Communications to the Editor

SYNTHESIS OF NEW *N*-ALKYL DAUNORUBICIN DERIVATIVES *VIA* MICHAEL ADDITION TO SUBSTITUTED MALEIMIDES

Sir:

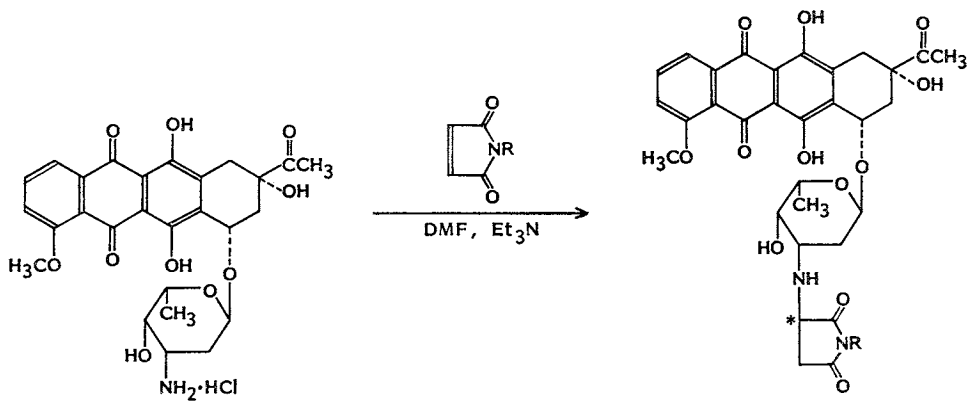
Daunorubicin and doxorubicin, members of anthracycline group of antibiotics, have well-established antitumor activitiy and clinical importance in the treatment of a range of human malignancies[1]. However, their use is associated with a number of undesirable effects, the most serious being a dose-dependent cumulative cardiotoxicity.

To improve the chemotherapeutic efficiency of the drugs, a broad program of analog synthesis has been undertaken. Among the daunorubicin modifications, changes at the amino group appeared to modify the biological properties to the greatest extent[2]. It has been concluded that the retention of activity of such derivatives requires the nitrogen atom to have a basic character. Therefore *N*-alkyl derivatives are of interest[3~5]. This paper describes the synthesis of new compounds belonging to this class and obtained *via* nucleophilic addition of daunorubicin amino group to the active double bond of *N*-substituted maleimides. The latter substances, with the most popular representative *N*-ethylmaleimide, are used in biochemical experiments for modification of sulfhydryl-containing bioactive molecules under mild reaction conditions[6,7]. They have also been shown to react with amino groups and form the corresponding addition products[6].

Synthesis of *N*-alkyl derivatives of daunorubicin is presented in Chart 1.

In a typical synthesis, 0.056 ml of triethylamine was added with stirring to a cooled solution of daunorubicin hydrochloride (225 mg, 0.4 mmol) in DMF (2.5 ml). Then, *N*-ethylmaleimide (300 mg, 2.4 mmol) was added in 3 portions within 5 hours and the reaction allowed to proceed at room temp for 24 hours. To the resulting solution H_2O (10 ml) was added and the product extracted with EtOAc (2×100 ml). The separated organic layer was washed twice with H_2O (10 ml),

Chart 1.



1 R = C_2H_5
2 R = C_6H_5
3 R = CH_2OCOCH_3
4 R = $(CH_2)_6N$
5 R = $(CH_2)_3N(CH_3)_2$

Table 1. Yields, physico-chemical and biological properties of daunorubicin derivatives **1**~**5**.

	1	**2**	**3**	**4**	**5**
Yield (%)	87.0	79.3	45.4	51.4	71.5
MP (°C)	149~151	155~156	144~145	132~134	147~150
Anal (Calcd)					
C	57.36 (57.51)	60.20 (60.28)	55.79 (55.70)	58.38 (58.60)	54.17 (54.00)
H	5.47 (5.41)	5.16 (5.06)	5.00 (5.09)	5.59 (5.52)	5.82 (5.92)
N	4.00 (4.07)	3.89 (3.80)	3.78 (3.82)	4.92 (5.00)	5.31 (5.25)
Formula	$C_{33}H_{37}N_2O_{12}Cl$	$C_{37}H_{37}N_2O_{12}Cl$	$C_{34}H_{37}N_2O_{14}Cl$	$C_{41}H_{46}N_3O_{14}Cl$	$C_{36}H_{45}N_3O_{12}Cl_2 \cdot H_2O$
(+) FAB-MS (*m/z*)	653 ($MH^+ - HCl$)	701 ($MH^+ - HCl$)	697 ($MH^+ - HCl$)	804 ($MH^+ - HCl$)	710 ($MH^+ - 2HCl$)
(−) FAB-MS (*m/z*)	652 ($M^{\cdot -} - HCl$)	700 ($M^{\cdot -} - HCl$)	696 ($M^{\cdot -} - HCl$)	803 ($M^{\cdot -} - HCl$)	709 ($M^{\cdot -} - 2HCl$)
TLC[a], Rf	0.42	0.39	0.33	0.46; 0.43[b]	0.00
					0.75; 0.69[b,c]
EC_{50}[d] (μg/ml)	0.30±0.03	3.84±1.33	4.22±1.83	0.27±0.07	16.47±7.68

[a] Silica gel; $CHCl_3$ - MeOH (15 : 1).

[b] Diastereoisomers.

[c] BuOH - pyridine - AcOH - H_2O (6 : 2 : 3 : 5).

[d] Activity against murine L1210 leukemia in tissue culture. Cells were maintained in RPMI-1640 medium supplemented with 5% fetal calf serum, penicillin (100 u/ml) and streptomycin (100 μg/ml) at 37°C in a controlled humid atmosphere of 5% CO_2 - 95% air. EC_{50} values were determined after 48 hours of incubation of cells with tested compounds[8]. For comparison, EC_{50} value obtained for daunorubicin hydrochloride was 0.02±0.002 μg/ml. (Mean±MSD).

Table 2. ^{1}H NMR chemical shifts of daunorubicin derivatives **1**~**5**.

Assignment	1	2	3	4	5
N-Ar	—	7.2~7.5 (5H)	—	—	—
CH=CH	—	—	—	6.96 (2H, s)	—
1-H[a]	—	—	5.32 (2H, s)	—	—
CH[b]	4.50 (1H, br s), 4.40 (1H, br s)	4.64 (1H, br s), 4.55 (1H, br s)	4.58 (1H, br s), 4.35 (1H, br s)	4.52 (1H, br s), 4.43 (1H, br s)	4.59 (1H, br s), 4.43 (1H, br s), 4.35 (1H, br s)
1-H, 6-H	—	—	—	3.31 (4H, m)	—
CH_2[b]	3.1~2.8 (2H)	3.3~3.0 (2H)	3.2~2.8 (2H)	3.1~2.8 (2H)	3.2~2.8 } (4H)
3-H	—	—	—	—	3.2~2.8 }
$N(CH_3)_2$	—	—	—	—	2.69 (6H, m)
CH_3COO	—	—	1.94 (3H, s)	—	—
2-H, 5-H	—	—	—	1.39 (4H, m)	—
3-H, 4-H	—	—	—	1.21 (4H, m)	—
2-H	1.01 (3H, t)	—	—	—	—

^{1}H NMR data were measured in DMSO-d_6 at 400 MHz. Chemical shifts are in ppm downfield of TMS. Other NMR signals recorded are in agreement with those obtained for the parent compound.

[a] 1~6 numbers of carbon atoms in the alkyl side chain R.

[b] Protons of the dioxopyrrolidine ring.

dried and evaporated *in vacuo*. The residue dissolved in $CHCl_3$ was subjected to column chromatography on silica gel and eluted with a mixture of $CHCl_3$ - MeOH (50 : 1) to give the free base of *N*-(*N'*-ethylsuccinimido)daunorubicin. The compound was dissolved in $CHCl_3$ (5 ml) and Et_2O (70 ml) saturated with HCl was added. The resultant precipitate was separated by centrifugation, washed with Et_2O and dried to give **1** (227 mg, 87%) (Table 1). The other derivatives were obtained in a similar manner. The exception was compound **5**, which was extracted from the reaction mixture with 1-butanol and purified by gel filtration on Sephadex LH-20 in MeOH.

Yields, physico-chemical and biological properties and ^{1}H NMR data for daunorubicin derivatives obtained are given in Tables 1 and 2, respectively.

Additional confirmation of the structures assumed was obtained from examination of the synthesized compounds using negative ion fast atom bombardment mass spectrometry (FAB-MS). In all the cases, including the parent antibiotic, the abundant ion at *m/z* 380 ($M^{\cdot}$ — aminosugar) was observed indicating the presence of unchanged aglycone and modified aminosugar moiety in the derivatives examined.

In the daunorubicin *N*-alkylation reaction a new asymmetric center is formed as a result of the addition of the amino group to the olefinic double bond of *N*-substituted maleimide. The analysis of appropriate signals (methine protons on the dioxopyrrolidine ring and anomeric protons) showed the presence of the corresponding diastereoisomers in a ratio of 1 : 1.

Although retain the basic character of a modified daunosamine amino group, the synthesized compounds generally exhibit decreased cytotoxic activity, compared to that of daunorubicin (Table 1). This activity is strongly dependent on the alkyl (R) substituent.

The reaction product of daunorubicin with maleimide bearing small alkyl group at nitrogen atom (compound **1**) exhibits quite good activity. Replacement of the ethyl group by a bulky substituent (compound **2**) or by moieties containing additional functional groups induces significant loss of activity, possibly due to a steric hindrance effect or ability to produce undesirable hydrogen bonds, respectively. An exception in this group is derivative **4**, which regardless of bulky and polyfunctional substituent exhibits cytotoxic activity comparable to that of compound **1**. This might suggest that in this case a different type of binding to biomolecules is involved based on nucleophilic attack on the reactive terminal maleimide moiety.

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