DNA Topoisomerase as Drug Target in unicellular protozoan parasite *Leishmania donovani*

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Abstract

Visceral leishmaniasis or Kala-Azar is a neglected tropical disease caused by the unicellular kinetoplastid parasite *Leishmania donovani*. The treatments available have various shortcomings like toxicity and high cost. The development of resistance in the strains makes the treatment less effective. Hence, there is a need for new and better treatments. DNA topoisomerases are a group of enzymes present ubiquitously in all organisms from unicellular protozoan parasites to humans. These enzymes control the cell's topological problems caused by DNA double helix during nucleic acid metabolism. However, the leishmanial topoisomerase (LdTOPILS) is very different from the human topoisomerase in structure, expression, and function. Therefore, LdTOPILS acts as a potent target for newer drugs. A diverse range of compounds has been developed to date against Leishmanial topoisomerases. This review article describes Leishmania topoisomerase enzyme's structural and catalytic activity and Topo-targeted antileishmanial drug development. Therefore, this review article would be beneficial for researchers to understand the structure-function relationship of topoisomerases with their targeted inhibitors.

Keywords: Anti-leishmanial drugs, Catalytic inhibitor, Drug development, *Leishmania donovani*, *Leishmania* topoisomerase I (LdTOP1LS), Programmed cell death (PCD), Topo poison, Visceral leishmaniasis.

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Introduction

DNA is a 2-meter-long[1] polymer of a single cell that is accommodated in a cell nucleus with a volume of approximately 10^{-17} m^3; high compaction is necessary for incorporating such a long molecule in a small nucleus. This is accomplished by supercoiling and folding DNA. This supercoiling and the anchorage of DNA to the nuclear matrix make the rotation and separation of the strands very difficult. For various metabolic activities including replication, transcription, recombination, repair, and chromosomal decatenation, the strands of DNA duplex have to be separated to serve as templates.[2] Moreover, during replication and transcription, the regions of DNA flanking the complex which generate supercoiling of DNA. This supercoiling of DNA duplex needs to be relaxed from positive or negative supercoiling of DNA by DNA topoisomerases solving the above topological problems.[3]

DNA Topoisomerases are ubiquitous enzymes that play a crucial role in maintaining the dynamic structure of cellular DNA. These enzymes catalyze the cleavage of DNA strands; the breaks induced allow the strands to rotate through the other strand, which resolves to supercoil. Topoisomerases are divided into two broad categories, including Type-I and Type-II DNA topoisomerases. Type-I topoisomerases catalyze only one strand break and rotation of this strand through the other strand. Type-II topoisomerases catalyze the cleavage of two strands, and both the strands then pass the other segment through the transient cut and are then re-ligated.[4] The trans-esterification reaction cleave all known topoisomerases and re-ligate the DNA phosphodiester bonds. DNA first binds to the tyrosine residue of the enzyme and is cleaved by the formation of a transient cleavage intermediate (complex cleavable formation), which forms a gate that allows the other strand to pass through.

The human topoisomerase I (hTopI) enzyme produces transient single-stranded breaks of double-stranded DNA. The mechanism of action involves a nucleophilic attack at the phosphodiester bond by a tyrosyl residue of topoisomerase. The attachment of the "controlled rotation" implies that this enzyme relaxes DNA by swiveling the 5' hydroxyl end of the broken strand around the intact strand; this reaction does not require ATP or divalent metal (Mg^{2+}). This enzyme can relax positive and negative supercoiled DNA at a significant efficiency even at 0°C.[5]

Like mammalian organisms, the protozoan parasites also possess DNA topoisomerase enzymes, and these are essential to maintain the integrity of the genomic DNA since one of the most important activities carried out by these protozoa is nucleic acid metabolism. This activity necessitates the presence of the topoisomerases. These protozoan parasites cause various infections, which may lead to the death of individuals. Examples of protozoan parasites include Leishmania, Trypanosoma, Giardia, and Plasmodium. These

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parasites cause infections like leishmaniasis, trypanosomiasis, giardiasis, malaria, etc. To protect lives from such fatal diseases, the physiology of the infectious agents needs to be studied so that the pathways essential for the survival of these parasites can be targeted. Some subcategories of topoisomerases are absent in mammalian cells. Therefore, such enzymes can act as targets to kill the parasites**.** DNA topoisomerases were previously proven to be a potent anti-cancer and anti-parasitic drug target for drug development.

DNA Topoisomerases

James J. Champoux and Renato Delbecco discovered the first eukaryotic topoisomerase in the year 1972. This group also found that the nuclear extract of the mouse embryo had the enzymatic properties of resolving negative as well as positive supercoils from circular DNA.[6] Topoisomerases are the DNA binding enzymes that make strand scission, manipulation and re-joining activities to modulate DNA topology directly. These actions of the enzyme provide a powerful way to influence the topological changes of DNA by altering the supercoiling levels. DNA topoisomerases play an important role in vital cellular processes like replication, transcription, repair, integration, recombination, chromosomal segregation, etc.[7]

DNA Topoisomerase resolves the supercoiling of DNA into the relaxed form during DNA metabolisms like replication and transcription. The enzyme resolves positive supercoils generated during the unwinding by DNA helicases. The enzyme is required for strand breakage during recombination. Topoisomerases play an important role in DNA compaction along with Structural Maintenance of Chromosome (SMC) proteins. These help in stabilizing long-range contact between segments of chromosomes. During mitosis, these enzymes disentangle the intertwined DNA.[8] The presence or absence of topoisomerase has been linked to the activation or repression of certain transcription factors and nucleosome remodeling.[9,10] Eukaryotic topoisomerases (TopIB) have been involved in controlling the gene expression through kinase activity, phosphorylating splicing factor, and therefore regulating their localization and enhancement of their activity.[11-13] DNA topoisomerases are pivotal enzymes in embryonic development in mammals.[14] In humans, Top1 and Top2 both enzymes bind to the regions of the pre-replicative complex in cells during the various phases, including M, early G1, and G1/S, to control the initiation of replication from the origins.[15-17] DNA topoisomerase II plays an essential part in chromosomal decatenation.[8]

Types of DNA Topoisomerases

There are two main classes of topoisomerases. Type I topoisomerase makes transient single strand breaks of double-stranded DNA, and type II topoisomerase breaks transient double strands in the presence of ATP as a cofactor.7 Type-I topoisomerases have three general mechanistic steps: First is binding an enzyme to the substrate DNA, then cleavage by trans-esterification reaction accompanied by the formation of a transient phosphodiesterase bond and finally strand religation.[7,18]

Type-I Topoisomerases

Type-I topoisomerases are divided into two groups - type IA and IB. This division is based on the end of the DNA strand where the enzymes bind. Type IA enzymes form a 5′-phosphotyrosyl covalent bond, whereas type IB enzymes form a covalent bond with the 3′ ends of the DNA. These enzyme-linked transient intermediates are called cleavage complexes.[19] Type-IA topoisomerases resolve only negatively supercoiled DNA, whereas type-IB can resolve positively and negatively supercoiled DNA both with equal productivity.

Type-II Topoisomerases

These enzymes cleave both the strands of the duplex, changing the linking number by a factor of 2. Unlike the top1, top2 undergoes controlled strand rotation or strand passage. Topoisomerase II resolves the supercoils by binding to two segments of DNA: the G (gate) segment, both strands are cleaved; and a T (transport) segment, is captured by a clamp (ATP-operated) and passed through the double-stranded break in the G segment which is stabilized by the enzyme.[20] Topoisomerase II cleaves both the strands of the DNA duplex. TOP2 protein has two isoforms: TOP2A and TOP2B. The enzymatic reaction of these topoisomerases involves - DNA binding; in this step the enzyme binds at a preferential binding site on DNA, then DNA is cleaved by nucleophilic attack on phosphodiester bond of DNA duplex leading to the formation of transient 3' or 5' phosphotyrosyl bond(s). This enzyme requires both ATP and divalent metal (Mg^{2+}). Top2 during mitosis is present throughout the entire length of the chromosome as it is involved in maintaining the structure of the chromosome and segregation. From prophase to anaphase, Top 2α accumulates at the mitotic centromere.[21-27] This centromeric top 2 plays the role of removal of the residual catenation, along with the direction of the separating sister chromatids.[28] The TopII enzymes have been recognized in protozoan parasites including *Trypanosoma, Leishmania, Plasmodium, Giardia, Toxoplasma*, etc. Trypanosoma and leishmanial topoisomerase have been studied thoroughly.[28] These enzymes cannot introduce supercoiling in the DNA. The topoisomerase of these kinetoplastid parasites decatenates the kinetoplastid DNA. These enzymes cannot relax supercoiled DNA. The nuclear topoII and mitochondrial topoII are the two type II topoisomerases encoded by the kinetoplastid DNA.[29] These enzymes are essential in nucleic acid metabolism. If they fail to work, it will lead to disruption in the replication of the minicircles, loss of the kDNA network, and eventually, death of the parasite.[29]

Leishmanial DNA Topoisomerases (LdTOPILS)

Topoisomerases are the DNA-acting enzymes that make strand scission, manipulation, and re-joining activities to modulate DNA topology (7, 18) directly. These actions of the

enzyme provide a powerful way to influence the topological changes of DNA by altering the supercoiling levels. These ubiquitous enzymes play a pivotal role in modulating the dynamic nature of DNA topological isomers. LdTOPILS, the leishmanial topoisomerase-I, acts by breaking and re-sealing a single strand of double-stranded DNA, thereby modulating essential biological functions in Leishmania like replication, transcription, repair, integration, recombination, chromosomal segregation, etc. LdTOPILS is an unusual bi-subunit enzyme composed of hetero-dimer of a large subunit (LdTOPIL) and small subunit (LdTOPIS). LdTOPIL is made up of 635 amino acids having a molecular mass of 73 kDa, and LdTOPIS is made up of 262 amino acids having a molecular mass of 29 kDa.[18,30] LdTOPIL is encoded by the gene located on chromosome 34 and is closely homologous to the core domain of human topoisomerase I. LdTOPIS is obtained from the gene present on chromosome 4, which contains the phylogenetically conserved 'SKXXY' motif at the C-terminal domain. Inhibitors of enzyme topoisomerase LdTOPILS are important therapeutics against leishmaniasis.

Mode of Action: There are two main classes of topoisomerases. The type I topoisomerase makes transient single strand breaks of double stranded DNA and type II topoisomerase breaks transiently the two strands of the double-stranded DNA supercoils in presence of ATP as cofactor.7 Type-I topoisomerases have three general mechanistic steps: First is binding an enzyme to the substrate DNA, then cleavage by trans-esterification reaction accompanied by the formation of a transient phosphodiester bond, and finally strand relegation.[7,18]

Topoisomerase Inhibitors

Topoisomerase is an essential enzyme for the survival of organisms. All organisms have at least one of the enzymes Top I or Top II. Studies have shown the topoisomerase present in parasites is sufficiently different from human topoisomerase. Hence acts as an attractive drug target for treating various parasite-associated diseases. Generally, the enzyme performs two transesterification reactions. In the first transesterification reaction, the DNA backbone is cleaved by a nucleophilic attack.[31] The enzyme tyrosine residue is attached either to the 3' termini or the 5' termini. The nucleophilic attack leads to a covalent enzyme-DNA complex called Topoisomerase cleavage complex (Top cc). Top cc is a transient catalytic intermediate. After the first transesterification reaction, the DNA supercoiling is relaxed. In the second trans-esterification reaction, the religation of the cleaved DNA occurs (the hydroxyl sugar acts as a nucleophile attacking the phosphotyrosyl bond).[31] This mechanism of action of the enzyme is hindered by the inhibitors. The drugs against DNA topoisomerases are divided into two classes - Class I and class II inhibitors.

Class I Inhibitors

Topoisomerase class I inhibitors are also called topoisomerase poisons. These inhibitors hinder the second transesterification reaction and do not allow religation of the broken strands, stabilizing the Top cc.[31] These Topoisomerase cleavage complexes are stabilized; hence their half-life increases, which might lead to cell death. The cleavage complexes transform into lesions during replication or transcription. When the replication fork collides with these stabilized cleavage complexes, it generates DNA double-strand breaks.[32] In non-replicating cells, the collision of Top cc with transcription complexes leads to cell death.[33] This conversion of single-strand breaks to double-strand breaks is a primary determinant of cell cytotoxicity.

Camptothecin: This drug comes under the class of DNA topoisomerase I poison. It is an uncompetitive inhibitor that stabilizes DNA-enzyme complexes and slows the religation step. It improves cleavage-complex formation in the presence of low salt concentration.[34] The 1-39 residues of the large subunit of enzyme play a modulating role in the interaction of DNA and sensitivity to CPT. CPT inhibits the action of the enzyme in the nucleus as well as in the kinetoplast (in parasites *T. brucei, T. cruzi, and L. donovani*). Besides, it induces programmed cell death in both stages of *Leishmania* amastigotes and promastigotes. Mechanism: Inhibition of the enzyme by CPT induces oxidative stress in the cell.35 The oxidative stress leads to the depletion in the level of GSH and an increase in lipid peroxidation. Further, this leads to an escalation in Ca^{2+} levels from intracellular and extracellular sources.[35] The elevated Ca^{2+} levels were accountable for the loss of membrane potential in the mitochondria of the Leishmanial cells. Because of the inhibition of the topoisomerase, there is impairment in Na^{+}/K^{+} pumps resulting in the decline of intracellular K^{+} (an essential parameter for maintenance of ionic balance).[36] The decrease in K+ leads to apoptosis. After the loss of membrane potential, there is a release of cytochrome c and activation of caspase-like proteases heading to apoptosis-like death of the parasite. Metacaspases found in the *Trypanosoma cruzi* exhibit caspase-like activity.[37] Point mutations (Gly 185 Arg and Asp325 Glu) in the large subunit (LdTOP1L) of topoisomerase developed in *L.donovani* strain (LdRCPT.160) by stepwise exposure to CPT, which is highly resistant to CPT. Mutant enzymes show reduced activity and lesser sensitivity to CPT.[38]

Class II Inhibitors

Class II inhibitors are referred to as catalytic inhibitors. These are the drugs that interfere with the catalytic activity of the enzyme. They do not trap the enzyme-DNA complex. These inhibitors block the enzyme's active site, forbidding the interaction between DNA and enzyme. Since these drugs do not allow the binding of DNA and enzymes, it leads to defects in major activities like replication, transcription, etc. The class I inhibitors are directly proportional to the amount of enzyme present, whereas the class II inhibitors are inversely proportional to the concentration of the enzyme present.

Dihydrobetulinic Acid (DHBA)

It is a derivative of betulinic acid. DHBA is a dual inhibitor as it can inhibit both the topoisomerases of the parasite.[39] This drug is an example of a catalytic inhibitor and prevents

interaction between DNA and enzyme. The drug does not induce dyskinetoplastidy in *L. donovani* promastigotes.[39] Treating the leishmanial cells with this drug (5 µM for 8 hours) causes chromatin margination and compaction towards the nuclear periphery of the genome, forming cup-shaped masses. Changes in the cell's shape, disruption of the outer membrane, and cell membrane blebbing could be observed (features of apoptosis). This results in the loss of cellular morphology and the formation of compartments like apoptotic bodies.

Leishmania Topoisomerase I Inhibitors

Diamidines

Evidence suggests that the site of action of pentamidines is mitochondria in the case of Leishmania. The pentamidines and DB75 (a diamidine analog of pentamidines) bind to the minor groove of AT-rich DNA.[40,41] Especially pentamidines linearize the minicircles in catenated kDNA.[42] In the case of pentamidines, the EC50 value was 1.46 µM; for DB75 the EC50 value was 20.3 µM[43] (the EC50 in the case of DB75 ranged from 2.8 to 32 µM depending on the form or strain of parasite). Pentamidines, DB75 exhibited a dose-dependent inhibition of LdTOPI activity via DNA relaxation assays. DB75 presents a higher inhibition capacity with the complete blockade of relaxation at 30 µM. Compounds 1 showed complete inhibition at 1 µM. Compound 3 at concentrations 10 and 50µM complete inhibition was observed, and compound 5 inhibition at concentrations 10 and 50 µM but not as prominent as compound 1.44 Therefore, the diamidines localize in the mitochondria and inhibit the replication of kDNA. The property of this compound to bind DNA interferes with the action of the proteins involved in kDNA replication, like LdTOPI interference hampers the growth of the parasite.[44]

Bisbenzylisoquinoline alkaloid isolated from Thalictrum Foliolosum

Four compounds isolated from the stem of *Thalictrum foliolosum* include two new bisbenzylisoquinoline alkaloids 1, 2, thalifendine 3, and berberine 4. Out of all the compounds, compound 2 **(**6, 5', 6', 7', 12- pentamethoxy-2, 2'-dimethyloxycanthan) inhibited LdTOP1B activity in a concentration-dependent manner through plasmid relaxation assay.[45] Compound 2 did not stabilize the enzyme-DNA complex. It acted as a catalytic inhibitor of the enzyme. Compound 2 was cytotoxic against wildtype and SAG-resistant promastigotes. In 12 hours, 95 and 93% of the AG83 and SAG resistant promastigotes were killed by 20 µM concentrations. These compounds exhibit toxicity against the mammalian cells at a concentration of 100 µM and kill only 20 to 22%. Therefore compound 2 is a potential candidate for a drug against the parasite as it selectively acts against LdTOP1B at low concentrations compared to mammalian cells.[45]

Copper Salisylaldoxime (CuSAL)

It has been reported that CuSAL has anti-cancer activity and induces topo II-mediated nicks in DNA.[46] Many anti-cancer drugs have been used as anti-leishmanial drugs because of the similarity between the metabolism of cancer cells and the kinetoplastid parasite.[47] In-vitro cell viability studies showed that 30µM CuSAL inhibits 97 (8 hours treatment) and 100 percent (12 hours treatment) growth of the parasite.[48] Viability test results indicate that CuSAL is non-toxic to hosts. Treatment of *L. donovani* infected hepatocytes with CuSAL showed no inflammatory response, no morphological alterations, and other various parameters like bilirubin and alkaline phosphatase in a normal range. CuSAL inhibits the enzymatic activity of the enzyme LdTopILS. It interacts with the enzyme and hence does not allow enzyme-DNA interaction, inhibits the catalytic reaction, and induces apoptosis.[48]

Trans-2-cis-8-Matricaria-ester from Essential Oil of Erigeron Multiradiatus

The composition of essential oil from the aerial parts of *Erigeron multiradiatus* was analyzed. One of the major constituents was trans-2-cis-8-Matricaria-ester (77.79%); the Leishmanicidal properties of the essential oil were related to the presence of trans-2-cis-8-Matricaria-ester. The EO and purified trans-2-cis-8-Matricaria-ester significantly decreased the growth of *Leishmania donovani* with IC_{50} 18.29 ± 2.1 µg/mL and 55.09 ± 6.4µM respectively for promastigotes.[49] EO and purified trans-2-cis-8-Matricaria-ester were safe for the hamster peritoneal macrophages without any cytotoxicity and cell deformity. In-silico studies suggested that trans-2-cis-8-Matricaria-ester is the constituent of EO that binds the four essential enzymes of the parasite L-asparaginase-1-like protein, metacaspase-2, metacaspase-1, and DNA topoisomerase II. ADME profile suggested that the absorption parameters like solubility and permeability of the ligand are as per the control's value (miltefosine).[49]

Cyclic Imides

This group of compounds can cross the cell membrane easily as they are electrically neutral and hydrophobic. These compounds show various pharmaceutical activities, including antitumor, anti-inflammatory, and antimicrobial activities, based on the size of the group present in the imidic ring.[50-54] The other compounds used to prepare the derivatives to include essential oils. It has the methylenedioxy group that contributes to the crucial characteristics of the derived molecules. Combined ligand- and structure virtual chemical screening of 33 cyclic imides was performed to pick molecules with a higher probability of showing anti-leishmanial effects against some selected *Leishmania* targets (*L. amazonensis* and *L. donovani*). The inhibitory activity of these compounds was evaluated against four enzymes present in the parasite, including topoisomerase I, N-myristoyl transferase, cyclophilin, and O-acetylserine

sulfhydrylase.55 The efficiency of the virtual studies was validated by performing in-vitro studies using promastigote forms of *L. amazonensis*. The pIC_{50} values for in-vitro studies were found to be less than 4.7, implying that these virtual studies were efficient in predicting true negative molecules. [55]

Isobenzofuranone derivative JVPH3

An isobenzofuranone derivative, namely 3,5-bis(4-chlorophenyl)-7-hydroxyisobenzofuran1(3H)-one (JVPH3),[56] is a synthetic catalytic inhibitor of LdTopII and can reduce the *L. donovani* parasite burden in an experimental model of visceral leishmaniasis (VL). Treatment with 15 µM of JVPH3 on promastigotes of *L. donovani* displays shrank morphology, loss in cell volume, and mitochondrial swelling accompanied by a loss of matrix content. Cells also show signs of disorganized kinetoplast.[57] With 20 µM of JVPH3, an atypical phenotype of *Leishmania* was observed, and signs of autophagy emerged. JVPH3 gave different results on cell surfaces of two species of *Leishmania*. But, the cellular organelles responded comparably. JVPH3 caused mitochondrial dysfunction. Further analysis in *T. cruzi* by microscopy and flow cytometry showed structural disintegrity in mitochondria, but no significant loss of kDNA topology, mitochondrial membrane depolarization.[57] JVPH3 is a catalytic inhibitor of LdTopII of *L. donovani*, perturbs the decatenation reaction of minicircles and maxicircles, which causes structural alteration in the mitochondrial structure of *Leishmania*.[57]

Arylidenefuropyridinediones

A molecular library of arylidene furo pyridinediones was designed in search of inhibitors against LdTop1. The library was designed by intuitive scaffold hopping and bioisosteric modification of the established topoisomerase one inhibitor like camptothecin, edotecarin, diflomotecan, and rosettacin. Scaffold hopping is remodeling the central core of already known active molecules to create architecturally new compounds. Bioisosteric modification is a strategy to design new drugs by replacing the chemical functionalities with other moieties that evoke better responses. The design rationalization was done by molecular docking studies of the representative molecule 13 with human and leishmanial topoisomerase. Among the other compounds, the 3,4-dihydroxy phenyl derivative 4 had the best potency with an EC_{50} of 4 µM. This compound did not show cytotoxicity against the mammalian cell line (COS7 cell line).

Further screening with extracellular promastigote gave IC50 of 4.21 ± 0.21 µM.[58] A 4 µM of compound 4 under the preincubation condition gave 100% inhibition and 92% inhibition under the simultaneous condition. The EC_{50} values in simultaneous and preincubation assays were 3.77 ± 0.27 and 1.72 ± 0.23 µM, respectively.[58] Therefore, it can be concluded that preincubation gave better results.

Voacamine

Voacamine has been reported that this indole alkaloid compound is effective against *Plasmodium falciparum*.[59-60] It is an antiprotozoal agent effective against many trypanosomatid parasites like *Leishmania donovani*, *Leishmania amazonensis*, and *Trypanosoma cruzi*. The relaxation assay found that it inhibits the relaxation activity of the LdTop1B, and the IC_{50} value was 14.702 ± 0.101 µM. Microscopic analysis suggested that the voacamine also caused cell body reduction and cell membrane alterations in *Leishmania*. Voacamine stabilizes the cleavage complex between the DNA template and LdTop1B within the parasitic cells and in vitro. The formation of such adducts leads to double-strand breaks. The compound does not react with the free enzyme and follows uncompetitive inhibition. It does not show any effect up to a concentration of 200µM on human topoisomerases.

N-benzyl-2,2′α-3,3′,5′,6′,7′,7α,α′octahydro-2methoxycarbonyl-spiro[indole-3,3′ pyrrolizidine]-2-one (compound 4c) - Spirooxindole Derivative

This drug compound 4c is a catalytic inhibitor. Catalytic inhibitors act in two ways: they prevent the enzyme from binding the DNA or inhibit the formation of covalent cleavage complex.[61,62] Compound 4c binds the free enzyme and does not allow the interaction of the DNA with the topoisomerase enzyme.63 This compound inhibits the growth of *Leishmania donovani*. In silico docking studies state that the drug binds at two sites on LdTopIB-1) small subunit and 2) the hinge region of the large subunit. This compound 4c has a benzyl moiety. The benzyl group in the drug penetrates the hydrophobic groove. This groove is present between large and small subunits of the enzyme.63 The benzyl group forms pi-stacking interaction with the Phe243 of the small subunit and strong H-bonding with the Asn444 in the large subunit. This compound leads to change in the mitochondrial membrane depolarization, formation of ROS, and eventually cell death.[63]

Partial Amino Acid Sequences of the Small Subunit of *Leishmania donovani* (Ld) TopIB and LRL-TP Compounds

LdTopIB has four conserved residues in the large subunit (R314, K352, R410, and H453) and a catalytic tyrosine (Y222) residue in the small subunit. Protein-protein interactions are necessary for the enzyme to relax the superhelix in DNA because the conserved catalytic residues are present in the small and large subunits of LdTopIB.[64] These interactions are absent in the human topoisomerase and hence act as a target to develop inhibitors against *Leishmania*.[64] Out of 151 compounds, LRL-TP-85 and LRL-TP-94, which share a common scaffold of 4- (3-piperidine-1-ylmethyl) phenyl) pyridine displayed potent activity in the LdTopIB assay. These compounds did not inhibit *Homo sapiens* topoisomerase I (HsTopIB) and were not toxic to BMDM cells. The compounds only inhibit promastigotes but not the amastigote form. Hence further investigations are needed.64 Peptides were synthesized that were composed of partial amino acid sequences of the small subunit of LdTopIB to decrease the

catalytic activity by interfering with the interaction of the two subunits.[64]

Amprenavir (APV)

APV is an HIV-1 protease inhibitor. The therapeutic effect of the HIV-1 protease inhibitors was analyzed against visceral leishmaniasis and HIV-VL co-infection. The effect of APV on *L. donovani* was observed. In-vitro cell viability analysis revealed the following IC_{50} and IC_{90} values of APV 8.0 ± 1.2 µM and 18.0 ± 1.4 µM at 12 h; and 4.5 ± 1.6 µM and 16.0 ± 2.2 µM at 24 h, respectively.[65] Treatment of BALB/C mice with 20mg/kg APV decreased the splenic and hepatic parasites burden, 97 and 98%, respectively, in the infected controls.[65] APV inhibits the catalytic activity of LdTOP1LS, similar to TopI inhibitor CPT. APV acts by stabilizing the LdTOP1LS-DNA cleavable complex. Further, APV induces the production of reactive oxygen species (ROS), which increases lipid peroxidation. Consequently, there is depolarization of mitochondrial membrane potential (ΔΨm). Then release of cytochrome c and activation of the CED3/CPP32 proteases lead to the formation of DNA lesions followed by DNA fragmentation.[65]

Conclusion

Visceral leishmaniasis caused by the protozoan parasite *L. donovani* is a major health issue due to lack of strategies for treatment. Topoisomerase enzymes are ubiquitously present in all organisms and therefore play an important role in maintaining the topology of the DNA. This enzyme also shows a unique architecture in the leishmanial parasite, which is different from that of humans; hence these enzymes act as a promising target to deal with the disease caused by the leishmanial parasite. The available therapeutic drugs in the market have many limitations regarding the development of resistance or side effects. The evolution of the protozoan parasites and the formation of resistant strains make developing effective inhibitors difficult. Therefore, new inhibitors are needed for the treatment. The future perspective is to explore different inhibitors so that alternatives are available in case of resistance. Many compounds have been reported specifically targeting the topoisomerase enzyme of the protozoan parasites without side effects but could not reach clinical trials.

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