

Effect of Structural Modifications of Anthracyclines on the Ability to Overcome Drug Resistance of Cancer Cells

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Abstract. In the search for new derivatives of anthracycline antibiotics with the ability to overcome the drug resistance barrier, a series of new analogs of these antibiotics, containing the amidino group at C-3' position of the daunosamine moiety, have been synthesized. The new compounds were tested for their cytotoxic activity *in vitro* against the sensitive LoVo, MES-SA and HL-60 human cancer cell lines as well as their resistant sublines: LoVo/Dx, MES-SA/Dx5 and HL-60/MX2, respectively. The majority of these derivatives appeared to be able, completely or partially, to overcome the drug resistance barrier of cancer cells. The effect of structural modification on this ability was determined. The obtained results indicated that introduction of the amidino group into the daunosamine moiety of anthracycline molecules appears to overcome the drug resistance of cancer cells.

Anthracycline antibiotics are effective agents widely used in cancer chemotherapy, however, the therapeutic activity of many drugs of this class is very low when the tumor cells express multidrug resistance (MDR). This term is classically used to define a phenotype where cells become simultaneously resistant to different drugs having no obvious structural resemblance and with different cellular targets. It affects patients with various blood cancers and solid tumors and, therefore, is a major factor in the failure of many chemotherapies.

MDR phenotypes have been identified in three separate forms characterized as: P glycoprotein (Pgp)-mediated

MDR, non-Pgp-mediated MDR [termed the multidrug resistance-associated protein (MRP)] and atypical MDR (at-MDR) due to an alteration in topoisomerase II (Topo II) activity.

Pgp and MRP, belonging to the ATP-binding cassette (ABC) superfamily of small molecules, are two transmembrane transporter molecules which act as pumps to remove toxic drugs from tumor cells. More recently, another protein, which may be involved in drug export from the nucleus, has been identified as the lung resistance-related protein (LRP).

It is now clear that MDR is always multifactorial, with at least two resistance mechanisms, simultaneously operating in the same tumor cell. These may include: decreased drug accumulation and/or altered intracellular distribution, increased detoxification, increased DNA repair, altered cell cycle regulation and uncoupling of the pathways linking cellular damage with programmed cell death (2-5).

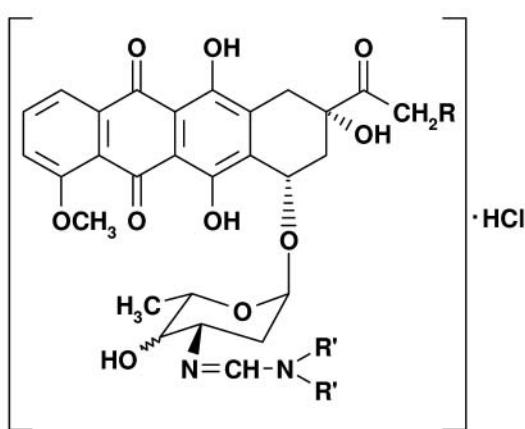
The resistance of cancer cells to multiple chemotherapeutic agents remains a major clinical challenge in cancer treatment. Therefore, much effort has been invested in the search for novel drugs to overcome drug resistance.

In the search for new derivatives of anthracycline antibiotics with better biological properties, a series of new analogs of daunorubicin, doxorubicin, epidaunorubicin and epidoxorubicin, containing the amidino group at the C-3' position of the daunosamine moiety, have been synthesized (compounds **1-16**, Figure 1). In our previous work (1) we showed that the majority of these amidinoanthracyclines, in comparison to the parent anthracycline antibiotics, exhibited lower toxicity, particularly cardiotoxicity and similar or even higher antiproliferative activity.

The aim of the present work was to evaluate the ability of the new anthracycline derivatives (**1-16**) to overcome the drug resistance barrier of cancer cells and to relate this to their structural characteristics.

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No.	R	OH*	NR'R'
1	H	a	Pyr
2	H	a	Mor
3	H	a	Pip
4	H	a	HX
5	OH	a	Pyr
6	OH	a	Mor
7	OH	a	Pip
8	OH	a	HX
9	H	e	Pyr
10	H	e	Mor
11	H	e	Pip
12	H	e	HX
13	OH	e	Pyr
14	OH	e	Mor
15	OH	e	Pip
16	OH	e	HX

*a = axial, e = equatorial

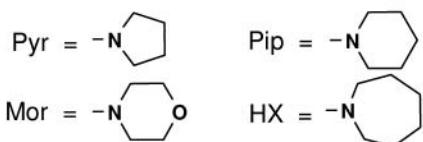


Figure 1. Structures of the derivatives of anthracycline antibiotics.

Materials and Methods

All the tested compounds, denoted as **1–16** (1), and their parent anthracyclines, *i.e.*, daunorubicin, doxorubicin, epidaunorubicin and epidoxorubicin, with purities $\geq 97.5\%$ according to HPLC method, were obtained from the Institute of Biotechnology and Antibiotics in Warsaw, Poland.

Antiproliferative assay in vitro. Cell lines. The cells of the following human cancer lines and their drug-resistant variants were used: LoVo – colon carcinoma and LoVo/Dx – variant resistant to doxorubicin with multidrug cross-resistance. The mechanism of drug resistance depends on the expression of the MDR1 gene product P-gp, MRP and LRP.

MES-SA – uterine sarcoma and MES-SA/Dx5 – variant resistant to doxorubicin with multidrug cross-resistance. The mechanism of drug resistance depends on the expression of P-gp.

HL-60 – promyelocytic leukemia and HL-60/MX2 – variant resistant to mitoxanthrone with multidrug cross-resistance. The mechanism of drug resistance does not depend on the expression of P-gp but seems to be related to the differential activity of Topo II.

All lines were obtained from the American Type Culture Collection (Rockville, Maryland, USA) and were cultured in the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. The colon carcinoma line (LoVo), the doxorubicin-resistant subline (LoVo/Dx), the uterine sarcoma (MES-SA) and the doxorubicin-resistant subline (MES-SA/Dx5) were cultured in RPMI 1640+Opti-MEM (1:1) medium supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 5% fetal bovine serum (all from Gibco, Scotland, UK). The LoVo/Dx and MES-SA/Dx5 cell cultures were supplemented with 15 nM doxorubicin (Institute of Biotechnology and Antibiotics, Warsaw). The promyelocytic leukemia HL-60 and resistant-to-mitoxantrone subline HL-60/MX2 were cultured in RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mM sodium pyruvate and 10% fetal bovine serum (all from Gibco). All the culture media were supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin (Polfa, Tarchomin S.A., Poland). All the cell lines were grown at 37°C with a 5% CO₂ humidified atmosphere.

The antiproliferative assay *in vitro* was carried out according to the method described previously (1). The ID₅₀ values (the dose of the tested compounds which inhibits proliferation of the cancer cells by 50% as compared to the control untreated cells) for the compounds (**1–16**) and for the parent anthracyclines were determined using the SRB (LoVo, MES-SA,) (6) or MTT (HL-60) method (7). For independent samples, the Student's *t*-test was applied to determine statistical significance.

Using the obtained ID₅₀ values, the resistance index (RI) values were calculated as the ratio of the ID₅₀ value for the resistant cell line to the IC₅₀ value for the sensitive one. The RI values of the derivatives (**1–16**) and for their parent anthracycline antibiotics are presented in Figure 2.

According to Harker *et al.* (8), three categories of cells could be distinguished:

- if the ratio approaches 1 (0-2), the cells are drug-sensitive;
- if the ratio ranges from 2 to 10, the cells are moderately drug-resistant;
- if the ratio is higher than 10, the cells are markedly drug-resistant.

Results and Discussion

The cells of all the resistant variant lines (Figure 2) were moderately (HL-60/MX2) or markedly (LoVo/Dx and MES-SA/Dx5) resistant to the parent anthracycline antibiotics, *i.e.*, daunorubicin, doxorubicin, epidaunorubicin and epidoxorubicin. The resistance index (RI) values for these antibiotics were in the range of 7.3–1389.0.

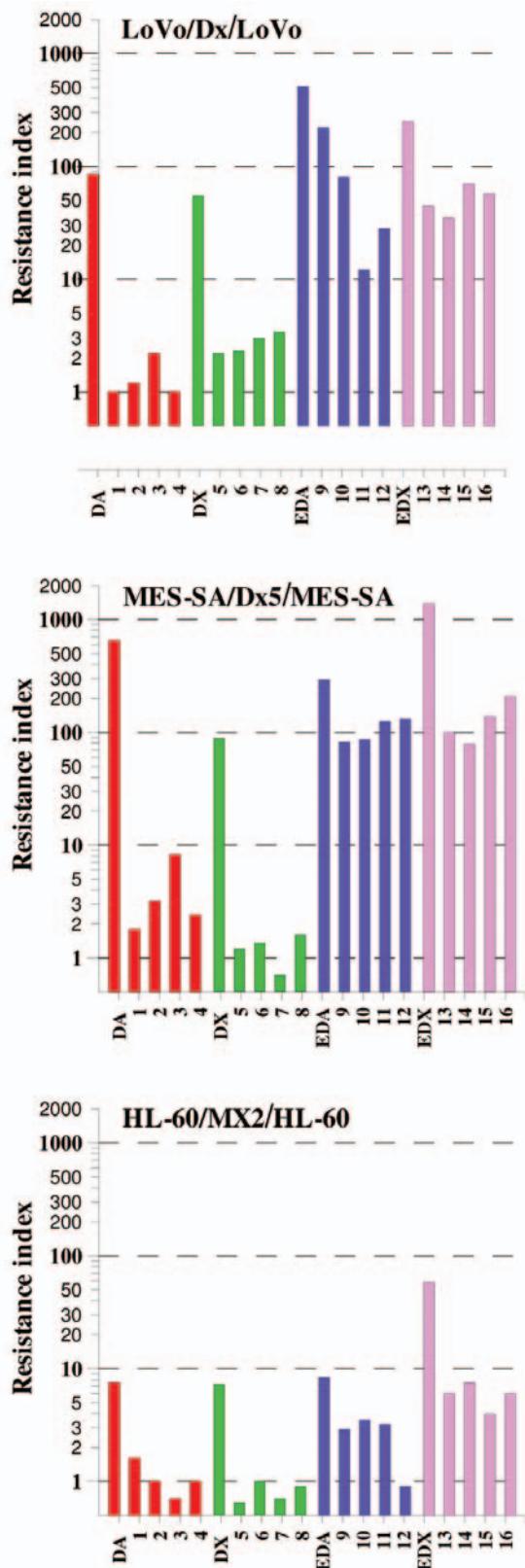


Figure 2. The resistance index (RI) values for derivatives (1–16) and for their parent antibiotics.

Among the new derivatives (1–16), the analogs of daunorubicin (1–4) appeared to be able to completely overcome the barrier of drug resistance in the LoVo/Dx and HL-60/MX2 cell lines, with RI values in the range of 0.7–2.0. The cells of the MES-SA/Dx5 line were moderately resistant to these derivatives (except for analog 1, with an RI = 1.8), with RI values ranging from 2.4 to 8.3 and, therefore, the analogs (2–4) were able to partially overcome the drug resistance barrier. It should be pointed out that the resistance of the MES-SA/Dx5 cell line was 78.0–270.0-fold lower compared to that of the parent daunorubicin.

The analogs of doxorubicin (5–8) were also able to completely overcome the barrier of drug resistance in the MES-SA/Dx5 and HL-60/MX2 cell lines with RI values ranging from 0.4 to 1.6. The cells of the LoVo/Dx line appeared to be moderately resistant (RI values in the range of 2.2–3.4), but this resistance was 16.3–25.0-fold weaker than that of the parent doxorubicin. It should be noted that all derivatives of doxorubicin reach RI values lower than 1.0, particularly in the case of the HL-60/MX2 cell line.

The derivatives of epidaunorubicin (9–12) and epidoxorubicin (13–16) were not able to completely overcome the drug resistance barrier (except for analog 12) in the LoVo/Dx and MES-SA/Dx5 cell lines. The RI values were in the range of 28.0–209.0, but this resistance was 3.4–139.0-fold lower than that of the parent antibiotics. Only the HL-60/MX2 cell line appeared to be moderately resistant to the compounds (9–11 and 13–16), with RI values in the range of 3.0–7.5. These analogs were, therefore, able to partially overcome the barrier of drug resistance. The only exception appeared to be the derivative of epidaunorubicin (12) with an RI of 0.9, which was able to completely overcome the drug resistance barrier.

On the basis of the obtained results, the influence of the orientation of the hydroxyl group at the C-4' position on the ability to overcome the drug resistance barrier is observed. All derivatives of daunorubicin and doxorubicin (1–8), in which the hydroxyl group at C-4' was in the axial orientation, proved to be able to overcome the barrier completely or partially in all tested cell line models with different mechanisms of resistance. On the contrary, the derivatives of epidaunorubicin and epidoxorubicin (9–16), where the hydroxyl group at C-4' was in the equatorial orientation, did not exhibit such an ability, with the exception of analog (12). Only in the case of the HL-60/MX2 cell line, analogs (9–11 and 13–16) were able to partially overcome drug resistance.

It seems that the mechanism of the antiproliferative activity of new amidinoanthracyclines against drug-resistant variant sublines of cancer cell depends neither (at least not fully and not only) on the MDR gene product

(P-gp) expression (LoVo/Dx and MES-SA/Dx5 cells), nor on Topo II activity (HL-60/MX2). The cytotoxic mechanisms of these derivatives are actually under investigation in our laboratories.

In a recent study (9), we found that the introduction of the amidino group into the daunosamine moiety of anthracycline antibiotics changed the mode of their cytotoxic action. The new derivatives, on the contrary to the parent anthracycline antibiotics, induced two types of DNA breaks by two separate processes. The first one is related to the poisoning of Topo II and the second presumably reflects the covalent binding of drug metabolites to DNA.

The obtained results indicated that the new anthracycline antibiotics, synthesized by transformation of the amino group at position C-3' into the amidino group, acquire the ability to partially or completely overcome drug resistance, unlike the parent drugs. Therefore, it can be concluded that chemical modification at the C-3' position is a good way to increased activity against resistant cancer *in vitro*.

The presented results provide an impetus to apply the new derivatives, mainly those of daunorubicin and doxorubicin (**1-8**), in the treatment of patients with cancer, which has acquired resistance to the parent anthracyclines.

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