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Sequence-Selective DNA Recognition: Natural Products and Nature's Lessons

Review

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Biologically active, therapeutically useful, DNA binding natural products continue to reveal new paradigms for sequence-selective recognition, to enlist beautiful mechanisms of in situ activation for DNA modification, to define new therapeutic targets, to exploit new mechanisms to achieve cellular selectivity, and to provide a rich source of new drugs. These attributes arise in compact structures of complex integrated function.

Introduction

DNA-interacting small molecules are of exceptional importance in medicine, accounting for a significant portion of all anticancer drugs [1, 2]. Over 60% of the clinical anticancer drugs introduced through 2002 are natural products or natural product derivatives, and most exert their effects by acting on DNA [1, 2]. Since the clinical introduction of mustards in the 1940s and Sidney Farber's use of the natural product actinomycin D (1954), when the mechanism of actions were unknown, much progress has been made toward understanding small molecule-DNA recognition. It was the landmark elucidation of the DNA structure by Watson and Crick that allowed the seminal conceptualization of DNA intercalation by Lerman in 1961 [3] and the elucidation of the origin of actinomycin's properties [4, 5]. In the subsequent 40 years, extensive studies have defined a number of small molecule DNA binding modes and recognition paradigms [6]. Most have arisen as a consequence of defining the site of action (DNA) of biologically active natural products and the subsequent elucidation of their binding selectivity and bound structure. Although general patterns of recognition are now appreciated, subtle structural features important to the DNA binding affinity or selectivity and the ensuing effects are still being unraveled. The advances may be attributed in part to the continued introduction of increasingly powerful new tools (e.g., footprinting, chemical synthesis, computational tools, gene profiling) as well as improvements in methods used for nucleic acid structure determination (e.g., X-ray, NMR).

The cumulative understanding of ligand-DNA recognition and the availability of such tools have provided the foundation for the design targeting of DNA and RNA in drug discovery, a strategy intuitively appealing in this postgenomic era. Most promising are the advances in the design of sequence-specific DNA binding compounds for the inhibition of transcription. Such efforts have been recently reviewed [7–11]. In spite of the advances, the de novo design of sequence-selective DNA binding agents is not yet straightforward, and the derivation of therapeutic compounds (e.g., antitumor drugs) remains an even more complex task. It is of special note that the most successful de novo approach to date entails the systematic elaboration of the hairpin polyamides that emerged from the examination of the natural product distamycin. In a large measure, this difficulty in de novo design may be attributed to the constellation of properties that must be embodied in a single structure to provide a biologically active, therapeutically useful, sequence-selective DNA binding agent. It is in this regard that natural products continue to be especially valuable. In addition to providing new paradigms for sequence-selective recognition and beautiful mechanisms of activation for DNA modification, new therapeutic targets have been defined (e.g., topoisomerase inhibition [12–16]), unanticipated sources of cellular selectivity discovered, and a series of important drugs introduced. The latter attributes were selected by screening, and even today many such lessons on integrated function within natural products remain unrecognized.

What is not addressed herein but is of equal importance is the central role that such natural products have and will continue to play in driving technological advances in chemistry and biology. Complementing the advances in biology that emerge from their study, the rich histories surrounding each natural product's identification and isolation, the remarkable science leading to their structure determination, and the beautiful semisynthetic and the landmark total synthesis studies used for structure confirmation, material access, and sophisticated SAR investigations have been conducted on structures so complex that they push the frontiers of the science, spawning countless advances for the field of organic synthesis and chemistry (for a detailed coverage of the historical importance that natural products played in the discovery of biologically active DNA binding compounds and the impact this had on chemistry, see reference [17]). This may be a direct consequence of the complex integrated function assembled in the compact structure of naturally occurring DNA binding compounds required to provide the rich constellation of chemical, physical, and functional properties.

Modes of DNA Binding

Intercalation

For small molecules that associate with DNA, three modes of binding are used to classify the interaction: intercalation, minor groove binding, and major groove binding [6]. The concept of intercalation was first recognized in studies on the aminoacridines by Lerman [3]. The proposal resulted from the observation of physical changes in DNA upon binding proflavin, including changes in DNA X-ray diffraction patterns, an increase in viscosity, and the lowering of the sedimentation coef-

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ficient. This physical distortion of DNA has become the hallmark of intercalation, of which the extension of the double helix and its local unwinding at the site of binding are most prominent. The connection between intercalation and the structural nature of the DNA distortion provided the basis for the correlation between simple intercalation (misrecognized as a base pair) and mutagenic potential and brought to preeminence DNA-targeted chemotherapy [18, 19].

An extensive range of natural products bind to DNA with intercalation [20, 21] (Table 1, Intercalation; Figure 1A), and most make additional groove contacts contributing to their sequence preference. A number of the natural products categorized herein as minor or major groove binders are further stabilized by intercalation of a pendant chromophore or quinone. In fact, an important de novo design application of intercalators is their analogous attachment to sequence-selective groove binding molecules to enhance their affinity, selectivity, or temporal stability.

The structural features that drive intercalation and the selectivity of binding, although appearing fairly simple, are complex and have been the subject of extensive study [6, 20, 21, 66]. The major driving forces are thought to be π-stacking and stabilizing electrostatic interactions, where the chromophore seeks maximum overlap with the flanking DNA base pairs (parallel intercalation). A typical example is provided by the tricyclic heterocycle of actinomycin positioned within DNA to make maximal base pair (bp) overlap such that the cyclic peptide substituents reside in the minor groove [67]. However, a number of perpendicular intercalators (e.g., anthracyclines) are known that thread end to end through DNA from the minor to major groove. From these studies, a generalized preference has emerged for intercalation to occur preferentially at an alternating pyrimidine-purine site, perhaps with a weak selectivity for 5′-CG versus 5′-TA. Offsetting the stabilizing π-stacking and electrostatic interactions is a distortion of the helix and disruption of existing bp-stacking interactions. Simplistically, it is now thought that the inherent π-stacking stabilization at a pyrimidine-purine (Py-Pu) site is weaker and easier to disrupt than that of a purine-purine (pyrimidine-pyrimidine) site [6].

A second, fundamental concept that emerged from the examination of naturally occurring DNA binding molecules is bisintercalation. A now classic series of natural products have been identified since the seminal studies on echinomycin [34] and triostin A [68, 69] that have been shown to bind by bisintercalation spanning a 2 bp site. Of these, the binding of sandramycin (Figure 2) is perhaps the most straightforward to appreciate [37, 38, 70]. It embodies three fundamental features that dictate preferential binding at a 5′-CATG site: (1) bisintercalation of the pendant chromophores spanning 2 bp, (2) binding of the linking cyclic depsipeptide in the more accessible deeper, AT-rich minor groove spanning the intercalation sites, and (3) preferential intercalation at two 5′-PyPu sites. However, nature's design is far from simple, requiring adoption of a disfavored sandramycin conformation bearing two *cis* amides in order to span 2 versus 3 bp and binding stabilization that is derived principally from the depsipeptide minor groove contacts that drive rather than follow from the sequential intercalations that provide a temporal stability to the complex which defies intuition ($t_{1/2}$ = 10 hr) [37, 38].

Following the very early clinical success with actinomycin D [71], arguably many of the most important antitumor drugs introduced into the clinic over the last 30 years have emerged from this class, including the anthracyclines (e.g., daunorubicin, doxorubicin) [23], the camptothecins (e.g., topotecan, irinotecan) [72, 73], and the podophyllotoxins (e.g., teniposide, etoposide) [30]. Studies surrounding the former defined DNA as an effective site of action [4, 5], and the latter two defined new and now classic therapeutic targets (trap of the topoisomerase I or II cleavable complex with DNA [14, 15]).

Minor Groove Binding

Groove binding small molecules are more recent additions to the field [6]. Although not yet heavily represented with drugs in clinical use, minor groove binding molecules are thought to hold the most promise for de novo design [6–11] (Table 1, Minor Groove Binding; Figure 1B). Interactions with individual bp along the groove floor and the deoxyribose groove walls provide cooperative binding contacts, extended recognition specificity, and the potential for a high degree of target selectivity. Binding affinity, originally thought to be weak, now has demonstrated affinities exceeding that of the classical intercalators and approaching that of regulatory proteins [10].

The structural features found to be most critical to small molecule recognition are groove width, groove depth, floor functionality, and electrostatic potential, and are all sequence dependent. Minor groove widths vary from 3–4 Å for AT-rich tracts to over 8 Å for GC tracts. Groove depth is more shallow for GC sequences than for AT tracts due to the presence of the G exocyclic C2-amine. A compensating feature of this groove floor functionality is the nucleophilic character of the G C2-amine (an alkylation site) and the additional opportunity it provides for recognition through H bonding. In contrast to intercalation, where compound binding induces a large change in DNA conformation, minor groove binding is characterized by little or no apparent DNA distortion and often occurs with induced changes in the bound compound conformation.

Distamycin and netropsin exemplify a large class of minor groove binding molecules better known for their antiviral than anticancer properties. They are characterized by repeating pyrrole subunits connected by amide bonds and usually terminate with one or more positively charged (protonated) nitrogen atoms. They were among the first compounds that displayed selectivity for AT sequences, and it is their characterization that provided the foundation for much of what we now understand about minor groove binding selectivity and affinity [8, 10, 11, 74]. The molecules possess a curved shape that can closely match the helical pitch of the B-DNA minor groove. The origin of their binding selectivity, often referred to as "shape-selective binding," entails preferential binding in the narrower, deeper AT-rich minor groove, where the stabilizing van der Waals contacts are optimized [59]. Further stabilizing the complex formation are accommodated H bonds of the linking amides with the floor bps and stabilizing electrostatic interactions of the terminal protonated amines [59].

Table 1. Representative Anticancer Natural Products that Interact with DNA by Intercalation, Minor Groove, and Major Groove Binding

Intercalation Binding Compound	Selectivity	Action	Activation	Reference
Daunomycin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[22]
Doxorubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[4, 5, 14]
Aclarubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[23]
Idarubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[23]
Epirubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[23]
Pirarubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[24]
Valrubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[23]
Amrubicin[a]	5′-WCG	inhibition of topoisomerase II	none (reductive?)	[23]
Nogalamycin	5′-TG, 5′-GT	unknown	none	[25]
Actinomycin D[a]	5′-PyGCPu	inhibition of topoisomerase II	none	[4, 5]
Camptothecin	ND	inhibition of topoisomerase I	none	[26, 27]
Topotecan[a]	ND	inhibition of topoisomerase I	none	[12]
Irinotecan[a]	ND	inhibition of topoisomerase I	none	[12]
Rebeccamycin	ND	inhibition of topoisomerase I	none	[28]
Ellipticine	GC-rich	inhibition of topoisomerase II	none (oxidative?)	[29]
Podophyllotoxin	ND	inhibition of topoisomerase II (?)	none	[30]
Etoposide[a]	ND	inhibition of topoisomerase II	none	[30, 31]
Teniposide[a]	ND	inhibition of topoisomerase II	none	[30]
Elsamicin	5′-PuG	inhibition of topoisomerase II	none	[32]
Dynemicin	5′-PuPy	ds DNA cleavage	reductive	[33]
Echinomycin	5′-CG	bisintercalation	none	[34]
Triostin A	5′-CG	bisintercalation	none	[35]
Luzopeptins	5′-CATG	bisintercalation	none	[36]
Sandramycin	5′-CATG	bisintercalation	none	[37, 38]
Quinoxapeptin	5′-AT, 5′-TA	bisintercalation	none	[36]
Thiocoraline	none	bisintercalation	none	[39]
Mitoxantrone[c]	5′-PuPy	inhibition of topoisomerase II	none	[40]
Bisantrene[c]	5′-PuPy	inhibition of topoisomerase II	none	[41]
m-AMSA[c]	5′-PuPy	inhibition of topoisomerase II	none (oxidative?)	[42]
Minor Groove Binding				
Bleomycin[a]	5′-GC, 5′-GT	ds DNA cleavage	oxidative (Fe^{+2} chelation)	[43]
Mitomycin C[a]	5′-CG	alkylation/crosslinking	reductive	[44]
FR66979	5′-CG	alkylation/crosslinking	reductive	[45, 46]
Mithramycin[a]	G-rich	RNA synthesis inhibition	none (Mg^{+2} chelation)	[47]
Duocarmycins	5′-WWWA	alkylation	target selective	[48, 49]
CC-1065	5′-WWWWA	alkylation	target selective	[48, 49]
Yatakemycin	5′-WWAWW	alkylation	target selective	[50]
Neocarzinostatin	5′-GT	DNA cleavage	nucleophilic	[51]
Calicheamicins[b]	5′-TPyPyT	ds DNA cleavage	nucleophilic	[52]
Retrorsine	5′-CG	alkylation/crosslinking	oxidative	[53]
Anthramycins	5′-PuGPu	alkylation	none	[54]
Saframycins	5′-GGS	alkylation	reductive	[55, 56]
Ecteinascidin 743	5′-PuGS	alkylation	dehydration	[57]
Isochrysohermidin	5′-CG	reversible crosslinking	none	[58]
Distamycin	A/T-rich	RNA synthesis inhibition	none	[59]
Netropsin	A/T-rich	RNA synthesis inhibition	none	[59]
Major Groove Binding				
Pluramycins	5′-PyG	intercalation/alkylation	oxidative	[60]
Aflatoxins	5′-GGG	intercalation/alkylation	oxidative	[61]
Azinomycins	5′-GNT, 5′-GNC	intercalation (?)/crosslinking	none	[62]
Leinamycin	ND	intercalation/alkylation	nucleophilic	[63, 64]
Ditercalinium	5′-CGCG	bisintercalation	none	[65]

ND, not determined. W = A or T; S = C or G.
[a] Clinically approved antitumor drug.
[b] Clinically used as an antibody conjugate.
[c] Synthetic drug.

Despite the extensive studies with distamycin, it was not until 1989 that the discovery was made that it can bind in a cooperative 2:1 as well as 1:1 complex with DNA [75]. The impact of this observation on synthetically designed agents (hairpin polyamides) has been remarkable. When this binding mode was recognized, the expanded groove width requirements could be combined with ongoing design modifications used to extend the binding recognition to a GC bp (incorporate H-bond acceptor, lexitropsins) to provide linked hairpin polyamides capable of reading specific DNA sequences. The subject of distamycin-inspired minor groove binders has been reviewed elsewhere [8, 10, 11, 74].

The variable conformational shape of the minor groove not only provides the opportunity for such shape-selective binding, but also for "shape-dependent catalysis."

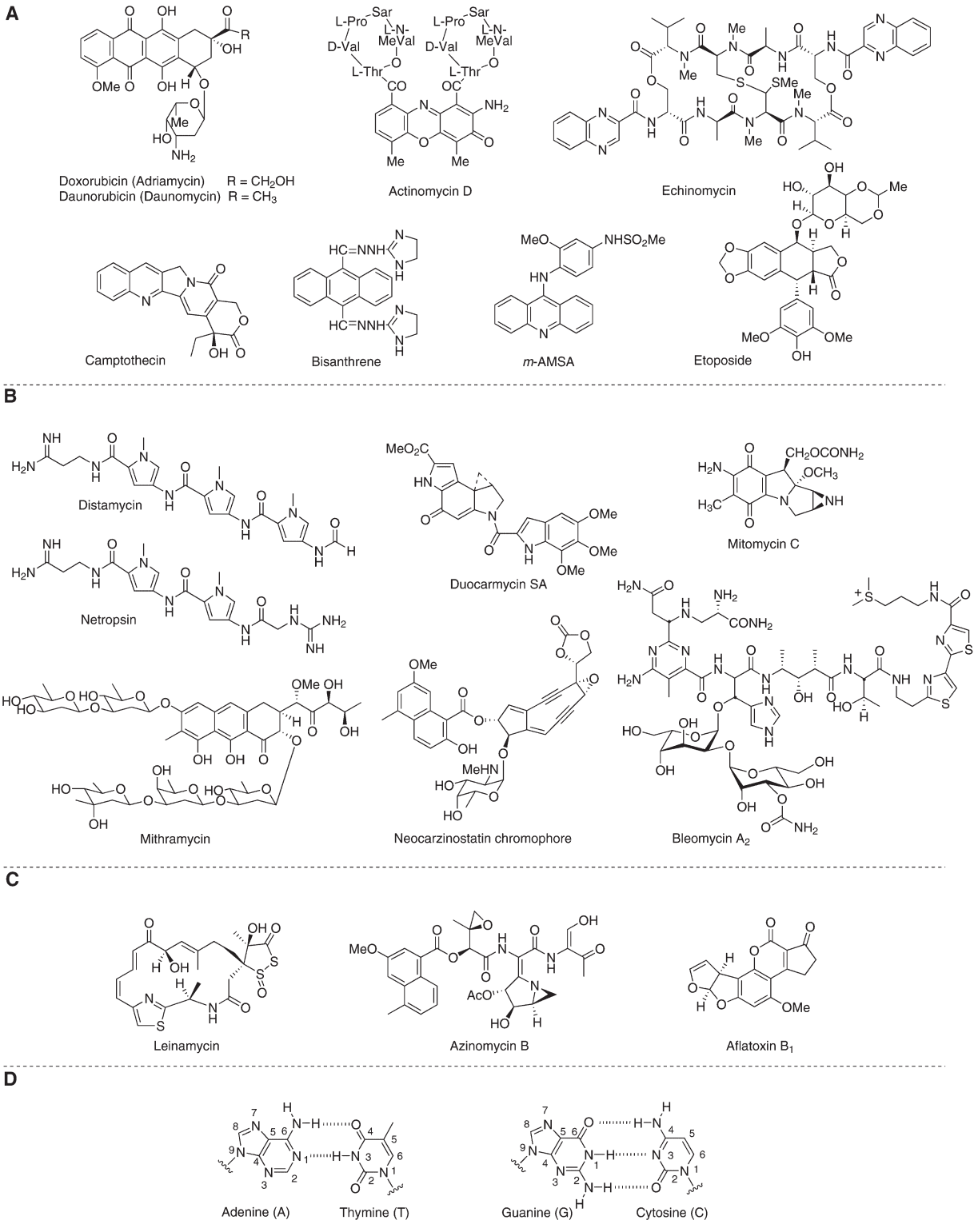

Figure 1. Structures of Selected Antitumor Natural Products that Bind DNA

(A), intercalation; (B), minor groove binding; (C), major groove binding; (D), Watson-Crick base pairing of DNA nucleotides.

The duocarmycins are exceptionally potent antitumor agents that derive their properties through a sequence-selective minor groove alkylation [48]. Not only have they been shown to exhibit an AT-rich binding selectivity like that of distamycin (shape-selective binding), but superimposed on this binding selectivity is a shape-

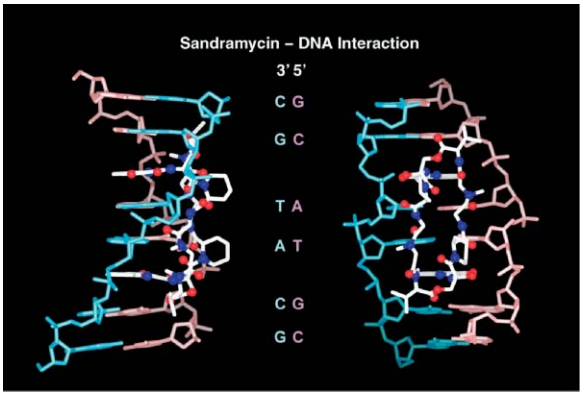

Figure 2. Two Views of the 5′-d(GCATGC)$_2$-Sandramycin Complex

Two views of the 5′-d(GCATGC)$_2$-sandramycin complex as determined by NMR, illustrating the symmetrical minor groove binding of the cyclic decadepsipeptide and the bisintercalation sandwiching the central 2 A-T bp [37].

dependent catalysis [49, 76], which also occurs preferentially in the narrower, deeper AT-rich minor groove. Thus, although the duocarmycins are unreactive toward conventional nucleophiles at pH 7, the DNA alkylation is exceptionally facile. This target selective reactivity is derived from a DNA binding induced conformational change in the molecule that twists the linking amide, disrupting the crossconjugated vinylogous amide stabilization of the cyclohexadienone and activating the cyclopropane for nucleophilic attack (Figure 3). This unique target-derived activation requires no chemical reaction. Rather, the increased reactivity derived from the binding-induced conformational change in the compound, which is greatest in the narrower AT-rich minor groove, is sufficient to accelerate (catalyze) the alkylation reaction [48, 49, 76].

An exquisite minor groove binding natural product is bleomycin [43, 77, 78]. The bulk of its DNA binding affinity resides with the C terminus sulfonium salt (major groove), bithiazole (perpendicular intercalation), and the linker valerate-threonine subunits (minor groove). The bithiazole serves as a swivel point for 180° rotation, permitting association with either strand of DNA from a single intercalation site (double-strand DNA cleavage), and each substituent on the linker region contributes to adoption of a single, rigid, compact conformation (Figure 4), productive for double-strand (ds) versus single-strand (ss) DNA cleavage. The N terminus chromophore chelates metals (Fe^{+3}, Cu^{+2}) and activates O_2, producing a powerful oxidant, and the 4-aminopyrimidine forms key triplex-like H bonds with G at the 5′-GC, 5′-GT cleavage sites, anchoring the metal-bound oxidant proximal to DNA for H-atom abstraction (Figure 4). Every subunit and nearly every functional group or substituent within bleomycin contributes to its functional binding and cleavage of DNA. In addition to its clinical use as an anticancer drug, bleomycin served as the inspiration and design template for the chemical footprinting [79] and affinity cleavage [80] tools used today, and it has emerged as one of nature's most exquisitely designed natural products of integrated function.

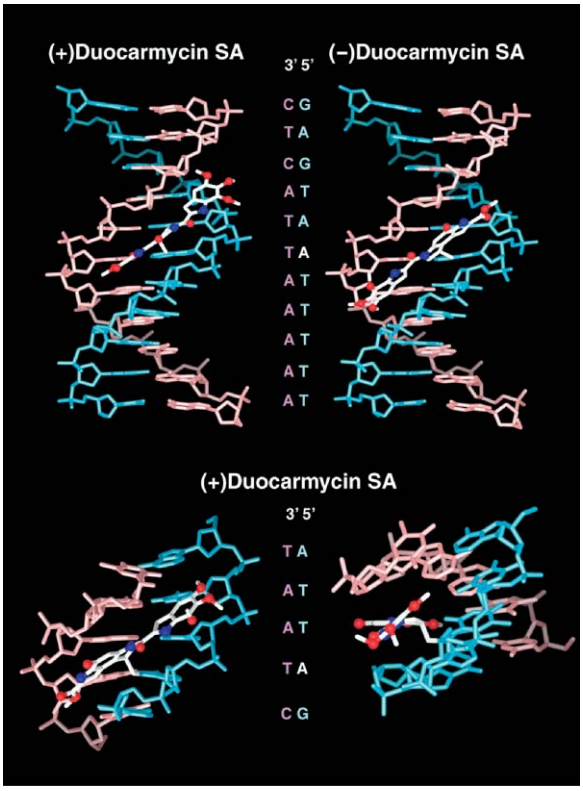

Figure 3. Models Illustrating the Alkylation at the Same Site within Duplex 5′-d(GACTAATTTTT) DNA by (+)-Duocarmycin SA and *ent*-(–)-Duocarmycin SA

Upper left, (+)-duocarmycin SA; upper right, *ent*-(–)-duocarmycin SA. The binding of the natural enantiomer extends in the 3′ to 5′ direction from the adenine-N3 alkylation site across the sequence 5′-CTAA, whereas that of the unnatural enantiomer binds in the reverse 5′ to 3′ direction across the site 5′-AATT [95]. Below, groove view NMR structure of (+)-duocarmycin SA bound to 5′-GATTA highlighting the DNA binding-induced intersubunit twist (conformational change) leading to alkylation catalysis [49].

Major Groove Binding

DNA major groove binding has not been exploited with small molecules or natural products to the same extent as the minor groove [6]. This is surprising since the major groove contains more H-bond donor and acceptor sites and consequently more information. In fact, the majority of proteins contact and recognize (read) this face of DNA. It has been suggested that the wider major groove provides a much larger, shallower binding pocket less effective for small molecule binding than the narrower, deeper minor groove [6].

To date, there are only a few examples of natural products that bind selectivity in the major groove (Table 1, Major Groove Binding; Figure 1C). Most such compounds bind by intercalation and make further H-bond contacts in the major groove. Examples include distercalinium and leinamycin, where the major groove interactions provide some degree of sequence specificity, but the binding affinity is provided principally by the intercalation event. The vast majority of the major groove binding natural products further alkylate DNA (G or A N7) via epoxide or aziridine electrophiles through

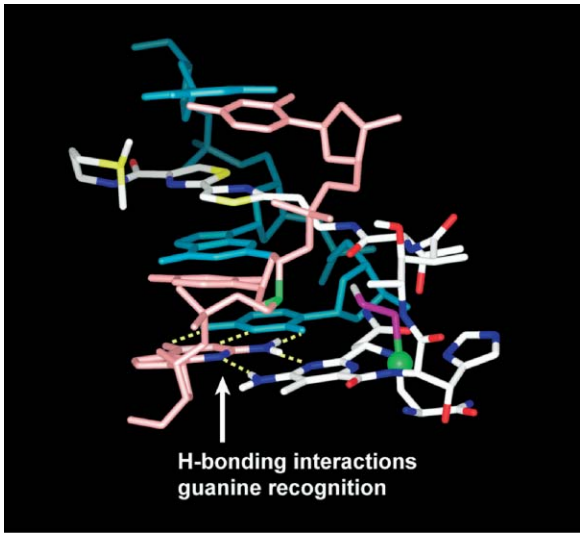

Figure 4. Expanded View of Co^{III}-OOH Bleomycin A_2 Bound to DNA Determined by NMR

This view illustrates two key H bonds between the pyrimidoblamic acid subunit (C4 amino group and N3 atom) and the guanine at the cleavage site. Also highlighted are the C4′-H abstraction site (green), the Co^{III}-OOH subunit (green ball and violet stick), and the rigid, compact conformation of the linker domain [106]. For clarity, the disaccharide subunit is not shown.

proximity-induced reactivity (affinity-induced reactivity). A large measure of this selectivity can be attributed to G N7 being the most reactive nucleophilic site in DNA rather than to inherent noncovalent binding selectivity.

DNA Modification

Three fundamental features often arise in the study of DNA binding natural products: binding affinity, binding mode and selectivity, and reaction or effector selectivity, including DNA cleavage, alkylation, or crosslinking. Each can independently assert levels of control on the sequence-selective recognition of DNA [81], and the establishment of the relative role of these effects remains a primary objective of most studies. In fact, most of what is known today about the chemical modification of DNA emerged from such studies on natural products. The majority of all reactions can be grouped into two categories: (1) reaction of electrophiles with nucleophilic sites on DNA or (2) reaction of radicals with DNA, and these have been reviewed elsewhere [46, 82, 83]. However, reactions of carbenes, nitrenes, singlet oxygen, strong nucleophiles, and photoexcited molecules relevant to some DNA-damaging natural products have been observed [83]. Common sites of DNA modification by electrophilic natural products include G N7, A N3, A N7, G C2-NH_2, and occasionally G N3 (see Figure 1D). Significant reaction selectivities among such nucleophilic sites, occasionally via reversible reactions partitioning to the thermodynamically most stable adducts, are observed and often contribute to the apparent sequence selectivity [81]. Electrophilic modification of Pu N7/N3 results in labilization of the glycosidic bond, leading to depurination, formation of an abasic site, and readily detectable strand scission. Alkylation at the exocyclic nitrogens or carbonyl oxygens of the bases or the phosphate oxygens affords stable adducts and has not been observed, or at least detected, as frequently. By contrast, H-atom abstraction from the deoxyribose backbone represents the most prevalent radical-induced mode of natural product DNA damage and almost always leads to DNA cleavage. In contrast to alkylation, this occurs without an inherent sequence or base selectivity, and the selectivity observed is typically intrinsic to the natural product-DNA interaction.

More Complex Binding Modes

Not highlighted in the discussion above are natural products that bind or differentiate unique DNA structures (e.g., A, B, or Z-DNA, quadraplexes, bulges [6]), target and bind DNA-protein complexes more strongly than DNA itself (e.g., topoisomerases [12, 13]), or bind RNA [84], including the myriad of antibiotics that bind the nucleic acid (RNA) embedded in ribosomes [85]. Each of these areas constitutes an exciting direction for natural product nucleic acid recognition, for which lessons and generalizations are only now beginning to emerge.

Embedded Structural Features Contributing to Biological Activity

Beyond the fundamental aspects of sequence-selective DNA binding, there are many additional functional features embedded in natural product structures that contribute to their properties.

Mechanisms of Activation

The concept of and many beautiful methods for in situ activation have been discovered that protect and then release an embedded reactive species capable of DNA alkylation or cleavage. Typically, these employ remarkable tactics that one could not imagine emerging from de novo design efforts, fascinating reaction cascades for release of the reactive species, and reveal rich and enlightening chemistry that can be exploited on the DNA structure. Importantly, most such in situ activation methods have been discovered through investigations of naturally occurring antitumor compounds that defined their site and mechanism of action [86]. The provocative mechanisms of DNA modification continue to provide an endless source of inspiration for de novo design.

In addition to the subtle shape-dependent catalysis of the duocarmycins, which does not entail a chemical or enzymatic activation, cascades that enlist oxidation (e.g., bleomycin, aflatoxin, pyrrolizidine alkaloids), reduction (e.g., mitomycin and quinone-containing natural products, FR-66979, dynemicin), nucleophilic addition (e.g., enediynes, leinamycin, illudins), elimination (e.g., ptaquiloside), photochemical reactivity (e.g., porphyrins, furocoumarins), and metal chelation (e.g., bleomycin, mithramycin) have been delineated. The classic reductive activation of the mitomycins [44, 87], the exquisite metal chelation and O_2 activation by bleomycin [43, 77], the subtle conformational activation of CC-1065/duocarmycins [48, 76], and the fascinating enediyne stabilization and subsequent triggering mechanisms for Berg-

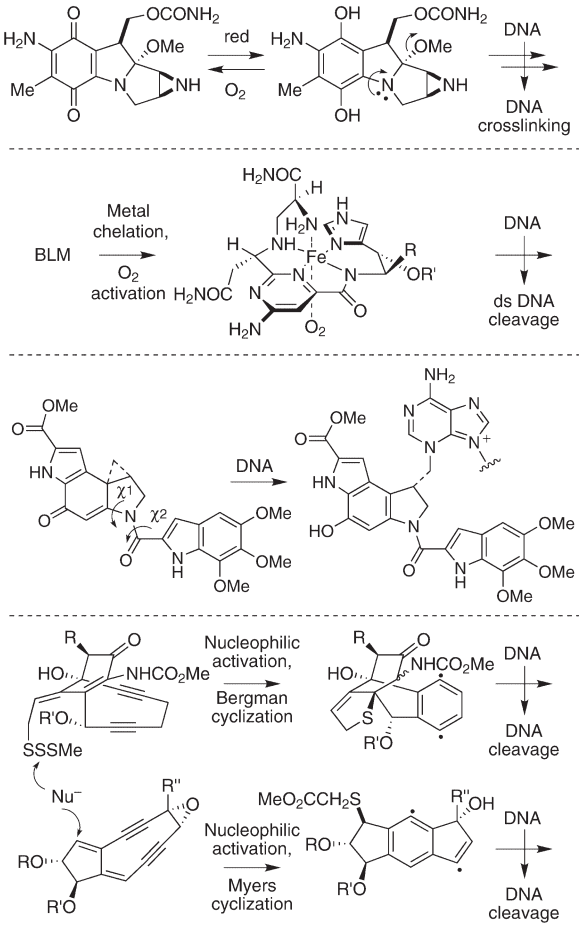

Figure 5. Nature's Creativity in Natural Product Activation

From top to bottom: mitomycin (reductive activation), bleomycin (metal chelation and O_2 activation), duocarmycin (shape-dependent catalysis), calicheamicin (Bergman cyclization), and neocarzinostatin (Myers cyclization).

man [88] or Myers [89] cyclization to reactive diradicals attest to nature's unparalleled creativity (Figure 5). These have been reviewed elsewhere [86], but the hallmark of nature's design is that the structural features required for in situ or target-selective activation are embedded in a structure that also possesses sequence-selective DNA binding properties. That is, the necessary functional features are integrated into the compact structure, not attached as a separate functional domain.

Of the endogenous activations, only reductive activation has been linked to the selective cytotoxic action of the molecules [86]. Similarly, of all the activation strategies recognized, only photochemical activation has been used exogenously to spatially define a site of treatment accounting for selective activity [86]. For the remainder, links between the activation method and selective biological activity have not yet been made. It may be that many natural products rely on other unrelated parameters for their selective action (e.g., selective cellular uptake).

Functional Activity and Selectivity

Although the understanding and structural depiction of small molecule-DNA recognition by natural products has advanced rapidly, the manner in which this translates into selective biological activity rarely has been defined. Rather, this productive activity was selected for in functional screens that often also led to the natural product identification. What is often defined with the examination of additional semisynthetic derivatives or synthetic analogs is that the DNA binding properties (affinity, alkylation, crosslinking, and ss or ds cleavage) correlate with biological activity, and this, along with suitable functional assays, has been sufficient to advance many drug discovery programs. The exceptional instances where the origin of the selective activity was defined have led to the validation of new therapeutic targets or an even greater appreciation of the integrated functional features of the natural product. The former is illustrated nicely with the discoveries that camptothecin and podophyllotoxin are potent topoisomerase I and II inhibitors [30, 72, 73], respectively, trapping a cleavable complex of the enzymes bound to DNA through formation of ternary DNA-enzyme-drug complexes, preventing religation following enzyme-mediated DNA cleavage and unwinding. This paved the way for rationale drug-discovery programs, the clinical introduction of a least ten semisynthetic derivatives, and validated two important antineoplastic targets, which are the focus of continued efforts today. The latter can be beautifully illustrated with the mitomycins. Mitomycin C is unreactive toward DNA at pH 7–8 [90] and has little inherent DNA binding affinity or selectivity [91]. However, in situ reduction of the quinone initiates an activation cascade that proceeds initially through its semiquinone and hydroquinone, resulting in efficient DNA crosslinking. Under aerobic conditions, the semiquinone and hydroquinone are reoxidized to the quinone by O_2, preventing its further entry into the activation cascade [92]. This forms the basis for its selective toxicity and effective use in the treatment of solid tumors that are characteristically hypoxic (O_2 deficient) [93, 94]. Thus, while cytotoxicity is a consequence of crosslinked DNA damage, its biologically relevant selective cytotoxicity is provided by the in situ activation mechanism.

There are many other features of the natural products that are integrated into their structures that make them attractive which have not been discussed. Many have multiple mechanisms of action that contribute to their composite biological activity (e.g., anthracyclines) and help alleviate the potential for acquired resistance. In addition, having emerged from a biological milieu, they often possess satisfactory ADME properties in spite of their complex and often large structures.

The Unnatural Enantiomers

Advances in chemical synthesis have not only accelerated increasingly detailed structure-activity studies of the complex natural products, permitting deep-seated structural changes needed to probe issues of recognition or reactivity, but they have also allowed access to their unnatural enantiomers. One of the most provocative observations to emerge in the last decade is that the unnatural enantiomers often possess DNA

Table 2. DNA Binding Selectivity and Biological Activity of the Natural and Unnatural Enantiomers of Selected Natural Products

Natural Enantiomer	Selectivity	Activity	Unnatural Enantiomer	Selectivity	Activity	Reference
(+)-CC-1065	5′-WWWWA	20 pM (IC_{50}, L1210)	*ent*-(–)-CC-1065	5′-WAWWW	20 pM (IC_{50}, L1210)	[48]
(+)-Duocarmycin SA	5′-WWWA	6–10 pM (IC_{50}, L1210)	*ent*-(–)-duocarmycin SA	5′-WAWW	100 pM (IC_{50}, L1210)	[48, 95]
(+)-Duocarmycin A	5′-WWWA	200 pM (IC_{50}, L1210)	*ent*-(–)-duocarmycin A	5′-WAWW	>20 nM (IC_{50}, L1210)	[48, 96]
(+)-Yatakemycin	5′-WWAWW	5 pM (IC_{50}, L1210)	*ent*-(–)-yatakemycin	5′-WWAWW	5 pM (IC_{50}, L1210)	[50, 108]
Fredericamycin A	ND	0.03 μg/ml (IC_{50}, L1210)	*ent*-fredericamycin A	ND	0.04 μg/ml (IC_{50}, L1210)	[97]
Mitomycin C	5′-CG crosslinks	0.06 μM (IC_{50}, HeLa S_A)	*ent*-mitomycin C	5′-CG crosslinks	0.11 μM (IC_{50}, HeLa S_A)	[98]
(+)-Roseophilin	ND	0.2 μM (IC_{50}, L1210)	*ent*-(–)-roseophilin	ND	0.1 μM (IC_{50}, L1210)	[99]
(+)-Daunorubicin	B-DNA	0.4 μM (KB3.1 sensitive), >100 μM (KB-VI MDR)	*ent*-(–)-daunorubicin	Z-DNA	8.3 μM (KB3.1 sensitive), 19 nM (KB-VI MDR)	[100]
Bleomycin B2	binds both D- and L-DNA but cleaves only natural D-DNA					[101]
(+)-Camptothecin	ND	0.04 μM (IC_{50}, KB), 0.03 μM (IC_{50}, SPS), 52% inhibition of topo I at 10 μM	(–)-camptothecin	ND	0.4 μM (IC_{50}, KB), 2 μM (IC_{50}, SPS), 20% inhibition of topo I at 10 μM	[102, 103]

ND, not determined.

binding properties in their own right and exhibit comparable, or at least interesting, levels of biological activity. Although the number of instances where this has been examined is still limited (Table 2), the observations are remarkable and their full ramifications are yet to be exploited. In the short term, the natural/unnatural enantiomer comparisons have proven key to establishing the fundamental features responsible for DNA binding affinity and selectivity (see Figure 4) and, in some instances, have provided effective, biologically active compounds [48].

Nature's Evolution versus Synthetic Optimization

One of the most misguided generalizations associated with this field and natural products in general is that they must constitute optimal structures, since they emerged from nature's evolutionary selection. The flaw in this rationalization is that nature rarely selected the candidates on the same basis for which we find them useful. Thus, there is no reason to expect natural product leads to constitute an optimized candidate. This is illustrated nicely with the two extreme examples of bleomycin and isochrysohermidin. There is little doubt that bleomycin evolved in bacteria as a potent, *catalytic*, ds DNA (or RNA) cleaving molecule to induce cell death *selectively* against invasive organisms, since nearly each feature, substituent, and functional group in this molecule contributes to this function [43, 77]. Nonetheless, its useful qualities as an antitumor drug are derived in part from both its selective cellular uptake and atypical metabolic deactivation (lack of bleomycin hydrolase) in sensitive tumor cell lines [104], features that were selected through screening and are unlikely to be optimal. In contrast to bleomycin, the presence of isochrysohermidin in *Mercurialis perennis* has nothing to do with its DNA binding properties (reversible crosslinking [58]). It is the oxidative degradation product of a purple pigment found in the flower, which, through screening, was found to possess the interesting DNA binding properties [58]. There is no reason to suspect that its DNA crosslinking and biological properties have somehow been optimized.

Rather, the challenge is to fully understand the subtle design elements that nature provided in the form of a natural product and work to extend the solution through rational design elements to provide more selective, potent, or efficacious compounds designed specifically for the problems under study.

Conclusions

Nucleic acids occupy a position of central importance in biological systems. It is the site at which genetic information is stored, accessed, and replicated in the form of a linear nucleotide code. DNA is transcribed into RNA, which is ultimately translated into proteins that provide much of the structure and carry out the function of life itself. Today, most therapeutics act by selectively targeting proteins, often the products of aberrant gene expression. However, it is reasonable to anticipate a time when therapeutics target the source (DNA) as well as the product (protein) of aberrant gene transcription. Fundamental to such opportunities is a detailed understanding of gene expression [105] and the development of small molecules that can selectively modulate it. Imaginative paradigms for the sequence-selective targeting and modification of nucleic acids by small molecules and some of the first insights into their modulation of aberrant gene transcription have emerged largely from the examination of biologically active natural products. In the short term, the discovery of biologically active DNA binding natural

products has and will continue to provide a rich source of new therapeutics (anticancer, antiviral, antibiotic); reveal new mechanisms of achieving cellular selectivity; define unprecedented DNA recognition motifs and chemical reactions; reveal unforeseen biological pathways and validate new therapeutic targets; reveal new mechanisms of in situ chemical or enzymatic activation; inspire new experimental tools (e.g., footprinting and affinity cleavage); and serve as prototypes for design of therapeutics embodying compact structures of integrated function.

For those who might suspect that the important DNA binding features of such molecules have long ago been discovered, it is important to note that the 2:1 binding of distamycin was only revealed in 1989 [75], the key minor groove triplex H bonding of bleomycin responsible for the sequence-selective cleavage was first disclosed in 1996 [106], and the source of the catalysis for the duocarmycin DNA alkylation reaction was first recognized in 1997 [107], despite their 20–40 year period of investigation. Undoubtedly, there are many more lessons yet to be learned from the existing natural products and new paradigms yet to be revealed by as yet undiscovered natural products.

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