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Review

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Interaction between natural compounds and human topoisomerase I

Abstract: Eukaryotic topoisomerase I (Top1) is a monomeric enzyme that catalyzes the relaxation of supercoiled DNA during important processes including DNA replication, transcription, recombination and chromosome condensation. Human Top1 I is of significant medical interest since it is the unique cellular target of camptothecin (CPT), a plant alkaloid that rapidly blocks both DNA and RNA synthesis. In this review, together with CPT, we point out the interaction between human Top1 and some natural compounds, such us terpenoids, flavonoids, stilbenes and fatty acids. The drugs can interact with the enzyme at different levels perturbing the binding, cleavage, rotation or religation processes. Here we focus on different assays that can be used to identify the catalytic step of the enzyme inhibited by different natural compounds.

Keywords: enzymatic catalytic steps; inhibition mechanism; natural compounds; topoisomerase I.

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Introduction

The dimension of a single mammalian genome corresponds to approximately 2 m, which is squeezed into a cell nuclear volume of approximately 10^{-17} m^3. Cellular DNA is therefore highly compacted and this creates many points of contact and many curvatures over the DNA strands. Moreover, during the continuous transcription, replication, recombination and repair processes, the two strands of the duplex must be separated one from the other and the large dimension of the replication and transcription complexes does not permit a free rotation of the flanking DNA domain, leading to the formation of DNA supercoiling. DNA therefore tends to be overwound (positively supercoiled) upstream of replication or transcription forks and underwound (negatively supercoiled) downstream of these forks. Such supercoiled DNA needs to be relaxed by topoisomerases (Tops).

DNA TOPs are essential and ubiquitous enzymes, belonging to two classes (type I and type II), both characterized by a catalytic mechanism that involves a nucleophilic attack of a DNA phosphodiester bond by a tyrosyl residue from the enzyme, but type I cleaves only one DNA strand, whereas type II cleaves both strands (Champoux, 2001). These enzymes have been shown to be essential in nearly all processes of DNA metabolism, such as replication, transcription, recombination and chromosomal segregation (Chen and Liu, 1994; Wang, 1996; Nitiss, 1998). Mice, knocked-out by the topoisomerase I (*TOP1*) gene, die early during embryogenesis (Morham et al., 1996) and the same happens in *Drosophila melanogaster* (Lee et al., 1993b). Yeast cells, however, can survive without the endogenous *TOP1*, although in this case yeasts show a high genomic instability (Reid et al., 1998) and their survival is probably due to compensation by the other TOPs.

Due to its essentiality and its crucial role in transcription and replication, Top1 is an attractive clinical molecular target and many laboratories are developing drugs with the aim of selectively hitting this enzyme. Several natural and non-natural compounds, such as terpenoids, flavonoids, stilbenes, fatty acids and transition metal complexes have been shown to interact with Top1, inhibiting it at different levels (Bailly, 2000; Pommier et al., 2010; Tesauro et al., 2010; Castelli et al., 2011). Natural products are particularly interesting since they have been selected

during evolution to interact with biological targets. Furthermore, their high degree of chemical diversity makes them attractive as lead compounds for the development of new drugs. There are numerous examples in pharmacology, not restricted to the field of antitumor drugs, where nature has provided the original principle that has been transformed by researchers to make compounds more active, less toxic and/or more easily manageable at the clinical level (Pezzuto, 1997).

Enzymatic catalytic steps and inhibitor interactions

The human Top1 enzyme is composed of 765 amino acids and has four distinct domains: the N-terminal domain (1–214), the core domain (215–635), the linker domain (636–712) and the C-terminal domain (713–765) (Stewart et al., 1996, 1998; Redinbo et al., 1998). The three-dimensional structure of the reconstituted N-terminal truncated version of human Top1 in complex with a 22-bp DNA molecule shows the enzyme organized in multiple domains that 'clamp on' to the DNA molecule (Figure 1; Redinbo et al., 1998). The active site of the human enzyme is composed of a catalytic pentad made by R488, K532, R590, H632 and Y723 (Pommier et al., 2010). The active site tyrosine (Y723) starts the catalytic cycle of the enzyme through a nucleophilic attack on the DNA backbone, resulting in the breakage of one DNA strand between the upstream (-1) and downstream (+1) base pairs, with the enzyme covalently attached to the 3′-phosphate to form the cleavage complex. After spontaneous DNA relaxation, a second nucleophilic attack driven by the 5′-hydroxy DNA end restores an intact double-stranded DNA, and the enzyme is released (Figure 2; Stewart et al., 1998). Based on this description, the catalytic cycle has been divided into five steps namely: 1) DNA substrate recognition and non-covalent binding; 2) cleavage with formation of the protein–DNA covalent complex; 3) substrate relaxation through a DNA strand rotation controlled by protein regions, such as the linker domain; 4) religation of the nicked strand; and 5) release of the relaxed substrate (Stewart et al., 1998).

The potential drug can interact with the enzyme at different levels impeding the binding, cleavage, rotation or religation processes. The drugs are classified as inhibitors when they impede the cleavage reaction and as poisons when they impede the religation process. Different enzymatic assays have been developed to identify at which level of the catalytic cycle the drug exerts its function. These will briefly be described here, taking camptothecin (CPT), a natural alkaloid extracted from the Chinese tree *Camptotheca acuminata* that poisons Top1 inhibiting the religation (Pommier et al., 1998), as the reference poisonous compound.

Relaxation assay

The most common activity assay is the 'relaxation assay', which permits us to observe the variation in DNA linking number induced by the purified enzyme as a function of time (Pommier et al., 1987). The enzyme is incubated with a supercoiled DNA substrate and the reaction is stopped by adding 0.5% sodium dodecyl sulphate (SDS).

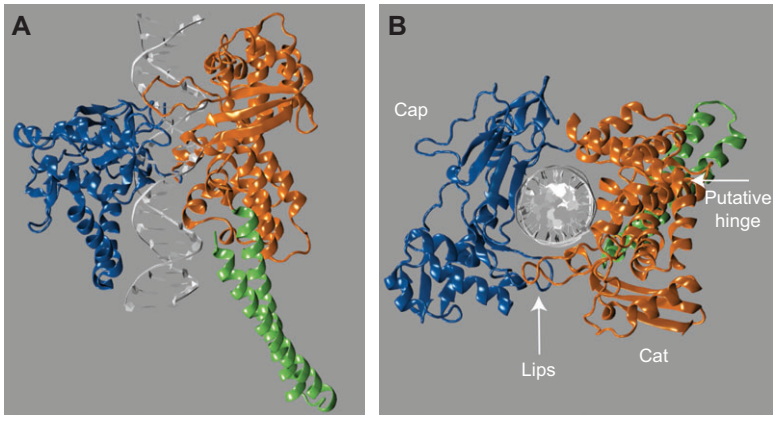

Figure 1 Side (A) and upper view (B) of human Top1 in complex with a 22 bp DNA (PDB 1A36).
The cat domain, harboring the catalytic pentad, is shown in orange, the cap domain in blue and the linker domain in lime. The putative hinge, for the opening and closing of the protein, and the lips, which keep the protein wrapped around the DNA, are highlighted in panel B.

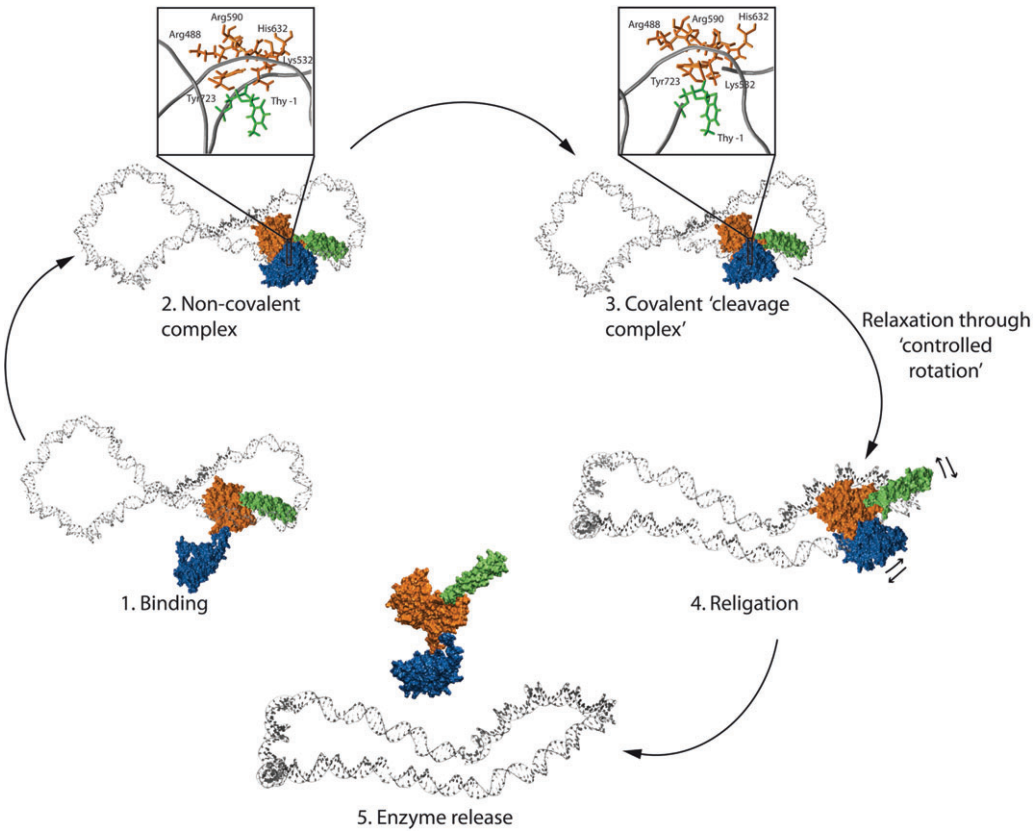

Figure 2 Scheme of the catalytic cycle.
The protein color code is the same as in Figure 1. The insert panel at points 2 and 3 shows a particular of the catalytic pentad in orange, and of the scissile base thymine -1 in green.

Electrophoresis of the samples, carried out in 1% agarose gel, permits us to highlight the presence of different topoisomers (Ireton et al., 2000). A typical gel, where the relaxation is followed in presence or absence of CPT (Figure 3), permits us to highlight the effect of the drug but it still leaves some ambiguity concerning the step of the catalytic cycle inhibited by the drug itself. In fact, in this case the effect of the drug is reversible so the inhibition is transient, leading to doubt as to whether the inhibition occurs at the level of the cleavage or of the religation.

Equilibrium assay

Another useful assay is the 'equilibrium assay', which is created by reaction of the enzyme with a double-stranded

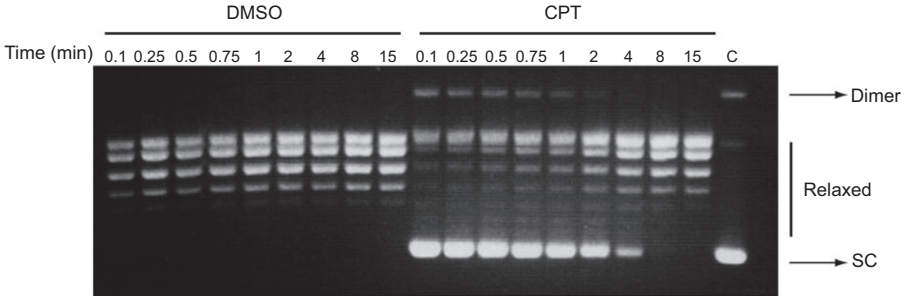

Figure 3 Relaxation of supercoiled DNA.
Gel electrophoresis of the relaxation of negative supercoiled plasmid DNA after incubation with the Top1 in a time course experiment in the absence or presence of CPT. C, no enzyme added.

DNA substrate consisting of two oligonucleotides, each one usually containing 25 nucleotides. The CL25 strand (GAAAAAAGACTTAGAAAAATTTTTA-3′) is radiolabeled with [γ^{32}P] ATP at its 5′ end. The CP25 complementary strand (5′-TAAAAATTTTTCTAAGTCTTTTTTC-3′) is phosphorylated at its 5′ end with unlabeled ATP. The two strands are annealed at a two-fold molar excess of CP25 over CL25. A final concentration of 20 nM duplex CL25/CP25 is then incubated with an excess of enzyme at 25°C in 20 mM Tris [or *ris*(hydroxymethyl)aminomethane] pH 7.5, 0.1 mM Na_2EDTA, 10 mM $MgCl_2$, 50 μg/ml acetylated BSA, and 50 or 150 mM KCl, in the presence or absence of 50 μM CPT. After 30 min, the reaction is stopped by adding 0.5% SDS and digested with trypsin after ethanol precipitation. Reaction products are resolved in 16% acrylamide–7 M urea gels (Chillemi et al., 2005). A typical gel obtained after 30 min incubation of the enzyme in presence or absence of CPT is shown in Figure 4. In the absence of the drug, the equilibrium is shifted toward religation and no band is observed; while in the presence of CPT, the equilibrium is shifted toward cleavage and the band due to the cleaved strand (Figure 4). The results of this experiment do not always have a straightforward interpretation, since the absence of the band could be due to a drug that inhibits cleavage or that does not perturb the cleavage or the religation reaction.

More direct and unambiguous results are obtained with experiments aimed at measuring the rate of cleavage or religation in the absence or presence of a drug.

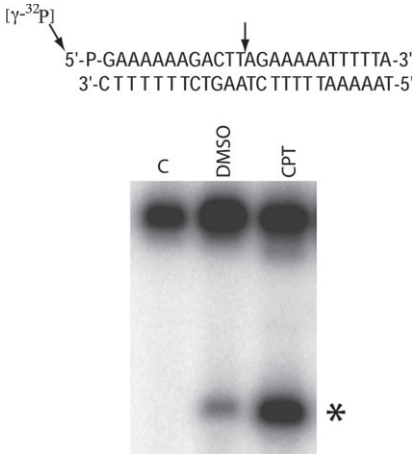

Figure 4 Cleavage/religation equilibrium of a full duplex DNA substrate.
Gel electrophoresis of the products coming from the incubation of the Top1 with the [γ-^{32}P] end-labeled duplex DNA, shown at the top of the figure in the absence and presence of CPT. The arrow at the DNA sequence indicates the CL1 preferred cleavage site. The asterisk indicates the band corresponding to the CL1 site. C, no enzyme added.

Cleavage assay

To measure the kinetics of cleavage, a specific substrate (called a suicide substrate) must be used that is made of two oligonucleotides of different lengths. A typical substrate with strand of 14 nucleotides (CL14) and the other 15 nucleotides (CP25) is depicted in Figure 5. The CL14 (5′-GAAAAAAGACTT*AG-3′), contains the preferred Top1 cleavage sequence (marked by an asterisk) radiolabeled with -γ^{32}P ATP at its 5′ end and annealed to the CP25 complementary strand (5′-TAAAAATTTTTCTAAGTCTTTTTTC-3′) to produce a duplex with an 11-base 5′ single-strand extension. The suicide cleavage reaction is carried out by incubating 20 nM of the duplex with an excess of enzyme in 20 mM Tris (pH 7.5), 0.1 mM Na_2EDTA, 10 mM $MgCl_2$, 50 μg/ml acetylated BSA, and 150 mM KCl at 23°C in a final volume of 50 μl, as described by Yang and Champoux (2002). When the enzyme cuts the suicide substrate at its preferential site, indicated by an arrow (Cl1) in Figure 5, the religation step is precluded because the AG-3′ oligonucleotide is too short to be religated, leaving the enzyme covalently attached to the 12-oligonucleotide 3′-end. The cleavage kinetics can then be analyzed using denaturing urea/polyacrylamide gel electrophoresis. At different time points, 5 μl aliquots are removed and the reaction is stopped with 0.5% SDS, after ethanol precipitation samples are resuspended in 5 μl of 1 mg/ml trypsin to remove all but a short trypsin-resistant peptide from the Top1–DNA covalent complexes, which makes the cleaved fragment run slower than the uncleaved one. Samples are analyzed in a sequencing gel. The percentage of cleavage 1 is determined by PhosphoImager and ImageQuant software and normalized on the total amount of radioactivity in each lane (Fiorani et al., 2003). A typical result is shown in Figure 5.

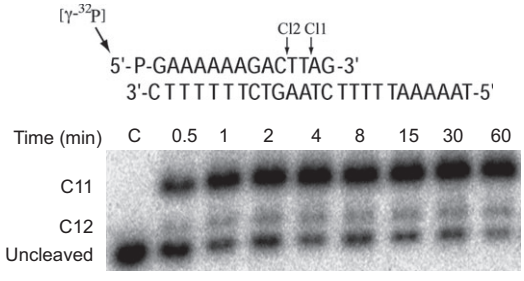

Figure 5 Suicide cleavage experiment.
Gel electrophoresis of the products coming from the incubation of the Top1 with the [γ-^{32}P] end-labeled suicide cleavage substrate shown at the top of the figure. CL1 and CL2 identify the cleaved complexes at the site indicated by the arrows. C, no enzyme added.

Religation assay

The religation kinetics can be followed by incubating the suicide CL14/CP25 substrate with an excess of Top1 enzyme for 1 h at 25°C and then for 30 min at 37°C in a standard reaction buffer. A 5 µl sample of the reaction mixture is then removed and used as the zero time point. Religation reactions are initiated by adding a 200-fold molar excess of R11 oligonucleotide (5′-AGAAAAATTTT-3′) over the duplex CL14/CP25 in the presence or absence of a drug, permitting the enzyme to perform the religation step restoring a full duplex oligonucleotide as the final product. At various points in the time-course, 5 µl aliquots are removed and the reaction stopped with 0.5% SDS after ethanol precipitation samples are resuspended in 5 µl of 1 mg/ml trypsin and incubated at 37°C for 1 h. Samples are analyzed by denaturing urea/polyacrylamide gel electrophoresis (Yang and Champoux, 2002). A typical experiment in the presence and absence of CPT, as shown in Figure 6, demonstrates that this drug strongly reduces the religation reaction. The percentage of religation is determined by PhosphoImager and ImageQuant software, normalized to the total amount of radioactivity in each lane and relative to the highest amount of substrate converted to reaction product by human Top1 in the absence of the drug (Fiorani et al., 2003).

Yeast viability assay

A powerful assay that can be used to test the targeting of endogenous Top1 *in vivo* is based on the evidence that yeast cells deficient for Top1 can survive and are resistant to CPTs (Reid et al., 1998). The same strain, once transformed with the gene expressing human Top1, becomes CPT-sensitive and so a viability assay in the presence of different drugs may show the efficiency of the drug in poisoning Top1. The same assay can be successfully used to identify Top1 mutants that are CPT resistant (Benedetti et al., 1993; Fiorani et al., 2006).

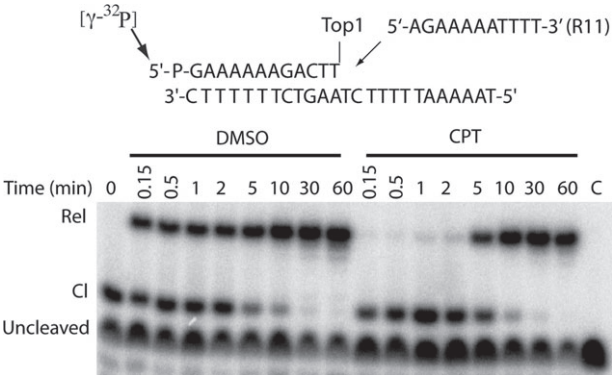

Figure 6 Religation experiment.
Gel electrophoresis of the products coming from the incubation of the Top1 with the substrate described on the top of the figure. The gel shows the time course of the religation experiment carried out between the R11 substrate and the wild type in the absence or presence of CPT. The R11 oligonucleotide is selectively religated to the CL1. C, no enzyme added.

Molecular docking

Finally, the availability of the three-dimensional structure of the Top1–DNA binary complex permits molecular docking of the complex with the drug leaving the possibility of identifying the most likely groups with chemical interacts. The method has been applied to dock several drugs and useful information concerning the interacting hot points has been obtained (Castelli et al., 2009; Tesauro et al., 2010).

The use of these assays permits us to discriminate the level at which the drug interacts with Top1, but such a procedure has not been always applied and in several works only the inhibitory effect of the compound on the relaxation reaction is followed. This has led to ambiguity about the mechanism of action of the drug. Here we will briefly review the natural compounds that have been reported to target Top1, dividing them into different chemical families and starting with CPT, which up until now has shown itself to be the most effective compound in selectively interacting with this enzyme.

Compounds that target Top1

Alkaloids

CPT was first identified from the Chinese tree *C. acuminata* (Wall et al., 1966), and only about 20 years later it was demonstrated that it was able to specifically target Top1 (Hsiang et al., 1985). It reversibly binds to the Top1–DNA cleaved complex, inhibiting religation. There are four unambiguous lines of evidence that CPT selectively poisons Top1:

- the natural CPT isomer is active against Top1 (Jaxel et al., 1989);
- genetically-modified yeast deleted for Top1 is immune to CPT (Eng et al., 1988; Nitiss and Wang, 1988);

– cells selected for CPT resistance show a point mutation in the *TOP1* gene (Pommier et al., 1999);
– CPT-producing plants have a point mutation in Top1 (N722S) that renders the enzyme immune to CPT (Sirikantaramas et al., 2008).

The cytotoxicity of CPT is due to the Top1–DNA cleavage complex being trapped, inhibiting the resealing of the cleaved strand and transforming the enzyme into something poisonous to the cell. During the S-phase of the cell cycle, collision of the replication fork with the drug-stabilized complexes converts a transient single-strand break into an irreversible double-helix break, resulting in cell death (Holm et al., 1989; Hsiang et al., 1989; Pommier et al., 1998; Liu et al., 2000). CPT then displays the interfacial inhibition paradigm that was firstly hypothesized in 1991 (Jaxel et al., 1991), and was demonstrated by the crystallization of the Top1–DNA–topotecan ternary complex (Staker et al., 2002). Topotecan, together with irinotecan, is one of the two water-soluble CPT derivatives approved by the US Food and Drug Administration that are in clinical use. Topotecan is used to treat ovarian and small-cell lung cancers, and irinotecan is used for colorectal tumors. The three-dimensional structure of the ternary complex has shown that topotecan mimics a DNA base pair and binds at the site of DNA cleavage by intercalating between the upstream (-1) and downstream (+1) base pairs (Figure 7). Intercalation displaces the downstream DNA, thus preventing religation of the cleaved strand. Topotecan and all the CPT derivatives then act as uncompetitive inhibitors, specifically binding to the enzyme–substrate complex and trapping this intermediate. CPTs exhibit a broad spectrum of antitumor activity but also have several limitations. The most important one concerns the fact the α-hydroxylactone E-ring of CPT is readily converted into a carboxylate giving, a form which is inactive against Top1 and binds to serum albumin (Burke and Mi, 1994).

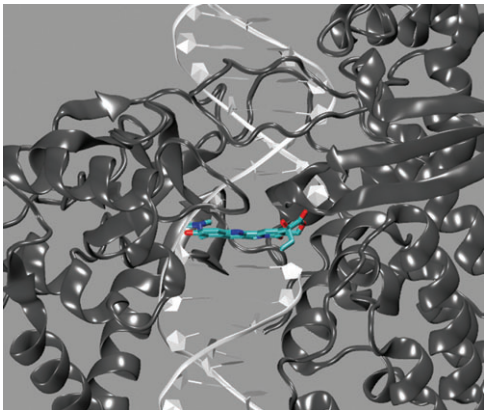

Figure 7 Three-dimensional structure of the topotecan–DNA–Top1 ternary complex (PDB 1K4T).
Topotecan is intercalated in the DNA bases at the level of the active site. The color code for topotecan atoms is as follows: C = cyan, O = red, N = blue.

The benzophenanthridine alkaloid nitidine (Figure 8), isolated from the Chinese plant *Zanthoxylum nitidum* and from the *Toddalia asiatica* found in Kenya, is more effective than CPT at inhibiting the relaxation of supercoiled plasmid DNA by Top1 (Wang et al., 1993). Also the closely related analog coralyne inhibits Top1 (Gatto et al., 1996), and is also found to stabilize triple helical DNA (Lee et al., 1993a). Analogs of coralyne (Figure 8) have been synthesized and their activity against Top1 has been evaluated (Makhey et al., 1996). Despite the pronounced activity of several of these coralyne derivatives as Top1 poisons, these compounds were generally less potent than CPT in cellular systems. Possible differences in cellular absorption between these coralyne analogs, which possess a quaternary ammonium group, and CPT may be responsible for the differences observed in their relative cytotoxicity. Cellular studies suggest that Top1 is the major cytotoxic target for nitidine (Makhey et al., 1996). However, it is worth bearing in mind that benzophenanthridine alkaloids have been reported to mediate a variety of biological activity, including potent and selective inhibition of protein kinase C, inhibition of cholinesterase systems and many others (Herbert et al., 1990; Wolff and Knipling, 1993), so that the main cause of its cytotoxic effect is still unclear. Other derivatives of a natural alkaloid compound, colchicine (Figure 8) derived from *Colchicum autumnale*, have been found to be active on human ovarian cell lines and on cisplatin-resistant cells. Thiocolchicine dimers (Figure 8) have a high degree of activity and were found to inhibit Top1, a property not shared by thiocolchine. The dimers do not produce the cleavage complex indicating that the inhibition occurs upstream of the religation step, however the dimers have also the ability to interact with tubulin so that the cytotoxic effect can be attributed to this dual activity (Raspaglio et al., 2005).

A CPT-like mechanism has been demonstrated for luotonin (Figure 8), a pyrroloquinazoquinoline alkaloid extracted from the Chinese plant *Peganum nigellastrum*. Luotonin is cytotoxic toward leukemia cell lines and produces an effect similar to CPT, as tested by a cleavage/religation equilibrium assay and by the effect observed in a *Saccharomyces cerevisiae* strain lacking yeast Top1, but harboring human Top1 (Cagir et al., 2003). This finding has brought to the synthesis of several modified and substituted luotonins, some of which have greater antiproliferative activity than the parent compound (Cagir et al., 2004; Dallavalle et al., 2004), and to the synthesis of

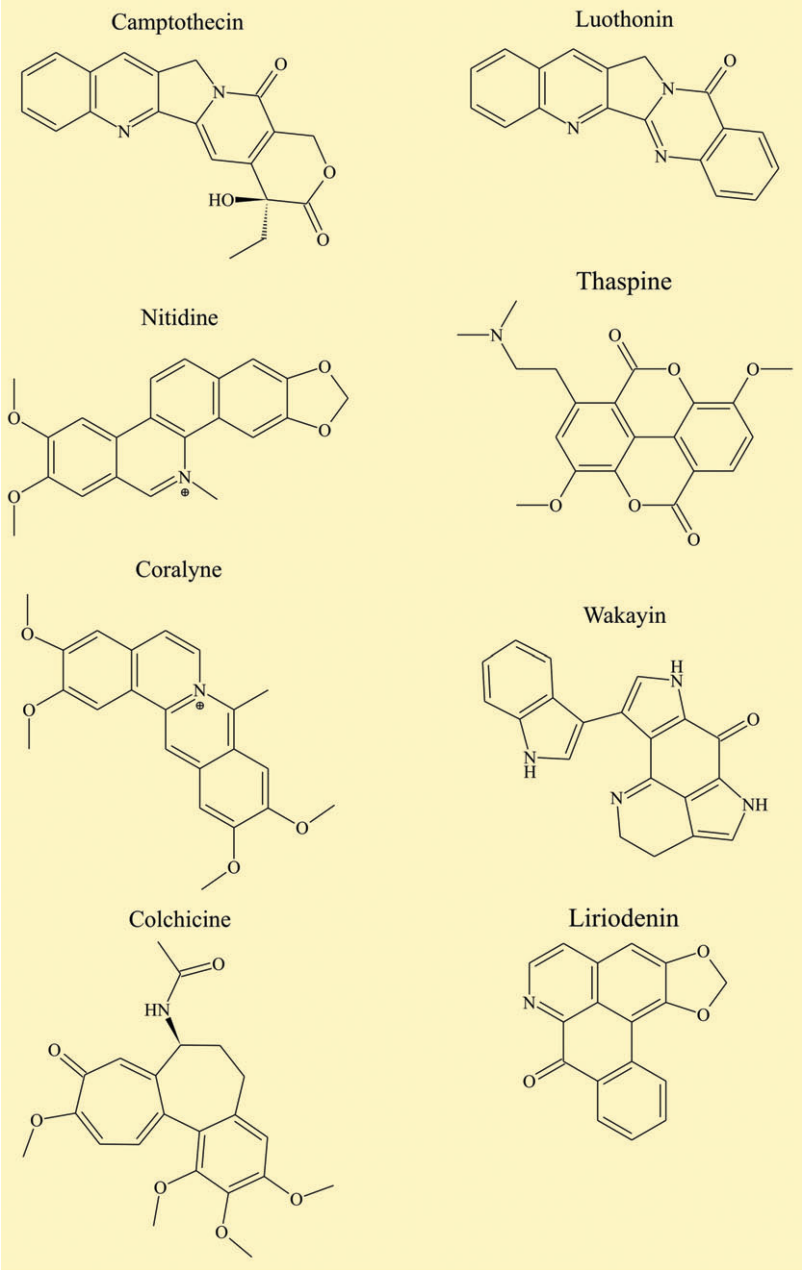

Figure 8 Chemical formulas of some alkaloids known to target Top1.

aromathecins, compounds with similarities to the natural product obtained from hybrids between indenoisoquinolines and CPT (Cinelli et al., 2008).

Upon screening a library of natural compounds, thaspine (also referred to as taspine, Figure 8), an alkaloid present in the cortex of the South American tree *Croton lechleri* and used in traditional medicine for different purposes, was shown to induce apoptosis in HCT116 colon carcinoma cells (Fayad et al., 2009). In the same study, thaspine was shown to induce a gene expression profile similar to that induced by CPT, suggesting a similar cytotoxic mechanism. Analysis of the interaction between thaspine and purified Top1, investigating the different steps of the enzyme catalytic cycle, has shown that thaspine has a dual activity inhibiting both the cleavage and the religation processes and suggesting that chemical modifications of the thaspine molecule may be able to confer specificity toward a selective inhibition of either of these two processes and may also increase the potency of the drug (Castelli et al., 2012).

The bispyrroloiminoquinone metabolite wakayin (Figure 8) isolated from the ascidian Clavelina in the Fiji

islands, enhances the formation of DNA–Top1 covalent complexes (Kokoshka et al., 1996). This finding has led to the synthesis of some analogs that also have some anti-Top1 activity (Legentil et al., 2006; Delfourne, 2008). The mechanism of interaction is not clear. Wakayin is believed to intercalate into DNA but, given its unusual structure, it is most likely that a portion of the molecule protrudes outside the double helix and establishes direct molecular interaction with the enzyme.

The natural compound liriodenine (Figure 8), an oxoaporphine alkaloid isolated from the traditional Chinese herb *Z. nitidum*, has been also reported to be cytotoxic and to target Top1 when chelated to gold (III) (Chen et al., 2012). The compound significantly poisons Top1 *in vitro* but the cytotoxic mechanism has not been clarified.

Flavonoids

Interaction between Top1 and several flavonoids has been demonstrated, but different interactions are reported to occur depending on the flavonoids' structure and on the Top1 investigated. Quercetin (Figure 9) and related flavonoids are known to inhibit tumor cells (Yoshida et al., 1992) and to increase the cytotoxicity of anticancer drugs such as cisplatin (Scambia et al., 1990; Shen and Weber, 1997). Investigation concerning the interaction of recombinant human Top1 and several natural flavones has indicated that quercetin and other flavones such as acacetin and kaempferol (Figure 9) are able to inhibit the enzyme religation reaction, although with a mechanism different from CPT (Boege et al., 1996). Flavones extracted from *Lethedon tannaensis*, a tree from New Caledonia, were also shown to inhibit Top1. In this case, the effect was only monitored following the relaxation assay (Zahir et al., 1996). Myricetin and myricetin-3-galactoside (Figure 9) have been also proposed to be Top1 poisons, but again their effect on the enzyme was followed through a DNA supercoiled relaxation assay (López-Lázaro et al., 2002). Luteolin (Figure 9) has been proposed to stabilize the Top1–DNA cleavage complex with a mechanism similar to CPT, but unlike CPT luteolin interacts with both free enzyme and DNA substrate (Chowdhury et al., 2002b). Actually the ability of

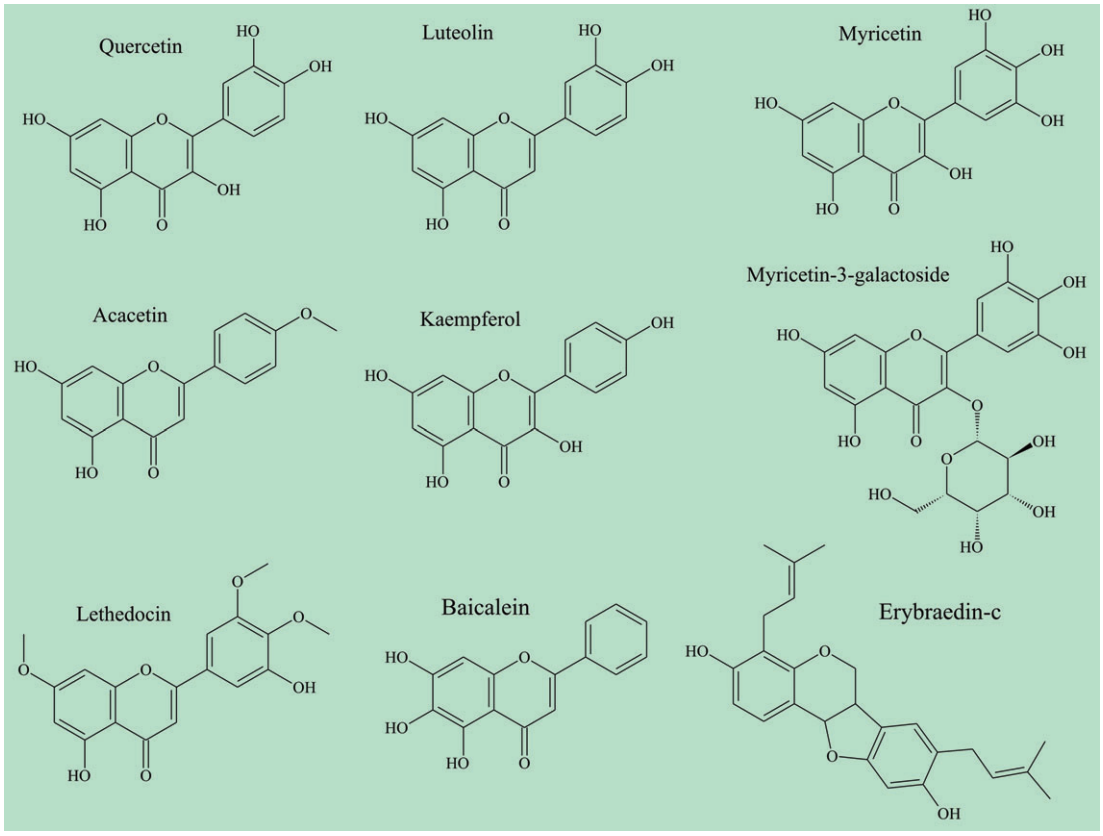

Figure 9 Chemical formulas of some flavonoids known to target Top1.

several flavonoids to intercalate DNA has probably led to some misinterpretation concerning their effect on Top1 and an association of Top1 inhibition with intercalation has been proposed (Webb and Ebeler, 2004).

It has been suggested that Top1 from *L. donovani* may be a promising target of the flavonoid family of drugs. Baicalein, luteolin and quercetin (Figure 9) have been proposed to stabilize the Top1–DNA covalent complex, besides being able to bind to the free enzyme and to intercalate in the DNA (Das et al., 2006). A dual inhibition activity is displayed by erybraedin C (Figure 9), a natural pterocarpan. This second group of isoflavones, purified from the plant *Bituminaria bituminosa*, is able to inhibit both the cleavage and the religation reaction (Tesauro et al., 2010). Molecular docking provides an explanation for this dual activity, showing that with the enzyme alone, the preferential drug binding site is localized in the proximity of the active Tyr723 residue, with one of the two prenilic groups close to the active-site residues Arg488 and His632, essential for the catalytic reaction. When interacting with the cleavage complex, erybraedin C interacts with both the enzyme and DNA in a way similar to topotecan. A catalytic inhibition of human Top1 has been also shown by flavonol glycosides extracted from *Vicia faba* and *Lotus edulis*, as evidenced by a DNA supercoiled relaxation assay (Tselepi et al., 2011).

Naphtoquinones

Lapachol (Figure 10) is a prenyl naphthoquinone isolated from plants of the Bignoniaceae family, such as several species of the *Tabebuia* genus growing mainly in Brazil (Díaz et al., 2004). Some of its derivatives have been shown to be cytotoxic against tumor cells (Li et al., 1995). However, it has been shown that β-lapachone, obtained by simple sulfuric treatment of the natural lapachol does not induce formation of the Top1–DNA cleaved complex, but it binds directly to the enzyme preventing DNA unwinding (Li et al., 1993). A similar mechanism occurs with isodiospyrin (Figure 10), a natural product from *Diospyros morrisiana*, which consists of an asymmetrical 1,2-binaphthoquinone chromophore. The compound displays cytotoxic activity to tumor cell lines (Kuo et al., 1997) and it inhibits

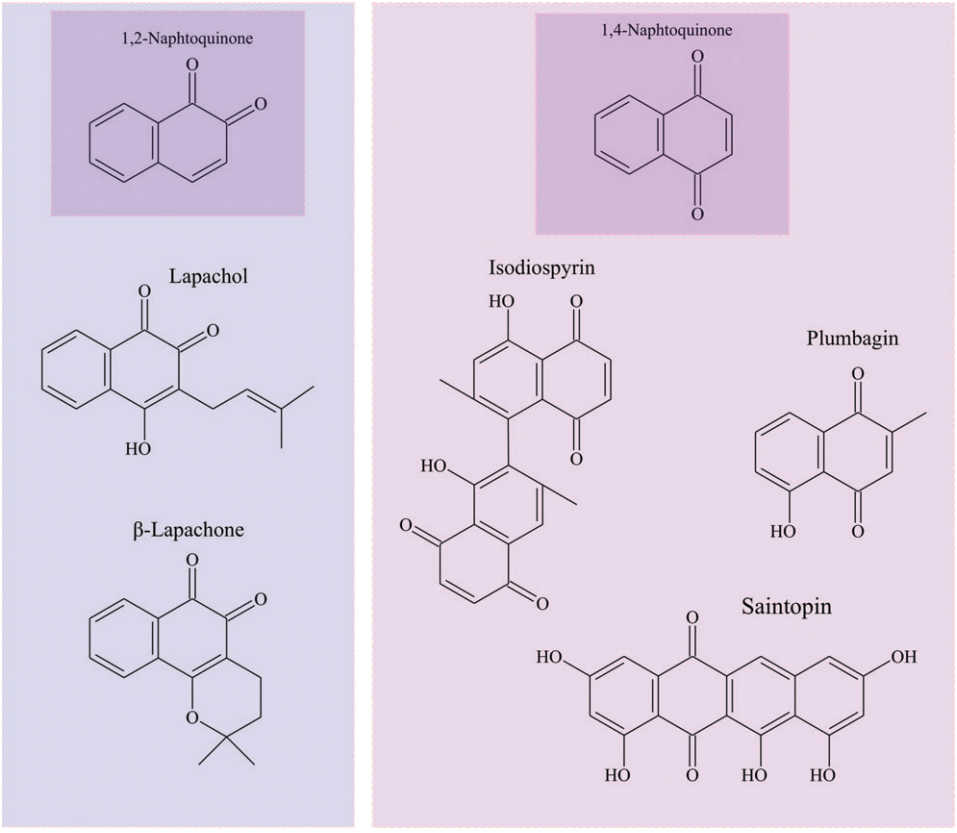

Figure 10 Chemical formulas of some napthoquinones known to target Top1.

Top1 by directly binding to it and limiting the access of the enzyme to the DNA substrate (Ting et al., 2003). There is no evidence for the formation of the Top1–DNA cleaved complex. In a previous work investigating the interaction between Top1 from *Leishmania donovani* and diospyrin, however, the authors claim that the compound stabilizes the Top1–DNA cleaved complex and that it is 10 times more active on the enzyme from *L. donovani* than on any other eukaryotic Top1 (Ray et al., 1998).

A fungal naphtoquinone metabolite isolated from *Paecilomyces* sp. known as saintopin (Figure 10) has been reported to be an inducer of Top1-mediated DNA cleavage (Fujii et al., 1997). As observed with CPT, DNA cleavages induced by saintopin occur preferentially at sites having a G located 3′ to the Top1-induced break (+1 position; Leteurtre et al., 1994). Derivatives having higher cytotoxic activities have been also synthesized (Martin et al., 2002), however it must be considered that these compounds are also Top2 inhibitors and that their cytotoxicity may also be due to the formation of a semiquinone radical (Verma, 2006).

Plumbagin (Figure 10), a naptoquinone used in the traditional Chinese medicine extracted from *Plumbago zeylanica*, has been shown to be cytotoxic and to target Top1 when chelated to copper (II) (Chen et al., 2009). However the compound has been only shown to inhibit the relaxation process, suggesting that it mainly interferes with the cleavage mechanism of the enzyme.

Triterpenes

Acetyl-boswellic acids (acetyl-BA), pentacyclic triterpenes (Figure 11) derived from the gum resin of *Boswellia serrata*, are effective cytotoxic agents, acting through a mechanism that appears to involve the inhibition of Top1 activity (Hoernlein et al., 1999). The inhibition does not involve stabilization of the cleaved complex or the intercalation of DNA, but competition with DNA for binding to the enzyme (Syrovets et al., 2000). Top1 inhibition by other triterpenoids, designated fomitellic acids (Figure 11), found in the higher plants *Tabebuia caraiba* and *Campsis radicans*, has been also reported and again a direct acid–enzyme interaction has been suggested to explain the inhibition mechanism (Mizushina et al., 2000). Similarly betulinic acid (Figure 11), a pentacyclic triterpenoid, was reported to inhibit Top1 by preventing Top1–DNA interaction and analysis of several derivatives indicated that specific hydrogenation of the side chain at C-20 enhanced the

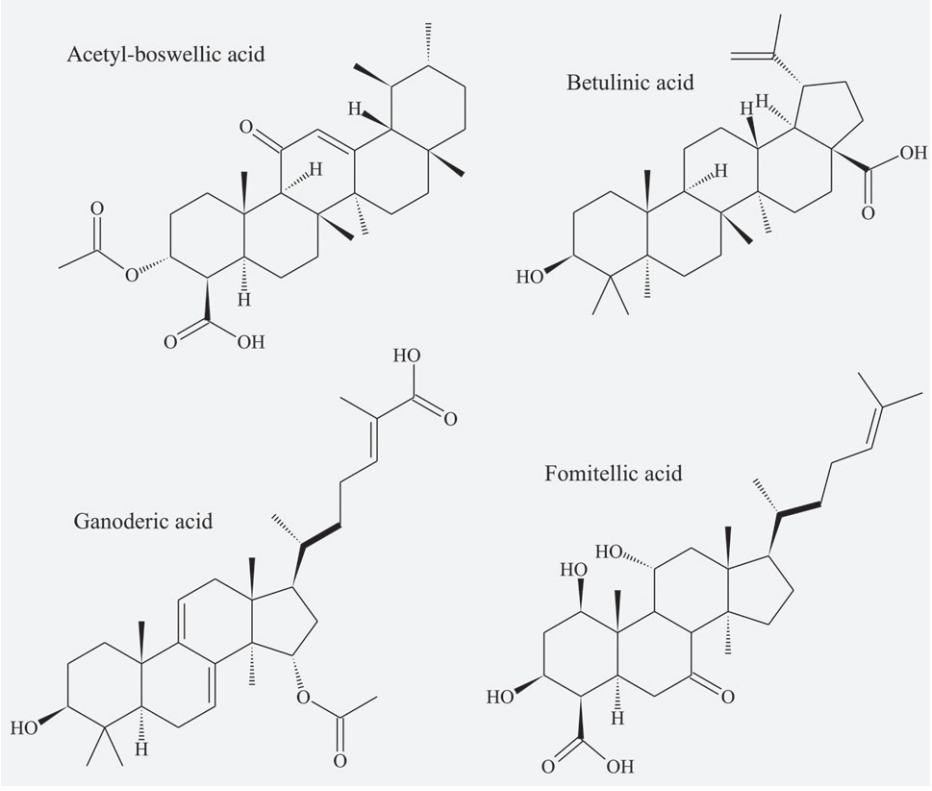

Figure 11 Chemical formulas of some triterpenes known to target Top1.

inhibitory activity of the compound (Chowdhury et al., 2002a). An inhibitory effect on the growth of cancer cells was also observed for tripertenes isolated from the fruit bodies of *Ganoderma amboinense* (Li et al., 2005). The triterpenes (Figure 11) inhibit Top1 relaxation activity but are also able to modulate the activities of protein kinase C and mitogen-activated protein kinases, suggesting that several mechanisms may participate in its cytoxicity.

Fatty acids

Natural C27-C30 fatty acids (Figure 12), isolated from an Australian sponge from the genus *Amphimedon*, are able to inhibit Top1 (Nemoto et al., 1998). Another Top1 inhibitor related to lipids is topostatin, isolated from the soil organism *Thermomonospora alba*, which posses a long lipid-type side chain attached to an unusual cyclic pseudo peptide (Suzuki et al., 1998). Similarly, topostatin B567, isolated from a culture broth of *Flexibacter topostinus*, inhibits Top1, suggesting that both lipophilic and anionic groups are necessary for enzyme inhibition. Conjugated eicosapentaenoic acid (cEPA), which is found in seaweeds such as red and green algae, has an inhibitory effect on human cancer cells (Yonezawa et al., 2007) and it inhibits supercoiled DNA relaxation by Top1 (Yonezawa et al., 2005). The acid inhibits the cleavage reaction and it is not able to stabilize the enzyme–DNA cleavage complex (Castelli et al., 2009). Molecular docking simulations indicate that the preferred cEPA binding site is proximal to the active site, with the carboxylic group strongly interacting with the positively charged K443 and K587 and the hydrophobic tail branching out over the protein surface arranged in many different hydrophobic clefts, confirming that both the hydrophilic and hydrophobic regions are important for the Top1 interaction (Castelli et al., 2009).

Fatty acids have been also shown to target Top1 from *L. donovani* and both a long chain and unsaturation are important factors for efficient leishmanicidal activity (Carballeira et al., 2009). In line cEPA has an anti-*L. donovani* promastigotes effect, confirming the importance of unsaturation for the antileishmanial activity of fatty acid compounds (Vassallo et al., 2011).

Conclusion

The vast number of natural compounds targeting Top1 that have been isolated or modified in the past few years attests to the fact that the area of Top1-targeted drugs is very active. The most interesting compounds are the ones acting at the level of the enzyme–DNA covalent complex, but compounds directly interacting with the enzyme may rapidly reveal themselves to be useful. Natural compounds, besides directly acting toward the molecular target, may provide a useful scaffold that can be modified or mimicked through chemical synthesis.

Although this review has considered natural compounds targeting Top1 for cancer therapy, it is important to note that other applications may be envisioned. Some

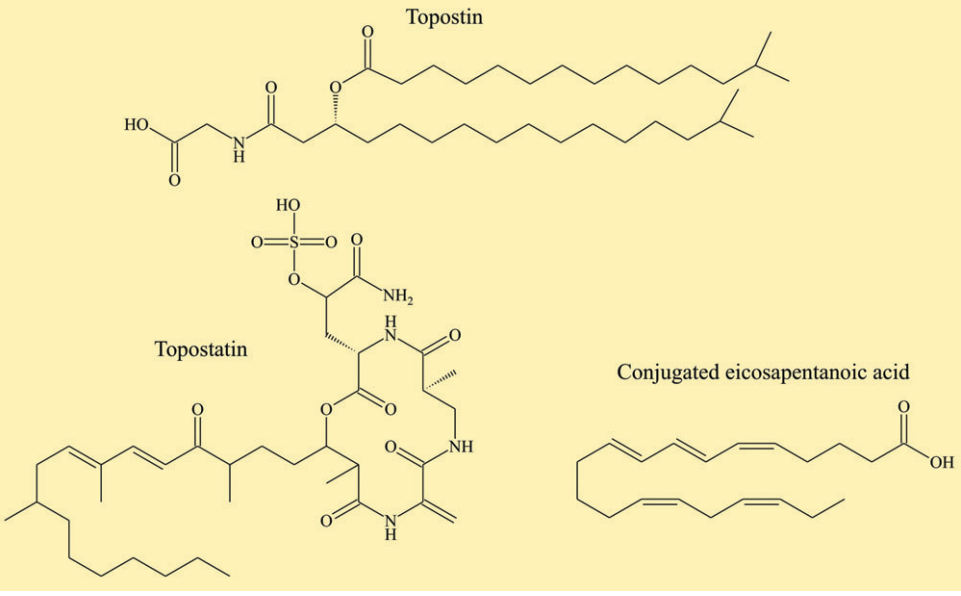

Figure 12 Chemical formulas of some fatty acids known to target Top1.

of these compounds are in fact able to target the Top1 of diverse widespread parasites such as *Plasmodium falciparum* and *L. donovani* (Bodley et al., 1998; Roy et al., 2008). A detailed comparative investigation of the functional structural properties of the enzymes of parasites against human Top1 would permit the development of selective drugs. Finally, topotecan at nanomolar concentrations has been shown to unsilence dormant genes in neurons (Huang et al., 2011), opening the route for another challenging application besides the anticancer one.

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