

# Voreloxin as a Topoisomerase II Poison: Role of DNA Intercalation

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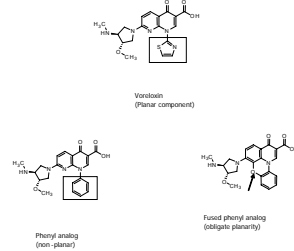


## Abstract

Voreloxin is a novel naphthyridine analog that is structurally related to quinolone-based antibacterials. The drug currently is in clinical trials in acute myeloid leukemia and platinum-resistant ovarian cancer. Voreloxin induces site-selective double-stranded DNA breaks and G2 arrest in treated cells. The cytotoxic properties of the drug have been attributed in part to its ability to poison (i.e., increase DNA cleavage by) human topoisomerase II $\alpha$  and  $\beta$ . In contrast to drugs like etoposide, voreloxin enhances enzyme-mediated cleavage at a restricted number of sites. Voreloxin intercalates DNA with an affinity that is  $\sim 1/2$  that of ethidium bromide. Intercalation is observed at  $<1 \mu\text{M}$  drug, with complete intercalation being seen at  $\sim 10 \mu\text{M}$ . Current data indicate that intercalation is necessary for the cytotoxicity of voreloxin, but detailed relationships between intercalation and the topoisomerase II activity of the drug have not been defined. To address this important issue, we assessed the effects of voreloxin and two derivatives with differing intercalative properties on the activity of human topoisomerase II $\alpha$  and  $\beta$ . One derivative (phenyl) contains a phenyl ring in the N-1 position that disrupts drug planarity. The second (fused phenyl) contains a fused phenyl at N-1 that enforces planarity. While the phenyl derivative displayed no ability to intercalate at concentrations as high as  $50 \mu\text{M}$ , the fused phenyl compound intercalated with an affinity that was  $\sim 2$ -fold higher than the parent compound and was more cytotoxic in proliferation and colony forming assays. Levels of voreloxin-induced DNA cleavage were highest at  $\sim 1 \mu\text{M}$ , a concentration at which intercalation was initially observed, but decreased as greater intercalation was seen. Such a "bell-shaped activation" curve is typical for the actions of intercalative topoisomerase II poisons. The phenyl compound did not enhance enzyme-mediated DNA cleavage. Although the fused phenyl derivative increased DNA cleavage by topoisomerase II $\alpha$  and  $\beta$  *in vitro* and in cultured human cells, it was no more active as a topoisomerase II poison than voreloxin. However, a more pronounced loss of topoisomerase II poisoning was observed at higher drug concentrations *in vitro* than was seen with the parent compound. These results indicate that intercalation is required for voreloxin activity against topoisomerase II, but that an increased DNA affinity does not necessarily enhance the ability to poison the enzyme. Finally, voreloxin inhibited the relaxation activity of human topoisomerase II $\alpha$  and  $\beta$  at drug levels that were below those required to alter the apparent topological state of the plasmid substrate. This suggests that the drug has a direct interaction with topoisomerase II that affects the activity of the enzyme. Supported by NIH grant GM33944 and funds from Sunesis Pharmaceuticals, Inc.

## Voreloxin

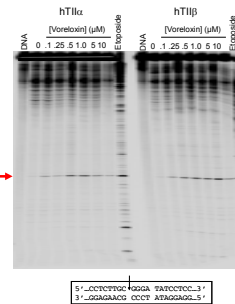
### Structures of Voreloxin and Its Derivatives



**Figure 1: Structures of voreloxin and its derivatives, voreloxin-P and voreloxin-FP.** The differences in chemical structure are marked in red. Voreloxin-P contains a phenyl group that is free to rotate. Voreloxin-FP contains a fixed phenyl group that results in a planar structure. While voreloxin-FP is 5-fold more potent at inhibiting cell proliferation than voreloxin, voreloxin-P does not significantly block cell growth [Stockett, Byl, *et al.* (2008) AACR Meeting Abstract 1860].

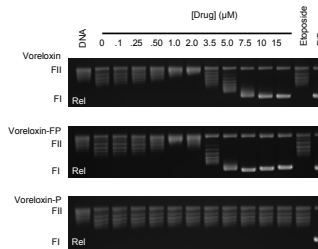
## Results

### DNA Cleavage Map



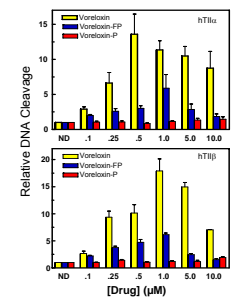
**Figure 2: Voreloxin enhances topoisomerase II-mediated DNA cleavage in a site-specific manner.** In contrast to the anticancer drug etoposide, voreloxin stimulates DNA cleavage in a highly site-specific manner (*red arrow*). The sequence of the DNA cleavage site is shown at the bottom. The *arrow* indicates the scissile bond.

### Intercalation of Voreloxin and Its Derivatives



**Figure 3: DNA intercalation of voreloxin and its derivatives.** Intercalation was monitored using a topoisomerase I-based assay. Complete voreloxin intercalation is observed at  $\sim 10 \mu\text{M}$ . The planar structure of voreloxin-FP results in stronger intercalation. Complete intercalation is observed at  $\sim 5 \mu\text{M}$ . No intercalation is seen with voreloxin-P, even at concentrations as high as  $100 \mu\text{M}$  (not shown).

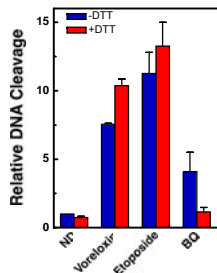
### Site-specific DNA Cleavage Titration



**Figure 4: The ability to intercalate DNA is required for voreloxin to poison topoisomerase II.** Voreloxin enhances site-specific topoisomerase II-mediated DNA cleavage to a greater extent than its two derivatives. Voreloxin-P, which does not intercalate, does not stimulate DNA cleavage. Results suggest that intercalation is necessary but not sufficient for actions of voreloxin against human type II topoisomerases since the strongest DNA intercalator, voreloxin-FP, does not enhance DNA cleavage as much as the parent compound.

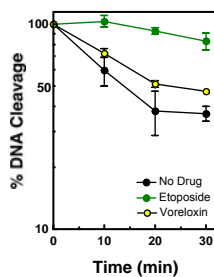
## Results

### Voreloxin Acts as a Traditional Poison of Topoisomerase II $\alpha$



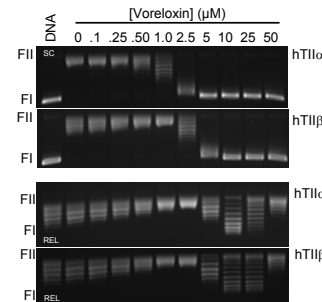
**Figure 5: Voreloxin acts as a traditional poison of topoisomerase II $\alpha$ .** The actions of "traditional" topoisomerase II poisons, such as etoposide are not affected by reducing agents such as DTT. Conversely, compounds such as benzoquinone (BQ) that require redox cycling to poison topoisomerase II lose their activity following exposure to reducing agents. Incubation with DTT did not inhibit the ability of voreloxin to enhance DNA cleavage mediated by human topoisomerase II $\alpha$ . Therefore, voreloxin acts as a traditional topoisomerase II poison.

### Effect of Voreloxin on DNA Ligation



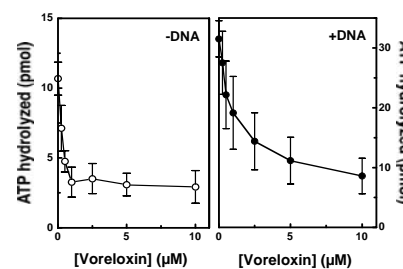
**Figure 6: Effect of voreloxin on topoisomerase II $\alpha$ -mediated DNA ligation.** Drugs can enhance topoisomerase II-mediated DNA cleavage in two ways. While drugs such as etoposide strongly inhibit ligation, quinolones such as CP-115,953 have little effect on ligation and presumably act by stimulating the forward rate of scission. Ligation was initiated by transferring the reaction from  $37^\circ\text{C}$  to  $4^\circ\text{C}$ . Compared to etoposide, voreloxin had minimal effect on rates of ligation of the DNA site shown in Figure 2. Thus, the mechanistic basis for the actions of voreloxin on topoisomerase II $\alpha$  appears to be similar to that of other quinolone-based drugs.

### Voreloxin Inhibits Topoisomerase II Catalysis



**Figure 7: Inhibition of topoisomerase II catalysis by voreloxin.** In addition to its action as a topoisomerase II poison, voreloxin inhibits the overall catalytic activity of type II topoisomerases. Levels of topoisomerase II-catalyzed DNA relaxation (*top two panels*) decreased at drug concentrations that were lower than those observed in assays that utilized topoisomerase I. Furthermore, DNA supercoiling in the presence of the intercalative drug (*bottom two panels*) was less than that observed in topoisomerase I assays (see Figure 3) at voreloxin concentrations up to  $\sim 5 \mu\text{M}$  and was blocked at concentrations  $\geq 10 \mu\text{M}$ . These findings indicate that voreloxin impairs the catalytic DNA strand passage reaction of topoisomerase II.

### Voreloxin Inhibits ATP Hydrolysis by Topoisomerase II $\alpha$



**Figure 8: Effect of voreloxin on topoisomerase II $\alpha$ -mediated ATP hydrolysis.** Voreloxin inhibits topoisomerase II $\alpha$ -mediated ATP hydrolysis in the absence (*left*) and presence (*right*) of DNA. The fact that voreloxin affects ATP hydrolysis in the absence of DNA suggests a direct interaction between the drug and topoisomerase II $\alpha$ .

## Conclusions

1. DNA intercalation appears to be required for the actions of voreloxin as a topoisomerase II poison. However, the enhanced intercalation of voreloxin-FP does not result in greater DNA cleavage activity.
2. Voreloxin enhances site-specific topoisomerase II $\alpha$ -mediated DNA cleavage without significantly inhibiting ligation.
3. Voreloxin acts as a "traditional" as opposed to a "redox-dependent" topoisomerase II poison.
4. Voreloxin inhibits the catalytic DNA relaxation activity of topoisomerase II. The drug acts at least in part by impairing the ability of the enzyme to hydrolyze ATP.
5. Voreloxin inhibits topoisomerase II $\alpha$ -catalyzed ATP hydrolysis in the absence of DNA. This suggests that there is a direct interaction between the drug and the type II topoisomerase.