Abstract: Topotecan (TPT) is in clinical use as an antitumor agent. It acts by binding to the covalent complex formed between nicked DNA and topoisomerase I, and inserts itself into the single-strand nick, thereby inhibiting the religation of the nick and acting as a poison. In this study, we have investigated the interaction of topotecan with chromatin in solution to elucidate the mechanism of its action, employing fluorescence spectroscopy technique. Chromatin, in the absence of the drug, at 278 nm exhibited a peak with a maximum at 334 nm. Addition of topotecan to chromatin solution decreased the fluorescence emission intensity without any red shift in the emission maxima (Imax) as the drug concentration was increased. As drug concentration is increased, the binding affinity is increased and reaches to a maxima (at 40 µg/ml of drug) providing saturation state of the binding. Quenching of TPT with phenylalanine residues of the protein components of the chromatin, employs that the histone proteins may play a role in TPT-chromatin interaction process. The results clearly suggest that apart from topoisomerase I, chromatin can be considered as a new target for this drug which opens a new insight into its anticancer activity in tumor cells. Topotecan interacts with chromatin components and probably its binding occurs through both interaction with chromatin components and intercalation into base pairs of DNA.