Chemistry and biology of DNA-binding small molecules

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Regulation of the transcription machinery is one of the many ways to achieve control of gene expression. This has been done either at the transcription initiation stage or at the elongation stage. Different methodologies are known to inhibit transcription initiation via targeting of double-stranded (ds) DNA by: (i) synthetic oligonucleotides, (ii) ds-DNA-specific, sequence-selective minor-groove binders (distamycin A), intercalators (daunomycin) combilexins and (iii) small molecule (peptide or intercalator)–oligonucleotide conjugates. In some cases, instead of ds-DNA, higher order G-quadruplex structures are formed at the start site of transcription. In this regard G-quadruplex DNA-specific small molecules play a significant role towards inhibition of the transcription machinery. Different types of designer DNA-binding agents act as powerful sequence-specific gene modulators, by exerting their effect from transcription regulation to gene modification. But most of these chemotherapeutic agents have serious side effects. Accordingly, there is always a challenge to design such DNA-binding molecules that should not only achieve maximum specific DNA-binding affinity, and cellular and nuclear transport activity, but also would not interfere with the functions of normal cells.

Keywords: DNA-binding molecules, duplex DNA, G-quadruplex structures, transcription.

Introduction

IN 1970, Crick[1] enunciated the primary basis of molecular biology, which dictated the one-way flow of genetic information from DNA to RNA to protein. It was further implied that proteins, which could produce observable physical and biochemical characteristics of an individual (phenotype), are determined by an individual's genome or DNA sequence (genotype). In order to decode the DNA, information-carrying RNA intermediary molecules are copied from the DNA in a process called transcription[2]. Transcription is a major determinant of the gene expression that allows cells to proliferate, differentiate and maintain proper homeostasis. These processes serve as a major molecular 'on/off' switch for the expression of genes[3].

Thus, a failure to generate the initial RNA transcript renders unnecessary the regulatory steps that follow, such as transcript processing, transport and translation of the RNA transcript into protein. So the central role played by DNA in biological systems has made it a long-standing target for the diagnosis and treatment for human illness. Small molecules which are capable of recognizing predetermined DNA sequences are powerful tools for interpreting the human genome. These molecules may ultimately prove valuable as therapeutic agents[4].

Structural features of duplex DNA

Double-helical DNA consists of two complementary, anti-parallel, sugar–phosphate poly-deoxyribonucleotide strands which are associated with specific hydrogen-bonding between nucleotide bases[5]. The backbone 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[6]. The chemical feature of the molecular surfaces in a given DNA sequence is distinct in either groove. This forms the basis for molecular recognition of duplex DNA by small molecules and proteins.

Interaction of duplex DNA with small organic molecules

Covalent interaction of duplex-DNA with small organic molecules

Cancer chemotherapy was found on agents that interact with DNA or alkylate it, and such compounds continue to be clinically important today. Over the last two decades, wide ranges of alkylating agents (Figure 1) have been synthesized in an attempt to control their inherent chemical reactivity. Alkylating agents are involved in reaction with the preferential N-7 position of guanine and N-3 of adenine in DNA. Thus the base pairing of the DNA could be inhibited and this leads to miscoding of DNA. In the first mechanism, an alkylating agent attaches alkyl groups

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to DNA bases. This alteration results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. A second mechanism by which alkylating agents cause DNA damage is the formation of cross-bridges, bonds between atoms in the DNA. In this process, two bases are linked together by an alkylating agent that has two DNA-binding sites. Cross-linking prevents DNA from being separated for synthesis or transcription. The third mechanism of action of alkylating agents causes mis-pairing of the nucleotides leading to mutations. The nitrogen mustards were the first alkylating agents used medically[7], as well as the first modern cancer chemotherapies. Cyclosporamide is a classic example for the role of the host metabolite in the activation of an alkylating agent and is one of the most widely used agents of this class. It was hoped that the cancer cells might possess enzymes capable of accomplishing the cleavage, thus resulting in the selective production of activated nitrogen mustard in the malignant cells. Another well known covalent DNA binder used as an anticancer drug is *cis*-platin (*cis*-diammine-dichloroplatinum), which makes an intra/interstrand cross-link with the nitrogens on the DNA bases and used extensively in testicular, ovarian, head, and neck cancers[8]. The early success of *cis*-platin as an anticancer drug has led to the development of other less toxic derivatives such as carboplatin. However, these agents are mostly non-specific in their action[9].

Non-covalent interactions of small molecules with duplex-DNA

Duplex-DNA intercalators: Molecules that bind to double-stranded DNA (ds-DNA) by intercalative mode have been significantly used as drugs (Figure 2). The binding of these molecules to DNA is characterized by insertion of planar aromatic rings between the DNA base pairs. This interaction can be quite strong despite the fact that energy is consumed to unwind the helix and unstack the base pairs to allow the complex formation. The stability of intercalation complexes is governed by van der Waals, hydrophobic and electrostatic forces. Intercalation is generally independent of base-pair sequence. The two major types of intercalation-binding modes are: (1) classical intercalation and (2) threading intercalation. Binding by the classical mode is typified by the much-studied DNA stain ethidium bromide and the antimalarial quinacrine[10]. An important contributor to the binding affinity of ethidium bromide and quinacrine to DNA is the stacking interaction of the respective heteroaromatic rings with the DNA base pairs. In case of quinacrine, the cationic side-chain contributes to complex stability by electrostatic interactions with the negative phosphate units of the groove. A number of other classical intercalators are present which are used as antitumour agents, including the anthracyclines and actinomycins[11,12]. Intercalation preferentially occurs at G/C-rich sequences (CpG sites), because these sequences get unstacked easily. Intercalators generally cause more significant distortion of the native conformations of DNA, a factor that contributes to the disruption of protein binding.

As threading intercalators typically have two side-chains on opposite sides of a planar aromatic ring system, the process of complex formation with DNA is more complicated. In such cases, one of the side-chains must slide through the intercalation cavity in order to form the complex. Favourable interactions of the side-chains with both the major and minor grooves contribute to the complex stability of the threading intercalators. The significant affinity and base-pair specificity of the antineoplastic agent, nogalomycin, has been attributed to the presence of side-chains in each groove in the DNA complex[12]. Details of the molecular intercalation process and structural requirements of the small-molecule intercalators are now well understood, and these fundamental aspects have been carefully and comprehensively reviewed[10]. Daunomycin is an example of the threading intercalators.

Duplex-DNA groove-binding molecules

Groove binders are another major class of small molecules that bind to ds-DNA and play an important role in drug development. In principle, molecules can bind to both the major or minor groove of ds-DNA. As the

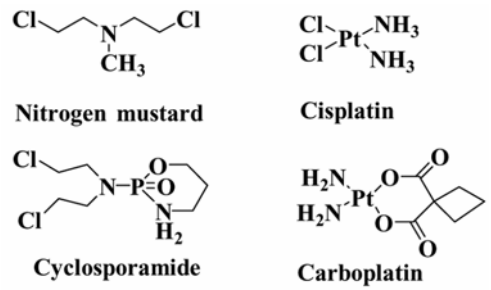

Figure 1. Chemical structure of some DNA alkylating agents.

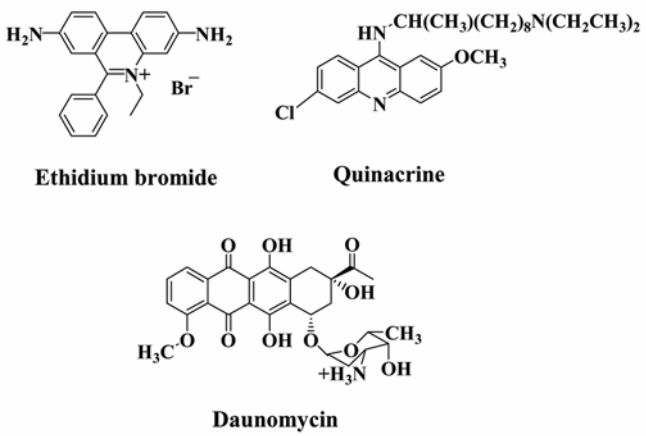

Figure 2. Chemical structure of some DNA intercalators.

dimensions of the two grooves are different, targeting them requires vastly dissimilar and different shaped molecules. The major groove, as the name implies, is much wider than the minor groove; the groove width values for averaged-sequence B-form ds-DNA are 11.6 and 6.0 Å respectively[13]. Due to this dimensional difference, the major grooves are the site for binding of many DNA-interacting proteins[14]. To date there are limited reports of non-protein molecules that bind to the DNA major groove[15].

Duplex-DNA major-groove binding small organic molecules: Proteins can recognize and bind to ds-DNA by reading the sequence information in either groove, but most often by major groove recognition. However, non-peptidyl compounds show a reverse preference, they bind with the minor groove, thus potentially allowing simultaneous major-groove recognition by proteins[16]. It is desirable to have a major-groove binding molecule that could block access to proteins that recognize the same groove. This could be done if sufficient affinity and sequence selectivity could be achieved. Duplexes that are made up of polypurine–polypyrimidine sequences can be read by oligomers that bind in the major groove and form hydrogen bond with bases of the purine strand. These are called triplex-forming oligonucleotides (TFOs)[17,18]. In this case the orientation of the third strand relative to the duplex is dependent on the sequence[19]. An alternative form of major-groove recognition could be achieved by peptide nucleic acids (PNAs; Figure 3)[20]. These are different from TFOs in two respects; first, the backbones of TFOs are oligonucleotides or their modified analogues, whereas PNAs have a peptide-like backbone. Second, TFOs can bind within the existing major groove of ds-DNA, whereas PNAs generally invade the helix to form a triplex[21], which then results in the displacement of non-complementary oligopyrimidine DNA strand. In order to target the major groove it is necessary to artificially modulate biological processes, for example, by transcription. TFOs and PNAs could be changed with sufficient sequence selectivity and affinity. Inhibition by transcription can either result from competition with transcription-factor binding or from physical arrest of the RNA polymerase. For activation of the transcription, the TFOs should be covalently linked to an activation domain. Alternatively, PNAs can be used to induce strand displacement and formation of a D-loop, which is then used to initiate transcription *in vitro* and in cell culture.

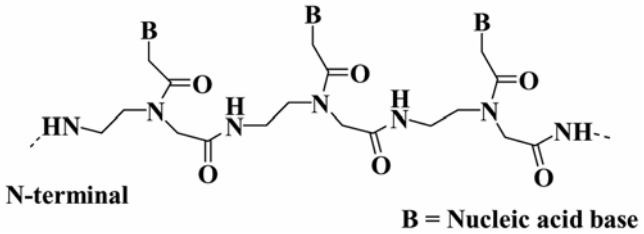

Figure 3. Chemical structure of peptide nucleic acid.

DNA minor-groove binding molecules: The amenability of the minor-groove to bind with small molecules (Figure 4) has led many investigators to focus on this aspect[13,22,23]. It has been speculated that the evolution of antibiotic minor-groove binders that target the DNA of competing organisms is related to the more attractive dimensions of the minor groove for small molecules[24]. On the other hand, minor-groove binding usually involves greater binding affinity and higher sequence specificity than that of intercalator binding.

Minor-groove binding has been demonstrated for neutral, mono-charged and multicharged ligands. Detailed discussions on the mechanism of minor-groove binders have been reviewed[22]. The forces that dominate small molecule–minor-groove binding interactions are electrostatic, van der Waals, hydrophobic and hydrogen-bonding.

Sequence specificity is often attributed to key hydrogen-bonds between the base pairs and the small molecule. Another crucial structural requirement of the small molecule is its crescent shape that is complementary to that of the minor-groove (radius of curvature). Frequently, minor-groove binders show pronounced AT selectivity. Several factors contribute to this commonly noted preference. The electrostatic potential is higher in AT-rich grooves than in the GC-filled ones[25]. This generally leads to AT selectivity for the dicationic molecules. On the other hand, the dimensions of the minor groove at AT sites are narrower and deeper than at the GC locations. A major contributor to this difference in dimension has been attributed to the differences in the ionic interactions in the two types of base-pair tracts[26]. The topology at AT sites allows for easier filling and greater van der Waals contacts by small molecules. Furthermore, the amino group of G in GC locations protrudes into the groove, thus prohibiting van der Waals contacts comparatively to those achievable at the AT sites[27]. The cationic minor-groove binders include the natural products netropsin and

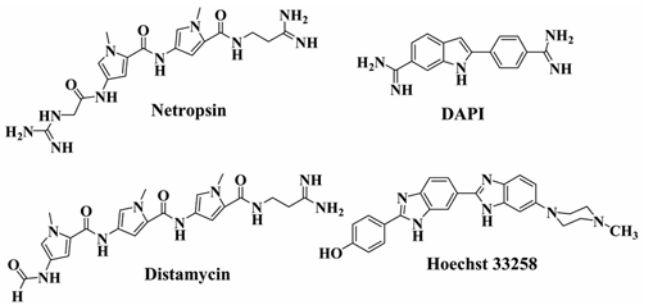

Figure 4. Chemical structure of some DNA minor groove-binding agents.

distamycin[28], and the synthetic molecules DAPI[29] and berenil[30], which have been intensively studied.

Nearly two decades of research in the laboratory of Dervan have engendered a new paradigm for the sequence-specific recognition of the DNA minor groove[4,23,31]. DNA-binding polyamides composed of *N*-methylimidazole (Im), *N*-methylpyrrole (Py), and *N*-methyl-3-hydroxypyrrole (Hp) are crescent-shaped molecules that bind to the minor groove as antiparallel dimers. The DNA association is driven by a combination of van der Waals and hydrogen-bonding interactions. Side-by-side pairings of aromatic residues stack five-membered heterocycles against each other and the walls of the minor groove, positioning the polyamide backbone and aromatic 3-substituents for intimate contacts with the edges of nucleotide bases on the adjacent DNA strand. The co-facial arrangement of the side-by-side pairings exploits the specific pattern of hydrogen-bond donors and acceptors, as well as subtle variations in the molecular shape of the minor-groove floor to distinguish the Watson–Crick base pairs[32]. Bhattacharya and co-workers[33] have reported some longer distamycin analogues without the leading amides, which resulted in high affinity and specificity towards AT-specific stretches of ds-DNA.

The synthetic bis-(benzimidazole) derivative Hoechst 33258 (Figure 4) is widely used in chromosome staining[34], and also possesses antitumour activity and is an inhibitor of DNA topoisomerase I[35]. DNA foot-printing studies have shown that Hoechst 33258 recognizes 3–4 consecutive A/T base-pairs by hydrogen bonding to base edges in 1 : 1 complex in the minor groove of duplex DNA. A number of crystal structure analysis and NMR studies of Hoechst 33258 complex to various oligonucleotide duplexes containing stretches of AT base pair have been reported[36,37].

Design of low molecular mass compounds, which bind with high affinity and specificity to pre-determined DNA sequences that are 10–16 base pairs long, is a key issue in chemical biology[38]. Hence the first strategy is to generate new DNA targetable compounds, e.g. the groove-binding agents. In order to increase their length of the binding site, one approach is to covalently connect such minor-groove recognizing units via optimized linkers[39]. Dervan and co-workers reported various types of multimeric AT/GC minor-groove targeting molecules based on *N*-methylpyrrole oligopeptides (distamycin analogues) or *N*-methylimidazole synthetic frames[40,41]. These authors achieved this by changing the connectivity, nature and length of the linker units[42]. The side-by-side 2 : 1 DNA minor-groove binding mode of the monomer[42] led them to devise an interesting strategy, that is, to connect two polypeptides at the ends via a suitable 'turn peptide' that would not disturb the side-by-side binding of the monomeric peptide with DNA. They used amino acids like glycine, β-alanine and γ-aminobutyric acid as linkers that allowed the linear peptide 'dimer' to fold back upon itself, thereby forming hairpin dimmers (Figure 5)[43]. High-affinity recognition up to ~ 10 base-pair sites has been achieved, for example, with β-alanine-linked polyamides for 1 : 1 recognition[44] and also with head-to-head hairpin dimmers[41]. On the other hand, 'tail-to-tail'-linked distamycin analogues devised by Bhattacharya and co-workers, bind ds-DNA with high enhanced affinity and cover approximately 8–10 base pairs in its binding site[39].

Several research groups have concentrated on the strategy that utilizes the pharmacophore-like benzimidazole motif derived from the well-established bis-benzimidazole drug, Hoechst 33258. In an attempt to increase the binding site-size affinity, several derivatives of Hoechst 33258 have been synthesized (Figure 5)[45]. Symmetric dimeric bisbenzimidazole derivatives have been independently developed by Joubert *et al.*[46], and Chaudhuri *et al.*[47]. Recently, Janada *et al.*[48a] have reported new bidentate constructs of Hoechst 33258. A number of benzimidazole trimers have also been synthesized and evaluated for various biological activities. These trimers are capable of recognizing up to seven base pairs still as 1 : 1 complexes. Attempts to target longer sequences with extended molecules, synthesized by incorporating a larger number of monomeric units, failed because the curvature of these extended molecules did not match with the natural curvature of the minor groove of DNA[48b]. Bruice *et al.*[49] reported a tripyrrole peptide–Hoechst 33258 conjugate, which resulted in high affinity and specificity over nine base pairs still as 1 : 1 complex (Figure 5).

Intercalator–minor-groove binding hybrid molecules

Compounds most commonly bind to DNA by intercalating between the DNA base pairs or by binding in the DNA minor groove. Natural products such as netropsin and distamycin A are crescent-shaped molecules that contain an oligo (pyrrole carboxamide) chain and cationic end side chains that result in high affinity for the AT-rich sequences of DNA[22,50]. In principle, hybrid molecules that contain both intercalating and minor-groove binding functionalities should be able to interact more strongly with DNA than those having either of the individual functionality, and thus should have a prolonged residence time on DNA allowing them to interfere with DNA-processing enzymes[50,51]. These hybrid molecules, because of their dual binding mode of action, have been called combilexins, and are composed of intercalators based on analogues of amsacrine, ellipticine, anthraquinones and mitoxantrone and minor-groove binders based on analogues of netropsin or distamycin A, etc.[22,51]. Combilexins should, in principle, also have enhanced DNA sequence specificity compared to mono-intercalators or minor-groove binders. The well-known and potent

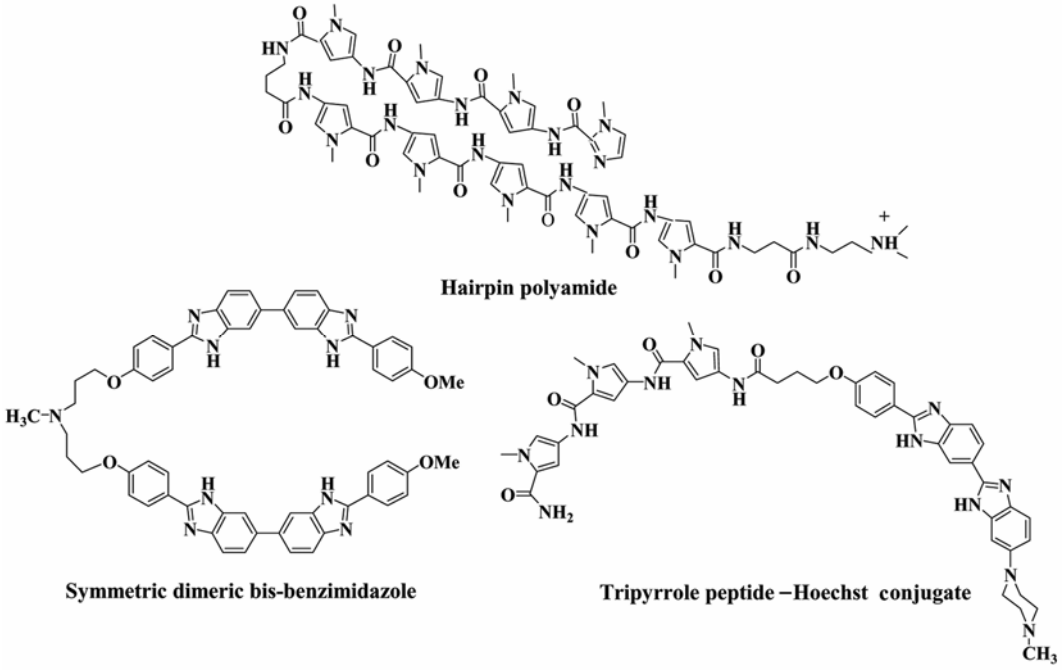

Figure 5. Chemical structure of some DNA minor groove-binding dimers.

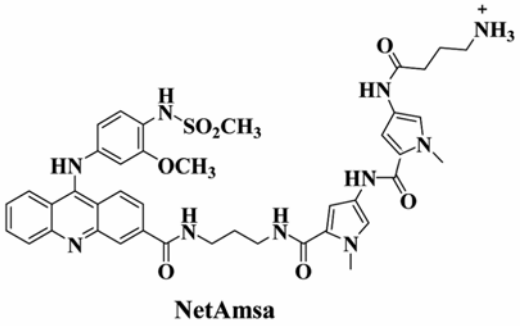

Figure 6. Example of a combilexin.

antitumour drug, NetAmsa (Figure 6), is a good example of the combilexins. This is derived from a covalent combination of the minor-groove binder, netropsin and the intercalator, amsacrine. This molecule shows three modes of binding with duplex DNA: (i) sequence-specific recognition of the minor groove of the DNA double-helix via the netropsin moiety; (ii) intercalation of the acridine chromophore inside the duplex DNA, and (iii) threading of the methane sulfonanilino group into the major groove[50]. The design of DNA-threading combilexins provides an original route for the development of sequence-specific ligands capable of forming stable complexes with DNA.

Small molecule–oligonucleotide conjugates

Another approach to regulate gene expression specifically and selectively is to conjugate deoxy-oligonucleotide (ODN) to the small molecules that are either minor-groove binders or intercalators or DNA-recognizing peptides. There are many reports where different cell targeting[52], cell signalling[53] and cell receptor[54]-based or structure-specific peptides were efficiently conjugated with a particular ODN to enhance cellular uptake as well as to improve cell structure[55] or conformation-specific[56] oligonucleotide delivery. The other approach lies in the design of covalent attachment of minor-groove binders (e.g. Hoechst 33258, distamycin derivative, $CDPI_3$, etc.; Figure 7)[57,58] or intercalators (e.g. naphthalene tetra carboxylic diimides, acridine, oxazolopyridocarbozole, benzophenone, etc.)[59,60] to ODNs in order to enhance target specificity and stabilizing the resultant duplex or triplex structures[61]. Many reports are known where ODNs were conjugated with DNA cross-linking substituent[60] (psoralen, dimethylanthraquinone, mitomycin, and 2-amino-6-vinylpurine), alkylators[62], fluorophores[63] and cleavable groups[64] to achieve oligomer-targeted or hybridization-induced cross-linking, alkylation[65], fluorescence[66] and cleavage[67]. However, the nature and length of the linker between the ODN and small molecules are crucial to get the maximum specificity and selectivity of each fragment.

DNA-interacting organometallic compounds

Transition metal complexes that are suitable for binding and cleaving nucleic acids are of significant current interest due to their various applications in nucleic acid chemistry, like foot-printing studies and sequence-specific binding agents and also as a putative anticancer drugs[68].

The discovery of *cis*-platin in 1965 has given an impetus to study transition metal complexes as anticancer agents[69]. Transition metal complexes with their varied coordination geometry, and versatile redox and spectral properties are often suitable for designing metal-based anticancer drugs[70]. Since the last few decades, *cis*-platin and its analogues have been clinically used as effective chemotherapeutic drugs[71]. Iron-bleomycins (Fe-BLMs) are natural antitumour chemotherapeutic agents that oxidatively cleave cellular DNA by targeting the deoxyribose sugar moiety[72]. The transition metal-based chemotherapeutic drugs have, however, various side effects and they also suffer from problems related to intrinsic drug resistivity and prolonged dark toxicity[73]. Further, an alternative strategy is required to circumvent the problems associated with the chemotherapeutic drugs. In order to do so, there is a need of designing and synthesizing metal-based anticancer agents that can be selectively activated by light in the cancer cells, while they remain non-cytotoxic in dark. Therefore, photodynamic therapy (PDT) has emerged as a method of non-invasive treatment of cancer with an advantage of having localized photoactivation of the drug at the targeted tumour cells leaving the healthy cells, unaffected from the photo-toxicity of the PDT agent[74].

Types of DNA cleavage

DNA cleavage can be achieved by targeting its basic components like phosphodiester linkages, deoxyribose sugar or nucleobases. Hydrolysis of the phosphodiester bond leads to the DNA strand scission, which is known as the hydrolytic cleavage of DNA. Lewis acidity of the central metal atom facilitates the hydrolytic cleavage of DNA. However, oxidative DNA cleavage occurs due to the oxidation of deoxyribose sugar or nucleobases. Oxidative DNA cleavage requires the presence of co-reactants like reducing or oxidizing agents or light or molecular oxygen, in addition to the principal agents.

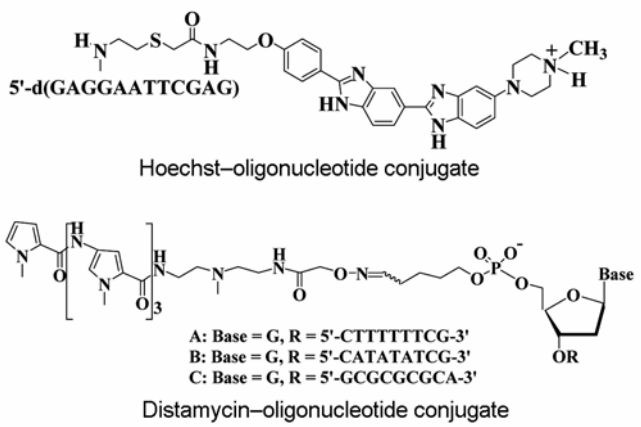

Figure 7. Chemical structure of Hoechst–oligonucleotide and distamycin–oligonucleotide conjugates.

Oxidative DNA cleavage can be induced by singlet oxygen, hydroxyl radical, photo oxidation, hydroperoxides, ionizing radiation, or many other transient radical species[75].

Oxidative DNA damage by sugar degradation process involves abstraction of hydrogen atom of the deoxyribose since the aliphatic nature of deoxyribose does not allow oxidation by electron abstraction. However, oxidative DNA damage generally occurs at the guanine (G) nucleobase, which has the lowest oxidation potential among all the natural nucleobases[76]. Guanine is more susceptible to oxidation by singlet oxygen ($^1O_2$) or electron transfer mediated by DNA-bound metal complexes. Moreover, oxidative nucleobase damage can also be accomplished by oxo-transfer by metal complexes activated by persulfate or other oxygen donors[77]. Oxidative damage of DNA mediated by the diffusible reactive oxygen species (ROS) can lead to severe cytotoxic effect. The oxidative damage is irreversible and hence cannot be repaired by cellular repair mechanism, which leads to apoptosis[78]. This in fact offers a great challenge to the therapeutic applications.

Metal-based DNA photocleavers

Transition metal complexes with their varied coordination environments and tunable redox and spectral properties are able to interact with duplex DNA in various ways. Transition metal complexes that bind to DNA in a covalent or non-covalent fashion or induce DNA strand scission have potential applications as DNA structural probes and as anticancer agents[79]. The unique properties of metal complexes offer various pathways to cause DNA lesion by modulating the nature of both the metal and the ligand system. Many transition metal (Rh, Ru, Os, Re, Pt, Cu, Co, Fe, Mn, etc.) complexes have been investigated as photonucleases. Due to their low-energy, electronic bands in the visible region offer promising alternatives to porphyrins and their analogues[80].

Chow and Barton[81] have extensively studied ds-DNA cleavage activity by Rh (II) complexes containing polypyridyl aromatic ligands. $[Rh(phen)2(phi)]^{3+}$ having 9,10-phenanthrenequinone diimine (phi) binds to B-DNA by intercalation of the phi ligand from the major groove and in the photo-excited state abstracts a hydrogen atom from C-3′ at the intercalation site (Figure 8)[82]. These complexes show wide variation in sequence selectivity by utilizing the nature of the aromatic ligand and the stereochemistry of the complexes. Turro and co-workers have reported a series of dirhodium(II) dppz complexes, e.g. *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppz)(\eta^1\text{-}O_2CCH_3)(CH_3OH)]^+$ and *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppz)_2]^{2+}$ showing photolytic cleavage of the plasmid and cellular DNA *in vitro* (Figure 8). These complexes have the ability to damage DNA in visible light ($\lambda \geq 395$ nm) via both oxygen-dependent and independent pathways attributed to the labile equatorial group[83].

Polypyridyl ruthenium(II) complexes, viz. $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$ and $[Ru(dsdp)_3]^{4-}$ (bpy = 2,2′-bipyridine, ddz = dibenzo[*h*,*j*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine, phen = 1,10-phenanthroline and dsdp = 1,10-phenanthroline-4,7-diphenylsulfonate) are well-known DNA photocleavers that generate singlet oxygen[84]. $[Ru(bpy)_2(dppz)]^{2+}$ having dipyrido[3,2-*a* : 2′,3′-c]phenazine (dppz) binds to B-DNA by intercalation of the dppz ligand from the major groove and causes oxidative damage of DNA by guanine oxidation[85].

$[Ru(bpy)_2(dpb)]^{2+}$ (dpb = 2,3-*bis*(2-pyridyl)-benzo[g]quinoxaline) exhibits DNA photocleavage activity in anaerobic conditions at very long wavelength MLCT absorption (≤ 500 nm)[86]. This compound is promising for potential application in the PDT of hypoxic tumours. Rose *et al.*[87] have recently reported a Ru(II) nitrosyl complex that shows PDT effect causing cellular damage on photo release of nitric oxide (NO) at the cancer cells on photolysis in visible light ($\lambda \geq 465$ nm).

The cytotoxicity has been found to be due to the photo-delivery of NO, which induces apoptosis to the MDA-MB-231 human breast cancer cells[88]. Mackay *et al.*[89] have shown that a Pt(IV) complex of *trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ having two photo-labile *trans* azide ligands forms a Pt(II) *cis*-platin analogue on photo-activation ($\lambda = 420$ nm). This generates a species *in situ*, which is cytotoxic towards a variety of cancer cells such as human HaCaT keratinocytes, A2780 human ovarian carcinoma cells and A2780 *cis*-platin resistant cells[89]. Cleavage activity of a variety of Cu(II) complexes has also been reported[90]. The photo-induced DNA cleavage activity of ternary Schiff base (salSMe) Cu(II) complexes [Cu(salSMe)B] (B = phenanthroline bases), having Cu–S bond has been reported by Reddy *et al.*[91] (Figure 8). The photo-induced DNA cleavage reaction proceeds via the formation of singlet oxygen on excitation of the sulphur-to-copper(II) charge transfer transition, and weak Cu(II) d–d band (~ 600–750 nm). The binary complex $[Cu(dpq)_2(H_2O)](ClO_4)_2$ (dpq = dipyridoquinoxaline) exhibits DNA cleavage activity[92] via type-II pathway in UV–vis and photo-redox pathway under red light of 694 nm. A ferrocene-conjugated Cu(II) complex which shows visible, light-induced DNA cleavage activity via photo-redox mechanism has been reported[93].

Chang *et al.*[94a] have reported Co(III)-bleomycin as a first compound of cobalt which shows DNA cleavage activity in UV or visible light irradiation. The DNA cleavage mechanism was proposed that HOO–Co(III) BLM, produced by photoirradiation in the presence of oxygen, initiated DNA strand scission through C4′−H abstraction resulting in site-selective damage of DNA. Water-soluble Co(II) salen complexes were shown to activate oxygen under ambient conditions that led to pronounced double-strand scission of DNA[95]. There are other reports on oxidative cleavage of plasmid DNA by several metal complexes of different functionalized salen derivatives[96]. Barton and co-workers[97] have reported Δ and Λ enantiomers of $[Co(dip)_3]^{3+}$ that show stereospecific ds-DNA cleavage activity on photoactivation. DNA photocleavage activity of methylcobalamin (coenzyme B_{12}) and B_{12} analogue, $[(DH)_2(CH_3)Co(py)]$ ($(DH)_2$ = bis(dimethylglyoximate), py = pyridine) has been studied by Tanaka *et al.*[98a] under anaerobic conditions (Figure 9). Homolytic cobalt–carbon bond cleavage of coenzyme B_{12} and organocobaloximes occurs on UV-A light irradiation producing methyl radical, which is

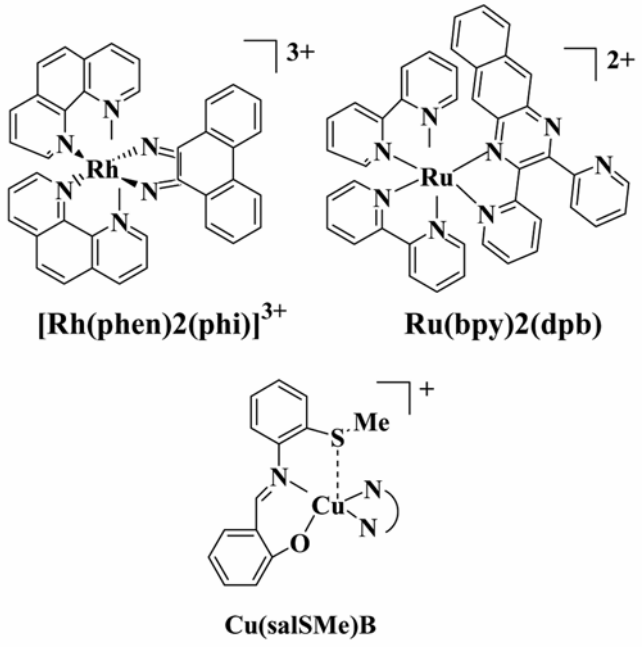

Figure 8. Chemical structure of some organometallic compounds.

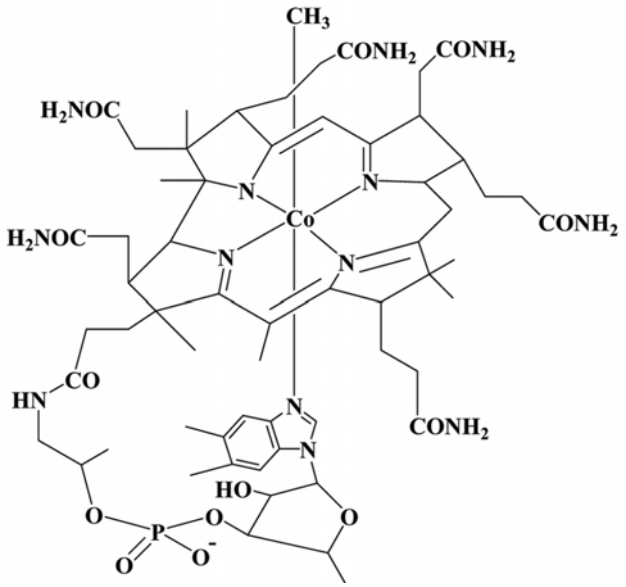

Figure 9. Chemical structure of coenzyme B_{12}.

responsible for showing DNA strand scission under anaerobic conditions. The dipyridophenazine (dppz) complex of cobalt(III) shows UV-A, light-induced DNA cleavage activity by forming $^{\bullet}OH$ radical as the cleavage-active species[98b]. Another example of the cleavage of plasmid DNA pTZ19R under visible light by cobalt–bis-picolylamine complexes has been reported by Bhattacharya and Mandal[99].

There are few reports on photo-induced DNA cleavage activity of iron complexes. Maurer *et al.*[100] reported visible light-induced DNA cleavage activity of *tris*(3-hydroxy-1,2,3-benzotriazine-4(3H)-one]iron(III) complex[100]. This complex releases nitrogen radical and causes DNA strand scission on photoactivation at visible wavelength of ligand-to-metal (LMCT) band. Di-iron complexes of 1,4- and 1,3-$Fp_2C_6H_4$ cleave plasmid DNA upon photolysis through a Pyrex filtered lamp[101]. These complexes show DNA cleavage activity by forming carbon centred radicals as the reactive species.

There is another recent report on *rac*-[Fe(diimine)$_3$]-$(ClO_4)_2$ complexes[102]. These complexes exhibit DNA cleavage abilities at 10 mM concentration in the presence of 100 mM H_2O_2. The anticancer activity of these complexes against human breast cancer cell line (MCF-7) has also been studied, and these are toxic towards cancer cells at very low concentration (0.8–20 μM).

Ansari *et al.* have recently reported Mn^{III}-salen and -salophen complexes[103] that show selectivity toward different cancer cells such as MCF7 (breast cancer) and CCL228 (colon cancer) over a normal non-malignant cell MCF10 (breast epithelial cells).

Topoisomerase enzyme and supercoiled DNA

DNA topoisomerases are ubiquitous enzymes that play a pivotal role in modulating the dynamic nature of supercoiled DNA, and thus provide essential functions inside the cell. Thus, topoisomerases play an important role in DNA replication, transcription and recombination. DNA topoisomerases are the targets of a number of antibacterial and anticancer chemotherapy agents, such as fluoroquinolones, pentamidines, acridines, camptothecins and epipodophyllotoxins[104].

Supercoiling of DNA

The topology of closed circular DNA is characterized by its linking number (L). This represents the number of times one strand of the duplex passes over the other when viewed on a planar surface. The linking number is a sum of twist and writhe ($L = T_w + W_r$), where twist represents the local pitch of the helix and writhe is the geometric in space[105]. Therefore, for a completely relaxed molecule ($L = L^o$), since the writhe is zero, its twist is the number of base pairs (N) divided by the pitch; consequently $T_w = N/10.5$. In covalently closed circular ds-DNA isolated from natural sources, supercoiling originates due to deficiency of duplex turns relative to its relaxed state where ($L < L^o$), and the DNA is positively supercoiled (Figure 10). The linking number of closed, circular molecules is a topological invariant. However, this can be altered by transient breakage of one of the duplex strands. Under these conditions, the extent of twist and writhe is modified by environmental parameters such as temperature, ionic strength and concentration of certain DNA-intercalating ligands[106].

Topoisomerase enzymes

DNA topoisomerases (Topo) are involved in the regulation of DNA topology and its degree of supercoiling[107,108]. Two types of the enzymes are known: type I Topos change the degree of supercoiling of DNA by causing single-strand breaks and religation; type II Topos (including bacterial gyrase)[109] cause double-strand breaks. Topo I is dispensable for a living cell, whereas Topo II is not. The different roles of DNA Topo I and Topo II may reflect the need for fine regulation of DNA supercoiling efficiency. The functions of Topo I and Topo II are especially crucial during DNA transcription and replication, when the DNA helix must be unwound to allow the large enzymatic machinery to operate properly. Topos have indeed been shown to maintain both transcription and replication. Recently, some new members of the family of both types I and II have been discovered. These may contribute to even more specialized roles for the topoisomerases, with some enzymes implicated in the regulation of recombination events, chromatin remodelling, chromatin condensation or decondensation, recombination, segregation of newly replicated chromosomes and DNA repair[110].

Topoisomerase I

Topo I participates in the relaxation of negative supercoils and has also been implicated in knotting and

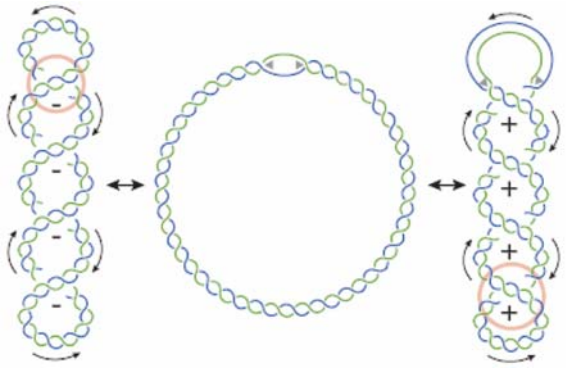

Figure 10. Negative supercoiled DNA (left), closed circular DNA (middle) and positive supercoiled DNA (right). Adapted from Schvartzman, J. B. and Stasiak, A., *EMBO Reports*, 2004, **5**(3), 256–261.

unknotting DNA and in linking complementary rings of single-stranded DNA into double-stranded rings[107]. The relaxation process catalysed by the enzyme essentially consists of a transient break of a segment of single-stranded DNA. This is followed by passing an intact strand of DNA through the gate (type I-A) or allowing rotation of the broken strand around the intact strand (type I-B), and finally rejoining of the broken segment. Topo I has several distinct features and it appears to fully perform its functions as a monomer. In addition, it does not require ATP hydrolysis to catalyse the complex topological rearrangements of DNA.

Catalytic process

The enzymatic cycle of Topo I can be divided into the following steps[108]: (i) binding of the enzyme to DNA; (ii) cleavage of a single DNA strand; (iii) passage of an intact strand through the gate, and (iv) DNA re-ligation. Once the initial protein–DNA complex is formed, a transesterfication process occurs between a key catalytic Tyr residue and a phospho-diester bond in the backbone of the single-stranded DNA segment. In type IA Topos, the 3′-oxygen in the backbone is displaced by a tyrosine oxygen, covalently linking the tyrosine oxygen to phosphorus at the 5′-backbone nick. Thus, the 5′-nick is covalently linked to Topo I in domain III. In type IB enzymes the 5′-oxygen is displaced by Tyr and the protein is bound at the 3′-backbone nick. Besides tyrosine, an important catalytic role is played by a highly conserved histidine residue[111]. Studies on mutated proteins indeed show that it does not act as a general acid to protonate the leaving 5′-oxygen during the cleavage reaction, but participates in stabilizing the pentavalent transition state through hydrogen bonding to one of the non-bridging oxygens. The covalent linkage to DNA causes a severe structural deformation of the protein in the 'closed' form, which strongly modifies the non-covalent interfaces between different domains containing many of the active-site residues. The rigid part formed by domains II and III moves away from the remainder of the structure, generating the 'open' conformation of the enzyme. The 3′-nick remains non-covalently associated with the DNA-binding domain I, whereas the 5′-nick will move up with domains II and III, thus creating a gate in the single-stranded segment. Now, either the passing element travels through the transient gate or a controlled rotation of the covalent enzyme–DNA complex around an intact DNA strand occurs changing the relative positions of the cleaved and non-cleaved DNA fragments. At this point, the open form reverts to the closed form to allow rejoining of the broken DNA ends through nucleophilic attack of the nick phosphorus by the nick OH group. The passing DNA segment is released from the complex, completing the strand passage event. The rejoined single-stranded DNA element may remain non-covalently associated with the enzyme for reiteration of the mechanism.

Topoisomerase I interacting organic molecules (Topo I poison)

Many of the topoisomerase-targeting drugs act by converting the enzyme to a DNA-damaging agent by stabilizing the covalent enzyme–DNA intermediate known as the cleavage complex. In this complex, the re-ligation step of the topoisomerase reaction is inhibited. The presence of the cleavage complex interferes with DNA metabolism and ultimately leads to irreversible DNA damage[112,113]. Camptothecins (Cpt) and epipodophyllotoxins (Figure 11) are examples of anticancer drugs that target topoisomerases I and II respectively[113]. Cpt was later shown to stabilize the covalent Topo I–DNA cleavage complex by preventing DNA re-ligation. In fact, Cpt both inhibits DNA relaxation and induces cleavage complexes (protein-linked DNA single-strand breaks)[114]. The drug-stabilized cleavable complex formation is a reversible molecular event. In addition, the breakage of single strand due to Topo I–Cpt intercalators could in principle be easily repaired. Hence, it is difficult to explain the outstanding anti-proliferative activity exhibited by Topo I poisons to account for cell-death stimulation. It is believed that collisions between ternary (drug–Topo I–DNA) complexes and advancing replication forks generate double-strand breaks. These lesions are much more toxic than single-stranded breaks, and thus by recruiting other factors like p53, could trigger the cell-death programme[115]. The tropotecan, camptothecin analogues are uncompetitive inhibitors of Topo I and bind to the complex by intercalating between the ds-DNA bases of both strands at the enzyme-induced nick[116].

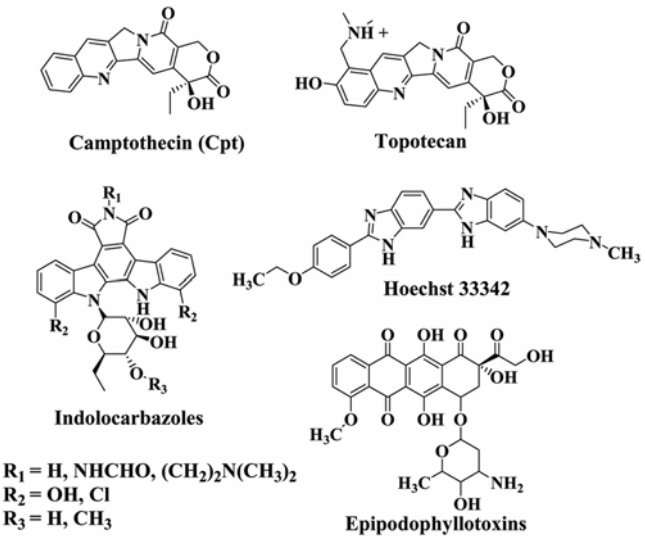

Figure 11. Chemical structure of topoisomerase I interacting small molecules.

Several DNA minor-groove binding drugs interfere with the catalytic activity of the enzyme. Among them minor-groove binding bisbenzimidazole derivatives Hoechst 33258, Hoechst 33342 and trisbenzimidazole 5PTB deserve to be mentioned[117]. Many of these drugs inhibit topoisomerase I action in a manner similar to Cpt. This occurs via stabilization of enzyme–DNA cleavable complex. Hoechst 33342 traps Topo I into reversible cleavable complex both *in vitro* and in human tumour cells[118].

The work on parasite topoisomerases has become a growing area of interest in the field of medicinal research[119]. Its significance as a human pathogen has made *Leishmania donovani*, the causative agent of human visceral leishmaniasis, the focus of intensive study. Toxic chemotherapy, increasing drug resistance of some parasite strains to classical drugs and co-infection of leishmaniasis with HIV, have made these parasites a severe threat to public health in the developing countries. Development of vaccines is still under trial and improved therapy is desirable[120].

Several organic small molecules which have emerged as potent inhibitors of Topo I of parasitic pathogen *L. donovani* were studied. Recently Chaudhuri *et al.*[121] have reported anthra [1,2-d]imidazole-6.11-dione derivatives as highly potent *L. donovani*, topoisomerase I inhibiting new molecular entities.

Targeting to G-quadruplex DNA: telomerase inhibitors

Another selective DNA anti-cancer target that has recently emerged is the telomeric DNA. This arises from the tandem repeats of short guanine tracts at the end of the chromosome. Somatic cells have a finite lifespan because normal DNA polymerase is unable to fully replicate the ends of telomeric DNA. Therefore, their telomeres progressively shorten with successive rounds of replication until they become critically short. When the cells enter irreversible replicative senescence, cell apoptosis begins. Tumour cells are able to stabilize their telomeric ends. This occurs by the action of the reverse transcriptase enzyme telomerase, which is not normally expressed in somatic cells. This catalyses the synthesis of telomeric DNA in order to balance those lost during replication[122]. Proof-of-principle experiments using antisense, dominant-negative telomerase or active-site inhibitors have shown that the inhibition of telomerase in cancer cells can cause senescence and apoptosis of the cancer cells[123]. From these paradigms, telomere and telomerase have emerged as the interesting therapeutic targets for cancer therapy, allowing restoration of a replicative senescence to cancer cells[124].

Telomeric sequences can adopt a wide range of topologies when folded into four-stranded guanine G-quadruplex (G4) arrangements and other complex folded structures. The full range of their structures is yet to be understood[125]. However, all contain several units of an essential building block, the guanine (G) quartet (Figure 12). This highly stable arrangement comprises of four coplanar guanine bases linked by pairs of Hoogsteen-type hydrogen bonds. The G-quartet requires the association of four DNA strands, where both intra- and intermolecular arrangements, which have distinct relative polarities, are possible. Stabilization of this structure by ligands would render the 3′-overhangs unavailable for hybridization with the telomerase template for the extension of the telomeric ends[126].

Structural features of G-quadruplex DNA

In 1910, it was found that gelatinous aggregates form from the guanine-rich DNA solution. However, their exact nature was discovered only in 1962 by Gellert and co-workers[127], who proposed that these gels form planar guanine tetramers which stack into cyclic arrangements. G-quadruplexes, as the name suggests, have a core that is made up of guanine bases with four guanines arranged in a symmetric manner, making Hoogsteen hydrogen bonds from N1–O6 and N2–N7 around the edges of the resulting square (Figure 12). These planar structures are called G-quartets. These G-quartets have large π-surfaces, and hence they tend to stack on each other due to π–π stacking, as well as to enable cations to intercalate between the G-quartets. In particular, ODNs with contiguous runs of guanine, such as $d(TGGGT)_n$ can form stacked structures with the G-quartets that are linked by the sugar–phosphate backbone. These are called G-quadruplexes and can form from either DNA or RNA strands (or other variants, such as synthetic PNA). They are helical in nature due to the constraints of π–π stacking, although for convenience they are often depicted without the helicity. This planar scaffold may adopt different structures in the presence of different cations, in particular K^+ and to a lesser extent NH_4^+ and N_a^+. These cations interact with the lone pairs on the O6 atoms surrounding the central core[128]. The K^+ form is considered to be biologically more relevant due to its higher intracellular concentration (~ 140 mM) than that of Na^+ (5–15 mM). The precise location of cations between tetrads depends on the nature of the ions: Na^+ ions are observed in a range of geometries, whereas K^+ ions are always equidistant from each tetrad plane. K^+ in general is preferred over Na^+ by G-quadruplexes as K^+ has a better coordination with eight guanine O6s and lower dehydration energy[128]. Since there is directionality to the strands customarily described as from the 5′-end to the end, there are topological variants possible for these four strands. All four strands may be parallel, three parallel and one in the opposite direction (anti-parallel), or there may be two in one direction and

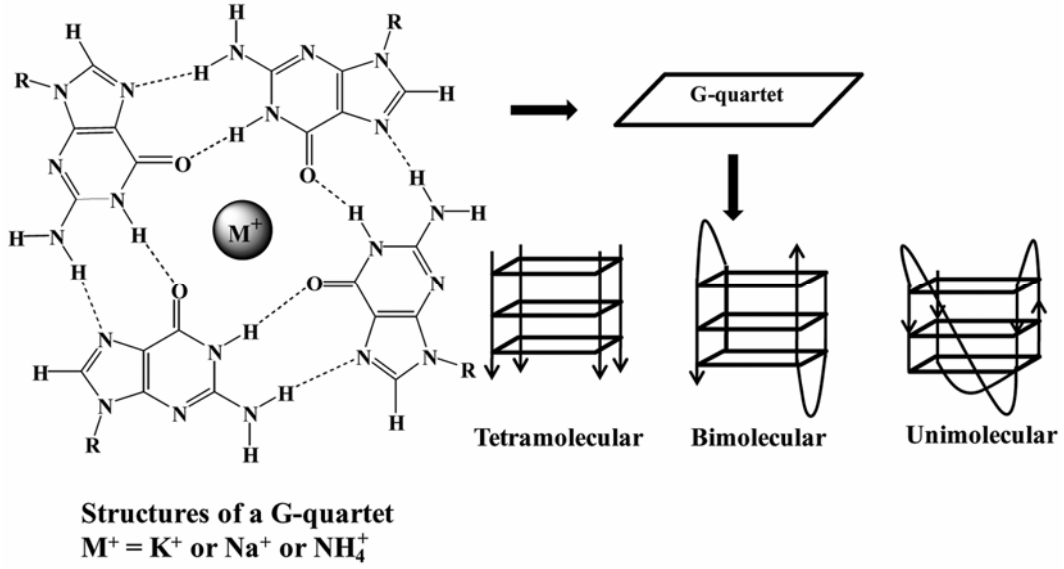

Figure 12. (Left) G-tetrad, a square-planar alignment of four guanines connected by cyclic Hoogsteen hydrogen bonding between the N1, N2 and O6, N7 of guanine bases. (Right) Tetrameric, dimeric and monomeric G-quadruplex composed of three G-tetrads.

two in the other, either with the parallel pairs adjacent to each other or opposite to each other (Figure 12).

G-quadruplexes may comprise of four separate strands, as in the example above, forming tetramolecular G-quadruplexes, which are always found in the all-anti parallel form[129]. Alternatively, they may form from two strands, each with two sets of contiguous guanines, or just from one strand, folding back on itself to form an intramolecular structure. In either of these cases, there will be loops that serve to connect the strands of the structure together. Depending on which strands are connected, these loops may cross diagonally across the topology of the structure, joining diagonally opposed anti-parallel strands, go across a side, linking adjacent anti-parallel strands or may loop around the side of the structure linking parallel strands and forming a double-strand reversal loop.

Intramolecular G-quadruplexes formed by single-stranded DNA are of intensive current research interest due to their potential formation in telomeres[130]. In contrast to tetramolecular quadruplexes, intramolecular structures form quickly and are more complex. This intermolecular structure exhibits great conformational diversity, such as in folding topologies, loop conformations and capping structures. On the other hand, different sequences not only adopt distinct topologies, but in addition, a given sequence can fold into a variety of other conformations, as in the case of the *Tetrahymena thermophilia* and human telomeric DNA sequence. With the recent progress in the structural studies of G-quadruplexes, numerous rules governing G-quadruplex folding have been formulated. However, complete prediction of a G-quadruplex conformation appears to be difficult. Thus structural characterization of G-quadruplexes seems to be necessary to understand each G-quadruplex structure and its ligand interactions.

Telomeres in ageing and cancer

Telomere length is a critical determinant of cellular lifespan. In primary cultured human cells and in most human somatic cells, telomere length decreases with each cell division event. The reversion of this degradation is brought about by a specialized enzyme called telomerase. Telomerase activity is high in germ cells, developing embryos and very young children, but it is down-regulated in most tissues starting at approximately 4 years of age[131]. Diminished telomerase activity is due to down-regulation of expression of the catalytic subunit of telomerase. In cells that must proliferate, such as B and T lymphocytes, reactivation of telomerase accompanies cell activation. The possibility that telomeres do not simply reflect but actually control cellular lifespan was articulated in the 'Hayflick hypothesis'. This hypothesis suggests that telomeres are a clock that counts the number of mitotic divisions allowed to each cell[132]. This was first borne out by experiments which showed that ectopic expression of telomerase permits cells to avoid senescence and proliferate indefinitely in culture[133]. Tumour cells must maintain telomere length in order to divide indefinitely. Most tumours (85–90%) contain telomerase activity, which supports cell proliferation. The importance of telomerase to tumour proliferation has been established by a simple experiment, which demonstrates that transfection with a construct expressing a dominant-negative telomerase catalytic subunit would halt cell proliferation[134]. Telomerase is therefore an exciting and promising target for

therapeutics[135]. In a minority of tumours, telomerase activity is absent, and recombination-dependent mechanisms are responsible for the telomere maintenance[136]. This pathway for telomere lengthening is called the alternative lengthening of telomeres, or 'ALT' pathway. This substitutes for telomerase in about 10–15% of human cancers. This raises the possibility that therapeutics directed against telomerase in cancer cells might inadvertently select for a population of cells, which use the ALT pathway, and are therefore resistant to therapy.

G-quadruplexes formed in human telomeres

Telomeres are specialized DNA–protein complexes that cap the ends of linear chromosomes and provide protection against gene erosion at cell divisions, chromosomal non-homologous end-joinings and nuclease attacks[137]. Human telomeres consist of tandem repeats of the hexanucleotide d(TTAGGG)$_n$ (5–10 kb in length), which terminate in a single-stranded 3′-overhang of 35–600 bases[138–140]. Telomeric DNA is extensively associated with various proteins, such as Pot1, TRF1 and TRF2, as well as TIN2, Rap1 and TPP1 (refs 136, 141, 142). The structure and stability of telomeres are closely related to cancer[130,143], ageing[133] and genetic stability[144]. In normal cells, each cell replication results in a 50- to 200-base loss of the telomere[145]. After a critical shortening of the telomeric DNA is reached, the cell undergoes apoptosis[145]. However, telomeres of cancer cells do not shorten on replication, due to the activation of a reverse transcriptase, telomerase, that extends the telomeric sequence at the chromosome ends[146]. Telomerase is activated in 80–85% of human cancer cells[147] and has been suggested to play a key role in maintaining the malignant phenotype by stabilizing telomere length and integrity[148]. The G-rich sequence of human telomeric DNA has a strong propensity to form the DNA G-quadruplex secondary structure, which inhibits the activity of telomerase[149]. In addition to the formation at the telomere end, which most likely involves intramolecular G-quadruplex structures, intermolecular G-quadruplex formation may also be involved in the T-loop invasion complex[150], and in meiotic chromosome synapses and homologous recombination[151].

Human telomeric G-quadruplex structures and polymorphism

Understanding of the human telomeric G-quadruplex structure under physiologically relevant conditions has been the subject of intense investigation. A 22-mer ODN human telomeric sequence 5′-AGGG(TTAGGG)$_3$-3′ (wtTel22) has been shown by NMR to form a basket-type G-quadruplex in Na^+ solution[152], which is a mixed antiparallel–parallel stranded intramolecular structure with three G-tetrads connected by one diagonal and two lateral (edge-wise) TTA loops. The same 22-mer ODN human telomeric sequence has more recently been shown in the crystalline state in the presence of K^+ to form a parallel-stranded intramolecular G-quadruplex consisting of three G-tetrads connected with three symmetrical propellers (double-chain-reversal) TTA loops[153]. However, when examined in K^+ solution by NMR, this wtTel22 sequence does not form a single G-quadruplex structure[154]. Recent structural studies have shown that the hybrid-type intramolecular G-quadruplex structures appear to be the major conformations formed in the human telomeric sequences in K^+ solution[154–156] even in the presence of high concentrations of Na^+ (ref. 154). The telomeric G-quadruplexes are always in dynamic equilibrium between two conformations, the hybrid-1 and hybrid-2 structures (Figure 13)[155]. Each hybrid-type G-quadruplex structure has distinct capping structures that may provide specific drug-binding sites. The hybrid-type conformations suggest a straightforward means for multimer formation with effective packing in the human telomeric sequence. It is important to note that the structural polymorphism appears to be intrinsic to the highly conserved human telomeric sequences and may be exploited in several biological systems.

Long human telomeric G-quadruplex DNA sequences

The next step toward understanding the structure of the 'real' telomeres is to address the question on the structure of long human telomeric DNA sequences[153,157–159]. Literature survey[157,158,160] suggests that the structures of long

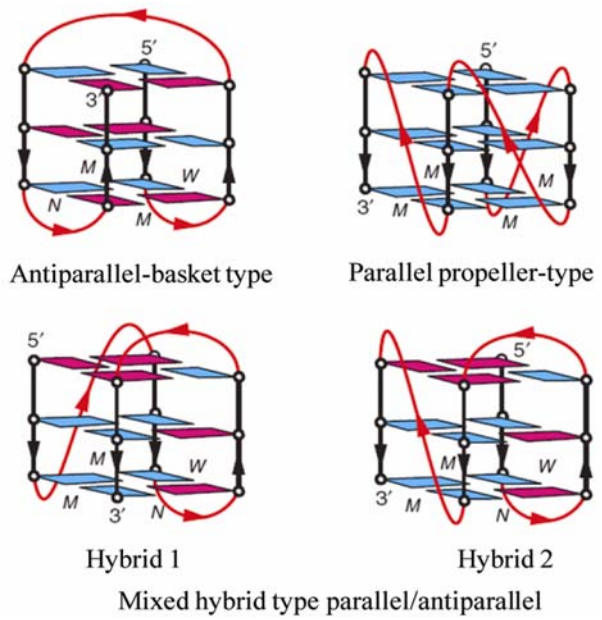

Figure 13. Folding topology of some monomeric intramolecular G-quadruplex DNA shown by NMR or X-ray crystal structures. Adapted from ref. 155b.

human telomeric DNA sequences are based on multiple G-quadruplex blocks, each formed by a four-repeat segment. Several models have been proposed regarding the arrangements of these G-quadruplex blocks[153,158] (Figure 14). In one of the models, G-quadruplex blocks are arranged like 'beads-on-a-string'[158], i.e. they can move relatively independent of each other and are constrained only by the connecting linkers. On the other hand, in another model, G-quadruplex blocks are proposed to stack on each other to form a higher-order structure. There may be three possible stacking modes between two parallel-stranded G-quadruplex blocks: (i) 5′-to-5′, in which the stacking interface is formed between the 5′-end of each block; (ii) 3′-to-3′, in which the stacking interface is formed between the 3′-end of each block and (iii) 5′-to-3′, in which the stacking interface is formed between the 5′-end of one block and the 3′-end of the other. In the 'same-direction stacking' model proposed for long human telomeric DNA, successive propeller-type parallel-stranded G-quadruplex blocks, which are oriented in the same direction, stack 5′-to-3′ continuously[153].

It has been suggested that a 200-nucleotide human telomeric DNA sequence, if folded into a stack of G-quadruplex, would form a rod of 60 Å (compared with a 680 Å long B-DNA helix)[153]. In the case of the hybrid-type human telomeric G-quadruplex structures, both hybrid-type structures provide an efficient scaffold for a compact-stacking structure of multimers in human telomeric DNA. Human telomeric DNA terminates with a 3′-single-stranded overhang of 35–600-mer ODN. The 5′- and 3′- ends of the hybrid-type structures point in opposite directions, allowing the hybrid-type G-quadruplex to be readily folded and stacked end-to-end in the elongated, linear, telomeric DNA strand. The capping structures (the adenine triple in hybrid-1 structure and the T : A : T triple in hybrid-2 structure) may provide stacking interactions between the two adjacent telomeric G-quadruplexes and at the same time they may provide specific binding sites for small molecules to target G-quadruplexes in human telomeres. A recent study indicates that the most plausible structure of a 44-mer ODN single-stranded human telomeric DNA is a dimer structure with each G-quadruplex in a different hybrid form[161].

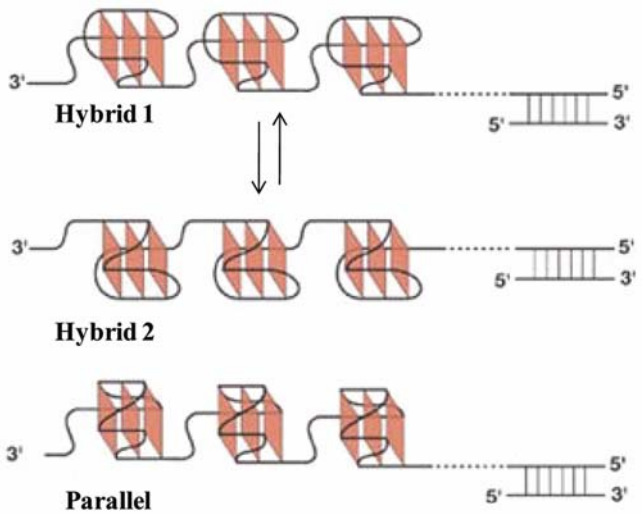

Figure 14. Proposed structure of the multimeric G-quadruplex DNA formed from longer human telomeric repeats. Adapted from ref. 159b.

Telomeric G-quadruplex DNA as a therapeutic target

It is hypothesized that drugs that interact with G-quadruplex DNA might interfere with the telomere replication and thus inhibit cell proliferation. In this regard a number of researchers have worked to identify or design small-molecule ligands which specifically bind to the G-quadruplex DNA. A number of molecules have been identified which have high affinity for G-quadruplex DNA and inhibit cell proliferation. Indeed some of these compounds have been shown to inhibit telomerase or to affect telomere lengthening[130,162,163]. Evidence that regulated G-quadruplex formation occurs at the telomeres, will undoubtedly provide further impetus to the development of drugs that interact with or promote formation of the G-quadruplex DNA. It is important to design ligands for target specificity. The mammalian telomeric repeat which is able to induce a distinctive G-quadruplex structure[153] will allow the design of ligands that can specifically target telomeric G-quadruplex DNA[164]. A desired ligand should bind a human G-quadruplex structure with high affinity and recognize specifically the G-quadruplex DNA in preference to the duplex DNA. Due to the different structural morphology of G-quadruplex DNA, it will allow different G-quadruplex recognition site for binding G-quadruplex interacting ligands: (i) stacking on the ends of the G-tetrad core, (ii) groove binding, (iii) taking the place of one or more strands in the core, (iv) interacting with the backbone (core and loops), and (v) interacting with the loop bases.

Structural diversity of G-quadruplex-targeted ligands

Most of the G-quadruplex-binding ligands[164–171] contain planar aromatic rings, which can interact with the human telomeric G-quadruplex by stacking on the terminal G-tetrads[164–168,171]. Literature survey shows that there is no conclusive evidence of the intercalation of a planar ligand between two G-tetrad layers. In addition of the end-stacking binding aromatic ligands, some ligands are able to recognize loops by stacking with loop bases or forming intermolecular hydrogen bonds[164,166–168] or recognize the backbone with electrostatic or hydrophobic interactions[170–172] of the G-quadruplex DNA. Alternatively, the

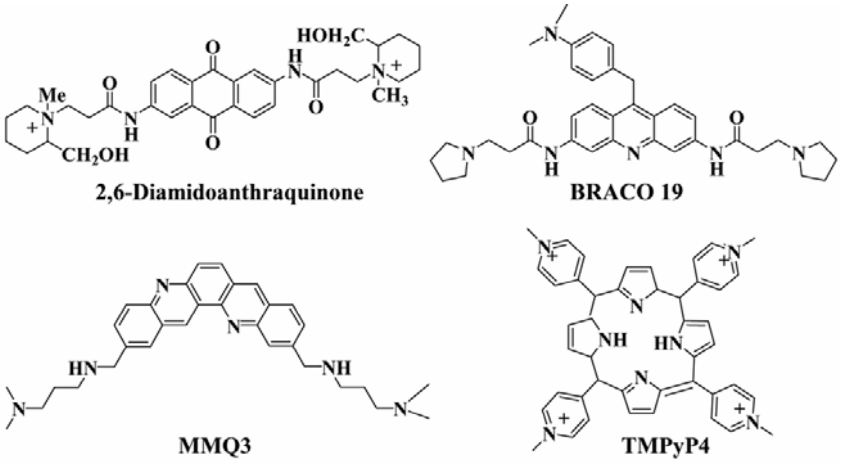

Figure 15. Chemical structure of G-quadruplex DNA stacking organic small molecules.

human telomeric DNA strand can be trapped in a G-quadruplex structure with a linear guanine-containing molecule based on a different backbone, such as PNA[173]. Researchers have designed some organic ligands which can stabilize real long human telomeric sequence by position between two consecutive G-quadruplex blocks[174]. Finally, fluorescent ligands have been designed to probe the formation and the ligand-induced stabilization of telomeric G-quadruplexes in the cell[175,176].

A number of recent reports have captured the range of G-quadruplex-targeted ligands derived from structure-based design approaches that turn out to be potent telomerase inhibitors[177]. Typical of DNA intercalators in general (as illustrated from ethidium bromide), are polyaromatic heterocycles designed for extensive π-stacking interactions with the G-tetrads (Figure 15). This offers a possibility for either intercalation within the stack of G-tetrads or through stacking on the end of the G-quadruplex. The anthraquinone derivative represents the first example from 1997, with numerous others following in rapid succession, including the dibenzophenanthroline derivatives[178] and tri-substituted acridines[179], which were reported to inhibit telomerase action in tumour cell lines with IC_{50} values of up to 28 and 60 nM respectively. The tri-substituted acridines were developed from the simple acridine on the basis of structure-based design principles to maximize the G-quadruplex binding affinity. Thus, inhibition of telomerase by these compounds appears to be correlated to selective stabilization of the human DNA G-quadruplex structure. Tetra-(*N*-methyl-4-pyridyl)-porphyrins (Figure 15)[180], 2,7-disubstituted fluorenones[181] and a dicationic perylene tetracarboxylic diimide derivative (PIPER) (Figure 16)[182] also inhibit telomerase activity with low IC_{50} values.

A novel fluorinated polycyclic quinoacridinium cation, RHPS4 (Figure 16), demonstrated enhanced binding to higher-ordered DNA structures (triplex/quadruplex) and induced telomere shortening with an IC_{50} value of 0.33 mM, while decreasing tumour cell proliferation of breast 21NT cells[183] at concentrations as low as 0.2 mM. RHPS4 is weakly cytotoxic (mean GI_{50} value in the NCI 60 human tumour cell panel is 13.18 mM), giving a therapeutic index (GI_{50}/IC_{50})[184] of 40. This activity does not appear to be associated with *Taq* polymerase and topoisomerase II inhibition. This phenomenon strongly suggests that RHPS4 is an inhibitor of telomerase function. The dimeric macrocycle in contrast to the related monomeric forms selectively binds with the G-quadruplex DNA, as demonstrated by competition dialysis experiments[185]. The triazine derivatives induce ODNs to fold into higher-order G-quadruplex structures, and they inhibit the telomerase activity in nanomolar concentrations[186]. Other new ligands are emerging based upon biarylpyrimidines[187], porphyrin–aminoquinoline conjugates[188] or indoloquinoline templates[189]. Schouten *et al.*[190] used the heterocyclic hemicyanine (HC) core motif and a tetrapeptide library to identify three peptide conjugates (HC–XXXX–$CONH_2$, where XXXX represents RKKV, KRSR and FRHR, one-letter amino acid code) with K_d ~ 50 mM and a high quadruplex over duplex selectivity. The peptide recognition motifs were for high-affinity binding to novel DNA targets. Recently, a zinc-finger library has successfully identified clones that bind in a sequence-dependent and structure-specific manner to the single-stranded human telomeric repeat sequence when folded into a G-quadruplex structure. However, the full structural details of the recognition process are yet to be described[191]. By analogy, ribosomal display using the human combinatorial antibody library has also identified single-chain antibody fragments that recognize the G-quadruplexes with high affinity (K_d = 125 pM). This also demonstrates at least 1000-fold specificity between parallel and antiparallel conformations[192]. Last, but not least, the most potent natural product known to act as a selective telomerase inhibitor is telomestatin, isolated from streptomyces anulatus[193]. This macrocycle binds with G-quadruplex structures with high specificity and indeed influences the type of G-quadruplex fold formed in solution[194].

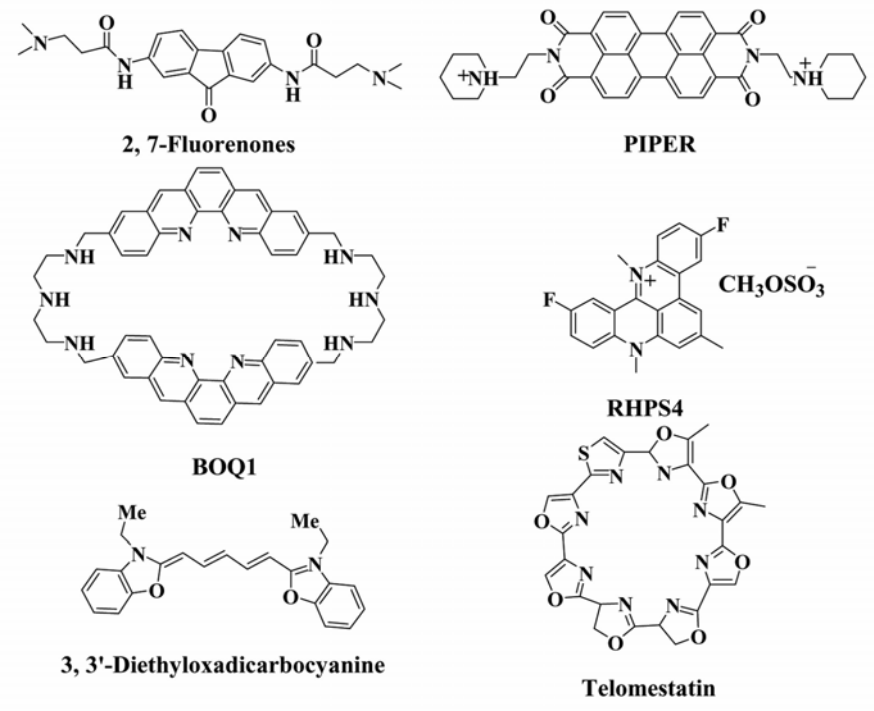

Figure 16. Chemical structure of 2,7-fluorenones, PIPER, BOQ1, RHPS4, 3,3′-diethyloxa-dicarbocyanine and telomestatin.

Conclusion

In this article we have discussed the different types of small organic molecules which target DNA and DNA-associated processes. But many of these when used as chemotherapeutic agents manifest one or more side effects. Therefore, there is always a challenge remaining with these designer DNA-binding molecules, to achieve maximum specific DNA-binding affinity, and cellular and nuclear transport activity without affecting the functions of the normal cells. For many of the newer targeted therapeutics that are under development for the treatment of cancer, it is however, expected that these new putative drugs will be used in combination with the more traditional drugs molecules such as *cis*-platin or doxorubicin. In combination with a DNA-interactive drug, the chemotherapeutic agent might exert considerably enhanced clinical efficacy as anticancer agents. The future challenge will be to 'conjugate' these agents appropriately on the basis of firm scientific principles. Combination of the other tools of genomics and proteomics might provide a new opportunity towards this end.

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