Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 11, pp. 1215-1220, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00203-X

Synthesis and Antitumor Activity of Novel Duocarmycin Derivatives

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Abstract: A series of Duocarmycin B_2 analogs bearing simplified right hand segments (Seg-Bs) with the protected phenolic hydroxyl group in left hand segment (Seg-A) was synthesized. Among them, the cinnamoyl derivatives **6c** and **6d** exhibited potent antitumor activity against *in vivo* murine tumor models in the wider range of doses without detectable toxic effects than $DUMB_2$.

A new class of antitumor antibiotics produced by *Streptomyces* sp., including duocarmycin (DUM) A[1-3], B_1[4], B_2 [4], C_1 [3], C_2 [2-3] and SA [5-7] possess exceptionally potent cytotoxicity. Since $DUMB_1$, B_2, C_1 and C_2 readily yield DUMA in aqueous solution, DUMA bearing a electrophilic cyclopropane is thought to be an active form among these antibiotics (Fig.1). [8] DUMA shows its cytotoxicity through a sequence-selective minor groove alkylation of double-stranded DNA mediating N3 adenine covalent adduct formation. [9-12,14] In the course of our efforts of synthesizing new derivatives of DUMs, KW-2189 was explored, [13-14] which exhibits a broad spectrum antitumor activity in a series of experimental tumor models. It is currently in phase I clinical trials.

The Seg-A containing a spirocyclopropylhexadienone moiety, is necessary for the formation of covalent bonding with DNA (Fig.1). Our previous results indicate that the A-ring structure influences the electrophilicity of cyclopropane. [14] On the other hand, the Seg-B has been considered to play some important roles for noncovalent binding to DNA. [15-18] With the objective to identify novel promising candidates, we have synthesized a series of DUM analogs

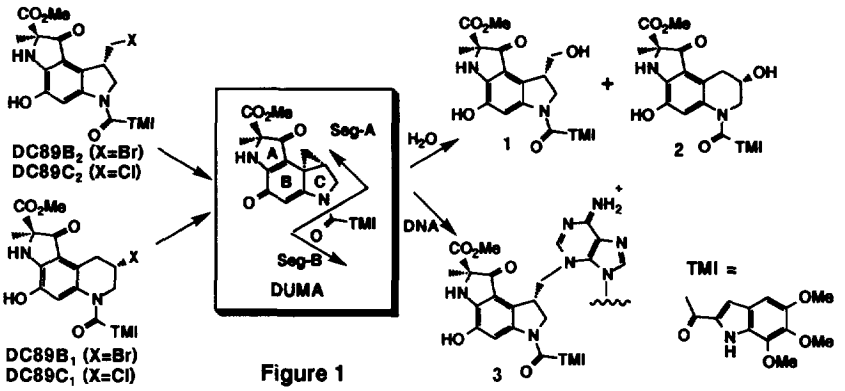


Figure 1

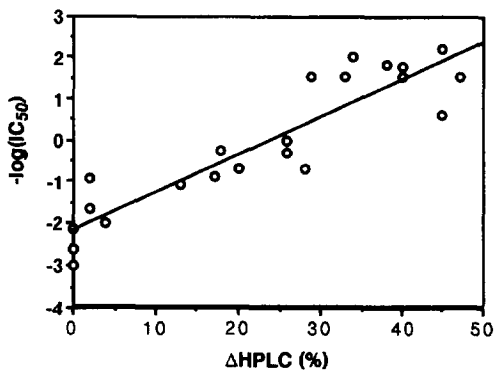


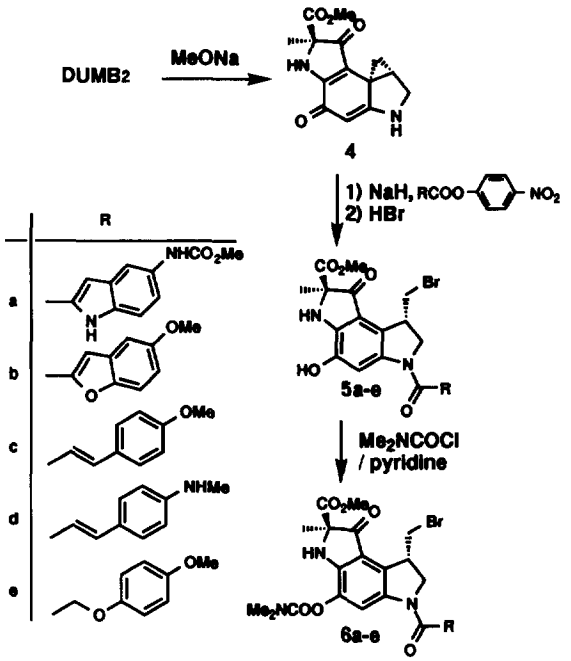
Figure 2. Correlation between DNA binding activity and cytotoxicity.
A sample (61μM) was incubated with or without calf thymus DNA (0.5mM bp) in 10% DMF / 10mM phosphate buffer (pH7.0) at 35 °C for one hour. The amount of residual drug was estimated by reverse phase HPLC analysis. ΔHPLC value was determined by subtraction of residual drug in the presence of DNA from that in the absence of DNA. Cytotoxicity was evaluated as previously described.[19] The concentration of drug required for 50% inhibition of cell growth (IC_{50} value) was determined after one hour of drug exposure.

bearing the simplified DNA-binding moieties such as acetyl, indole-2-carbonyl, benzofuran-2-carbonyl, cinnamoyl or phenoxyacetyl group.[19] These compounds exhibited varied cytotoxiciy. We also developed a simple assay based on HPLC analysis for detecting the covalent reaction of DUMs with calf thymus DNA.[19] As shown in Fig.2, the examination of the DNA-alkylating activity of these Seg-B derivatives revealed a good correlation between the DNA-alkylating activity and the cytotoxic potency. Along with the previous reports,[15,20-21] these results strongly indicate that a principal target of DUMs in cells is DNA and the Seg-B is a rate regulating-subunit for noncovalent binding to DNA. Some of these analogs with high affinity to DNA exhibited potent cytotoxicity and their *in vivo* antitumor activity was as potent as that of DUMB2. Though the spirocyclopropylhexadienone moiety of DUMs is necessary for DNA-alkylation, this subunit is unstable in aqueous solution to give the inactive hydrolysis products **1** and **2** (Fig.1). This hydrolytic conversion seems to decrease the efficacy of these compounds *in vivo*. In order to enhance *in vivo* activity of Seg-B analogs that showed potent

activity *in vitro*, the C8-phenolic hydroxyl group was protected with a chemically stable group. This protection is anticipated to prevent spontaneous formation of the cyclopropane. Our previous results revealed that dialkyl carbamoyl moiety on the phenolic hydroxyl group is effective to suppress the formation of DUMA from $DUMB_2$ and to enhance *in vivo* efficacy.[22]

The starting material utilized for the synthesis of cited compounds is Seg-A **4** obtained from $DUMB_2$ by methanolysis with sodium methoxide (Scheme 1). Synthesis of compounds **5** was performed by reacting the intermediate **4** with a suitable acylating agent, *p* -nitrophenyl esters, followed by cyclopropane cleavage with hydrobromic acid. Subsequent acylation with N,N-dimethylcarbamoyl chloride in pyridine afforded the desired derivatives **6**. For **6d**, *p* -nitrophenyl 4-(N-*t* -butoxycarbonylmethylamino)cinnamate was used for acylation. After obtaining the carbamoyl derivative, deprotection of *t* -butoxycarbonyl group was conducted with TFA.

The activity of all synthesized compounds has been tested *in vitro* on HeLaS3 and *in vivo* on murine sarcoma 180. The cytotoxicity and antitumor activity have been evaluated as previously described.[22] All of the N,N-dimethylcarbamoyl derivatives showed 10^3-10^4 times inferior cytotoxicity to that of $DUMB_2$. However, they exhibited potent antitumor activity *in vivo* (Table 1). These results are consistent with the activity of N,N-dimethylcarbamoyl derivative of $DUMB_2$ **7**. Higher doses of **6a-e** than that of $DUMB_2$ were employable to inject into mice without detectable toxicity. **6a** and **6b** exhibited the same efficacy as that of **7**. Unexpectedly, the cinnamoyl and phenoxyacetyl derivatives were more potent and lower toxic than the indole-2-carbonyl or benzofuran-2-carbonyl derivatives including the trimethoxyindole derivative, the natural type of Seg-B. Particularly, the cinnamoyl derivatives, **6c** and **6d**, showed sufficient efficacy (T/C <0.2) in the wide range of doses from 4mg/kg to 16mg/kg without detectable toxic effect. **6c** also showed an efficacy against human xenographt lung carcinoma LC-06 in nude mice. Its T/C value was 0.25 in 31.8mg/kg dose. Examination of DNA-alkylating activity of these carbamoyl derivatives by HPLC resulted in no evidence of the alkylating activity (data not shown). We reported before that KW-2189 alkylates DNA without loss of carbamoyl moiety, and proposed that the rate-determining formation of reactive cyclopropane is necessary to alkylate DNA.[14] These results imply that the formation of cyclopropane is essential for DNA-alkylation. Compounds presented here are basically stable in aqueous buffer solution and any decarbamoyl or hydrolyzed compounds were not produced. However, we observed the



Scheme 1

Table 1. Cytotoxicity and antitumor activity

NO	*in vitro* IC_{50} (nM)	*in vivo* dose (mg/kg)	T/C
6a	584	4.0	0.13
		2.0	0.31
6b	400	1.0	0.13
		0.5	0.26
6c	510	16	0.057
		8.0	0.14
		4.0	0.19
6d	720	16	0.055
		8.0	0.10
		4.0	0.18
6e	98	16	0.076
		8.0	0.16
		4.0	0.24
7	56	1.0	0.087
		0.5	0.13
		0.25	0.31
DUMB2	0.028	0.25	0.24

conversion of **5c** from **6c** in murine serum by HPLC analysis. These results indicate that **6c** is chemically stable but produces **5c** *in vivo* murine system via enzymatic hydrolysis of the carbamoyl moiety. Thus, we propose that the DUM derivatives presented here work *in vivo* as prodrugs and this activation mechanism is useful for potentiating the activity of DUMs *in vivo*. We previously reported that DNA-alkylating reaction of DUMs is reversible, and showed different reversibility of the cynnamoyl derivatives from the trimethoxyindole derivatives [18]. Other groups also proposed the importance of reversibility of this kind of agents for biological property. [16-18] Lower toxicity of **6c** might explain this hypothesis. Further evaluation of the mode of action for **6c** including its derivatives is underway.

Acknowledgment We thank Dr. Mayumi Yoshida and Mr. Shingo Kakita for measuring NMR spectra.

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23) ^{1}H-NMR data of all new compounds are given below (Bruker AM400 spectrometer, δ in ppm, TMS as internal standard, $CDCl_3$) **6a** : δ 9.40 (1H, br), 8.43 (1H, s), 7.82 (1H, br), 7.37 (1H, d, J=8.8Hz), 7.21 (1H, dd, J=8.8, 1.7Hz), 6.98 (1H, brs), 6.65 (1H, brs), 5.53 (1H, brs), 4.62 (1H, dd, J=10.6, 9.4Hz), 4.57 (1H, dd, J=10.7, 4.5Hz), 4.23 (1H, m), 4.02 (1H, dd, J=10.1, 3.4Hz), 3.80 (3H, s), 3.79 (3H, s), 3.62 (1H, dd, J=10.0, 8.7Hz), 3.14 (3H, s), 3.05 (3H,s), 1.68 (3H, s). **6b** : δ 8.34 (1H, br), 7.52 (1H, d, J=0.9Hz), 7.49 (1H, d, J=9.0Hz), 7.11 (1H, d, J=2.4Hz), 7.07 (1H, dd, J=9.0, 2.4Hz), 5.50 (1H, br), 4.71 (2H, m), 4.22 (1H, m), 3.98 (1H, dd, J=3.3, 10.1Hz), 3.87 (3H, s), 3.78 (3H, s), 3.66 (1H, dd, J=8.4, 10.1Hz), 3.15 (3H, s), 3.05 (3H, s), 1.88 (3H, s). **6c** : δ 8.49 (1H, brs), 7.78 (1H, d, J=15.3Hz), 7.55 (2H, d, J=8.7Hz), 6.93 (2H, d, J=8.7Hz), 6.69 (1H, d, 15.3Hz), 5.50 (1H, brs), 4.41 (1H, dd, J=10.7, 10.7Hz), 4.34 (1H, dd, J=4.4, 10.7Hz), 4.19 (1H, m), 4.04 (1H, dd, J=3.2, 10.0Hz), 3.86 (3H, s), 3.77 (3H, s), 3.57 (1H, dd, J=9.5, 9.5Hz), 3.14 (3H, s), 3.05 (3H, s), 1.67 (3H, s). **6d** : δ 8.47 (1H, br), 7.75 (1H, d, J=15.2Hz), 7.48 (2H, d, J=8.5Hz), 7.22 (1H, s), 6.72 (2H, d, J=8.5Hz), 6.61 (1H, d, 15.2Hz), 4.39 (1H, dd, J=10.2, 10.2Hz), 4.31 (1H, dd, J=4.4, 10.2Hz), 4.17 (1H, m), 4.02 (1H, dd, J=3.2, 10.0Hz), 3.87 (1H, br), 3.78 (3H, s), 3.57 (1H, dd, J=10.0, 10.0Hz), 3.15 (3H, s), 3.05 (3H, s), 2.91 (3H, s), 1.67 (3H, s). **6e** : δ 8.33 (1H, br), 6.93 (2H, d, J=9.1Hz), 6.84 (2H, d, J=9.1Hz), 5.48 (1H, br), 4.73 (1H, d, J=14.1Hz), 4.72 (1H, d, J=14.1Hz), 4.29 (2H, m), 4.15 (1H, m), 3.95 (1H, d, J=3.4, 10.1Hz), 3.76 (3H, s), 3.75 (3H, s), 3.57 (1H, dd, J=10.1, 8.8Hz), 3.12 (3H, s), 3.03 (3H, s), 1.66 (3H, s).

(*Received in Japan* 22 *March* 1996; *accepted* 24 *April* 1996)