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Review paper

DNA topoisomerase targeting drugs: mechanisms of action and perspectives

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The nuclear enzymes DNA topoisomerases I and II appeared as cellular targets for several antitumor drugs: campthotecin derivatives interacting with topoisomerase I, and actinomycin D, anthracycline derivatives, elliptinium acetate, mitoxantrone, epipodophyllotoxine derivatives, amsacrine and a new olivacine derivative, NSC-6596871 (S 16020-2), which interact with topoisomerase II. The functions of these enzymes are numerous and important since they are critical for DNA functions and cell survival. Despite the fact that they share the same target, topoisomerase II inhibitors have different mechanisms of action. Two principle types of induced alterations are involved in cellular resistance to topoisomerase II drugs: qualitative or quantitative alteration of the enzyme and/or increased drug efflux due to overexpression of P-glycoprotein. S 16020-2, a new olivacine derivative with a high antitumor activity against solid tumors, shows a potent cytotoxic effect against tumor cells expressing P-glycoprotein. This observation suggests that the comprehension of the respective effects of topoisomerase inhibitors and the precise knowledge of their mechanisms of resistance would improve the use of this therapeutic class in the clinic within rational chemotherapeutic combinations.

Key words: DNA, mechanism of action, topoisomerase.

Introduction

Since the discovery of *Escherichia coli* topoisomerase I,[1] researchers have isolated other DNA topoisomerases from both prokaryotes and eukaryotes.[2–4] Investigations of the mechanism of catalysis of DNA topoisomerases have led to the classification of those nuclear enzymes in two types.[5] Type I DNA topoisomerases catalyze DNA relaxation via a transient single-stranded DNA break. Type II DNA topoisomerases catalyze the topological crossing of double-stranded DNA segments via a transient double-stranded DNA break.[5–7] Although we still do not fully understand catalysis of ATP-dependent strand passing by topoisomerases II, a working model has been suggested.[8] Topoisomerase II can break and rejoin the DNA double helix by forming an equilibrium mixture of, at least, two types of complexes: non-cleavable and cleavable.[9] The presence of two DNA segments at the interface of the two protein subunits presumably results in strand passing. Thus, topoisomerases are known to be involved in many important DNA metabolism reactions including replication, recombination, transcription and chromosome segregation during mitosis.[10] It is also well known that topoisomerase II is the molecular target of many anticancer agents. They belong to anthracyclines (adriamycin), epipodophyllotoxines (VP16), anthracinediones (mitoxantrone), acridines (*m*-AMSA) and ellipticines (2-methyl-9-hydroxy-elliptinium).[11] Recently, new inhibitors have been identified. Some of them act as the above-mentioned compounds by trapping the cleavable complex, such as amonafide,[12] genistein,[13,14] saintopine,[15] terpentecin and clerocidin,[16] while others inhibit topoisomerase II catalytic activity without stabilizing the cleavable complex, such as merbarone,[17] fostriecin[18] and bis-2,6-dioxopiperazine derivatives.[19] More recently, azatoxin was designed as an analog hybrid between VP16 aglycone and ellipticine. This molecule does not intercalate into DNA while it induces the largest number of topoisomerase II cleavage sites among topoisomerase II inhibitors in both SV40 and c-*myc* DNA.[20] Despite the fact that all of the drugs share the same target, they have different experimental and clinical antitumor properties which could be due to different modes of action or to specific sites of interactions.[21–24] This may explain the continuous interest of investigators to study and develop new topoisomerase II inhibitors

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showing higher antitumor effect in resistant tumors and reduced side effects.

Topoisomerase II targeting drugs

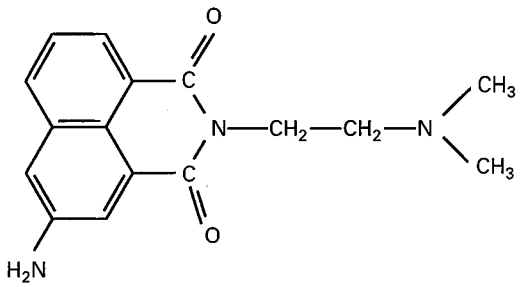
High levels of topoisomerase II in tumor cells parallel the high proliferative potential of these cells.[25,26] This fact is consistent with the important role of the enzymes in DNA replication and emphasizes the interest of targeting the enzymes in order to design new anticancer agents.

In the last decade, some investigators have dedicated special efforts to study and develop new topoisomerase II inhibitors. Despite the fact that they were analogs of ellipticine, they had different antitumor profiles.[27-33] Drugs that affect DNA topoisomerase II (Table 1 and Figures 1-3) could have distinct mechanisms since DNA intercalating and non-intercalating agents are known to trap topoisomerase II, which suggests that different binding sites on the DNA and/or the enzyme may be involved.[11] Studies on the local DNA sequence dependence on doxorubicin stimulation of topoisomerase II DNA cleavage have led to the proposal of a molecular model of the ternary complex DNA-drug-topoisomerase II, which may be

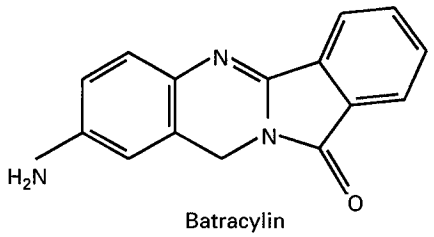
Table 1. Topoisomerase II targeting antitumor drugs

Chemical class	Specific agent	Reference
DNA intercalators and stabilizers of the cleavable complex (Topoisomerase II poisons)		
Acridines	*m*AMSA; 4′-(9-acridinylamino) methanesulfon-*m*-anisidide	10
	N-[2(dimethylamino) ethyl] acridine-4-carboxamide	37
Anthracyclines	Doxorubicin, Daunorubicin, Idarubicin	38 34
Actinomycines	Actinomycin D	38
Anthracenes	Mitoxantrone, Bisanthrene, Piroxanthrone	38 39
Ellipticines	2-Methyl-9-hydroxy elliptinium	40
7H-Benzo[*e*]pyrido[4,3-*b*] indoles	Intoplicine (RP60475)	41
Olivacines	NSC-6596871 (S16020-2)	42
Isoindolo[1,2-b]quinazolines and benzo[4,5]isoquinolino[1,2-*b*]quinazolines	Batracylin	43
Flavones	Quercetine, Pisetine	14
Benzisoquinolinediones	Amonafide, Nafidimide	12, 44
Benzanthracenes	Saintopin	15
Benzo[*c*]phenanthridines	Fagaronine	45
Thiazoles	BE10988	46
Stabilizers of the cleavable complex, non-intercalative drugs (Topoisomerase II poisons)		
Epipodophyllotoxines	VP16 (etoposide) and VM26 (Teniposide)	47
Quinolones	CP-67,804 and CP-115,953	48
Isoflavones	Genistein, Orobol	14
Terpenoides	Terpentecin, Clerocidin	16
2-Nitroimidazoles	Ro 15-0216	49
Indoloquinolinediones	Azal QD	50
Quinoline-5,8 diones	Streptonigrin	51
5-(3,5-Dimethoxy-4-hydroxyphenyl) oxazolo (3′,4′:1,6)pyrido(3,4-*b*)indoles	Azatoxin	20
Naphtoquinones	Menadione	52
Non-intercalative, non-stabilizer of the cleavable complex drugs (Topoisomerase II suppressors)		
Bis-2,6-dioxopiperaxines	ICRF-193, ICRF-154	19, 23
Anthracyclines	Aclarubicin	53
Dihydropyranones	Fostriecin and analogs	18
Pyrimidine carboxamides	Merbarone	17
Naphtylurea polysulfonates	Suramine	55

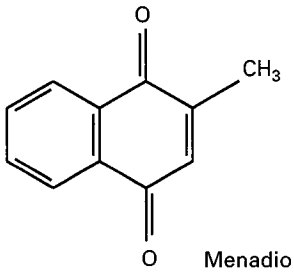
common to intercalating and non-intercalating agents and to topoisomerase I.[34-36] Recent studies have shown that some of the topoisomerase II targeting drugs (Table 2) had a dual topoisomerase I and II stimulating DNA cleavable function.[15,45,50,54,56] They strongly hinder the DNA relegation step and induce replication fork arrest which may be responsible for cell death. Actually, it is well known that intercalation is necessary but not sufficient for antitumor activity[58] and for topoisomerase II-mediated cleaving activity of



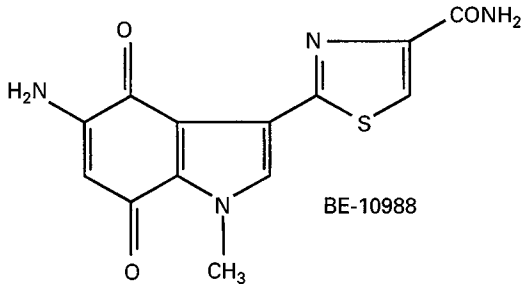
Amonafide



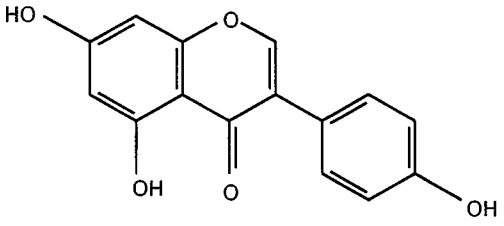
Batracylin



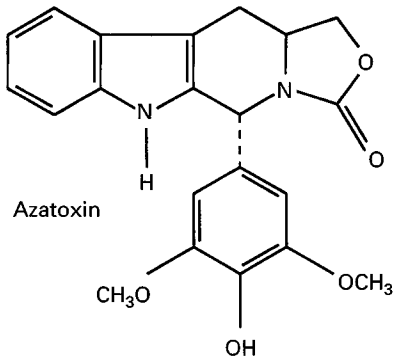
Menadione

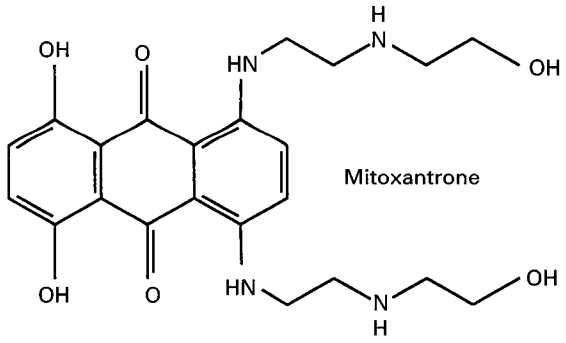


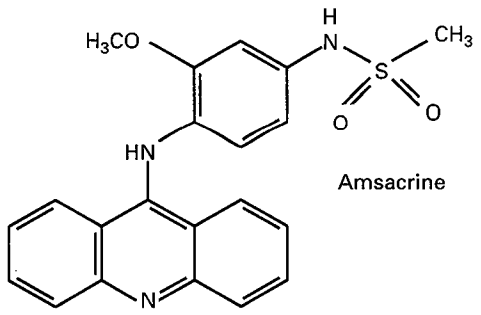
BE-10988



Genistein







Amsacrine

anthracyclines.[60] The same holds for their DNA sequence selectivity—the role of this selectivity in drug cytotoxicity needs further investigation. Indeed, a very recent study showed that DNA binding affinities to anthracycline derivatives were not related to drug sequence specificity. This study established a clear relationship between a specific drug substituent and base sequence selectivity, and indicated putative DNA and enzyme interacting domains of the anthracycline molecule.[24] However, how mechanistic differences between anthracycline derivatives influence the antitumor activity of these agents has yet to be determined. Moreover, derivatives of bis-2,6-dioxopiperazines may be inhibitors of mammalian type II DNA topoisomerases at low concentrations (IC_{50} = 2 μM) without being inhibitors of topoisomerase I at high concentrations (300 μM). This observation, together with the finding that ICRF-193, the most potent inhibitor in this class of chemicals, did not intercalate into DNA and did not stimulate the formation of a cleavable complex between DNA and topoisomerase II but rather inhibited the formation of enzyme-mediated DNA cleavage induced by etoposide or acridines,

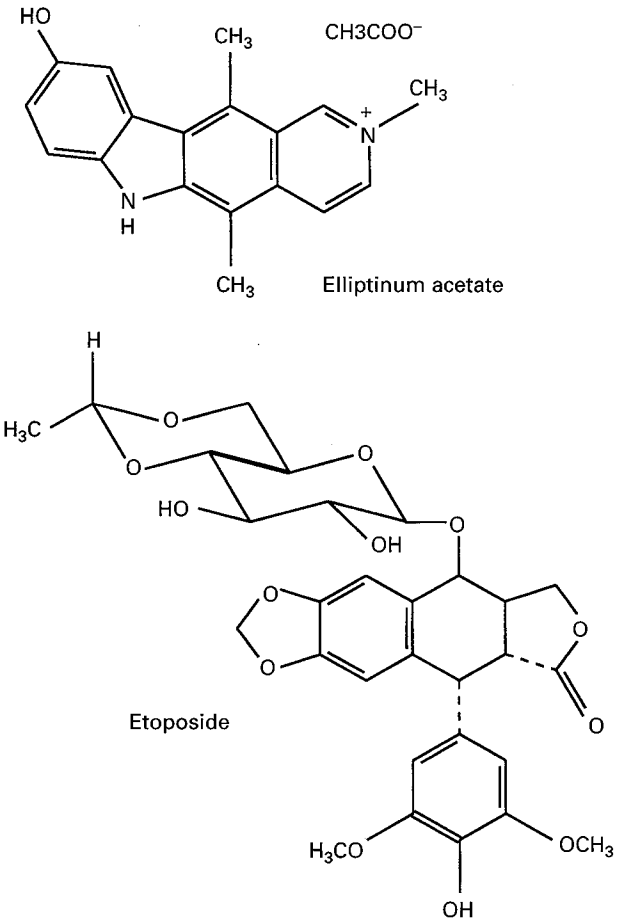


Figure 1. Chemical structures of topoisomerase II targeting drugs.

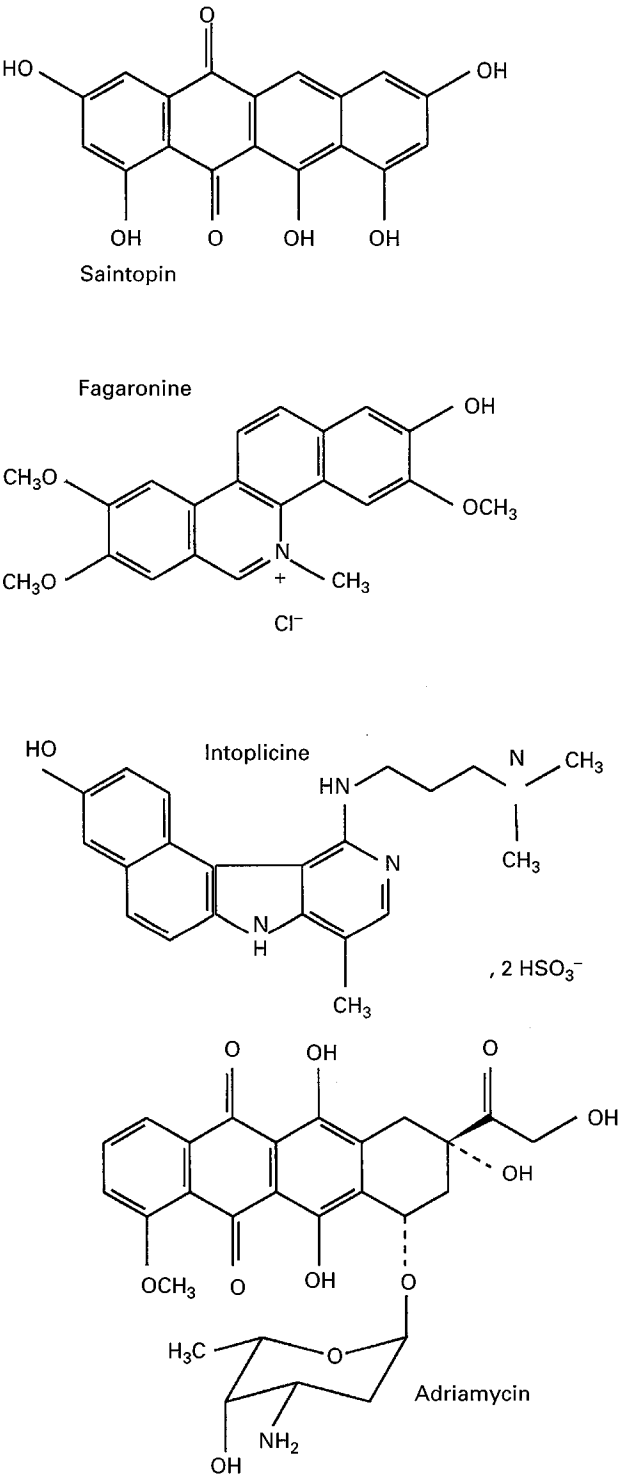
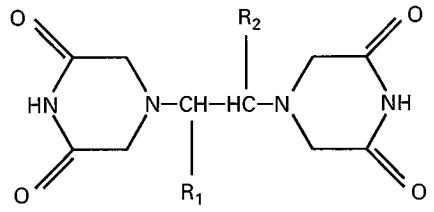
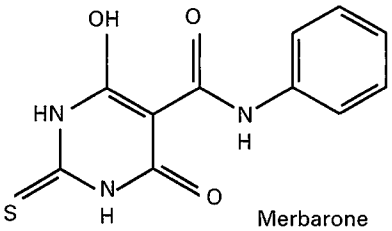


Figure 2. Chemical structures of topoisomerase I and II targeting drugs.

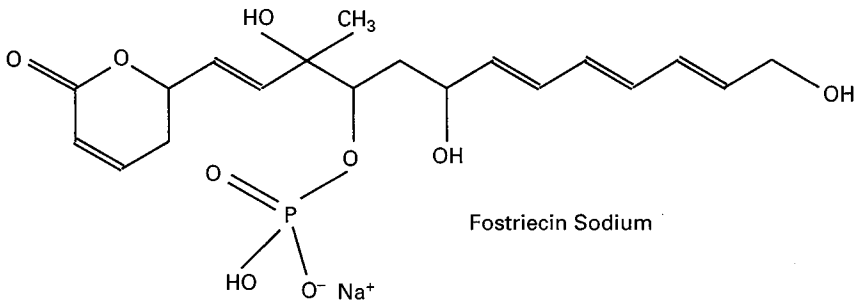
suggests that bis-2,6-dioxopiperazine derivatives are specific inhibitors of topoisomerase II with different modes of action.[19] They probably interfere with some steps before the formation of the intermediate cleavable complex in the catalytic cycle by trapping the closed-clamp conformation of the enzyme in the



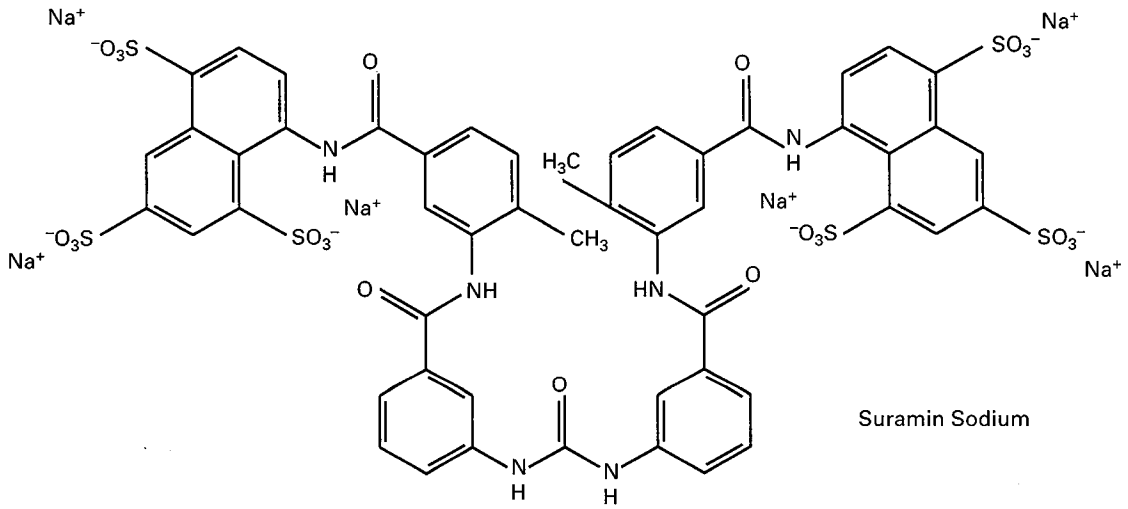
ICRF-154: R_1=H, R_2=H
ICRF-159: R_1=CH_3, R_2=H
ICRF-193: R_1=CH_3, R_2=CH_3



Merbarone



Fostriecin Sodium



Suramin Sodium

Figure 3. Chemical structures of topoisomerase II targeting drugs (non-intercalative and non-stabilizer of the cleavable complex drugs).

Table 2. Dual topoisomerase I and II targeting drugs

Chemical class	Specific agent	Reference
Anthracyclines	Adriamycin	54
Actinomycines	Actinomycin D	56
Benzanthracenes	Saintopin	15
Benzo[*c*]phenanthridines	Fagaronine	45
7H-Benzo[*e*]pyrido[4,3-*b*] indoles	Intoplicine (RP60475)	57

presence of ATPase.[59] This mechanism seems to be shared by a group of inhibitors that include merbarone, aclarubicin and fostriecin.[17,18,53] Since compounds of this class are cytotoxic agents with antitumor activity, cleavable complex formation may not be the only mechanism of cell killing by topoisomerase II targeting drugs—some other mechanisms may be operating that lead to cell death. Alteration of the nuclear architecture induced by an inhibition of topoisomerase II may contribute to apoptosis-related gene expression, since topoisomerase II is a major component of the nuclear matrix that can regulate gene expression. Indeed, the bis-2,6-dioxopiperazine derivatives, which do not form the cleavable complex, induced thymocyte apoptosis as observed in etoposide-treated thymocytes and they did not interfere with etoposide-induced apoptosis.[23,64] It was reported that the maximal cytotoxicity was achieved when cells were exposed to amsacrine or etoposide during the S phase,[61,62] while the bis-2,6-dioxopiperazine compounds showed their optimal cytotoxicity when the cells were in the G_2/M phase.[64] The fact that this difference in phase specificity of cytotoxicity among these different classes of topoisomerase II targeting drugs is due to their mechanism of action on the enzyme has not been established yet.

Drug resistance to topoisomerase II targeting drugs

The development of resistance of human cancer to potent anticancer drugs has been ascribed to the selection and outgrowth of pre-existing or newly occurring subpopulations of resistant tumor cells.[65] The mechanisms by which tumor cells become resistant to multiple chemotherapeutic agents has been the subject of extensive investigation.[66] In many drug-resistant cultured cell lines, multiple drug resistance (MDR) is correlated to the overexpression of a 170 000–180 000 mass glycoprotein, the P-glycoprotein encoded by the *MDR1* gene. This protein is found in plasma membranes and in the luminal side of the Golgi stacks,[67] and is thought to function as an energy-dependent efflux pump.[68]

As for the other anticancer drugs, resistance to various topoisomerase II inhibitors has been documented in cultured tumor cell lines with respect to *MDR1* expression.[69] However, other mechanisms have been described in both P-glycoprotein negative and positive MDR cell lines where cross-resistance to the full range of anti-topoisomerase II drugs was observed. This cross-resistance may be attributed to lengthened cell cycle time,[70] altered DNA repair function,[71] expression of a dominant negative genetic suppresser element[72] and mainly to alteration of topoisomerase II: reduced enzyme levels[73] or enzyme mutation.[74] In fact, there are two highly homologous isoforms of human topoisomerase II which have been designated topoisomerase IIα (170 kDa) and topoisomerase IIβ (180 kDa).[75] They are encoded by two co-migrating mRNAs but their cognate genes are located on different chromosomes.[76] There is some evidence that these isoenzymes carry out different cellular functions and the role of each isoform in drug resistance may differ. A recent study on doxorubicin and etoposide in human lung cancer cell lines indicates a minor role for topoisomerase IIα content and catalytic activity in determining drug sensitivities to topoisomerases II inhibitors.[78] Other biochemical mechanisms are thought to exist in lung cancer, including the *MRP* gene,[79] subcellular drug distribution,[80] decreased drug accumulation,[81] c-*myc* expression,[82] different susceptibility to apoptosis[83] and the state of topoisomerase II phosphorylation.[84]

Understanding these mechanisms has been helpful in developing strategies to overcome resistance by blocking relevant pathways through drug design. A variety of pharmacological agents[77,85] including structural analogs of anthracyclines[86] were used as substrates to inhibit the transport pump, P-glycoprotein, and thus reverse the multidrug resistance.

Cytotoxic analogs of anthracyclines with lack of resistance to classical anthracyclines were developed.[53] This suggests that some topoisomerase II inhibitors (Table 3) may not be recognized by P-glycoprotein and overcome partially, if not totally, the *MDR1*-mediated resistance. Interestingly, menadione[52] and batracylin,[43] two inhibitors of topoisomerase II, are efficient in treating *MDR1*-expressing tumor cells.[87,88] This suggests that the development of topoisomerase II anticancer drugs which can overcome one of the mechanisms of resistance related to topoisomerase II targeted drugs may be useful in the treatment of some human solid tumors.

More interestingly, S 16020-2 (NSC-6596871) (Figure 4), a new olivacine derivative,[89] was rationally designed in order (i) to keep the 6H-pyrido[4,3-*b*]carbazole nucleus with a 9-OH group which appears to play a crucial role in the antitumor effect since it is a necessary condition to intercalate into DNA and interact with the topoisomerase II, (ii) to maintain a monobasic side chain which allows salification and therefore water solubility in order to obtain a better bioavailability and increase the antitumor activity, (iii) to methylate the indole nitrogen in order to prevent the possibility of producing quinone imine species—as in 9-hydroxy ellipticine—which is responsible for the covalent binding of elliptinium acetate to macromolecules, rendering this latter molecule antigenic and generating immuno- and general toxicity, and (iv) to avoid the substitution of the pyridine nitrogen atom, the quaternary amonium ion of elliptinium acetate being suspected to be a potent immunogenic determinant.

S 16020-2 was identified as a topoisomerase II poison. It was shown to intercalate into DNA and stabilize the DNA-topoisomerase II cleavable complex using 5P65 DNA as substrate and topoisomerase II purified from calf thymus.[42] This structure was efficient in treating *MDR1*-expressing tumor cells, and overcame the MDR in cultured cells and animal model system.[90] The most striking aspect of the pharmacotherapeutic properties of S 16020-2 is its very high antitumor activity against the relatively resistant and metastatic murine solid tumor, the Lewis lung carcinoma. Whatever the site of tumor cell inoculation, subcutanously (s.c.), intramuscularly (i.m.) or intravenously (i.v.), the tumor cells metastasize regularly in the lung, resulting in the rapid death of mice. S 16020-2 administered i.v. against the s.c. model following an intermittent schedule on days 3, 6 and 9 post-implant was curative (100% cure) at the dose levels of 20, 40 and 60 mg/kg, while adriamycin was marginally active even at the optimal dose of 10 mg/kg.[90,91] S 16020-2 administered i.v. on days 5, 9 and 13 (early treatment) or on days 11, 15 and 19 (delayed treatment) was highly effective and more active than adriamycin against the i.v. implanted Lewis lung carcinoma.[92,93] Moreover S16020-2 showed important antitumor activity in a panel of human tumor xenografts including lung and ovary carcinomas.[94] Recently, it was demonstrated that S16020-2 displays a preferential cytotoxicity on tumor cells harboring activated *ras* oncogenes.[95]

Perspectives and conclusion

Designing new drugs for various resistant tumors requires fundamental information on multiple drug resistance mechanisms since drug resistance which is associated with topoisomerase II may result from any process that reduces the catalytic function of the enzyme or reduces the formation of the cleavable complex.[96] This may happen via a decrease in the amount of topoisomerase II or an enzymatic modification resulting in an alteration of the interaction of the enzyme with either drug or DNA. Some investigators have found a direct relation between levels of the nuclear enzyme topoisomerase II and chemosensitivity to anticancer drugs whose toxicity is mainly mediated by inhibiting the enzyme (such as amsacrine, adriamycin and etoposide) in human testis and bladder tumor cell lines.[97] Others attribute the emergence of a resistant phenotype in

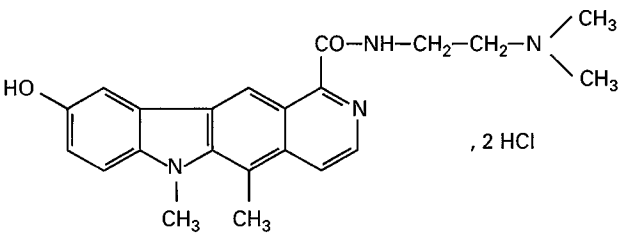


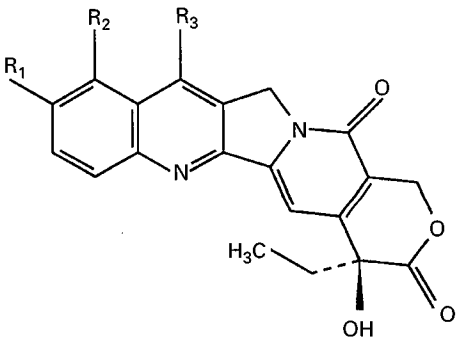
Figure 4. Chemical structure of NSC-6596871 (S 16020-2).

Table 3. Topoisomerase II inhibitors active against resistant tumor cell lines

Chemical class	Specific agent	Reference
Anthracylines	Menogaril, Aclacinomycine	53
Isoindolol[1,2-b]quinazolines and benzo[4,5]isoquinolino[1,2-*b*]quinazolines	Batracylin	43
Thiazoles	BE10988	46
Naphtoquinones	Menadione	52
Olivacines	NSC-6596871 (S16020-2)	94

H209/V6 small cell lung cancer by selection in etoposide to a quantitative and qualitative alteration in topoisomerase IIα.[98] More recently, it was reported that the doxorubicin-selected multidrug resistant small cell lung cancer cell line H69AR contained lower levels of both topoisomerase IIα and topoisomerase IIβ protein and mRNA, and that reduced level of isoenzymes may contribute to the resistance of H69AR cells to etoposide and other drugs that target these isoenzymes.[72] This data would suggest that a quantitative rather than a qualitative alteration in topoisomerase II contributes to drug resistance in H69AR cells. However, the relatively modest (2- to 5-fold) decreases in topoisomerase IIβ are probably not sufficient to account for the 35- and 50-fold resistance in H69AR cells to etoposide and doxorubicin.[73] They also cannot be responsible for the resistance of these cells to non-topoisomerase II targeting drugs, a second (or more) resistance mechanism(s) must be involved. Indeed, the expression of a novel transporter gene, *MRP*, may be implicated.[99] From the other side, it was also reported that drug resistance to topoisomerase II drugs is associated with alteration in topoisomerase IIβ.[100,101] This differential sensitivity of topoisomerase IIα and topoisomerase IIβ to various topoisomerase II inhibitors[104] and the various distribution patterns of topoisomerase IIα and topoisomerase IIβ in different tissues and phases of the cell cycle[102,103] add some difficulties to studies on the mechanisms of topoisomerase II inhibitors. It is clear that further studies are necessary to elucidate the role of each isoenzyme in drug resistance. In a very recent article[104] on topoisomerase inhibitors, the authors reported that topoisomerase I is a more favorable cellular target for anticancer drug development because, unlike topoisomerase II, topoisomerase I is not a cell cycle-dependent enzyme.[97] However, the superiority of the selective inhibitor of topoisomerase II, S 16020-2, compared to adriamycin, an inhibitor of both topoisomerase I and II, on the treatment of Lewis lung carcinoma *in vivo* contradicts this statement. Moreover, topoisomerase I inhibitors such as camptothecin derivatives (Figure 5) are only active during the S phase of the cell cycle and they are treatment-schedule dependent.[105]

It is true that high levels of topoisomerase II have been detected in proliferating cells and that these levels decrease when cells are induced to differenti-



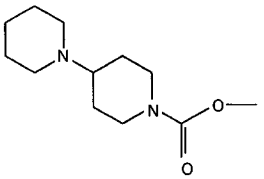
	R_1	R_2	R_3
Camptothecin	H	H	H
9-Amino-camptothecin	H	NH_2	H
Topotecan	OH	$-CH_2-N(CH_3)_2$	H
Irinotecan		H	$-C_2H_5$

Figure 5. Chemical structures of topoisomerase I inhibitors.

ate.[25] On the other hand, topoisomerase I is present at high levels in both proliferating and quiescent human fibroblasts, suggesting that this function may be independent of the cellular growth rate.[26] The implication of these differences in clinical antitumor activity is not yet established and one may argue that a high level of topoisomerase I in quiescent cells may increase the incidence of side effects induced by topoisomerase I targeted drugs and constitutes a serious limiting factor. This, added to the emergency of cross-resistance related to topoisomerase I inhibitors, should help us realize that only clinical activity would help us to appreciate the quality of the targets. Indeed, it was reported that two isoenzymes of topoisomerase I (molecular weight 100 and 66 kDa, respectively) may be involved in drug resistance.[106] In addition, recent studies have indicated that topotecan, which stabilizes the DNA-topoisomerase I complex causing DNA strand breaks, may be a substrate for P-glycoprotein.[107]

From the other side, it is consistent to think that the inhibition of one form of topoisomerase results in an increase of other topoisomerase activity to compensate for DNA synthesis and cell survival. This may suggest that a sequential treatment using first an inhibitor of topoisomerase I, which will deplete this enzyme in the transaction of cells during the G_0 phase while the activity of topoisomerase II will be increased and may be targeted consecutively by a topoisomerase II inhibitor, may be of great interest. Our growing understanding of the molecular structure and function of type II and type I topoisomerases is very helpful in developing new topoisomerase inhibitors and in choosing specific associations of those drugs which may have relevance in cancer treatment. For instance, recombinant human tumor necrosis factor (rHuTNF) synergistically potentiates the cytotoxicity of topoisomerase I and II inhibitors on the A2780 human ovarian cancer cell line, while similar synergy did not occur in a combination of rHuTNF and cisplatin or mitomycin C.[108] Tubulin interacting agents such as vincristine or vinblastine synergistically potentiate the topoisomerase II inhibitor S16020-2 on A549, a non-small cell lung carcinoma cell line, when pre-treatment with microtubule poisons is followed by S16020-2 treatment.[109] Finally, when resistance to topoisomerase II inhibitors is related to hypophosphorylation of the targeted enzyme, it is consistent to associate such inhibitors to a protein kinase C (PKC) modulator such as briostatine which stimulates PKC activity and thus may increase the phosphorylation of the target enzyme. Recent data supports this idea that briostatin may circumvent etoposide resistance to topoisomerase II inhibitors.[110]

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