

Topoisomerase I Poisons and Suppressors as Anticancer Drugs

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Abstract: Inhibitors of topoisomerase I constitute a novel family of antitumor agents. The camptothecin derivatives topotecan and irinotecan represent new weapons in our arsenal for battling human cancer. These two drugs act specifically at the level of the topoisomerase I-DNA complex and stimulate DNA cleavage. This mechanism of action is not restricted to the camptothecins. Numerous topoisomerase I poisons including DNA minor groove binders such as Hoechst 33258 and DNA intercalators such as benzophenanthridine alkaloids and indolocarbazole derivatives have been discovered and developed. Another important group of topoisomerase I inhibitors contains drugs which prevent or reverse topoisomerase I-DNA complex formation. Many of these topoisomerase I suppressors are natural products (-lapachone, diospyrin, topostatin, topostin, favonoids) which are believed to interact directly with the enzyme. This review is concerned with the different families of topoisomerase I poisons and suppressors. Their origin, chemical nature and mechanism of action are presented. The relationships between drug binding to DNA and topoisomerase I inhibition are discussed.

A large number of anticancer drugs used in the clinic were discovered before the 1970's (Fig. 1). For example, the antitumor drugs 5-fluorouracil, cyclophosphamide and cisplatinum have been routinely used for more than 25 years. The anthracyclines also have a long history in the treatment of cancer. Doxorubicin (adriamycin) and daunomycin were isolated in the 1960s from *Streptomyces*. Almost forty years after their discovery, they are still amongst the most widely prescribed and effective anticancer drugs [1]. The clinical formulations, administration schedules and drug combinations have often changed but the active principle remains the same. Only two main categories of drugs have been introduced into clinical practise during the past ten years. The first category contains the taxoids and the second one the camptothecins. In both cases, the original molecule is a natural product isolated from plants, as was the case for the vincaalcaloids and the epipodophyllotoxins. There are numerous examples in pharmacology, not restricted to the field of antitumor drugs, where Nature provided the original principle which Man was able to exploit and transform to make it more active, less toxic and/or more easily manageable at the clinical level [2].

Researchers in molecular and cellular biology regularly identify novel potential targets which are specific to or selective for the cancer cell [3]. Biologists have characterized biochemical differences between cancer and normal cells which may be exploited by medicinal chemists for the design of tumor-specific drugs [4]. The problem is that these biochemical differences are generally very subtle and varied in nature and thus are difficult to exploit. However, there are a few examples where the opportunities opened up by molecular biology have been successfully exploited by medicinal chemists to design new drugs or to find the specific target of existing drugs. In this context, the development of the camptothecins targeting topoisomerase I is exemplary.

Topotecan (TPT, Hycamtin®) and irinotecan (CPT-11, Camppto®) were introduced in the clinic in 1997, i.e. more than thirty years after the isolation of camptothecin (CPT) from an ethanolic extract of the plant *Camptotheca acuminata* (Decaisne, Nyssaceae) known as the tree Xi Shu in Southeastern provinces of China [5,6]. The alkaloid has also been found in the Asian Tree *Mappia foetida* [7]. CPT was discovered during a National Cancer Institute cytotoxic drug screening in 1966 [8]. Soon after the preclinical results revealed a marked antineoplastic activity, the drug was tested clinically in the form of a water soluble sodium carboxylate but its clinical development was discontinued in the early 1970s due to the appearance of unacceptable side effects such as hemorrhagic

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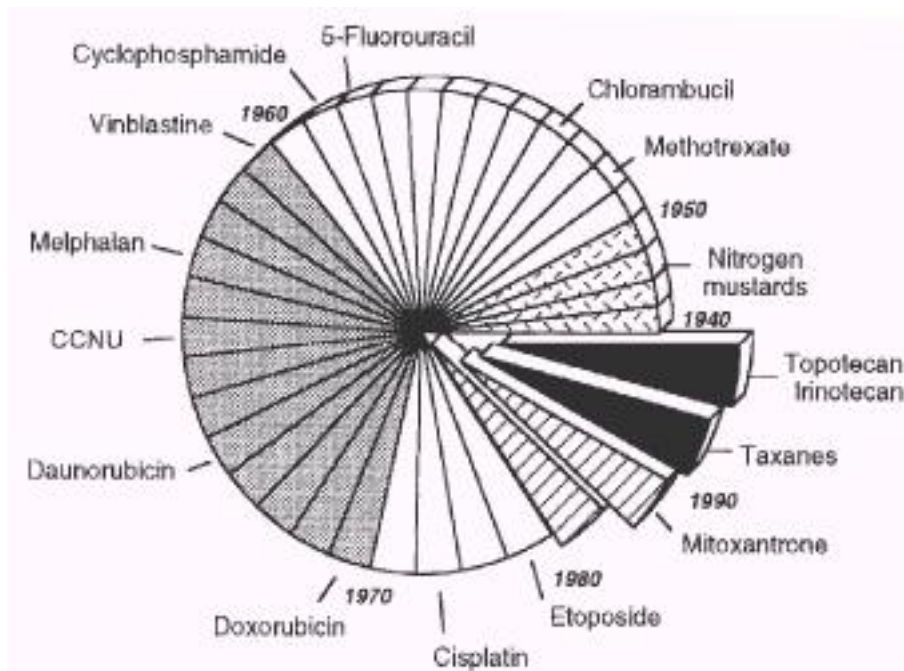


Fig. (1). Discovery of antitumor drugs.

cystitis and severe myelosuppression [9]. The major discovery in 1985 of the molecular target of CPT, the nuclear enzyme DNA topoisomerase I [10,11], re-initiated the development of camptothecins as anticancer drugs. Over the last ten years, a large number of CPT analogs have been developed [12-16]. Extensive structure-activity data on CPT have given a coherent picture that may help in the development of improved versions of these drugs. Topotecan and irinotecan are approved for the treatment of fluoropyrimidine-refractory ovarian and colorectal cancer, respectively [18-22]. Other CPT analogs such as 9-amino-CPT, 9-nitro-CPT, DX-8951f, GG-211, CKD602 and the homocamptothecin derivative BN 80915 are currently undergoing clinical trials (Fig. 2). Although all these drugs share a strong structural similarity and a common mechanism of action, they present interesting differences. For example, CPT-11 distinguishes itself by being a prodrug (releasing SN-38), 9-nitro-CPT and BN 80915 are non-water soluble compounds while the other molecules can be formulated as salts. These drugs share the unstable six-membered lactone ring of CPT, except BN 80915 which contains a stabilized seven membered lactone. Due to the generally narrow therapeutic index of anticancer agents, differences in cytotoxicity, as well as tissue distribution and metabolism can be expected to translate into distinct clinical profiles. The on-going development of CPT analogs should therefore provide novel chemotherapeutic agents to fight a variety of solid tumors and disseminated malignant cells.

DNA topoisomerases are ubiquitous enzymes alleged to resolve topological problems that arise

during various nuclear processes including transcription, recombination, chromatin assembly and chromosome partitioning at cell division [23,24]. Topoisomerase I triggers transient single-strand breaks in DNA and passes the other strand through the cleavage site before resealing the break. The reaction between double-stranded DNA and topoisomerase I produces a covalent 3'-phosphotyrosyl adduct, usually referred to as the cleavable complex. Under physiological conditions, the DNA cleavage and ligation reactions catalysed by the enzyme are tightly coordinated and the covalent intermediate is barely detectable. The cleavage is coupled with the religation to restore continuity to the DNA duplex [25]. A number of drugs such as CPT can convert topoisomerase I into a cell poison by blocking the religation step, thereby enhancing the formation of persistent DNA breaks responsible for cell death [26]. The single-strand breaks induced by topoisomerase I are considered non-toxic to the cells because they can be efficiently and rapidly repaired. But their conversion into double-strand breaks, formed when the cleaved complex is encountered by a moving replication fork, generates potentially lethal lesions [27-29].

The reaction of topoisomerase I with DNA is schematically illustrated in Fig. 3. The topoisomerization cycle begins with the binding of the enzyme to the double-stranded superhelical DNA. At this point the interaction is controlled to a large extent by the surface and charge complementarity of the two reacting species. The conformational adaptation of the two partners results in the formation of a tight "kissing" complex whereby the active site tyrosine of human

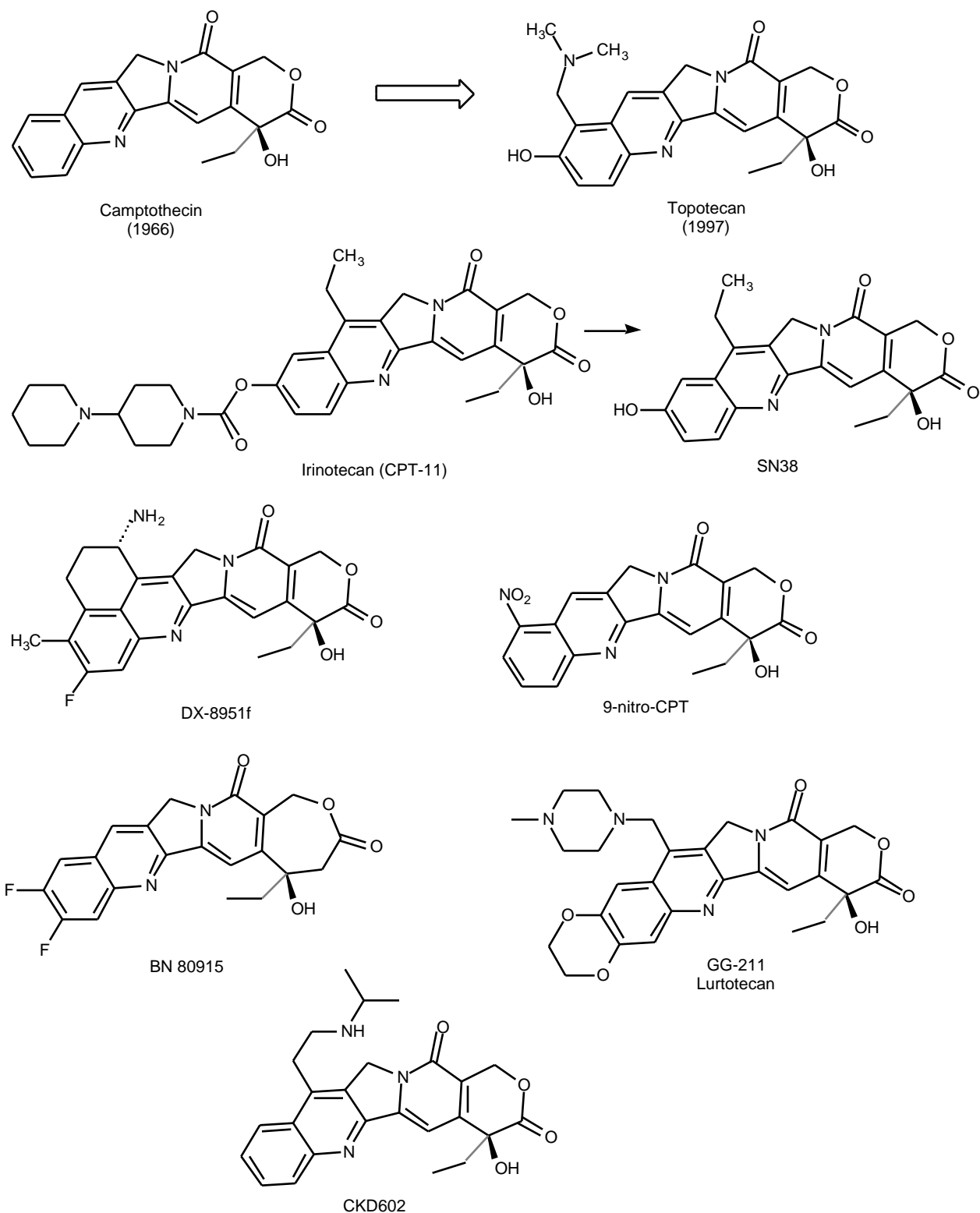


Fig. (2). CPT and its derivatives.

topoisomerase I (Y723) is brought into a favorable position for attack of the facing DNA strand and subsequent formation of a covalent adduct. The "bite" of topoisomerase I induces the release of the superhelical tension in DNA which is relaxed by a mechanism called controlled rotation [30]. Once the DNA is partially relaxed, the covalent intermediate is

religated, leaving intact the Y723 residue ready for a subsequent "kiss and bite" catalytic cycle. Depending on the point of the reaction cycle at which the drug will act, i.e. before or after the "bite", topoisomerase I inhibitors will reduce or increase the extent of DNA cleavage.

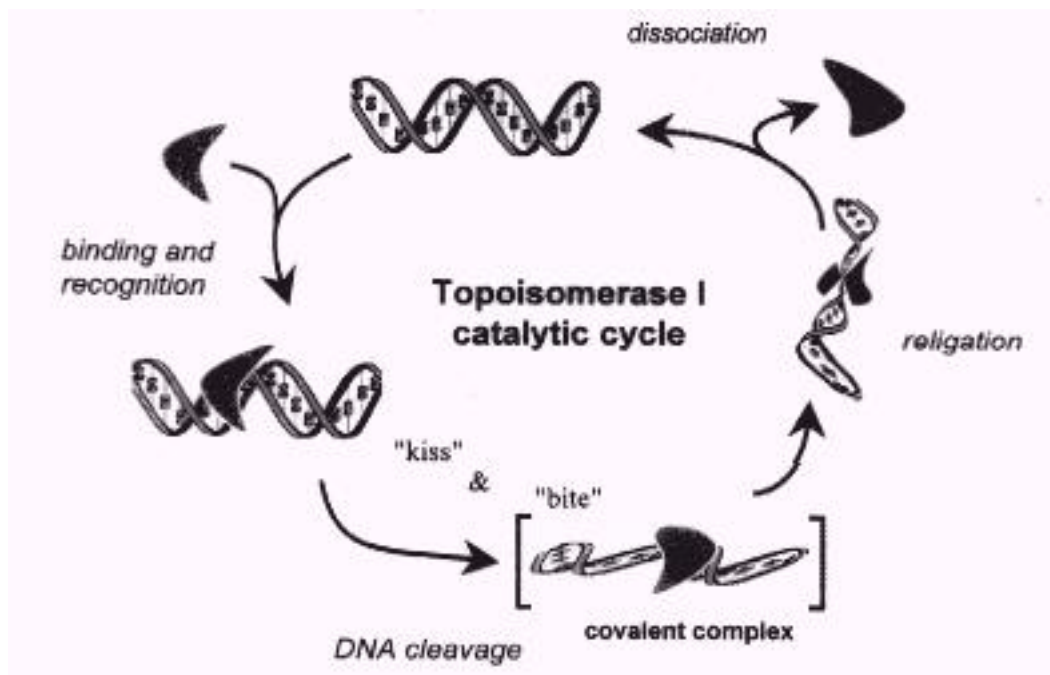


Fig. (3) The topoisomerization reaction. Schematically, the cyclic "kiss and bite" process involves binding of topoisomerase I to DNA, followed by DNA cutting and then religation.

CPT is arguably one of the most potent topoisomerase I inhibitors. However, in addition to CPT and its derivatives, a relatively important number of topoisomerase I inhibitors with diverse structures and origins have been identified. The drugs, which include a variety of natural products, can be divided into two classes. Topoisomerase I suppressors correspond to compounds which inhibit the enzyme but do not stabilize the intermediate DNA-topoisomerase I covalent complex. Their interaction with the free enzyme inhibits binding of topoisomerase I to the DNA cleavage site, thus preventing all subsequent steps in the catalytic cycle. In contrast, the topoisomerase I poisons, like CPT, act after the cleavage of DNA by the enzyme and inhibit the religation. In this case, the drug may freeze the topoisomerase I-DNA complex via three possible routes: (i) the enzyme binds to the preformed drug-DNA complex, (ii) the drug specifically recognizes the enzyme-DNA binary complex, and (iii) the drug-enzyme association interacts with DNA [31]. The mechanism of action of CPT and the advantages and disadvantages associated with the camptothecin-based inhibitors that are targeted to the topoisomerase I-DNA cleavable complex have been discussed in several review articles [32-34]. These articles are generally focussed on CPT and its derivatives with little mention of the other classes of inhibitors. In this review I hope to reverse this situation by describing the handful of non-CPT topoisomerase I inhibitors that have been discovered as suppressors or poisons. It is impossible within a constrained space to provide an

encyclopedic overview of the current state of the topoisomerase I field. However, in this review I wish to present an overview of the different families of inhibitors known so far and to provide a framework and some guidelines for pursuing the design of anticancer drugs targeting the enzyme.

Inhibition of Topoisomerase I via Direct Binding to the Enzyme

-Lapachone (Fig. 4), which is present in the bark of the South American Lapacho tree (*Tabebuia avellanedae*), is not a conventional topoisomerase I inhibitor. Unlike CPT, this compound does not stabilize the DNA-topoisomerase I covalent complex [35]. It is suspected that -lapachone binds directly to the enzyme to prevent DNA unwinding by topoisomerase I. The cytotoxicity of -lapachone against tumor cells, in particular prostate and breast cancer cells, is a direct result of drug-induced apoptosis [36,37]. A similar mechanism of enzyme inhibition has been reported with other natural products (Fig. 4) from plants such as a variety of naphthoquinone derivatives including alkannin and shikonin isolated from roots of the plant *Lithospermum erythrorhizon* [38,39] and with AKBA (acetyl-11-keto- -boswellic acid), the main compound of frankincense which is the gum resin of *Boswellia serrata* and *Boswellia carterii* [40]. In contrast, the antitumor bis-naphthoquinone diospyrin isolated from the stem bark of *Diospyros montana* Roxb. [41,42] induces topoisomerase I-mediated DNA cleavage *in*

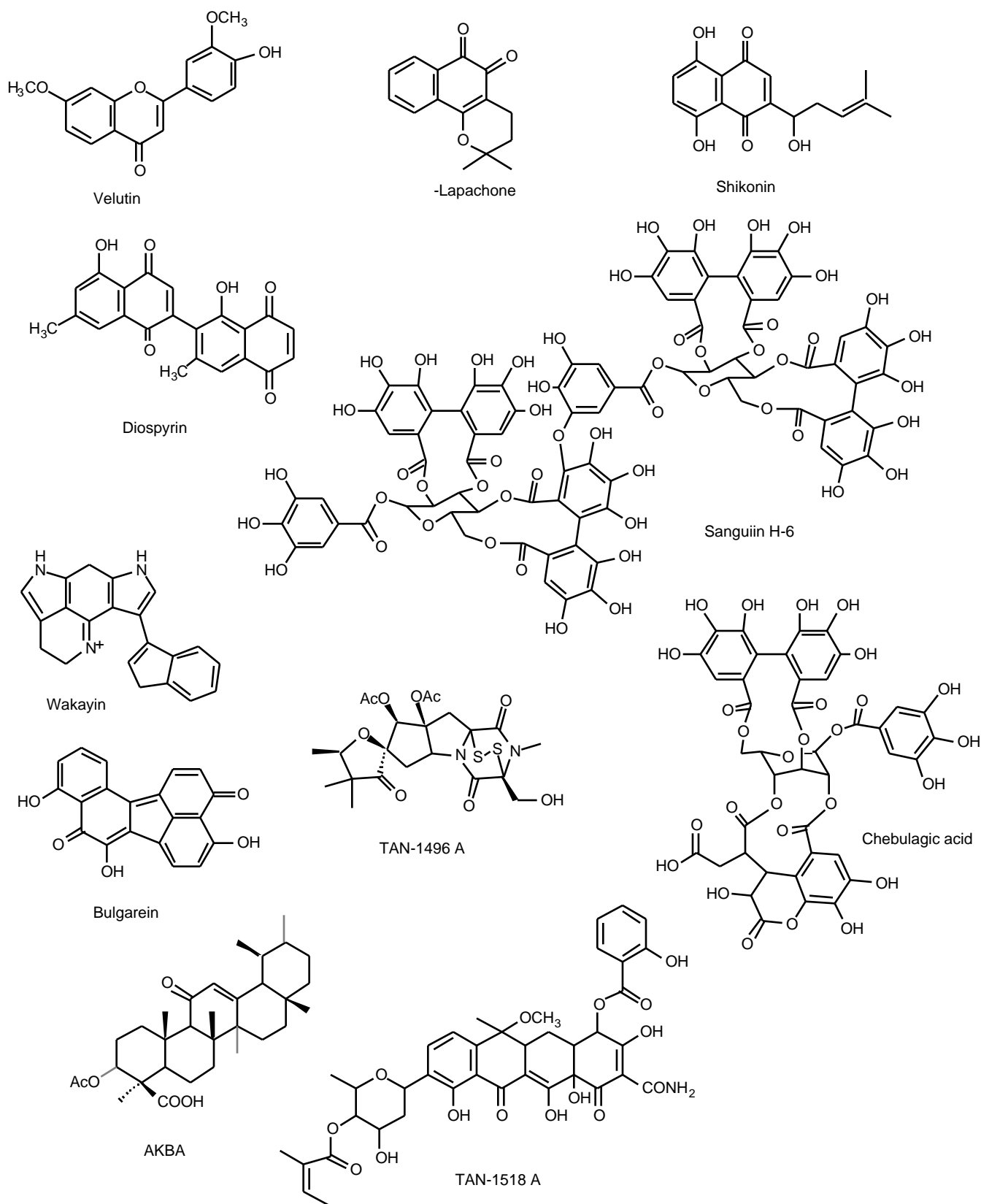


Fig. (4). Naturally-occurring topoisomerase I suppressors and poisons.

in vitro [43]. This plant-derived naphthoquinoid which exerts both anticancer and antiparasitic activities, is 10-times more efficient against the topoisomerase I from *Leishmania donovani* than against other eukaryotic

topoisomerase I and has no effect on topoisomerase II [43]. Like -lapachone, diospyrin is presumed to bind directly to the enzyme, but unlike the other naphthoquinone its stabilizes the covalent complexes.

Hydroxynaphthoquinones are effective chelators of divalent metal ions. For this reason, these compounds may inhibit topoisomerases by binding to a zinc finger domain of the protein. A recent study supports this hypothesis because a correlation has been found between the ability of shikonin analogs to complex Zn^{2+} and their topoisomerase I inhibition properties [44]. Other suppressors of topoisomerase I activity have been reported. These include corilagin and the ellagitannin chebulagic acid (Fig. 4) both isolated from the plant *Erodium stephanianum* found in China [45,46]. The dietary anticancer agent ellagic acid [C47] and the dimeric ellagitannin isolated from *Sanguisorba officinalis*, sanguin H-6, were also shown to inhibit DNA relaxation by topoisomerase I and to prevent CPT from stabilizing the covalent intermediate, presumably via direct interaction with the enzyme [48]. Velutin extracted from the tree *Lethedon tannaensis* found in New Caledonian, certain curcuminoids [49] and TAN-1518 A (Fig. 4) which is a naphthacenecarboxamide antibiotic with suppressive activity against Meth A fibrosarcoma *in vivo* [50,51], interfere with the DNA relaxation activity of topoisomerase I. All these natural products do not stimulate DNA cleavage by topoisomerase I like CPT and presumably act via an inhibition of the covalent attachment of the enzyme to the double helix. Their inhibitory action may not be specific to topoisomerase I. For example, -lapachone not only suppresses topoisomerase I activity but also inhibits topoisomerase II, reverse transcriptase and DNA polymerase [52].

Another group of topoisomerases I inhibitors are structurally related to lipids. Topostatin is a dual topoisomerase I and II inhibitor isolated recently from the culture filtrate of the soil organism

Thermomonospora alba. Although it inhibits the relaxation of DNA by topoisomerase I, this compound does not stabilize covalent DNA-enzyme complexes and is not toxic to tumor cells [53]. Topostatin possesses a long lipid-type side chain attached to an unusual cyclic pseudopeptide (Fig. 5). This is not a unique case. Andoh and co-workers have isolated lipid-containing amino acids and peptides from several bacteria. These compounds, such as topostin B567 (Fig. 5) isolated from a culture broth of *Flexibacter topostinus*, were shown to inhibit topoisomerase I [54-57]. A variety of acidic lipids including phospholipids, fatty acids and ceramide sulfate can interfere with topoisomerase I suggesting thus that both lipophilicity and anionic nature are important for the enzyme inhibition [58,59]. These topoisomerase I suppressors may interact with topoisomerase I even in the absence of DNA.

Different analogs of tyrphostin which is essentially a potent and selective blocker of protein tyrosine kinases, might bind directly to topoisomerase I. The reaction of topoisomerase I with DNA results in the formation of a covalent bond between the tyrosine residue at position 723 of human topoisomerase I and a 3' phosphate group along the DNA backbone. This chemical reaction resembles the protein kinase-mediated tyrosine phosphorylation process. It is therefore not entirely surprising that tyrosine kinase inhibitors such as the tyrphostin derivative AG-555 (Fig. 6) interacts directly with topoisomerase I thus preventing its DNA relaxation activity [60]. The antiproliferative activity of some tyrphostin derivatives may be due to their inhibitory activity against both protein tyrosine kinases and topoisomerase I. The interaction of AG-555 with topoisomerase I could block

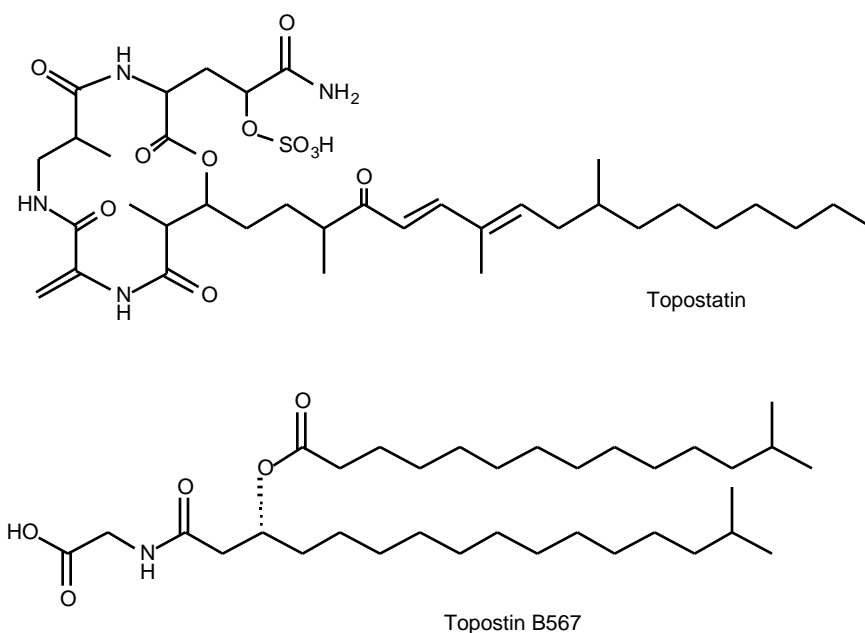


Fig. (5). Lipid-type suppressors.

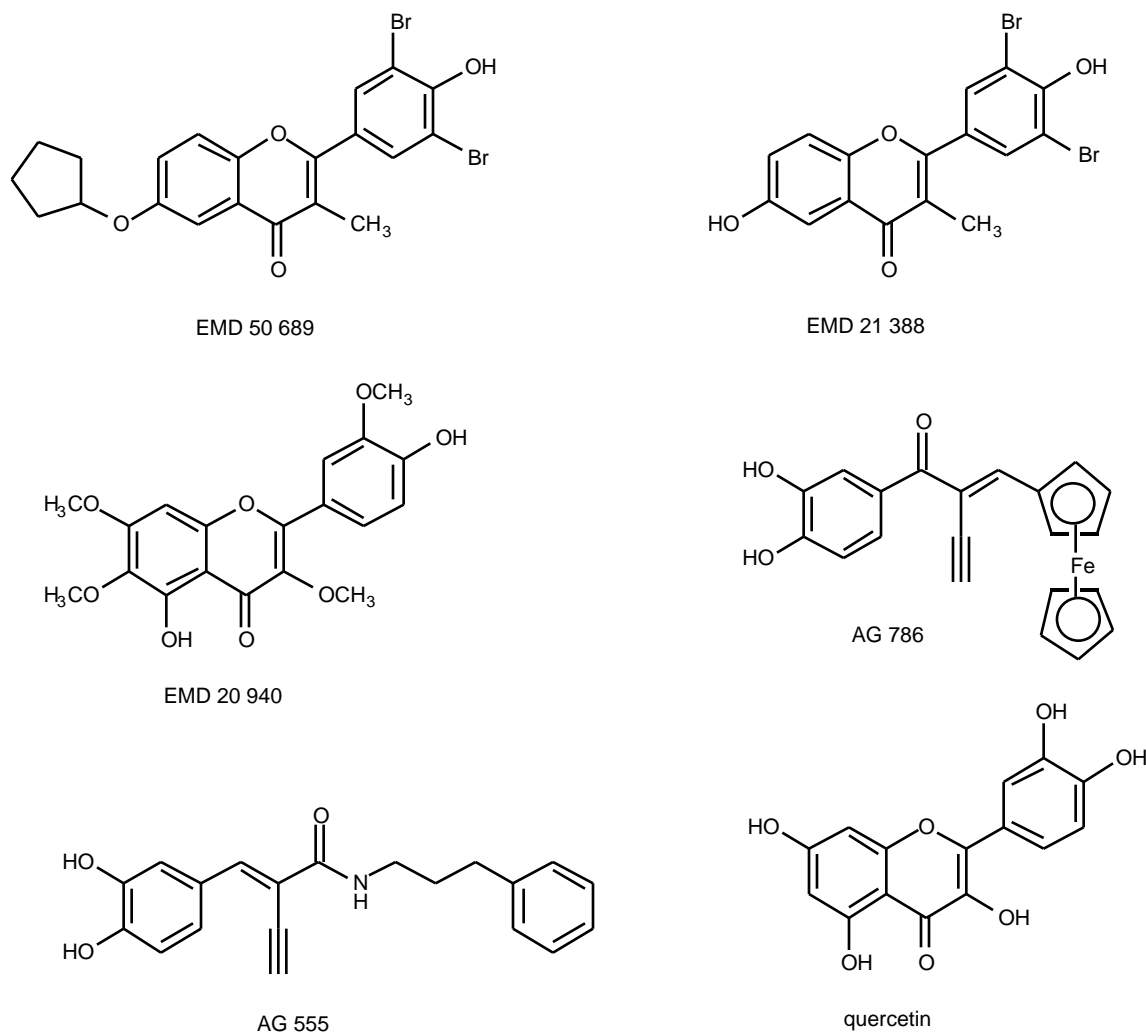


Fig. (6). Tyrphostin and quercetin analogs.

the catalytic site of the enzyme and/or may cause conformational changes in the enzyme so as to reduce its ability to bind to DNA. Interestingly, the action of AG-555 is specific to topoisomerase I. Other DNA binding enzymes such as DNA polymerase I, DNA ligase and Mo-MuLV reverse transcriptase are not affected [60]. However, other tyrphostin derivatives have no effects on topoisomerase I but inhibit HIV-1 integrase [61]. Markovits et al., [62] also found that an analog of tyrphostin, AG-786 which has a ferrocene structure (Fig. 6), totally inhibited topoisomerase I at a concentration of 20 μM . In addition, they found that certain analogs of the tyrosine kinase inhibitor erstatin inhibited topoisomerase I but at a concentration of 100 μM . Neither the erbstatin nor the tyrphostin derivatives stabilized topoisomerase I-DNA covalent complexes.

Direct binding to the free form of topoisomerase I, but not the DNA-bound form, has been evidenced with flavonoids structurally related to the natural product quercetin found in many vegetables and fruits. Quercetin and related flavonoids are toxic to tumor cells and potentiate the anticancer activity of DNA-

damaging drugs such as cisplatin [63,64]. Phase I clinical trials with quercetin itself revealed evidence of antitumor activity [65]. The flavone quercetin and the isoflavone genistein are potent inhibitors of topoisomerase II but generally exert a much weaker effect on topoisomerase I [66-68]. The brominated flavones EMD 21 388 and EMD 50 689 (Fig. 6) were found to trap topoisomerase I in its free form blocking the access to the DNA substrate and thus preventing the catalytic activity. Analogs of these two compounds lacking the Br atoms do not bind to the enzyme. The bromo substituent together with the methyl group are forcing these compounds into nonplanar structures that cannot intercalate into the DNA double helix. The hydrophobic substituent at position 6 is also essential for interaction with topoisomerase I [69]. In contrast, quercetin and a few natural flavones (e.g. acacetin, kaempferol) stabilize the covalent enzyme-DNA complex. These drugs do not affect the cleavage reaction by the enzyme but prevent the subsequent religation reaction [69]. 2-Phenylthiochromen-4-one derivatives related to flavones have also been shown to inhibit topoisomerase I [70].

In the following section, different series of topoisomerase I inhibitors which bind to DNA in the absence of the enzyme are presented. It is possible that the drug-DNA interaction is responsible for the poisoning of the enzyme. However, it is not excluded that in addition some of these drugs can interact also with topoisomerase I in its free, not DNA-bound, form.

Inhibition of Topoisomerase I by DNA Binding Drugs

Certain DNA minor groove binders are topoisomerase I inhibitors. This is the case with the bisbenzimidazole dyes Hoechst 33258 (also named pibenzimole) and Hoechst 33342 (Fig. 7). This later compound, which contains an ethoxy group on the 4-

phenyl ring, is more lipophilic than the former drug and is frequently used for histochemical staining and flow cytometric analysis of DNA content in viable cells. The ethoxy substituent of Hoechst 33342 increases the membrane permeability and the cytotoxicity of the drug. These two benzimidazole derivatives bind very strongly and preferentially to AT-rich sequences in DNA. Initially, Beerman and co-workers [71] found that Hoechst 33258 and derivatives inhibited topoisomerase I. The following year, Liu and co-workers reported that Hoechst 33342 share a similar mechanism of action with CPT in trapping topoisomerase I cleavable complexes [72]. The drug traps topoisomerase I but not topoisomerase II into reversible cleavage complexes both *in vitro* and in human tumor cells [72]. Until recently, it was not clear whether specific binding of the Hoechst compounds to

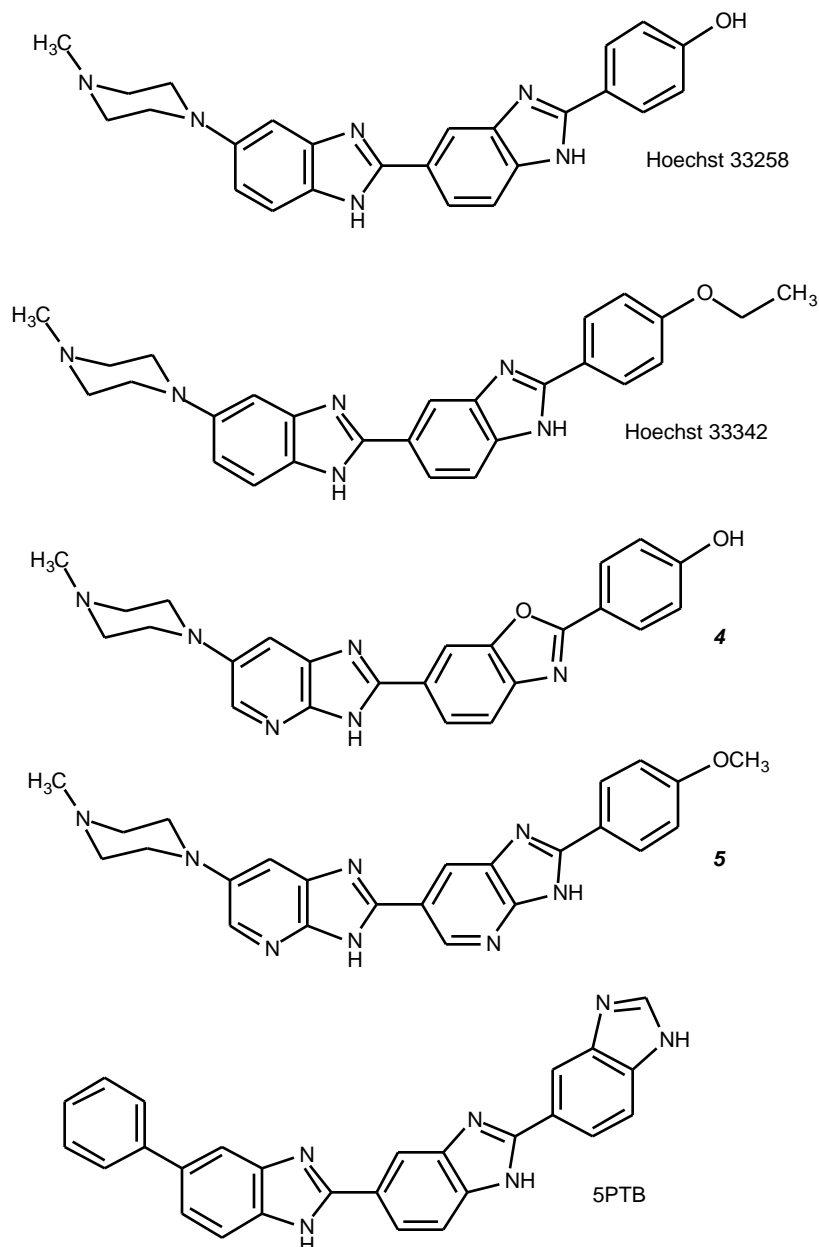


Fig. (7). DNA-minor groove binders inhibitors of topoisomerase I.

the minor groove of AT-rich sequences was responsible for the trapping of topoisomerase I-DNA complexes. On the one hand, certain drug-induced cleavage sites have been located in regions with extremely high A+T content. But on the other hand, other DNA minor groove binders such as netropsin and distamycin, also exhibit a pronounced AT-selectivity but do not stimulate DNA cleavage by topoisomerase I [72]. Distamycin prevents binding of topoisomerase I to DNA, irrespective of the topological state of the DNA [73], but does not act as a CTP-like poison. In their structure-activity relationship study, Beerman et al. [71] observed a linear correlation between DNA binding affinity (K_a) and topoisomerase I inhibition for several analogs of Hoechst 33258. They also concluded that retention of the two nitrogens of the benzimidazole ring was associated with increased inhibitory potency. The addition of a nitrogen atom (benzimidazole pyridoimidazole) may also increase enzyme inhibition [71].

Hoechst 33258 has unusual DNA binding properties. In addition to fitting into the minor groove of AT sequences, the drug can intercalate into GC-rich sequences [74,75]. Similar DNA sequence-dependent binding modes have been evidenced with DAPI, berenil and diphenylfuran derivatives [76-80]. Therefore inhibition of topoisomerase I by Hoechst 33258 may depend, at least partially, on the intercalative binding mode rather than the DNA-minor groove complex

formation. To test this hypothesis, we recently investigated the effect of two Hoechst analogs on topoisomerase I activity (compounds **4** and **5** in Fig. 7). For compound **4**, the two benzimidazole rings of Hoechst 33258 were replaced with a benzoxazole and a pyridoimidazole, the rest of the molecule being identical. Compound **5** contains two pyridoimidazole rings and a 4-methoxy group. Previous DNA binding studies using electric linear dichroism showed that the two compounds do not present the dual sequence-dependent binding mode characteristic of Hoechst 33258 [81]. Compound **5** is a pure DNA minor groove binder whereas compound **4** behaves as a typical DNA intercalating agent, even in the presence of polynucleotides containing exclusively A•T base pairs. Interestingly, we found that compound **4** has no effect on the relaxation of supercoiled plasmid by topoisomerase I. In contrast, compound **5** strongly inhibited the enzyme. The results are presented in Fig. 8. The level of nicked DNA molecules is considerably increased in the presence of compound **5**, as observed with CPT, reflecting significant stabilization of the DNA-topoisomerase I covalent complex. The results suggest that the inhibition of topoisomerase I by benzimidazoles is connected with their capacity to bind to the minor groove of DNA.

In the course of structure-activity study, it was found that compounds with three juxtaposed benzimidazoles could also inhibit topoisomerase I [82]. The terbenzimidazole 5PTB emerged as a promising human topoisomerase I poison essentially because, unlike Hoechst 33342, this drug is toxic to tumor cells expressing the multidrug resistance phenotype [83]. 5PTB (Fig. 7) preferentially binds to the minor groove and alters the conformation of the duplex $d(GA_4T_4C)_2$ which is bend or bendable but not the sequence-isomeric duplex $d(GT_4A_4C)_2$. In contrast, other minor groove binders that do not inhibit topoisomerase I such as netropsin and DAPI neither exhibit a greater affinity for $d(GA_4T_4C)_2$ relative to $d(GT_4A_4C)_2$, nor alter their conformations. These observations suggested that the stabilization of DNA-topoisomerase I covalent complexes may depend on the capacity of the drug to induce DNA bending or to stabilize a bent DNA conformation [84,85]. The cytotoxic activity of 5PTB can be increased by addition of a lipophilic substituent at position 5 on the terbenzimidazole. For example, a 5-phenyl derivative is as potent as 5PTB at inhibiting topoisomerase I *in vitro* but is considerably more cytotoxic than 5PTB against a human lymphoblastoma cell line. A 5-methoxy derivative is more active both in terms of topoisomerase I inhibition and cytotoxicity [86].

Studies with bis and ter-benzimidazoles have shown that the interaction between the drug and the

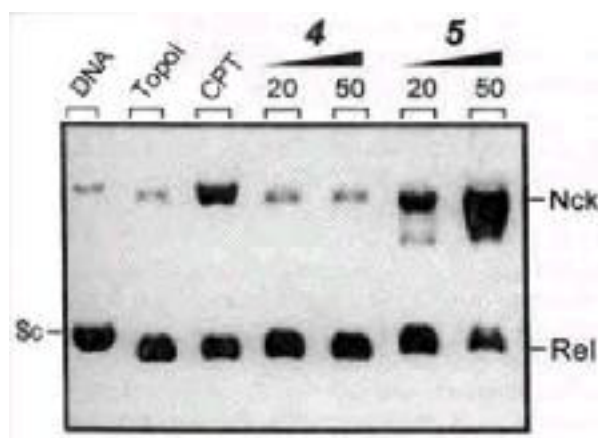


Fig. (8). Effect of the Hoechst 33258 analogs **4** and **5** on the relaxation of plasmid DNA by human topoisomerase I. Native supercoiled pKMP27 DNA (0.5 μ g) (lane DNA) was incubated with 4 units topoisomerase I in the absence (lane Topol) or presence of drug at the indicated concentration (μ M). Camptothecin (lane CPT) was used at 20 μ M. Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on an agarose gel containing ethidium bromide. The gel was photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled.

minor groove of DNA is essential to the poisoning of topoisomerase I. It is therefore not surprising to observe that intercalating drugs containing with various side chains that locate into the minor groove of DNA can stabilize topoisomerase I-DNA complexes. The two bulky cyclic peptide portions of the antitumor drug

actinomycin D (Fig. 9) are likely involved in the interaction of the drug with the topoisomerase I-DNA complex [87]. Crystallographic studies on actinomycin-DNA complexes provide compelling evidence of minor groove changes [88]. NMR studies also showed that upon binding of actinomycin to the oligonucleotide

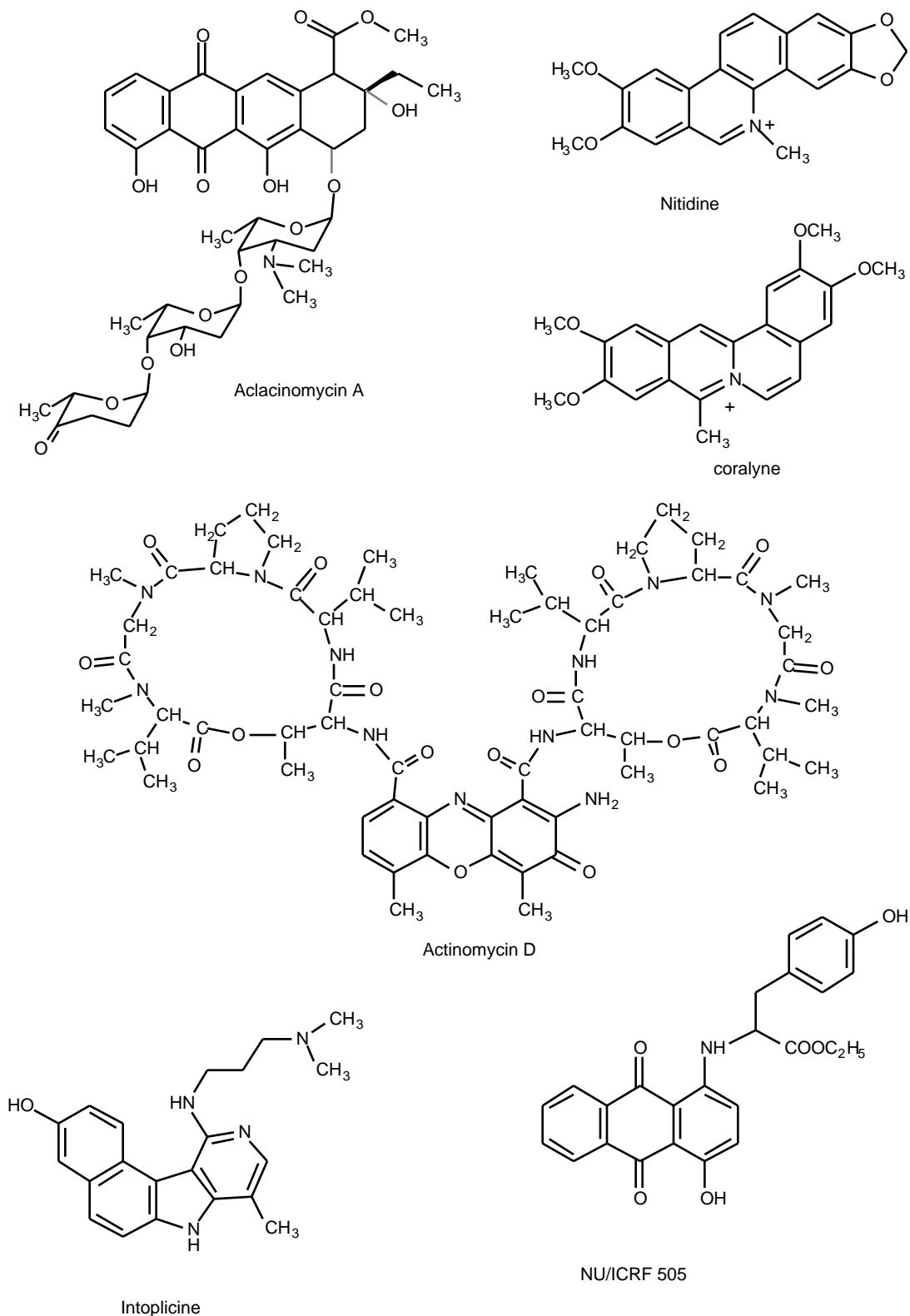


Fig. (9). DNA-intercalating topoisomerase I poisons.

d(AAAGCTTT)₂ the double helix develops a pronounced kink and is fully unwound at the central GpC site, thereby inducing an opening and widening of the minor groove at adjacent sequences [89]. Such actinomycin-induced variation in the width of the minor groove could easily account for the observed alteration of DNA cutting by topoisomerase I or by the antitumor drug bleomycin [90]. The minor groove width is an important determinant for sequence recognition by both bleomycin and topoisomerase I [91]. Another factor which may contribute to the poisoning of topoisomerase I by actinomycin D is DNA bending. Intercalation of actinomycin into DNA causes the double helix to bend towards the major groove [89] and the interaction of topoisomerase I with a bent fragment induces the DNA helix to kink toward the major groove [92]. This causes a local opening of the opposite minor groove which may facilitate attack by topoisomerase I [93]. The insertion of a curve in DNA markedly changes the reaction properties of surrounding sequences both for cleavage and topoisomerization [93]. Accordingly it is reasonable to postulate that the stimulation of DNA cleavage by topoisomerase I in the presence of actinomycin is a manifestation of actinomycin-induced bending of the helix.

The mechanism of cytotoxic action of the anthracyclines involves intercalation into DNA and topoisomerase II-mediated strand breaks. Doxorubicin and daunomycin have relatively little, if any, effects on topoisomerase I [94] but the enzyme inhibition is essentially non-specific and does not involve the trapping of covalent DNA-topoisomerase I cleavable complexes [95]. In contrast a few other anthracyclines, such as morpholinodoxorubicin, can act as topoisomerase I poisons [96]. Aclacinomycin A (Fig. 9), which is a trisaccharide anthracycline, efficiently stabilized DNA-topoisomerase I covalent complexes [97]. The drug inhibits the enzyme in a cell-free assay and inhibits DNA-protein cross-linking in tumor cells [98]. Aclacinomycin A is a typical DNA intercalating agent and a dual topoisomerases I and II poison. The fact that this drug kills both exponentially growing and plateau phase cells by a non-cell cycle-selective mechanism (not involving G2 phase arrest as observed with CPT) may be a consequence of its ability to poison the two types of topoisomerases [98]. NMR studies of the drug complexed with the hexanucleotide duplex d(CGTCAG)₂ indicated that the intercalation of the anthraquinone chromophore at the CpG disposes the deoxyfucose ring of the trisaccharide close to the DNA backbone at the central A•T pair, forcing the DNA helix to kink toward the major groove [99]. This causes a local opening of the opposite minor groove which may facilitate attack by topoisomerase I. The kinking of the DNA double helix near the intercalation site for aclacinomycin A is not seen with daunomycin or

doxorubicin and may explain why aclacinomycin inhibits topoisomerase I in addition to interfering with topoisomerase II. The studies with terbenzimidazole derivatives [84,85] and actinomycin D mentioned above and those with aclacinomycin concur to suggest that drug-induced structural distortion of the DNA helix may be recognized in a unique way by topoisomerase I which is highly sensitive to the topological context and conformation of DNA [93,100].

Inhibition of topoisomerase I by the antitumor drug intoplicine (Fig. 9) may also be a result of the structural consequences introduced by intercalation of its benzopyridoindole chromophore into the helix. Spectroscopic studies using surface-enhanced Raman scattering revealed that the formation of DNA-topoisomerase I-intoplicine ternary complexes induces local destabilization of the double helix at AT-rich regions [101]. Like most intercalating drugs, intoplicine contains a cationic side chain which projects from one of the grooves of DNA, likely the minor groove, allowing for direct contact with topoisomerases [102,103]. The importance of side chain substituents and their role in modulating drug activity has been well documented for ellipticines as well as for anthracenedione derivatives. For example, the anthraquinone drug NU/ICRF 505 (Fig. 9) is a potent topoisomerase I poison whereas closely related analogs with, for example, a phenylalanine in place of the tyrosine residue have no effect on topoisomerase I activity [104-107]. Binding of NU/ICRF 505 to DNA involves intercalation of the anthraquinone chromophore coupled with minor groove anchorage of the appended amino acyl moiety [108]. This cytotoxic compound induces apoptosis in human cancer cell lines [109-110].

The benzophenanthridine alkaloid nitidine (Fig. 9), isolated from the Chinese plant *Zanthoxylum nitidum* [111] and from the plant *Toddalia asiatica* found in Kenya [112], is more effective than CPT at inhibiting the relaxation of supercoiled plasmid DNA by topoisomerase I [113]. Nitidine also inhibits topoisomerase II whereas its closely related analog coralyne (Fig. 9) only inhibits topoisomerase I [114]. These two benzophenanthridine alkaloids intercalate into DNA and bind preferentially to regions of mixed sequence, especially those containing alternating purines and pyrimidines [115]. Nevertheless, DNA intercalation would not be the underlying mechanism for poisoning topoisomerase I [114]. In addition, coralyne was found to stabilize triple helical DNA [116]. Cellular studies suggest that topoisomerase I is the major cytotoxic target for nitidine [114,117]. However, it is worth to bear in mind that benzophenanthridine alkaloids have been reported to mediate a variety of biological activity, including potent and selective inhibition of protein kinase C, binding to vasopressin

V1 receptor, inhibition of taxol-mediated polymerization of tubulin and inhibition of cholinesterase systems, to cite only a few of them [118-121]. Therefore the antileukemic activity of nitidine may be due to the inhibition of topoisomerases and/or microtubule function. Structure-activity studies have shown that the 3,4-methylenedioxy substituent is important for topoisomerase I inhibition [122,123]. A similar ortho-dimethoxy-substituted phenolic ring is found in the quercetin analog EMD 20940 [69] and the synthetic compound NSC 314622 (Fig. 10) which is related to nitidine and acts as a very potent topoisomerase I poison but apparently does not intercalate into DNA [124]. Interestingly, the cleavage complexes trapped by NSC 314622 are more persistent than those induced by CPT both *in vitro* and in cancer cells [124]. It seems likely that the anti-topoisomerase I activity of nitidine and related products is not associated with intercalative binding to DNA.

In the preceding paragraphs, I mentioned a number of DNA intercalating drugs which inhibit both topoisomerases I and II: aclacinomycin A, actinomycin D, saintopin, intopicine and coralyne. Recently, two other synthetic DNA intercalating drugs have also been shown to act as dual topoisomerases I and II poisons: TAS-103 and DACA (Fig. 10). The quinoline derivative TAS-103 is slightly more efficient *in vitro* against topoisomerase I than against the type II enzyme (IC_{50} : 2 μ M and 6.5 μ M, respectively) [125]. In KB cells (to which it is very toxic: IC_{50} : ~10 nM), the drug generates a similar amount of topoisomerase II-DNA complex to that induced by the reference inhibitor etoposide but a smaller amount of topoisomerase I-DNA complex to that produced by CPT. TAS-103 has shown remarkable activity against various lung metastatic tumors and presents a broad antitumor activity against 12 of 13 subcutaneously implanted solid tumor xenografts including cancer of the lung, colon and pancreas, with generally greater efficacy than CPT-11 and etoposide [126]. Interestingly, TAS-103 does not

show any cross-resistance in several resistant phenotypes such as cisplatin resistance, multidrug resistance or topoisomerase inhibitor resistance [127]. In addition, a very recent study of TAS-103 in combination with various established anticancer agents showed that simultaneous use of TAS-103 and cisplatin had a supraditive effect which may prove beneficial for the treatment of small-cell lung cancer [128]. TAS-103 thus represents a promising anticancer agent which is currently undergoing clinical trials. The DNA binding properties of TAS-103 have not yet been investigated in details but on the basis of its structure, it is most likely that the indenoquinolinone chromophore intercalates into DNA. It will be interesting to determine the position of the (dimethylamino)ethylamino side chain in the drug-DNA complex. As for other drugs, the positively charged aminoalkyl substituent may be an important contributor to topoisomerases inhibition.

TAS-103 bears a cationic side very similar to that of DACA which is also a dual topoisomerase I/II poison. This acridine-4-carboxamide derives from the well-known antileukemic drug amsacrine which is one of the most potent topoisomerase II poison [129,130]. DACA preferentially poisons topoisomerase II whereas its chlorinated analog 7-chloro-DACA preferentially poisons topoisomerase I [131]. The addition of the 4-carboxamide chain is known to reinforce significantly the drug-DNA interaction (affinity), to increase the residence time of the drug on DNA (kinetic) and to confer a selectivity for GC-rich sequences of DNA (specificity) [132,133]. But in addition, this chain seems to be an important factor affecting the drug transport rate through cell membranes. In particular, a 4-carboxamide chain with a (dimethylamino)ethylamino moiety, as in DACA, results in a more efficient penetration into cells and a higher DNA-damaging activity both contributing to a higher cytotoxicity [134]. The antitumor activity of DACA is extremely high in certain tumor models *in vivo* [135]. For example, the drug is curative against transplantable Lewis lung

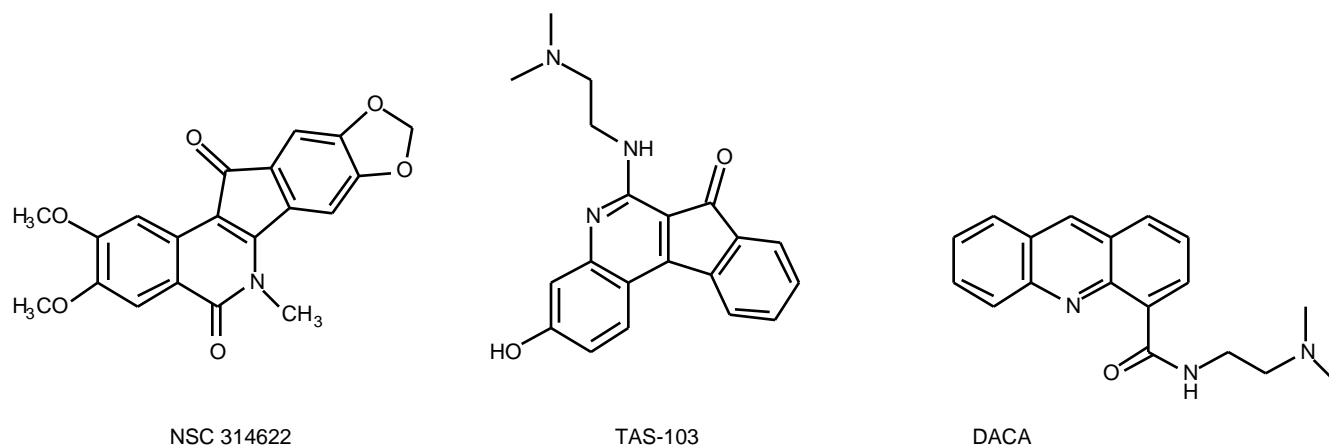


Fig. (10). Synthetic topoisomerase I poisons.

adenocarcinoma growing as lung tumor nodules in mice and is more effective than standard drugs in various xenografted cancers [136,137]. DACA is currently undergoing phases I/II clinical trial as an anticancer agent. One of the most interesting point about this acridine compound is its unusual mode of binding to DNA. A recent cristallographic study of a DACA derivative (9-amino-DACA) bound to the hexanucleotide duplex d(CG(BrdU)ACG)₂ revealed that the intercalation of the acridine ring is accompanied with a major groove binding of the carboxamide side chain [138]. Major groove binding is commonly observed with proteins and peptides but it is rarely seen with small molecules. Drugs for which major groove binding has been evidenced or suspected include DNA-threading agents such as nogalamycin [139] and a few bis-intercalating drugs such as ditercalinium [140] or bis-naphthalimides [141,142]. The cationic terminal group of DACA specifically interacts with the N-7 of guanine thus providing a steric hindrance in the major groove, and changes of the local electrostatic potential. The steric and electronic effects may be essential to the topoisomerase I/II poisoning activity of DACA.

Topoisomerase I poisons have also been isolated from fungus and from marine organisms. The bispyrroloiminoquinone metabolite wakayin (Fig. 4) isolated from the ascidian *Clavelina* in the Fiji islands, enhances the formation of DNA-topoisomerase covalent complexes [143]. Although the mode of binding to DNA has not been studied in detail, wakayin is believed to intercalate into DNA but given its unusual structure, it is most likely that a portion of the molecule protrudes outside the double helix so as to establish direct molecular interaction with the enzyme. The

metabolites TAN-1496 A (Fig. 4), C and E isolated from *Microsphaeropsis* sp. [144] and bulgarein (Fig. 4) extracted from *Heteroconium* sp. [145] are potent inhibitors of the enzyme. The TAN-1496 components belonging to the epi-oligothiadiketopiperazine class of fungal metabolites do not stabilize the topoisomerase I-DNA covalent complex but inhibit the relaxation activity of the enzyme. This action may be responsible for their high antiproliferative activities and their capacities to induce apoptosis in tumor cells [144]. The blue pigment bulgarein is an interesting compound in term of DNA binding because it induces a widening of DNA whereas all intercalating agents unwind the double helix. Upon binding to DNA, perhaps via minor groove interaction, bulgarein produces positively supercoiled DNA and efficiently stabilizes topoisomerase I-DNA covalent complexes. Here again it is most likely that the unique effect of bulgarein on DNA structure and conformation is responsible for the drug-induced inhibition of DNA religation after topoisomerase I cleavage. The DNA binding mode of bulgarein differ significantly from that of a family of fungal metabolites isolated from *Paecilomyces* sp. Saintopin and its water-soluble analog UCE1022 (Fig. 11) possessing a sulfate ester are considered as intercalating agents [146]. Both compounds are potent inducers of topoisomerase I-mediated DNA cleavage, as is the case with another antitumor antibiotic isolated from actinomycetes, UCE6 (Fig. 11) which also contains a tetrahydroxynaphthacenedione structure [147,148]. At low concentrations (<1µM) UCE6 is more potent than saintopin and UCE1022 at stimulating DNA cleavage. Saintopin belongs to the rapidly expanding group of poisons that inhibit the catalytic activities of both topoisomerases I and II [149,150]. As observed with CPT, DNA cleavages induced by saintopin occur

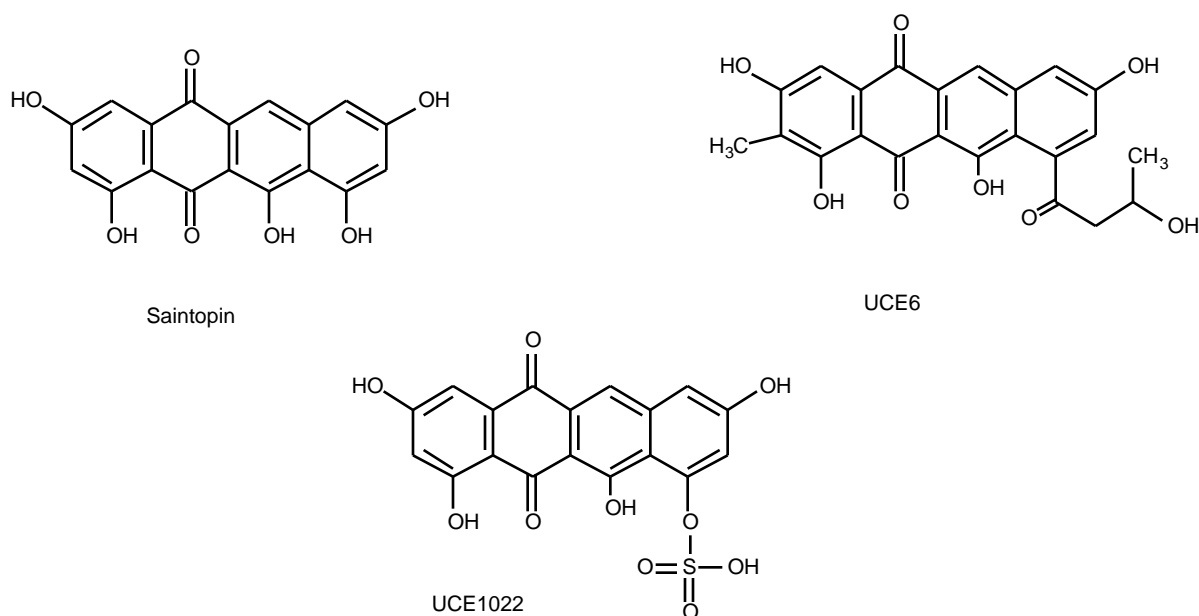


Fig. (11). Naphthacene-dione topoisomerase I poisons.

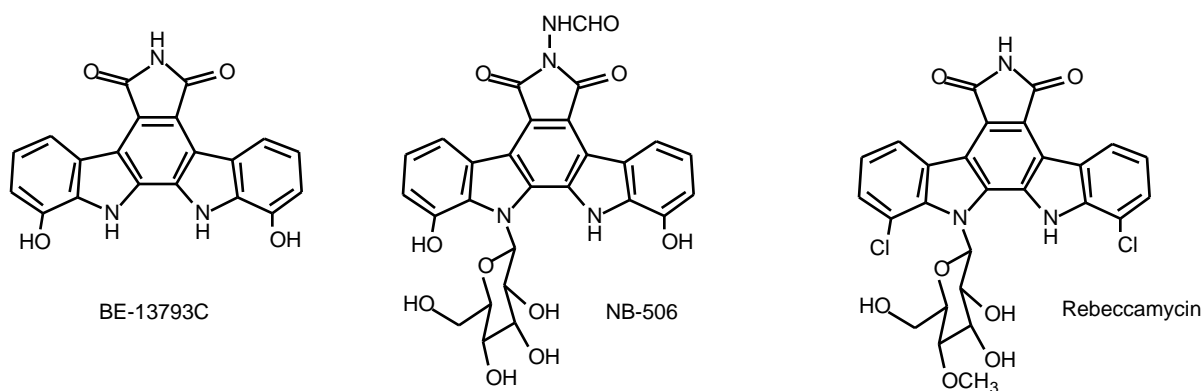


Fig. (12). Indolocarbazole antitumor agents.

preferentially at sites having a G located 3' to the topoisomerase I-induced break (+1 position) [151]. Moreover, a CPT-resistant enzyme with a Asn722 Ser mutation adjacent to the catalytic Tyr723 residue is cross-resistant to CPT suggesting that both CPT and saintopin recognize identical features of the topoisomerase I-DNA complex [151].

The preference for topoisomerase I-mediated cutting at TG sites has been observed with CPT [152], saintopin and more recently with glycosylated indolocarbazoles (IND) related to the antibiotic rebeccamycin (Fig. 12) [153]. Therefore drugs with diverse structure can exhibit comparable sequence selectivity. The existence of a specific pharmacophore for topoisomerase I inhibitors has been proposed [151]. In a recent study, CPT and IND were shown to recognize similar structural elements of the topoisomerase I-DNA covalent. A molecular model showing that CPT and IND share common steric and electronic features was proposed [154]. Fig. 13 shows the superimposed structures of IND and CPT. Three overlapping sites can be distinguished: the two aromatic structures are extended; the position of the N1 nitrogen atom of CPT coincides with the position of

one of the nitrogen indole of R-3 and one of the carbonyl group of the naphthalimide moiety of the IND is positioned like the CPT carbonyl group at position 16. The correlation coefficient between the two structures is relatively high: 0.74. These overlapping sites could be the simplest explanation for the similar effects of the drugs on topoisomerase I [154].

Indolocarbazoles deserve a particular attention because of their very promising anticancer activities. Originally, the antibiotic BE-13793C (Fig. 12) isolated from cultures of *Streptovercillium* species was identified as a potent topoisomerase I inhibitor [155]. To overcome its low aqueous solubility, BE-13793C was glycosylated to give the compound ED-110 which is a potent topoisomerase I poison endowed with marked antitumor activities *in vitro* and *in vivo* [156-158]. Further developments lead to the formyl-amino derivative NB-506 (Fig. 12) which exhibits remarkable antineoplastic activities in cell culture systems and in xenograft models of human tumors [159-163]. Shortly after the preclinical experimentation, NB-506 entered phase I clinical trial in Japan and the first results are encouraging [164,165]. It must be said, however, that in general indolocarbazoles strongly bind to plasma proteins. For example, the strong and specific binding of the indolocarbazole derivatives UCN-01 and CGP41251 (inhibitors of protein kinases C, not topoisomerase I) to the human α_1 -acid glycoprotein may hamper the clinical development of these anticancer drugs [166,167].

The antitumor activity of NB-506 and related rebeccamycin analogs has been attributed to their inhibitory effects on topoisomerase I [168]. The drug has no effect on topoisomerase II and unlike the indolocarbazoles of the staurosporine family (e.g. UCN-01, CGP41251), NB-506 has no effects on protein kinases such as PKA, PKC and PTK [159]. The mechanism by which the drug poisons topoisomerase I is comparable to that of the camptothecins and consists in the stabilisation of the abortive reaction

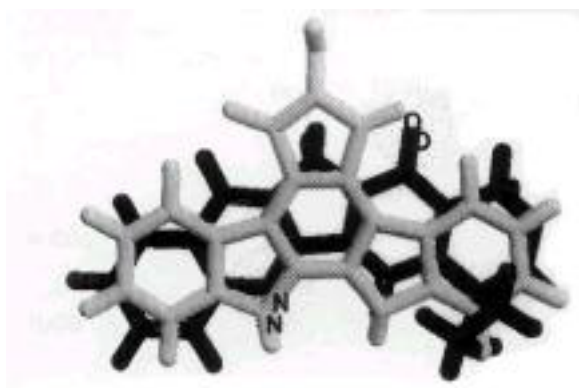


Fig. (13). Superimposition of the CPT and IND structures. The nitrogen indole and the CO group of IND overlap with the same groups in CPT.

intermediate between topoisomerase I and DNA. In terms of inhibition of the religation step of topoisomerase I catalytic cycle, NB-506 has shown superior activity compared to camptothecin or its derivative topotecan [169].

In addition to their very distinct chemical structures, rebeccamycin-type compounds differ considerably from the camptothecins in terms of binding to DNA. CPT has no significant interaction with DNA in the absence of topoisomerase I [170]. However, the presence of DNA stabilizes the active camptothecin lactone form [171,172]. In sharp contrast, most indolocarbazoles bind quite well to DNA in the absence of the DNA-relaxing enzyme [153,173,174]. The chlorine atoms at positions 1 and 12 on the chromophore of rebeccamycin hinder binding to DNA but dechlorinated analogues behave as typical intercalating agents [153]. NB-506 also forms stable intercalation complexes with DNA (Fig. 14) but its affinity for DNA ($K_a = 2.6 \times 10^4$ (M bp) $^{-1}$, in low salt phosphate buffer at neutral pH) is considerably weaker compared to classical intercalating agents such as the anthracyclines [175]. A large number of IND drugs related to NB-506 have been synthesized in recent years [176-185]. The structure-activity relationship studies in the IND series have provided important information for future drug design. One of the most important aspect is the chemical nature and conformation of the sugar residue. Indeed, as with the anthracycline antibiotics, the stereochemistry of the glycoside residue is essential. The attachment of the sugar residue to the IND chromophore via a β -glycosidic bond permits tight fitting of the carbohydrate into the minor groove of the duplex without affecting intercalation of the adjacent chromophore. In contrast, the linkage via an α -glycosidic bond is detrimental for correct drug binding. Only the β -compounds are able to recognize GC-rich sequences in DNA and are potent inhibitors of topoisomerase I. Altering the stereochemistry of the sugar attachment changes the mode of binding to DNA and the capacity of the drug to interfere with topoisomerase I [186]. The cytotoxicity of rebeccamycin and IND analogs depends on their capacity to interact with DNA and to inhibit topoisomerase I. But this does not necessarily means that the two events are inter-dependent. A recent study with two regio-isomeric forms of NB-506 reveals that DNA-binding and topoisomerase I poisoning activities of NB-506 must be viewed as two separate mechanisms. Indeed, an isomeric analog of NB-506 having two hydroxyl groups at positions 2,10 instead of positions 1,11 has lost its capacity to intercalate into DNA but remains an extremely potent topoisomerase I poison. Moreover, the newly designed drug, which stimulates cleavage by topoisomerase I at the same sites in DNA as CPT, is considerably (up to 100-fold)

more toxic to tumor cells than the parent drug NB-506 [187].



Fig. (14). Model for the binding of NB-506 to DNA. The IND chromophore intercalates between two base pairs disposing the sugar residue in the minor groove of the double helix.

Recent data suggest that NB-506 and analogs share the rare property of CPT to recognize specifically the topoisomerase I-DNA complex. But in addition, we cannot exclude the possibility that the drug may also bind directly to topoisomerase I even in the absence of DNA. This possibility seems plausible if one take into account the kinase activity of topoisomerase I [188]. Certain rebeccamycin analogs completely inhibit the phosphorylation of SF2/ASF (a member of the SR protein family) by topoisomerase I. Unlike CPT, the kinase inhibitory effect of IND is observed in the absence of DNA [189]. In conclusion, NB-506-type IND drugs can inhibit topoisomerase I by three complementary mechanisms: (1) specific trapping of topoisomerase I-DNA covalent complexes, (2) non-specific inhibition of a DNA-processing enzyme via DNA binding and (3) inhibition of the kinase activity of topoisomerase I. The drug effects at the DNA cleavage site and the protein kinase site proceed independently [189].

Conclusion

The vast the number of inhibitors that have been isolated or developed in the past few years attests that the area of topoisomerase I-targeted drugs is very active. The majority of inhibitors endowed with anticancer activities are topoisomerases I poisons acting at the level of the enzyme-DNA covalent complex. But topoisomerase I suppressors interacting directly with the enzyme may rapidly reveal useful as of

anticancer drugs. Together with the taxanes, topoisomerase I inhibitors are presumably the most important classes of anticancer drugs introduced into clinical practise. The CPT derivatives topotecan and irinotecan are now used regularly in cancer chemotherapy and it is possible that a few non-CPT inhibitors such as intoplicine, saintopin, indolocarbazole or related compounds will also be valuable for the treatment of cancer in near future. In addition to cancer chemotherapy, certain topoisomerase I inhibitors display a useful spectrum of antiparasitic and antiviral activity. For example, CPT show significant activity against diverse widespread parasites which cause serious diseases such as *Plasmodium falciparum* [190,191]. The efficacy, even limited, of different topoisomerase I poisons as agents to treat infection by African parasites such as *Plasmodium* and *Leishmania* offers promising therapeutical perspectives. Because topoisomerase I is a vulnerable target for anticancer, antibacterial and antimalarial drug development, the search for topoisomerase I inhibitor will remain very active in years to come.

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List of Abbreviations

IND = Indolocarbazole
CPT = Camptothecin.

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