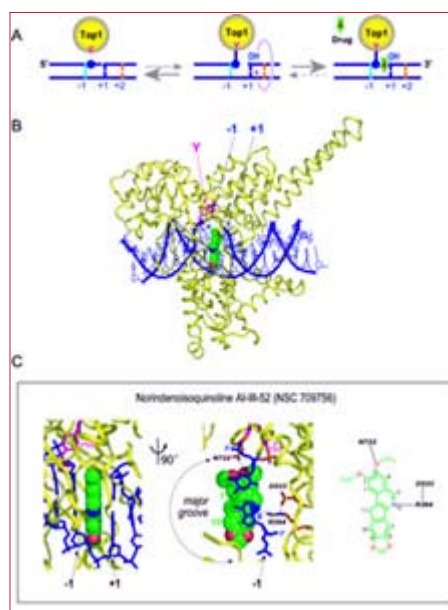


## ■ MOLECULAR BIOLOGY/PHARMACOLOGY

### Novel Interfacial Inhibitors of Topoisomerase I

Marchand C, Antony S, Kohn KW, Cushman M, Ioanoviciu A, Staker BL, Burgin AB, Stewart L, and Pommier Y. A novel norindenoisoquinoline structure reveals a common interfacial inhibitor paradigm for ternary trapping of the topoisomerase I-DNA covalent complex. *Mol Cancer Ther* 5: 287–95, 2006.

**H**uman DNA topoisomerase I (Top1) is a ubiquitous and essential enzyme because it relaxes DNA supercoiling during replication and transcription. Top1 generates DNA single-strand breaks and allows rotation of the cleaved strand around the double helix axis. During relaxation, the 3' end of the cleaved DNA strand is covalently linked to a Tyr residue on the protein. After relaxation, Top1 religates the cleaved strand and regenerates intact duplex DNA. Under normal conditions, the covalent Top1-cleaved DNA intermediates, referred to as “cleavage complexes,” are transient and remain at a very low level because the religation (“closing”) step is much faster than the cleavage (“nicking”) step (Figure 1, part A).



**Figure 1.** (A) The human DNA topoisomerase I (Top1)–mediated cleavage and religation of DNA. (B) Structure of the norindenoisoquinoline AI-III-52 in a Top1 cleavage complex. The 3' end of the cleaved strand is covalently linked to the catalytic Tyr residue 723 (Y). (C) An expanded view of AI-III-52 bound inside the Top1 cleavage complex in the same orientation as in part B (left panel) or with a 90° rotation (center panel). The –1 base pair (capped sticks) covers and stacks against the

entire drug (space filling).

Top1-specific inhibitors, such as camptothecins, can trap Top1 cleavage complexes. These potent anticancer drugs bind at the Top1-DNA interface in a ternary complex and prevent DNA religation. Their anticancer activity is therefore not directly driven by the inhibition of Top1 catalytic activity *per se*, but by the generation of lethal DNA lesions. Many camptothecin derivatives have been synthesized for clinical development, and two have been approved by the U.S. Food and Drug Administration: topotecan (Hycamtin) for ovarian and lung cancer and irinotecan (CPT11, Campto) for colon carcinomas. To circumvent the clinical limitations of camptothecins, other Top1 inhibitors have been developed. Among them, the indolocarbazole edotecarin is the most advanced in clinical development.

Indenoisoquinolines are a novel family of Top1 inhibitors with several advantages over camptothecins. First, they do not require metabolic activation and have a prolonged half-life. Second, they induce Top1 cleavage complexes at different sites and therefore offer a different biological profile. Third, Top1 cleavage complexes induced by indenoisoquinolines are markedly more stable and persistent than those trapped by camptothecins.

We recently analyzed the co-crystal structures of Top1-mediated DNA cleavage complexes with five potent and highly specific Top1 inhibitors: AI-III-52 norindenoisoquinoline, MJ-238 indenoisoquinoline, SA315F indolocarbazole, topotecan, and camptothecin. The norindenoisoquinoline AI-III-52 is bound deeply inside the protein and intercalated between the base pairs flanking the cleavage site (positions -1 and +1) (Figure 1, part B). The 3' end of the cleaved strand is covalently linked to the catalytic Tyr residue 723 (Y). The -1 base pair covers and stacks against the entire drug (Figure 1, part C). Extensive stacking is also observed for the +1 base pair, which is completely covered by the drug (and therefore not shown in Figure 1, part C). The drug is stabilized by two critical hydrogen bonds with residues Asn 722 and Arg 364 on Top1 (Figure 1, part C, center and right panels).

We found that all five inhibitors exhibit a common mechanism of action. They all bind at the Top1-DNA interface by intercalating between the base pairs flanking the DNA cleavage site and by forming a network of hydrogen bonds with Top1, as observed for the norindenoisoquinoline AI-III-52 (Figure 1, part B). Our results add critical information to the understanding of the biological effect of Top1 inhibitor substitutions and offer new insights for the rational design of novel Top1 inhibitors.

The camptothecins (camptothecin, topotecan, and irinotecan) and non-camptothecins (indenoisoquinolines and indolocarbazole) represent a paradigm for interfacial inhibition because they bind at the interface of two macromolecules (Top1 and its DNA substrate) as these macromolecules undergo a catalytic conformational change (cleavage complex) (Pommier Y and Cherfilis J. *Trends Pharmacol Sci* 26: 138–45, 2005). Interfacial inhibition is reversible and uncompetitive. Interfacial inhibitors trap a catalytic intermediate of the macromolecular complex in a specific conformation. The interfacial inhibitors identified so far are natural products that stabilize a wide range of macromolecular complexes: brefeldin

A (for the Arf-GTP exchange factor), colchicine, vinblastine, Taxol, epothilones (for alpha- and beta-tubulin), rapamycin (for the FKBP-TOR complex), antibiotics within the ribosome, and alpha-amanitin (within the elongating RNA pol II complex) (Pommier Y and Marchand C. *Curr Med Chem Anticancer Agents* 5: 421–9, 2005). The mode of interfacial inhibition for these camptothecins and non-camptothecins has implications for drug discovery because interfacial inhibitors stabilize rather than inhibit the formation of macromolecular complexes. Hence, screening through the use of methods to measure the stabilization of macromolecular complexes has the potential to lead to the discovery of highly selective interfacial inhibitors.

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