REVIEWS

Pyrrolo(1,4)benzodiazepine Antitumor Antibiotics: Chemistry, Interaction with DNA, and Biological Implications

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Abstract: The pyrrolo(1,4)benzodiazepine (P(1,4)B) antitumor antibiotics, anthramycin, tomaymycin, sibiromycin and the neothramycins A and B, are potent anticancer agents that form covalent adducts through the exocyclic amino group of guanine in DNA. This review describes the chemistry important for both the DNA reactivity and synthesis of the carbinolamine containing drugs and the strategy for elucidation of the three-dimensional form of the adduct with DNA. The high DNA sequence specificity as well as some of the observed biological consequences of DNA damage caused by these agents in human and yeast cells are rationalized through the proposed structure of the drug-DNA adducts. Parallel toxicological studies have led to a proposal for the underlying mechanism for the cardiotoxicity of certain members of this group of agents. A rationale for designing drugs which should retain their potent antitumor activity without the associated cardiotoxicity is also proposed. Lastly, the application of the P(1,4)B's as probes for monitoring drug binding to DNA and drug-induced conformational changes is described.

Characterization of highly specific interactions of antitumor antibiotics (bleomycins, actinomycins and anthramycins) with DNA and recent dramatic advances in understanding DNA structure provide exciting opportunities in antitumor drug design. The pyrrolo(1,4)benzodiazepine (P(1,4)B) antitumor antibiotics, anthramycin, tomaymycin, sibiromycin and the neothramycins, belong to a unique class of DNA reactive drugs for which the structure of the DNA adduct is known in some detail. Therefore, it may become possible to understand some of the biological responses to DNA damage on the basis of the structure of the DNA-adduct. Although the P(1,4)B antibiotics have been used experimentally in the treatment of human cancer, their clinical acceptance, at least in the case of anthramycin and sibiromycin, has been seriously compromised by cardiotoxicity (1). However, renewed interest has been sparked by the clinical observation that while the neothramycins are effective antitumor agents they appear to lack cardiotoxicity (2).

The formulation of a three-dimensional model for the structure of the P(1,4)B-DNA adducts and the consequent insight into the mechanism of their biological effects can serve as a basis for drug design and as a means to probe DNA structure and function. This paper describes the strategy for elucidating the structure of the P(1,4)B-DNA adducts, their remarkable DNA sequence specificity and some of the biological consequences of DNA damage. The application of this information to drug design and synthesis of analogs as probes for drug-induced DNA conformational changes is also discussed.

The Chemistry of the Pyrrolo(1,4)benzodiazepine Antitumor Antibiotics

An appreciation of the chemistry and reactivity of the P(1,4)B's is critical for understanding the molecular basis of both the antitumor and the cardiotoxic effects of this family of drugs. Anthramycin and related drugs (Fig. 1) all possess the P(1,4)B nucleus, but differ in the type and position of the aromatic and pyrrolo-substituents and in the degree of unsaturation of the five-membered ring (1). X-ray crystallographic data on anthramycin (3) and tomaymycin (4) have shown that both compounds possess a right-handed twist when viewed along the lengths of the molecules; the five-membered rings are positioned at 45° and 9° respectively to the plane of the aromatic rings. A carbinolamine or the chemical equivalent at N10-C11 (e.g. the methyl ether of tomaymycin or the imine function of the neothramycins) is essential for covalent binding to DNA (1). In the case of anthramycin and tomaymycin, the

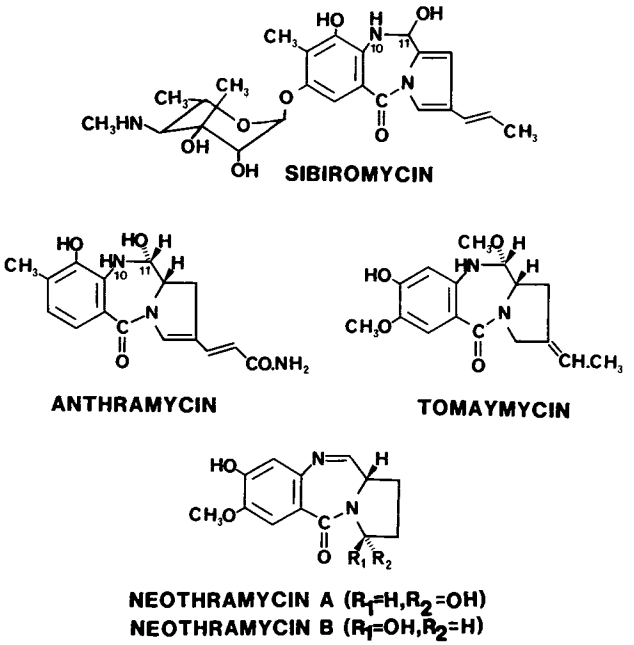

Fig. 1 Structures of the pyrrolo(1,4)benzodiazepine antitumor antibiotics.

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Parker *et al.* have recently revised the structure of the aminosugars of sibiromycin as shown in Figure 1. Parker, K. A. and Babine, R. E. (1982) J. Amer. Chem. Soc. *104*, 7330–7331, and references within.

carbinolamine can undergo reversible dehydration to form the imine (1). However, formation of anhydrosibiromycin is irreversible (5) presumably due to conjugation of the aromatic ring through the imine to the substituted pyrrolo ring. This extended conjugation is difficult to disrupt, and the compound is biologically inactive (5). While compounds such as anthramycin (6, 7) and sibiromycin (8, 9) which possess 9-phenolic hydroxy groups are cardiotoxic (11), the neothramycins (2) appear to lack cardiotoxicity. We suggest that the cardiotoxicity of anthramycin and sibiromycin, which is similar to that produced by the anthracyclines, doxorubicin and daunorubicin (7, 10, 11), is linked to the potential of anthramycin and sibiromycin to undergo oxidation or tautomerization to orthoquinone imines (Fig. 2, II and IV), a process which cannot occur in the case of the neothramycins or tomaymycin because they lack 7- or 9-phenolic hydroxyl groups.

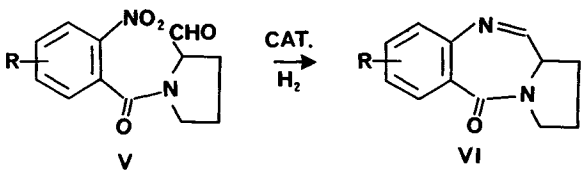

Fig. 3

Our attention therefore turned to the second literature approach proposed by Leimgruber and others (16, 17, 18) which involves the hydride reduction of dilactams. In agreement with their work, we have found that dilactam compounds of type VII (Fig. 4) which lack N-10 substituents (R=H) do not react with sodium borohydride or lithium aluminium hydride under reflux conditions in aprotic solvents. For this

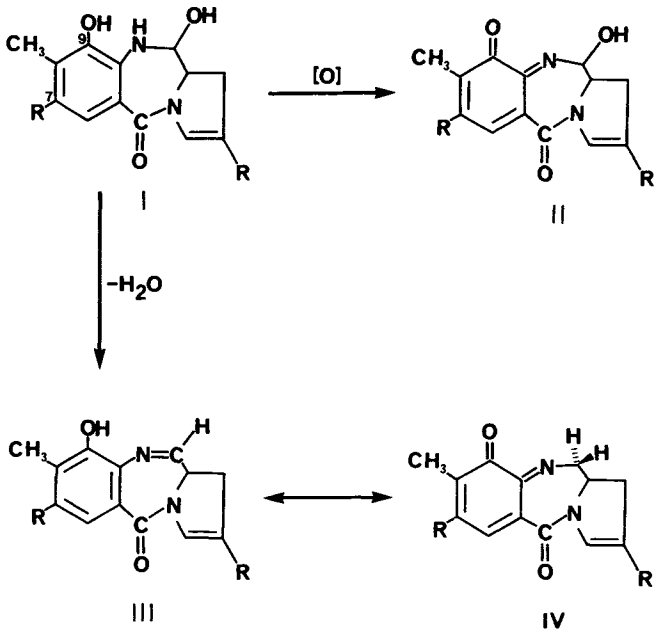

Fig. 2 Proposed conversion of anthramycin or sibiromycin to orthoquinone imines *via* oxidation or tautomerisation routes.

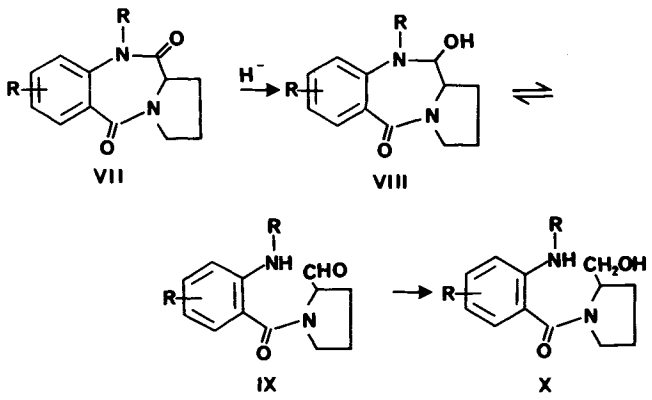

Fig. 4

reason, we investigated (19) a series of N-substituted compounds (VII; R=alkyl) and found that hydride reduction gave predominantly the open chain alcohols of type X. This type of behavior has been previously documented (20, 21) for succinamide type compounds and is due to initial formation of the carbinolamines (VIII), followed by ring opening to the corresponding amino aldehydes (IX) which undergo further reduction to the amino alcohols (X). One possible explanation is that rotation about the amino-aryl bond in IX is unfavorable for reformation of tautomer VIII, and the equilibrium lies essentially to the side of the amino aldehyde IX. The synthetic sequence of Leimgruber for the preparation (16) of anthramycin is therefore unique in that the nitrogen is "anchored" to the aromatic ring via a benzal bridge (Fig. 5, XI). The equilibrium of the resulting carbinolamine (XII) and amino aldehyde (XIV) therefore lies predominantly to the left (XII). The relatively stable carbinolamine may then be treated with methanolic HCl at room temperature to remove the bridge and form the carbinolamine methyl ether (XIII). An alternative explanation is that electron donating substituents in the aromatic ring stabilize the cyclic (XII) versus the acyclic (XIV) tautomer. Efforts are currently underway to investigate the precise mechanism of this reaction which perhaps may lead to wider application of this approach. We have thus far been able to prepare analogs that contain a C-9 hydroxyl substituent by both the reductive cyclization and the hydride reduction approach. However, for compounds lacking a C-9 hydroxyl we are currently limited to the reductive cyclization technique. A more detailed account of the synthetic chemistry is published elsewhere (19).

Formation and Reactivity of the Carbinolamine System of the P(1,4)B's

Two main synthetic approaches leading to compounds possessing the DNA-reactive imine, carbinolamine or carbinolamine alkyl ether moieties have been reported in the literature (12–18). The first approach involves catalytic reductive cyclization of an open chain nitro aldehyde of type V as reported by Lown and Joshua (13) for preparation of the model compound VI, by Miyamoto and co-workers (14) for the total synthesis of neothramycin and more recently by Tozuka and Takaya (15) for the total synthesis of tomaymycin (Fig. 3). In the first two cases, extremely low yields of products were reported; however, in similar reactions we have found that by refining the experimental procedure, yields can approach 60%, which appears to be in agreement with the work of Tozuka and Takaya (15). In addition, we have been unable to prepare the imine VI by using the method of Lown and Joshua, isolating instead the corresponding methyl ether.

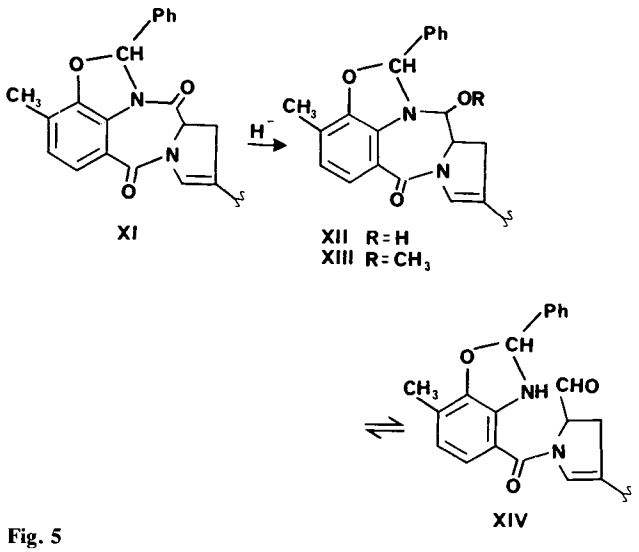

Fig. 5

The isolation of acyclic amino alcohols from the overreduction of dilactams is good evidence for the tautomeric equilibrium of type VIII ⇌ IX in the anthramycin type drugs. We previously speculated (22) that the reaction of anthramycin with DNA involves nucleophilic displacement of a conjugate acid by attack of the exocyclic amino group of guanine at C-11 of the drug molecule (Fig. 6, pathway "a"). While this mechanism may still be valid, existence of a small percentage of the tautomeric acyclic amino aldehyde suggests an alternative pathway "b" in which the open chain aldehyde species initally forms a Schiffs base which undergoes subsequent nucleophilic attack by the adjacent aryl amine, leading to recyclization. We have also previously discussed (22) the possibility of an imine intermediate.

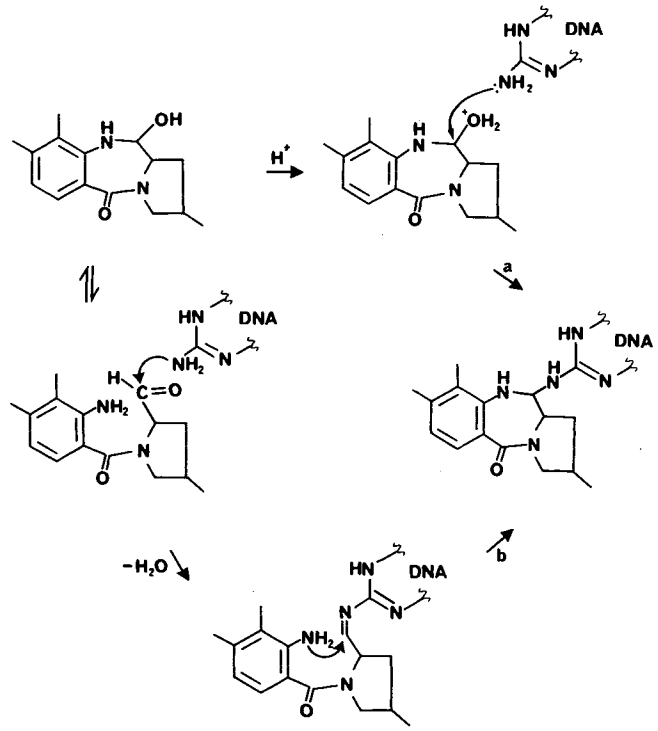

Fig. 6 Alternative mechanisms for formation of the P(1,4)B-DNA adducts.

Elucidation of the Structure of the Anthramycin-DNA adduct

The interaction of anthramycin with DNA is unique in several ways. The reaction is slow, taking about 60 minutes to reach saturation binding (22), it has a strict specificity for guanine in double stranded polydeoxynucleotides (23), and it produces a labile covalent adduct (24) which is stabilized in double stranded DNA by specific hydrogen bonding interactions (25). The chemical lability of the covalent adduct has so far frustrated attempts to isolate a discrete anthramycin-deoxynucleotide adduct and has led to alternative procedures for the structural elucidation of this unique DNA adduct.

A. Initial Characterization of the Anthramycin-DNA Adduct

Investigations by several groups including Stefanovic (26), Bates (27), Kohn (23, 28–30) and Horwitz (31–33) have provided important information on the character of the anthramycin-DNA adduct, such as the instability of the adduct to pH's below 5 (28), the requirement for a carbinolamine (26, 33), and the specificity for deoxyguanosine in a double helix (23). The physical characteristics of the adduct, including viscosity measurements which indicated that anthramycin produces stiffening while not lengthening of the DNA (30), were suggestive of an interaction within one of the grooves of DNA. Firm evidence that anthramycin does not bind to DNA by intercalation was provided from fluorescence studies to PM2-DNA (13) and unwinding measurements using SV-40 DNA (25). Further stability studies on the adduct showed that anthramycin was released in an unchanged form from DNA upon denaturation either by heat (85°C at pH 7.4 for 5 mins) or enzymatic treatment (V.P. plus DNase I, pH 7.4 for 48 hrs) (24).

B. Circumstancial Evidence for the Structure of the Anthramycin-DNA Adduct

The inability to isolate a discrete anthramycin-nucleotide adduct led us to seek indirect evidence for the points of covalent attachment between anthramycin and DNA. Earlier work by Kohn (23) had demonstrated that anthramycin is specific for G-C pairs. Proof that deoxyguanosine was the anthramycin reactive base was provided by an experiment in which (^{3}H)anthramycin-polydG.polydC was denaturated under alkaline conditions and the drug was found to be exclusively bound to polyG (25). Experiments were then designed to pinpoint the anthramycin covalent linkage site on guanine. Of the possible alkylation positions, N-7 and C-8 were eliminated using an alkylation assay with (8-^{3}H)guanine-labelled DNA (25–34), O-6 was eliminated using T-4 DNA (25) and N-3 discounted since anthramycin modified DNA did not undergo depurination by heating at neutral pH (25). This left N-2 of guanine, which is situated in the minor groove of DNA, as the most likely covalent linkage point for anthramycin. In accordance with this hypothesis, anthramycin was unable to bind to polydI.polydC which lacks the N-2 function (23). Structure activity relationships (1) suggested that anthramycin alkylation occurred *via* the carbinolamine at C-11, and therefore, an aminal linkage to N-2 of guanine seemed a likely possibility (Fig. 6). An X-ray crystallographic structure of anthramycin 11-methyl ether (3) revealed that anthramycin has a 45° right handed twist through the long axis and also suggested an interaction within one of the grooves of DNA. CPK models of anthramycin in the X-ray determined conformation with covalent DNA binding *via* an aminal linkage

(C-11 of anthramycin to N-2 of guanine) produced a drug-DNA adduct in which the drug molecule followed the twist of the minor groove of DNA without distortion or protrusion outside the helical axis (Fig. 7) (35).

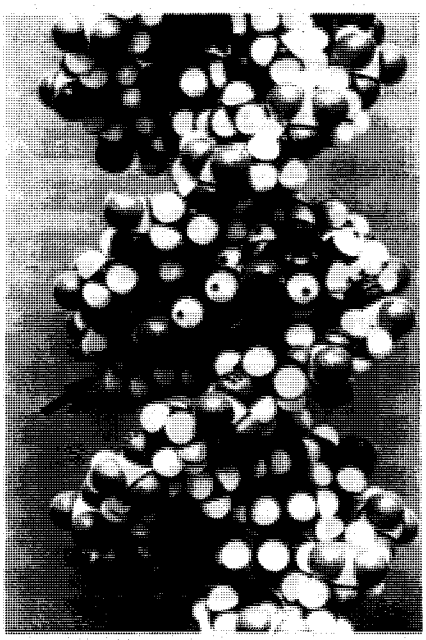

Fig. 7 CPK model of the anthramycin-DNA adduct (35).

This model led to a number of predictions which were tested by experimentation (25, 35). For example, the aminal linkage between C-11 of anthramycin and N-2 of guanine should be labile at neutral and acid pH, but is stabilized by hydrogen bonding interactions with DNA in the *double stranded form.* Therefore, denaturation of the anthramycin-DNA adduct by acid (pH4), heat (85°C for 5 mins) or enzymatic degradation (V.P. plus DNase I) leads to release of anthramycin (24). However, under alkaline conditions which prevent protonation and cleavage of the aminal linkage, denaturation leaves the drug bound to the single stranded DNA (25, 30). An unusual feature of this adduct, relative to most other covalent adducts with DNA, is that anthramycin neither appears to distort DNA or protrude outside the helix (35). Experiments using S_1-nuclease and BND-cellulose chromatography are in excellent agreement with these predictions (35). An important prediction of the CPK model is that because of the snug fit of the drug molecule within the minor groove of DNA, drug binding should show a high sensitivity to the secondary structure of DNA. This has proven to be the case and most probably is the basis for the high sequence specificity of these drugs (see later). The CPK model of the anthramycin-DNA adduct has been extended to all other known members of the P(1,4)B antitumor-antibiotic series and is also predictive of relative binding strengths (25). Equally important is the prediction of structure-activity relationships for derivatives of the P(1,4)B antibiotics (25). Fortunately, a wide array of derivatives of anthramycin, tomaymycin, sibiromycin and neothramycins are available, which have been evaluated for their ability to react with DNA. Derivatives with minor changes in structure (e.g. stereoisomeric forms) show drastic differences in their ability to react with DNA (36), and without exception these can be rationalized based upon our CPK models (25).

C. Confirmation of the Covalent Binding Sites

While strong circumstancial evidence for the three dimensional structure of the anthramycin-DNA adduct has been provided by the previously described studies, direct evidence for the aminal linkage was still lacking. With the availability of biosynthetically prepared (11-^{13}C)anthramycin (37) and DNA of defined length prepared by nuclease digestion of calf thymus chromatin (38), we have been able to show that the car-

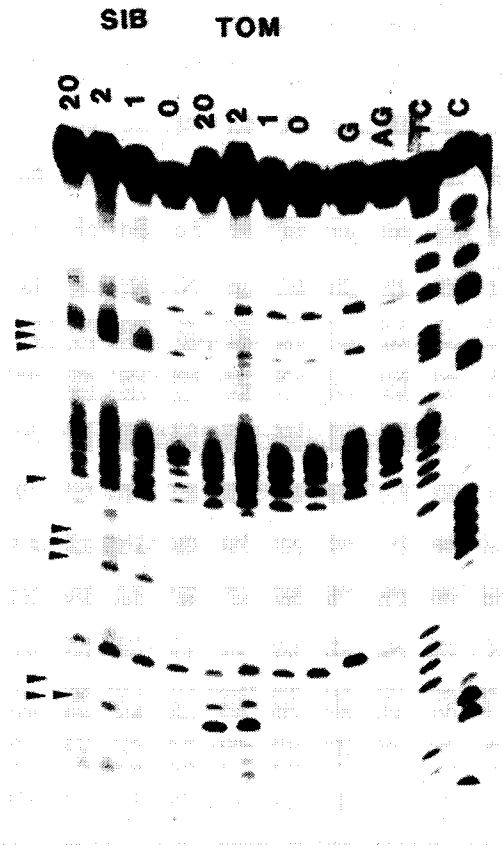

Fig. 8 Sequencing gel analysis of drug-modified oligonucleotides obtained when a 70 bp restriction fragment of T-7 phage is used as the substrate. The figure is an autoradiograph of an 8 per cent sequencing gel. Lanes, left to right, are sibiromycin and tomaymycin treated (four lanes of each drug corresponding to 1.5 n mol (20), 0.15 n mol (2) and 0.075 n mol (1) and control (0)) and Maxam Gilbert sequencing reactions for guanine, adenine-guanine, thymine-cytosine and cytosine. The drug modified restricted fragments were treated by a normally G-specific methylene blue reaction. The gel shows the occurrence of methylene blue induced DNA breaks (see arrow to left of gel) in drug-treated lanes, in addition to that normally produced by this G-specific reagent. (Compare sibiromycin and tomaymycin control lanes to drug treated lanes.) Analysis of the position of the drug-directed methylene blue induced breaks in these fragments indicates that although the breaks can be produced at any nucleotide they are always a defined number of bases removed from a cytosine at the 3' side of the break. Consensus sequences for binding of tomaymycin and sibiromycin are 5'$CPy^{A}_{T}A \blacktriangleright PuPu^{G}_{C}$ and 5'$(Py)_3CPyPy \blacktriangleright$, in which the arrows indicate breakage sites. Since these breaks only occur when both the drug and methylene blue are present, we conclude that these drugs direct the interaction of a methylene blue molecule. The break occurs adjacent to a covalently bound drug, which binds through N-2 of guanine on the opposite strand and is 2 or 3 bases removed from sibiromycin and tomaymycin respectively.

binolamine carbon of anthramycin (C-11) undergoes, a 15.5 ppm up-field shift in the ^{13}C-NMR spectrum upon formation of the DNA adduct. This is consistent with conversion of the carbinolamine (N-C-O) linkage to an aminal (N-C-N) linkage and provides excellent evidence that anthramycin is attached *via* C-11 to a nitrogen atom of guanine (39). Proof that it is the N-2 position of guanine which is attached to C-11 was obtained from experiments in which the self complementary hexadeoxynucleotide pd(ATGCAT) was shown to form an adduct with anthramycin in which one of the 2-amino protons of guanine is replaced by the covalent linkage from anthramycin (39).

Sequence Specificity

Various lines of evidence suggested that members of the P(1,4)B group might have a specificity greater than just for a guanine in a double stranded DNA template. For example, the reactivity of anthramycin differed considerably from polydG-.polydC to polyd(G-C).polyd(G-C) (25), supercoiled DNA is less reactive than linear form DNA (25), and likewise core DNA is less reactive than linker DNA in isolated chromatin (40). The sensitivity of the P(1,4)B's for the sequence specific microheterogenity in DNA has now been confirmed with the use of a modified Maxam Gilbert sequencing procedure (41). Utilizing drug modified restriction enzyme fragments in combination with a methylene blue DNA sequencing reaction, we have demonstrated that tomaymycin and sibiromycin have a surprisingly high sequence specificity. Under normal (i.e. non-drug induced) conditions, this methylene blue reaction is guanine specific, but the presence of drug molecules directs the production of new methylene blue induced strand breaks at bases other than just guanine (see Fig. 8). By comparing the DNA sequences at which drug directed methylene blue induced breaks occur we have been able to pinpoint the site of drug binding and its specificity for defined sequences in DNA. In the case of tomaymycin, there is always a "C" four bases to the 3' side of the cut, which is complementary to a "G". Since this is the only G-C pair which is reproducibly near the cut site, it is reasonable to assume that this is the base pair to which tomaymycin is attached. A consensus analysis of all such cut sites (Fig. 8) reveals a remarkably high sequence specificity for such a small DNA ligand. A similar analysis for sibiromycin is also shown in Fig. 8.

Reaction of Anthramycin with Cellular DNA and Biological Consequences

Our inability to isolate a discrete anthramycin-deoxyguanosine adduct, because of its inherent chemical instability, precluded attempts to examine directly the structure of the cellularly formed adduct. Consequently, although admittedly less definitive, we have compared the characteristics of the *in vitro* and cellular DNA adducts of anthramycin. In summary, adducts prepared in both cases released equivalent amounts of chemically unchanged anthramycin or its N10-C11 chemical equivalent upon thermal denaturation, and no drug induced distortion of DNA was apparent in either the *in vitro* or cellular DNA adducts (40). Thus it seems highly likely that both methods of reacting anthramycin with DNA produce similar products, and consequently the *in vitro* adduct characterization can be applied to the cellular anthramycin-DNA adduct. Additional support that metabolic activation of anthramycin is not required for DNA binding stems from the observation that addition of S-9 mix to anthramycin reduces the toxicity with no enhancement of the mutational frequency in any of the Ames salmonella strains tested (42).

Anthramycin treatment of both procaryotic and eucaryotic cells leads to a potent inhibition of nucleic acid synthesis (43), a presumed consequence of covalent modification of DNA by anthramycin. Moreover, persistent single and double strand breaks were shown to occur in human cell lines (44, 45). The anthramycin induced strand breaks appear to be dependent on excision repair, since xeroderma pigmentosum cells in complementation group A (XPA) fail to recognize or respond to anthramycin damage, while strand breaks did occur in excision repair normal fibroblasts (44, 45). These results have been confirmed using partially purified DNA endonuclease protein fractions from both normal and XPA cells (46). The induction of repair pathways in response to anthramycin damage involving both ultraviolet excision and double-strand break repair is also supported by data from studies using various repair deficient mutant strains of *Saccharomyces cerevisiae* (47). However, XP cells show a 10 fold increase in sensitivity to anthramycin demonstrating that failure to remove anthramycin is a more lethal event than the production of persistent single or double strand breaks (45). Anthramycin is non-mutagenic but highly recombinogenic (42); this effect is very similar to that produced by bleomycin (48) and may be related

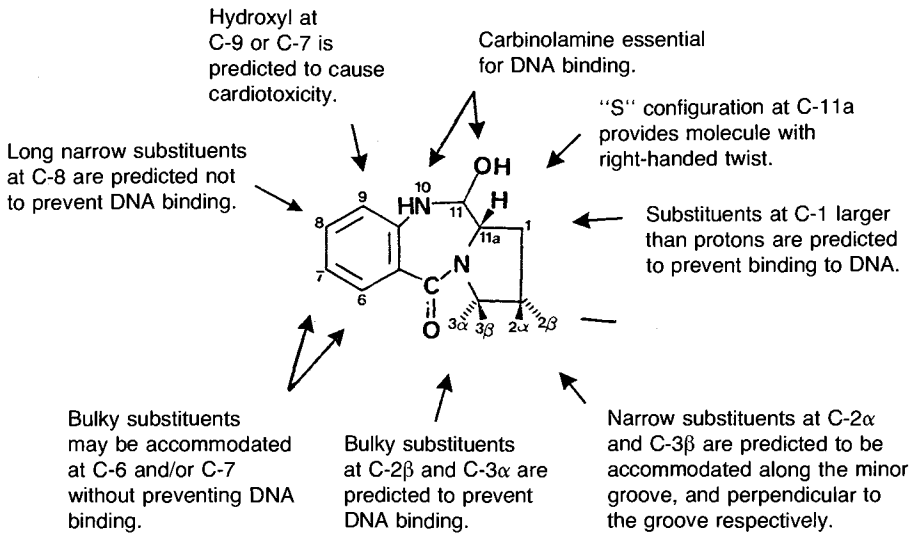

Fig. 9 Structure of pyrrolo(1,4)benzodiazepine nucleus showing predicted structure activity relationships.

to the common production of double strand breaks by both agents. Moreover, the lack of significant mutagenicity may be a consequence of an anthramycin modified DNA template which prevents replicative synthesis.

While there is not an obvious way to relate the persistence of excision-dependent single and double strand breaks to the anthramycin lesion on DNA, it seems possible that the "invisibility" or non-DNA distortive nature of the adduct may be related to a possible difficulty of an endonuclease in recognizing which DNA strand is modified by anthramycin. This could directly result in a "patch" on the "wrong strand" or alternatively, breaks on opposite strands around an anthramycin lesion, resulting in both the persistent single and double DNA strand breaks.

Structural and Biological Applications of the P(1,4)B-DNA Adducts

A logical extension of our appreciation of the manner in which the P(1,4)B's interact with DNA, is to use this insight both as a basis for drug design and as a means to probe for conformational changes in DNA induced by other DNA binding ligands. The latter is particularly attractive since anthramycin seems highly sensitive to DNA conformational variation and also appears to produce a single type of lesion on DNA.

A. The P(1,4)B-DNA Adduct as a Basis for Drug Design

The clinical acceptance of anthramycin and sibiromycin as antitumor agents has been severely restricted by a dose limiting cardiotoxicity (6, 10, 11) and acute tissue necrosis at the site of injection (6). On the basis of our CPK models of the P(1,4)B antibiotic-DNA adducts, insight into the structural features responsible for cardiotoxicity, and the application of pro-drug design, it seems a realistic possibility to design a new generation of anthramycin analogs which will maintain antitumor activity, while lacking both of these dose-limiting toxicities. Hence, anthramycin is not myelosuppressive, but actually appears to produce an elevation of WBC count (49), an attribute that could make an anthramycin analog a useful drug for combination cancer chemotherapy.

The cardiotoxicity of anthramycin mimics very closely that produced by the anthracyclines, doxorubicin and daunorubicin (7), although they appear to be structurally dissimilar. In the case of the anthracyclines, the p-quinone ring appears to be related to the cardiotoxicity and may mediate the formation of free radicals which lead to membrane dysfunction and mitochondrial disturbances (50, 51). We have been able to demonstrate that protective therapy with Co-enzyme Q_{10} (52), vitamin E (53) or a combination of these agents (54) against anthramycin induced cardiotoxicity, mimics that of the same agents in protection against the anthracycline induced cardiotoxicity. These results suggest that the underlying mechanism for cardiotoxicity is the same for both anthramycin and the anthracyclines. Since the P(1,4)B antibiotics lack quinone structures we have considered the possibility that tautomerisation or oxidation of anthramycin leads to ortho quinone-imine products (Fig. 2). Support for this concept is available from existing SAR's, since while anthramycin and sibiromycin (o-OH) are cardiotoxic (6, 7, 10, 11), neither tomaymycin or the neothramycins (m-OH) are reported to be cardiotoxic (2).

Anthramycin and related drugs produce a severe tissue necrosis at the site of injection (6) which if caused by interaction of the drug with cellular DNA in and around the site of administration, may be preventable. Prodrugs of anthramycin in which the carbinolamine is blocked from reaction with DNA should show markedly reduced necrotic activity relative to the free drug. Therefore, a prodrug approach in which the drug is chemically or sterically blocked from interaction with DNA until the free drug is released seems a reasonable approach to solving this problem.

The design of new pyrrolo(1,4)benzodiazepine antitumor antibiotics lacking both cardiotoxicity and tissue necrosis must at the same time preserve the potential for initial noncovalent interaction with DNA and subsequent formation of a covalent adduct between C-11 of the drug molecule and N-2 of guanine. The following structural requirements are predicted from information already available to be necessary for DNA binding and antitumor activity:

1. A tricyclic pyrrolo(1,4)benzodiazepine nucleus;
2. An R configuration at C-11a of the antibiotics to provide a right handed twist to the molecule;
3. A carbinolamine or its equivalent at N10-C11 of the nucleus which is necessary for covalent adduct formation;
4. Possibly, substituents on the aromatic ring necessary to provide secondary stabilizing forces to maintain the structural integrity of the drug-DNA adduct.

It is also possible to predict steric interactions that are likely to prevent access of the drug molecule to the site of interaction on the inside of the minor groove of DNA and thereby prevent covalent binding through the 2-amino group of guanine. For example, substituents larger than hydrogen on C-1, C-2β, C-11, C-11a and N-10 or larger than hydroxyl on 3α (Fig. 9) cannot be accommodated according to our CPK model since these positions lie in close proximity to the inside of the groove of DNA. Opportunities therefore exist at these positions to design prodrugs which will prevent reaction with DNA until the blocking groups are released.

Conversely, much larger substituents can be accommodated at C-2α, C-3β, C-6 and C-7, since these positions either point along the groove (C-2α) or are orientated perpendicular to the groove (C-3β), (C-6 and C-7). It is therefore in these positions that conjugate groups can be attached which may modify the physical, chemical or biological properties of the DNA reactive nucleus.

In order to avoid cardiotoxicity, we propose that phenolic hydroxy groups at C-7 and C-9 should be avoided since these may be tautomerised or oxidized to the p- and o-quinone imines, respectively, which are predicted to be cardiotoxic. A schematic representation of these ideas is presented in Fig. 9.

B. P(1,4)B's as Probes for Monitoring Drug Binding to DNA and Drug Induced Conformational Changes in DNA

The availability of the P(1,4)B's as highly selective and sensitive probes for monitoring other DNA ligand binding characteristics has been exploited by us to examine the modes of CC-1065 and D mitomycin-C binding to DNA.

CC-1065 is a very potent antitumor agent (55) which has some similarities in its DNA binding characteristics to anthramycin. For example, both drugs possess a notable right-handed twist through the long axis and bind covalently to DNA without detectable distortion of the helix (56). They differ, however, in their base-pair specificity, since while anthramycin is G-C specific, CC-1065 is A-T specific (56). Since neither drug distorts DNA, we would not expect anthramycin to affect the binding of CC-1065 to DNA, provided the drugs bind in

opposite grooves, i. e., anthramycin-minor groove, CC-1065-major groove. However, if the reverse is true and CC-1065 also binds in the minor groove of DNA, then even though both drugs are specific for different base pairs, we would expect at least a partial inhibition of CC-1065 binding by anthramycin. The results of experiments with CC-1065 binding to anthramycin-DNA adducts show that at low anthramycin binding ratios there is no overlap of binding sites, but at higher drug binding levels up to a 50 per cent inhibition of CC-1065 binding occurs. We interpret this to mean that both drugs bind in the minor groove, but competition for binding sites on DNA occurs only at the higher anthramycin binding ratios where perhaps there are less stringent requirements for DNA binding.

In a somewhat more complex case, we have used anthramycin as a probe to determine both the groove specificity and the effects on DNA of the binding of mitomycin C (MC) (54). Mitomycin C has been shown to bind predominantly through O-6 of guanine (58) within the major groove of DNA, inducing coiling, bending or kinking of the DNA structure (59). The stoichiometry of anthramycin inhibition of MC binding strongly suggests that MC binding does indeed occur within the major groove of DNA and that anthramycin inhibition of MC binding is due to prevention of a conformational change required for MC binding.

Summary and Future Directions for Research

Just as our earlier biosynthetic work on the P(1,4)B antibiotics (60) provided us with the means to elucidate the manner in which anthramycin and related drugs interact with DNA, we are now in a position to capitalize on our insights into interaction of anthramycin with DNA for probing nucleic acid structure and for drug design. Since we have recently developed a new synthetic approach to the P(1,4)B nucleus containing the required DNA-reactive moiety, we can now test our drug design postulates. Through the availability of synthetic oligodeoxynucleotides we anticipate further refinement of the structure of P(1,4)B-DNA adducts, as we enter the molecular biology of the drug-nucleic acid research area.

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Drug Interactions Through Binding to Cytochrome P 450: The Experience with H_2-Receptor Blocking Agents[3]

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Abstract: H_2-receptor blocking agents, such as cimetidine, ranitidine or oxmetidine, are consumed in large amounts often together with a variety of other drugs. There is increasing evidence that cimetidine interferes with the hepatic elimination of several drugs, thereby aggravating the effects of the comedication. Microsomal studies in vitro revealed that cimetidine binds in therapeutic concentrations to cytochrome P 450, which may represent the primary mechanism for its ability to inhibit drug metabolism and thereby interact with other drugs. The structurally different ranitidine (replacement of the imidazole in cimetidine by a furan ring) is about five times as potent as a H_2-receptor blocker and displays low affinity for binding sites on cytochrome P 450. Therefore, therapeutic doses of ranitidine do not impair the metabolism of other drugs. Preliminary data with oxmetidine suggest that it too does not interfere at the level of hepatic elimination. Thus, it is concluded that new therapeutic agents should be tested for their ability to bind to cytochrome P 450 to determine possible risks of drug interactions.

H_2-receptor blocking agents are among the most widely used drugs. They decrease basal and stimulated acid secretion (1, 2) and are effective drugs in the treatment of peptic ulcer (for review see 3, 4). In the Western world this disease afflicts approximately 12 % of the adult population, and about 1 % of total costs for all diseases are spent for peptic ulcer treatment (5). Thus, many patients will be taking H_2-blockers over prolonged periods. Hence, the potential of drug interactions exists and needs to be investigated.

H_2-blockers elevate the pH of the gastric juice to values between 3 and 5. Consequently, the absorption (bioavailability) of other drugs might be modified (for review see 6). Cimetidine and ranitidine are partly excreted unchanged by tubular secretion. Therefore, competition for this active transport process with endogenous creatinine (7) or other basic drugs (8) can also result in interactions. However, the prolongation of hexobarbital sleeping time in rats (9) and the clinical aggravation of warfarin action by cimetidine (10–12) have stimulated the most attention to interactions with H_2-blockers.

Cimetidine was released to the European market at the end of 1976 (USA: August 1977) followed by ranitidine 5 years later. Oxmetidine is still under clinical investigation. Since the initial reports on drug interactions with cimetidine (9, 12, 13) a

Table I. Inhibition of Hepatic Elimination of Different Drugs by Cimetidine.

Drug	References
Warfarin	12
Acenocoumarol	12
Phenandion	12
Phenytoin	36, 37, 38
Carbamazepine	39
Diazepam	40, 41, 42
Desmethyldiazepam	21
Chlordiazepoxide	43, 44
Alprazolam	45
Triazolam	45
Theophylline	46, 47, 48, 49, 50, 63
Coffeine	51, 52
Propranolol	29, 53, 54
Labetalol	55
Metoprolol	56
Penbutolol	57
Lidocaine	58, 64
Chlormethiazole	59
Imipramine	60, 81
Ethanol	61, 62

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