63.

18. (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.

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

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

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