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Chapter

Anticancer Drugs' Deoxyribonucleic Acid (DNA) Interactions

Saad Hmoud Alotaibi and Awad Abdalla Momen

Abstract

The deoxyribonucleic acid (DNA) is the molecule of life that controls all the chemical changes that take place in cells. The interaction of drugs with DNA is among the most important aspects of biological studies in drug discovery and pharmaceutical development processes. Moreover, the knowledge of specific targets in rational design of chemotherapeutics is a fundamental factor, principally, for the design of molecules that can be used in the treatment of oncologic diseases. Observing the pre- and postsigns of drug-DNA interaction provides good evidence for the interaction mechanism to be elucidated. Also, this interaction could be used for the quantification of drugs and for the determination of new drugs targeting DNA. Approaches can provide new insight into rational drug design and would lead to further understanding of the interaction mechanism between anticancer drugs and DNA. The intention of this chapter is to provide several examples of anticancer drugs, DNA interaction, and the mechanisms of interaction in order to understand the influence of several interaction factors in the capacity and selectivity of the anticancer drugs to interact with DNA. In addition, different experimental and theoretical approaches to detect and to evaluate the anticancer drugs' interactions with DNA were also discussed.

Keywords: anticancer drugs, DNA, interactions, proteins, hydrogen bond

1. Introduction

DNA information is stored in the form of a code that constitutes four chemical bases namely: cytosine (C), adenine (A), thymine (T), and, lastly, guanine (G). The human DNA has approximately 3 billion bases, and not <99% of these bases are similar in all individuals. The sequence of these bases governs the available information for maintaining and building an organism, similar to the manner in which alphabetical letters are arranged to form sentences and words [1].

The chemical bases in a DNA pair up (C with G and A with T), in order to produce units known as base pairs. In addition, each base is attached to a phosphate molecule and a sugar molecule. Together, a phosphate, sugar, and base are referred to as a nucleotide. The nucleotides are organized in two long strands thereby forming a spiral known as a double helix. A double helix's structure

resembles a ladder, with the phosphate and sugar molecules forming the ladder's vertical sidepieces. On the other hand, the base pairs form the rungs of the ladder.

Many anticancer drugs in clinical use 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 [2, 3].

2. Structural features of DNA

DNA consists of two complementary anti-parallel sugar phosphate poly-deoxyribonucleotide strands that are associated with specific hydrogen bonding between nucleotide bases. The two strands are held together primarily through Watson-Crick hydrogen bonds where A forms two hydrogen bonds with T and C forms three hydrogen bonds with G (**Figure 1**). The structure of these paired strands defines the helical grooves, within which the edges of the heterocyclic bases are exposed. The biologically relevant B-form of the DNA double helix is characterized by a shallow-wide major groove and a deep-narrow minor groove. The chemical structure (feature) of the molecular surfaces in a given DNA sequence is well known in either groove. This forms the basis for molecular recognition of duplex DNA by small molecules and proteins [4, 5].

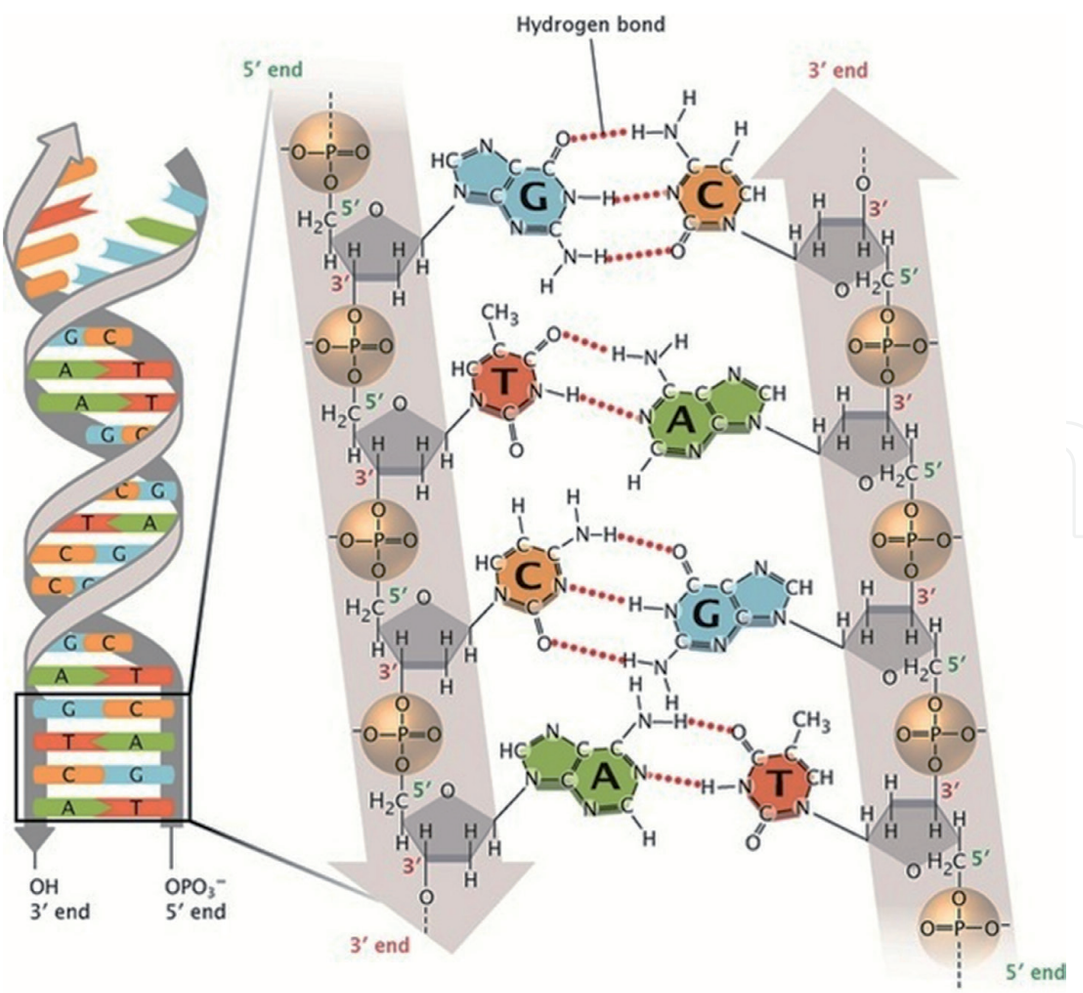


Figure 1.
Watson-Crick pairing between purine and pyrimidine bases in complementary DNA strand.

3. Anticancer drug-DNA interaction

DNA as carrier of genetic information is a major target for anticancer drug interaction because of the ability to interfere with transcription and DNA replication, a major step in cell growth and division. There are three principally different ways of anticancer drug binding. First is through control of transcription factors and polymerases. Here, the anticancer drugs interact with the proteins that bind directly to DNA. Second is through RNA binding to DNA double helices to form nucleic acid triple helical structures or RNA hybridization to exposed DNA single strand regions that will be forming DNA-RNA hybrids and it may interfere with transcriptional activity. Third is through small aromatic ligand molecules that bind to DNA double helical structures through non-covalent interaction either by intercalating binder or by minor groove binders (**Figure 2**) [6, 7]. Therefore, intercalation 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 [8]. In addition, intercalation binding involves the insertion of a planar molecule between DNA base pairs, which results in a decrease in the DNA helical twist and lengthening of the DNA. While groove binding, unlike intercalation, does not induce large conformational changes in DNA and may be considered similar to standard lock-and-key models for ligand-macromolecular binding. In addition, Groove binders are usually crescent-shaped molecules that bind to the minor groove of DNA [7].

In order to accommodate the binder (like intercalation binder), DNA must undergo a conformational change to create a cavity for the incoming chromophore. The double helix is therefore partially unwound, which leads to distortions of the sugar-phosphate backbone and changes in the twist angle between successive base pairs (**Figure 3**) [8]. Once the drug has been sandwiched between the DNA base

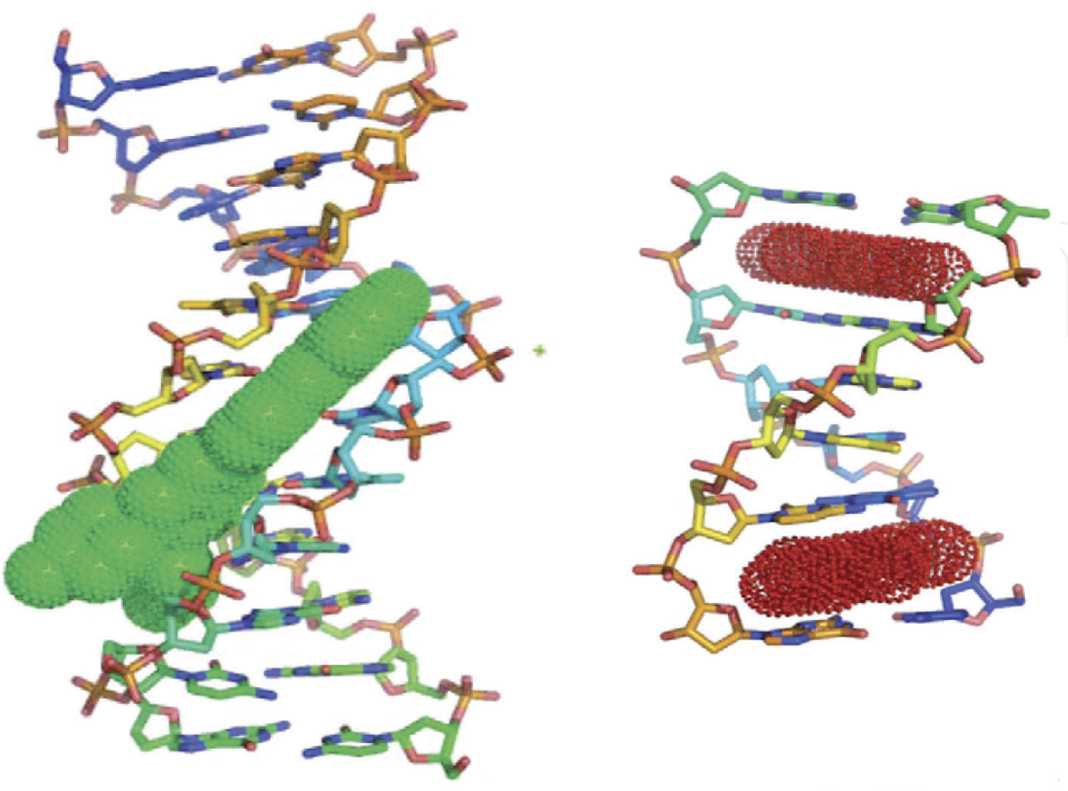


Figure 2.
Groove binding to the minor groove of DNA (left) and the intercalation into DNA (right).

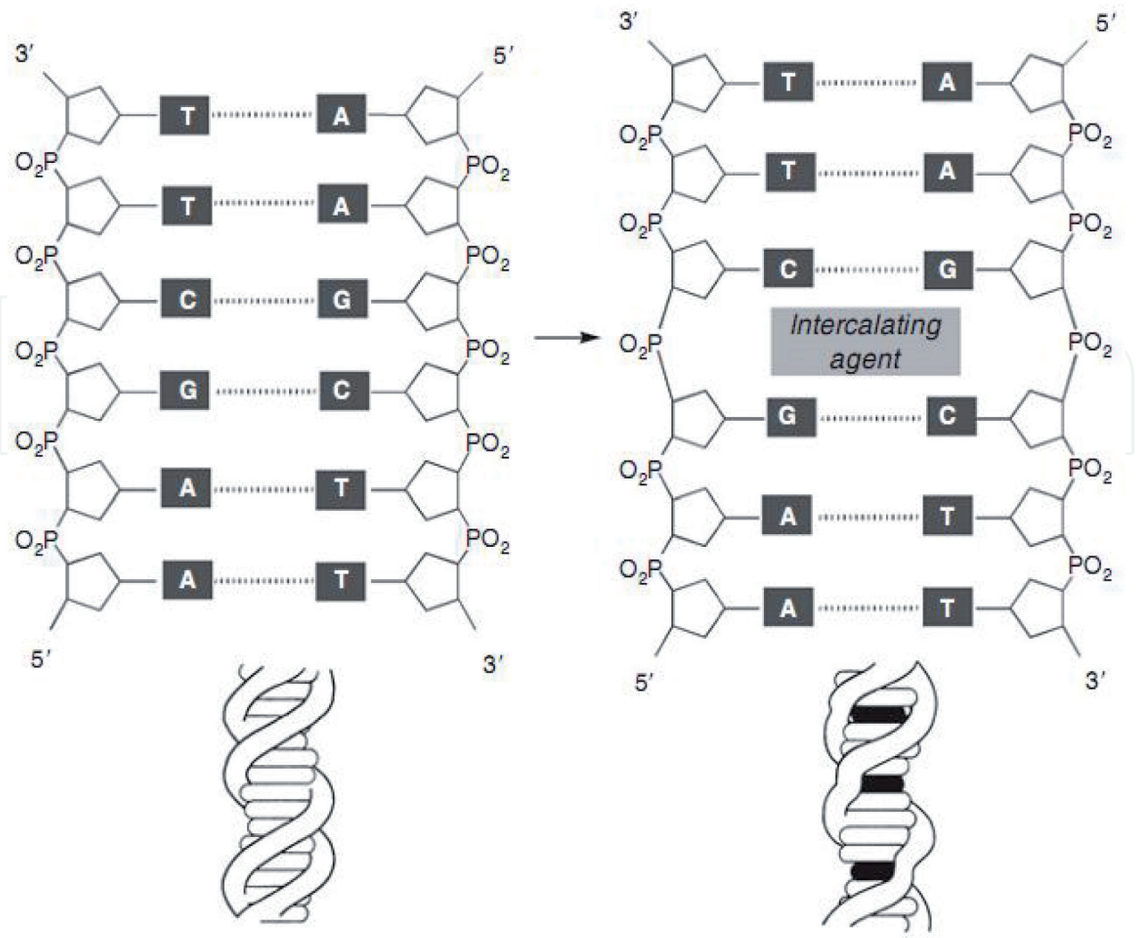


Figure 3.
Deformation of DNA by an intercalating agent.

pairs, several non-covalent interactions such as Van der Waals interaction and hydrogen bonding optimizes the stability of the complex.

4. Types of drug-DNA interactions

The study of interaction between drug molecules and DNA is very exciting and significant not only in understanding the mechanism of interaction, but also for the design of new drugs. However, the mechanism of interactions between them is still relatively little known. By understanding the mechanism of interaction between them, designing of new DNA-targeted drugs and the screening of these in vitro will be possible [9]. Many of the most valuable anticancer drugs currently used in therapy interact with DNA either by a covalent or non-covalent mechanism. Unfortunately, several of them show a considerable toxicity when the DNA molecular target is present in both normal and tumor cells [10]. The covalent type of binding of drug-DNA is irreversible and invariably causes the complete inhibition of DNA processes and subsequent cell death. A major advantage of covalent binders is the high binding strength. However, covalent bulky adducts can cause DNA backbone distortion, which affect both transcription and replication (disrupting protein complex recruitment). The covalent binders are also called alkylating agents due to adduct formation because they are used in cancer treatment to attach an alkyl group (C_nH_{2n+1}) to DNA [11]. **Table 1** lists the different types of drug-DNA interactions with suitable examples. In addition, some important examples of a cross-linking agent covalent and non-covalent binder were shown in **Figure 4** [5, 12].

No.	Type of interaction	Example
1	Covalent bonding	Nitrogen mustard, carboplatin and cyclophosphamide
2	Non-covalent bonding	Ethidium bromide and quinacrine

Table 1.
Listing the different types of drug-DNA interactions with suitable examples.

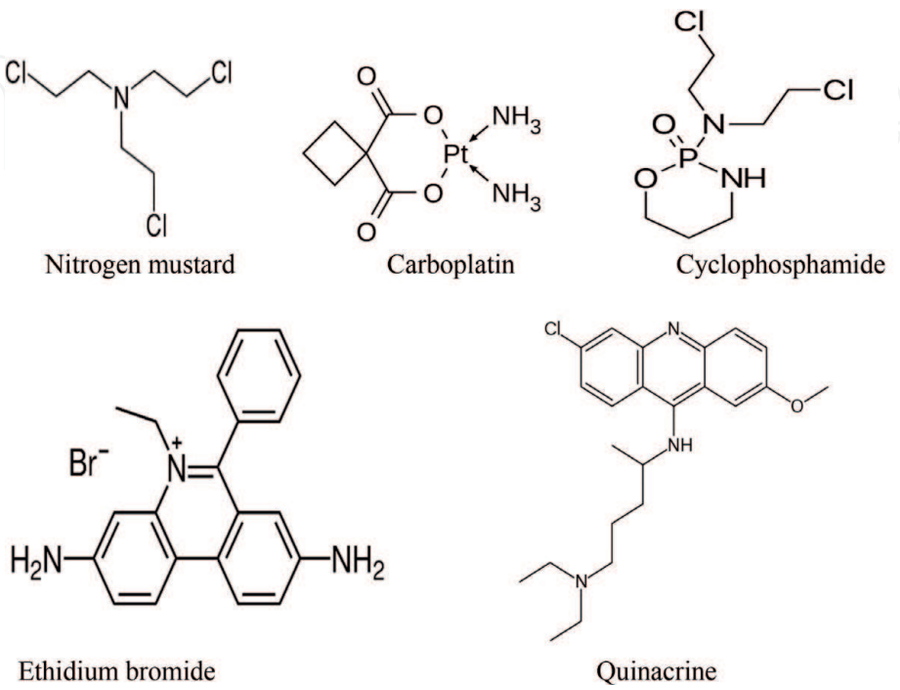


Figure 4.
Chemical structure of some covalent and non-covalent binders of DNA.

Non-covalent DNA interacting agents (groove, intercalators, and external binders) are generally considered less cytotoxic than agents producing covalent DNA adducts and other DNA damage. The non-covalent binding type is reversible and is typically preferred over covalent adduct formation keeping the drug metabolism and toxic side effects in mind. In addition, non-covalent DNA interacting agents can changes DNA conformation, DNA torsional tension, interrupt protein-DNA interaction, and potentially lead to DNA strand breaks [11].

5. Modeling of hairpin minor groove binders

Hairpin minor grove binding molecules have been identified and synthesized that bind to G-C reach nucleotide sequences. Hairpin polyamides are linked systems that exploit a set of simple recognition rules for DNA base pairs through specific orientation of imidazole (Im) and pyrrole (Py) rings (**Figure 5**) [13]. They originated from the discovery of the three-ring Im-Py-Py molecule that bound to minor groove DNA as an antiparallel side by side dimer.

The solid phase synthesis of polyamides of variable length has produced efficient ligands. The advantage of polyamide ligand design has been reached with finding structures able to recognize DNA sequences of specific genes. Moreover, a new strategy of rational drug design exploits the combination of polyamides with bis-intercalating structures. The new synthetic compound showed a resistant against multidrug resistance in which small aromatic compounds are efficiently

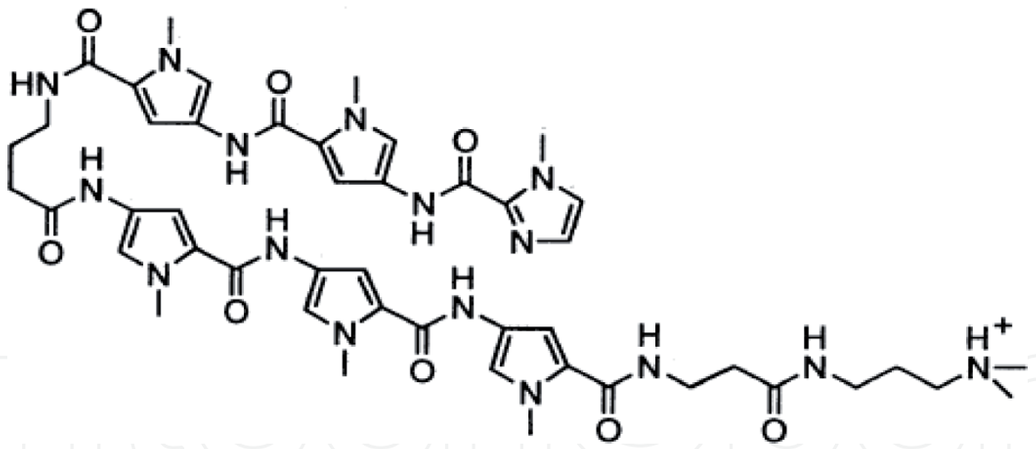


Figure 5.
Structure of hairpin polyamide Im-Py-Py.

expelled from the cell-by-cell membrane transport proteins that commonly referred to as ABC transporters or ATP binding cassette proteins [14].

6. Rational for drug design

When a compound intercalates into nucleic acids, there are changes, which occur in both the DNA and the compound during complex formation that can be used to study the ligand DNA interaction. The binding is of course an equilibrium process because no covalent bond formation is involved. The binding constant can be determined by measuring the free and DNA bound form of the ligand. In addition, DNA double helix structures are found to be more stable with intercalating agents present and show a reduced heat denaturation. Correlating these biophysical parameters with cytotoxicity is used to support the antitumor activity of these drugs as based on their ability to intercalate in DNA double helical structures. [15].

Improvement of anticancer drugs based on intercalating activity is not only focused on DNA-ligand interaction, but also on tissue distribution and toxic side effects on the heart (cardiac toxicity) due to redox reduction of the aromatic rings and subsequent free radical formation. Free radical species are thought to induce destructive cellular events such as enzyme inactivation, DNA strand cleavage and membrane lipid peroxidation [16, 17].

7. Cisplatin-DNA interactions

Cisplatin (cis-$[PtCl_2(NH_3)_2]$) is the most widely used anticancer drug today. Since the development of cisplatin became one of the main biological targets for the antitumor compounds. It is used against ovarian, cervical, head and neck, esophageal and non-small cell lung cancer. However, chemotherapy treatment by cisplatin comes with a price of severe side effects including nausea, vomiting and ear damage, as cisplatin not only attacks cancer cells, but also healthy cells. It is therefore important to elucidate the details of the cisplatin mode of action to design new cisplatin analogs that specifically target cancer cells. Furthermore, most cancer cells are insensitive towards cisplatin or develop resistance. There is therefore, also a need for cisplatin analogues with a broader range of cytotoxicity. The search for new analogues and the elucidation of the complete mode of action have been going on for more than 40 years and there is an enormous amount of data available for researchers. Still, the picture of how cisplatin works is incomplete [11, 18].

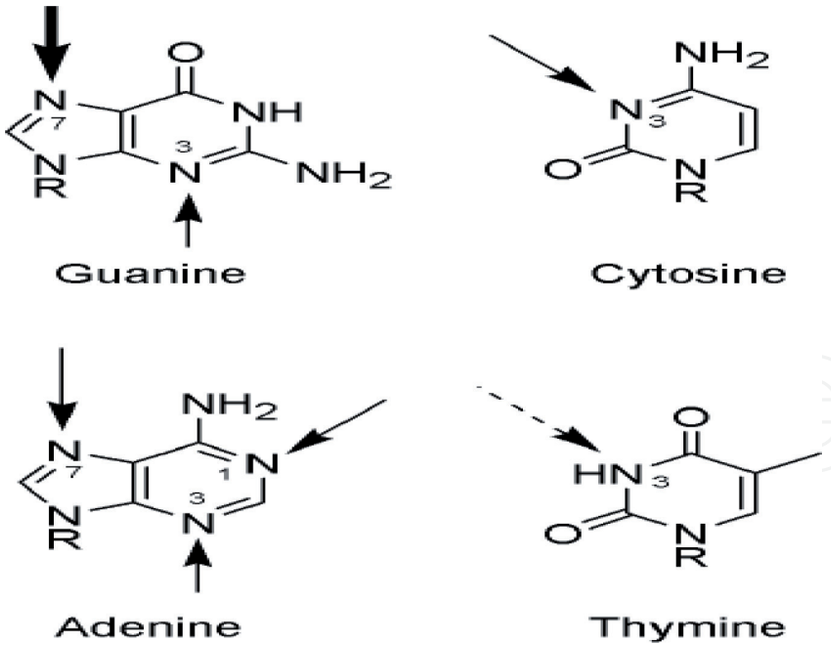


Figure 6.
The structure of the most common binding sites on the nucleobases for Pt. The big arrow on guanine indicates the overall favorable coordination site in DNA, the arrow towards thymine is dotted because the proton has to be removed before Pt association.

Cellular DNA has been shown to be the primary target for cisplatin, although cisplatin can react with several other cellular components. In the cell, the salt concentration is significantly lower (~20 mM) and cis-$[PtCl_2(NH_3)_2]$ is hydrolyzed by high salt concentration (>100 mM) to the probable active species cis-$[PtCl(OH_2)(NH_3)_2]^+$. The hydrolyzed product binds to DNA and preferentially to guanine N7> > adenine N7 > cytosine N3, first as a monoadduct, then forming a bidentate adduct. The primary products are 1,2-intrastrand cross-links of GpG (60–65%) or ApG (20–25%) sequences. A smaller amount corresponds to 1,3-intrastrand or G N7–G N7 interstrand adducts. The most common binding sites on the nucleobases for Pt are shown in **Figure 6** [18]. The big arrow on guanine indicates the overall favorable coordination site in DNA, the arrow towards thymine is dotted because the proton has to be removed before Pt association.

The formation of these 1,2-intrastrand cross-links alters the duplex conformation. The most dramatic effect is unwinding of the two strands and bending of the DNA double helix (several values for the bend angle are reported in the range 20–80°). The platinated adducts are assumed to be recognized by proteins, followed either by stabilization of the distorted DNA structure or removal of the lesion through repair [18]. The deformation of the DNA structure can interfere with the normal functions of DNA, such as replication and transcription, leading to cellular death by apoptosis or necrosis. The ineffective isomer of cisplatin, transplatin (trans-$[PtCl_2(NH_3)_2]$), is not able to form 1,2-intrastrand cross-links [19]. Transplatin forms only 1,3-intrastrand and interstrand cross-links and this might be the reason why transplatin is antitumor inactive [18].

In addition, a sensing system based on the photoinduced electron transfer of quantum dots (QDs) was also designed to measure the interaction of anticancer drug and DNA, taking mitoxantrone (MTX) as a model drug. The MTX adsorbed on the surface of QDs and this, can quench the photoluminescence (PL) of QDs through the photoinduced electron-transfer process, then the addition of DNA will bring the restoration of QDs PL intensity, as DNA can bind with MTX and remove it from QDs.

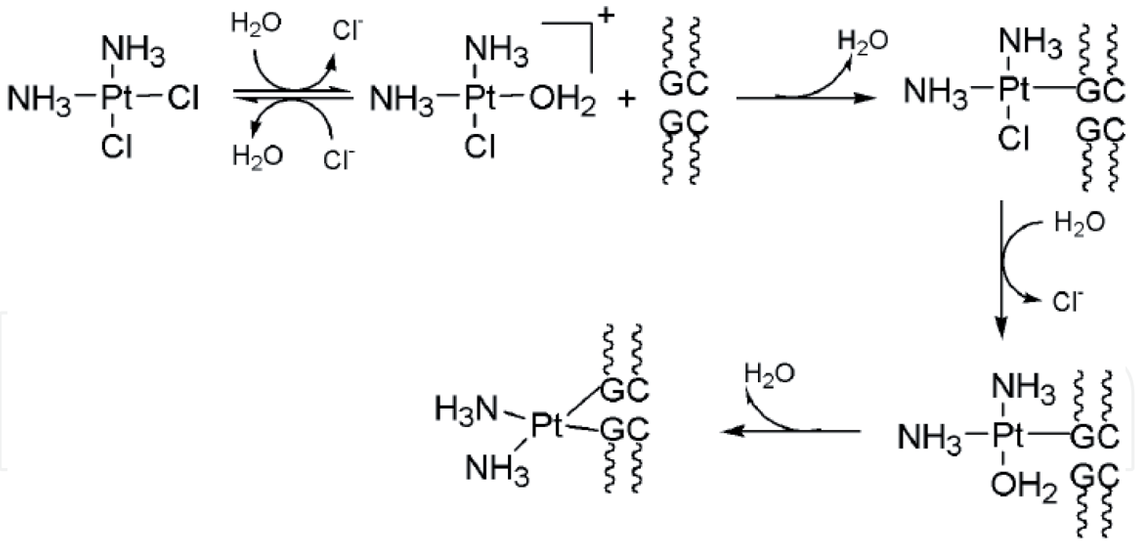


Figure 7.
Assumed mechanism for the formation of cisplatin-DNA adducts.

Cisplatin-DNA sequence selectivity has been given great attention from the research community. Several studies show that cisplatin first binds monofunctionally to guanine N7 and is particularly reactive towards Gn-runs ($n \geq 2$) (**Figure 7**) [18, 20, 21]. The high nucleophilicity of Gn-runs attracts the positively charged cisplatin monoaqua specie. The lifetime of the monoadduct is relatively long and it has therefore been suggested that the initial monoadduct is crucial for the type of cross-linked adduct formed and thus for the cytotoxic properties of the Pt complex. The main factors influencing the mono-functional binding affinity in DNA are thought to be [18] the type of bound nucleotide and of the adjacent residues, the steric effects of the Pt complex, the hydrogen binding properties of the Pt-DNA adduct and the DNA conformation.

8. Sequence specific structural perturbation

The formation of a cisplatin adduct with the GpG bases requires a significant tilting of the bases leading to a perturbation of the regular B-DNA conformation. The structural perturbation has been shown to be specifically recognized by a number of cellular proteins, including proteins with high-mobility group (HMG) binding domains and the TATA box binding protein [22]. It is believed that (some of) these recognition proteins mediate the cellular response which finally induces cell death by apoptosis or necrosis. In some cases, relatively subtle changes in the adduct structure can affect the recognition and the biological effects in a major way. This is exemplified by the cisplatin analogue oxaliplatin which forms similar G*G*-Pt adducts as cisplatin [18]. However, the oxaliplatin-G*G adducts differ in repair efficiency, mutagenesis and translesion synthesis, believed to be related to the differential activity of the two drugs (oxaliplatin is used, in combination with 5-fluorouracil, for the treatment of colorectal cancers against which cisplatin is inactive). The evaluation of the structural details of the platinum-DNA adducts and of their effects on protein, recognition can therefore help to understand why the biological activities of two similar platinum compounds (e.g., cisplatin versus oxaliplatin) are different. So far only nine cisplatin-DNA adducts have been characterized by NMR and/or x-ray crystallography. These structures were extensively reviewed by Ano et al. and found to be basically similar in structure. The cisplatin-GG adduct kinks the double helix approx. Approximately 60 towards the major groove and induces N sugar pucker for X of 5′ XG*, 5′ G* and the C complementary to 3′ G* [18].

This metal-based compound or coordination compounds that bind to DNA have been an active area of research since the discovery of cisplatin and the platinum-based drugs. The transition-metal compounds bind to DNA through several ways and different factors that promote it, such as the intercalant ligand and the nature and position of the substituent over it. Several techniques to follow metal-based drugs interactions with DNA are used as a powerful tool in order to reach a deep knowledge of the parameters involved in the stabilization of coordination compound-DNA adduct.

9. Methods for elucidation of DNA-anticancer drug interactions

DNA damaging agents (drugs that interfere with DNA function by chemically modifying specific nucleotides) includes mitomycin-C and echinomycin.

9.1 Mitomycin-C

Mitomycin-C is a well-characterized antitumor antibiotic that forms a covalent interaction with DNA after reductive activation (**Figure 8**). The activated antibiotic forms a cross-linking structure between guanine bases on adjacent strands of DNA therefore inhibiting single strand formation [8].

9.2 Echinomycin

Several studies have proved that both echinomycin quinoxaline rings bisintercalate into DNA, with CG selectivity, while the inner part of the depsipeptide establishes H-bonds with the DNA bases of the minor groove region of the two base pairs comprised between the chromophores (**Figure 9**) [8].

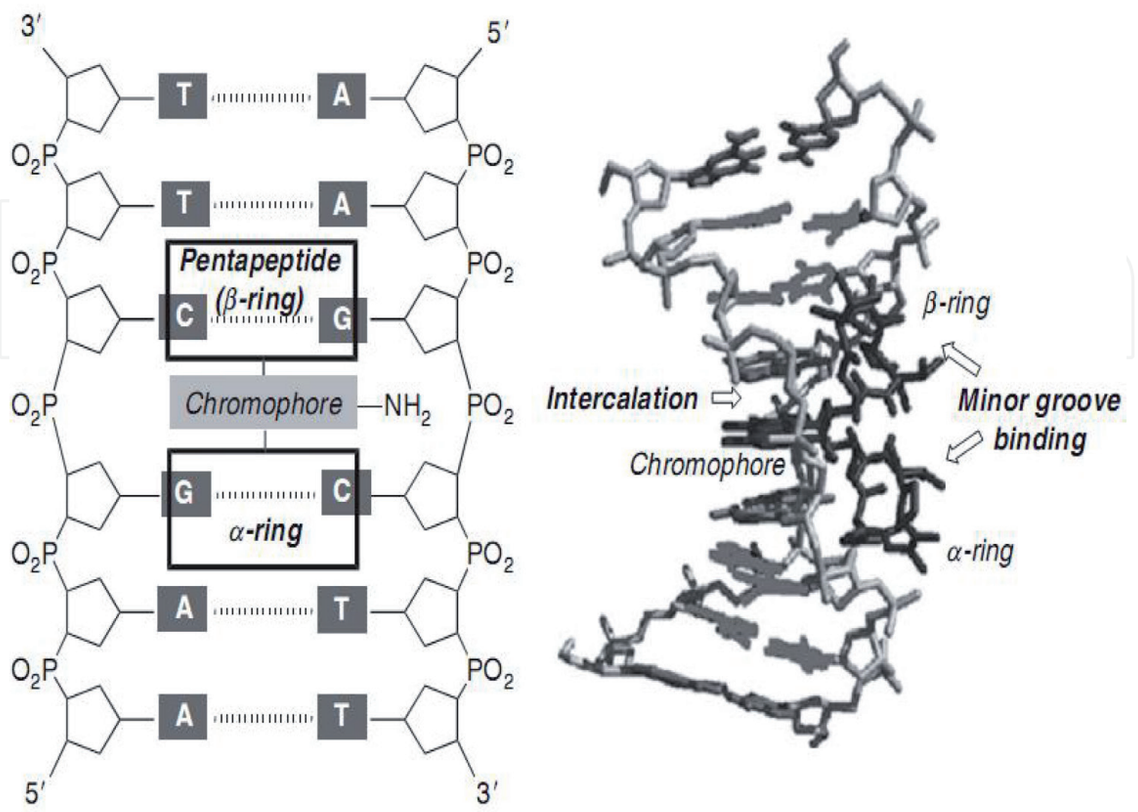


Figure 8.
Schematic interaction between DNA and mitomycin-C.

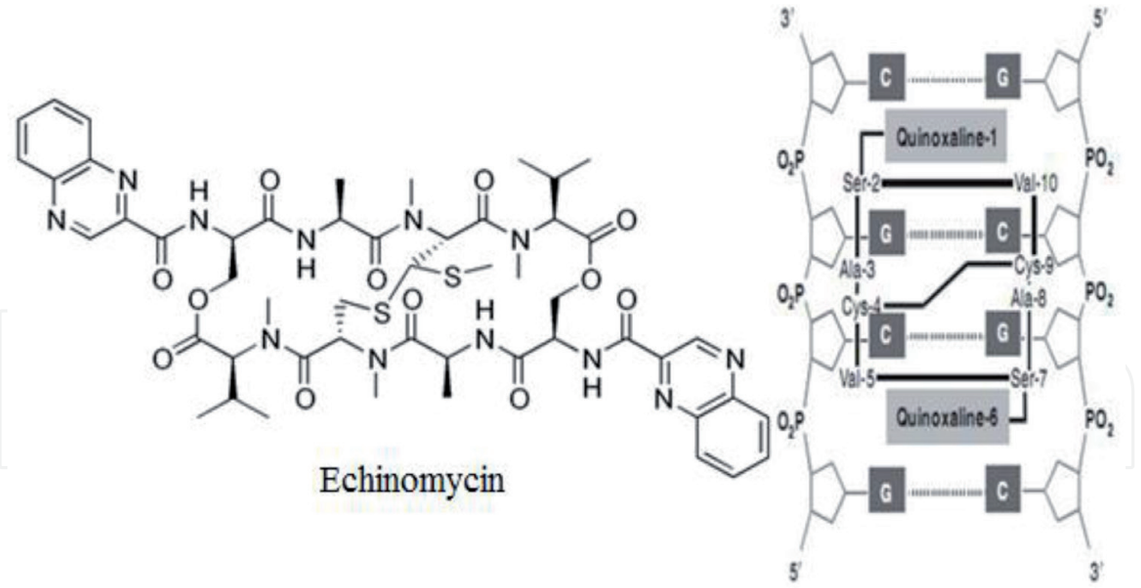


Figure 9.
Schematic interaction between DNA and echinomycin.

10. Mechanisms of anticancer drug-DNA interaction

The addition of anticancer drugs to a DNA molecule creates a new bond. Some examples for these mechanisms include intercalating agents, intercalating reagents (II), and bleomycins.

10.1 Intercalating agent

This agent contains planar aromatic or heteroaromatic ring systems (dactinomycin as an example), binding to sugar phosphate backbone by cyclic peptide or by NH_3. The planar systems slip between the layers of nucleic acid pairs and disrupt the shape of the helix. The preference is often shown for the minor or major groove. The intercalation prevents replication and transcription. In addition, the intercalation inhibits

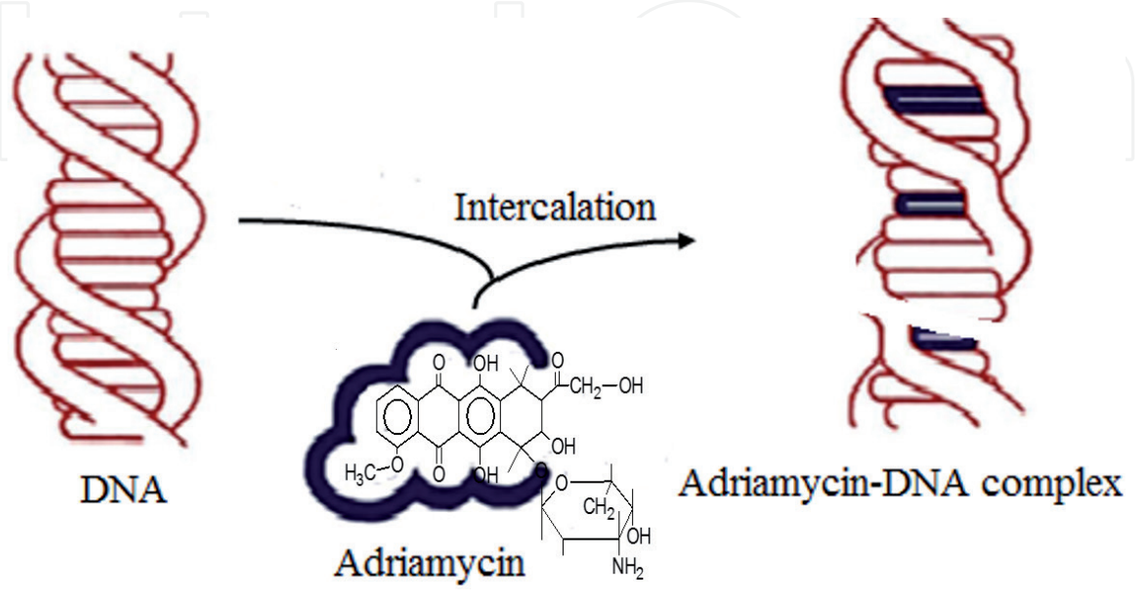


Figure 10.
Diagrammatic model illustrating intercalation of the flat part of the molecule of adriamycin (in black) into DNA, presenting the local unwinding of the helical structure.

topoisomerase II (an enzyme that relieves the strain in the DNA helix by temporarily cleaving the DNA chain and crossing an intact strand through the broken strand). Another example is the intercalation of the flat part of the molecule of Adriamycin into DNA, presenting the local unwinding of the helical structure (**Figure 10**) [23].

10.2 Intercalating reagents (II)

During replication, supercoiled DNA is unwound by the helicase. The thereby created tension is removed by the topoisomerase II (topo II) that cuts and rejoins the DNA strands. When doxorubicin is bound to the DNA it stabilizes the DNA-topo (II) complex at the point where the enzyme is covalently bound (**Figure 11**) [1, 24].

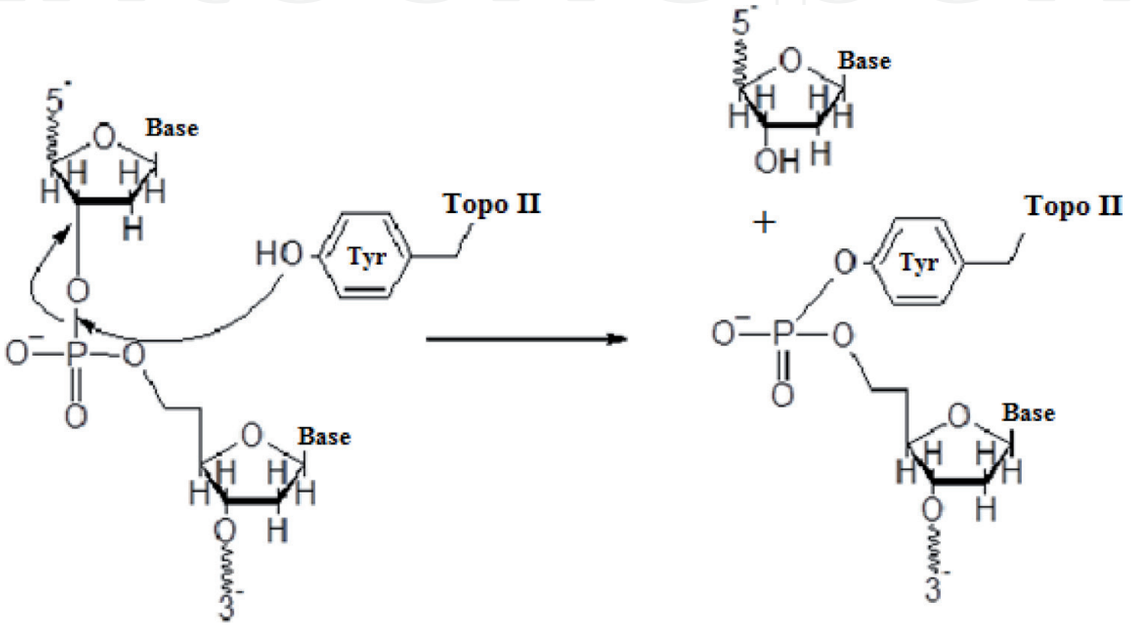


Figure 11.
Stabilizations of DNA-topo (II) complex.

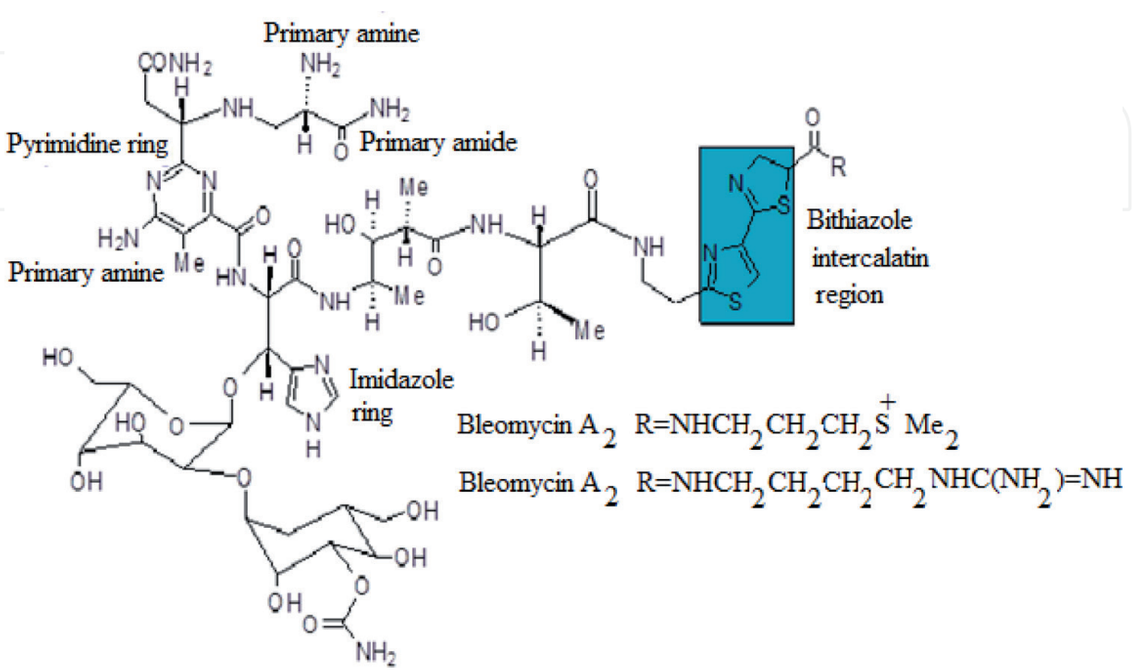


Figure 12.
The intercalating region (in blue color) of bleomycin A2 via the bithiazole moiety to DNA.

10.3 Bleomycin A_2

The bleomycin A_2 intercalate via the bithiazole moiety (DNA-binding domain) (**Figure 12**). The bithiazole moiety intercalates into the double helix and the attached side chain containing a sulfonium ion is attracted to the phosphodiester backbone. In addition, the N-atoms of the primary amines, pyrimidine ring and imidazole ring chelate Fe, which is involved in the formation of superoxide radicals, which subsequently act to cut DNA between purine and pyrimidine nucleotides [25].

11. Techniques for studying drug-DNA interactions

Various analytical techniques have been used for studying drug-DNA interactions (interaction between DNA and small ligand molecules that are potentially of pharmaceutical importance). Several instrumental techniques (emission and absorption spectroscopic) such as infrared (IR), UV-visible, nuclear magnetic resonance (NMR) spectroscopies, circular dichroism, atomic force microscopy (AFM), electrophoresis, mass spectrometry, viscosity measurements (viscometry), UV thermal denaturation studies, and cyclic, square wave and differential pulse voltammetry, etc., were used to study such interactions. These techniques have been used as a major tool to characterize the nature of drug-DNA complexation and the effects of such interaction on the structure of DNA. In addition, these techniques are regularly applied to monitor interactions of drugs with DNA because these optical properties are easily measured and tend to be quite sensitive to the environment. Moreover, these techniques provide various types of information (qualitative or quantitative) and at the same time complement each other to provide full picture of drug-DNA interaction and aid in the development of new drugs. In addition, the information gained from this part might be useful for the development of potential survey for DNA structure and new therapeutic reagents for tumors and other diseases. In this part of the chapter, we will focus on FT-IR, UV-Visible, NMR, AFM and viscosity measurements [5].

11.1 Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy is a widely used technique to study interactions of nucleic acids (DNA and RNA) and proteins with anticancer drugs and other cytotoxic agents in solutions [26, 27]. In addition, it can generate structural information of the whole molecule in a single spectrum as a photograph of all conformations present in the sample that can distinguish among A-, B- and Z-forms of DNA, triple stranded helices, and other structural patterns. In addition, it is a powerful tool to study interactions of DNA with drugs and the effects of such interactions in the structure of DNA, and providing some insights about the mechanism of drug action. The technique is ideal for systematic studies of nucleic acids (e.g., sequence variations, covalent modifications), since it is fast, nondestructive, and requires only small amount of sample [28].

IR spectrum can be divided into four characteristic spectral ranges. The region between 1800 and 1550 cm^{-1} corresponds to the in-plane double bond vibrations of the nucleic bases (C═O, C═N, C═C and N▬H bending vibrations of bases). These bands are sensitive to changes in the base stacking and base pairing interactions. Bands occurring in the interval 1500–1250 cm^{-1} assigned to vibrations of the bases and base-sugar connections are strongly related to the conformational changes of the backbone chain and glycosidic bond rotation. The range 1250–1000 cm^{-1} involves sugar phosphate vibrations, such as, PO_2 symmetric and asymmetric stretching

vibrations and C═O stretching vibrations. These vibrations show high sensitivity to conformational changes in the backbone. The range 1000–800 cm^{-1} is characteristic for bands associated with vibrations of sugars which correlate with the various nucleic acid sugar puckering modes (C2'-endo and C3'-endo) [29, 30].

Due to interfering absorption bands of water at 1650 cm^{-1} and below 950 cm^{-1}, spectra are generally recorded also in D_2O, where these bands move to 1200 cm^{-1}, and below 750 cm^{-1}. Combination of results from both spectra allows obtaining a complete spectrum. The use of D_2O also causes shifts in nucleic acid absorptions, resulting from deuterium exchange of labile NH protons, and these can be used to monitor H–D exchange processes. A method to remove water signals in the spectra is water subtraction, using a sodium chloride (NaCl) solution as reference. D_2O is used to allow shifts in the absorption of nucleic acid in order to monitor H–D exchange processes. Four regions, each having marker bands showing either nucleic acid interactions or conformations, are presented in **Figure 13** [31, 32].

The ring vibrations of nitrogenous bases (C═O, C═N stretching), PO_2 stretching vibrations (symmetric and asymmetric) and deoxyribose stretching of DNA backbone are confined in the spectral region between 1800 and 700 cm^{-1}. The vibrational bands of DNA at 1710, 1662, 1613 and 1492 cm^{-1} are assigned to guanine (G), thymine (T), adenine (A) and cytosine (C) nitrogenous bases, respectively. Bands at 1228 and 1087 cm^{-1} denote phosphate asymmetric and symmetric vibrations, respectively. These are the prominent bands of pure DNA, which are monitored during carboplatin-DNA interaction at different ratios. Changes in these bands are shown in **Figure 14** [33]. After carboplatin addition to DNA solution, G-band at 1710 shifts to 1702–3, T-band at 1662 shifts to 1655 and A-band at 1613 shifts towards lower wave number 1609–10 cm^{-1}. These shifting can be attributed to direct platin binding to G (N7), T (O_2), and A (N7) of DNA bases. No major shifting is observed for phosphate asymmetric and symmetric vibrations indicating no external binding. The plots of the relative intensity ($R\,i$) of several peaks of DNA in-plane vibrations related to A–T, G–C base pairs and the PO_2^- stretching vibrations such as 1717 (G), 1663 (T), 1609 (A), 1492 (C), and 1222 cm^{-1} (PO_2^- groups), against the compound concentrations can be obtained after peak normalization using formula (1) [5, 34]:

$$R_i = \frac{I_i}{l_{968}} \tag{1}$$

where R_i is the relative intensity, I_i is the intensity of absorption peak for pure DNA and DNA in the complex with i concentration of compound, and l_{968} is the intensity of the 968 cm^{-1} peak (internal reference) [35].

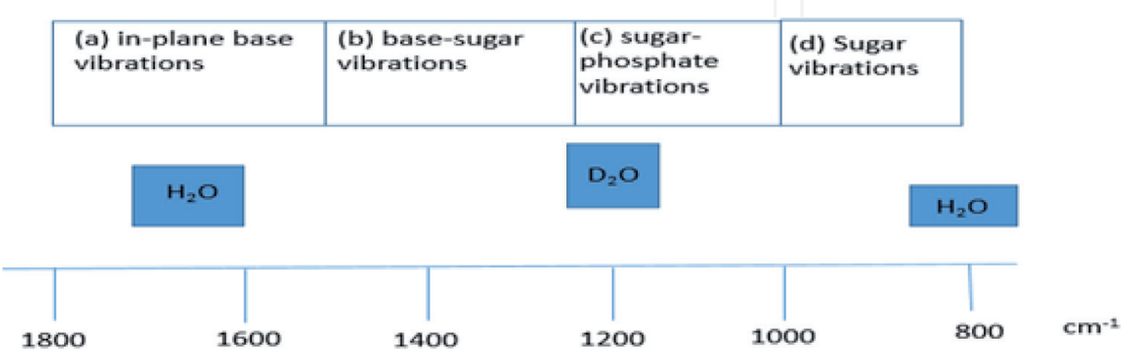


Figure 13.
The characteristics IR bands of DNA and aqueous solvents. (a) 1800–1500 cm^{-1} region is sensitive to effects of base pairing and base stacking; (b) 1500–1250 cm^{-1} region is sensitive to glycosidic bond rotation, backbone conformation, and sugar pucker; (c) 1250–1000 cm^{-1} region is sensitive to backbone conformation; and (d) 1000–800 cm_{-1} region is sensitive to sugar conformation.

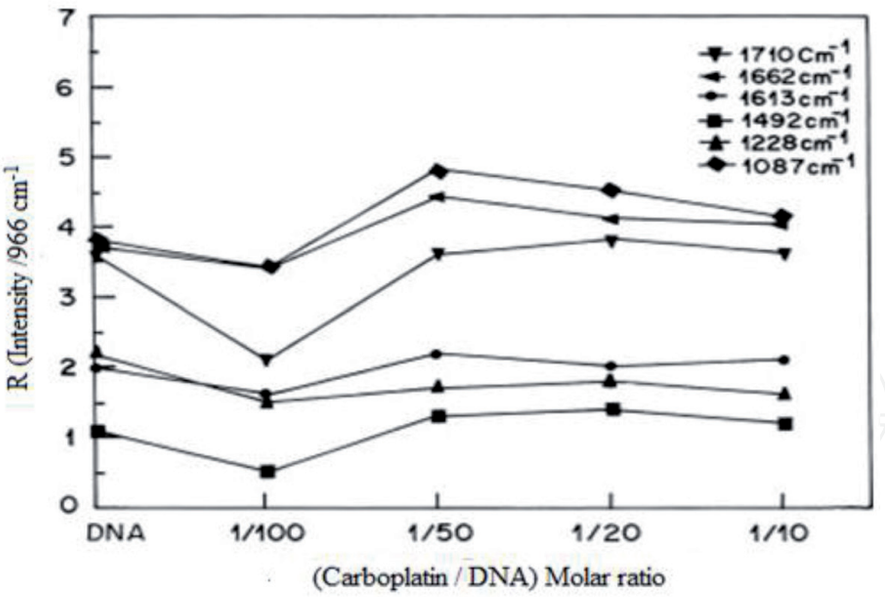


Figure 14.
Intensity ratio variations for DNA as a function of different carboplatin/DNA molar ratios.

Similarly, Raman spectroscopy, which also depends on characteristic group vibrational frequencies, can be ᵘsed together with infrared spectra to study vibrations in DNA. It is useful because Raman and IR spectroscopy provide complementary information.

11.2 UV-visible spectroscopy

UV-visible absorption spectroscopy can be utilized to detect the DNA-ligand interaction by measuring the changes in the absorption properties of the DNA molecules or the ligand. The UV-vis absorption spectrum of DNA displays a broad band in the range of 200–350 nm in the UV region, with a maximum situated at 260 nm. The maximum is due to the chromophoric groups in pyrimidine and purine moieties responsible for the electronic transitions. The utilization of this simple and versatile technique enables an accurate estimation of the DNA molar concentration based on absorbance measurement at 260 nm. To measure the interaction between ligands and DNA, a hypochromic shift is utilized because the monitoring of the values of absorbance enables studying of the melting action of DNA. Apart from versatility, other major advantages of UV-vis absorption spectroscopy include simplicity, reproducibility, and good sensitivity [36, 37].

11.3 Nuclear magnetic resonance spectroscopy

Binding between ligands and the molecules of DNA causes a significant change in the chemical shift of the values presented in **Table 2** [32]. For example, applying thermal denaturing in order to un-stack the base-pair double-helical DNA to form two ss-DNAs is often accompanied by the ^{1}H resonances' downfield shift for non-exchangeable protons.

The broadening of ^{1}H NMR resonances of DNA upon addition of an appropriate minor groove binding compound is one type of evidence of complex formation in DNA ^{31}P-NMR spectroscopy has also been used to provide important information concerning the binding of intercalators to DNA. The ^{31}P chemical shifts are sensitive DNA conformational changes, and hence intercalating drugs cause downfield shift, while divalent cations causes up field shifts in the ^{31}P signal [38].

Proton type	Expected chemical shift[a] (ppm)	Proton type	Expected chemical shift[a] (ppm)
T5 (CH_3)	1.00–2.00	A 2 (CH); A 8 (CH); G 8 (CH) T 6 (CH) C 6 (CH)	6.50–8.20
Sugar 2′ (CH_2)	2.00–3.00		
Sugar 5′ terminal (CH_2)	3.70		
Sugar 5′ (CH_2); 4'(CH)	4.00–4.50	C 4 (NH_2) (H-1)[b]	6.40–6.80
Sugar 3′ (CH)	4.50–5.20	C 4 (NH_2) (H-2)[b]	8.30–8.50 ppm
Sugar 1′ (CH)	5.30–6.20	G 1 (NH)	12.50–13.00 ppm
C 5 (CH)	5.30–6.20	T 3 (NH)	13.50–14.00 ppm

[a] *Chemical shifts relative to internal TSP (3-(trimethylsilyl)propionic acid).*
[b] *For Watson-Crick base pairs (CG).*

Table 2.
Typical ranges of chemical shifts for ^1H NMR spectra of nucleic acids.

11.4 Mass spectrometry

Mass spectrometry (MS) has become one of the most common techniques adopted to study interactions between DNA and small ligand molecules. The ability of mass spectrometry to investigate drug-DNA interactions have been reviewed recently. The binding stoichiometry, the relative binding affinities and the binding constants for DNA double helices of various sequences may be determined. Electrospray ionization (ESI) is the most common ionization method used in the study of biomolecules due to its soft ionization. Using ESI techniques, biomolecules can be transferred from the solution to the mass spectrometer with minimal fragmentation and, so, both the mass of the DNA and the mass of the DNA-ligand complex can be determined, as the non-covalent interactions that formed the complex are not altered during the electrospray process [39–41]. Focusing on the use of ESI-MS to study complexes, MS gives a signal for each species with a different mass and so it is very straightforward to establish the stoichiometry of the complexes. ESI-MS signals enable several calculations to be performed. The number of DNA strands involved, the number of bound cations (if present) and the number of bound ligands, among others. Taking into account the structure of the nucleic acids, ESI-MS studies are performed using negative polarity. It is well known that the phosphodiester backbone of DNA is fully deprotonated under usual working conditions. In general, in order to preserve their structure, nucleic acid solutions are prepared with monovalent ions. Perylene derivatives, such as, N,N-bis-(2-(dimethylamino)ethyl)-3,4,9,10-perylenetetracarboxylic acid diimide, favor π-π interactions with the G-tetrad surface. Moreover, 5,10,15,20-tetrakis-(1-methyl-4-pyridyl)-21H,23H-porphine is an effective telomerase inhibitor, also binds to the G-quadruplex in the c-myc promoter [42].

11.5 Atomic force microscopy

Atomic force microscopy (AFM) can be used to distinguish proteins bound to nucleic acid templates. One of the great advantages of the atomic force microscope, particularly with respect to the imaging of biological specimens, is that it can work in fluid, so that experiments can be performed under near physiological conditions and allowing the imaging of interactions and transactions between molecules in real time [43]. AFM techniques will play a larger role in studying interactions between biological specimens, such as ligand-receptor and protein-DNA systems,

and can be applied to the study of drug interactions with a variety of biological specimens [5].

Drug-DNA complexes have been studied with AFM to determine the binding force between them. This is of considerable interest since nucleic acid ligands are commonly used as anticancer drugs and in the treatment of genetic diseases. However, determining whether they bind to DNA by intercalation within major and/or minor grooves, by normal modes, or by a combination of these modes can often be difficult. AFM was used to study drug binding mode, affinity, and exclusion number by comparing the length of DNA fragments that have and have not been exposed to the drug. It is well known that if intercalative binding is occurring, the DNA strand increases in length. Moreover, the degree of lengthening is informative in determining the binding affinity and the site-exclusion number. AFM was shown to be an effective means of seeing and measuring any changes in the DNA strand. For example, when it exposed to ethidium, the DNA strand was shown through AFM to have increased in length from 3300 to 5250 nm, this indicating the intercalative mode of binding. Similarly, AFM intercalative binding studies showed the increase in the DNA strand, from 3300 to 4670 nm, upon exposure to daunomycin. This technique has also successfully been applied to new drugs in which the mode of binding was unclear. For example, exposure of 2,5-bis(4-amidinophenyl) (APF), did not produce lengthening of the DNA strands, indicating that the drug binds by non-intercalative modes. The different structural changes and binding processes of the DNA occur because of interactions with these two components [5].

11.6 Viscosity measurements

DNA viscosity is sensitive to DNA length change, for this reason, its measurement upon the addition of a compound is often concerned as the least ambiguous and most critical method to clarify the interaction mode of a compound with DNA and this will provide reliable evidence for the intercalative binding mode. Relative viscosity measurements have proved to be a reliable method for the assignment of the mode of binding compounds to DNA. In the case of classical intercalation, DNA base pairs are separated in order to host the bound compound resulting in the lengthening of the DNA helix and subsequently increased DNA viscosity. On the other side, the binding of a compound exclusively in DNA grooves by means of partial and/or non-classic intercalation, under same conditions, causes a bend or kink in the DNA helix and reducing its effective length and, as a result, DNA solution viscosity is decreased, or it remains unchanged.

Figure 15 show the interaction of three Schiff base compounds of N′-substituted benzohydrazide and sulfonohydrazide derivatives: (1) N′-(2-hydroxy-3-methoxybenzylidene)-4-tert-butylbenzohydrazide, (2) N′-(5-bromo-2 hydroxy-benzylidene)-4-tert-butylbenzohydrazide and (3) N′-(2-hydroxy-3-methoxy-benzylide-ne)-4-methylbenzenesulfonohydrazide with SS-DNA [44]. This can be explained by the insertion of the compounds in between the DNA base pairs, leading to an increase in the separation of base pairs at intercalation sites and, thus, an increase in DNA length [45].

The viscosity data show that there are at least two phases of binding between the complex and CT-DNA. At lower concentration of the complex, the viscosity first decreases and then increases at higher concentration of complex. This slow increase in viscosity is an indication of groove binding [11].

Figure 16 indicate that with increasing amount of (3-(3,5 dimethyl-phenylimino) methyl)benzene-1,2-diol (HL), the relative viscosity of DNA first remains constant and then increases [46]. This observation supports that HL bind through intercalation mode but with different affinity, i.e., also show some affinity for

binding with grooves of DNA through hydrogen bonding, typically to N_3 of adenine and O_2 of thymine. However, strong binding is presumably due to intercalation with DNA [11].

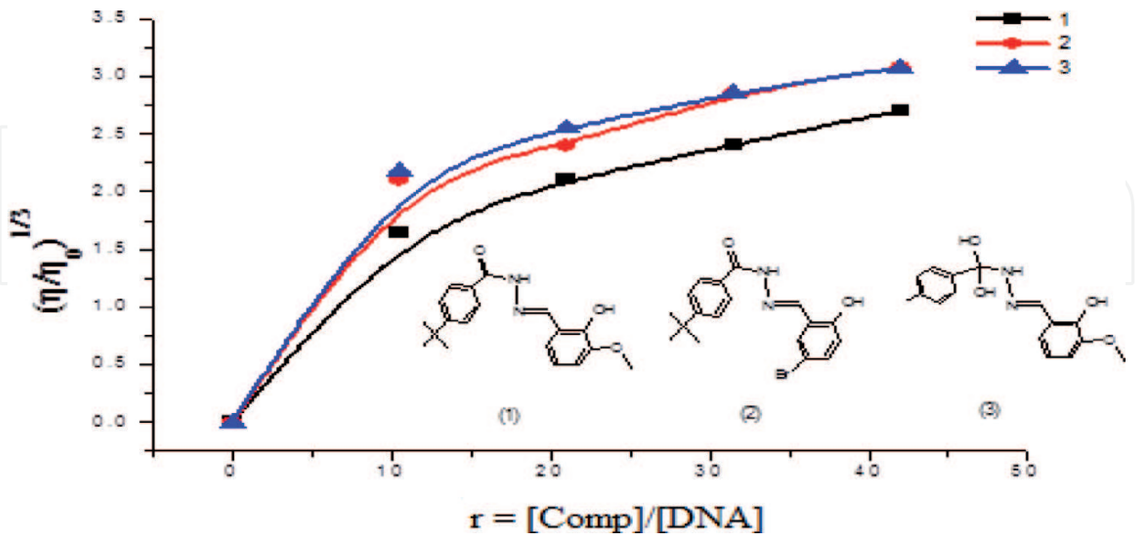


Figure 15.
Effects of increasing amount of compounds (1–3) on relative viscosity of SS-DNA at 25 ± 0.1°C. [DNA] = 7.2 μM, r = 0, 6.9, 13.9, 20.8, 27.8.

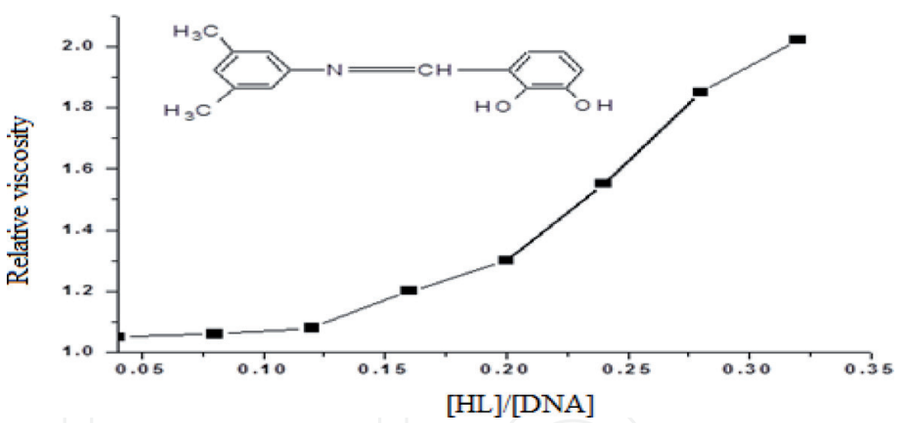


Figure 16.
Effects of increasing amount of HL on relative viscosity of CT-DNA at 25 ± 0.1°C. [DNA] = 2.37 × 10^{-5} M.

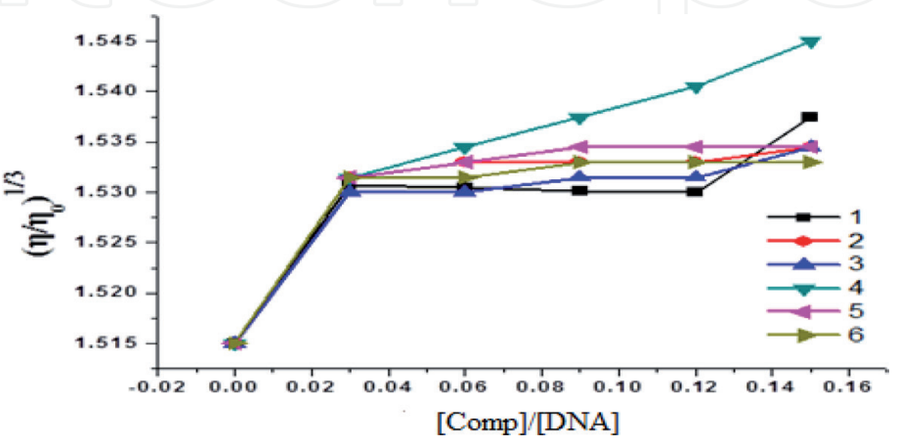


Figure 17.
(1) Effect of increasing amount of the complexes [Ni(hhmh)$_2$], (2) [Ni(bhmh)$_2$], (3) [Ni(ihmh)$_2$], (4) [Ni(PPh$_3$)(hpeh)], (5) [Ni(PPh$_3$)(bpeh)] and (6) [Ni(PPh$_3$)(ipeh)] on the relative viscosity of HS-DNA at 16(±0.L)°C.

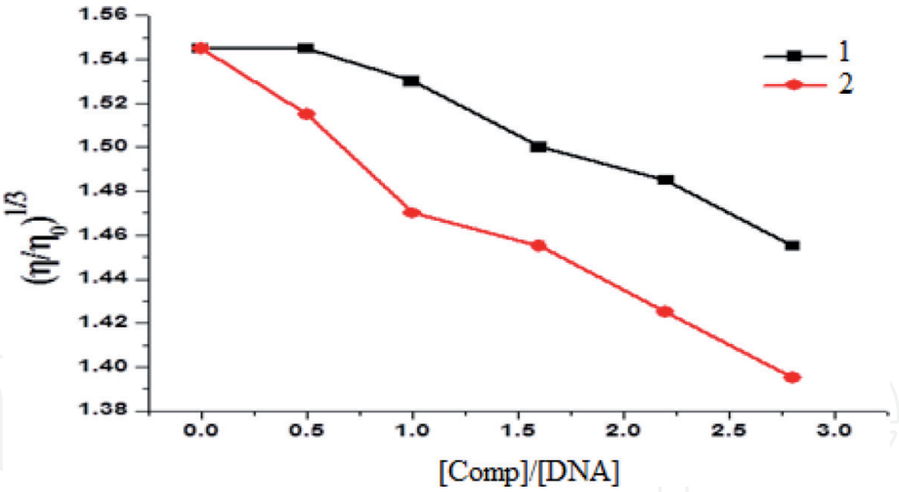


Figure 18.
(1) Effects of increasing amount of tri-n-butyltin (IV) 3-[(3',5'dimethylphenylamino)] propanoate and (2) triphenyltin(IV) 3-[(3',5'dimethylphenylamino)]propanoate on relative viscosity of SS-DNA at 25 ± 0.1°C, [DNA] = 1.86 × 10^{-4} M.

Figure 17 [47] and **Figure 18** [48] shows the electrostatic binding mode of nickel and organotin(IV) complexes with DNA, respectively. The viscosity of DNA remains essentially unchanged on the addition of the nickel complexes while it decreases in case of organotin(IV) complexes [11].

12. Conclusions

This chapter has focused on drug-DNA interactions and their study by various analytical techniques such as IR spectroscopy, viscosity measurements, MS and AFM. These techniques are used to evaluate the binding mode as well as binding strength of the complex formed between drug and DNA. The study should be useful for the development of potential survey for DNA structure and new therapeutic reagents for tumors and other diseases. Fundamentally, drugs interact with DNA through two different ways, covalent and/or non-covalent modes. Covalent binders act as alkylating agents as they alkylate the nucleotides of DNA, while, the non-covalent binders interact by three different ways: (i) intercalation, (ii) groove binding, and (iii) external binding (on the outside of the helix). Different spectroscopic techniques are generally, powerful tools to study interactions of DNA with drugs and the effects of such interactions in the structure of DNA, providing some insights about the mechanism of drug action. The binding stoichiometry, the relative binding affinities and the binding constants for DNA double helices of various sequences.

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