

Metal-Based Drug-DNA Interactions

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Abstract. Coordination compounds that bind to DNA have been an active area of research since the discovery of cisplatin and the platinum based drugs. In this review we offer a general overview about transition-metal compounds that binds DNA through several ways and some examples are given to understand the role played by the different factors that promotes 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 mentioned as well as a brief description of computational techniques that can be used as a powerful tool in order to reach a deep knowledge of the parameters involved in the stabilization of coordination compound-DNA adduct. **Keywords:** Coordination compounds, transition metals, DNA-interaction, antitumoral, experimental methods, theoretical methods

Resumen. El estudio de los compuestos de coordinación que se unen directamente al ADN ha sido un área activa de investigación desde el descubrimiento del cisplatino y compuestos relacionados con éste. En esta revisión se ofrece una visión general sobre los compuestos de metales de transición que se une el ADN a través de varias formas y damos algunos ejemplos para comprender el papel de los diferentes factores que lo promueven, como el ligante intercalante así como de la naturaleza y la posición del sustituyente sobre el mismo. Varias técnicas para seguir las interacciones compuesto de coordinación-ADN son mencionadas, así como una breve descripción de las técnicas computacionales que se pueden utilizar como una herramienta poderosa con el fin de llegar a un conocimiento profundo de los parámetros involucrados en la estabilización del aducto compuesto de coordinación-ADN. **Palabras clave:** Compuestos de coordinación, metales de transición, interacción con ADN, anticáncer, métodos experimentales, métodos teóricos.

Introduction

DNA is the library of life and all the knowledge encrypted therein becomes more accessible every day. It has been 10 years now since the Human Genome Project announced that the total human genome was decoded providing us a huge quantity of new specific therapeutic targets [1]. The knowledge of specific targets in rational design of therapeutics is a fundamental factor, principally, for the design of molecules that can be used in the treatment of oncologic diseases. Since the development of cisplatin, DNA became one of the main biological targets for the antitumor compounds [2, 3], even though some researchers consider that this strategy seems unproductive today for the area of new drug development where the knowledge on cancer genomics is suggesting the use of targets selectively expressed, or over-expressed by cancer cells [4]. The intention of this review is to provide several examples of coordination compounds that possess an intercalating ligand in its coordination sphere in order to understand the influence of several factors such as the preferential geometry of the metal center, the nature of the intercalating ligand and the nature, number and position of the substituents over the intercalating ligand in the capacity and selectivity of the coordination compounds to interact with DNA. Also, experimental and theoretical approaches to evaluate the metal-based drugs interactions with DNA are discussed.

DNA structure

A simple unit of DNA known as “nucleotide” is constituted by a five-membered carbohydrate deoxyribose bonded in its C1' to a heterocyclic base that can be either adenine (A), guanine (G), cytosine (C) or thymine (T), and on the other side of the carbohydrate ring, at C5'. A phosphodiester linker bonds the 5'-end of one sugar and the 3'-end of the other. Accordingly, the polymerization of nucleotides gives rise to long, single-stranded polyanionic chains with a well-defined directionality (traditionally described in the 5'- to 3'- direction) which essentially constitutes the primary structure of the nucleic acid [5].

The first DNA structure was published in 1953 by James Watson and Francis Crick [6] without the appropriate recognition to the Rosalind Franklin contributions. Franklin was the first to discover that DNA fibers yield two different diffraction patterns: the “crystalline” or A-form and the “wet” or B-form. John Randall gave Franklin the A-form and Maurice Wilkins the B-form, assigning them each the task of elucidating their molecular structure. However, it was a titanic job due that the samples contain both structures and provided “muddy” diffraction patterns that were near impossible to interpret. After discovering the existence of the A and B forms of DNA, Rosalind Franklin also succeeded in developing an ingenious and laborious method to separate the two forms, providing the first DNA crystals pure enough to yield interpretable diffraction

patterns. She then went on to obtain excellent X-ray diffraction patterns of crystalline B-form DNA and, using a combination of crystallographic theory and chemical reasoning, discovered important basic facts about its structure. She discovered that the sugar-phosphate backbone of DNA lies on the outside of the molecule, not the inside as was previously thought. She also discovered that the helical structure of DNA has two strands, not three as proposed in competing theories. She gave quantitative details about the shape and size of the double helix, but the missing piece of the puzzle, how the base paired on the inside of the helix, and thus the secret of heredity itself, was the part provided by Watson and Crick to complete the famously structural model [7]. DNA adopts a double-stranded arrangement as their secondary structure. The two strands of the duplex are held together by hydrogen bonding between bases on opposite strands: the most common “base-pairing” scheme (often called “Watson-Crick base pairing”) involves G-C and A-T purine-pyrimidine inter-strand pairs. The overall stability of the double-helical arrangement is further supported by π - π interactions between the stacked hydrophobic aromatic rings of adjacent bases on the polynucleotide chains. This structure forms two distinct grooves labeled major and minor, the major groove is narrow and deep, while the minor groove is wide and shallow [8, 9].

Conformations

DNA is able to adopt a variety of different conformations that are influenced by environmental factors (hydration and ionic strength) and the primary structure (base sequence) of the polynucleotide [10] and are classified as A-, B-, C-, D- and Z-form [11]. However, under physiological conditions, the B-form of DNA is predominant. A-form is a right-handed helix that consists of 11 base residues per full turn with a closer distance (2.25 Å) between the bases and a helix diameter of 23 Å. A-DNA is also characterized by a 20° tilting of the base

pairs and their net migration away from the central axis. This produces a more compacted structure with a hollow core. On the other hand, the base pairs of the right-handed B-DNA are stacked 3.4 Å apart with ten and a half base pairs residues per full rotation of the helix and a helix diameter of 20 Å. The minor groove in this structure is 4.8 Å while the major groove is 10.5 Å wide (Figure 1).

A third (C-DNA) and fourth (D-DNA) allomorphs, both B-like in structure but with reduced rise per residue (3.31 and 3.05 Å, respectively) and increased helical twists (38.6-40.0 and 45.0°, respectively), are obtained with Li and Na ions under low hydration conditions, which is when the salt content is between the A- or B-DNA states. Alternating purine and pyrimidine sequences also produces D-DNA form [11].

The Z-DNA conformation is adopted at high salt concentration and is characterized by the adoption of left-handed geometry. The helix in this form is elongated and slender (diameter of 18 Å) and can be induced by the covalent bindings of various metal complexes. It has twelve base pairs per turn with the sugar phosphate adopting a zigzag shape. The orientation of the carbohydrates groups alternates every second unit, revealing a departure from the standard mononucleotide repeating unit of B-DNA in favor of a dinucleotide motif. The minor groove in this arrangement is deeper than that observed in the B-DNA extending all the way down to the axis of the molecule, thus, the major groove is absent here (Figure 1).

DNA can also adopt other conformations such as hairpin, triplex (H-DNA), cruciform, or tetraplex (G-quadruplex and *i*-motif). In order to form those structures, DNA strands should be folded in a different manner from B-DNA or make unusual base pairs such as Hoogsteen base pairs that are the unusual pairs of hydrogen bonding among nucleic bases compared with Watson-Crick base pairs. Self-complementary sections of single strand DNA can form hairpin structures or cruciform structures, where two opposite hairpins are interacting [12]. Hairpin structures have been identified in genomic DNA and

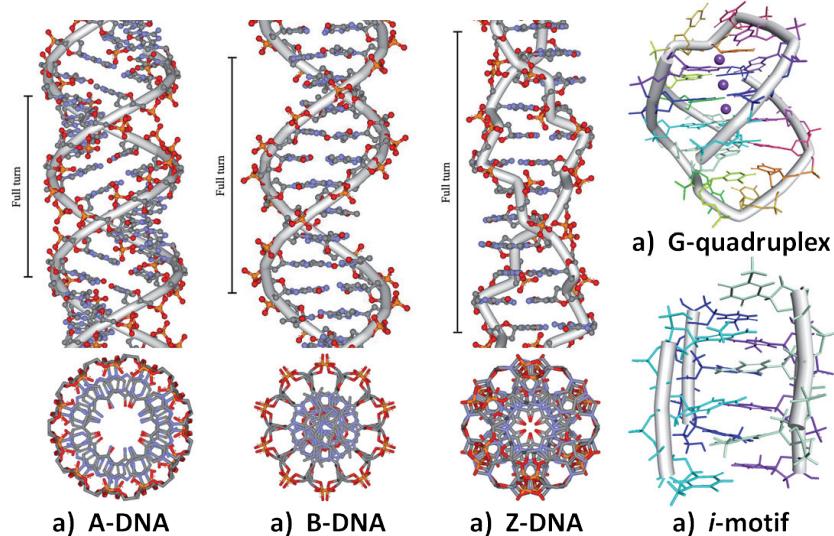


Fig. 1. Different conformations adopted by DNA.

are considered potential sites for controlling gene expression. So, drugs that can target hairpin and cruciform structures in promoter regions may be able to modify transcription and control gene expression [13-16]. Guanine (G)-rich sequences can form G-quadruplex structures consisting of π - π stacking of planar G-tetrads, cyclically bound to each other through eight hydrogen bonds according to the Hoogsteen base pairs [17-19]. G-rich sequences are observed frequently in the promoter region of oncogene and human telomeric DNA [20-22]. The *i*-motif structure is formed from a cytosine (C)-rich strand at slightly acidic pH or even neutral pH [23]. It is known that C-rich sequences are present in or near the regulatory regions of >40% of all genes, especially in the promoter region of oncogene and human telomeric DNA [24]. The *i*-motif structure consists of two parallel-stranded C: C+ hemi protonated base-paired duplexes that are intercalated in an antiparallel manner. The *i*-motif structures are significantly affected by the number of cytosine bases [25], loop length [26], environmental condition [27, 28], and attached or interacting material with the DNA strands [28-30].

Double stranded DNA can also bind to a third strand under acidic conditions to form a triple helix, also known as H-DNA [31-33]. The bases of the third strand bind to the existing base pairs via Hoogsten base pairing, where T binds to A in a novel fashion and protonated C binds to G, forming the triplets T•AT and C•GC complementary sequence. Triple helices are not as stable as duplex, and are thought to relieve torsional stress caused by supercoiling.

Modes of binding of bioinorganic structures to DNA

A search using the Nucleic Acids database [34] produces 247 crystal and NMR structures of DNA with various types of binders, from which only a handful relates to any kind of bioinorganic or metal-containing drug. Some examples of experimental drug-DNA interactions using transition metal compounds include Ru [35], Co [36], Ni, Cu, Zn [36], and rare-earths compounds [37]. Since DNA offers many potential binding sites due to its size and complexity, coordination compounds are able to bind to DNA via two main interaction modes: irreversible (covalent or coordination binding) and reversible (intermolecular association). The latter binding mode can be further divided into electrostatic interactions, groove binding and intercalation.

Covalent binding

Coordination compounds may exhibit a preference for a particular binding mode or nucleotide sequence depending on the size and shape of the molecule. Cisplatin is a well-known antitumor drug which principal target is DNA. It is responsible for the cure of over 90% of testicular cancer [38] and plays an important role in the treatment of other kinds of cancer such as ovarian, bladder, head and neck, lymphomas and melanomas [39]. Even though the great knowledge achieved, cisplatin is only effective in a limited range of cancer and also produce

severe side-effects: often causing severe nausea and vomiting, bone marrow suppression and kidney toxicity. In spite of that, cisplatin is still one of the world's best-selling anticancer drugs.

The action mechanism of cisplatin has been extensively studied as well as the solvation process that it suffers to produce the "activated" molecules $\text{cis-}[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)]^+$ and $\text{cis-}[\text{Pt}(\text{H}_2\text{O})_2(\text{NH}_3)_2]^{2+}$ [39-46]. Antitumor activity of solvated cisplatin derives from its capability to form bifunctional DNA cross-links [47]. The predominant adducts formed by cisplatin with DNA are guanine-guanine (GG) intrastrand cross-links throughout the coordination of Pt to N7 of guanine (Figure 2). The coordination to N7 of guanines is not surprising as nitrogen N7 atoms of purines are the most electron-dense and accessible sites in DNA for electrophilic attack by platinum. They are exposed in the major groove of the double helix, and not involved in base pair hydrogen-bonding. This particular platinum-induced kink in the DNA is considered to be the critical lesion due to the chain of events that the biomolecular recognition eventually provokes in the cell [47-51].

The significant prevalence of intrastrand GG cross-links over any other adduct has been examined in the context of contributions of H-bonding, the electrostatics in the vicinity of the binding site, and the steric effects of adduct conformation. It has been argued that hydrogen bonding between the NH_3 of the DNA-cisplatin complex and O6 of a nearby guanine—not possible for adenine—has a large impact on the adduct configuration, being essential for stability and eventual cytotoxic activity [52-54].

Some other platinum derivatives present a covalent interaction with DNA. Platinum (IV) complexes with octahedral geometry are believed to be reduced to platinum (II) in vivo [55]. Satraplatin ((OC-6-43)-bis(acetato)amminedichloro(cycl ohexylamine) platinum, JM216) is the most successful of the

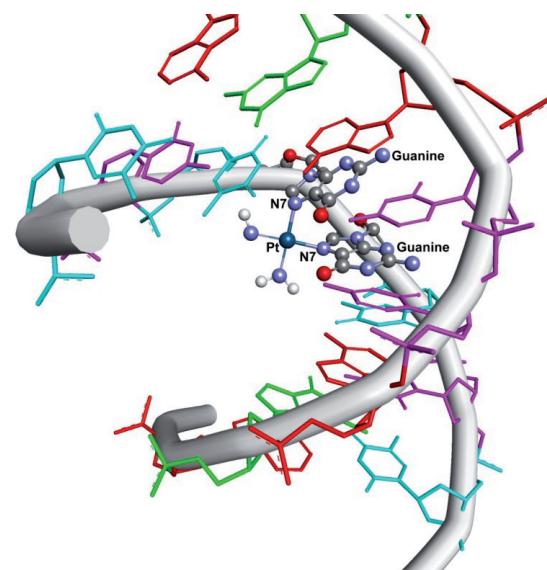


Fig. 2. Cisplatin forms adducts with the N7 nitrogen from guanine bases (adapted from PDB entry code 3LPV).

platinum(IV) complexes [56], on the other hand, iproplatin (cis-dichloro-trans-dihydroxy-bis-isopropylamine platinum(IV)) and tetraplatin (1,2-diaminocyclohexane tetrachloroplatinum (IV)) [57] are also undergoing clinical development. A number of multinuclear platinum complexes have also been developed [58, 59], some of which have shown activity in both cisplatin-sensitive and cisplatin resistant cell lines.

All mononuclear platinum complexes could form intrastrand and interstrand adducts with DNA. When interstrand lesion is formed, massive distortions of the B-DNA are observed. Similarly, the intrastrand lesion, while it forms much more readily than the interstrand lesion, it induces mutational events via the distortion of its nucleic acid target [60]. These detrimental issues have been addressed by a generation of dinuclear Pt complexes which possess two Pt (II) centers, each of which carries only one chloride ligand thus relegating each Pt (II) capable of only one coordination event. Farrell and his group designed and synthesized binuclear platinum (II) complexes and studied their interactions with calf thymus DNA and a small 49 base pair oligodeoxyribonucleotide, see Figure 2 [61]. Owing to the presence of the pyridyl ligands, this compound induces a much higher degree of DNA unwinding than that seen with either of the ammonia-bound complexes, as well as the mononuclear trans-[PtCl₂-(py)₂]. Similarly, to the mononuclear compound [(Pt(*trans*-(py)₂Cl)₂-μ-(diamminobutane)]²⁺ stabilizes B-form DNA while the other complexes with amino groups instead pyridine rings induce B-Z transitions upon DNA binding. These alterations likely involve the ability of [(Pt(*trans*-(py)₂Cl)₂-μ-(diamminobutane)]²⁺ to undergo π-stacking interactions upon DNA association which in turn, disfavors the Z-DNA conformation. Importantly, interstrand-cross links formation is very efficient for all three complexes with yields of 80%, 52%, and 41% for [(Pt(*cis*-(NH₃)₂Cl)₂-μ-(diamminobutane)]²⁺, [(Pt(*trans*-(NH₃)₂Cl)₂-μ-(diamminobutane)]²⁺, and [(Pt(*trans*-(py)₂Cl)₂-μ-(diamminobutane)]²⁺ respectively. Further, only [(Pt(*trans*-(NH₃)₂Cl)₂-μ-(diamminobutane)]²⁺ was capable of intrastrand adduction.

DNA binding of BBR3464 was the formation of {Pt,Pt} inter-strand cross-links (IXL), expected to be structurally distinct from those formed by cisplatin because each Pt unit will bind to one strand of the DNA duplex [62]. For this compound the crosslinks occur not only in the “normal” 5'-5' direction, since the DNA helix is normally read from the 5'-side, but also in the “opposite” antiparallel 3'-3' direction [63]. The directionality is dependent on the nature of the cross-link. Interestingly and according to Farrell, this is the unique example of anti-cancer drugs behaving in this manner [64, 65].

Trinuclear platinum complex BBR3464 is one of the most studied of this kind [66-69]. This compound has shown a higher potency than cisplatin *in vitro* [70-72], while *in vivo* studies have shown it to be effective towards human xenograft tumors with intrinsic or acquired resistance to cisplatin [73]. In phase II clinical trials only partial responses have been observed with cisplatin-resistant ovarian and non-small cell lung cancers [74]. Even though Phase I and II clinical studies gave preliminary indications of activity in melanoma, pancreatic, lung and ovar-

ian cancers, further phase II studies have shown to have minimal antitumor activity against gastric carcinomas, with significant hematological toxicities encountered by patients, the authors conclude that further studies with BBR3464 in gastric or gastro-esophageal adenocarcinoma are not recommended [75].

Groove binding

Molecules normally reach DNA through one of the grooves and react to either the backbone or the nucleobases. Reversible intermolecular associations such as electrostatic binding occur due the interaction between cations with the negatively charged phosphate backbone at the exterior surface of the DNA helix [76]. Some of the most common ligands which have been studied as intercalator are shown in Fig. 3.

Major groove

Ruthenium (II) polypyridyl complexes can bind by direct interactions in either the major or minor groove of DNA due to their great diversity in overall shape, size, hydrogen-bonding potential, polarizability of ligands and charge. Even though if the structure has an adequate intercalation motif, compounds with bulky substituent preferentially occupy the major groove. Examples of this kind of complexes include the ruthenium derivatives with the ability to hydrogen-bond that appear to favor major-groove binding like Λ-[Ru(TMP)₃]²⁺ (TMP = 3,4,7,8-tetramethyl-1,10-phenanthroline) [77] and [Ru(phen)₂(11-(9-acridinyl)dipyrido[3,2-a:20,30-c]phenazine)]²⁺ which present a preference to intercalate on GC rich region of the major groove than the AT rich one [78]. Copper compounds also present this binding mode, like [Cu(ferrocenylmethylbis(2-pyridylmethylamine)(dppz)](ClO₄)₂ which shows dual chemical nuclease activity involving both metal centers and displays efficient photo-induced DNA cleavage activity in visible laser light of 458 and 568 nm and also in red light of 647 nm within the photodynamic therapy (PDT) window [79] or [Cu(salicylaldehyde-N(4)-phenylthiosemicarbazone) (dppz)] which also present nuclease activity [79].

Tetracationic triple helical supramolecular cylinder [Fe₂(C₂₅H₂₀N₄)₃]Cl₄ was one of the first reported examples of this type of complexes that interact with DNA major groove. Major groove interaction was suggested by the DNA kinking observed in atomic force microscopy (AFM) images of the interaction between the helicate and plasmidic DNA pBR322. The major groove binding was confirmed by NOESY experiments [80]. Further studies of the interaction of this helicate structure with DNA show that exist an enantiomeric preference for the binding process that is reflected in the DNA deformation degree [81]. Binding strength and groove selectivity can be modified adding methyl groups to the helicate that produce width increase or lengthening of the structure. Increasing the width reduces the DNA binding strength, the bending and coiling effect and the groove selectivity of the enantiomers compared with the parent compound, while the lengthening results

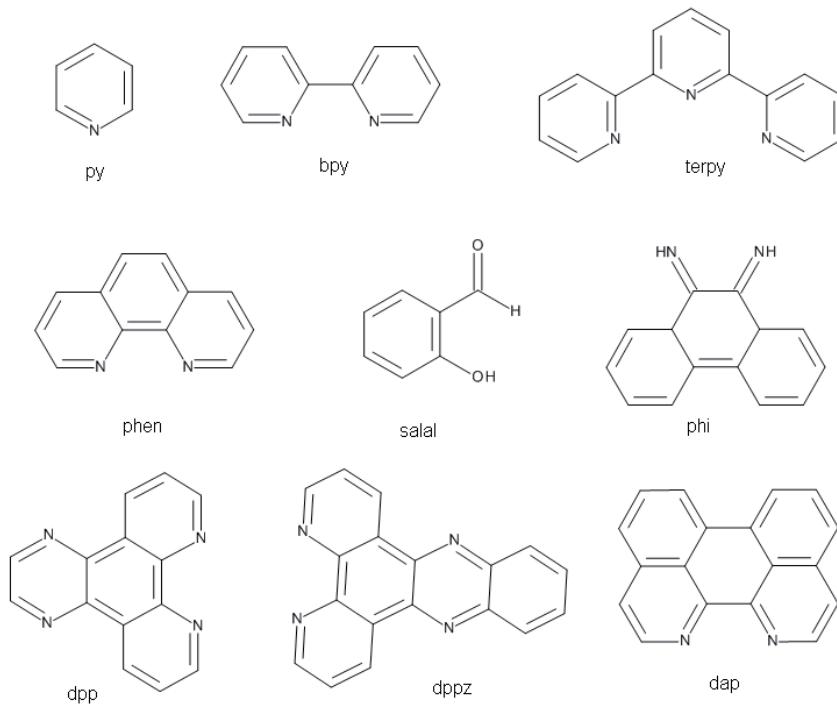


Fig. 3. Some intercalating ligands mentioned in this review py = pyridine, bpy = bipyridine, terpy = terpyridine, phen = phenanthroline, salal = salicylaldehyde, phi = phenanthrene-9,10-diimine, dpp = 2,3-bis(2-pyridylpyrazine) , dppz = dipyridyl[3,2-a:2'3'-c]phenazine, dap = 1,12-diazaperylene.

in DNA interactions similar to those of the parent compounds, with an increased preference of the P enantiomer for the minor groove indicating an enhancement of mode (P enantiomer of these iron supramolecular helicates deviates polarized light to the left) [82]. Another examples of helicate structures that provide certain groove selectivity degree are the enantiomerically pure dinuclear complexes of the form $[\text{Ru}_2(1,10\text{-phen})_4\text{L}1]^{4+}$ and $[\text{Ru}_2(\text{bpy})_4\text{L}1]^{4+}$, where $\text{L}1 = (\text{C}_5\text{H}_4\text{N})\text{C}=\text{N}(\text{C}_6\text{H}_4)_2\text{CH}_2$. In contrast to the di-iron helicates, the phen complexes show DNA sequence effects with Δ , Δ - $[\text{Ru}_2(\text{phen})_4\text{L}1]^{4+}$ binding preferentially to GC and Λ , Λ - $[\text{Ru}_2(\text{phen})_4\text{L}1]^{4+}$ to AT [83].

Intercalation

Intercalation is distinguished by the insertion of generally planar polycyclic aromatic systems between the stacked base pairs of DNA. This interaction is stabilized by the overlap of the π -clouds of the intercalator and the nearby nucleobases [84] and can occur from either the major [85] or the minor groove [86]. Intercalation increases the separation of adjacent base pairs, and the resulting helix distortion is compensated by adjustments in the sugar-phosphate backbone and, generally, by an unwinding of the duplex. These systems have a planar surface area of approximately 28 \AA^2 [87] and they must be positively charged so that electrostatic interactions can occur between the intercalating molecule and the negatively charged DNA [88]. The inclusion of a metal center into the design of intercalating compounds gives rise to a greater range of geometric diversity

and structural flexibility than any purely organic molecules can achieve.

Due the remarkable results obtained with cisplatin, for much time was believed that compounds with square geometries possessing an intercalator moiety were the perfect candidates for the accomplishment of the interaction with DNA. In 1974, the intercalative binding of platinum (II) complexes containing the bidentate ligands bpy, phen and terpy were investigated using X-ray diffraction and electrophoresis [59, 89, 90]. However, intercalation is not restricted to square planar complexes, a series of chiral octahedral complexes using zinc [91], iridium [92], iron [93, 94], cobalt [95, 96], osmium [97, 98], rhodium [99-101] and ruthenium [75-105, 123, 124, 141-148] as well as tetrahedral complexes such as $[\text{Co}(\text{phen})_2]^{2+}$ [102], $[\text{Cu}(\text{phen})_2]^{2+}$ [103], were synthesized and their interactions with DNA investigated.

A series of related platinum (II) compounds containing bpy, phen and TMP as intercalating ligand and ethylenediamine (en) or diaminopropane completing the coordination sphere were tested against the L1210 murine leukaemia cell line to assess their cytotoxicity.

The effect of the intercalating moiety in metallodrugs was investigated by comparing the cytotoxicity of different methyl-substitution on the phenanthroline of $[\text{Pt}(\text{R-phen})(\text{en})]^{2+}$ complexes [104], where R-phen = phen, 4-Me-phen, 5-Me-phen, 4,7-Me₂-phen, 5,6-Me₂- phen and TMP in the L1210 cell line. These achiral complexes present IC_{50} values in the range 0.7-2 mM that suggest to the investigation group that the combination of intercalating and ancillary ligand coordinated to the

platinum has a significant effect on the biological activity of these complexes [105]. These experiments also did show that methyl substitution on specific endocyclic carbon atoms of phen can affect the biological activity of a complex. The order of activity is 5,6-Me₂-phen > 5-Me-phen > phen > 4-Me-phen = 4,7-Me₂-phen = TMP. NMR studies revealed that they all insert into the hexanucleotide d(GTCGAC)₂ from the minor groove, predominantly between the T2A5 and C3G4 base pairs. The NMR evidence also indicated that the intercalator is inserted into the DNA with the ethylenediamine moiety in the minor groove. It is probable that the observed enhancement in biological activity is due to favorable hydrogen bonding or steric interactions. For the complexes [Pt(4-Me-phen)(en)]²⁺, [Pt(4,7-Me₂-phen)(en)]²⁺ and [Pt (TMP)(en)]²⁺, results from DNA-NMR binding experiments suggest that the hydrogens of the methyl groups on C4 and C7 are close to the base pairs and possibly interacting unfavorably and this may result in the reduced biological activity of these complexes. In addition, it is possible that the methyl groups interact with phosphodiester groups of the sugar-phosphate backbone, affecting the depth of intercalation [104]. Most platinum (II) intercalators binding have association constants in the range of 10⁴-10⁷ M⁻¹. Variance in binding strength appears to be due to differences in the charge and extent of the aromatic system of individual molecules, with the general trend being phen > terpy > bpy [106-108].

Similar results were reported by Chikira [109] employing ternary Cu(II) complexes with phenanthroline and amino acids, [Cu(phen)(α -aminoacidate)], shown that the three-fused aromatic rings in phenanthroline are critical for the intercalative binding of the complexes. The intercalative binding was promoted by 5,6-dimethyl groups on the phenanthroline ring, whereas it was disturbed by 2,9-dimethyl groups, indicating that the planarity of the coordination sphere is important for the intercalative binding. For these compounds, the inhibitory concentrations are in the micromolar range in several cell lines such as cervix (HeLa and SiHa), breast (MCF-7), and colon (HCT-15). Even though these ternary Cu(II) coordination compounds have shown a remarkable cytotoxic, cytostatic, genotoxic and antitumoral activity [110], the paramagnetism of these molecules made hard the study of interaction with oligonucleotides, studies that were done as a routine analysis with metals such as Pt(II) and Ru(II) employing NMR spectroscopy.

This is the case of mononuclear ruthenium (II) complexes that were extensively studied in the middle of the 1980s and during the 1990s [85, 111-140]. One of the first examples of transition metal complexes that interact with DNA extensively studied was Λ - and Δ -[Ru(phen)₃]²⁺. Different biological activity was established for both isomers by studies carried out on mice [141]. The results of these experiments allowed setting out the hypotheses that the interaction of these complexes with the biological system was physical instead chemical, and that the biological effect must be due to the overall three-dimensional shape of the complex, rather than simply the constituent ligands (Figure 4). Association constants are reported to be of the order of 10⁵-10⁶ M⁻¹ [102].

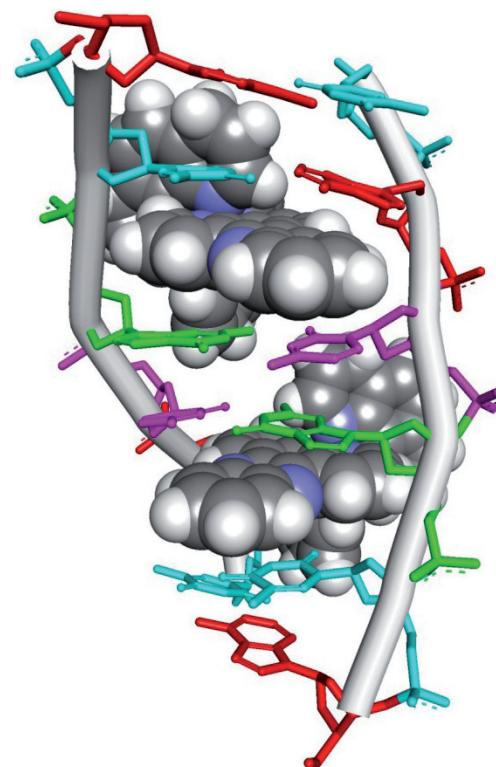


Fig. 4. Intercalation binding of a ruthenium complex with B-DNA form (adapted from PDB entry code 4E87).

Barton [142-144] suggested that binding of [Ru(phen)₃]²⁺ could occur by two possible modes: intercalation and surface interaction. However, other studies published some years later employing linear and circular dichroism studies suggested that both isomers do not intercalate [144]. DNA interaction discrepancies were solved by posterior NMR studies where were established that [Ru(phen)₃]²⁺ exhibit different binding modes, depending on the chirality of the enantiomer of the complex, the DNA sequence and, for the Δ -enantiomer, the concentration of the complex, at high metal complex to DNA ratios, the minor groove is saturated by surface bound complexes, whereas the major groove is partly filled in a partially intercalative geometry [137, 139, 145].

On the other hand, bis-intercalating compounds consisting of two intercalating moieties joined by a linking chain or spacer were developed to improve cytotoxicity. Bis intercalating compounds may have higher affinities due to their increased charge and ability to span a greater number of DNA base pairs unobtainable by mono intercalators [146]. For platinum derivatives of this kind of compounds the cytotoxicity was reported to depend on three variables; linker length, charge density and counter ions [147]. Complexes with shorter linkers displayed higher activity than those with longer linkers [147]. The more highly charged complexes were also more cytotoxic [146, 147]. The high charge density and greater electrostatic stress of the shorter linked complexes is believed to increase the platination of DNA, which leads to their greater cytotoxicity [147].

Dimers of rhodium (III) complexes such as $[\text{Rh}(\text{bpy})_2(\text{phi})]^{3+}$ ($\text{phi} = 9,10\text{-phenanthrenequinone diimine}$) and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ have also been found to show some DNA recognition. Dimers of $\Delta\text{-}[\text{Rh}(\text{4,4'-diphenylbpy})_2(\text{phi})]^{3+}$ ($4,4'\text{-diphenylbpy} = 4,4'\text{-diphenyl-2,2'-bipyridine}$) specifically targeted the palindromic DNA sequence 5'-CTCTAGAG- 3' [122, 148]. These rhodium (III) complexes demonstrated the significance of shape complementarity for specific binding between the octahedral complexes and DNA. Complexes with sterically bulky methyl or phenyl groups on the non-intercalating ligands showed sequence preferences [149].

As well as in platinum complexes, Rh(III) derivatives combining intercalating and coordinated easily leaving groups have also been studied [150-151]. 2D NMR shows combined coordinative and intercalative interactions between the dirhodium(II) complex $\text{cis-}[\text{Rh}_2(\text{dap})(\mu\text{-O}_2\text{CCH}_3)_2(\eta^1\text{-O}_2\text{CCH}_3)(\text{CH}_3\text{OH})](\text{O}_2\text{CCH}_3)_{14}$ ($\text{dap} = 1,12\text{-diazaperylene}$) and the duplex (5'-GGAAGTTGAGAG-3')/(5'-CTCTCAACTTCC-3'). One rhodium atom binds coordinatively to the N7 of A6 base, while the dap ligand intercalates classically into the central A6pA7 base step.

Besides NMR spectroscopy and X-ray diffraction, the atomic force microscopy has been employed in the study of metallodrugs-DNA interactions in recent years with remarkable results. In this field the indispensable reference is the group of Professor Virtudes Moreno, who has studied the interaction of an endless number of coordination compounds. Among the studied compounds some of them present antitumor activity such as derivatives of Pt (II) and Pd (II) [152-154], Ru (II) [155-157], Co (III) [158], Cu (I) [159], Cu (II) [160]. This technique made possible to identify the interaction of paramagnetic compounds such as Casiopeínas (Cas III-ia, $[\text{Cu}(4,4'\text{-dimethyl-2,2'-bipyridine})(\text{acetylacetone})]\text{NO}_3$; Cas III-Ea, $[\text{Cu}(4,7\text{-dimethyl-1,10-phenanthroline})(\text{acetylacetone})]\text{NO}_3$, and Cas II-gly, $[\text{Cu}(4,7\text{-dimethyl-1,10-phenanthroline})(\text{glycinate})]\text{NO}_3$) with plasmidic DNA pBR322 establishing interaction differences derived from the structural modifications [160].

In addition to the antitumor compounds, some metallodrugs have been designed to be used in the treatment of other diseases also trying to reach DNA as main target. Some examples are oxovanadium compounds tested as antileishmanial [161] and antitrypanosomal [162, 163] agents. Metals usually employed to get antitumor agents such as Pt(II), Pd(II) were recently tested against *Trypanosoma cruzi* with very good results [164]. Ru(II) compounds with phosphine and diimine ligands were tested against tuberculosis [165], while Au(III) compound $[\text{Au}(\text{dppz})_2]\text{Cl}_3$, $[\text{Cu}(\text{dppz})_2]\text{BF}_4$ and trans-platinum (II) compounds with chloroquine have shown noteworthy results in the proliferative inhibition of *Leishmania mexicana* cultures [166-168].

Although a lot of experimental work has been done studying the interaction of coordination compounds with DNA, much of this knowledge is still the peak of the iceberg, due that in many cases the information obtained with experimental designs that do not involve expensive procedures and equip-

ments is extremely poor. However, in recent years the computational methods have been suffering an incredible growth related with the size of the systems that can be calculated and the time expended to get results. The next part of this review is focused in the description of some computational approaches employed in the investigation of metal based drugs-DNA interactions.

Computational Chemistry approach of coordination compounds binding to DNA

While extensive research has been done about drug-DNA binders, work about coordination compounds interacting with DNA is still an exciting and active field of research. Experimental techniques and analysis used for routine DNA studies can be extrapolated to coordination complexes in most cases and this is also true with Computational Chemistry tools, except by the fact that transition metals are still complex to simulate due to their multiple oxidation states and resulting geometry change, therefore, special care has to be accounted when generating force field parameters or applying a Quantum Mechanical method to these molecules. Extensive work has been done to understand the interaction of metals and transition-metals with DNA as cations present in the solvent (for an in-depth review, refer to the work by Sponer and co-workers [169]). In this manuscript, we focus on research about metal-containing compounds that interact directly with DNA.

Molecular Dynamics studies

Studies using Molecular Dynamics (MD) are numerous and provide insight about the general structure of the mechanism of binding. Even if no electronic effect is taken into account, these simulations provide useful information of the dynamics not only in the place where the metal-compound is binding but also the conformational changes of the whole system. The AMBER and the CHARMM family of force fields for nucleic acids are able to simulate a stable B-DNA chain in the micro second realm and realistic sampling of the global and local parameters is now possible [170]. MD also allows refinement based on Nuclear Overhauser Effect (NOE) restraints of distance, angle and penalty functions based on NMR spectra. This type of analysis tools is probably the most efficient way to study the structure of a metal compound and the way it binds to nucleic acids, but it requires the restrain information obtained by NMR spectroscopy. The technique has been used to build models and study the structure of many systems [171-173] and also in depth study of DNA-protein binding and triple-helix DNA [174]. In the field of metal-DNA binding, recent work includes studies of the ruthenium complex $[\text{Ru}_2\text{bidppz}(\text{bipy})_4]^{4+}$ and the dodecamer $[\text{d}(\text{CGCGAATTGCG})_2]$ using NMR spectroscopy showed a minor-groove binding interaction that was stable after 10ns of simulation (Figure 4) [175]. Force Field (FF) parameters of small organic molecules are available and provide acceptable results, however, these general force fields do not have the parameters to describe a bioinorganic compound which includes

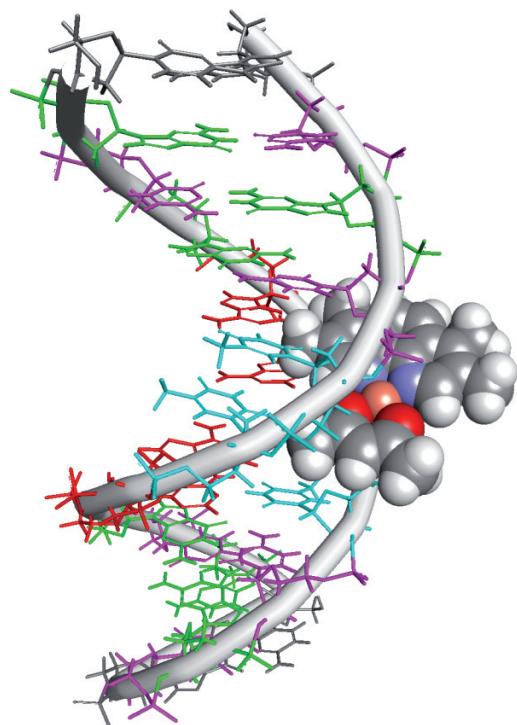


Fig. 5. Intercalation binding of Casiopeína III-Da $[\text{Cu}(2,2'\text{-bipyridine})(\text{acetylacetone})(\text{H}_2\text{O})]\text{NO}_3$ with B-DNA form.

a transition metal, so this is a concern that has to be resolved to be able to run a MD simulation. There are several options available that will generate the bonded and non-bonded parameters around a metal center, one of this is the Metal Center Parameter Builder [176, 177] available in the Amber programs [178]. If the X-ray or NMR structure of the metal-compound is available, then it will be relatively easier to generate the required FF parameters using the bond, angle and dihedral values from the experimental structure. Non-bonded parameters are available for a number of metals [179]. The resulting FF values can be used to recreate the metal-compound and run MD for several nanoseconds to guarantee its stability and correct representation for the simulation. An example of this methodology is shown in our previous work using the copper-compound $[\text{Cu}(2,2'\text{-bi pyridine})(\text{acetylacetone})(\text{H}_2\text{O})]^+$ (Casiopeína®, [180]. Since an X-ray of the binding of this copper compounds is still not available, we manually docked the molecule in 4 possible starting binding configurations with the B-DNA and run MD for 100ns to study the energetics of each resulting trajectory (Fig. 5) [181].

If no experimental structure is available, Quantum Mechanical (QM) methods can be used to optimize an initial structure and with this structure, generate the appropriate FF parameters. In a recent work from Liu, Chen and Tang with a new family of mononuclear copper and dinuclear copper-platinum nucleases Density Functional Theory (DFT) methodology was used to generate the optimized geometries. FF parameters for the nucleases, minus the copper and platinum atoms, were obtained from the Generalized Amber Force Field (GAFF) using

the programs available in AMBER11. The charge calculation for each atom was obtained following the Restrained Electrostatic Potential (RESP) procedure that allows a multi-conformation, multi-orientation approach, yielding a more accurate representation of the electronic distribution of the molecule [182]. As mentioned above, van-der-Waals and Lennard-Jones parameters for both metals were adopted from available databases and previous work. B-DNA was generated using the *nucgen* program available in AMBER11. To generate the initial structure prior to the MD simulation, docking methodology was used over 1000 docking predictions using Autodock4.0. Docking methodology will sample through different conformations between two molecules evaluating the total interaction energy for every conformation until a certain threshold is reached. In this case, the metal binders were located among the minor groove of the B-DNA chain. With this starting structure, MD was run for 40ns. Information about the structural changes and movement of the binders along the groove is extracted from the trajectories and information about the global and local inter and intra base conformation.

Quantum Mechanical studies

The use of Quantum Mechanical methods allow full electronic study of the system and considerable reduction of empirical approximations, although, these methods are extremely computational and memory demanding and the representation of solvent interactions are limited. Explicit solvent representation is simply not reachable with current methods, but one can use a previously generated Molecular Dynamics simulation with explicit solvent and transfer specific water molecules for the QM treatment. It is important to mention that transition metals contain a considerable amount of electrons which will take a lot of computing time. Although it is important to include every electron of the metal atom, in most cases the core electrons are not used for reactions or energetics. This can be simulated using effective core potentials (ECP) which “freezes” core electrons and facilitate the electronic representation of the metal, requiring less time to achieve conversion. A simple approach to study these interactions is using isolated bases and nucleotides, basically, stripping apart the DNA or RNA chain. This allows the use of high-level computational methods and analysis procedures. An example is the work done by Hirao and Matsubara using divalent cyclen-coordinated metal complexes (Zn, Cu and Ni) where the metal center is treated with the LANL2dz ECP basis set, the rest of the atoms using the 6-31G* and using the B3LYP functional. They study the binding of DNA bases guanine, thymine and uracil to the metal complexes and the effect of the solvent using the polarized-continuum-model (PCM) approximation. They report detailed information about the charge transfer and electrostatic interactions using this methodology [183]. Another advantage about QM methods is the possibility to obtain spectroscopic data (for example: UV-VIS, fluorescence and RAMAN), this allows a way to compare between experiments and simulations starting with specific configurations [184].

Hybrid QM/MM studies

Quantum Mechanics and Molecular Dynamics are both methods that give detailed information about some aspect of a molecular system, each with its own advantages and flaws. In an attempt to bring up together the best of both worlds, Warshel and Levitt [185] created a hybrid approach in which the system was split between two zones or layers. The high layer or QM part is where the reaction is taken place, as it occurs in the active site inside enzymes, and the low layer or MM part, which in this case is the rest of the tertiary structure of the same protein. In most cases, the dynamics and fluctuations of the whole protein are not directly involved in the reaction mechanism; therefore, it is not required to employ such a detailed level of calculation like the ones attainable with a QM method. On the contrary, on the active site, we want to study bond breaking, bond forming, electronic interactions, etc., and if possible avoid the use of empirical approximations. The main idea of the QM/MM approach is to split the system of study so that the binder and the area involved in the binding process are simulated with QM and the rest of the system is modeled with MD. The energy of the system is then given by equation:

$$E_{\text{ele}} \approx E_{\text{QM}} + E_{\text{MM}} + E_{\text{QM/MM}}$$

E_{QM} and E_{MM} can be obtained using any of the methodologies already mentioned. The differences between the available QM/MM implementations reside in how to calculate the term $E_{\text{QM/MM}}$. Most common methods include ONIOM [186, 187], Car-Parinello QM/MM [188], effective fragment potential [189] and empirical valence bond method [190].

One of the earliest works using hybrid methods to study the binding of coordination compounds to DNA was done using the cisplatin system with the Car-Parinello/MM scheme [191, 192]. This methodology has been proved efficient to study the non-covalent interactions and dynamics of new cisplatin derivatives with DNA [193]. The ONIOM partitioning scheme was applied in the study of Ruthenium compounds binding to B-DNA. First, the DNA molecule is generated and solvated using explicit solvent and counter ions are added. The system is then heated and equilibrated over a 100ps time frame using MD simulations. The resulting structure is extracted from the solvent and the ruthenium complex is manually placed near two guanine bases. The QM layer was defined as the metal complex, including the two guanine bases, and the MM part is the rest of the DNA chain. This allowed to generate a fully optimized geometry of the placement of the metal complex and the guanine bases and then, use only those three molecules to run a series of transition states to study two possible reaction mechanisms [194].

Even though the information of the rest of the DNA chain is lost, following this methodology allows to study a possible reaction mechanism starting from an explicitly solvated structure.

Final remarks

The development of new coordination compounds that specifically interact with DNA—one of the main targets used in the treatment of several diseases, principally cancer—is the Holy Grail for all the researchers devoted to medicinal chemistry. The treatment of cancer focusing on the interruption of reproductive cycle has been a main objective for many years. Now, with the knowledge of complete genome and the fast growth experimented by the epigenetic, nucleic acids have to be one of the main targets of which chemists have to aim when developing new compounds. The use of transition metals gives a superb tool to drug chemists to develop and study molecules capable of obtaining specific DNA-drug interactions considering the multiple options of d-block metals available in the periodic table. Transition metals are dynamic in geometry, electron affinity and reactivity, making them excellent choices to feed the ongoing field of antineoplastic drug development. Even though the enormous advantages provided by the employment of transition metals related with the three-dimentional arrangement and with the knowledge of the fundamental role played by the intercalating moieties to provide a reversible interaction with DNA, the fundamental factors of this interactions still posses greatest gaps inasmuch as the results provided by experimental designs that do not involve expensive protocols and equipments are extremely poor to identify the specific interactions due the lower energetic changes involved, making clear the use of methodologies such as computational chemistry to help solve these problems. In this review we try to provide a general scope about experimental and theoretical approaches that are used in current research and some of the limitations and success stories found. We consider this area of research a hot and current topic considering the improved experimental and analysis techniques available to synthesize novel coordination compounds designed to accomplish determined task, such as direct and specific interaction with a particular region of DNA.

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