Novel Findings on Trypanosomatid Chemotherapy Using DNA Topoisomerase Inhibitors

Rosario Díaz-González, Yolanda Pérez-Pertejo, Christopher Fernández Prada, Celia Fernández-Rubio, Rafael Balaña-Fouce* and Rosa M. Reguera

Dpto. Farmacología y Toxicología (INTOXCAL), Universidad de León, Campus de Vegazana s/n, 24071 León, Spain

Abstract: Trypanosomatid (order *Kinetoplastida*)-borne neglected tropical diseases – African and American trypanosomiasis and leishmaniasis - are amongst the most devastating health threats of underdeveloped, developing and poor countries. Climatic changes due to global warming, tourism exchange and increasing migratory fluxes are re-distributing the endemic subtropical location of these diseases to a new scenario with a rising presence in developed countries during the last decades. In addition, the proved opportunistic transmission of these diseases through contaminated syringes shared by drug users, in combination with immunosuppression processes linked to HIV infections and the poor response to the typical treatments, point to AIDS patients as a sensitive sub-population prone to suffer from these diseases.

DNA topoisomerases are the "molecular engineers" that unravel the DNA during replication and transcription. The mechanism of DNA unwinding includes the scission of a single DNA strand - type I topoisomerases - or both DNA strands - type II topoisomerases - establishing transient covalent bonds with the scissile end. Camptothecin and etoposide – two natural drugs whose semi-synthetic derivatives are currently used in cancer chemotherapy – target types I and II DNA-topoisomerases respectively, stabilizing ternary topoisomerase-DNA-drug covalent complexes, which irreversibly poison the enzymes. Several differences between parasite and host DNA topoisomerases have pointed to these enzymes as potential drug targets in Trypanosomatids. The unusual localization inside the mitochondria-like organellum – the kinetoplast - linked to mini and maxicircles, as well as the uncommon heterodimeric structure of the DNA topoisomerase IB subfamily, make these proteins unquestionable targets for drug intervention against trypanosomatids.

Key Words: DNA-topoisomerases, chemotherapy, camptothecin, parasitic protozoa, trypanosomatids, tropical diseases.

INTRODUCTION

Neglected tropical diseases are one of the most painful consequences of poverty. Poor prophylactic measures, null or inadequate sanitary conditions and a difficult access to effective medicines or therapies, along with no effective vaccines against trypanosomatids - despite the important role of the host's immune system in resistance and healing - are giving rise to an enormous incidence of these diseases in Asian, African and South-American countries [1-3].

The pharmacological management of these parasitoses is at crossroads nowadays. On the one hand, the old fashion first-line drugs – arsenic and antimony derivatives, diamidines, macrolide antibiotics, benznidazole, amongst others – are losing efficiency due to their massive use during decades and the emergence of resistant strains [4-6]. On the other hand, new hit drugs are being marketed after successful trials in humans. This was the case of α-difluoromethylornithine (DFMO) - an irreversible inhibitor of ornithine decarboxylase [7]– developed in the early 90s against late stages of African trypanosomiasis [8], or more recently the alkylphospholipid – miltefosine – with unquestionable effects against visceral leishmaniasis [9, 10]. However, miltefosine exhibited variable efficacies in trials against *Leishmania* Viannia mucocutaneous leishmaniasis [11]. Reproductive toxicity remains a problem and is an important part of post-marketing miltefosine surveillance [12]. Therefore, the lack of efficient antiparasitic drugs strongly justifies the characterization and validation of new molecular targets for drug intervention [13].

A common feature of all trypanosomatids is the existence of a single mitochondrion, which extends towards the cell. The part that is attached to the basal body contains an intricate mesh of disc-shaped extranuclear DNA called kinetoplast DNA (kDNA), which represents the 10-20% of the total cellular DNA [14]. kDNA is arranged in thousands of interlocked DNA rings called mini- and maxicircles. Minicircles are the most abundant form in kDNA (5.000-10.000 molecules per cell). They consists of circular DNA molecules, from 100 to 2.500 bp in length, encoding guide RNAs involved in the edition of mitochondrial nascent RNAs [15]. Maxicircles are much larger rings - 30.000-50.000 bp in legth - but less abundant than minicircles - 50 copies per cell. Maxicircles are equivalent to the mitochondrial DNA from other eukaryotes, since they codify mitochondrial proteins, ribosomal RNA and tRNAs [16]. Replication of kDNA occurs synchronously with genomic DNA during cell division. kDNA components must be decatenated from the network and catenated later to the nascent mesh when DNA is replicated [17]. DNA replication from both mini and

*Address correspondence to this author at the Dpto. Farmacología y Toxicología, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain; Tel: 34987291257; Fax: 34 987291252; E-mail: rbalf@unileon.es

1389-5575/09 $55.00+.00

maxicircles takes place in a *theta* (θ)-shape intermediaries, where DNA-topoisomerases play a key role [17, 18]. The data reviewed below is focused on the structural differences existing between trypanosomatid's topoisomerases and their mammalian homologues, and how these enzymes can be effectively targeted to treat the diseases associated with these microorganisms.

DNA TOPOLOGY AND TOPOISOMERASES PROTEINS

Classical electronic microphotographies of the SV40 polyoma virus DNA, revealed the different spatial configurations displayed by a close circular DNA *in vitro* [19]. Relaxation of supercoiled DNA requires an increase in the amount of distinct topological isomers (topoisomers), differing their "linking number" in one or several integers. The "linking number" (Lk) is a topological constant that represents the sum of twist (Tw) and writhe (Wr). The twist is the number of helical turns in the DNA and the writhe is the number of times the axis of the double helix crosses itself in interwound structures. To produce a change in Lk it is necessary to break at least one of the phosphodiester strands in the DNA backbone. This process is notably important, because the genetic information encoded in DNA is not accessible for replication, transcription or recombination processes under compact supercoiled superstructures [20].

Relaxation of supercoiled DNA is achieved through the introduction of transient single or double strand breaks in the double helix, action that is carried out by DNA topoisomerases (Top), a group of enzymatic molecular machines discovered by Wang in 70s [21]. Top produce topological changes in DNA by a coordinated three-step sequence: cleavage, rotation and religation. The product of these Top-mediated activities is a more relaxed DNA specimen, due to the Lk modification in one or several integers [22-24]. Moreover, some Top have additional activities, such as knotting/unknotting and catenation/decatenation (see below). A common feature of all Top described so far, is the presence of tyrosine residues at the active center of the enzyme. Tyrosines are responsible for the *trans*-esterification reaction with the DNA phosphate backbone, thus creating a transient covalent-bonded DNA-Top intermediary.

The coordinated three-step sequence exerted by Top to manipulate DNA can be fuelled either by the energy stored in the supercoiled DNA torque or by ATP hydrolysis. This feature in combination with the number of DNA strands broken during *trans*-esterification, have served to classify Top in two different families. Type I Top (TopI) are monomeric enzymes – with the remarkable exception of the enzymes revised in this report – that introduce transitory single-strand breaks in DNA followed by passage and rejoining. TopI are ATP-independent enzymes that exhibit relaxation activity for both positively and negatively supercoiled DNA. Type II Top (TopII) are multimeric proteins that hydrolyze ATP to generate temporary double-strand breaks in DNA followed by passage and rejoining [25]. TopII enzymes relax not only positively supercoiled DNA, but also knot/unknot and catenate/decatenate closed circular DNA [26].

Structural and mechanistic characteristics permit a further classification of both Top families: The TopIA subfamily was initially classified as an exclusive bacterial TopI [27]. TopIA introduce positive supercoils, decatenate single-stranded DNA and unwind supercoiled DNA through covalent binding to the 5' end of the broken DNA strand, allowing changes in the Lk by steps of 1. This subfamily includes bacterial TopI, eukaryotic TopIII and reverse gyrases [28]. Unlike TopIA, TopIB are exclusively eukaryotic enzymes that can relax both negatively and positively supercoiled DNA, establishing transitory covalent bonds with the 3' end of the broken DNA strand and allowing multiple turns – up to five – per reaction cycle [29]. An extremely important difference between TopIA and TopIB is the metallic requirement of TopIA to complete the enzymatic cycle [30]. Eukaryotic TopI, *Methanopyrus kandleri* TopV and the vaccinia TopI, are TopIB enzymes [31].

The TopIIA subfamily includes homodimeric A_2 (eukaryotic TopII) or heterotetrameric A_2B_2 (bacterial TopII) enzymes, producing changes in the Lk by steps of +/- 2 [32, 33]. TopIIB are heterotetrameric A_2B_2 enzymes sharing common catalytic properties with bacterial type IIA enzymes. TopIIB were discovered for the first time in the extreme thermophilic archaebacterium *Sulfobolus shibate* and they were called TopVI [34]. TopIIB have also been described in plants including green and red algae [35]. TopIIA form double-stranded breaks with four base pairs overhangs, whereas TopIIB form double-stranded breaks with two base pairs overhangs [36]. Unlike type IIB [22], type IIA topoisomerases are able to simplify DNA topology [37].

TOPI OF TRYPANOSOMATIDS

Table **1** shows the specific structural and biochemical features of Top proteins exhibited by trypanosomatids, according to the annotations done in their corresponding Genome Projects [38]. All trypanosomatids contain a typical eukaryotic TopIB, which have been studied in different species of trypanosomes and leishmanias (see below). Furthermore, a TopIA open reading frame (ORF), as well as a putative TopIII-encoding gene have been annotated in the genomes of all trypanosomatids. *T. brucei* TopIA has been recently characterized and gene silencing by RNAi, showed a loss of kDNA and cell growth arrest [39]. Unfortunately, TopIA and TopIII inhibitors have not been described so far.

Despite TopIB had been initially pointed out as a putative target in trypanosomatids [40], no information about its structure was elucidated up to late 90s due to its atypical nature. In 1999, Broccoli and co-workers reported the presence of one ORF encoding a 636 amino acids TopIB-like protein in *L. donovani*. The sequence of this protein resembled well the central core domain of other TopIB, but lacked the "SKxxY" motif placed at the C-terminal end that supplies the catalytic Tyr [41]. The recombinant polypeptide failed to unwind supercoiled DNA *in vitro*, pointing to an anomalous TopIB structure or to the existence of a second ORF encoding a protein that may supply the C-terminal end [42]. Later, Villa and co-workers– found in the *L. major* Genome database - in progress during late 2002 – and within chromosome 4, a second ORF encoding a protein that harbored the missing active site. Cloning of both genes in a biscistronic vector for yeast expression, yielded substantial

Table 1. Classification of Type I and Type II DNA Topoisomerases Families and Subfamilies in Trypanosomatids

	Type I Subfamily		Type II Subfamily
	TopIA	**TopIB**	**TopII**
Structure			
Oligomeric status	monomer (A)	heterodimer (AB)	homodimer (A_2)
ATPase domain	no	no	GHKL
Toprim fold	yes	no	yes
Biochemistry			
metal dependence	Mg^2	no	Mg^{2+}
Cleavage	5' Single strand	3' Single strand	5' Double strand
ΔLk	+1	+/-n	+/-2
Enzymes			
	TopIA and TopIII	TopIB	TopIIα, TopIIβ and mtTopII

relaxation activity *in vitro* and permitted the purification of the recombinant enzyme [43]. According to these results, the structure of *L. donovani* TopIB (LdTopIB) was established as a heterodimeric bi-subunit enzyme made up of a 636-amino acid large subunit (LdTopIL) containing a putative core domain and a 262-amino acid polypeptide (LdTopIS) that conserves the catalytic Tyr [43-46]. Soon after, Bodley and co-workers reported similar findings in African trypanosomes using a classical protein purification procedure [47]. This unique molecular organization of TopIB, which combines two heterologous protomers to build up an active enzyme, seems to be exclusive of trypanosomatids (Fig. **1A**).

Due to its bi-subunit nature, interactions between protomers appear to be necessary to reconstitute active TopIB in trypanosomatids. These interactions can be addressed by the overall charge difference between subunits. The different pI existing between the large (9.47) and the small (5.21) subunits, together with the unusual salt sensitivity of LdTopIB, led to raise that the protein regions bearing these differences may contribute to establish ionic interactions to hold the subunits together [48]. Recently, Majumders' group has proposed, using site-directed mutagenesis, that the Lys-455 present in the large subunit interacts with the Asp-261 of the small subunit to constitute a minimal functionally active LdTopIB [49].

There is another remarkable difference between trypanosomatid TopIB and their eukaryotic homologues. In mammals, the existence of a separate TopIB acting exclusively in the mitochondria, was reported [50]. The human mitochondrial TopIB-encoding gene presumably arose by gene duplication and modification of the gene encoding the nuclear enzyme [51]. However, immunocytochemical localization experiments carried out in trypanosomatids showed dual localization of the enzyme associated with both genomic DNA in the nucleus and kDNA minicircles in the kinetoplast [45, 52].

With the exception of yeasts [53], TopIB is essential for eukaryotic organisms [54, 55]. RNAi silencing of either TopIB subunits, results in a drastic reduction of both DNA and RNA synthesis in African trypanosomes, mimicking the inhibition of nucleic acid biosynthesis observed when bloodstream-form trypanosomes are treated with CPT [56]. In addition, double replacement of the gene encoding the small TopIS subunit produced a non-viable phenotype in *L. major* [57].

TOPII OF TRYPANOSOMATIDS

Due to the lack of genomic evidence for TopIIB-encoding genes in trypanosomatids and in order to simplify, type IIA topoisomerases will be referred hereafter as TopII. Multiple forms of TopII have been described in both the nucleus and the mitochondrion of eukaryotic organisms (Table **1**). Two forms of nuclear TopII - TopIIα and TopIIβ - participate in mitosis and/or meiosis of mammalian cells. Similarly, two tandemly arranged genes - located on chromosome 11 and separated by a region of 1.7 kb - encode the nuclear α and β forms of *T. brucei* TopII [58]. Furthermore, an additional gene encoding the mitochondrial *T. brucei* TopII (mtTopII) is placed on chromosome 9 and its product is associated with kDNA [59]. This feature is unique in trypanosomatids, although TopII activity serving the mitochondrial genome has been described in mammals. Nevertheless, it appears to be a truncated form of nuclear TopIIβ [60]. Separate nuclear and mitochondrial TopII-encoding genes have been annotated in the genome Projects of all trypanosomatids described so far, and they seem to be essential for kDNA replication. A phylogenetic tree including multiple TopII-encoding genes from different organisms, concluded that all nuclear TopII have a common ancestral eukaryotic origin, whereas the trypanosomatids genes encoding the mitochondrial forms, are closer to the prokaryotic DNA-gyrase branch [58].

L. donovani TopII (LdTopII) has been extensively studied by Majumder's group. From a structural point of view, the monomer conserves all the characteristics of the eukaryotic enzyme; *viz*. an N-terminal ATPase domain, a linker, a central DNA-binding and catalytic core domain and a

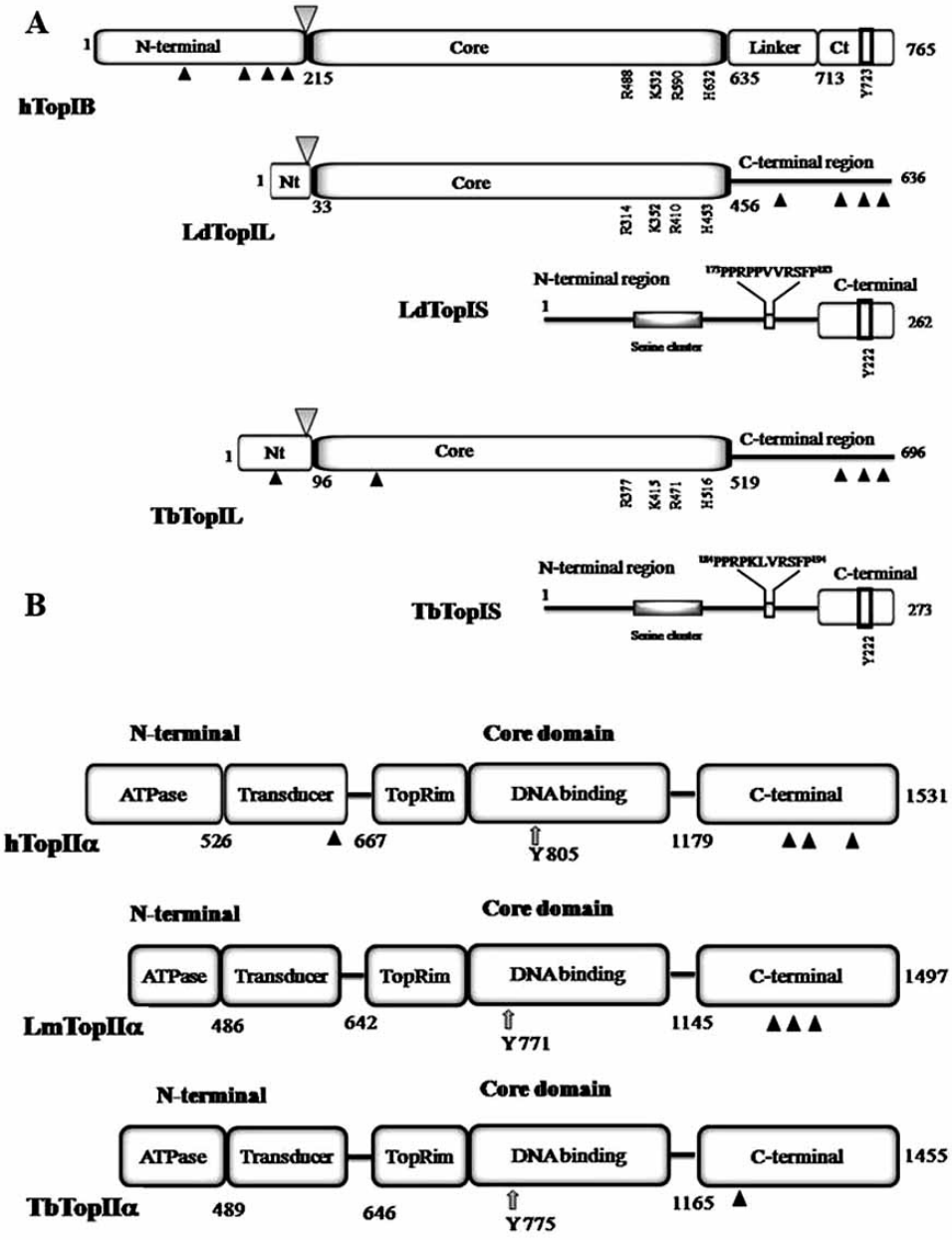


Fig. (1). (A) Schematic and comparative description of hTopIB regarding Trypanosoma and Leishmania counterparts. hTopIB is structured in four domains, a poorly conserved N-terminal domain that harbours the NLS motifs in the human enzyme (arrowheads), and a conserved Trp motif, represented with an inverted triangle. The central core domain contains most of the residues interacting with DNA - R488, K532, R590 and H632: "catalytic tetrad" - that are directly involved in the controlled rotation. The "SKxxY" motif of the C-terminal domain bears the catalytic Tyr, and it is connected with the core by a linker, a region with no homology with other TopIBs. A comparison between human and trypanosomatids TopIB reveals a similar disposition for the "catalytic tetrad" and active Tyr, although these domains are shared by both subunits: the core domain is present in the parasitical large subunit, whereas the C-terminal is allocated to the small subunit. Furthermore, there are two regions with no resemblance to any other TopIB: the C-terminal extension in the large subunit and the N-terminal extension in the small subunit. Their role in topoisomerization remains unclear. **(B)**: Schematic comparison of nuclear TopIIα proteins in human and trypanosomatid species. In contrast with TopIB, the homology is highly conserved among the different proteins, and the active Tyr is pointed in all enzymes. The putative NLS (tagged with arrowheads) is present in all ortologues, although positions are not concordant. Distances represented in the drawings are not in scale.

variable C-terminal region [61, 62]. The N-terminal domain is extended 489 amino acids from the start-Met and contains a characteristic ATP-hydrolizing domain. A transducer motif communicates the nucleotide state of the ATPase domain to the rest of the protein. The conserved core domain contains a Toprim fold (an unusual Rossman fold that coordinates two magnesium ions) and a DNA binding core that contains the catalytic tyrosine (Fig. **1B**).

Shapiro's group silenced the expression of both nuclear and mitochondrial TopII by RNAi in *T. brucei* showing profound defects in cell and nuclear morphology. Since mitochondrial TopII is involved in decatenation of the kDNA network into individual minicircles prior to replication [17], enzyme silencing produces a singular kDNA lacking phenotype, called dyskinetoplasty, that leads to cell death [63]. On the other hand, nuclear TopIIα silencing gives rise to growth arrest and severe defects in nuclear (but not mitochondrial) DNA, unlike TbTopIIβ silencing, which shows no apparent phenotype Nuclear isozyme silencing produced a faster cell proliferation than silencing of the mitochondrial isozyme, fact that led the authors to suggest this protein as a more suitable target for drug development [58].

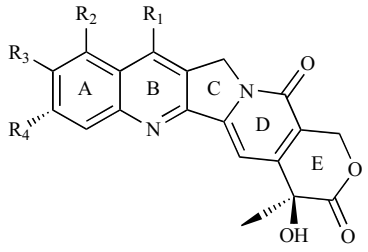
TOPIB INHIBITORS

Since its discovery [64], TopIB has been pointed out as an attractive target for therapeutic intervention in proliferative processes because of its strategic position in replication, transcription and recombination [65, 66]. Due to this pivotal role in preserving the fidelity of genetic information, its structure has remained phylogenetically conserved. Thereby, drugs do not usually discriminate between the invading parasite´s enzyme and the host´s enzyme. Trypanosomatids TopIB is a remarkable exception because it differs from that of the mammalian host in the structure and expression level [46, 48, 67]. TopIB inhibitors fall into two categories: i) compounds stabilizing the enzyme/DNA cleavage complex; they have been generically called class I inhibitors or enzymatic poisons; ii) compounds interfering with the catalytic role of the enzyme; or class II inhibitors.

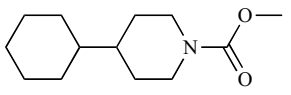
CPT **(1a)** and its derivatives are the best examples of poison class I inhibitors. Structurally CPT is a natural pentacyclic alkaloid, firstly isolated from the bark and stem of *Camptotheca acuminata*, a tree of the *Nyssaceae* family originated in China and Tibet [68]. Despite CPT was ruled out as anti-tumour agent because of toxic side effects, this molecule has generated an enormous series of compounds with anti-tumour activity, some of them integrated into current cancer chemotherapy [69, 70]. Biochemically speaking, CPT is a potent sub-micromolar non-competitive inhibitor of TopIB that establishes irreversible ternary complexes with nicked DNA (cleavage complex), which cannot be sealed [71]. This mechanism of action has been confirmed in protozoan parasites of medical interest.

Structure/activity studies with series of CPT analogues carried out with *T. brucei* showed that parasite death correlated well with the formation of cleavage-complexes [72, 73]. More recent studies have revealed that water-soluble derivatives of CPT, irinotecan **(1b)** and topotecan **(1c)** – which are clinically used as antitumour drugs – have a reduced cytotoxic effect against *T. brucei* trypomastigotes. The authors concluded that this reduced effectiveness may be consequence of its low intake into the parasite [74].

Docking studies carried out by Staker and co-workers based on X-ray analysis of hTopIB, DNA and CPT co-crystals, describe a model of interaction between the components of the cleavage complex that could be assumed in trypanosomatids (assuming their bi-subunit structure) [75]. According to this model, CPT intercalates into the DNA cleavage site mimicking one DNA base pair. Within the intercalation pocket site, the side chain of Asp-353 present in the large LdTopIL subunit, establishes a hydrogen bond with the 20(S)-hydroxyl moiety of the lactone form of CPT E-ring. On the other hand, Arg-190 is hydrogen-bonded to the nitrogen atom at the CPT B-ring. Asn-221, the amino acid adjacent to the DNA-cleaving Tyr of the small subunit, is also needed for CPT inhibition, although it does not establish hydrogen bonds with the drug.



camptothecin (CPT)

	R_1	R_2	R_3	R_4
CPT **(1a)**	H	H	H	H
Topotecan **(1b)**	H	$CH_2NH(CH_3)_2$	OH	H
Irinotecan **(1c)**	C_2H_5	H		H

Very few studies have been carried out to determine the activity of CPT and their analogues in experimental infections with trypanosomatids. In 2001 Proulx and co-workers studied the efficacy of a formulation of liposome-encapsulated CPT in a mouse model of visceral leishmaniasis. The authors found a significant reduction in parasitic burden in spleen and liver with an intraperitoneal dose of 2.5 mg/kg body weight but the complete healing was not achieved [76].

Indole alkaloids with antiprotozoal activity – harmaline derivatives **(2)** [77] – and poliheterocyclic indolocarbazoles (rebeccamycin; REB **(3)** and analogues) - are able to intercalate with DNA producing cell arrest. However, unlike harmaline, REB derivatives belong to a promising group of class I TopIB inhibitors. Many semi-synthetic REB derivatives have been tested *in vitro* to evaluate QSAR against a variety of human tumour cell lines and animal models [78]. In trypanosomatids, Deterding and co-workers found that REB has cytotoxic effects against *T. brucei* trypomastigotes at sub-micromolar concentrations *in vitro* [74], results that have been corroborated by our group in *Lesihmania* promastigotes (Balana-Fouce and coworkers, unpublished results), although *in vivo* studies have not been performed at present (Fig. **2**).

L. donovani promastigotes are very sensitive to natural naphthoquinone derivatives such as lapachol **(4a)**, β-lapachone **(4b)** and diospyrin **(5)** (Fig. **2**). Lapachol and β-lapachone are hydroxyl-derivatives of napthoquinone with proved trypanocide and leishmanicide *in vitro* activity. Unlike lapachol, β-lapachone interacts with TopIB preventing the formation of cleavable complexes with DNA [79]. Diospyrin - a bis-naphtoquinone from plant origin – however, behaves as a class I inhibitor [80]. Diospyrin shows a

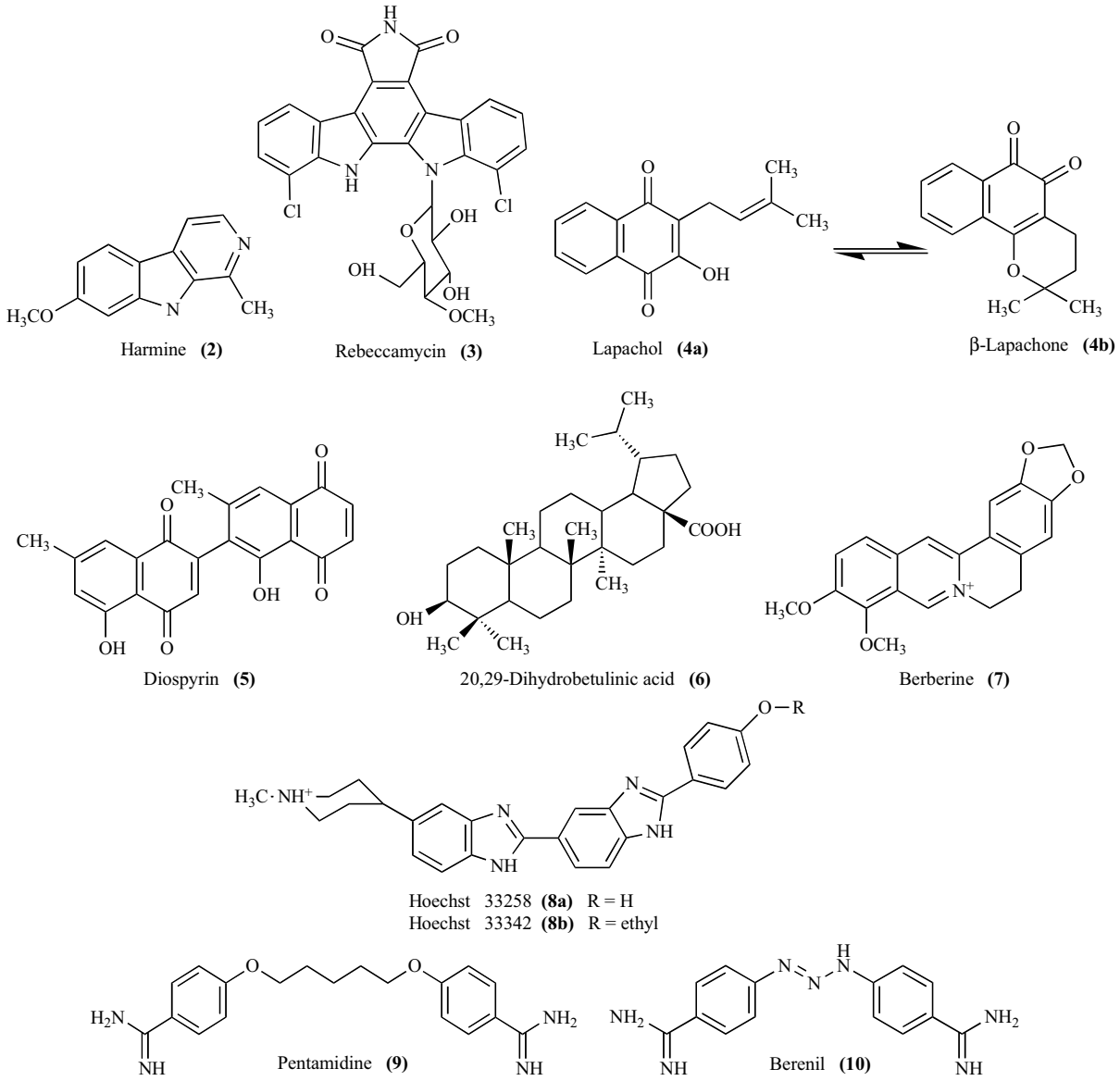


Fig. (2). Chemical structure of TopIB inhibitors.

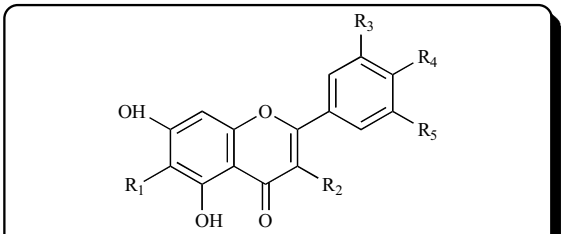
selectivity index of ten when the inhibition of the leishmanial and the human enzyme are compared [81]. Acetyl boswellic acid derivatives - betullinic acid **(6)** - inhibit both TopI and TopII activities through competition with DNA for enzyme binding [82]. These compounds have a dramatic inhibitory effect on *L. donovani* promastigotes proliferation *in vitro*, inducing chromatin marginalization and subsequent dechromatination without impact on kDNA [83]. Protoberberine analogs - quaternary isoquinolinic alkaloids – act as a class I inhibitor of TopIB. Protoberberine and some of its analogues possess significant activity against several species of *Leishmania* and *Trypanosoma* both *in vitro* and *in vivo* [84]. Berberine **(7)** partially clears the *L. donovani* amastigote burden from liver macrophages and reduces the lesion size of *L. braziliensis panamensis* infections *in vivo* [85].

Finally, several compounds that have been used for decades against parasites, can behave as TopIB inhibitors in *in vitro* tests. The pentavalent antimony derivative Pentostam® can stabilize the cleavage complex with DNA *in vitro* [86] at much higher concentrations than CPT. However, it does not seem to constitute its primary leishmanicide mechanism of action *in vivo* [87, 88] and it should be simply regarded as an epiphenomenon.

DNA minor groove binders [89], such as the bisbenzimide derivatives Ho-33342 **(8a)** and Ho-33258 **(8b)** or the proved trypanocide drugs pentamidine **(9)** and berenil **(10)** [90], – aromatic diamidines - are dual TopI and TopII inhibitors *in vitro* [91]. These compounds bind selectively to the AT-rich regions at the minor groove of DNA interfering with the enzyme catalysis, but with the notable exception of Ho-33342 and Ho-33258, they do not promote DNA cleavage [92]. However, a recent work shows that inhibition of Top by these compounds is only a marginal side effect, indicating that their leishmanicidal effect corresponds to a more complex pleiotropic behavior [93] (Fig. **2**).

TOPII INHIBITORS

Similarly to TopIB, TopII was proposed as a putative drug target against trypanosomatid parasites since late 80's [40, 48, 67, 94]. Since transient tyrosine-mediated cleavage in the phosphodiester backbone is required to change DNA topology, the drug-mediated irreversible stabilization of the ternary intermediates is the main objective of most of the developed compounds.

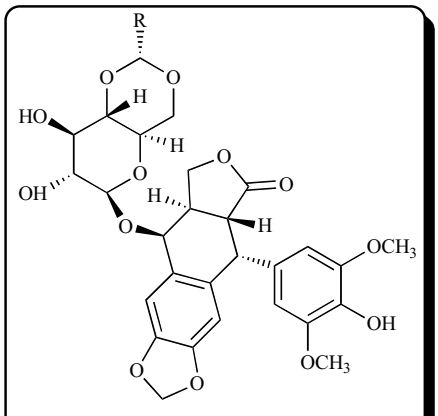


Natural flavones

Baicalein	**(11a)**	R_1=OH;	R_2=H;	R_3=H;	R_4=H;	R_5=H
Quercetin	**(11b)**	R_1=H;	R_2=OH;	R_3=OH;	R_4=OH;	R_5=H
Luteolin	**(11c)**	R_1=H;	R_2=H;	R_3=OH;	R_4=OH;	R_5=H

Naturally occurring flavonoids are current constituents of all foods and edibles from plant origin and herbal-containing dietary supplements. The use of these compounds has been increased because of their powerful antioxidant properties *in vitro*, but their actual mode of action is still unknown. Flavonoids are aromatic planar flavones that can intercalate with DNA due to the presence of the double bond between carbons 2 and 3 in the C-ring. However, intercalation does not fully explain the potent antiproliferative effect of these compounds. Flavonoids more likely bind to the enzymes involved in DNA replication rather than participate in intercalative processes that only occurred at very high concentrations. Baicalein **(11a)**, luteolin **(11b)** and quercetin **(11c)** inhibit the growth of *L. donovani* and other trypanosomatids *in vitro* at micromolar concentrations. These compounds have shown dual TopI and TopII inhibitory effect in eukaryotic cells. Boege and coworkers showed in HL-60 cells that quercetin and some other derivatives stabilize the covalent enzyme-DNA post-cleavage complex by a mechanism different from CPT. These compounds establish ternary complexes with TopIB and DNA, which do not affect the cleavage itself, preventing the subsequent religation step [95, 96]. These compounds promote the TopII-mediated linearization of kDNA minicircles *in vitro*, inhibiting the ATPase linked TopII activity in *L. donovani*. As a consequence, flavonoids produce apoptosis-like death in *Leishmania* promastigotes. A systematic QSAR study with more than 100 flavones and flavonoids showed the efficacy of these compounds against trypanosomatids *in vitro* [97]. The therapeutic potential of luteolin and quercetin was evaluated in a model of visceral leishmaniasis. A significant ~90% reduction in the spleen amastigote burden was observed in both treatments, but quercetin produced a 4-fold higher reduction in the parasite load than that provided by luteolin [98].

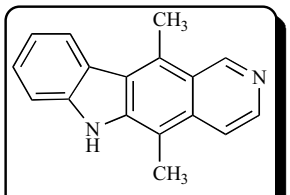
The semisynthetic podophyllotoxin-derived antineoplasic agent etoposide **(12a)** (VP-16) – a recognized mammalian TopII inhibitor – and teniposide **(12b)** cause profound changes in the structure of kDNA networks in *C. fasciculata* promastigotes and bloodstream forms of *T. equiperdum* [99]. Etoposide incubation gave rise to θ-shaped kDNA minicircles release from the kDNA network, with the enzyme linked to the 5′-end of the cleaved minicircle catenane. In the presence of etoposide, decatenation and segregation are blocked and DNA synthesis proceeds, promoting the transformation



Etoposide **(12a)** R=methyl
Teniposide **(12b)** R=thiophene

of θ-shaped structures into multiple interlocked dimers that remain linked to the original small catenane. These authors later confirmed that etoposide also promotes a notable increase in free maxicircles [100].

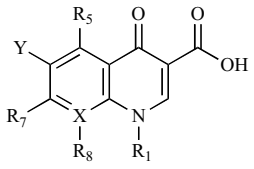
Etoposide-treated cells yield a remarkable - more than 20-fold - increase in free maxicircle DNA, suggesting that maxicircles were linearized and released from the kDNA mesh. The mechanism of TopII poisoning is due to the competition with ATP at the ATPase site in the N-terminal domain. A notable difference between hTopII and LdTopII is the lack of stimulation of the parasite protein in the presence of DNA. In addition, docking and point mutation studies have confirmed that an amino-acid "tetrad" consisting of three asparagines and one aspartic acid, is involved in the interaction of LdTopII with ATP and etoposide [62].



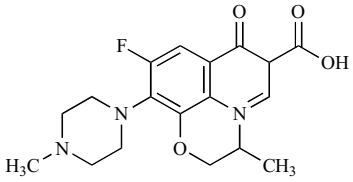
Ellipticine **(13)**

Ellipticine **(13)** is a natural plant product widely used in cancer therapy [101]. Although it remains still unclear, the planar polycyclic structure allows the interaction with DNA through intercalation, exhibiting a high DNA binding affinity. Ellipticine derivatives showed inhibitory effects on relaxation and catenation activities in *T. cruzi*, at concentrations similar to those observed for the mammalian enzymes [102]. The involvement of ellipticine derivatives in trypanosomal TopII inhibition was found later by Shapiro and coworkers, who showed that m-hydroxy-ellipticine inhibited kDNA decatenation by a purified *T. equiperdum* TopII preparation within a low micromolar range [103].

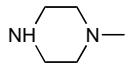
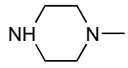
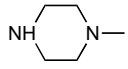
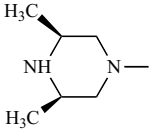
Quinolone-based antibiotics - fluoroquinolones - are potent antibiotic drugs that target bacterial DNA gyrases – mostly in Gram (–) microorganisms – and TopIV in Gram(+) cells. Fluoroquinolones trap both enzymes establishing ternary covalent cleavage complexes with DNA, which actively kill the bacteria [104, 105]. Early studies with nalidixic acid **(14)** – a first-generation quinolone – showed no effect on both TopII mediated kDNA decatenation and DNA cleavage assays and poor cytostatic effect over *T. equiperdum in vitro* cultures [103]. Later, González-Perdomo and coworkers [106] observed the role played by the C7 substituents in the toxicity of second generation fluoroquinolones on *T. cruzi* epimastigotes and amastigotes. The authors showed that ofloxacin **(15)** and norfloxacin **(16)** had a strong trypanocidal activity in those processes dependent on kDNA replication, such as metacyclogenesis and epimastigote proliferation, but not in amastigote differentiation. These results suggested an involvement of mtTopII in fluoroquinolone toxicity. A series of second generation fluoroquinolones were tested against bloodstream forms of *T. brucei*, showing scarce toxic differences with regard to L1210 leukemia cells [107]. New



General structure



Ofloxacin **(15)**

Name	Y	X	R_1	R_5	R_7	R_8	Number
Nalidixic acid	H	N	$-CH_2CH_3$	H	CH_3	H	14
Norfloxacin	F	CH	$-CH_2CH_3$	H		H	16
Ciprofloxacin	F	CH		H		H	17
Enoxacin	F	N	$-CH_2CH_3$	H		H	18
Sparfloxacin	F	CH		NH_2		F	19

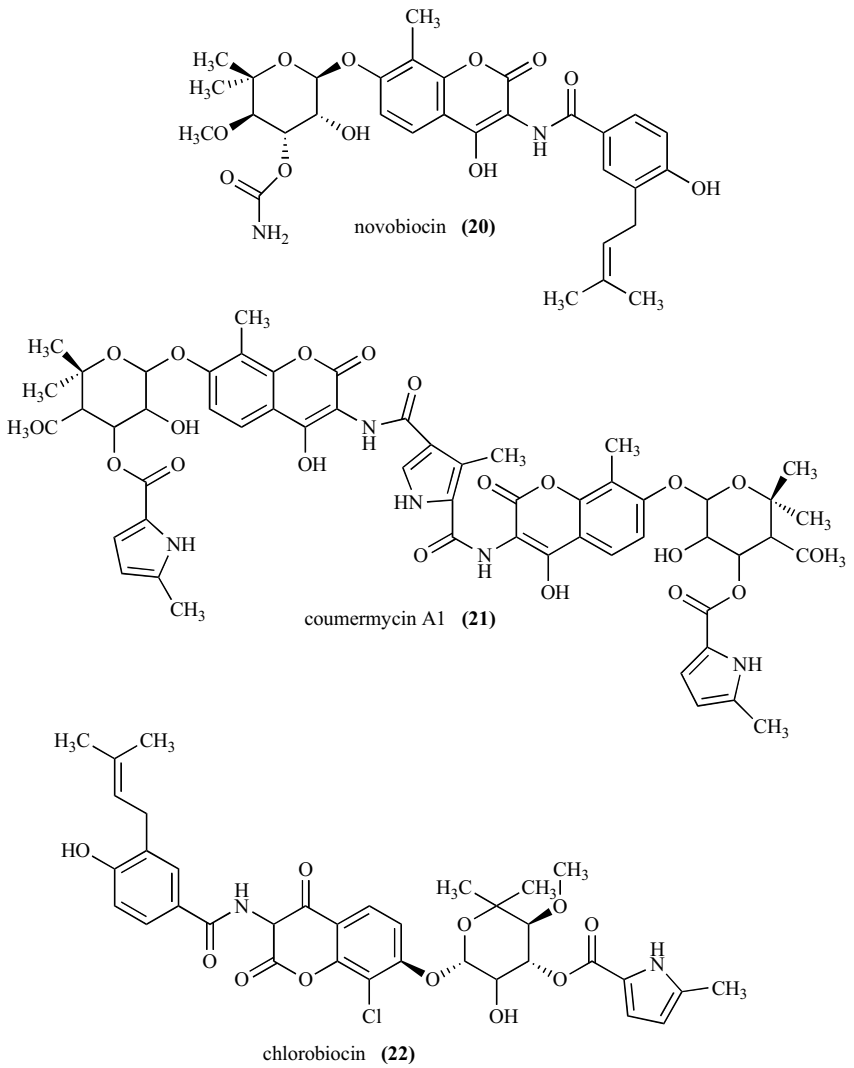


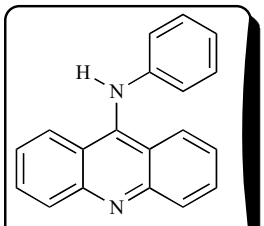
Fig. (3). Chemical structure of aminocoumarins inhibitors of TopII.

fluoroquinolone compounds resultant after the substitution of the piperazinyl group of second generation ciprofloxacin **(17)** and sparfloxacin **(19)** with 3-aminopyrrolidine, produced a fivefold improvement in trypanocidal activity and TopII mediated DNA cleavage complexes with regard to the parent compounds, but no progress in selective toxicity with mammalian cells was achieved [108]. Recently, a series of commercial second generation fluoroquinolones has been tested against *L. panamensis in vitro* showing high selectivity in TopII kDNA decatenation regarding the human enzyme. Enoxacin **(18)** and ciprofloxacin showed high correlation between TopII inhibition and leishmanicidal activity *in vitro*. Unlike previous reports, the authors showed very low differences between R7 substitutions and activity, thus indicating the minimal contribution of this position to the selectivity observed [92].

Aminocumarin antibiotics – novobiocin **(20)**, coumermycin A1 **(21)** and chlorobiocin **(22)** - are *Streptomyces* products used for a long time as β-lactamic alternatives with much stronger inhibitory power on bacterial DNA gyrases than fluoroquinolones (Fig. **3**). Novobiocin competitively inhibits the ATPase function of GyrB subunit in DNA gyrase and bacterial TopIV. Furthermore, aminocoumarins prevent ATP hydrolysis at higher concentrations in other eukaryotic TopII enzymes. Novobiocin has not been reported to be a good TopII inhibitor in trypanosomatids. Early results showed that novobiocin was able to prevent kDNA decatenation by *C. fasciculata* TopII at very high concentrations [109]. This result was later corroborated *in vitro* with *L. donovani* TopII at similar concentrations [110]. These results correlate well with the poor efficacy to kill *T. cruzi* epimastigotes in culture. However, novobiocin had a discrete effect on *T. cruzi* metacyclogenesis and prevented the proliferation of axenic amastigotes [106, 111]. The authors concluded that novobiocin is a very unlikely treatment against Chagas disease, because the concentration of the antibiotic that antagonize with the parasite, is in the limit of achievable safe drug level in the sera of patients.

Other aminocoumarins - coumermycin A1 and chlorobiocin - were found to be effective against *T. cruzi* epimas-

tigotes and amastigotes, but exhibited no effects against the trypomastigote forms of the parasite [111]. More recently, Singh and coworkers [112] have shown that novobiocin induces programmed cell death in *L. donovani* promastigotes in a process mediated by TopII inhibition. In addition, these authors described that a *L. donovani* arsenite-resistant strain increased TopII expression and exhibited higher resistance to aminocoumarins.



9-anilinoacridines **(23)**

9-Anilinoacridines **(23)** have long been used to arrest DNA replication and transcription in antitumor therapy. Several reports confirm that it is possible to modify existing anticancer drugs and improve their activities and specificities against trypanosomes and leishmanias [11, 113-115]. 9-Anilinoacridines derivatives act as TopII poisons stabilizing cleavage complexes with both nuclear and kinetoplast DNA in *L. chagasi* [116]. Several compounds bearing 1'-NH-alkyl substituents produced a deep inhibition of macrophage-infected *L. major* amastigotes at submicromolar concentrations, producing low toxicity to human Jurkat cells [117]. Further studies confirmed that members of the 9-anilino-acridine class of antitumor agents, which bear lipophilic electron-donating 1'-anilino substituents, are active against both *L. major* promastigotes and amastigotes [118].

CONCLUSION

DNA topoisomerases were recognized as effective drug targets for cancer therapy since late 80's, but their suitability against parasitic protozoa-causing diseases has not been sufficiently exploited. Some striking differences between trypanosomatids and hosts Top have put forward the extremely important properties of these enzymes as putative therapeutic targets for drug intervention. In the last few years, several reports have established the unique bi-subunit structure of leishmanial TopIB. In addition, three TopII – two localized to the nucleus and one to the mitochondrion in association with kDNA – have been characterized and knocked down in African trypanosomes. Thanks to molecular and docking studies, novel structural insights have been revealed in both enzymes of trypanosomatids [119-121]. These differences can be useful in the near future as a good starting point for the development of new lead compounds, reinforcing the hypothesis of their suitability as promising targets for antiparasitic drug development. However, due to the scant information regarding the effect of these compounds on experimental infections, there is a pressing need to perform systematic *in vivo* trials to evaluate the actual efficiency of drug treatments targeting both TopIB and TopII. In conclusion, new molecular biology techniques as well as combinatorial chemistry will be helpful to accelerate the R&D of new drugs in this amazing field.

ACKNOWLEDGEMENTS

This manuscript as well as the research developed by the authors in this field was supported by Ministerio de Ciencia y Tecnología (grants AGL2006-07420/GAN) and Instituto de Salud Carlos III (grant PI06302 and RICET) from Ministerio de Salud y Consumo from the Spanish Kingdom.

ABBREVIATIONS

Top	=	Topoisomerase
CPT	=	Camptothecin
Lk	=	Linking number
Tw	=	Twist
Wr	=	Writh
TopI	=	Type I DNA-topoisomerase
TopII	=	Type II DNA-topoisomerase
NLS	=	Nuclear localization signal
GFP	=	Green fluorescent protein
ORF	=	Open reading frame
kDNA	=	Kinetoplast DNA
RNAi	=	Small interference RNA
REB	=	Rebeccamycin

REFERENCES

[1] Kioy, D.; Jannin, J.; Mattock, N. Human African trypanosomiasis. *Nat. Rev. Microbiol.,* **2004**, *2*, 186-87.

[2] Teixeira, A.R.; Nitz, N.; Guimaro, M.C.; Gomes, C.; Santos-Buch, C.A. Chagas disease. *Postgrad. Med. J*., **2006**, *82,* 788-98.

[3] Desjeux, P. Leishmaniasis: Current situation and new perspectives. *Comp. Immunol. Microb.,* **2004**, *27,* 305-18.

[4] Legros, D.; Ollivier, G.; Gastellu-Etchegorry, M.; Paquet, C.; Burri, C.; Jannin, J. Treatment of human African trypanosomiasis - present situation and needs for research and development. *Lancet Infect. Dis.,* **2002**, *2*, 437-40.

[5] Kennedy, P.G. Human African trypanosomiasis of the CNS: current issues and challenges. *J. Clin. Invest.,* **2004**, *113*, 496-504.

[6] Balaña-Fouce, R.; Cubría, J.C.; Reguera, R.M.; Ordóñez, D. The pharmacology of leishmaniasis. *Gen. Pharmacol.,* **1998**, *30*, 435-43.

[7] Bacchi, C.J.; Nathan, H.C.; Hutner, S.H.; McCann, P.P.; Sjoerdsma, A. Polyamine metabolism: a potential therapeutic target in trypanosomes. *Science,* **1980**, *210,* 332-4.

[8] Burri, C.; Brun, R. Eflornithine for the treatment of human African trypanosomiasis. *Parasitol. Res.,* **2003**, *90,* Supp 1, S49-52.

[9] Berman, J. Miltefosine to treat leishmaniasis. *Expert. Opin. Pharmacol.,* **2005**, *6,* 1381-88.

[10] Bhattacharya, S.; Sinha, P.K.; Sundar, S.; Thakur, C.P.; Jha, T.K.; Pandey, K.; Das, V.R.; Kumar, N.; Lal, C.; Verma, N.; Singh, V.P.; Ranjan, A.; Verma, R.B.; Anders, G.; Sindermann, H.; Ganguly, N.K. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. *J. Infect. Dis.,* **2007**, *196*, 591-98.

[11] Fuertes, M.A.; Nguewa, P.A.; Castilla, J.; Alonso, C.; Pérez, J.M. Anticancer compounds as leishmanicidal drugs: challenges in chemotherapy and future perspectives. *Curr. Med. Chem.,* **2008**, *15*, 433-39.

[12] Berman, J.; Bryceson, A.D.; Croft, S.; Engel, J.; Gutteridge, W.; Karbwang, J.; Sindermann, H.; Soto, J.; Sundar, S.; Urbina, J.A. Miltefosine: issues to be addressed in the future. *Trans. R. Soc. Trop. Med. Hyg.,* **2006**, *100*, S41-4.

[13] Croft, S.; Coombs, G.H. Leishmaniasis - current chemotherapy and recent advances in the search for novel drugs. *Trends Parasitol.,* **2003**, *19*, 502-8.

[14] Shapiro, T.A.; Englund, P.T. The structure and replication of kinetoplast DNA. *Annu. Rev. Microbiol.,* **1995**, 49, 117-43.

[15] Stuart, K.; Panigrahi, A.K. RNA editing: complexity and complications. *Mol. Microbiol.,* **2002**, *45*, 591-6.

[16] Shlomai, J. The structure and replication of kinetoplast DNA. *Curr. Mol. Med.,* **2004**, *4*, 623-47.

[17] Liu, B.; Liu, Y.; Molyka, S.A.; Agbo, E.E.C.; Englund, P.T. Felowships of the rings: the replication of kinetoplast DNA. *Trends Parasitol.,* **2005**, *21*, 363-9.

[18] Carpenter, L.R.; Englund, P.T. Kinetoplast maxicircle DNA replication in Crithidia fasciculata and Trypanosoma brucei. *Mol. Cell. Biol.,* **1995**, *15*, 6794-803.

[19] Vinograd, J. L.J.; Radloff, R.; Watson, R.; Laipis, P. The twisted circular form of polyoma viral DNA. *Proc. Natl. Acad. Sci. USA,* **1965**, *53*, 1104-11.

[20] De Witt, S. Lifting the curtain: using topology to probe the hidden action of enzymes. *Not. Am. Math. Soc.,* **1995**, *42*, 528-37.

[21] Wang, J.C. Interaction between DNA and an *Escherichia coli* protein omega. *J. Mol. Biol.,* **1971**, *55*, 523-33.

[22] Corbett, K.; Berger, J.M. Structure, molecular mechanisms, and evolutionary relationships in DNA topoisomerases. *Annu. Rev. Biophys. Biomol. Struct.,* **2004**, *33*, 95-118.

[23] Champoux, J.J. DNA topoisomerases: Structure, function, and mechanism. *Annu. Rev. Biochem.,* **2001**, *70*, 369-413.

[24] Wang, J. DNA topoisomerases. *Annu. Rev. Biochem.,* **1996**, *65*, 635-92.

[25] Bates, A.D.; Maxwell, A. Energy coupling in type II topoisomerases: why do they hydrolyze ATP? *Biochemistry,* **2007**, *46*, 7929-41.

[26] McClendon, A.K.; Osheroff, N. DNA topoisomerase II, genotoxicity, and cancer. *Mutat. Res.,* **2007**, *623*, 83-97.

[27] Viard, T.; de la Tour, C.B. Type IA topoisomerases: a simple puzzle? *Biochimie,* **2007**, *89*, 456-67.

[28] Tse-Dinh, Y.C. Bacterial and archeal type I topoisomerases. *Biochim. Biophys. Acta,* **1998**, *1400*, 19-27.

[29] Leppard, J.B.; Champoux, J.J. Human DNA topoisomerase I: relaxation, roles, and damage control. *Chromosoma,* **2005**, *114*, 75-85.

[30] Aravind, L.; Leipe, D.D.; Koonin, E.V. Toprim-a conserved catalytic domain in type IA and II topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. *Nucleic Acids Res.,* **1998**, *26*, 4205-13.

[31] Shuman, S. Vaccinia virus DNA topoisomerase: a model eukaryotic type IB enzyme. *Biochim. Biophys. Acta,* **1998**, *1400*, 321-37.

[32] Berger, J.; Gamblin, S.J.; Harrison, S.C.; Wang, J.C. Structure and mechanism of DNA topoisomerase II. *Nat. Rev. Microbiol.,* **1996**, *379*, 225-32.

[33] Brown, P.O.; Cozzarelli, N.R. A sign inversion mechanism for enzymatic supercoiling of DNA. *Science,* **1979**, *206*, 1081-3.

[34] Bergerat, A.; Gadelle, D.; Forterre, P. Purification of a DNA topoisomerase II from the hyperthermophilic archaeon Sulfolobus shibatae: a thermostable enzyme with both bacterial and eucaryal features. *J. Biol. Chem.,* **1994**, *269*, 27663-9.

[35] Forterre, P.; Gribaldo, S.; Gadelle, D.; Serre, M.C. Origin and evolution of DNA topoisomerases. *Biochimie,* **2007**, *89*, 427-46.

[36] Buhler, C.; Lebbink, J.H.; Bocs, C.; Ladenstein, R.; Forterre, P. DNA topoisomerase VI generates ATP-dependent double-strand breaks with two-nucleotide overhangs. *J. Biol. Chem.,* **2001**, *276*, 37215-22.

[37] Rybenkov, V.V.; Ullsperger, C.; Vologodskii, A.V.; Cozzarelli, N.R. Simplification of DNA topology below equilibrium values by type II topoisomerases. *Science,* **1997**, *277*, 690-3.

[38] Reguera, R.M.; Díaz-González, R.; Pérez-Pertejo, Y.; Balaña-Fouce, R. Characterizing the bi-subunit type IB DNA topoisomerase of Leishmania parasites; a novel scenario for drug intervention in trypanosomatids. *Curr. Drug Targets,* **2008**, *9*, 966-78.

[39] Scocca, J.R.; Shapiro, T.A. A mitochondrial topoisomerase IA essential for late theta structure resolution in African trypanosomes. *Mol. Microbiol*., **2008**, *67*, 820-9.

[40] Burri, C.; Bodley, A.L.; Shapiro, T.A. Topoisomerases in kinetoplastids. *Parasitol. Today,* **1996**, *12*, 226-31.

[41] Broccoli, S.; Marquis, J.F.; Papadopoulou, B.; Oliver, M.; Drolet, M. Characterization of a Leishmania donovani gene encoding a protein that closely resembles a type IB topoisomerase. *Nucleic Acids Res.,* **1999**, *27,* 2745-52.

[42] Das, A.; Mandal, C.; Dasgupta, A.; Sengupta, T.; Majumder, H.K. An insight into the active site of a type I topoisomerase from the kinetoplastid protozoan Leishmania donovani. *Nucleic Acids Res*., **2002**, *30*, 794-802.

[43] 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.; Ordóñez, D. A novel active DNA topoisomerase I in Leishmania donovani. *J. Biol. Chem.,* **2003**, *278*, 3521-6.

[44] Das, B.B.; Sen, N.; Ganguly, A.; Majumder, H.K. Reconstitution and functional characterization of the unusual bi-subunit type I DNA topoisomerase from Leishmania donovani. *FEBS Lett.,* **2004**, *565*, 81-8.

[45] Das, B.B.; Sengupta, T.; Ganguly, A.; Majumder, H.K. Topoisomerases of kinetoplastid parasites: why so fascinating? *Mol. Microbiol.,* **2006**, *62*, 917-27.

[46] Balaña-Fouce, R.; Redondo, C.M.; Pérez-Pertejo, Y.; Díaz-González, R.; Reguera, R.M. Targeting atypical trypanosomatid DNA topoisomerase. *Drug Discov. Today,* **2006**, *15-16*, 733-40.

[47] Bodley, A.; Chakraborty, A.K.; Xie, S.; Burri, C.; Shapiro, T.A. An unusual type IB topoisomerase from African trypanosomes. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*, 7539-44.

[48] Bakshi, R.P.; Shapiro, T.A. DNA topoisomerases as targets for antiprotozoal chemotherapy. *Mini Rev. Med. Chem.,* **2003**, *3*, 597-608.

[49] Bosedasgupta, S.; Das, B.B.; Sengupta, S.; Ganguly, A.; Roy, A.; Tripathi, G.; Majumder, H.K. Amino acids 39-456 of large subunit and 210-262 of small subunit constitute the minimal, functionally interacting fragments of the unusual heterodimeric topoisomerase IB of Leishmania. *Biochem. J.,* **2008**, *409*, 481-9.

[50] Zhang, H.; Barcelo, J.M.; Lee, B.; Kohlhagen, G.; Zimonjic, D.B.; Popescu, N.C.; Pommier, Y. Human mitochondrial topoisomerase I. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*, 10608-13.

[51] Zhang, H.; Meng, L.H.; Pommier, Y. Mitochondrial topoisomerases and alternative splicing of the human TOP1mt gene. *Biochimie,* **2007**, *89*, 474-81.

[52] Bosedasgupta, S.; Ganguly, A.; Das, B.B.; Roy, A.; Khalkho, N.V.; Majumder, H.K. The large subunit of Leishmania topoisomerase I functions as the 'molecular steer' in type IB topoisomerase. *Mol. Microbiol.,* **2008**, *67*, 31-46.

[53] Uemura, T.; Morino, K.; Uzawa, S.; Shiozaki, K.; Yanagida, M. Cloning and sequencing of Schizosaccharomyces pombe DNA topoisomerase I gene, and effect of gene disruption. *Nucleic Acids Res.,* **1987**, *15*, 9727-39.

[54] Lee, M.; Brown, S.D.; Chen, A.; Hsieh, T.S. DNA topoisomerase I is essential in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*, 6656-60.

[55] Morham, S.; Kluckman, K.D.; Voulomanos, N.; Smithies, O. Targeted disruption of the mouse topoisomerase I gene by camptothecin selection. *Mol. Cell. Biol.,* **1996**, *16*, 6804-9.

[56] Bakshi, R.; Shapiro, T.A. RNA interference of Trypanosoma brucei topoisomerase IB: Both subunits are essential. *Mol. Biochem. Parasitol.,* **2004**, *136*, 249-55.

[57] Balaña-Fouce, R.; García-Estrada, C.; Pérez-Pertejo, Y.; Reguera, R.M. Gene disruption of the DNA topoisomerase IB small subunit induces a non-viable phenotype in the hemoflagellate Leishmania major. *BMC Microbiol.,* **2008**, *8*, 113.

[58] Kulikowicz, T.; Shapiro, T.A. Distinct genes encode type II Topoisomerases for the nucleus and mitochondrion in the protozoan parasite Trypanosoma brucei. *J. Biol. Chem.,* **2006**, *281*, 3048-56.

[59] Shapiro, T.A. Mitochondrial topoisomerase II activity is essential for kinetoplast DNA minicircle segregation. *Mol. Cell. Biol.,* **1994**, *14*, 3660-7.

[60] Low, R.L.; Orton, S.; Friedman, D.B. A truncated form of DNA topoisomerase IIbeta associates with the mtDNA genome in mammalian mitochondria. *Eur. J. Biochem*., **2003**, *270*, 4173-86.

[61] Sengupta, T.; Mukherjee, M.; Mandal, C.; Das, A.; Majumder, H.K. Functional dissection of the C-terminal domain of type II DNA topoisomerase from the kinetoplastid hemoflagellate Leishmania donovani. *Nucleic Acids Res.,* **2003**, *31*, 5305-16.

[62] Sengupta, T.; Mukherjee, M.; Das, A.; Mandal, C.; Das, R.; Mukherjee, T.; Majumder, H.K. Characterization of the ATPase activity of topoisomerase II from Leishmania donovani and identification of residues conferring resistance to etoposide. *Biochem. J.,* **2005**, *390*, 419-26.

[63] Wang, Z.; Englund, P.T. RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. *EMBO J.,* **2001**, *20*, 4674-83.

[64] Champoux, J.; Dulbecco, R. An activity from mammalian cells that untwists superhelical DNA-a possible swivel for DNA replication. *Proc. Natl. Acad. Sci. USA,* **1972**, *69*, 143-6.

[65] D'Arpa, P.; Liu, L.F. Topoisomerase-targeting antitumor drugs. *Biochim. Biophys. Acta,* **1989**, *989*, 163-77.

[66] Pommier, Y.; Pourquier, Ph.; Fan, Y.; Strumberg, D. Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme. *Biochim. Biophys. Acta,* **1998**, *1400*, 83-106.

[67] Das, A.; Dasgupta, A.; Sengupta, T.; Majumder, H.K. Topoisomerases of kinetoplastid parasites as potential chemotherapeutic targets. *Trends Parasitol.,* **2004**, *20*, 381-7.

[68] Wall, M.; Wani, M.C.; Cook, C.E.; Palmer, K.H.; Mc Pail, A.T.; Sim, G.A. Plant antitumour agents: the isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca accuminata. *J. Am. Chem. Soc.,* **1966**, *88*, 3888-90.

[69] Champoux, J.J. Structure-based analysis of the effects of camptothecin on the activities of human topoisomerase I. *Ann. NY. Acad. Sci.,* **2000**, *922*, 56-64.

[70] Pommier, Y. Topoisomerase I inhibitors: Camptothecins and beyond. *Nat. Rev. Cancer,* **2006**, *6*, 789-802.

[71] Liu, L.F.; Desai, S.D.; Li, T.K.; Mao, Y.; Sun, M.; Sim, S.P. Mechanism of action of camptothecin. *Ann. NY. Acad. Sci.,* **2000**, *922*, 1-10.

[72] Bodley, A.L.; Shapiro, TA. Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibitor, on trypanosomes and Leishmania. *Proc. Natl. Acad. Sci. USA,* **1995**, *92*, 3726-30.

[73] Bodley, A.; Wani, M.C.; Wall, M.E.; Shapiro, T.A. Antitrypanosomal activity of camptothecin analogs. Structure-activity correlations. *Biochem. Pharmacol.,* **1995**, *50*, 937-42.

[74] Deterding, A.; Dungey, F.A.; Thompson, K.A.; Steverding, D. Anti-trypanosomal activities of DNA topoisomerase inhibitors. *Acta Trop.,* **2005**, *93*, 311-6.

[75] Staker, B.; Hjerrild, K.; Feese, M.D.; Behnke, C.A.; Burgin, A.B. Jr.; Stewart, L. The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 15387-92.

[76] Proulx, M.; Desormeaux, A.; Marquis, J.F.; Olivier, M.; Bergeron, M.G. Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin. *Antimicrob. Agents Chemother.,* **2001**, *45*, 2623-7.

[77] Cao, R.; Peng, W.; Chen, H.; Ma, Y.; Liu, X.; Hou, X.; Guan, H.; Xu, A. DNA-binding properties of 9-substituted harmine derivatives. *Biochem. Biophys. Res. Comm.,* **2005**, *338*, 1557-63.

[78] Prudhomme, M. Rebeccamycin analogues as anti-cancer agents. *Eur. J. Med. Chem.,* **2003**, *38*, 123-40.

[79] Chiang, J.L.; Averboukh, L.; Pardee, A.B. β-Lapachone, a novel DNA-toposiomerase I inhibitor with a mode of action different from camptothecin. *J. Biol. Chem.,* **1993**, *268*, 22463.

[80] Hazra, B.; Saha, A.K.; Ray, R.; Roy, D.K.; Sur, P.; Banerjee, A. Antiprotozoal activity of diospyrin towards Leishmania donovani promastigotes *in vitro*. *Trans. R. Soc. Trop. Med. Hyg.,* **1987**, *81*, 738-41.

[81] Ray, S.; Hazra, B.; Mitra, B.; Das, A.; Majumder, H.K. Diospyrin, a bisnaphthoquinone: a novel inhibitor of type I DNA topoisomerase of Leishmania donovani. *Mol. Pharmacol.,* **1998**, *54*, 994-9.

[82] Syrovets, T.; Buchele, B.; Gedig, E.; Slupsky, J.R.; Simmet, T. Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and II. *Mol. Pharmacol.,* **2000**, *58*, 71-81.

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

[84] Merschjohann, K.; Sporer, F.; Steverding, D.; Wink, M. *In vitro* effect of alkaloids on bloodstream forms of Trypanosoma brucei and T. congolense. *Planta Med.,* **2001,** *67*, 623-7.

[85] Vennerstrom, J.; Lovelace, J.K.; Waits, V.B.; Hanson, W.L.; Klayman, D.L. Berberine derivatives as antileishmanial drugs. *Antimicrob. Agents Chemother.,* **1990**, *34*, 918-21.

[86] Lucumi, A. ; Robledo, S. ; Gama, V. ; Saravia, N.G. Sensitivity of Leishmania viannia panamensis to pentavalent antimony is correlated with the formation of cleavable DNA-protein complexes. *Antimicrob. Agents Chemother*., **1998**, *42*, 1990-5.

[87] Chakraborty, A.; Majumder, H.K. Mode of action of pentavalent antimonials: specific inhibition of type I DNA topoisomerase of Leishmania donovani. *Biochem. Biophys. Res. Commun.,* **1988**, *152*, 605-11.

[88] Walker, J.; Saravia, N.G. 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.,* **2004**, *90*, 1155-62.

[89] Wilson, W.D.; Tanious, F.A.; Mathis, A.; Tevis, D.; Hall, J.E.; Boykin, D.W. Antiparasitic compounds that target DNA. *Biochimie,* **2008**, *90*, 999-1014.

[90] Johnson, R.; Cubría, J.C.; Reguera, R.M.; Balaña-Fouce, R.; Ordóñez, D. Interaction of cationic diamidines with Leishmania infantum DNA. *Biol. Chem*., **1998**, *379*, 925-30.

[91] Chen, A.; Yu, C.; Gatto, B.; Liu, L.F. DNA minor groove-binding ligands: a different class of mammalian DNA topoisomerase I inhibitors. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*, 8131-5.

[92] Cortazar, T.M.; Coombs, G.H.; Walker, J. 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*., **2007**, *116*, 475-82.

[93] Jean-Moreno, V.; Rojas, R.; Goyeneche, D.; Coombs, G.H.; Walker, J. Leishmania donovani: differential activities of classical topoisomerase inhibitors and antileishmanials against parasite and host cells at the level of DNA topoisomerase I and in cytotoxicity assays. *Exp. Parasitol.,* **2006**, *112*, 21-30.

[94] Cheesman, S.J. The topoisomerases of protozoan parasites. *Parasitol. Today,* **2000**, *16*, 277-81.

[95] Boege, F.; Straub, T.; Kehr, A.; Boesenberg, C.; Christiansen, K.; Andersen, A.; Jakob, F.; Köhrle, J. Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. *J. Biol. Chem.,* **1996**, *271*, 2262-70.

[96] Das, B.B.; Sen, N.; Roy, A.; Dasgupta, S.B.;, Ganguly, A.; Mohanta, B.C.; Dinda, B.; Majumder, H.K. 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.,* **2006**, *34*, 1121-32.

[97] Tasdemir, D.; Kaiser, M.; Brun, R.; Yardley, V.; Schmidt, T.J.; Tosun, F.; Rüedi, P. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrob. Agents Chemother*., **2006**, *50*, 1352-64.

[98] Mittra, B.; Saha, A.; Chowdhury, A.R.; Pal, C.; Mandal, S.; Mukhopadhyay, S.; Bandyopadhyay, S.; Majumder, H.K. 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.,* **2000**, *6*, 527-41.

[99] Shapiro, T.A.; Englund, P.T. Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. *Proc. Natl. Acad. Sci. USA,* **1990**, *87*, 950-4.

[100] Shapiro, T.A.; Showalter, A.F. *In vivo* inhibition of trypanosome mitochondrial topoisomerase II: effects on kinetoplast DNA maxicircles. *Mol. Cell. Biol.,* **1994**, *14*, 5891-7.

[101] Garbett, N.C.; Graves, D.E. Extending nature's leads: the anticancer agent ellipticine. *Curr. Med. Chem. Anticancer Agents,* **2004**, *4*, 149-72.

[102] Douc-Rasy, S.; Kayser, A.; Riou, G.F. Inhibition of the reactions catalysed by a type I topoisomerase and catenating enzyme of Trypanosoma cruzi by DNA-intercalating drugs. Preferential inhibition of the catenating reaction. *EMBO J.,* **1984**, *3*, 11-6.

[103] Shapiro, T.A.; Klein, V.A.; Englund, P.T. Drug-promoted cleavage of kinetoplast DNA minicircles. Evidence for type II topoisomerase activity in trypanosome mitochondria. *J. Biol. Chem.,* **1989**, *264*, 4173-8.

[104] Drlica, K. Mechanism of fluoroquinolone action. *Curr. Opin. Microbiol.,* **1999**, *2*, 504-8.

[105] Drlica, K.; Malik, M. Fluoroquinolones: action and resistance. *Curr. Top. Med. Chem.,* **2003**, *3*, 249-82.

[106] Gonzales-Perdomo, M.; Lisboa de Castro, S.; Meirelles, M. N. S. L.; Goldenberg, S. Trypanosoma cruzi proliferation and differentiation are blocked by topoisomerase II inhibitors. *Antimicrob. Agents Chemother.,* **1990**, *34*, 1707-14.

[107] Nenortas, E.; Burri, C.; Shapiro, T.A. Antitrypanosomal activity of fluoroquinolones. *Antimicrob. Agents Chemother.,* **1999,** *43*, 2066-8.

[108] Nenortas, E.; Kulikowicz, T.; Burri, C.; Shapiro, T.A. Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions. *Antimicrob. Agents Chemother.,* **2003**, *47*, 3015-7.

[109] Shlomai, J.; Zadok, A. Reversible decatenation of kinetoplast DNA by a DNA topoisomerase from trypanosomatids. *Nucleic Acids Res*., **1983**, *11*, 4019-34.

[110] Chakraborty, A.K.; Majumder, H.K. Decatenation of kinetoplast DNA by an ATP-dependent DNA topoisomerase from the kinetoplast hemoflagellate Leishmania donovani. *Mol. Biochem. Parasitol.,* **1987**, *26*, 215-24.

[111] Pate, P.G.; Wolfson, J.S.; McHugh, G.L.; Pan, S.C.; Swartz, M.N. Novobiocin antagonism of amastigotes of Trypanosoma cruzi growing in cell-free medium. *Antimicrob. Agents Chemother*., **1986**, *29,* 426-31.

[112] Singh, G.; Jayanarayan, K.G.; Dey, C.S. Mol. Novobiocin induces apoptosis-like cell death in topoisomerase II over-expressing arsenite resistant Leishmania donovani. *Biochem. Parasitol*., **2005**, *141*, 57-69.

[113] Werbovetz, K.A.; Lehnert, E.K.; Macdonald, T.L.; Pearson, R.D. Cytotoxicity of acridine compounds for Leishmania promastigotes *in vitro*. *Antimicrob. Agents Chemother*., **1992**, *36*, 495-7.

[114] Figgitt, D.; Denny, W.; Chavalitshewinkoon, P.; Wilairat, P.; Ralph, R. *In vitro* study of anticancer acridines as potential antitrypanosomal and antimalarial agents. *Antimicrob. Agents Chemother*., **1992**, *36*, 1644-7.

[115] Obexer, W.; Schmid, C.; Galy, J.P.; Brun, R. Activity and structure relationship of acridine derivatives against African trypanosomes. *Trop. Med. Parasitol*., **1995**, *46*, 49-53.

[116] Werbovetz, K.A.; Spoors, P.G.; Pearson, R.D.; Macdonald, T.L. Cleavable complex formation in Leishmania chagasi treated with anilinoacridines. *Mol. Biochem. Parasitol*.,**1994**, *65*, 1-10.

[117] Mauël, J.; Denny, W.; Gamage, S.; Ransijn, A.; Wojcik, S.; Figgitt, D.; Ralph, R. 9-Anilinoacridines as potential antileishmanial agents. *Antimicrob. Agents Chemother*., **1993**, *37*, 991-6.

[118] Gamage, S.A.; Figgitt, D.P.; Wojcik, S.J.; Ralph, R. K.; Ransijn, A.; Mauel, J.; Yardley, V.; Snowdon, D.; Croft, S.L.; Denny, W.A. Structure-activity relationships for the antileishmanial and antitrypanosomal activities of 1'-substituted 9-anilinoacridines. *J. Med. Chem*., **1997**, *40*, 2634-42.

[119] Davies, D.; Mushtaq, A.; Interthal, H.; Champoux, J.J.; Hol, W.G. The structure of the transition state of the heterodimeric topoisomerase I of Leishmania donovani as a vanadate complex with nicked DNA. *J. Mol. Biol*., **2006**, *357*, 1202-10.

[120] Díaz González, R.; Pérez-Pertejo, Y.; Ordóñez, D.; Balaña-Fouce, R.; Reguera, R.M. Deletional study of DNA topoisomerase IB from Leishmania donovani: searching for a minimal functional heterodimer. *PLoS ONE,* **2007**, *2*, e1177.

[121] Díaz-González, R.; Pérez-Pertejo, Y.; Redondo, C.M.; Pommier, Y.; Balaña-Fouce, R.; Reguera, R.M. Structural insights on the small subunit of DNA topoisomerase I from the unicellular parasite Leishmania donovani. *Biochimie,* **2007**, *89*, 1517-27.

Received: 07 February, 2009 Revised: 24 March, 2009 Accepted: 30 March, 2009