DNA Topoisomerases of Kinetoplastid Parasites: Brief Overview and Recent Perspectives

Sourav Saha†, Somenath Roy Chowdhury† and Hemanta K. Majumder*

Infectious Diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India.

*Correspondence: hkmajumder@iicb.res.in, hemantamajumder@yahoo.co.in

†Both authors contributed equally.

DOI: https://dx.doi.org/10.21775/cimb.031.045

Abstract

Topoisomerases are a group of enzymes that resolve DNA topological problems and aid in different DNA transaction processes viz. replication, transcription, recombination, etc. inside cells. These proteins accomplish their feats by steps of DNA strand(s) scission, strand passage or rotation and subsequent rejoining activities. Topoisomerases of kinetoplastid parasites have been extensively studied because of their unusual features. The unique presence of heterodimeric Type IB topoisomerase and prokaryotic 'TopA homologue' Type IA topoisomerase in kinetoplastids still generates immense interest among scientists. Moreover, because of their structural dissimilarity with the host enzymes, topoisomerases of kinetoplastid parasites are attractive targets for chemotherapeutic interventions to kill these deadly parasites. In this review, we summarize historical perspectives and recent advances in kinetoplastid topoisomerase research and how these proteins are exploited for drug targeting.

Introduction

DNA topoisomerases are ubiquitous enzymes that help to maintain proper topological states of the double helical DNA molecules and prevent genomic instability inside cell. During replication, transcription, recombination, and other vital nucleic acid metabolic processes, permanent or temporary separations of DNA strands cause different topological constraints. These topological constraints, if not removed, become disastrous and greatly affect cellular health (Chen *et al.*, 2013). DNA topoisomerases are the molecular machines inside all kinds of living cells that resolve these kinds of topological tensions. Not only that, DNA topoisomerases sometimes introduce measured topological tensions in the double helical DNA molecules to aid DNA transaction processes (Champoux, 2001).

The mechanism of action of DNA topoisomerases involves following major steps: (1) binding of topoisomerase to the substrate DNA, (2) cleavage by trans-esterification reaction accompanied by the formation of a transient phosphodiester bond between a tyrosine residue in the protein and one of the ends of the broken strand, (3) strand passage/rotation through/around the break leading to change in the linking number and (4) strand religation and release of the enzyme as the DNA is religated (Stewart *et al.*, 1998; Koster *et al.*, 2005; Champoux, 2001).

Based on structure and mechanism of action, topoisomerases are classified as type I and type II. Those enzymes that cleave a single strand of DNA and pass or rotate another strand through or about the resultant gap are defined as type I

topoisomerases and these enzymes change the linking number of the substrate DNA in discrete steps of one or more than one (Champoux, 2001). They are further classified either as type IA or as type IB enzyme. These two subfamilies have no sequence similarities between them and exhibit dissimilar structures and distinct region characteristics. Type IA topoisomerase makes a transient covalent complex with the 5′-end of the broken DNA strand. On the other hand, type IB topoisomerase forms a covalent intermediate with the 3′-end of the DNA (Champoux, 2001). The type II topoisomerases act in a manner fundamentally different from either of the type I enzymes. They require divalent metal ions for their activity and use ATP to actively transport DNA duplex through a transient enzyme-mediated break in the second duplex of the same DNA strand thus changing linking numbers in steps of two. Prokaryotes and lower eukaryotes encode only a single type II topoisomerase whereas vertebrates generally express two discrete forms of the enzyme, topoisomerase IIA and IIB (Champoux, 2001). Both Type IIA and IIB enzymes break the DNA by attacking and bonding to the 5′-phosphate, and have sequence similarities. They are encoded by separate genes, have different molecular masses, show distinct patterns of expression and perform different cellular functions. Topoisomerase IIA helps mainly in the DNA replication and chromosome segregation in proliferating cells and topoisomerase IIβ is mainly required for proper neural development (Deweese and Osheroff, 2009; Yang *et al.*, 2000; Wang, 2002; Champoux, 2001; Velez-Cruz *et al.*, 2004; McClendona and Osheroff, 2007).

Different topoisomerases of kinetoplastid parasites

Topoisomerases of kinetoplastid parasites are of following kinds:

- type IB topoisomerase;
- type IA topoisomerase;
- type II topoisomerase.

Type IB topoisomerase of kinetoplastid parasites

Type IB topoisomerases are generally monomeric enzymes. But in kinetoplastid parasites, type IB topoisomerase is heterodimeric in nature (Das *et al.*, 2004a). The discovery of bi-subunit type IB topoisomerase in kinetoplastid parasites opened up a new avenue in topoisomerase research related to evolution of type IB topoisomerase family and its relevance as a drug target for trypanosomiasis therapeutic interventions (Das *et al.*, 2006).

Type IB DNA topoisomerase activities were first purified from *Leishmania donovani* (Chakraborty and Majumder, 1988; Chakraborty *et al.*, 1993), *Trypanosoma cruzi* (Riou *et al.*, 1983) and *Crithidia fasciculata* (Melendy and Ray, 1987). The purified active enzymes (65–79 kDa) were ATP independent and found to be sensitive to the topoisomerase I-specific inhibitor camptothecin (Melendy and Ray, 1987).

Topoisomerase IB-like gene from the kinetoplastid *L. donovani* was first cloned and reported by Broccoli *et al.* (1999). The amino acid sequence of this gene showed homology with the N-terminal and central core of other eukaryotic type IB topoisomerases, but had a variable C-terminus. Interestingly, this ORF was lacking the SKINYL motif that supplies the catalytic tyrosine (Broccoli *et al.*, 1999). Villa and co-workers first reported the presence of a second small subunit harbouring the SKINYL motif in *Leishmania* parasites (Villa *et al.*, 2003). Similar results were obtained in case of African trypanosomes in the same year (Bodley *et al.*, 2003).

In general, type IB DNA topoisomerases of kinetoplastid parasites are expressed from two different genes and form a heterodimeric active enzyme (Table 10.1). In *L. donovani*, gene for large subunit (LdTOP1L) is present on chromosome 34 encoding a 636 amino acid polypeptide with an estimated molecular weight of 73 kDa and the gene for the small subunit (LdTOP1S) is situated on chromosome 4 encoding a 262 amino acid polypeptide with a molecular weight of 29 kDa (Das *et al.*, 2004a). Bodley *et al.*, 2003 have also identified a heterodimer topoisomerase I in *Trypanosoma brucei* which is comprised of the 90 kDa large subunit and 36 kDa small subunit (Bodley *et al.*, 2003). Large subunit (LdTOP1L) is closely homologous to the core domain of human topoisomerase I and small

Table 10.1 Different topoisomerase genes of kinetoplastid parasites as annotated in GeneDB database (www.genedb.org/Homepage) (Logan-Klumpler *et al.*, 2012)

Protein coding gene	Gene DB systematic IDs	Length
DNA topoisomerase IB large subunit (L)	LmjF.34.3440 (*L. major* Friedlin)	1905 base pairs
	LdBPK_343220.1 (*L. donovani* BPK282A1)	1908 base pairs
	Tb927.4.133 (*T. brucei* 927)	2091 base pairs
	TcCLB.508693.20 (*T. cruzi*)	1995 base pairs
DNA topoisomerase IB small subunit (S)	LmjF.04.0060 (*L. major* Friedlin)	789 base pairs
	LdBPK_040070.1 (*L. donovani* BPK282A1)	789 base pairs
	Tb927.9.6940 (*T. brucei* 927)	822 base pairs
	TcCLB.506625.110 (*T. cruzi*)	819 base pairs
DNA topoisomerase IA	LmjF.21.0125 (*L. major* Friedlin)	2439 base pairs
	LdBPK_210180.1 (*L. donovani* BPK282A1)	2430 base pairs
	Tb927.10.1900 (*T. brucei* 927)	2421 base pairs
	TcCLB.510121.160 (*T. cruzi*)	2493 base pairs
	TcCLB.506493.80 (*T. cruzi*)	2490 base pairs
DNA topoisomerase III	LmjF.28.1780 (*L. major* Friedlin)	2601 base pairs
	LmjF.36.3200 (*L. major* Friedlin)	2844 base pairs
	LdBPK_281900.1 (*L. donovani* BPK282A1)	2604 base pairs
	LdBPK_363350.1 (*L. donovani* BPK282A1)	2844 base pairs
	Tb927.11.9520 (*T. brucei* 927)	2757 base pairs
	Tb927.11.9170 (*T. brucei* 927)	2559 base pairs
	TcCLB.510901.100 (*T. cruzi*)	2766 base pairs
	TcCLB.508851.170 (*T. cruzi*)	2526 base pairs
	TcCLB.511589.120 (*T. cruzi*)	2766 base pairs
DNA topoisomerase II	LmjF.28.2280 (*L. major* Friedlin)	4494 base pairs
	LdBPK_282450.1 (*L. donovani* BPK282A1)	4497 base pairs
	BSAL_28010 (*B. saltans*)	3741 base pairs
	Tb927.11.11550 (*T. brucei* 927)	4368 base pairs
	Tb927.11.11560 (*T. brucei* 927)	4275 base pairs
	TcCLB.508699.10 (*T. cruzi*)	4431 base pairs
	TcCLB.509203.70 (*T. cruzi*)	4455 base pairs
Mitochondrial DNA topoisomerase II	LmjF.15.1290 (*L. major* Friedlin)	3711 base pairs
	LdBPK_151310.1 (*L. donovani* BPK282A1)	3711 base pairs
	Tb927.9.5590 (*T. brucei* 927)	3666 base pairs
	TcCLB.508277.370 (*T. cruzi*)	3696 base pairs
	TcCLB.506445.60 (*T. cruzi*)	3693 base pairs

subunit LdTOP1S contains the phylogenetically conserved 'SKXXY' motif placed at the C-terminal domain of all topoisomerases IB (TOPIB) which harbours a conserved tyrosine residue playing a crucial role in DNA cleavage. Overall, the structure and catalytic machinery of the two enzymes (LdTOPIB and human TOPIB) are highly conserved, despite the fact that LdTOPIB is a heterodimer whereas the human TOPIB is a monomer.

The *in vitro* reconstitution of the two recombinant proteins LdTOP1L and LdTOP1S corresponding to the large and small subunit forms active bi-subunit LdTOP1LS/LdTOPIB. LdTOP1L and LdTOP1S form a direct 1 : 1 heterodimer complex

through protein–protein interaction (Das *et al.*, 2004a). Interaction between the two subunits leading to the formation of an active complex could be exploited for development of new therapeutic agents with specific selectivity (Das *et al.*, 2004a). This observation leads to the concept that non–covalent interaction of both subunits is necessary for the activity. Interestingly, deletion of 99 amino acids from the N-terminus of LdTOP1L results in a protein which failed to interact with the smaller subunit. This could be attributed to the presence of many polar residues in this region. Polar interactions are common between the subunits of dimeric proteins. (Das *et al.*, 2005). It was also found that the deletion of the first 39 amino acids from N-terminus of LdTOP1L results in a protein with decreased cleavage activity and sensitivity to CPT (Das *et al.*, 2005). These data argue in favour of the interpretation that N-terminal amino acids of the large subunit regulates DNA dynamics during relaxation by controlling noncovalent DNA-binding or by coordinating DNA contacts by the other parts of the enzyme. Further, it was shown that the amino acids 39–456 of LdTOP1L and 210–262 of LdTOP1S constitute the minimal functionally interacting fragments of LdTOP1LS (Bosedasgupta *et al.*, 2008a) and the LdTOP1L navigates the heterodimeric LdTOP1LS to its cellular DNA targets (Bose Dasgupta *et al.*, 2008b). A recent report also identified the regions of both subunits that interact to each other to form a functional linker in *L. donovani* topoisomerase IB. Amino acids 175 and 180 (RPPVV) of the small subunit interact with a region (amino acids 525–581) of the large subunit (LdTopIL) and have role in sensitivity to camptothecin in *Leishmania* (Prada *et al.*, 2012). Recently it was also shown that LdTOP1LS activity is stimulated by ATP (generally Type IB topoisomerases are ATP independent) in the absence of Mg^{2+} and this stimulation does not need ATP hydrolysis. The ATP binds to a Arg190 residue on LdTOP1L and stimulates the rate of strand rotation by the enzyme (Sengupta *et al.*, 2011).

LdTOP1B localizes in both nucleus and kinetoplast of *L. donovani*. Multiple nuclear localization signals (NLSs) have been mapped in the large subunit of *Leishmania* TOP1B but no NLS has been found in smaller subunits of the enzyme. So it is likely that the subunits interact in the cytosol before nuclear and kinetoplast importation (Das *et al.*, 2004a; Das *et al.*, 2008) and LdTOP1L navigates LdTOP1S by acting as a 'molecular steer' (Bose Dasgupta *et al.*, 2008b). Another recent study reported three nuclear localization signals (NLSs) in LdTOP1B, one in LdTOP1L and two in LdTOP1S and both subunits are transported to the nucleus separately. They also found that LdTOPIB–GFP constructs do not localize to mitochondria (Prada *et al.*, 2013a).

From bioinformatic analysis (multiple sequence alignment) and crystal structure of LdTOP1LS (*L. donovani* topoisomerase IB) captured as a vanadate complex linking the enzyme to a nicked DNA, The crystal structure of the reconstituted *L. donovani* topoisomerase IB (PDB ID 2B9S) reveals that the three-dimensional configuration of the active site is almost similar to that of human topoisomerase I. Five amino acids have been identified as active site residues (Davies *et al.*, 2006). These are Arg314, Lys352, Arg410, His453 and Tyr222. Functions of these amino acids in the *L. donovani* topoisomerase IB have been confirmed by single site directed mutation studies (Díaz González *et al.*, 2007b; Ganguly *et al.*, 2009). Among these, Tyr222 resides in the SKXXY motif in the small subunit and is responsible for the nucleophilic attack on the scissile phosphate group in the DNA backbone and subsequent formation of phosphotyrosine linkage. Arg410 activates Tyr222 for this nucleophilic attack with water acting as a specific base. Mutation of the histidine at position 453 has been shown to alter DNA binding of the enzyme. Of the remaining three, Arg314 and Lys352 stand as potential candidates for acting as the general acid and thereby play a vital role in transesterification reaction (Davies *et al.*, 2006; Ganguly *et al.*, 2009).

Type IB topoisomerases including human topoisomerase I and vaccinia virus topoisomerase I share a common catalytic domain with the tyrosine recombinases that includes bacteriophage HP1 and certain phage integrases and the XerC/D, Cre and Flp recombinases (Corbett and Berger, 2004). In prokaryotic tyrosine recombinases XerD, Cre, HP1 integrase and eukaryotic topoisomerases the active site residues RKHR (H/W)Y and RKRHY resides on a single monomer. In eukaryotic Flp recombinase and *Leishmania* heterodimeric topoisomerase IB, the active site tyrosine is provided by one subunit and the other residues implicated in catalysis comes from another subunit (Chen and Rice, 2003). It may

be possible that the active site in case of kinetoplastid topoisomerase I is created by the association of subunits similar to Flp recombinase. The structural diversity of the amino-terminal domain and the linker domain in various eukaryotic topoisomerase IB argues that they have probably evolved by independent gene fusion. Krogh and Shuman described that nuclear topoisomerase I enzymes evolved from the bacterial/poxvirus precursor (Krogh and Shuman, 2002). However, in contrast to vaccinia topoisomerase I, the catalytic tyrosine of human topoisomerase I resides on a small 6 kDa domain and is connected to the central core domain by a 7 kDa coil–coil linker element (Champoux, 2001). It now seems simpler to posit that during evolution of eukaryotic IB topoisomerase, the kinetoplastid parasites (*Leishmania*, *Trypanosoma*) which arose earlier than multicellular eukaryotes gained some regulatory sequences (nuclear localization signals and sequences for CPT sensitivity) as split domain architecture. Marcotte *et al.* (1999) demonstrated that the fusion of the subunits and insertion of additional domains allow for the rapid evolution of new signalling pathways by the incorporation of a novel interaction domain into a pre-existing peptide. Horizontal gene transfer has been shown to have an important role in the evolution of composite proteins (Simonson *et al.*, 2005). It is possible that horizontal gene transfer did play a key role in propelling the topoisomerase IB protein among the eukaryotes (Krogh and Shuman, 2002). Thus we can suggest that eukaryotic monomeric type IB topoisomerase evolved from the common ancestral bi-subunit enzyme by fusion of the two subunits at genetic level.

Type IA topoisomerases of kinetoplastid parasites

Type IA topoisomerases differ from type IB counterpart in their structures, requirement of Mg^{2+} for activity, substrate specificity (type IA topoisomerases only relax negatively supercoiled DNA) and unlike type IB topoisomerases, type IA topoisomerases form 5′-phosphotyrosine catalytic intermediate (Stewart *et al.*, 1997; Champoux, 2001; Wang, 2002). Apart from type IB topoisomerase, three type IA topoisomerases are there in the parasite genome (Table 10.1), termed as topoisomerase IA, and two topoisomerase III (topoisomerase IIIα and IIIβ). Topoisomerase IA mainly prefers catalysing relaxation reaction whereas topoisomerase III favours catenating/decatenating DNA molecules (Li *et al.*, 2000).

Topoisomerase IA

The presence of topoisomerase IA homologue in the kinetoplastid parasites is very intriguing (Table 10.1). Topoisomerase IA is of prokaryotic origin and is not present in eukaryotes. However, kinetoplastid parasites are exceptions in the eukaryotic world in this issue since they contain topoisomerase IA. This makes topoisomerase IA of these parasites a fantastic drug target due to its absence in the human host and warrants a thorough functional and mechanistic investigation of the type IA topoisomerases of kinetoplastid parasites. Our laboratory is currently involved in characterizing topoisomerase IA of *L. donovani*.

Another group reported topoisomerase IA from *T. brucei*. *T. brucei* topoisomerase IA gene is present on chromosome 10 (NCBI Reference Sequence: XP_822446/Systematic Name: Tb927.10.1900), 2418 bp in size and encodes an 88.9 kDa predicted protein. This enzyme is mitochondrial, essential for late theta structure resolution during kinetoplast DNA (kDNA) replication process and is important for parasite survival (Scocca and Shapiro, 2008).

Topoisomerase IIIα and topoisomerase IIIβ

Like other higher eukaryotes, kinetoplastid parasites also have two putative DNA topoisomerase III (paralogous isoforms of type IA topoisomerase) genes. Very recently, topoisomerase IIIα from *T. brucei* has been shown to play a critical role in antigenic variation by monitoring expression-site-associated VSG switching (Kim and Cross, 2010).

DNA topoisomerase IIIβ from kinetoplastid parasite *L. donovani* (LdTopIIIβ) has been well studied (Banerjee *et al.*, 2011). LdTopIIIβ has high sequence homology with human and drosophila topoisomerase IIIβ. It has features typical for topoisomerase IIIβ proteins including the CXXC type of motifs and a long stretch of G and R residues at its C-terminus. The recombinant protein was purified from *in vitro* transcription-translation reaction and relaxation activity of the enzyme was characterized. GFP-fused LdTopIIIβ localizes both in nucleus and kinetoplast of *L. donovani* parasites indicating the involvement of LdTopIIIβ in DNA processing inside both the parasite organelle.

Wild-type LdTopIIIβ could complement the topoisomerase III mutant yeast with slow-growth phenotype whereas the C-terminal deletion construct of LdTopIIIβ lacking its Zn-binding domain was unable to rescue the mutant yeast revealing that the C-terminal 258 amino acids were indispensable for functional complementation of LdTopIIIβ *in vivo*. Further investigations are required to determine the role of C-terminal end of the LdTopIIIβ protein in DNA binding (Banerjee *et al.*, 2011).

The presence of functionally active topoisomerase III proteins in parasites indicates towards its role in DNA metabolism in the parasites, which requires further studies and might emerge as a new therapeutic target that can be exploited against the deadly parasites (Kim and Cross, 2010; Banerjee *et al.*, 2011).

Type II topoisomerases of kinetoplastid parasites

Eukaryotic type II topoisomerases are mainly involved in chromosome segregation and condensation via their decatenation activity. Eukaryotic topoisomerase II is available in two forms, topo IIα and topo IIβ throughout the phylogeny which exhibits strikingly different distributions among the three domains of life (Gadelle *et al.*, 2003). The distribution of the enzyme among the kinetoplastid parasites is rather trivial. The kinetoplastid parasites harbours two type II topoisomerases viz. topoisomerase II and mitochondrial DNA topoisomerase II (Kulikowicz and Shapiro, 2006) (Table 10.1).

Genes of topoisomerase II have been isolated, cloned and sequenced from *C. fasciculata*, *T. brucei*, *T. cruzi*, *L. donovani*, *L. infantum*, *L. chagasi*, and *B. saltans* and also its activity has been purified from many kinetoplastid parasites (Das *et al.*, 2001, 2004b; Pasion *et al.*, 1992; Strauss and Wang, 1990; Fragoso and Goldenberg, 1992; Gaziová and Lukes, 2003). Compared to other higher eukaryotes topoisomerase II proteins of the parasites were found to be smaller, still they share the same functional domains and are more homologous to eukaryotic than prokaryotic type II enzymes. Unlike *E. coli* DNA gyrase (Wang *et al.*, 1996) none of the parasitic type II enzymes are endowed with supercoiling activity and the enzymatic activities are nearly same as other eukaryotic counterparts (Das *et al.*, 2004b). Though all type IIA topoisomerases are identical in one way that they change the linking number of DNA in an ATP-dependent manner, the eukaryotic type II enzymes are homodimers, while their bacterial counterparts such as gyrase and topo IV are A_2B_2 tetramers; the B and A subunits being the N and C-terminal halves of their eukaryotic counterparts (Lynn *et al.*, 1996).

Topoisomerase II proteins of *L. donovani* and *B. saltans* were found to be localized both in the nucleus and kinetoplast whereas topoisomerase II activity isolated from *C. fasciculata* was shown to be immunolocalized in kinetoplast (Das *et al.*, 2001; Gaziová and Lukes, 2003). Two nuclear topoisomerase II genes from *T. brucei brucei* namely TbTOP2α and TbTOP2β which are separated by 1.7 kb intergenic regions have been reported. TbTOP2α is ATP-dependent and is localized in the nucleus. Silencing of this gene by RNAi leads to growth arrest and causes severe defects in nuclear DNA although mitochondrial DNA remains unaltered. However, silencing of TbTOP2β has no effect on the apparent phenotype of the cell and precise role of TbTOP2β remains unclear (Kulikowicz and Shapiro, 2006). TbTop2α is also essential in bloodstream form *T. brucei* as it is responsible for centromere-specific topoisomerase cleavage activity and has roles in chromosome segregation. TbTop2β gene does not have an obvious role in chromosome segregation (Obado *et al.*, 2010). Mitochondrial topoisomerase II of *Trypanosoma* is involved in replication of the massive kinetoplast DNA network. RNAi of mitochondrial topoisomerase II leads to the progressive degradation of mitochondria (kinetoplast) in *Trypanosoma* (Wang and Englund, 2001). During kDNA replication mini-circle is individually released from the network and this phenomenon creates holes in the kDNA network. After depletion of TbTOP2mt by RNAi these holes continue to exist, indicating that this enzyme is responsible for remodelling the kDNA network during replication to maintain a proper mini-circle density and network structure (Lindsay *et al.*, 2008).

Kinetoplastid parasites diverged early in the eukaryotic evolution at the base of the evolutionary tree well before the emergence of the metazoan kingdom. In spite of having a similarity and identity of 31% and 23% with yeast topoisomerase II, *L. donovani* topoisomerase II (LdTOP2) was found to complement a temperature-sensitive mutant yeast strain (Sengupta *et al.*, 2003).

Just like other eukaryotic topoisomerase II, the *L. donovani* enzyme can also be divided into an N-terminal ATPase, a central DNA-binding domain and an unconserved C-terminal domain (Sengupta *et al.*, 2003, 2005a,b). Though unconserved, the nuclear localization signal and the dimerization domain of this homodimeric enzyme have been mapped in the C-terminus. The C-terminus also contains a stretch of 60 amino acids not present in the human host. Therefore this region can be exploited to develop antileishmanial targets (Sengupta *et al.*, 2003). The parasite enzyme has a greater affinity for DNA and was also stable at a very high salt concentration compared with its human host. These findings were quite consistent with the greater susceptibility of the parasite protein to the anti-topoisomerase II agents. This is because of the fact that an enzyme with more affinity towards DNA would perform more DNA cleavage and thus a greater chance of being trapped in that state by an anti-topoisomerase II drug (Sengupta *et al.*, 2005b). The N-terminal 385 amino acid residues of LdTOP2 were found to possess the ATPase activity. Although the ATPase activity resides in the first 385 amino acid residues, only a larger protein was found to mimic the full-length enzyme kinetics in *in vitro* assay (Sengupta *et al.*, 2005a). This characteristic of the parasite protein was found to be in contrast to the human enzyme where a smaller protein also has ATPase kinetics similar to the full length enzyme (Campbell and Maxwell, 2002) except for the fact that a smaller fragment (1–420 amino acids) fails to be hyper stimulated by DNA. Most interestingly, the N-terminal 385 amino acids of the parasite protein were also found to be inhibited by etoposide which showed that etoposide in addition to being a poison for the parasite enzyme was also a catalytic inhibitor. The study identifies specific amino acids such as Asn^{65}, Asn^{69}, Asn^{96} and Asp^{130} of the parasite protein that are involved in the interaction with ATP and etoposide (Sengupta *et al.*, 2005a). The active site tyrosine implicated in DNA breakage and rejoining for *L. donovani* topoisomerase II has been mapped to be Tyr^{775} (Sengupta *et al.*, 2005b).This tyrosine is the only residue in the parasite protein, which is involved in the trans-esterification reaction and is also homologous to the Tyr^{804} of human (Tsai-Pflugfelder *et al.*, 1988). Surprisingly, the C-terminal truncation mutants of the parasite protein failed to be inhibited by etoposide (Sengupta *et al.*, 2005b) while the full-length protein generates more cleavable complex in the presence of etoposide. Like the human enzyme, the core domain of LdTOP2 contains all the elements essential for sequence preference in protein–DNA interaction but unlike the human enzyme, the C-terminus of the parasite enzyme plays an important role in the protein–drug interaction for the *in vitro* topoisomerase II cleavage reaction.

Exploiting the topoisomerases of kinetoplastid parasites for therapeutic interventions

During its catalytic cycle, topoisomerase generates breaks in the DNA and attaches itself to the newly generated DNA 3′ (type IB topoisomerases) or 5′ termini (all other topoisomerases) via phosphotyrosyl bonds. Under normal physiological conditions, these covalent enzyme–DNA cleavage complexes are short-lived catalytic intermediates and are tolerated by the cell. However, conditions that significantly increase the physiological concentrations or lifetime of these breaks unleash a multitude of deleterious side-effects. Thus, all topoisomerases are fundamentally dualistic in nature. Although they catalyse essential reactions in the cell, they possess an inherent dark side capable of inflicting great harm to the genome of an organism. Topoisomerase-targeting therapeutics currently in use act by stabilizing these covalent topoisomerase–DNA complexes.

The known topoisomerase targeting compounds can be divided into two classes, class I and class II (Liu, 1989; Wang, 1994). The class I inhibitors have been referred to as 'topoisomerase poisons' where as the class II compounds are referred to as 'catalytic inhibitors'. The class I drugs act by stabilizing the covalent topoisomerase-DNA covalent complexes. The class II drugs interfere with catalytic function of DNA topoisomerase without trapping the covalent complexes. A major determinant of cytotoxicity for the class I drug is the conversion of a latent single- or double-stranded break in a drug–topoisomerase-DNA complex into an irreversible double-stranded break. Replication and transcription are the key cellular processes that drive this conversion. However, for class II topoisomerase II drugs, processes other than replication might also be involved. Cell killing by class II topoisomerase II

drugs may involve arresting cell cycle progression. Traversing of eukaryotic cells through cell division in the absence of functional DNA topoisomerase II can lead to aneuploidy and chromosomal breakage. For class I drug, cytotoxicity increases with increasing cellular level of target enzyme where as for class II drugs opposite is true. Thus increased levels of topoisomerases render cells hypersensitive to enzyme poisons but resistant to inhibitors. Conversely, decreased enzyme levels render cells resistant to poison but hypersensitive to inhibitors.

Topoisomerase IB inhibitors

Topoisomerase IB of kinetoplastids, being a heterodimeric enzyme, has added a new paradigm in the quest of antitrypanosomatid agents as we have already discussed in the earlier section of this chapter. Continuous effort is being made to identify inhibitors, both from natural and synthetic origin, which selectively target bi-subunit topoisomerase IB of trypanosomatids with negligible or no impact upon host enzyme. The extent of enzyme inhibition varies with structure and nature of the compound. Unfortunately, reports on specific inhibitors of *Trypanosoma* topoisomerase IB remain scarce. 2–6 dimethyl-9-hydroxyellipticinium was shown to inhibit the relaxation activity of *Trypanosoma cruzi* topoisomerase IB (Douc-Rasy *et al.,* 1983). Nonetheless, few studies have been made to observe the effect of different eukaryotic and bacterial topoisomerase I inhibitors upon the growth of *Trypanosoma* species (Zuma *et al.,* 2011; Jobe *et al.,* 2012). Mostly, contribution has been made to develop topoisomerase IB inhibitors targeted to *Leishmania* species. Such inhibitors come from a vast spectrum of chemical identity (Table 10.2). The mode of inhibition primarily follows two distinct mechanisms as conferred in the preceding section. They either hamper the catalytic activity of the enzyme (termed as 'catalytic inhibitors') or stabilize DNA–topoisomerase cleavage complex (termed as 'poisons'). Though such kind of classification is not available for all the inhibitors reported to date, few sporadic theories pertaining to the extent of inhibition have been established. Fatty acids are one of the major groups of compounds studied thoroughly in this regard (Carballeira *et al.,* 2009). Two parameters viz. (1) carbon chain

Table 10.2 Compounds (natural/synthetic) targeting kinetoplastid topoisomerase IB

Compound	Chemical identity	Kinetoplastid	Reference
(2*R*,5*Z*,9*Z*)-2-METHOXY-25-methyl-5,9-hexacosadienoic acid and (2*R*,5*Z*,9*Z*)-2-methoxy-24-methyl-5,9-hexacosadienoic acid	α-Methoxylated Δ5,9 Fatty Acids	*L. infantum*	Carballeira *et al.* (2016)
16α-Hydroxycleroda-3,13(14)Z-dien-15,16-olide	Clerodane diterpenoids	*L. donovani*	Misra *et al.* (2010)
2-methoxy-5,9-eicosadienoic acid, 2-methoxy-5,9-eicosadiynoic acid	α-methoxylated fatty acid and acetylinic analogue	*L. donovani*	Carballeira *et al.* (2013)
2-Octadecynoic acid, 2-hexadecynoic acid, 2-tetradecynoic acid	2-alkylnoic fatty acid	*L. donovani*	Carballeira *et al.* (2012a,b)
3,4-Dihydroxyphenyl, derivative of arylidenefuropyridinediones	Furopyridinediones	*L. donovani*	Mamidala *et al.* (2016)
3–3′-Di-indolylmethane	Acid condensation product of indole-3-carbinol	*L. donovani*	Roy *et al.* (2008)
3β,6β,16β-trihydroxylup-20(29)-ene	Lupane triterpene	*L. amazonensis*	Teles *et al.* (2015)
5-Nitrofuran and 5-nitroimidazole analogues of N-substituted-piperazinyl-1,3,4-thiadiazoles	1,3,4-Thiadiazole derivatives	*Leishmania* spp.	Poorrajab *et al*. (2009)
Anthra[1,2-d]imidazole-6,11-dione derivatives	Imidazole fused anthraquinone derivatives	*L. donovani*	Chaudhuri *et al.* (2007)
Baicalein, luteolin, quercetin	Flavonoids	*L. donovani*	Das *et al.* (2006)
Berberin	Poliheterocyclics	*L. braziliensis* *L. donovani*	Vennerstrom *et al.* (1990), Marquis *et al.* (2003)

Table 10.2 Continued

Compound	Chemical identity	Kinetoplastid	Reference
Bisbenzylisoquinoline alkaloid	bisbenzylisoquinoline alkaloid	*L. donovani*	Kumar *et al.* (2016)
Camptothecin	Quinoline alkaloid	*T. brucei*, *L. donovani*	Bodley and Shapiro (1995)
Copper salisylaldoxime (CuSAL)	Transition metal complex	*L. donovani*	Singh *et al.* (2017)
Diamidine compounds including pentamidine and its analogue DB75	Diamidines	*L. donovani*	Yang *et al.* (2016)
Dichloroacetamido bromobenzyl bromoindolyl bromoquinoline	Indolyl quinolines	*L. donovani*	Ray *et al.* (1997)
Dihydrobetulinic acid	Pentacyclic triterpenoid	*L. donovani*	Chowdhury *et al.* (2003)
Diospyrin, naphthalene derivatives	Naphthoquinones	*L. donovani* *T. brucei* *T. cruzi*	Ray *et al.* (1998); Ganapaty *et al.* (2006)
Disuccinyl betulin, Diglutaryl dihydrobetulin, Disuccinyl dihydrobetulin	Derivatives of betulin, a natural triterpene	*L. donovani*	Chowdhury *et al.* (2011)
Hoechst-33258, Hoechst-33342	Bis-benzimidazoles	*L. donovani*	Walker and Saravia (2004)
Indotecan (LMP400), AM13–55	Indenoisoquinolines	*L. infantum*	Balaña-Fouce *et al.* (2012)
N-6 3-aminopropyl indenoisoquinolines, N-6 3-imidazolylprolyl Indenoisoquinolines, alkylamine repeats N-6	Indenoisoquinolines	*T. brucei*	Bakshi *et al.* (2009)
N-benzyl 2, 2′α3,3′,5′, 6',7′,7α,α′-octahydro-2methoxycarbonyl-spiro [indole-3, 3′-pyrrolizidine]-2 one	Spirooxindole derivative	*L. donovani*	Saha *et al.* (2016)
Niranthin, Lyoniside, Saracoside	Lignan glycosides	*L. donovani*	Chowdhury *et al.* (2012), Saha *et al.* (2013)
Peganine hydrochloride dihydrate	Peganine hydrochloride dihydrate	*L. donovani*	Misra *et al.* (2008)
Pentostam, glucantime	Pentavalent antimony	*L. donovani*	Chakraborty and Majumder (1988)
Putranoside-D, putranoside-A, putranoside-A methyl ester	Saponin	*L. donovani*	Kumar *et al.* (2014)
Rebeccamycin	Indocarbazoles	*T. brucei*	Deterding *et al.* (2005)
Tetrahydro indeno-1,5-naphthyridines and indeno[1,5]naphthyridines	Indeno-1,5-naphthyridines	*L. infantum*	Tejería *et al.* (2016)
Topotecan, gimatecan, pro-drug irinotecan and active metabolite SN38	Quinoline alkaloid	*L. infantum*	Prada *et al.* (2013b)
Voacamine	Indole alkaloid	*L. donovani* *L. amazonensis* *T. cruzi*	Chowdhury *et al.* (2017)

length and, (2) amount of unsaturation are crucial that govern the DNA relaxation activity by fatty acids. If the nature of unsaturation is kept constant, inhibition capability becomes proportional with the carbon chain length. This correlation is exemplified by 2-alkanoic acids. 2-Octadecynoic acid shows highest inhibition followed by 2-hexacecynoic acid and 2-tetradecynoic acid (Carballeira *et al.*, 2011). Similarly, icosenoic acid is more potent than heptadecenoic acid (Carballeira *et al.*, 2009). On the other hand, if the chain length is kept constant, unsaturated fatty acids show more inhibition than their saturated counterparts. For this reason, 2-methoxy-heptadecynoic acid shows higher inhibition than 2-methoxy-heptadecenoic acid. Their saturated counterpart 2-methoxy-heptadecanoic

acid shows no inhibition at all. Probably the weak intermolecular interactions between enzyme active site and unsaturated bonds are responsible for this. In contrast, there are factors that negatively regulate the enzyme inhibition capability of fatty acids. Presence of ciscyclopropane group is one such factor. Another theory suggests that rational modification of core structure of known inhibitors improves inhibition efficacy. Chaudhuri *et al.* (2007) reported that if imidazole group is fused with anthraquinone derivatives, it improves the efficiency. This improved efficacy is probably due to the variation in pK_a value and electronegativity of the side-chain nitrogen. But it is to be remembered all such inhibitors do not necessarily impart cytotoxicity upon parasites. Though good inhibitors generally reflect the same trend with promastigotes and amastigotes, probability still exists that they may not be fruitful in all cases. Normally, fatty acids, being amphipathic, get incorporated into lipid membranes. They perturb the structural integrity of lipid bilayer resulting in cytotoxicity (Carballeira *et al.*, 2016). This argument is corroborated by the finding that presence of triple bond along with α-methoxylation enhances enzyme inhibition. But surprisingly, α-methoxylation decreases the cytotoxicity towards promastigotes. It is reported that despite being potential inhibitors of topoisomerase IB, methoxylated fatty acids are not significant enough as far as parasite viability is concerned. There is no general theory to establish the correlation between fatty acids and their antiproriferative effect upon parasite. Few studies suggest that this effect is probably due to inhibited uptake of glucose or leucine by the protozoa (Chaudhuri *et al.*, 1986). Finally, the most important concern is the host cytotoxicity. Situations often prevail where a particular inhibitor shows significant inhibition of parasite enzyme and induces cell death. But at the same time, the compound exhibits similar or more toxicity to host topoisomerase IB. Carballeira *et al.* (2016) recently reported that few methoxylated fatty acids show more inhibition of human topoisomerase IB than the parasite enzyme. They showed that preference of an inhibitor is shifted from parasite enzyme towards host enzyme due to increase in alkyl chain length. Short chain fatty acids are better inhibitors of *Leishmania* enzyme than human topoisomerase and the case gets reversed for long chain fatty acids. However, more investigation is required to establish these findings in a form of a theory. In the succeeding subsections, we will now deal with two fundamental models of topoisomerase IB inhibition.

Catalytic inhibitors of topoisomerase IB

Catalytic inhibitors show a general trend of interacting with the free enzyme. Their inhibitory effect markedly increases when they are preincubated with the free enzyme prior to DNA substrate addition under *in vitro* conditions. These kinds of inhibitors increase the K_M and follow competitive model of inhibition. However, they cannot bind with DNA–topoisomerase cleavage complex and hence do not alter V_{max} value. Several compounds come under the aegis of catalytic inhibitors (Table 10.2). Derivatives of betulin such as disuccinyl betulin, diglutaryl dihydrobetulin, and disuccinyl dihydrobetulin (Chowdhury *et al.*, 2011), putranoside-D, putranoside-A, putranoside-A methyl ester (Kumar *et al.*, 2014), spirooxiindole derivative (Saha *et al.*, 2016), few bisbenzylisoquinoline alkaloids (Kumar *et al.*, 2016) are some of the examples. All these compounds interact with topoisomerase IB of *Leishmania*. However their binding stoichiometry varies. As an example, three betulin derivatives developed by Chowdhury *et al.* (2011) show 1:1 binding stoichiometry with the enzyme whereas another catalytic inhibitor, *N*-benzyl-octahydro-2-methoxycarbonyl-spiro[indole-3,3′-pyrrolizidine]-2-one, shows a 1:2 stoichiometry (Saha *et al.*, 2016). These catalytic inhibitors reversibly bind with the enzyme and abrogate the DNA-enzyme covalent complex formation.

Topoisomerase IB poisons

Inhibitors falling under this group tend to bind either solely with DNA–enzyme covalent complex or with both free enzyme and DNA–enzyme complex. They may decrease both K_m and V_{max} values acting as uncompetitive inhibitor or may decrease only V_{max} value keeping K_m unaltered acting as non-competitive inhibitor. Many such compounds, including camptothecin, have been established to date as *Leishmania* topoisomerase IB poisons (Table 10.2). Unlike catalytic inhibitors, topoisomerase poisons do not abrogate the DNA-enzyme covalent complex formation, rather stabilize the complex. Such kind of DNA–enzyme adducts ultimately cause DNA single-strand breaks. These breaks are

subsequently converted to double-strand breaks upon collision with transcription or replication machineries. Double-strand breaks are detrimental to the parasite. The cell cycle progression is arrested triggering programmed cell death of the parasite. All these poisons can be further classified into two subclasses depending upon their capability to inhibit the religation reaction. Camptothecin (Das *et al.,* 2006), lyoniside, saracoside (Saha *et al.,* 2013), niranthin (Chowdhury *et al.,* 2012), DIM (Roy *et al.,* 2008) are some of the poisons which inhibit the religation action. In contrast, baicalein, luteolin cannot inhibit this topoisomerase IB-mediated religation step (Das *et al.,* 2006). Moreover, there are few examples of topoisomerase poisons which not only binds with DNA-enzyme covalent complex but also intercalate into the DNA itself at a very high concentration. Quercetin, bicalein, luteolin have been established to possess DNA intercalation property at 300 μM (Das *et al.,* 2006).

Topoisomerase II inhibitors

Topoisomerase II of the kinetoplastid parasites are homodimeric enzymes similar to higher eukaryotes including human. But it is a crucial enzyme for the parasites because of its involvement in replication of kinetoplast DNA network (kDNA) inside mitochondria (Shapiro *et al.,* 1995; Das *et al.,* 2008). Hence, topoisomerase II has also been explored as a promising target for antiparasitic therapeutics (Table 10.3). Interestingly, pentamidine was later found to also be an inhibitor of topoisomerase II (Singh *et al.,* 2007). A distinct aspect of this enzyme in trypanosomatids is the discrete existence of nuclear and kinetoplast topoisomerase II (Kulikowicz and Shapiro, 2006). Some compounds

Table 10.3 Compounds (natural/synthetic) targeting kinetoplastid topoisomerase II

Compound	Chemical identity	Kinetoplastid	Reference
3,5-Bis(4-chlorophenyl)–7-hydroxyisobenzofuran-1(3H)-one and (4-bromo)-30-hydroxy-50-(4-bromophenyl)-benzophenone	Isobenzofuranone derivatives	*L. donovani*	Mishra *et al.* (2014)
5-Nitrofuran and 5-nitroimidazole analogues of N-substituted-piperazinyl-1,3,4-thiadiazoles	1,3,4-Thiadiazole derivatives	*Leishmania* spp.	Poorrajab *et al.* (2009)
Anilinoacridine compounds	Anilinoacridine	*L. chagasi*	Werbovetz *et al.* (1994)
Anilinoacridine compounds	Anilinoacridine	*T. brucei*	Gamage *et al.* (1997)
Dichloroacetamido bromo benzyl bromoindolyl bromo quinoline	Indolyl quinolines	*L. donovani*	Ray *et al.* (1997)
Dihydrobetulinic acid	Pentacyclic triterpenoid	*L. donovani*	Chowdhury *et al.* (2003)
Doxorrubicin	Anthracyclins	*L. donovani*	Singh and Dey (2007)
Enoxacin, Ciprofloxacin	Fluoroquinolones	*L. panamensis*	Romero *et al.* (2005), Cortázar *et al*. (2007)
Etoposide, teniposide	Podophylotoxins	*L. donovani*	Sangeeta *et al.* (2005a,b)
Lute Olin	Flavonoids	*L. donovani*	Muttra *et al.* (2000)
Monoacid and monoacid analogues	4-nitro-benzo-isoquinolinedione and derivatives	*L. chagasi*	Slant *et al.* (1996)
Offloading, ciprofloxacin, offloading	Fluoroquinolones	*T. brucei*	Neonates *et al*. (1999, 2003)
Norfloxacin, ofloxacin	Fluoroquinolones	*T. cruzi*	Gonzales-Perdomo *et al.* (1990)
Novobiocin	Aminocoumarin antibiotic	*L. donovani*	Singh *et al.* (2005)
Novobiocin	Aminocoumarin antibiotic	*T. cruzi*	Gonzales-Perdomo *et al.* (1990)
Pentamidine	Diamidines	*L. donovani*	Singh and Dey (2007)

differentially target nuclear and kinetoplast topoisomerase II whereas some have similar preferences for both. As for example, one subset of mitonafide analogues promotes kDNA linearization in *Leishmania* and another subset affects activity of nuclear topoisomerase II (Slunt *et al.*, 1996). Moving onto anilinoacridines, they have similar effect for both the enzymes. This differential activity is primarily controlled by the presence and position of the nitro groups in the compounds. Now moving onto host toxicity, similar scenario is encountered with topoisomerase II just like we saw with topoisomerase IB. Host toxicity is a critical issue as far as topoisomerase II targeted therapeutic development is concerned. Few researches have shown that parasitic topoisomerase II is more closely related to mammalian counterpart rather than prokaryotic topoisomerase II which has made the situation more complex. This similarity increases the possibility for a protozoan enzyme inhibitor to inhibit host enzyme (Werbovetz *et al.*, 1992). Few compounds tend to inhibit both parasite and host enzyme at comparable range. Compounds such as anilinoacridine, protoberberine coralyne, bisbenzimidazoles, diamidine diminazene, etc. show equal effect on both parasite and human enzymes whereas ellagic acid has preference for macrophage topoisomerase II. Nevertheless second generation fluoroquinolones such as enoxacin and ciprofloxacin, flavonoid quercetin target *L. panamensis* DNA topoisomerase II with substantially less toxicity towards macrophage enzyme (Cortazar *et al.*, 2007). Moving onto *Trypanosoma*, just like topoisomerase IB, reports on *Trypanosoma*-specific topoisomerase II inhibitors are substantially meagre. Effects of few bacterial (ofloxacin, novibiocin, etc.) and eukaryotic (aclarubicin, idarubicin, mitoxantrone, merbarone, etc.) topoisomerase II inhibitors on growth of *Trypanosoma* have been studied. But whether these compounds inhibit *Trypanosoma* topoisomerase II is not known so far. Topoisomerase II inhibitors are also fundamentally subdivided into two subclasses; namely, catalytic inhibitors and poisons. In the succeeding section, we will take a closer view of these two kinds of inhibitors mostly in a *Leishmania* purview.

Catalytic inhibitors of topoisomerase II

Topoisomerase II is fundamentally different from topoisomerase IB in its requirement of ATP to perform its catalytic cycle. Catalytic inhibitors of topoisomerase II have two different mechanisms. They either bind with the free enzyme preventing it to sit on DNA substrate or inhibit the ATPase activity of the enzyme by interacting with ATPase domain. Since they abrogate topoisomerase–DNA interaction, naturally cleavage complex formation is blocked. Mishra *et al.* (2014) reported that two derivatives of isobenzofuranone inhibit *L. donovani* topoisomerase II by interfering at its catalytic centre but neither have any effect in ATPase activity nor do they intercalate within substrate DNA.

Topoisomerase II poisons

Topoisomerase II poisons stabilize enzyme-kinetoplast DNA cleavable complex and disrupt cleavage-religation equilibrium. This irreversible covalent association between topoisomerase II and DNA ultimately cause double-strand breaks which are extremely cytotoxic for the parasites. Luteolin, quercetin (Mittra *et al.*, 2000), some mitonafide analogues fall under this category. They are further classified as intercalating and non-intercalating poisons. Some poisons can intercalate into DNA by virtue of their planar aromatic region (Slunt *et al.*, 1996). During interaction, a local distortion occurs which resembles an intermediate of the topoisomerase II catalytic cycle. If an inhibitor favours that intermediate formation, cleavage complex is stabilized. Amonafide and one mitonafide analogue have been reported to possess this property. Naturally, all poisons do not have this capacity to get intercalated. A classical example of non-intercalating *Leishmania* topoisomerase II poison is etoposide (Sengupta *et al.*, 2005b), which is a well-known anticancer drug. Surprisingly, etoposide can also inhibit the ATPase activity of the recombinant N-terminal domain of *L. donovani* topoisomerase II (Sengupta *et al.*, 2005a). But etoposide has no impact upon parasite viability.

Dual inhibitors of topoisomerase IB and topoisomerase II

There are few compounds which inhibit both type IB and II topoisomerases of *Leishmania*. Nonetheless, there is no theory available to date to functionally connect the structure of an inhibitor with dual inhibition potential. Few indolyl quinoline analogues namely 2-(2′-dichloroacetamidobenzyl)-3-(3′-indolyl)-quinoline,

2-(2′-dichloroacetamido-5′-bromobenzyl)-3′-[3′-(5′-bromoindolyl]-6-bromo quinoline, and 2-(2′-acetamido benzyl)–3-(3′-indolyl)-quinoline (Ray *et al.*, 1997), a pentacyclic triterpenoid viz. dihydrobetulinic acid (DHBA) (Chowdhury *et al.*, 2003) inhibit both the enzyme. DHBA is a catalytic inhibitor of both the enzymes. It is also cytotoxic for the parasites. Though there are no reports on *Trypanosoma*-specific dual inhibitors, antiproliferative effects of several eukaryotic dual inhibitors have been investigated upon *T. cruzi* (Lacombe *et al.*, 2014).

Conclusion and future perspectives

There has been immense progress in the understanding of the biology of kinetoplastid parasites especially of parasitic topoisomerases. The availability of the Tritryp genome database has provided significant impetus towards the study of these enzymes and also provided necessary momentum to the much needed identification of new targets for anti-leishmanial therapeutics. Scientists throughout the world including the author's laboratory have contributed significantly towards the understanding of structures and activities of these parasitic topoisomerases and established *Leishmania* topoisomerases as potential drug targets for leishmaniasis (kala-azar). Topoisomerase genes and proteins characterized from these lower eukaryotes appear to share many characteristics associated with their human homologues. But certain striking differences, including different enzyme activity requirements, variable catalytic sites and different sensitivities to topoisomerase poisons, provide insight for the development of topoisomerase-directed antiparasitic therapeutics. It has been established by several studies that the inhibitors of topoisomerases convert these essential enzymes into intracellular proliferating cell toxins and thereby provide a good tool for preferentially killing of the highly replicative parasite cells within the host. However, future challenges are to overcome the problem related to drug resistance for development of novel anti-leishmanial therapeutics. In this context, the approach of combinatorial therapy becomes very effective and identification of new drugs and new targets is absolutely warranted to combat parasite menace in the foreseeable future.

The search also continues for novel RNA topoisomerase activities in species of *Leishmania* and *Trypanosoma.* This kind of non-canonical function of type IA topoisomerases was first reported for *Escherichia coli* topoisomerase III (Wang *et al.*, 1996) and later in human topoisomerase 3β (Stoll *et al.*, 2013; Xu *et al.*, 2013). An extensive genetic recombination among the 10,000 mini-circles, creating novel guide RNAs followed by RNA editing, might require distinct RNA topoisomerase.

The major obstacle of kinetoplastid topoisomerase research is over-expressing purified recombinant topoisomerases and elucidating their crystal structure. In this way, structural information gained from crystallographic studies can contribute to a better understanding of the molecular mechanisms of enzyme action *in vivo*, including their interaction with inhibitors and poisons screened from natural or synthetic sources. This will cause a paradigm shift in the quest to target the topoisomerases selectively as a means of therapeutic control of the parasites.

Acknowledgements

We thank Dr Samit Chattopadhyay, the Director of our Institute (CSIR-Indian Institute of Chemical Biology), for his interest in this work. This work was supported by Department of Science and Technology (DST) Indo-Brazil project (DST/INT/Brazil/RPO-01/2009/2), Raja Ramanna Fellowship (DAE, Government of India) and NASI-Senior Scientist Platinum Jubilee Fellowship to HKM.

References

Bakshi, R.P., Sang, D., Morrell, A., Cushman, M., and Shapiro, T.A. (2009). Activity of indenoisoquinolines against African trypanosomes. Antimicrob. Agents Chemother. *53*, 123–128. https://doi.org/10.1128/AAC.00650-07.

Balaña-Fouce, R., Prada, C.F., Requena, J.M., Cushman, M., Pommier, Y., Álvarez-Velilla, R., Escudero-Martínez, J.M., Calvo-Álvarez, E., Pérez-Pertejo, Y., and Reguera, R.M. (2012). Indotecan (LMP400) and AM13–55: two novel indenoisoquinolines show potential for treating visceral leishmaniasis. Antimicrob. Agents Chemother. *56*, 5264–5270. https://doi.org/10.1128/AAC.00499-12.

Banerjee, B., Sen, N., and Majumder, H.K. (2011). Identification of a functional type IA topoisomerase, LdTopIIIβ, from Kinetoplastid parasite *Leishmania donovani*. Enz. Res. 2011 (Article ID 230542). https://doi.org/10.4061/2011/230542.

Berger, J.M., Gamblin, S.J., Harrison, S.C., and Wang, J.C. (1996). Structure and mechanism of DNA

topoisomerase II. Nature 379, 225–232. https://doi.org/10.1038/379225a0.

Bodley, A.L., and Shapiro, T.A. (1995). Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibitor, on trypanosomes and *Leishmania*. Proc. Natl. Acad. Sci. U.S.A. *92*, 3726–3730.

Bodley, A.L., Chakraborty, A.K., Xie, S., Burri, C., and Shapiro, T.A. (2003). An unusual type IB topoisomerase from African trypanosomes. Proc. Natl. Acad. Sci. U.S.A. *100*, 7539–7544. https://doi.org/10.1073/pnas.1330762100.

Bosedasgupta, S., Das, B.B., Sengupta, S., Ganguly, A., Roy, A., Tripathi, G., and Majumder, H.K. (2008a). Amino acids 39–456 of the large subunit and 210–262 of the small subunit constitute the minimal functionally interacting fragments of the unusual heterodimeric topoisomerase IB of *Leishmania*. Biochem. J. 409(2), 481–489. https://doi.org/10.1042/BJ20071358.

BoseDasgupta, S., Ganguly, A., Das, B.B., Roy, A., Khalkho, N.V., and Majumder, H.K. (2008b). The large subunit of *Leishmania* topoisomerase I functions as the 'molecular steer'in type IB topoisomerase. Mol. Microbiol. *67*, 31–46. https://doi.org/10.1111/j.1365-2958.2007.06002.x.

Broccoli, S., Marquis, J.F., Papadopoulou, B., Olivier, M., and Drolet, M. (1999). Characterization of a *Leishmania donovani* gene encoding a protein that closely resembles a type IB topoisomerase. Nucleic Acids Res. *27*, 2745–2752.

Campbell, S., and Maxwell, A. (2002). The ATP-operated clamp of human DNA topoisomerase IIalpha: hyperstimulation of ATPase by 'piggy-back' binding. J. Mol. Biol. *320*, 171–188. https://doi.org/10.1016/S0022-2836(02)00461-8.

Carballeira, N.M., Cartagena, M., Li, F., Chen, Z., Prada, C.F., Calvo-Alvarez, E., Reguera, R.M., and Balaña-Fouce, R. (2012a). First total synthesis of the (±)-2-methoxy-6-heptadecynoic acid and related 2-methoxylated analogs as effectiveinhibitors of the *Leishmania* topoisomerase IB enzyme. Pure Appl. Chem., *84*, 1867–7185. https://doi.org/10.1351/PAC-CON-11-10-21.

Carballeira, N.M., Cartagena, M., Sanabria, D., Tasdemir, D., Prada, C.F., Reguera, R.M., and Balaña-Fouce R. (2012b). 2-Alkynoic fatty acids inhibit topoisomerase IB from *Leishmania donovani*. Bioorg. Med. Chem. Lett. *22*, 6185–6189. https://doi.org/10.1016/j.bmcl.2012.08.019.

Carballeira, N.M., Cartagena, M.M., Prada, C.F., Rubio, C.F., and Balaña-Fouce, R. (2009). Total synthesis and antileishmanial activity of the natural occurring acetylenic fatty acids 6-heptadecynoic acid and 6-icosynoic acid. Lipids *44*, 953–961. https://doi.org/10.1007/s11745-009-3345-z.

Carballeira, N.M., Montano, N., Alvarez-Velilla, R., Prada, C.F., Reguera, R.M., and Balaña-Fouce, R. (2013). Synthesis of marine α-methoxylated fatty acid analogs that effectively inhibit the topoisomerase IB from *Leishmania donovani* with a mechanism different from that of camptothecin. Marine Drugs, 11, 3661–75. https://doi.org/10.3390/md11103661.

Carballeira, N.M., Montano, N., Amador, L.A., Rodríguez, A.D., Golovko, M.Y., Golovko, S.A., Reguera, R.M., Álvarez-Velilla, R., and Balaña-Fouce, R. (2016). Novel very long-chain α-methoxylated Δ5, 9 fatty acids from the sponge *Asteropusniger* are effective inhibitors of topoisomerases IB. Lipids, 51(2), 245–256. https://doi.org/10.1007/s11745-015-4114-9.

Carballeira, N.M., Montano, N., Cintrón, G.A., Márquez, C., Rubio, C.F., Prada, C.F., and Balaña-Fouce, R. (2011). First total synthesis and antileishmanial activity of (Z)-16-methyl-11-heptadecenoic acid, a new marine fatty acid from the sponge Dragmaxia undata. Chem. Phys. Lipids, *164*, 113–117. https://doi.org/10.1016/j.chemphyslip.2010.11.006.

Chakraborty, A.K., and Majumder, H.K. (1988). Mode of action of pentavalent antimonials: Specific inhibition of type I DNA topoisomerase of *Leishmania donovani*. Biochem. Biophys. Res. Commun. *152*, 605–611.

Chakraborty, A.K., Gupta, A., and Majumder, H.K. (1993). A type 1 DNA topoisomerase from the kinetoplast hemoflagellate *Leishmania donovani*. Ind. J. Biochem. Biophys. 30(5), 257–263.

Champoux, J.J. (2001). DNA topoisomerases: structure, function, and mechanism. Annu. Rev. Biochem. *70*, 369–413. https://doi.org/10.1146/annurev.biochem.70.1.369.

Chaudhuri, G., Ghoshal, K., and Banerjee, A.B. (1986). Toxic effects of fatty acids on *Leishmania donovani* promastigotes. Ind. J. Med. Res. *84*, 361–365.

Chaudhuri, P., Majumder, H.K., and Bhattacharya, S. (2007). Synthesis, DNA binding, and leishmania topoisomerase inhibition activities of a novel series of anthra [1, 2-d] imidazole-6, 11-dione derivatives. J. Med. Chem. *50*, 2536–2540. https://doi.org/10.1021/jm0610604.

Chen, S.H., Chan, N.L., and Hsieh, T.S. (2013). New mechanistic and functional insights into DNA topoisomerases. Annu. Rev. Biochem. *82*, 139–170. https://doi.org/10.1146/annurev-biochem-061809-100002.

Chen, Y., and Rice, P.A. (2003). New insight into site-specific recombination from Flp recombinase-DNA structures. Annu. Rev. Biophys. Biomol. Struct. *32*, 135–159. https://doi.org/10.1146/annurev.biophys.32.110601.141732.

Chowdhury, A.R., Mandal, S., Goswami, A., Ghosh, M., Mandal, L., Chakraborty, D., Ganguly, A., Tripathi, G., Mukhopadhyay, S., Bandyopadhyay, S., and Majumder, H.K. (2003). Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by targeting DNA topoisomerase I and II: implications in antileishmanial therapy. Mol. Med. 9(1–2), 26–36.

Chowdhury, S., Mukherjee, T., Mukhopadhyay, R., Mukherjee, B., Sengupta, S., Chattopadhyay, S., Jaisankar, P., Roy, S., and Majumder, H.K. (2012). The lignan niranthin poisons *Leishmania donovani* topoisomerase IB and favours a Th1 immune response in mice. EMBO Mol. Med. 4(10), 1126–1143. https://doi.org/10.1002/emmm.201201316.

Chowdhury, S., Mukherjee, T., Sengupta, S., Chowdhury, S.R., Mukhopadhyay, S., and Majumder, H.K. (2011). Novel betulin derivatives as antileishmanial agents with mode of action targeting type IB DNA topoisomerase. Mol. Pharmacol. *80*, 694–703. https://doi.org/10.1124/mol.111.072785.

Chowdhury, S.R., Kumar, A., Godinho, J.L.P., Silva, S.T.D.M., Zuma, A.A., Saha, S., Kumari, N., Rodrigues, J.C.F., Sundar, S., Dujardin, J.C. *et al.* (2017). Voacamine alters *Leishmania* ultrastructure and kills parasite by poisoning unusual bi-subunit topoisomerase 1B. Biochem. Pharmacol. *138*, 19-30. https://doi.org/10.1016/j.bcp.2017.05.002.

Corbett, K.D., and Berger, J.M. (2004). Structure, molecular mechanisms, and evolutionary relationships in DNA topoisomerases. Annu. Rev. Biophys. Biomol. Struct. *33*, 95–118. https://doi.org/10.1146/annurev.biophys.33.110502.140357.

Cortázar, T.M., Coombs, G.H., and Walker, J. (2007). *Leishmania panamensis*: comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine. Exp. Parasitol. 116(4), 475–482. https://doi.org/10.1016/j.exppara.2007.02.018.

Das, A., Dasgupta, A., Sengupta, T., and Majumder, H.K. (2004). Topoisomerases of kinetoplastid parasites as potential chemotherapeutic targets. Trends Parasitol. *20*, 381–387. https://doi.org/10.1016/j.pt.2004.06.005.

Das, A., Dasgupta, A., Sharma, S., Ghosh, M., Sengupta, T., Bandopadhyay, S., and Majumder, H.K. (2001). Characterisation of the gene encoding type II DNA topoisomerase from *Leishmania donovani*: a key molecular target in antileishmanial therapy. Nucleic Acids Res. 29(9), 1844–1851.

Das, B.B., Ganguly, A., and Majumder, H.K. (2008). DNA topoisomerases of *Leishmania*: the potential targets for anti-leishmanial therapy. Adv. Exp. Med. Biol. *625*, 103–115. https://doi.org/10.1007/978-0-387-77570-8_9.

Das, B.B., Sen, N., Dasgupta, S.B., Ganguly, A., and Majumder, H.K. (2005). N-terminal region of the large subunit of *Leishmania donovani* bisubunit topoisomerase I is involved in DNA relaxation and interaction with the smaller subunit. J. Biol. Chem. *280*, 16335–16344. https://doi.org/10.1074/jbc.M412417200.

Das, B.B., Sen, N., Roy, A., Dasgupta, S.B., Ganguly, A., Mohanta, B.C., Dinda, B., and Majumder, H.K. (2006). Differential induction of *Leishmania donovani* bi-subunit topoisomerase I–DNA cleavage complex by selected flavones and camptothecin: activity of flavones against camptothecin-resistant topoisomerase I. Nucleic Acids Res. 34(4), 1121–1132. https://doi.org/10.1093/nar/gkj502.

Das, B.B., Sengupta, T., Ganguly, A., and Majumder, H.K. (2006). Topoisomerases of kinetoplastid parasites: why so fascinating? Mol. Microbiol. *62*, 917–927. https://doi.org/10.1111/j.1365-2958.2006.05428.x.

Das, B.B., Sen, N., Ganguly, A., and Majumder, H.K. (2004). Reconstitution and functional characterization of the unusual bi-subunit type I DNA topoisomerase from *Leishmania donovani*. FEBS Lett. *565*, 81–88. https://doi.org/10.1016/j.febslet.2004.03.078.

Davies, D.R., Mushtaq, A., Interthal, H., Champoux, J.J., and Hol, W.G. (2006). The structure of the transition state of the heterodimeric topoisomerase I of *Leishmania donovani* as a vanadate complex with nicked DNA. J. Mol. Biol. *357*, 1202–1210. https://doi.org/10.1016/j.jmb.2006.01.022.

Deterding, A., Dungey, F.A., Thompson, K.A., and Steverding, D. (2005). Anti-trypanosomal activities of DNA topoisomerase inhibitors. Acta Trop. 93,311–316. https://doi.org/10.1016/j.actatropica.2005.01.005.

Deweese, J.E., and Osheroff, N. (2009). The DNA cleavage reaction of topoisomerase II: wolf in sheep's clothing. Nucleic Acids Res. 37,738–748. https://doi.org/10.1093/nar/gkn937.

Díaz, González, R., Pérez Pertejo, Y., Redondo, C.M., Pommier, Y., Balaña-Fouce, R., and Reguera, R.M. (2007). Structural insights on the small subunit of DNA topoisomerase I from the unicellular parasite *Leishmania donovani*. Biochimie, 89, 1517–1527. https://doi.org/10.1016/j.biochi.2007.07.015.

Douc-Rasy, S., Kayser, A., and Riou, G. (1983). A specific inhibitor of type I DNA-topoisomerase of *Trypanosoma cruzi*: dimethyl-hydroxy-ellipticinium. Biochem. Biophys. Res. Commun. 117(1), 1–5.

Fragoso, S.P., and Goldenberg, S. (1992). Cloning and characterization of the gene encoding *Trypanosoma cruzi* DNA topoisomerase II. Mol. Biochem. Parasitol. *55*, 127–134. https://doi.org/10.1016/0166-6851(92)90133-5.

Gadelle, D., Filée, J., Buhler, C., and Forterre, P. (2003). Phylogenomics of type II DNA topoisomerases. Bioessays. *25*, 232–242. https://doi.org/10.1002/bies.10245.

Gamage, S.A., Figgitt, D.P., Wojcik, S.J., Ralph, R.K., Ransijn, A., Mauel, J., Yardley, V., Snowdon, D., Croft, S.L., and Denny, W.A. (1997). Structure–activity relationships for the antileishmanial and antitrypanosomal activities of 1′-substituted 9-anilinoacridines. J. Med. Chem. *40*, 2634–2642. https://doi.org/10.1021/jm970232h.

Ganapaty, S., Steve Thomas, P., Karagianis, G., Waterman, P.G., and Brun, R. (2006). Antiprotozoal and cytotoxic naphthalene derivatives from *Diospyros assimilis*. Phytochem. *67*, 1950–1956. https://doi.org/10.1016/j.phytochem.2006.05.039.

Ganguly, A., Sengupta, S., BoseDasgupta, S., Roy, A., and Majumder, H.K. (2009). Mutational studies reveal lysine 352 on the large subunit is indispensable for catalytic activity of bi-subunit topoisomerase I from *Leishmania donovani*. Mol. Biochem. Parasitol. 165(1), 57–66. https://doi.org/10.1016/j.molbiopara.2009.01.002.

Gaziová, I., and Lukes, J. (2003). Mitochondrial and nuclear localization of topoisomerase II in the flagellate *Bodo saltans* (Kinetoplastida), a species with non-catenated kinetoplast DNA. J. Biol. Chem. *278*, 10900–10907. https://doi.org/10.1074/jbc.M202347200.

Gonzales-Perdomo, M., de Castro, S.L., Meirelles, M.N., and Goldenberg, S. (1990). *Trypanosoma cruzi* proliferation and differentiation are blocked by topoisomerase II inhibitors. Antimicrob. Agents Chemother. *34*, 1707–1714.

Jobe, M., Anwuzia-Iwegbu, C., Banful, A., Bosier, E., Iqbal, M., Jones, K., Lecutier, S.J., Lepper, K., Redmond, M., Ross-Parker, A., and Ward, E. (2012). Differential *in vitro* activity of the DNA topoisomerase inhibitor idarubicin against *Trypanosoma rangeli* and *Trypanosoma cruzi*. Memórias do Instituto Oswaldo Cruz, *107*, 946–950.

Kim, H.S., and Cross, G.A. (2010). TOPO3alpha influences antigenic variation by monitoring expression-site-associated VSG switching in *Trypanosoma brucei*. PLOS

Pathog. 6(7), e1000992. https://doi.org/10.1371/journal.ppat.1000992.

Koster, D.A., Croquette, V., Dekker, C., Shuman, S., and Dekker, N.H. (2005). Friction and torque govern the relaxation of DNA supercoils by eukaryotic topoisomerase IB. Nature 434, 671–674. https://doi.org/10.1038/nature03395.

Krogh, B.O., and Shuman, S. (2002). A poxvirus-like type IB topoisomerase family in bacteria. Proc. Natl. Acad. Sci. U.S.A. 99,1853–1858. https://doi.org/10.1073/pnas.032613199.

Kulikowicz, T., and Shapiro, T.A. (2006). Distinct genes encode type II topoisomerases for the nucleus and mitochondrion in the protozoan parasite *Trypanosoma brucei*. J. Biol. Chem. *281*, 3048–3056. https://doi.org/10.1074/jbc.M505977200.

Kumar, A., Chowdhury, S.R., Chakrabarti, T., Majumdar, H.K., Jha, T., and Mukhopadhyay, S. (2014). A new ellagic acid glycoside and DNA topoisomerase IB inhibitory activity of saponins from *Putranjiva roxburghii*. Nat. Prod. Commun. *9*, 675–677.

Kumar, A., Chowdhury, S.R., Sarkar, T., Chakrabarti, T., Majumder, H.K., Jha, T., and Mukhopadhyay, S. (2016). A new bisbenzylisoquinoline alkaloid isolated from *Thalictrum foliolosum*, as a potent inhibitor of DNA topoisomerase IB of *Leishmania donovani*. Fitoterapia,= *109*, 25–30. https://doi.org/10.1016/j.fitote.2015.11.021.

Lacombe, O.K., Zuma, A.A., da Silva, C.C., de Souza, W., and Motta, M.C.M. (2014). Effects of camptothecin derivatives and topoisomerase dual inhibitors on *Trypanosoma cruzi* growth and ultrastructure. J. Negat. Results Biomed. 13(1), 11. https://doi.org/10.1186/1477-5751-13-11.

Li, Z., Mondragón, A., Hiasa, H., Marians, K.J., and DiGate, R.J. (2000). Identification of a unique domain essential for *Escherichia coli* DNA topoisomerase III-catalysed decatenation of replication intermediates. Mol. Microbiol. *35*, 4888–4895.

Lindsay, M.E., Gluenz, E., Gull, K., and Englund, P.T. (2008). A new function of *Trypanosoma brucei* mitochondrial topoisomerase II is to maintain kinetoplast DNA network topology. Mol. Microbiol. *70*, 1465–1476. https://doi.org/10.1111/j.1365-2958.2008.06493.x.

Liu, L.F. (1989). DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. *58*, 351–375. https://doi.org/10.1146/annurev.bi.58.070189.002031.

Logan-Klumpler, F.J., De Silva, N., Boehme, U., Rogers, M.B., Velarde, G., McQuillan, J.A., Carver, T., Aslett, M., Olsen, C., Subramanian, S. *et al.* (2011) GeneDB—an annotation database for pathogens. Nucl. Acids Res. *40*, 98-108. https://doi.org/10.1093/nar/gkr1032.

Lynn, R., Giaever, G., Swanberg, S.L., and Wang, J.C. (1986). Tandem regions of yeast DNA topoisomerase II share homology with different subunits of bacterial gyrase. Science, *233*, 647–649. http://www.jstor.org/stable/1698013.

Mamidala, R., Majumdar, P., Jha, K.K., Bathula, C., Agarwal, R., Chary, M.T., Majumder, H.K., Munshi, P., and Sen, S. (2016). Identification of *Leishmania donovani* Topoisomerase 1 inhibitors via intuitive scaffold hopping and bioisosteric modification of known Top 1 inhibitors. Sci. Rep., 6. https://doi.org/10.1038/srep26603.

Marcotte, E.M., Pellegrini, M., Ng, H.L., Rice, D.W., Yeates, T.O., and Eisenberg, D. (1999). Detecting protein function and protein–protein interactions from genome sequences. Science 285, 751–753. https://doi.org/10.1126/science.285.5428.751.

Marquis, J.F., Makhey, D., LaVoie, E.J., and Olivier, M. (2003). Effects of topoisomerases inhibitors protoberberine on *Leishmania donovani* growth, macrophage function, and infection. J. Parasitol. 89(5), 1048–1052. https://doi.org/10.1645/GE-3161.

McClendon, A.K., and Osheroff, N. (2007). DNA topoisomerase II, genotoxicity, and cancer. Mutat. Res. *623*, 83–97. https://doi.org/10.1016/j.mrfmmm.2007.06.009.

Melendy, T., and Ray, D.S. (1987). Purification and nuclear localization of a type I topoisomerase from *Crithidia fasciculata*. Mol. Biochem. Parasitol. *24*, 215–225.

Mishra, A., Vinayagam, J., Saha, S., Chowdhury, S., Roychowdhury, S., Jaisankar, P., and Majumder, H.K. (2014). Isobenzofuranone derivatives exhibit antileishmanial effect by inhibiting type II DNA topoisomerase and inducing host response. Pharmacol. Res. Perspect. 2(6). e00070. https://doi.org/10.1002/prp2.70.

Misra, P., Khaliq, T., Dixit, A., SenGupta, S., Samant, M., Kumari, S., Kumar, A., Kushawaha, P.K., Majumder, H.K., Saxena, A.K., and Narender, T. (2008). Antileishmanial activity mediated by apoptosis and structure-based target study of peganine hydrochloride dihydrate: an approach for rational drug design. J. Antimicrob. Chemother. 62(5), 998–1002. https://doi.org/10.1093/jac/dkn319.

Misra, P., Sashidhara, K.V., Singh, S.P., Kumar, A., Gupta, R., Chaudhaery, S.S., Gupta, S.S., Majumder, H.K., Saxena, A.K., and Dube, A. (2010). 16alpha-Hydroxycleroda-3,13 (14)Z-dien-15,16-olide from *Polyalthia longifolia*: a safe and orally active antileishmanial agent. Br. J. Pharmacol. *159*, 1143–1150. https://doi.org/10.1111/j.1476-5381.2009.00609.x.

Mittra, B., Saha, A., Chowdhury, A.R., Pal, C., Mandal, S., Mukhopadhyay, S., Bandyopadhyay, S., and Majumder, H.K. (2000). Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol. Med. *6*, 527–541.

Nenortas, E., Burri, C., and Shapiro, T.A. (1999). Antitrypanosomal activity of fluoroquinolones. Antimicrob. Agents Chemother. *43*, 2066–2068.

Nenortas, E., Kulikowicz, T., Burri, C., and Shapiro, T.A. (2003). Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions. Antimicrob. Agents Chemother. 47(9), 3015–3017. https://doi.org/10.1128/AAC.47.9.3015-3017.2003.

Obado, S.O., Bot, C., Echeverry, M.C., Bayona, J.C., Alvarez, V.E., Taylor, M.C., and Kelly, J.M. (2010). Centromere-associated topoisomerase activity in bloodstream form *Trypanosoma brucei*. Nucleic Acids Res. *39*, 1023–1033. https://doi.org/10.1093/nar/gkq839.

Pasion, S.G., Hines, J.C., Aebersold, R., and Ray, D.S. (1992). Molecular cloning and expression of the gene encoding the kinetoplast-associated type II DNA topoisomerase of *Crithidia fasciculata*. Mol. Biochem. Parasitol. 50(1), 57–67.

Poorrajab, F., Ardestani, S.K., Foroumadi, A., Emami, S., Kariminia, A., Behrouzi-Fardmoghadam, M., and Shafiee, A. (2009). Selective leishmanicidal effect of 1, 3, 4-thiadiazole derivatives and possible mechanism of action against *Leishmania* species. Exp. Parasitol. *121*, 323–330. https://doi.org/10.1016/j.exppara.2008.12.004.

Prada, C.F., Álvarez-Velilla, R., Balaña-Fouce, R., Prieto, C., Calvo-Álvarez, E., Escudero-Martínez, J.M., Requena, J.M., Ordóñez, C., Desideri, A., Pérez-Pertejo, Y., and Reguera, R.M. (2013). Gimatecan and other camptothecin derivatives poison *Leishmania* DNA-topoisomerase IB leading to a strong leishmanicidal effect. Biochem. Pharmacol. *85*, 1433–1440. https://doi.org/10.1016/j.bcp.2013.02.024.

Prada, C.F., Álvarez-Velilla, R., Diaz-González, R., Prieto, C., Pérez-Pertejo, Y., Balaña-Fouce, R., and Reguera, R.M. (2012). A pentapeptide signature motif plays a pivotal role in *Leishmania* DNA topoisomerase IB activity and camptothecin sensitivity. Biochim. et Biophys. Acta, *1820*, 2062–2071. https://doi.org/10.1016/j.bbagen.2012.09.005.

Prada, C.F., Álvarez-Velilla, R., Díaz-Gozález, R., Pérez-Pertejo, Y., Balaña-Fouce, R., and Reguera, R.M. (2013). Identification and characterization of the regions involved in the nuclear translocation of the heterodimeric leishmanial DNA topoisomerase IB. PloS One, 8(9), e73565. https://doi.org/10.1371/journal.pone.0073565.

Ray, S., Hazra, B., Mittra, B., Das, A., and Majumder, H.K. (1998). Diospyrin, a bisnaphthoquinone: a novel inhibitor of type I DNA topoisomerase of *Leishmania donovani*. Mol. Pharmacol. *54*, 994–999. https://doi.org/10.1124/mol.54.6.994.

Ray, S., Sadhukhan, P.K., Mandal, N.B., Mahato, S.B., and Majumder, H.K. (1997). Dual inhibition of DNA topoisomerases of *Leishmania donovani* by novel indolyl quinolines. Biochem. Biophys. Res. Commun. *230*, 171–175. https://doi.org/10.1006/bbrc.1996.5874.

Riou, G.F., Gabillot, M., Douc-Rasy, S., Kayser, A., and Barrois, M. (1983). A type I DNA topoisomerase from *Trypanosoma cruzi*. Eur. J. Biochem. *134*, 479–484. https://doi.org/10.1111/j.1432-1033.1983.tb07592.x.

Romero, I.C., Saravia, N.G., and Walker, J. (2005). Selective action of fluoroquinolones against intracellular amastigotes of *Leishmania* (Viannia) *panamensis in vitro*. J. Parasitol. *91*, 1474–1479. https://doi.org/10.1645/GE-3489.1.

Roy, A., Das, B., Ganguly, A., Bose Dasgupta, S., Khalkho, N.V., Pal, C., Dey, S., Giri, V., Jaisankar, P., Dey, S., and Majumder, H.K. (2008). An insight into the mechanism of inhibition of unusual bi-subunit topoisomerase I from *Leishmania donovani* by 3, 3′-di-indolylmethane, a novel DNA topoisomerase I poison with a strong binding affinity to the enzyme. Biochem. J. *409*, 611–622. https://doi.org/10.1042/BJ20071286.

Saha, S., Acharya, C., Pal, U., Chowdhury, S.R., Sarkar, K., Maiti, N.C., Jaisankar, P., and Majumder, H.K. (2016). A novel spirooxindole derivative inhibits the Growth of *Leishmania donovani* parasites both *in vitro* and *in vivo* by targeting type IB Topoisomerase. Antimicrob. Agents Chemother. *60*, 6281–6293. https://doi.org/10.1128/AAC.00352-16.

Saha, S., Mukherjee, T., Chowdhury, S., Mishra, A., Chowdhury, S.R., Jaisankar, P., Mukhopadhyay, S., and Majumder, H.K. (2013). The lignan glycosides lyoniside and saracoside poison the unusual type IB topoisomerase of *Leishmania donovani* and kill the parasite both *in vitro* and *in vivo*. Biochem. Pharmacol. *86*, 1673–1687. https://doi.org/10.1016/j.bcp.2013.10.004.

Scocca, J.R., and Shapiro, T.A. (2008). A mitochondrial topoisomerase IA essential for late theta structure resolution in African trypanosomes. Mol. Microbiol. *67*, 820–829. https://doi.org/10.1111/j.1365-2958.2007.06087.x.

Sengupta, S., Ganguly, A., Roy, A., Bosedasgupta, S., D'Annessa, I., Desideri, A., and Majumder, H.K. (2011). ATP independent type IB topoisomerase of *Leishmania donovani* is stimulated by ATP: an insight into the functional mechanism. Nucleic Acids Res. *39*, 3295–3309. https://doi.org/10.1093/nar/gkq1284.

Sengupta, T., Mukherjee, M., Das, A., Mandal, C., Das, R., Mukherjee, T., and Majumder, H.K. (2005a). Characterization of the ATPase activity of topoisomerase II from *Leishmania donovani* and identification of residues conferring resistance to etoposide. Biochem. J. *390*, 419–426. https://doi.org/10.1042/BJ20042128.

Sengupta, T., Mukherjee, M., Mandal, C., Das, A., and Majumder, H.K. (2003). Functional dissection of the C-terminal domain of type II DNA topoisomerase from the kinetoplastid hemoflagellate *Leishmania donovani*. Nucleic Acids Res. *31*, 5305–5316. https://doi.org/10.1093/nar/gkg727.

Sengupta, T., Mukherjee, M., Das, R., Das, A., and Majumder, H.K. (2005b). Characterization of the DNA-binding domain and identification of the active site residue in the 'Gyr A' half of *Leishmania donovani* topoisomerase II. Nucleic Acids Res. *33*, 2364–2373. https://doi.org/10.1093/nar/gki527.

Shapiro, T.A., and Englund, P.T. (1995). The structure and replication of kinetoplast DNA. Annu. Rev. Microbiol. *49*, 117–143. https://doi.org/10.1146/annurev.mi.49.100195.001001.

Simonson, A.B., Servin, J.A., Skophammer, R.G., Herbold, C.W., Rivera, M.C., and Lake, J.A. (2005). Decoding the genomic tree of life. Proc. Natl. Acad. Sci. U.S.A. *102* (Suppl. 1), 6608–6613.

Singh, G., and Dey, C.S. (2007). Induction of apoptosis-like cell death by pentamidine and doxorubicin through differential inhibition of topoisomerase II in arsenite-resistant *L. donovani*. Acta Trop.*103*, 172–185. https://doi.org/10.1016/j.actatropica.2007.06.004.

Singh, G., Jayanarayan, K.G., and Dey, C.S. (2005). Novobiocin induces apoptosis-like cell death in topoisomerase II over-expressing arsenite resistant *Leishmania donovani*. Mol. Biochem. Parasitol. *141*, 57–69. https://doi.org/10.1016/j.molbiopara.2005.01.014.

Singh, M.K., Bhaumik, S.K., Karmakar, S., Paul, J., Sawoo, S., Majumder, H.K., and Roy, A. (2017). Copper salisylaldoxime (CuSAL) imparts protective efficacy against visceral leishmaniasis by targeting *Leishmania donovani* topoisomerase IB. Exp. Parasitol. *175*, 8–20. https://doi.org/10.1016/j.exppara.2017.02.010.

Slunt, K.M., Grace, J.M., Macdonald, T.L., and Pearson, R.D. (1996). Effect of mitonafide analogs on topoisomerase II

of *Leishmania chagasi*. Antimicrob. Agents Chemother. *40*, 706–709.

Stewart, L., Ireton, G.C., and Champoux, J.J. (1997). Reconstitution of human topoisomerase I by fragment complementation. J. Mol. Biol. *269*, 355–372. https://doi.org/10.1006/jmbi.1997.1056.

Stewart, L., Redinbo, M.R., Qiu, X., Hol, W.G., and Champoux, J.J. (1998). A model for the mechanism of human topoisomerase I. Science *279*, 1534–1541. https://doi.org/10.1126/science.279.5356.1534.

Stoll, G., Pietiläinen, O.P., Linder, B., Suvisaari, J., Brosi, C., Hennah, W., Leppä, V., Torniainen, M., Ripatti, S., Ala-Mello, S. *et al.* (2013). Deletion of TOP3β, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders. Nature Neurosci. *16*(9), 1228–1237. https://doi.org/10.1038/nn.3484.

Strauss, P.R., and Wang, J.C. (1990). The TOP2 gene of *Trypanosoma brucei*: a single copy gene that shares extensive homology with other TOP2 genes encoding eukaryotic DNA topoisomerase II. Mol. Biochem. Parasitol. *38*, 141–150.

Tejería, A., Pérez-Pertejo, Y., Reguera, R.M., Balaña-Fouce, R., Alonso, C., Fuertes, M., González, M., Rubiales, G., and Palacios, F. (2016). Antileishmanial effect of new indeno-1, 5-naphthyridines, selective inhibitors of *Leishmania infantum* type IB DNA topoisomerase. Eur. J. Med. Chem. *124*, 740–749. https://doi.org/10.1016/j.ejmech.2016.09.017.

Teles, C.B.G., Moreira-Dill, L.S., de Almeida Silva, A., Facundo, V.A., de Azevedo, W.F., da Silva, L.H.P., Motta, M.C.M., Stábeli, R.G., and Silva-Jardim, I. (2015). A lupane-triterpene isolated from *Combretum leprosum* Mart. fruit extracts that interferes with the intracellular development of *Leishmania (L.) amazonensis in vitro*. BMC Complement. Alt. Med. *15*, 165. https://doi.org/10.1186/s12906-015-0681-9.

Tsai-Pflugfelder, M., Liu, L.F., Liu, A.A., Tewey, K.M., Whang-Peng, J., Knutsen, T., Huebner, K., Croce, C.M., and Wang, J.C. (1988). Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21–22. Proc. Natl. Acad. Sci. U.S.A. *85*, 7177–7181.

Velez-Cruz, R., and Osheroff, N.D. (2004). DNA topoisomerases: type II. Encyclopedia of Biological Chemistry, 1, 806À811.

Vennerstrom, J.L., Lovelace, J.K., Waits, V.B., Hanson, W.L., and Klayman, D.L. (1990). Berberine derivatives as antileishmanial drugs. Antimicrob. Agents Chemother. *34*, 918–921.

Villa, H., Otero Marcos, A.R., Reguera, R.M., Balaña-Fouce, R., García-Estrada, C., Pérez-Pertejo, Y., Tekwani, B.L., Myler, P.J., Stuart, K.D., Bjornsti, M.A., and Ordóñez, D. (2003). A novel active DNA topoisomerase I in *Leishmania donovani*. J Biol. Chem. *278*, 3521–6. https://doi.org/10.1074/jbc.M203991200.

Walker, J., and Saravia, N.G. (2004). Inhibition of *Leishmania donovani* promastigote DNA topoisomerase I and human monocyte DNA topoisomerases I and II by antimonial drugs and classical antitopoisomerase agents. J. Parasitol. *90*, 1155–1162. https://doi.org/10.1645/GE-3347.

Wang, H., Di Gate, R.J., and Seeman, N.C. (1996). An RNA topoisomerase. Proc. Natl. Acad. Sci. U.S.A. *93*, 9477–9482.

Wang, J.C. (1994). DNA topoisomerases as targets of therapeutics: an overview. Adv. Pharmacol. 29A, 1–19.

Wang, J.C. (2002). Cellular roles of DNA topoisomerases: a molecular perspective. Nat. Rev. Mol. Cell Biol. *3*, 430–440. https://doi.org/10.1038/nrm831.

Wang, Z., and Englund, P.T. (2001). RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. EMBO J. *20*, 4674–4683. https://doi.org/10.1093/emboj/20.17.4674.

Werbovetz, K.A., Lehnert, E.K., Macdonald, T.L., and Pearson, R.D. (1992). Cytotoxicity of acridine compounds for *Leishmania* promastigotes *in vitro*. Antimicrob. Agents Chemother. *36*, 495–497.

Werbovetz, K.A., Spoors, P.G., Pearson, R.D., and Macdonald, T.L. (1994). Cleavable complex formation in *Leishmania chagasi* treated with anilinoacridines. Mol. Biochem. Parasitol. 65(1), 1–10.

Xu, D., Shen, W., Guo, R., Xue, Y., Peng, W., Sima, J., Yang, J., Sharov, A., Srikantan, S., Yang, J., *et al.* (2013). Top3β is an RNA topoisomerase that works with fragile X syndrome protein to promote synapse formation. Nat. Neurosci. *16*, 1238–1247. https://doi.org/10.1038/nn.3479.

Yang, G., Choi, G., and No, J.H. (2016). Antileishmanial Mechanism of Diamidines Involves Targeting Kinetoplasts. Antimicrob. Agents Chemother. 60(11), 6828–6836. https://doi.org/10.1128/AAC.01129-16.

Yang, X., Li, W., Prescott, E.D., Burden, S.J., and Wang, J.C. (2000). DNA topoisomerase IIbeta and neural development. Science *287*, 131–134. https://doi.org/10.1126/science.287.5450.131.

Zuma, A.A., Cavalcanti, D.P., Maia, M.C., De Souza, W., and Motta, M.C. (2011). Effect of topoisomerase inhibitors and DNA-binding drugs on the cell proliferation and ultrastructure of *Trypanosoma cruzi*. Int. J. Antimicrob. Agents, 37, 449–56, https://doi.org/10.1016/j.ijantimicag.2010.11.031.