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Inhibitory activity of four demethoxy fluorinated anthracycline analogs against five human-tumor cell lines

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ABSTRACT

Four anthracycline analogs synthesized in our laboratory were evaluated in comparison with adriamycin (doxorubicin) for their growth-inhibitory effect against five human-tumor cell lines, including lung carcinoma, colon adenocarcinoma, breast adenocarcinoma, melanoma, and glioblastoma. The compounds included 4-demethoxy-7-*O*-(2,6-dideoxy-2-fluoro-L-talopyranosyl)daunomycinone (**2**), its 3′,4′-diacetate (**1**), its 14-bromo derivative **3**, and its 14-hydroxy analog, namely 4-demethoxy-7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)adriamycinone (**4**). Compounds **1, 2**, and **3** showed moderate cytotoxic effect in most of the cell lines, while compound **4** had a strong effect, comparable to or better than that of adriamycin in most of the cell lines.

Analogs of the natural anthracycline antibiotics daunorubicin and doxorubicin having diminished toxicity and/or enhanced antitumor activity have been targets of synthesis in this laboratory[1] and others.[2–7] Replacement of the 3′-amino group by hydroxyl via semisynthesis through appropriate glycosylation of daunomycinone has yielded products of lower toxicity,[8] and hydroxylation of the aglycon at the 14-position has permitted conversion into doxorubicin analogs of enhanced antitumor activity.[9] Introduction of an axially oriented halogen atom at the 2-position of the sugar has afforded products demonstrating higher activity,[10,11] the synthesis of which has been achieved via glycal intermediates for the 2′-chloro,[11] 2′-bromo-,[11] and 2′-iodo[10] structures; alternative strategy has been required to obtain the 2′-fluoro compounds.[12,13]

Although daunomycinone and adriamycinone, the aglycones of the natural antibiotics, possess a methoxy group at the 4-position of the aromatic ring, this group does not appear to play any essential role in the biological activity of their 7-glycosylated derivatives.[12,14,15] Envisaging the target of a fully synthetic analog incorporating attributes favorable to high activity, a component aglycon lacking the 4-methoxy group would be easier to synthesize, as there would be no requirement for a regiospecific step to correctly orient the methoxy group. We have already demonstrated[15] that a chiral glycon can be used as a resolving agent in coupling to synthetic, racemic 4-demethoxydaunomycinone[16] to furnish separable, enantiomerically pure diastereomers, one of which has the stereochemistry of the natural aglycon.

We have earlier reported[17] the glycosidic attachment to the 7-position of (+)-(7*S*,9*S*)-4-demethoxydaunomycinone (idarubicinone)[18] of a 2,6-dideoxy-2-fluoro-α-L-talopyranosyl group, to afford a daunorubicin analog (**2**) via its 2′,3′-diacetate (**1**), and its subsequent conversion via hydroxylation at the 14-position into the corresponding doxorubicin analog **4** by way of a 14-bromo intermediate **(3)**. Compounds **2** and **4** are 4-demethoxy-3′-hydroxy-3′-deamino-2′axial-fluoro analogs of the parent natural antibiotics.

It is noteworthy that computational studies on DNA binding[19] suggest that removal of the 4-methoxy group from the aglycone of anthracycline antibiotics correlates with increase in antitumor potency and selectivity.

The four anthracycline glycosides **1–4** were evaluated in vitro, along with adriamycin, for antitumor activity against five human-tumor cell lines, including lung, colon, and breast carcinoma, melanoma, and glioblastoma multiforme CNS tumor. All showed activity, and details are reported here.

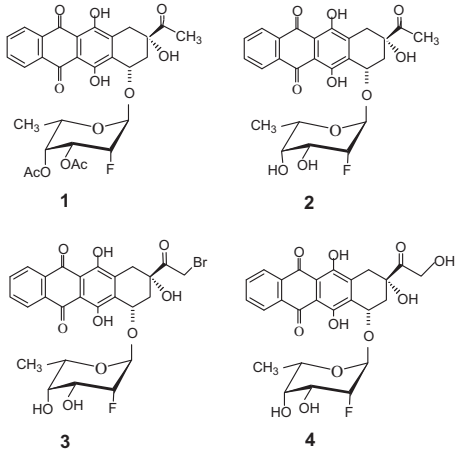

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Table 1
Effect of drug treatment on the survival of a panel of cancer cell lines: comparison of ED_{50} values for analogs (**1**–**4**) of adriamycin

Sample	ED_{50} values (μg/mL)				
	A549 (lung carcinoma)	HT29 (colon adenocarcinoma)	MCF-7 (breast adenocarcinoma)	RPMI (melanoma)	U251 (glioblastoma)
Adriamycin	0.03	>0.000	0.03	0.02	0.02
1	0.07	0.001	0.06	0.05	0.1
2	0.008	>0.000	0.03	0.03	0.04
3	0.6	>0.000	0.4	Ndt[a]	Ndt[a]
4	0.008	>0.001	0.02	0.02	0.02

[a] Ndt, no detectable toxicity.

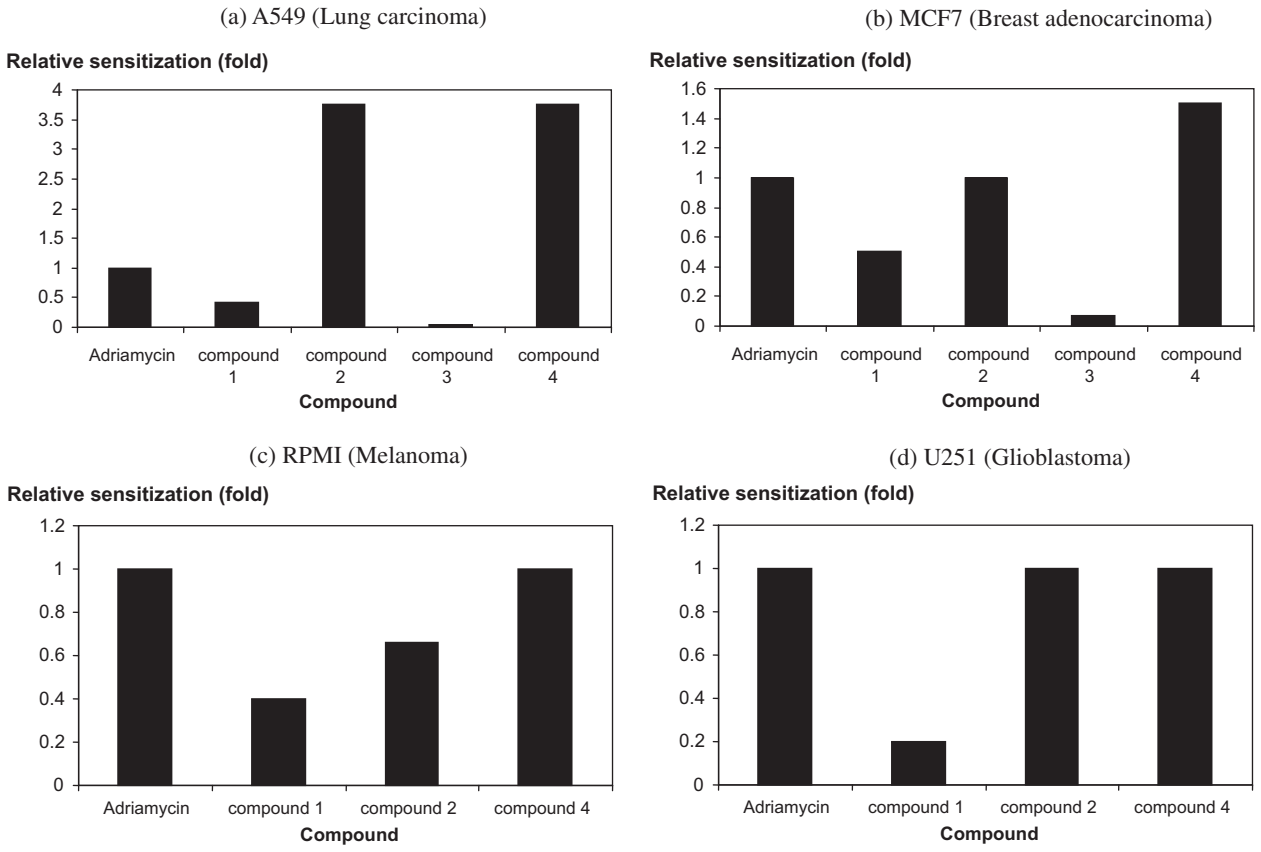

Figure 1. Relative sensitization of a panel of cancer cell lines by adriamycin and analogs (a) A549; (b) MCF-7; (c) RPMI; (d) U251.

Compounds. The four compounds evaluated, 4-demethoxy-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-2-fluoro-α-L-talopyranosyl)daunomycinone (**1**), 4-demethoxy-7-*O*-(2,6-dideoxy-2-fluoro–L-talopyranosyl)daunomycinone (**2**), 14-bromo-4-demethoxy-7-*O*-(2,6-dideoxy-2-fluoro–L-talopyranosyl)daunomycinone (**3**), and 4-demethoxy-7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)adriamycinone (**4**), were synthesized in our laboratory as detailed in Ref. 17.

Cell lines. The study was performed on a panel of human-tumor cell lines that included A-549 (lung carcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), RPMI-7951 (melanoma), and U251 MG (glioblastoma) obtained from the American Type Culture Collection (Rockville, MD). Cells of A-549 were cultured in Ham's F12K medium supplemented with 10% fetal bovine serum with 2 mM L-glutamine. Cells of HT-29 were cultured in McCoy's **5a** medium (modified) supplemented with 10% fetal bovine serum and 1.5 mM L-glutamine. Cells of MCF-7 were grown routinely in RPMI-1640 medium supplemented with 5% fetal bovine serum and 2 mM L-glutamine. Cells of RPMI-7951 were cultured in Minimum Essential Medium (Eagle) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Cells of U251 MG were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20 mM L-glutamine and 10% fetal calf serum. Cells were used in the exponential growth phase for all experiments.

In vitro chemosensitivity assay. The quantitative sulforhodamine B (SRB) assay was used to determine the growth-inhibitory effect of drugs on the tumor cells. Briefly, cells were seeded at 1000–2000 cells/well in a 96-well plate and grown for 24 h. The cells were then treated with increasing concentrations of the test compounds or dimethyl sulfoxide solvent control. At the end of drug exposure, cells were fixed in 50% trichloroacetic acid for 1 h, washed once with water, and then stained with 0.4% SRB (Sigma Aldrich, MO) dissolved in 1% acetic acid for 10 min. Finally the stained cells were rinsed with 1% acetic acid to remove unbound stain, air dried, and the bound dye was solubilized with 10 mM Tris [tris(hydroxymethyl)aminomethane] solution. The optical density of treated cells was detected at 490 nm (reference 450 nm) with a Dynatech 700 plate reader. The data were electronically captured and analyzed by Quattro Pro (Borland, Corel).

Drug exposure. Five log dilutions of the compound (beginning with the maximum soluble concentration) were tested on each of the cell lines. Each sample was run in sextuplet. Simultaneously, negative controls (samples processed in drug-free medium) and adriamycin-treated (at 5 log dilutions of cell-line specific concentrations) positive controls were also run. Evaluation of the cytotoxic effect was performed immediately after the end of drug exposure. The data were captured electronically and analyzed by QuattroPro. Individual points were reference-corrected, averaged, and linear regressions of the experimental and control data calculated for determination of the ED_{50} (effective dose at which the cell growth is retarded to 50% of the control growth). Data from a representative experiment are shown in Table 1, and relative sensitization in various cell lines for the compound analogs are shown in Figure 1. In the case of HT29, the ED_{50} value for all compound analogs is comparable to that of adriamycin, and therefore these data are not presented in the figure.

Cytotoxic activity. The cytotoxic activity of compounds **1**, **2**, **3**, and **4** in comparison with that of adriamycin is reported in Table 1. Compounds **1, 2**, and **3** show a moderate cytotoxic effect in most of the cell lines, while compound **4** evinced a strong cytocidal effect in most cell lines, and this effect was either comparable to or greater than that observed after adriamycin treatment.

The daunorubicin analog **2** and its precursor diacetate (**1**) were both active in each of the screens, but the dihydroxy analog **2** was consistently more active than the diacetate **1**. In contrast, the 14-bromo derivative **3** was of low or negligible activity, whereas the 14-hydroxy analog **4** showed comparable or greater activity than adriamycin. All of the compounds tested were inactive in the HT29 colon carcinoma screen.

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