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STUDIES ON THE ACTIVE METABOLITE (DU-86) OF KW-2189, A NOVEL DERIVATIVE OF DUOCARMYCIN

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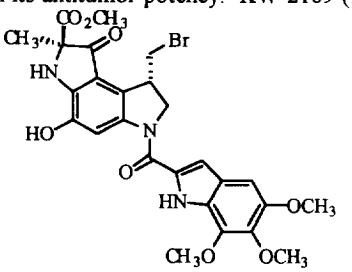
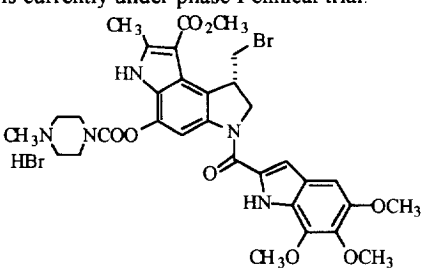
Abstract: Some derivatives of DU-86 as an active metabolite of KW-2189, which is a novel duocarmycin derivative, have been synthesized and evaluated for the biological activity. The results suggested that the methylation of aromatic NH moiety showed extremely decreased anticellular and antitumor activity, demonstrating that the free NH moiety play an important role in the biological activity.

A new class of antitumor antibiotics produced by *Streptomyces sp.*, including duocarmycin A, B1, B2, C1, C2, and SA possess exceptionally potent cytotoxicity.[1] Duocarmycin A and SA have a unique cyclopropane ring responsible for the sequence-selective and reversible alkylation of duplex DNA.[2] The structure of their pharmacophore (cyclopropane dienone) is similar to that of CC-1065.[3] We have synthesized duocarmycin analogs with the aim of enhancing and broadening the spectrum of the antitumor activity, and improving the solubility.[4] Recently, KW-2189 (**1**), a novel derivative of duocarmycin B2, was synthesized and demonstrated excellent *in vivo* antitumor activity, aqueous solubility greater than 10 mg/mL.[5] It was designed as a prodrug which requires enzymatic hydrolysis followed by regeneration of DU-86 (**2**) as an active metabolite. However, we unexpectedly found that KW-2189 itself alkylates calf thymus DNA without release of **2** in a buffer solution (pH 7.0) at 35 °C. As the detailed studies about the mechanism of **1**, it can be concluded that **2** as a metabolite, is responsible for the *in vivo* antitumor activity of **1**.[6] KW-2189 (**1**) itself seems not to be dominant in its antitumor potency. KW-2189 (**1**) is currently under phase I clinical trial.



duocarmycin B2



KW-2189 (1)

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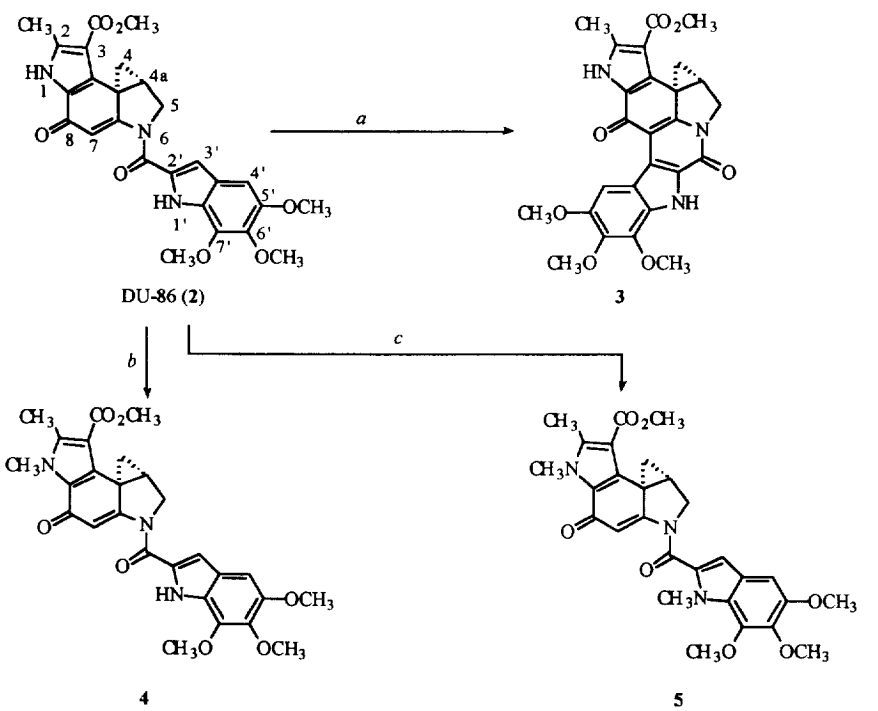
Therefore, it is interesting to study the structure-activity relationship of DU-86. In this communication, we would like to report the synthesis of the analogues of DU-86 to explore the role of the conformation and the aromatic NH moiety on their anticellular and antitumor activity.

DU-86 (**2**) was prepared by employing the Wagner-Meerwein type rearrangement of 8-*O*-protected-3-hydroxy-duocarmycin B2 followed by deprotection of the protecting group under basic conditions.[7] Compound **2** was dissolved in $CHCl_3$, and stirred at room temperature for 10 days under fluorescent lamp. Under this condition, an interesting cyclization happened to afford the compound **3**, which was derived from a bonding between the C-7 position and the C-3' position (45% yield) as shown in Scheme. Compound **3** is less polar than **2**, and the structure was elucidated on the basis of mass spectrometry and NMR, especially two-dimensional heteronuclear multiple bond connectivity spectroscopy (HMBC).[8] The mechanism was considered to be a photochemical cyclization between the enamide and the indole, as reported by Hutchins *et al.*.[9] When this reaction was achieved without light, the cyclized product was not obtained. This result also supports that this reaction is photochemical cyclization.

DU-86 (**2**) was also treated by slightly excess iodomethane in the presence of K_2CO_3 in DMF to afford the compound (**4**) methylated at the N-1 position selectively. The structure of **4** was proved by the observation of the coupling between the N-1' proton and the C-3' proton in the ^1H NMR spectrum. When this reaction was achieved with 3eq of iodomethane, compound **5** methylated both at the N-1 position and at the N-1' position was produced.



Scheme. *a*: *hv*, $CHCl_3$; *b*: CH_3I (1.1eq), K_2CO_3, DMF; *c*: CH_3I (3eq), K_2CO_3, DMF.

The antitumor activity of these derivatives was evaluated primarily by assays of growth inhibition against HeLa S_3 cells (*in vitro*), and antitumor activity against murine sarcoma 180 (*in vivo*).[10] As shown in Table 1, the efficacy *in vivo* is expressed as *T/C*, which is defined as treated versus control value of tumor volume. Tumor volume was calculated according to the method described previously.[4, 5] Then, the stability of these compounds was measured in 0.01 M phosphate buffer (pH 7) containing 50% CH_3CN at 35 °C by HPLC-analysis.[11]

Table 1

compound	Stability[a]	HeLa S_3 IC_{50} (nM)[b]		S-180 (s.c. - i.v.)[c]	
	$T_{1/2}$ (h)	1 h	72 h	dose (mg/kg)	T/C
2	340	0.045	0.0052	0.13	0.18
3	880	36	6.6	NT	
4	990	1.4	0.069	1.0	0.22
5	580	890	230	8.0	0.96
duocarmycin SA	>1000	0.0045	0.0069	0.10	0.21

[a] A half-life at 35 °C. Drug concentration was 0.02 mg/ml.
[b] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.
[c] Mice (five mice/group) were implanted subcutaneously (s.c.) with tumor cells, and the drug was dosed (mg/kg) intraveneously (i.v.).

These estimated compounds were solvolyzed to the corresponding diols, which were produced by a nucleophilic addition of H_2O to the cyclopropane ring. As shown in Table 1, the *N*-methyl compounds demonstrated increased stability greater than the *N*-H compounds (**4** and **5** vs. **2**).

The cyclized compound (**3**) showed decreased anticellular activity about 1×10^3 times inferior to DU-86 (**2**). The IC_{50} value at 72 h exposure was 6.6 nM. This result suggests that compound **3** is not a complementary shape of the DNA minor groove enough to permit and promote the association with DNA. The methylation of the aromatic NH group seems not to contribute to an increase of the biological activity. The compounds **4** and **5** exhibited 10 and 40,000 times less potent *in vitro* anticellular activity than DU-86, respectively. They did not exhibit superior *in vivo* antitumor activity to **2**. Moreover, the dimethyl derivative (**5**) showed no *in vivo* activity. These results exhibit that the free NH moiety in the aromatic ring is essential for anticellular and antitumor activity. In the examination of the simple derivatives of tetrahydrocyclopropa[1,2-c]benz[1,2-e]indole (CBI), the solvolytically more stable derivatives of CBI proved to be the most potent in *in vitro* assay systems.[12] In our test of the stability (pH7), however, a correlation between solvolytic chemical stability and biological potency was not observed among the *N*-methyl compounds (**2**, **4**, and **5**). These finding may prove useful in the predictable design of new duocarmycin derivatives; especially in the modification of the trimethoxy indole part.

Acknowledgment

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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) Ichimura, M.; Muroi, K.; Asano, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Nakano, H. *J. Antibiot.* **1988**, 41, 1285. (c) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, 43, 1037. (d) Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, 44, 1045.

(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.

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

(5) (a) Nagamura, S.; Asai, A.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. *Chem. Pharm. Bull.* **1996**, *in press*. (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.

(6) (a) Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem.* **1996**, *in press*. (b) 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. (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.

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

(8) Spectral data for compound **3**: ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.67 (1 H, br s), 12.12 (1 H, s), 8.85 (1 H, s), 4.49 (1 H, dd, *J*=12.8, 12.8 Hz), 4.34 (1 H, dd, *J*=12.8, 5.7 Hz), 3.92 (3 H, s), 3.91 (3 H, s), 3.86 (3 H, s), 3.80 (1 H, m), 3.77 (3 H, s), 2.53 (3 H, s), 2.19 (1 H, dd, *J*=7.7, 3.4 Hz), 1.43 (1 H, dd, *J*=4.5, 3.4 Hz). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 173.3, 164.1, 154.3, 153.9, 147.8, 142.0, 141.4, 138.6, 129.5, 129.5, 129.1, 126.7, 121.4, 117.9, 108.4, 106.8, 103.5, 61.2, 60.8, 52.1, 55.7, 50.7, 32.6, 26.4, 25.2, 13.7. SIMS *m/z* 489 $(M)^+$. *Anal.* Calcd for $C_{26}H_{23}N_3O_7$: C, 63.80; H, 4.74; N, 8.58. Found: C, 63.99; H, 4.55; N, 8.71.

(9) Hutchins, M.; Sainsbury, M.; Scopes, D. *Tetrahedron* **1984**, 40, 1511.

(10) (a) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, 3, 1. (b) Inaba, M.; Kobayashi, T.; Tashiro, T.; Sakurai, Y.; Maruo, K.; Ohnishi, Y.; Ueyama, Y.; Nomura, T. *Cancer* **1989**, 64, 1577.

(11) In the test of stability, the condition of an aqueous solution (pH7) containing 20 % CH_3CN had been so far used. Under this condition, a half life of **2**, **4** and duocarmycin SA is 130, 150 and 330 h, respectively. However, **3** and **5** were not dissolved in this aqueous solution.

(12) (a) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, 116, 5523. (b) Boger, D. L.; McKie, J. A.; Han, N.; Tarby, C. M.; Riggs, H. W.; Kitos, P. A. *Bioorg. Med. Chem. Lett.* **1996**, 6, 659.

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