Current Topics in Pharmacology
Vol. 17, *1*, 2013

Review

Covalent poisons of topoisomerase II

Elizabeth G. Gibson[1] and Joseph E. Deweese[1,2,*]

[1]Department of Pharmaceutical Sciences, Lipscomb University College of Pharmacy and Health Sciences, One University Park Drive, Nashville, Tennessee 37204-3951, [2]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, USA

ABSTRACT

DNA topoisomerase II is an essential enzyme that regulates DNA topology to facilitate critical cellular processes such as transcription, replication and cell division. Topoisomerase II creates double-stranded DNA breaks and passes an intact double helix through the break in order to alleviate supercoiling and untangle interlinked DNA helices. Topoisomerase II accomplishes this by cleaving the DNA four base pairs apart on opposite strands generating a transient, enzyme-linked double-stranded break. These transient enzyme-DNA complexes, termed cleavage complexes, pose a threat to genomic integrity with the possibility of permanent double- and single-stranded breaks. A number of compounds target topoisomerase II and take advantage of this cellular threat for the treatment of cancer and bacterial infections. Unfortunately, some of these agents lead to translocations, which have been associated with the development of specific types of leukemias. Most anticancer agents use a more traditional interfacial mechanism to physically block ligation of the cleaved DNA. Accumulation of cleavage complexes presents a physical obstruction on the genetic material, which can lead to permanent strand breaks when DNA tracking systems collide with the cleavage complexes. However, a growing class of compounds causes an increase in DNA cleavage through a covalent mechanism. Formerly referred to as redox poisons, these covalent poisons share many common characteristics including covalent adduction to the enzyme. These compounds come from a number of sources including dietary and plant-derived, industrial chemicals, and drug metabolites. In this review, we will focus on these covalent poisons of topoisomerase II.

KEYWORDS: topoisomerase II, cancer, covalent poisons, interfacial poisons, DNA damage, translocations

INTRODUCTION

The genetic information in cells is encoded within the DNA molecule in a linear array of bases along two complementary, antiparallel strands wound around one another. This double-helical structure of DNA provides for compaction and storage while posing significant challenges for accessing and utilizing this information [1, 2].

Processes such as replication, transcription, and repair often require separating the individual strands of the double-helix [2, 3]. While the genomes of most organisms are slightly negatively supercoiled (underwound), replication and transcription cause positive supercoiling (overwinding), which must be relieved in order for these processes to continue [2-4]. Due to the extreme length of linear chromosomes or the circular nature of other chromosomes, these forms of strain cannot be resolved without assistance. Further, replication causes catenation (interlinking) of the sister chromatids that must be resolved prior

*Corresponding author: joe.deweese@lipscomb.edu

to mitosis [4]. All of these forms of strain can be resolved by altering the topological state of DNA [3, 5]. Alteration of DNA topology requires the breaking of one or both strands of the DNA [3, 5].

In order to overcome these challenges, cells employ enzymes known as DNA topoisomerases [2, 3, 5-7]. These essential enzymes resolve topological strain in DNA using transient single-stranded (type I) or double-stranded (type II) breaks. These enzymes act as "molecular scissors" by generating strand breaks in order to remove positive supercoils, knots, and catenanes. Topoisomerases form transient, covalent intermediates, known as cleavage complexes, with the DNA [3, 5-8]. This deliberate strand break mechanism comes at a significant risk to genomic integrity, as will be discussed below [8]. Since DNA topoisomerases are part of essential cellular processes, several widely-used anticancer and antibacterial agents target these ubiquitous enzymes in order to hijack this critical function to the detriment of the cell [8, 9]. This review will focus on a specific class of compounds that target mammalian type II topoisomerases.

DNA topoisomerases

DNA topoisomerases are divided into two categories based upon mechanism. Type I topoisomerases are monomeric enzymes that generate transient single-stranded DNA breaks, which allows these enzymes to relieve torsional strain from overwinding due to transcription and replication [10]. Type I enzymes are generally named using odd numbers (e.g., topoisomerase I and topoisomerase III) and broken into subtypes based upon differences in structure and mechanism.

Type II topoisomerases are homodimeric (eukaryotic) or heterotetrameric (prokaryotic) enzymes that form transient double-stranded DNA breaks [11]. Thus, these enzymes can relieve torsional strain while also decatenating and unknotting DNA. Type II enzymes are generally named using even numbers (e.g., topoisomerase II and topoisomerase IV) and are grouped into subtypes based upon structural and mechanistic distinctions (i.e., type IIA and type IIB).

Both type I and type II enzymes utilize active site tyrosine residues that participate in the transesterification reaction [10, 11]. Organisms typically express one or more forms of both types of topoisomerases either as isoforms or splicing variants [5, 11]. Type II enzymes can be broken into subclasses of type IIA and IIB topoisomerases. However, type IIB enzymes are found in archaeal organisms and some plants and will not be discussed in this review [11]. Since the current focus is on agents that target the type II enzyme, we will review the catalytic mechanism of type IIA topoisomerases and examine the specific isoforms briefly.

Type IIA topoisomerases are symmetrical enzymes that share functional groups between subunits. The mirror-image active sites share residues from both halves of the enzyme and cleave DNA four base-pairs apart on opposite strands using a two-metal-ion mechanism [12]. The enzyme also undergoes significant conformational changes to move the cleaved DNA ends apart, pass an intact strand through the break, bring the cleaved ends back together, and release the strands of DNA [13]. These movements are powered by ATP binding and hydrolysis at the two ATPase domains found on the enzyme [13].

The catalytic cycle of topoisomerase II involves the following steps (Figure 1). *Step 1*: the enzyme binds two segments of DNA ("Gate" or "G-segment" and "Transport" or "T-segment") [14-16]. In the presence of Mg^{2+} ions in the active site, the enzyme determines the malleability of the DNA segment, which is a factor in cleavage site selection [17]. *Step 2*: the enzyme uses a two-metal-ion mechanism and the active site tyrosine residues to form an enzyme-bound double-stranded DNA break in the G-segment with a four base-pair 5' overhang [12, 14, 18-23]. The binding of two molecules of ATP induces conformational changes that close the N-terminal domain around the T-segment [13, 24-27]. *Step 3*: following DNA cleavage, conformational changes in the enzyme open the G-segment and pass the T-segment through the G-segment [13, 16, 25, 26, 28-30]. Strand passage appears to occur more efficiently after the hydrolysis of one of the two ATP molecules, though hydrolysis may not be required [24, 25, 31]. *Step 4*: the enzyme ligates the G-segment and the ATPase domains rotate around one another preventing the T-segment

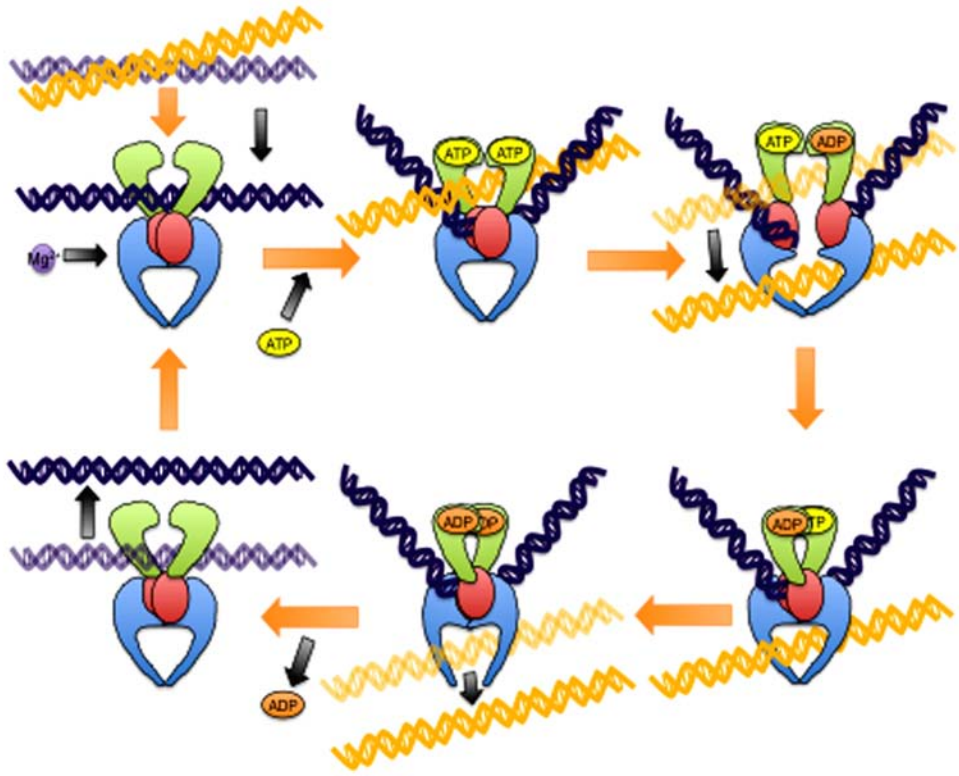

Figure 1. DNA topoisomerase II homodimer is modeled in blue (C-terminal gate), red (TOPRIM domain), and green (N-terminal gate and ATPase domain). Two DNA double helices are in purple (G-segment) and gold (T-segment). Magnesium ions (purple) are required for DNA bending and cleavage. ATP molecules (yellow) and ADP molecules (orange) are included showing approximate binding, hydrolysis, and release. Movement of DNA is depicted by faded DNA segments and arrows.

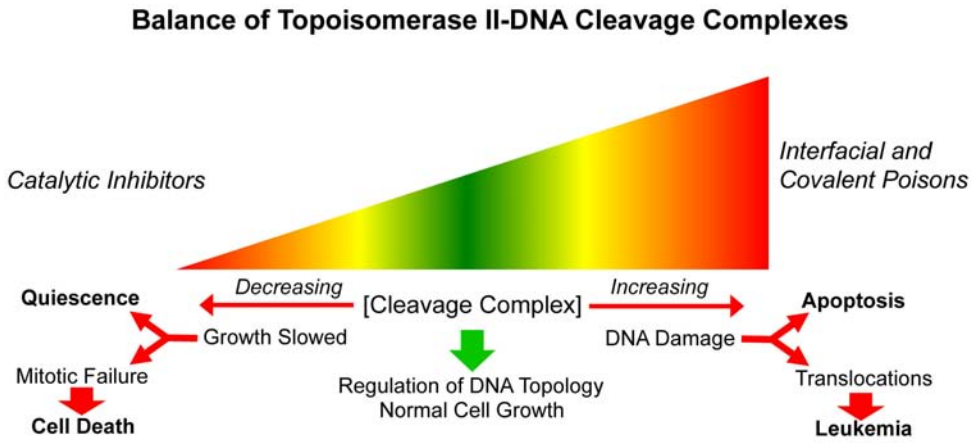

Figure 2. Topoisomerase II DNA cleavage complexes must be maintained at a sufficient level (green) to support the regulation of DNA topology and normal cell growth. When cleavage complex concentration decreases, growth is slowed resulting in quiescence or mitotic failure (resulting in cell death). Catalytic inhibitors decrease cleavage complex concentration. An increase in cleavage complex concentration leads to DNA damage which may lead to apoptosis or repair of damage. In some cases, repair of damage leads to translocations associated with the development of leukemia. Interfacial and covalent poisons increase cleavage complex concentration.

from returning back through the G-segment [13, 32-34]. *Step 5*: the enzyme releases the T-segment and the second molecule of ATP is hydrolyzed [24, 25, 31]. *Step 6*: the enzyme releases the ADP molecules and the G-segment and resets for another round of catalysis.

Vertebrate species have two isoforms of type II topoisomerases known as topoisomerase IIα and IIβ [11, 35-41]. These orthologous enzymes are expressed from different chromosomes [11, 35-41]. Topoisomerase IIα expression increases in response to cell growth and peaks during mitosis [42-45]. In contrast, topoisomerase IIβ expression does not appear to fluctuate during the cell cycle [46-48]. Topoisomerase IIβ cannot fully compensate for a loss of topoisomerase IIα [45, 47, 49]. Topoisomerase IIβ knockout results in neural development abnormalities and death shortly after birth [48, 50, 51]. Evidence indicates that topoisomerase IIα decatenates chromosomes during and after replication [45, 48]. Topoisomerase IIβ is involved in relieving topological strain as a result of transcription [52-54].

The covalent enzyme-DNA intermediate, known as the cleavage complex, preserves the bond energy of the DNA backbone while also preventing dissociation of the DNA. While this transient cleavage complex is critical to the survival of the cell, it also poses a significant threat to the genome [5, 7, 8, 55-57]. As depicted in Figure 2, cells maintain a sufficient level of topoisomerase activity to control DNA topology and allow for normal cell growth and division [5, 7, 8, 55-57]. If this activity level drops too low (reducing the concentration of cleavage complexes), cells will become quiescent and/or die as a result of mitotic failure [5, 7, 55-57]. On the other hand, a rise in cleavage complex concentration may result from an increase in DNA cleavage or an inhibition of DNA ligation. If replication or transcription machinery collides with these cleavage complexes, permanent single- or double-stranded breaks will result and must be repaired by the cell [5, 7, 8, 55-57]. These breaks may overwhelm the cell and cause cell death [5, 7, 8, 55-57]. This is the premise behind modern chemotherapeutic targeting of topoisomerases to treat cancer and bacterial infections [9, 58, 59].

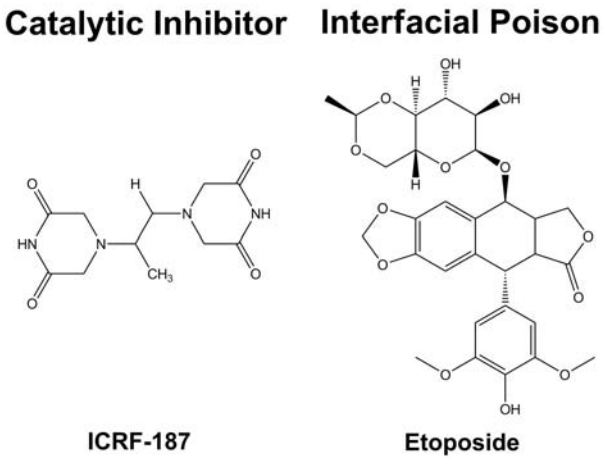

Figure 3. DNA topoisomerase II can be targeted by catalytic inhibitors or interfacial poisons. ICRF-187 inhibits catalytic activity of topoisomerase II while etoposide inhibits ligation of DNA cleaved by topoisomerase II.

Further, it should also be noted that if the DNA breaks are not sufficient to kill the cell, cells may attempt to repair the damage and survive. In some cases, these repairs result in translocations that are associated with secondary leukemias [9, 58-61]. Several therapy-associated translocations have been linked to drugs targeting topoisomerases [60, 61]. For these reasons, it is critical to understand the mechanism of compounds that impact topoisomerase II activity in order to determine the potential for therapeutic value and the risk of adverse events.

Therapeutic targeting

Compounds that impact topoisomerase II are divided into three different classes based upon mechanism of action: catalytic inhibitors, interfacial poisons, and covalent poisons. First, catalytic inhibitors are compounds that block catalytic activity and generally do not lead to accumulation of strand breaks. These agents often impact the function of the ATPase domain and thereby abrogate the catalytic activity of topoisomerase II. For example, ICRF-187 (Figure 3) binds to and stabilizes the dimer interface between the ATPase domains of the protomers [26, 27]. As a result, ICRF-187 induces a closed-clamp conformation of topoisomerase II and blocks enzyme turnover [26, 27].

Second, interfacial topoisomerase II poisons are compounds that block the ability of the enzyme to ligate DNA once it is cleaved [8]. Structural and biochemical evidence indicates that interfacial poisons act at the enzyme-DNA interface and physically block the enzyme from ligating the cleaved DNA ends [33, 62-66]. The cellular consequence is the stabilization and accumulation of cleaved DNA complexes. These stabilized complexes serve as "road blocks" to DNA tracking systems and result in single- and double-stranded breaks, which overwhelm the cell with damage [8, 59, 67]. This is the most common mechanism for chemotherapeutic agents targeting topoisomerase II. For example, etoposide (Figure 3) is a widely-used anticancer agent that poisons topoisomerase II using the interfacial mechanism [33, 62-66].

Third, the interfacial poisons are distinct from another class of compounds known as covalent poisons [68]. Formerly referred to as redox-dependent poisons, these compounds act on topoisomerase II by forming a covalent adduct to the enzyme and in some cases crosslink enzyme protomers [68-73]. Rather than inhibiting ligation, the covalent adduction event abrogates enzyme activity and generally promotes the stabilization of high levels of DNA cleavage [70, 74-78]. As discussed below, several covalent poisons have been implicated in contributing to the development of leukemia while others are potentially chemopreventative. Thus, topoisomerase II poisons can serve as both a cure and a cause of cancer [8, 59]. These compounds will be examined further in the next section.

Covalent poisons

Covalent poisons of topoisomerase II were previously described as redox-dependent topoisomerase II poisons due to the fact that some of these agents underwent redox cycling prior to impacting enzyme function [69, 70]. However, covalent adduction is common to these compounds regardless of redox cycling [69, 72, 76]. Covalent poisons trap the enzyme and in some cases, crosslink enzyme protomers. This action may also prevent the release of DNA if the DNA is already bound. This abrogation of function can be tested using an inactivation assay where the enzyme is incubated with the compound prior to adding DNA [70, 78]. Further, covalent adduction has also been observed in other ways including mass spectrometry and electrophoretic mobility shift assays [72, 74]. The consequences of covalent poisons of topoisomerase II appear to be covalently stabilized cleavage complexes with high levels of DNA breaks.

Covalent poisons of topoisomerase II come from many different sources including dietary and plant derived (Figure 4), industrial chemicals and byproducts (Figure 5), and drug metabolites (Figure 6). Compounds in each of these classes will be discussed below.

Dietary and plant derived

The structural isomers, α and β lapachone (Figure 4) are pyranonapthoquinones derived from Elutherin found in the bulb of the flowering plant *Eleutherine Americana* [71, 79]. These compounds have been studied in cells and *in vitro*. Results appear to demonstrate that these compounds can "inactivate" the enzyme and block further catalytic activity [71, 79]. Based upon high levels of stable cleavage complexes isolated in cells, these compounds do not appear to be catalytic inhibitors [79]. There is also evidence that these compounds undergo redox cycling before inducing irreversible cleavage complexes with topoisomerase II [79].

The thiol-reactive quinone 2-methyl-1,4-napthoquinone, known as menadione (Figure 4), is a metabolite of vitamin K (K_3). Menadione stimulates topoisomerase II-mediated DNA cleavage *in vitro* that is not readily reversible by shifts in temperature [69]. As with other covalent poisons, menadione inactivates DNA cleavage when incubated with the enzyme prior to addition of DNA [69]. Interestingly, stimulation of DNA cleavage in the presence of thiol-reactive quinones appears to be dependent upon the presence of thiol groups in the enzyme, which is consistent with a covalent adduction mechanism [69].

Curcumin (diferuloylmethane, Figure 4) is the major active component of the spice turmeric isolated from the root of *Curcuma longa L*. [80]. This compound has been used in traditional Asian medicine for many years and is recognized to have cancer chemopreventative properties, which are the focus of ongoing clinical trials. Evidence from cellular studies indicates that this compound

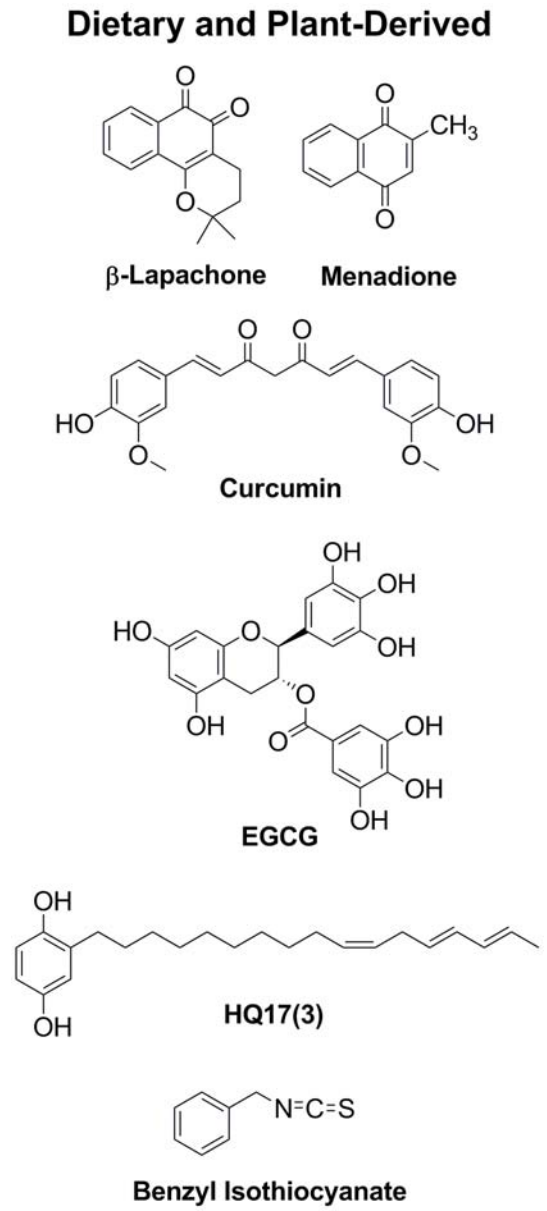

Figure 4. Dietary and plant-derived covalent poisons of topoisomerase II. EGCG = (-)-epigallocatechin gallate. HQ17(3) = 10'(Z),13'(E),15'(E)-heptadecatrienylhydroquinone.

induces cleavage complexes and is sensitive to the presence of reducing agents [81]. *In vitro* assays support these results showing that the oxidation of curcumin using potassium ferricyanite yields a highly active poison of topoisomerase II, which enhances DNA cleavage levels [80]. Co-incubation of topoisomerase II and curcumin in the presence of the oxidizing agent prior to DNA leads to

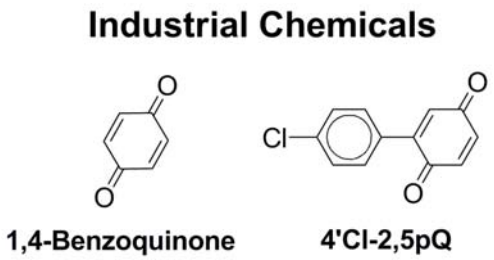

Figure 5. Industrial chemicals that are covalent poisons of topoisomerase II. 1,4-benzoquinone is a metabolite of benzene. 4'Cl-2,5pQ = 2-(4-chloro-phenyl)-[1,4]benzoquinone, a polychlorinated biphenyl.

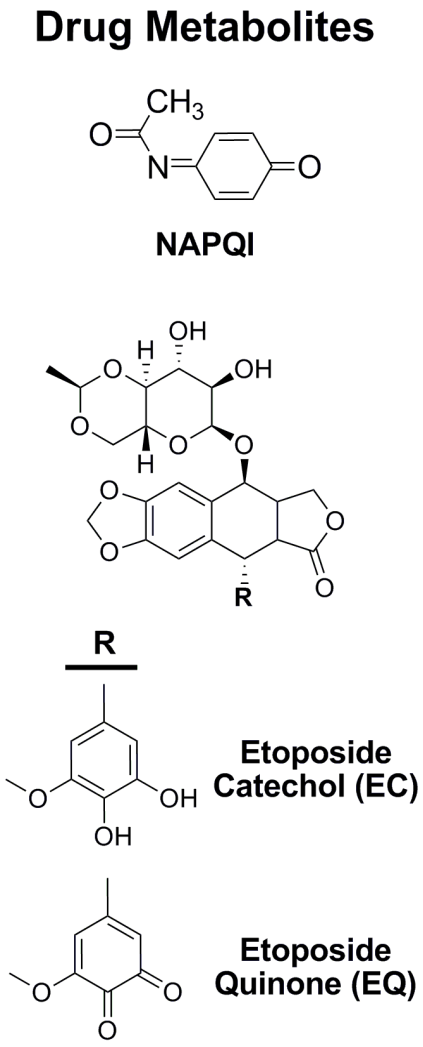

Figure 6. Drug Metabolites that are covalent poisons of topoisomerase II. NAPQI: N-acetyl-p-benzoquinone imine, metabolite of acetaminophen. Etoposide catechol and quinone are metabolites of etoposide.

inactivation of DNA cleavage [80]. Oxidation of curcumin causes the formation of several reactive intermediates [80]. While these intermediates have not all been isolated for analysis, these reactive compounds are likely responsible for the effects on topoisomerase II since the stable bicyclopentadione end-product does not enhance DNA cleavage on its own [80].

The most bioactive polyphenolic component of green tea is (–)-epigallocatechin gallate (EGCG, Figure 4). Most of the health effects of green tea are ascribed to EGCG. Experimental results indicate a number of effects on cellular pathways including topoisomerase II-mediated DNA cleavage [82]. EGCG enhances enzyme-mediated DNA cleavage and also displays the ability to inactivate the enzyme when co-incubated with the enzyme prior to DNA [82]. These effects are redox-dependent as shown by experiments involving reducing agents and order-of-addition [82]. Epimerization of this compound during brewing yields (–)-gallocatechin gallate (GCG), which displays the same abilities to covalently poison topoisomerase II [83]. The related compound (–)-epigallocatechin (EGC) and its epimer gallocatechin (GC) were also found to poison topoisomerase II in a redox-dependent manner consistent with other covalent poisons [83, 84]. In related work, the flavanol myricetin also displayed properties of a covalent poison [84].

A number of cytotoxic alkyl hydroquinones have been isolated from the sap of the *Rhus succedanea L.* tree including 10'(Z),13'(E),15'(E)-heptadecatrienylhydroquinone [HQ17(3), Figure 4] [85]. Evidence from cellular and *in vitro* work indicates that HQ17(3) inhibits topoisomerase II-mediated DNA relaxation and increases enzyme-mediated DNA cleavage [85]. As with other covalent poisons, HQ17(3) inactivates topoisomerase II when added prior to DNA and is sensitive to the presence of reducing agents [85]. Recent evidence demonstrates that this hydroquinone reacts with Cys residues on topoisomerase IIα including Cys-427, -733, and -997, further supporting a covalent mechanism of action [86].

Isothiocyanates (ITCs) are cruciferous vegetable-derived thiol-reactive compounds [87-89]. Evidence from animal and cellular studies indicates a role for ITCs in cancer prevention [87-89]. ITCs induce apoptosis in transformed or initiated cancer cells [89]. Cellular sensitivity to ITCs is reduced when topoisomerase IIα is knocked down [89]. Further, three ITCs, benzyl-ITC (Figure 4), phenethyl-ITC, and sulforaphane (4-methyl-sulfinylbutyl-ITC), have the ability to induce topoisomerase II-mediated DNA cleavage complex accumulation *in vitro* [89]. Cleavage complex formation is reduced in the presence of glutathione [89]. These effects are likely mediated by covalent interactions with Cys residues of topoisomerase II. Proteomic analysis demonstrated an interaction between benzyl-ITC and 10 of 13 Cys residues on topoisomerase IIα [89]. Of these residues, Cys-300 was the most reactive followed by several other highly-reactive positions (Cys-104, -170, -392, -455, and -733) [89]. Given this evidence, ITCs use a covalent modification mechanism to poison topoisomerase II.

Industrial chemicals and byproducts

Benzene is a prevalent industrial production chemical used to manufacture a wide variety of common products including synthetic fibers and plastic [90]. This chemical is also found in crude oil and cigarette smoke and has been linked to the formation of human hematopoietic cancers, specifically acute myelogenous leukemia (AML) and acute non-lymphocytic leukemia [91].

Carcinogenic effects of benzene are attributed to several toxic oxidative metabolites [92-94]. Of these, 1,4-benzoquinone (Figure 5) and 1,4-hydroquinone are known covalent poisons of topoisomerase II [70, 77, 78]. These compounds both increase topoisomerase II-mediated DNA cleavage *in vitro*, inactivate the enzyme when added prior to DNA, and cause cleavage complex accumulation in cultured cells [70, 77, 78]. DNA cleavage levels are decreased in the presence of dithiothreitol (DTT) or glutathione indicating these compounds are sensitive to reducing agents. Proteomic and mutational analysis provide evidence that benzene metabolites can adduct to Cys residues on topoisomerase IIα including Cys-170, Cys-392, Cys-405, and Cys-455 [72].

Polychlorinated biphenyls (PCBs) have been banned for more than 30 years in most countries, but these highly stable industrial chemicals remain

in the environment and pose significant health hazards [95, 96]. PCB exposure is associated with human health problems including, but not limited to, neurotoxicity and hepatotoxicity. Evidence also indicates severe genotoxic effects and chromosomal abnormalities resulting from exposure to PCBs [95-98]. This class of compounds has a pair of connected benzene rings with one or more chlorine atoms attached to the rings. Due to redox cycling, reactive PCB metabolites are generated that are known to form protein adducts [99-103]. Several PCB metabolites, including 2-(4-chloro-phenyl)-[1,4]benzoquinone (4'Cl-2,5pQ, see Figure 5), display the ability to poison topoisomerase II and increase DNA cleavage levels [74]. These compounds inhibit ligation, inactivate the enzyme when added prior to DNA, and block the N-terminal gate of topoisomerase II [74]. The ability to covalently poison topoisomerase II may, in part, explain the observed genotoxicity of these chemicals.

Drug metabolites

Acetaminophen is the most widely-used analgesic and antipyretic agent. Acetaminophen has been associated with liver failure due to either chronic or acute overexposure. Acetaminophen poisoning is one of the most common causes of poisoning reported to poison control centers. The toxic effects of this drug are attributed to the N-acetyl-*p*-benzoquinone imine (NAPQI, Figure 6) metabolite [104]. NAPQI is toxic to cells and causes genotoxic damage including DNA strand breaks [104-106]. While acetaminophen displays no ability to poison topoisomerase IIα, NAPQI increases levels of enzyme-mediated DNA cleavage and inhibits DNA ligation and plasmid DNA relaxation [75]. Treatment of cells with NAPQI leads to an accumulation of topoisomerase II-DNA cleavage complexes [75]. Based upon the ability of NAPQI to inactivate topoisomerase IIα when incubated with the enzyme prior to adding DNA, this compound is categorized as a covalent poison [75]. Given the above evidence, at least some of the toxic effects of NAPQI are a result of its action against topoisomerase II.

Etoposide is a widely-used anticancer agent that has been in use for more than 40 years. While etoposide is an effective anticancer agent, it is also associated with secondary leukemias in 2-3% of patients [107-112]. The mechanism for leukemogenesis resulting from etoposide therapy has not been fully elucidated. Etoposide impacts topoisomerase II function by inhibiting DNA ligation using a traditional interfacial poisoning mechanism [33, 62-66]. However, etoposide is metabolized by CYP3A4 and cellular oxidases (myeloperoxidase) to catechol and quinone forms (Figure 6) that also poison topoisomerase II [113-116]. There is evidence that these metabolites are involved in DNA damage associated with leukemogenesis [117]. While initial experimental evidence supported an interfacial poisoning mechanism [115, 117-119], more recent results under non-reducing conditions are consistent with a covalent mechanism [76]. The presence of reducing agents such as DTT prevents the quinone form from reacting with topoisomerase II in a covalent manner [76]. Etoposide quinone causes an increase in topoisomerase II-mediated DNA cleavage with a higher ratio of double-stranded breaks than seen with etoposide [76]. Etoposide quinone also inactivates topoisomerase II when incubated with the enzyme prior to DNA [76]. Etoposide catechol can also oxidize to the quinone and act as a covalent poison [73].

CONCLUSIONS

DNA topoisomerases are essential enzymes in living organisms due to the nature of the double-helix. The catalytic activity of type II topoisomerases are impacted by a number of natural and synthetic compounds. As discussed in this review, the damage promoted by topoisomerase II poisons (either interfacial or covalent) can lead to cell death, which may be therapeutic and/or chemopreventative. However, there is evidence that the action of some compounds on topoisomerase II can promote strand breaks leading to translocation events. Specific translocation events that lead to the development of leukemia have been identified and linked to the exposure to topoisomerase II poisons. This raises an important area of study: why do some poisons lead to chemoprevention and/or cancer cell death while others are associated with cancer induction?

Based upon the involvement of the α and β isoforms in relieving torsional strain in either

replicative or transcription associated processing, it is thought that targeting of topoisomerase IIα may be more important in killing cancer cells and in chemoprevention, while impacting topoisomerase IIβ may mediate negative side effects in healthy and non-proliferative tissues [54, 120]. Though this information has been available for some time, it has been difficult to identify agents that preferentially target one isoform over the other due to the highly similar structure and mechanism of these enzymes. Further, many of the compounds reviewed above have not been examined with both isoforms to determine selectivity. Given several high-resolution structures of eukaryotic topoisomerases published in recent years, there are now very focused searches taking place to identify compounds with selectivity between the α and β isoforms.

Another important issue that must be examined revolves around environmental and dietary exposure to topoisomerase II poisons. In particular, how can individuals be protected from the cancer-causing properties of environmental or dietary exposure to covalent poisons? What agents or strategies may be employed to minimize the impact of these compounds? As the list of covalent topoisomerase II poisons continues to grow, it will be critical to identify how these compounds are working in order to properly assess the threat to public health.

ACKNOWLEDGEMENTS

We would like to thank Kellie M. Regal for her review of this manuscript.

REFERENCES

1. Watson, J. D. and Crick, F. H. 1953, Nature, 171, 964.
2. Bates, A. D. and Maxwell, A. 2005, DNA Topology, Oxford University Press, New York.
3. Deweese, J. E., Osheroff, M. A. and Osheroff, N. 2009, BAMBED, 37, 2.
4. Kanaar, R. and Cozzarelli, N. R. 1992, Curr. Opin. Struct. Biol., 2, 369.
5. Wang, J. C. 2002, Nat. Rev. Mol. Cell Biol., 3, 430.
6. Wang, J. C. 1996, Annu. Rev. Biochem., 65, 635.
7. Champoux, J. J. 2001, Annu. Rev. Biochem., 70, 369.
8. Deweese, J. E. and Osheroff, N. 2009, Nucleic Acids Res., 37, 738.
9. Nitiss, J. L. 2009, Nat. Rev. Cancer, 9, 338.
10. Leppard, J. B. and Champoux, J. J. 2005, Chromosoma, 114, 75.
11. Nitiss, J. L. 2009, Nat. Rev. Cancer, 9, 327.
12. Schmidt, B. H., Burgin, A. B., Deweese, J. E., Osheroff, N. and Berger, J. M. 2010, Nature, 465, 641.
13. Schmidt, B. H., Osheroff, N. and Berger, J. M. 2012, Nat. Struct. Mol. Biol., 19, 1147.
14. Osheroff, N. 1987, Biochemistry, 26, 6402.
15. Zechiedrich, E. L. and Osheroff, N. 1990, EMBO J., 9, 4555.
16. Roca, J., Berger, J. M. and Wang, J. C. 1993, J. Biol. Chem., 268, 14250.
17. Lee, S., Jung, S. R., Heo, K., Byl, J. A., Deweese, J. E., Osheroff, N. and Hohng, S. 2012, Proc. Natl. Acad. Sci. USA, 109, 2925.
18. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M. and Chen, G. L. 1983, J. Biol. Chem., 258, 15365.
19. Sander, M. and Hsieh, T. 1983, J. Biol. Chem., 258, 8421.
20. Zechiedrich, E. L., Christiansen, K., Andersen, A. H., Westergaard, O. and Osheroff, N. 1989, Biochemistry, 28, 6229.
21. Mueller-Planitz, F. and Herschlag, D. 2008, J. Biol. Chem., 283, 17463.
22. Deweese, J. E. and Osheroff, N. 2009, Biochemistry, 48, 1439.
23. Deweese, J. E. and Osheroff, N. 2010, Metallomics, 2, 450.
24. Roca, J. and Wang, J. C. 1992, Cell, 71, 833.
25. Lindsley, J. E. and Wang, J. C. 1993, J. Biol. Chem., 268, 8096.
26. Classen, S., Olland, S. and Berger, J. M. 2003, Proc. Natl. Acad. Sci. USA, 100, 10629.
27. Vaughn, J., Huang, S., Wessel, I., Sorensen, T. K., Hsieh, T., Jensen, L. H., Jensen, P. B., Sehested, M. and Nitiss, J. L. 2005, J. Biol. Chem., 280, 11920.

28. Wei, H., Ruthenburg, A. J., Bechis, S. K. and Verdine, G. L. 2005, J. Biol. Chem., 280, 37041.
29. Bendsen, S., Oestergaard, V. H., Skouboe, C., Brinch, M., Knudsen, B. R. and Andersen, A. H. 2009, Biochemistry, 48, 6508.
30. Osheroff, N. 1986, J. Biol. Chem., 261, 9944.
31. Harkins, T. T., Lewis, T. J. and Lindsley, J. E. 1998, Biochemistry, 37, 7299.
32. Osheroff, N. and Zechiedrich, E. L. 1987, Biochemistry, 26, 4303.
33. Robinson, M. J. and Osheroff, N. 1991, Biochemistry, 30, 1807.
34. Bromberg, K. D., Hendricks, C., Burgin, A. B. and Osheroff, N. 2002, J. Biol. Chem., 277, 31201.
35. Drake, F. H., Zimmerman, J. P., McCabe, F. L., Bartus, H. F., Per, S. R., Sullivan, D. M., Ross, W. E., Mattern, M. R., Johnson, R. K. and Crooke, S. T. 1987, J. Biol. Chem., 262, 16739.
36. Tsai-Pflugfelder, M., Liu, L. F., Liu, A. A., Tewey, K. M., Whang-Peng, J., Knutsen, T., Huebner, K., Croce, C. M. and Wang, J. C. 1988, Proc. Natl. Acad. Sci. USA, 85, 7177.
37. Drake, F. H., Hofmann, G. A., Bartus, H. F., Mattern, M. R., Crooke, S. T. and Mirabelli, C. K. 1989, Biochemistry, 28, 8154.
38. Chung, T. D., Drake, F. H., Tan, K. B., Per, S. R., Crooke, S. T. and Mirabelli, C. K. 1989, Proc. Natl. Acad. Sci. USA, 86, 9431.
39. Austin, C. A. and Fisher, L. M. 1990, FEBS Lett., 266, 115.
40. Jenkins, J. R., Ayton, P., Jones, T., Davies, S. L., Simmons, D. L., Harris, A. L., Sheer, D. and Hickson, I. D. 1992, Nucleic Acids Res., 20, 5587.
41. Tan, K. B., Dorman, T. E., Falls, K. M., Chung, T. D., Mirabelli, C. K., Crooke, S. T. and Mao, J. 1992, Cancer Res., 52, 231.
42. Heck, M. M. and Earnshaw, W. C. 1986, J. Cell Biol., 103, 2569.
43. Hsiang, Y. H., Wu, H. Y. and Liu, L. F. 1988, Cancer Res., 48, 3230.
44. Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K. and Drake, F. H. 1991, Cell Growth Differ., 2, 209.
45. Grue, P., Grasser, A., Sehested, M., Jensen, P. B., Uhse, A., Straub, T., Ness, W. and Boege, F. 1998, J. Biol. Chem., 273, 33660.
46. Isaacs, R. J., Davies, S. L., Sandri, M. I., Redwood, C., Wells, N. J. and Hickson, I. D. 1998, Biochim. Biophys. Acta, 1400, 121.
47. Austin, C. A. and Marsh, K. L. 1998, BioEssays, 20, 215.
48. Linka, R. M., Porter, A. C., Volkov, A., Mielke, C., Boege, F. and Christensen, M. O. 2007, Nucleic Acids Res., 35, 3810.
49. Sakaguchi, A. and Kikuchi, A. 2004, J. Cell Sci., 117, 1047.
50. Yang, X., Li, W., Prescott, E. D., Burden, S. J. and Wang, J. C. 2000, Science, 287, 131.
51. Lyu, Y. L., Lin, C. P., Azarova, A. M., Cai, L., Wang, J. C. and Liu, L. F. 2006, Mol. Cell. Biol., 26, 7929.
52. Ju, B. G., Lunyak, V. V., Perissi, V., Garcia-Bassets, I., Rose, D. W., Glass, C. K. and Rosenfeld, M. G. 2006, Science, 312, 1798.
53. Cowell, I. G. and Austin, C. A. 2012, Cell Cycle, 11, 3143.
54. Cowell, I. G. and Austin, C. A. 2012, Int. J. Environ. Res. Public Health, 9, 2075.
55. Osheroff, N. 1998, Biochim. Biophys. Acta, 1400, 1.
56. Nitiss, J. L. 1998, Biochim. Biophys. Acta, 1400, 63.
57. Wilstermann, A. M. and Osheroff, N. 2003, Curr. Top. Med. Chem., 3, 321.
58. Bender, R. P. and Osheroff, N. 2008, DNA Topoisomerases as Targets for the Chemotherapeutic Treatment of Cancer, in Checkpoint Responses in Cancer Therapy, W. Dai (Ed.) Humana Press, Totowa, New Jersey.
59. McClendon, A. K. and Osheroff, N. 2007, Mutat. Res., 623, 83.
60. Felix, C. A. 2001, Med. Pediatr. Oncol., 36, 525.
61. Felix, C. A., Kolaris, C. P. and Osheroff, N. 2006, DNA Repair, 5, 1093.

62. Osheroff, N. 1989, Biochemistry, 28, 6157.
63. van Hille, B., Perrin, D. and Hill, B. T. 1999, Anticancer Drugs, 10, 551.
64. Bromberg, K. D., Burgin, A. B. and Osheroff, N. 2003, J. Biol. Chem., 278, 7406.
65. Deweese, J. E., Burgin, A. B. and Osheroff, N. 2008, Biochemistry, 47, 4129.
66. Wu, C. C., Li, T. K., Farh, L., Lin, L. Y., Lin, T. S., Yu, Y. J., Yen, T. J., Chiang, C. W. and Chan, N. L. 2011, Science, 333, 459.
67. D'Arpa, P., Beardmore, C. and Liu, L. F. 1990, Cancer Res., 50, 6919.
68. Ketron, A. C. and Osheroff, N. 2013, Phytochem. Rev., 12, in press.
69. Wang, H., Mao, Y., Chen, A. Y., Zhou, N., LaVoie, E. J. and Liu, L. F. 2001, Biochemistry, 40, 3316.
70. Lindsey, R. H. Jr., Bromberg, K. D., Felix, C. A. and Osheroff, N. 2004, Biochemistry, 43, 7563.
71. Krishnan, P. and Bastow, K. F. 2000, Biochem. Pharmacol., 60, 1367.
72. Bender, R. P., Ham, A. J. and Osheroff, N. 2007, Biochemistry, 46, 2856.
73. Jacob, D. A., Gibson, E. G., Mercer, S. L. and Deweese, J. E. 2013, Chem. Res. Tox., 26, 1156.
74. Bender, R. P., Lehmler, H. J., Robertson, L. W., Ludewig, G. and Osheroff, N. 2006, Biochemistry, 45, 10140.
75. Bender, R. P., Lindsey, R. H. Jr., Burden, D. A. and Osheroff, N. 2004, Biochemistry, 43, 3731.
76. Jacob, D. A., Mercer, S. L., Osheroff, N. and Deweese, J. E. 2011, Biochemistry, 50, 5660.
77. Lindsey, R. H., Bender, R. P. and Osheroff, N. 2005, Chem. Biol. Interact., 153-154, 197.
78. Lindsey, R. H. Jr., Bender, R. P. and Osheroff, N. 2005, Chem. Res. Toxicol., 18, 761.
79. Krishnan, P. and Bastow, K. F. 2001, Cancer Chemother. Pharmacol., 47, 187.
80. Ketron, A. C., Gordon, O. N., Schneider, C. and Osheroff, N. 2013, Biochemistry, 52, 221.
81. Lopez-Lazaro, M., Willmore, E., Jobson, A., Gilroy, K. L., Curtis, H., Padget, K. and Austin, C. A. 2007, J. Nat. Prod., 70, 1884.
82. Bandele, O. J. and Osheroff, N. 2008, Chem. Res. Toxicol., 21, 936.
83. Timmel, M. A., Byl, J. A. and Osheroff, N. 2013, Chem. Res. Toxicol., 26, 622.
84. Bandele, O. J., Clawson, S. J. and Osheroff, N. 2008, Chem. Res. Toxicol., 21, 1253.
85. Huang, C. P., Fang, W. H., Lin, L. I., Chiou, R. Y., Kan, L. S., Chi, N. H., Chen, Y. R., Lin, T. Y. and Lin, S. B. 2008, Toxicol. Appl. Pharmacol., 227, 331.
86. Lin, T. Y., Huang, C. P., Au, L. C., Chang, Y. W., Hu, C. Y. and Lin, S. B. 2012, Chem. Res. Toxicol., 25, 2340.
87. Zhang, Y., Talalay, P., Cho, C. G. and Posner, G. H. 1992, Proc. Natl. Acad. Sci. USA, 89, 2399.
88. Herr, I. and Buchler, M. W. 2010, Cancer Treat. Rev., 36, 377.
89. Lin, R. K., Zhou, N., Lyu, Y. L., Tsai, Y. C., Lu, C. H., Kerrigan, J., Chen, Y. T., Guan, Z., Hsieh, T. S. and Liu, L. F. 2011, J. Biol. Chem., 286, 33591.
90. ATSDR, 2011, Toxicological Profile for Benzene, US Dept of Health and Human Services, Public Health Service.
91. IARC, 2012, Benzene, IARC Monographs on the Evaluation of the Carinogenic Risk of Chemicals to Humans, 100, 249.
92. Lovern, M. R., Cole, C. E. and Schlosser, P. M. 2001, Crit. Rev. Toxicol., 31, 285.
93. Snyder, R. and Hedli, C. C. 1996, Environ. Health Perspect., 104, 1165.
94. Ross, D. 2000, J. Toxicol. Environ. Health A., 61, 357.
95. Silberhorn, E. M., Glauert, H. P. and Robertson, L. W. 1990, Crit. Rev. Toxicol., 20, 440.
96. Kimbrough, R. D. 1987, Annu. Rev. Pharmacol. Toxicol., 27, 87.
97. Safe, S. 1984, Crit. Rev. Toxicol., 13, 319.
98. Kimbrough, R. D. 1985, Environ. Health. Perspect., 59, 99.
99. Amaro, A. R., Oakley, G. G., Bauer, U., Spielmann, H. P. and Robertson, L. W. 1996, Chem. Res. Toxicol., 9, 623.

100. Oakley, G. G., Devanaboyina, U., Robertson, L. W. and Gupta, R. C. 1996, Chem. Res. Toxicol., 9, 1285.
101. Oakley, G. G., Robertson, L. W. and Gupta, R. C. 1996, Carcinogenesis, 17, 109.
102. Srinivasan, A., Lehmler, H. J., Robertson, L. W. and Ludewig, G. 2001, Toxicol. Sci., 60, 92.
103. Srinivasan, A., Robertson, L. W. and Ludewig, G. 2002, Chem. Res. Toxicol., 15, 497.
104. Bergman, K., Muller, L. and Teigen, S. W. 1996, Mutat. Res., 349, 263.
105. Bae, M. A., Pie, J. E. and Song, B. J. 2001, Mol. Pharmacol., 60, 847.
106. Dybing, E., Holme, J. A., Gordon, W. P., Soderlund, E. J., Dahlin, D. C. and Nelson, S. D. 1984, Mutat. Res., 138, 21.
107. Pui, C.-H., Ribeiro, R. C., Hancock, M. L., Rivera, G. K., Evans, W. E., Raimondi, S. C., Head, D. R., Behm, F. G., Mahmoud, M. H., Sandlund, J. T. and Crist, W. M. 1991, N. Engl. J. Med., 325, 1682.
108. Winick, N. J., McKenna, R. W., Shuster, J. J., Schneider, N. R., Borowitz, M. J., Bowman, W. P., Jacaruso, D., Kamen, B. A. and Buchanan, G. R. 1993, J. Clin. Oncol., 11, 209.
109. Pedersen-Bjergaard, J., Philip, P., Larsen, S. O., Andersson, M., Daugaard, G., Ersboll, J., Hansen, S. W., Hou-Jensen, K., Nielsen, D. and Sigsgaard, T. C. 1993, Leukemia, 7, 1975.
110. Smith, M. A., Rubinstein, L. and Ungerleider, R. S. 1994, Med. Ped. Oncol., 23, 86.
111. Relling, M. V., Yanishevski, Y., Nemec, J., Evans, W. E., Boyett, J. M., Behm, F. G. and Pui, C. H. 1998, Leukemia, 12, 346.
112. Smith, M. A., Rubinstein, L., Anderson, J. R., Arthur, D., Catalano, P. J., Freidlin, B., Heyn, R., Khayat, A., Krailo, M., Land, V. J., Miser, J., Shuster, J. and Vena, D. 1999, J. Clin. Oncol., 17, 569.
113. van Maanen, J. M., de Vries, J., Pappie, D., van den Akker, E., Lafleur, V. M., Retel, J., van der Greef, J. and Pinedo, H. M. 1987, Cancer Res., 47, 4658.
114. Relling, M. V., Nemec, J., Schuetz, E. G., Schuetz, J. D., Gonzalez, F. J. and Korzekwa, K. R. 1994, Mol. Pharmacol., 45, 352.
115. Kagan, V. E., Kuzmenko, A. I., Tyurina, Y. Y., Shvedova, A. A., Matsura, T. and Yalowich, J. C. 2001, Cancer Res., 61, 7777.
116. Zhuo, X., Zheng, N., Felix, C. A. and Blair, I. A. 2004, Drug Metab. Dispos., 32, 993.
117. Lovett, B. D., Strumberg, D., Blair, I. A., Pang, S., Burden, D. A., Megonigal, M. D., Rappaport, E. F., Rebbeck, T. R., Osheroff, N., Pommier, Y. G. and Felix, C. A. 2001, Biochemistry, 40, 1159.
118. van Maanen, J. M., Lafleur, M. V., Mans, D. R., van den Akker, E., de Ruiter, C., Kootstra, P. R., Pappie, D., de Vries, J., Retel, J. and Pinedo, H. M. 1988, Biochem. Pharmacol., 37, 3579.
119. Gantchev, T. G. and Hunting, D. J. 1998, Mol. Pharmacol., 53, 422.
120. Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L. S., Lyu, Y. L., Liu, L. F. and Yeh, E. T. 2012, Nat. Med., 18, 1639.