Synthesis and Antiproliferative Effects of a 4'-Morpholino-9-methyl Anthracycline

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The anthracyclines daunorubicin and doxorubicin are among the most valuable cytostatic agents in clinical use.[1] Their usefulness is somewhat limited by the occurrence of tumour cells with the multidrug resistance[2] which is associated with the presence of a plasma membrane glycoprotein (P-170 or P-glycoprotein (P-gp)) encoded by the *mdr* genes. In MDR cells, the level of expression of the P-glycoprotein is proportional to the level of drug resistance and there is a strong evidence that P-glycoprotein acts as an unidirectional drug efflux pump.[3]

A range of compounds have been identified as possessing modulating capacity of MDR and have been used to restore the sensitivity of resistant cells.[4] However, the use of modulators of P-gp cannot be critically assessed without taking into account the effect that these agents may have on normal physiological processes, since it is now clear that the *mdr* gene product (P-gp) is also expressed in a large range of normal tissues. Therefore, the use of compounds that alter multidrug resistance should probably be limited to certain key clinical areas. Meanwhile, it should be also remembered that circumvention of P-gp mediated drug resistance can clearly be achieve experimentally and perhaps clinically by a different means, *i.e.* the use of non-cross resistant cytotoxic agents.[5]

Indeed, among the modifications, an alkyl substitution at position 9 of the A-ring and/or the incorporation of the daunosamine amino group within a morpholinyl ring seemed to be the most notable.[6] In many instances, anthracyclines with retain activity in MDR cells are modified in more that one position simultaneously. For example, the compound MX-2[7] possesses both a 9-alkyl substitution (ethyl group) and morpholinyl ring whereas aclacinomycin[8] contains a 9-alkyl group and a trisaccharide moiety.

Following these observations, 9-alkyl anthracyclinones became our priority as leading targets in our general program aimed towards the chiral pool synthesis of aglycones using glucosaccharinolactone as a chiral template.[9,10] More recently, we published[11] an enantioselective synthesis of 9-alkyl anthracyclinones based upon highly diastereoselective alkylation of 4-cyanofuranosugars. Now, we report the cytotoxic activity of the first anthracycline in this series bearing a 9-methyl side-chain and a 4'-morpholino sugar derivative which was used instead of the more classical 3'-morpholino-analog (daunosamine-like).

4-Demethoxy-feudomycinone C **1** was obtained from α-D-glucosaccharino-1,4-lactone[10] or alternatively from 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose.[11]

The 3-*O*-benzoyl-4-trifluoroacetamido-2,4,6-trideoxy-L-*lyxo*-hexopyranose (**2**) easily obtained in few steps from L-rhamnal[12] was converted into the corresponding bromo derivative **4** *via* the 1-*O*-acetyl sugar **3**.

Glycosidation of **1** with a two-fold excess of 3-*O*-benzoyl-4-trifluoroacetamido-2,4,6-trideoxy-L-hexopyranose bromide (**4**) was carried out under Koenigs-Knorr conditions (yellow HgO, $HgBr_2$, molecular sieves 4 Å, dry CH_2Cl_2). This afforded (60% yield) the glycoside **5** as a mixture of unseparable α and β anomers (ratio ≈ 2:1). However, pure α-L-anomer **6** could be separated

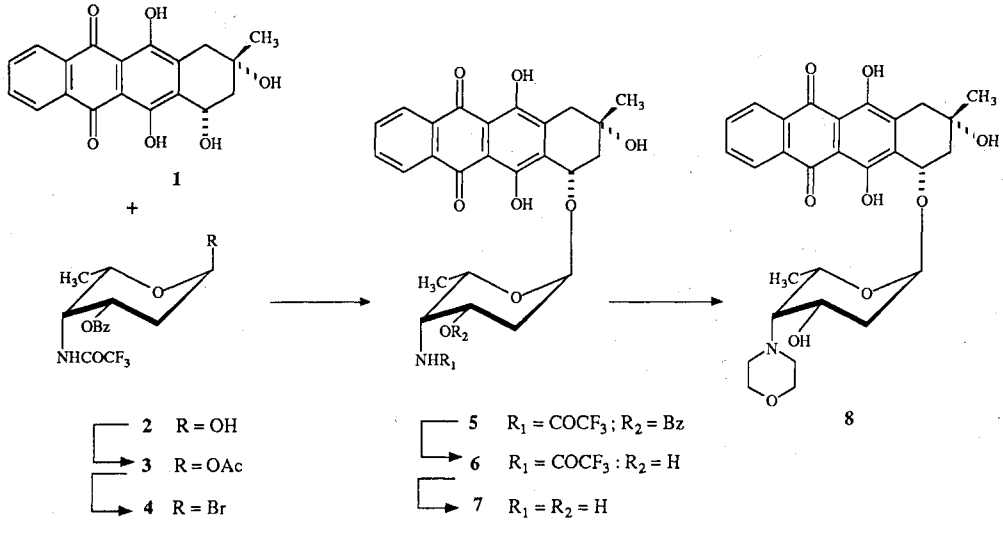

Table 1. Effects of anthracycline **8** on HL-60, MCF-7 and A 549 cells *in vitro* (IC_{50} μg/ml).

Cell lines	Compound **8**	Daunorubicin (DNR)	Doxorubicin (DXR)
HL_{60} (parental)	0.16	0.021	
HL_{60} (DNR-resistant)	2.5 (15)[a]	5.3 (252)[a]	
MCF-7 (parental)	5.4		0.09
MCF-7 (DXR-resistant)	5.4 (1)[a]		1.4 (16)[a]
A 549 (parental)	0.22		0.015

[a] Resistance factor values: IC_{50} for drug-resistant cell line/parental cell line.

(≈50% yield) after partial deprotection with aqueous 0.25 N NaOH in a mixture of MeOH-CH_2Cl_2 at 0°C. Removal of the trifluoroacetamido function (NaOH, 0.1 N in THF, r.t.) quantitatively led to the glycoside **7** which was subsequently converted (≈60%) in the morpholino derivative **8** according to the procedure of ACTON *et al.*[13].

The antiproliferative activity of the new anthracycline derivative **8** was tested against three tumor cell lines and compared to that of doxorubicin used as reference. Furthermore, the capacity of **8** to overcome multidrug resistance was evaluated by using two resistant tumor cell lines.

Comparison of the IC_{50} value between doxorubicin and **8** showed that the antiproliferative activity of **8** was approximately ten-fold lower than that of doxorubicin on both leukemic cells and A_{549} tumor cells and six-fold inferior to that of doxorubicin on MCF_7 cells (Table 1). However, when comparing the IC_{50} values obtained on the resistant cell lines, the difference of antiproliferative effect between **8** and the reference anthracyclines was reduced. Calculation of the resistance factor (RF) showed a low degree of resistance for **8** compared to doxorubicin.

Following this study, it appears that this new analogue was weakly active in inhibiting the proliferation of tumor cells *in vitro*. Nevertheless according to the remaining activity against resistant cell lines, this result confirms the favourable role of a morpholino substitution in the sugar moiety in the circumvention of multidrug resistance. On the other hand, since it has been very recently reported that i) the isomeric 4'-morpholino analog of doxorubicin is significantly less active *in vivo* that the 3'-morpholino doxorubicin itself[14] ii) the resistance factor clearly decreased with increasing 9-alkyl chain length[15] iii) the associated lipophilic character of anthracyclines plays an important role in the activity[16] syntheses of anthracyclines bearing an isobutyl or benzyl group at C-9 and a 3-morpholino sugar are under consideration.

Acknowledgments

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