REVIEW

Recent developments in topoisomerase-targeted cancer chemotherapy

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Abstract The DNA topoisomerase enzymes are essential to cell function and are found ubiquitously in all domains of life. The various topoisomerase enzymes perform a wide range of functions related to the maintenance of DNA topology during DNA replication, and transcription are the targets of a wide range of antimicrobial and cancer chemotherapeutic agents. Natural product-derived agents, such as the camptothecin, anthracycline, and podophyllotoxin drugs, have seen broad use in the treatment of many types of cancer. Selective targeting of the topoisomerase enzymes for cancer treatment continues to be a highly active area of basic and clinical research. The focus of this review will be to summarize the current state of the art with respect to clinically used topoisomerase inhibitors for targeted cancer treatment and to discuss the pharmacology and chemistry of promising new topoisomerase inhibitors in clinical and pre-clinical development.

1. Human topoisomerases—structure and function

DNA topoisomerases present ubiquitous chemotherapeutic drug targets and are under continuous investigation in the development of novel antibacterial and anticancer agents. Essential for all domains of life, the multiple types and sub-types of DNA topoisomerases have been the target of marketed drug classes for several decades. The various topoisomerases enzymes deal with the topological issues related to DNA transcription and replication and are capable of relaxing positive or negatively supercoiled DNA, introducing negative or positive supercoils into DNA, and catenating or decatenating (disentangling) circular and linear DNA[1,2]. Within the last decade, developments in the structural biology and biochemistry of these enzymes have enabled the advancement of the new avenues of drug discovery targeting these agents, included structure-guided methods and novel compound screening strategies[3–8]. This has resulted in the discovery of several new chemical classes of topoisomerase inhibitors with both antibacterial and anticancer properties. This review will discuss new agents that have been introduced for the treatment of cancers within the last several years and discuss their novel chemistry, pharmacology, and clinical efficacy, where relevant.

Table 1 summarizes the function and mechanism of the various types and sub-types of DNA topoisomerases found in eukaryotic organisms. The DNA topoisomerases can be categorized into two general subfamilies, type I and type II topoisomerases. Type I topoisomerases affect DNA topology by passing a single DNA strand through a break in the opposing single strand using an active site tyrosine residue to cleave the DNA strand, forming a phosphodiester bond with the protein. Type II topoisomerase create double-stranded breaks using similar active site tyrosine residues, through which another double-strand DNA segment is then passed. Further sub-divisions of the topoisomerases, types IA, IB, and IIA are categorized based upon the polarity of the DNA-protein bond (i.e., tyrosine attachment to the 5′- or 3′-phosphate), the mechanism (strand passage or rotation), and the number of overhanging DNA bases in the staggered double strand cleavage of the type II topoisomerases. Structurally, eukaryotic type I topoisomerases are monomeric protein, single-gene products, while eukaryotic type II topoisomerases are homodimers, composed of the products of a distinct gene. Functionally, type I topoisomerases are not dependent on ATP hydrolysis to power the energy required for their reaction, instead they derive the energy required from the intrinsic strain energy of the supercoiled DNA itself[9]. Type II topoisomerases are typically ATP-dependent and possess an ATP-binding domain separate from the DNA-binding domain. Additionally, the DNA topoisomerases can also be distinguished by their dependence, or lack of dependence, on

Mg^{2+} as a requirement for catalytic activity. The type IA and all type II topoisomerases appear to require Mg^{2+}, while the type IB topoisomerases are catalytically active in the absence of Mg^{2+}[10]. The targets of the currently marketed cancer chemotherapeutic agents are topoisomerase I, IIα, and IIβ.

Several excellent reviews have summarized the current state of knowledge pertaining to the structure and function of the type I and type II topoisomerases[1,9,11–13]. Fig. 1 shows the overall structures of representative human type I and type II DNA topoisomerases[14,15]. The type IA topoisomerases represented by eukaryotic topoisomerase IIIα and IIIβ consist of four domains that coordinate DNA binding, cleavage, and strand passage[16–18]. Domain I contains the so-called TOPRIM fold, a Rossman-fold like structure that can bind magnesium ions[18,19]. Domain II consists primarily of β-strands that forms the central core and links domain III, which contains the catalytic tyrosine residue, to domain IV. The type IB topoisomerases, represented by eukaryotic topoisomerase I, is also composed of four domains including an N-terminal domain, a linker domain, a core domain, and a C-terminal domain[14,20]. The core domain is responsible for DNA binding and appears to be highly conserved. The catalytic tyrosine is in the C-terminal domain and operates as part of a catalytic pentad with four residues located in the core domain. Type IIA topoisomerases, represented by eukaryotic topoisomerase IIα and IIβ, consist of a three-domain structure spanning the A and B subunits that form the homodimer (or heterotetramer in prokaryotes). The N-terminal domain contains the ATB-binding region (ATPase domain), a core domain that contains a TOPRIM fold and DNA-binding region, and a C-terminal domain with unclear function[21–23].

The sub-types of topoisomerases are mechanistically distinct with respect to how they act on their DNA substrates. Type IA topoisomerases operate using a "strand-passage" mechanism, whereby a single strand of DNA is passed through a break in a second single DNA stand. In this mechanism, the first DNA single strand binds to domain I and III of the topoisomerase and is cleaved by the catalytic tyrosine residue located on domain III, creating a 5′-phosphodiester bond between the enzyme and the DNA. Domain II is then believed to act as a hinge, separating the cleaved DNA strand and permitting a second strand to pass through, after which domains I and III come back together and the cleaved DNA strand is re-joined. The type IB topoisomerases operate using a "hindered rotation mechanism", whereby the enzyme binds to DNA and cleaves a single strand via a 3′-phosphodiester bond using an active site tyrosine residue. The 5′-end then rotates about the second DNA strand, relaxing the DNA. As discussed above, both type IA and type IB topoisomerases use the DNA torsional strain (torque) to drive the uncoiling process rather than ATP hydrolysis. Type IA topoisomerases relax

Table 1 Summary of eukaryotic DNA topoisomerase enzyme function and mechanism.

Type	Subtype	Protein	Gene	Function	Mechanism	Multimericity	Metal dependence	Cleavage polarity
I	A	Topoisomerase IIIα	*top3A*	(−) Supercoil relaxation	Strand passage	Monomer	Yes (Mg^{2+})	5′
		Topoisomerase IIIβ	*top3B*	Unknown				
	B	Topoisomerase I	*top1*	(−) and (+) supercoil relaxation	Strand rotation	Monomer	No	3′
II	A	Topoisomerase IIα	*top2A*	Decatenation during replication	Strand passage (ATPase)	Homodimer	Yes (Mg^{2+})	5′
		Topoisomerase IIβ	*top2B*	Various, neuronal transcription				
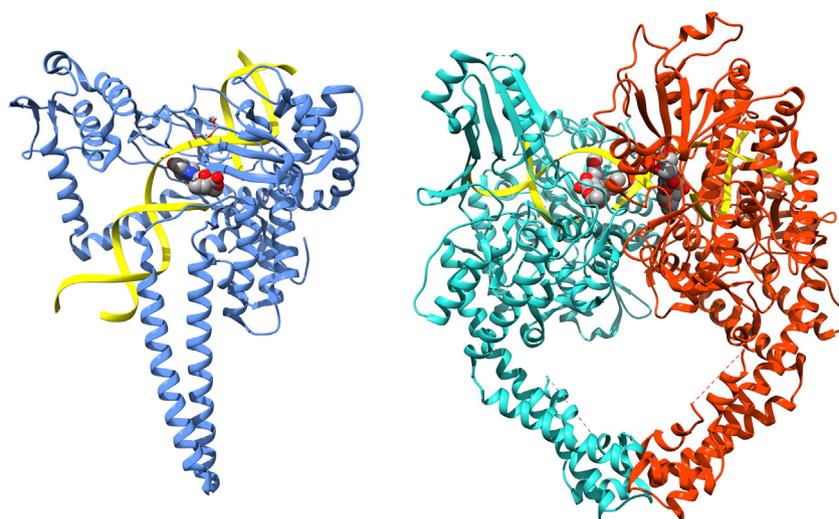
Figure 1 Structures of human topoisomerases. Shown are the structures of the full length human topoisomerase I (left, PDB ID 1k4t) and topoisomerase IIα (right, PDB ID 5qwk) enzymes with bound DNA, representative of the overall structure and domains of the two sub-family types (DNA ribbon is colored yellow; chain A of topoisomerase I is blue; chain A of topoisomerase IIα is green; chain B of topoisomerase IIα is red).[14,15]

negatively supercoiled DNA and perform DNA decatenation, while type IB topoisomerases can relax both negatively and positively supercoiled DNA. Like the type IA topoisomerases, the type IIA topoisomerases exert their effect through a strand passage mechanism, in this case using what is known as a "two-gate" mechanism[24]. Double-stranded DNA, called the "G-segment", binds to the topoisomerase in a central DNA-binding region called the "DNA-gate". A second double-stranded DNA, the "T-segment", binds to the N-terminal ATPase domain facilitated by ATP binding and domain dimerization. ATP hydrolysis and the release of inorganic phosphate causes a double-stranded break with 4-base overhang in the G-segment DNA facilitated by the formation of 5′-phosphodiester bonds between two tyrosine residues and individual strands of the G-segment DNA. The DNA-binding gate separates, and the T-segment is passed through the G-segment into the C-terminal region, or "C-gate". The C-gate opens and releases the T-segment DNA, and ADP product is released, resetting the enzyme for another catalytic cycle.

2. Targeting topoisomerases for cancer chemotherapy

Inhibition of the DNA topoisomerase enzymes can occur by one of several generally accepted molecular mechanisms[5,25–27]. One potential mechanism is substrate competitive inhibition, the binding of an inhibitor compound to the topoisomerase active site that prevents the binding of the DNA substrate. There are no notable examples of topoisomerase-specific inhibitors that work by this mechanism, but recent reports of DNA-competitive inhibitors of other DNA-binding proteins suggest that this mechanism may be possible in DNA topoisomerases as well[28]. Another common mechanism is the formation of so-called "topoisomerase poisons", that are composed of a ternary protein-DNA-drug complex that prevents DNA re-ligation and locks the enzyme into a "cleavage complex". This complex results in prevention of enzyme turnover and the build-up of high levels of the cytotoxic cleavage complex within the cell. A third mechanism is by competitive inhibition of the ATP binding site, seen only in the type II topoisomerases, which prevents the ATP-hydrolysis driven enzymatic action (discussed above). Examples of ATP-site binders include

novobiocin and coumermycin, which are not used clinically due to issues with potency, specificity, and poor pharmacokinetic properties[29,30]. Compounds that can bind to DNA and prevent topoisomerase binding, such as the compounds aclarubicin and suramin, present another potential inhibitory mechanism, though specificity is again an issue with agents such as these[31,32]. Agents such as the compound merbarone, that can bind to the DNA-protein complex and prevent cleavage, represent yet another mechanism of catalytic inhibition[32,33]. Lastly, the ATP-dependent type II topoisomerases can be inhibited by agents that prevent ATP hydrolysis and DNA release after strand passage, as exemplified by the bisdioxopiperazine agent dexrazoxane[34–36]. These agents result in a "closed clamp complex" that is analogous to the cleavage complex generated by the topoisomerase poisons. In this section, we will review in some detail the inhibitory mechanism of the more common classes of topoisomerase inhibitors from a structural and biochemical perspective, and provide examples of clinically used agents, where applicable.

Of highest clinical relevance is the mechanism of the topoisomerase poisons[27]. This mechanism involves the stabilization of the cleavage complex by creation of a locked ternary complex of cleaved DNA, protein, and drug that builds up and causes a cytotoxic effect[37]. The mechanism is exemplified by the camptothecin-derived agents that act upon type IB topoisomerases, and the anthracycline, anthracenedione, and epipodophyllotoxin agents that act upon type IIA topoisomerases for the treatment of cancer (discussed in further detail below). These agents bind to the cleaved-DNA/protein complex and prevent the re-ligation of DNA, locking the enzyme into the cleavage complex and preventing enzyme turnover. A build-up of the cleavage complex results in DNA strand breaks and ultimately cellular death[27]. Most known topoisomerase poisons act via an interfacial mechanism by intercalation between the −1 and +1 DNA base pairs in the protein-DNA cleavage complex. Additional hydrophobic and electrostatic interactions with both the DNA and protein stabilize the binding of the poison and prevent DNA re-ligation by the topoisomerase[37–39]. A second topoisomerase poison mechanism is through the redox-dependent, covalent formation of a drug-enzyme complex. The covalent class compounds bind to a site distal to the DNA active site and work to stabilize the enzyme-
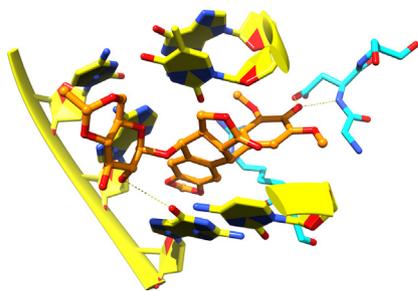
Figure 2 Etoposide binding to human topoisomerase IIα. Shown is the topoisomerase poison, etoposide, bound to the active site of human topoisomerase IIα. DNA is represented with yellow ribbons, yellow carbons, and filled rings. Protein is represented with cyan carbons, and the drug is shown with ball and stick representation with orange carbons. Hydrogen bonds to protein and DNA are shown as dashed yellow lines.

Figure 3 ATP binding site of Human Topoisomerase IIα. Depicted is the ATP binding site of the human topoisomerase IIα enzyme. ADP is bound to the binding site (yellow carbons) with hydrogen bonds to the protein shown in yellow, dashed lines. The active site residues are shown with cyan carbons and a translucent gray surface is used to show the shape of the binding site.

DNA cleavage complex, resulting in a similar build-up and ultimate cell death[39]. Shown in Fig. 2, is the binding of the drug etoposide to human topoisomerase IIα. This interfacial topoisomerase poison can be seen intercalating two DNA-base pairs in the active site, facilitated by stacking interactions between the DNA bases and the connected, 4-ring system of the drug. The drug's adjoining ring systems are seen hydrogen bonding to the nearby residues from the protein and local DNA bases. A similar binding event (not shown) takes place 4 base pairs distal on the opposite DNA strand, reflecting the double strand cleavage of this type II topoisomerase.

A second, highly relevant topoisomerase inhibition mechanism is catalytic inhibition by competitive binding of small molecules to the ATP binding site found in the N-terminal of region of type II topoisomerases[5,40]. As discussed above, the energy required for the action of type I topoisomerases is derived from the torsional strain of the supercoiled DNA itself, while the energy powering the action of the type II topoisomerases is derived from ATP hydrolysis. Competitive inhibition of ATP hydrolysis by small molecules prevents the progression of the DNA T-segment through the G-gate into the C-terminal domain, resulting in a catalytic inhibition of the topoisomerase, halting DNA transcription and replication, and ultimately leads to cell death[25]. This mechanism does not result in the DNA damage and cellular damage responses seen with the topoisomerase poisons (discussed further below). Catalytic inhibitors of this type were first seen with the aminocoumarin antibiotics, represented by the compound novobiocin, which is no longer clinically marketed[25,41]. Though there are no currently marketed anticancer agents possessing this mechanism of inhibition, there have been fairly recent reports discussing agents in development that show promise in the area[42,43]. Fig. 3 shows the ATP binding site of human topoisomerase IIα with ADP bound[44]. The key binding interactions are highlighted. An interesting, related mechanism that bears mention is that of the bisdioxopiperazine compounds represented by the drug dexrazoxane (ICRF-187)[34]. This class of agents shows catalytic inhibition by binding to the enzyme in an ATP-dependent, uncompetitive manner and inhibiting the ATP to ADP conversion. This appears to lock the topoisomerase in a closed clamp conformation. Though the inhibition is catalytic in nature, there appears to be some evidence of a resulting DNA damage and cellular damage response, like that induced by the topoisomerase poisons[36]. Dexrazoxane is marketed under the

brand name Zinecard in the USA and Cardioxane in the EU and other countries as a protectant agent to mitigate cardiotoxicity caused by anthracyclines (discussed further below).
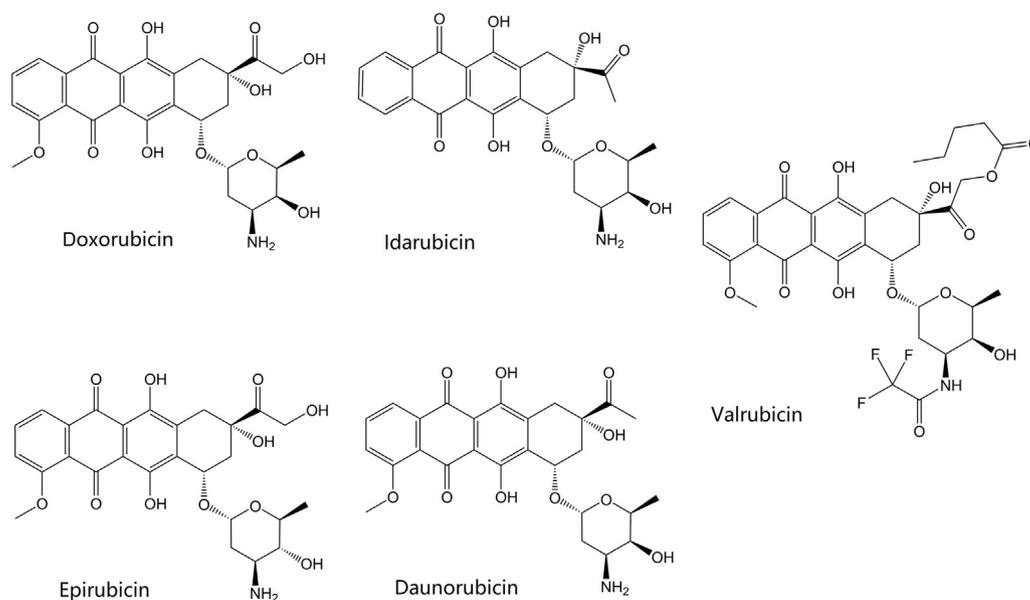
3. Clinically marketed topoisomerase inhibitors

Table 2 summarizes the currently marketed topoisomerase active agents used for cancer chemotherapy[45–47]. The first known class of topoisomerase inhibitors used for the treatment of cancer was the anthracycline agents. The anthracyclines were first extracted from bacterial *Streptomyces* species and discovered to possess antibiotic and antitumor activity[48]. The clinically marketed anthracycline derivatives include doxorubicin, epirubicin, valrubicin, daunorubicin, and idarubicin. Their structures are shown in Fig. 4. Doxorubicin has several current indications including treatment of breast cancer, various types of leukemia, lymphoma, sarcomas, carcinomas, and other tumors. Other anthracyclines which have indications for treatment of leukemia include daunorubicin and idarubicin. Epirubicin is indicated in breast cancer following resection, while valrubicin is indicated in urinary bladder carcinoma. The anthracycline agents affect their cytotoxic activity by acting as topoisomerase "poisons", as discussed in the previous section. They primarily affect type IIa topoisomerases, topoisomerase IIα and topoisomerase IIβ indiscriminately, by intercalation into the bound and cleaved DNA, stabilizing the DNA and topoisomerase complex and preventing DNA re-ligation by the topoisomerase. Interestingly, this appears to be one of the mechanisms that the anthracyclines induce cell death[49]. The production of free radical species in an iron-dependent manner appears to be another mechanism behind the anthracyclines' cytotoxic effects in cancer cells. Unfortunately, this mechanism also appear to be responsible for additional toxicities associated with this class of agents[50]. Anthracyclines are well known for their cardiotoxic properties, causing both acute and chronic cardiac complications in a dose-dependent manner. Menna and colleagues[51]. discuss the mechanisms behind this toxicity, including anthracyclines' production of superoxide anions and hydrogen peroxide in cardiac muscle cells, which contribute to oxidative stress and eventually apoptosis. Anthracyclines also disrupt

Table 2 Summary of marketed topoisomerase inhibitors and indications.

Drug	Class	Approval date	Mechanism/Target	Indication[a]
Doxorubicin	Anthracycline	8/7/1974 (U.S.)	Type IIA poison	In combination with other chemotherapy agents to treat women after surgical resection of breast cancer with axillary lymph node involvement; acute lymphoblastic and myeloblastic leukemias; Hodgkin and non-Hodgkin lymphomas; metastatic neuroblastoma, Wilms′ tumor, cancers of the breast, soft tissue sarcoma, and bone sarcomas; metastatic ovarian, transitional cell bladder, thyroid, gastric, and bronchogenic carcinomas
Epirubicin	Anthracycline	9/15/1999 (U.S.)	Type IIA poison	Combination with other chemotherapy agents to treat women after surgical resection of breast cancer with axillary lymph node involvement
Valrubicin	Anthracycline	9/25/1998 (U.S.)	Type IIA poison	Intravesical administration for urinary bladder carcinoma refractory to BCG therapy in patients who are not candidates for cystectomy
Daunorubicin	Anthracycline	12/19/1979 (U.S.)	Type IIA poison	In combination with other approved chemotherapy agents to induce remission in acute myelogenous, monocytic, and erythroid lymphocytic leukemias in adults and in acute lymphocytic leukemia in children
Idarubicin	Anthracycline	9/27/1990 (U.S.)	Type IIA poison	In combination with other approved agents to treat adults with acute myeloid leukemia, including French-American-British M1–M7 classifications
Mitoxantrone	Anthracenedione	12/23/1987 (U.S.)	Type IIA poison	For patients with secondary (chronic) multiple sclerosis with significantly abnormal neurologic status between relapses to reduce neurologic disability and or/the frequency of relapses; in combination with corticosteroids as initial chemotherapy to treat pain related to advanced hormone-refractory prostate cancer; in combination with other approved agents to as initial therapy of acute nonlymphocytic anemia in adults
Pixantrone	Anthracenedione	2/16/2012 (E.U.)	Type IIA poison	Monotherapy for the treatment of adult patients with multiply relapsed or refractory aggressive Non-Hodgkin B-cell lymphomas
Etoposide	Epipodophyllotoxin	11/10/1983 (U.S.)	Type IIA poison	Refractory testicular tumors in combination with other chemotherapy agents; first-line treatment in combination with cisplatin for small-cell lung cancer
Teniposide	Epipodophyllotoxin	7/14/1992 (U.S.)	Type IIA poison	Induction of remission in patients with refractory childhood acute lymphoblastic leukemia
Amsacrine	Miscellaneous	12/31/1983 (Canada)	Type IIA poison	Induction of remission in acute adult leukemia refractory to conventional therapy
Topotecan	Camptothecin	5/28/1996 (U.S.)	Type IB poison	Small-cell lung cancer after failure of first-line chemotherapy; combination with cisplatin for persistent or recurrent stage IV-B carcinoma of the cervix not cured by surgery and/or radiation
Irinotecan	Camptothecin	6/14/1996 (U.S.)	Type IB poison	First-line chemotherapy in combination with 5-fluorouracil and leucovorin in patients with metastatic carcinoma of the colon or rectum; recurrent or progressive metastatic carcinoma of the colon or rectum following initial fluorouracil-based therapy
Belotecan	Camptothecin	12/10/2003 (S. Korea)	Type IB poison	Non-small-cell lung cancer; ovarian cancer

[a]Drug indications are taken from the approving country's drug regulatory agency websites[45–47].

Figure 4 Structures of the marketed anthracycline topoisomerase inhibitors.

calcium and iron levels in cardiac cells, which leads to even more free-radical generation[51]. Some recent studies have implied another potential mechanism of the cardiotoxicity[52,53]. These studies suggest that inhibition of topoisomerase IIβ, which is overexpressed in the heart, by the anthracycline agents may result cardiotoxicity from apoptosis and ROS. Dexrazoxane, discussed above, is an FDA-approved agent indicated to reduce adverse cardiac affects in women who have received a total dose of 300 mg/m^2 of doxorubicin and are continuing to receive doxorubicin[54]. Interestingly, the mechanism of cardiotoxicity mitigation appears to be related, at least partially, to the ability of the drug to chelate iron. The drug is also known to catalytically inhibit topoisomerase IIβ, as discussed above, which may reduce cardiotoxicity related to generation of the topoisomerase IIβ poisons formed by anthracyclines. Other investigational measures to prevent cardiotoxicity include the use of the beta blocker carvedilol. Spallarossa and colleagues found a decrease in free radical production and apoptosis in heart muscle cells when treating cells with carvedilol prior to treatment with doxorubicin, due to carvedilol's unique antioxidant effect[55].

The anthracenedione agents include the drugs mitoxantrone and pixantrone. The anthracenedione (anthraquinone) agents are synthetic agents, designed to act similarly to the anthracycline drugs, with fewer adverse effects and toxicities. Mitoxantrone was approved in 1987 in the U.S. and pixantrone, a newer agent, was approved in 2012 in Europe. The structures of these agents are shown in Fig. 5. Mitoxantrone can cross the blood brain barrier and is indicated for the reduction of the frequency and intensity of multiple sclerosis relapses in addition to its indication as a chemotherapeutic agent in leukemia and prostate cancer[56]. Similar to the anthracyclines, the anthracenedione agents are topoisomerase poisons, primarily affecting type II topoisomerases. Like the previously discussed agents, mitoxantrone intercalates topoisomerase-bound DNA, preventing DNA re-ligations, and ultimately resulting in DNA strand breakage and disruption of DNA repair. Pixantrone, an aza-anthracenedione approved in treatment of non-hodgkin B-cell lymphoma, exhibits its cytotoxic effects by intercalating into DNA like the anthracyclines, but also causes long term cell damage and eventual death by causing errors

in mitosis and segregation of chromosomes[57]. Pixantrone has shown less toxicity than doxorubicin in cardiac muscle cells because of its inability to bind iron and contribute to free radical production in the heart; as a result, animal models showed decreased heart weight in animals treated with doxorubicin as compared to pixantrone[58,59].

Clinically marketed camptothecin derivatives include topotecan, irinotecan, and belotecan. Their structures are shown in Fig. 6. The camptothecin alkaloid was first derived from the Chinese tree, *Camptotheca acuminata*. The camptothecin-derived agents are topoisomerase poisons which primarily affect type I topoisomerases. *In vitro* studies showed camptothecin, a cytotoxic alkaloid, was capable of inhibiting topoisomerase I and causing DNA strand breaks, thus preventing DNA replication[60]. The water-soluble forms of camptothecin include the clinically marketed irinotecan and topotecan, which reversibly bind and form a ternary complex with topoisomerase I and DNA, as discussed above[61]. Topotecan is approved as second-line small cell lung cancer and, in combination with cisplatin, for patients with stage IV-B cervical carcinoma not treated by surgery or radiation. Irinotecan is approved following failure or progression following treatment with fluorouracil or in combination with 5-fluorouracil and leucovorin for patients with metastatic colon or rectal carcinoma. Belotecan is a relatively new camptothecin derivative agent, approved in South Korea for treatment of non-small-cell lung cancer and ovarian cancer in 2003[62,63]. The mechanism of action is the same as other agents in this class. Compared with older camptothecin agents, belotecan is reported to have a similar efficacy profile, with reduced toxicities[64].

The drugs etoposide and teniposide are epipodophyllotoxin-derived agents. Epipodophyllotoxins are natural substances derived from the Mayapple plant (wild mandrake), *Podophyllum peltatum*[65,66]. The drugs have been available in the U.S. and other countries since the early 1980's. Their structures are shown in Fig. 7. Both agents act as topoisomerase poisons and cause DNA strand breaks by binding to type II topoisomerases, similar to the agents described above[67]. Etoposide is indicated as part of a multi-drug chemotherapy regimen for refractory testicular tumors and in combination with cisplatin to treat small-cell lung cancer.
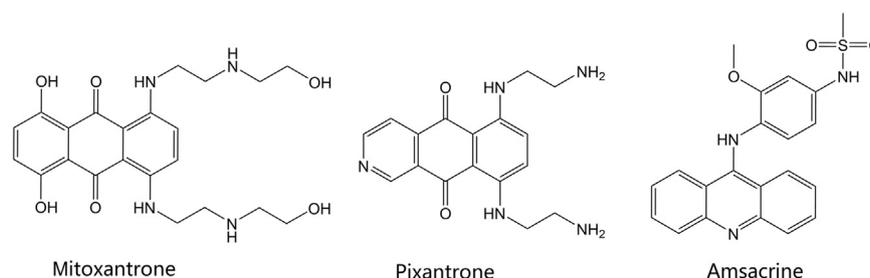
Figure 5   Structures of the marketed anthracenedione and acridine-derived topoisomerase inhibitors.
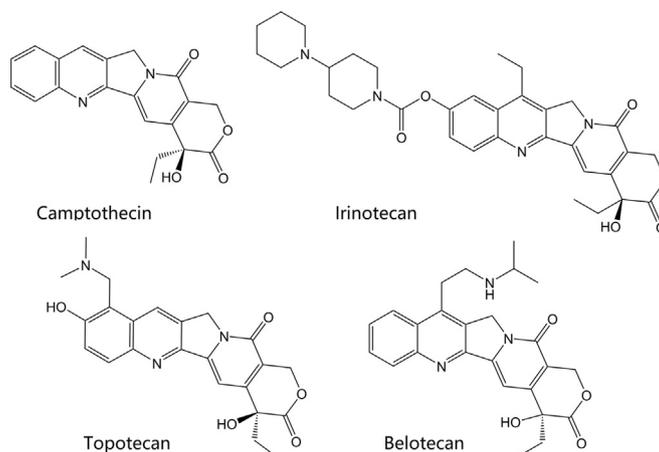
Figure 6   Structures of the camptothecin-derived topoisomerase inhibitors.

Teniposide is approved in patients with refractory childhood acute lymphoblastic leukemia in combination with other chemotherapy drugs.

The sole marketed agent it its chemical class, amsacrine (*m*-AMSA, Fig. 4), is a synthetic agent composed of a planar, acridine ring system. Like the agents discussed above, amsacrine is a topoisomerase poison targeting the type II topoisomerases. Interestingly, amsacrine was the first drug proven to poison eukaryotic topoisomerase II[68]. The acridine ring system is the component of the drug that intercalates DNA and contributes to the activity of the drug, while the non-intercalative 4′-amino-methane-sulfon-*m*-anisidide (*m*-AMSA) headgroup imparts specificity for the DNA-topoisomerase cleavage complex[69]. Amsacrine is approved in Canada to induce remission in adults with acute leukemia resistant to conventional therapy[70].

4.   Topoisomerase inhibitors in clinical trials

This section discusses novel topoisomerase inhibitor compounds that have been investigated in human clinical trials. Table 3 summarizes the trials discussed in this section and several other trials of note that are not discussed in the section. In the table, clinical trials are categorized as phase 1, 2 and 3, and listed with the relevant National Clinical Trial (NCT) identifier, or another regulatory agency identifier where applicable. Clinical trials that are summarized here are those listed in the U.S. National Library of Medicine's Clinical Trials database and the WHO/ICMJE ISRCTN Registry[71,72].

4.1.   Phase 1 clinical trials

The U.S. National Cancer Institute (NCI) has conducted a phase 1 clinical trial for neoplasm lymphoma using agents from a novel class of non-camptothecin type I topoisomerase inhibitors known as indenoisoquinolines (NCT-01794104, Fig. 8). Indenoisoquinolines create a stable DNA-topoisomerase cleavage complex, similar to the camptothecin derivatives, but preferring specific DNA cleavage sites which allows them to gain efficacy against camptothecin-resistant cell lines[73]. These compounds are chemically stable and act upon cells over-expressing ATP-binding cassette transporters ATP-binding cassette (ABC) transporters ABCG2 and P-glycoprotein (MDR1)[74]. Stabilization of the cleavage complex induces DNA damage, demonstrating the efficacy of these inhibitors as potent anticancer therapies. Further, indenoisoquinolines delay DNA repair, which leads to cell death. This study aimed to demonstrate that patients can respond to topoisomerase I inhibitor therapy if their tumor biopsies show topoisomerase I expression. Twenty-one adult patients with refractory solid tumors and lymphomas were enrolled in this study of the indenoisoquinoline, LMP400. LMP400 has linear pharmacokinetics (PK) with drug accumulation after five days of dosing[75]. It is hypothesized that weekly dosing will increase the drug's peak levels and lead to improvements in clinical safety and efficacy[76].

Namitecan (ST1968) is a topoisomerase I inhibitor with superior antitumor activity along with a better safety profile than irinotecan and topotecan[77,78]. PK studies with repeated dosing schedules demonstrated a lack of both metabolite production and accumulation based on its short half-life. Present studies have
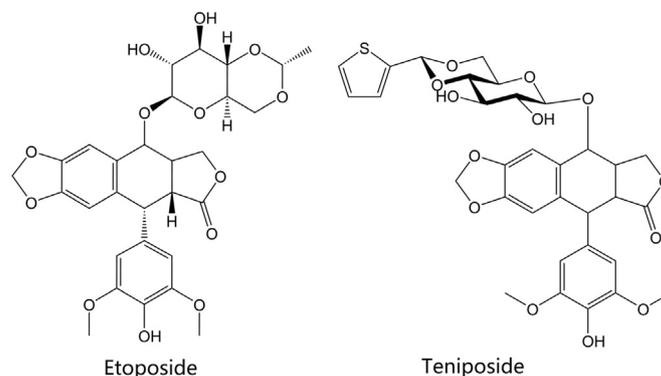
Figure 7 Structures of epipodophyllotoxin-derived topoisomerase inhibitors.

confirmed the safety and PK profile of namitecan, including manageable neutropenia and successful antitumor activity with response in bladder and endometrium cancers[79,80].

4.2. Phase 2 clinical trials

The Vanderbilt-Ingram Cancer Center is currently recruiting patients for a phase II trial study to demonstrate how well vosaroxin and cytarabine work in treating patients with untreated acute myeloid leukemia (NCT-02658487). Vosaroxin is an anti-cancer quinolone derivative (AQD), which affects type II topoisomerases (Fig. 8)[81–83]. AQDs target the DNA-topoisomerase cleavage complex and intercalate DNA at specific GC rich sites to prevent DNA re-ligation by the topoisomerase. This results in site-specific DNA damage and S-phase prolongation along with G2 phase cell cycle arrest, which ultimately triggers apoptosis. Vosaroxin has a stable quinolone core, rendering it less reactive than other classes of topoisomerase inhibitors (see above)[84]. The class produces less toxic metabolites and reactive oxygen species decreasing the potential for off-target organ damage and cardio-toxicity. This quinolone core further allows vosaroxin to evade cellular drug efflux because it is not a substrate for the P-glycoprotein efflux pump[84]. Vosaroxin can also induce p53-independent apoptosis, allowing for it to combat mechanisms of drug resistance associated with the inactivation of p53[85]. Lastly, the stable quinolone structure of vosaroxin is not well metabolized by enzymes including the major p450 isoforms nor does it readily inhibit or induce p450 activity, reducing the potential for drug–drug interactions and even allowing for the potential to enhance anti-cancer drug activity, such as cytarabine[85]. The primary objective of this study is to assess the rate of complete remission after induction therapy using this drug combination in patients with newly diagnosed as well as previously untreated acute myelogenous leukemia. Secondary objectives include the following: frequency of adverse events, evaluation of the presence of minimal residual disease after induction phase(s), determination of the incomplete blood count recover rate after each treatment cycle, determination of the time to neutrophil and platelet recovery following the induction phase(s), assessing disease-free and overall survival after one year following treatment, along with determining the correlation of hematopoietic stem cell transplant comorbidity index and Wheatley index scores with response to disease.

NewLink Genetics Corporation completed a Phase 2 clinical trial to study the impact of CRLX101 on average survival of patients with advanced non-small cell lung cancer (NSCLC) compared to patients receiving best supportive care (NCT-01380769)[86]. CRLX101 is a camptothecin nanoparticle conjugated to a cyclodextrin-based polymer, designed as a targeted therapy regimen to increase the exposure of tumor cells to camptothecin meanwhile minimizing its side effects[87–89]. The cyclodextrin-based polymer improves the water-solubility of camptothecin and is designed to be hydrolyzed *in vivo* after localization to the tumor. Tumor-specific targeting is facilitated by the size of the drug nanoparticle, which has been designed to extravasate from the "leakier" blood vessels found in tumors[90].

4.3. Phase 3 clinical trials

CTI BioPharma has conducted a phase 3 study to compare the efficacy of pixantrone with rituximab to gemcitabine, a nucleoside analog, with rituximab in 260 patients with relapsed or refractory diffuse large B-cell lymphoma or follicular grade 3 lymphoma (NCT-01321541)[91]. The primary outcome of this study was progression free survival (PFS), with the time frame being a randomized time to the date of disease progression or death. Secondary outcome measures were evaluated from randomization to death and included overall survival, complete, and overall response rate, as well as safety evaluation regarding the number of laboratory values falling outside of predetermined ranges and the frequency of adverse events.

Doxorubicin is currently the main treatment for soft tissue sarcomas, but its pro-drug aldoxorubicin is a promising option according to expert opinion[92]. Aldoxorubicin contains a carboxylic hydrazine that covalently binds to albumin in blood to reach the acidic tumor environment, which then dissolves the hydrazone linker to release doxorubicin into the tissue[93]. A phase 3 study sponsored by CytRx involved administering aldoxorubicin on the first day of every 21-day cycle of treatment in patients with soft tissue sarcomas until there was either tumor progression or an unacceptable toxicity occurred (NCT-02049905). The active comparator was the investigator's choice among darcabazine, pazopanib, gemcitabine with docetaxel, doxorubicin, or ifosfamide. Aside from overall survival over 36 months, the safety of aldoxorubicin compared to the investigator's choice will be assessed using the following parameters: frequency and severity of adverse events, abnormal findings during physical examinations, laboratory tests, vital signs, echocardiogram evaluations, electrocardiogram results, disease control rate, and tumor response. Preliminary results from this phase 3 study demonstrated a PFS advantage in patients with leiomyosarcoma and liposarcoma treated with aldoxorubicin[92].

Other topoisomerase inhibitors of note that have entered clinical trials include silatecan, a camptothecin derivative (Fig. 8)[94,95]. This agent represents a unique silicon-containing class of topoisomerase I

Table 3   Summary of clinical trials discussed.

Study	Study purpose	Time frame	Sample size	Outcome measures	Relevant findings	NCT
Phase 1						
Indenoisoquinoline LMP400 for advanced solid tumors and lymphomas	Safety and efficacy of Indenoisoquinoline LMP400 for advanced solid tumors or lymphomas	February 2013–October 2017	21 participants	To establish the safety, tolerability, and PK profiles of weekly LMP400 in patients with refractory solid tumors and lymphomas	LMP776 is overall well tolerated	1794104
A phase I study of indenoisoquinolines LMP400 and LMP776 in adults with relapsed solid tumors and lymphomas	Study how LMP400 and LMP776 are processed by the body and how effective they are in treating difficult-to-treat types of cancer	January 2010–June 2017	55 participants	Define the MTD, dose-limiting toxicities, and PD endpoint (gamma-H2AX in tumor biopsy pre- and post-treatment) of LMP400 and LMP776 administered intravenously daily for 5 days	N/A	1051635
Phase-I dose finding and pharmacokinetic study of the novel hydrophilic camptothecin ST-1968 (namitecan) in patients with solid tumors	First-in-human, dose-escalation study to determine the MTD of intravenous, flat-dosed ST-1968 (namitecan), a new hydrophilic camptothecan derivative	June 2007 – December 2011	62 participants	MTD of ST1968 given intravenously once every week for 2 consecutive weeks every 3 weeks and MTD of ST1968 given intravenously once every 3 weeks for 21 days	Neutropenia was the drug-limiting toxicity, with 15 mg being defined as the recommended dose for one group and 23 mg for the other group. Non-hematological toxicity was negligible. Namitecan exhibited fully dose-proportional PK	1748019
Phase 2 clinical trials						
Vosaroxin and infused cytarabine in treating patients with untreated acute myeloid leukemia (VITAL)	Study how well vosaroxin and cytarabine work in treating patients with untreated acute myeloid leukemia	March 2016-Ongoing (estimated to complete in July 2019)	61 participants	Assess the rate of complete remission after induction therapy with the vosaroxin and standard dose infused cytosine arabinoside for patients with newly diagnosed, previously untreated acute myelogenous leukemia	N/A	2658487
A phase 2 Study of CRLX101 in patients with advanced non-small cell lung cancer	Compare median overall survival of patients with advanced non-small cell lung cancer treated with CRLX101 to patients treated with best supportive care (BSC)	June 2011–October 2014	157 participants	Compare overall survival of patients treated with CRLX101 and BSC to patients treated with BSC only for up to 18 months	No statistical analysis provided for to compare overall survival of patients in both treatment arms	1380769
	Study the efficacy and determine the 6-month progression free survival	December 2009–February 2015	58 participants	Determine the 6-month PFS of AR-67 administered in	N/A	1124539

Study of AR-67 in adult patients with recurrence of glioblastoma multiforme (GBM) or gliosarcoma	(PFS) of AR-67 (7-*t*-butyldimethylsiltyl-10-hydroxy-camptothecin) in patients with GBM			adults with confirmed recurrence of GBM who have not had experienced a recurrence within 90 days after receiving bevacizumab or temazolamide for treatment		
Rebeccamycin analog in treating children with relapsed or refractory neuroblastoma	Study effectiveness of rebeccamycin analog in treating children diagnosed with relapsed or refractory neuroblastoma	January 1999–September 2006	30 participants	Determine the response rate and toxicity to rebeccamycin analogue in children with relapsed or refractory neuroblastoma	N/A	3737
Rebeccamycin analog in treating patients with metastatic or locally recurrent colorectal cancer	Study effectiveness of rebeccamycin analog in treating patients diagnosed with metastatic or locally recurrent colorectal cancer	February 2000–June 2002	37 participants	Determine response rate, toxicity, and overall survival of patients with metastatic or locally recurrent colorectal cancer treated with rebeccamycin analogue	N/A	5085
Rebeccamycin analogue in treating women with stage IIIB or stage IV breast cancer	Compare effectiveness of two different rebeccamycin analogue regimens in treating women diagnosed with stage IIIB or stage IV breast cancer	March 2000–May 2006	42 participants	Assess activity of rebeccamycin analog as therapy for advanced breast cancer administered in two different treatment regimens	N/A	5817
Intravenous edotecarin in patients with advanced gastric cancer that has progressed or recurred after chemotherapy	Study the efficacy of edotecarin in adult patients with advanced gastric cancer, reasonable performance status, good organ function, lack of serious concomitant medical conditions in repeated 3-week cycles of treatment	April 2004–June 2005	28 participants	Assess antitumor activity of edotecarin using repeated radiographic assessments at 6-week intervals	N/A	87503
Phase 3						
Comparison of pixantrone + rituximab with gemcitabine + rituximab in patients with aggressive B-cell non-Hodgkin lymphoma or follicular grade 3 lymphoma who have relapsed after therapy and are not eligible for stem cell transplant (PIX-R)	Evaluate the efficacy of Pixantrone with rituximab compared to Gemcitabine with Rituximab in patients with relapsed or refractory diffuse large B-cell lymphoma or follicular grade 3 lymphoma	April 2011–December 2017	260 participants	Progression free survival from randomization to the date of disease progression or death	N/A	1321541

Table 3 (*continued*)

Study	Study purpose	Time frame	Sample size	Outcome measures	Relevant findings	NCT
Gemcitabine and docetaxel *versus* doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomized controlled phase 3 trial	Compare gemcitabine and docetaxel *versus* doxorubicin as first-line treatment for advanced or metastatic soft-tissue sarcoma	January 2010–January 2013	250 participants	Progression-free survival, assessed using the RECIST Criteria every six weeks, after each set of two cycles; 2-monthly following treatment assessment	The proportion of patients alive and free of cancer progression after 24 weeks did not differ between the treatment arms. The most common adverse effects were neutropenia and febrile neutropenia in approximately the same percentage of patients who received either treatment arm. Percentage of patients who died during or after this study was also similar for both treatment arms; none of the deaths were related to the treatment. The study concludes that there is significant evidence for clinicians to consider doxorubicin as a single agent in patients with locally advanced or metastatic soft-tissue sarcoma	07742377 in ISRCTN registry
Phase 3 study to treat patients with soft tissue sarcomas	Determine the efficacy and safety of aldoxorubicin in subjects with metastatic, locally advanced, or unresectable soft tissue sarcomas	January 2014–May 2017	433 participants	Progression-free survival over 24 months	Aldoxorubicin has minimal cardiac toxicity and survival advantage in patients with leiomyosarcoma and liposarcoma	2049905
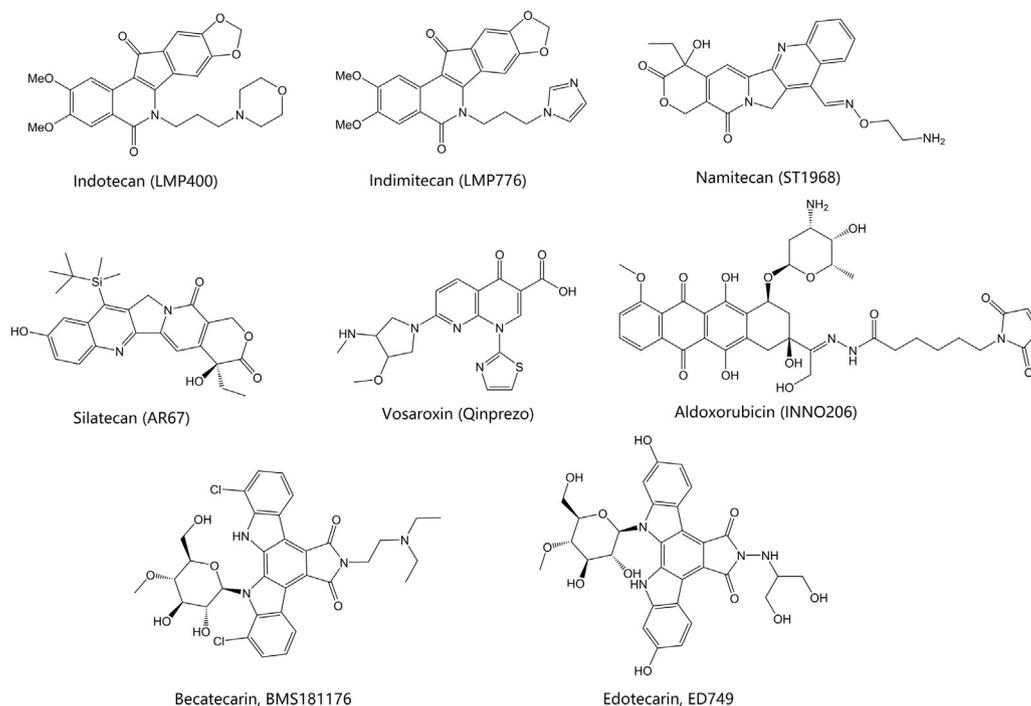
Figure 8   Representative topoisomerase inhibitors in clinical trials for cancer.

Table 4   Summary of pre-clinical studies reported.

Citation	Compound(s) tested	Enzyme activity (µmol/L)[a]	Cell activity (µmol/L)[a]
Kwon et al.[99]	Benzo-furo-pyridine (Fig. 9, series A, $n=0$)	Topo I—65.2 (IC_{50}) Topo IIα—13.4 (IC_{50})	HEK293—4.93 (IC_{50}) DU145—1.90 (IC_{50}) HCT15—0.15 (IC_{50}) T47D—0.83 (IC_{50})
Kwon et al.[99]	Chromeno-pyridine (Fig. 9, series A, $n=1$)	Topo I—not reported Topo IIα—60.2 (IC_{50})	HEK293—5.69 (IC_{50}) DU145—1.43 (IC_{50}) HCT15—0.005µM (IC_{50}) T47D—0.54 (IC_{50})
Shrestha et al.[100]	Benzo-furo-pyridine (Fig. 9, series B, compound I)	Topo I—22.4% inhibition (100) Topo IIα—100% inhibition (100)	HCT15—1.22 (IC_{50}) T47D—0.59 (IC_{50}) HeLA—0.86 (IC_{50})
Khadka et al.[101]	1,3-diarylisoquinoline[b] (Fig. 9, series C, compound II)	Topo I—1.22x camptothecin (100) Topo IIα—0.06x camptothecin (100)	MCF10A—5.74 (IC_{50}) T47D—0.74 (IC_{50}) HeLA—5.06 (IC_{50}) HCT15—2.77 (IC_{50})
Wang et al.[102]	Thio-evodiamine (Fig. 9, series D, compound III)	Topo I—gel assay results shown only[c] Topo IIα—gel assay results shown only[c]	A549—0.02 (IC_{50}) MDA-MB-435—<0.003 (IC_{50}) HCT116—<0.003 (IC_{50})

[a]Activity is reported for the most active compound if a series of analogs was reported.
[b]Topoisomerase inhibition relative to camptothecin activity was reported for 1,3-diarylisoquinolone compounds.
[c]Gel assay results at 100 indicate inhibition of both Topo I and Topo IIα.

inhibitors that have progressed into clinical trials. A phase 2 study of this compound for use in gliosarcoma has been registered by Arno Therapeutics, but no results have been posted to date (NCT-01124539). Rebeccamycin analogs have similarly progressed to phase 2 clinical trials (Fig. 8)[96–98]. Derived from the natural product

rebeccamycin, these compounds possess an indolocarbazole ring system with an attached sugar moiety. The synthetic compounds becatecarin and edotecarin are two examples that have progressed to Phase II trials (Table 3). Rebeccamycin analogs have shown activity as dual topoisomerase I and topoisomerase II poisons. Clinical

development of both becatecarin and edotecarin appears to have ceased and it remains to be seen whether any additional rebecca-mycin compounds will be tested clinically.

5. Pre-clinical topoisomerase inhibitors under investigation

Several interesting new chemical classes of topoisomerase inhibitor are currently under pre-clinical investigation (summarized in Table 4[99–102]). In this section, we review some of the more notable examples and discuss their chemistry, mechanism, activity, and selectivity. Discussion is focused on agents that have been reported in the literature within the last 5 years, with an emphasis on agents that have shown favorable results in animal models, but that have not yet progressed to clinical studies.

A recent study by Kwon and coworkers[99] investigated a novel terpyridine scaffold for inhibitory activity against topoisomerase I and II. An α-terpyridine molecule served as the starting point for the development of these compounds (Fig. 9). Twenty-nine compounds were synthesized and tested for inhibition of eukaryotic topoisomerase I and II. Two compounds with unique scaffolds were identified as potent, non-intercalative catalytic inhibitors. The benzo[4,5]-furo[3,2-b]-pyridine compound (Fig. 9, Series A, $n=0$) was identified as a dual topoisomerase I and II catalytic inhibitor, while the chromeno[4,3-b]-pyridine compound (Fig. 9, Series A, $n=1$) was identified as a specific topoisomerase IIα inhibitor. Both compounds showed potent anticancer activity against MCF7, T47D, and HCT15 cells and induced apoptosis and G1 arrest in T47D human breast cancer cells. Results from xenograft mouse tumor growth model tests showed that the benzofuro-pyridine compound significantly reduced both volume and weight of the tumors, but the benzochromeno-pyridine compound, while showing a clear reduction, did not demonstrate a statistically significant difference. DNA toxicity, measured by the accumulation of DNA strand breaks, compared to etoposide in MCF7 and T47D cell lines was also significantly less for both compounds.

Expanding on their work discussed above, Shrestha and coworkers[100] recently published the synthesis and testing of a 2nd generation of benzofuro-pyridine compounds with activity against type II topoisomerases (topoisomerase IIα). Sixteen compounds based on the benzofuro[3,2-b]pyridin-7-ol scaffold (Fig. 9, Series B) were synthesized and tested for topoisomerase I and IIα inhibition as well as antiproliferative activity using several cancer cell lines. Activity testing against topoisomerase IIβ was not reported. The cancer cell lines used for the testing were HCT15 (colorectal adenocarcinoma cell line), T47D (ductal breast cancer cell line), and HeLa (cervix tumor cell line). Camptothecin, etoposide, and doxorubicin were used as the controls. Hydroxyl ring substitutions at the R-position were investigated and compounds with *ortho* and *para* hydroxyl groups showed greater topoisomerase IIα inhibition than etoposide at both concentrations tested against all cell lines. The meta hydroxyl substituted analog (Fig. 9, compound I) showed the greatest selectivity and most potent inhibitory activity against topoisomerase IIα in the HeLa cell line[100]. All ring-substituted analogs, and the unsubstituted analog showed potent topoisomerase IIα inhibition and strong antiproliferative activity against the HCT15 and T47D cell lines.

The diarylisoquinolone scaffold presents another novel chemical scaffold currently under investigation for topoisomerase

inhibitory potential. Expanding previous efforts in the investigation of 3-arylisoquinolones and 3,4-diarylisoquinolines (Fig. 9, Series C), Khadka and coworkers[101,103] have recently reported the synthesis and evaluation of 1,3-diarylisoquinoline compounds. The C_4-aromatic ring of 3,4-diarylisoquinolone was shifted to design 1,3-diarylisoquinoline and various small substituents on both rings were investigated. Overall, 22 compounds were synthesized, tested for activity, and compared with camptothecin and etoposide as controls for topoisomerase I and topoisomerase IIα inhibition. The compounds were also compared to doxorubicin for cytotoxic activity against non-cancerous cell lines. Though the preliminary cytotoxic profile of the 1,3-diarylisoquinolines showed concerning cytotoxic activity against non-cancerous cell lines, the IC_{50} values for the lines tested were typically at or above those shown for doxorubicin. Compound II (Fig. 9) was shown to be the most potent inhibitor of topoisomerase I and, importantly, demonstrated non-intercalative, catalytic inhibitory activity. The authors did not report testing against topoisomerase IIβ and selectivity for IIα over IIβ is unknown. The novel mechanism of action and reported activity of this new chemical class shows significant promise.

Natural compounds have also been investigated for their potential inhibitory activity against the DNA topoisomerases[104]. The quinazoline-carboline alkaloid, evodiamine (Fig. 9, series D), is a naturally occurring compound that is used as a dietary supplement for many potential benefits. It is also been reported to be a catalytic inhibitor of both eukaryotic topoisomerase I and II[105–107]. Evodiamine has been reported to exhibit anticancer activity in cancer cells resistant to camptothecin[107]. A recent study by Wang and coworkers[102] tested the topoisomerase inhibitor activity of compounds derived by modification of the evodiamine scaffold. These researchers reported the synthesis and testing of 11 compounds against topoisomerase I and II, and tubulin. 3-Chloro-10-hydroxyl thio-evodiamine (Fig. 9, compound III) was found to be a moderate inhibitor of topoisomerase I and II and a potent inhibitor of tubulin and was reported by the investigators to be a first-in-class triple inhibitor. The lead compounds in this study, including compound III, were tested *in vivo* using a mouse tumor model and the tumor growth inhibition rate was favorable (up to 48% growth inhibition). The toxicity of the compounds was compared to topotecan in the *in vivo* studies and were found to be lower, based upon a measure of body weight loss. The authors concluded that thio-evodiamine derivatives were a promising new class of topoisomerase/tubulin inhibitors. Notwithstanding the favorable results discussed above, another recent study by Christodoulou and coworkers[108] investigating the *S*-enantiomer of evodiamine, and derivatives thereof, showed that the compounds they synthesized and tested were unable to affect the catalytic activity of topoisomerase I. These researchers ultimately showed that their compounds possessed selective inhibitory activity against the sirtuin, SIRT2. The studies discussed here provide justification for the further investigation of evodiamine and derivatives as potential topoisomerase inhibitors with anti-cancer potential.

Other notable reports in the recent literature of novel topoisomerase inhibitors include the chalcone-linked carbazole derivatives reported by Li and coworkers[109] (Fig. 10). These compounds are reported to possess topoisomerase II inhibitory activity with a non-
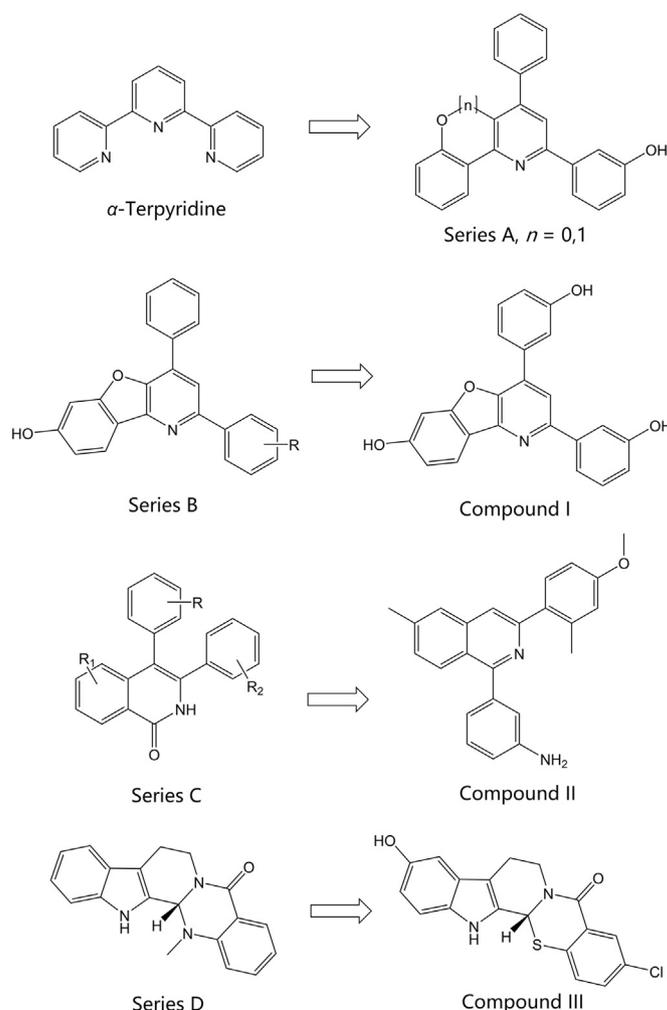
Figure 9 Preclinical topoisomerase inhibitors in recent literature.

intercalative catalytic inhibitory mechanism. Sathish and coworkers[110] have reported the synthesis of a series of podophyllotoxins linked to beta-carbolines with demonstrated anticancer activity against the A549 (lung cancer), DU-145 (prostate cancer), MDAMB-231 (breast cancer), HT-29 (colon cancer), and HeLa (cervical cancer) cell lines. Their investigations to date suggest a topoisomerase II inhibitory mechanism. Jadomycins, an interesting class of natural products, have recently been reported by Hall and coworkers[111] to inhibit topoisomerase IIα. Several jadomycin analogs were tested and showed activity against a drug-resistant breast cancer cell line. Mechanistic studies showed that several of the compounds acted as selective topoisomerase IIβ poisons, a concern from a cardiotoxicity standpoint, but that jadomycin S did not act through a poisoning mechanism. Finally, the polyphenol compound, resveratrol, derived from plant sources and red wine, has recently been reported to possess topoisomerase II inhibitory activity[43]. The chemical similarity of resveratrol to ICRF-187 (dexrazoxane), a known catalytic inhibitor of topoisomerase II, is notable (Fig. 10). Lee and coworkers reported[43], based upon a series of biochemical assays, the catalytic inhibitory activity of resveratrol against topoisomerase II *via* a mechanism related to ICRF-187, though resveratrol appears to prevent ATPase domain dimerization rather than stabilize it, as is seen with the latter compound.

6. Concluding remarks

The DNA topoisomerases continue to represent highly relevant targets for the treatment of various types of cancer and are under continuous investigation as targets for novel classes of inhibitory agents. While nearly all clinically marketed topoisomerase inhibitors used to treat cancer fall within the topoisomerase poison mechanistic category, several very promising catalytic inhibitors have been disclosed recently and offer promise as potentially novel anti-cancer agents with significantly reduced toxicity profiles. Of note are the novel chemical classes with activity against type I topoisomerases, as there is currently only one marketed class of inhibitors in clinical use. This review has presented and discussed several notable examples of novel topoisomerase inhibitors under investigation for cancer indications, both in clinical trials and in pre-clinical studies.
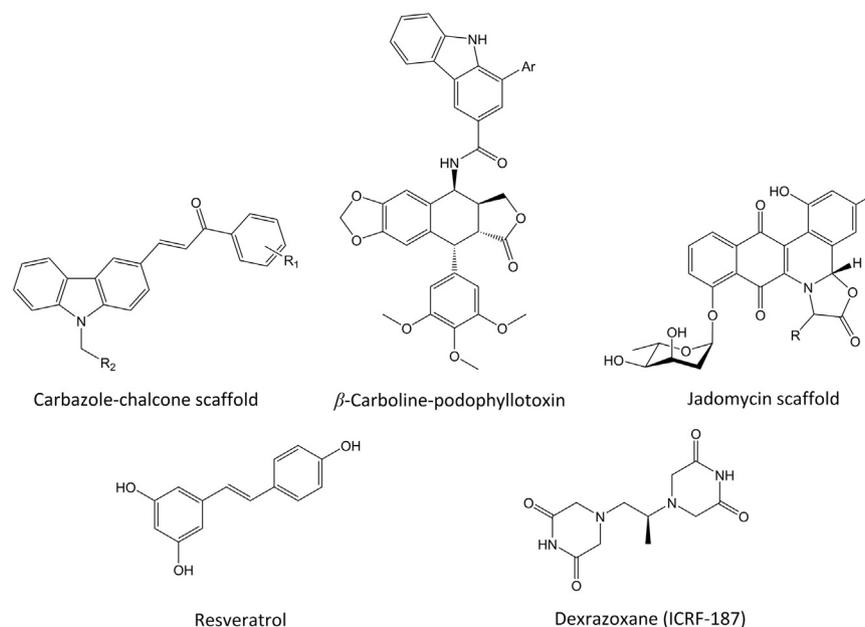
Figure 10 Additional topoisomerase-active agents in recent literature.

Acknowledgments

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