The Azinomycins. Discovery, Synthesis, and DNA-binding Studies

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24.1 Introduction

Nature is a rich source of potential antitumor agents that can act as a paradigm for the development of synthetic agents that retain the biological activity of the natural product but are targeted to the tumor, have increased bioavailability or are simply prodrug forms that are inactive until administered. Examples of potent antitumor antibiotics that interact with DNA include the clinically used anthracyclines [1] and bleomycins [2], while agents under investigation include CC-1065 and the duocarmycins [3], ecteinascidin 743 [4], and rebeccamycin [5]. The effective exploitation of the biological activity of natural products requires a combination of synthesis, computational studies, an array of DNA-binding studies and both in vitro and in vivo biological studies. Such a combination has led to the development of agents such as mitoxantrone [6] and, more recently, the pro-drug AQ4N [7], based upon extensive knowledge of the biological activity of the anthracycline antibiotics.

Synthetic alkylating agents have a long history in cancer chemotherapy [8]. With their origins as the sulfur mustards developed for chemical attacks during the First World War, they were first used clinically in the fourth decade of the twentieth century. Typically, synthetic alkylating agents derive their biological activity from an ability to alkylate and crosslink DNA, and extensive studies have been made of relationships between structure, sequence selectivity, and antitumor activity. Nature has also developed agents that can bind covalently to nucleic acids and that have potential therapeutic value, particularly in the treatment of cancer. Currently used clinically in the treatment of gastrointestinal, breast and bladder cancers, mitomycin C is an example of an antitumor antibiotic that can bind to and crosslink DNA [9]. CC-1065 and the duocarmycins are minor groove binding alkylating agents that are amongst the most potent antitumor antibiotics discovered to date. Their mode of action, through alkylation of DNA in the minor groove, has been extensively studied and reviewed [3].

The azinomycins A (1) and B (2) (Fig. 24.1) [10, 11] and the truncated analog 3 represent novel structures that have been extensively explored from a synthetic
viewpoint [12], but for which studies of structure–activity relationships from a DNA-binding and biological viewpoint have been few and widely spaced chronologically [12]. To date, little advance has been made in progressing these agents into a clinical setting. In this chapter, we aim to bring together the details of the isolation, synthesis, structural, and DNA-binding studies of the azinomycins, to generate a starting point from which to begin to understand the properties of these natural products that could lead to new, therapeutically relevant compounds.

24.2 Isolation of the Azinomycins 1–3

The azinomycins A (1) and B (2), along with the truncated analog 3, which consists of the naphthalene and the epoxide fragments, were isolated from *Streptomyces griseofuscus* S42227 by researchers at the SS Pharmaceutical company in Japan [10]. The structures and the *in vivo* biological activities of the compounds in a number of murine tumors were described [13]. Interestingly, this group reported no cytotoxicity for 3, although more recently this compound has been reported by a number of other groups to have potent antitumor activity (see below, Section 24.3.2). The structures of the three compounds were ascertained by inspection of the mass, $^1$H and $^{13}$C NMR spectral data and these workers noted that the spectral data were similar to that for carzinophilin, an antitumor antibiotic that was isolated in 1954 from *Streptomyces sahachiroi* [14]. Armstrong later disclosed that the $^1$H and $^{13}$C NMR of carzinophilin and azinomycin B were superimposable [15] and since that date, the two compounds have been regarded as being the same.

Carzinophilin had an interesting history, as attempts to assign a structure to the compound had been dogged by revisions until the discovery of the azinomycins. The structure originally proposed by Lown and co-workers to be a bisintercalating bisalkylator with the structure 4 [16], was later revised by Onda [17] to an N-acety laziridine intercalator possessing a highly oxygenated structure 5 (Fig. 24.2). These early studies led to Shibuya’s synthesis of the naphthalene ring structure, at the beginning of a synthetic route to Lowns compound [18], which has been used with small variations, by all workers in the area (see Section 24.3).
Studies of the Truncated Analog 3

24.3.1 Stereoselective Synthesis of the Truncated Azinomycin 3

The first synthetic studies of the azinomycins [18] were based upon the erroneous structure of carzinophilin suggested by Lown and co-workers [16]. Although it has some historical interest, it also forms the basis of the synthesis of the azinomycin chromophore that has been utilized by all workers since this time. In essence, Shibuya synthesized one of the degradation products of azinomycin A (compound 6), which formed the C1–C18 segment of the structure 4 [18]. 3-Methoxy-5-methylnaphthalene-1-carboxylic acid 10 was synthesized via a condensation reaction of 1-(2-methylphenyl)-2-propanone 7 with diethyl oxalate to give the enol 8, which was cyclized to the naphthol 9 under acidic conditions. The naphthol was methylated and hydrolyzed in high yield, under basic conditions to give 10 (Scheme 24.1).

The side chain synthesis noted in this work is of interest in that Shibuya went on to use a similar approach in the synthesis of the truncated azinomycin analog 3, the first description of the synthesis of this compound [19]. To achieve this aim, Shibuya used the “chiral pool” and began from commercially available diacetone d-glucose, using a five-step synthesis to generate the aldehyde 11, which was reduced and refluxed with catalytic acid to generate the γ-lactone 12 (Scheme 24.2). This lactone could be coupled directly with the acid chloride of naphthalene 10 and ring-opened with ammonia to give the amide 14 in excellent yield and without cleavage of the ester bond. Removal of the benzyl group, mesylation of the primary alcohol and base-induced cyclization generated 3.
This stereospecific synthesis of 3, while somewhat lengthy compared with later efforts, established the stereochemistry of the epoxide in the natural products, as the (18S, 19S) diastereoisomer (azinomycin numbering) matched exactly that of the isolated natural product of 3 and, crucially, had the same optical rotation. Shibuya and co-workers also used a second route to the epoxide via the chiral pool, beginning from fructose [20].

The majority of other studies on the synthesis of the epoxide fragment of the azinomycins and on the synthesis of 3 have focused upon the use of the Sharpless asymmetric epoxidation, for which the fragment is ideally suited. First reports of the use of this reaction were by Armstrong and co-workers [21] and Shishido et al. [22], but more direct efforts on the precursor 17 were reported by Konda [23]. Kinetic resolution of racemic 17 under Sharpless AE conditions using d-(-)-diisopropyl tartrate gave a 48% isolated yield and 98% ee of (2S, 3S)-18, along with
recovered \([R]-17\) (Scheme 24.3). Due to errors in the reporting of optical rotation in Shibuya's earlier work [20], Konda mis-assigned the stereochemistry in his original paper and Shipman and co-workers [24] later revised his results to show that the above was correct. Konda believed that he had obtained the opposite to the expected stereochemistry.

The use of the Sharpless AE in the synthesis of the epoxide, which has been further investigated by Coleman [25], is a rapid route into the epoxide fragment and to the synthesis of 3. However, in an elegant approach, Shipman and co-workers used stereocontrol throughout the synthesis by the development of a Sharpless asymmetric dihydroxylation/asymmetric epoxidation methodology [24]. Using the readily available benzyl ester of dimethyl acrylic acid 19, they performed an asymmetric dihydroxylation using AD-mix-\(\alpha\) to generate 20. Mesylation, epoxide formation and acid catalyzed epoxide opening gave the intermediate \((S)-17\), which was now correctly functionalized for Sharpless AE. Use of \(-\)(-)-diethyl tartrate generated the required \((2S,3S)-18\) with minor separable amounts of the \((2S,3R)-18\) (Scheme 24.4). Reaction with the acid chloride of 10, deprotection and reaction with ammonia under coupling conditions gave a high yielding route to 3. Single crystal X-ray structures of intermediates firmly established the stereochemistry of 18. One of the strengths of this approach is the ability to generate all four stereoisomers of 18. Shipman reported a 37:63 mixture of diastereoisomers of 18 when MCPBA was used as oxidizing agent and in the authors’

![Scheme 24.3. Konda synthesis of the epoxide fragment [23].](image1)

![Scheme 24.4. Shipman synthesis of the epoxide fragment [24].](image2)
laboratory, we have used this method to generate all four diastereoisomers of 3, including the (2S, 3R) and (2R, 3S) isomers of 18, which are easily separated by chromatography and which have not been previously studied. Such investigations may aid in our understanding of factors that contribute to the biological activity of the natural products (M. Casely-Hayford and M. Searcey, unpublished observations).

24.3.2 DNA-binding and Biological Activity Studies of the Truncated Fragment and Synthetic Analogs

The synthesis of the left-hand fragment of the azinomycins, in itself a natural product with some demonstrated, although controversial, antitumor activity, allowed the study of its interactions with DNA and further assessment of its antitumor activity in vitro. Zang and Gates have disclosed the most comprehensive study of this compound and its interactions with its nucleic acid target (Fig. 24.3) [26]. Using a 5'-3P-labeled 145-base-pair restriction fragment, they showed that 3 alkylated DNA at guanine residues with little, if any, sequence selectivity but at much lower concentrations than a simple epoxide alkylating agent 23. This strongly supports the hypothesis that 3 may bind non-covalently to DNA. They also demonstrated that addition of ethidium bromide inhibits DNA alkylation, that DNA binding is accompanied by bathochromic and hypochromic shifts in the UV-visible spectrum and that in a T4 ligase DNA-winding assay 3 requires a concentration 200–500 times that of daunomycin to induce similar effects.

![Fig. 24.3. Zang and Gates results confirm intercalation and guanine alkylation by 3 [26].](image-url)
All of these experiments point to an intercalative mode of binding for the naphthalene chromophore, a mode that has been suggested for azinomycin but that is somewhat surprising in view of the generally held belief that naphthalene derivatives are poor DNA intercalators. Crucially, these authors used viscometric experiments that showed increases in viscosity of double-stranded DNA-containing solutions upon the addition of both 3 and the non-alkylating analog 24, strongly indicative of an intercalative mode of binding for these compounds. These findings correlate with, and help to explain, the observations of other groups with regard to the biological activity of analog structures of the natural product. Upon completion of the stereoselective synthesis of 3, Shipman and co-workers began a synthetic effort to identify analogs of the natural product that may be useful antitumor agents (Fig. 24.4) [27]. They began a small structure–activity survey of the substituents to the left (the naphthalene) and to the right (the amide) of the epoxide. Replacement of the 3-methoxy-5-methylnaphthalene with a phenyl group (compound 28, Fig. 24.4), which would be expected to show little affinity for DNA through intercalation, effectively removes the biological potency of the epoxide in a variety of cell lines. Intriguingly, both 1- and 2-naphthalene-carboxylate analogs, while losing significant (up to 20-fold) activity, remain relatively potent with biological activities as low as 100–200 nM in some cell lines. Introduction of the quinoxaline chromophore, the intercalating moiety of the cyclic depsipeptide antibiotics such as triostin A, led to a complete loss of biological activity confirming, perhaps, that the chromophore alone, even in this well-studied family of bisintercalators, is not sufficient for DNA intercalation.

In a further effort to generate more potent analogs of 3, Shipman has also designed, synthesized, and studied a crosslinking dimer of the epoxide that can form interstrand crosslinks in DNA (Fig. 24.5, compounds 30–32) [28]. Using an alkyl linker through the right-hand carboxylic acid group, they demonstrated that an optimum linker length appeared to be four methylene groups and that all of these

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**Fig. 24.4.** Effect of intercalator structure on antitumor activity [27].

<table>
<thead>
<tr>
<th>Cytotoxicity IC₅₀ Values (µM)</th>
<th>A2780</th>
<th>A2780cisR</th>
<th>CH1</th>
<th>SKOV-3</th>
<th>HT29</th>
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<tbody>
<tr>
<td>3</td>
<td>&lt;0.05</td>
<td>0.076</td>
<td>&lt;0.05</td>
<td>1.25</td>
<td>0.33</td>
</tr>
<tr>
<td>25</td>
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<td>3.5</td>
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<td>4.3</td>
</tr>
<tr>
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<td>1.1</td>
<td>0.55</td>
<td>5.1</td>
<td>2.15</td>
</tr>
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<td>&gt;25</td>
<td>&gt;25</td>
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<td>&gt;25</td>
</tr>
</tbody>
</table>
agents ($n = 2, 4, 6$ in Fig. 24.5) can crosslink DNA and have potent cytotoxic activity, although none of the compounds had significantly greater activity than the non-crosslinking 3 (see Section 24.6). An interesting study in this context would be the effect of stereochemistry on the biological activity of these compounds. Presumably a dimer formed from the ($S,S$) and ($R,R$) diastereoisomers would be able to form intrastrand crosslinks more easily than interstrand ones.

24.4 Studies on the Total Synthesis of the Azinomycin A

24.4.1 Synthesis of the 1-Azabicyclo[3.1.0]hex-2-ylidene Dehydroamino Acid Subunit

Most of the synthetic work on the azinomycins has been focused upon the densely functionalized 1-azabicyclo[3.1.0]hex-2-ylidene dehydroamino acid subunit, chiefly as the research was driven by the enquiries of synthetic organic chemists for whom the chemistry was a significant and attractive challenge. It is outside the scope of this review to comprehensively cover all of the efforts on this unit and the reader is referred to reviews by Shipman [12] (who also covers Tereshima’s work in more detail than here) and Coleman [29] (covering his own work) for a more synthetic approach. Here, we will briefly review efforts that ultimately led to the total synthesis of the azinomycins.

Both Armstrong [30] and Coleman [31] have, independently and concurrently, described a route to the aziridine core unit that involves disconnection of the intact aziridine from the alkene, and of the alkene from the backbone (Fig. 24.6). In the forward direction, this requires the reaction of a glycine phosphonate with a functionalized aldehyde, carrying the intact aziridine, a stereoselective bromination and a Michael addition–elimination reaction to complete the synthesis. Drawbacks to this approach became apparent as the reactivity of the 12-OH meant that selection
of the protecting groups was a key issue and must be contemplated early in the synthesis [29].

24.4.1.1 Synthesis of the aldehyde unit
In initial studies, Armstrong utilized both L-serine and D-arabinose as starting points for the synthesis of the aldehyde [32]. Coleman, in a similar fashion to Armstrong, initially utilized the natural “chiral pool” to make the aldehyde side chain, beginning his synthesis from D-glucosamine [33]. However, it is clearly of benefit to differentiate between the 12- and 13-oxygens early in the synthesis and while Coleman achieved this end using glucosamine, the protecting group strategy proved eventually to be unworkable. This group has more recently described two elegant routes to the differentiated aldehyde, one involving asymmetric allylation of an organoborane [33] followed by Sharpless AE and ring opening of the resulting epoxide with azide to put in place all the required functionality [34]. Similarly, chiral aldehyde underwent a Lewis acid-promoted addition reaction with allyl stannane, thus differentiating the two alcohols and allowing the introduction of an enzyme cleavable protecting group at position 12 in the final compound [35].

24.4.1.2 Wadsworth–Horner–Emmons reaction, bromination, and ring closure
Armstrong’s initial work utilized the reaction of the aldehyde with a glycine phosphonate into which he also introduced the left-hand ketone group of azinomycin A (Scheme 24.6) [30]. Reaction of the phosphonate with the aldehyde gave a mixture of (Z)/(E)-stereoisomers (4:1) which was subsequently hydrolyzed to give only the (Z)-isomer, the (E)-isomer being destroyed under the reaction conditions. Following coupling to the ketoamine, bromination with bromine at low temperatures, followed by addition of DABCO gave only the (Z)-isomer, which upon deprotection and ring closure under basic conditions gave the unnatural (Z)-isomer (Scheme 24.6).

Armstrong and co-workers later discovered that the conditions of the bromination were key to the successful production of the compound with the correct (natural) stereochemistry [36]. Thus, bromination of the (Z)-isomer at −78°C with bromine, as noted, gave the (Z)-isomer, while treatment with NBS in dichloromethane gave exclusively the (E)-isomer (Scheme 24.7).
Scheme 24.5. Coleman's syntheses of the aldehyde with differentiated 12- and 13-OH groups [34, 35].

Scheme 24.6. Armstrong synthesis of the Z-isomer of the aziridine subunit [30].
Early in their studies, Coleman and co-workers noted the isolable nature of the bromoimine intermediate 49 in the bromination of the double bond in 48 by NBS [31]. They later showed that treatment of this compound with hindered bases led to, at best, a stereocontrol of only 1.5:1 \( (E)/(Z) \)-product when the 12-OH was protected as a phenylacetyl group, but as high as 10:1 \( (E)/(Z) \) with a \( p \)-methoxybenzyl 12-OH protecting group [37] (Scheme 24.8).

Deprotection and final ring closure of the aziridine has been shown by both Armstrong and Coleman’s groups to occur with complete stereocontrol [30, 31], emphasizing that introduction of the bromine with correct stereochemistry is key to the production of the natural product stereochemistry.

24.4.2 The Total Synthesis of Azinomycin A

The total synthesis of one of the azinomycins proved elusive, in spite of extensive studies of both 3 and the densely functionalized aziridine moiety, until the recent report of the synthesis of azinomycin A by Coleman and co-workers [38]. To finally
achieve their aim, this group had to rethink the segment-based synthesis approach originally identified and turn to a slightly less convergent, but elegant and, eventually, successful approach.

Studies of the aziridine fragment of the azinomycins had suggested that the dense functionality of this group, and in particular the 12-OH group of the full structure, leads to marked instability in the natural products and in synthetic analogs [29]. In early studies of carzinophilin (azinomycin B), Terashima and co-workers synthesized the skeleton of the azinomycins up to the aziridine (compound 50, Scheme 24.9) but without the problematic substituents on the pyrrolidine ring [39]. In a similar, recent synthesis, Shipman and co-workers made the same ester by a different route in order to demonstrate its DNA-crosslinking ability [40].

Prior to Coleman’s description of the total synthesis of azinomycin A, Tereshima had described the synthesis of an advanced structure en route to the synthesis of the more complex azinomycin B (Scheme 24.10) [41]. This route cleverly utilized a masked fragment 54 that corresponds to the epoxide and aziridine amino acids. The fragment was synthesized in five steps from methyl 3-hydroxymethyl-2-butenoate and the aziridine precursor fragment 52 previously described by the same authors [42]. Sharpless asymmetric dihydroxylation, followed by epoxide formation and inversion of configuration at the secondary hydroxyl in 53 via an oxidation/stereoselective reduction sequence, gave the complete epoxide/aziridine fragment 54 ready for coupling to the chromophore. The sequence was completed
by reaction of the 2-oxazolin-5-one with the protected compound 55 derived from methyl L-threonine. A series of manipulations gave the fully protected precursor to azinomycin B 56. At this stage, the authors discovered that it was not possible, under a variety of conditions, to remove the benzyl protecting groups from the aziridine fragment, presumably due once more to the instability of the unprotected compound at the 12-OH position.

To complete the total synthesis of an azinomycin and to access sufficient material for biological studies, Coleman and co-workers realized that an alternative approach to the construction of the natural product would be required [38]. Most synthetic efforts had concentrated on synthesizing the parts of the azinomycins separately and then bringing the final pieces together. This was justified, as each piece represented a different synthetic challenge, both for the stereochemically defined epoxide and the stereochemically challenging and functionally dense aziridine. However, it is clear that in a demanding natural product synthesis, introduction of sensitive functional groups should lie as close to the end of the synthesis as possible and with the observation in hand that the 12-OH caused extensive problems, Coleman and co-workers adjusted their approach to a late stage introduction of this group. Key to the total synthesis was the assembly of the backbone of the natural product, including the epoxide moiety, followed by the late stage introduction of the azabicyclic system through a Wadsworth–Horner–Emmons reaction (Scheme 24.11).

Scheme 24.10. Tereshima synthesis of fully protected azinomycin B analog 56 [42].
The backbone was formed through reaction of the epoxide acid 51, synthesized as described above, with the phosphonate alcohol 58, formed from reaction of the glycine phosphonate 56 with racemic 1-amino-2-propanol. Swern oxidation gave the backbone in the correct oxidation state and the resulting compound was reacted directly with the azabicyclic precursor prepared with the 12-OH protected as a triethylsilyl ether via a route previously described by the authors [34]. Bromination, tautomerization, aziridine deprotection, and cyclization gave 59, the protected precursor to azinomycin A, which upon deprotection displayed similar characteristics (NMR and chromatography) to the natural product, including an instability that wasn't detected when the 12-OH group was fully protected. There are a number of problems with the synthesis, including poor yields for a number of steps, but this cannot detract from the elegance of the approach and the fact that this remains, to date, the only total synthesis of one of the azinomycins.

24.5 Computational Studies of DNA Binding of the Azinomycins

As considerable amounts of the azinomycins remained elusive and DNA-binding studies were therefore difficult, Coleman and co-workers also carried out a number of computational studies of the binding of the natural product to DNA. Having
developed force-field parameters for the natural products [43], a model was developed for DNA crosslinking by azinomycin B [44]. Two approaches were considered. The first involved generating a DNA duplex containing an intercalation site, based upon the 9-aminoacridine-4-carboxamide crystal structure [45], whilst the second used a non-intercalative model. Both models suggested that initial alkylation should occur at the N7 of adenine through alkylation by the aziridine followed by N7-G alkylation on the opposite strand by the epoxide group. This model has been supported by experimental observations by Fujiwara and Saito [48] and is discussed in more detail in the following section.

24.6 Experimental DNA-binding Studies and Antitumor Activities of the Full Azinomycin Structures – Is Crosslinking Required for Biological Activity?

In early studies on the biological activity of azinomycin B, Lown and co-workers noted the ability of the natural product to form covalent links between complementary strands of DNA [46] and this was confirmed by Armstrong’s group who used 32P-labeled synthetic oligonucleotides to demonstrate crosslinking [47]. Essentially, they took synthetic DNA strands containing a 5'-GCT and modified only this sequence, suggested as an excellent crosslinking sequence for azinomycin B, to try to understand the requirements for DNA alkylation. The results are summarized in Fig. 24.7. Azinomycin B formed crosslinks most efficiently in the

![Fig. 24.7. Crosslinking of DNA by azinomycin B demonstrated by Armstrong and co-workers [47].](image-url)
5'-GCT sequence, as well as 5'-GCC, whereas crosslinking decreased to trace amounts with a central A or T in the sequence. Crosslinking of 5'-GC was not observed. These authors also noted that piperidine-sensitive base-induced lesions were seen, to some extent, at all G’s within the fragments they were analyzing and suggested a model in which initial G-alkylation is followed by a crosslinking reaction at suitably positioned A and G two bases to the 3'-side of the original lesion and on the opposite strand.

More recently, Fujiwara and Saito [48] have suggested an alternative crosslinking reaction, which supports the hypothesis of Coleman’s modeling studies [44]. Using a self-complementary short oligonucleotide containing a 5'-GCT sequence, HPLC analysis revealed that incubation with azinomycin B at 0°C gave two products, one corresponding (by MS) to the crosslinked adduct and the other to the monoalkylated product. The latter compound was found to convert to the crosslinked product on further incubation. Heating the monoadduct to 90°C for 5 min induced depurination of the oligonucleotide at the 5'-A (sequence 5'-TAGCTA2), whereas heating of the diadduct under the same conditions also gave cleavage at the central G. Analysis of the same reaction using the methylated analog 60 of azinomycin B allowed the authors to suggest that epoxide alkylation occurred at the N7 of guanine and thus to propose that initial alkylation occurred through aziridine reaction with the N7 of adenine followed by efficient crosslinking through a second reaction of the N7 of guanine with the epoxide (Fig. 24.8).

In a recent review, Hodgkinson and Shipman gave a comprehensive listing of the tissue culture-based antitumor activities of the azinomycins and their various derivatives [12]. The principal question to arise from this is of the requirement for interstrand crosslinking for biological activity. As Lown [46], Armstrong [47], and Saito [48] have demonstrated crosslinking for the natural products, whatever the order of adduct formation, it has been implied that this is required for biological activity. However, Shipman has shown that the truncated azinomycin analog 3 has potent antitumor activity in a variety of cell lines that matches and in some cases surpasses the activity of a crosslinking derivative synthesized and studied by the same group [12]. In fact, both the natural (S,S)- and unnatural (R,R)-diastereoisomers have potent antitumor activity (see Tab. 24.1 for comparison of the natural agent with a crosslinking analog 50). Compound 3 has been demonstrated to be an effective DNA alkylator at the N7 of guanine in the major groove [26] and it is of interest that Armstrong and co-workers noted during their crosslinking studies that azinomycin B alkylated at all Gs within the sequences under study, in spite of forming crosslinks only where the guanine or adenine was correctly position two base pairs to the 3'-side and on the opposite strand [47].
natural products, notwithstanding Coleman’s elegant total synthesis, deem them to be unlikely agents for use in the clinic. Synthetic efforts to date have identified a number of routes both to the truncated analog 3 and to the densely functionalized aziridine and at least one of these routes has yielded a total synthesis along with a number that have given very late stage agents or crosslinking analogs. Target

![Diagram of DNA crosslinking by azinomycin B](image)

**Fig. 24.8.** Crosslinking of DNA by azinomycin B as shown by Fujiwara and Saito suggests a different mechanism to Armstrong (Fig. 24.7) [48].

<table>
<thead>
<tr>
<th>No.</th>
<th>Crosslinker?</th>
<th>Cyotoxicity, IC(_{50}) ((\mu)M)</th>
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<td>1</td>
<td>Yes</td>
<td>0.12    – – – – – – – – – – – –</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>0.18    – – – – 0.076 &lt; 0.05 0.078</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>0.011  0.011 0.076 &lt; 0.05 0.078 1.25 0.33</td>
</tr>
<tr>
<td>26</td>
<td>No</td>
<td>– 0.44  1.1 0.55 0.078 0.087 2.1 0.52</td>
</tr>
<tr>
<td>27</td>
<td>No</td>
<td>– 0.39  1.35 0.17 0.39 0.39 13 2.6</td>
</tr>
<tr>
<td>50</td>
<td>Yes</td>
<td>0.047  0.076 0.28 0.078 0.087 2.1 0.52</td>
</tr>
<tr>
<td>56</td>
<td>Yes</td>
<td>0.50    – – – – – – – – – – – –</td>
</tr>
</tbody>
</table>

From Ref. [12].

**Tab. 24.1.** Cytotoxicities for crosslinkers and non-crosslinkers.
binding and biological activity studies have lagged behind the synthesis effort with only a small number of disclosures of DNA alkylation, crosslinking, and antitumor activity. This lack of fundamental studies stems in part from the lack of natural product to study and the synthetic efforts will contribute extensively to our future understanding of the activity of this class of compound. To date, there would appear to be several conflicting observations with regard to the biological activity and these problems need to be addressed before analog development can lead to useful agents:

1. Is intercalation required for biological activity? Coleman’s computational studies have suggested that intercalation of the napthalene chromophore and DNA crosslinking are unlikely to be compatible due to constraints on the extended structure of the azinomycins [44]. Gates and co-workers have presented strong evidence for intercalation of the truncated agent 3 [26] and the lower activity of both the epoxide and aziridine moieties alone suggests a role in DNA binding for the chromophore that is difficult to explain in any other fashion. More attention to the structure of the chromophore, including the introduction of classical intercalating functions such as acridines, may build on the initial studies of Shipman and co-workers [27] and define the requirement, if any, for intercalation.

2. Is crosslinking required for activation? Any controversy within the study of azinomycins has focused upon the different crosslinking mechanisms suggested by Armstrong [47] and Saito [48]. A more telling consideration is the requirement for crosslinking for biological activity. As noted above, the truncated analog 3 retains high antitumor activity in cell culture in spite of an inability to form crosslinks and Armstrong’s observation of non-crosslinking alkylation by azinomycin B [47] suggests that extensive monoalkylation occurs even with the natural products. Although classical antitumor alkylating agents exert their effects through crosslinking DNA, the minor groove binding alkylating agents such as CC-1065 and the duocarmycins form only monoalkylated products [3]. It is possible that major groove alkylation by the azinomycins combined with intercalation of the chromophore is sufficiently disruptive to initiate cell death.

The azinomycins are natural products with potent antitumor activity and yet have not progressed to a stage where they are clinically useful. Considerable synthetic efforts have been successful in synthesizing the truncated analog, advanced intermediates in the total synthesis and, in at least one case, the full natural product. DNA-binding and biological studies have established that the azinomycins can both monoalkylate and crosslink DNA and that they, and some of their analogs, have potent antitumor activity in various cell lines. However, the questions of whether the activity of the azinomycins stems from DNA crosslinking and of how these agents can be made into clinically useful agents remain unanswered. Further key studies of the natural products, analog structures, including potential pro-drug forms, and their effects on biological systems remain to be performed before these questions can be answered.
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