

作用於DNA拓樸異構酶之抗癌藥物

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摘要

DNA拓樸異構酶是細胞核內的重要蛋白質,分爲第一、第二兩型,可經由切或接DNA而調節DNA的超螺旋結構。故此酶幾乎參予了所有DNA的生物反應如複製、轉錄及重組。近年來更發現DNA拓樸異構酶是許多抗細菌、真菌、病毒或抗癌藥物的標的物。本文主要介紹以DNA拓樸異構酶爲標的之抗癌藥物,例如喜樹鹼作用在第一型拓樸異構酶(Topo I),而VM26則作用在第二型拓樸異構酶(Topo II),它們的作用均是抓住DNA和拓樸異構酶所形成的切割複合體,使斷裂的DNA無法接合而干擾DNA的複製或轉錄等重要生物反應。然而如同其他的抗癌藥物,癌細胞會產生多種耐藥性機制來逃過這些藥物的作用,例如在細胞表面大量表現多重耐藥蛋白(multiple drug resistance protein 1,MDR1),將抗癌藥物運送出細胞;或降低細胞內拓樸異構酶的濃度,或產生具耐藥性的拓樸異構酶突變種等。因此研究抗癌藥物標的物及耐藥性機制,將有助於我國發展新而有效的抗癌藥物。

前言

1953年Watson和Crick提出DNA雙螺旋構造的模型,後經其他科學家證明DNA是由兩股長鏈去氧核糖核酸繞著一個共同的軸,以右轉螺旋相互旋繞而成。其後更發現DNA雙螺旋的軸還會互繞,將DNA堆擠的更緊密,這種較高層次的結構被命名爲超螺旋(supercoil),無論是質體或病毒的環形DNA或哺乳動物細胞內DNA幾乎均有相似程度之超螺旋結構⁽¹⁾。王倬博士於1971年在大腸桿菌中發現第一型DNA拓樸異構酶(*E.coli* DNA topoisomerase I, or ω protein),同時具有切DNA的作用如DNase和接DNA的作用如ligase,在不需能量供給下可切斷並接回一股DNA,而改變DNA之超螺旋結構⁽²⁾。此後在原核和真核細胞中又陸續發現了數種不同的DNA拓樸異構酶。1979年王倬博士等人正式將這一類會改變DNA立體構造的酶稱爲DNA拓樸異構酶(DNA topoisomerase)⁽³⁾。目前

DNA拓樸異構酶主要分爲兩類,分別是第一型DNA拓樸異構酶(Topo I),每次切或接一股DNA。人類的Topo I是分子量爲100 KD之單體蛋白質,可鬆解正超螺旋及負超螺旋DNA之其中一股。另一爲第二型DNA拓樸異構酶(Topo II)每次同時切或接雙股之DNA,Topo II爲分子量170 KD之雙體蛋白質,其催化反應需要ATP,除可鬆解正超螺旋及負超螺旋外,還可解結(unknot)及解環(Decatenation)⁽⁴⁾。經由這兩種不同的反應機轉,可調節DNA的超螺旋結構。而細胞內DNA最基本之生物反應是進行複製、轉錄及DNA重組,這些反應的進行都需要分開彼此相互環繞的兩股DNA,所以DNA拓樸異構酶幾乎參與了所有DNA的複製、轉錄及重組^(5,6,7)。因此一旦DNA拓樸異構酶發生變異,功能喪失或受到藥物抑制時,細胞生理狀況必受到影響,甚而導致細胞死亡。近年來科學家更發現多種抗細菌⁽⁸⁾、真菌⁽⁹⁾、病毒⁽¹⁰⁾和抗癌藥物⁽¹¹⁾均是以DNA拓樸異構酶做爲標的物,因而拓樸異構酶在疾病治療上的重要性更爲顯著。

一、抑制第一型DNA拓樸異構酶的抗癌藥物

(一)喜樹鹼(camptothecin)及其衍生物:

抑制第一型DNA拓樸異構酶的藥物,目前以喜樹鹼為主,它是一種植物鹼,是Wall等人於1966年從喜樹(*Camptotheca acuminata*)的材心、樹皮及果實中分離所得⁽¹²⁾。它可抑制DNA和RNA的合成,將細胞留滯於G₂ phase,因細胞無法繼續分裂而導致死亡。此藥物無論在活體外(in vitro)或活體內(in vivo)都具有很強的抗腫瘤作用。1985年劉昉等人首先發現喜樹鹼殺死細胞的機制是作用在Topo I上⁽¹³⁾。如前所述當細胞在進行DNA複製或經由轉錄作用合成RNA時,細胞核內Topo I的任務是和DNA結合,先切開一股DNA,讓緊密纏繞的DNA旋轉一次後,再迅速把斷裂的DNA連接起來。在整個反應中,Topo I的酪氨酸會與斷裂DNA的3-磷酸基先形成一個切割複合體(Cleavable complex),再進行DNA的连接作用⁽¹⁴⁾。喜樹鹼進入細胞後會抓住Topo I與DNA形成之切割複合體而形成一個三體複合物(ternary complex)繼而干擾斷裂DNA的再連接作用⁽¹⁵⁾。劉昉博士曾依據養殖細胞內或試管中Topo I之酵素活性分析提出Fork collision model來解釋喜樹鹼殺死細胞的機制⁽¹⁶⁾。如圖一所示,當DNA進行複製時Topo I會先與DNA結合,但當加入喜樹鹼後,Topo I-喜樹鹼-DNA會形成三體複合物,如果此三體複合物形成於DNA複製叉(replication fork)前,結果會導致DNA的斷裂,使DNA無法再接合,有趣的是Topo I在切斷一股DNA時是有方向性的,斷裂的那股DNA和DNA複製時的領導鏈(leading strand)互補,結果經常導致雙股DNA在複製叉附近斷裂,使得正在複製的細胞,因DNA嚴重受損而引發細胞死亡訊息⁽¹⁷⁾。喜樹鹼不會單獨和DNA或Topo I結合,只認得Topo I和DNA之切割複合體。

喜樹鹼的毒殺作用取決於細胞內Topo I之含量,而人類癌細胞內常表現較高濃度之Topo I⁽¹⁸⁾,故喜樹鹼具有很強的抗腫瘤作用。如圖二可看出喜樹鹼為一幾乎平面的分子,不溶於水,也難溶於普通有機溶劑,高毒性及低溶解度,使喜樹鹼的臨床實驗只停留在第一階段⁽¹⁹⁾。目前依據喜樹鹼及其衍生物之化學構造和抗癌功效關係之研究,可知A、B、C、D、E五環之結構為保持抑制Topo I活性及體內抗腫瘤活性所必需,但可在A環,E環導入各種不同之取代基,近年來日本Terasawa更合成一系列對A、B、C環骨架本身進行了修飾的衍生

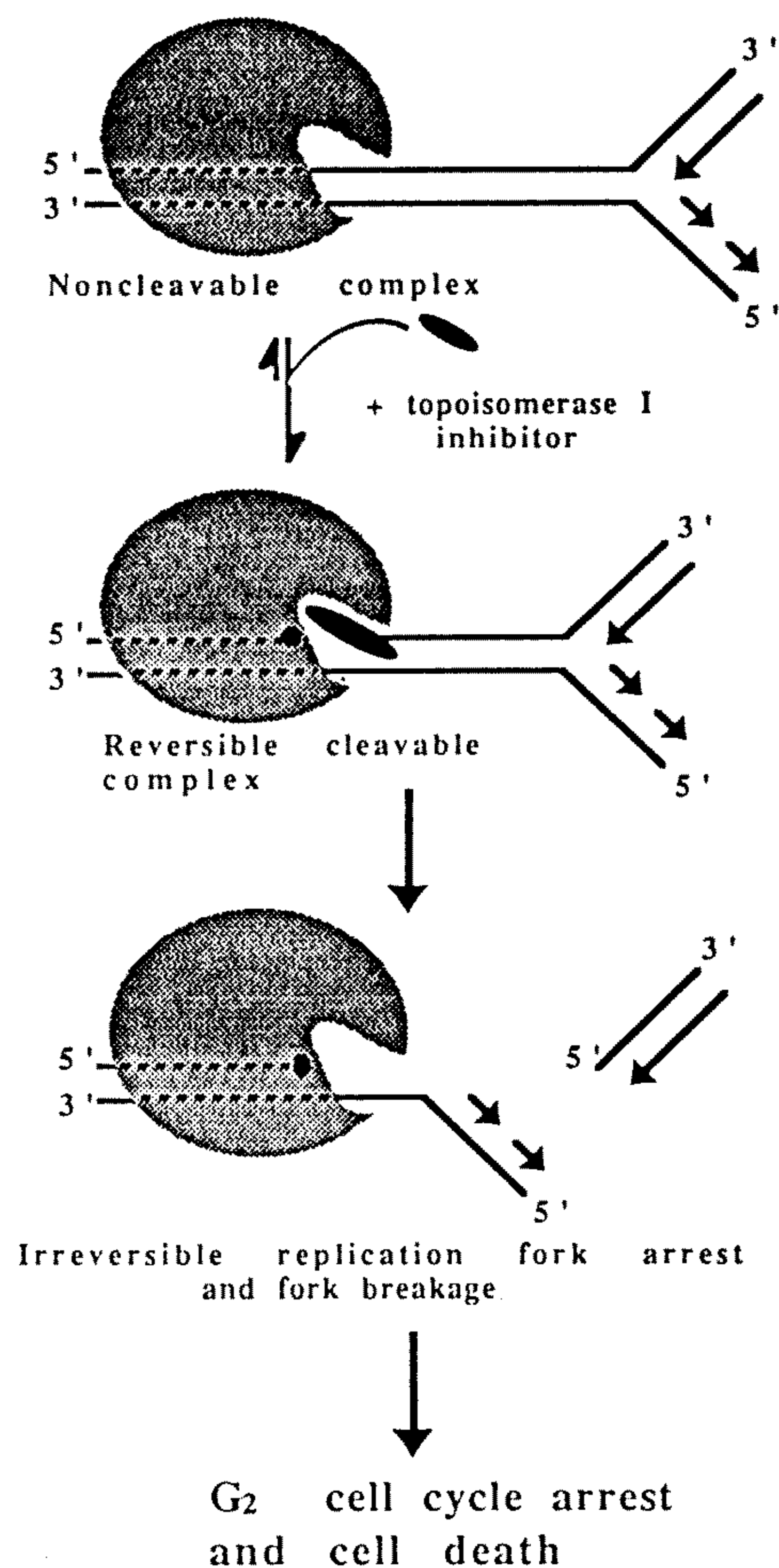


Figure 1. A fork collision model for Camptothecin-induced cytotoxicity. During the DNA replication, the topoisomerase I will form a reversible DNA-topoisomerase I cleavable complex, the cleavable complex and the noncleavable complex are at equilibrium. Camptothecin perturbs this equilibrium by trapping the cleavable complex and forming a topoisomerase I-camptothecin-DNA ternary complex on DNA. In this model, the topoisomerase I-mediated transient break is made on the strand that is complementary to the leading strand of DNA synthesis. Topoisomerase I is also represented as an asymmetric enzyme in which the major protein-DNA contact is upstream of the site of cleavage. This polarity-dependent collision triggers cell death and cell cycle arrest at the G₂ phase. (Adapted from reference 21)

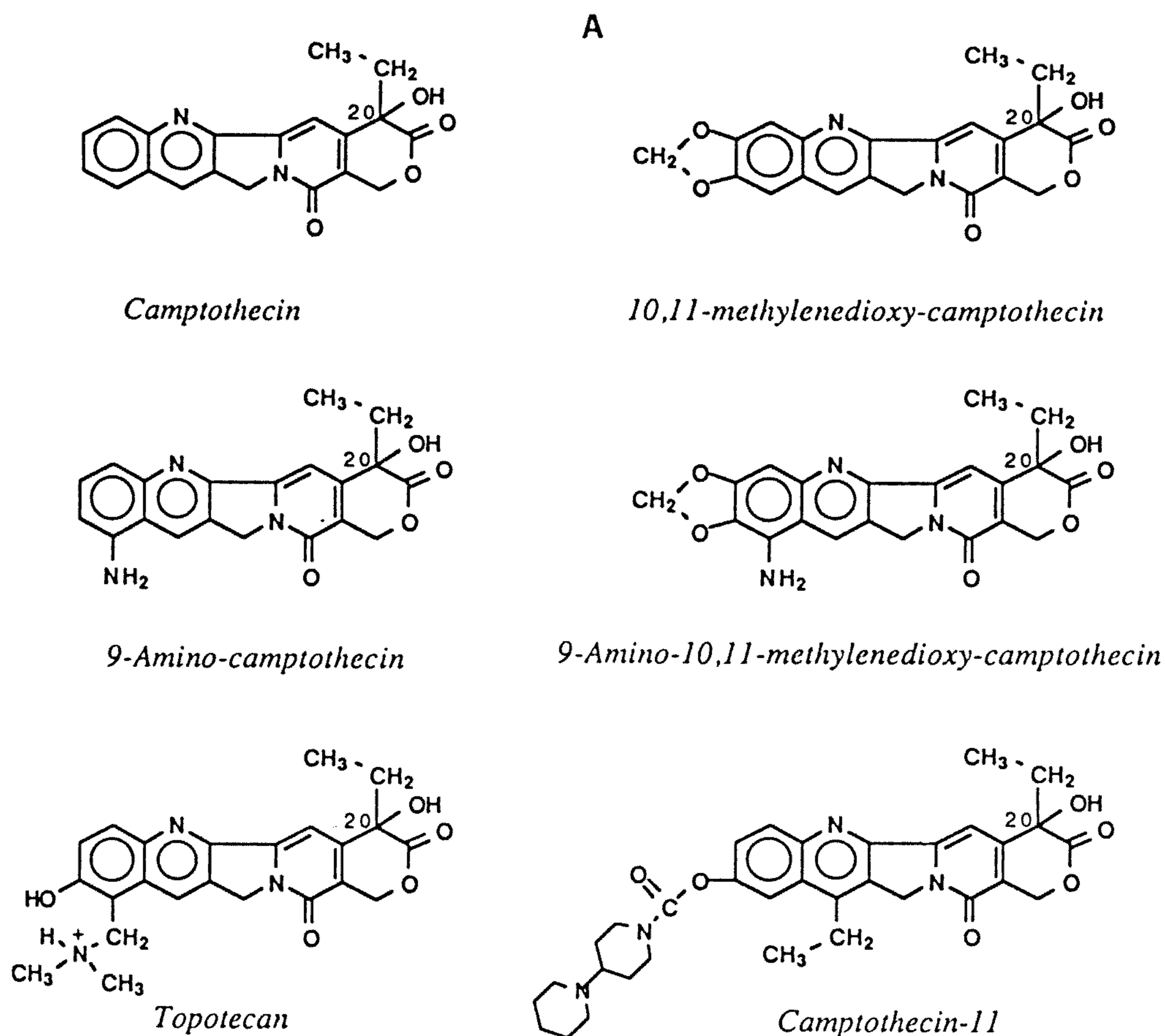


Figure 2. Chemical structure of Camptothecin and some promising camptothecin derivatives. 9-amino-camptothecin, Topotecan and Camptothecin-11 (CPT-11) are at various stages of clinical development in Japan, European and the United States. Among these, 9-amino-10,11-methylenedioxy-camptothecin is the most potent, followed by 10,11-methylenedioxy-camptothecin. (Adapted from reference 11)

物,其目的則是積極開發低毒、高效、水溶之衍生物⁽²⁰⁾。圖二列出了喜樹鹼和數個較有上市潛力之喜樹鹼衍生物的化學構造,其中抑制Topo I 活性最強的是9-amino-10,11-methylenedioxy-camptothecin,其次為10,11-methylenedioxy-camptothecin⁽²¹⁾。CPT-11為首例之水溶性喜樹鹼衍生物,毒性低,其本身不會抑制Topo I 活性,但在活體內會分解生成SN-38,此時方能與Topo I-DNA切割複合體作用,才具抗癌活性,CPT-11目前已在日本進行臨床二期試驗⁽²²⁾。Topotecan為另一新近開發的水溶性喜樹鹼衍生物,雖然其在試管內抑制Topo I 之活性僅為原喜樹鹼之四分之一,但在動物體內抗腫瘤的活性則大為增加,目前在美國亦已進入臨床二期試驗⁽²⁰⁾。

(二)能與DNA小凹溝(minor groove)結合之第一型DNA拓模異構酶抑制物:

除了喜樹鹼可抑制第一型DNA拓模異構酶活性,如圖三所示,可和DNA的minor groove結合並嵌入DNA中的化合物,如Hoechst 33258和33342亦可抑制Topo I 的活性⁽²³⁾。目前已知Hoechst dye抑制第一型DNA拓模異構酶之機轉和喜樹鹼類似,均會阻礙DNA和Topo I 所形成的切割複合體之再接合的作用,使DNA的複製因而受阻。1993劉昉博士的實驗室更進一步確認出Hoechst dye所辨認的DNA序列為5'-T^{*}CATTTT-3'(*所指為Topo I 切割DNA的位置)⁽²³⁾。由Hoechst dye之研究可知Topo I 會結合在DNA切割點的5'端,Hoechst dye則結合在3'端的TTTT,因而阻止斷裂

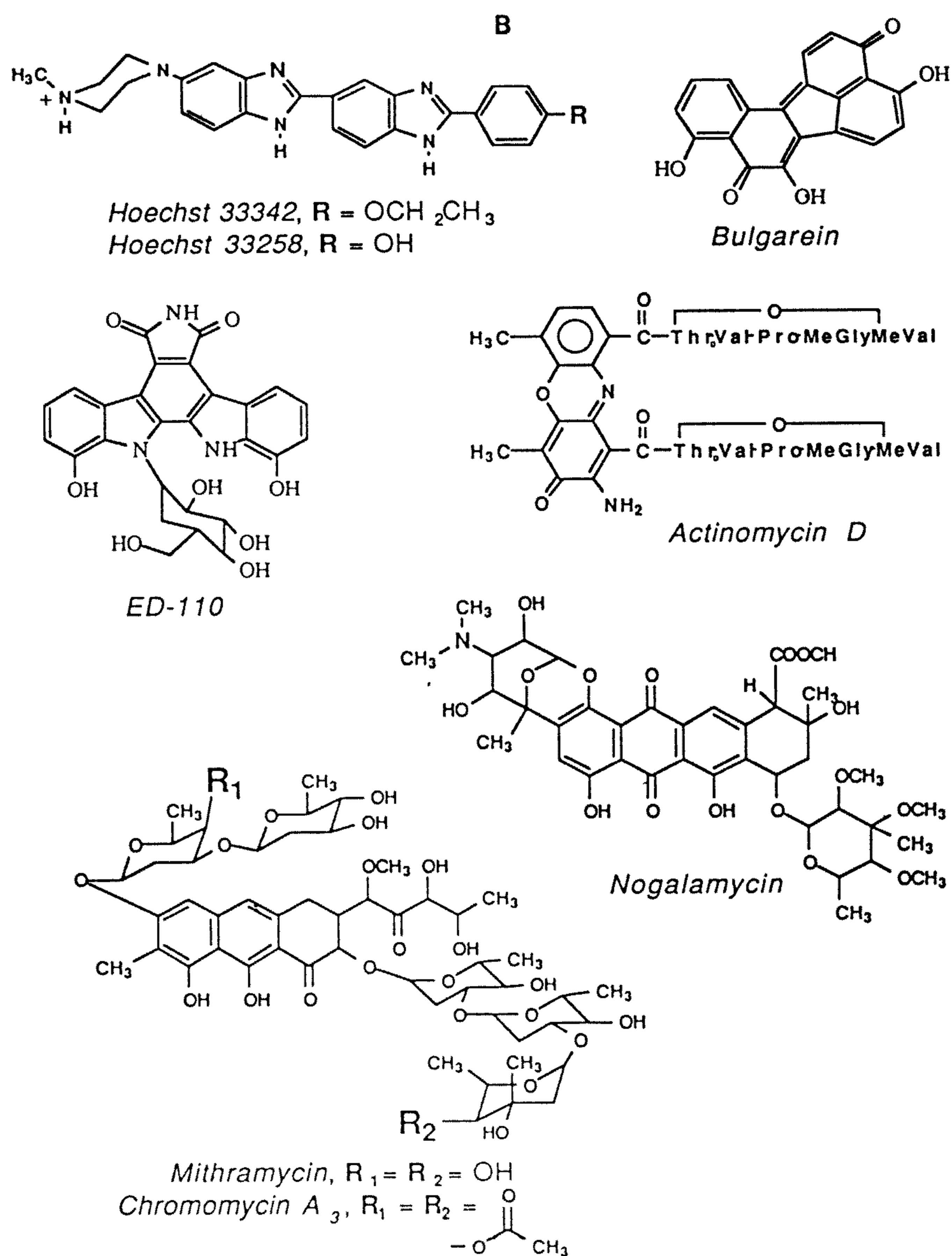


Figure 3. DNA minor groove-binding drugs represent other topoisomerase I poisons. DNA minor groove-binding drugs represents a major class of compounds with broad spectrum antimicrobial and antitumor activities. Their binding to the minor groove of DNA with A+T specificity, which causes widening of the minor grooves and prevents ligation of the two transiently disjointed ends. (Adapted from reference 23)

的DNA再接合。

Actinomycin D是很早即發現會嵌入DNA之藥物，會抑制哺乳動物DNA Topo I 和Topo II 的活性，由圖三Actinomycin D之化學結構中發現phenoxazine環可使此藥嵌入DNA中，而五個多肽環(pentapeptide rings)則會和DNA之鹼基形成氫鍵而結合至DNA之小凹溝⁽²⁴⁾，但目前對於Actinomycin D會抑制Topo I 活性之主因是否源於其和DNA小凹溝之結合或因此藥會嵌入DNA中

則尚無定論。而nogalamycin, mithramycin, 和chromomycin A₃這三種Topo I 抑制物經由NMR之分析研究，可知其和DNA小凹溝結合是在糖基(sugar moieties)部份。Bulgarein和indole carbazole衍生物ED-110亦是會和DNA小凹溝結合之第一型DNA拓模異構酶之抑制物。Yamashita曾證明indole carbazole衍生物嵌入DNA中的強度和抑制Topo I 接合DNA的能力無關⁽²⁵⁾。這一類藥物因為同時具有和DNA之小凹溝結合及嵌入

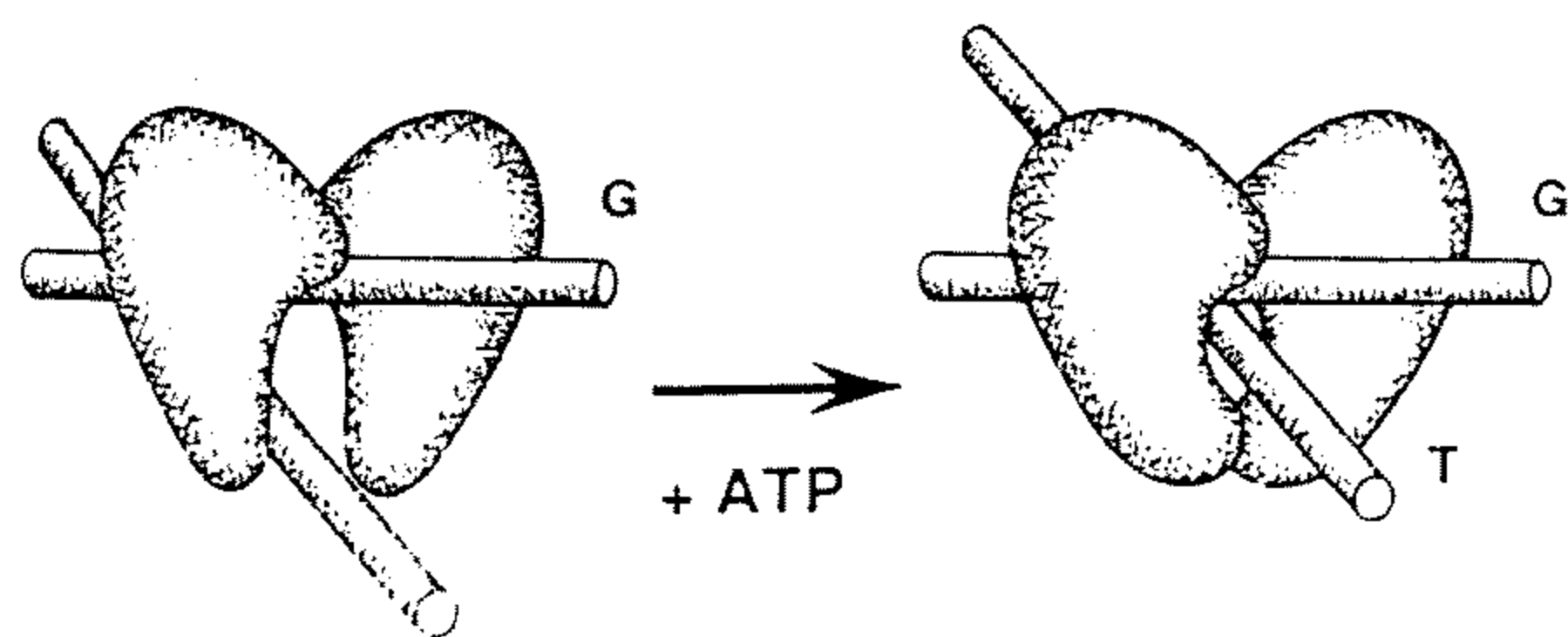


Figure 4. An ATP-dependent protein clamp model for the DNA topoisomerase II. In the absence of ATP (left), the G-segment-bound protein clamp is open and a second DNA segment, the T-segment, can enter the molecular trap. The binding of ATP to the type II enzyme closes the clamp, trapping the T-segment if one is present (right). Topological transformation of DNA is accomplished by transporting the captured T-segment through the enzyme-mediated DNA gate in the G-segment. (Adapted from reference 27)

DNA之特性,故其抑制Topo I 接合作用的機制仍需進一步證明。

二、抑制第二型DNA拓樸異構酶(Topo II)的抗癌藥物

第二型DNA拓樸異構酶的分子量是170KD,可是需由雙體(dimer)結合才具酵素活性。其催化反應需要ATP,雖然目前我們對於ATP如何影響Topo II的催化作用,還有許多問題尚待解決,但由圖四,王倬博士所提出的模型中⁽²⁶⁾,我們推測ATP的結合可控制Topo II雙體蛋白質似夾子般張開或關閉,夾住DNA並進而修飾DNA之超螺旋結構。目前已知許多抗癌藥物是以Topo II為標的⁽²⁷⁾,其作用機制雖未完全清楚,但主要仍如喜樹鹼般會抓住DNA-拓樸異構酶切割複合物而阻礙DNA的再接合。由圖四中我們知道ATP會改變Topo II的結構⁽²⁸⁾。而有些以Topo II為標的之抗癌藥物其作用會受ATP的影響如Adriamycin, VP-16和VM-16,但有些則不受ATP影響如menadione,故推測VP-16和VM-16等可能作用在關閉狀態(closed-gate)的Topo II,而menadione則作用在張開狀態(open-gate)之Topo II⁽²⁹⁾。除此之外,亦有一些以Topo II為標的之抗癌藥物並非抓住DNA拓樸異構酶之複合物,而是干擾Topo II之ATPase之作用,或是直

接抑制Topo II之催化活性如Suramin⁽³⁰⁾或ICRF 193⁽³¹⁾。

三、癌細胞對抗DNA拓樸異構酶抑制物之機制

耐藥性的產生在癌症的化學療法上是最讓人困擾的問題,目前所知,癌細胞抗藥性的生成可能有五種機制:

(一)藥物運送(drug transport)的增強:耐藥性癌細胞常在細胞膜上大量表現一種糖蛋白稱為多重耐藥性蛋白(multiple drug resistant protein、MDR 1),抗癌藥物進入細胞後,會被細胞膜上的多重耐藥性蛋白運送出細胞外,以降低細胞內藥物的量。所以這種耐藥性癌細胞,常可同時對抗數種化學構造不同的抗癌藥物如Adriamycin, vinblastine等⁽³²⁾。

(二)藥物代謝(drug metabolite)之增快,抗癌藥物雖能進入細胞內,但在還沒發生殺細胞作用前,即很快被代謝而失去了藥效⁽³³⁾。如癌細胞內Glutathione的含量及相關藥物代謝的酵素活性增加,使癌細胞內解毒系統活化,藥物快速失效而產生耐藥性。

(三)藥物標的物的改變(drug target alternation):有些癌細胞會降低細胞內Topo I或Topo II的表現量,甚至產生Topo I或Topo II的結構突變,使藥物無法辨識而癌細胞產生耐藥性。

(四)細胞內DNA修補能力增強而使癌細胞產生耐藥性。

(五)細胞自戕(Apoptosis)作用的改變,大部分的抗癌藥物均是造成癌細胞DNA損壞而刺激細胞產生一連串的反應,來誘發細胞死亡,稱為programmed cell death或細胞自戕⁽³⁴⁾。在細胞自戕一連串的反應中如其中有一步驟無法順利延續,均會延緩癌細胞的死亡進而使細胞產生耐藥性⁽³⁵⁾。如p53為誘發細胞自戕的因子,帶有p53突變基因之腫瘤細胞,DNA受損後卻無法誘導細胞自戕現象因而呈現耐藥性⁽³⁶⁾。癌細胞耐藥機制的探討,將有助於我們從各種角度謀求對策,以化學療法戰勝頑強之癌細胞。

結 語

自從1980年以後,陸續發現許多抗癌藥物是以DNA拓樸異構酶為標的,這方面研究的蓬勃展開,已為抗癌藥物的篩選及作用機制的研究拓展了一個新的領域。DNA拓樸異構酶在細胞核內同時具

有切、割作用的重要酵素，但許多以其為標的之抗癌藥物卻可將此重要酵素轉變為只能切斷DNA因而阻礙DNA之複製、轉譯等重要活動，最後導致細胞死亡。這一類的抗癌藥物常可同時殺死多種癌細胞，所以目前欲由天然植物或化學合成藥物中開發新的抗癌藥物時，通常是以能抑制DNA拓樸異構酶活性為首要篩選的目標。然而這些抗癌藥物是如何抓住DNA和拓樸異構酶的複合物？如何阻礙了拓樸異構酶的再接合作用？至今還有許多疑點尚未釐清。本實驗室為了探討致癌細胞耐藥性的發展機制，曾以變異誘導劑(EMS)處理人類卵巢癌A2780細胞株，並在培養基中逐步增加喜樹鹼的濃度，而成功的篩選出能夠耐喜樹鹼的突變株。經由深入研究知道其耐藥性乃由於細胞內第一型DNA拓樸異構酶基因發生了突變，胺基酸Gly⁷¹⁷突變為Val，及Thr⁷²⁹突變成Ile。因這雙點突變非常接近酵素的活化中心(Tyr⁷²³)，因此我們猜測喜樹鹼可能作用在酵素活化中心，或其鄰近區域。此外其他的實驗室亦在不同腫瘤細胞中發現，當第一型DNA拓樸異構酶的某些胺基酸發生突變，亦可產生對喜樹鹼之耐藥性⁽³⁷⁾。依據這些結果我們可以推斷喜樹鹼阻斷第一型DNA拓樸異構酶應與這些胺基酸參與作用有關。我們深信這方面的研究，不但可讓我們在分子層次上瞭解喜樹鹼如何干擾Topo I的活性以及對癌細胞的耐藥機制謀求對策，更可幫助我們設計藥物和標的物的分子模型，來進一步改造已有的藥物為低毒性且具高效性的抗癌藥物。

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DNA Topoisomerases as Targets of Anticancer Drugs

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ABSTRACT

Eukaryotic DNA topoisomerases are ubiquitous nuclear enzymes that alter DNA topology by breaking and rejoining DNA strands. There are two classes of DNA topoisomerases; DNA topoisomerase I introduces a transient single-strand DNA break, while topoisomerase II introduces transient double-strand DNA breaks for each catalytic reaction. Both are important for solving topological problems arising during DNA replication, transcription, recombination and other cellular functions. Recently, scientists have realized the importance of topoisomerases as new therapeutic targets for antibacterial, antifungal, antiparasitic, antiviral and anticancer drugs. The present review focuses on anticancer drugs targeting mammalian DNA topoisomerases, which are named DNA topoisomerase poi-

son. Camptothecin and VM26 (teniposide) are representative DNA topoisomerase poisons that target DNA topoisomerase I and topoisomerase II, respectively. These drugs alter the breakage-reunion reactions of DNA topoisomerases by trapping topoisomerase-DNA cleavable complexes in both the purified system and cultured cells. However, resistance to various DNA topoisomerase poisons has been documented in cancer cells with respect to MDR1 overexpression, reduced topoisomerase levels, drug-resistant mutant topoisomerase, lengthened cell cycle time and altered DNA repair function. A better understanding of the molecular targets for anticancer drugs and the various drug-resistance mechanisms may help us to discover and tailor new drugs for particular drug-resistant tumors.

Key Words : DNA Topoisomerase poison, DNA Topoisomerase I , DNA Topoisomerase II.

