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Invited Review

Trypanosomatids topoisomerase re-visited. New structural findings and role in drug discovery

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ABSTRACT

The Trypanosomatidae family, composed of unicellular parasites, causes severe vector-borne diseases that afflict human populations worldwide. Chagas disease, sleeping sickness, as well as different sorts of leishmaniases are amongst the most important infectious diseases produced by *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., respectively. All these infections are closely related to weak health care services in low-income populations of less developed and least economically developed countries. Search for new therapeutic targets in order to hit these pathogens is of paramount priority, as no effective vaccine is currently in use against any of these parasites. Furthermore, present-day chemotherapy comprises old-fashioned drugs full of important side effects. Besides, they are prone to produce tolerance and resistance as a consequence of their continuous use for decades. DNA topoisomerases (Top) are ubiquitous enzymes responsible for solving the torsional tensions caused during replication and transcription processes, as well as in maintaining genomic stability during DNA recombination. As the inhibition of these enzymes produces cell arrest and triggers cell death, Top inhibitors are among the most effective and most widely used drugs in both cancer and antibacterial therapies. Top relaxation and decatenation activities, which are based on a common nicking–closing cycle involving one or both DNA strands, have been pointed as a promising drug target. Specific inhibitors that bind to the interface of DNA-Top complexes can stabilize Top-mediated transient DNA breaks. In addition, important structural differences have been found between Tops from the Trypanosomatidae family members and Tops from the host. Such dissimilarities make these proteins very interesting for drug design and molecular intervention. The present review is a critical update of the last findings regarding trypanosomatid's Tops, their new structural features, their involvement both in the physiology and virulence of these parasites, as well as their use as promising targets for drug discovery.

Contents

Abbreviations: Top, DNA topoisomerase; CL, cutaneous leishmaniasis; VL, visceral leishmaniasis; DALYs, disability-adjusted life years; kDNA, kinetoplast DNA; NTD, neglected tropical diseases; NGO, non-governmental organization; lk, linking number; NLS, Nuclear Localization Signal.

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1. Introduction

The family Trypanosomatidae (order Kinetoplastida) comprises several unicellular flagellated-protozoan parasites responsible for many human infectious diseases in low-income populations of less developed and least economically developed countries (WHO, 2008). Two species of the genus *Trypanosoma*, *Trypanosoma brucei* (subspecies *gambiense* and *rhodesiense*) and *Trypanosoma cruzi*, as well as the different species of the genus *Leishmania*, are collectively known as the "tritryps". They are responsible for some of the most harmful neglected diseases that were tackled by WHO in the 2003 meeting held in Berlin. Human African trypanosomiasis, sleeping sickness or Congo trypanosomiasis is a lethal infection caused by *T. brucei*, which is transmitted by tse–tse flies (Glossina) in Sub-Saharan African countries. Sleeping sickness is a biphasic disease. The first phase is clinically characterized by the presence of fever, headache, joint pain and itching. Whilst in the second one, which can be lethal if left untreated, the parasite causes anaemia and neurological symptoms owing to the aggressive pathogen-invasion of the host central nervous system (Lejon et al., 2013). American trypanosomiasis (Chagas disease), caused by *T. cruzi* in Central and South American countries, is transmitted by the faeces of blood-sucking "kissing bugs" of the subfamily Triatominae. In addition, indirect transmission through blood transfusions or organ transplantation from infected patients has also been reported. Chagas disease symptoms may vary during the course of infection. Local swelling can be noticed in early stages, but disease chronification leads to a cardiomyopathy, which may result in sudden death. The occurrence of damage in the gastrointestinal tract is also very common, with the presence of megacolon and megaesophagus, thereby leading to an inevitable weight loss (Teixeira et al., 2011). Leishmaniasis is transmitted by sandflies of the genera *Phlebotomus* and *Lutzomyia*. Cutaneous leishmaniasis (CL), whose etiological agent is *Leishmania major* in the Old World – among other species – and *Leishmania panamensis* and *Leishmania braziliensis* in the New World, should be considered the mildest clinical presentation of this disease. CL presents with erythema, edema and permanent ulcers at the bite site. Although CL is not fatal, it is extremely disfiguring when located on the host's face. American tegumentary leishmaniasis (also known as mucocutaneous leishmaniasis) arises as a consequence of an inadequately treated New-World CL (WHO, 2007). This clinical form presents with severe facial disfiguration, including nose and lips, which can derive to cancer processes. Last, there is a very aggressive visceral form of leishmaniasis (VL) produced by *Leishmania donovani* in Asia and *Leishmania infantum* in the countries of the Mediterranean basin. VL produces organ swelling (specifically targeting liver and spleen) and may be deadly if left untreated (Ashford, 2000; Murray et al., 2005; Antinori et al., 2012).

The impact of these diseases can be measured in terms of prevalence, fatal outcomes, as well as disability-adjusted life years (DALYs) lost as a direct consequence of both pathologic effect and treatments (Goto and Lindoso, 2010). Sleeping sickness is an infection exclusively concentrated in South-Saharan countries. Nowadays, this scourge is prevalent in 36 countries, whose endemic infection involves around 60-million people at risk (Aksoy et al., 2003). The number of reported cases was estimated to be *ca.* 10,000 but it represented only one-third of the real infections occurred in 2009. In addition, an estimated annual DALYs lost of 1.7-million has a profound impact in the economy of these countries (Simarro et al., 2011). For its part, Chagas disease has presence in 18 Central and South American countries with 17 million individuals currently infected and 100 million people at risk. Statistical annual records show the emergence of 41,200 new cases every year, as well as 12,500 related deaths. Chagas calculated DALYs reach annual values over 570,000 (Moncayo and Silveira, 2009). Lastly, leishmaniases are prevalent in 98 countries with presence in four continents (Oceania is the only one free of these maladies) with approximately 360-million people at risk. Chagas disease has resurged in USA and Europe due to the incoming tourism and migratory fluxes from prevalent countries of South America (Schmunis, 2007; Gascon et al., 2010). The annual estimation of leishmaniasis is close to 1.6 new million cases each year, 20,000–40,000 of which become fatal. Over 50% of the 2-million worldwide-appraised DALYs is mainly focused in South-East Asia, with Bihar (India) as the epicenter of *Leishmania*-related deaths (Alvar et al., 2012). VL is a re-emerging opportunistic disease in immunosuppressed patients owing to three main facts: (i) the immunosuppressive effect of drugs used in organ transplantation; (ii) as a consequence of HIV infections in South-European countries or (iii) climate change due to global warming (Alvar et al., 2008; van Griensven et al., 2014; Koltas et al., 2014).

Although prevention is considered the best approach to fight against these diseases, any possible vaccine is in a very early stage of development. The main reason is that these infections cause a deregulation of the host immune system. In fact, it has been shown that both human patients, as well as experimental animals, become susceptible to infections caused by these pathogens when Th1/Th2 balance shifts toward Th2-mediated humoral immunity (Alexander and Bryson, 2005). However, when there is a shift toward Th1-dominant immune responses the host becomes resistant and develops (life-span) natural immunity against re-infection. Nowadays, some new therapies take advantage of this circumstance to treat these infectious diseases by means of Th1 stimulants or auto vaccines developed from infected hosts. However, these promising approaches are being only developed for *Leishmania*-infected dogs but not for humans, making chemotherapy the most feasible alternative against these neglected tropical diseases (NTDs) at present (for a comprehensive review see: Palatnik-de-Sousa, 2012).

Current chemotherapy against these diseases was developed many years ago, is plenty of toxic side effects and it often requires parenteral treatments. These inconveniences make the use of this chemotherapy in field conditions difficult, and since the administration of repeated doses is needed, the appearing of relapses is extremely frequent due to a poor adherence to treatments (Leprohon et al., 2011). African trypanosomiasis is a good example of these statements. Pentamidine (an aromatic diamidine) and suramin are the chosen drugs against the first blood-stage infection (Burri, 2010). However, when the neurological phase is established, only arsenicals (melarsoprol) or the ornithine decarboxylase suicide inhibitor eflornithine can ameliorate the symptoms (Lutje et al., 2013). Recently, the combination of nifurtimox and eflornithine has prompted the change from the old-fashioned melarsoprol to the best treatment available (Simarro et al., 2012). For the Chagas disease, the current pharmacopoeia is reduced to two drugs, nifurtimox and benznidazole, with a 60–90 days long treatment and with noticeable side effects (Rassi et al., 2010).

First choice drugs against leishmaniases are old-fashioned pentavalent antimonium-based drugs (Glucantime and Pentostam),

which are the current therapy against these diseases since the 50s all over the World (Balaña-Fouce et al., 1998). These drugs have several flaws, including the need of parenteral administration, the occurrence of many undesirable side effects, as well as the appearing of resistances due to the over-usage (Croft and Olliaro, 2011). Drug combinations of antimonials with allopurinol (a xanthine oxidase inhibitor) very much improve the curative outcomes (Manna et al., 2009). In a second instance, macrolide antibiotics such as Amphotericin B, are a good alternative to the former compounds (Monge-Maillo and López-Vélez, 2013). This antibiotic formulated in liposomes (AmBisome) has resulted in a good therapy against VL, but it is extremely expensive for developing countries and requires intravenous administration (Mohamed-Ahmed et al., 2012). Lipid-ether compounds – miltefosine and edelfosine – are promising oral drugs first synthesized as anti-tumourals but with potent anti-leishmanial effect (Bhattacharya et al., 2007; Berman, 2008). For its part, paromomycin – an aminoglycoside antibiotic – has been approved in 2006 in India against VL (Sundar et al., 2007). These compounds require repeated dosing during 20 days or more, and with the exception of miltefosine, they must be parenterally administered to the patients.

At this point, the need of new drugs against these diseases is clearly justifiable (Barrett and Croft, 2012). However, since NTDs mostly affect low-income countries, the expectable economic profits of the big pharmaceutical trusts are negligible compared to those obtained with other pathologies of the more developed World. Therefore, basic academic research groups have assumed new drug target discovery and drug design research. Interestingly, drug discovery initiatives (i.e., DNDi), promoted by world-wide NGOs and supported by public and private funds are aimed to eliminate NTDs during the current decade. Meanwhile, Glaxo Smith Kline has done the economic effort of offering thousands of compounds from different drug libraries to be tested by basic research groups in order to find new hits against NTDs pathogens.

Within the novel targets for drug intervention, DNA topoisomerases (Top) have attracted attention since their discovery. The role played by Top in cell physiology is of paramount importance and since the beginning many research groups pointed to these enzymes as putative targets against proliferative processes. This is the reason why some Top inhibitors are being marketed against cancer (Pommier et al., 2010) or as promising antiparasitic drugs (Das et al., 2008; García-Estrada et al., 2010).

In the present review we update novel information about Top enzymes in trypanosomatids, making special emphasis in the novel findings on their peculiar structure as well as in the molecules used to target them.

2. DNA topoisomerases

Since the length of the DNA helix containing the genetic information of an organism is several millions times longer than the size of the cellular nucleus, it is a physical pre-requisite to fold it thousands of times in order to fit it inside this organelle (Annunziato, 2008). DNA packaging can be solved by simple folding of the circular chromosome in prokaryotic organisms, or can be condensed around nucleosomes, where histones play a key role in gene expression and structure of eukaryotic chromosomes. Trypanosomatids are ancient eukaryotes that contain a unique disc-shaped extranuclear DNA called kinetoplast inside their single mitochondrion. Adjacent to the flagellar basal body there is an intricate mesh of thousands of interlocked DNA rings called mini- and maxicircles that represents 10 to 20% of the total DNA in the cell (Shapiro and Englund, 1995; Chen et al., 1995a). Minicircles are the most abundant form in kDNA (5.000–10.000 molecules per cell). They are circular DNA molecules with a length perimeter of 100–2500 bp encoding guide RNAs involved in editing mitochondrial nascent RNAs (Stuart and Panigrahi, 2002). Maxicircles are bigger in perimeter – 30,000–50,000 bp – but less abundant than minicircles – 50 copies per cell. Maxicircles are the equivalent of mitochondrial DNA from other eukaryotes, as they codify mitochondrial proteins, ribosomal RNA and tRNAs (Shlomai, 2004). Replication of kDNA occurs synchronously with genomic DNA during cell division. kDNA components must be decatenated from the network before their DNA replication and then catenated again to the nascent mesh when this process is finished (Liu et al., 2005). DNA replication from both mini- and maxicircles takes place in theta (θ)-shaped intermediaries, where Top play a key role (Jensen and Englund, 2012). Since kinetoplast is unique to trypanosomatids, it has been pointed as an important target for drug discovery.

DNA appears in nature as underwound form, acquiring a compact negatively supercoiled organization that is not accessible to most physiological cell processes when needed. For this reason, during DNA replication or transcription to RNA, as well as during DNA repair, DNA supercoils should be resolved to a more relaxed form that permits access to polymerases, transcriptional factors or DNA repair proteins. Top are the proteins responsible for relaxing supercoiled DNA introducing breaks in single or both strands of the double helix (Champoux and Dulbecco, 1972). At this moment, DNA can rotate freely alleviating the tension accumulated by supercoiling, powering the rotation of one strand over the other. This task can be fuelled by ATP hydrolysis or not. Since these enzymes are molecular machines, a support of energy should be necessary to exert the mechanical function. According to these characteristics; the number of DNA strand breaks introduced and the requirement of metabolic energy, Top are divided into two Families. Type I Top (TopI) are ATP-independent enzymes that break one DNA strand, whereas Type II Top (TopII) need ATP hydrolysis to break both DNA strands (Champoux, 2001). Differences in the mode of action suppose differences in changes introduced in DNA topology. TopI are solely involved in the relaxation of supercoiled DNA (Cretaio et al., 2007). TopII, however, are not only involved in supercoiled DNA relaxation, but they are also implicated in the catenation/decatenation and knotting/unknotting of complex DNA topological networks (for reviews see: Wang, 1998, 2002, 2009; Champoux, 2001; Forterre et al., 2007; Pommier et al., 2010).

TopIA was considered, since long time ago, as a prokaryotic-borne enzyme that resolves positive supercoils through the transient covalent binding to the 5′-end of the broken DNA strand followed by passage and rejoining (Corbett and Berger, 2004). Regardless TopIA, TopIB is a true Mg^{2+}-independent eukaryotic enzyme that introduces both positive and negative supercoils, establishing a temporary covalent bond to the 3′-end of cut DNA releasing a 5′-OH free end (Cheng et al., 1998). The catalytic Tyr at the active site of TopIB introduces a nucleophilic attack on a phosphate group of the nucleotide chain, creating a transient phosphodiester bond with the broken DNA strand (Stewart et al., 1998; Pommier, 2009). This allows TopIB to change the linking number (Lk) in multiples of 1 (n), unlike TopIA, which introduces changes strictly in steps of one in the Lk of a single-stranded circular DNA (Lindsley, 2005).

TopII are multimeric proteins that break both DNA strands fuelled by the hydrolysis of ATP. TopII bind transiently to the 5′-ends of both DNA strands relaxing positive and negative supercoils, changing the Lk in steps of +2 (Watt and Hickson, 1994). The transient break at both DNA strands permits the passage of another duplex through the gap and subsequent rejoining. TopII enzymes relax positively supercoiled DNA, but also have additional catenation/decatenation and knotting/unknotting activities that make them needed for chromosomal segregation in the nucleus and for

kDNA disassembly in individual minicircles in the kinetoplast during cell division (Chen et al., 1995b).

A common feature to all Top enzymes is the presence of a Tyr residue in the catalytic site of the enzyme (Champoux, 2001). Tyr is the amino acid that produces a nucleophilic attack to the phosphodiester DNA backbone introducing a transient covalent bond with the enzyme. This transient bonding has been exploited to design compounds able to target it. Since the time required to religate cleaved DNA is extremely short, many compounds act stabilizing this enzymatic step introducing an enzyme-DNA adduct that undergoes irreversible damage (Hsiang et al., 1985). When the replication fork collides with one of these stabilized complexes, a double-strand break can be produced, starting the irreversible fragmentation (Hsiang et al., 1989). Since the stabilization of DNA-Top complexes can have a natural origin, eukaryotic cells have developed a DNA repair systems that act removing these tyrosyl-DNA bonds. Tyrosyl-DNA-phosphodiesterase-1 (TDP1) has been regarded as a potential therapeutic co-target of TopIB (Dexheimer et al., 2008; Banerjee et al., 2010).

A full set of Top enzymes is currently available in the TriTryp Genome database http://tritrypdb.org/tritrypdb/; (i) nuclear TopIB and TopIIA enzymes involved in DNA replication and repair that have been fully described as putative drug targets; (ii) mitochondrial TopIA and TopII that have been described closely linked to kDNA replication; (iii) two putative members of the TopIA subfamily TopIII (α and β) involved in resolving DNA entanglements that can arise during DNA recombination (Table 1).

2.1. Type IB topoisomerases

Unlike other enzymes characterized to date, kinetoplastid TopIB is a heterodimer (Reguera et al., 2008). The first description was done in *L. infantum* where the functional bisubunit enzyme was composed of a large (L) subunit of 635 amino acids with a MW of 73 kDa (LiTopIL), which contains the N-terminal and core domains, and a small (S) subunit of 262 amino acids with a MW of 28 kDa (LiTopIS), which retains the Tyr catalytic residue. Genes encoding these proteins are located on different chromosomes in the leishmanial genome (Villa et al., 2003). In this very year, similar results were published in African trypanosomes using a classical platform of protein purification. However, a controversy about the oligomeric status (L_2S_2) is still under debate (Bodley et al., 2003). It seems that this unique genomic organization, in which the genes encoding both TopIB protomers are located on different chromosomes, occurs solely in trypanosomatids (Reguera et al., 2006).

Multiple alignments of DNA sequences and X-ray analysis of a co-crystal containing a truncated LdTopIB bound to nick double stranded DNA at a resolution of 2.27 Å, permitted the identification of specific protein domains in the leishmanial enzyme (Davies et al., 2006). The large LdTopIL subunit contains a short and poorly conserved N-terminal domain, whose function has not yet been clarified so far. The central core domain conserves a high homology with other TopIB proteins (Stewart et al., 1996; Cretaio et al., 2007). It contains several amino acids interacting with DNA; i.e., Arg314 (Arg488 in the human enzyme), Lys352 (Lys532 in the human enzyme), Arg410 (Arg590 in the human enzyme) and His453 (His632 in the human enzyme) (Diaz-González et al., 2008). Finally, this subunit displays a C-terminal extension, which lacks homology with other ortholog proteins (Diaz-Gonzalez et al., 2007a). The small LdTopIS subunit contains a long and non-conserved N-terminal extension enriched in Ser residues, which is susceptible of post-translational phosphorylation. However, the C-terminal domain conserves high homology with the SKINY signature, where Tyr222 (Tyr723 in human enzyme) is the fifth amino acid of the catalytic pentad that plays a role in the nucleophilic attack to DNA. The functionality of these amino acids in the leishmanial enzyme has been confirmed by single site mutation analysis and expression in a TopIB-null yeast mutant (Diaz-Gonzalez et al., 2007b) (Fig. 1A).

However, the 3D model obtained from the X-ray analysis fails in describing the linker domain, a poorly conserved region that connects the core and C-terminus end, because it was removed to prepare the crystal (Davies et al., 2006). The linker domain is not merely a connector but it contributes to both DNA binding and sensitivity to TopIB poisons, probably increasing the rigidity of the structure and slowing down the rotation process prior to the religation of the cleaved DNA strand (Stewart et al., 1997). A recent study has disclosed the regions of both subunits that interact to each other to constitute a functional linker in *Leishmania* (Prada et al., 2012). Using a predictive 3D model combined with a sequential set of internal and lineal truncations on both monomers, the authors were able to identify within the N-extension domain of LdTopIS a conserved heptapeptide motif (RPPVVRS), which is spatially close enough to interact with a region (amino acids 525–581) of the large LdTopIL subunit. Remarkably, the pentapeptide RPPVV introduces a loop in the secondary structure of LdTopIS that permits the interaction with the large subunit. These results agree well with those reported by Stewart and co-workers (1999), who reconstituted enzyme species from hTopIB fragments that lack the linker domain and observed that any alteration on this region produces modifications in the religation step and an impairment to be poisoned by Top inhibitors (Ireton et al., 2000; Fiorani et al., 2003).

TopIB are nucleus-operating enzymes that need to display recognizable Nuclear Localization Signals (NLS) for nucleo-cytoplasmic trafficking. Three NLS motifs have been recently identified in LdTopIB by sequential deletion analyses of the genes encoding the two subunits fused to the GFP-encoding ORF (Prada et al., 2013b). NLS1 is located at the C-terminal extension of LdTopIL and consists of a 44-amino acids region enriched in Lys residues (easily identifiable by nuclear importins). NLS2 and NLS3 are located at the N-terminal and C-terminal domains of the small LdTopIL subunit, respectively. NLS2 is a well-identified 10-amino acids signature preceding a well-conserved cluster of Ser in trypanosomatids. This fact is important since this NLS requires at least one Ser residue to be functional; suggesting that hypothetical phosphorylation of Ser is necessary for nuclear localization (Teng et al., 2011). Finally, NLS3 is a 28-amino acid region that includes the catalytic Tyr222. The amino acid sequence of NLS3 bears no resemblance at all to other canonical NLS signals described so far. However, it looks like the PY-NLSs signals proposed by Lee and co-workers (2006) in human proteins, where proximal Pro and Tyr residues are recognized by a β-importin to drive the protein inside the nucleus. Furthermore, regardless previous results (BoseDasgupta et al., 2008), it was demonstrated that both sub-

Table 1
Tritryps Top-encoding genes annotated into GeneDB database (http://www.genedb.org/Homepage). TopIA, TopIB (large and small subunits), TopII and TopIII genes from different species: *L. major* Friendlin, *T. brucei* 927 and *T.cruzi*.

Protein	*L. major* Friendlin	*T. brucei* 927	*T. cruzi*
GeneDB database			
TopIA	LmjF21.0125	Tb927.10.1900	TcCLB.510121.160
TopIB (L)	LmjF34.3440	Tb927.4.1330	TcCLB.508693.20
TopIB (S)	LmjF04.0060	Tb927.9.6940	TcCLB.506625.110
TopII	LmjF28.2280	Tb927.11.11550	TcCLB.508699.10
		Tb927.11.11560	TcCLB.509203.70
mtTopII	LmjF.15.1290	Tb927.9.5590	TcCLB.508277.370
TopIII	LmjF28.1780	Tb927.11.9170	TcCLB.510901.100
	LmjF36.3200	Tb927.11.9520	TcCLB.511589.120
			TcCLB.508851.170

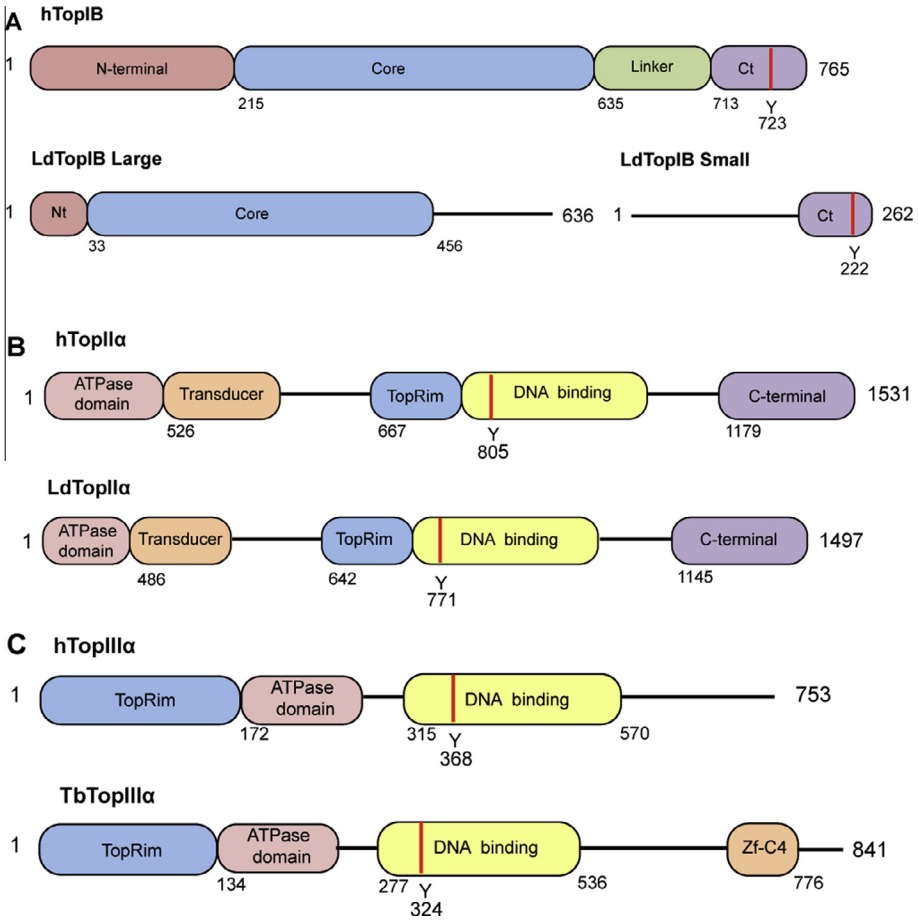

Fig. 1. Schematic lineal representations of TopIB, TopII and TopIII from tritryps compared to human counterparts. (A) Human (top) and bisubunit *L. donovani* (down) TopIB, structural domains: Non-conserved hydrophilic N-terminal domain (Nt); central "core" domain with the representative active site amino acids ("tetrad"); non-conserved "linker" subdomain that connects "core" and the C-terminal domain and C-terminal domain (Ct) containing the DNA cleaving Tyr (red strip). (B) Human (top) and *L. donovani* TopII α (down) structural domains: N-terminal domain containing the ATPase and transducer domains; the Mg^{2+} binding site at the TopRim motif; a DNA binding that contains the DNA cleaving Tyr (red strip); C-terminal domain. (C) Human (top) and *T. brucei* TopIII α (down) structural domains: N-terminal domain containing the Mg^{2+} binding site at the TopRim motif; the ATPase domain and C-terminal domain; DNA binding site that contains the DNA cleaving Tyr (red strip); Znf-C4 (zinc-finger domain). Distances represented in the drawings are not in scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

units can reach the nuclear compartment independently (Prada et al., 2013b). Although some authors pointed to dual nucleus/kinetoplast LdTopIB localization, no specific mitochondrial localization signals have been identified in any of the subunits of the dimer. In addition, none of the chimeras fused to GFP co-localize with kDNA, thus proving that none of LdTopIB subunits are bound to DNA minicircles, which in fact rules out the hypothesis of a TopIB operating in the kinetoplast (Das et al., 2006a,b).

2.2. Type II topoisomerases

Type II topoisomerases (Top II) are ubiquitous and essential enzymes required for the regulation of DNA topology (Buck and Zechiedrich, 2004). Top II are multimeric enzymes that hydrolyze ATP to generate transient phosphotyrosine bonds at the 5′ end of both broken strands of the DNA helix. After cutting, the ends of the DNA are separated and another DNA duplex is passed through this break, which is finally religated (reviewed in Berger et al., 1996; Wang, 1996; Champoux, 2001).

Type II enzymes are organized into two families, Top IIA and IIB. The former includes, among others, the homodimeric (A_2) eukaryotic Top II, which relaxes positively supercoiled DNA. Type IIB topoisomerases are heterotetrameric (A_2B_2) enzymes that only include one family member, topoisomerase VI. Although its role is less understood, it shares several features with the bacterial type IIA topoisomerase (reviewed in Nitiss, 2009).

Eukaryotic Top II enzymes participate in the segregation and maintenance of the chromosome structure (Gasser et al., 1986; Rose et al., 1990) and are involved in chromosome condensation and decondensation events (Adachi et al., 1991). In trypanosomatids, besides the essential role in DNA nuclear metabolism Top II are also important for the segregation, replication, remodeling and maintenance of kDNA (Shapiro, 1994; Shapiro and Showalter, 1994; Lindsay et al., 2008). Mitochondrial TopII (mtTopII) is of paramount importance to disaggregate kDNA network during cell division in kinetoplastids previous to cell division (Jensen and Englund, 2012). Early studies carried out with the antitumour drug etoposide in *T. brucei*, showed that replication of free minicircles was disrupted by the drug, giving rise to multiple interlocked minicircle-dimers that contained newly synthesized DNA (Shapiro et al., 1989). In fact, a loss in kDNA network (diskinetoplasty) and subsequent cell death was reported upon the induction of an RNAi-mediated silencing event in mtTop II, a phenomenon that was preceded by a gradual shrinkage of the network and accumulation of gapped free minicircle replication intermediates (Wang and Englund, 2001). There are other likely functions for mtTopII, such as the mending of kDNA after releasing minicircles for replication and the reattachment of nicked or gapped minicircles to the

periphery of the replicating network (Lindsay et al., 2008), where a type I Top enzyme could also play a critical role (Scocca and Shapiro, 2008).

Only one mtTop II, localized primarily to the antipodal sites of the kinetoplast (Melendy et al., 1988), is predicted after the analysis of the genome sequence of several trypanosomatids (Strauss and Wang, 1990; Fragoso and Goldenberg, 1992; Das et al., 2001; Hanke et al., 2003; De Sousa et al., 2003). In addition, two other Top II enzymes, which are encoded by the tandemly-linked single-copy genes Top II α and Top II β, were found in the chromosome 11 of *T. brucei* (Kulikowicz and Shapiro, 2006). These Top II (74% identical and differing only in the C-terminal region) share a common node with other nuclear topoisomerases. The latter are clearly different from the clade that includes the mtTop II, which is positioned near the DNA gyrase branch that is more closely related to the prokaryotic type II enzymes. RNAi-mediated silencing of the ATP-dependent nuclear Top II α in *T. brucei* caused abnormalities in nuclear DNA and cell growth arrest at early stages of cell cycle, pointing to this protein as an attractive therapeutic target (Wang and Englund, 2001). Although the localization and function of the putative Top II β protein is unknown, it is phylogenetically related to other eukaryotic nuclear Top II (Kulikowicz and Shapiro, 2006). These researchers produced knock-down mutants in the gene encoding TbTopII β showing that there was no detectable effect on growth rate, remaining its function in trypanosomatids still unclear.

Regarding other trypanosomatids, a single ortholog of Top II α β (but not of TopII β) is present in the *L. major* chromosome 28. Although two additional ORFs were found in *T. cruzi*, they appeared to be alleles of the same gene rather than α and β isoforms of Top II (Kulikowicz and Shapiro, 2006).

Top IIA activity has been purified from several trypanosomatids and some of the corresponding coding genes have been cloned from *Crithidia fasciculata* (Shlomai and Zadok, 1983; Melendy et al., 1988; Melendy and Ray, 1989; Pasion et al., 1992; Hines and Ray, 1997), *T. brucei* (Strauss and Wang, 1990; Wang and Englund, 2001; Kulikowicz and Shapiro, 2006), *T. cruzi* (Douc-Rasy et al., 1986; Fragoso et al., 1998), *Trypanosoma equiperdum* (Shapiro, 1994), *L. donovani* (Chakraborty and Majumder, 1987, 1991; Das et al., 2001), *Leishmania chagasi* (De Sousa et al., 2003) and *L. infantum* (Hanke et al., 2003).

Basically, trypanosomatids TopII α are homodimeric proteins, each subunit ranging in size from 137 to 160 kDa. Similarly to other eukaryotes, each subunit is constituted by the fusion of domains from bacterial DNA gyrase (GyrB and GyrA) and TopIV (ParE and ParC) building a GyrB/ParE fused-domain at the N-terminal-end and a GyrA/ParC fused-domain at the C-terminal-end, respectively (Champoux, 2001).

Three structural domains, namely ATPase, central DNA binding (B′ and A′), and C-terminal domain, which are present in a single polypeptide chain, constitute eukaryotic Top II. These domains are connected to each other by a flexible hinge region (Sengupta et al., 2003). The ATPase domain is found at the enzyme N-terminal end, which spans the first 489 amino acids in *T. brucei* and the first 384 amino acids in *L. donovani* (Sengupta et al., 2005a,b). ATP binding to the nucleotide-binding cassette promotes the dimerization of the N-terminal ATPase domains, which control the open/close movements of the protein that are involved in DNA releasing (Wei et al., 2005). Following the ATPase domain the core region comprises the B′ domain (Toprim or unusual Rossman fold that contains the constant Asp-X-Asp motif and coordinates two Mg^{2+} ions per each subunit) (Aravind et al., 1998) and the N-terminal region of A′ (a central heart-shaped DNA-binding core), the latter containing the catalytic Tyr residue involved in the trans-esterification reaction of DNA (Bjergbaek et al., 2000). The transducer helical domain, which links the ATPase domain to B′, is believed to communicate the nucleotide state of the ATPase domain to the rest of the protein (Bendsen et al., 2009). Finally, after the A′ domain, eukaryotic Top II have an unconserved C-terminal hydrophilic domain that is dispensable for topoisomerization, although it plays in *L. donovani* an important role in the *in vitro* TopII cleavage reaction (Das et al., 2006b). In addition, this domain may also include signals for nuclear localization and interaction with other proteins (Reece and Maxwell, 1991). In fact, a potent nuclear localization signal was found within the C-terminus of the *L. donovani* Top II (Sengupta et al., 2003) (Fig. 1B).

In general, Top II are ATP-dependent enzymes that require the presence of Mg^{2+} ions to fulfill their catalytic cycle (Bates and Maxwell, 2007). Mg^{2+} ions seem to facilitate the interaction between the enzyme and substrate in at least two ways: (i) facilitating the formation of TopII/DNA cleavage complexes; (ii) forming the ATP-Mg^{2+} substrate required for ATPase activity (Sissi and Palumbo, 2009). As it was indicated above, Top II contain the conserved Mg^{2+}-interaction domain called Toprim. Since Top II perform two sequential phosphoryl transfer reactions that promote profound conformational enzymatic changes, the high affinity of Toprim for Mg^{2+} may be related to the need to keep the free 3′-OH end produced by the cleavage reaction strongly connected to the Toprim acidic sequence, hence in place to perform religation (Zhu et al., 1997).

2.3. Type IA topoisomerases

Subfamily Top IA differs in structure, sequence and catalytic mechanism from TopIB. TopIA enzymes are essentially DNA relaxing enzymes (relaxases) introducing changes in negatively supercolied DNA in discrete numbers (Lk in multiples of 1) (Byrd and Matson, 1997). However, TopIII enzymes appear to be more active catenating/decatenating DNA (decatenases) due to a specific 17-amino insertion located close to the central cavity within the enzyme (Li et al., 2000; Forterre et al., 2007; Viard and Bouthier, 2007). Trypanosomatids have a full set of TopIA subfamily enzymes compartmentalized both in the nucleus and kinetoplast. The *T. brucei* genome contains three type IA topoisomerase-encoding genes: one in chromosome 10 (annotated as true TopIA) and two in chromosome 11, both annotated as TopIII (http://www.genedb.org/genedb/tryp). For its part, *L. major* Friedlin has a putative TopIA-encoding gene in chromosome 21 and two putative TopIII ORFs at chromosomes 28 and 36, respectively. A similar composition of putative TopIA orthologous genes is deposited in the *T. cruzi* genome database.

Trypanosomatid TopIA along with mtTopII are mitochondrial enzymes that play a role in kDNA minicircles segregation during cell division. Unlike TopIB, the catalytic tyrosine establishes the transient phosphotyrosine intermediate bond at the 5′-end of the scissile DNA strand (Champoux, 2001). Structurally *T. brucei* TopIA (TbTopIA) was characterized as an 88.9 kDa-monomeric protein encoded by a gene of 2418 bp with a high degree of phylogenetic conservation. The *L. donovani* ortholog consists of a protein of 90 kDa encoded by an ORF of 2453 bp. TbTopIA contains an N-terminal domain, which has a mitochondrial localization signal that drives the protein to kDNA, followed by a phylogenetically conserved region similar to prokaryotic TopIA called core (Scocca and Shapiro, 2008). This region contains all the conserved domains of the protein with two DNA binding topofolds (amino acids 186–660) (Duguet et al., 2006) and a C-terminal end variable in size and sequence. Four structural domains constitute the core region that give rise to a toroidal-shaped protein with a central motif filled of basic amino acids that allows the passage of a single- or double-stranded DNA molecule (Duguet et al., 2006). Between domains II and IV a hinge motif allows two spatial open and close configurations. TbTopIA has the catalytic Tyr-362 residue within

domain III near a Mg^{2+} binding domain enriched in acidic amino acids named toprim (amino acids 10–170), which is involved in the conformational changes of the protein during the relaxation reaction. Unlike prokaryotic TopIA proteins, TbTopIA lack Zn-binding domains (Scocca and Shapiro, 2008).

Immunolocalization assays clearly demonstrated that mtTbTopIA operates inside the single mitochondrion of *T. brucei* linked to flagellar basal body and associated to the enzymes in charge of DNA replication (SSE-1, PIF5, Pol β, ligase β and mtTopII) at the antipodal sites of kDNA disc (Jensen and Englund, 2012). These studies showed that the position of mtTbTopIA is changing from one pole to the other pole of the kDNA disk as replication is taking place. Silencing studies of mtTbTopIA by RNAi under tetracycline control, showed that after induction with the antibiotic, growth arrest and almost a complete disappearance of the kDNA occurred with no changes in nuclear DNA (Scocca and Shapiro, 2008). A deeper analysis of minicircle network replication after RNA silencing showed an accumulation of late theta forms but not of daughter catenates, suggesting that mtTbTopIA plays a role in the resolution on the entwined parental strands during the resolution step of minicircle replication (Scocca and Shapiro, 2008). Finally, late free minicircles containing gaps are used by mitochondrial TopII enzymes to reattach them to the replicating kDNA network (Wang, 2002). Since mitochondrial trypanosomatid TopIA has no orthologs in the definitive host, and as a result of the deadly effects that its abolition has (as described above), there is no doubt that this enzyme is an ideal target to be explored for the development of novel drugs (Tang and Shapiro, 2010).

Top III are TopIA subfamily proteins, which in turn are subdivided into TopIII α and TopIII β isoforms (Viard and Bouthier, 2007). Trypanosomatids contain phylogenetically well-conserved genes encoding both TopIII α and TopIII β (Kim and Cross, 2010). Genetic analysis revealed the presence of a 2754-bp ORF encoding the 102.5-kDa *T. brucei* TopIII α (TbTopIII α) and a putative 2523-bp ORF encoding the 937-kDa *T. brucei* TopIII β (TbTopIII β) (Kim and Cross, 2010). Similarly, the putative gene encoding *L. donovani* TopIII α (LdTopIII α) is 2844 bp long and encodes a 104-kDa monomer, whereas the 2601-bp gene encoding TopIIIb (LdTopIII β) produces a 95-kDa protein (http://www.genedb.org/Homepage/Ldonovani_BPK282A1). The deduced amino acid sequence of LdTopIII β has the active Tyr at position 327, which is located within a highly conserved GYISYPRTES signature. This protein contains seven CXXC sequences instead of the eight motifs found in other TopIIIβ proteins (Banerjee et al., 2011). In a similar way, TbTopIII α has been characterized and contains a Zn-binding domain at the C-terminus. The sequences of tritryps TopIIIα are very well conserved in *T. brucei*, *T. cruzi* and *L. major* and contain Zn-binding motif(s) at their C-terminal regions. A LdTopIII β fused to GFP chimera was used to identify the localization of this protein, revealing that LdTopIIIβ is targeted inside both the parasite nucleus and kinetoplast (Banerjee et al., 2011) (Fig. 1C).

TopIII proteins show decatenase rather than relaxase activity by their own. TopIII α proteins play a role in concert with RecQ helicases maintaining genomic stability and integrity by controlling recombination events (Kim and Wang, 1992). TbTopIII α acts as a single-stranded DNA decatenase and release non-crossover products resulting from the dissolution of recombinogenic structures, such as double Holliday junctions. Regarding other eukarya organisms, TbTopIII α may be part of a putative RecQ-TopIII-Rmi1 (RTR) complex that is involved in the recombination-mediated antigenic switching in *T. brucei*, removing undesirable recombination intermediates (Yang et al., 2010; Knoll et al., 2014). Null-TbTopIII α mutants have been shown to play a critical role in homologous recombination processes and antigenic switching. Double replacement of the two alleles encoding the TbTopIII α produces no changes in the proliferation rate of the parasites, but generates an elevated frequency of recombinant events and hyper-switcher phenotype always linked to RAD51. These experiments concluded that TopIII α plays a crucial role in homologous recombination processes and is closely linked to adaptive VSG switching that allows blood forms of *T. brucei* to evade from the host immune response (Kim and Cross, 2010).

Very few studies have been carried out with TopIII β enzymes in trypanosomatids. Although TopIII β is not essential for cell survival, null-mutants display unique phenotypes in eukarya. Knock-out mice lacking the TopIII β -encoded gene develop normally up to maturity, but have a shorter lifespan and exhibit decreased fertility (Kwan and Wang, 2001; Kwan et al., 2003). In addition, these mutants have a deficient activation of p53, pointing to an important role of TopIIIβ in apoptosis induced by DNA damage (Mohanty et al., 2008). Deficient β *top3* mutant yeasts are viable but display a slow-growth phenotype (Gangloff et al., 1999). Experiments with the LdTopIII β gene, showed a partial recovery of phenotype (Banerjee et al., 2011). In addition, this property is lost when rescuing is produced by a partially deleted ORF lacking the Zn-binding domain of the protein. However, no experiments regarding genome stability and programmed cell death have been performed in trypanosomatids, thus the role of this enzyme remaining a mystery in these parasites.

3. DNA topoisomerases as potential drug targets

Both TopIB and TopII are validated targets for antineoplastic drugs in human medicine. TopIB inhibitors, such as topotecan (a water soluble derivative of CPT) and irinotecan (Camptosar) – a prodrug that must be processed hydrolytically by intracellular esterases – are currently in clinical use against ovarian carcinoma (Wethington et al., 2008) and colorectal cancer, respectively (Kuppens et al., 2004). On the other hand, TopII inhibitors are also part of the antitumour drug Vademecum, such as the epipodophyllotoxin derivatives (etoposide and teniposide) (Hande, 1998) or acridine analogues (amsacrine, doxorubicin, etc.) (Louie and Issell, 1985). Last but not least, the bacterial DNA gyrase has been used for years as a target for a number of antibiotics of the fluoroquinolone family (Drlica and Malik, 2003).

Top inhibitors are classified into two categories: (i) Class I or enzyme poisons are those compounds that displace the cleaving/ligation balance toward cleavage, thus stabilizing the cleavage complexes with DNA. This selectively kills the cells found in S phase by collision with the advancing replication fork during DNA replication (Tsao et al., 1993); (ii) Class II or "true inhibitors" are those compounds that interfere with the catalytic functions of the enzyme without trapping the covalent complexes (Pommier, 2006).

With this favourable background, the opportunity to explore these enzymes as potential drug targets in trypanosomatids was early underlined, even when their molecular structures had not yet been described (Burri et al., 1996; Cheesman, 2000). The presence of decatenase and relaxase activities in the single mitochondria of these parasites and their role on the structure of the kinetoplast minicircle network, pointed to these enzymes as extremely attractive targets to design specific drugs to deconstruct kDNA. In 1995 the group led by Shapiro showed that CPT and etoposide triggered kDNA linearization and further disappearance in *T. brucei*, a phenomenon called diskinetoplasticity, which led to parasite death within hours (Bodley and Shapiro, 1995).

With the exception of yeast, TopIBs are essential for eukaryotic organisms (Uemura et al., 1987). Double gene replacement of the gene encoding LdTopIS, originates a non-viable phenotype in *L. major* (Balaña-Fouce et al., 2008). In addition, gene silencing of each of the *T. brucei* TopIB subunits, results in a drastic reduction

Table 2
TopIB inhibitors assayed in trypanosomatids.

Class	TopIB inhibitors	Trypanosomatid	References
Camptothecins	CPT, SN38, topotecan, gimatecan	*L. infantum*	Prada et al. (2013a)
	CPT, irinotecan, topotecan	*T. brucei*	Bodley and Shapiro (1995)
			Deterding et al. (2005)
Indocarbazoles	Rebeccamycin	*T. brucei*	Deterding et al. (2005)
Napthoquinones	Diospyrin	*L. donovani*	Hazra et al. (1987)
	diospyrin, naphthalene derivatives	*T. brucei*, *T.cruzi*	Ray et al. (1998)
			Ganapaty et al. (2006)
Flavonoids	Baicalein, luteolin, quercetin	*L. donovani*	Das et al. (2006a,b)
Poliheterocyclis	Berberin	*L. braziliensis*	Vennerstrom et al. (1990)
		L. donovani	Marquis et al. (2003)
		T. brucei, *T. congolense*	Merschjohann et al. (2001)
Pentavalent antimony	Pentostam, glucantime	*L. donovani*	Chakraborty and Majumder (1988)
Indenoisoquinolines	Indotecan, AM 13-55	*L. infantum*	Balaña-Fouce et al. (2012)
	alkylamine N-6, N-6 3-aminopropyl, N-6 3-imidazolylprolyl	*T. brucei*	Bakshi et al. (2009)
Bis-benzimidazoles	Hoechst-33258, Hoechst-33342	*L. donovani*	Walker and Saravia (2004)
Triterpenoids	Betulinic acid and derivatives	*L. donovani*, *L. amazonensis*,	Domínguez-Carmona et al. (2010)
	dihydrobetulinic acid	*L. braziliensis*, *T. cruzi*	Chowdhury et al. (2003)
		L. donovani	
Insaturated fatty acids	6-Heptadecynoic acid and 6-icosynoic acid	*L. donovani*	Carballeira et al. (2009)
	(±)-17-methyltrans-4,5-methyleneoctadecanoic acid		Carballeira et al. (2010)
	2-Alkynoic fatty acids		Carballeira et al. (2012)
Lignan glycosides	Lyoniside, sacaroside	*L. donovani*	Saha et al. (2013)

in DNA and RNA synthesis *in vitro* (Bakshi and Shapiro, 2004). These findings and the structural differences from host orthologs pointed to these enzymes as putative targets for drug discovery (Balaña-Fouce et al., 2006). First, the uncompetitive inhibitor CPT and its derivatives have been used as inhibitors against *T. brucei* and *Leishmania* spp. TopIB with promising results (Das et al., 2004). The primary effect of the formation of single strand breaks (SSBs) can lead to the induction of the TDP1 repair system enzyme studied by Pommier in mammalian cells (Dexheimer et al., 2008) and recently described in trypanosomatids (Banerjee et al., 2010). The collision of the replication fork during DNA replication transforms SSBs into irreversible double-stranded DNA breaks (DSBs), which can be repaired only by homologous DNA recombination mechanisms. The persistence of no repaired DSBs may cause a process of programmed cell death both in amastigotes and promastigotes, which has been described in detail as a result of the exposure of *L. donovani* to Top poisons (Sen et al., 2004).

In addition to CPT, topotecan and Camptosar were experimentally tested against *ex vivo* cultures of experimentally infected murine splenocytes with *L. infantum* amastigotes, showing that gimatecan, an orphan drug approved by the European Medicines Agency for the treatment of glioblastoma (Hu et al., 2013), has a strong leishmanicidal activity and good selective index (Prada et al., 2013a). An interesting group of non-CPT TopIB poisons are indenoisoquinolines, which were initially developed as antitumour compounds with an improved ability to stabilize cleavage complexes (Antony et al., 2005). Some of these compounds have shown interesting therapeutic profiles against *L. infantum* and *T. brucei* in experimental infections of mice (Bakshi et al., 2009; Balaña-Fouce et al., 2012). Indotecan, administered intraperitoneally every 2 days for 15 days (a total of eight doses) had a similar cure rate that paromomycin alone or in combination with 200-mg/kg-body-weight Glucantime in experimental infections of VL in BALB/c mice (Gangneux et al., 1997). Furthermore, indotecan was more effective than the same dose schedule of CPT either free or liposome encapsulated, in a murine model of *L. donovani* leishmaniasis (Proulx et al., 2001). Some naturally occurring molecules have shown to be TopIB poisons and have anti-leishmanial effects. Amongst others, the bisnapthaquinone dyospirin (Ray et al., 1998), the pentacyclic triterpenoid dihydrobetulinic acid

Table 3
TopII inhibitors assayed in trypanosomatids.

Class	TopII inhibitors	Trypanosomatid	References
Fluoroquinolones	Norfloxacin, ciprofloxacin, ofloxacin	*T. brucei*	Nenortas et al. (1999, 2003)
	Ciprofloxacin, enoxacin	*L. panamensis*	Cortazar et al. (2007)
	Norfloxacin, ofloxacin	*T. cruzi*	Gonzales-Perdomo et al. (1990)
Podophylotoxins	Etoposide, teniposide	*L. donovani*	Sengupta et al. (2005a,b)
		T. equiperdum	Shapiro and Showalter (1994)
Ellipticines	2-Methyl-9-OH-ellipticin	*T. equiperdum*	Shapiro et al. (1989)
Flavonoids	Baicalein, luteolin, quercetin	*L. donovani*	Tasdemir et al. (2006)
		L. panamensis	Mittra et al. (2000)
			Cortazar et al. (2007)
Anthracyclins	Doxorrubicin	*L. donovani*	Singh and Dey (2007)
Diamidines	Pentamidine	*L. donovani*	Singh and Dey (2007)
		L. panamensis	Cortazar et al. (2007)
Aminocoumarins	Novobiocin	*T. cruzi*	Gonzales-Perdomo et al. (1990)
		L. donovani	Singh et al. (2005)
Acridines	Anilinoacridines	*L. chagasi*	Werbovetz et al. (1992)
		T. brucei	Gamage et al. (1997)
Triterpenoids	Dihydrobetulinic acid	*L. donovani*	Chowdhury et al. (2003)

(Chowdhury et al., 2003) or the naturally occurring flavones (baicalein, luteolin and quercetin) are potent LdTopIB poisons *in vitro* (Mittra et al., 2000; Das et al., 2006a,b). For their part, unsaturated fatty acids from marine organisms have shown to be selective Class II inhibitors of recombinant *L. infantum* TopIB (Carballeira, 2013) (Table 2).

Similarly to TopIB, TopII silencing in *T. brucei*, leads to the progressive degradation of kDNA network and parasite death (see above). Eukaryotic TopII was proposed as a putative target for anti-parasitic drugs since the 1990s (Burri et al., 1996; Cheesman, 2000). One of the first groups of compounds that showed therapeutic potential against trypanosomatids was derived from the 9-anilinoacridine backbone, and had potential anti-tumour activity owing to TopII inhibition (Werbovetz et al., 1994). Linearization of kDNA minicircles has also been reported in parasites treated with these compounds because of TopII inhibition, preventing the growth of *L. major* promastigotes and amastigotes (Gamage et al., 1997). Epipodophyllotoxins are semisynthetic derivatives of non-alkaloid toxins from the root extracts of plants from the genus *Podophyllum* (Table 3). The two leading compounds, etoposide and teniposide, stabilize the double-stranded cleavage mediated by TopII and inhibit the religation of DNA breaks (Yang et al., 2009). These compounds are clinically in use against different types of cancer (Anonymous, 2000), and have been proven effective against different parasites (García-Estrada et al., 2010), inducing apoptotic-like cell death (Meslin et al., 2007). However, etoposide have not shown any effect on trypanosomatids, pointing to important structural differences between this enzyme and that from the host counterpart (Sengupta et al., 2005a,b).

4. Concluding remarks

Trypanosomatids have a complete set of six classes of Top enzymes encoded in their respective genomes. Each class, defined by structure and mechanism of action, has distinct but sometimes overlapping cellular localizations and functions. TopIB enzymes are structurally dissimilar to the rest of proteins described so far, but they conserve the ability to pass a single DNA strand in a processive manner to relief torsional stress. TopIA, including TopIII α and TopIII β enzymes have decatenase rather than relaxase activity and work in concert with helicases to resolve noncrossover products by dissolution of recombination intermediates. TopII enzymes, both nuclear and mitochondrial, carry out strand passage through a double-strand break, including decatenating and unknotting activities and are key enzymes in kDNA replication. TopIB and TopII are important targets for drug intervention in cancer. Many of the drugs used as antitumourals have been used in a "piggy backing" strategy against tritryps with different success. A full structural knowledge of tritryps Top proteins based on 3D X-ray analysis of Top crystals, will allow *in silico* high-throughput virtual screening of potential inhibitors in a structure-based drug discovery strategy.

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