Biological Evaluation of an Antibiotic DC-81–Indole Conjugate Agent in Human Melanoma Cell Lines

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Pyrrolo[2, 1-*c*][1, 4]benzodiazepines (PBDs) are potent inhibitors of nucleic acid synthesis because of their ability to recognize and bind to specific sequences of DNA and form a labile covalent adduct. DC-81, an antitumor antibiotic produced by *Streptomyces* species, is a PBD. We combined DC-81 and an indole carboxylate moiety to synthesize a hybrid designed to have much higher sequence selectivity in DNA interactivity. In this paper, the cytotoxic potency of the hybrid in human melanoma cell lines was studied. XTT assay demonstrated that the DC-81–indole conjugate possessed cytotoxicity against human melanoma cell lines.

Key Words: pyrrolo[2, 1-*c*][1, 4]benzodiazepines, PBDs, DC-81, indole carboxylate moiety, melanoma, XTT assay
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Many compounds have been discovered that bind to and interact with the B-form of DNA and can inhibit nucleic acid synthesis and block DNA transcription. Pyrrolo[2, 1-*c*][1, 4]benzodiazepines (PBDs) are a group of potent, naturally occurring antitumor antibiotics produced by *Streptomyces* species [1]. The cytotoxic and antitumor effects of these compounds are believed to arise from the modification of DNA, which leads to the inhibition of nucleic acid synthesis and the production of excision-dependent single- and double-strand breaks in cellular DNA [2, 3]. These antibiotics have been proposed to covalently bond to N2 of guanine to form a neutral minor groove adduct (Figure 1) [4–7]. Anthromycin [8, 9], tomaymycin [10, 11], and DC-81 [12] are the best-known examples of PBDs. We have previously reported a very short (6 steps) and efficient synthesis of DC-81 [13] with an overall yield of about 35% (67% yield based on recovered starting material at the fifth step). The reaction can be carried out on a much larger scale (10 g) than previously reported syntheses. Furthermore, in the first three steps, the products are easily recrystallized and pure enough for subsequent reactions.

Although PBDs have high antitumor activity, they are cardiotoxic, which has precluded their continued clinical application [14]. Therefore, hybrid compounds with active moieties of known antitumor and antiviral agents are being designed and synthesized to provide highly sequence-selective DNA-interactive properties and antitumor activity [15]. Thurston et al reported a PBD-ethylene diaminetetraacetic acid (EDTA) conjugate that covalently binds to DNA at 5′-PuGPu (Pu = purine, G = guanine) sequences leading to site-specific cleavage [16]. Because the PBD-EDTA hybrid is GC-specific, we designed and synthesized hybrid **3** (Figure 2) from DC-81 (**1**) coupled with an indole carboxylate moiety (**2**). DC-81 and indole carboxylate are commonly used as medicinal compounds, e.g. as antimitotic drugs [17–19]. We expected that hybrid **3** would

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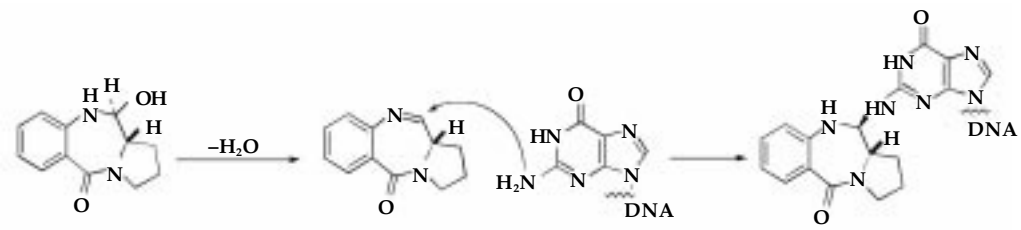

Figure 1. *Possible mechanism of the formation of the pyrrolo[2, 1-c][1, 4]benzodiazepine (PBD)-DNA adduct.*

recognize more DNA base pairs and bind sequence-selectively to the macromolecule.

Melanoma is a rapidly proliferating and highly metastatic tumor that is resistant to radio- and chemotherapy. Melanoma provides a classic example of multistage carcinogenesis, showing well-defined stages of progression with distinct clinical, biologic, and histologic characteristics [20].

In the present study, we evaluated the cytotoxicity of the DC-81–indole conjugate (**3**), DC-81 (**1**), and indole carboxylate (**2**), either alone or a combination of **1** and **2**, by determining the inhibition of cell growth in various human melanoma cell lines (A2058, A375, RPMI7951, and Hs695T) in culture. The aim of our study was to evaluate whether hybrid **3** had more antitumor activity than DC-81 (**1**) and indole carboxylate **2**, either alone or combination.

Materials and Methods

Dulbecco's modified Eagle medium (DMEM), fetal calf serum (FCS), penicillin/streptomycin (100 U/ml and 100 μg/ml), Dulbecco's minimal essential medium (MEM), non-essential amino acids, sodium pyruvate, Dulbecco's phosphate buffered saline (PBS), trypsin-EDTA, and dimethyl sulfoxide (DMSO) were obtained from Gibco BRL (Gaithersburg, MD, USA). The XTT assay kit was purchased from Promega (Madison WI, USA).

Human melanoma cell lines and cell culture

Four human melanoma cell lines, A2058, A375, Hs695T and RPMI7951, were purchased from American Type Culture Collection (Manassas, VA, USA). The cell lines

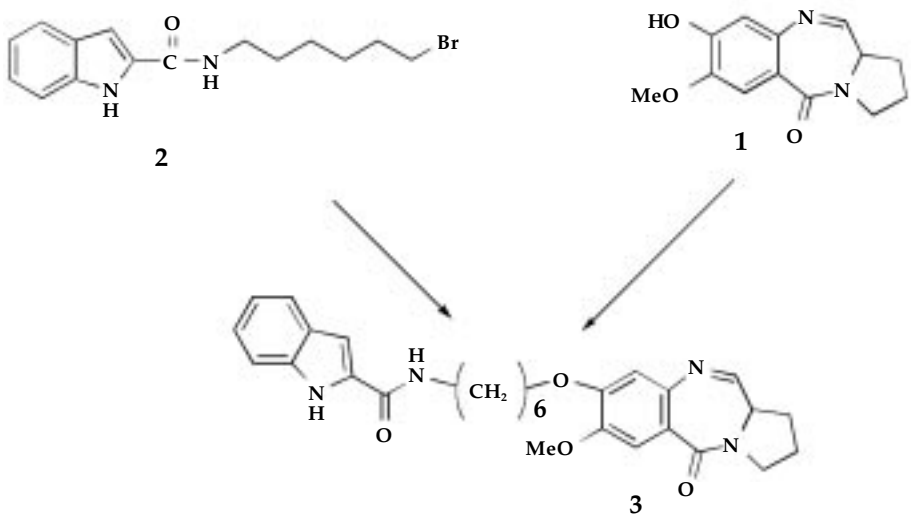

Figure 2. *Proposed reaction of DC-81 (**1**) with the indole carboxylate moiety **2** to form hybrid **3**.*

were maintained in culture medium (MEM: A2058 and A375; MEM with 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate: Hs695T and RPMI7951) supplemented with 10% FCS, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate. Melanoma cells were passaged at confluence after treatment with 5 mM EDTA (Gibco BRL) and incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide. Following trypsinization and cell counting, cells in 100 µl culture medium were seeded into 96-well microtiter plates (2 x 10^3 cells/well).

Cytotoxicity

Antitumor compounds were dissolved in DMSO at a final concentration of 1 mg/ml and stored at 4°C. After the cells adhered to the plate, the supernatant was aspirated and 100 µl of media containing various concentrations of different test agents were added to the wells for 24-hour incubation. Each experiment was repeated three times. After incubation, the XTT assay was performed as described below. Inhibiting concentration (IC) was determined by plotting compound concentration versus cell viability. The mean IC_{50} value was calculated for each cell line.

XTT assay

We determined the relationship between the number of cells and formazan production. XTT solution (20 µl) was added to each well. Following 2 hours' incubation, absorbance at 490 nm was measured in an enzyme-linked immunosorbent assay (ELISA) reader to determine proliferation by quantifying cellular metabolic activity. The results were expressed as the optical density of the control in triplicate cultures [21]. The unpaired Student's *t*-test was used for statistical evaluation of the relationship between the control and experimental groups; $p < 0.05$ was considered statistically significant.

Results

Results are shown in Figures 3 to 6 for A2058, A375, RPMI7951, and Hs695T, respectively. The cytotoxic effects were dependent on drug concentration. Hybrid **3** was the most potent inhibitor of A2058, A375, and RPMI7951. However, all compounds tested had equal potency against Hs695T cells. The Table shows the IC_{50} values; treatment with DC-81 (**1**) or a combina-

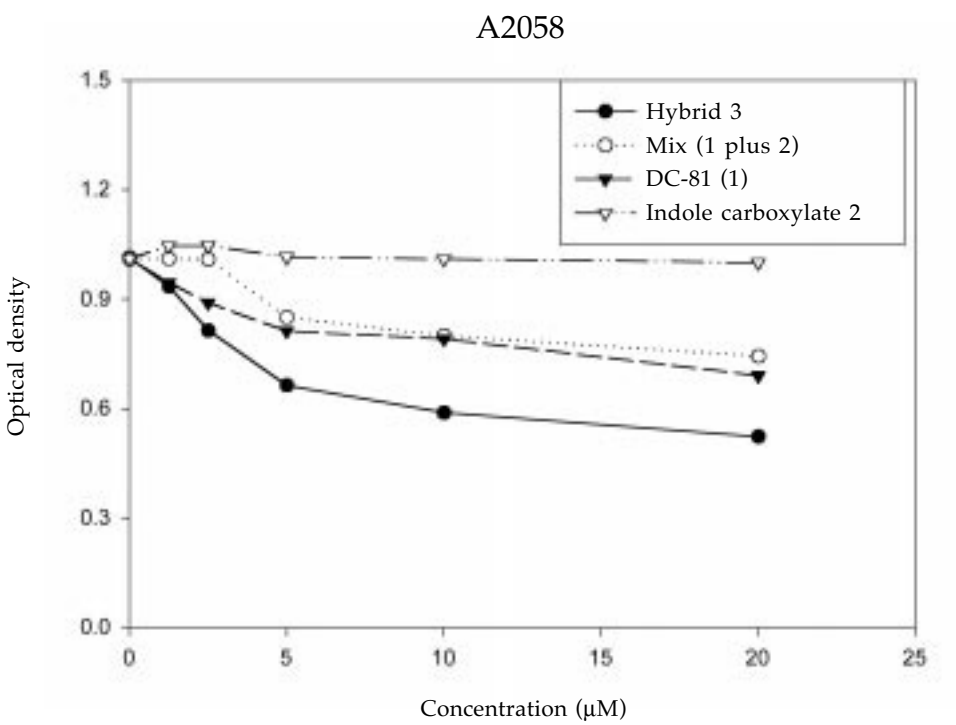

Figure 3. *Dose-response curves for compounds tested against A2058 cells (2 x 10^3 cells/well, 2-day culture, 1-day treatment, 2-hour incubation with XTT). Results are expressed as mean ± standard deviation.*

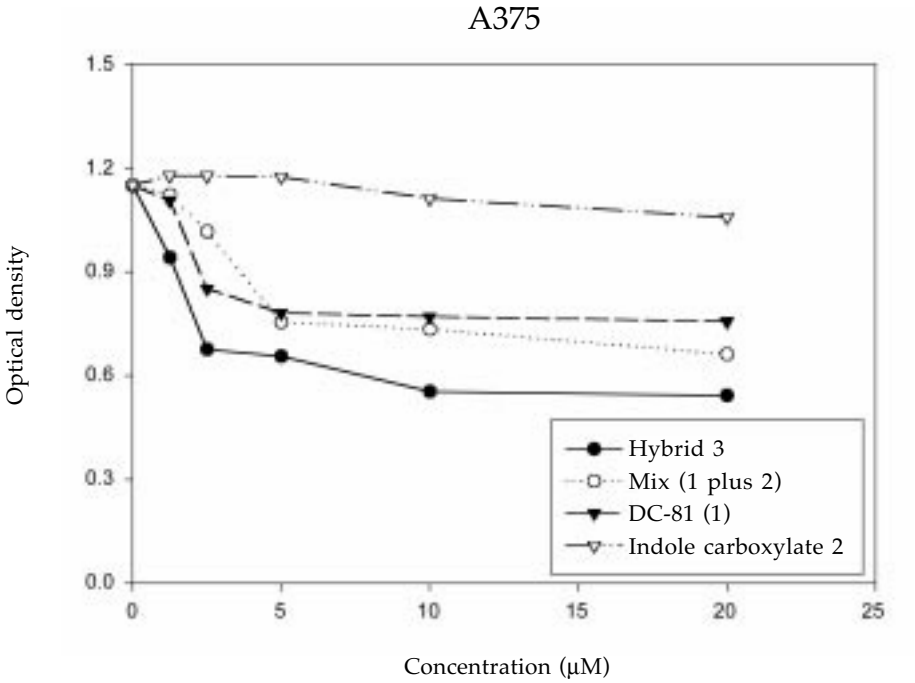

Figure 4. *Dose–response curves for compounds tested against A375 cells (same experimental conditions as in Figure 3).*

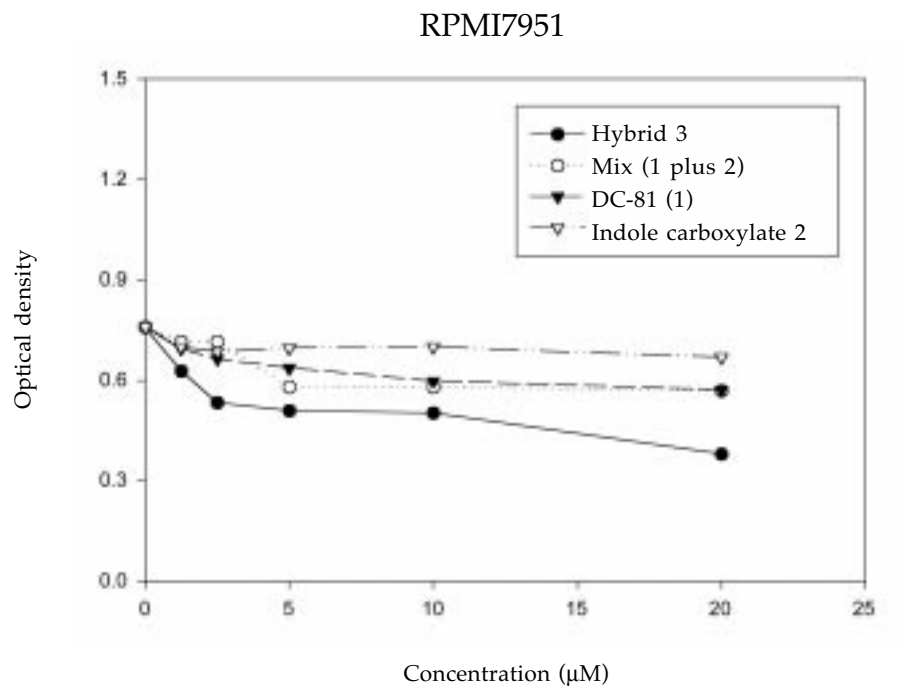

Figure 5. *Dose-response curves for compounds tested against RPMI7951 cells (same experimental conditions as in Figure 3).*

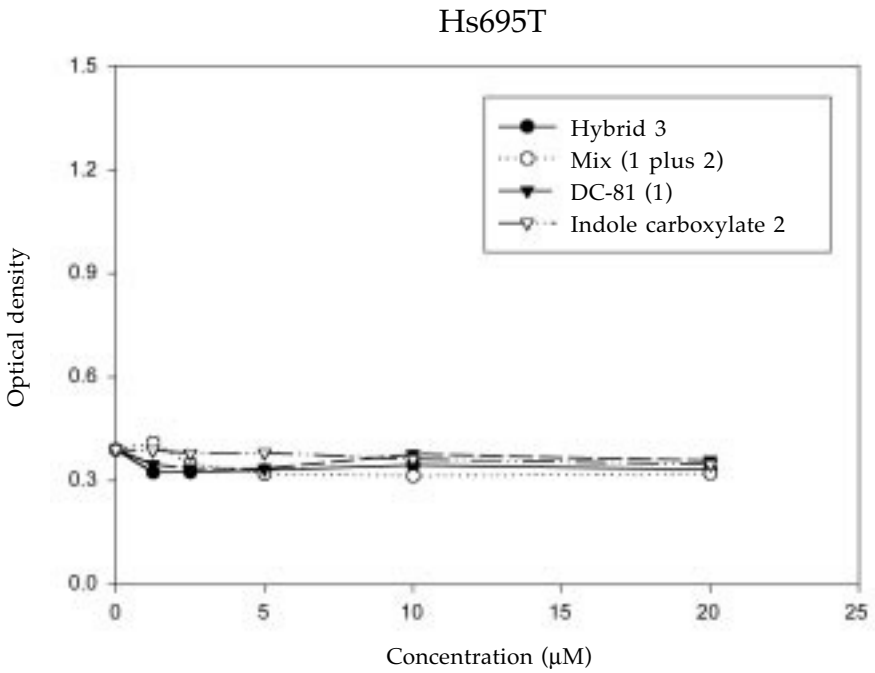

Figure 6. *Dose-response curves for tested compounds against Hs695T cells (same experimental conditions as in Figure 3).*

tion of **1** and **2** gave very similar results on all cell lines except Hs695T.

Discussion

DNA is a target for many antitumor drugs currently used in the clinic. However, there are only a few DNA-interactive agents that bind to DNA with high sequence selectivity. Selectivity is generally thought to favor the targeting of rapidly growing tumor cells, so the development of low molecular weight molecules with highly sequence-selective DNA-interactive properties is of interest. One approach to this involves the design and synthesis of molecules with predictable affinity-cleavage properties that might function as artificial restriction enzymes to inhibit tumor cell proliferation and metastasis. DC-81 (**1**) exhibits its biological activity by covalently binding to the N2 of guanine in the minor groove of DNA, via the electrophilic carbinolamine functionality at N10-C11. The (S) configuration at the chiral C11a position pro-

Table. *In vitro* cytotoxicity of tested compounds on human melanoma cell lines A2058, A375, RPMI7951, and Hs695T

	IC_{50} (μM)			
Cell line	Hybrid **3**	Mix (**1** plus **2**)	DC-81 (**1**)	Indole carboxylate (**2**)
RPMI7951	18.4	36.9	41.5	128.6
Hs695T	NC	NC	NC	NC
A2058	17.4	33.5	31.0	294.2
A375	14.1	20.8	18.5	25.7

NC = not calculable. IC_{50} (50% inhibitory concentration) represents the mean from dose-response curves of three experiments.

vides the molecules with the necessary right-hand twist to fit properly within the minor groove of DNA, spanning three base pairs for 5′-PuGPu sequences. In this study, we used the XTT assay to evaluate the cytotoxicity of tested compounds in human melanoma cell lines. Our results indicated that hybrid **3** was more effective as an antiproliferative agent than DC-81 (**1**) and indole carboxylate (**2**), sufficient to strongly inhibit A2058, A375, and RPMI7951 cell growth. One can speculate that this was because hybrid **3** recognized more DNA-binding sites, increasing the stability of the drug/DNA complex. We will investigate the structure of the drug/DNA complex using molecular modeling. It was surprising that hybrid **3** had no inhibitory effect on Hs695T. Based on our results, we suggest that hybrid **3** may be a cell-selective antitumor agent, which encourages us to design more diverse antitumor DC-81 analogues.

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