3,3’-Diindolylmethane Is a Novel Topoisomerase IIα Catalytic Inhibitor That Induces S-Phase Retardation and Mitotic Delay in Human Hepatoma HepG2 Cells

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ABSTRACT

Epidemiological evidence suggests that high consumption of Brassica genus vegetables, such as broccoli, cabbage, and Brussels sprouts, is very effective in reducing the risks of several types of cancers. 3,3’-Diindolylmethane (DIM), one of the most abundant and biologically active dietary compounds derived from Brassica genus vegetables, displays remarkable antitumor activity against several experimental tumors. In the present study, we demonstrate for the first time that DIM is a novel catalytic topoisomerase IIα inhibitor. In supercoiled DNA relaxation assay and kinetoplast DNA decatenation assay, DIM strongly inhibited DNA topoisomerase IIα and also partially inhibited DNA topoisomerases I and IIβ. DIM did not stabilize DNA cleavage complex and did not prevent etoposide-induced DNA cleavage complex formation. Further experiments showed that DIM inhibited topoisomerase IIα-catalyzed ATP hydrolysis, which is a necessary step for the enzyme turnover. In cultured human hepatoma HepG2 cells, DIM blocked DNA synthesis and mitosis in a concentration-dependent manner, which was consistent with the outcome of topoisomerase inhibition in these cell-cycle phases. Our results identified a new mode of action for this intriguing dietary component that might be exploited for therapeutic development.

Certain indole derivatives from Brassica genus vegetables are under study for their potential cancer-protective activities (Dashwood et al., 1994; Firestone and Bjeldanes, 2003; Chang et al., 2005). Indole-3-carbinol, found in the Brassica genus vegetables such as broccoli, cabbage, and Brussels sprouts, inhibited tumor development in mammary gland, liver, uterus, stomach, and other organs when administered orally to rodents before or during carcinogen exposure (Dashwood et al., 1994; Takahashi et al., 1995; He et al., 1997; Oganesian et al., 1997). 3,3’-Diindolylmethane (DIM) is one of the major products of indole-3-carbinol that is formed in gastric acid (De Kruif et al., 1991; Staub et al., 2002; Leibelt et al., 2003). DIM induces the expression of phase 1 and phase 2 xenobiotic metabolizing enzymes in the liver and extrahepatic tissues, resulting in the enhanced capacity for the detoxification of carcinogens (Chen et al., 1996; Lake et al., 1998; Renwick et al., 1999; Sanderson et al., 2001; Gross-Steinmeyer et al., 2004). Studies clearly showed that DIM administered orally at the dose of 5 mg/kg body weight for 21 days effectively inhibited mammary gland tumor growth by 80% in female Sprague-Dawley rats that were initiated with mammary gland carcinogen, the 7,12-dimethylbenz(a)anthracene (Chen et al., 1998). Results of studies conducted in our laboratory further demonstrated that DIM significantly inhibited the growth of human breast tumor xenografts (MCF-7) in athymic mice (Chang et al., 2005). Because the growth of xenograft tumors does not rely on carcinogen exposure, detoxification of carcinogen does not explain the growth-inhibitory effects of DIM on xenograft tumors. Thus, other mechanisms must be considered.

DNA topoisomerases (topos) are nuclear enzymes that regulate conformational changes in DNA topology by catalyzing the breakage and resealing of DNA strands. In humans, there are six topoisomerase genes encoding for nuclear topoisomerase: I (topo I); mitochondrial topo I; topo II, α and β; and topo III, α and β (Champoux, 2001; Wang, 2002). Although the functions of some of these enzymes are still not clear, they are extremely important in relaxing supercoiled DNA generated during major cellular processes such as DNA replication or transcription.

ABBREVIATIONS: DIM, 3,3’-diindolylmethane; DAPI, 4’,6-diamidino-2-phenylindole; topo, topoisomerase; m-AMSA, amsacrine; [3H]dAT, [3H]deoxythymidine; PBS, phosphate-buffered saline; KDNA, kinetoplast DNA; DMSO, dimethyl sulfoxide; MST-16, sobuzoxane; ICRF-187, dexrazoxane.
replication, RNA transcription, and DNA recombination. In addition, topo II is indispensable during mitosis in separating the extensively intertwined sister chromatids because of its decatenation activity (Clarke et al., 1993; Chang et al., 2003). Topoisomerase inhibitors are one of the most widely studied and clinically used classes of anticancer agents (Pommier et al., 1998; Wang, 2002; Denny and Baguley, 2003; Larsen et al., 2003). Topoisomerase inhibitors can be divided into two classes according to their mechanisms of action (Topcu, 2001; Larsen et al., 2003). The class I drugs, including camptothecin, etoposide, anthracyclines, ellipticines, epipodophyllotoxins, and others, are also called topoisomerase poisons because they are able to stabilize a reversible, covalent DNA-topoisomerase complex (called the DNA cleavage complex), which is a normal reaction intermediate in the catalytic cycle of the enzymes. Class II topoisomerase inhibitors, which include a variety of structurally diverse compounds, interfere with different steps in the catalytic cycle of the enzymes without trapping the covalent complex (Larsen et al., 2003). The drugs in this class are referred to as topoisomerase catalytic inhibitors, such as aclarubicin, merbarbone, and bisdioxopiperazines.

In the present study, we demonstrate for the first time that DIM strongly inhibited DNA topo IIα and partially inhibited DNA topo I and IIβ, without stabilizing DNA cleavage complex. To illustrate the mechanism of DIM-induced topo IIα inhibition, we found that DIM did not inhibit the steps before DNA cleavage in the enzyme catalytic cycle; instead, it inhibited IIα-catalyzed ATP hydrolysis, which is a necessary step for enzyme turnover. The topoisomerase inhibition was further confirmed in cultured HepG2 cells by demonstrating that DIM inhibited S-phase progression and chromosomal segregation.

Materials and Methods

Chemicals. Salmon sperm DNA and all of the cell-culture reagents except for fetal bovine serum (Omega Scientific Inc., Tarzana, CA) were from GIBCO/Invitrogen (Carlsbad, CA). DIM was purchased from LKT Laboratory Inc. (St. Paul, MN). RNase, propidium iodide, aclarubicin, amascrine (m-AMSA), thymidine, 4’,6-diamidino-2-phenylindole (DAPI), and nocodazole were from Sigma (St. Louis, MO). [3H]Deoxythymidine ([3H]dT) and [3H]-32P]ATP (3000 Ci/mmol stock; PerkinElmer), and different concentrations of DIM (in 1% final DMSO) in a total volume of 20 μl. Reactions were started by the addition of topo IIα and incubated at 37°C for 15 min. The reactions were stopped by 10 μl of stopping buffer containing 4 M formic acid, 2 M LiCl, and 36 mM ATP. Samples (3 μl) were spotted onto polyethyleneimine-cellulose thin-layer chromatography sheets (Analtech, Newark, DE). The sheets were developed in freshly made 1 M formic acid and 0.5 M LiCl solution and analyzed by autoradiography. The areas corresponding to the free inorganic phosphate were cut out and quantified by scintillation counting.

Single-Cell Electrophoresis Assay/Comet Assay. HepG2 cells were seeded at a density of 5 × 10^5/ml into six-well plates and were treated as indicated. After the indicated incubation time, cells were collected and gently trypsinized and were resuspended in cold PBS. The following steps were carried out using Trevigen Comet Assay kit. In brief, cells were mixed with low-melting-point agarose, lysed at 4°C for 60 min, denatured in alkaline solution for 30 min, and then electrophoresed in Tris borate-EDTA buffer at 1 V/cm for 10 min. After air-drying, the slides were stained with SYBR Green DNA fluorescent dye (494/521 nm) and observed. Digital images were captured on a Zeiss Axioshot microscope equipped with Qimaging camera (Carl Zeiss Inc., Thornwood, NY).

Cell Synchronization and Flow Cytometry. Cells were synchronized at G1-S boundary by a double thymidine block. In brief, exponentially growing HepG2 cells were sequentially treated with 2 mM thymidine for 16 h, fresh complete medium for 10 h, and 2 mM thymidine for another 16 h. Metaphase cells were synchronized by treating HepG2 cells with 0.04 μg/ml nocodazole for 24 h. The mitotic cells were collected by gentle shaking.

To perform flow cytometry cell-cycle analysis, HepG2 cells were seeded at a density of 5 × 10^5/ml into six-well plates and were treated as described. After the indicated incubation times, cells were collected and resuspended in PBS containing 200 μg/ml RNase, 50 μg/ml propidium iodide, 0.1% sodium citrate, and 0.1% (v/v) Triton X-100. Flow cytometry was performed on an EPICS XL flow cytometer (Beckman Coulter), and data were processed by WinMDI 2.8 software.

Thymidine Incorporation Assay. Cells were seeded at a density of 2 × 10^6/ml into 24-well plates and were treated as indicated. At the end of the treatment, 3 μCi of [3H]dT was added into the medium and was allowed to incubate for 1 h. After three gentle washes with cold 10% trichloroacetic acid, 500 μl of 0.3 N NaOH was
added. The lysate was transferred into opaque scintillation vials after 1-h incubation. The amount of \(^3\text{H}\)dT incorporated into DNA was counted in a scintillation counter.

**Confocal Microscopy.** HepG2 cells were seeded into coverslip-containing six-well plates and were treated as indicated. After rinsing with PBS, slides with attached cells were immersed in 4% paraformaldehyde solution for 10 min at room temperature followed by 10 min of permeabilization with 0.2% Triton X-100 solution. Slides were mounted in antifading solution with DAPI (2 \(\mu\)g/ml), and the digital images were obtained with a Zeiss LSM510 Meta confocal microscope.

**Statistic Analysis.** The statistical differences between groups were determined using Student’s \(t\) test. Statistical significances are noted at the level of \(p < 0.05\). The results are expressed as means \(\pm\) S.D. for at least three replicates for each assay.

## Results

**DIM Exerts Cytostatic Effect in HepG2 Cells.** To determine the effects of DIM on cell proliferation, human hepatoma HepG2 cells were treated with 0 to 75 \(\mu\)M DIM for up to 72 h. The results shown in Fig. 1A indicated that DIM exhibited concentration-dependent growth inhibitory effects in HepG2 cells. DAPI staining showed that the nuclei of 75 \(\mu\)M DIM-treated cells were shrunken and irregular in comparison with the normal round-shaped nuclei seen in control cells (Fig. 1B).

**DIM Interacts with DNA In Vitro.** Previous studies in our laboratory showed that DIM was rapidly taken into the cells and was highly concentrated in the nuclear fraction (R. Staub and L. F. Bjeldanes, unpublished data). Therefore, it was of interest to determine whether DIM could interact with DNA. Fluorescence titration assay was carried out to determine the DIM-DNA interaction. With an excitation wavelength of 280 nm, we observed that DIM gave maximal fluorescence at the wavelength of 370 nm in a concentration-dependent manner (Fig. 2A). The addition of increasing concentrations of sheared salmon sperm DNA gradually decreased the fluorescence intensity of DIM by 30\% (Fig. 2B). The emission wavelength exhibited a blue shift of \(~5\) to 10 nm upon increasing DNA concentration. From the above fluorescent titration experiment, we concluded that DIM could interact with DNA in vitro.

To further examine whether DIM intercalated into DNA, we performed an ethidium bromide displacement experiment, in which the binding of another DNA intercalator will competitively displace ethidium bromide from DNA, thus decreasing the ethidium bromide fluorescence. As shown in Fig. 2C, increasing concentrations of m-AMSA, a well-known DNA intercalator, gradually displaced ethidium bromide, as indicated by a strong concentration-dependent reduction in ethidium bromide fluorescence. However, the addition of

![Fig. 1. DIM inhibited HepG2 cell proliferation. A, DIM concentration-dependently inhibited the growth of HepG2 cells as measured by cell number counting every 24 h for up to 72 h. Reported values were mean \(\pm\) S.D. from one experiment with three replicates. The results are representative of two duplicate runs. B, confocal microscopy of the morphological changes in HepG2 nuclei after 24 h of 75 \(\mu\)M DIM treatment. The slides were prepared as described under Materials and Methods.](image)

![Fig. 2. DIM interacted with DNA in vitro. A, DIM fluoresced concentration-dependently when excited at 280 nm. From 1 to 6, respectively: DIM (50, 30, 10, 5, and 0 \(\mu\)M). B, salmon sperm DNA concentration-dependently quenched DIM fluorescence at 370 nm. DIM concentration was 30 \(\mu\)M. From 1 to 8, respectively: 0 to 14 \(\mu\)g of DNA at 2-\(\mu\)g intervals. C, DIM was a weak DNA intercalator compared with m-AMSA, as determined by ethidium bromide displacement assay. *, significant difference versus vehicle control DMSO at \(p < 0.05\).](image)
DIM had a small effect on ethidium fluorescence (up to a maximum of 20% reduction). This result indicated that DIM is only a weak DNA intercalator.

**DIM Inhibits Topoisomerases.** DNA topoisomerases are among the most sensitive targets of DNA-interacting molecules. In our study, the effects of DIM on human topo I, IIα, and IIβ were investigated using supercoiled DNA relaxation assay and KDNA decatenation assay. Results presented in Fig. 3A showed that camptothecin, a positive control, strongly inhibited topo I-mediated supercoiled pBR322 relaxation. DIM showed some inhibitory effect on topo I, but the inhibition was not complete, even at the highest DIM concentrations examined. In contrast, DIM exhibited a much stronger inhibitory effect on topo IIα, as shown in Fig. 3, B and C. At concentrations greater than 10 μM, DIM concentration-dependently inhibited topo IIα-catalyzed supercoiled pRYG relaxation and KDNA decatenation, as did the positive control, etoposide. The inhibition reached almost 100% at DIM 25 μM. However, in topo IIβ-mediated KDNA decatenation.

![Fig. 3. Effects of DIM on human topo I, IIα, and IIβ activities.](image)

**Fig. 4.** DIM inhibited synchronized HepG2 cell-cycle progression. HepG2 cells were synchronized at G1-S boundary by double-thymidine block as described under Materials and Methods. Two hours after release from G1-S block, HepG2 cells were treated with either DMSO (0.1%) or different concentrations of DIM. The samples were collected for flow cytometry DNA content analysis at 0, 5, 9, and 12 h after the release. The positions of 2N and 4N DNA are indicated.

**Fig. 5.** DIM inhibited DNA synthesis in HepG2 cells. DIM inhibited DNA synthesis within 1 h of treatment, and the effects were reversible after the removal of DIM. HepG2 cells were treated with DMSO (0.1%) or different concentrations of DIM. [3H]dUT incorporation was measured within the 1st h of treatment. To examine whether the DNA synthesis inhibition was reversible, after 1 h of DIM exposure, cells were washed with PBS and reincubated in DIM-free fresh medium. [3H]dUT incorporation was measured again during the 1st or 4th h of recovery. *, significant decrease versus vehicle control DMSO at p < 0.05; †, significant increase versus no DIM removal at p < 0.05; ‡, significant increase versus 1 h after DIM removal at p < 0.05.
DIM Blocks Cell-Cycle Progression in HepG2 Cells. Next, we determined whether the inhibition of topoisomerases by DIM would lead to cell-cycle perturbation. For the experiment, HepG2 cells were synchronized at the G1-S boundary by double thymidine block. Two hours after the block was released, cells were treated with different concentrations of DIM and were collected at the indicated times for flow cytometry DNA content analysis. The results presented in Fig. 4 showed that DIM concentration-dependently blocked cell-cycle progression. Most of the control cells progressed into G2/M phase 9 h after the release; DIM-treated cells, however, were concentration-dependently retarded in S phase. DIM 100 μM almost completely abolished the S-phase progression. Twelve hours after the release, when control cells had finished mitosis and progressed back into G1, DIM 50 μM-treated cells were delayed in G2/M phase, and DIM 75 and 100 μM-treated cells were still in S phase. The response of synchronized cells to DIM clearly showed that DIM inhibited S-phase progression and mitosis.

DIM Inhibits DNA Synthesis in Cultured Cells. In a further experiment, we performed a [3H]dT incorporation assay to quantitatively measure the negative impact of DIM on DNA synthesis. The results presented in Fig. 5 show that during the first hour of exposure, DIM rapidly and strongly inhibited DNA synthesis in a concentration-dependent manner (IC50 = ~30 μM). We conducted a further experiment to determine whether the inhibition was reversible. One hour after DIM exposure, cells were gently washed with PBS and reincubated in DIM-free medium. Considering that it takes time for DIM to be completely removed from cells because of its lipophilic nature, the rates of DNA synthesis were measured 1 and 4 h after recovery. The results showed that DNA synthesis was gradually and significantly restored in all DIM-treated groups. For DIM 100 μM-treated cells, [3H]dT incorporation rate increased from 4 to 75% of control after 4 h of recovery. Taken together, the results indicate that DIM was very effective in inhibiting DNA synthesis and that the effect of short-term DIM treatment was reversible.

DIM Interferes with Mitosis. It is well established that topo IIα, especially topo IIα, are critical for successful mitotic chromosome condensation and segregation (Clarke et al., 1993; Chang et al., 2003; Cortes et al., 2003). Because DIM strongly inhibited topo IIα in vitro, we next asked whether DIM affected mitotic chromosome segregation. For the experiment, we synchronized HepG2 cells in early mitosis by nocodazole treatment. Immediately after release, cells were treated with different concentrations of DIM or 10 μM aclarubicin, a known topo II inhibitor (Sorensen et al., 1992). Four hours later, the remaining mitotic cells were counted after DAPI staining. The results in Fig. 6 show that more than 90% of the control cells finished mitosis in 4 h, but DIM-treated cells were concentration-dependently retarded in mitosis. They were arrested in the different stages of mitosis, and in some extreme cases, the chromosomes seemed abnormal, such as with entangled sister chromatids during anaphase (in Fig. 7). Therefore, DIM indeed adversely affected the separation of sister chromatids, which is consistent with the outcome of topo IIα inhibition.

DIM Does Not Stabilize DNA-Cleavage Complex In Vitro or in Cells. Topoisomerase poisons such as camptothecin and etoposide interfere with the religation step of topoisomerases by stabilizing the DNA-cleavage complex (Osheroff, 1986; Pommier et al., 1998; Champoux, 2001). To investigate whether DIM is a topoisomerase poison, a cleavage complex assay was performed (Fig. 8, A and B). The
positive control camptothecin stabilized the topo I cleavage complex as expected, resulting in the formation of open circular DNA that migrated more slowly during electrophoresis than did supercoiled DNA. However, no open circular DNA was formed after incubation with different concentrations of DIM. Similar results were obtained in the experiments with topo IIα. The positive control etoposide stimulated the formation of cleavage complex after incubation with topo IIα, as indicated by the appearance of both open circular and linear DNA, neither of which was seen in DIM-containing reaction mixtures. These results indicated that DIM did not stabilize the DNA-cleavage complex in vitro.

This conclusion was further justified by the results of single-cell electrophoresis assay (Comet assay). It is known that DNA-cleavage complexes produced by topoisomerase poisons are converted into DNA strand breaks in cells through collision with DNA replication or RNA transcription machinery (Pommier et al., 1998). To evaluate whether DIM generated DNA damage in cultured cells, single-cell gel electrophoresis assay was used. The results presented in Fig. 8, C to E, show that only 1 h of 10 μM etoposide treatment caused obvious comet-shaped etoposide replication that is characteristic of ex-

Discussion

In this study, we provide evidence for the first time that DIM is a human topo IIα inhibitor. Our results showed that...
DIM could completely block topo IIα-catalyzed supercoiled DNA relaxation and KDNA decatenation and partially inhibit topo I-mediated supercoiled DNA relaxation. The effect of topoisomerase inhibition was also characterized in cultured HepG2 cells by demonstrating that DIM arrested G1-S synchronized HepG2 cells at S phase and at G2/M phase. In a further [3H]dT incorporation assay, we showed that DIM inhibited DNA synthesis in a clear concentration-dependent manner; the inhibition occurred rapidly after DIM exposure, and the effect was reversible after DIM removal. These observations support our findings that DIM directly inhibited topoisomerase activity. Topoisomerases have long been recognized as important enzymes in DNA replication to relieve DNA supercoiling caused by the moving replication fork. Therefore, the inhibition of topoisomerases will lead to the blockade of DNA synthesis (Berger et al., 1996; Champoux, 2001; Topcu, 2001; Wang, 2002; Cortes et al., 2003).

DIM is less active toward human topo IIβ than topo IIα. It is known that human topo IIα and IIβ are encoded by different genes, with 68% homology at amino acid level. Although they share ATP-dependent strand passage activity, topo IIβ relaxes DNA in a more processive manner (Austin and Marsh, 1998). Previous experiments have shown that the two enzyme isoforms have different sensitivities to topoisomerase-targeting drugs. For merbarone, a catalytic topo II inhibitor, topo IIβ is 8 to 10 times less sensitive than IIα (Austin and Marsh, 1998; Fortune and Osheroff, 1998). The inhibitory effect of DIM against topo IIα is important, given crucial role of this enzyme in mitotic chromosome segregation (Osheroff et al., 1983; Osheroff, 1986; Berger et al., 1996; Chang et al., 2003). Between the two isoforms of vertebrate topo II, topo IIα has been more widely studied and is believed to be more essential for mitotic chromosome separation because of its peak expression in mitosis and its association with chromosomes from metaphase to telophase. Our results clearly showed that DIM strongly inhibited topo IIα. Not only was mitosis severely retarded by DIM, but the abnormal chromosome morphology was seen after DIM treatment. These observations, together with the strong inhibitory effect of DIM on DNA synthesis, provide evidence that DIM functions as topoisomerase inhibitor in vivo.

We propose that DIM inhibits human topo IIα by targeting the step of ATP hydrolysis (Osheroff et al., 1983; Osheroff, 1986; Berger et al., 1996). A protein clamp model has been proposed for eukaryotic topo II in which a homodimeric enzyme acts as an ATP-modulated clamp to hold the DNA substrate. ATP binding closes the clamp, and ATP hydrolysis reopens the clamp to release the DNA. Antitumor drugs such as bisdioxopiperazines inhibit topo II by inhibiting ATP hydrolysis, thus trapping the enzyme in the form of a closed protein clamp (Osheroff, 1986; Roca et al., 1994; Berger et al., 1996). We performed topo IIα-mediated ATPase assay using thin-layer chromatography and found that DIM concentration-dependently inhibited topo IIα-mediated ATP hydrolysis, and the inhibition was more complete when a lower amount of enzyme was present in the reaction mixture. This observation also helps to explain why DIM 50 μM could completely inhibit topo IIα activity in the supercoiled DNA relaxation assay, in which only 1 unit of topo IIα was used.

Our observation that topo IIα is a target of DIM action is consistent with available information on the distribution of the compound in cultured cells. In a previous study to determine the subcellular distribution of DIM, we found that DIM was very rapidly taken into the cultured cells and was highly concentrated in the nuclear and the lipid membrane fractions of the cells (R. Staub and L. F. Bjeldanes, unpublished data). These observations suggested that DIM could reach its target: nuclear topoisomerases.

RAPIDLY PROLIFERATING AND TRANSFORMED CELLS ARE KNOWN TO CONTAIN HIGHER LEVELS OF TOPOISOMERASES, WHICH MAKES THEM MORE SENSITIVE TO TOPOISOMERASE INHIBITION (van der Zee et al., 1994; Bronstein et al., 1996; Lynch et al., 1997; Nakopoulou et al., 2001). Two classes of topoisomerase inhibitors have been characterized—topoisomerase poisons, which stabilize DNA cleavage complex and induce DNA damage, and the catalytic inhibitors, which include structurally diverse compounds interfering with different stages of the catalytic cycle but do not induce DNA cleavage complex formation (Larsen et al., 2003). We provide both in vitro and cell-culture evidence to classify DIM as a catalytic inhibitor. Catalytic topoisomerase inhibitors are now being clinically used as anti-neoplastic agents (aclacinomycin and MST-16), cardioprotectors (ICRF-187), or modulators to increase the efficacy of other agents (suramin and novobiocin) (Larsen et al., 2003). Increasing evidence suggests that topoisomerase inhibition can regulate gene promoter activity by altering local DNA topology (Narayana et al., 1998; Collins et al., 2001). As revealed by the studies of topoisomerase II inhibitor etoposide and salvicine, topoisomerase II inhibition may be able to regulate the expression of genes involved in tumor migration, invasion, and metastasis (Mashimo et al., 2000; Larsen et al., 2003; Chang et al., 2005; Lang et al., 2005). In our preliminary study, DIM inhibited human primary endothelial cell migration in culture and decreased blood vessel formation in xenograft solid human breast tumors (Chang et al., 2005). Although the mechanisms of this inhibition are under further investigation, it is possible that inhibition of DNA topoisomerase changes the expression of genes involved in tumor metastasis.

In conclusion, the results of our study indicate that DIM is a catalytic topo IIα inhibitor that exerts cytostatic effects on hepatoma cells and interferes with several phases of the cell cycle. Our study identified a further important mode of action for this intriguing dietary component that might be exploited for therapeutic development.

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