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A NEW DESIGNED TUMOR SELECTIVE DAUNOMYCIN DERIVATIVE

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*Abstract: The synthesis and biological profile of the new daunomycin derivative **2** as an antitumor agent is described.*

Daunomycin hydrochloride (**1**, Scheme I, B) is a well known anticancer agent, particularly useful in the treatment of leukemia.[1] The accepted mechanism of action of daunomycin involves intercalation of the anthraquinone moiety between base pairs of DNA and interaction of the amino group with the negatively charged phosphate groups.[2] Bioreduction, reaction with oxygen, radical formation and DNA cleavage[3] then results in cell death. Protection of the amino group is known to reduce the cytotoxicity of the drug.[4]

In a recent study in these laboratories involving designed enediynes[5] the phenylsulfonylethoxy carbonyl group was found to be a useful triggering device for activation of these compounds within the cell. The chemistry involved in the mechanism of activation of these systems is shown in Scheme 1A. Thus, base- or intracellular-induced β-elimination is followed by carbon dioxide extrusion generating the active amine on the site of action. Because of the high selectivity against tumor cells exhibited by systems equipped with this triggering device we decided to install it on the nitrogen of daunomycin.

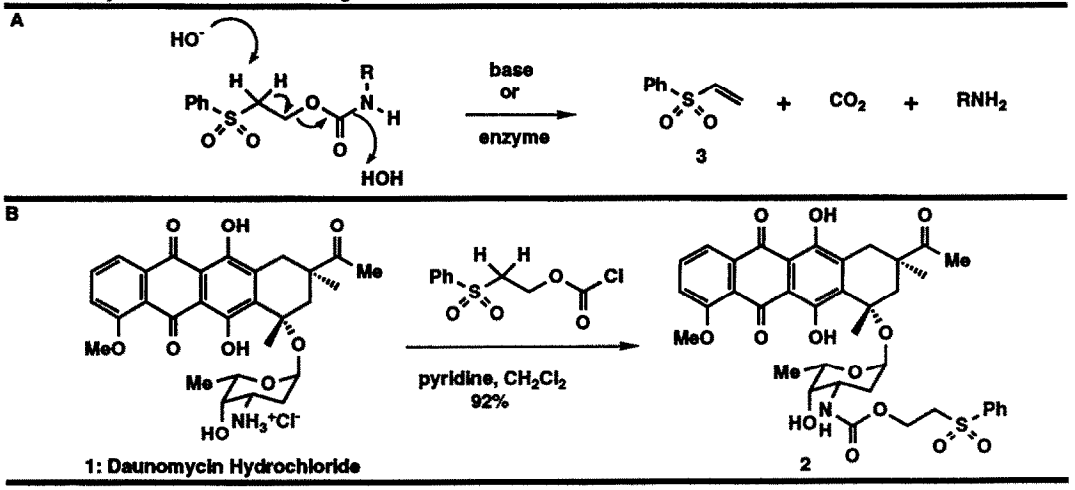
With the above considerations in mind we proceeded to design phenylsulfonylethoxy-formyl daunomycin **2** as a potential prodrug with selective anticancer properties. Compound **2** was prepared in 94% yield by treatment of daunomycin hydrochloride (**1**) with stoichiometric amounts of phenylsulfonyl ethoxycarbonyl chloride and excess pyridine in dichloromethane at -20 °C (Scheme IB).

The cytotoxicities of carbamate **2** against a panel of cell lines was compared to that of daunomycin hydrochloride (**1**) (Table 1). The cytotoxicity of **2** after incubation for 24 hours was found to be less than that of daunomycin for normal cells (by a factor of 300 for HMEC and nearly a factor of 150 for NHDF), while the cytotoxicities of the two compounds were comparable (by a factor of less than 20) for tumor cells, thus fulfilling our expectations. Furthermore, carbamate **2** significantly inhibited tumor growth in experimental mouse mammary tumors showing a profile similar to daumonycin itself (Figure 1). The LD_{50} for the intraperitonially administered drugs in Balb C mice was found to be 38 mg/kg[6] for carbamate **2** and 15 mg/kg for daunomycin hydrochloride (**1**).[7]

When exposed to conditions which simulated a physiological environment (pH = 7.3, 37 °C), derivative **2** was converted to daunomycin **1** and phenyl vinyl sulfone **3**, both of which were characterized spectroscopically and by HPLC. This is strong evidence that **2** is acting as

a prodrug of daunomycin **1**. Although it is a potential alkylating agent, phenyl vinyl sulfone **3** exhibited almost no cyctoxicity.

In conclusion, a potent antitumor compound has been conveniently obtained in a single step from the readily available daunomycin hydrochloride (**1**) and was shown to have biological activity comparable to that of daunomycin while showing considerably lower cystemic toxicity in mice. This compound may have clinical potential as a therapeutic anticancer agent and is currently under further investigation.



Scheme 1. A: Proposed mechanism of activation of phenylsulfonylethoxy carbamate derivatives.
B: Synthesis of daunomycin phenylsulfonylethoxy carbamate derivative (**2**) from daunomycin hydrochloride (**1**).

Table 1. Cytotoxicities of daunomycin hydrochloride (**1**) and pro-daunomycin derivative (**2**).

Cell type	Cell line	IC_{50} (M) after 24 h 1	IC_{50} (M) after 24 h 2	IC_{50} (M) after 72 h 1	IC_{50} (M) after 72 h 2
TUMOR CELLS					
pancreatic carcinoma	Capan-1	3.90×10^{-7}	7.80×10^{-7}	3.10×10^{-6}	3.10×10^{-6}
Chinese hamster ovary	CHO	3.90×10^{-7}	6.30×10^{-6}	2.94×10^{-7}	2.56×10^{-6}
promyelocytic leukemia	HL-60	$<9.80 \times 10^{-8}$	9.80×10^{-8}	9.80×10^{-8}	9.80×10^{-8}
colon carcinoma	HT-29	7.80×10^{-7}	7.80×10^{-7}	3.57×10^{-7}	3.50×10^{-6}
breast carcinoma	MCF-7	7.80×10^{-7}	7.80×10^{-7}	1.35×10^{-5}	1.17×10^{-5}
T-cell leukemia	Molt-4	1.00×10^{-9}	1.00×10^{-9}	8.55×10^{-13}	7.53×10^{-9}
ovarian carcinoma	Ovcar-3	3.90×10^{-7}	1.60×10^{-6}	2.87×10^{-7}	1.58×10^{-7}
mouse leukemia	P-388	3.90×10^{-7}	7.80×10^{-7}	$<9.8 \times 10^{-8}$	$<9.8 \times 10^{-8}$
melanoma	SK-Mel-28	1.60×10^{-6}	3.10×10^{-6}	1.41×10^{-6}	1.05×10^{-5}
lung carcinoma	UCLA P-3	7.30×10^{-7}	3.10×10^{-6}	---	---
NORMAL CELLS					
human mamm epithelial cells	HMEC	3.90×10^{-7}	1.30×10^{-5}	$<9.8 \times 10^{-8}$	1.50×10^{-6}
norm human dermal fibroblast	NHDF	1.90×10^{-7}	1.30×10^{-5}	4.04×10^{-7}	2.39×10^{-6}

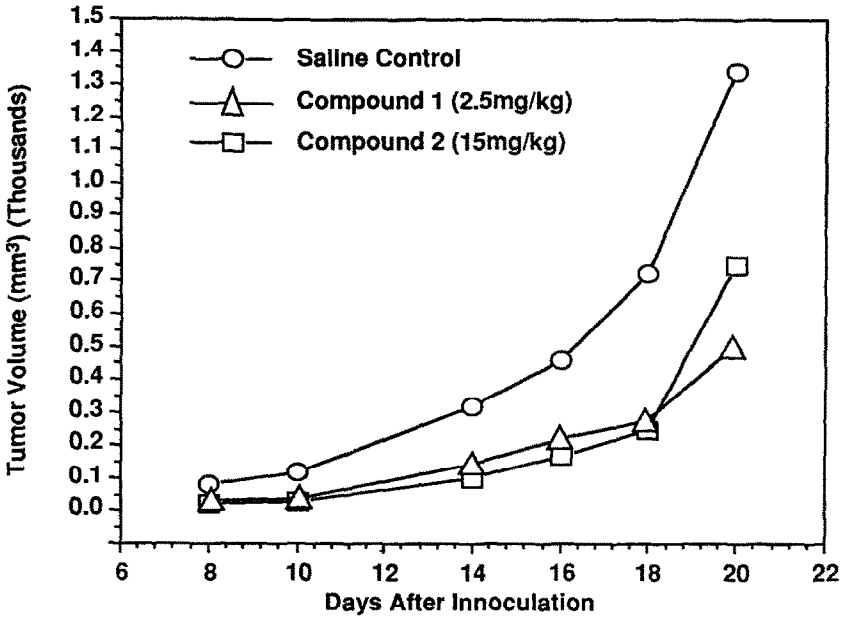


Figure 1. Antineoplastic Activity of Compound 1 and 2 on EMT6 Tumor Growth (Injections on Day 1, 3, 5 and 7)

Experimental:

Preparation of Compound 2: To a solution of 2-phenylsulfonylethanol (223 mg, 1.20 mmol) and bis(trichloromethyl)carbonate (triphosgene) (119 mg, 0.40 mmol) in dichlormethane (5.0 mL) cooled to 0 °C was added pyridine (97 µL, 1.20 mmol) dropwise over 10 min with stirring. Stirring was continued for 1 h, allowing the temperature to rise to 10 °C. Then 2.0 mL of this solution of 2-(phenylsulfonyl)ethoxycarbonylchloride was added dropwise over 2 h to a solution of daunomycin hrydrochloride (243 mg, 0.443 mmol) and pyridine (0.215 mL, 2.66 mmol) in dichloromethane (0.3 mL) with stirring at -20 °C. Stirring was continued for a further 10 min at room temperature, and the reaction mixture was diluted with chloroform (5 mL). The reaction mixture was extracted with 0.25 M aqueous HCl (25 mL). The organic layer was washed with 0.1 M aqueous HCl (50 mL), water (50 mL), and brine (25 mL). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by flash chromatography (silica, 25% acetone in chloroform) to give 242 mg (94%) of compound **2**: bright red gum; *Rf* = 0.19 (silica, 20% acetone in chloroform; IR (thin film) ν_{max} 3498, 2973, 2936, 1713, 1617, 1580, 1524, 1443, 1412, 1289, 1232, 1209, 1145, 1078, 1033, 986, 732 cm-1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.97 (s, 1 H, ArO*H*), 13.24 (s, 1 H, ArO*H*), 8.02 (d, *J* = 7.7 Hz, 1 H, anthraquinone), 7.89 (d, *J* = 7.5 Hz, 2 H, phenyl), 7.77 (t, *J* = 8.2 Hz, 1 H, anthraquinone), 7.65 (t, *J* = 7.5 Hz, 1 H, phenyl), 7.56 (t, *J* = 7.5 Hz, 2 H, phenyl), 7.38 (d, *J* = 8.2 Hz, 1 H, anthraquinone), 5.47 (d, *J*

= 3.6 Hz, 1 H, OC*H*O-anomeric), 5.24 (br s, 1 H, N*H*), 4.92 (d, *J* = 8.6 Hz, 1 H, ArC*H*O-sugar), 4.43 (s, 1 H, (C=O)CO*H*), 4.33 (m, 2 H, SO2CH2C*H*2OCO), 4.18 (br q, *J* = 6.4 Hz, 1 H, CH3C*H*O), 4.07 (s, 3 H, ArOCH3), 3.76 (m, 1 H, C*H*OH), 3.60 (br d, *J* = 5.4 Hz, 1 H, CHO*H*), 3.41 (t, *J* = 5.9 Hz, 2 H, SO2C*H*2CH2OCO), 3.20 (br d, *J* = 18.8 Hz, 1 H, ArC*H*2C(OH)(C=O)), 2.88 (d, *J* = 18.8 Hz, 1 H, ArC*H*2C(OH)(C=O), 2.41 (s, 3 H, C*H*3CO), 2.30 (br d, *J* = 14.9 Hz, 1 H, C*H*NH), 2.11 (dd, *J* = 14.9, 4.0 Hz, 1 H, C*H*2CHNH), 1.85 (d, *J* = 7.8 Hz, C*H*2), 1.76 (dd, *J* = 13.2, 5.0 Hz, 1 H, C*H*2), 1.64 (td, *J* = 13.0, 4.0 Hz, 1 H, C*H*2), 1.26 (d, *J* = 6.4 Hz, 3 H, C*H*3COCOH); ^{13}C NMR (125 MHz, CDCl3) δ 212.0, 187.0, 186.6, 161.0, 156.3, 155.8, 154.5, 139.4, 135.7, 135.4, 134.4, 133.9, 129.3, 128.1, 128.0, 120.8, 119.8, 118.4, 111.4, 111.2, 100.5, 70.0, 69.2, 67.0, 58.0, 56.6, 55.4, 47.0, 34.9, 33.4, 30.0, 29.7, 24.9, 16.7; HRMS (FAB+) for C36H37NO14SCs (M + Cs), calcd 872.0989, found 872.0936.

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References

1. Di Marco, A.; Gaetani, M.; Scarpinato, B *Cancer Chemother. Rep.* **1969**, *53*, 33.
2. Frederik, C. A.; Willimas, L. D.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Rich, A.; Wang, A. H.-J. *Biochem.* **1990**, *29*, 2538; Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci.* **1980**, *77*, 7204; Gabbay, E. J.; Grier, D.; Fingerle, R. E.; Reimer, R.; Levy, R.; Pearce, S. W., Wilson, W. D. *Biochem. Biophys. Res. Comm.* **1977**, *76*, 705.
3. Lown, J. W. *Mol. Cell Biochemistry* **1983**, *55*, 17.
4. Bodley, A.; Liu, L. F.; Israel, M.; Sedhadri, R.; Koseki, Y.; Giuliani, F. C.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 5969.
5. Nicolaou, K.C.; Dai, W.-M. *Angew Chem. Int. Ed. Engl.* **1991**, *30*, 1387 (review); Nicolaou, K.C.; Dai, W.-M.; Wendeborn, S. V.; Smith, A. L.; Torisawa, Y.; Maligres, P.; Hwang, C.-K. *Angew Chem. Int. Ed. Engl.* **1991**, *30*, 1032; Nicolaou, K.C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; *Science* **1992**, *256*, 1172; Nicolaou, K. C.; Maligres, P.; Suzuki, T.; Wendeborn, S. V.; Dai, W.-M.; Chadha, R.K. *J. Amer. Chem. Soc.* **1992**, *114*, in press; Nicolaou, K.C.; Dai, W.-M. *J. Am. Chem. Soc.* **1992**, *114*, in press.
6. Wrasidlo, W., The Scripps Research Institute, unpublished results.
7. Kunz, K. *Chem. Ber.* **1976**, *109*, 3693; Tesser, G. I.; Balver-Geers, I. C. *Int. J. Pept. Proteins Res.* **1975**, *7*, 295.