CONCLUSIONS
The work embodied in this thesis evaluates the functional significance of topoisomerase II antagonism by organometallic complexes of iron and ruthenium for anticancer therapy. The major conclusions of our study are enumerated below, which argue that these topoisomerase II targeting drugs have the potential to be exploited for cancer chemotherapy.

1. The molecular analysis of topoisomerase II poisoning suggests the requirement of N-donor, O-donor and S-donor groups on the ligands (attached to the iron or ruthenium atom) to interact with the enzyme. These electronegative centers may be involved in non-covalent interaction with the electropositive groups on the enzyme.

2. Unsubstituted ferrocene has no effect on topoisomerase II activity and also shows no anticancer action, while the mono-substituted acetyl and carboxaldoxime derivatives of ferrocene (AcFecp and FecpOx) inhibit the topoisomerase II-catalyzed relaxation of supercoiled DNA but do not poison the enzyme by cleavage complex formation. On the other hand, the di-substituted derivatives (DacFecp & FecpDox) effectively poison topoisomerase II by cleavage complex formation. This, coupled with the molecular modeling analysis suggests that though the iron atom and the cyclopentadiene rings do not directly involve in topoisomerase II poisoning, the structural conformation provided by this backbone to the double substitution (and not the single substitution) may be important for topoisomerase II poisoning by these compounds.
3. FecpDox was more potent than DacFecp suggesting that the N-donor interaction of the carboxaldoxime group with topoisomerase II may be more effective for poisoning the enzyme compared to the O-donor interaction of the acetyl group. The DNA non-binding nature of the ferrocene molecules and the organometallic linkages on the metal atom could minimize iron induced toxicity. These observations suggest that the di-substituted ferrocene compounds are attractive anticancer drug candidates, and merit further investigation.

4. Our work on the RuBen drugs introduces novel and effective topoisomerase II poisons, which poison the enzyme through formation of a ternary 'cleavage complex’ The first compound in this class, RuBen(dmso), served as a good lead molecule for the design of new derivatives with enhanced anticancer action.

5. A noteworthy point in understanding the molecular mechanism of action of these compounds is that a pyridine ring in place of the dimethyl sulfoxide group (in RuBenPy) abolishes topoisomerase II poisoning. In RuBenPy, the electronegative center of the pyridine ring, the nitrogen atom, is already coordinated to the ruthenium atom and there is no other group on the ring for enzyme interaction. Introduction of a single amino group on the pyridine ring (RuBenAPy) greatly increased topoisomerase II poisoning and anticancer activity. This result and the molecular modeling analysis suggest that the coordinated ligands, ‘dms0’, ‘aminopyridine’, ‘aminoguanidine’ and ‘aminobenzoic’ acid may be major enzyme interacting domains on the RuBen drugs.
6. Based on the molecular analysis of topoisomerase II poisoning by the ferrocene and RuBen drugs, the following putative mechanism of action may be proposed -

"The di-substituted ferrocene drug binds to the topoisomerase II and following DNA interaction and cleavage by the enzyme, the drug freezes the enzyme and cleaved DNA in a 'closed clamp conformation' called the cleavage complex, thus abrogating the DNA religation step.

The RuBen drugs follow a slightly different mechanism. They interact bi-directionally with both enzyme and DNA- the ruthenium atom interacts with DNA while the coordinated ligands interact with the enzyme, ultimately leading to the formation of the cleavage complex."

7. The anticancer studies using the iron and ruthenium drugs correlate with their topoisomerase II poisoning ability, arguing that topoisomerase II poisoning may be the major mechanism involved in their anticancer action.

8. Our studies show that the spatial conformation of the organometallic iron and ruthenium molecules is an important determinant for topoisomerase II poisoning. The enzyme interacting groups must extend out of the molecular structure in order to interact strongly with the enzyme. This is especially true in the case of the coordination ruthenium complexes of RuIm and RuInd. A detailed X-ray crystallographic analysis on the topoisomerase II-drug association could determine the exact drug interacting
sites on the enzyme. This information could be immensely useful for understanding the molecular events leading to cleavage complex formation and in the development of superior organometallic therapeutics targeted to topoisomerase II.

9 Our studies on the transferrin mediated delivery of the RuBen drugs provides a methodology for targeting topoisomerase II antagonistic anticancer metal complexes to cancer cells. Because transferrin is a natural delivery system and has a good affinity for binding to ruthenium containing compounds, it is an attractive approach for delivery of such compounds. The results of this study show that apotransferrin holds promise as a potential delivery vehicle for ruthenium containing compounds.