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THE STABILIZATION OF DNA TOPOISOMERASE II CLEAVABLE COMPLEX BY MITONAFIDE ANALOGS

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Abstract: Amonafide (4-aminobenzoisoquinolinedione) and its structural analog, mitonafide **1**, have been shown to stabilize topoisomerase II cleavable complexes. The position of the nitro group and structural modifications of the side chain influence the interactions between drug, enzyme, and DNA. It was shown that the analogs with the nitro in the 5-position, as in **1**, are the most potent inhibitors in this structural class.

DNA topoisomerase II (topo II) has been identified as the biochemical target for several classes of clinically valuable antitumor agents, including the anthracyclines, ellipticines, epipodophyllotoxins, anthracenediones, and bisantrenes (reviewed in ref. 1-3). DNA topoisomerase II alters the topological status of DNA through a catalytic sequence involving DNA double-strand cleavage, DNA strand passage through the protein-associated strand break, and subsequent DNA religation. The antitumor agents noted above interfere with the cleavage-religation reaction of the enzyme by stabilizing a reversible covalent enzyme-DNA cleavable complex.[4-6]

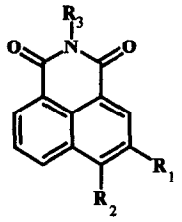
Amonafide (4-aminobenzoisoquinolinedione) and its structural analog, mitonafide **1**, have been shown to exhibit cytotoxicity in cultured human leukemia cells and HeLa cells.[7-8] Braña *et al.* demonstrated that mitonafide reversibly inhibited DNA and RNA replication in Ehrlich ascites tumor cells.[9] It was later shown that the toxic effects of these drugs may be correlated with the stabilization of the topo II cleavable complex.[10]

We have hypothesized that in the ternary drug-DNA-enzyme "cleavable complex" the principal interaction of the drug is with DNA and that drug-DNA complexation distorts the DNA duplex in a fashion similar to an intermediate in the enzyme's catalytic cycle.[11] The molecular model for the interaction of drugs with topo II and DNA has been refined by Capranico.[12] The Capranico model suggests that doxorubicin intercalates between the base pairs in the enzyme-DNA complex and that this intercalation interferes with the enzyme's activity and prevents the resealing of DNA. It has been proposed that specific interactions with DNA by the particular drug causes the differences in sequence preference observed with various classes of inhibitors.

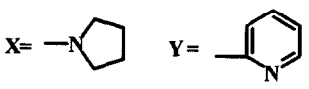
We have investigated a number of mitonafide analogs with different side chains and placements of the nitro group in an attempt to clarify the structural requirements for this new class of topo II inhibitors. Stimulation of cleavable complex formation as well as DNA binding affinities were investigated. Various analogs of mitonafide shown in the Table were synthesized by refluxing the appropriate napthalic anhydride with the amine containing the desired R_3 side chain in absolute ethanol as described previously.[9] The compounds were analyzed for their ability to induce topo II cleavable complex with pUC 18 DNA and purified human topo II[13,14] as described by Rowe, *et al.*[15]; the relative activity[16] is shown in the Table. The

TABLE

Stimulation of Topoisomerase II (Topo II) Cleavage Activity and DNA Binding (C_{50} Values) by Various Mitonafide Analogs



Compound	R_1	R_2	R_3	Stabilization of TopoII Cleavable Complex[16]	C_{50} (μM)[17]
1	NO_2	H	$(CH_2)_2N(CH_3)_2$	++++	11
2	NO_2	H	$(CH_2)_3N(CH_3)_2$	++++	16
3	NO_2	H	$(CH_2)_2N(CH_2CH_3)_2$	++	35
4	NO_2	H	$(CH_2)_2NH_2$	+++	>100 (27%)
5	NO_2	H	$(CH_2)_2NHCH_3$	++++	14
6	NO_2	H	$(CH_2)_2OH$	+++	>100 (25%)
7	NO_2	H	$(CH_2)_2X$	++++	10
8	NO_2	H	$(CH_2)_2Y$	+	>100 (10%)
9	NO_2	H	$(CH_2)_2N(CH_2CH_2)_2N$	inactive	>100 (39%)
10	H	NO_2	$(CH_2)_2N(CH_3)_2$	inactive	>100 (33%)
11	H	NO_2	$(CH_2)_3N(CH_3)_2$	inactive	>100 (36%)
12	H	NO_2	$(CH_2)_2N(CH_2CH_3)_2$	+	28
13	H	NO_2	$(CH_2)_2NH_2$	+	17
14	H	NO_2	$(CH_2)_2NHCH_3$	+	19
15	H	NO_2	$(CH_2)_2OH$	++	>100 (34%)
16	H	NO_2	$(CH_2)_2X$	++	11
17	H	NO_2	$(CH_2)_2Y$	inactive	>100 (0%)
18	H	H	$(CH_2)_2N(CH_3)_2$	+	100
19	H	H	$(CH_2)_2N(CH_2CH_3)_2$	inactive	100
20	H	H	$(CH_2)_2NH_2$	+	50
21	H	H	$(CH_2)_2NHCH_3$	+	>100 (41%)
22	H	H	$(CH_2)_2OH$	+	>100 (12%)



DNA binding affinity of the compounds was determined by a decrease in fluorescence of an ethidium bromide-calf thymus DNA solution as described previously.[18] The C_{50} values (Table) indicate the concentration of drug at which the fluorescence of the complex is decreased by 50 percent.

The compounds can be divided into three series based on the position of the nitro group. Each series will be discussed separately, followed by a discussion of the overall specific structural requirements for inhibition of topo II and for DNA binding.

The position and presence of the nitro group appears to dictate the activity of the analogs. The 4-nitro series (nitro group position = R_1) possesses the greatest activity and allows for a larger degree of predictability regarding the structure-activity relationships. Presumably in this group of analogs, the planar aromatic region of the molecule intercalates between DNA base pairs, as indicated by the C_{50} values, and the 4-nitro group appears to serve to position the drug in an orientation in the ternary complex that allows for the enhanced activity. By correlation with the model for interaction of topo II agents in the cleavable complex, the side chain, R_3, would be anticipated to reside in the minor groove and clearly influences the DNA association ability of the drug. A correlation between the size of the amine in the side chain, R_3, and the cleavage inducing activity is manifested in our study. As shown in the Table, compounds **1**, **2**, **5**, and **7**, which show the strongest stimulation of topo II cleavage sites, have a tertiary or secondary amine in the side chain which would exist as an ammonium salt and would be predicted to lie in the center of the minor groove in hydrated form, as shown by x-ray crystallography for the amine in the side chain of daunomycin.[19] Compound **4** shows moderate activity and contains a primary amine in the side chain, R_3, which would have less steric bulk and concomitant hydrophobicity to influence the spatial orientation of the molecule in the cleavable complex. Compound **6** also shows moderate activity and has an alcohol in the side chain, R_3; presumably the alcohol moiety could act as hydrogen bond donor or acceptor in the DNA minor groove and these interactions serve to position the drug in the cleavable complex. It has been previously observed with other classes of inhibitors that a dimethlyamino side chain (as in **1**) is more effective in stimulation of topo II activity relative to the alcohol side chain (as in **6**) or the primary amine (as in **4**).[18,19] This observation suggests that van der Waals interactions and/or hydrophobicity play roles in positioning the side chain in the minor groove, although electrostatic effects may facilitate DNA association. The positioning of the side chain in the minor groove must affect the overall drug-DNA conformation, thus affecting cleavable complex formation. In the case of **3**, the decrease in activity may be due to an orientation of the ethyl groups, which alters the interaction between the drug and DNA or alters the recognition of the cleavage site by topo II. Activity can be restored by restraining the conformationally mobile ethyl groups into a ring system, as in compound **7**.

The second series with a nitro group in the 5 position (R_2) exhibits a structure-activity relationship that is strongly contrasted with and is generally less predictable than the 4-nitro series. Activity appears to be correlated with compounds that have a R_3 substituent containing a secondary or primary amine, with the exception of **12** and **16**. Generally the compounds exhibiting activity intercalate with DNA, as indicated by the C_{50} values. The 5-nitro group presumably dictates the binding orientation of the planar aromatic region of the molecule into the ternary complex, perhaps placing the side chain, R_3, such that it contacts the minor groove in a manner different from the 4-nitro series. This would explain the opposite structural substitutions required on the nitrogen in the side chain between the 4-nitro and 5-nitro series; indicating that the nitro group forces the side chain into a smaller cleft within the minor groove therefore preferring side chains with less steric bulk. The strongest activity in this series is observed with compounds **13**, **14**, and **15**, which have side

chains R_3 that are smaller in size than the tertiary amines. Compounds **12** and **16** have slight activity and contain side chains with tertiary amines, demonstrate that the side chain, as directed by the nitro group, is important in orienting the drug in the cleavable complex.

The series without a nitro group appear to have a diminished activity, but follows the SAR trends noted for the 4-nitro series. It is likely that without the "directing" influence of the nitro group, the compounds may adopt several modes of intercalation. It has been shown that some topo II inhibitors can associate with DNA in multiple modes of intercalation or DNA interaction, which produce an array of DNA deformations.[22,23] Only one of these association modes may stabilize cleavable complex formation. Since this series lacks a nitro group which would help to "lock" it into one conformation, several modes of interaction may be feasible and the effective drug concentration of the requisite DNA-drug association mode with consequent DNA deformation would be small, thus leading to diminished topo II activity. This implies a profound importance of the nitro group in orientating the interaction of the drug with DNA.

Another point of interest is that these families of analogs induce the same cleavage sites, but the major cleavage bands differ between the three series (Figure 1). Such similarity in cleavage sites is often characteristic of a structurally related family of agents. The explanation for this behavior is thought to be that structurally related analogs interact at the same site in DNA, but that subtle differences in the orientations of the drug affect the induction of topo II cleavage at each site.

Molecular modeling studies suggests that the compounds in the 4-nitro series may intercalate parallel to the base pairs, such that the R_3 side chain lies in the minor groove and the nitro group (R_2) is penetrating into the hydrophilic major groove. Modeling studies were carried out according to protocols developed during earlier studies conducted in our laboratory.[11] Placement of the nitro group at the 5 position shifts the drug in DNA slightly, so that the R_3 side chain encounters steric interactions in accommodation to the minor groove. The difference in position of intercalation with DNA could explain the difference in activity and size requirements for the amines in the side chains, R_3, and indicates that the nitro group is essential for orientation of the drug into an active conformation in the enzyme-DNA complex.

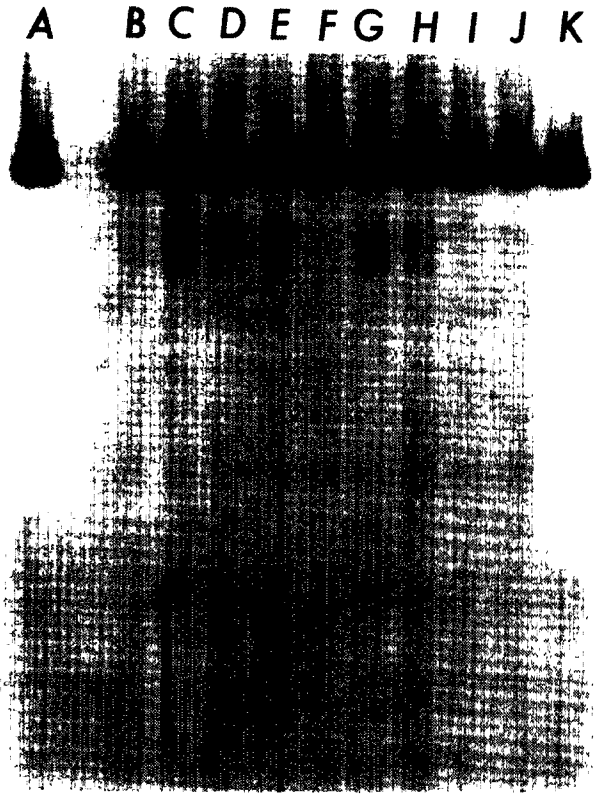
The different orientations within the enzyme-DNA complex may explain the differences in the induction of specific topo II cleavage sites as shown in Figure 1. If the side chains (R_3) are interacting differently in the minor groove, depending on the position of the nitro group, then the enzyme may cleave the site as influenced by the drug. Thus one series may induce more single strand breaks than double strand, which could lead to a difference in the degree of induction of cleavage sites. The drugs would still interact within a common site in the DNA, but the difference in orientation may contribute to a different induction of topo II cleavage sites. This would support the hypothesis that each class of inhibitors stimulates a different set of cleavage sites based on the specific ways the drug interacts with the DNA.

The series without a nitro group does not have the directing influence of the nitro group and may therefore adopt several modes of intercalation. The drug will cause an array of DNA distortions, but only one may stimulate cleavable complex formation. This would lead to a decrease in the actual concentration of drug that is available to inhibit the enzyme. This may explain why the activity is weak and that there appears to be no specific preference for any of the cleavage sites.

In conclusion it appears that the nitro group affects the orientation of the drug within the cleavable complex of topo II. The side chains appear to interact with the minor groove in a specific fashion as dictated by the nitro group which influences its stimulation of topo II cleavable complex. The

information deduced from these compounds may shed some light into the importance of specific interactions with DNA in order to stimulate cleavable complex formation.

Figure 1 - Site-Specific Topo II Cleavage of pUC 18 plasmid DNA by Mitonafide Analogs. Topo II cleavage activity was carried out as described in the methods. Lane A, DNA only; Lane B, topo II added, Lanes C-E, topo II and 10, 50, and 100 μM **6** added respectively; Lanes F-H, topo II and 10, 50, and 100 μM **15** added respectively; Lanes I-K, topo II and 10, 50, and 100 μM **22** added respectively.



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