



SNS-595 is a potent anti-tumor agent that has a dual mechanism of action: DNA intercalation and site-selective topoisomerase II poisoning

David Stockett*, Jo Ann Byl*[‡], Rachael E. Hawtin, Nguyen Tan, Michelle R. Arkin, Yonghong Zhu, Wenjin Yang, Robert McDowell, Neil Osheroff[‡] and Judith A. Fox

Sunesis Pharmaceuticals, Inc., South San Francisco, CA and [‡]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146 *Co-first authors

ABSTRACT

SNS-595 is a replication-dependent agent that induces DNA damage, irreversible G2 arrest and apoptosis. SNS-595 is a naphthyridine analog, closely related to the quinolone family of antibiotic compounds. The purpose of this study was to further investigate the mechanism of action of SNS-595 for identification of potential pharmacodynamic and patient stratification biomarkers. Here we report direct evidence of SNS-595-induced damage to DNA and in vitro and cell-based studies that demonstrate that SNS-595 intercalates DNA and poisons topoisomerase II.

Direct evidence of SNS-595-mediated DNA damage was generated using pulsed-field gel electrophoresis. Six-hour treatment of CCRF-CEM cells (a human acute lymphocytic leukemia cell line) with SNS-595 identified a dose-dependent induction of DNA damage, detectable at and above 1 μM.

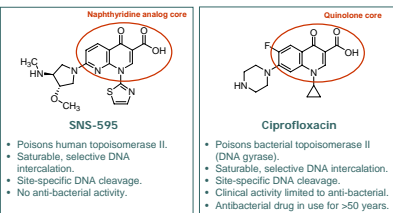
We investigated whether the DNA damage caused by SNS-595 involved topoisomerase II. In biochemical assays, SNS-595 was a poor topoisomerase II poison and little induction of global DNA cleavage was seen with either topoisomerase IIα or β. However, DNA cleavage at specific sites was enhanced ~5-fold by SNS-595 with both topoisomerase II isoforms. Treatment of cultured human CEM cells with 1 μM SNS-595 increased physiologic levels of DNA cleavage mediated by topoisomerase IIα or β approximately 3-5X. This increase was approximately 50% of that seen with 1 μM etoposide. Topoisomerase IIα knockdown studies in A549 lung cancer cells abrogated the SNS-595-induced G2 arrest at concentrations below 1 μM. A small (< 2-fold) decrease in SNS-595 inhibition of colony formation was observed, and the DNA damage signaling response to SNS-595 also was diminished. The impact of topoisomerase IIα knockdown on the activity of etoposide was more pronounced, indicating that SNS-595 is less dependent than etoposide on poisoning topoisomerase II for its activity.

DNA intercalation was observed in vitro with SNS-595. This observation was extended to cell-based studies using a planar fixed-ring analog of SNS-595 that is predicted to be a DNA intercalator, and a non-planar phenyl analog that is predicted to be a poor intercalator. Proliferation (MTT) and colony-forming assays in multiple cell lines indicated that the cytotoxicity of the planar analog was >5-fold greater than SNS-595, while the non-planar analog was more than 100-fold less potent. These data support the hypothesis that SNS-595 cytotoxicity involves DNA intercalation.

Collectively these observations suggest that the SNS-595 mechanism of action is similar to the quinolone antibiotics, and involves both DNA intercalation and a novel poisoning of topoisomerase II that causes site-selective DNA damage and shows selectivity for proliferating cells. The DNA damage signaling pathways involved in the repair of SNS-595 induced lesions are under investigation. SNS-595 is under clinical investigation in acute leukemia and ovarian cancer. Clinical responses have been observed in these indications (Lancet et al., ASH 2007; McGuire et al., SGO 2008), as well as in non-small cell (NSCLC) and small cell lung cancers (Burns et al., ECCO 2007).

SNS-595 MECHANISM DERIVES FROM STRUCTURE

- SNS-595 is a naphthyridine analog that induces site-selective DNA damage and poisoning of topoisomerase II. These targeted DNA-protein interactions may contribute to the broad therapeutic window observed in patients treated with SNS-595.
- SNS-595 is currently in Phase 1b and 2 clinical studies in relapsed, refractory AML, both as a single agent and in combination with cytarabine, and in platinum-resistant ovarian cancer.
- Clinical activity observed in both solid and hematologic tumors.



* SNS-595 targets the eukaryotic equivalent of bacterial DNA gyrase

SNS-595 INDUCES DOUBLE STRAND BREAKS AND CLEAVAGE COMPLEXES

Figure 1. SNS-595 induces dose-dependent DNA damage in human CCRF-CEM leukemia cells.

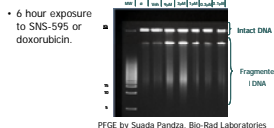
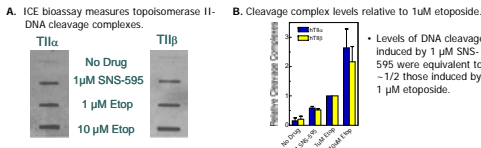
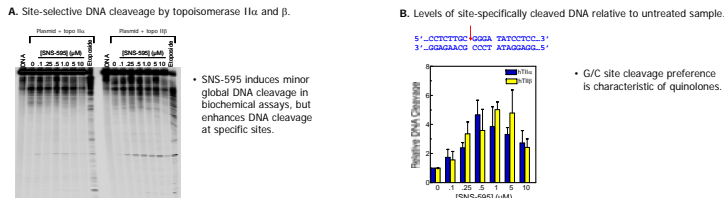


Figure 2. SNS-595 induces topoisomerase II-DNA cleavage complexes in CEM cells.



SNS-595 INDUCES SITE-SELECTIVE DNA DAMAGE WITH TOPOISOMERASE II

Figure 3. SNS-595 induces site-selective DNA cleavage by topoisomerase IIα and β in vitro.



TOPOISOMERASE IIα KNOCKDOWN INHIBITS SNS-595-INDUCED G2 ARREST

Figure 4. Knockdown of topoisomerase IIα inhibits dose-dependent G2 arrest induced by SNS-595.

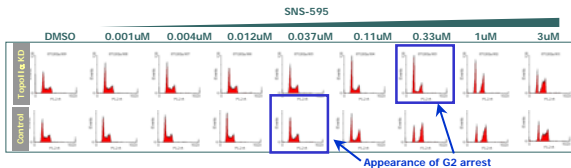


Table 1. Topoisomerase IIα knockdown has a greater effect on etoposide-induced G2 arrest than on SNS-595 or doxorubicin.

	SNS-595 μM	Doxorubicin μM	Etoposide μM
Topo IIα competent	0.037	0.004	0.11
Topo IIα knockdown	0.33	0.037	3
Fold-decrease in sensitivity	9	9	27

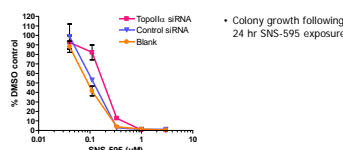
* Etoposide is more dependent upon the expression of topoisomerase IIα for drug-induced G2-arrest.

• Appearance of G2 in topoisomerase IIα-knockdown cells, arrest requires a 9-fold increase in SNS-595 concentration compared to control cells.

Figure 5. siRNA knockdown of topoisomerase IIα in A549 cells.



Figure 6. Knockdown of topo IIα moderately decreases colony growth inhibition by SNS-595.



SNS-595 CYTOTOXIC ACTIVITY INVOLVES DNA INTERCALATION

Figure 7. SNS-595 intercalates plasmid DNA in a biochemical assay

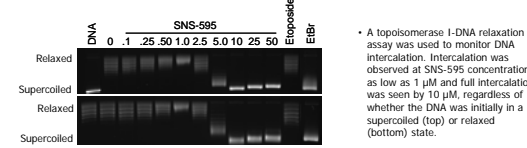
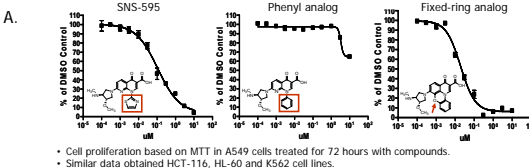


Figure 8. Planar fixed-ring analog of SNS-595 shows enhanced cytotoxicity while phenyl ring analog shows diminished cytotoxicity by both MTT (A) and colony forming (B) assays.



• Cell proliferation based on MTT in A549 cells treated for 72 hours with compounds.
• Similar data obtained HCT-116, HL-60 and K562 cell lines.

Table 2. Cytotoxicity of SNS-595 analogs correlates with intercalation potential

Compound	EC50 μM	Potency Relative to SNS-595
SNS-595	0.11	1
Phenyl analog	>10	>100X decrease
Fixed-ring analog	0.02	5.5X increase

• Colony forming assay in A549 cells treated for 16 hours with compounds.

SUMMARY & CONCLUSIONS

- SNS-595's targeting of human topoisomerase II in cancer cells parallels the mechanism of action of quinolones on bacterial type II topoisomerases in bacteria.
 - Site-specific intercalation and poisoning of topoisomerase II.
 - Site-selective DNA damage, preferential for G/C regions.
 - These targeted DNA-protein interactions may contribute to the broad therapeutic window observed in patients treated with SNS-595.
 - Both the SNS-595 structure and molecular mechanism provide advantages over other topoisomerase II poisons.
- SNS-595 mechanism of action supports and directs clinical focus to indications where topoisomerase II poisons are active.
 - Clinical responses to SNS-595 observed in relapsed/refractory AML and platinum-resistant ovarian cancers, as well as in lung cancers (ECCO 2007, ASH 2007, SGO 2008).
 - Objective responses have been observed in patients who have failed anthracycline-based therapies.
 - Broad activity has been seen in primary breast cancer biopsy samples (Poster #2830).
- SNS-595 is currently in a clinical phase 1b trial in relapsed, refractory AML in combination with cytarabine, and in phase 2 trials as a single agent in both platinum resistant ovarian cancer and in elderly, untreated AML patients.