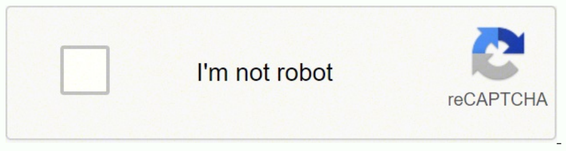

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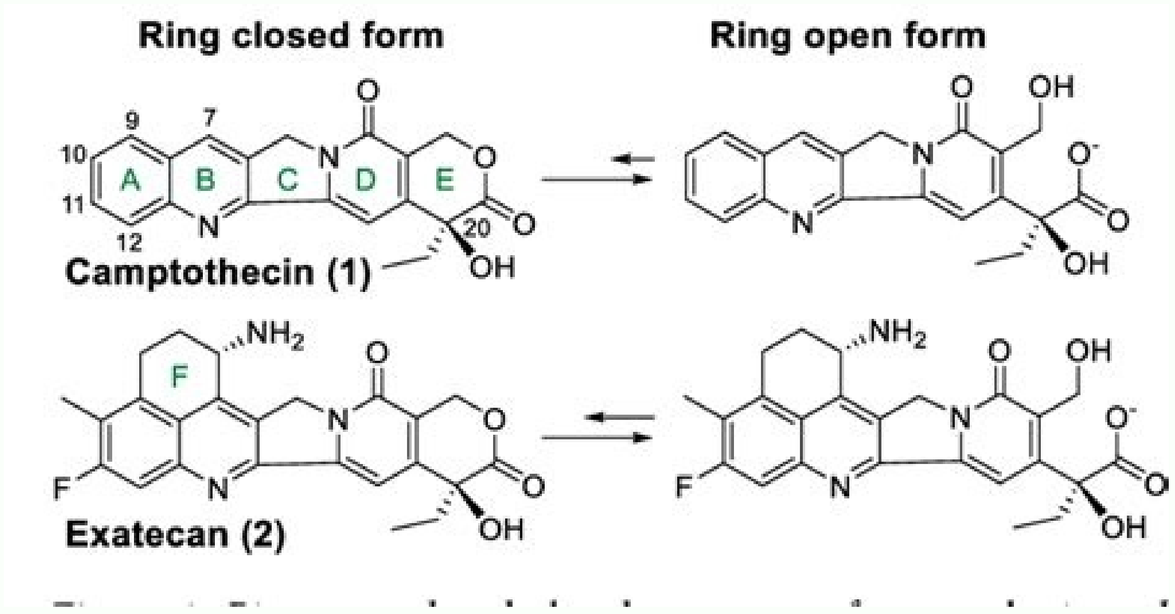
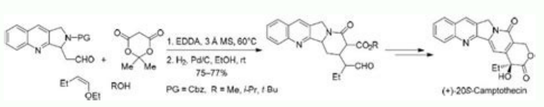
Camptothecin synthesis pdf





Proc. Nat. Acad. Sci. USA
Vol. 68, No. 12, pp. 3009–3014, December 1971

Ribosome Formation is Blocked by Camptothecin, a Reversible Inhibitor of RNA Synthesis

(HeLa cells/precursor RNA/ribosomal proteins/nucleolus/reversible inhibition)

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Communicated by Abraham White, September 30, 1971

ABSTRACT **A new drug, camptothecin, has been used to study the regulation of ribosome synthesis in HeLa cells. 5 μM camptothecin inhibits the synthesis of heterogeneously sedimenting nuclear RNA by about 70%. Camptothecin also blocks a specific step in the processing of ribosomal precursor RNA, allowing the conversion of 43S RNA to 32S RNA, but inhibiting the conversion of 32S RNA to 28S RNA. The action of camptothecin, unlike that of actinomycin D, is rapidly reversible. Within 3 min after the removal of the drug, ribosomal precursor RNA synthesis and maturation resume at the normal rate.**

Ribosomal proteins made in the presence of camptothecin do not accumulate in the nucleolus, and are not subsequently used to form ribosomes if RNA synthesis is allowed to resume.

The plant alkaloid, camptothecin, has shown antitumor activity against Walker 256 rat carcinosarcoma, murine leukemia L1210, K1964, L5178Y (1–4), and solid tumors (5). Horwitz, Chang, and Grollman (6, 7) reported that camptothecin rapidly inhibited RNA and DNA synthesis in HeLa cells, and that the inhibition of RNA synthesis was readily reversed by removal of the drug from the medium. A closer examination revealed that camptothecin inhibited the labeling of ribosomal RNA; transfer RNA was not affected (7). Other preliminary reports indicate that camptothecin inhibits the incorporation of thymidine and uridine into those nucleic acids of cultured L1210 murine leukemia cells that are made in the nucleus, but not those of the nucleolus (8,*†). It is also inactive against *Escherichia coli* RNA polymerase (9, 8). The present study examines the effect of camptothecin on ribosomal RNA synthesis and on ribosomal protein accumulation and utilization in the nucleoli of HeLa cells. Some data concerning the effect of camptothecin on the synthesis of heterogeneously sedimenting nuclear RNA are also presented.

MATERIALS AND METHODS

Cell Culture. HeLa S3 cells were grown in spinner culture at 4–5 × 10^5/ml in Eagle's minimal essential medium (9) supplemented with 5% fetal calf serum.

Cell Fractionation, RNA and Protein Extraction, and RNA Electrophoresis Analysis. Cells were fractionated into cytoplasm and detergent-cleared nuclei, and nuclei into supernatant and pellet (nucleolar) fractions (10–12). The nucleoli were prepared by the method of Penman (10, 11) for the isolation of ribosomal precursor RNA, and by the method of Weisbach et al., (12) for the isolation of nucleolar proteins. The RNA of cytoplasmic extracts was released by 1% sodium dodecyl sulfate (SDS) and analyzed by sucrose-gradient zonal sedimentation (13). Nucleolar RNA was released for sedimentation analysis by dissolving the nucleolar pellet in 0.5 M urea–0.5% SDS–0.01 M sodium phosphate (pH 7.2). HnRNA (see below) was isolated from the nuclear supernatant fraction with 1% SDS, followed by 2 volumes of cold 95% ethanol. After 18 hr at −20°C, the RNA was collected by centrifugation at 25,000 × *g* for 10 min. The RNA was then dissolved in 0.01 M Tris–HCl–0.01 M EDTA–0.1 M NaCl–0.2% SDS and analyzed by sucrose-gradient zonal sedimentation. Ribosomes were prepared by Mg^{++} precipitation and the subunits were isolated by sucrose gradient sedimentation (14, 15). Preparation and acrylamide gel analysis of proteins from nascent ribonucleoprotein particles and 80S ribosomal subunits have been described (16).

Drugs. The sodium salt of camptothecin was kindly provided by Dr. H. B. Wood, Jr. of the National Cancer Institute. 4-mg portions were dissolved in 10 ml of water to make 1 mM solutions and kept at −20°C until use.

RESULTS

Effects of Camptothecin on RNA and Protein Synthesis in HeLa Cells. Two major classes of RNA are synthesized in the nucleus, heterogeneously sedimenting nuclear RNA (HnRNA) and ribosomal precursor RNA (precursor rRNA) (10). They are made in different locations (17) by different enzymes (18), and can be distinguished by cell fractionation and sucrose gradient analysis (19). 5 μM camptothecin causes a severe inhibition of HnRNA synthesis (Fig. 1*B*). The inhibition is about 30% at 1 μM, 70% at 5 μM, and 90% at 10 μM. The major effect of moderate doses of the drug is to inhibit specifically the synthesis of larger molecules of HnRNA (Fig. 1*C*). Although the role of HnRNA in protein synthesis is unclear, we do find, in confirmation of the results of Harris et al. (7), that protein synthesis continues unabated for at least 3 hr in the presence of 5 μM camptothecin. Furthermore, there is no loss of polyribosomes after incubation for 48 min with 10 μM camptothecin.

Abbreviations: HnRNA, heterogeneously sedimenting nuclear RNA; SDS, sodium dodecyl sulfate.

* Moore, B. G., H. S. Schwartz, and H. Stein, *Abstracts of Tenth Meeting, American Soc. Cell Biol., San Diego (1970); J. Cell Biol.*, 47, 144A (1970).

† Wood, H., *Abstracts of 62nd Annual Meeting Pac. Proceedings*, San Francisco, 30, 1178Abe (1971).

The International Journal of Biochemistry & Cell Biology 41 (2009) 1165–1172

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

De novo ceramide synthesis is responsible for the anti-tumor properties of camptothecin and doxorubicin in follicular thyroid carcinoma

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ARTICLE INFO

Article history:
Received 5 June 2008
Received in revised form 14 October 2008
Accepted 22 October 2008
Available online 5 November 2008

Keywords:
Ceramide
Apoptosis
Thyroid
Cancer
Doxorubicin
Camptothecin

ABSTRACT

Doxorubicin and camptothecin are two cytotoxic chemotherapeutic agents triggering apoptosis in various cancer cells, including thyroid carcinoma cells. Recent studies revealed a critical role of ceramide in chemotherapy and suggested that anti-cancer drugs may kill tumor cells through sphingomyelinase activation. However, in comparison to sphingomyelin hydrolysis, the relative involvement of de novo ceramide synthesis remained poorly explored and highly controversial. Here, we evidenced that both doxorubicin and camptothecin triggered ceramide accumulation in thyroid carcinoma cells. We demonstrated that ceramide increase occurred via the de novo pathway without neither acidic nor neutral sphingomyelinase contribution. Interestingly, de novo ceramide generation was responsible for the drug-induced malignant cell apoptosis through a caspase-3-dependent pathway and a decrease of thrombospondin amount. Furthermore, blocking ceramide metabolism by inhibiting glucosylceramide synthase strengthened the camptothecin and doxorubicin-dependent effects. Altogether, we evidenced that de novo ceramide synthesis mediates the anti-tumor properties of doxorubicin and camptothecin in thyroid carcinoma and suggested that glucosylation of ceramide may contribute to the drug-resistance phenotype in thyroid malignancies.

1. Introduction

Thyroid malignant diseases represent the majority of deaths from endocrine cancers (Correa and Chen, 1995). After the Chernobyl tragedy in April 1986, the incidence rate of thyroid cancers rose more than 10 times between 1990 and 2000 in Western Europe. In U.S., over 23,000 new cases of thyroid cancers are diagnosed annually and the incidence of thyroid malignancies has risen nearly 50% since 1973 (Jemal et al., 2004). Although thyroidectomy remains the standard protocol for patients with medullary carcinoma, radioactive iodine is a major modality for the detection and treatment of recurrent differentiated thyroid carcinomas, e.g., papillary and follicular carcinomas (Jossart and Clark, 1994; Gagel et al., 1996; Pagano et al., 2004). However, 20–30% of these tumors do not fix radioactive iodine and are frequently associated with aggressive clinical behavior (Sarlis, 2001). Chemotherapy is used as a single or as part of combined modality treatment against metastatic or locally advanced thyroid cancers when other conventional treatments (e.g., surgery and radiation therapy) failed. Anticancer chemotherapy leading to tumor cell apoptosis is predominantly employed in thyroid carcinomas that do not concentrate iodine (Mandal et al., 2005; Furuya et al., 2007). Follicular thyroid carcinoma (FTC), a well-differentiated form of thyroid cancer, accounts for up to one-third of thyroid cancer cases. Patients suffering from lung or bone metastases of follicular thyroid carcinoma have a poor prognosis and efficient treatments are currently lacking (Schlumberger, 1998; Lin et al., 1999). Our recent results evidenced that efficient FTC cell death triggered by anticancer drugs occurred in a TSP-1-dependent manner. Indeed, we demonstrated for the first time that both camptothecin and doxorubicin-induced apoptosis were constantly associated with TSP-1 expression down regulation in FTC-133 malignant cells (Rath et al., 2006a). TSP-1 therefore appeared as an interesting molecular marker of camptothecin and doxorubicin-induced apoptosis in FTC.

Abbreviations: CPT, camptothecin; DOX, doxorubicin; FB1, fumonisin B1; FTC, follicular thyroid carcinoma; GCS, glucosylceramide synthase; LASS, longevity assurance homolog; PDMP, phenyl-2-decanoylamino-3-morpholino-1-propanol; SMase, sphingomyelinase; TSP-1, thrombospondin-1.

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doi:10.1016/j.biocel.2008.10.021

Vol. 68, No. 12, pp. 3009–3014, December 1971

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Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, New York, 10461

Communicated by Abraham White, September 20, 1971

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Cell Fractionation, RNA and Protein Extraction, and RNA Sedimentation Analysis. Cells were fractionated into cytoplasm and detergent-cleaned nuclei, and nuclei into supernatant and pellet (nucleolar) fractions (10–12). The nucleoli were prepared by the method of Penman (10, 11) for the isolation of ribosomal precursor RNA, and by the method of Weinberg *et al.*, (12) for the isolation of nucleolar proteins. The RNA of cytoplasmic extracts was released by 1% sodium dodecyl sulfate (SDS) and analyzed by sucrose-gradient zonal sedimentation (13). Nucleolar RNA was released for sedimentation analysis by dissolving the nucleolar pellet in 0.5 M urea–0.5% SDS–0.01 M sodium phosphate (pH 7.2). HnRNA (see below) was isolated from the nuclear supernatant fraction with 1% SDS, followed by 2 volumes of cold 95% ethanol. After 18 hr at −20°C, the RNA was collected by centrifugation at 15,000 × *g* for 10 min. The RNA was then dissolved in 0.01 M Tris·HCl–0.01 M EDTA–0.1 M NaCl–0.2% SDS and analyzed by sucrose-gradient zonal sedimentation. Ribosomes were prepared by Mg^{++} precipitation and the subunits were isolated by sucrose gradient sedimentation (14, 15). Preparation and acrylamide gel analysis of proteins from nascent ribonucleoprotein particles and 50S ribosomal subunits have been described (14).

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The conversion of DXP to isopentenyl diphosphate (IPP), which is the common terpenoid biosynthesis precursor involves 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) and 1-hydroxy-2-methyl-2(E)-butenyl-4-diphosphate reductase (HDR). PMIDÃ Â1995300.{{cite journal}}: CS1 maint: multiple names: authors list (link) ^ Y. BNP1350 which belongs to the series of karenitecins exhibits cytotoxic activity and ability to overcome drug resistance. Sissi; B. One of the most potent compounds is the oxyiminomethyl derivative ST1481 that has the advantage to overcome drug resistance caused by transport systems.[20] Basic nitrogen in a carbon chain at position 7 makes the compound more hydrophilic and hence more water-soluble. These groups are able to react with the DNA in the presence of topoisomerase I which leads to more tumor activity. Redon; V.A. Rao; J.A. Seiler; O. PMIDÃ Ã30218487. Samuelsson (2004). Chemical Biology & Drug Design. ^ "Camptothecin". Its planar structure is thought to be one of the most important factors in topoisomerase inhibition.[13][14] Binding of CPT to topoisomerase I and DNA Binding CPT binds to the topoisomerase I and DNA complex (the covalent complex) resulting in a ternary complex, and thereby stabilizing it. Beretta; L. Enlargement of the lactone ring by one CH2 unit also enhances its abilities, as in homocamptothecin. PMIDÃ Â20108971. Retrieved from " 2008. I. First, chorismate is converted to anthranilate by the alpha-subunit of anthranilate synthase (ASA). ^ M. The reason is the unfavorable steric interactions of ethylenedioxy analogues with the enzyme.[13][20] Adding amino or chloro group at 9th position or chloromethyl group at 7th position to these 10, 11-methylenedioxy or ethylenedioxy analogues results in compounds with even greater cytotoxicity but weaker solubility in water. 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[25] strictosidine then suffers intermolecular cyclization to produce estremosamide, which Sllec Recnac Ynam Ni Si Ti SA, Noitidnoc Cidica Ni Deroval Si Mrof Desolc Eht .302Â "â € ¢ 371:) 2â" â € ¢ 1 (235.5-8000-500-08200s / 7001.01: IOD .DEZISEHTNYS NEEB evah seugolana ynam TPC fo yrevocsid eht ecniS seugolana TPC] 02 [] 31 [.TPC naht sllec doolb der rof ytiniffa erom dna gnidnib nietorp desaerced fo esuaceb ytilibats amsalp namuh decnahne tibihxe sTPCh.) 2791 (South nahtanwsiV, RT irahcadnivoG ^ secnerefeR] 62 [.adisolimupykshoad-) Agricultural (3 dna edisolimup-) Agricultural (3, edimasotcirts ary enidisotcirts morf decudorp si nicehtotpmac taht detalutsop neeb sah ti, devloser ylluf ton si ti hguohtlA Kg; Tsrihved. 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Reduced drug-HSA interactions could result in improved activity.[16][20] Structure-activity relationship Camptothecin Studies have shown that substitution at position 7, 9, 10 and 11 can have positive effect on CPT activity and physical properties, e.g. potency and metabolic stability. Stewart; P. doi:10.1007/s11676-018-0794-3. Substitution at position 12 and 14 leads to inactive derivative.[20] A- and B-ring modification Alkyl substitution Alkyl substitution at position 7 has shown increased cytotoxicity, such as ethyl (C2H5) or chloromethyl (CH2Cl). Y. PMCÃ Â316296. Methoxy group at both position 10 and 11 simultaneously leads to inactivity.[13][20] Hexacyclic CPT analogues Hexacyclic CPT analogues have shown great potency. 93 (6): 986¢ÃÂÂ992. Impact of the tumor pH gradient". PMIDÃ Â16001167. 31: 27¢ÃÂÂ43. "Current status and perspectives in the Development of Camptothecins". II. Antony; L. ^ a b c d e f g H. 62 (2): 2039¢ÃÂÂ2057. Redinbo; L. "Influencing in vitro clonal propagation of Chonemorpha fragrans (moon) Alston by culture media strength, plant growth regulators, carbon source and photo periodic incubation". Anthranilate reacts with 5-phosphoribose pyrrophosphate to produce 5-phosphoribosylanthranilate. Journal of Chromatography B. Exatecan is an example of hexacyclic CPT that has a 6 membered ring over position 7 and 9, and is 10-methyl, 11-fluoro substituted [4]. "Camptothecins, a review of their chemotherapeutical potential". It Isolated from the bark and stem of Camptotheca acuminata (Camptotheca, Happy tree), a tree native to China used in traditional Chinese medicine. [1] [2] It has been used clinically more recently in China for the treatment of gastrointestinal tumors. [3] CPT showed anti-cancer activity in preliminary clinical trials, especially against cancer of the chest, ovarian, colon, lung and ³. [4] However, it has low solubility and adverse effects have been reported when used ââ 8] and medicinal products have developed numerous Camptothecine [6] [7] [7] [8] and derivative viruses to increase the benefits of chemical substtion, with good results. 134 (1): 161 Ã¢ â¬ "170. Merlini; G. The hydroxyl group at position 20 forms a hydroxide binding side chain in Aspertic acid No 533 (ASP533) in the enzyme. 57 (2): 145 Ã¢ â¬ "154. Doi: 10.1016 / j.phytochem.2004.09.001. "Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine". Two: 10.1021 / JA00968A057. 88 (16): 3888 Ã¢ â¬ "3890. HOL (1998). It is fundamental that the chiral carbon configuration is (s) because (r) it is inactive. Journal of forest research. 8 (27): 2505 â¬ 2520. The most important part of the structure is the electron ring that interacts with the enzyme from µ different positions. 45 (3): 273 - 281. Retrieved October 9, 2016. The HCPT e-ring opens more slowly and the opening is open. C. I am, Tasiu; Umar, Shahid (September 2018). TWO: 10.1016 / 0014-4827 (91) 90534-2. ^ a b yamazaki, Yasuyo; Kitajima, Mariko Arita, masanori; Takayama, Hiromitsu; Sudo, hiroshi; Yamazaki, Mami; Aimi, Norio; Saito, Kazuki (2004). "The S-phase cytotoxicity of Camptothecine". L. Based on studies with specific radioactive labeling inhibitors and spectroscopic pathways, the MEP pathway is the major source of secologanin. [29] Shikimate path and secologanin of the MVA or MEP path are converted to estricosidine stricosidine .94896221Â ÃDIMP .)6002(miK .X ;uoZ .42071811Â ÃDIMP .dnob negordyh gnimrof yb xelpmoc tnelavoc AND-I esaremosiopot eht sezilibats dna dnarts devaelc-non no enisotyc 1+ eht htiw stcaretni gnir-D ehT .Z ;uL .).deÂ Ã72(ngiseD lacituecamrahP tnerruC ."esoculG]C31-1[morf ydutS recarT oviV ni dna ociliS nI .)7002(M kniW ,SV allamiknoK ,G zrawhcS ,GY uZ ,JY uF ,T htreffE ^ .50-90-9002 no lanigiro eht morf devihcrA .7 noitisop ta tneutitsbus gnisilibulos retaw a ecudortni ot si yaw doog a ytilibulos retaw doog htiw seugolana yxoidenelyhte ro yxoidenelyhtem-11 ,01 dleiy oT .sisylordyh ot elbitpecsus ylhgih si TPC ni gnir enotcal ehT yrtsimehC]91[.TPC dna AND yb demrof sexelpmoc egavaelc eht htiw sedilloc krof noitacilper eht nehw skaerb dnarts-elbuod otni skaerb dnarts-elgnis fo noisrevnoc fo tluser a yliramirp si yticixot sti dna]81[esahp S gnirud AND gnitacilper sllec eht ot cixototyc ylevitceles si TPC]71[]61[.enisotyc 1+ fo gnir enidimiryp eht no puorg onima dna gnir-D eht no 71 noitisop ni puorg lynobrac neewteb si dnob negordyh sihT .065474515Â ÃDIMP .11 ro 01 noitisop ta puorg lyxordyh dna 01 dna 9 noitisop ta orolhc ro omorb ,ortin ,onima ekil spuorg gniwardhtiw-nortcele gnittup yb deveihca eb nac ytivitca retaerg ylbaredisnoC]12[]02[.TPC htiw nees ytiticixot dna ytilibulos retaw roop eht semocrevo yllufsseccus dna rotibihni I esaremosiopot tnetop a si hcihw ,206-DKC dellac etavired a si ,elpmaxe roF .stcepsorP erutuF dna sisehtnysoiB diolaklA no thgiL weN ^ ."AND htiw sexelpmoc tnelavocnon dna tnelavoc ni I esaremosiopot namuh fo erutcurts latsyrC" .)7002(gnaiJ .H ;ouL ."nicehtotpmaC - sgurD fo yranoitciD - esabtenmehC" ^ .ycnetop desaercni dna setavired elbulos-retaw erom ot sdael hcihw gnir derebmem 6 ro 5 a mrof 11 dna 01 neewteb detcennoc puorg yxoidenelyhte ro yxoidenelyhtem ,elpmaxe roF .)9102(H naiX ,gnaW ^ .63434641Â ÃDIMP .esahtnys enidisotcirts yb dezylatac noitcaer noitasnednoc a si tnemecalper elbissop enO] 32 [.I POT fo etis evitca eht ot gnidnib rof yrassecen si ti esuaceb ytivitca TPC gnisol tuohtiw segnahc larutcurts ynam wolla tÂ Ã Ã ¢ nseod gnir-E ehT snoitacifidom gnir-E nicehtotpmacomoH] 31 [.yassa yticixototyc rehto ni dnuopmoc tnerap naht dnuopmoc tnetop ssel hcum ni stluser noitisop yna ni tnemecalpeR .M; smadA .ygolocamrahP dna ygolocixoT yrotalugeR.) 2002 (isetarP .snoitartsinimda ni ytluciffid sesuac hcihw, snoitulos suoeuqa ni elbulosni ylevitaler era sdnuopmoc eseht tuB .01 ta spuorg onima ro lyhtem dna 11 noitisop ni tup era spuorg gniwardhtiw-nortcele nehw evitca erom emoceb TPC cilcycaxeh esehT .reirrab niarb doolb eht ssorc osla nac yeht dna ytilibats doolb sti ot setubirtnoc hcihw snoitcaretni ASH-gurd decuder nwohs evah snicehtohtpmaclylis-7 ro snacetaliS .50475641Â Ã, DIMP .snacetalis evitca tsom eht gnoma si dna Evitavired YXordyh-01 A SI 76-BD .983920.301.PP / 4011.01: IOD .23â "â € Ã ¢ 4202:) 91 (41 .400.50.6002.HPTry.j / 6101.01: iod .j; lhaw .c; ob mulap .EVITCAER YREV ENTCAL EHT SEKAM HCIHW PUORG LYXOBRAC EHT NO TCEFFE EVITCUDNI SSEL SAH LYXORDYH SÂ ™ â € Ã ¢ TPCH EHT.) 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The isolation and structure of camptothecin, a new alkaloid leukemia and camptotheca acuminate tumor inhibitor". Gatto S. Wani in the systematic screening of natural products for anti-cancer drugs. pp. 143Ã Â149. pp. Ã 143Ã

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