Multitarget Drugs: No-Donor Doxorubicins

MULTITARGET DRUGS: NO-DONOR DOXORUBICINS

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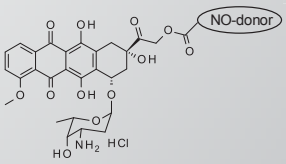
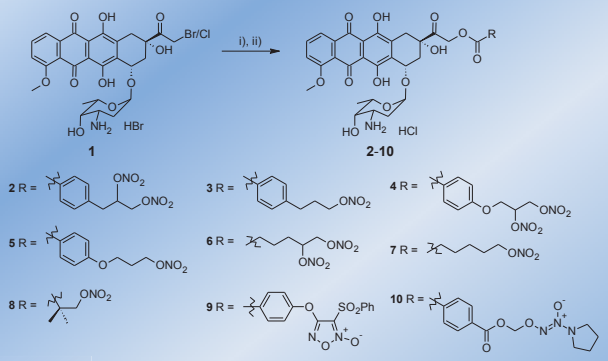


Figure 1

Introduction. Doxorubicin (DOXO) is an antibiotic belonging to the class of anthracyclines, used in treating a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas. However, there are some serious limitations on DOXO's efficacy in cancer therapy mainly due to Multidrug Resistance (MDR) following the overexpression of ATP-Binding Cassette (ABC) transporters, such as P-glycoprotein (Pgp), Multidrug Resistance Related Proteins (MRPs) and Breast-Cancer Resistance Related Protein (BCRP).[1] In previous research our group showed that exogenous nitric oxide (NO) reduced the activity of Pgp and MRPs, by nitrating tyrosine residues crucial for the protein functions, with consequent increase of intracellular DOXO concentration and toxicity in MDR tumor cells.[2] On this basis, new semisynthetic DOXOs were studied, in which the antibiotic is joined through an ester linkage to NO-donor moieties (***Figure 1***).[3] These compounds can accumulate in doxorubicin-resistant human colon cancer cells (HT29/dx), inducing cytotoxicity. In order to extend this new class of semisynthetic DOXOs, a small library of novel chimeras of DOXO were designed, bearing different NO-donor groups at the C-14 position (***Scheme 1***). A series of experiments were carried out in order to evaluate their cytotoxicity profile and to explore their different biological behaviour.

Synthesis. The designed NO-DOXOs were prepared from a mixture of 14-bromo/chloro daunorubicine hydrobromide (**1**) [4], by the reaction with carboxylic acid bearing NO-donor in the presence of KF as a weak base in DMF solution (***Scheme 1***).



Scheme 1. i) RCOOH, KF, DMF dry, rt; ii) HCl in dioxane dry, THF dry.

Intracellular accumulation. All the compounds except **10** were retained at least as much as DOXO in HT29 and HT29/dx cells; compounds **2-5**, **7**, **8** accumulated to a greater extent than DOXO in HT29/dx cells (***Figure 2***). This trend was in line with the different degree of nitration of MRP1 and BCRP present on the plasma-membrane (***Figure 3***).

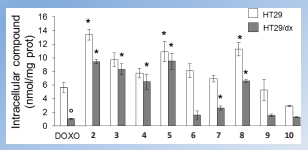


Figure 2. Intracellular accumulation of compounds in HT29 and HT29/dx cells

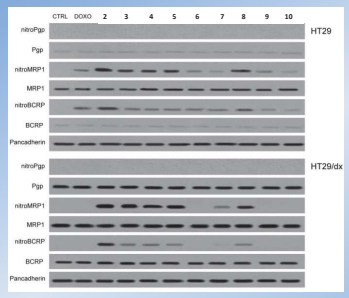


Figure 3. Nitration of plasma-membrane associated ABC transporters.

Cytotoxicity. DOXO is cytotoxic only in sensitive HT29 cells, Surprisingly, only compounds **2**, **8** and **9** were more cytotoxic than DOXO in both sensitive HT29 and resistant HT29/dx cells (***Figure 4***).

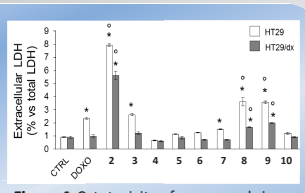


Figure 4. Cytotoxicity of compounds in HT29 and HT29/dx cells

Hydrolytic stability. One possible explanation for the lack of correlation between intracellular accumulation and cytotoxicity is the different susceptibility to esterase enzymes, which gives the different compounds different hydrolytic stabilities (***Table 1***).

Table 1. Hydrolytic stability of NO-DOXOs in human serum

Compound	$t_{½}$ (h) human serum	Compound	$t_{½}$ (h) human serum
2	16.0	**7**	4.9
3	24.6	**8**	21.7
4	34.7	**9**	>>24 h [a]
5	26.7	**10**	2.8
6	6.0		

[a] 73.5% conc. at 24h

These results could explain the lack of efficacy of compounds **6**, **7** and **10**, and the good efficacy of compounds **8** and **9**. However, the highly stable compounds **3-5** were characterized by the lowest cytotoxic efficacy in the biological assays.

Intramitochondrial accumulation. Compound **2, 8** and **9** had more marked intramitochondrial retention than DOXO in both HT29 and HT29/dx cells (***Figure 5***). These results are in line with mitochondrial ABC transporters nitration (***Figure 6***). The compounds **3-5** do not nitrate ABC pumps and do not accumulate in mitochondrion.

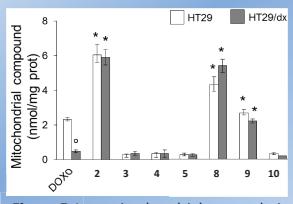


Figure 5. Intramitochondrial accumulation of compounds in HT29 and HT29/dx cells

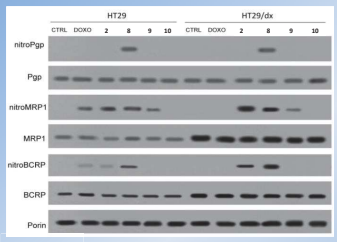


Figure 6. Nitration of mitochondrial ABC transporters in HT29 and HT29/dx cells

These results suggest that the cytotoxicity of the NO-releasing DOXOs is closely dependent on their ability to localize within the mitochondria. Indeed, compounds **2**, **8** and **9** increased the activation of caspase 9 and 3 while the compounds that accumulated little within the mitochondria did not activate the caspase 9/caspase 3 axis. These data may provide a rational explanation for the different cytotoxic efficacy of the NO-releasing DOXOs of the compounds studied here.

Conclusions. This study shows that the synthetic NO-releasing drugs with physico-chemical properties and/or conjugated with specific moieties favoring **intramitochondrial delivery** are very effective against DOXO-resistant cells. These features should be considered in the design of future **NO-releasing DOXOs** as effective **MDR-reversing tools**.

References.

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[2]. Riganti, C.; Miraglia, E.; Viarisio, D. et. al., *Cancer Res.* **2005**, *65(2)*, 516-525.
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[4]. Fernandez, A.-M.; Dubois, WO 2004/011033, **2004**.