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Targeting atypical trypanosomatid DNA topoisomerase I

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Tropical diseases produced by kinetoplastid protozoa are among humanity's costliest banes, owing to high mortality and the economic burden resulting from morbidity. Drug resistant strains of parasites, together with insecticide-resistant vectors, are contributing to their increased prevalence in the developing world. Their extension now threatens industrialized countries because of opportunistic infections in immuno-compromised individuals. Current chemotherapy is expensive, has undesirable side effects and, in many patients, is only marginally effective. Based on the clinical success of camptothecin derivatives as anticancer agents, DNA topoisomerases have been identified as targets for drug development. The substantial differences in homology between trypanosome and leishmania DNA topoisomerase IB compared with the human form provides a new lead in the study of the structural determinants that can be targeted.

Species of the family Trypanosomatidae (order Kinetoplastida) are amongst the world's most dangerous eukaryotic parasites. Trypanosomatid protozoa are characterized by the presence of one flagellum and a single mitochondrion housing a specialized organelle known as a kinetoplast that contains maxicircles and minicircles of DNA (kDNA) [1]. African trypanosomiasis (sleeping sickness) is a parasitic disease transmitted to humans by tsetse flies. *Trypanosoma brucei gambiense*, found in western and central Africa, causes chronic infections that can be asymptomatic for months, or even years. *Trypanosoma brucei rhodesiense*, by contrast, is a more dangerous strain, causing acute and virulent outbreaks mostly in southern and eastern Africa [2].

American trypanosomiasis (Chagas' disease) is caused by *Trypanosoma cruzi*, a parasite widespread in Central and South America. Humans contract Chagas' disease when bitten by infected triatomine (order Hemiptera) insects. After penetration, the parasite invades the bloodstream and multiplies inside host cells, particularly heart and smooth muscle, provoking severe cardiomyopathies and intestinal mega syndromes [3].

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The term human leishmaniasis, covers a complex of zoonotic diseases transmitted by the bite of female phlebotominae mosquitoes, causing cutaneous, mucocutaneous and visceral leishmaniasis (kala azar) in humans. Kala azar is, clinically speaking, the most harmful form of human leishmaniasis. Characterized by fever, anaemia, and swelling of the spleen and liver, it is usually fatal if not diagnosed and treated in time. More than 90% of *Leishmania donovani*-mediated visceral leishmaniasis is found in India, Bangladesh, Indonesia and Sudan [4].

Infection by these microorganisms constitutes a serious problem for the immunosuppressed community. *Leishmania*–HIV coinfection causes cumulative immunodeficiency because *Leishmania* parasites and HIV infect and destroy the same types of cell. Severe acute leishmaniasis is a serious problem in south-western European countries, where needle sharing by drug users is the most widespread form of transmission of the disease [5].

Anti-trypanosomatid chemotherapy is often toxic, and several decades of use have led to the development of resistant strains. The treatment of African trypanosomiasis is stage-specific. Pentamidine and suramin are the first-line drugs used for the first stage, whereas melarsoprol, an old-fashioned arsenic derivative, is the drug of choice for treating late-stage sleeping sickness. In 1990,

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eflornithine [difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase] was registered as an alternative to melarsoprol treatment [6]. There are currently no drugs available to cure American trypanosomiasis. Benznidazole and nifurtimox are used to counter blood circulating parasites, but are only relatively effective in young people [7]. Pentavalent antimonials (Pentostam, GlaxoSmithKline, and Glucantime, Sanofi Aventis) and amphotericin B are the first-line drugs against different presentations of leishmaniasis [8]. A Phase IV trial of Miltefosine, recently registered by the Indian government to treat visceral leishmaniasis, is currently underway [9].

These drugs are not easy to manage, generally speaking, for the lengthy and costly treatment involved, nor are they free from undesirable side effects. The genome sequences for *T. brucei* [10], *T. cruzi* [11] and *L. major* [12] have been publicly available since May 2005, with the potential that this might uncover novel chemotherapeutic targets for future drug discovery.

DNA topoisomerases are ubiquitous enzymes, catalyzing changes in the topological state of duplex DNA during replication, transcription, recombination and DNA-repair processes [13–15]. There are three categories of such enzymes: DNA topoisomerases types IA and IB (EC 5.99.1.2), and type II (EC 5.99.1.3). Type I DNA topoisomerases are monomeric ATP-independent enzymes with relaxation activity for positively and negatively supercoiled DNA. They introduce single-stranded breaks in DNA followed by passage and rejoining, thereby allowing single step changes in the linking number of circular DNA. They are subdivided into two distinct classes: type IA enzymes that bind covalently to the 5′ end and type IB enzymes that form covalent bonds with the 3′ end of the broken DNA strand. Type II DNA topoisomerases are homodimeric ATP-dependent enzymes that introduce transient double-stranded breaks in the double helix, followed by passage and rejoining. These enzymes can relax, catenate–decatenate, knot–unknot or introduce supercoils in the DNA molecule [16].

The identification of DNA topoisomerases as a promising drug target is based on the clinical success of camptothecin derivatives as anticancer agents. Recent studies show that kinetoplastid topoisomerases are drastically distinct from their human counterparts supporting the belief that topoisomerase-based antiparasitic drugs might be a potential objective for future therapies.

Trypanosomatidae type IB DNA topoisomerases

Type IB DNA topoisomerases include eukaryotic topoisomerases, archaebacterial topoisomerase V and vaccinia topoisomerase I. They have a five-step catalytic cycle: (i) binding of the DNA; (ii) cleavage of one of the DNA strands at the 3′ terminus, thereby establishing a transient, covalently bonded enzyme–DNA intermediate complex; (iii) relaxation of superhelical tension; (iv) resealing of the DNA break; and (v) release of the DNA, thereby restoring the integrity of the double-stranded duplex [17]. The covalent phosphodiester bond between the DNA and the enzyme is established by a tyrosine residue found in the active site of all topoisomerase I molecules described to date. Human DNA topoisomerase I is a monomeric protein consisting of 765 residues with a predicted molecular mass of 91 kDa. Crystallographic studies have confirmed the existence of four structural and functional domains required for the protein to be operational [14,15,18]. The N-terminal domain is highly (positively) charged, is not phylogenetically conserved and contains four putative nuclear localization signal motifs. This portion of the enzyme is not required for DNA-relaxation activity – truncated proteins lacking this region are fully active [18]. The core domain is essential for relaxation of supercoiled DNA and shows a high degree of phylogenetic conservation, particularly with respect to residues that interact closely with the double helix. In this region of human topoisomerase I, an amino acid tetrad, consisting of Arg-488, Lys-532, Arg-590 and His-632, constitutes the active site of the enzyme [19]. The amino acid that establishes a transient covalent phosphodiester bond with DNA is found in the C-terminal domain. All type IB topoisomerases contain a conserved 'SKXXY' signature in this region in which a tyrosine residue (Tyr-723 in the human topoisomerase I) is the DNA-cleaving amino acid. Finally, the core and C-terminal domains are connected to one another by a poorly conserved linker, not functionally involved in DNA relaxation activity [18].

Leishmanial topoisomerase heterodimeric structure

Trypanosomal and leishmanial type I DNA topoisomerases are substantially different from the homologues described in all other organisms studied to date. Trypanosmatid type I topoisomerases are heterodimers in which the genes encoding each protein subunit are located on different chromosomes [20]. Genetic analyses have identified a gene for the large subunit, namely *LdTOPIA*, on *L. donovani* chromosome 34, encoding for a 636 amino acid polypeptide with an estimated molecular mass of 73 kDa. This subunit is closely homologous to the core domain of human topoisomerase I. In turn, the gene encoding for the small subunit, *LdTOPIB*, is found on *L. donovani* chromosome 4 and encodes for a 262 amino acid polypeptide with a predicted molecular mass of 28 kDa. This protein contains the phylogenetically conserved signature 'SKXXY' containing Tyr-222 that has a role in DNA cleavage. The molecule's ability to relax negative DNA supercoils was reconstituted only when the two subunits were co-expressed in *Saccharomyces cerevisiae* with a biscistronic yeast expression vector [20]. This heterodimeric structure explains the existence, reported by some authors, of an *L. donovani* topoisomerase-like polypeptide lacking the active site [21], and definitively rules out an earlier hypothesis identifying it as an unusual monomeric enzyme bearing a Ser as the DNA-cleaving amino acid instead of the C-terminal domain Tyr observed in all previously described type I topoisomerases [22]. However, the presence of a dimeric IB DNA topoisomerase is not an isolated event in nature. The fact that type I DNA topoisomerases from African and American trypanosomes are also encoded by two separate genes for the core and catalytic domains is indicative that this might be a feature common to kinetoplastids [23,24].

Early experiments have shown that a single linear structure is not absolutely necessary to reconstitute the relaxation activity of eukaryotic topoisomerases, whose catalytic core and C-terminal domains can interact with DNA. Stewart *et al.* [25] confirmed this finding when they reconstituted the relaxation activity of human topoisomerase by adding the core domain to a series of C-terminal peptides, in a proportion of 1:1, in the presence of DNA. Similarly, Park and Sternglanz [26], using a two-hybrid expression system, were able to identify proteins that supplement the catalytic core of *S. cerevisiae* topoisomerase I, the domain containing part of the linker and the C terminus of the yeast enzyme.

Two hypotheses can be put forward to explain the reconstitution of dimeric type I DNA topoisomerases. The first stated that the two fragments are not properly folded and that their association goes hand-in-hand with the conformational changes required to form the active enzyme – this hypothesis can bee ruled out, as shown by Davies *et al.* [27] in their recent work. According to the second hypothesis, the core and C-terminal domains are independent folding units that form an active enzyme when united by non-covalent bonds. In any event, the presence of multiple putative nuclear localization signals at the C-terminal extension of the LdTOP1A subunit only, and not in the LdTOP1B sequence, provides evidence that enzyme assembly takes place in the cytosol before translocation to the nuclear compartment [20,24].

Leishmanial topoisomerase: domain characterization.

The composite sequence of the kinetoplastid heterodimer bears a close resemblance to the conventional eukaryotic type IB enzyme sequence, although significant differences have been reported. Figure 1 compares the linear structure of the two leishmanial type I topoisomerase monomers to their human and yeast homologues. The LdTOPIA protein contains a short nonconserved N-terminal domain (start-Met-Glu-43) as well as the conserved core domain (Arg-44-Ser-456). Whereas homology is wholly absent at the C-extension end (Val-457-Val-635), the conserved core region contains all the amino acids involved in relaxation activity, such as Arg-314 (Arg-488 in the human enzyme), Lys-352 (Lys-532 in the human enzyme), Arg-410 (Arg-590 in the human enzyme) and His-453 (His-632 in the human enzyme). The small LdTOPIB subunit, in turn, contains a large nonconserved N-terminal extension (start-Met-Asn-210); the C-terminal domain is closely homologous to the catalytic domain of eukaryotic topoisomerases and contains the Tyr-222 (Tyr-723 in the human enzyme) required for DNA cleavage [20]. The trypanosomal enzyme has a similar structure to all the conserved amino acids (with Arg-377, Lys-415, Arg-471 and His-514 constituting the conserved amino acid tetrad in *T. brucei*) [22].

As noted above, although the N-terminal and core domains are found on the LdTOPIA subunit and the C-terminal domain on the small LdTOPIB subunit, the location of the nonconserved linker domain in dimeric topoisomerases is merely speculative. According to the scheme in Figure 1, all or part of the C-terminal and N-terminal ends on the large LdTOPIA and small LdTOPIB subunits can interact to generate a functional linker and establish a cleavable complex involving the core, active site and DNA. One possibility is that this linker-like structure is shared by the two subunits that interact to stabilize the enzyme. Another is that part of the long carboxyl or amino extensions on the subunits are removed by post-translational modifications before assembly.

A systematic partial deletion study of the two subunits to assess the regions needed for activity in the leishmanial enzyme has

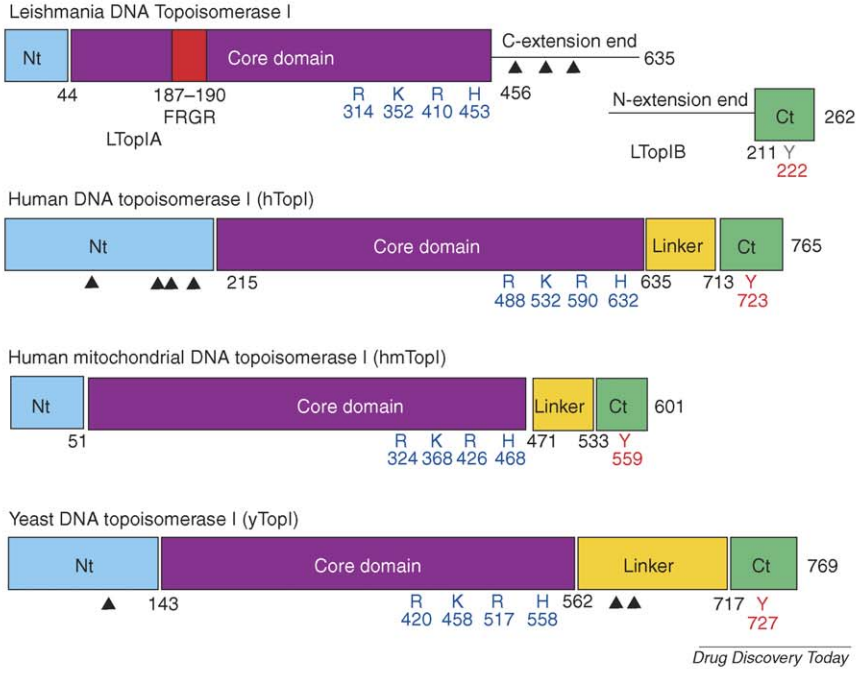

FIGURE 1

Scheme of the *Leishmania* topoisomerases IA and IB structures compared with their human topoisomerase I, human mitochondrial topoisomerase I and yeast topoisomerase I counterparts. GeneBank accession numbers are as follows: *LTOPIA* gene AF303577; *LTOPIB* gene AY062908; *Homo sapiens* nuclear, K03077; *Homo sapiens* mitochondrial, AAL10791; *Saccharomyces cerevisiae* J03250. Sequence comparisons of cellular eukaryotic type I DNA topoisomerases proteins demonstrate that the human topoisomerase I can be divided into four domains. Residues start-Met-Gly-214 comprise the unconserved N-terminal domain. Residues Ile-215-Ala-635 (54 kDa) form the conserved core domain, which is followed by a small positively charged linker domain of unconserved residues Pro-636-Lys-712 (5 kDa). Finally, residues Gln-713-Phe-765 (8 kDa) make up the highly conserved C-terminal domain, which contains the active site tyrosine at position 723. Arrowheads denote the nuclear localization signals corresponding to each of the type I DNA topoisomerases.

found that more than 70 amino acid residues sited at the C-terminus of the large subunit are not required for relaxation or cleaving activities, although this region contains the multiple putative nuclear localization signals required to direct the assembled protein to the nucleus (Figure 1). By contrast, nearly the entire N-terminal end of the small subunit is unnecessary for relaxation or cleaving activity and lacks any putative nuclear or mitochondrial localization signal motifs. On the other hand, Das *et al.* [28] – using a leishmanial type I DNA topoisomerase truncated at the N terminus by 39 amino acids – have suggested that the loss of residues 1–39 from the large subunit leads to slow cleavage and relaxation rates, pointing to their possible role in coordinating DNA contacts by other parts of the enzyme. Furthermore, a deletion of the first 99 amino acids from the N terminus resulted in an enzyme lacking relaxation activity. This suggests that amino acids 40–99 might be involved in the interaction between the large and small subunits of the heterodimer, in which their role would be to correctly position the active site tyrosine for its nucleophilic attack on the DNA.

There is a second important difference between trypanosomatid type I topoisomerase and its eukaryotic homologues. Immunocytochemical localization experiments in all three representative species of trypanosomatids show dual localization of the enzyme, associated both with genomic DNA in the nucleus and DNA in the kinetoplast (kDNA). Although the trypanosomatid kDNA relaxation, knotting–unknotting and catenating–decatenating activities attributed to type II DNA topoisomerase are well defined, the role played by type I topoisomerase in the organelle is not sufficiently clear. As in most eukaryotic cells, topoisomerases I and II are essential to cell life. Enzyme inhibition or gene disruption of trypanosomatid type II DNA topoisomerase produces a singular phenotype lacking kDNA, called dyskinetoplastidy that leads to cell death [29]. Moreover, RNA-interference-mediated disruption of gene expression of either subunit of topoisomerase IB results in a drastic reduction of DNA and RNA synthesis in African trypanosomes, mimicking the inhibition of nucleic acid biosynthesis observed when bloodstream-form trypanosomes are treated with the specific inhibitor camptothecin (CPT) [30].

Inhibition of DNA topoisomerase I

The structural differences between human and kinetoplastid type I DNA topoisomerases make this enzyme an attractive target for chemotherapeutic intervention [31–33]. Topoisomerase inhibitors fall into two general categories: compounds that stimulate the formation of covalent enzyme-DNA complexes or topoisomerase poisons (class I inhibitors), and products that interfere with the enzymatic functions of the enzyme (class II inhibitors) [34].

Camptothecin is a good example of a class I topoisomerase inhibitor. CPT is a pentacyclic alkaloid (Figure 2a) produced by the plant *Camptotheca accuminata*. In addition to their potential role in the treatment of parasitic diseases, the camptothecins are widely used for the treatment of cancer [35–37]. (An in-depth discussion of their role in cancer is, however, beyond the scope of this review.) CPT is a non-competitive inhibitor, active in the micromolar and submicromolar range, which, by binding to DNA and the enzyme to form a ternary complex, prevents DNA religation. CPT generates covalent DNA–topoisomerase complexes with both nuclear and kinetoplastic preparations of DNA from trypanosomes, leishmanias [38] and other protozoan parasites of medical importance [39].

QSAR studies conducted by Bodley *et al.* [40] test a series of CPT analogues for their *in vitro* effectiveness against African trypanosomes showed that their cytotoxicity was closely correlated to the ability to promote the formation of covalent protein–DNA complexes. This would indicate that the sole cellular target of these agents is topoisomerase I. Moreover, antitrypanosomal activity is increased by 9-substituted-10,11-methylenedioxy analogues, which are selectively less cytotoxic to mammalian cells. Recent results revealed that the water-soluble derivatives of CPT, irinotecan and topotecan hydrochlorides (two anti-tumour drugs [36,37]), are weakly effective against the bloodstream forms of *T. brucei*. The authors concluded that the reduced efficacy of these compounds is due more to the low permeability of the parasite plasma membrane to the drugs, than to any inability to establish the cleavage complex with DNA. Furthermore, 7-ethyl-10-hydroxy-CPT, a metabolite formed during the hydrolysis of irinotecan by a carboxylesterase [41] not active in the parasite, exhibits substantial trypanocidal activity [42].

Based on the copurification of human topoisomerase I recombined with CPT and DNA, Staker *et al.* [43,44] proposed a series of interactions amongst the different components of the cleavage complex that, in the absence of the crystalline structure of the parasitic heterodimer, can be assumed in this last model. Derived from this model, CPT intercalates at the site of DNA cleavage mimicking a DNA base pair. Within the intercalation pocket site, the side chain of Asp-533 in the human enzyme (corresponding to Asp-353 on the large subunit in leishmanial topoisomerase I) can establish a hydrogen bond with the 20(*S*)-hydroxyl moiety of the lactone form of CPT, whereas Arg-364 (corresponding to Arg-190 in *Leishmania*) establishes a second hydrogen bond with the nitrogen at the CPT B-ring. Asn-722, the amino acid adjacent to the DNA-cleaving Tyr, is also needed for CPT inhibition, although it does not establish hydrogen bonds with the drug. Other important residues involved in CPT interaction – Phe-361, Gly-363 and Arg-364 [19] (corresponding to a conserved Phe-X-Gly-Arg motif in the core domain of *Leishmania*) – are conserved in the trypanosomatid enzyme. These authors observed that other point mutations in the human topoisomerase, namely Glu-418 and Ala-653, lead to resistance to the drug, although their position with respect to the cleavage site is not close enough to interact directly with CPT. In yeast and *Leishmania*, these amino acids are not conserved, providing a possible explanation for the paradoxical behaviour of these enzymes in the presence of CPT. Further, partially truncated human type I topoisomerases lacking the linker domain [45] conserve enzymatic activity but lose sensitivity to CPT in standard relaxation assays, which confirms the crucial role of this domain in the interaction with the drug.

Marquis *et al.* [46] recently described two amino acid substitutions in the large subunit of type I DNA topoisomerase from CPT-resistant strains of *L. donovani*. The LdTOPIA large unit became highly CPT-resistant when two substitutions, G185R and D325E, were made in the core domain of the protein. *L. donovani* topoisomerase I residues Gly-185 and Asp-325 are conserved in all known members of the eukaryotic topoisomerase family. These amino acid substitutions prevent the formation of the

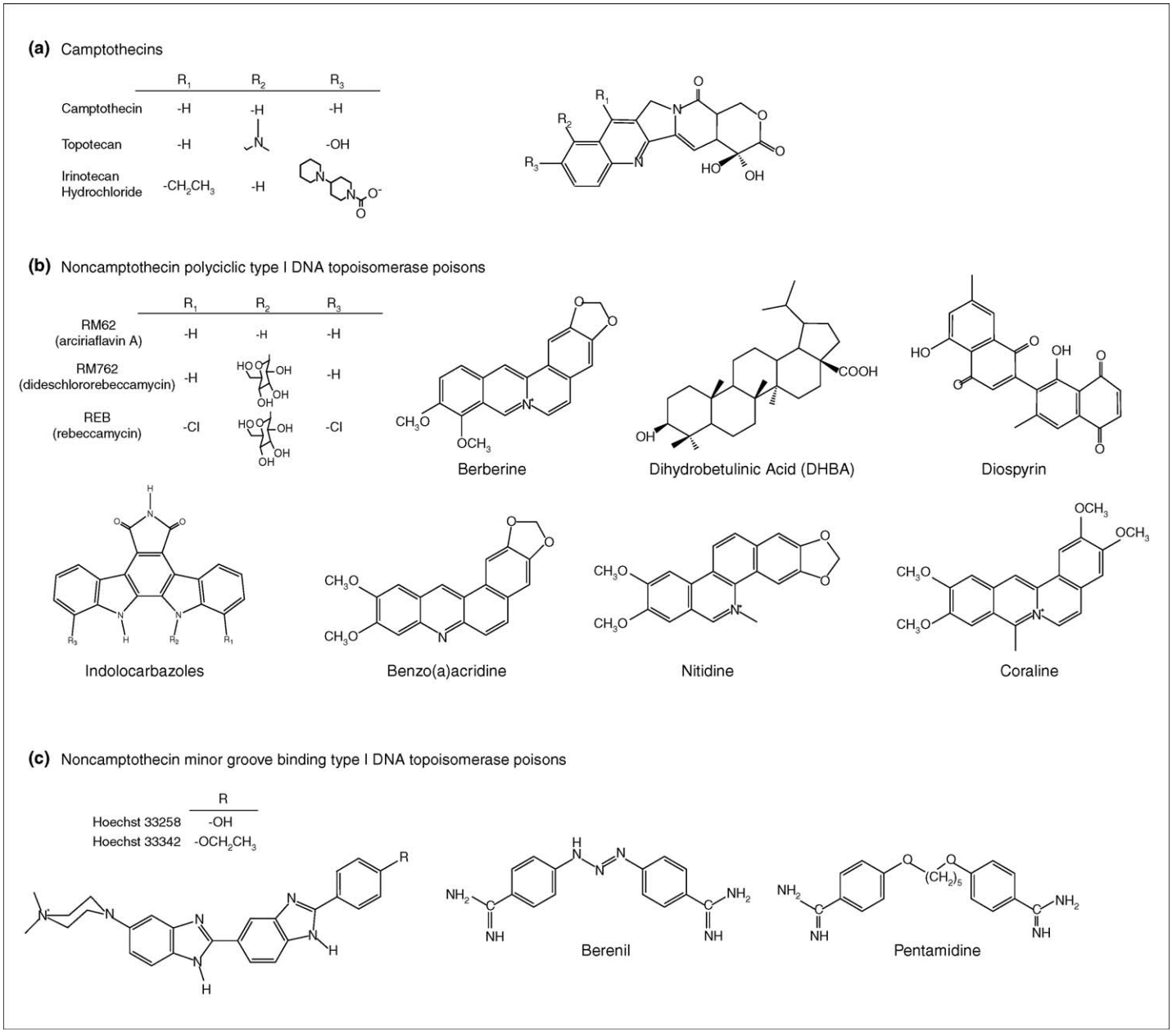

FIGURE 2

Chemical structure of DNA–topoisomerase I poisons. (a) CPT and derivatives; **(b)** non-camptothecin polycyclic chemicals; and **(c)** minor-groove binding compounds.

CPT–topoisomerase-I–DNA complexes normally observed in wild-type CPT-treated kinetoplastids.

Like other DNA-damaging agents, class I topoisomerase poisons are efficient inducers of apoptosis [47], which would explain the cytotoxic effect of these compounds in kinetoplastids. Sen *et al.* [48] found that CPT-mediated leishmanicidal effect appears after the mitochondrial function is inhibited, which is followed by an increase in mitochondrial membrane potential. These authors showed that CPT raises the intracellular concentration of reactive oxygen species, with the concomitant rise in lipid peroxidation and decline in the concentration of the free-radical scavenger glutathione. As in mammalian systems, caspase-3-like protease activation, poly(ADP–ribose) polymerase cleavage and an increase in cytosolic Ca^{2+} released from cellular stores and followed by the rapid formation of the cytochrome-C-mediated complex are common developments in CPT-induced apoptosis in *L. donovani* promastigotes [49].

Very few studies have been conducted on the efficacy of CPT and analogues in *in vivo* kinetoplastid infection. Proulx *et al.* [50] tested the efficacy of free and liposome-encapsulated CPT in a murine model of visceral leishmaniasis. The parasite burden was significantly reduced when infected mice were treated with intraperitoneal injections of free and liposomal CPT. One particularly relevant finding was that encapsulation of CPT in liposomes neither increases the *in vitro* or *in vivo* efficacy of the drug, nor decreases its toxicity profile.

Indolocarbazole derivatives (Figure 2b) constitute a promising group of class I topoisomerase poisons with a polyheterocyclic

aromatic structure. Many rebeccamycin analogues have been tested in *in vitro* QSAR studies for efficacy against tumour cells and some of them selected for clinical trials and development [51]. The planar structure of these molecules enables them to intercalate the pile of DNA base pairs, where they eventually establish DNA–topoisomerase I cleavage complexes. The reaction between crystalline human topoisomerase I and rebeccamycin analogues shows that one of the two carbonyl groups from the maleimide ring can interact with Arg-364 (conserved as Arg-190 in *Leishmania*) at the minor groove side of the intercalation complex. Indolocarbazole molecules are symmetrical molecules except for the sugar substituent bond to the nitrogen atom of one of the indolyl ring. This asymmetry situates the ternary intercalation pocket in such a way that the glycosylated indole stacks with bases on the undamaged DNA strand, whereas the nonglycosylated indole interacts with the cleaved strand of duplex DNA [43]. By contrast, the Asn-722, which is crucial for CPT inhibition, is irrelevant to the establishment of the indolocarbazole-induced DNA–topoisomerase I cleavage complex [52]. Deterding *et al.* [42] found rebeccamycin to have an *in vitro* cyctoxic effect on African trypanosomes at submicromolar concentrations.

L. donovani has been shown to be sensitive to diospyrin, a naphthoquinone derivative that acts like a class I inhibitor [53]. Diospyrin inhibition is relatively specific, requiring a tenfold concentration to inhibit calf thymus DNA topoisomerase I, and failing to inhibit *L. donovani* DNA topoisomerase II [54]. Betulinic acid derivatives (DHBA) are pentacyclic triterpenoids that inhibit parasite DNA types I and II topoisomerases by preventing enzyme–DNA binary complex formation. DHBA does not interact with substrate DNA and therefore is unable to stabilize the ternary cleavable complex [55]. DHBA pertains to a rare group of topoisomerase inhibitors that antagonize with CPT and etoposide (a type II DNA topoisomerase inhibitor) in establishing DNA cleavage. This compound has a drastic inhibitory effect on the growth of *L. donovani* promastigotes in the micromolar range. DHBA derivatives induce chromatin margination and dechromatinization of the leishmanial genome but not dyskinetoplastidy, providing support for the direct correlation between topoisomerase inhibition and apoptosis in *L. donovani* [56].

Other type I topoisomerase activity inhibitors include the first-line leishmanicidal pentavalent antimonials [57] and berberine analogues [58]. Sodium stibogluconate (Pentostam) but not meglumine antimoniate (Glucantime) can stabilize DNA–topoisomerase I cleavable complexes in the purified leishmanial enzyme and in intact promastigotes [59]. However, the drug's cytotoxicity is negligible at this stage in the parasitic life cycle, suggesting that the stabilization of DNA-topoisomerase I cleavable complexes is a side-effect rather than the actual mode of action of the compound [60].

Berberine is a polyheterocyclic class I topoisomerase inhibitor with a structure resembling the intercalant drug benzo[a]acridine. Several berberine analogues, tested against *L. donovani* [61] and bloodstream forms of *T. brucei* and *T. congolense* [62], exhibited micromolar range efficacy. *In vivo* trials with protoberberine analogues have also been shown to effectively treat golden hamster visceral leishmaniasis. Coralyne, a berberine derivative, has likewise been shown to effectively combat *T. cruzi* infection [63]; nitidine, also a berberine derivative, is not effective.

DNA minor groove binders (Figure 2c) such as certain bisbenzimidazole dyes (Ho-33342 and its parent compound, Ho-33258) and a series of aromatic diamidines, including the antiparasitic drugs pentamidine and berenil, can inhibit DNA topoisomerase I *in vitro* [64]. These compounds bind to AT-rich regions of DNA, interfering with enzyme catalysis but, with exception of Ho-33342 and Ho-33258, do not stabilize the cleavable complexes between enzyme and DNA. A recent report reveals that the leishmanicidal effect of these compounds is poorly related to type I DNA topoisomerase inhibition, pointing to a more complex pleiotropic effect [65].

Recently, a new group of topoisomerase inhibitors has been described [66]. It has been shown that the naturally occurring flavones baicalein, luteolin and quercetin stabilize the covalent

TABLE 1

The efficacy of DNA I topoisomerase inhibitors in models of kinetoplast infective disease

Compound class	Compound	Activity against[a]		
		African trypanosomiasis	Chagas' disease	Leishmaniasis
Camptothecins	Camptothecin	✓✓✓ [40][b] [41][b]	✓✓ [38][b]	✓✓ [38][b] [50][c]
	Topotecan	✓✓✓ [41][b]		
	Irinotecan	✓ [41][b]		
Non-camptothecin type I DNA topoisomerase poisons	Berberine	✓✓ [62][b]		✓✓ [58][c] [61][b]
	Coralyne		✓ [63][c]	✓ [65][b]
	DHBA			✓✓ [56][c]
	Diospyrin			✓✓ [53][b]
	Indolocarbazoles	✓✓✓ [41][b]		
	Pentavalent antimonials			✓✓ [57][b] [59][b] [60][b]
	Ho-33342 Pentamidine Berenil			✓✓ [64][b] [65][b]
	Flavones			✓✓ [66][b]

[a] Definitions: ✓, weakly effective; ✓✓, moderately effective; ✓✓✓, highly effective; Blank squares, not known or not investigated.
[b] *In vitro* test.
[c] In animal models.

DNA–topoisomerase I complex preventing the religation step. Baicalein and luteolin have proved to be effective with highly CPT-resistant *L. donovani* strain.

A summary of the efficacy of the different kinds of topoisomerase inhibitors in models of kinetoplast infective diseases is shown in Table 1.

Conclusion

Type I DNA topoisomerase is a promising drug target for cancer therapy whose effectiveness against the diseases caused by parasitic protozoa has not been sufficiently exploited. The phylogenetically unique, anomalous bisubunit structure of the enzyme and its dual location – associated with genomic DNA and kDNA – makes it an auspicious target for new antiparasitic drug development. Despite the multiple topoisomerase inhibitors tested against tumor cells, only scant QSAR information on the effect of these compounds on trypanosomatidae is available. There is, moreover, a pressing need to perform *in vivo* trials to evaluate the actual efficiency of drug treatments targeting topoisomerase I. Recent findings relating to topoisomerase poison-induced apoptosis make such compounds important tools in cell biology research focusing on these primitive eukaryotes. The crystalline structure of this protein will provide information of cardinal importance on the structural determinants involved in processes, such as the assembly of the active heterodimer, enzyme kinetic machinery and interaction with inhibitors, that will play a key role in future drug discovery.

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