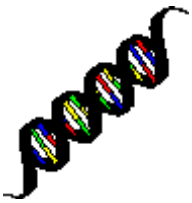
Anthracycline DNA Interactions

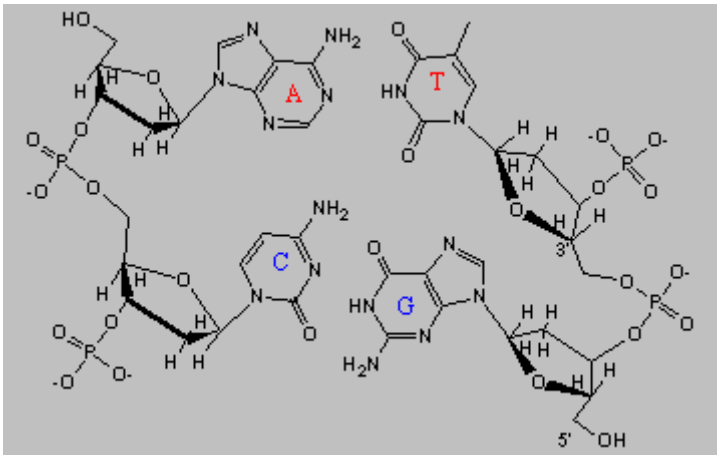
This is a scientific account of how anthracyclines interact with cancer cells (biology section), and a summary of anthracycline synthesis (chemistry section).

Anthracycline Biology

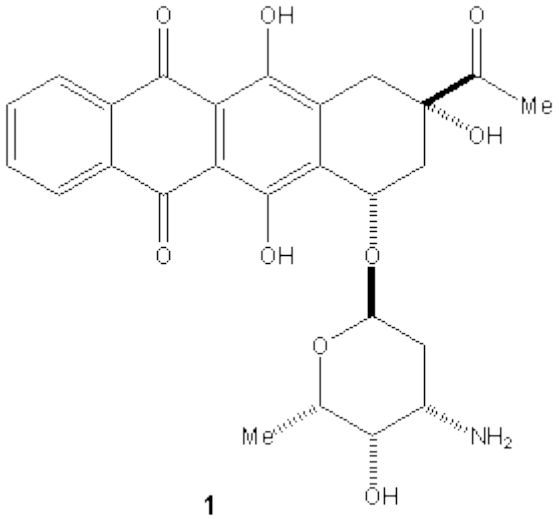
Consider a portion of the very long DNA double helix, which comprises the genetic material used in cell replication:



Here is a DNA *Watson-Crick* base-pair interaction, where the bases form the coloured bands in the structure above:



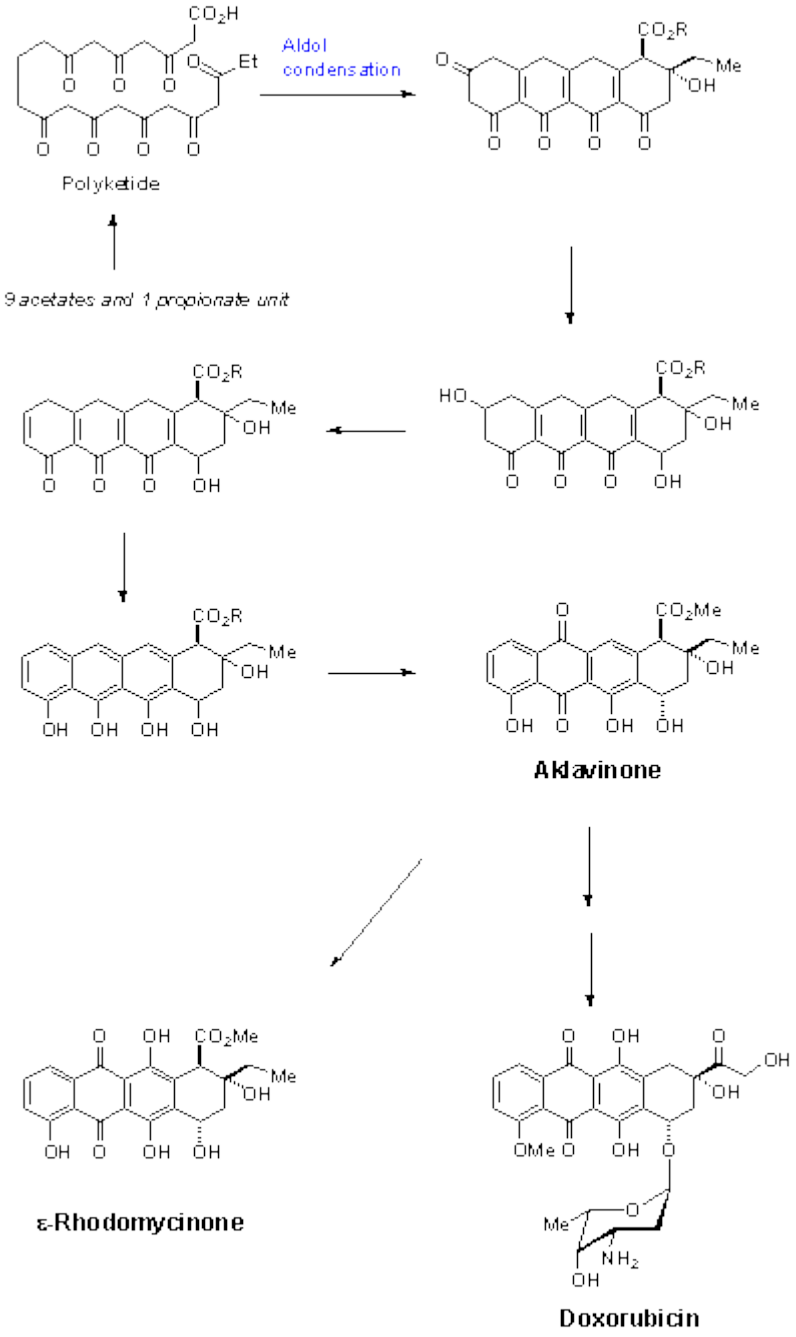
This diagram shows part of the DNA, whereby adenine (A) is hydrogen bonded to thiamine (T) and cytosine (C) is hydrogen bonded to guanine (G). The bond angles and lengths are approximate to show clarity of diagram. More about the structure of DNA is reviewed[1] below.



1

The structure of an anticancer chemotherapeutic agent, idarubicin (1), for the treatment of cancer, such as leukaemia is shown above. The ring-D is on the far left and the ring-A is on the far right of the structure. It is a member of the class of natural molecules called anthracyclines[2], which were originally discovered from bacterial sources (*Streptomyces*) several decades ago.

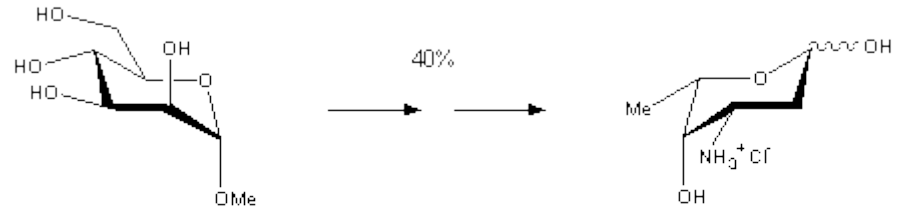
Biosynthetically, the anthracyclinones (and hence anthracyclines) are derived initially from acetate and propionate units to undergo an aldol condensation to afford a polyketide intermediate which is further transformed by the enzymes in *Steptomyces* to doxorubicin as shown in the scheme below. Advances in biotechnology have lead to enhancements in doxorubicin production[3] (greater than four-fold) by co-overexpression of certain recombinant genes.



Biosynthesis of anthracyclinones

Interestingly, *L*-Daunosamine (the sugar moiety in the parent anticancer anthracyclines) has been synthesised from a *D*-mannose sugar:

Synthesis of the Aminosugar, Daunosamine

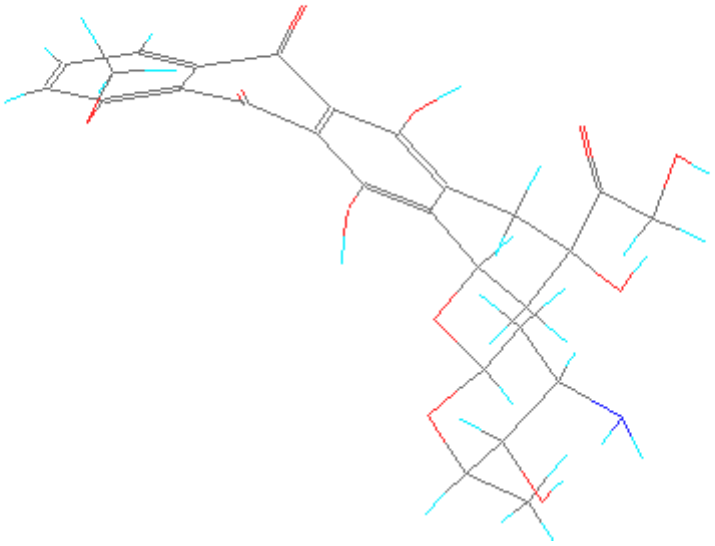


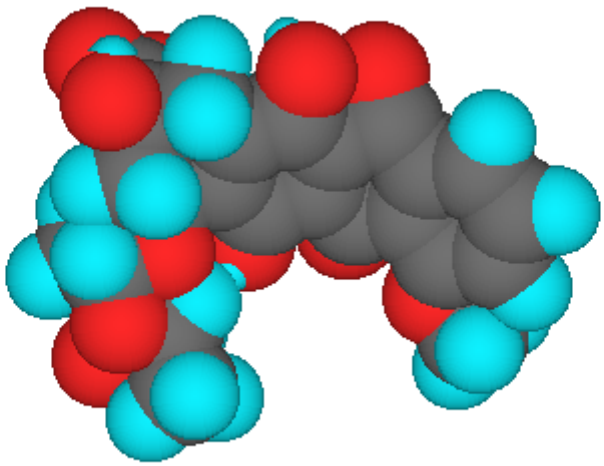
methyl-α-D-mannopyranoside

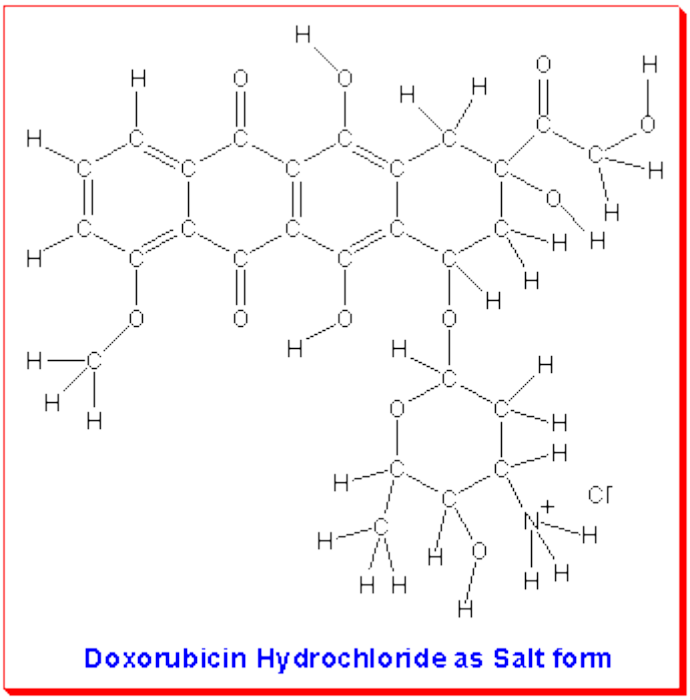
3-amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose-daunosamine hydrochloride

D. Horton and W. Weckerle, *Carbohydrate Research*, 1975, **44**, 227

Click here to download a zip file (5.80Kb). *Key:* Carbon atom=grey ball, hydrogen=light blue, nitrogen=dark blue and oxygen=red.



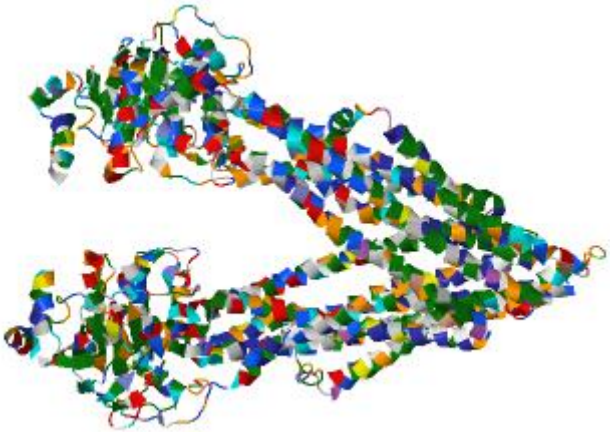




Doxorubicin Hydrochloride as Salt form

Daunorubicin and doxorubicin (adriamycin) are still in use. However, most chemotherapeutic drugs have unpleasant side effects and are often administered with other agents. For a general medical site, including links to cancer treatments, click here.

P-glycoprotein was purified in 1979, and strong evidence in support of its role in pleiotropic drug resistance[4] came in 1982. See image below for its crystal structure[5] (rotated ca. 90°). MDR-1 is the gene required for its synthesis and is a target in chemotherapy. MRP is another protein involved in drug resistance. Preliminary phase I/II data were encouraging, showing that Incel (biricodar dicitrate, VX-710) could restore or enhance the activity of the anticancer agent doxorubicin in small-cell lung cancer (STS) patients who had documented aggressive disease, and who had intrinsic or acquired resistance to doxorubicin. Doxorubicin is the standard chemotherapy for this disease, accounting for up to one quarter of newly diagnosed lung cancers. Approximately 70% of patients do not respond to initial chemotherapy, relapse is frequent, and the five-year survival rate for patients who are refractory to chemotherapy is a low 10-30%. Cisplatin with the drug etoposide are alternatively used in the treatment and 31% can survive one year.

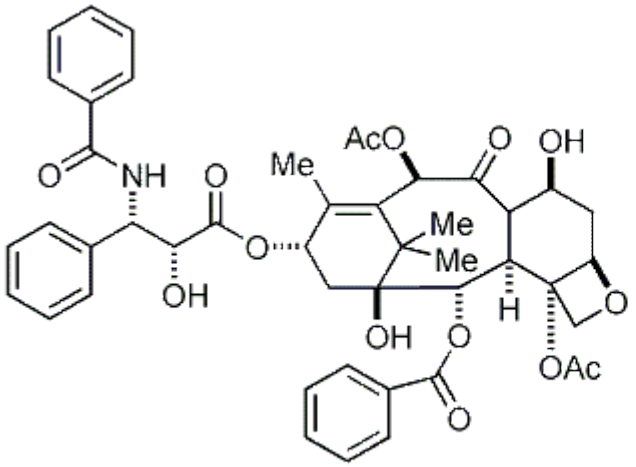


P-glycoprotein

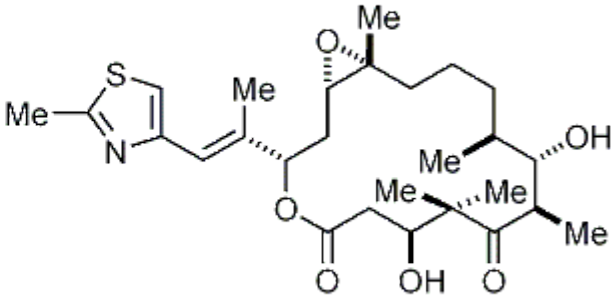
Mitosis is the process by which dividing cells control the separation of genetic material accurately into the two resulting daughter cells. Genes controlling mitosis in cancer and other proliferating cells are also the focus of cancer research. Proteins produced by gene targets interact with and regulate many aspects of the mitotic process, many interact with the mitotic spindle, which is the target of *paclitaxel*, the world's largest-selling cancer drug. These genes encode for many different classes of proteins such as enzymes, structural and scaffolding proteins, thereby increasing the likelihood of identifying new cancer targets amenable to small molecule drug development. The p53 gene found on chromosome 17, is a tumour-suppressor gene. In the cell, the p53 protein binds DNA at specific locations and stimulates another gene to produce a protein called p21. In turn, p21 suppresses a division-stimulating protein (cdk2) to prevent the cell from passing through to the next stage of cell division. When p53 is mutant and can no longer bind DNA effectively, the p21 protein is not available to act as the stop signal for cell division. Thus cells may divide uncontrollably and form tumours. The p53 gene plays a key role in the pathogenesis or etiology of human cancers and clearly is an important component in a network of events that culminate in tumour formation. Mutations in p53 are found in most tumour types.

An article[6] studied the use of anthracyclines (doxorubicin, epirubicin) with the more recently discovered taxanes (docetaxel [Taxotere], paclitaxel [Taxol]) which are in phase III clinical trials might be more effective in treatment of advanced metastatic breast cancer than used on their own. Further steps toward optimising the treatment schedules will include combining chemotherapy with the therapeutic principles of very different modes of action, such as target-specific antibodies against cell surface receptors of growth factors or inhibitors of tyrosine kinases in pathways of signal transduction. Genetic factors would be included in breast cancer studies. However, the epothilones[7] are a new class of cancer drugs. In a similar manner to the taxanes, they prevent cancer cells from dividing by

interfering with tubulin, but in early trials epithilones have better efficacy and milder adverse effects than taxanes. Epothilone B was first synthesised by Danishefsky[8] in 1997.

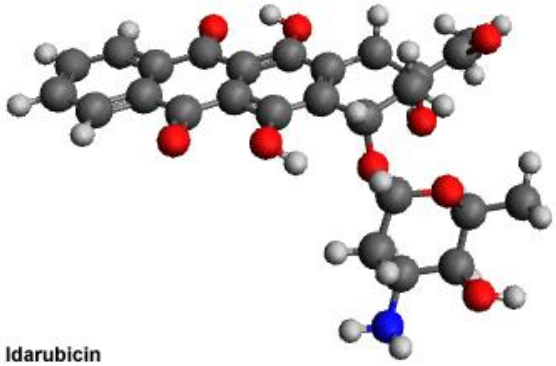


Taxol

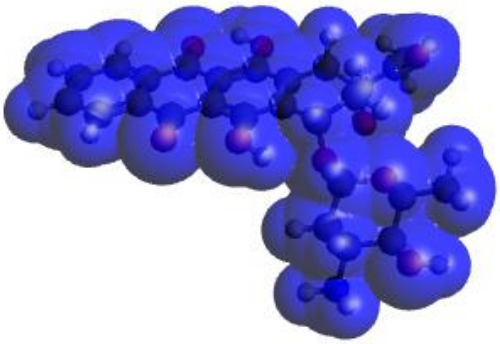


Epothilone B

Anthracyclines intercalate with nucleolar DNA (to form a sandwich, where the drug is the filling in the sandwich and the base pairs are the bread) to form a stabilised interaction, which is reversible. The DNA base pairs buckle in order to accept the molecule; the aromatic D-ring enters first (at the major groove) and the substituted A-ring is at the minor groove. The antracycline is at right angles to the direction of the DNA bases. The amino-sugar attached to the A-ring form further H-bonding with the DNA molecule including bonding via water molecules. There is considered to be Π-bonding above and below the aromatic rings in the complex.



Idarubicin



Idarubicin showing Van der Waals surfaces

Nogalamycin, with its ring-D bicyclic sugar substituent actually intercalates with nucleic acids, and the DNA bases buckle even more. This has been proved by X-ray analysis of drug-DNA hexamers[9].

Another mode of action[2] of anthracyclines is via free radicles which attack the DNA backbone. Oxygen is required to initialize this. Anthracyclines do

not appear to form covalent bonds with DNA, although in the presence of formaldehyde this can occur.

Anthracyclines attack fast growing heart tissue, mucal (e.g. loss of hair) and light intestine, resulting in their side effects. However, if administered in controlled doses, fatal heart cardiomyopathy is uncommon. For a mutagenic cancer, it is better to give the drug a chance, before it gets too late to treat it! Taxol[10], a taxane (e.g. tamoxifen) and *dynemycin* agents are becoming more popular too in the search of a cure for cancer. There are several families of different molecules that are used in hospitals or clinical trials, in addition to those mentioned above, and with different modes of action. These include (amongst others) the monoclonal antibodies, **Rituxan** (MabThera/rituximab): a chimeric monoclonal antibody that binds to the CD20 protein of malignant B cell membranes, **Avastin** (Bevacizumab): inhibits the function of a natural protein called vascular endothelial growth factor that stimulates new blood vessel formation or **Hercetpin** (trastuzumab): targets the HER2 receptor in HER2+ breast cancer patients who have HER2 protein overexpression. Tyrosine kinase inhibitors such as **Gleevec** (imatinib) have also gained global prominence and Gleevec has also been found to reverse doxorubicin resistance in melanoma cells[11] including inhibition of P-glycoprotein function amongst other changes. Crizotinib, inhibits an enzyme called anaplastic lymphoma kinase (ALK), another tyrosine kinase. Some non-small-cell lung cancer cells have an overactive version of ALK and crizotinib can be used for this. The structure[12] of G1269A mutant ALK in complex with crizotinib is shown (see image: crizotinib.png) but with the hydrogen atoms omitted.

Of current interest within the scientific community, gold nanoparticles[13] show promise for effective delivery systems for cancer-target therapeutic drugs. Cancer immunotherapy is an area of considerable current interest[14] with the aim of achieving higher specificity and therefore less toxicity. Genetically engineered anticancer (oncolytic) viruses[15] can trigger an immune response involving cancer-seeking T cells in reported cases. Traditional techniques such as radiotherapy or surgery are also used in some cancer treatment.

Finally, to put some of this into perspective, a popular review article[16] should be of interest discussing the biological complexities of cancer and tumour progression.

Anthracycline Chemistry

Numerous laboratory synthetic endeavours have been devised by chemists and the drugs are now commercially available. Stoodley's group[16] at the University of Manchester (formerly UMIST), England has developed a *stereoselective* route to (1); however this molecule did not contain the aminosugar on ring-A (there are methods to attach it[2]).

Interestingly and unique to Stoodley's group, a D-glucose based diene (a modified Danishefsky's diene) set up the stereochemistry in the molecule. Continuing with this methodology, myself[17], I have tried to synthesise modified anthracyclines, although the projects are incomplete and difficult, though providing interesting chemistry.

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Some of the scientific content in this article **has previously been published within my original research articles** referenced within: Miller, J.P. In *Recent Advances in Asymmetric Diels-Alder Reactions* in Advances in Chemistry Research; Taylor, J.C.; Ed.; vol. 18, Nova: New York, 2013, pp. 179-220. (ISBN: 978-1-62257-911-2); see also J. P. Miller, *ChemInform*, 2013, **44** (48) DOI: 10.1002/chin.201348243.

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