



DNA intercalators and using them as anticancer drugs

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ABSTRACT

Many anticancer drugs in clinical use interact with DNA through intercalation, which is process that starts with the transfer of the intercalating molecule from an aqueous environment to the hydrophobic space between two adjacent DNA base pairs. In general, intercalating agents are two types: monofunctional and bifunctional. Monofunctional intercalators contain one intercalating unit and Bifunctional intercalators (bis-intercalators) contain two intercalating units, normally cationic, separated by a spacer chain that must be long enough to allow double intercalation taking into account the neighbour exclusion principle.

Key words: DNA intercalation, anticancer drug, monofunctional intercalator, bifunctional intercalator, bis-intercalator

1) INTRODUCTION

Many anticancer drugs in clinical use (e.g. anthracyclines, mitoxantrone, dactinomycin) interact with DNA through intercalation, which can be defined as the process by which compounds containing planar aromatic or heteroaromatic ring systems are inserted between adjacent base pairs perpendicularly to the axis of the helix and without disturbing the overall stacking pattern due to Watson–Crick hydrogen bonding. Since many typical intercalating agents contain three or four fused rings that absorb light in the UV–visible region of the electromagnetic spectrum, they are usually known as chromophores. Besides the chromophore, other substituents in the intercalator molecule may highly influence the binding mechanism, the geometry of the ligand–DNA complex, and the sequence selectivity, if any.

2) DNA INTERCALATION AND ITS CONSEQUENCES

The intercalation process (Graves et al, 2000) starts with the transfer of the intercalating molecule from an aqueous environment to the hydrophobic space between two adjacent DNA base pairs. This process is thermodynamically favoured because of the positive entropy contribution associated to disruption of the organized shell of water molecules around the ligand (hydrophobic effect). In order to accommodate the ligand, DNA must undergo a conformational change involving an increase in the vertical separation between the base pairs to create a cavity for the incoming chromophore. The double helix is thereby partially unwound, (Berman et al, 1981) which leads to distortions of the sugar-phosphate backbone and changes in the twist angle between successive base pairs (Fig. 1). Once the drug has been sandwiched between the DNA base pairs, the stability of the complex is optimized by a number of non-covalent interactions, including van der Waals and π -stacking interactions, (Gago et al, 1998) reduction of coulombic repulsion between the DNA phosphate groups associated with the increased distance between the bases because of helix unwinding, ionic interactions between positively charged groups of the ligand and DNA phosphate groups, and hydrogen bonding. Generally speaking, cationic species are more efficient DNA intercalators because they interact better with the negatively charged DNA sugar-phosphate backbone in the initial stages and also because intercalation releases counterions associated to phosphate group, such as Na, leading to the so-called polyelectrolyte effect. This is a very important driving force for intercalation, since it diminishes repulsive interactions between the closely spaced charged counterions. In fact, most intercalating agents are either positively charged or contain basic groups that can be protonated under physiological conditions.

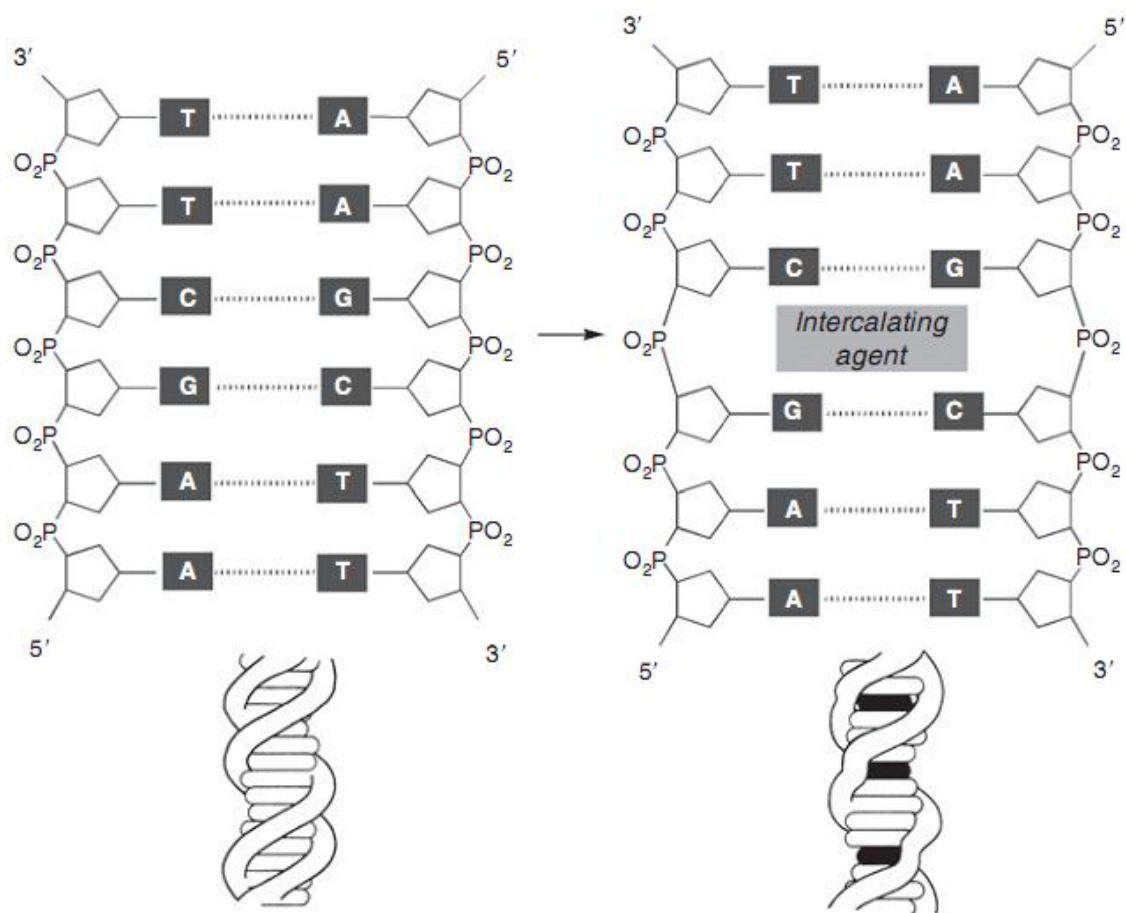


Figure 1. Deformation of DNA by an intercalating agent.

DNA intercalators are less sequence selective than minor groove binding agents, and, in contrast with them, show a preference for G-C regions. This selectivity is mainly due to complementary hydrophobic or electrostatic interactions, which are due to substituents attached to the chromophore within the major or minor grooves. DNA intercalation is also governed by the nearest-neighbour exclusion principle, which states that both neighbouring sites on each site of the intercalation remain empty, that is, they bind, at most, between alternate base pairs (Kapur et al, 1999).

This is an example of a negative cooperative effect, whereby binding to one site induces a conformational change that hampers binding to the adjacent base pair. Intercalation of a drug molecule into DNA is only the first step in a series of events that eventually lead to its biological effects (Brana et al, 2001). Structural changes induced in DNA by intercalation lead to interference with recognition and function of DNA-associated proteins such as polymerases, transcription factors, DNA repair systems, and, specially, topoisomerases.

3) MONOFUNCTIONAL INTERCALATING AGENTS

3.1. *Ellipticine and its analogues*

Ellipticine, an alkaloid isolated from the leaves of *Ochrosia elliptica* and other Apocynaceae plants, is the prototype of intercalators based on the pyridocarbazole system and displays a broad spectrum of antitumor activity (Garbett et al, 2004). Although at physiological pH values it can exist both as a neutral species and as a monocation (Fig. 2), it is the latter form that seems responsible for DNA intercalation, which leads to RNA polymerase inhibition. Other DNA-related enzymes that are inhibited by ellipticine include DNA polymerase, RNA methylase, and topoisomerase II, although it is not known whether these effects are a consequence of intercalation.

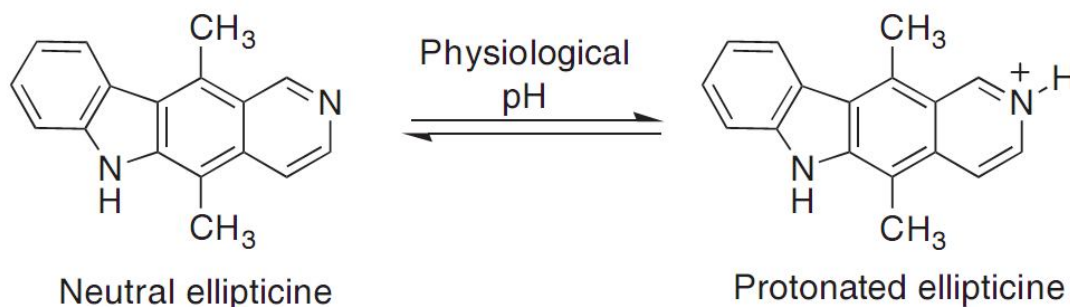


Figure 2. Neutral and protonated forms of ellipticine.

3.2. *Actinomycins*

Actinomycin D (dactinomycin) is a member of the actinomycin family of compounds, which was isolated from several *Streptomyces* strains. It contains a phenoxazine chromophore attached to two cyclic depsipeptides containing five amino acid residues. It can be considered as a hybrid compound that behaves both as a DNA intercalator and a minor groove binding agent. Although it differs from most intercalating drugs in that it lacks a positive charge, it has been suggested that this is compensated by its high dipole moment, arising from a non-symmetrical distribution of polar substituents (Gallego et al, 1997). Dactinomycin is used to treat sarcomas, pediatric solid tumors (e.g. Wilms' tumor, a type of renal tumor), germ cell cancers (testicular cancer), and choriocarcinoma.

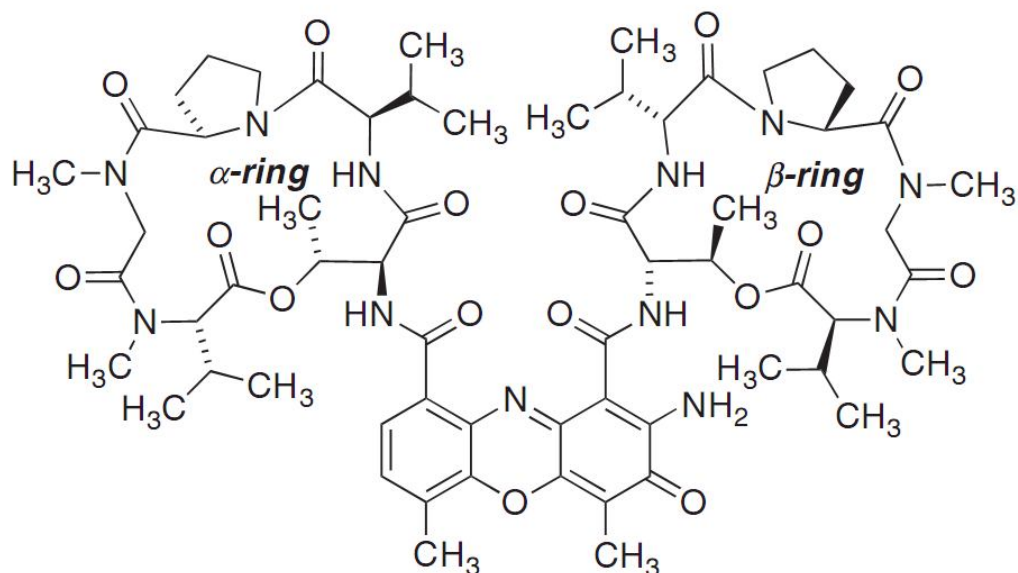


Figure 3. Actinomycin D (dactinomycin)

3.3. Fused quinoline compounds

TAS-103 is a dual topoisomerase I and II inhibitor that has marked efficacy against various lung metastatic cancers and a broad antitumor spectrum in human xenografts, and it has reached clinical trials for the treatment of solid tumors (Ewesuedo et al, 2001).

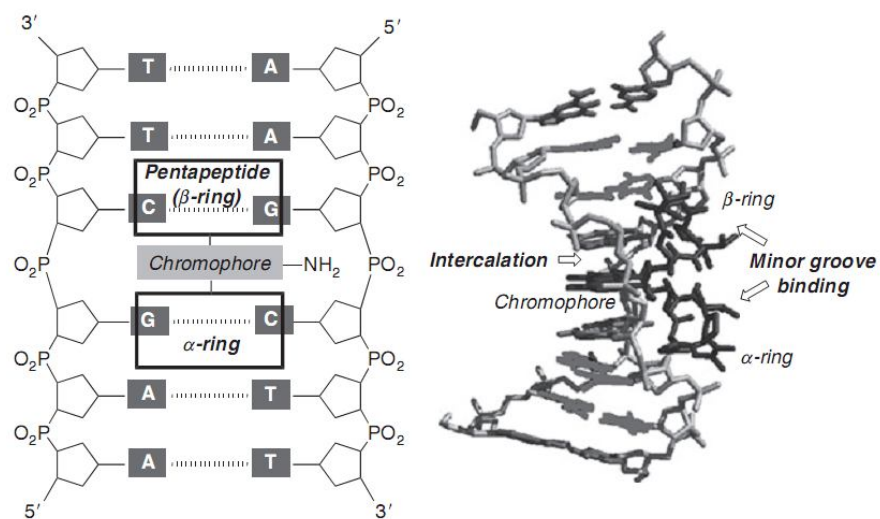


Figure 4. DNA intercalation by actinomycin D.

DNA binding and unwinding assays indicate that TAS-103 intercalates into DNA, although spectroscopic studies show that outside binding is also important (Ishida et al, 1999).

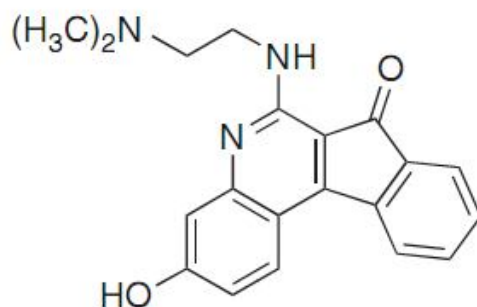


Figure 5. TAS-103

4.3. Naphthalimides and related compounds

Naphthalimide derivatives bearing an aminoalkyl side chain such as mitonafide and amonafide have shown interesting cytotoxic activity (Brana et al, 2001), which is due to intercalation and topoisomerase II inhibition (De Isabella et al, 1995). Both mitonafide (Rosell et al, 1992) and amonafide (Allen et al, 1994) have been extensively tested in clinical trials but have not been employed in therapeutics, although they have been used as leads in the design of bis-intercalators (Fig. 6).

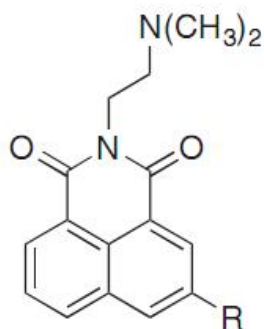


Figure 6. R = NO_2 Mitonafide, R = NH_2 Amonafide

5.3. Chartreusin, elsamicin A, and related compounds

Chartreusin and elsamicin A are structurally related antitumor antibiotics that were isolated from *Streptomyces chartreusis* and from an unidentified actinomycete strain, respectively. Chartreusin suffered from unfavourable pharmacokinetic properties (slow oral absorption and biliar excretion) which prevented its clinical development. Semi-synthetic chartreusin analogues with improved pharmacokinetics have been developed. One of them, IST-622, is under Phase II clinical trials for the oral treatment of breast cancer (Asai et al, 2002).

On the other hand, elsamicin A, one of the most potent known inhibitors of topoisomerase II, has entered Phase I clinical studies for relapsed or refractory non-Hodgkin's lymphoma. The activity was modest, but the compound was nevertheless considered promising because of the absence of myelosuppression (Portugal et al, 2003).

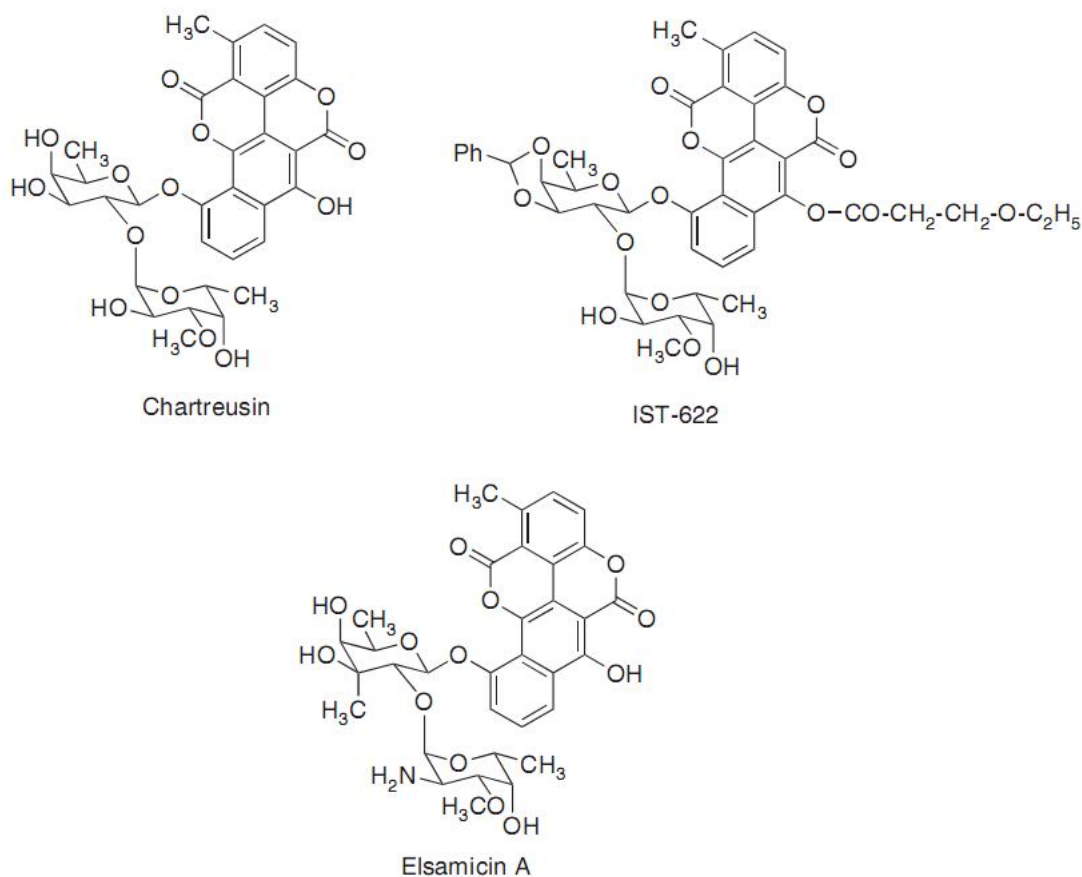


Figure 7. IST-622, Chartreusin and Elsamicin A

4. BIFUNCTIONAL INTERCALATING AGENTS

In efforts to increase the binding constant of intercalating compounds, bifunctional or even polyfunctional compounds have been designed. Bifunctional intercalators (bis-intercalators) contain two intercalating units, normally cationic, separated by a spacer chain that must be long enough to allow double intercalation taking into account the neighbour exclusion principle (Fig. 8).

Ditercalinium is an interesting bis-intercalator derived from ellipticinium with a novel mechanism of action different from that of its monomer, since topoisomerase II inhibition is not involved. Ditercalinium causes inhibition of enzymes that locate and repair damaged DNA sites, specially the nucleotide excision repair (NER) system (Lambert et al, 1988), due to the unstacking and bending that it induces on DNA because of the rigidity of the linker chain (Gao et al, 1991). Elnafide is a bis-intercalator derived from the naphthalimide pharmacophore (Brana et al, 1993) that exhibited excellent antitumor activity and reached Phase I clinical trials (Villalona et al, 2001), showing anti-neoplastic activity in ovarian cancer, breast cancer, and mesothelioma. Mechanistic studies on elinafide and its analogues are still in (Bailly et al, 2003) progress, but this drug suffers from neuromuscular dose-limiting toxicity that has halted its clinical development.

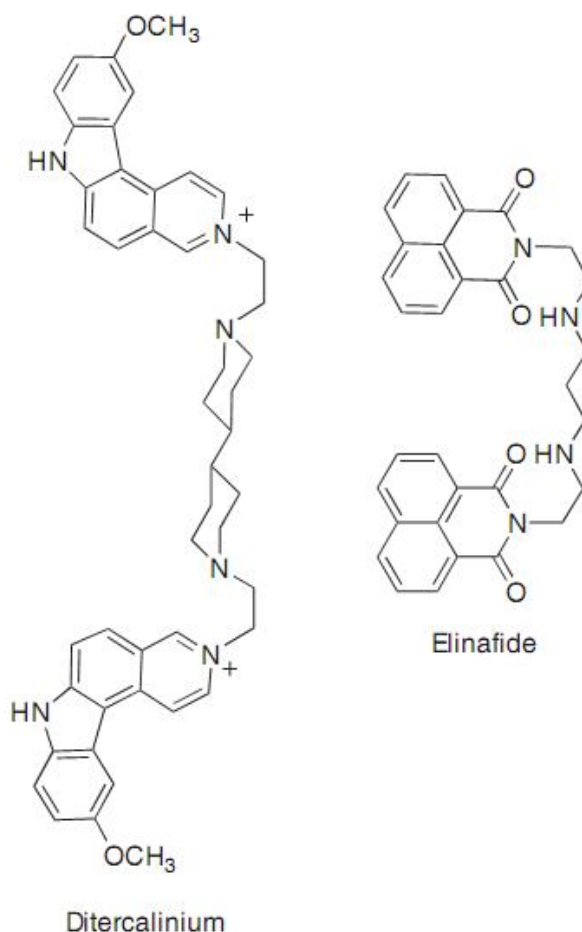


Figure 8. Ditercalinium and Elnafide

Echinomycin is an antitumor antibiotic isolated from *S. echinatus*, which consists of two quinoxaline chromophores attached to a cyclic octadepsipeptide ring, with a thioacetal cross-bridge. Because of its potent antitumor activity, this compound has been advanced to several Phase II clinical studies (Wadler et al, 1994), although it was eventually withdrawn from further clinical trials because it showed a high toxicity without any marked therapeutic benefit. More recently, echinomycin has been characterized as a very potent inhibitor of the binding of HIF-1 (hypoxia-inducible factor 1) to DNA. This is an interesting feature because HIF-1 is a transcription factor that controls genes involved in processes important for tumor progression and metastasis, including angiogenesis, migration, and invasion.

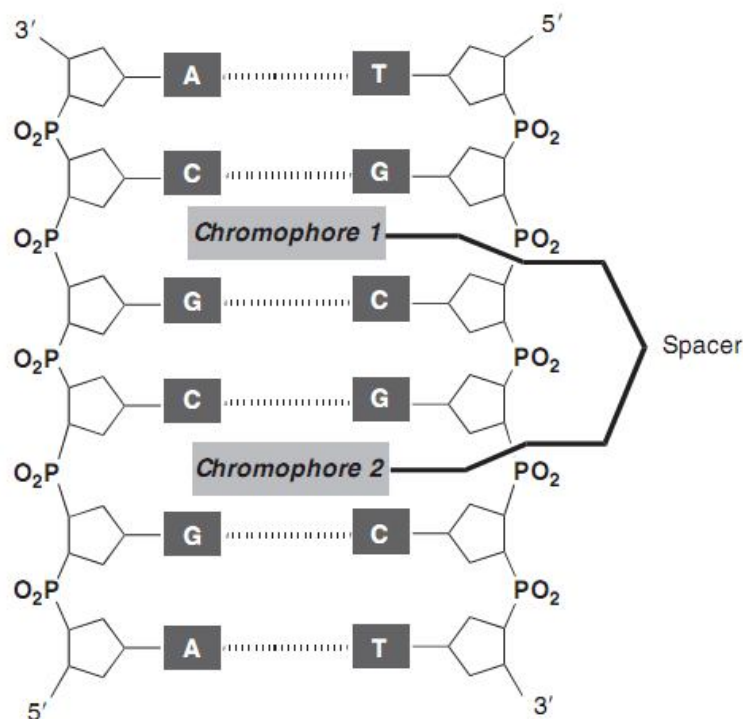


Figure 9. Schematic interaction between a bis-intercalator and DNA.

Several studies have proved that both echinomycin quinoxaline rings bisintercalate into DNA, with CG selectivity, while the inner part of the depsipeptide establishes hydrogen bonds with the DNA bases of the minor groove region of the two base pairs comprised between the chromophores (Fig. 9).

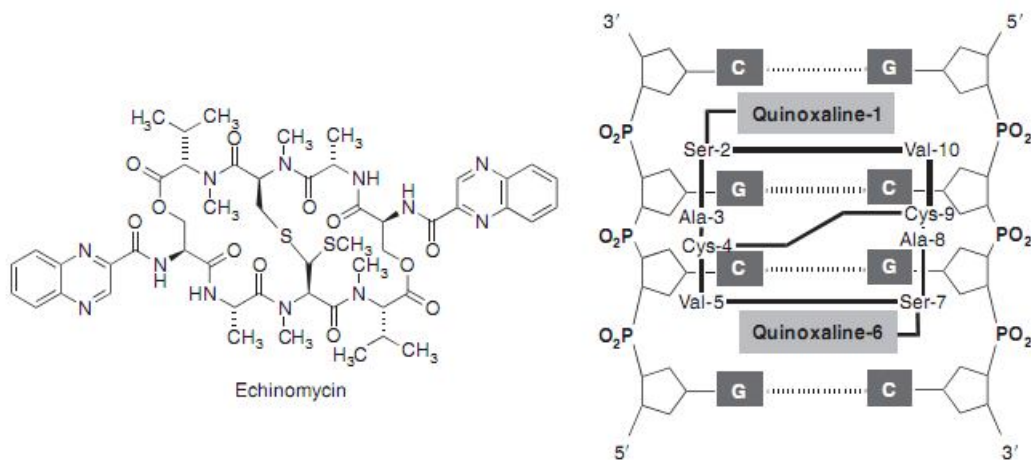


Figure 10. Schematic interaction between echinomycin and DNA.

A calorimetric study has proved that the binding reaction is entropically driven, showing that the complex is predominantly stabilized by hydrophobic interactions, although direct molecular recognition between echinomycin and DNA, mediated by hydrogen bonding and van der Waals contacts, also plays an important role in stabilizing the complex.

Conclusion

Many anticancer drugs in clinical use (e.g. anthracyclines, mitoxantrone, dactinomycin) interact with DNA through intercalation, which can be defined as the process by which compounds containing planar aromatic or heteroaromatic ring systems are inserted between adjacent base pairs perpendicularly to the axis of the helix and without disturbing the overall stacking pattern due to Watson–Crick hydrogen bonding. Ellipticine, Actinomycin D (dactinomycin), Fused quinoline compounds, Naphthalimide, Chartreusin, elsamicin A are the monofunctional intercalator which contain one intercalating unit and Ditercalinium, Elinafide and echinomycin are the bifunctional intercalator that contain two intercalating units.

References

- Graves, D. E.; and Velea, L. M. Intercalating binding of small molecules to nucleic acids. *Curr. Org. Chem.*2000; 4: 915-929.
- Berman; H. M., and Young, P. R. the interaction of intercalating drugs with nucleic acids. *Annu. Rev. Biophys. Bioeng.*1981; 10: 87-114.
- Gago, F. stacking interactions and intercalative DNA binding. *Methods.*1998; 14: 277-292.
- Kapur, A.; Beck, J. L.; and Sheil, M. M. Rapid Commun. Observation of daunomycin and nagolamycin complexes with duplex DNA using electrospray mass spectroscopy. *Mass Spectrom.*1999; 13: 2489-2497.
- Brana, M. F.; Cacho, M.; Gradillas, A.; de Pascual-Teresa, B. and Ramos, A. Intercalators and anticancer drugs. *Curr. Pharm. Des.*2001; 7: 1745-1780.
- Garbett, N. C.; and Graves, D. E. Entending nature's leads: the anticancer agent ellipticine. *Curr. Med. Chem. Anticancer Agents.*2004; 4: 149-172.
- Gallego, J.; Ortiz, A. R.; de Pascual-Teresa, B.; and Gago, F. J. computational methods on intercalators phenomena. *Comput. Aided Mol. Des.* 1997; 11: 114-122.
- Ewesuedo, R. B.; Iyer, L.; Das, S.; Koenig, A.; Mani, S.; Vogelzang, N. J.; Schilsky, R. L.; Brenckman, W.; and Ratain, M. J. J. anticancer drugs. *Clin. Oncol.* 2001; 19: 2084-2098.

Ishida, K.; and Asao, T. Observation of daunomycin and nagolamycin complexes with duplex DNA. Nucl. Acids Symp. Ser. 1999; 42: 129-143.

Brana, M. F.; and Ramos, A. naphtalimides as anticancer agents: synthesis and biological activity. Curr. Med. Chem. Anticancer Agents. 2001; 1: 237-255.

De Isabella, P.; Zunino, F. and Capranico, G. sequence selectivity of topoisomerase DNA cleavage. Nucleic Acids Res. 1995; 23: 223-254.

Rosell, R.; Carles, J.; Abad, A.; Ribelles, N.; Barnadas, A.; Benavides, A.; and Martin M. Phase study of mitonafide in 120 hour continuous. Invest. New Drugs. 1992; 10: 171-175.

Allen, S. L.; Budman, D. R.; Fusco, D.; Kolitz, J.; Kreis, W.; Schulman, P.; Schuster, M.; DeMarco, L.; Marsh, J.; Haptas, K. and Fischkoff, S. Proc. Am. Assoc. Cancer Res. 1994; 35: 325-331.

Asai, G.; Yamamoto, N.; Toi, M.; Shin, E.; Nishiyama, K.; Sekine, T.; Nomura, Y.; Takashima, S.; Kimura, M., and Tominaga, T. Cancer Chemother. Pharmacol. 2002; 49: 468-478.

Portugal, J. Curr. Med. Chem. Elsamicine A and related anti-cancer antibiotics. Anticancer Agents. 2003; 3: 411-420.

Lambert, B.; Rocques, B. P.; and Le Pecq, J.B. DNA intercalation. Nucleic Acids Res. 1988; 16: 1063-1070.

Gao, Q.; Williams, L. D.; Egli, M.; Rabinovich, D.; Le Chen, S.; Qugley, G.; and Rich, A. DNA induced DNA repair X-ray structure of a DNA-ditercalinium complex. Proc. Natl. Acad. Sci. USA. 1991; 88: 2422-2431.

Brana, M. F.; Castellano, J. M.; Moran, M.; Perez de Vega, M. J.; Romerdahl, C. R.; Qian, X.-D.; Bousquet, P.; Emling, F.; Schlick, E.; and Keilhauer, G. bis naphtalimides: a new class of antitumor agents. Anticancer Drug Des. 1993; 8: 257-268.

Villalona-Calero, M. A.; Eder, J. P.; Toppmeyer, D. L.; Allen, L. F.; Fram, R.; Velagapudi, R.; Myers, M.; Amato, A.; Kagen-Hallet, K.; Razvillas, B., Kufe, D. W., Von Hoff, D. D. DNA-ditercalinium complex. J. Clin. Oncol. 2001; 19: 857-864.

Bailly, C.; Carrasco, C.; Joubert, A.; Bal, C.; Wattez, N.; Hildebrand, M. P.; Lansiaux, A.; Colson, P.; Houssier, C., Cacho, M., Ramos, A., and Brana, M. F. topoisomerase DNA cleavage. Biochemistry. 2003; 42: 4136-4141.

Wadler, S.; Tenteromano, L.; Cazenave, L.; Sparano, J. A.; Greenwald, E. S.; Rozenblit, A.; Kaleya, R., and Wiernik, P. H. naphtalimides as anticancer. Cancer Chemother. Pharmacol. 1994; 34: 266-272.