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Synthesis and Antitumor Activity of Water-soluble Duocarmycin B1 Prodrugs

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Abstract: The water-soluble duocarmycin B1 prodrugs such as glycoside **3**, phosphate **4** and carbamate **5** were synthesized for improving biological and pharmaceutical profiles of duocarmycin. Among these prodrugs, *N*-methylpiperazinylcarbamoyl derivative **5** exhibited potent antitumor activity against several human tumors *in vivo*.

A new class of antitumor antibiotics produced by *Streptomyces* sp., including duocarmycin (DUM) A[1-3], B1 [4], B2 [4], C1 [3], C2 [2-3] and SA [5-7] possess exceptionally potent anticellular activity. Since DUMB1, B2, C1 and C2 readily yield DUMA in aqueous solution, DUMA bearing an electrophilic cyclopropane is thought to be an active form among these antibiotics [8]. DUMA shows its anticellular activity through a sequence-selective minor groove alkylation of double-stranded DNA mediating N3 adenine covalent adduct formation [9-13]. In the course of our efforts of synthesizing new derivatives of DUMA, A-ring pyrrole derivatives **1** and **2** were discovered (Fig. 1). Interestingly, **1** and **2** showed different DNA-alkylating property from that of DUMA[13]. KW-2189, a prodrug of **1**, which exhibits a broad spectrum of antitumor activity in a series of experimental tumor models was discovered [14, 15] and it is currently under phase II clinical trials.

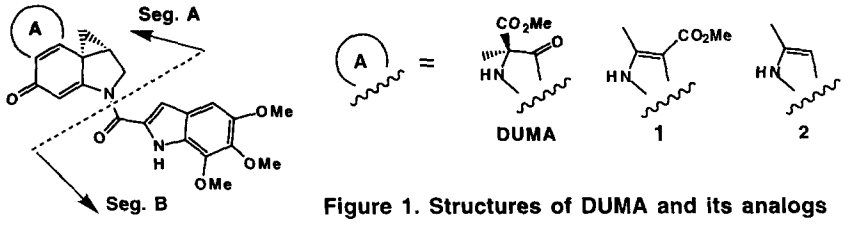

Figure 1. Structures of DUMA and its analogs

Synthesis of prodrugs and their conversion to DUMB1

We have reported the synthesis and antitumor activity of several kinds of analog prepared from DUMB2 or DUMSA[15-20]. We report here the initial studies on synthesis and biological evaluation of DUMB1 analogs. The limited antitumor activity of DUMs *in vivo* was supposed to be due to the instability and poor solubility in aqueous conditions. The free 8-phenolic hydroxy group of left hand segment (Seg. A) of DUMB1 and B2 is responsible to generate DUMA in aqueous conditions. Our previous investigations have shown that the protection of this phenolic hydroxy group is anticipated to prevent spontaneous formation of the cyclopropane and leads to potentiate antitumor activity of

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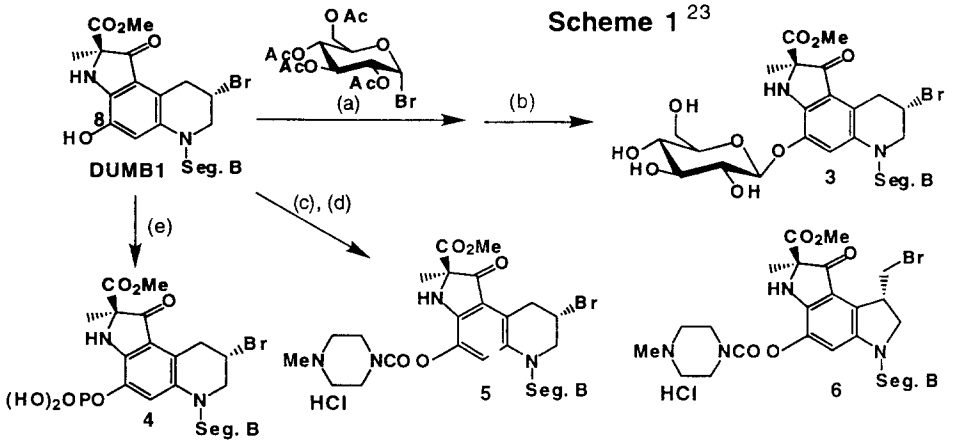
DUMB2 *in vivo* [15-20]. We chose three chemically stable hydrophilic substituents for the protection of 8-phenolic hydroxy group of DUMB1. The protective groups include electronically neutral sugar moiety, anionic phosphoryl group and cationic *N*-methylpiperazine. *O*-Glycosidation of DUMB1 with commercially available tetra-*O*-acetyl–α-D-glucopyranosyl bromide gave β-D-glucoside with high selectively [21] (Scheme 1). Following deacetylation under the basic conditions afforded *O*-β-glucoside **3**. The water-solubility of **3** was 0.2 mg/mL. Phosphorylation of DUMB1 was carried out with $POCl_3$ in pyridine [22] to yield phosphorylated DUMB1 **4**. The Hydrochloride **5** of *N*-methylpiperazinylcarbamoyl derivative was prepared as previously described [15]. Both **4** and **5** showed excellent water-solubility of >20 mg/mL.

Scheme 1 [23]



Reagents and conditions: (a) $SnCl_2$, $AgClO_4$, MS4A, CH_2Cl_2, –20 °C, 86%; (b) MeONa, MeOH, –20 °C, 93%; (c) *p*-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, –78 °C, then *N*-methylpiperazine, 0 °C, 79%; (d) 1N HCl / MeOH, 100%; (e) $POCl_3$, pyridine, CH_3CN, H_2O, 4 °C, 38%

Stability of drugs including conversion to DUMB1 was analyzed by HPLC. All of analogs presented here were stable and were not converted to DUMB1 or DUMA in phosphate buffer (pH7.4) at 37 °C (Table 1). Longer incubation of **5** (>3 days) resulted in rather formation of an inactive olefin **7** by the elimination of HBr. Next we examined the conversion from these derivatives to DUMB1 in mouse serum. Both **4** and **5** were partially converted to DUMB1 after the incubation for one minute in mouse serum. In this assay system longer incubation resulted in no more conversion to DUMB1 and the regained DUMB1 was further converted to DUMA. The conversion was not observed in the pre-boiled mouse serum at all (Fig. 3). These results indicate that enzymes in serum such as phosphatases or esterases hydrolyze a phosphoryl group and a carbamoyl group at the 8-position. On the other hand, glycoside **3** was stable and did not generate DUMB1 under these conditions. We examined the conversion of **3** into DUMB1 in the presence of recombinant bacterial β-glucosidase (Sigma6906). Incubation of **3** with the glucosidase resulted in 28% formation of DUMB1 (Table 1). These results indicate that all of DUMB1 analogs described here are prodrugs which require the enzymatic activation to generate DUMB1. Among three derivatives, compound **5** showed most potent efficacy as described below.

Table 1. Regeneration of DUMB1 from the prodrugs *in vitro*

Conditions	Drug residue (%) 3	4	5
Buffer (1 hr)	100	100	98
Serum (1 min)	98	76 (16)*	73 (20)*
Gulcosidase (30 min)	63 (28)*	not tested	not tested

Amount of drug was estimated by HPLC after incubation in phosphate buffer (pH7.4) for 1hr, mouse serum for 1min or sodium acetate buffer (pH5) in the presence of β-glucosidase for 30min at 37 °C. ( )* : Yield of regenerated DUMB1.

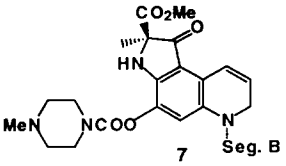


Figure 2. Degradation product of **5** in phosphate buffer (pH7.4)

Table 2. Anticellular and antitumor Activity of DUMB1 derivatives

NO	HeLa S3, IC_{50} (nM) 1 hr	72 hr	Sarcoma 180 Dose *	T/C	Colon 26 Dose *	T/C
3	2000	830	8.0	0.55		N.T.
			16.0	0.38		
4	40	3.2	0.5	0.57		N.T.
			1.0	0.29		
5	2000	70	1.0	0.19	1.0	0.23
			2.0	0.083	2.0	0.045
6	170	15	0.5	0.26	0.25	0.37
			1.0	0.13	0.5	0.13
DUMB1	0.13	0.038	0.5	0.22		N.T.

*, mg/kg; N.T., not tested

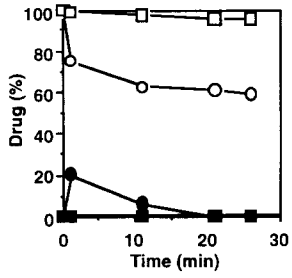


Figure 3. Conversion of **5** into DUMB1 in mouse serum
—○— : **5** in serum
—●— : DUMB1 in serum
—□— : **5** in boiled serum
—■— : DUMB1in boiled serum

Biological activity

The activity of all synthesized compounds have been tested *in vitro* on the alkylating activity to calf thymus DNA and anticellular activity against HeLaS3, and *in vivo* on murine sarcoma 180 and colon 26 according to previous reports [13, 15]. All of the derivatives did not alkylate DNA *in vitro* (data not shown) and showed 10^2-10^4 times inferior anticellular activity to that of DUMB1. However, they exhibited potent antitumor activity against sarcoma 180 *in vivo* (Table 2), suggesting that these prodrugs were activated by enzymatic system *in vivo*. In particular, carbamate **5** exhibited significant activity against both sarcoma 180 and colon 26 (T/C=0.083 and 0.045 respectively). Although carbamate derivative of DUMB2 **6** also showed potent activity [15], compound **5** was selected for further evaluation because **5** exhibited more potent efficacy than **6** (Table 2) in addition to the superior water-solubility. In further evaluation **5** showed statistically significant antitumor activity against human solid tumors such as St-4 (T/C=0.16), LC-6 (T/C=0.008) and Co-3 (T/C=0.34) that were insensitive to most chemotherapeutic drugs. Antitumor activity of **5** against these human tumors was comparable to that of KW-2189[14]. However, in contrast to KW-2189, **5** did not induce peripheral blood toxicity (reduction of the number of WBC and PL), suggesting that it does not affect hematopoetic cells or that it has limited access to such cells. **5** and KW-2189 are thought to generate the active species, DUMA and **1**, respectively *in vivo*. DUMA showed different DNA-alkylating property from that of **1** *in vitro* [13]. Difference in their toxicological profiles might be due to the difference in their DNA-alkylating properties. Thus, compound **5** could be a new lead compound that possesses significant antitumor activity and different toxicological profile from that of KW-2189.

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