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Phytochemicals as Anticancer and Chemopreventive Topoisomerase II Poisons

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Abstract

Phytochemicals are a rich source of anticancer drugs and chemopreventive agents. Several of these chemicals appear to exert at least some of their effects through interactions with topoisomerase II, an essential enzyme that regulates DNA supercoiling and removes knots and tangles from the genome. Topoisomerase II-active phytochemicals function by stabilizing covalent protein-cleaved DNA complexes that are intermediates in the catalytic cycle of the enzyme. As a result, these compounds convert topoisomerase II to a cellular toxin that fragments the genome. Because of their mode of action, they are referred to as topoisomerase II poisons as opposed to catalytic inhibitors. The first sections of this article discuss DNA topology, the catalytic cycle of topoisomerase II, and the two mechanisms (interfacial *vs.* covalent) by which different classes of topoisomerase II poisons alter enzyme activity. Subsequent sections discuss the effects of several phytochemicals on the type II enzyme, including demethyl-epipodophyllotoxins (semisynthetic anticancer drugs) as well as flavones, flavonols, isoflavones, catechins, isothiocyanates, and curcumin (dietary chemopreventive agents). Finally, the leukemogenic potential of topoisomerase II-targeted phytochemicals is described.

Keywords

Demethyl-epipodophyllotoxins; Bioflavonoids; Catechins; Isothiocyanates; Curcumin

Introduction

Topoisomerase II is the target for a broad spectrum of anticancer and chemopreventive agents. Many of these agents are dietary phytochemicals or are derived from these compounds. Topoisomerase II-targeted chemicals kill cells in a unique and insidious fashion: rather than depriving cells of the essential activities of the enzyme, they convert topoisomerase II into a toxic enzyme that fragments the genome. Hence, these compounds are referred to as “topoisomerase II poisons.”

In order to fully appreciate how phytochemicals are able to impact human health through their interactions with topoisomerase II, it is necessary to understand why cells need the type II enzyme, how it functions, and how topoisomerase II poisons alter the activity of the

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enzyme. Therefore, the early sections of this article will address these important issues. The later sections will discuss phytochemicals that are topoisomerase II poisons, including widely prescribed anticancer drugs as well as dietary compounds with chemopreventive (and potentially chemotherapeutic) properties.

DNA Topology

The human genome is encoded in a series of forty-six linear chromosomes that range in size from ~50 million to ~250 million DNA base pairs. Furthermore, the genetic information is stored in the form of a double helix in which the two DNA strands are plectonemically coiled around one another (Bates and Maxwell, 2005; Deweese et al., 2008; Liu et al., 2009). Although these structural features contribute to the physical integrity of the genome, they impose a number of topological constraints on the genetic material that affect all of its physiological functions (Bates and Maxwell, 2005; Deweese et al., 2008; Liu et al., 2009).

Topological properties of DNA, including under- and overwinding, knotting, and tangling, can only be changed when one or both strands of the double helix are broken (Bates and Maxwell, 2005; Deweese et al., 2008; Liu et al., 2009; Ketron and Osheroff, 2013). DNA underwinding (*i.e.*, negative supercoiling) is important because the two strands of the double helix must be separated in order to replicate or express the genetic information. Because negative supercoiling destabilizes the double-stranded nature of DNA, it facilitates strand separation. Conversely, DNA overwinding (*i.e.*, positive supercoiling) that occurs ahead of replication forks and transcription complexes inhibits strand separation and blocks these critical cellular processes (Bates and Maxwell, 2005; Deweese et al., 2008; Liu et al., 2009; Ketron and Osheroff, 2013).

Essential nucleic acid functions, such as DNA recombination and replication, generate knots and tangles within the double helix (Bates and Maxwell, 2005; Deweese et al., 2008; Liu et al., 2009; Ketron and Osheroff, 2013). DNA knots impair the ability to separate the two strands of the genetic material and intermolecular DNA tangles prevent segregation of chromosomes during mitosis. Consequently, these topological obstacles can be lethal to cells if they are not resolved. Enzymes that regulate the topological structure of DNA are called topoisomerases (Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2008; Vos et al., 2011; Ketron and Osheroff, 2013). Topoisomerases can be separated into two major classes, which are distinguished by the number of DNA strands they cleave and ligate. Type I topoisomerases act by cleaving one strand of the double helix. Thus, they are able to regulate levels of DNA supercoiling (Champoux, 1994; Leppard and Champoux, 2005; Pommier, 2009). In contrast, type II topoisomerases act by cleaving both strands of the double helix (Deweese and Osheroff, 2009; Nitiss, 2009a; Vos et al., 2011). As a result, they can regulate the superhelical density of DNA and also can resolve tangles and knots in duplex DNA. In order to maintain the integrity of the genome during the required DNA cleavage event, all topoisomerases form covalent bonds between active site tyrosyl residues and the DNA termini generated during the reaction (Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2008; Vos et al., 2011; Ketron and Osheroff, 2013). This covalent enzyme-cleaved DNA complex (known as the “cleavage complex”) is a hallmark of topoisomerases.

This article will focus on eukaryotic type II topoisomerases. A number of recent review articles that discuss type I topoisomerases (Baker et al., 2009; Pommier, 2009, 2013) or bacterial type II enzymes are available (Sissi and Palumbo, 2010; Collin et al., 2011).

Topoisomerase II

Eukaryotic type II topoisomerases are homodimeric enzymes. Humans express two isoforms of topoisomerase II, α and β (Austin and Marsh, 1998; Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009a; Vos et al., 2011). These two isoforms share extensive amino acid sequence identity (~70%), but are encoded by separate genes (located at chromosomal bands 17q21-22 and 3p24 in humans, respectively). Topoisomerase IIα and IIβ also can be distinguished by their protomer molecular masses (~170 kDa and ~180 kDa, respectively).

Topoisomerase IIα and IIβ have distinct patterns of expression and separate nuclear functions (Austin and Marsh, 1998; Champoux, 2001; Wang, 2002; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009a; Vos et al., 2011). Topoisomerase IIα is essential for the survival of proliferating cells and protein levels rise dramatically during periods of cell growth. The enzyme is further regulated over the cell cycle, with protein concentrations peaking in G2/M. Topoisomerase IIα unlinks tangled daughter chromosomes following replication and resolves DNA knots that are formed during recombination. It also helps to alleviate the torsional stress that accumulates ahead of replication forks and transcription complexes. Furthermore, topoisomerase IIα is required for proper chromosome condensation, cohesion, and segregation and appears to play roles in centromere function and chromatin remodeling. Finally, the enzyme is important for the maintenance of proper chromosome organization and structure and is the major non-histone protein of the mitotic chromosome scaffold and the interphase nuclear matrix.

Topoisomerase IIβ is dispensable at the cellular level, and its presence cannot compensate for the loss of topoisomerase IIα in mammalian cells (Austin and Marsh, 1998; Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009a; Vos et al., 2011). However, the β isoform is required for proper neural development in mice (Yang et al., 2000). In contrast to topoisomerase IIα, the concentration of topoisomerase IIβ is independent of the cell cycle, and high levels of this isoform are found in most cell types regardless of proliferation status (Austin and Marsh, 1998; Isaacs et al., 1998; Linka et al., 2007). Furthermore, topoisomerase IIβ dissociates from chromosomes during mitosis. Ultimately, the physiological functions of the β isoform have yet to be fully defined. However, it has been suggested that topoisomerase IIβ plays an important role in the transcription of hormonally- or developmentally-regulated genes (Haince et al., 2006; Ju et al., 2006).

Much of what we understand regarding the mechanism of action of type II enzymes comes from experiments with topoisomerase II from eukaryotic species that express only a single form of the protein. Consequently, eukaryotic type II topoisomerases will be referred to collectively as topoisomerase II, unless the properties being discussed are specific to either the α or β isoform.

Topoisomerase II catalytic cycle

Topoisomerase II regulates the superhelical density of DNA and removes tangles and knots from the genetic material by the double-stranded DNA passage reaction depicted in Figure 1 (Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2009; Deweese and Osheroff, 2009; Liu et al., 2009; Deweese and Osheroff, 2010; Vos et al., 2011; Ketron and Osheroff, 2013). The enzyme requires divalent metal ions (Mg^{2+} appears to be the physiological ion) and ATP in order to carry out the complete catalytic cycle.

Step 1: Topoisomerase II binds two segments of DNA. The first segment bound by the enzyme is the double helix that will be cleaved and is referred to as the “Gate-” or “G-

segment." The second segment is the double helix that will be transported through the transient DNA gate and is referred to as the "Transport-" or "T-segment." DNA binding requires no cofactors. *Step 2:* In the presence of the active site Mg^{2+} ions, topoisomerase II samples the DNA for malleability (Lee et al., 2012). Sequences that can be cleaved are bent to an angle of ~150° (Dong and Berger, 2007; Schmidt et al., 2010; Hardin et al., 2011; Lee et al., 2012). Conversely, sequences that cannot be bent are not cleaved (Lee et al., 2012). *Step 3:* A double-stranded break is generated in the G-segment using a noncanonical two-metal-ion mechanism (Deweese and Osheroff, 2010; Schmidt et al., 2010). Cleavage is initiated by the nucleophillic attack of the two active site tyrosyl residues (one in each subunit of the homodimeric enzyme; Tyr805 and Tyr821 in human topoisomerase IIα and topoisomerase IIβ, respectively) on the DNA backbone, each of which makes a single-stranded DNA break. The resulting transesterification reaction results in the formation of a covalent phosphotyrosyl bond that links the protein to each of the newly generated 5'-DNA termini. It also generates a 3'-hydroxyl moiety on the opposite terminus of each cleaved strand. The scissile bonds in the two strands of the double helix are staggered and are located across the major groove from one another. Thus, topoisomerase II generates cleaved DNA molecules with 4-base 5'-single-stranded cohesive ends, each of which is covalently linked to a separate protomer subunit of the enzyme. *Step 4:* Two molecules of ATP are bound by the enzyme, which triggers the closing of the N-terminal protein gate, the opening of the DNA gate, and the translocation of the T-segment through the gate. Although hydrolysis of the cofactor is not a prerequisite for DNA translocation, it appears that this step proceeds more rapidly if it is preceded by hydrolysis of one of the bound ATP molecules. *Step 5:* Topoisomerase II ligates the cleaved DNA strands. *Step 6:* The T-segment is released through the C-terminal protein gate. *Step 7:* Upon hydrolysis of the second ATP molecule, topoisomerase II regains the ability to initiate a new round of catalysis.

The covalent enzyme-DNA linkage formed during DNA scission (*Step 3*) plays two important roles in the topoisomerase II reaction mechanism (Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2009; Deweese and Osheroff, 2009; Liu et al., 2009; Deweese and Osheroff, 2010; Vos et al., 2011; Ketron and Osheroff, 2013). First, it conserves the bond energy of the sugar-phosphate DNA backbone. Second, because it does not allow the cleaved DNA chain to dissociate from the enzyme, the protein-DNA linkage maintains the integrity of the genetic material during the cleavage event. The covalent topoisomerase II-cleaved DNA reaction intermediate is referred to as the "cleavage complex" and is critical for the pharmacological activities of phytochemicals on the enzyme, which are discussed later in this article. The DNA cleavage/ligation equilibrium of the enzyme greatly favors ligation (Champoux, 2001; Corbett and Berger, 2004; Deweese et al., 2009; Deweese and Osheroff, 2009; Liu et al., 2009; Deweese and Osheroff, 2010; Vos et al., 2011; Ketron and Osheroff, 2013). Thus, topoisomerase II-DNA cleavage complexes normally are short-lived and are readily reversible. As described below, compounds that increase the longevity of cleavage complexes can have serious cellular consequences.

Topoisomerase II as a cellular toxin

Because topoisomerases generate DNA strand breaks as obligate reaction intermediates, they are intrinsically dangerous proteins (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010). Thus, while necessary for cell viability, these enzymes also have the capacity to fragment the genome (Figure 2). As a result of this dual "Dr. Jekyll/Mr. Hyde" persona, cells maintain levels of cleavage complexes in a critical balance. If topoisomerase IIα cleavage drops below threshold levels, daughter chromosomes remain entangled following replication (Wang, 1991; Champoux, 2001; Deweese et al.,

2008; Nitiss, 2009a; Vos et al., 2011). As a result, chromosomes cannot segregate properly, and cells die as a result of catastrophic mitotic failure (Figure 2).

Increased levels of topoisomerase IIα- or IIβ-DNA cleavage complexes also cause deleterious physiological effects, but for different reasons (Figure 2) (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010). When replication forks, transcription complexes, or other DNA tracking proteins attempt to traverse covalently bound protein "roadblocks" in the genetic material, accumulated cleavage intermediates are converted to strand breaks that are no longer tethered by proteinaceous bridges. The ensuing damage induces recombination/repair pathways that can trigger mutations and other chromosomal aberrations. If the number of DNA breaks overwhelms the repair process, it can initiate cell death pathways (D'Arpa et al., 1990; Kaufmann, 1998; Fortune and Osheroff, 2000; McClendon and Osheroff, 2007; Bender and Osheroff, 2008). Conversely, if cells are not killed, DNA breaks can be converted to permanent chromosomal translocations that lead to specific forms of leukemia (Felix et al., 2006; Joannides and Grimwade, 2010).

Topoisomerase II poisons

Compounds that alter topoisomerase II activity can be separated into two categories. Chemicals that decrease the overall activity of the enzyme are known as catalytic inhibitors (Andoh and Ishida, 1998; Fortune and Osheroff, 1998; Bailly, 2012; Pommier, 2013). Conversely, chemicals that increase levels of topoisomerase II-DNA cleavage complexes are said to "poison" the enzyme and convert it to a cellular toxin that initiates the mutagenic and lethal consequences described in Figure 2 (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010; Bailly, 2012; Chen et al., 2012; Pommier, 2013). Because of their actions, these latter compounds are referred to as "topoisomerase II poisons" to distinguish them from catalytic inhibitors that do not increase the concentration of cleavage complexes (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010; Bailly, 2012; Chen et al., 2012; Pommier, 2013). Although some topoisomerase II poisons also inhibit overall activity, the "gain of function" induced by these compounds in the cell (*i.e.*, increased levels of cleavage complexes) is a dominant phenotype. Thus, they kill cells by a fundamentally different mechanism than that of most protein-targeted drugs (which act by robbing the cell of an essential function). As discussed below, a number of phytochemicals that display anticancer or chemopreventive properties act as topoisomerase II poisons.

Chemicals that function as topoisomerase II poisons act by two distinct mechanisms (Figure 3). Compounds utilizing the first mechanism are referred to as interfacial topoisomerase II poisons (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010; Pommier, 2013). These chemicals form non-covalent interactions with topoisomerase II at the protein-DNA interface in the vicinity of the active site tyrosine. They also interact with DNA within the ternary enzyme-DNA-poison complex and inhibit ligation by intercalating into the double helix at the cleaved scissile bond. Thus, they present a physical barrier to ligation and act as "molecular doorstops." It is notable that the actions of interfacial topoisomerase II poisons are not affected by reducing agents, such as dithiothreitol, and that these compounds induce similar levels of enzyme-mediated DNA cleavage whether they are added to the binary topoisomerase II-DNA complex or are incubated with the enzyme prior to the addition of nucleic acid substrates.

Unlike interfacial poisons, compounds that use the second mechanism contain protein reactive groups. Because many of the original compounds that were examined underwent redox cycling as a prerequisite for activity, compounds that utilize this second mechanism originally were referred to as redox-dependent topoisomerase II poisons (Wang et al., 2001; Lindsey et al., 2004; Bender et al., 2006; Deweese and Osheroff, 2009; Lin et al., 2011). However, recent studies indicate that some of these compounds can be activated in the absence of redox cycling (Wang et al., 2001; Lindsey et al., 2004; Bender et al., 2006; Deweese and Osheroff, 2009; Lin et al., 2011; Ketron et al., 2013). Therefore, because the common feature of these poisons is the covalent adduction of the enzyme (see below), we suggest that it is more correct to call them covalent topoisomerase II poisons.

Covalent topoisomerase II poisons all incorporate sulfhydryl-reactive groups such as quinones, isothiocyanates, or maleimides (Wang et al., 2001; Lindsey et al., 2004; Bender et al., 2006; Deweese and Osheroff, 2009; Lin et al., 2011; Ketron et al., 2013). In contrast to interfacial topoisomerase II poisons, covalent poisons modify the enzyme at amino acid residues outside of the active site (Wang et al., 2001; Lindsey et al., 2004; Bender et al., 2006; Deweese and Osheroff, 2009; Lin et al., 2011), and their ability to poison topoisomerase II can be abrogated by reducing agents. In addition, compounds within this second group enhance DNA cleavage when added to the protein-DNA complex, but display the distinguishing feature of inhibiting topoisomerase II activity when incubated with the enzyme prior to the addition of DNA.

There is evidence that some covalent topoisomerase II poisons function (at least in part) by crosslinking or closing the N-terminal protein gate of topoisomerase II (Bender et al., 2006). Such an action could provide a mechanistic basis for stabilizing pre-existing cleavage complexes, while excluding DNA binding to unoccupied enzymes. However, the precise details by which covalent topoisomerase II poisons increase levels of DNA cleavage complexes have yet to be determined.

A number of topoisomerase II poisons described to date are derived from natural sources. Among these, phytochemicals have proven to be a rich source of topoisomerase II-active compounds. As discussed below, some phytochemical-based topoisomerase II poisons are widely utilized anticancer drugs. Others are normal components of the human diet and display chemopreventive (and potentially chemotherapeutic) properties.

Demethyl-epipodophyllotoxins

Etoposide and the related compound teniposide (Figure 4) are two of the most successful anticancer drugs in the world. They are used to treat a variety of cancers, including small cell lung cancer, germ-line malignancies, sarcomas, leukemias, and lymphomas (Hande, 1998a, b; Baldwin and Osheroff, 2005; Deweese and Osheroff, 2009; Bailly, 2012; Chen et al., 2012).

Etoposide is derived from podophyllotoxin (Figure 4) (Stahelin and von Wartburg, 1991; Baldwin and Osheroff, 2005). This natural product is produced by *Podophyllum peltatum*, more commonly known as the mayapple or American mandrake plant. Podophyllotoxin has been used as a folk remedy for over a thousand years and is an antimitotic drug that acts by preventing microtubule formation (Hande, 1998b, a; Baldwin and Osheroff, 2005). The clinical use of this compound as an antineoplastic agent was prevented by high toxicity. In the 1950's, investigators at Sandoz Pharmaceuticals initiated a discovery program in the hope of overcoming the prohibitive toxicity of the parent compound (Stahelin and von Wartburg, 1991; Hande, 1998b; Baldwin and Osheroff, 2005). As a result, a series of semi-synthetic podophyllotoxin derivatives was generated. Ultimately two analogs, etoposide and

teniposide, displayed increased antineoplastic activity and decreased toxicity. Further analysis revealed, however, that these drugs no longer interacted with microtubules (Loike and Horwitz, 1976). It was determined several years later that they act as topoisomerase II poisons (Chen et al., 1984; Ross et al., 1984; Yang et al., 1985). Etoposide was approved for clinical use in the mid-1980s and for several years was the most widely prescribed anticancer drug in the world (Hande, 1998b, a; Baldwin and Osheroff, 2005).

Of all the known topoisomerase II poisons, etoposide is by far the best characterized (Baldwin and Osheroff, 2005). Studies with this drug have provided important conceptual frameworks that paved the way for later work on other anticancer agents. Etoposide was one of the first chemotherapeutic drugs demonstrated to kill cells by targeting topoisomerase II and was the first shown to inhibit the DNA ligation activity of the type II enzyme (Chen et al., 1984; Ross et al., 1984; Yang et al., 1985; Osheroff, 1989). It is an interfacial topoisomerase II poison that enters the binary enzyme-DNA complex primarily through interactions with the protein (Burden et al., 1996; Kingma et al., 1999).

Etoposide remains the only topoisomerase II poison for which we have structural data that describes its interactions with the enzyme. Studies that coupled saturation transfer difference NMR spectroscopy of the binary human topoisomerase IIα-etoposide complex with drug activity/binding strongly suggest that the binding of etoposide by topoisomerase IIα is driven by interactions with the A– and B–rings, and potentially by stacking interactions with the pendent E–ring (Figure 5) (Wilstermann et al., 2007; Bender et al., 2008; Pitts et al., 2011). In addition, the 3'-, 4'-, and 5'-substituents on the E-ring are important for drug function and interact with the enzyme, but do not appear to contribute significantly to binding (Wilstermann et al., 2007; Bender et al., 2008). Finally, portions of the D-ring and (to a lesser extent) the C-4 carbohydrate moiety mediate interactions between etoposide and DNA (Pitts et al., 2011). These findings largely have been substantiated by a recent structure of the ternary human topoisomerase IIβ-DNA-etoposide complex (Wu et al., 2011).

The two critical modifications of podophyllotoxin that convert it from a tubulin-targeted drug to a topoisomerase II poison are the substitution of a hydroxyl moiety for the methoxy group at C-4' and the inclusion of the carbohydrate group at C-4 (Figure 4). The C-4' position appears to sit in a constrained pocket in topoisomerase II (Bender et al., 2008; Wu et al., 2011). Consequently, the presence of the C-4' methoxy prevents the parent compound from binding to the type II enzyme (Bender et al., 2008). The inclusion of a bulky group at C-4 (although not necessary for interactions with topoisomerase II) appears to prevent the binding of etoposide to tubulin (Loike and Horwitz, 1976).

Because the C-4 carbohydrate of etoposide does not interact with topoisomerase II (Wilstermann et al., 2007; Bender et al., 2008), it is possible to substitute other functional groups at this position (Bailly, 2012) (Figure 4). For example, TOP-53 is an etoposide derivative that contains a C-4 aminoalkyl side chain (Kitamura et al., 1997). In contrast to the carbohydrate moiety of etoposide, every proton associated with the side chain of TOP-53 contacts topoisomerase IIα in the binary enzyme-drug complex (Wilstermann et al., 2007). The inclusion of the aminoalkyl group increases the binding affinity of TOP-53 for topoisomerase IIα and significantly enhances the potency and efficacy of the drug against the type II enzyme (Byl et al., 2001; Wilstermann et al., 2007). Alternatively, F14512 is a novel etoposide derivative that contains a spermine group in place of the C-4 glycosidic moiety (Barret et al., 2008). The presence of the spermine enhances the selectivity of the drug for cancers that overexpress an active polyamine transport system (Annereau et al., 2008; Barret et al., 2008; Kruczynski et al., 2009). In addition, because the spermine converts the drug into a DNA binder (Barret et al., 2008), F14512 is a more potent and efficacious topoisomerase II poison than etoposide (Gentry et al., 2011). F14512 forms

highly stable ternary topoisomerase II-DNA-drug complexes (Gentry et al., 2011) and currently is in clinical trials.

Bioflavonoids

Bioflavonoids are a diverse group of polyphenolic compounds that are constituents of many fruits, vegetables, legumes, and plant leaves (Kurzer and Xu, 1997; Scalbert and Williamson, 2000; Galati and O'Brien, 2004; Yao et al., 2004; Kandaswami et al., 2005; Siddiqui et al., 2006). They are an integral component of the human diet and represent the most abundant natural source of antioxidants (Kurzer and Xu, 1997; Scalbert and Williamson, 2000; Dragsted, 2003; Galati and O'Brien, 2004; Yao et al., 2004; Sang et al., 2005a; Siddiqui et al., 2006).

It is believed that the dietary intake of bioflavonoids provides a number of health benefits to adults (Adlercreutz et al., 1993; Kurzer and Xu, 1997; Lamartiniere, 2000; Scalbert and Williamson, 2000; Galati and O'Brien, 2004; Yao et al., 2004; Kandaswami et al., 2005; Siddiqui et al., 2006). Epidemiological studies suggest that these compounds help protect against cancer, cardiovascular disease, osteoporosis, age-related diseases, and inflammation. The mechanistic basis for the physiological actions of bioflavonoids is not fully described, as they have a variety of effects on human cells. Beyond their antioxidant properties, many of these polyphenols are potent inhibitors of tyrosine kinases (Akiyama et al., 1987; Hagiwara et al., 1988; Geahlen et al., 1989; Cushman et al., 1991; Yang et al., 2001; Hollosy and Keri, 2004; Kandaswami et al., 2005), display anti-proliferative, pro-apoptotic, and genotoxic effects, and decrease the expression or function of several proteins that are involved in cell-cycle progression (Ren et al., 2003; Williams et al., 2004; Kandaswami et al., 2005; Fresco et al., 2006; Sarkar et al., 2006; Siddiqui et al., 2006).

A variety of flavones, isoflavones, and flavonols have been examined for their abilities to enhance DNA cleavage mediated by human topoisomerase IIα and IIβ, and several were found to be potent topoisomerase II poisons *in vitro* and in cultured human cells (Figure 6) (Austin et al., 1992; Constantinou et al., 1995; Bandele and Osheroff, 2007; Lopez-Lazaro et al., 2010). Among the bioflavonoids, genistein appears to have the highest activity against the human type II enzymes (Austin et al., 1992; Bandele and Osheroff, 2007). Many of the chemopreventive, cytotoxic, and genotoxic properties of flavones, isoflavones, and flavonols are consistent with their activities as topoisomerase II poisons. To this point, the sensitivity of cells to genistein has been correlated to the activity of the type II enzyme (Markovits et al., 1995; Lopez-Lazaro et al., 2007a).

With the exception discussed below, flavones, isoflavones, and flavonols are interfacial topoisomerase II poisons and increase levels of cleavage complexes primarily by inhibiting enzyme-mediated DNA ligation (Austin et al., 1992; Bandele and Osheroff, 2007). Structure-activity studies indicate that (like etoposide) the presence of a C-4’ hydroxyl on the pendant ring (in this case, the B-ring) does not mediate binding of bioflavonoids to topoisomerase II, but is important for the activity against the type II enzyme (Bandele and Osheroff, 2007). Once again, the C-4’ hydroxyl appears to interact with a constrained pocket in topoisomerase II, as the inclusion a methoxy group at this position impairs the ability of compounds to bind the enzyme (Bandele and Osheroff, 2007). The C-5 hydroxyl also appears to be important for bioflavonoid function and binding (Austin et al., 1992; Constantinou et al., 1995; Bandele and Osheroff, 2007). It has been proposed that this substituent forms a pseudo ring with the C-4 keto group that helps to maintain the planarity of the A- and C-rings (Kozerski et al., 2003).

In general, flavones, isoflavones, and flavonols appear to be more efficacious against topoisomerase IIβ than the α isoform (Bandele and Osheroff, 2007). Furthermore, cells that are depleted of topoisomerase IIβ are resistant to genistein (Lopez-Lazaro et al., 2007a). Therefore, it is believed that many of the cellular effects of flavones, isoflavones, and flavonols as topoisomerase II poisons are mediated primarily by the β isoform (Lopez-Lazaro et al., 2007a).

Catechins represent another major and important class of bioflavonoids (Galati and O'Brien, 2004; Yao et al., 2004; Kanwar et al., 2012). Green tea, which is one of the most commonly consumed beverages in the world, is a rich source of catechins and has been suggested to reduce the incidence of breast, prostate, colorectal, and lung cancer in humans (Sang et al., 2005a; Isbrucker et al., 2006a; Isbrucker et al., 2006b; Yang et al., 2007). The most abundant catechins in green tea are (−)-epigallocatechin gallate (EGCG) and the related compounds (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) (Figure 7) (Sang et al., 2005a; Isbrucker et al., 2006a; Isbrucker et al., 2006b; Yang et al., 2007). Although EGCG and EGC are potent topoisomerase II poisons, neither ECG nor EC (which contain only two hydroxyl groups on their B-rings) display any substantial activity against the human type II enzymes (Austin et al., 1992; Bandele et al., 2008). Thus, the ability of green tea catechins to poison topoisomerase II reflects the presence of three hydroxyl groups on the B-ring, with the D-ring having little relevance.

Surprisingly (and in major contrast to the flavones, isoflavones, and flavonols), EGCG and EGC appear to be covalent (rather than interfacial) topoisomerase II poisons (Bandele et al., 2008; Bandele and Osheroff, 2008). The mechanistic differences between bioflavonoid classes appear to be related to structural elements in the B- and C-rings (Figure 7) (Bandele et al., 2008). Although the C-4’ hydroxyl of the B-ring is critical for bioflavonoids to act as interfacial topoisomerase II poisons (Austin et al., 1992; Constantinou et al., 1995; Bandele and Osheroff, 2007), the inclusion of two additional B-ring hydroxyl groups increases redox activity (Valcic et al., 1999; Valcic et al., 2000) and is required for compounds to act as covalent topoisomerase II poisons (Bandele et al., 2008; Bandele and Osheroff, 2008). In addition, the C-ring in flavones, isoflavones, and flavonols is aromatic, planar, and includes the C-4 keto group that allows the formation of the proposed pseudo ring with the C-5 hydroxyl (Kozerski et al., 2003). All of these elements are required for binding to human type II topoisomerases (Bandele and Osheroff, 2007; Bandele et al., 2008). Because EGCG and EGC contain the catechin C-ring, they are unable to act as interfacial topoisomerase II poisons and function exclusively as covalent poisons. Furthermore, because ECG and EC lack the critical third hydroxyl group on their B-rings that would allow them to function as covalent poisons, they show virtually no activity against topoisomerase II. As predicted from the above, if three hydroxyl groups are included on the B-ring of a flavonol such as myricetin (see Figure 6), the compound acts as a dual function topoisomerase II poison and displays both interfacial and covalent characteristics (Bandele et al., 2008).

During the brewing process, a portion of EGCG (and related green tea catechins) undergoes epimerization. As a result, the stereochemistry of the bond linking the B- and C-rings inverts, converting EGCG to (−)-gallocatechin gallate (GCG) (Sang et al., 2005b; Ishino et al., 2010). Despite this stereochemical alteration, GCG poisons topoisomerase II with an activity that is similar to that of EGCG (Timmel et al., 2013).

Isothiocyanates

Dietary glucosinolates are found in cruciferous vegetables, including broccoli, cabbage, cauliflower, and kale (Stan et al., 2008). They are converted to bioactive isothiocyanates such as benzyl-isothiocyanate, phenethyl-isothiocyanate, and sulforaphane (Figure 8), upon

hydrolysis by myrasinase (Herr and Buchler, 2010). Many of these compounds inhibit cell proliferation, display chemopreventive properties, and inhibit tumor growth in xenograft models (Chung et al., 2000; Singh et al., 2004; Warin et al., 2009).

Isothiocyanates are topoisomerase II poisons *in vitro*, and silencing topoisomerase IIα in cultured mouse embryonic fibroblasts decreases DNA damage induced by these compounds (Lin et al., 2011). As found for reactive quinone-based topoisomerase II poisons (Bender et al., 2007), isothiocyanates act as covalent poisons and modify several cysteine residues in human topoisomerase IIα (Lin et al., 2011). Consistent with a mechanism that requires cysteine modification, the ability of isothiocyanates to induce topoisomerase II-mediated DNA cleavage is abolished when compounds are co-incubated with excess glutathione (Lin et al., 2011).

Curcumin

Curcumin (Figure 9) is the principal flavor and color component of turmeric, a common spice used in curries and a variety of other Asian cuisines (Goel et al., 2008; Gupta et al., 2012). Beyond its culinary uses, curcumin is believed to positively impact human health and commonly is used in traditional Chinese herbal medicine and Ayurvedic medicine (Goel et al., 2008; Gupta et al., 2012). The compound has chemopreventive properties against a variety of human malignancies and currently is in clinical trials as an anticancer agent (Satoskar et al., 1986; Sharma et al., 2001; Mahady et al., 2002; Dhillon et al., 2008; Hatcher et al., 2008; Patel et al., 2010).

Curcumin has poor oral bioavailability and is unstable under physiological conditions (Tonnesen and Karlsen, 1985; Wang et al., 1997; Pfeiffer et al., 2003; Anand et al., 2007; Griesser et al., 2011). Thus, it has been suggested that some of its biological effects are mediated by metabolites (Ireson et al., 2001; Anand et al., 2008; Shen and Ji, 2009, 2012). Curcumin can undergo spontaneous autoxidation in aqueous solutions at physiological pH (Figure 9) (Griesser et al., 2011), and this reaction gives rise to novel quinone-containing products that have potential for biological activity. Treatment of human cells with curcumin induces DNA cleavage complexes formed by topoisomerase IIα and IIβ (Lopez-Lazaro et al., 2007b). Cleavage complex formation is prevented by the addition of an antioxidant, suggesting the importance of oxidative pathways in curcumin activity against the type II enzymes (Lopez-Lazaro et al., 2007b).

A recent study demonstrated that reactive oxidized metabolites of curcumin, but neither the parent compound nor the stable bicyclopentadione end product (Figure 9), are covalent topoisomerase II poisons (Ketron et al., 2013). These metabolites display similar abilities to induce DNA cleavage mediated by human topoisomerase IIα and IIβ. Although breakdown products of curcumin, including vanillin, ferulic acid, and feruloylmethane, are bioreactive, none display activity against the human type II enzymes (Ketron et al., 2013).

Basis for the selectivity of topoisomerase II poisons

Topoisomerase II poisons are in wide use as anticancer chemotherapeutics (Pommier and Marchand, 2005; McClendon and Osheroff, 2007; Deweese et al., 2008; Deweese and Osheroff, 2009; Nitiss, 2009b; Pommier, 2009; Pommier et al., 2010; Bailly, 2012; Chen et al., 2012; Pommier, 2013). Considering that all cells in the human body contain type II topoisomerases, an obvious question arises: why do topoisomerase II poisons preferentially kill cancer cells?

First, due to the mechanism of action of these agents (*i.e.* the generation of DNA strand breaks), the higher the cellular level of topoisomerase II, the more lethal they become. Most

cancer cells proliferate rapidly and contain higher than normal levels of type II enzymes (Nitiss, 2009b). Therefore, treatment with topoisomerase II poisons generates more DNA strand breaks and induces greater toxicity.

Second, the transient DNA breaks generated by type II topoisomerases are converted to permanent strand breaks upon collision with DNA tracking machinery, such as replication and transcription complexes. Since cancer cells are distinguished by high rates of metabolism and frequent replication, cleavage complexes stabilized by topoisomerase II poisons in these cells are more likely to be converted to permanent (and potentially lethal) DNA strand breaks.

Third, cancer cells display the hallmark characteristics of genomic instability and impaired DNA damage response pathways (Friedberg et al., 2006). Thus, they are more susceptible than normal cells to the effects of DNA damaging agents, such as topoisomerase II poisons.

As discussed above, many dietary topoisomerase II poisons appear to have chemopreventive properties. It is likely that the above criteria apply (at least in part) to these compounds as well. Ingested at low levels, topoisomerase II-targeted phytochemicals may preferentially affect cells that are progressing toward malignancy. To this point, compounds such as genistein, curcumin and green tea catechins have been investigated in animal and human trials as anticancer agents (Sarkar and Li, 2004; Von Low et al., 2007; Dhillon et al., 2008; Hatcher et al., 2008; Patel et al., 2010; Kanwar et al., 2012).

Topoisomerase II-associated leukemias

Despite the importance of topoisomerase II as a target for anticancer drugs and chemopreventive agents, evidence suggests that DNA strand breaks generated by the enzyme can trigger chromosomal translocations associated with specific types of leukemia (Felix et al., 2006; McClendon and Osheroff, 2007; Deweese and Osheroff, 2009; Joannides and Grimwade, 2010; Joannides et al., 2011; Cowell and Austin, 2012). To this point, 2–3% of patients who receive regimens that include etoposide subsequently develop acute myeloid leukemias (AMLs) (Baldwin and Osheroff, 2005; Felix et al., 2006; McClendon and Osheroff, 2007; Deweese and Osheroff, 2009; Joannides and Grimwade, 2010; Joannides et al., 2011; Cowell and Austin, 2012). Most of these leukemias are characterized by translocations with breakpoints in the *MLL* (mixed lineage leukemia) gene at chromosomal band 11q23. The MLL protein is a histone methyltransferase that regulates (among other substrates) the *Hox* genes, which control proliferation in hematopoietic cells. Several breakpoints in *MLL* have been identified and are located in close proximity to topoisomerase II-DNA cleavage sites that are induced by etoposide (Felix et al., 1995; Lovett et al., 2001a; Lovett et al., 2001b; Whitmarsh et al., 2003; Robinson et al., 2008).

In addition to treatment-related leukemias, ~80% of infants with AML or acute lymphoblastic leukemia (ALL) display translocations that involve the *MLL* gene (Strick et al., 2000; Felix et al., 2006; McClendon and Osheroff, 2007; Deweese and Osheroff, 2009). The chromosomal translocations associated with these cancers have been observed *in utero*, indicating that infant leukemias are initiated during gestation. Epidemiological studies indicate that the risk of developing these infant leukemias increases >3-fold by the maternal consumption (during pregnancy) of foods that are rich in bioflavonoids and other naturally occurring topoisomerase II poisons (Ross et al., 1994; Greaves, 1997; Spector et al., 2005). Consistent with this finding, treatment of cultured human cells with dietary bioflavonoids induces cleavage within the *MLL* gene (Strick et al., 2000). Furthermore, compounds that display the highest activity in *in vitro* topoisomerase II-DNA cleavage assays show the greatest propensity to generate breaks in the *MLL* gene in cultured cells (Strick et al., 2000).

Thus, the same topoisomerase II-active phytochemicals that help to maintain health in human adults appear to have harmful effects on developing embryos.

Conclusions

Topoisomerase II poisons play critical roles in curing and preventing cancer. However, in some cases, they are linked to the development of specific forms of the disease. Many topoisomerase II poisons are derived from natural sources, and some of the most important compounds that affect the type II enzyme originate in plants. Many of these compounds are routinely consumed as part of the human diet. Clearly, phytochemicals are a rich source of structurally and mechanistically diverse topoisomerase II poisons. It is likely that plants will continue to yield novel anticancer agents that function through the type II enzyme, and drug discovery efforts in this area are strongly encouraged.

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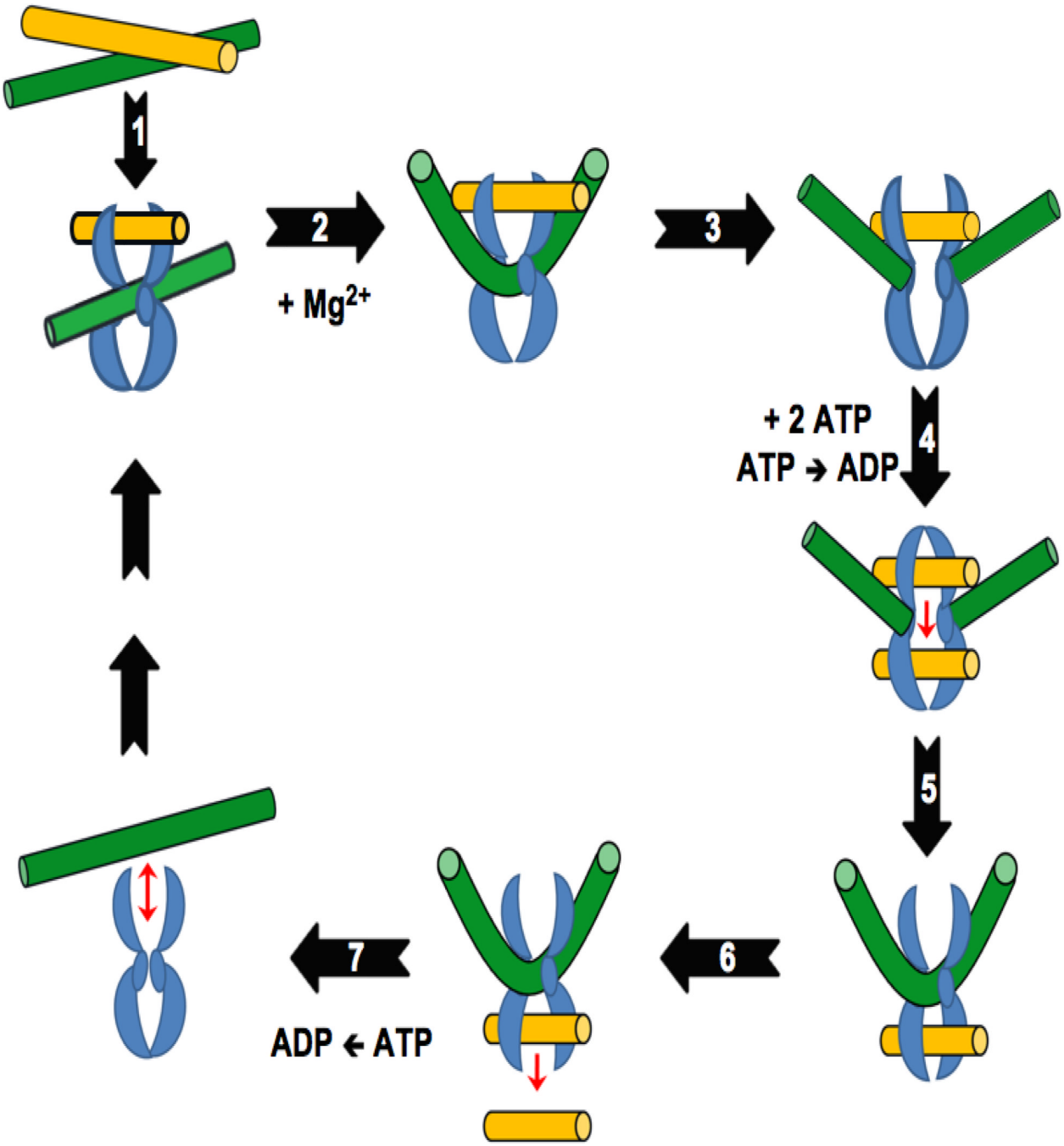


Figure 1.
Catalytic cycle of type II topoisomerases. Adapted from (Ketron and Osheroff, 2013). The homodimeric enzyme is shown in blue, the DNA double helix that is cleaved and acts as the DNA gate (G-segment) is shown in green, and the double helix that is transported through the DNA gate (T-segment) is shown in yellow. Details of the individual reaction steps are described in the text.

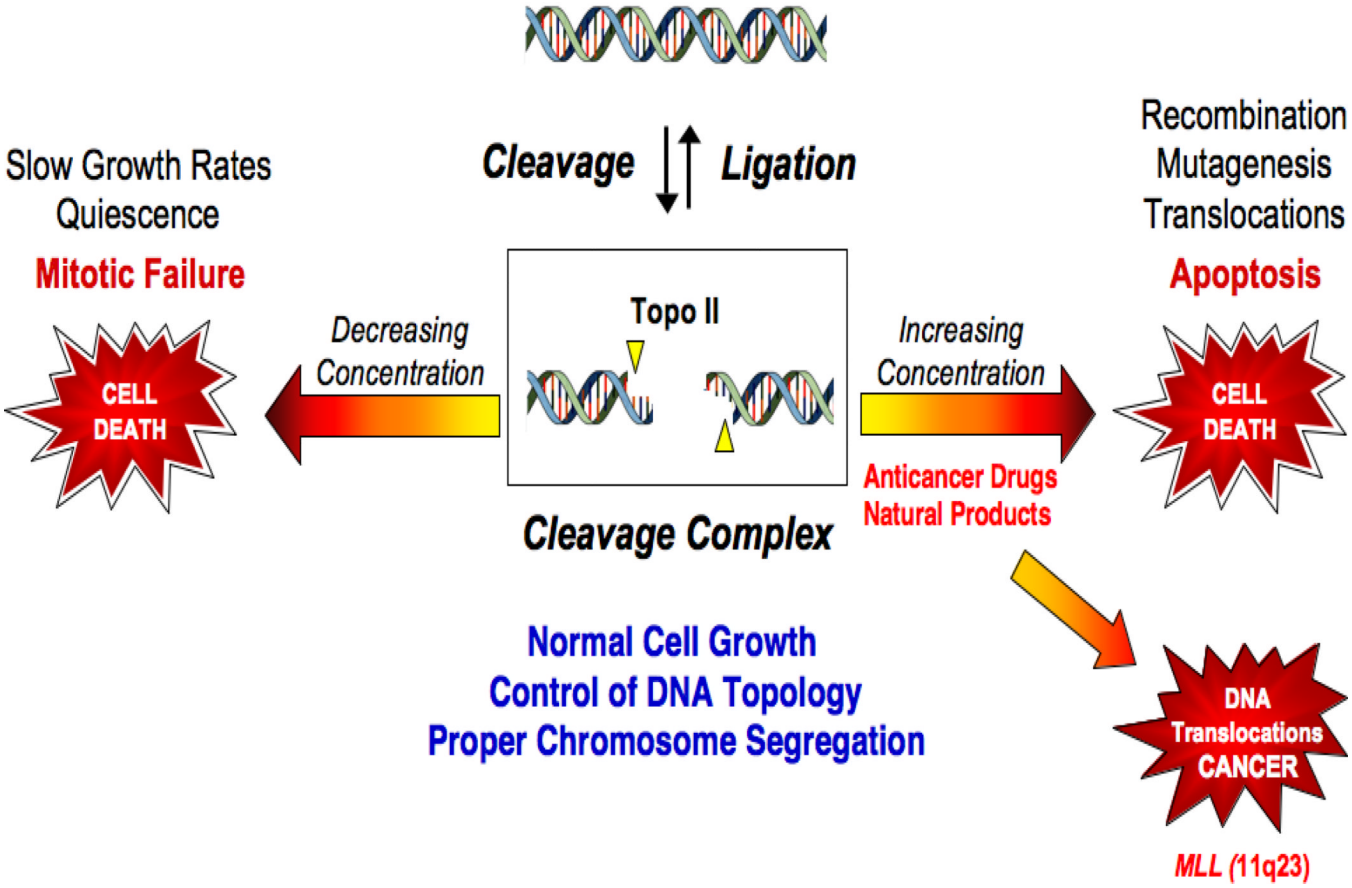


Figure 2.

Topoisomerase II-DNA cleavage complex equilibrium. Adapted from (Deweese and Osheroff, 2009). The formation of covalent DNA cleavage complexes is required for topoisomerases to perform their critical cellular functions. If the level of topoisomerase II-DNA cleavage complexes falls below threshold levels (left arrow), cells are unable to segregate their chromosomes and ultimately die of mitotic failure. If the level of cleavage complexes becomes too high (right arrow) the actions of DNA tracking systems can convert these transient complexes to permanent double-stranded breaks. The resulting DNA breaks, as well as the inhibition of essential DNA processes, initiate recombination/repair pathways and generate mutations, chromosome translocations, and other DNA aberrations. If the strand breaks overwhelm the cell, they can trigger apoptosis. This is the basis for the actions of several widely prescribed anticancer drugs that target topoisomerase II. However, if the increase in enzyme-mediated DNA strand breaks does not kill the cell, mutations or chromosomal aberrations may persist in surviving populations. In some cases, exposure to topoisomerase II-targeted agents has been associated with the formation of acute myeloid leukemias that involve the *MLL* (mixed lineage leukemia) gene at chromosome band 11q23 (lower right arrow).

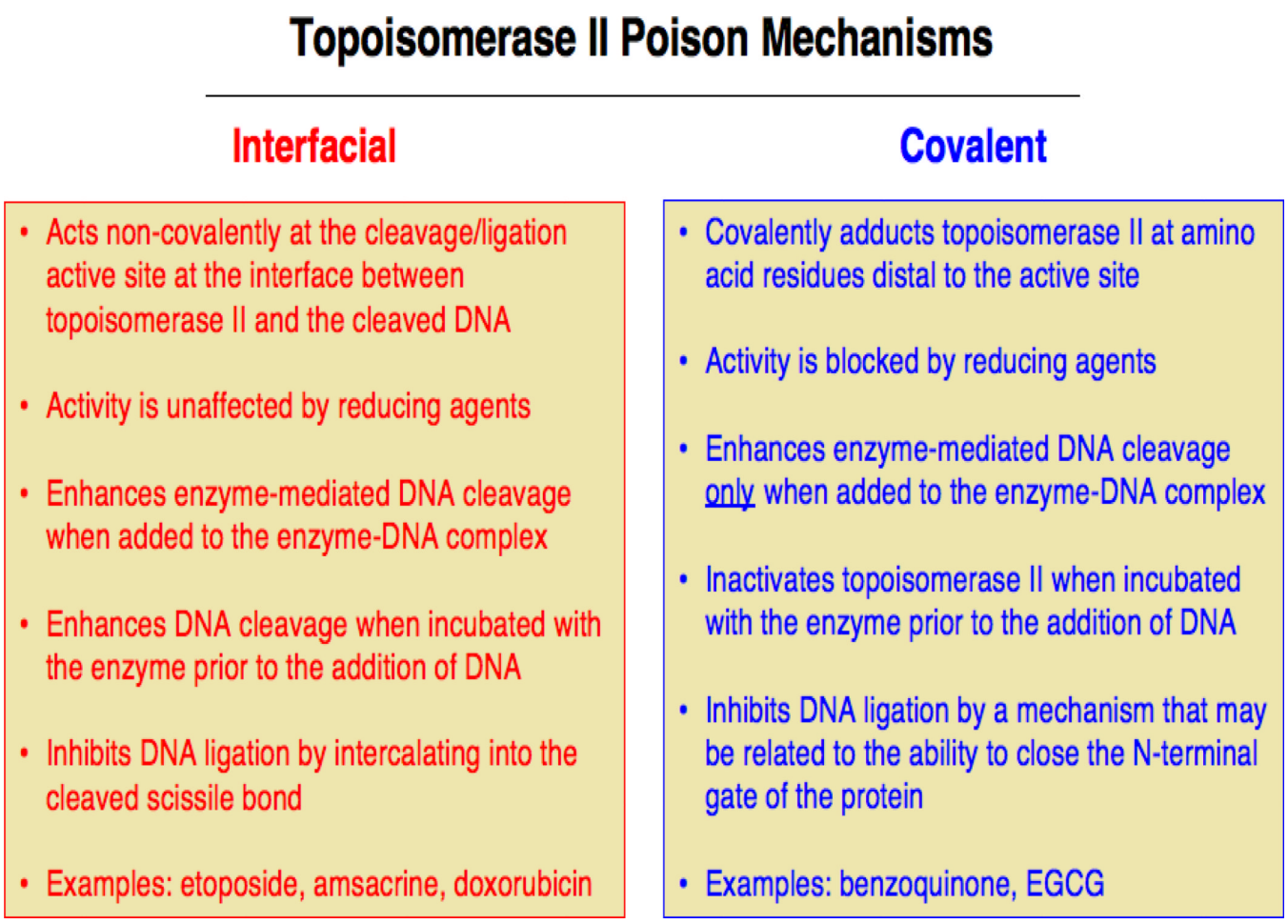


Figure 3.
Distinguishing characteristics of interfacial *vs.* covalent topoisomerase II poisons. Details are provided in the text.

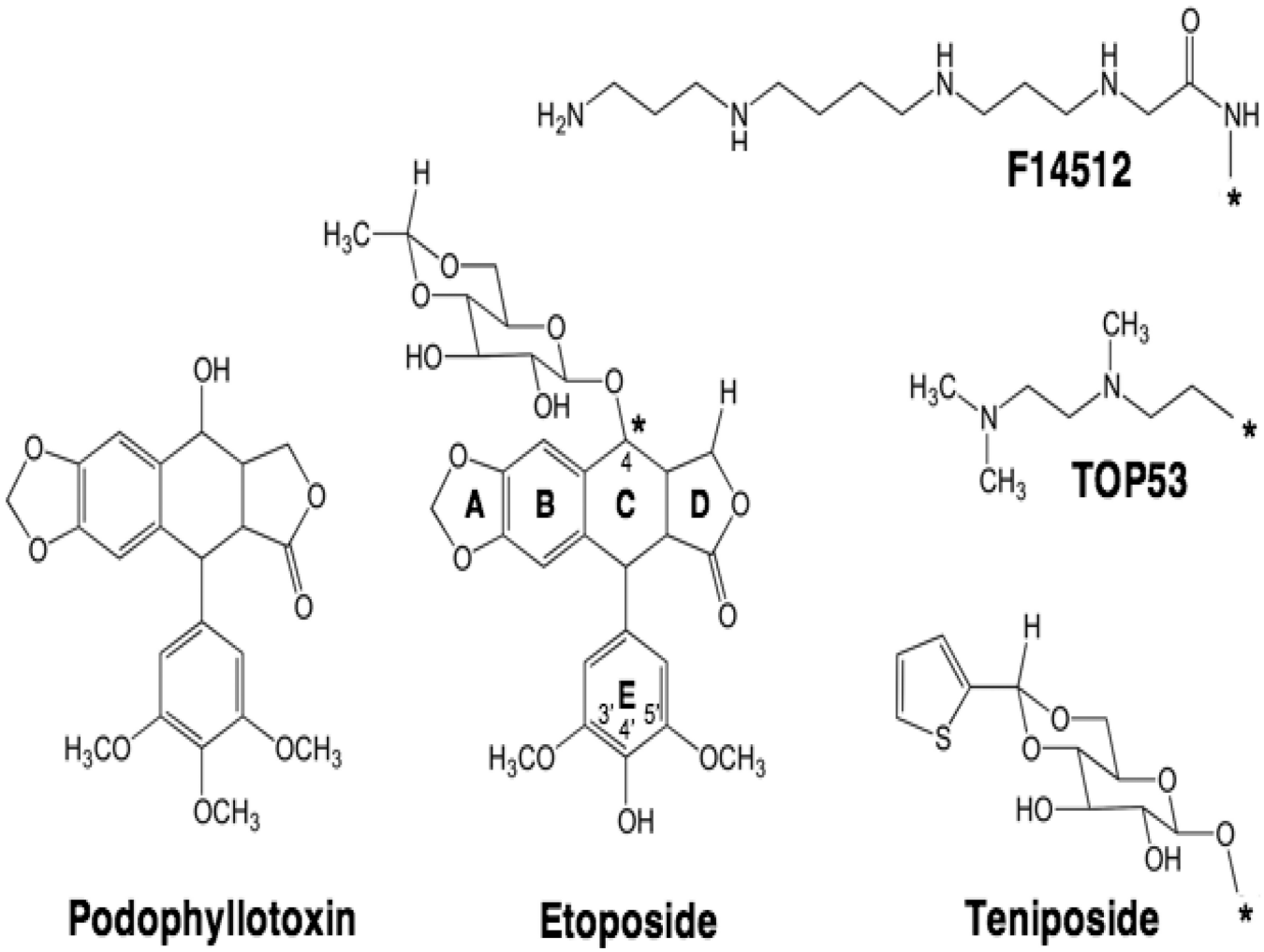


Figure 4.

Structures of podophyllotoxin and demethylated epimers. Podophyllotoxin and etoposide are shown. C-4 (*) substitutions of the glycosidic moiety on etoposide are shown for teniposide, TOP-53 and F14512.

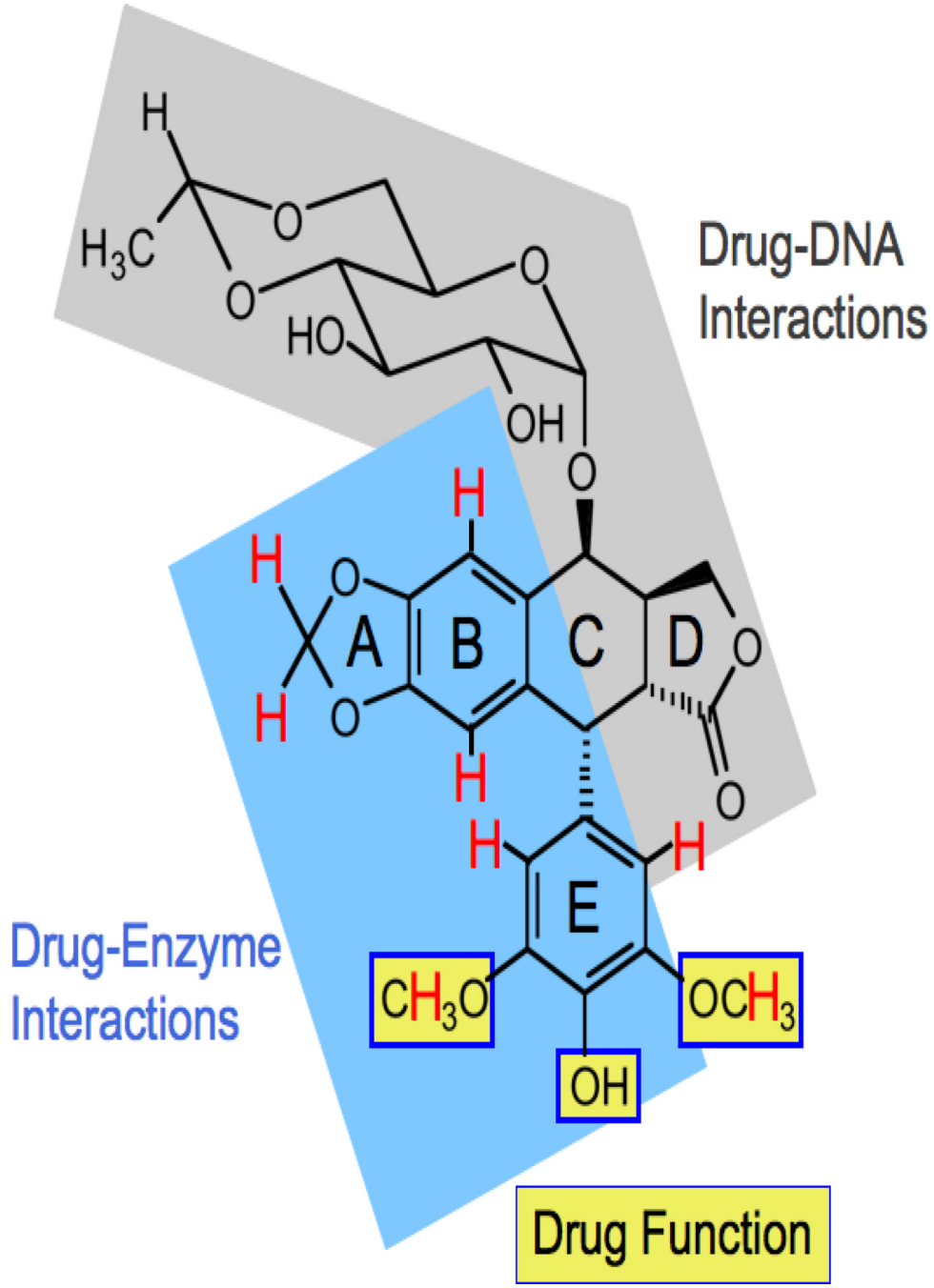
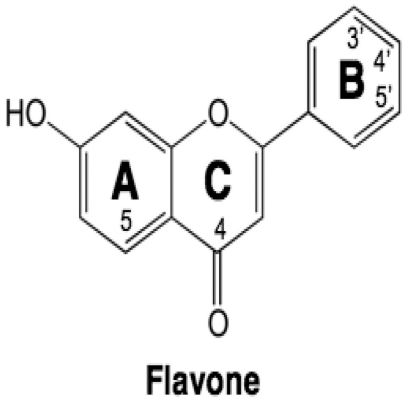


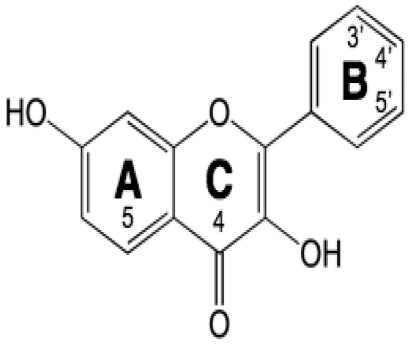
Figure 5.

Summary of etoposide substituents that interact with human topoisomerase IIα. Adapted from (Pitts et al., 2011). Protons that interact with the enzyme (as determined by saturation transfer difference NMR spectroscopy) are shown in red. The blue region on etoposide, including portions of the A–, B– and E–rings, has been proposed to interact with topoisomerase II in the binary drug-enzyme complex. E–ring substituents highlighted with yellow boxes are important for drug function and interact with the enzyme, but did not appear to contribute significantly to binding. It has been proposed that interactions between etoposide and DNA in the ternary complex (shaded in gray) are driven primarily by the D-ring, with additional contributions from the C-4 sugar.



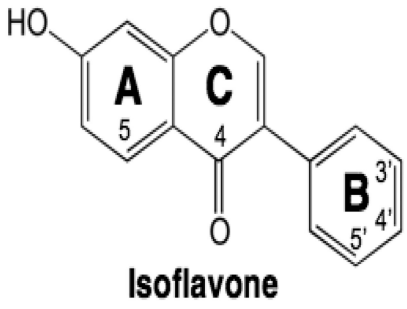
Flavone

Flavones	Position 5	3'	4'	5'	Topo II Activity
Luteolin	OH	OH	OH	H	++
Apigenin	OH	H	OH	H	+
Diosmetin	H	OH	OCH_3	H	+
Chrysin	OH	H	H	H	+/–



Flavonol

Flavonols	Position 5	3'	4'	5'	Topo II Activity
Myricetin	OH	OH	OH	OH	++
Quercetin	OH	OH	OH	H	++
Kaempferol	OH	H	OH	H	+
Fisetin	H	OH	OH	H	+
Isorhamnetin	OH	OCH_3	OH	H	–
Galangin	OH	H	H	H	–



Isoflavone

Isoflavones	Position 5	3'	4'	5'	Topo II Activity
Genistein	OH	H	OH	H	+++
Daidzein	H	H	OH	H	–
Biochanin A	OH	H	OCH_3	H	–

Figure 6.

Structures of selected bioflavonoids. Adapted from (Bandele and Osheroff, 2007). Flavones, flavonols, and isoflavones are shown, and the ability of each to enhance topoisomerase II-mediated DNA cleavage is indicated as >8-fold (+++), 6- to 8-fold (++), 3- to 6-fold (+), 2- to 3-fold (+/−), or <2-fold (−) over baseline.

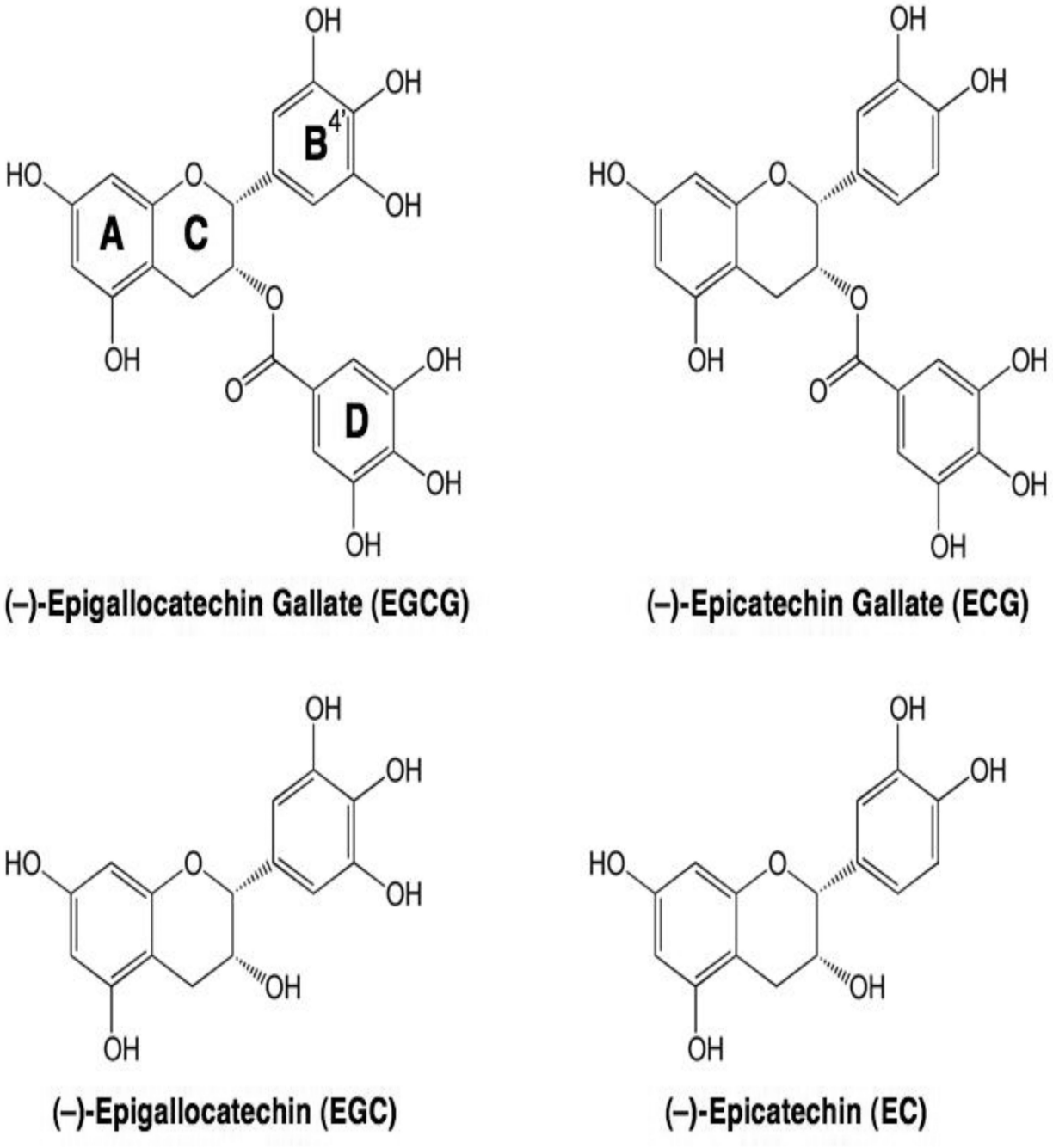


Figure 7.
Structures of EGCG and related catechins.

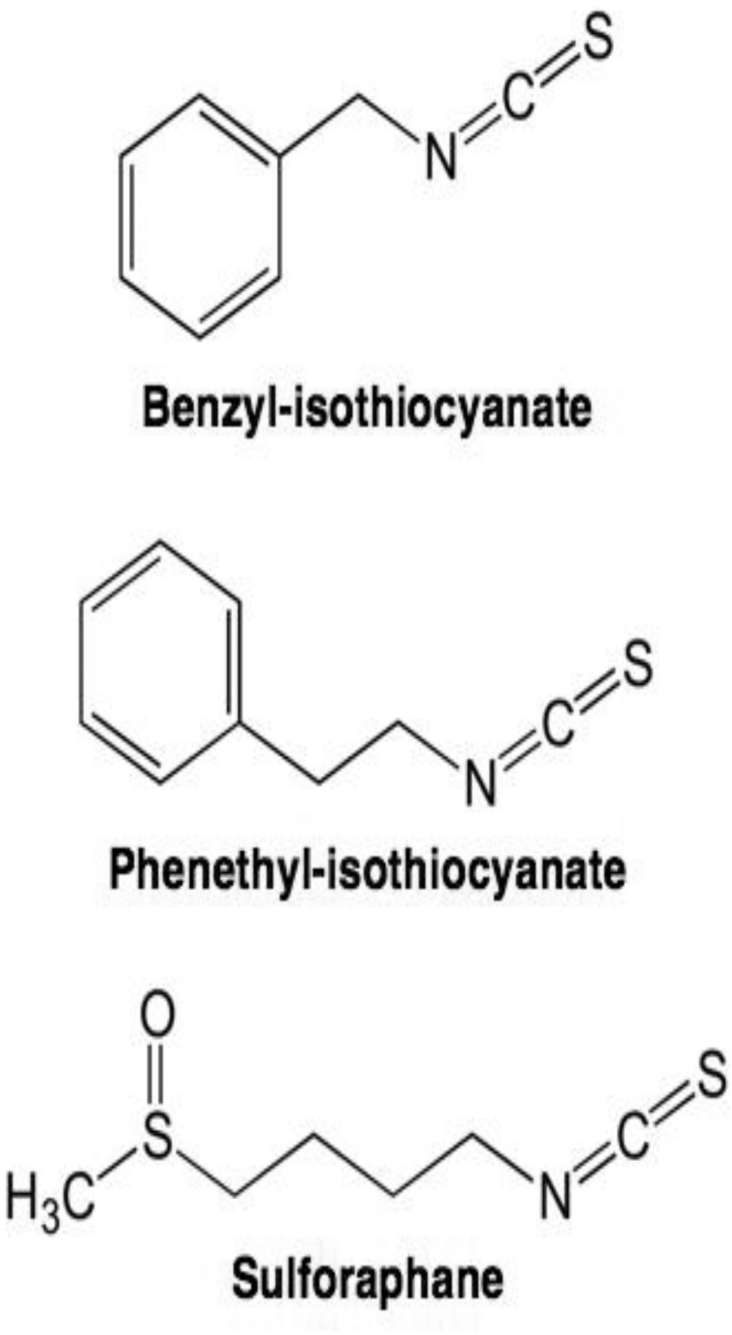


Figure 8.
Structures of selected isothiocyanate-based topoisomerase II poisons.

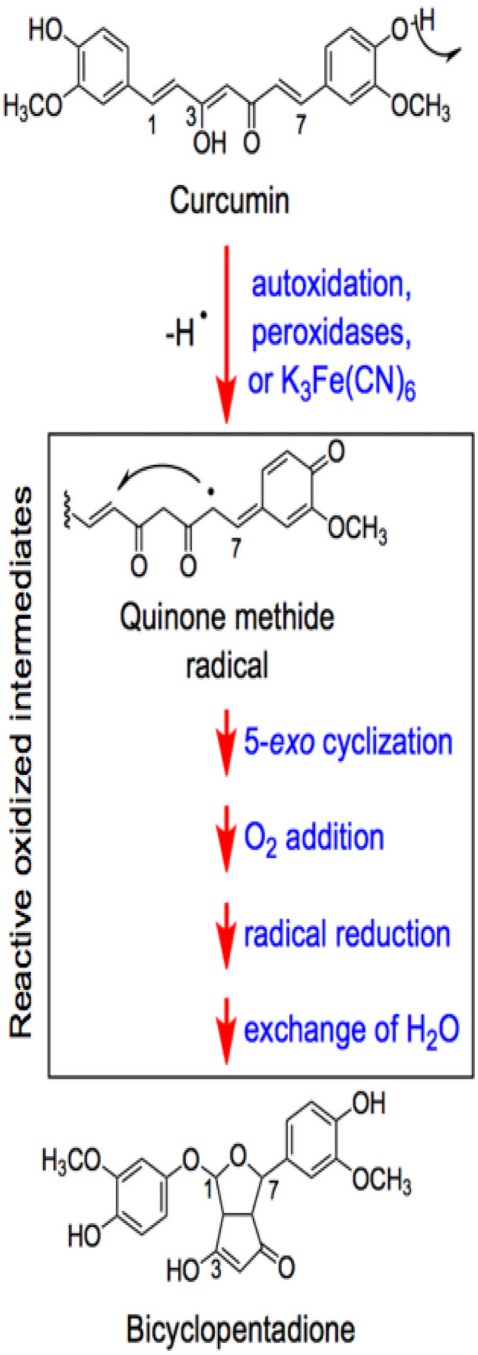


Figure 9.
Oxidative transformation of curcumin. Adapted from (Griesser et al., 2011)