REVIEW ARTICLE

Molecular biology of *Leishmania*: Kinetoplast DNA and DNA topoisomerases as novel therapeutic targets

Asit K. Chakraborty and Hemanta K. Majumder*

Molecular Parasitology Laboratory, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Calcutta 700 032, India

Human leishmaniasis is caused by the unicellular kinetoplastid protozoan parasites of the genus *Leishmania*. About 22 different species or sub-species of *Leishmania* are separately responsible for a complex set of deadly clinical symptoms worldwide. The related parasites belonging to the genus *Trypanosoma* also result in fatal diseases like sleeping sickness and Chagas' disease in humans, and economically significant diseases in cattle. *Trypanosoma* sp. can evade the immune response of human by changing the antigenic epitopes of surface glycoproteins. In *Leishmania* sp. the evasion of the immune response is even more complex. Thus, no suitable vaccine has been developed either for leishmaniasis or trypanosomiasis, and chemotherapy is the only way by which these parasitic diseases are being treated. Development of drug-resistant forms of these parasites suggest that these pose a major medical threat unless some potent nontoxic drugs or vaccines are developed in the near future. DNA topoisomerases that catalyse a variety of DNA topoisomerization reactions may well provide suitable targets for many antibacterial, antiparasitic and anticancer drugs. Due to complexity of the kinetoplast DNA (kDNA), being the mitochondrial DNA of the parasite, it has been shown that multiple DNA topoisomerases are necessary for the replication of kDNA and probably for its transcription and recombination as well. Many drugs used for the treatment of parasitic diseases like leishmaniasis (kala-azar), Chagas disease and sleeping sickness are found to be potent inhibitors of different types of topoisomerases isolated from those parasites. Thus, the complex mechanisms of kDNA replication and the novelty of topoisomerases in kinetoplastid parasites are subjects of intensive research to develop safer and more topo-active drugs, synthesized chemically or isolated from medicinal plants.

THE various problems of disentangling DNA strands or duplexes in a cell are all rooted in the double helical structure of the DNA. Distinct enzymes known as the DNA topoisomerases, have evolved to solve these problems[1]. These DNA topoisomerases control many vital cellular processes like replication, transcription, chromosomal segregation and recombination, by catalysing topological interconversion of DNA molecules either by nicking–closing the single-strand of the double-stranded DNA (type I topoisomerase), or both strands of the double-stranded DNA (type II topoisomerase)[2,3]. Structural and catalytic differences among the type I and type II topoisomerases, as well as their evolutionary divergence, have made it possible to discover many inhibitors[3–6]. Since the late 1970s, prokaryotic type II topoisomerase (gyrase) inhibitors have been mostly used as drugs for the treatment of a diverse range of diseases caused by gram-positive and gram-negative bacteria. In mid-1980s, the discovery of anticancer properties of many topoisomerase inhibitors quickly placed these compounds as the frontier molecular medicinal drugs, and as a subject of active molecular biological research[4,7–9]. Furthermore, topoisomerases have been discovered as the target of many antileishmanial and antitrypanosomal drugs, resulting in further impetus to topoisomerase research[10–12]. Human diseases like visceral leishmaniasis and sleeping sickness caused by *L. donovani* and *T. brucei* affecting millions, have become an international issue for the development of safer drugs for these deadly parasitic diseases[13]. Recently, the focus of research has been on studying the effect of many synthetic and bioactive natural compounds on the activities of these parasitic topoisomerases[6,14,15]. We will define here the state of the art of the current research and development in this area.

*For correspondence. (e-mail: hkmajum@calz.vsnl.net.in)

Unique molecular targets of kinetoplastid organisms, differing from host

Kinetoplastid parasites exist as two distinct morphological and biochemical forms: the flagellated promastigote forms in sandfly vectors and ovoid amastigote forms in the macrophages of the infected individuals. Human

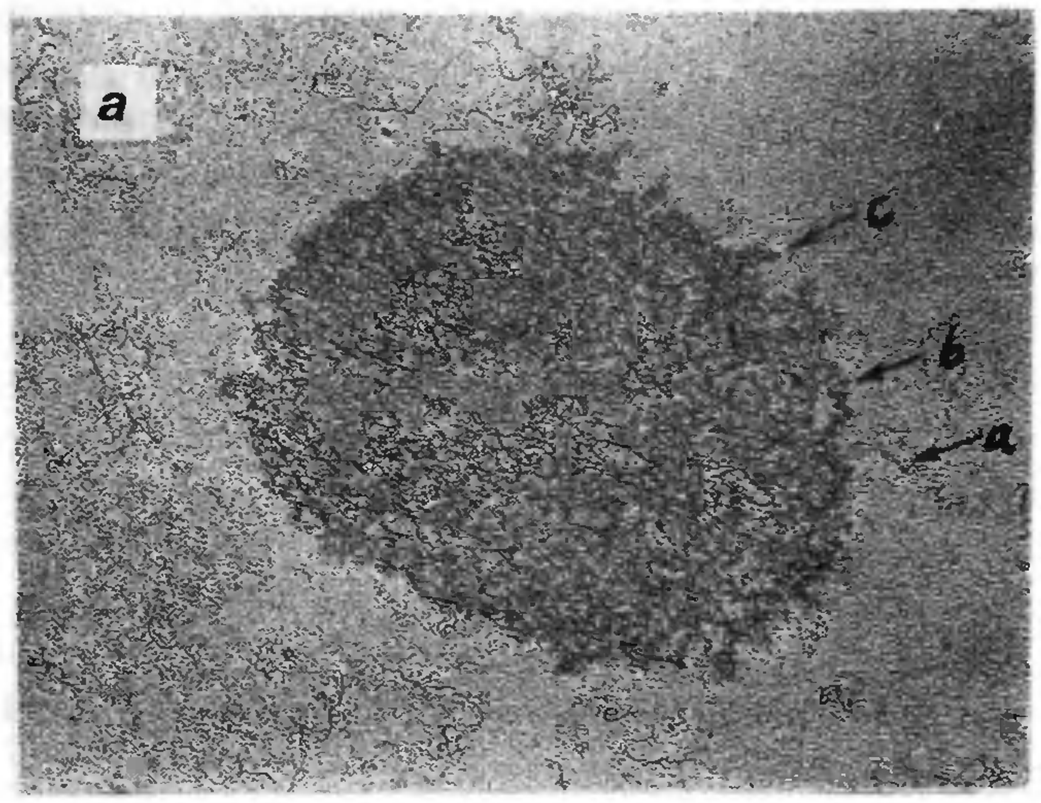
leishmaniasis is caused by several species of protozoan parasite *Leishmania*: cutaneous leishmaniasis is caused by *L. major, L. tropica*; mucocutaneous leishmaniasis by *L. braziliensis, L. mexicana*; and most devastating form, visceral leishmaniasis or kala-azar by *L. donovani, L. infantum* and *L. chagasi*[16,17]. The different types of leishmaniasis can be detected as distinct entities; clinically as well as pathologically[18,19]. Visceral leishmaniasis or kala-azar is a fatal disease which affects spleen and liver. It is prevalent in the Indian subcontinent and in many tropical and subtropical countries with increasing number of antimonial and pentamidine drug-resistant cases[18,20].

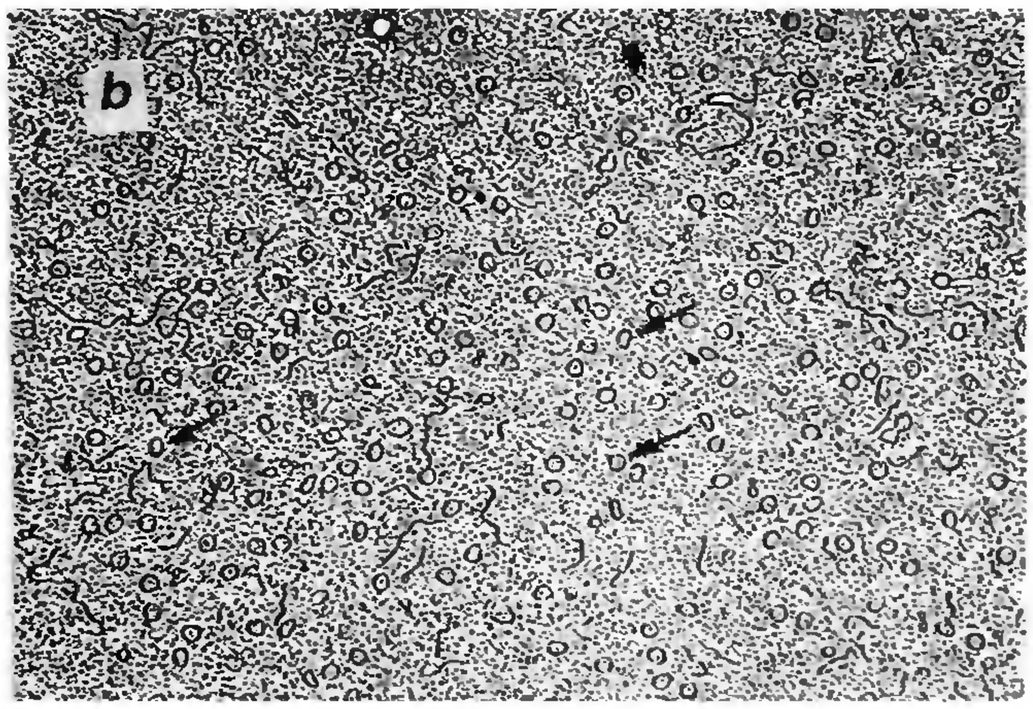
Kinetoplastid parasites have many unusual features that differ from their hosts. These are: (i) intercatenated kDNA networks within the mitochondria; (ii) extensive RNA editing; (iii) antigenic variation; (iv) novel way of nucleoside biosynthesis; and (v) glycosomes[15,21]. These differences may lead to new targets for chemotherapy and for vaccine development. The kDNA is unique as none of the host organisms of these parasites have DNA that resembles kDNA, and therefore, it may be possible to specifically inhibit the replication and expression of kDNA. This specific inhibition may result in selective killing of the parasites, as the kinetoplast gene products are necessary at all stages of the life cycles of some of the parasites, for example *L. donovani* and *T. cruzi*[22]. We shall focus our attention here mainly on the kDNA and DNA topoisomerases of the parasites that may act as targets of various therapeutic agents, either chemically synthesized or isolated from medicinal plants.

kDNA as targets of antiparasitic drugs

The unique kDNA (Figure 1 *a*) consists of five to ten thousands of minicircles (~0.8–2.5 kb) (Figure 1 *b*) and 30–50 (~ 30 kb) maxicircles (Figure 1 *c*), localized near the base of the flagellum in a disk-shaped body termed kinetoplast[21]. Maxicircles are equivalent to mitochondrial DNA of eukaryotes and encode genes for enzymes of oxidative phosphorylation. The functions of the 5000–10000 minicircles, which were mostly unknown, have recently been unraveled and some have been found to code for smaller (40–50 nucleotides) guide RNA molecules that are involved in RNA editing of mitochondrial genes[23].

Among the kinetoplastid parasites, minicircles are mostly 0.86–0.88 kb length in *Leishmania* sp., 1–1.2 kb in *Trypanosoma* sp., and 2.5 kb in *Crithidia*. In *Leishmania* sp. and *Trypanosoma* sp., minicircles are highly heterogeneous in sequence unlike in *Crithidia* which contains mostly homogenous minicircles sequences[21]. Because of the massive intercatenated network structure, the kDNA offer themselves as potential targets for various therapeutic agents. Many trypanoci-





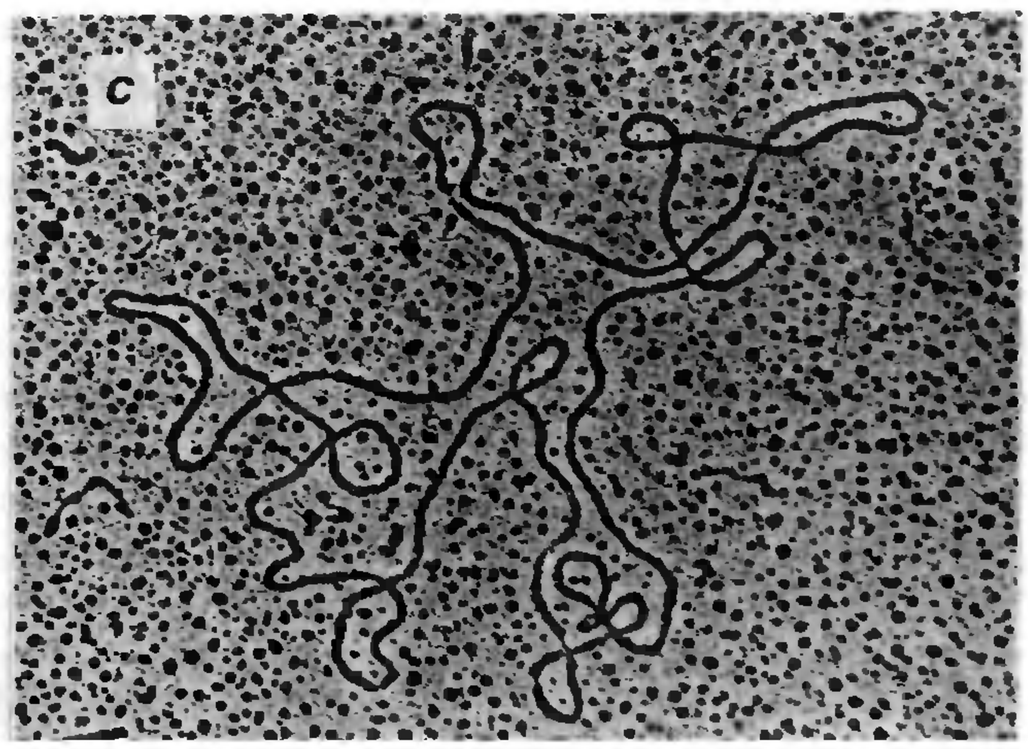


Figure 1. Electron micrograph of kDNA. *a*, kDNA networks of *Leishmania* strain UR6 promastigotes. Networks have an average size of 6.8 µM. Minicircles from rosettes. Long-looped maxicircle DNA is seen at the rim (arrow marked a). Tandem repeated chains of minicircles (arrow marked b) occur at the periphery. Minicircles can also be seen to be arranged in lampbrush-like structure (arrow marked c) (× 15000). kDNA networks were treated with *Leishmania* type II DNA topoisomerase under standard decatenation assay conditions as described[41]. The DNA products were extracted with phenol in presence of 0.1% SDS and precipitated with ethanol. *b*, Decatenated minicircles marked by arrows. (× 30000). *c*, A decatenated maxicircle (× 60000).

dal drugs like pentamidine, berenil, hydroxystibamidine (diamidines), ethidium bromide, acriflavine, proflavine, etc. interact with DNA producing various morphological alterations in the kDNA[24]. Ethidium bromide, an intercalating drug, produces conformational change in the kDNA by modifying the supercoiling of the DNA and blocks replication and transcription[24]. Nonintercalating drugs such as berenil, netropsin, distamycin all bind to the cellular DNA within the minor groove of the double helix[25]. Shapiro *et al.* have demonstrated that trypanocidal drugs pentamidine, berenil, suramin and ethidium bromide promote kDNA minicircle linearization in *T. equiperdum*[26]. The linearized minicircles are in a DNA–protein complex in which protein is bound to the 5′-termini. It was proposed that these drugs selectively inhibit mitochondrial type II DNA topoisomerase, preferentially disrupting mitochondrial DNA structures and generate dyskinetoplastic trypanosomes which lack mitochondrial DNA[26]. The dyskinetoplastic state found in trypanosomes including *T. brucei*, *T. equiperdum* and *T. evansi* is eventually lethal because of the impairment of the mitochondrial respiratory chain. *L. donovani* amastigotes when treated with acriflavin in tissue culture become dyskinetoplastic and loose their viability and ability to transform into promastigotes when removed from the host cell[27].

Over the past decade, interest in topoisomerases has expanded beyond the realm of basic science laboratory into the clinical arena. These enzymes are now known to be the primary cellular targets of some of the most widely prescribed antibiotics and anticancer drugs used in the treatment of human diseases. These enzymes have also been found to be targets of many antiparasitic and DNA intercalating drugs[11,28]. Many mammalian DNA topoisomerase inhibitors are potent anticancer drugs and are under clinical evaluation[6,8]. Many synthetic and bioactive organic molecules having antitopoisomerase activities are also under development as antiparasitic drugs for the treatment of leishmaniasis and trypanosomiasis[12,15].

Different types of DNA topoisomerases

In 1971 James C. Wang, and in 1976 Martin Gellert first isolated a type I and a type II DNA topoisomerase from bacteria, *Escherichia coli*. The type I topoisomerase changes super-twisted plasmid DNA into its relaxed form, and type II topoisomerase changes relaxed plasmid DNA into its negatively supercoiled form[1,2,29]. It has been demonstrated that every cell controls its DNA conformation, integrity, and functions by using these ubiquitous enzymes that perform many reactions *in vivo* including catenation–decatenation, knotting–unknotting, and relaxation–supercoiling of DNA[1,2,29] (Figure 2). The eukaryotic type I (EC#5.99.1.2) and type II

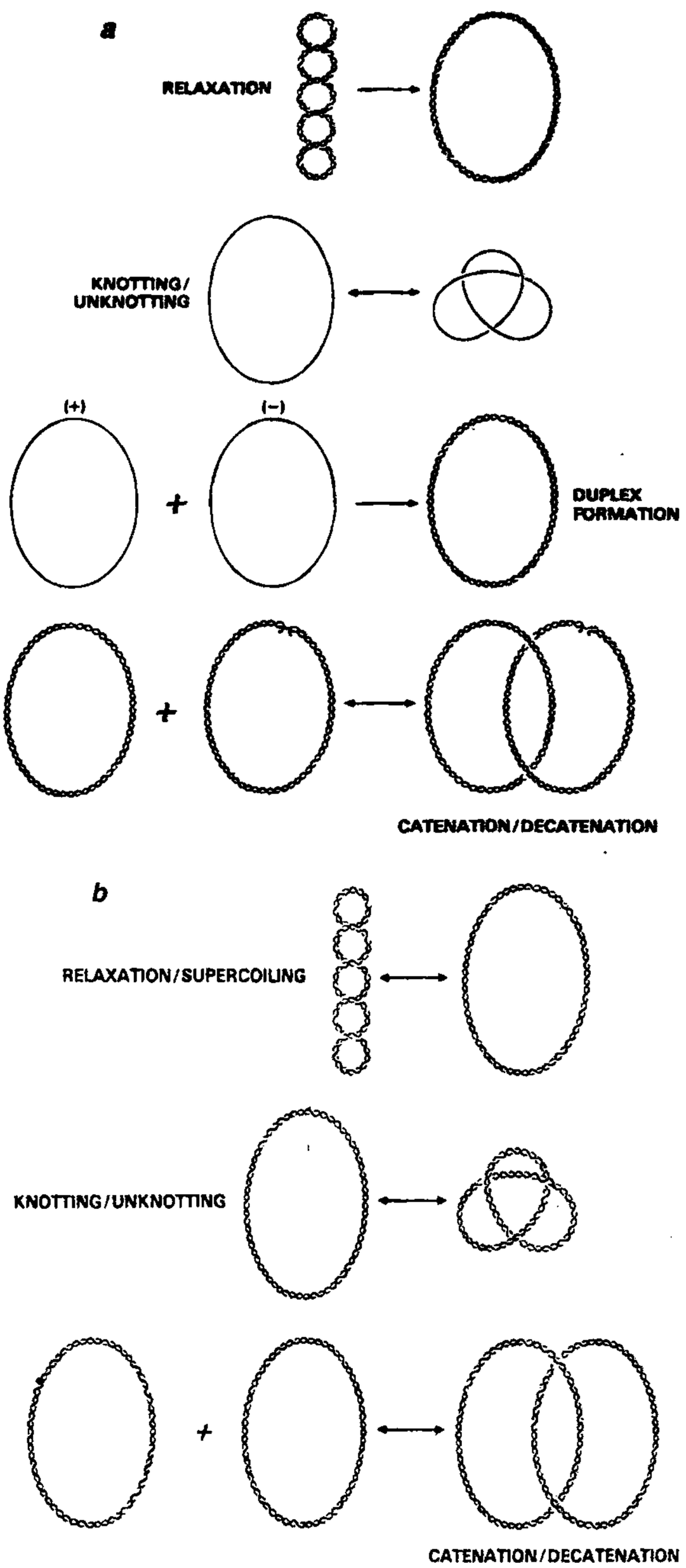


Figure 2. Topological reactions of DNA toposiomerases[63]: *a*, catalysed by type I; and *b*, by type II.

(EC#5.99.1.3) topoisomerases are best assayed by their ability to relax pBR322 DNA by changing the unique linking number in steps of one and in steps of two, re-

spectively (Figure 3). Figure 4 shows the different types of topoisomerases in different organisms named as topoisomerase I (ref. 2), II (ref. 30), III (ref. 31), IV (ref. 32), V (ref. 33), gyrase[1,2], reverse gyrase[34], etc. on the basis of their isolation and mode of action.

DNA topoisomerases in kinetoplastid parasites

DNA topoisomerases have been characterized in many kinetoplastid parasites[28] and also genes for these enzymes have been sequenced and compared[12,35] with bacterial[36] and mammalian DNA topoisomerase genes[37]. Topoisomerase II genes of kinetoplastid parasites share general eukaryotic topoisomerase II structure. Like eukaryotic topoisomerase II, the genes for topoisomerase II of kinetoplastid parasites also have four functional domains; ATP-binding domain (ATP), a linker region (L), a DNA cutting/joining (C/R) and a variable C-terminal region (VAR)[12]. The first report of topoisomerase I from kinetoplastid parasite was from *T. cruzi* and the enzyme was purified[38]. An ATP-independent catenating enzyme has been detected in *T. cruzi*. The enzyme is unable to catalyse decatenation of kDNA[39], and the same group has purified an ATP-independent topoisomerase II in *T. cruzi* and *T. equiperdum* which can catalyse both catenation and decatenation reactions[40]. The results suggest that the solubilization and purification procedures may result in the loss or gain of one or the other topoisomerase II activities or may be due to the loss or gain of topoisomerase-associated proteins that may regulate different topoisomerization reactions *in vivo*. However, an enzyme may behave functionally different due to formation of an active proteolytic product, a possibility which cannot be ruled out in case of *T. cruzi* or *L. donovani* ATP-independent topoisomerase[41].

DNA topoisomerases I and II have also been purified from *C. fasciculata*. The topoisomerase II is likely to be a homo-tetramer (60 kD monomer) and appeared to be kDNA associated[42]. A topoisomerase II from *L. donovani* has been partially purified which prefers covalently closed minicircles in kDNA networks over nicked circles, similar to that of *Crithidia* enzyme[43]. A kinetoplast-associated homodimeric topoisomerase II (132 kD monomer) has been purified by novobiocin-affinity chromatography[44]. This enzyme is located in the two sites of the kDNA periphery as judged by immunofluoresence with antibody against *Crithidia* topoisomerase II (ref. 45). The existence of two distinct mitochondrial topo II (one tetramer of 60 kD and other dimer of 132 kD) in *C. fasciculata* undoubtedly suggests that multiple topoisomerases control the kDNA breakage and rejoining reactions involving distinct topological isomers of kDNA minicircles as well as maxicircles. A type I topoisomerase has been purified from the same parasite[46]. Using antibody against *Crithidia* topoisomerase I, it has been found to cross react with extracts of *C. luciliae, L. tarentolae, H. samuelrenoai, P. davidi* and *L. seymouri*, suggesting evolutionary conservation of topoisomerases in these kinetoplastid parasites[46].

Leishmania topoisomerases have been studied in detail in our laboratory. An ATP-dependent topoisomerase II activity has been partially purified by assaying its ability to decatenate kDNA into monomeric circles[43]. This topoisomerase II fails on the catenation reaction. Our group has isolated another ATP-independent topoisomerase II, showing catenation/decatenation activity (Figure 5) using high salt extraction of *L. donovani* nuclear kinetoplast fraction[41]. A type I topoisomerase has also been purified to homogeneity. It has an apparent molecular weight of 67 kD (ref. 47). Whereas this *Leishmania* topoisomerase I relaxes only negatively supercoiled DNA by *E. coli* type I topoisomerase, the *T. cruzi* topoisomerase I enzyme relaxes both positive and negative supercoiled DNA similar to mammalian topoisomerase I enzyme.

Our understanding of kinetoplastid DNA topoisomerases is just emerging. Considering the complexities of kDNA structure, their replication and expression of maxi- and minicircles and extensive RNA editing, dual hosts, etc., it is hypothesized that several topoisomerase I and II enzymes may be present in trypanosomes. Thus the isolated ATP-independent topoisomerase II in *T. cruzi* and *L. donovani* may not be proteolytic products of ATP-dependent topoisomerase II as suggested by Burri *et al.*[12] but may represent unique type II enzymes which may associate with other necessary factors *in vivo* that reflect their activities *in vitro* due to loss or gain of such factors during enzyme isolation.

Currently search for novel RNA topoisomerase activity in species of *Leishmania* and *Trypanosoma* is being made since an extensive genetic recombination among the 10,000 minicircles, creating novel guide RNAs followed by RNA editing, may require distinct

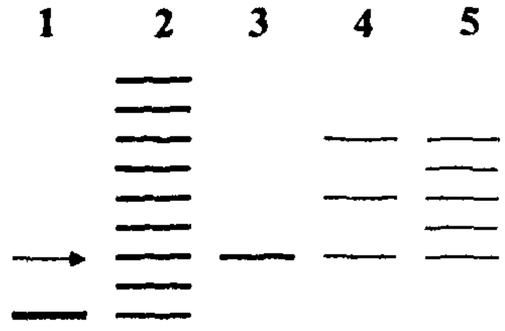


Figure 3. Assay to define topoisomerases I and II. Topoisomerase I changes the linking number in steps of one and topoisomerase II changes the linking number in steps of two. Supercoiled plasmid DNA (lane 1) is relaxed by topo I, and the topological isomers are separated by agarose gel electrophoresis (lane 2). A specific topoisomer (see arrow) is purified (lane 3) and treated with purified enzymes. The pattern of mobility is shown in lane 4 (topo II) and in lane 5 (topo I), respectively. (*Reproduced with permission from Current Drug*, London, ref. 6.)

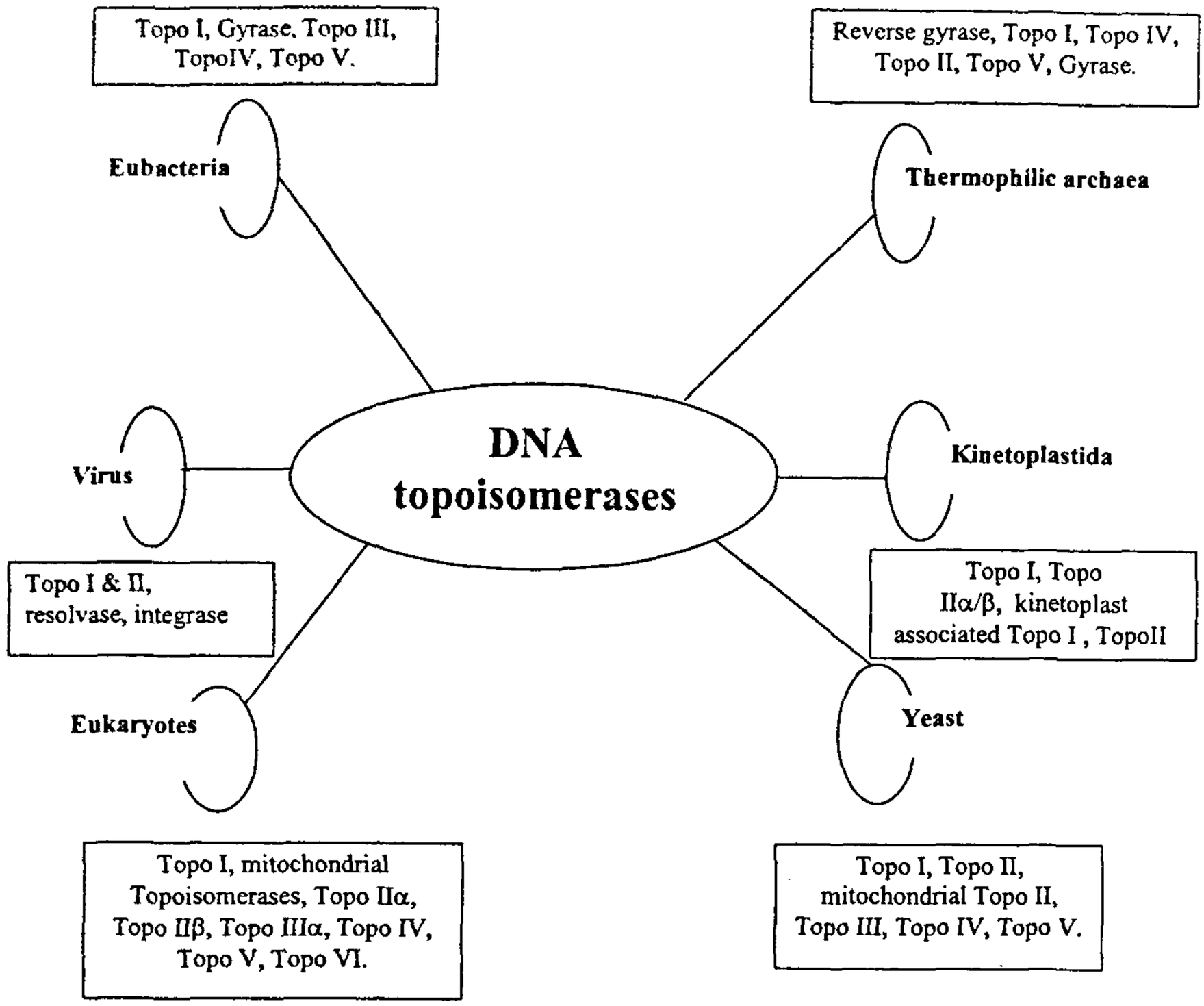


Figure 4. Types of DNA topoisomerases. DNA topoisomerases are from virus, eubacteria, thermophilic bacteria, fungi, yeast, protozoa, plant and many mammalian systems. DNA topoisomerases are named I to V according to their discovery and are specially named like gyrase (topo II type), reverse gyrase (topo I type), resolvase (topo II type), integrase (topo II type), etc.

RNA topoisomers. Such RNA topoisomerase activity has been reported recently[48].

Different classes of DNA topoisomerase inhibitors

The known DNA topoisomerase-targeted drugs can be divided into two classes. The class I drugs include the bacterial gyrase quinolone antibiotics; the eukaryotic topoisomerase I drug, camptothecin; and the eukaryotic topoisomerase II drugs doxorubicin, amsacrine, etoposide and teniposide. These compounds act by stabilizing the covalent topoisomerase I-DNA complexes. These drugs have been referred to as 'topoisomerase poisons'[29]. The class II drugs interfere with the catalytic functions of the DNA topoisomerases by binding with the enzymes and preventing enzyme–DNA binary complex formation. They have been referred to as 'topoisomerase inhibitors'[3]. Class I drugs include coumermycin family of antibiotics that act on bacterial DNA gyrase and eukaryotic topoisomerase II inhibitors suramin, merberone, indolylquinolines, etc.[49]. Several topoisomerase I inhibitors of class II type have been reported recently. Different types of topoisomerase inhibitors can be classified according to the chemical nature of the compounds (Table 1).

Oxolinic acid and nalidixic acid were found to be inhibitors of DNA gyrase[1]. Further improvement by introducing a 7-piperazinyl ring and 6-fluorine atom in the quinoline nucleus like norfloxacin, ciprofloxacin, lomefloxacin, fleroxacin, etc. or a 1,8-naphtyridine (enoxacin, tosulfloxacin) gave rise to gyrase inhibitors, fluoroquinolones[4]. Many isothioquinolones and ofloxacin analogues are potent gyrase inhibitors. These drugs appear to bind to gyrA subunit and interfere with the DNA strand breakage and rejoining reactions[4]. Novobiocin and coumermycin AI inhibit the gyrB subunit function (ATPase activity), and also inhibit many eukaryotic topoisomerase II enzymes at a high concentration (100 μg/ml).

Table 1. Different classes of DNA topoisomerase inhibitors

Class of drugs	Topoisomerase inhibitors	Types of topoisomerase
Quinolones	Nalidixic acid, Oxolinic acid, Piromidic acid	gyrase
Fluoroquinolone	Norfloxacin, Ciprofloxacin,Ofloxacin, Enoxacin	gyrase/topo II?
Coumarins	Novobiocin, Coumermycin A1, Chlorobiocin	gyrase/topo II?
Anthracyclines	Adriamycin, Doxorubicin, Daunorubicin	topo II and gyrase
Podophyllotoxins	Teniposide, Etoposide, Etopohos, NK611	topo II
Alkaloids	Camptothecin, Topotecan, CPT-11, Nitidine	topo I
MGDBs	Distamycin, Hoechest 33342, Berenil, Netropsin	topo I
Acridines	m-AMSA, 9-anilino acridines	topo II
Ellipticines	2-Me 9-OH Et, Pazelliptine, Anatoxin, Datelliptium	topo II
Antibiotics	Saintopin^{+}, Topostin^{+}, Streptonigrin	topo I^{+} and topo II
Benzophenazine	NC-190	topo II
Flavonoids	Genistein, Woodfruticosin	topo II
Terpenoids	Terpenticin, Cleroxidin	topo II
Dioxopiperazine	Sobuzoxane, Razoxane, ICRF-154, ICRF-193	topo II
Indolocarbazole	ED-110, KT-6528	topo I
Makaluvamines	Makaluvamine derivatives	topo II
Antimonials	Sodium stibogluconate, Urea stibamine	*Leishmania* topo I
Lignanolides	(-)-Arctigenin, (-)-Trachelogenin	topo II

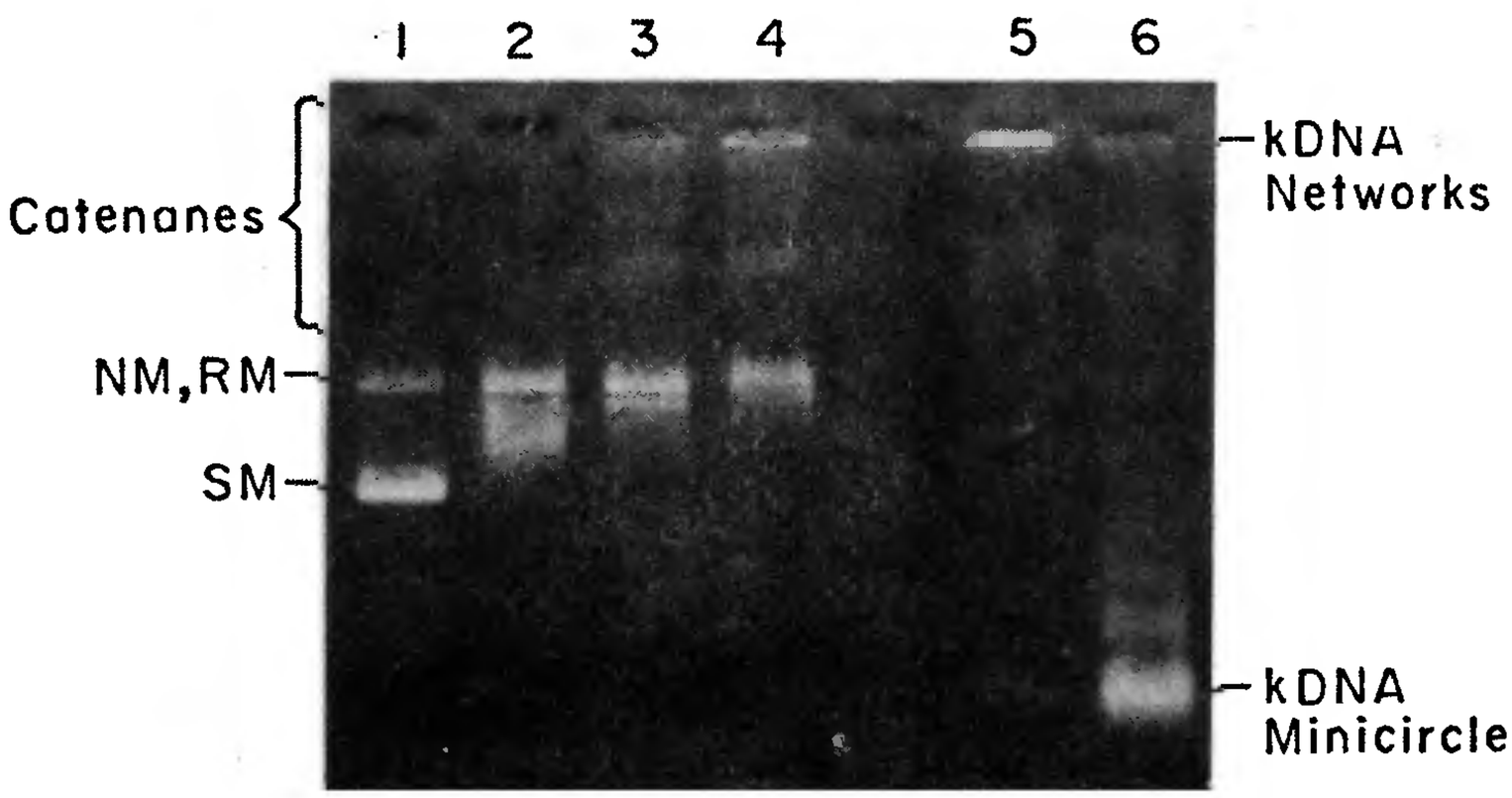


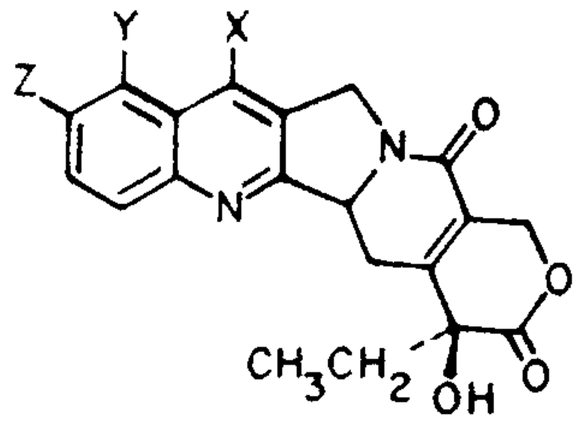
Figure 5. Decatenation and catenation reactions catalysed by ATP-independent type II DNA topoisomerase of *Leishmania*. One µg of plasmid pBR322 (lane 1) was incubated with 2 units of purified topoisomerase II (lane 2), 2 units of enzyme plus 10 µM spermidine (lane 3) and 2 units of enzyme plus 8 µg/ml histone H1 (lane 4) in standard catenation reaction mixture as described[11]. One µg of control kDNA (lane 5) was incubated with 2 units of enzyme (lane 6) in a standard decatenation reaction[43]. Electrophoresis was carried out in 1% agarose gel, stained at 1.5 V/cm for 14 h stained with ethidium bromide and photographed under UV light[43].

Etoposide (VP-16) and teniposide (VM-26) are strong inhibitors of type II DNA topoisomerases (Figure 6). These inhibitors are very active anti-cancer agents and are now at different stages of human clinical trial[3].

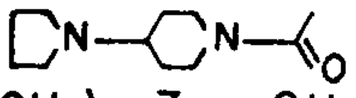
Many camptothecin derivatives like CPT-II, topotecan, 9-amino camptothecin are better inhibitors of mammalian type I topoisomerases and are anti-tumor agents[9,29]. These alkaloids (Figure 6) do not intercalate DNA, and have no inhibitory effect on type II topoisomerases. These drugs bind to the enzyme and inhibit the enzyme activity by stabilizing the enzyme-DNA cleavable complexes, and the enzyme is linked to the 5′-OH end of the broken DNA. Topoisomerase inhibitors and their uses as antibacterial and as anticancer agents have been described in many reviews[6,8,50].

DNA topoisomerase inhibitors as antitrypanosomal and antileishmanial agents

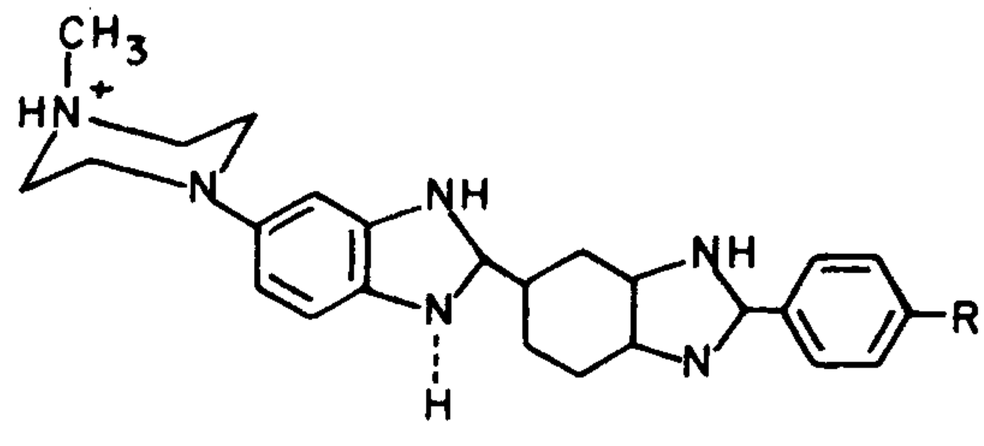
While many topo-reactive alkaloids, antibiotics, and organic compounds are used against acute bacterial infections and cancer, others are in various stages of clinical trials (Table 1 and Figure 6). In the following sections, we shall discuss the recent discovery of some topoisomerase inhibitors that block the life cycle of many pathogenic kinetoplastid organisms. Interestingly,



Camptothecin : X = H, Y = H, Z = H

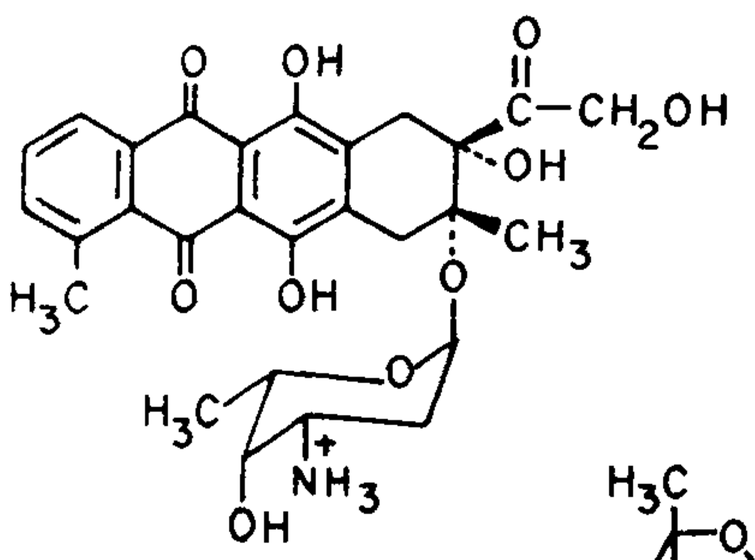
CPT-11: X = $-CH_2CH_3$, Y = H, Z = 

Topotecan : X = H, Y = $-CH_2-N(CH_3)_2$, Z = $-OH$

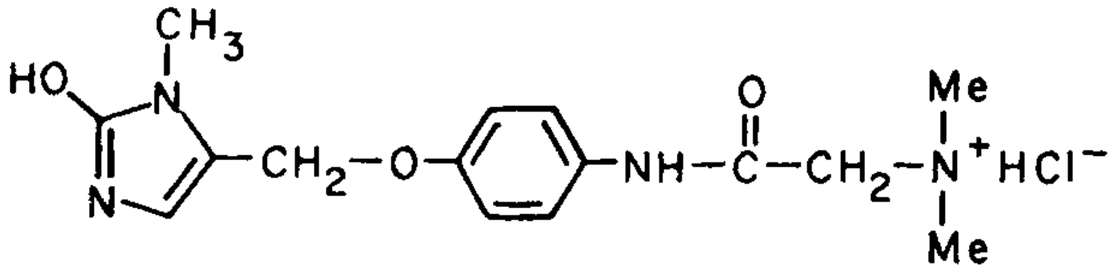


Hoechst 33342

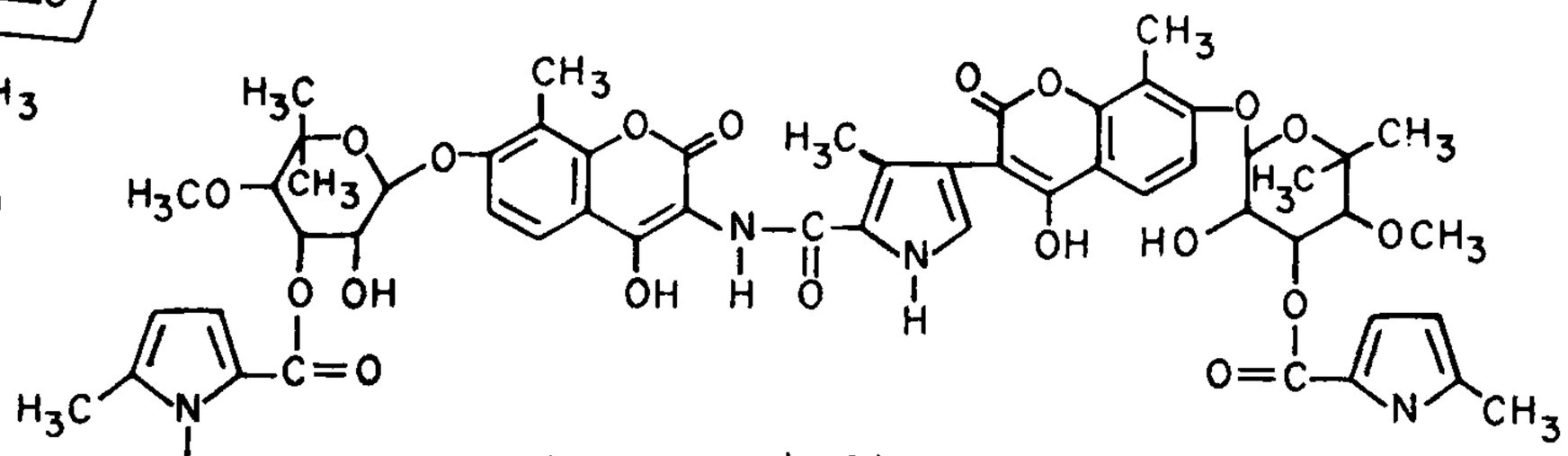
R = OCH_2CH_3



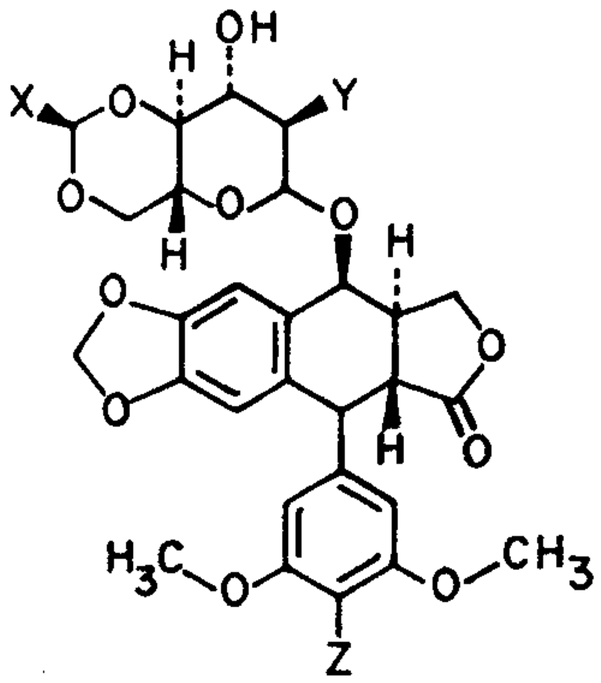
Doxorubicin



Ro 15-0216



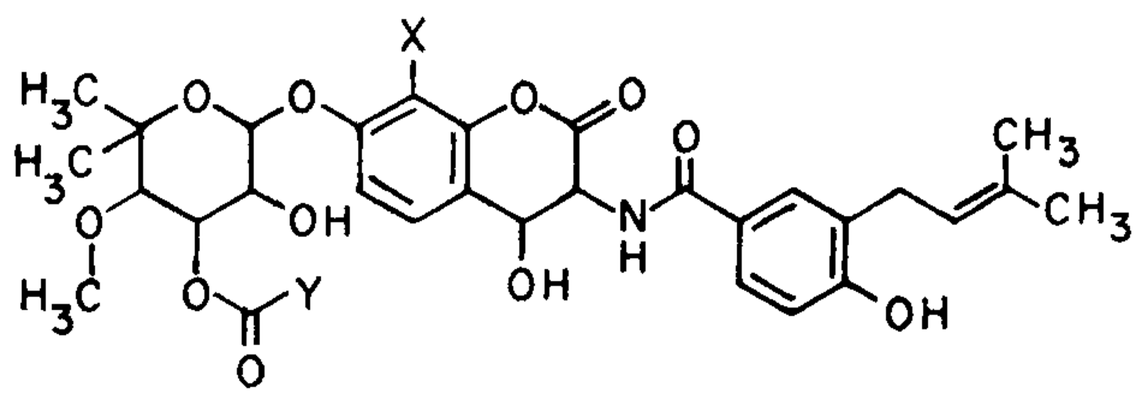
Coumermycin A1



Teniposide : X = , Y = OH, Z = OH

Etoposide : X = CH_3, Y = OH, Z = OH

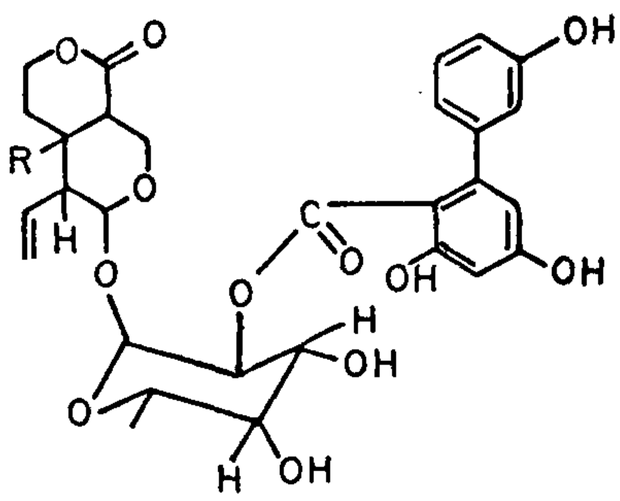
Etopohos : X = CH_3; Y = OH; Z = OPO_3H_2



Novobiocin : X = CH_3; Y = NH_2

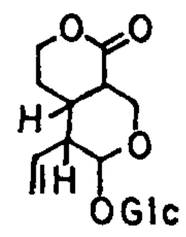
Chlorobiocin : X = C;
Y = 2-methyl-pyrrol-5-yl

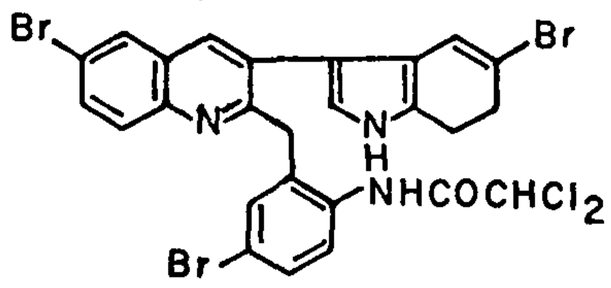
Figure 6. Chemical structure of some type I and II DNA topoisomerase inhibitors that also inhibit trypanosomal topoisomerases.



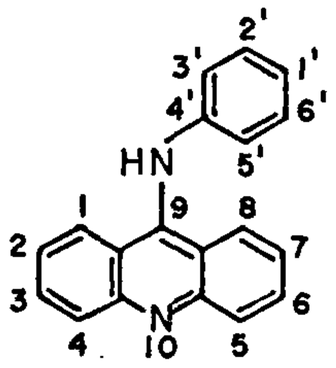
Amarogentin R = H, Amaroswerin R = OH

Sweroside =



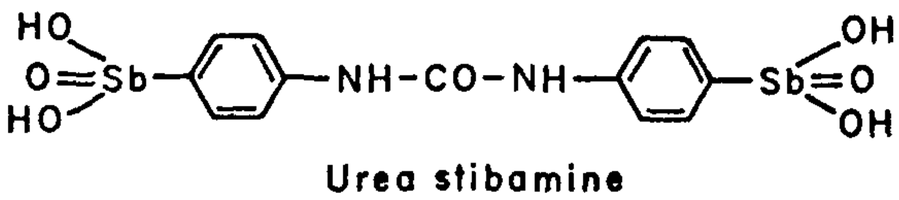


Indolyl Quinoline Bromo-derivative

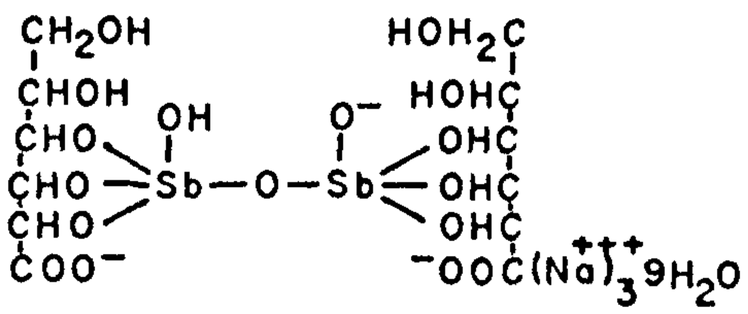


Active 9-anilinoacridines

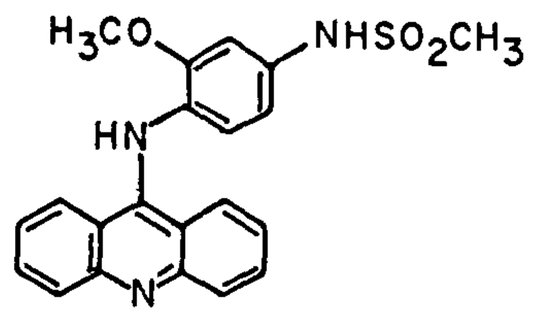
Compound	Substitutions
7282	C-1' = $NHCH_3$
8071	= $N(CH_2CH_3)(CH_3)$
19124	= $NH(CH_2)_2CH_3$
20084	= $NH(CH_2)_5CH_3$
22761	= NH_2 & C-6 = Cl



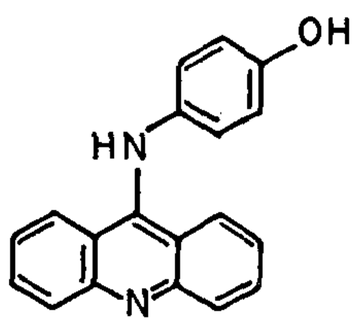
Urea stibamine



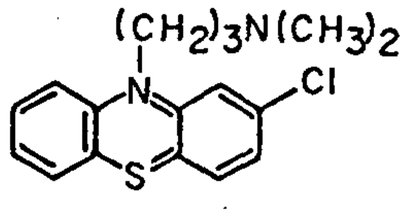
Sodium stibogluconate



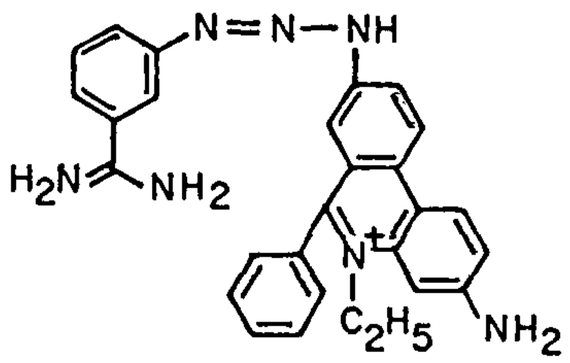
m-AMSA



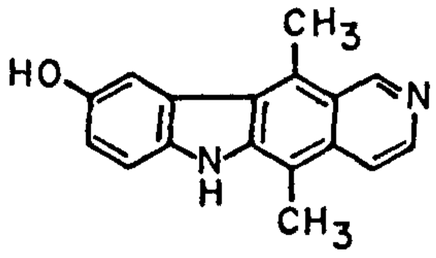
N-Paraphenol-9-Amino Acridine



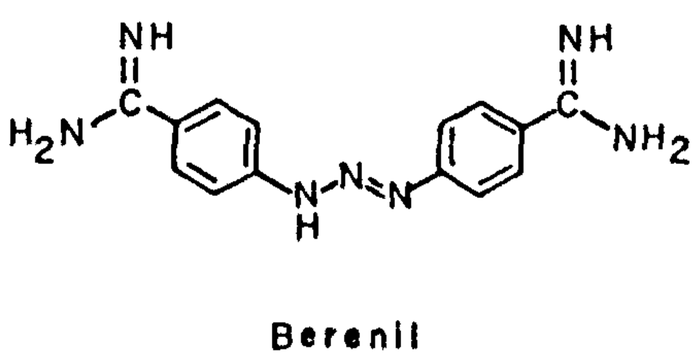
Chlorpromazine



Samorin



2,6 di-Me 9-hydroxy ellipticine



Berenil

Chemical structures of some known antileishmanial and antitrypanosomal drugs having target site on DNA topoisomerases.

some of the inhibitors have been in use for many years as conventional antileishmanial or antitrypanosomal drugs (Figure 7). Some eukaryotic topoisomerase I and II inhibitors are cytopathic to species of *Leishmania* and *Trypanosoma*. These compounds are synthesized or screened from bio-active plants which may provide initial leads for new drug design.

In 1984, Douc-Rasy *et al.* tested many DNA-intercalating drugs on the activity of *T. cruzi* DNA topoisomerase I and II (ref. 39). Interestingly, topoisomerase II only catalyses the catenation as well as relaxation reactions but does not perform decatenation or unknotting reaction. The catenation reaction is preferentially inhibited by many ellipticinium and isoellipticinium derivatives. The dimeric form of the intercalators has been shown to be more potent (20–30 times) inhibitors than the corresponding monomers. For example, acridine dimer inhibited the relaxation activity at 3.5 μM concentration, which is 20 times less than the minimal concentration of the monomer, acriflavine. 2,6 dimethyl-9-hydroxyl ellipticinium inhibits both catenation and relaxation activities at 1.5 μM concentration. The compound, at the same concentration, inhibits rat liver type II topoisomerase, but very high concentration is needed to inhibit rat liver type I topoisomerase (60 μM). However, the authors have pointed out that the efficiency of DNA intercalation is not always a requirement for their potency, since a nonintercalating compound, bromoellipticine, inhibited catenation reaction[39].

Riou *et al.* purified a type II topoisomerase from *T. cruzi* which does not support decatenation reaction[10]. Similar results have been obtained for *Leishmania* ATP-dependent topoisomerase II which however do not support catenation reaction[43]. The inhibitors coumarmycin A2 at 20–70 μM, and chlorobiocin at 28 μM concentrations strongly inhibit the catenating activity of trypanosomal topoisomerases[10]. The inhibitory activities of many antitrypanosomal drugs, like acriflavine and ellipticine derivatives, at very low concentrations (1.5–3.5 μM) have been demonstrated as well.

Earlier it was reported from our laboratory that a type I topoisomerase from the promastigotes of *L. donovani* is selectively inhibited by two commonly used antileishmanial drugs, sodium stibogluconate and urea stibamine[11]. The inhibition is dose-dependent, suggesting that both drugs interact with the enzyme or DNA-enzyme complex rather than the DNA alone (Figure 8). The target sites of these drugs were unknown and it has been suggested that antimony interacts with the -SH groups of many enzymes of different metabolic pathways, one or more of which may be specific for *Leishmania*. Although the antimonials are highly toxic, these are the most recommended drugs for the treatment of kala-azar and post kala-azar dermal leishmaniasis (PKDL) even today. Recently, sodium stibogluconate-

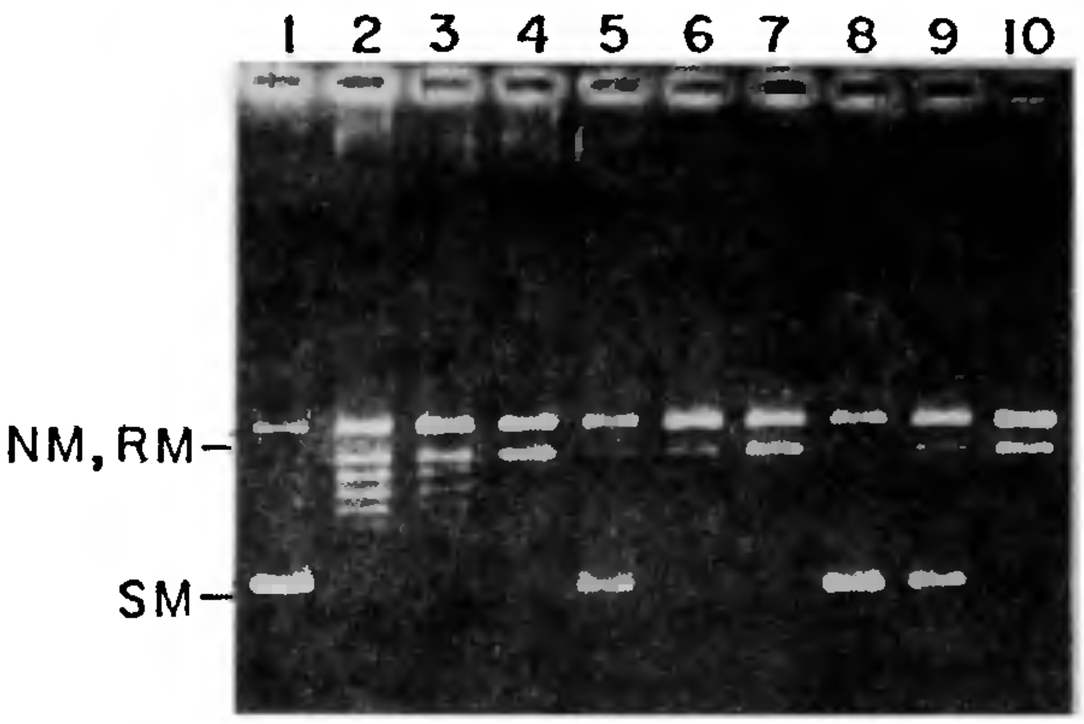


Figure 8. Dose-dependent inhibition of relaxation activity of type I DNA topoisomerase of *Leishmania* by sodium stibogluconate. Electrophoresis in 1% agarose gel. Lane 1, supercoiled pBR322 DNA (1 μg); lanes (2–4), relaxation of supercoiled pBR322 with 2, 4 and 8 units of enzyme; lanes (5–7), same as lanes (2–4) but in pesence of 16 μM sodium stibogluconate; lanes (8–10), same as lanes (2–4), but in presence of 40 μM sodium stibogluconate. After electrophoresis gel was stained with ethidium bromide and photographed as in Figure 5.

resistant *Leishmania* parasites have been isolated and cultured *in vitro* and *in vivo* as drug-resistant parasites (K. Kar, personal communication). Thus, it is possible to purify DNA topoisomerase I from such parasites and the inhibitory action of sodium stibogluconate on the relaxation activity of the purified topoisomerase I can be tested. If DNA topoisomerase I is the only or major target site of antimonial drugs then topoisomerase I from such parasites will be resistant to sodium stibogluconate. Also cloning and sequencing of this gene to identify the region of mutation for drug resistance may be interesting to study for the mechanism of drug resistance.

Inhibition of *Leishmania* topo I by antimonial drugs[11] justifies the testing of many bioactive and synthetic organic compounds on the topoisomerase I and II of *L. donovani*. Ray *et al.*[51] have identified a secoiridoid glycoside, amarogentin, which selectively inhibits the purified topoisomerase I activity of *L. donovani* promastigotes. Amarogentin is obtained by fractionation of methanol extract of Indian plant *Swertia chirata* and experiments are underway to test the activities of amarogentin, using animal model. Another development has been reported from our group that novel indolyl quinolines effectively inhibited the relaxation and decatenating activities of *Leishmania* topoisomerase I and II, respectively[43]. But the same drugs have no inhibitory effect on rat testis topo II, and the bromo derivatives are best inhibitors in this assay.

Shapiro and Englund[53] have shown that incubation of *T. equiperdum* cells in presence of mammalian topoisomerase II inhibitors like epipodophyllotoxins,

2-methyl-9-hydroxy ellipticinium, acriflavin, and m-AMSA resulted in the release of sufficient amounts of kDNA minicircles with the enzyme linked to 5′-end of the cleaved minicircles. These results clearly suggest that trypanosome mitochondrial DNA topoisomerase is the target of these drugs.

Shapiro and Englund[53] further demonstrated that pentamidine, berenil, samorin and other antitrypanosomal drugs promote linearization of *T. equiperdum* minicircles from the mitochondrial kDNA networks. These drugs act at minimum therapeutic concentrations and the enzyme is topoisomerase II that is linked to the 5′-end of the linearized minicircle DNA. Mode of action of these drugs may not be similar to mammalian topoisomerase II inhibitors, like etoposide, which acts on both chromosomal and mitochondrial DNA. Since antitrypanosomal drugs linearize kDNA minicircles, this kDNA network is a unique target for chemotherapy. These results also suggest the preferential roles of these drugs on kDNA metabolism and the mechanisms by which these drugs disrupt trypanosome mitochondria and generate dyskinetoplastic parasites.

Many 9-anilinoacridines have potent antileishmanial activities at 1 μM concentration. They exert their action probably by inhibiting topoisomerase II (ref. 54). Pyronacridine, a 9-anilino-aza-acridine, inhibits the *P. falciparum* topoisomerase II and was observed to be effective against drug-resistant human malaria, but such compounds have not been tested on *Leishmania* enzymes. Recently, many C-1′ alkyl aminoacridines and other acridine derivatives have been shown to be very potent antitrypanosomal and antimalarial agents[54]. *L. chagasi* DNA topoisomerase II was inhibited by mitonafide derivatives[55].

Recently, it has also been demonstrated from our laboratory that *Leishmania* topoisomerase I is inhibited by diospyrin, a *bis*-naphthoquinonoid compound isolated from the stem bark of the Indian medicinal plant *Diospyros montana*[56]. Like camptothecin, a class I inhibitor, diospyrin induces topoisomerase I-mediated DNA cleavage *in vitro*. However, it differs from camptothecin with respect to its mode of action. Camptothecin does not bind with the enzyme alone but diospyrin does and this interaction is reversible. Therefore, diospyrin is a novel 'topoisomerase I poison'. The finding that *Leishmania* topoisomerase I is more susceptible to this compound than other eukaryotic topoisomerases may be exploited in developing national approaches to chemotherapy of leishmaniasis. The unique nature of intercatenated kDNA network requires specific topoisomerases for replication and transcription, and hence it can be a special target for chemotherapy. However, many known topoisomerase inhibitors like β-lapachone[57], a fungal antibiotic saintopin[58], staurosporine[59], indolocarbazole derivatives of rebeccamycin[60], alkylamino anthraquinones and their N-oxides[61], and many others have not yet been tested on trypanosome and *Leishmania* DNA topoisomerases.

Conclusion

For last two decades, topoisomerase research has had a profound impact on modern medicine since antitopoisomerase drugs are mostly used for the treatment of drug-resistant bacterial pathogens, and many topoisomerase inhibitors are now used for the treatment of human cancer. DNA topoisomerases are involved in DNA replication, transcription, recombination, and segregation during cell division. It is clear that further studies on the different types of DNA topoisomerases will help to understand their functions *in vivo* for the development of more selective drugs. Topoisomerases and the accessory factors that are involved in kDNA replication and transcription, are very important targets for chemotherapy. Presence of two distinct types of kinetoplast-associated topoisomerases in *C. fasciculata* demands further studies on such topoisomerases in *Leishmania* and *Trypanosoma* to find novel target sites that will help to develop better topoisomerase-specific drugs. It is likely that more screening of bioactive compounds and synthesis of selective topoisomerase inhibitors will help in developing rational approaches to chemotherapy of kinetoplastida-associated parasitic diseases. Our laboratory is presently involved in cloning and characterization of type I and type II DNA topoisomerases of *Leishmania* in order to understand the structure of these enzymes for developing topoisomerase-directed antileishmanial compounds.

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