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DNA and the chromosome – varied targets for chemotherapy

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Abstract

The nucleus of the cell serves to maintain, regulate, and replicate the critical genetic information encoded by the genome. Genomic DNA is highly associated with proteins that enable simple nuclear structures such as nucleosomes to form higher-order organisation such as chromatin fibres. The temporal association of regulatory proteins with DNA creates a dynamic environment capable of quickly responding to cellular requirements and distress. The response is often mediated through alterations in the chromatin structure, resulting in changed accessibility of specific DNA sequences that are then recognized by specific proteins. Anti-cancer drugs that target cellular DNA have been used clinically for over four decades, but it is only recently that nuclease specific drugs have been developed to not only target the DNA but also other components of the nuclear structure and its regulation. In this review, we discuss some of the new drugs aimed at primary DNA sequences, DNA secondary structures, and associated proteins, keeping in mind that these agents are not only important from a clinical perspective but also as tools for understanding the nuclear environment in normal and cancer cells.

Introduction

Numerous alterations in the nuclear environment occur in the development of cancer. In the past several decades, a major focus of cancer research has been discovering and understanding these tumourigenic events. These include small-scale changes in DNA sequences such as point mutations; larger scale chromosomal aberrations such as translocations, deletions, and amplifications; and other changes affecting chromatin structure including aberrant DNA methylation and histone modification. In all cases, these alterations can have dramatic direct effects on general nuclear activities, including DNA replication and repair, or on more specific activities such as the expression of key growth regulatory genes.

Coincident with understanding tumourigenesis has been the development of agents to treat patients with cancer. The current focus in anti-cancer drug design attempts to mimic the uniqueness of the cancer with appropriate therapy, in the expectation that treatment regimes will become increasingly specific for the cancer type and less deleterious to the overall health of the patient.

The goal of this review is to discuss some of the current strategies that specifically exploit our increasing knowledge of the nuclear environment in cancer cells to target specific cell classes for death. Anticancer drugs that target DNA have been used in the clinic, with varying degrees of success, for more than 40 years[1]. These compounds vary in the type of chemical interaction with DNA, the degree of sequence selectivity, the extent of lesion reversibility

and/or ability to be repaired, and cancer cell susceptibility to their action. Classical DNA binding drugs have been considered as non-specific cytotoxic agents, with most of their therapeutic effects due to cytokinetic differences between normal and cancer cells. More recently, DNA interacting agents are being designed to affect specific nuclear functions, through interaction at designated primary DNA sequences, genomic locations, DNA secondary structures, or DNA-associated proteins[1].

Levels of structural organisation of DNA

The effects of DNA interacting drugs within cells can be experimentally observed at many different levels (Figure 1). The first, most basic level, is the chemical interaction of the drug with the DNA double helix. A variety of techniques have been developed to examine this level of interaction, and have shown that the chemistry of the interaction is very similar if studied on naked DNA or in cells in culture. Alkylation at specific sites and sequences in cells can be examined by using a modified thermal cleavage assay[2]. Following reaction of cells with the DNA alkylator, genomic DNA is extracted and heated to induce breaks at alkylation sites. The frequency of breaks can be ascertained by measuring the presence of modified nucleotide residues using HPLC or mass spectrometry. Time course experimentation can be performed to determine how the DNA adduct is maintained in the context of the nuclear environment. The site of alkylation and preferred sequence can be examined in cells using PCR stop assays wherein the DNA adduct prevents *Taq* polymerase elongation, or by ligation of the broken DNA with linker DNA molecules, followed by site-specific PCR and analysis of recovered products [3-7]. Compounds that bind tightly but reversibly to DNA can be analysed for their binding sites by footprinting assays, where short, defined DNA sequences (100–400 bp) are treated with random DNA-breaking agents in the presence of the compound, which protects from cleavage at its preferred binding sequences. This technique has been extensively used to study minor groove binding drugs such as distamycin A and the bis(benzimidazole) Hoechst 33258 [8-12].

A second degree of interaction is the disruption of DNA/protein or other nucleic acid interactions (such as important DNA secondary structures) that result from the drug/DNA interaction. Transcription factors, chromatin-associated proteins, and proteins involved in replication, recombination and repair are examples of proteins that recognize aspects of the DNA double helix (major versus minor groove, B versus non-B form DNA), certain DNA sequences and regions, or other DNA modifications, all of which could be affected by drug binding. The drug/DNA interaction might stabilize a transient DNA structure, introduce a new conformation or structure, or mask a DNA modification and in these ways alter the normal regulatory processes working at these sites [13-15]. Some of the proteins that have been examined include the high mobility group (HMG) proteins [16-20]. These chromatin-associated proteins induce DNA bending, recognize distorted DNA structures, and play a role in regulating gene expression, repair and recombination. Electromobility shift assays (EMSA) [21-25] and chromatin immunoprecipitation (ChIP) [26-30] are some of the methods used to assess DNA/protein interactions *in vitro* and *in vivo*. Expression microarrays and real-time reverse transcriptase PCR can be used to assess changes in transcription of genes in the presence of DNA-binding compounds, a likely end product of alterations in protein/DNA binding. Indeed, transcription of 21 genes with promoters containing match sites for the minor groove-binding hairpin polyamide/chlorambucil conjugates were significantly inhibited in the presence of these compounds[7].

DNA secondary structures, such as those that form during DNA recombination, or G-quadruplexes found at the ends of chromosomes and sometimes present within regulatory regions of genes, are other examples of possible targets for drug design. Several recent reviews have discussed some of these structures and their relevance for targeting[13,31,32]. Techniques that are used to investigate these DNA structures, their orientation and polarity, and stability *in vitro* include CD, IR, and NMR spectroscopy, X-ray crystallography, and molecular techniques such as native gel electrophoresis, chemical probing of isolated DNA species with nucleotide-specific cleavage reagents, PCR and primer extension reaction stop assays [33-37].

More global changes in the nuclear environment can be visualized using a combination of fluorescence in situ hybridization (FISH) and confocal microscopy techniques,"ImmunoFISH", a relatively new technology that allows coincident visualization of protein complexes and DNA in intact nuclei[38,39]. This type of microscopy enables visualization of nuclear complexes composed of DNA and protein, such as nuclear factories[40], the nuclear matrix attachment sites and organizational centres for processes of DNA replication and transcription. Alterations in these complexes that result from drug treatment might not be detected using other molecular techniques. This technology is particularly useful for examining the nuclear distribution of specific genomic locations or sequences which might change during the course of the cell cycle. For instance, this technique has recently been used to examine distribution and binding of membrane Tel binding protein (MTBP/TRF2) to telomeres during spermatogenesis and the cell cycle[41]. Deconvolution microscopy using fluorescently-labelled polyamide compounds has also been used to monitor drug uptake and cellular distribution [7]. These

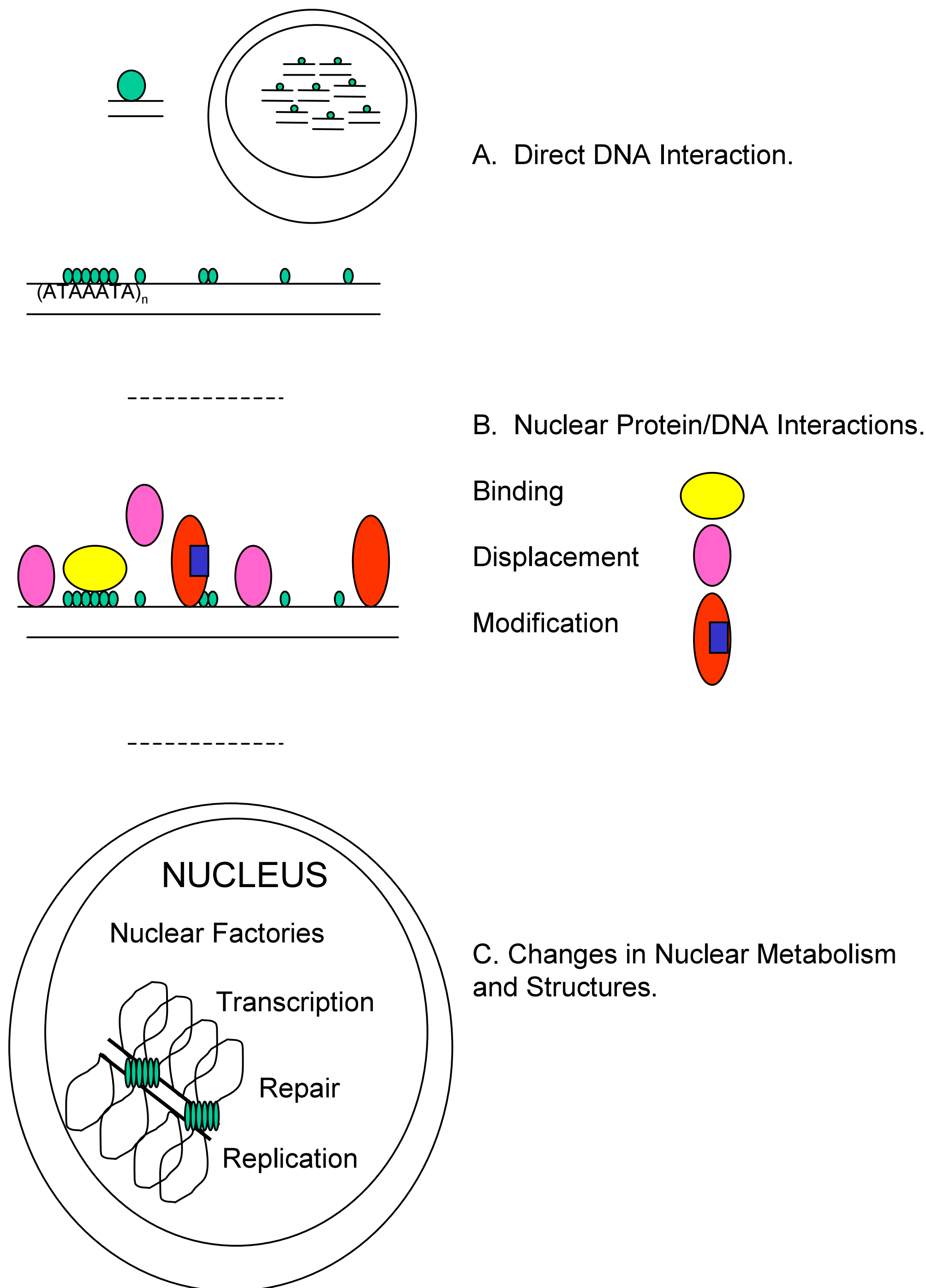


Figure 1

Levels of Drug/DNA Interactions: A) The first level of interaction is the direct drug interaction with the DNA. Drug binding frequency and sequence preferences are two examples of evaluations of this level. B) The second level involves the disruption of DNA interactions with other molecules that result from the drug/DNA interaction such as DNA/protein interactions. Another possibility (not shown) is the alteration or stabilization of DNA secondary structures. C) The third level is the changes in nuclear activities such as transcription, replication, and repair or disruption in complex ternary structures. Green circles, drug/DNA adduct; black lines, DNA strands, chromosome (with loops); yellow oval, red oval, and pink circle, DNA associated proteins; blue square, protein modification such as phosphorylation or methylation.

technologies will undoubtedly provide insight into the global effects of drug treatments and aid in future drug design.

Primary base sequence

The DNA primary base sequence is the simplest level of complexity governing the genetic code. However, its importance in regulating nuclear processes should not be underestimated, despite the inability to always predict outcomes based on observed patterns. For example, the consensus sequence for a given transcription factor in a promoter region does not necessary mean that protein is constitutively bound to that site. It is because of this unpredictability that the more complex nature of genomic DNA is considered in later sections of this review.

We have divided the discussion of DNA primary base sequence into four sections in order to introduce the types of anticancer drugs that interact with DNA. These include non-specific interactions and sequence-specific interactions with repetitive DNA, AT-rich DNA, or stringent consensus binding sites. The structures of the compounds vary as well as the way in which they interact with DNA. It is important to realize, however, that clear distinctions between these groups do not always exist.

Non-sequence-specific (global) DNA damage

One of the key observable differences between many types of cancer cells and normal tissues is that the former divide more rapidly. Treating cells with DNA damaging agents should perturb the cancer cell's ability to divide. The way in which this is achieved is determined by the type of DNA lesion, as well as the genetic makeup of the cell. From the point of view of cancer treatment the desired pathway is induction of cell death, as DNA repair can lead to the generation of mutations if the repair is not accurate. If a DNA lesion is not repaired prior to approach of a replication fork either by transcription coupled repair or global repair pathways, the replication fork will stall. A variety of signals are then sent out by the stalled fork so that the lesion is either repaired or bypassed, initiation of other replication origins is inhibited, and possibly apoptosis is induced [42-44].

Inducing apoptosis in anti-cancer therapy is not as straightforward as one would expect. The two major apoptotic pathways are the external death-receptor-induced pathway (which involves ligands and receptors, for example, FAS and TNF) and the mitochondria-apoptosome-mediated pathway, which is intrinsic and induced by insults such as chemotherapy and radiation [45]. Intrinsic programmed cell death is dependent on the activation of cellular checkpoint proteins. In the case of DNA damage, sensing proteins, such as RAD9, RAD1, RAD17, and HUS1, relay the damage signal to signal transducers (Mek1, ChK1, Rad53 in yeast; ATM, ATR, ChK1, and ChK2 in mammals) and effectors (p53, BRCA1, repair proteins, etc) [46]. In mammals the story is not as clear as it is in yeast, although many mammalian homologues of the yeast proteins have been identified. Other protein complexes may play roles in damage sensing such as the BRCA1-associated genome surveillance complex which includes BRCA1, ATM, the MRE11-RAD50-NBS1 (MRN) complex, MSH2/6 and MLH2 mismatch repair proteins, and Bloom's helicase[47]. Activation of the damage response pathway can lead to arrest of cells at various stages in the cell cycle, induction of DNA repair, and activation of specific gene expression, as well as apoptosis. P53 is an important G1 checkpoint protein that prevents passage of the cells into S-phase via transactivation of the cyclin-dependent kinase (CDK) inhibitor, $p21^{waf1/cip1}$ [48,49] but also plays a role in other cell cycle checkpoints. Other examples of proteins involved in the G1/S checkpoint include Rb-E2F pathway, G1 cyclins, and the ARF proteins[46,48,49].

The S-phase checkpoint involves ATM, ATR, ChK1 and ChK2 proteins [50-54] and leads to inhibition of initiation of replication at origins and stalling of replication forks. How this checkpoint manifests in upper eukaryotes is not entirely clear although in yeast, when a lesion is encountered by a progressing replication fork, replication protein A (RPA) binds to the single stranded DNA about the lesion which in turn recruits the Mec1/Dcd2 sensing complex (Mec1 is a homolog of mammalian ATR and ATM). This sensing complex associates with Rad24/Rfc2-5, and Rad53 (Rad53 is the ChK2 homolog) is then recruited and activated by phosphorylation in a Mec1 dependent manner [42,55]. In mammals, the DNA damage signal is sensed by ATM and ATR proteins and propagated directly or via the ChK1 and ChK2 kinases to downstream effectors including p53, BRCA1, Mus81 and CDC 25 [52]. It should be noted that the exact roles these damage response proteins play in yeast and mammalian cells is not necessarily the same. ATM has also been shown to phosphorylate the Nijmegen breakage syndrome gene (NBS-1) *in vitro* and *in vivo* in mammalian cells in response to γ-irradiation [56]. NBS-1 is a component of the MRN complex which is involved in recombination and repair, and thus provides a direct link between the checkpoint proteins and DNA repair[53]. BRCA1 has also been shown to be phosphorylated in an ATM-dependent manner following DNA damage and to bind to the MRN complex [57-59].

Induction of apoptosis involves activation of signalling pathways that often shift the balance from anti-apoptotic proteins to pro-apoptotic proteins, leading to cell cycle arrest and activation of caspase enzymes. The apoptotic cell is characterized by loss in cell volume, membrane

blebbing, nuclear condensation, chromatin aggregation and endonucleolytic DNA fragmentation [45]. A variety of techniques can be used to analyze apoptosis, including flow cytometry (annexin V labelling of externalized phosphatidylserine, caspase activation using fluorescently labelled caspase inhibitors, and PI-staining DNA content analysis), microscopy (morphological changes) and gel electrophoresis (DNA laddering). In most cases more than one method is employed to clearly define the apoptotic process. This is particularly important when studying induction of apoptosis in cancer cells compared to normal cells, because expression of growth factors, tumour suppressor proteins and other cell cycle inhibitors is deregulated leading to unexpected outcomes in these classical apoptosis assays. In addition, different cell types may not activate all the pathways that result in the apoptotic cell phenotype.

The first drugs to be used to treat highly proliferative cancers were the relatively non-specific nitrogen mustard DNA alkylating agents[1] such as chlorambucil [1], melphalan [2], and cyclophosphamide [3]. These form monoadducts primarily at any G-N7 site in the major groove. However, the biologically important initial lesion formed by mustards in cells is interstrand cross-links, primarily at 5'GPuC sequences[60]. There is also evidence that they cause termination of transcription [61]. Cyclophosphamide is the most widely used mustard clinically, and is a non-specific prodrug of the active metabolite phosphoramide mustard, requiring enzymic activation by cellular mixed function oxidases. The (necessarily) high chemical reactivity of mustards leads to rapid loss of drug by interaction with other cellular nucleophiles, particularly proteins and low molecular weight thiols. This results in the development of cellular resistance by increases in the levels of low molecular weight thiols (particularly glutathione) [62,63]. Of equal importance for efficacy, much of the drug can reach the DNA with only one alkylating moiety intact, leading to mono-alkylation events which are considered to be genotoxic rather than cytotoxic[64]. The fact that cross-linking is a two-step process adds to the proportion of (genotoxic) monoalkylation events, since the second step is very dependent on spatial availability of a second nucleophilic DNA site. Because of their genotoxicity, there is a risk of the development of second cancers from their mutagenic effects, with the most frequent alkylator-induced malignancy being acute leukemia[65], usually occurring a long period (3–7 years) after treatment.

Another class of even less selective alkylating agents is those which break down to very unstable intermediates that react indiscriminately. These include nitrosoureas such as streptozotocin [4], which has been used as a component of multi-drug protocols for Hodgkin's disease [66], and triazenes such as dacarbazine [5], widely used for malignant melanoma[67], and the more recent temozolomide [6], used increasingly for gliomas [68].

Mitomycin C [7] is an example of a more complex and sequence-specific DNA cross-linking agent. It is widely used clinically, perhaps most effectively now in bladder cancer [69], but its use is limited by myelosuppressive side-effects. The mitomycin C-related FR family of antibiotics, including FR900482 [8] and related compounds, are compelling potential replacements which may in some cases offer decreased toxicity[26,27]. The FR family of compounds undergo reductive activation to form reactive mitosene derivatives, which crosslink DNA preferentially at 5'CpG'3 steps[26]. Although generally considered a non-selective agent, there is some evidence that mitomycin and related compounds have shown some selective effects in cells. Using a modified ChIP assay with Jurkat T cells, FR900482 was shown to crosslink regions in the *IL-2* and *IL-2R*α promoters and the HMG I/Y, HMG 1, and HMG 2 minor groove binding proteins and not the major groove binding proteins (with overlapping DNA target sequences), Elf-1 and NFκB[26].

Repetitive DNA sequences

We now know that the human genome contains considerable areas of repetitive DNA sequences [70,71]. These are generally organized in heterochromatin, mainly in centromeres. These satellites consist of repeat units of several thousand base pairs. Minisatellites (also called variable number tandem repeats) and microsatellites (also called short tandem repeats) are distinctly different from satellites in that the repeat units are shorter and less complex, and they are dispersed across the genome. The difference between minisatellites and microsatellites is the latter repeat length is between 1 and 13 bp whereas the minisatellite is longer. In some cases mini- and microsatellites may serve important regulatory functions. For instance, a vast majority of the CGG trinucleotide repeats are located in the 5' untranslated regions of genes and are oriented with respect to the transcribed strand such that the mRNA contains the repeat. In addition, repetitive DNA often has the ability to take on non-B form DNA conformations which might recruit certain regulatory proteins that participate in control of gene expression.

"AT-islands", containing between 85–100% AT, are distinct minisatellite regions[3-6,15]. These islands consist of between 200 to 1000 bp of repetitive DNA. A number of critical nuclear processes are organized around AT-rich DNA sequences in the genome. In some instances, these islands function as matrix attachment regions (MARs) that organise DNA loops on the nuclear matrix and coordinate nuclear activities such as DNA replication, transcription, and mitosis[15]. Nuclear matrix binding ability

of DNA sequences can be demonstrated *in vitro* by preparing nuclear matrices and incubating with labelled DNA probes of sequences of interest and unlabeled competitor DNAs (either non-specific or MAR containing), followed by gel electrophoresis of washed matrices. *In vivo* MAR binding activity can be assessed by digestion of nuclear DNA with a number of enzymes that do not cleave within the potential MAR, followed by nuclear matrix preparation to separate the matrix-associated DNA from loop DNA. Labelled probes are then used to screen dot blots of prepared matrices and associated DNA. Enrichment of DNA sequences within the nuclear-matrix fractions versus loop DNA fraction is highly suggestive of MAR activity[72].

Repetitive sequences, like these "AT-islands", are notoriously unstable elements with changes occurring either through polymerase slippage or unequal recombination[71]. The types of rearrangements that often occur at these sites include expansion and deletion of the repetitive elements. Not surprisingly, mini- and microsatellite instability features in a number of human diseases and cancers, such as human colorectal cancer and a variety of leukaemias and lymphomas. Using a variety of experiments including *in vitro* and *in vivo* MAR binding assays, Jackson *et al*[72] have recently demonstrated that the AT-islands within the FRA16B fragile site are expanded and preferentially associated with the nuclear matrix in the CEM leukaemia T cell line as compared to normal WI-38 fibroblasts. This alteration in the organization of DNA in the leukaemia cell line correlates with a hypersensitivity to drugs which specifically alkylate in repetitive AT-rich regions [4,6,15].

Origins of replication and various promoter sequences are other examples of AT-rich sequences. Unlike simpler eukaryotes, mammalian origins of replication are not as clearly defined or localized on individual chromosomes. Sites have been identified however, such as the *c-MYC* origin that lies in the 5' region of the gene. This site is AT-rich although the *c-MYC* MAR located in the 3' region of the gene has a significantly higher AT content. Destabilization of these regions would undoubtedly affect the cell's ability to initiate DNA synthesis, although the impact this would have on cellular proliferation is not necessarily predictable. Genetic changes associated with instability could also affect gene expression.

Many genes have been identified that contain mini- and microsatellites of all sorts, expansions or other alterations of which have been implicated in deregulation and association with disease[70,71]. Examples include the CAG repeat in the Huntington's gene, the G/C rich repeat 600 bp upstream of the insulin gene ATG (insulin-dependent diabetes mellitus), the GAA repeat in the X25 intron associated with Friedreich's ataxia, and the G/C rich repeat downstream of the HrasI polyA signal, certain alleles of which are associated with increased cancer risk.

AT-rich regions (ORIs, MARs)

The metabolic processes at AT-rich minisatellites may be directly affected by drug-induced DNA lesions and/or hindered by the induction of cellular checkpoints and DNA damage response pathways. For example, drugs that interact specifically at such sites could interfere with essential protein/DNA interactions that then lead to delay in or inhibition of DNA synthesis or deregulation of gene expression. Drug interference could result from competition for factor binding to the sequence, deletion or alteration in the DNA sequence as a result of repair processes, and/or distortion in the local DNA confirmation[14,15].

The cyclopropyindoline compounds, including the natural product CC-1065[73,9] and related synthetic analogues like adozelesin [10] and bizelesin [11], are extremely cytotoxic DNA alkylators that target the N-3 of adenine in the minor groove of AT-rich DNA sequences. Even simpler analogues such as the hydroxyl- and aminoCBI compounds [12] and [13] show very similar patterns of DNA alkylation when compared on a section of the *gpt* gene, alkylating preferentially at 5'-A(A/T)$\underline{\text{A}}$N sequences, although the amino analogue was the more efficient alkylator[2], and showed similar levels of potency in a variety of cell lines[74]. A comparison of the monoalkylating derivative, adozelesin, and the related bifunctional analogue, bizelesin, showed that while both are highly AT-selective, the latter requires a target site with adenines spaced six base pairs apart, and most commonly alkylates by crosslinking adenines very preferentially at $T(A/T)_4A$ sites[75]. Monoadducts have also been observed at $A(A/T)_4A$ sites, although to a lesser extent [3,4,76]. *In silico* drug/DNA binding analysis predicts that the bizelesin binding motif occurs approximately 2.8 times every 250 bp. AT-island hotspots are present once every 10^6 bp, and within these hotspots bizelesin sites occur 99 times every 250 bp. These long AT-islands are suspected to be the major targets for bizelesin binding and responsible for its high toxicity. Using a model AT-island DNA, actual bizelesin binding sites were determined and confirmed the *in silico* predictions. Bizelesin was 100 times more reactive with the model AT-island DNA than the non-AT-island model[6,15].

Woynarowski in a recent review[15] has suggested that the potent cytotoxicity of the general indoline class of toxins is caused by disruption in critical nuclear processes that are organized around functional AT-rich DNA sequences in the genome. Matrix attachment regions, origins of replication, and candidate promoters are examples of AT-rich sequences that might be specifically targeted by these

compounds as a result of their alkylation preference and sequence specificity. Furthermore, hits to AT-rich sequences by the region-specific AT binding drugs such as bizelesin or other AT-specific drugs like tallimustine that are not organized in these regions would be much less deleterious to the cell because of a lack of functional consequence. The variation in cytotoxic potencies of different indoline analogues and other AT-specific drugs could result from the ability to target these regions effectively.

The degree of susceptibility of a cancer cell to these agents may depend on a number of additional factors including deregulated gene expression and genomic instability, and in this way these compounds may be more specifically toxic to cancer cells. Woynarowski and co-workers[6] have found that the AT-rich fragile sites Fra16B and Fra16D, and the *c-MYC* origin, a region commonly amplified in cancer cells, are targeted by bizelesin. In addition, we have found that 50% of the recovered mutations in the *gpt* gene of surviving AS52 Chinese hamster ovary cells treated with aminoCBIs are deletions in AT-rich regions (unpublished). Thus, specifically localized DNA damage can result from treatment with drugs of this class and may contribute to the potent cytotoxicity[15]. Given the increased level of genomic instability in cancer cells, drug potency may be enhanced due to expansion of these satellite regions and/or because of deletions in these critical regions following drug treatment.

CC-1065, bizelesin and adozelesin have been shown to inhibit DNA replication in cell-free and cell-based systems (yeast and mammalian) [77-82]; however, the mechanism of inhibition is not clearly understood. Based on the DNA/adduct distortion studies[83,84], replication initiation may be inhibited by distortion of specifically targeted MARs resulting in a block in origin complex assembly necessary for proper origin firing. This could explain the very high lethality of bizelesin (<10 adducts per cell leads to inhibition of growth) compared to that for the AT-specific alkylator tallimustine [14] (>200 per cell) and conventional mustards (several thousands per cell)[15] which do not demonstrate region-specific DNA binding.

Another reason why selective damage to AT-rich DNA might be important in the mechanism of drug action is that binding to these sequences affects specific gene expression. This may arise by preventing transcription factor binding, increasing the affinity of a transcription factor for its sequence, or creating unnatural binding sites. For example, CC-1065 has been shown by EMSA to inhibit TATA Box Binding protein (TBP) from binding to a DNA oligonucleotide containing the adenovirus major late promoter TATA box sequence[21]. In this case, drug binding was thought to directly hinder minor groove binding of TBP to the TATA box. Binding of Specificity Protein 1 (SP1), a member of the SP/KLF family of transcription factors[85], to 6 GC boxes present in the simian virus 40 (SV40) early promoter is also inhibited by CC-1065 binding to AGTTA* between the SP1 sites, where * indicates the site of covalent modification[83]. These authors propose that the inhibition in SP1 binding, particularly at the 3'-GC box, resulted from distortions in the DNA caused by adduct formation. High-field NMR studies of the adozelesin/DNA adduct have confirmed that drug binding distorts the DNA double-helix despite maintaining normal Watson-Crick base pairing[84]. AT-rich sequences found in regulatory regions in other genes associated with cancer, such as *c-MYC*, have also been identified as sites specifically alkylated in cells treated with related compounds[4]. Using a similar approach (real-time PCR stop assay) we have found that the aminoCBI compounds also target AT-rich sites located within the *c-MYC* gene in cell culture, and furthermore using real-time reverse transcriptase PCR analysis, we have found that *c-MYC* expression rapidly (within a few hours) decreases following treatment (unpublished). We are currently extending these studies to look at changes in protein expression levels.

Specific DNA sequences (oncogenes)

Hairpin polyamides

There are several drug classes that are able to span DNA and recognise a limited number of specific sequences. The most discriminatory sequence selective DNA binding compounds to be developed are the pyrrole-imidazole (Py-Im) polyamides[10,86,87]. These minor groove binding compounds are synthetic ligands that were developed based on the binding properties of the AT base selective drug distamycin A [15,88]. The dimeric hairpin Py-Im polyamides derivatives have been shown to inhibit transcription factor binding, such as TBP, NFκB, and ETS-1, to recognition sequences *in vitro* [89-91]. Another recent study demonstrated that Py-Im polyamides can derepress expression of the HIV long terminal repeat by inhibiting host factor LSF binding to the repressor complex sequence in the context of host cell chromatin [92]. Despite successful inhibition of transcription factor binding to naked DNA, the hairpin polyamides have not proved to be effective at inhibiting gene expression in cells[7].

A new approach is to conjugate polyamides with DNA alkylating agents such as chlorambucil (Py-Im-Chl) [16], in the expectation of increasing their biological potential and hence therapeutic use [91,93,94]. These derivatives were shown to inhibit *in vitro* replication of SV40, mammalian cell growth, and genomic DNA replication, and cells treated with Py-Im-Chl conjugate arrested in G_2/M[93]. More detailed analysis of the accessibility of nuclear chromatin and effects on gene expression have been performed using the Py-Im-Chl conjugate by

Dudouet *et al.*[7]. Using ligation-mediated PCR to examine alkylation sites, these investigators found that the Py-Im-Chl conjugate was capable of accessing target binding sites in the HIV-1 enhancer and promoter in lymphoid cells. Microarray analysis of cellular expression profiles indicated that a limited number of genes (21 genes using 2 conjugates) were affected by polyamide-conjugate treatment, and that in each case match sites were located within the 5'-flanking region of the gene. While it is still not known how effective these agents will be clinically, it is very promising that the expression of so few genes can be altered considering the number of potential polyamide binding sites for these agents within the entire human genome (experimentally determined to be approximately 1 in 1900 bp, expected frequency calculated to be present 1 in 2048 bp)[7].

Ecteinascidin (Et-743)

Et-743 [17] is a minor groove alkylating agent which was originally isolated from the sea squirt *Ecteinascidia turbinata*, and is currently in clinical development [95,96]. Alkylation by Et-743 of the N2 of the central guanine of the DNA binding triplet results in a conformational change in the DNA, with the minor groove widening and the double helix bending towards the major groove [97-101]. Et-743 does not have the same degree of DNA sequence specificity as the polyamide compounds. However, this compound does demonstrate a unique potential to alter gene expression of discrete loci based on the presence of GC boxes in the promoter regions. The potent cytotoxicity of Et-743 is thought to be due to inhibition of transcription factor binding, resulting in effects on transcription. For example, DNA binding of NF-Y to the CCAAT box and the transcriptional activation of MDR1 and HSP70 (genes regulated in part by NF-Y via the CCAAT motif) are affected by Et-743 [102-104]. Interestingly, constitutive expression of *MDR1* and *HSP70* is not affected[103]; therefore, Et-743 may work via inhibiting activated transcription in response to certain stimuli[105]. Microarray analysis of tumour cell lines treated with Et-743 and phthalascidin [18] (Pt 650)[106], a synthetic analog of Et-743, showed similar changes in gene expression including a decrease in expression of genes which bind to CCAAT-boxes which might contribute to the repression in activation of transcription of *MDR1* and *HSP70* [105,107].

Et-743 also inhibits the transcription of other genes, including *c-FOS*, *c-JUN*, *E2F1*, *H2B*, and *H4*. The mechanism is presumably by alkylation of the guanine bases in the GC boxes present in the promoter regions, resulting in a block in the binding of transcription factors such as NF-Y, SP1 and ERG1[108].*In vitro* inhibition of the transcription factors TBP, E2F, and SRF has also been observed[102]. Modelling studies suggest that head to tail binding of three Et-743 molecules to DNA resembles an RNA-DNA hybrid complex, and that the distortions mentioned above mimic those induced by zinc finger transcription factor binding[109]. These investigators have speculated that such changes in the DNA could not only inhibit factor recognition but also induce DNA/Et-743/protein interactions. Et-743 also seems to exert its cytotoxic effect on cells by inducing single-strand DNA breaks (ssDB) via an interaction with the transcription-coupled repair machinery, as cells resistant to Et-743 have defects in the xeroderma pigmentosum genes, and show reduced ssDBs following treatment[110,111]. Mutations in the DNA double-strand break repair pathway, however, sensitize cells to Et-743 cytotoxicity[110]. Taken together, these studies suggest that while inhibition of transcription factor binding to certain promoters is an important part of the Et-743 mode of action, the anti-tumour activity is also dependent on endogenous features of the cancerous cell such as certain DNA repair pathways.

Secondary DNA structures

In the previous section of this review we discussed the biological impact of several different DNA-interacting compounds including the nature of their interaction with DNA, mechanism of action, and known anti-cancer activity. In addition to small molecules that interact with specific bases and base sequences, a number of compounds are being developed which target DNA secondary structures such as DNA tetraplexes and quadruplexes, hairpins, and Holliday junctions. Some of these DNA structures have been implicated in regulating numerous nuclear activities, and represent an exciting new area of research into potential anti-cancer targets as well as for treatment of numerous other human diseases including diabetes and neurodegenerative disorders[112].

DNA quadruplexes (telomeres)

Telomeres are the repetitive DNA sequences (TTAGGG) at the ends of chromosomes that protect the 3' ends from degradation and inappropriate repair activities and interact with a number of different proteins forming the telomeric complex [113-115]. In normal proliferating cells, telomeres are shortened with each round of replication and telomerase expression is negligible. Eventually, telomeres become so short that they are no longer capable of protecting chromosome ends, leading to chromosome fusions and erosion. This results in the induction of "telomere-induced senescence" and loss of cell viability. In many cancer cells, however, a short telomere length is maintained during cell divisions in part because of increased telomerase activity. In fact more than 90% of all human cancers have increased expression of telomerase which is one reason why it has been suggested as a target for anti-cancer drug design efforts. In addition to the telomerase enzyme, the telomeric DNA structure is being

examined for its ability to be targeted by anti-cancer treatments[114,116-121].

Telomerase is a multi-component enzyme comprised of protein and nucleic acid. The two main components are the RNA moiety (hTER) and the catalytic subunit (hTERT), although there are several additional regulatory binding proteins (for example: HSP90, p23, and TEP1). There are numerous telomerase inhibitors in development[120]. The main strategies include anti-sense oligonucleotides, peptide nucleic acids and ribozymes targeting the RNA component of telomerase, dominant negative versions of the hTERT subunit, small molecule inhibitors of the enzyme complex, and disruption of the G-quartet (see below)[120,122,123]. While decreases in telomerase activity have been achieved using these approaches, this has not necessarily led to reduced cancer cell viability.

The G-quartets of the telomere (3' G-rich overhang of 150–200 bp that form the DNA secondary structure, the G-quartet) are stacked tetrads arising from planar associations of four guanines in a cyclic Hoogsteen hydrogen-bonding arrangement (Figure 5) [32]. G-quartets can be stabilized by sodium and potassium ions, and this stabilization can inhibit telomerase activity. As such, the ability of small molecules to interact with and presumably stabilize these secondary structures as a means of inhibiting telomerase has been a major drug design effort.

A number of small molecules have been identified that interact with G-quartets. Molecular modelling studies of anthraquinones predicted these compounds would interact with G-quartets by a threading intercalation model [124]. Nuclear magnetic resonance studies have confirmed that the 2,6-diamidoanthraquinone BSU1051 [19] interacts with and stabilizes the G-quartet, and inhibits telomerase activity[125]. A 3,6,9-trisubstituted acridine [20] was also a potent (IC_{50} 18 nM) inhibitor of telomerase[126]. In A431 human squamous cell carcinoma xenografts it showed a significant additional growth delay compared with paclitaxel alone, with no additional toxicity[127].

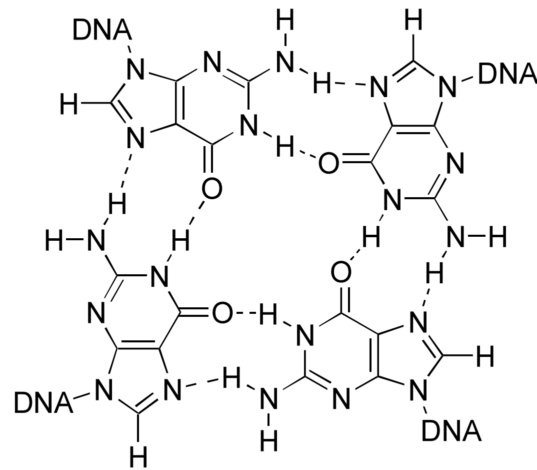
Cationic porphyrins, exemplified by TMPyP4 [21], are another class of agents that were predicted to bind to G-tetrads by interactive stacking[32]. Two independent research groups showed by a variety of methods (spectroscopy, CD, NMR) that these compounds do interact with parallel and anti-parallel G-quadruplexes[32,128]. The way in which the compounds interact with the DNA is not entirely clear, but most likely involves external stacking of the porphyrins relative to the G-quadruplex[129]. A third class of G-tetrad interacting compound is typified by the perylenetetracarboxylic diimide PIPER [22] that demonstrates similar binding attributes to the porphyrins. Interestingly these compounds may not merely bind to such DNA structures, but may also induce their formation in cells [130].

In addition to telomeres, G-quadruplex sequence motifs have been identified in other regions of the genome, particularly in the upstream promoter regions of a number of oncogenes [34]. Within the *c-MYC* promoter, the nuclease hypersensitive element III1 (NHE), corresponding to bases 2186–2212 in human *c-MYC* [131], has been known to play an important role in regulation of *c-MYC* expression. Insight into the regulatory nature of this region was first demonstrated when synthetic oligonucleotides with sequences complementary to the NHE *c-MYC* coding were capable of blocking *c-MYC* expression[132,133]. Further *in vitro* studies using *c-MYC* promoter DNA demonstrated this region was capable of forming quadruplex structures (G-rich strand) and *i*-tetraplexes (C-rich strand) [34-36]. These latter structures are formed based on hemiprotonated cytosine$^+$/cytosine base pairs containing three stabilizing hydrogen bonds between them. The four-stranded structure is composed of two parallel-stranded duplexes zipped together in an anti-parallel configuration [134-136]. In the case of the *c-MYC* promoter the formation of the G-quadruplex appears to be the biologically relevant structure.

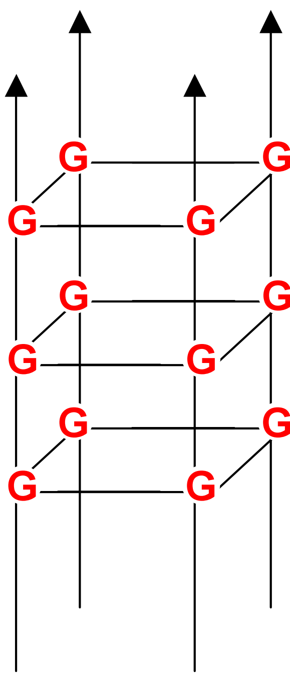
The use of G-quadruplex stabilizing compounds targeted at telomeres prompted the investigation of affects within the *c-MYC* locus. Using the cationic porphyrin, TMPyP4, Grand *et al.* (2002)[137] and Siddiqui-Jain *et al.* (2002)[33] have demonstrated repression of transcriptional activation of *c-MYC* in cells based on G-quadruplex stabilization. In addition, mutational analysis by replacement of a G to an A within this G-rich region which is predicted to destabilize quadruplex formation results in a 3-fold increase in *c-MYC* expression also points to a biological role for this secondary structure [33].

Hairpins and Holliday junctions (mini- & micro-satellites)

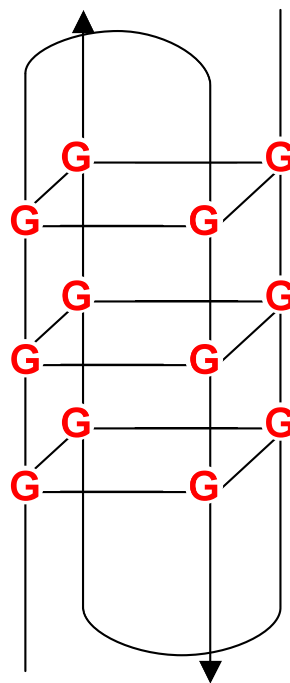
A variety of secondary structures (hairpins, cruciforms) have now been detected in the genomic DNA of a number of prokaryotic and eukaryotic species, including humans. These structures are associated with regulation of gene transcription, possibly as recognition binding sites, and may be targets for selectively binding drugs that could either block or enhance transcription[138]. Thus, the potent transcription inhibitor actinomycin D [23] and analogues bind at least 10-fold more tightly to the hairpin conformation formed from the single-stranded DNA 5'-$A_7TAGT_4A_3TAT_7$-3' than to same strand fully duplexed to its complementary sequence[139]. Similar results have been reported for actinomycin D binding to GC-rich hairpin sequences [140].



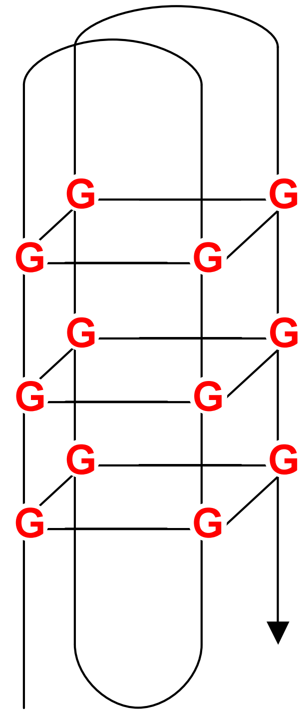
A. G-tetrad structure



B.



C.



D.

Figure 5
DNA Secondary Structures: G-Tetrad and G-quadruplex. A) Schematic illustration of G-tetrad, four guanine bases in a planar arrangement through Hoogsteen hydrogen bonds; B) G-quadruplex, parallel arrangement of four DNA strands; C) Intermolecular G-quadruplex, DNA sequences with G-rich repeats forming hairpins that dimerize to stablize bimolecular structure; and D) anti-parrallel intramolecular G-quadruplex (fold-over G-quadruplex), single DNA strand with four G-rich repeats that can fold upon themselves to form the quadruplex structure (adapted from Han and Hurley [32]).

DNA four-way junction structures (Holliday junctions)[141], often created by mismatches, are known to occur during DNA replication, repair and recombination, making them potential targets for the development of novel antiviral and antibacterial agents. Human TOPO IIβ also binds preferentially to four-way junction DNA[142], suggesting that it might operate via such structures. It has long been known that some intercalating agents show preferential binding to such branched DNA sequences[143]. Recently, a series of crystal structures of both mono-intercalators [24-26,144-146] and dimeric drugs [27,28,144,147], complexed with short oligonucleotides, have shown that the ligands can induce Holliday junction-like DNA structures. It remains unclear whether this reflects the situation in longer DNA.

Triple helices

DNA duplexes of certain sequences can bind a third DNA (or modified DNA) strand, to form a triple helix. The third strand binds to the existing base pairs in a manner called Hoogsteen base pairing; T binds to A (but in a different way to normal) and protonated C binds to G, to form the triplets T.AT and C^+.GC[148]. In this way a homopyrimidine third strand can bind to its complementary sequence of duplex DNA. Triple helices are generally less stable than the duplexes, and are thought to be induced naturally in the genome by supercoiling, as triple helix formation can relieve torsional stress[149]. However, some small molecules can stabilize triple helixes by preferentially binding to them. Many such compounds, like those that preferentially bind quadruplexes, tend to be intercalating agents of larger than usual planar area, with side chains in the correct disposition to bind in one or more of the three grooves of the triple helix. One of the best-studied is the benzo [e]pyridoindole BePI [29], that binds T.AT triplets[150]. The related antiviral indoloquinoxaline 9-OH-B220 [30] provides a very large stabilization of such triplet species, shifting the triplex-to-duplex equilibrium by up to 50°C [151]. The other major class of triplex stabilizers are the aminoglycosides. One of the most effective is neomycin [31], which binds preferentially in the larger Watson-Hoogsteen groove rather than one of the regular duplex grooves [152].

DNA-Associated Protiens

Proteins that associate with DNA vary in structure, sequence and structural binding motifs, degree of interaction, regulation, and role in regulating nuclear metabolism. Protein targets for drug design that will be discussed in this review include topoisomerase proteins, DNA methyltransferases, HMGs, TBP and histone deacetylases. Other transcription factors, repair proteins and other important nuclear proteins involved in replication and recombination are also important candidates to consider, but will not be discussed here.

Topoisomerases

Topoisomerase enzymes are involved in resolving topological problems in DNA, such as superhelical tension, that arise during most nuclear activities involving DNA. Topoisomerase I (TOPO I) acts by introducing one break in one strand of the DNA, whereas Topoisomerase II (TOPO II) acts by making a double strand break. Aside from several intercalating agents such as doxorubicin, TOPO I and II are the targets of numerous drugs, some of which function as poisons and others as competitive inhibitors[153].

The major class of TOPO I poisons is exemplified by camptothecin (CPT) [32,154], a cytotoxic alkaloid containing an essential six membered lactone ring. This is a natural product, originally isolated from *Camptotheca acuminata*. It binds to TOPO I[155] in association with DNA and stabilizes the single-stranded break, preventing strand passing through the break and subsequent resealing. Structure-activity studies have lead to the development of a number of closely related compounds that are also used clinically. TOPO II poisons can be classified into two major classes[153]. The DNA intercalators that bind primarily to DNA are exemplified by the anthracycline doxorubicin [33], while those that bind primarily to the protein can be exemplified by the epipodophyllotoxin etoposide [34]. Both classes of compounds work by formation of a ternary drug/DNA/enzyme complex that inhibits the DNA resealing activity of the enzyme, stabilizing the cleavable complex and resulting in DNA double-strand breaks[156]. Other inhibitors, such as fostriecin [35] and derivatives, inhibit the catalytic activity of topoisomerase without stabilizing the cleavable complex[157].

Changes in the nuclear environment that result from topoisomerase inhibitors vary. TOPO II is cell-cycle dependent and expression is higher in actively proliferating cells leading to their greater susceptibility[158,159]. TOPO II inhibitors generally disrupt the cell-cycle during S phase because the increased concentration of DNA double-strand breaks interferes with DNA replication and triggers apoptosis[153,160]. In the case of CPT, the generation of DNA breaks in addition to the collision of the stabilized cleavable complex with the DNA replication machinery is necessary for cell killing [160-162]. CPT also blocks the elongation step of transcription[163].

DNA methylases and DNA methylation

Nuclear DNA methylation patterns are often altered in human malignancies. In some cases, the genome-wide degree of methylation is reduced and in certain circumstances may be responsible for abnormal gene activation. In other cases, promoters of certain genes are hypermethylated and this leads to gene silencing [164]. There are at least three enzymes responsible for DNA methylation of

CpG dinucleotides (the transfer of methyl groups from S-adenosyl-L-methionine to cytosine to form 5-methylcytosine), DNA methyltransferase (DNMT) 1, DNMT3A, and DNMT 3B. DNMT1 is predominantly responsible for maintaining methylation patterns during replication, whereas DNMT3A and 3B are required for *de novo* methylation[165].

CpG islands are concentrated regions of CpG dinucleotides generally found in promoter regions[166,167]. Methylation of these regions in normal cells generally occurs on genes located on the inactivated X chromosome and on selectively silenced alleles[168,169]. In neoplastic tissue however, methylation can lead to silencing of key tumour suppressor genes[170]. Tumour suppressor and growth inhibitory genes, such as $p15^{INK4b}$, $p16^{INK4a}$, and $p21^{WAF1/CIP1}$, are often silenced in cancer cells by DNA hypermethylation of their promoter regions[165]. Reversing the degree of DNA methylation using azanucleoside DNA methyltransferase inhibitors such as 5-azacytidine [36] and 5-aza-2'-deoxycytidine [37] (decitabine)[171] reactivates transcription at previously silenced tumour suppressor promoters[172]. The mechanism by which these azanucleotides act is via incorporation into the cellular DNA and inhibition of DNMT1 via covalent interaction (thioether bond) at C-6[173] which forces replication to proceed without DNA methylation. Thus far these compounds have shown some success in clinical trials [174-177].

Two alternate strategies that have been employed to inhibit DNMT1 are the use of DNMT1 anti-sense oligonucleotides and the use of hairpin-structured oligonucleotide substrate mimics[164]. Fournel *et al.* [178]) demonstrated that use of antisense oligonucleotides led to loss of DNMT1 protein, decrease in methylation at the $p16^{INK4a}$ promoter, and expression of $p16^{INK4a}$ RNA. Although the hairpin mimics effectively inhibit purified DNMT1 *in vitro*, they have not been able to induce methylation changes in cells[179].

Histone acetylation/deacetylation (HDACs)

The regulation of chromatin structure and DNA sequence accessibility is the subject of extensive research because they play important roles in governing numerous nuclear activities including transcription, recombination, and replication. The basic nuclear DNA unit is the nucleosome; a 146 bp stretch of nuclear DNA wrapped around an octamer of histone (H) proteins (two each of H2A, H2B, H3 and H4) [180-182]. Arrays of nucleosomes are then folded into higher-order structures such as chromatin fibres. The three known mechanisms influencing chromatin structure are ATP-dependent chromatin remodelling, histone replacement, and covalent modification of either DNA or associated histones. In the last case, modifications include cytosine methylation and histone acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation[180]. The regulation of these mechanisms and their cross-talk in the nucleus affects whether DNA sequences will be accessible for nuclear processes.

Active transcription is associated with lysine acetylation of histones and methylation at Lys-9 of H3; whereas, transcriptional repression is associated with deacetylated histones, methylation of Lys-4 of H3 and methylated DNA[180,181,183,184]. The main mechanism by which HDACs repress transcription is likely to be the result of functional linkage between HDACs and DNA methylation via recruitment of HDAC to DNA by methyl binding proteins (for example MeCP2 and MBPs1–4)[184]. This in turn inhibits the recruitment transcriptional activators such as SWI/SNF, SAGA, and TBP proteins to the promoter regions preventing gene expression[185]. In addition, the deacetylated histone tails may actively recruit transcriptional silencers. For example in yeast, Sir3 preferentially binds to non-acetylated DNA and associates with Sir2/Sir4 to form a "silencing" complex that spreads heterochromatin formation [180,183,186]. Although Sir3 and Sir4 human homologues have not been identified, the potential for preferential binding to non-acetylated DNA is still a feasible mechanism to consider. In addition, it is important to consider that HDACs may act on other molecules, such as specific transcription factors and alter their activation states[180].

There are three major classes of HDACs, although known inhibitors only work on two of the three classes[187]. The HDAC I class is composed of proteins that are homologous to the yeast RPD3 gene; HDAC1,2,3,8, and 11. HDAC class II is subdivided into two; IIA proteins include HDAC4,5,7, and 9, IIB proteins are HDAC 6 and 10 and are characterized by sequence homology to yeast HDAI. Class III proteins are similar to the yeast repressor protein, Sir2, although they have not been extensively characterized in mammals. The HDAC inhibitors of both HDAC I and II classes bind and inhibit HDAC activity, induce acetylation of histones in cells, inhibit tumour cell proliferation *in vitro*, and several compounds have shown promising results in human xenograft experiments [187-190].

The HDACs are attractive targets for anti-cancer drug design because they are involved in gene silencing similar to the DNA methylase targets mentioned above, and a number of structurally diverse classes of inhibitors are in development. However, only the first two types of HDACs are sensitive to the inhibitors developed thus far. These fall into several structural classes; the short chain fatty acids, the hydroxamic acids, the cyclic tetrapeptides, the benzamides, and the epoxides[187].

A number of these HDAC inhibitors are at various stages of clinical study[187]. Phase I studies with suberoylanilide hydroxamic acid (SAHA) [38] concluded that it was well tolerated, inhibited the HDAC activity *in vivo* (in peripheral blood mononuclear cells and in post-therapy tumour biopsy), and has anti-tumour activity in solid (bladder) and haematological tumours[191]. SAHA is in phase II development for treatment of cutaneous T cell lymphoma, peripheral T cell lymphoma, and recurrent or metastatic squamous cell carcinoma of the head and neck[187]. The depsipeptide FR901228 (FK228) [39] is in trials for a variety of leukemias and lymphomas, and refractory or progressive small cell and non-small cell lung cancer[192].

The cellular response to HDAC inhibitors is becoming better characterized. For example, SAHA binds directly to the HDAC catalytic site, inhibits enzyme activity, inhibits cellular proliferation and promotes an apoptotic cellular response [193-195]. Oligonucleotide microarray analysis of multiple myeloma cells treated with SAHA demonstrated that commitment to apoptosis is associated with suppression of genes involved in promoting cell growth and survival, drug resistance, cell cycle control, DNA replication and repair, and proteosome function[196].

Combination therapy using HDAC inhibitors and other agents such as DNA methylase inhibitors and heat shock protein 90 (HSP90) antagonists have demonstrated a synergistic effect on cellular responses such as activation of gene expression and induction of apoptosis [197-202]. For example, using depsipeptide and 5-aza-2'-deoxycytidine treatment of breast cancer cell lines, Primeau *et al.*[197] demonstrated synergistic anti-neoplastic activity and the activation of *mapsin* and *gelsolin* gene expression, two metastatic tumour suppressor genes that are silenced by epigenetic mechanisms in breast cancer lines. In the study by Rahmini *et al*[202], co-administration of the HSP90 antagonist 17-allylamino-17-demethoxy-geldanamycin (17-AAG) and SAHA induced synergistic induction of mitochondrial damage, caspase activation (-3 and -8), and apoptosis in several different human leukaemia cell lines. These investigators found that co-administration of 17-AAG with SAHA blocked the SAHA mediated induction of the cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1}$, and that this novel finding might explain why the combination treatment was more effective. When cells were forced to express $p21^{WAF1/CIP1}$, the combination treatment was far less effective[202].

Proteins that recognize distorted DNA structures: Tata Box Binding Protein and High Mobility Group proteins

In many cases the interaction of small molecules with DNA results in a distortion in the normal DNA structure. This distortion may result in the inhibition or induction of protein binding at or in the vicinity of the perturbation. The outcomes of the alteration vary and may be more or less dependent on the primary nucleotide sequence. In this subsection, TBP and HMG proteins are discussed.

Cis-diamminedichloroplatinum [40] (cisplatin) is a DNA alkylator that forms predominantly intrastrand crosslinks by binding to the N_7 nitrogen of adjacent guanidine residues or guanidine-adenine residues. Cisplatin binding causes a structural distortion in the DNA (bend of 40–60°, and twist of 25–32°) as revealed by crystallography and NMR spectroscopy[203]. The cisplatin-distorted DNA activates binding of a number of nuclear proteins such as TBP, p53, HMG1, and H1[23,24,204-208]. In the case of TBP, binding is somewhat sequence dependent, although the preferred sequence is not the TBP consensus sequence[208]. It has been suggested that the affinity of TBP for cisplatin-modified DNA is because the DNA distortion caused by cisplatin crosslinking is similar to TATA box DNA structure when bound to TBP[209].

The effects of cisplatin treatment include inhibition of DNA synthesis and RNA transcription, effects on the cell cycle, and activation of apoptosis[207]. The binding of HMG1 to the cisplatin adduct has been shown to block translesion DNA synthesis *in vitro* [210]. Studies by He *et al.*[211] suggest that the interaction of HMG1 and the DNA adduct increases the cytotoxic potential of cisplatin. In these experiments, breast cancer cells were induced to over express HMG1 by pre-treatment with estrogen and were shown to be more sensitive to cisplatin than uninduced cells. This sensitization could be because DNA replication is halted either directly or because DNA adducts are no longer effectively recognized by cellular nucleotide excision repair machinery[212,213].

While cisplatin has been effective in treating a number of different cancer types including testicular cancer, ovarian cancer, head and neck cancer, the onset of resistance has limited its use[207,214]. In general, the degree of cytotoxicity of cisplatin is correlated with the formation and duration of DNA adducts, therefore processes that interfere with these will contribute to the development of resistance. The pathways that have been implicated in the development of resistance to cisplatin include pharmacologic-based mechanisms (reduced drug uptake and increased drug inactivation) as well as mechanisms that inhibit apoptotic signalling. Some of these anti-apoptotic mechanisms include increased repair of the cisplatin-DNA adducts and/or masking of the DNA damage by creating unnatural protein binding sites and/or inducing binding of proteins such as HMG1, and H1 [1,14,203,215]. Additionally, many studies point to the DNA adduct masking (repair shielding) as a process strongly involved in potentiating cytotoxicity[212].

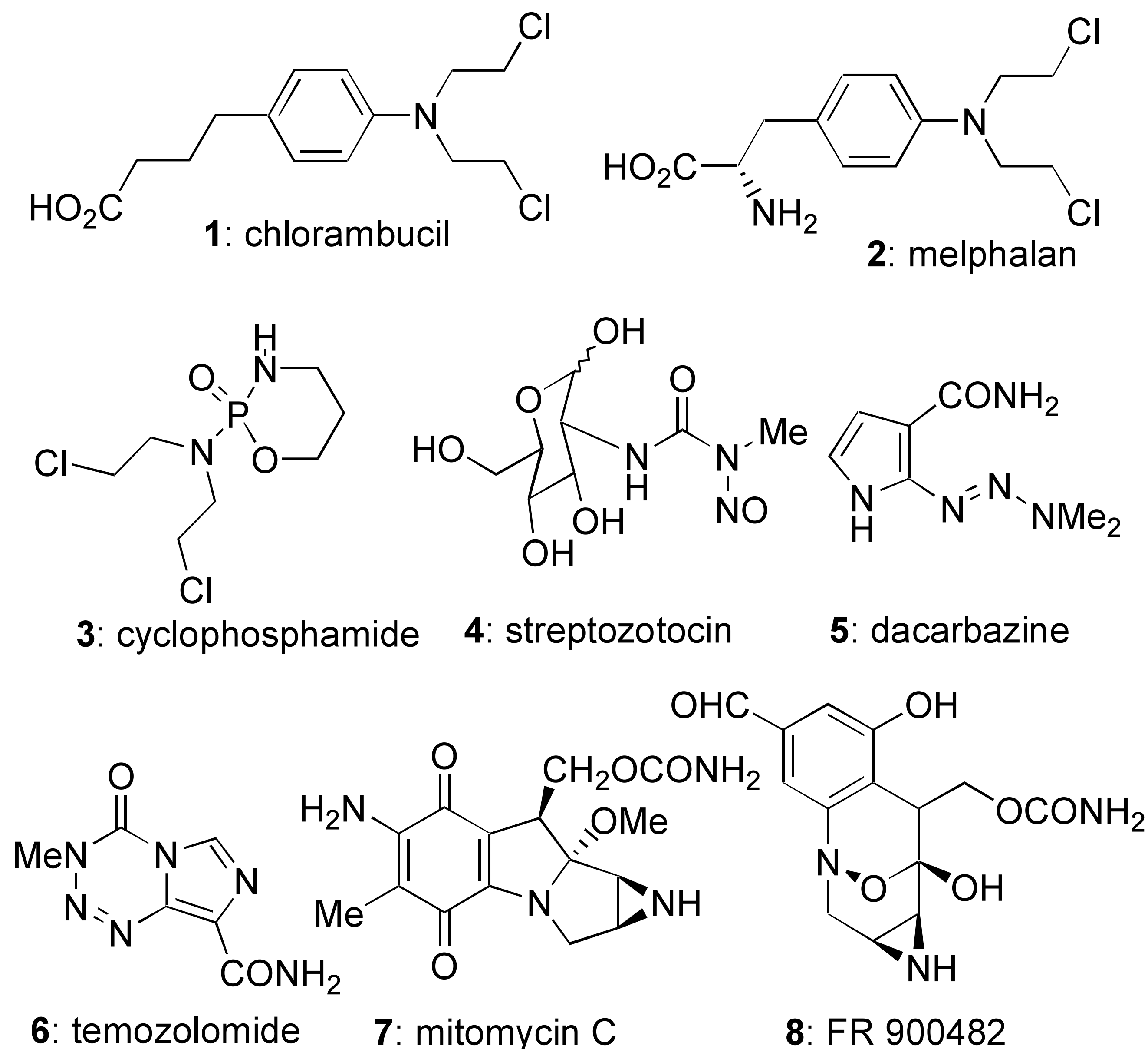


Figure 2
Chemical Structures 1–8. The chemical structures of chlorambucil, melphalan, cyclophosphamide, streptozotocin, dacarbazine, temozolomide, mitomycin C, FR 900482. Compounds are referred to in text by number in [#].

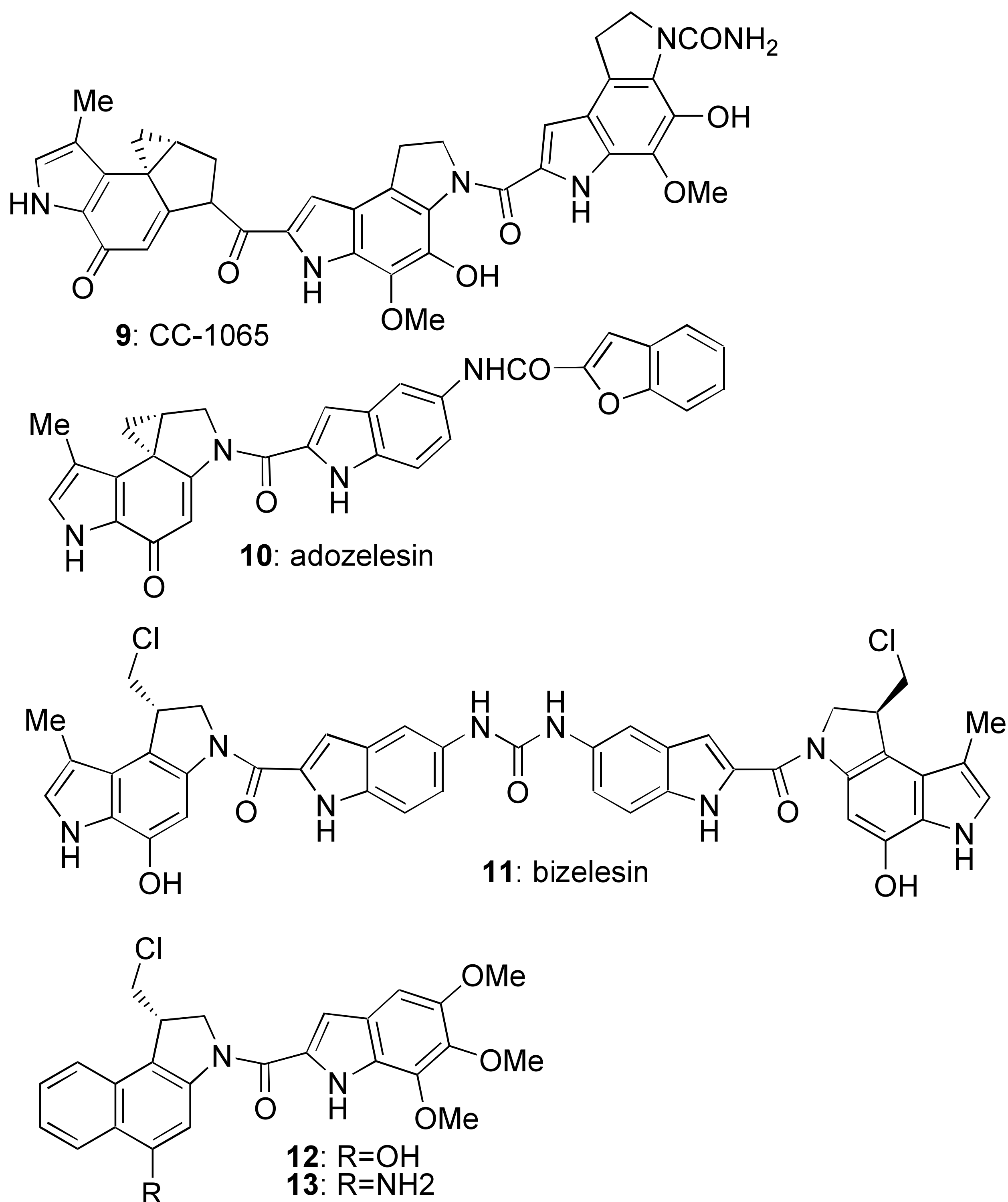


Figure 3
Chemical Structures 9–13. The chemical structures of CC-1065, adozelesin, bizelesin, and the aminoindoline compounds [12] and [13].

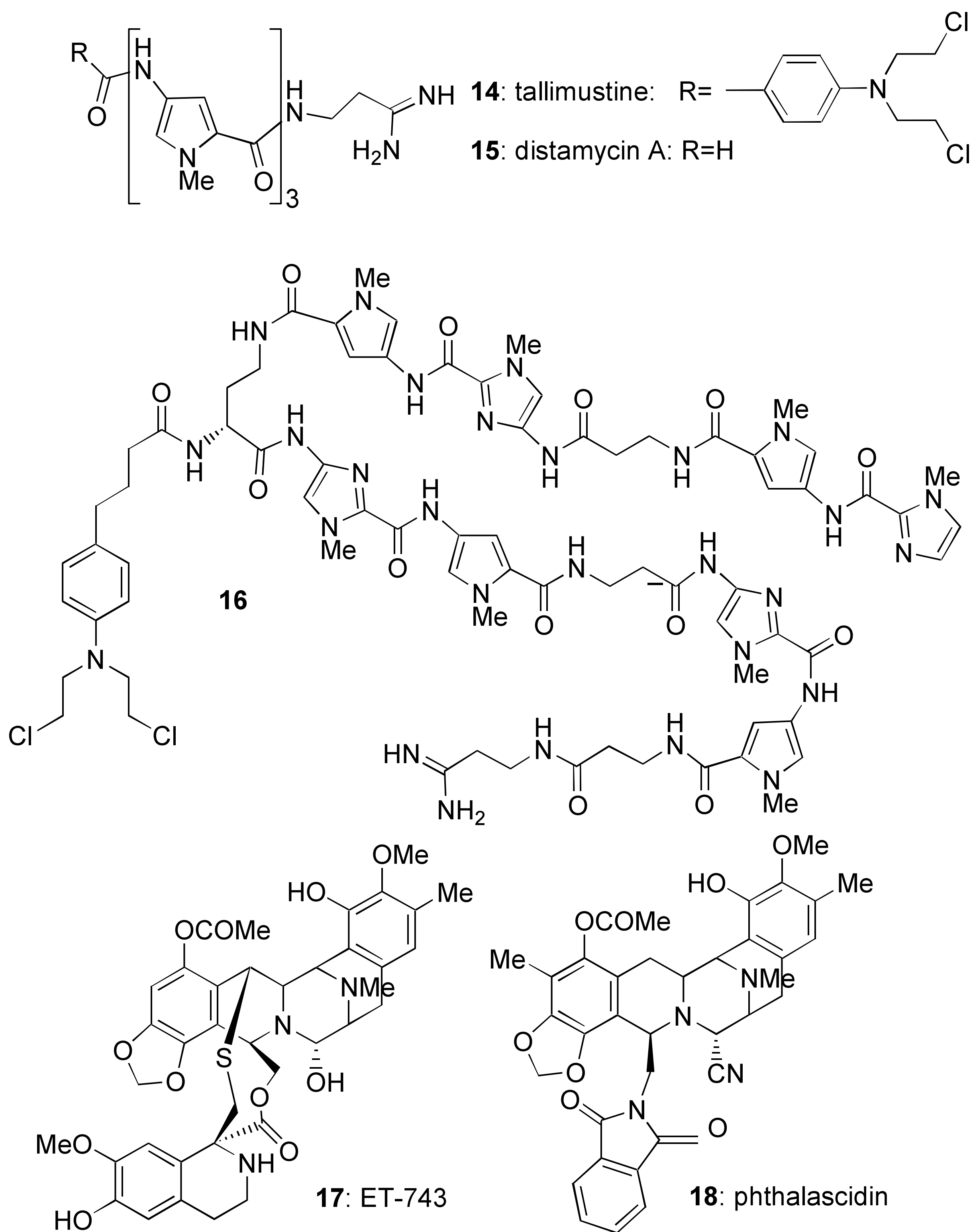


Figure 4
Chemical Structures 14–18. The chemical structures of tallimustine, distamycin A, [16], Et-743, and phthalascidin.

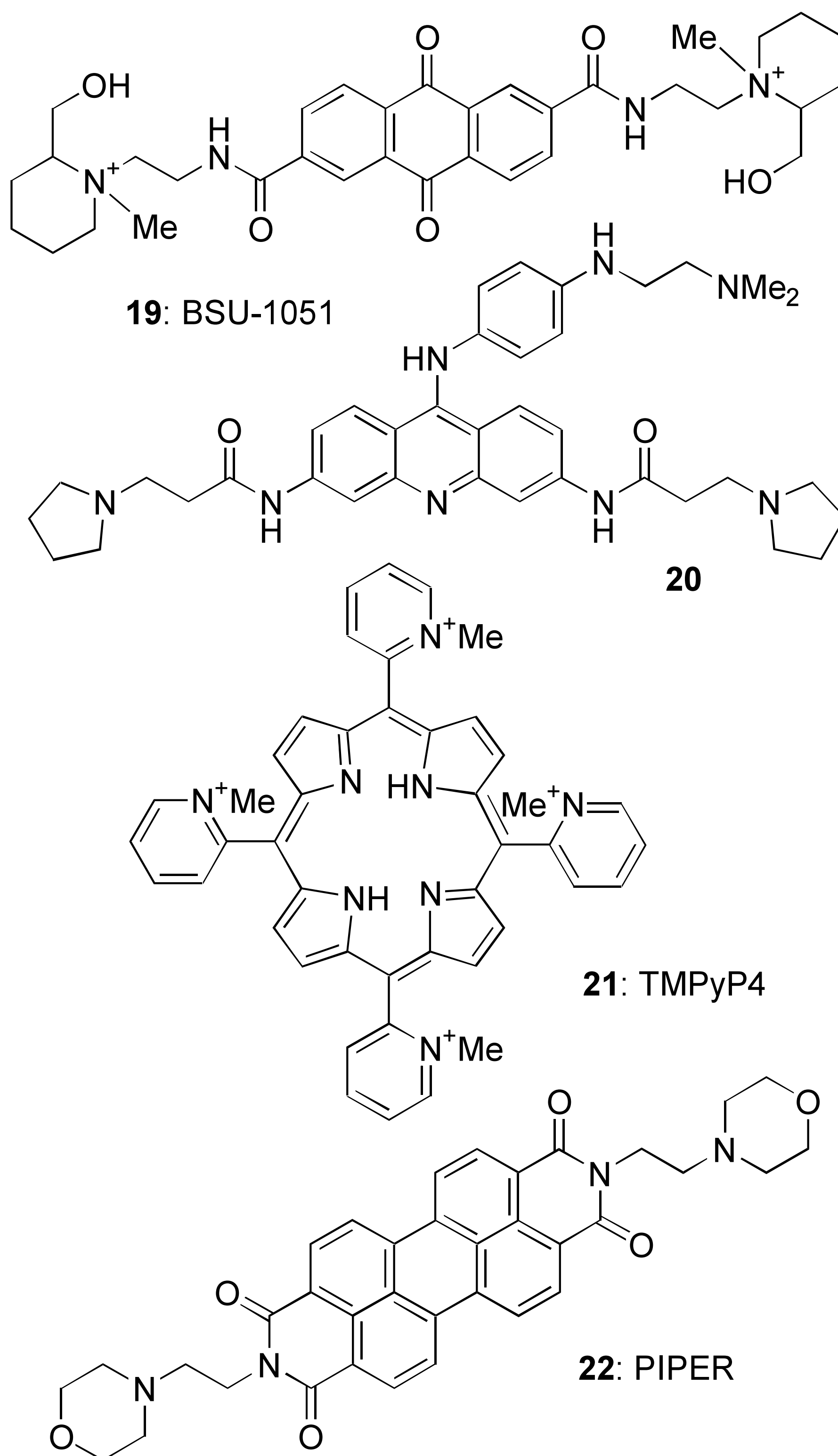


Figure 6
Chemical Structures 19–22. The chemical structures of BSU-1051, [20], TMPyP4, and PIPER.

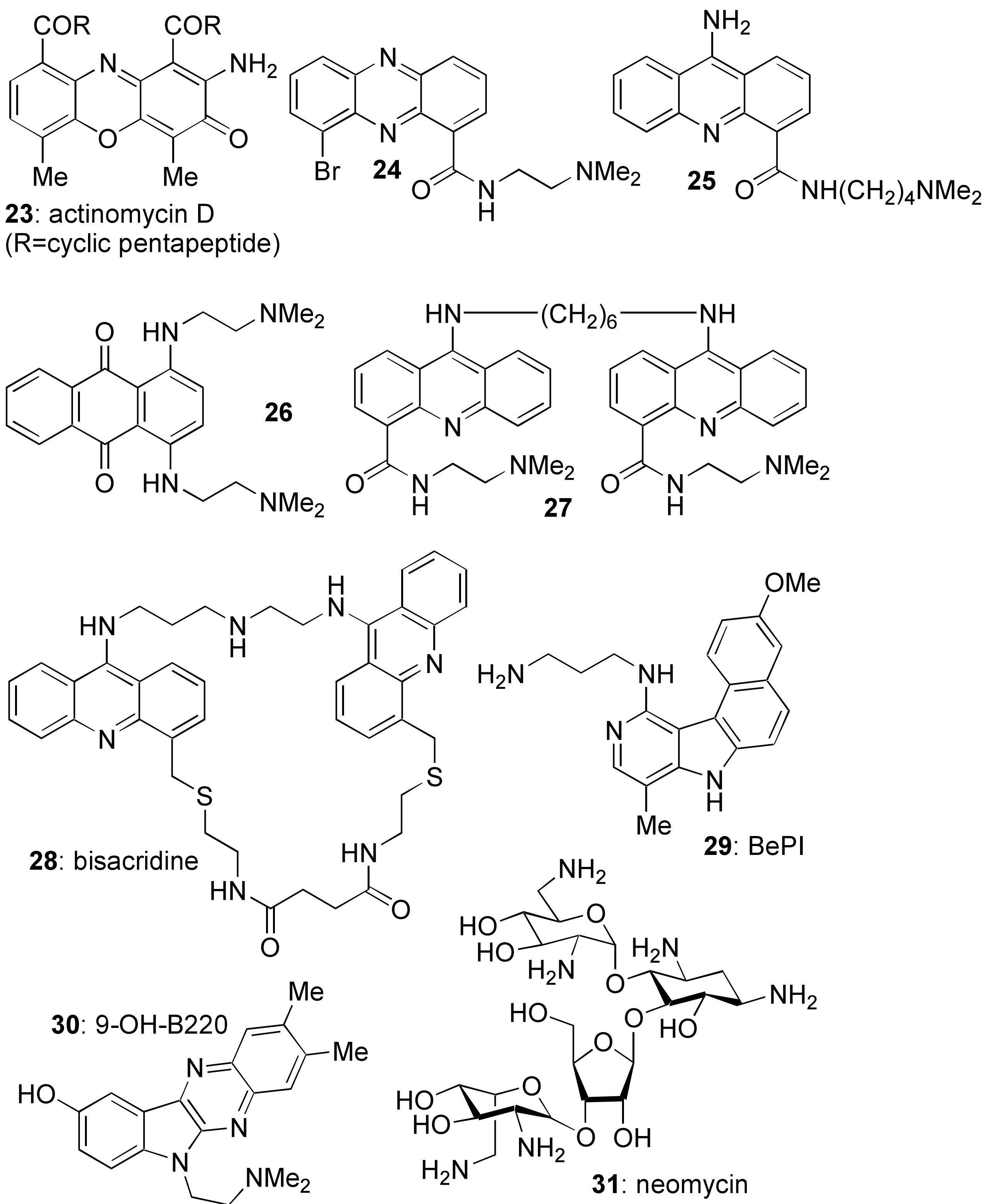


Figure 7
Chemical Structures 23–31. The chemical structures of actinomycin D, [24-27], bisacridine, BePI, 9-OH-B220, and neomycin.

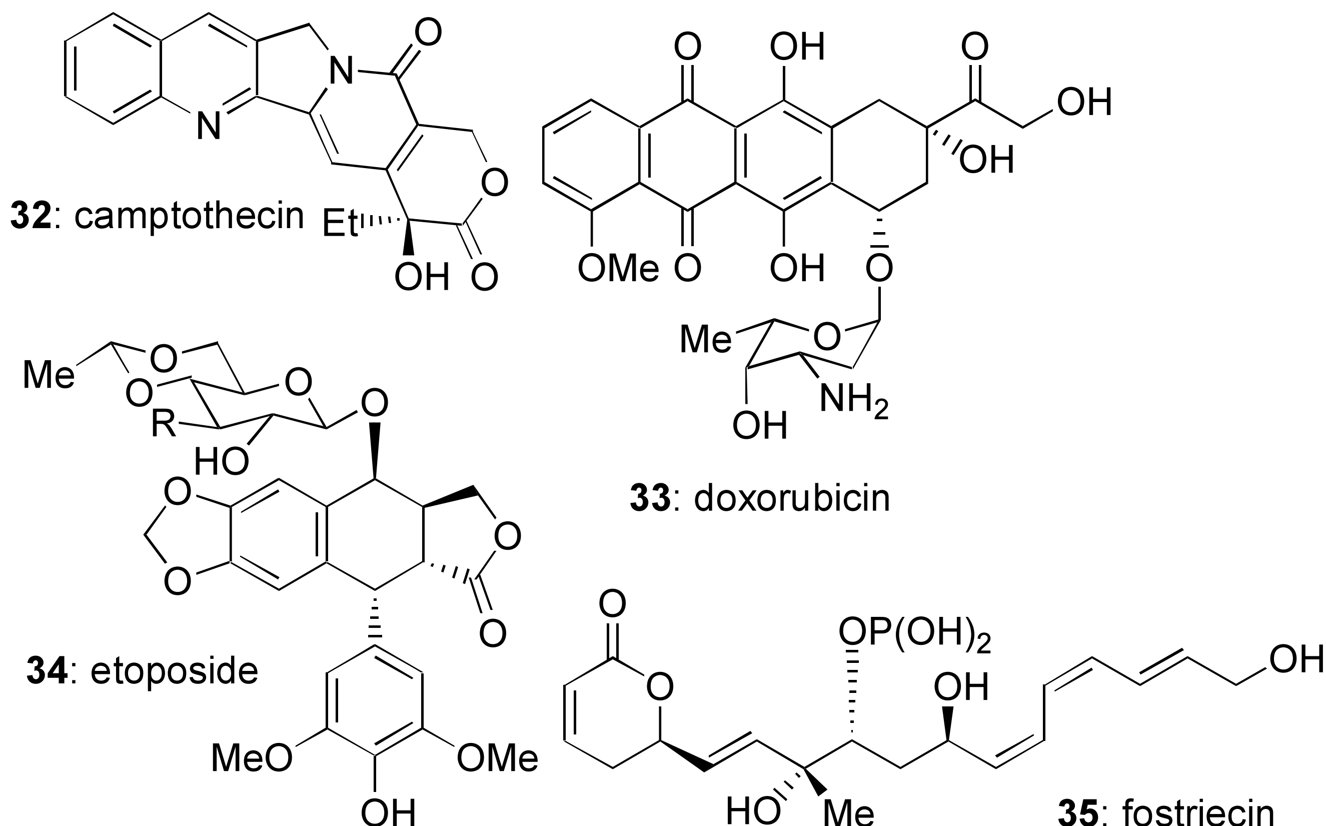


Figure 8
Chemical Structures 32–35. The chemical structures of camptothecin, doxorubicin, etoposide, and fostriecin.

Conclusions

The nucleus offers a variety of potential targets for anti-cancer drug design. Historically, most nucleus-specific agents have been designed to interact with DNA, causing extensive DNA damage leading to induction of cell death. The new strategy in drug design is to tailor the drug to the specific cancer diagnosed in an attempt to provide the most suitable treatment with the least deleterious side effects to the patient. The outcomes of this approach include drugs targeting specific signalling molecules such as BCR/ABL in the case of Glivec and chronic myelogenous leukemia[216]. In addition to reduced toxicity, using defined targets in combination therapy may help to reduce the development of drug resistance. Targeting specific key molecules in the nuclear environment, such as chromatin-associated proteins, proteins involved in replication, recombination, repair, and transcription is another level at which to disrupt requisite cancer-promoting pathways. The HDAC inhibitors in combination with the DMNTs shows promise of specifically activating genes that are important tumour suppressors silenced by hypermethylation more effectively than when used as single agents. Many topoisomerase targeted compounds have been successfully used in the clinic thus far, and certainly set the stage for the targeting of other proteins involved in DNA replication and recombination including some important helicases and repair proteins. The DNA-associated HMG proteins are another useful target and set a precedent for examining other DNA distortion- recognizing proteins and proteins that bind to specific DNA regions such as MARs.

Alternatively, as we understand more about genomic DNA itself, specific sequences and regions, secondary structures, nucleosome formation and higher-order compaction, and interactions with the nuclear matrix and associated proteins, drugs have been and will continue to be designed to disrupt these processes specifically. It is

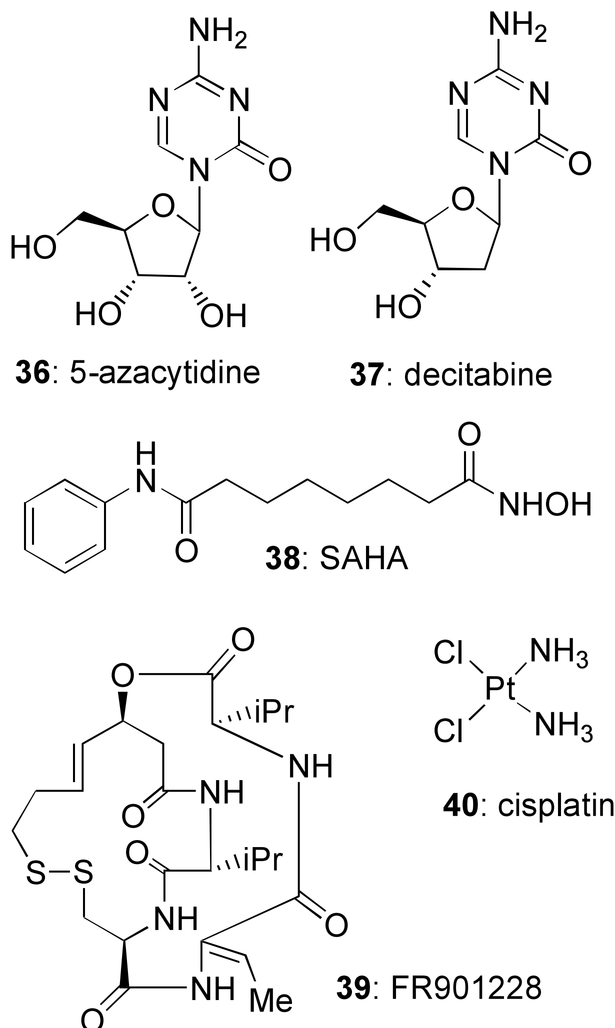


Figure 9
Chemical Structures 36–40. The chemical structures of 5-azacytidine, decitabine, SAHA, FR 901228, and cisplatin.

hoped that this approach will provide the same benefits as outlined above for targeting the associated proteins, but may be broader by interfering with multiple processes that occur at the affected locations.

An exciting area of research being developed is conjugation of compounds capable of recognizing certain sequences with compounds that exert another function. Examples of this are the polyamide-chlorambucil and CBI conjugates[93,94] that connect highly specific DNA sequence recognition with DNA alkylation and mostly work by inhibiting specific gene expression, but this approach could be extended to facilitate targeting of the DNMT and HDAC inhibitors, for instance, to specific tumour suppressor promoters to activate transcription. Lastly, another important area of research is focused on taking these small molecules and developing them into tumour-specific pro-drugs as another means of reducing deleterious side effects to the patient[217].

Abbreviations used

HPLC high performance liquid chromatography

PCR polymerase chain reaction

bp base pairs

HMG High Mobility Group

EMSA electromobility shift assay

ChIP chromatin immunoprecipitation

CD circular dichroism

IR infra red

NMR nuclear magnetic resonance

FISH fluorescence in situ hybridization

MTBP/TRF2 Membrane Tel binding protein

Tel Telomere

FAS APO-1, CD95, death receptor for FAS ligand

TNF Tumour Necrosis Factor

ATM Ataxia-telangiectasia Mutated

ATR Ataxia-telangiectasia Mutated and RAD3-related

ChK1,2 Checkpoint Kinase

BRCA-1 Breast Cancer gene 1

NBS Nijmegen breakage syndrome

MRE Meiotic recombination

MSH Mut S homologue

MLH Mut L homologue

CDK Cyclin-dependent kinase

Rb Retinoblastoma

E2F E2 Factor

RPA Replication Protein A

MRN MRE-11/RAD50/NBS-1

PI Propidium iodide

IL Interleukin

ELF member of the ETS family of trabscription factors

NFκB Nuclear factor of κ Chain from B cells

MAR Matrix attachment region

FRA Fragile site

c-MYC homologue to the oncogene *v-myc* isolated from MC29 myelocytomatosis virus

CBI Cyclopropylbenzindoline

gpt xanthine-guanine phophoribosyl transferase gene

TBP Tata Box Binding Protein

SP1 Specificty Protein

SV40 Simion virus 40

Py-Im pyrrole-imidazole

ETS E-26 avion erthroblastosis virus transformation specific protein

HIV Human immunodeficiency virus

LSF also known as LBP-1, UBP, CP-2

Py-Im-Chl Py-Im chlorambucil

Et-743 Ecteinascidin 743

NF-Y Nuclear Factor Y

MDR Multi-drug resistance

HSP Heat Shock Protein

FOS AP-1 protein

JUN AP-1 protein

H Histone

ERG1 Early Growth Response Factor

SRF Serum Response Factor

ssDB single-strand DNA break

TEP1 also known as PTEN, MMAC1

hTER RNA moiety of telomerase

hTERT Catalytic subunit of telomerase

NHE Nuclease hypersensitive element

TOPO Topoisomerase

CPT Camptothecin

DNMT DNA methyltransferase

HDAC Histone Deacetylase

MBP Methyl Binding Protein

MeCP Methyl Cytosine binding Protein

SWI/SNF global transcriptional activation multi-protein complex

SAGA Histone acetyltransferase complex (Spt/Ada/Gcn5/acetylase)

Sir2,3,4 yeast transcriptional silencer proteins

SAHA suberoylanilide hydroxamic acid

p53 Protein 53

BCR/ABL fusion protein resulting from a translocation of *BCR* and *ABL* genes

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References

1. Hurley LH: **DNA and its associated processes as targets for cancer therapy.** *Nat Rev Cancer* 2002, **2:**188-200.
2. Gieseg MA, Matejovic J, Denny WA: **Comparison of the patterns of DNA alkylation by phenol and amino seco-CBI-TMI compounds: use of a PCR method for the facile preparation of single end-labelled double-stranded DNA.** *Anticancer Drug Des* 1999, **14:**77-84.

3. Herzig MC, Trevino AV, Arnett B, Woynarowski JM: **Tallimustine lesions in cellular DNA are AT sequence-specific but not region-specific.** *Biochemistry* 1999, **38:**14045-14055.
4. Woynarowski JM, Napier C, Trevino AV, Arnett B: **Region-specific DNA damage by AT-specific DNA-reactive drugs is predicted by drug binding specificity.** *Biochemistry* 2000, **39:**9917-9927.
5. Herzig MC, Rodriguez KA, Trevino AV, Dziegielewski J, Arnett B, Hurley L, Woynarowski JM: **The genome factor in region-specific DNA damage: the DNA-reactive drug U-78779 prefers mixed A/T-G/C sequences at the nucleotide level but is region-specific for long pure AT islands at the genomic level.** *Biochemistry* 2002, **41:**1545-1555.
6. Woynarowski JM, Trevino AV, Rodriguez KA, Hardies SC, Benham CJ: **AT-rich islands in genomic DNA as a novel target for AT-specific DNA-reactive antitumor drugs.** *J Biol Chem* 2001, **276:**40555-40566.
7. Dudouet B, Burnett R, Dickinson LA, Wood MR, Melander C, Belitsky JM, Edelson B, Wurtz N, Briehn C, Dervan PB, Gottesfeld JM: **Accessibility of nuclear chromatin by DNA binding polyamides.** *Chem Biol* 2003, **10:**859-867.
8. Abu-Daya A, Brown PM, Fox KR: **DNA sequence preferences of several AT-selective minor groove binding ligands.** *Nucleic Acids Res* 1995, **23:**3385-3392.
9. Abu-Daya A, Fox KR: **Interaction of minor groove binding ligands with long AT tracts.** *Nucleic Acids Res* 1997, **25:**4962-4969.
10. Bremer RE, Szewczyk JW, Baird EE, Dervan PB: **Recognition of the DNA minor groove by pyrrole-imidazole polyamides: comparison of desmethyl- and N-methylpyrrole.** *Bioorg Med Chem* 2000, **8:**1947-1955.
11. Harshman KD, Dervan PB: **Molecular recognition of B-DNA by Hoechst 33258.** *Nucleic Acids Res* 1985, **13:**4825-4835.
12. Schultz PG, Dervan PB: **Distamycin and penta-N-methylpyrrolecarboxamide binding sites on native DNA. A comparison of methidiumpropyl-EDTA-Fe(II) footprinting and DNA affinity cleaving.** *J Biomol Struct Dyn* 1984, **1:**1133-1147.
13. Hurley LH: **Secondary DNA structures as molecular targets for cancer therapeutics.** *Biochem Soc Trans* 2001, **29:**692-696.
14. Gniazdowski M, Denny WA, Nelson SM, Czyz M: **Transcription factors as targets for DNA-interacting drugs.** *Curr Med Chem* 2003, **10:**909-924.
15. Woynarowski JM: **Targeting critical regions in genomic DNA with AT-specific anticancer drugs.** *Biochim Biophys Acta* 2002, **1587:**300-308.
16. Reeves R, Beckerbauer L: **HMGI/Y proteins: flexible regulators of transcription and chromatin structure.** *Biochim Biophys Acta* 2001, **1519:**13-29.
17. Reeves R, Beckerbauer LM: **HMGA proteins as therapeutic drug targets.** *Prog Cell Cycle Res* 2003, **5:**279-286.
18. Agresti A, Bianchi ME: **HMGB proteins and gene expression.** *Curr Opin Genet Dev* 2003, **13:**170-178.
19. Catez F, Lim JH, Hock R, Postnikov YV, Bustin M: **HMGN dynamics and chromatin function.** *Biochem Cell Biol* 2003, **81:**113-122.
20. Thomas JO: **HMG1 and 2: architectural DNA-binding proteins.** *Biochem Soc Trans* 2001, **29:**395-401.
21. Chiang SY, Welch J, Rauscher F. J., 3rd, Beerman TA: **Effects of minor groove binding drugs on the interaction of TATA box binding protein and TFIIA with DNA.** *Biochemistry* 1994, **33:**7033-7040.
22. Chow CS, Whitehead JP, Lippard SJ: **HMG domain proteins induce sharp bends in cisplatin-modified DNA.** *Biochemistry* 1994, **33:**15124-15130.
23. Jung Y, Mikata Y, Lippard SJ: **Kinetic studies of the TATA-binding protein interaction with cisplatin-modified DNA.** *J Biol Chem* 2001, **276:**43589-43596.
24. Jung Y, Lippard SJ: **Nature of full-length HMGB1 binding to cisplatin-modified DNA.** *Biochemistry* 2003, **42:**2664-2671.
25. Welch JJ, Rauscher F. J., 3rd, Beerman TA: **Targeting DNA-binding drugs to sequence-specific transcription factor.DNA complexes. Differential effects of intercalating and minor groove binding drugs.** *J Biol Chem* 1994, **269:**31051-31058.
26. Beckerbauer L, Tepe JJ, Cullison J, Reeves R, Williams RM: **FR900482 class of anti-tumor drugs cross-links oncoprotein HMG I/Y to DNA in vivo.** *Chem Biol* 2000, **7:**805-812.
27. Beckerbauer L, Tepe JJ, Eastman RA, Mixter PF, Williams RM, Reeves R: **Differential effects of FR900482 and FK317 on apoptosis, IL-2 gene expression, and induction of vascular leak syndrome.** *Chem Biol* 2002, **9:**427-441.
28. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP: **A DNA damage checkpoint response in telomere-initiated senescence.** *Nature* 2003, **426:**194-198.
29. Weinmann AS, Farnham PJ: **Identification of unknown target genes of human transcription factors using chromatin immunoprecipitation.** *Methods* 2002, **26:**37-47.
30. Wells J, Farnham PJ: **Characterizing transcription factor binding sites using formaldehyde crosslinking and immunoprecipitation.** *Methods* 2002, **26:**48-56.
31. Hurley LH, Wheelhouse RT, Sun D, Kerwin SM, Salazar M, Fedoroff OY, Han FX, Han H, Izbicka E, Von Hoff DD: **G-quadruplexes as targets for drug design.** *Pharmacol Ther* 2000, **85:**141-158.
32. Han H, Hurley LH: **G-quadruplex DNA: a potential target for anti-cancer drug design.** *Trends Pharmacol Sci* 2000, **21:**136-142.
33. Siddiqui-Jain A, Grand CL, Bearss DJ, Hurley LH: **Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription.** *Proc Natl Acad Sci U S A* 2002, **99:**11593-11598.
34. Simonsson T, Pecinka P, Kubista M: **DNA tetraplex formation in the control region of c-myc.** *Nucleic Acids Res* 1998, **26:**1167-1172.
35. Simonsson T, Pribylova M, Vorlickova M: **A nuclease hypersensitive element in the human c-myc promoter adopts several distinct i-tetraplex structures.** *Biochem Biophys Res Commun* 2000, **278:**158-166.
36. Simonsson T, Sjoback R: **DNA tetraplex formation studied with fluorescence resonance energy transfer.** *J Biol Chem* 1999, **274:**17379-17383.
37. Phillips K, Dauter Z, Murchie AI, Lilley DM, Luisi B: **The crystal structure of a parallel-stranded guanine tetraplex at 0.95 A resolution.** *J Mol Biol* 1997, **273:**171-182.
38. Brown K: **Visualizing nuclear proteins together with transcribed and inactive genes in structurally preserved cells.** *Methods* 2002, **26:**10-18.
39. Brown KE: **Chromatin folding and gene expression: new tools to reveal the spatial organization of genes.** *Chromosome Res* 2003, **11:**423-433.
40. Brown K: **Nuclear structure, gene expression and development.** *Crit Rev Eukaryot Gene Expr* 1999, **9:**203-212.
41. Dolnik AV, Kuznetsova IS, Voronin AP, Podgornaya OI: **Telomere-binding TRF2/MTBP localization during mouse spermatogenesis and cell cycle of the mouse cells L929.** *J Anti Aging Med* 2003, **6:**107-121.
42. Sidorova JM, Breeden LL: **Precocious G1/S transitions and genomic instability: the origin connection.** *Mutat Res* 2003, **532:**5-19.
43. Barbour L, Xiao W: **Regulation of alternative replication bypass pathways at stalled replication forks and its effects on genome stability: a yeast model.** *Mutat Res* 2003, **532:**137-155.
44. Holmquist GP, Maher VM: **The bypass of DNA lesions by DNA and RNA polymerases.** *Mutat Res* 2002, **510:**1-7.
45. Hu W, Kavanagh JJ: **Anticancer therapy targeting the apoptotic pathway.** *Lancet Oncol* 2003, **4:**721-729.
46. Zhou BB, Elledge SJ: **The DNA damage response: putting checkpoints in perspective.** *Nature* 2000, **408:**433-439.
47. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J: **BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures.** *Genes Dev* 2000, **14:**927-939.
48. Fei P, El-Deiry WS: **P53 and radiation responses.** *Oncogene* 2003, **22:**5774-5783.
49. Iliakis G, Wang Y, Guan J, Wang H: **DNA damage checkpoint control in cells exposed to ionizing radiation.** *Oncogene* 2003, **22:**5834-5847.
50. Goodarzi AA, Block WD, Lees-Miller SP: **The role of ATM and ATR in DNA damage-induced cell cycle control.** *Prog Cell Cycle Res* 2003, **5:**393-411.
51. Kastan MB, Lim DS: **The many substrates and functions of ATM.** *Nat Rev Mol Cell Biol* 2000, **1:**179-186.
52. McGowan CH: **Checking in on Cds1 (Chk2): A checkpoint kinase and tumor suppressor.** *Bioessays* 2002, **24:**502-511.
53. Bartek J, Falck J, Lukas J: **CHK2 kinase--a busy messenger.** *Nat Rev Mol Cell Biol* 2001, **2:**877-886.

54. Bartek J, Lukas J: **Chk1 and Chk2 kinases in checkpoint control and cancer.** *Cancer Cell* 2003, **3:**421-429.
55. Rhind N, Russell P: **Mitotic DNA damage and replication checkpoints in yeast.** *Curr Opin Cell Biol* 1998, **10:**749-758.
56. Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB: **ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway.** *Nature* 2000, **404:**613-617.
57. Kim ST, Lim DS, Canman CE, Kastan MB: **Substrate specificities and identification of putative substrates of ATM kinase family members.** *J Biol Chem* 1999, **274:**37538-37543.
58. Cortez D, Wang Y, Qin J, Elledge SJ: **Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks.** *Science* 1999, **286:**1162-1166.
59. Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH: **Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response.** *Science* 1999, **285:**747-750.
60. Hansson J, Lewensohn R, Ringborg U, Nilsson B: **Formation and removal of DNA cross-links induced by melphalan and nitrogen mustard in relation to drug-induced cytotoxicity in human melanoma cells.** *Cancer Res* 1987, **47:**2631-2637.
61. Pieper RO, Futscher BW, Erickson LC: **Transcription-terminating lesions induced by bifunctional alkylating agents in vitro.** *Carcinogenesis* 1989, **10:**1307-1314.
62. Wang AL, Tew KD: **Increased glutathione-S-transferase activity in a cell line with acquired resistance to nitrogen mustards.** *Cancer Treat Rep* 1985, **69:**677-682.
63. Suzukake K, Vistica BP, Vistica DT: **Dechlorination of L-phenylalanine mustard by sensitive and resistant tumor cells and its relationship to intracellular glutathione content.** *Biochem Pharmacol* 1983, **32:**165-167.
64. Brendel M, Ruhland A: **Relationships between functionality and genetic toxicology of selected DNA-damaging agents.** *Mutat Res* 1984, **133:**51-85.
65. Palmer RG, Denman AM: **Malignancies induced by chlorambucil.** *Cancer Treat Rev* 1984, **11:**121-129.
66. Schein PS, O'Connell MJ, Blom J, Hubbard S, Magrath IT, Bergevin P, Wiernik PH, Ziegler JL, DeVita VT: **Clinical antitumor activity and toxicity of streptozotocin (NSC-85998).** *Cancer* 1974, **34:**993-1000.
67. Cohen GL, Falkson CI: **Current treatment options for malignant melanoma.** *Drugs* 1998, **55:**791-799.
68. Friedman HS, Kerby T, Calvert H: **Temozolomide and treatment of malignant glioma.** *Clin Cancer Res* 2000, **6:**2585-2597.
69. Bradner WT: **Mitomycin C: a clinical update.** *Cancer Treat Rev* 2001, **27:**35-50.
70. Ramel C: **Mini- and microsatellites.** *Environ Health Perspect* 1997, **105 Suppl 4:**781-789.
71. Debrauwere H, Gendrel CG, Lechat S, Dutreix M: **Differences and similarities between various tandem repeat sequences: minisatellites and microsatellites.** *Biochimie* 1997, **79:**577-586.
72. Jackson JA, Trevino AV, Herzig MC, Herman TS, Woynarowski JM: **Matrix attachment region (MAR) properties and abnormal expansion of AT island minisatellites in FRA16B fragile sites in leukemic CEM cells.** *Nucleic Acids Res* 2003, **31:**6354-6364.
73. Hanka LJ, Dietz A, Gerpheide SA, Kuentzel SL, Martin DG: **CC-1065 (NSC-298223), a new antitumor antibiotic. Production, in vitro biological activity, microbiological assays and taxonomy of the producing microorganism.** *J Antibiot (Tokyo)* 1978, **31:**1211-1217.
74. Atwell GJ, Tercel M, Boyd M, Wilson WR, Denny WA: **Synthesis and cytotoxicity of 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbomyl]-1,2-dihydre-3H-bene[e]indole (amino-seco-CBI-TMI) and related 5-alkylamino analogues: new DNA minor groove alkylating agents.** *J.Org. Chem.* 1998, **63:**9414-9420.
75. Reddy BS, Sharma SK, Lown JW: **Recent developments in sequence selective minor groove DNA effectors.** *Curr Med Chem* 2001, **8:**475-508.
76. Woynarowski JM, McHugh MM, Gawron LS, Beerman TA: **Effects of bizelesin (U-77779), a bifunctional alkylating minor groove agent, on genomic and simian virus 40 DNA.** *Biochemistry* 1995, **34:**13042-13050.
77. McHugh MM, Woynarowski JM, Mitchell MA, Gawron LS, Weiland KL, Beerman TA: **CC-1065 bonding to intracellular and purified SV40 DNA: site specificity and functional effects.** *Biochemistry* 1994, **33:**9158-9168.
78. Cobuzzi R. J., Jr., Burhans WC, Beerman TA: **Inhibition of initiation of simian virus 40 DNA replication in infected BSC-1 cells by the DNA alkylating drug adozelesin.** *J Biol Chem* 1996, **271:**19852-19859.
79. Woynarowski JM, Beerman TA: **Effects of bizelesin (U-77,779), a bifunctional alkylating minor groove binder, on replication of genomic and simian virus 40 DNA in BSC-1 cells.** *Biochim Biophys Acta* 1997, **1353:**50-60.
80. Weinberger M, Trabold PA, Lu M, Sharma K, Huberman JA, Burhans WC: **Induction by adozelesin and hydroxyurea of origin recognition complex-dependent DNA damage and DNA replication checkpoints in Saccharomyces cerevisiae.** *J Biol Chem* 1999, **274:**35975-35984.
81. McHugh MM, Kuo SR, Walsh-O'Beirne MH, Liu JS, Melendy T, Beerman TA: **Bizelesin, a bifunctional cyclopropylpyrroloindole alkylating agent, inhibits simian virus 40 replication in trans by induction of an inhibitor.** *Biochemistry* 1999, **38:**11508-11515.
82. Wang Y, Beerman TA, Kowalski D: **Antitumor drug adozelesin differentially affects active and silent origins of DNA replication in yeast checkpoint kinase mutants.** *Cancer Res* 2001, **61:**3787-3794.
83. Sun D, Hurley LH: **Binding of Sp1 to the 21-bp repeat region of SV40 DNA: effect of intrinsic and drug-induced DNA bending between GC boxes.** *Gene* 1994, **149:**165-172.
84. Cameron L, Thompson AS: **Determination of the structural role of the linking moieties in the DNA binding of adozelesin.** *Biochemistry* 2000, **39:**5004-5012.
85. Black AR, Black JD, Azizkhan-Clifford J: **Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer.** *J Cell Physiol* 2001, **188:**143-160.
86. Dervan PB, Edelson BS: **Recognition of the DNA minor groove by pyrrole-imidazole polyamides.** *Curr Opin Struct Biol* 2003, **13:**284-299.
87. Gottesfeld JM, Melander C, Suto RK, Raviol H, Luger K, Dervan PB: **Sequence-specific recognition of DNA in the nucleosome by pyrrole-imidazole polyamides.** *J Mol Biol* 2001, **309:**615-629.
88. Zimmer C, Wahnert U: **Nonintercalating DNA-binding ligands: specificity of the interaction and their use as tools in biophysical, biochemical and biological investigations of the genetic material.** *Prog Biophys Mol Biol* 1986, **47:**31-112.
89. Ehley JA, Melander C, Herman D, Baird EE, Ferguson HA, Goodrich JA, Dervan PB, Gottesfeld JM: **Promoter scanning for transcription inhibition with DNA-binding polyamides.** *Mol Cell Biol* 2002, **22:**1723-1733.
90. Dickinson LA, Trauger JW, Baird EE, Dervan PB, Graves BJ, Gottesfeld JM: **Inhibition of Ets-1 DNA binding and ternary complex formation between Ets-1, NF-kappaB, and DNA by a designed DNA-binding ligand.** *J Biol Chem* 1999, **274:**12765-12773.
91. Wurtz NR, Dervan PB: **Sequence specific alkylation of DNA by hairpin pyrrole-imidazole polyamide conjugates.** *Chem Biol* 2000, **7:**153-161.
92. Coull JJ, He G, Melander C, Rucker VC, Dervan PB, Margolis DM: **Targeted derepression of the human immunodeficiency virus type 1 long terminal repeat by pyrrole-imidazole polyamides.** *J Virol* 2002, **76:**12349-12354.
93. Wang YD, Dziegielewski J, Wurtz NR, Dziegielewska B, Dervan PB, Beerman TA: **DNA crosslinking and biological activity of a hairpin polyamide-chlorambucil conjugate.** *Nucleic Acids Res* 2003, **31:**1208-1215.
94. Wang YD, Dziegielewski J, Chang AY, Dervan PB, Beerman TA: **Cell-free and cellular activities of a DNA sequence selective hairpin polyamide-CBI conjugate.** *J Biol Chem* 2002, **277:**42431-42437.
95. D'Incalci M, Erba E, Damia G, Galliera E, Carrassa L, Marchini S, Mantovani R, Tognon G, Fruscio R, Jimeno J, Faircloth GT: **Unique features of the mode of action of ET-743.** *Oncologist* 2002, **7:**210-216.
96. Schwartsmann G, Da Rocha AB, Mattei J, Lopes R: **Marine-derived anticancer agents in clinical trials.** *Expert Opin Investig Drugs* 2003, **12:**1367-1383.
97. Pommier Y, Kohlhagen G, Bailly C, Waring M, Mazumder A, Kohn KW: **DNA sequence- and structure-selective alkylation of guanine N2 in the DNA minor groove by ecteinascidin 743,**

a potent antitumor compound from the Caribbean tunicate Ecteinascidia turbinata. *Biochemistry* 1996, **35:**13303-13309.

98. Garcia-Nieto R, Manzanares I, Cuevas C, Gago F: **Increased DNA binding specificity for antitumor ecteinascidin 743 through protein-DNA interactions?** *J Med Chem* 2000, **43:**4367-4369.
99. Hurley LH, Zewail-Foote M: **The antitumor agent ecteinascidin 743: characterization of its covalent DNA adducts and chemical stability.** *Adv Exp Med Biol* 2001, **500:**289-299.
100. Zewail-Foote M, Hurley LH: **Differential rates of reversibility of ecteinascidin 743-DNA covalent adducts from different sequences lead to migration to favored bonding sites.** *J Am Chem Soc* 2001, **123:**6485-6495.
101. Zewail-Foote M, Hurley LH: **Ecteinascidin 743: a minor groove alkylator that bends DNA toward the major groove.** *J Med Chem* 1999, **42:**2493-2497.
102. Bonfanti M, La Valle E, Fernandez Sousa Faro JM, Faircloth G, Caretti G, Mantovani R, D'Incalci M: **Effect of ecteinascidin-743 on the interaction between DNA binding proteins and DNA.** *Anticancer Drug Des* 1999, **14:**179-186.
103. Jin S, Gorfajn B, Faircloth G, Scotto KW: **Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation.** *Proc Natl Acad Sci U S A* 2000, **97:**6775-6779.
104. Minuzzo M, Marchini S, Broggini M, Faircloth G, D'Incalci M, Mantovani R: **Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743.** *Proc Natl Acad Sci U S A* 2000, **97:**6780-6784.
105. Friedman D, Hu Z, Kolb EA, Gorfajn B, Scotto KW: **Ecteinascidin-743 inhibits activated but not constitutive transcription.** *Cancer Res* 2002, **62:**3377-3381.
106. Martinez EJ, Owa T, Schreiber SL, Corey EJ: **Phthalascidin, a synthetic antitumor agent with potency and mode of action comparable to ecteinascidin 743.** *Proc Natl Acad Sci U S A* 1999, **96:**3496-3501.
107. Martinez EJ, Corey EJ, Owa T: **Antitumor activity- and gene expression-based profiling of ecteinascidin Et 743 and phthalascidin Pt 650.** *Chem Biol* 2001, **8:**1151-1160.
108. Adams J, Elliott PJ: **New agents in cancer clinical trials.** *Oncogene* 2000, **19:**6687-6692.
109. Marco E, Garcia-Nieto R, Mendieta J, Manzanares I, Cuevas C, Gago F: **A 3.(ET743)-DNA complex that both resembles an RNA-DNA hybrid and mimicks zinc finger-induced DNA structural distortions.** *J Med Chem* 2002, **45:**871-880.
110. Damia G, Silvestri S, Carrassa L, Filiberti L, Faircloth GT, Liberi G, Foiani M, D'Incalci M: **Unique pattern of ET-743 activity in different cellular systems with defined deficiencies in DNA-repair pathways.** *Int J Cancer* 2001, **92:**583-588.
111. Takebayashi Y, Pourquier P, Zimonjic DB, Nakayama K, Emmert S, Ueda T, Urasaki Y, Kanzaki A, Akiyama SI, Popescu N, Kraemer KH, Pommier Y: **Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide-excision repair.** *Nat Med* 2001, **7:**961-966.
112. Usdin K, Grabczyk E: **DNA repeat expansions and human disease.** *Cell Mol Life Sci* 2000, **57:**914-931.
113. Rhodes D, Fairall L, Simonsson T, Court R, Chapman L: **Telomere architecture.** *EMBO Rep* 2002, **3:**1139-1145.
114. Rezler EM, Bearss DJ, Hurley LH: **Telomere inhibition and telomere disruption as processes for drug targeting.** *Annu Rev Pharmacol Toxicol* 2003, **43:**359-379.
115. Kanoh J, Ishikawa F: **Composition and conservation of the telomeric complex.** *Cell Mol Life Sci* 2003, **60:**2295-2302.
116. Meeker AK, De Marzo AM: **Recent advances in telomere biology: implications for human cancer.** *Curr Opin Oncol* 2004, **16:**32-38.
117. Saretzki G: **Telomerase inhibition as cancer therapy.** *Cancer Lett* 2003, **194:**209-219.
118. Saretzki G, von Zglinicki T: **Telomerase as a promising target for human cancer gene therapy.** *Drugs Today (Barc)* 2003, **39:**265-276.
119. Blasco MA: **Telomeres and cancer: a tale with many endings.** *Curr Opin Genet Dev* 2003, **13:**70-76.
120. Rezler EM, Bearss DJ, Hurley LH: **Telomeres and telomerases as drug targets.** *Curr Opin Pharmacol* 2002, **2:**415-423.
121. Sun D, Hurley LH: **Targeting telomeres and telomerase.** *Methods Enzymol* 2001, **340:**573-592.
122. Saretzki G, Ludwig A, von Zglinicki T, Runnebaum IB: **Ribozyme-mediated telomerase inhibition induces immediate cell loss but not telomere shortening in ovarian cancer cells.** *Cancer Gene Ther* 2001, **8:**827-834.
123. Shammas MA, Shmookler Reis RJ, Akiyama M, Koley H, Chauhan D, Hideshima T, Goyal RK, Hurley LH, Anderson KC, Munshi NC: **Telomerase inhibition and cell growth arrest by G-quadruplex interactive agent in multiple myeloma.** *Mol Cancer Ther* 2003, **2:**825-833.
124. Tanious FA, Jenkins TC, Neidle S, Wilson WD: **Substituent position dictates the intercalative DNA-binding mode for anthracene-9,10-dione antitumor drugs.** *Biochemistry* 1992, **31:**11632-11640.
125. Sun D, Thompson B, Cathers BE, Salazar M, Kerwin SM, Trent JO, Jenkins TC, Neidle S, Hurley LH: **Inhibition of human telomerase by a G-quadruplex-interactive compound.** *J Med Chem* 1997, **40:**2113-2116.
126. Harrison RJ, Cuesta J, Chessari G, Read MA, Basra SK, Reszka AP, Morrell J, Gowan SM, Incles CM, Tanious FA, Wilson WD, Kelland LR, Neidle S: **Trisubstituted acridine derivatives as potent and selective telomerase inhibitors.** *J Med Chem* 2003, **46:**4463-4476.
127. Gowan SM, Harrison JR, Patterson L, Valenti M, Read MA, Neidle S, Kelland LR: **A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting in vitro and in vivo antitumor activity.** *Mol Pharmacol* 2002, **61:**1154-1162.
128. Anantha NV, Azam M, Sheardy RD: **Porphyrin binding to quadrupled T4G4.** *Biochemistry* 1998, **37:**2709-2714.
129. Han H, Langley DR, Rangan A, Hurley LH: **Selective interactions of cationic porphyrins with G-quadruplex structures.** *J Am Chem Soc* 2001, **123:**8902-8913.
130. Han H, Cliff CL, Hurley LH: **Accelerated assembly of G-quadruplex structures by a small molecule.** *Biochemistry* 1999, **38:**6981-6986.
131. Gazin C, Dupont de Dinechin S, Hampe A, Masson JM, Martin P, Stehelin D, Galibert F: **Nucleotide sequence of the human c-myc locus: provocative open reading frame within the first exon.** *Embo J* 1984, **3:**383-387.
132. Cooney M, Czernuszewicz G, Postel EH, Flint SJ, Hogan ME: **Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro.** *Science* 1988, **241:**456-459.
133. Postel EH, Flint SJ, Kessler DJ, Hogan ME: **Evidence that a triplex-forming oligodeoxyribonucleotide binds to the c-myc promoter in HeLa cells, thereby reducing c-myc mRNA levels.** *Proc Natl Acad Sci U S A* 1991, **88:**8227-8231.
134. Kang CH, Berger I, Lockshin C, Ratliff R, Moyzis R, Rich A: **Crystal structure of intercalated four-stranded d(C3T) at 1.4 A resolution.** *Proc Natl Acad Sci U S A* 1994, **91:**11636-11640.
135. Gehring K, Leroy JL, Gueron M: **A tetrameric DNA structure with protonated cytosine.cytosine base pairs.** *Nature* 1993, **363:**561-565.
136. Chen L, Cai L, Zhang X, Rich A: **Crystal structure of a four-stranded intercalated DNA: d(C4).** *Biochemistry* 1994, **33:**13540-13546.
137. Grand CL, Han H, Munoz RM, Weitman S, Von Hoff DD, Hurley LH, Bearss DJ: **The cationic porphyrin TMPyP4 down-regulates c-MYC and human telomerase reverse transcriptase expression and inhibits tumor growth in vivo.** *Mol Cancer Ther* 2002, **1:**565-573.
138. Wadkins RM: **Targeting DNA secondary structures.** *Curr Med Chem* 2000, **7:**1-15.
139. Wadkins RM, Tung CS, Vallone PM, Benight AS: **The role of the loop in binding of an actinomycin D analog to hairpins formed by single-stranded DNA.** *Arch Biochem Biophys* 2000, **384:**199-203.
140. Chen FM, Sha F, Chin KH, Chou SH: **Unique actinomycin D binding to self-complementary d(CXYGGCCY'X'G) sequences: duplex disruption and binding to a nominally base-paired hairpin.** *Nucleic Acids Res* 2003, **31:**4238-4246.
141. Eichman BF, Vargason JM, Mooers BH, Ho PS: **The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions.** *Proc Natl Acad Sci U S A* 2000, **97:**3971-3976.
142. West KL, Austin CA: **Human DNA topoisomerase IIbeta binds and cleaves four-way junction DNA in vitro.** *Nucleic Acids Res* 1999, **27:**984-992.
143. Zhong M, Rashes MS, Marky LA, Kallenbach NR: **T-T base mismatches enhance drug binding at the branch site in a four-arm DNA junction.** *Biochemistry* 1992, **31:**8064-8071.

144. Yang XL, Robinson H, Gao YG, Wang AH: **Binding of a macrocyclic bisacridine and ametantrone to CGTACG involves similar unusual intercalation platforms.** *Biochemistry* 2000, **39:**10950-10957.
145. Thorpe JH, Hobbs JR, Todd AK, Denny WA, Charlton P, Cardin CJ: **Guanine specific binding at a DNA junction formed by d[CG(5-BrU)ACG](2) with a topoisomerase poison in the presence of Co(2+) ions.** *Biochemistry* 2000, **39:**15055-15061.
146. Adams A, Guss JM, Collyer CA, Denny WA, Wakelin LP: **A novel form of intercalation involving four DNA duplexes in an acridine-4-carboxamide complex of d(CGTACG)(2).** *Nucleic Acids Res* 2000, **28:**4244-4253.
147. Teixeira SC, Thorpe JH, Todd AK, Powell HR, Adams A, Wakelin LP, Denny WA, Cardin CJ: **Structural characterisation of bisintercalation in higher-order DNA at a junction-like quadruplex.** *J Mol Biol* 2002, **323:**167-171.
148. Fox KR: **Targeting DNA with triplexes.** *Curr Med Chem* 2000, **7:**17-37.
149. Sarkar PS, Brahmachari SK: **Intramolecular triplex potential sequence within a gene down regulates its expression in vivo.** *Nucleic Acids Res* 1992, **20:**5713-5718.
150. Duval-Valentin G, de Bizemont T, Takasugi M, Mergny JL, Bisagni E, Helene C: **Triple-helix specific ligands stabilize H-DNA conformation.** *J Mol Biol* 1995, **247:**847-858.
151. Sehlstedt U, Aich P, Bergman J, Vallberg H, Norden B, Graslund A: **Interactions of the antiviral quinoxaline derivative 9-OH-B220 [2, 3-dimethyl-6-(dimethylaminoethyl)- 9-hydroxy-6H-indolo-[2, 3-b]quinoxaline] with duplex and triplex forms of synthetic DNA and RNA.** *J Mol Biol* 1998, **278:**31-56.
152. Arya DP, Micovic L, Charles I, Coffee R. L., Jr., Willis B, Xue L: **Neomycin binding to Watson-Hoogsteen (W-H) DNA triplex groove: a model.** *J Am Chem Soc* 2003, **125:**3733-3744.
153. Topcu Z: **DNA topoisomerases as targets for anticancer drugs.** *J Clin Pharm Ther* 2001, **26:**405-416.
154. Wall ME, Wani MC: **Camptothecin. Discovery to clinic.** *Ann N Y Acad Sci* 1996, **803:**1-12.
155. Hsiang YH, Liu LF: **Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin.** *Cancer Res* 1988, **48:**1722-1726.
156. Berger JM, Gamblin SJ, Harrison SC, Wang JC: **Structure and mechanism of DNA topoisomerase II.** *Nature* 1996, **379:**225-232.
157. Larsen AK, Escargueil AE, Skladanowski A: **Catalytic topoisomerase II inhibitors in cancer therapy.** *Pharmacol Ther* 2003, **99:**167-181.
158. Hsiang YH, Wu HY, Liu LF: **Proliferation-dependent regulation of DNA topoisomerase II in cultured human cells.** *Cancer Res* 1988, **48:**3230-3235.
159. Prosperi E, Negri C, Marchese G, Ricotti GC: **Expression of the 170-kDa and 180-kDa isoforms of DNA topoisomerase II in resting and proliferating human lymphocytes.** *Cell Prolif* 1994, **27:**257-267.
160. Del Bino G, Lassota P, Darzynkiewicz Z: **The S-phase cytotoxicity of camptothecin.** *Exp Cell Res* 1991, **193:**27-35.
161. Goossens JF, Henichart JP, Dassonneville L, Facompre M, Bailly C: **Relation between intracellular acidification and camptothecin-induced apoptosis in leukemia cells.** *Eur J Pharm Sci* 2000, **10:**125-131.
162. Kaufmann WK, Boyer JC, Estabrooks LL, Wilson SJ: **Inhibition of replicon initiation in human cells following stabilization of topoisomerase-DNA cleavable complexes.** *Mol Cell Biol* 1991, **11:**3711-3718.
163. Zhang H, Wang JC, Liu LF: **Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes.** *Proc Natl Acad Sci U S A* 1988, **85:**1060-1064.
164. Karpf AR, Jones DA: **Reactivating the expression of methylation silenced genes in human cancer.** *Oncogene* 2002, **21:**5496-5503.
165. Claus R, Lubbert M: **Epigenetic targets in hematopoietic malignancies.** *Oncogene* 2003, **22:**6489-6496.
166. Gardiner-Garden M, Frommer M: **CpG islands in vertebrate genomes.** *J Mol Biol* 1987, **196:**261-282.
167. Cross SH, Bird AP: **CpG islands and genes.** *Curr Opin Genet Dev* 1995, **5:**309-314.
168. Barlow DP: **Gametic imprinting in mammals.** *Science* 1995, **270:**1610-1613.
169. Goto T, Monk M: **Regulation of X-chromosome inactivation in development in mice and humans.** *Microbiol Mol Biol Rev* 1998, **62:**362-378.
170. Jones PA, Laird PW: **Cancer epigenetics comes of age.** *Nat Genet* 1999, **21:**163-167.
171. Jones PA, Taylor SM: **Cellular differentiation, cytidine analogs and DNA methylation.** *Cell* 1980, **20:**85-93.
172. Bender CM, Zingg JM, Jones PA: **DNA methylation as a target for drug design.** *Pharm Res* 1998, **15:**175-187.
173. Ferguson AT, Vertino PM, Spitzner JR, Baylin SB, Muller MT, Davidson NE: **Role of estrogen receptor gene demethylation and DNA methyltransferase.DNA adduct formation in 5-aza-2'deoxycytidine-induced cytotoxicity in human breast cancer cells.** *J Biol Chem* 1997, **272:**32260-32266.
174. Leone G, Voso MT, Teofili L, Lubbert M: **Inhibitors of DNA methylation in the treatment of hematological malignancies and MDS.** *Clin Immunol* 2003, **109:**89-102.
175. Leone G, Teofili L, Voso MT, Lubbert M: **DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias.** *Haematologica* 2002, **87:**1324-1341.
176. Lubbert M: **DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.** *Curr Top Microbiol Immunol* 2000, **249:**135-164.
177. Santini V, Kantarjian HM, Issa JP: **Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications.** *Ann Intern Med* 2001, **134:**573-586.
178. Fournel M, Sapieha P, Beaulieu N, Besterman JM, MacLeod AR: **Down-regulation of human DNA-(cytosine-5) methyltransferase induces cell cycle regulators p16(ink4A) and p21(WAF/Cip1) by distinct mechanisms.** *J Biol Chem* 1999, **274:**24250-24256.
179. Bigey P, Knox JD, Croteau S, Bhattacharya SK, Theberge J, Szyf M: **Modified oligonucleotides as bona fide antagonists of proteins interacting with DNA. Hairpin antagonists of the human DNA methyltransferase.** *J Biol Chem* 1999, **274:**4594-4606.
180. Yang XJ, Seto E: **Collaborative spirit of histone deacetylases in regulating chromatin structure and gene expression.** *Curr Opin Genet Dev* 2003, **13:**143-153.
181. Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF: **Histone deacetylases: unique players in shaping the epigenetic histone code.** *Ann N Y Acad Sci* 2003, **983:**84-100.
182. Fischle W, Wang Y, Allis CD: **Histone and chromatin cross-talk.** *Curr Opin Cell Biol* 2003, **15:**172-183.
183. Grewal SI, Moazed D: **Heterochromatin and epigenetic control of gene expression.** *Science* 2003, **301:**798-802.
184. Jaenisch R, Bird A: **Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals.** *Nat Genet* 2003, **33 Suppl:**245-254.
185. Deckert J, Struhl K: **Targeted recruitment of Rpd3 histone deacetylase represses transcription by inhibiting recruitment of Swi/Snf, SAGA, and TATA binding protein.** *Mol Cell Biol* 2002, **22:**6458-6470.
186. Moazed D: **Common themes in mechanisms of gene silencing.** *Mol Cell* 2001, **8:**489-498.
187. Henderson C, Brancolini C: **Apoptotic pathways activated by histone deacetylase inhibitors: implications for the drug-resistant phenotype.** *Drug Resist Updat* 2003, **6:**247-256.
188. Arts J, de Schepper S, Van Emelen K: **Histone deacetylase inhibitors: from chromatin remodeling to experimental cancer therapeutics.** *Curr Med Chem* 2003, **10:**2343-2350.
189. De Schepper S, Bruwiere H, Verhulst T, Steller U, Andries L, Wouters W, Janicot M, Arts J, Van Heusden J: **Inhibition of histone deacetylases by chlamydocin induces apoptosis and proteasome-mediated degradation of survivin.** *J Pharmacol Exp Ther* 2003, **304:**881-888.
190. Vigushin DM, Coombes RC: **Histone deacetylase inhibitors in cancer treatment.** *Anticancer Drugs* 2002, **13:**1-13.
191. Kelly WK, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, Tong W, Klang M, Schwartz L, Richardson S, Rosa E, Drobnjak M, Cordon-Cordo C, Chiao JH, Rifkind R, Marks PA, Scher H: **Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously.** *Clin Cancer Res* 2003, **9:**3578-3588.

192. Marshall JL, Rizvi N, Kauh J, Dahut W, Figuera M, Kang MH, Figg WD, Wainer I, Chaissang C, Li MZ, Hawkins MJ: **A phase I trial of depsipeptide (FR901228) in patients with advanced cancer.** *J Exp Ther Oncol* 2002, **2:**325-332.
193. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP: **Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors.** *Nature* 1999, **401:**188-193.
194. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK: **Histone deacetylases and cancer: causes and therapies.** *Nat Rev Cancer* 2001, **1:**194-202.
195. Mitsiades N, Mitsiades CS, Richardson PG, McMullan C, Poulaki V, Fanourakis G, Schlossman R, Chauhan D, Munshi NC, Hideshima T, Richon VM, Marks PA, Anderson KC: **Molecular sequelae of histone deacetylase inhibition in human malignant B cells.** *Blood* 2003, **101:**4055-4062.
196. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, Akiyama M, Chauhan D, Munshi N, Gu X, Bailey C, Joseph M, Libermann TA, Richon VM, Marks PA, Anderson KC: **Transcriptional signature of histone deacetylase inhibition in multiple myeloma: Biological and clinical implications.** *Proc Natl Acad Sci U S A* 2004, **101:**540-545.
197. Primeau M, Gagnon J, Momparler RL: **Synergistic antineoplastic action of DNA methylation inhibitor 5-AZA-2'-deoxycytidine and histone deacetylase inhibitor depsipeptide on human breast carcinoma cells.** *Int J Cancer* 2003, **103:**177-184.
198. Zhu WG, Otterson GA: **The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells.** *Curr Med Chem Anti-Canc Agents* 2003, **3:**187-199.
199. Gagnon J, Shaker S, Primeau M, Hurtubise A, Momparler RL: **Interaction of 5-aza-2'-deoxycytidine and depsipeptide on antineoplastic activity and activation of 14-3-3sigma, E-cadherin and tissue inhibitor of metalloproteinase 3 expression in human breast carcinoma cells.** *Anticancer Drugs* 2003, **14:**193-202.
200. Yang L, Mei Q, Zielinska-Kwiatkowska A, Matsui Y, Blackburn ML, Benedetti D, Krumm AA, Taborsky G. J., Jr., Chansky HA: **An ERG (ets-related gene)-associated histone methyltransferase interacts with histone deacetylases 1/2 and transcription co-repressors mSin3A/B.** *Biochem J* 2003, **369:**651-657.
201. Zhu WG, Lakshmanan RR, Beal MD, Otterson GA: **DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors.** *Cancer Res* 2001, **61:**1327-1333.
202. Rahmani M, Yu C, Dai Y, Reese E, Ahmed W, Dent P, Grant S: **Coadministration of the heat shock protein 90 antagonist 17-allylamino- 17-demethoxygeldanamycin with suberoylanilide hydroxamic acid or sodium butyrate synergistically induces apoptosis in human leukemia cells.** *Cancer Res* 2003, **63:**8420-8427.
203. Ming LJ: **Structure and function of "metalloantibiotics".** *Med Res Rev* 2003, **23:**697-762.
204. Malina J, Kasparkova J, Natile G, Brabec V: **Recognition of major DNA adducts of enantiomeric cisplatin analogs by HMG box proteins and nucleotide excision repair of these adducts.** *Chem Biol* 2002, **9:**629-638.
205. Cohen SM, Mikata Y, He Q, Lippard SJ: **HMG-domain protein recognition of cisplatin 1,2-intrastrand d(GpG) cross-links in purine-rich sequence contexts.** *Biochemistry* 2000, **39:**11771-11776.
206. Pil PM, Lippard SJ: **Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin.** *Science* 1992, **256:**234-237.
207. Siddik ZH: **Cisplatin: mode of cytotoxic action and molecular basis of resistance.** *Oncogene* 2003, **22:**7265-7279.
208. Wei M, Cohen SM, Silverman AP, Lippard SJ: **Effects of spectator ligands on the specific recognition of intrastrand platinum-DNA cross-links by high mobility group box and TATA-binding proteins.** *J Biol Chem* 2001, **276:**38774-38780.
209. Vichi P, Coin F, Renaud JP, Vermeulen W, Hoeijmakers JH, Moras D, Egly JM: **Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP.** *Embo J* 1997, **16:**7444-7456.
210. Hoffmann JS, Locker D, Villani G, Leng M: **HMG1 protein inhibits the translesion synthesis of the major DNA cisplatin adduct by cell extracts.** *J Mol Biol* 1997, **270:**539-543.
211. He Q, Liang CH, Lippard SJ: **Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin.** *Proc Natl Acad Sci U S A* 2000, **97:**5768-5772.
212. Huang JC, Zamble DB, Reardon JT, Lippard SJ, Sancar A: **HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease.** *Proc Natl Acad Sci U S A* 1994, **91:**10394-10398.
213. Zamble DB, Mu D, Reardon JT, Sancar A, Lippard SJ: **Repair of cisplatin--DNA adducts by the mammalian excision nuclease.** *Biochemistry* 1996, **35:**10004-10013.
214. Siddik ZH: **Biochemical and molecular mechanisms of cisplatin resistance.** *Cancer Treat Res* 2002, **112:**263-284.
215. Boulikas T, Vougiouka M: **Cisplatin and platinum drugs at the molecular level. (Review).** *Oncol Rep* 2003, **10:**1663-1682.
216. Roskoski R., Jr.: **STI-571: an anticancer protein-tyrosine kinase inhibitor.** *Biochem Biophys Res Commun* 2003, **309:**709-717.
217. Denny WA: **Prodrug strategies in cancer therapy.** *Eur J Med Chem* 2001, **36:**577-595.

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