Chapter 4. The DNA intercalation story 220719aa3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

CHAPTER 4

The DNA Intercalation Story: Drug-DNA sandwiches.

In Chapters 1-3, we saw how alkylating agents and platinum complexes bind to DNA tightly and irreversibly (covalently). We come now to other DNA-binding anticancer drugs that bind tightly, but not covalently – which means that the DNA-binding of these drugs was spontaneously reversible, in contrast to the covalent binders of the previous three chapters, whose binding was irreversible. The non-covalent DNA-binding drugs of this chapter have a flat multi-ring system having size and shape resembling a DNA base-pair – which allows them to slip in between base-pairs of DNA in a sandwich-like configuration that is called "DNA intercalation" (Figures 4.1-4.3). The DNA helix unwinds slightly to open a space between adjacent base-pairs that is just sufficient in size to accommodate the intercalating ring system. The intercalation is stabilized in part by the electron distribution patterns of intercalator and the DNA base-pairs that are snuggly stacked against each other (Figure 4.3).

The intercalation story began in 1960. We had been discussing the notion of DNA intercalation in Paul Doty's laboratory, but it was first put on solid footing by Leonard Lerman in studies of the physical consequences of the DNA binding of the dye, proflavine (Figure 4.2). The DNA intercalation concept was to have unexpected applications in DNA studies, particularly to topoisomerase blocking agents (Chapter 10), and the mode of action of doxorubicin (Chapter 8).

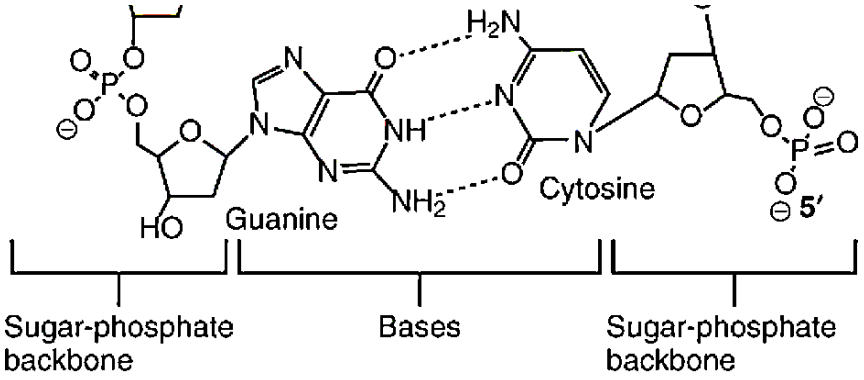

Figure 4.1. A guanine-cytosine base-pair and connections to the backbones of the 2 DNA strands. The dashed lines indicate the hydrogen bonds that allow the 2 bases (guanine-cytosine or adenine-thymine) to fit together. The base pairs are flat and stack one upon another in the DNA double helix. (*From Wikimedia Commons. File:0322 DNA Nucleotides*.)

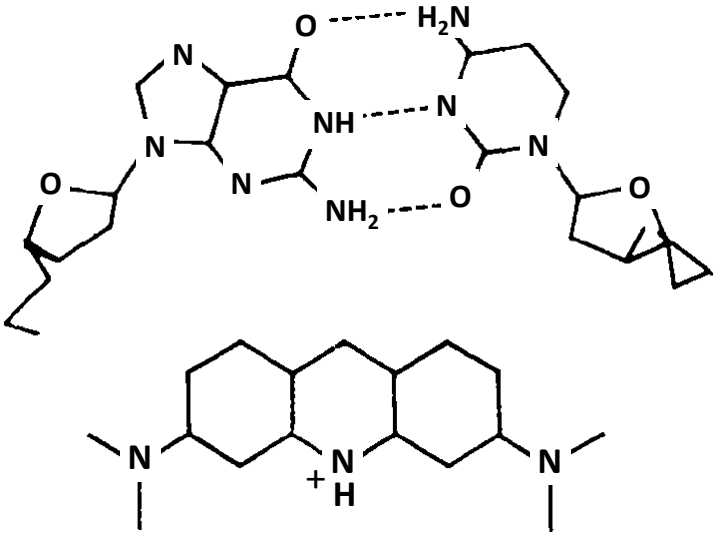

Figure 4.2. Outline structure of a base pair (above) and of the DNA intercalator, proflavine (below) (modified from (Lerman, 1961)). The double-bonds are not shown. The DNA base-pair and proflavine have similar size and shape, which allows proflavine to stack against the base-pair and to intercalate between base-pairs in a DNA double helix.

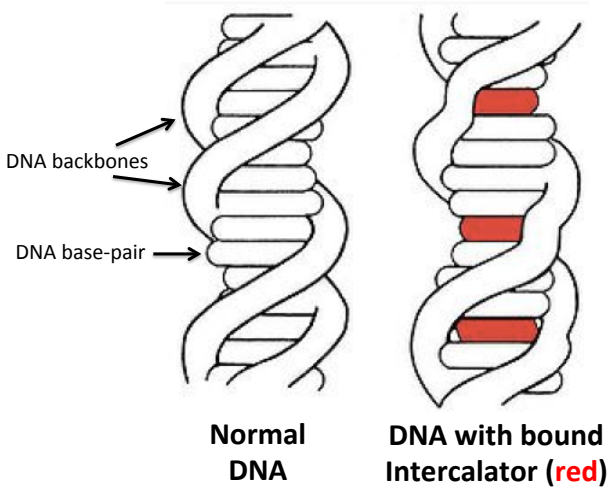

Figure 4.3. Simplified picture of a DNA intercalation structure as proposed by Lerman in 1961 (Lerman, 1963). The drug, with its flat multi-ring structure (red) is sandwiched between base-pairs of a slightly unwound DNA double helix. The DNA helix is unwound just enough for the intercalator to slip in between base-pairs

Another simple intercalator, ellipticine, derived from the bark of the Australian tree *Ochrosia elliptica*, was found to have substantial anticancer activity in mice in NCI screens (Figures 4.4 and 4.5). Although it has four rings, rather than the three in proflavine, ellipticine's size and shape closely approximates that of a base-pair. The drug is an effective DNA intercalator and was found to block topoisomerase II (Chapter 8) (Kohn et al., 1975; Ross et al., 1979; Ross et al., 1978).

On the down-side, however, ellipticine's ability to block topoisomerase II was considered mediocre compared with other more potent drugs. Moreover, it was difficult to use clinically because of low solubility, and problematic toxicities were encountered. The chemical structure of ellipticine was therefore modified in hope of producing novel effective drugs. That effort succeeded in producing more potent topoisomerase II blockers with ability to kill cancer cells in culture. However, toxicity still precluded their approval for general use in cancer chemotherapy (Auclair et al., 1987; Vann et al., 2016).

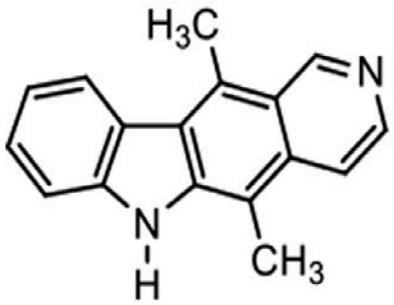

Figure 4.4. Chemical structure of ellipticine. The nitrogen in the 5-membered ring can pick up a proton (hydrogen ion) from water and become positively charged. The attraction of ellipticine's positive charge to the DNA's negative charge helps stabilize the binding.

Figure 4.5. Ellipticine is found in the bark of plants of the *Apocynaceae* family, such as *Ochrosia borbonica* (Tmejova et al., 2014). (Picture from Wikipedia.)

DNA intercalating drugs, such as doxorubicin, exert their anticancer action by blocking topoisomerase II (Chapter 8). But intercalating ability by itself does not guaranty action against topoisomerase. In point is the case of the anticancer drug, m-AMSA (amsacrine). An isomer of m-AMSA, called o-AMSA, intercalates equally well (Waring, 1976), but only m-AMSA blocked topoisomerase II and only *it* had anticancer activity. The structure of o-AMSA differs only in a small chemical group on a ring (not involved in the intercalation). The chemical group (H_3CO) is moved over by one carbon atom (Figure 4.6). Despite its ability to intercalate and its close structural similarity to its active isomer, o-AMSA was totally inactive. Evidently, the part of the drug that sticks out from the DNA intercalation structure must interact

with the topoisomerase protein. The configuration in o-AMSA presumably is incompatible with that essential interaction. Thus, we see that, although o-AMSA intercalates in DNA (Waring, 1976), it lacked the ability to block topoisomerase II, and had no antitumor activity (Zwelling et al., 1981). It seems that the repositioned group on the external ring prevented the interaction of the intercalated compound with the topoisomerase II enzyme, perhaps due to its effect on how the 6-membered external ring lies in the DNA minor groove (Jangir et al., 2013).

A few words about amsacrine (m-AMSA) as an anticancer drug. The DNA binding and anticancer activities of the drug were discovered in 1980 by Bruce Cain and his colleagues at the University of Auckland, New Zealand, as outcome of an intensive investigation of certain positively charged compounds that also had lipid-binding capability (Atwell and Cain, 1967; Baguley et al., 1981a, b). They carefully studied the relationship between chemical structure and ability to prolong the life of leukemic mice. This eventually led to m-AMSA as best of the set of compounds. Interestingly, m-AMSA was one of the first anti-cancer drugs to be designed by chemists, rather than biological organisms.

Clinical trials of amsacrine produced responses, especially in leukemias, but the responses relative to toxicity were not good enough to merit its general use (Jelic et al., 1997). Nevertheless, it was occasionally used in combination with other drugs.

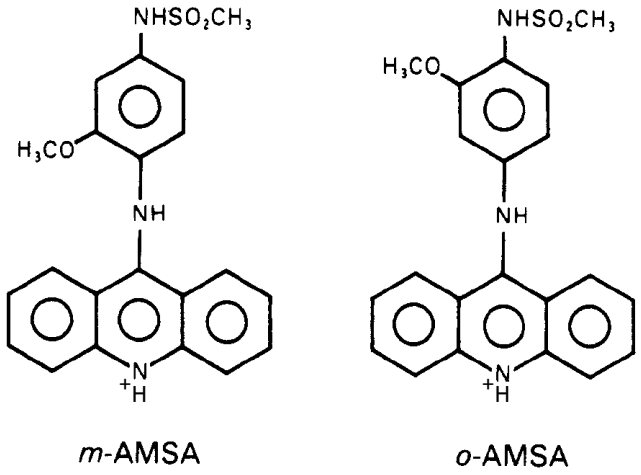

Figure 4.6. Chemical structure of amsacrine (m-AMSA) and its isomer o-AMSA, which differs only in the position of the H_3CO- group on the upper ring in the diagram. Both compounds intercalate in DNA (Waring, 1976), but only m-AMSA blocked topoisomerase II, and only this isomer was effective in killing cancer cells (Zwelling et al., 1981). The three-ring part of the molecule intercalates, while the external ring lies in the minor groove where it could interact with topoisomerase II.

An intercalator anticancer drug with a more complicated structure was actinomycin D, an effective inhibitor of RNA synthesis (transcription). Derived from *Streptomyces* soil bacteria (Figure 4.7), it was the first antibiotic shown to have anticancer activity; it is still used in combination with other anticancer drugs in the treatment of certain cancers (Cortes et al., 2016).

Actinomycin has a flat ring system that slips nicely between DNA base pairs. In addition, a part of the molecule fits compactly between the DNA backbone chain in the DNA minor groove (Figures 4.8 and 4.9). The intercalated ring system and the side chains in the minor groove combine to bind actinomycin tightly to DNA (Hollstein, 1974) (Sobell, 1973). Clinical use of actinomycin however was limited by excessive toxicity, perhaps due to its unusually strong inhibition of RNA synthesis.

Actinomycin's toxicity and strong RNA synthesis inhibition may well be related to its interaction with the amino (NH2) at position 2 of guanine, which it requires for strong DNA binding (Figure 4.10) (Sobell, 1973). This may account for its action differences from other DNA intercalating anticancer drugs.

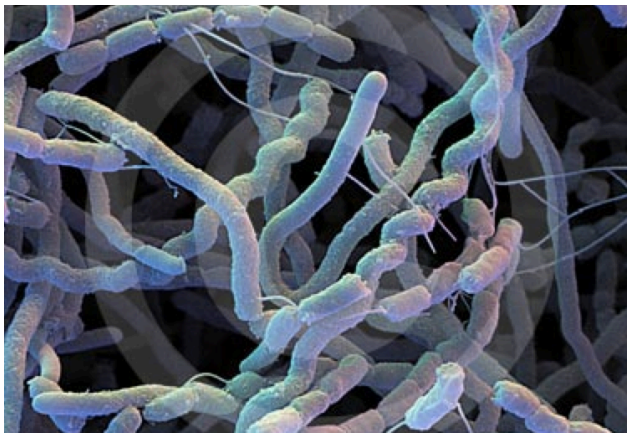

Figure 4.7. *Streptomyces* soil bacteria, the source of several important anticancer drugs, including actinomycin. (Scanning electron microscope image, from Wikipedia).

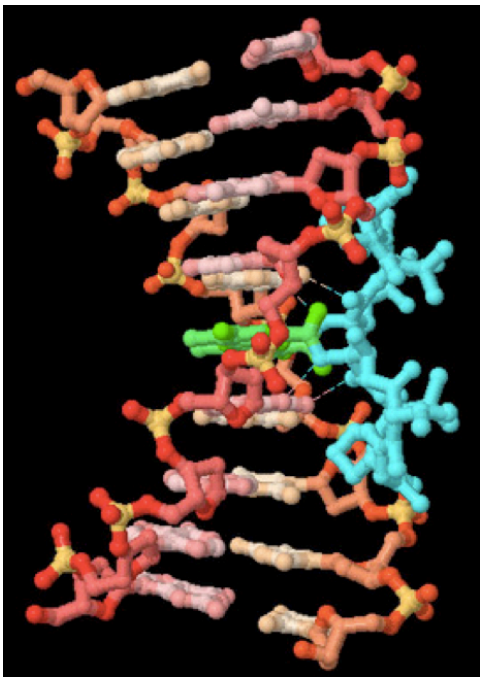

Figure 4.8. Model of actinomycin bound to DNA. The actinomycin ring system (green) is intercalated between DNA base-pairs, while the external part of the molecule (blue) fits nicely into the DNA minor groove. The intercalation and minor-groove binding cooperate to produce strong binding to DNA, even though actinomycin bears no positive charge (from educational portal PDB-101; http://pdb101.rcsb.org/motm/160).

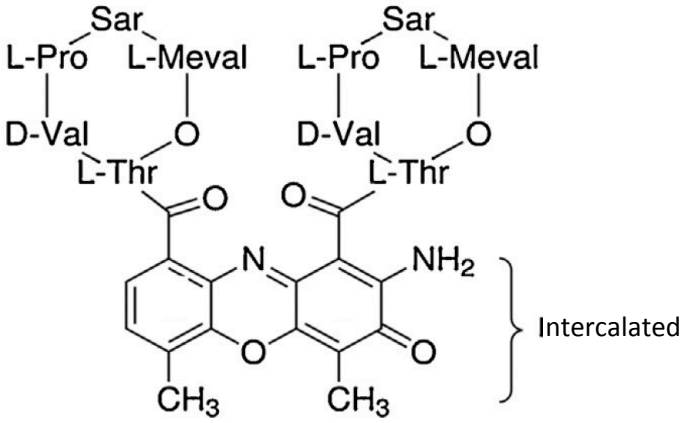

Figure 4.9. Chemical structure of actinomycin D. The 3-ring system at the bottom intercalates between base-pairs in DNA, and the two rings of amino acids at the top bind in the DNA minor groove (see Figure 4.8). Tight binding of actinomycin occurs preferentially to DNA regions that are rich in guanine-cytosine base-pairs (Lohani et

al., 2016), which might have something to do with why actinomycin is particularly effective in blocking RNA synthesis.

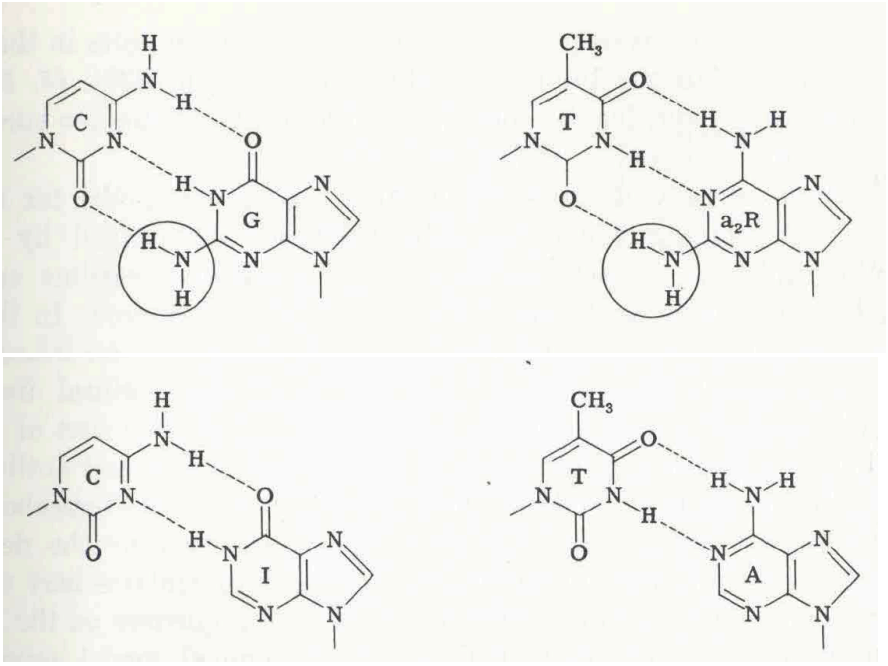

Figure 4.10. Strong binding of actinomycin to DNA required an amino group (NH_2) at position 2 of guanine (encircled in the structure at upper left). Replacement of guanine's oxygen by an NH_2, which allowed it to base-pair with thymine (T) instead of cytosine (C) retained strong actinomycin binding (upper right). But base pairs that lack the NH_2 at guanine position-2 did not bind actinomycin (lower two structures) (Sobell, 1973).

The discovery of doxorubicin.

The most important DNA intercalating drug, however, was doxorubicin. The story began with the discovery of daunomycin, a close chemical relative of doxorubicin, by Di Marco and his colleagues at the Instituto Nazionale dei Tumori in Milan, Italy (Di Marco et al., 1964). Members of the same research group demonstrated its binding to DNA, and noted that the drug increased the viscosity of DNA solutions (Calendi et al., 1965), but they did not surmise the cause: intercalation between base-pairs, lengthens the DNA helix and thereby increases its viscosity in solution. The full story is the subject of Chapter 8.

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