# Interactions of Novel Morpholine and Hexamethylene Derivatives of Anthracycline Antibiotics with DNA

Mariola K. Piestrzeniewicz[a], Dorota Wilmańska[a], Janusz Szemraj[b], Kazimierz Studzian[a], and Marek Gniazdowski[a,*]

[a] Department of Medicinal Chemistry, Institute of Physiology and Biochemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland. Fax (+48-42) 678-42-77. E-mail: magn@csk.am.lodz.pl

[b] Department of Medical Biochemistry, Institute of Physiology and Biochemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 739–748 (2004); received March 16/May 17, 2004

Doxorubicin (DOX), daunorubicin (DRB), epidoxorubicin (EDOX) and their analogues with a 3′-$NH_2$ group in daunosamine form a covalent bond with a 2-$NH_2$ group of guanine via a methylene group from formaldehyde ($CH_2O$). It is assumed that a Schiff base type intermediate is formed between $CH_2O$ and the 3′-$NH_2$ group in the reaction. This reaction is supposed to occur in the cell. New analogues of anthracyclines with formamidine functionality bound to C-3′ of daunosamine and containing the bulky morpholine (DRBM, DOXM and EDOXM) or hexamethyleneimine rings attached are studied in our laboratory. These substituents decrease the association of the drugs to DNA and potentially hinder the formation of Schiff base-intermediates. Our experiments indicate that the formation of the covalent complexes by DRB, DOX and EDOX under these conditions is confirmed by a high enhancement (17–40x) of the inhibition of overall RNA synthesis by *E. coli* RNA polymerase on T7 DNA. DRBM and DOXM exhibit a lower enhancement of the inhibition by $CH_2O$ (7–13x). The other analogues show a 1.6–3x increase of inhibition. Hence, their covalent binding is lower than that of the parent compounds. These conclusions are confirmed by spectrophotometric estimations following removal of non-covalently associated drugs. Electrophoretic analysis of drug-DNA complexes formed in the presence of $CH_2O$ indicates that DRBM and DOXM as their parent compounds induce labile cross-links in DNA. Comparison of the results obtained at the subcellular level with cytotoxicity estimations indicates that there is a correlation between cytotoxicity of the anthracyclines on L1210 cells and transcriptional template activity of drug-DNA complexes formed in the presence of $CH_2O$ ($r = 0.64$; $n = 9$). These data confirm a notion that covalent attachment of anthracyclines to DNA is an essential event leading to cytotoxicity.

*Key words*: Anthracyclines, DNA-Interactions