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DESIGN AND SYNTHESIS OF AN ANTITUMOR PRODRUG RELEASED BY THE REACTION WITH SULFHYDRYL COMPOUNDS

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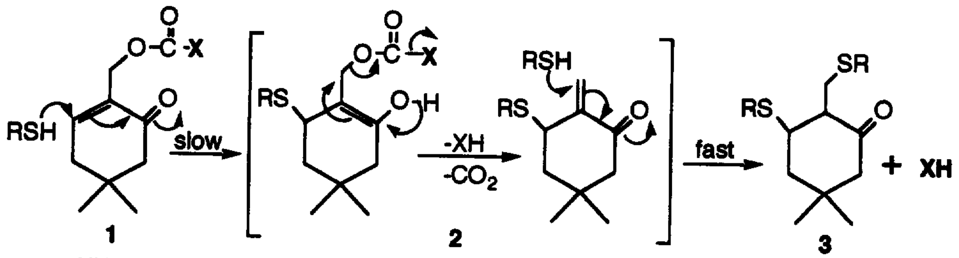
Abstract: The design and synthesis of carbamate-daunomycin prodrug **11** that can release free daunomycin by the reaction with sulfhydryl compounds is described. The compound **11** is more sensitive to adriamycin-resistant L1210 cell line than free daunomycin with relative resistance of 3.7 and 9.2, respectively.

The development of acquired resistance to antineoplastic drugs and the associated broad cross-resistance to other agents are the major obstacles to curative therapy of cancer. A variety of mechanisms for the development of resistance to antineoplastic agents have been explained by several factors including decreased drug accumulation, increased detoxification, and change of intracellular pharmacokinetics etc. Glutathione (GSH, nonprotein intracellular sulfhydryl compound)[1] and metallothionein (MT, protein intracellular sulfhydryl compound)[2] play important roles in the detoxification of a variety of antineoplastic agents such as alkylating drugs, quinone-containing drugs (such as adriamycin, daunomycin, mitomycin C), and sulfhydryl-reactive terpene lactones (such as vernolepin, jatrophane) mainly by nucleophilic thioether formation or oxidation-reduction reactions. An increase of GSH in tumor cell lines with the acquired resistance to melphalan, adriamycin, and cisplatin has been observed.[3] Furthermore, augmentation of their cytotoxicity in drug-resistant carcinoma cell lines by DL-buthionine-*R*,*S*-sulfoximine (BSO, a specific inhibitor of GSH synthesis) mediated GSH depletion has been reported.[4] It is also well known that sulfhydryl compounds react easily with crossed and non-crossed αβ-unsaturated ketones and esters in Michael type addition reaction. Based on the preceding experimental results, we speculated that the intracellular GSH should be able to cleave the carbon-acyloxy bond in **1** by double Michael addition reactions (Chart 1), ultimately resulting in the liberation of the free agent along with reduction of the concentration of GSH in drug-resistant cells.

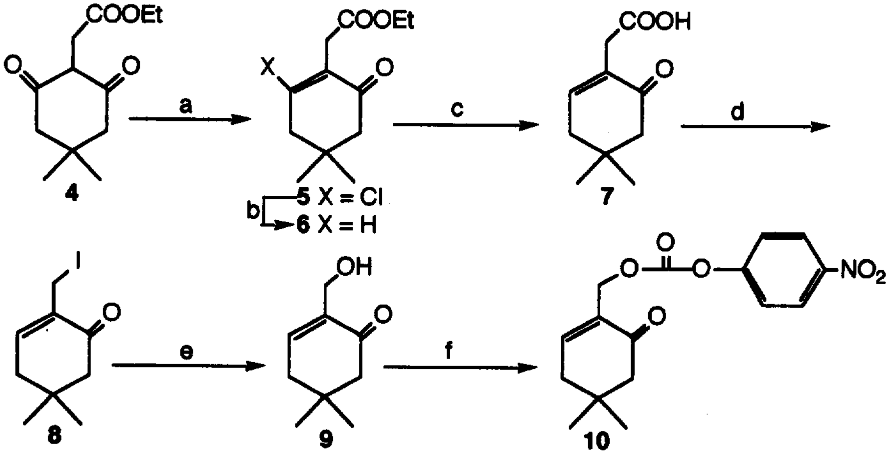


XH = antineoplastic agent, RSH = sulfhydryl compound such as glutathione

Chart 1. A plausible path for the release of free antineoplastic agent by the reaction with sulfhydryl compound

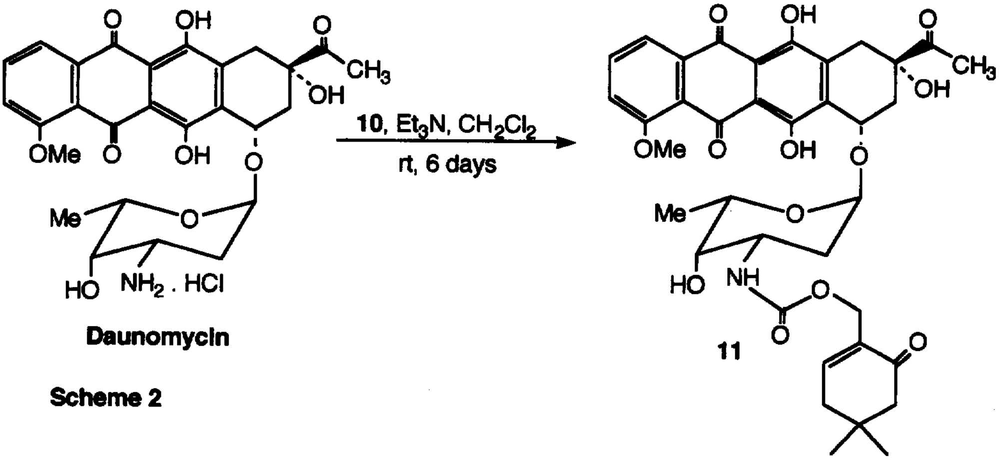
To testify the above mentioned conjecture, we synthesized the carbamate-type prodrug **11** of daunomycin, a potent anthracycline neoplastic agent, and evaluated its biological activity against adriamycin-resistant L-1210 cells.

Synthesis of hydroxy-enone 9: As shown in Scheme 1, the synthesis of key compound **9** began with diketo-ester **4** prepared from dimedone.[5] Reaction of **4** with oxalyl chloride followed by reduction with Zn-Ag in MeOH afforded enone-ester **6** in 54 % yield (2 steps). Hydrolysis of **6** with KOH in MeOH gave the acid **7**. Then, compound **7** was subjected to an oxidative decarboxylation reaction. Treatment of **7** with lead teraacetate-I_2 in CCl_4 under tungsten lamp irradiation gave iodo-enone **8** in 57 % yield. Reaction of **8** with $AgClO_4$ in acetone-water gave the target compound **9** in 86 % yield.



Scheme 1. Reagents and conditions: a) $(COCl)_2$, benzene, reflux, 3h; b) Zn-Ag, MeOH, rt, 16 h; c) KOH, MeOH, rt, 2 h; d) $Pb(OAc)_4$, I_2, CCl_4, W-lamp; e) $AgClO_4$, H_2O, acetone, rt, 30 min; f) p-nitrophenylchloroformate, Et_3N, benzene, rt, 16 h.

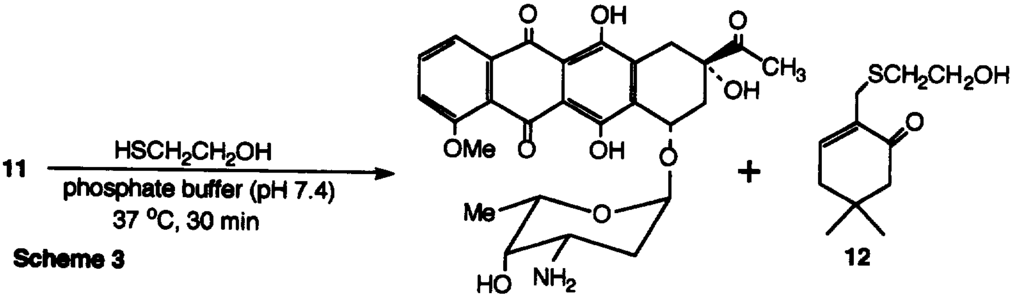
Synthesis of prodrug 11: Synthesis of carbamate-type prodrug **11** was accomplished as follows: the reaction of **9** with *p*-nitrophenylchloroformate [6] provided carbonate **10** in 62 % yield, which condensed upon treatment with daunomycin hydrochloride in CH_2Cl_2 to give the target compound **11** in 79 % yield (Scheme 2).



Scheme 2

Biological Activities:

Model studies on the release of free drug by sulfhydryl compound. Examination of the prodrug principle was achieved by treatment of compound **11** with mercaptoethanol as a sulfhydryl compound. As expected, **11** afforded free daunomycin and compound **12**[7] upon treatment with mercaptoethanol in phosphate buffer solution at 37 °C for 30 min (Scheme 3). In a control experiment in which mercaptoethanol was absent, **11** was stable to hydrolysis for over 1 h.



Scheme 3

Drug sensitivity against L-1210 parental and adriamycin resistant cell line. The compound **11** was less cytotoxic than the free drug against L-1210 cells, but resulted in cytotoxic activity equivalent to daunomycin against adriamycin-resistant L1210 cell line with relative resistance (IC_{50} of resistant cell line / IC_{50} of parental cell line) of 3.7 and 9.2, respectively.[8]

Table 1. Sensitivity against L-1210 and its adriamycin-resistant cell lines

	IC_{50} (nmol / ml)	
Agents	L-1210	L-1210 / ADR[a]
11	0.038	0.140 (3.7)[b]
Daunomycin	0.016	0.147 (9.2)
Adriamycin	0.022	0.315 (14.1)

[a] Adriamycin-resistant L-1210 cell line

[b] Relative resistance value: IC_{50} value of resistant cells / IC_{50} value of parental cells

In summary, we have designed and synthesized the antitumor prodrug released by the reaction with sulfhydryl compound. The prodrug principle outlined in this report seems to be efficient and applicable to other antineoplastic agents. Further studies on the syntheses of prodrugs of other agents and the results of biological testing will be reported in future publications.[9]

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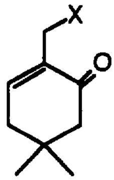
References and Notes:

1. Arrick, A. A.and Nathan, C. F. *Cancer Research* **1984**, *44*, 4224-4232.
2. Kelly, S. L.; Basu, A.; Teicher, B. A.; Hacker, M. P.; Hamer, D. H. and Lazo, J. S. *Science* **1988**, *241*, 1813-1815. Kasahara, K.; Fujiwara, Y.; Nishio, K.; Ohmori, T.; Sugimoto, Y.; Komoya, K.; Matsuda, T. and Saijo, N. *Cancer Research* **1991**, *51*, 3237-3242. Green, J. A.; Vistica, D. T. and Young R. *Cancer Research*, **1984**, *44*, 5427-5431.
3. Hamilton, T. C.; Winker, M. A.; Louie, K. G.; Batist, G.; Behrens, B. C.; Tsuruo, T.; Grotzinger, K. R.; Makoy, W. M.; Young, R. C and Ozols, R. F. *Biochem. Pharmacol.* **1985**, *34*, 2538-2586.
4. Lourie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A. and Ozols, R. F. *Cancer Research* **1985**, *45*, 2110-2115.
5. Rosenmund, K. W.; Herzberg, H. and Schutt, H. *Chemische Berichte*, **1954**, *87*, 1258-1267.
6. Letsinger, R. L. and Ogilvie, K. K. *J. Org. Chem.*, **1967**, *32*, 296-300.
7. It is most likely that compound **12** was a secondary product derived from the intermediate **3** during work-up.
8. Table 2 demonstrates that α-substitutedmethyl-enone compounds showed only moderate cytotoxicities against HeLa S_3 cells. It is noteworthy that the cytotoxicity of compound **9** (X = OH) was very weak.

Table 2. Cytotoxicity against HeLa S_3 Cells



X	IC_{50} μg / ml
I	2.3
OCOCH=CHMe	4.7
OAc	23.5
OH	>100
11	0.0096

9. In recent years, the design of antitumor-prodrug using antibody-directed enzyme prodrug therapy and antibody-directed catalysis has received considerable attention. Alexander, R. P.; Beeley, N. R. A.; O'Driscoll, M.; O'Neill, F. P.; Millican, T. A.; Pratt, A. J. and Willenbrock, F. W. *Tetrahedron Lett.* **1991**, *32*, 3269-3272. Hanessian, S. and Wang, J. *Can. J. Chem.* **1993**, *71*, 896-906. Jungheim, L. N.; Shepherd, T. A. and Kling, J. K. *Heterocycles*, **1993**, *35*, 339-348. Deonarain, M. P. and Epenetos, A. A. *Br. J. Cancer*, **1994**, *70*, 786-794. Jungheim, L. N. and Shepherd, T. A. *Chem. Rev.* **1994**, *94*, 1553-1566.

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