Cytotoxicities and Topoisomerase I Inhibitory Activities of 2-[2-(2-Alkynylphenyl)ethynyl]benzonitriles, 1-Aryldec-3-ene-1,5-diynes, and Related Bis(enediynyl)arene Compounds

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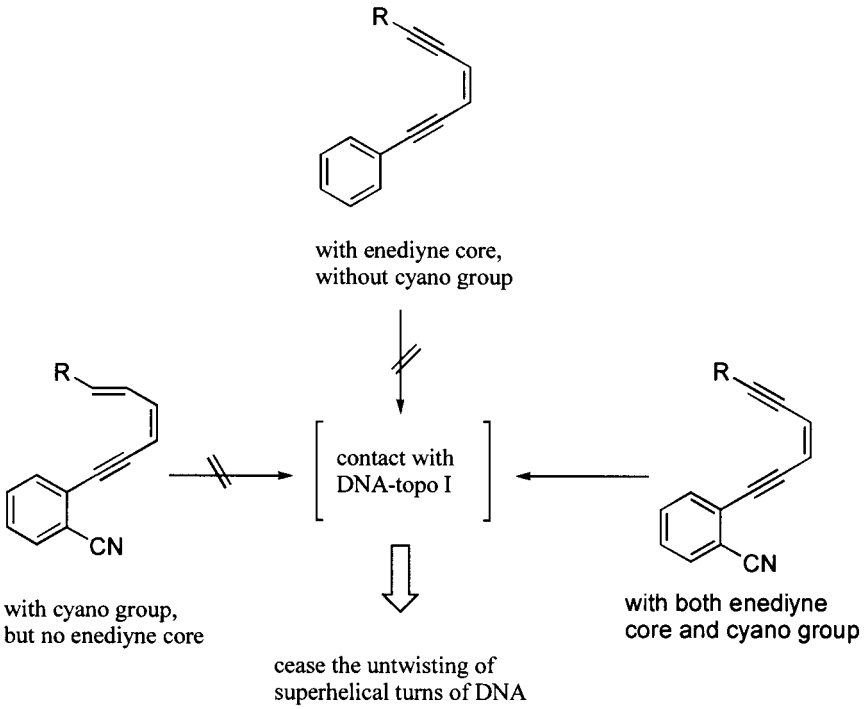
The activities of a series of acyclic enediynes, 2-(6-substituted hex-3-ene-1,5-diynyl)benzonitriles (**1**–**5**) and their derivatives **7**–**23** were evaluated against several solid tumor cell lines and topoisomerase I. Compounds **1**–**5** show selective cytotoxicity with Hepa cells, and 2-[6-phenylhex-3-ene-1,5-diynyl]benzonitrile (**5**) reveals the most-potent activity. Analogues **8**–**10** and **13**–**22** also have the same effect with DLD cells; 1-[(*Z*)-dec-3-ene-1,5-diynyl)-4-nitrobenzene **21** shows the highest activity among them. Moreover, 1-[(*Z*)-dec-3-ene-1,5-diynyl]-2-(trifluoromethyl)benzene (**20**) exhibits the strongest inhibitory activity with the Hela cell line. Derivatives **9**, **10**, **18**, and **23** display inhibitory activities with topoisomerase I at 87 μM. The cell-cycle analysis of compound **5**, which induces a significant blockage in S phase, indicates that these novel enediynes probably undergo other biological pathways leading to the cytotoxicity, except the inhibitory activity toward topoisomerase I.

Introduction. – Topoisomerases are important enzymes highly associated with the separation of DNA strands in many cellular metabolic processes that alter the topological state of duplex DNA. Topoisomerases can be classified into two types based on the mode of cleavage of duplex DNA [1]; topo I makes a transient nick on a single-strand of DNA and does not require an energy cofactor [2], while topo II acts by nicking both strands of the DNA and hydrolyzes ATP during its catalytic cycle [3]. Topoisomerase I can be isolated from some cellular organisms, including nuclear and mitochondria. Moreover, topo I is present throughout the cell, and its activity varies less than that of topo II during cell cycle [4], which makes a topo I inhibitor an attractive target for anticancer, antibacterial, and antiviral drug development.

Although a series of synthetic acyclic enediynes, 2-(6-substituted hex-3-ene-1,5-diynyl)benzonitriles **2**–**5**, exhibited cytotoxicity toward KB and Hep 2,2,15 cells, and significant topo I inhibitory properties [5] in low micro-molar concentration ranges (*Scheme 1*), the precise relationships between the cytotoxicities and these unique structures are still under investigation. This prompted us to further study the structure-activity relationships (SAR) of this new type of enediynes.

In continuation of our interests in the biological activities of 2-(6-substituted hex-3-ene-1,5-diynyl)benzonitriles, several derivatives thereof, *i.e.*, 2-[2-(2-alkynylphenyl)ethynyl]benzonitriles **7**–**10**, 1,4-bis(dec-3-ene-1,5-diynyl)benzene (**11**), 4,4′-bis-(dec-3-ene-1,5-diynyl)-1,1′-biphenyl (**12**), and 1-aryldec-3-ene-1,5-diynes **13**–**23**

Scheme 1



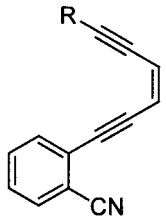
(*Scheme 2*), were designed and synthesized by modification of the enediyne core and the aryl group bearing it. The analogues were evaluated for cytotoxic activity against five human solid tumor cells and the topoisomerase I inhibitory activities, and the cell-cycle analysis was performed to provide more advanced understanding of the cytotoxicity.

Results and Discussion. – *Chemistry.* 2-[2-(2-Alkynylphenyl)ethynyl]benzonitriles **7**–**10** were synthesized from the Pd-catalyzed coupling reaction of 2-(2-substituted eth-1-ynyl)-1-iodobenzenes **24**–**27** with 2-ethynylbenzonitrile **28** [6] (*Scheme 3*). The yields obtained were 40–98%.

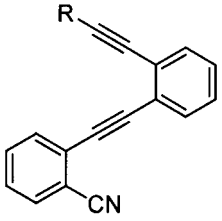
1,4-Bis(dec-3-ene-1,5-diynyl)benzene (**11**) and 4,4′-bis(dec-3-ene-1,5-diynyl)-1,1′-biphenyl **12** were prepared from 1-chlorooct-1-ene-3-yne (**29**) as the starting material (*Scheme 4*). Coupling **29** with (trimethylsilyl)acetylene with $Pd(PPh_3)_4$ as a catalyst gave **30** in 53% yield. Treatment of **30** with Bu_4NF (TBAF) produced compound **31** in 80% yield. Finally, Pd-catalyzed coupling reaction of dec-3-ene-1,5-diyne (**31**) with aryl diiodides **32** and **33** gave bis(enediynyl)arenes **11** and **12** in 65 and 35% yields, respectively.

Generation of 1-aryldec-3-ene-1,5-diynes **13**–**23** was accomplished under the same Pd-catalyzed coupling reactions between dec-3-ene-1,5-diyne (**31**) and aryl iodides **34**–**44** in 31–99% yields (*Table 1*).

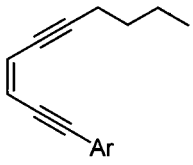
Scheme 2. *Structures of Enediyne Compounds* **1**–**23**



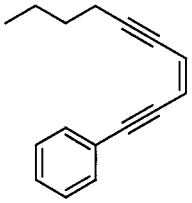
1 R = $THPOCH_2$
2 R = $THPO(CH_2)_3$
3 R = $Me(CH_2)_3$
4 R = $Me(CH_2)_4$
3 R = Ph



7 R = $THPOCH_2$
8 R = $THPO(CH_2)_3$
9 R = $Me(CH_2)_3$
10 R = $Me(CH_2)_4$



13 Ar = 4-ClC_6H_4
14 Ar = 4-CNC_6H_4
15 Ar = pyridin-2-yl
16 Ar = thionphen-2-yl
17 Ar = pyrazin-2-yl
18 Ar = 4-$CF_3C_6H_4$
19 Ar = 3-$CF_3C_6H_4$
20 Ar = 2-$CF_3C_6H_4$
21 Ar = 4-$NO_2C_6H_4$
22 Ar = 2-$CH_3OCOC_6H_4$
23 Ar = 4-$CH_3COC_6H_4$



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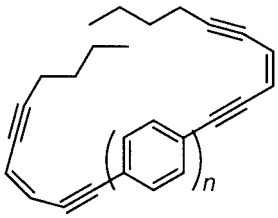


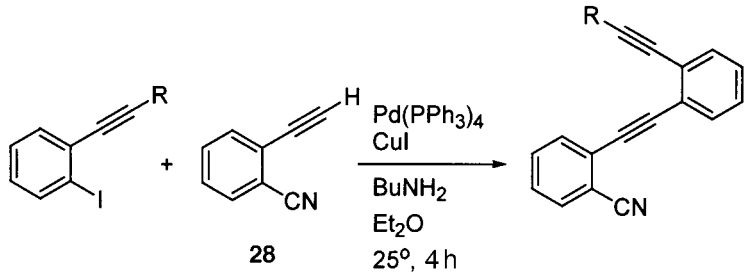
11 *n* = 1
12 *n* = 2

THP = 3,4,5,6-Tetrahydro-2*H*-pyran-2-yl

Cytotoxicity. The IC_{50} values of compounds **1**–**23** with five human solid tumor cells (KB, Hela, DLD, NCI, and Hepa) are listed in *Table 2*. Obviously, 2-(6-substituted hex-3-ene 1,5-diynyl)benzonitriles **1**–**5** display a tendency to inhibit the growth of Hepa cells. Among these compounds, 2-(6-phenylhex-3-en-1,5-diynyl)benzonitrile (**5**) exhibited the highest cytotoxic activity against Hepa cells at 1.09 μg/ml. Moreover, 2-[2-(2-alkynylphenyl)ethynyl]benzonitriles **8**–**10** and 1-aryldec-3-ene-1,5-diynes **13**–**22** also showed the same tendency with DLD cell line, in which 1-[(*Z*)-dec-3-ene-1,5-diynyl]-4-nitrobenzene (**21**) showed selective potency against DLD cells at the low IC_{50} value of 1.66 μg/ml. 1-[(*Z*)-dec-3-ene-1,5-diynyl)- 2-(trifluoromethyl)benzene (**20**) was more active than the other derivatives against Hela cells ($IC_{50} = 2.15$ μg/ml).

Inhibition Tests for Topoisomerase I. On the other hand, the cleavage of supercoiled DNA by topo I in the presence of 2-[2-(2-alkynylphenyl)ethynyl]benzonitriles **7**–**19**0, 1,4-bis(dec-3-ene-1,5-diynyl)benzene (**11**), 4,4′-bis(dec-3-ene-1,5-diynyl)-1,1′-biphenyl (**12**), and 1-aryldec-3-ene-1,5-diynes **13**–**23** was evaluated by gel electrophoresis, and

Scheme 3. *Preparation of 2-[2-(2-Alkynylphenyl)ethynyl)benzonitriles* **7**–**10**



24 R = $THPOCH_2$ **7** R = $THPOCH_2$ (98%)

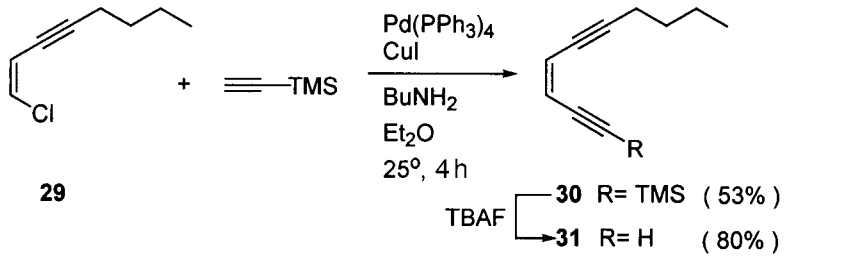
25 R = $THPO(CH_2)_3$ **8** R = $THPO(CH_2)_3$ (89%)

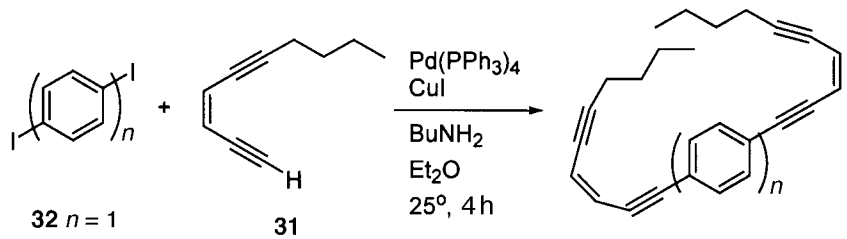
26 R = $Me(CH_2)_3$ **9** R = $Me(CH_2)_3$ (62%)

27 R = $Me(CH_2)_4$ **10** R = $Me(CH_2)_4$ (40%)

THP = 3,4,5,6-Tetrahydro-2*H*-pyran-2-yl

Scheme 4. *Generation of 1,4-Bis(dec-3-ene-1,5-diynyl)benzene* (**11**) *and 4,4′-Bis(dec-3-ene-1,5-diynyl)-1,1′-biphenyl* **12**





32 *n* = 1

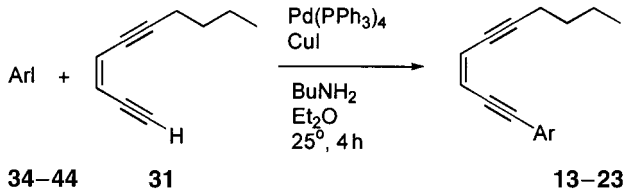
33 *n* = 2

31

11 *n* = 1 (65%)

12 *n* = 2 (35%)

Table 1. *Preparation of 1-Aryldec-3-ene-1,5-diynes* **13**–**23**



34–**44**	**13**–**23** (Yield [%])
34 Ar = 4-ClC_6H_4	**13** (31)
35 Ar = 4-CNC_6H_4	**14** (46)
36 Ar = Pyridin-2-yl	**15** (84)
37 Ar = Thiophen-2-yl	**16** (53)
38 Ar = Pyrazin-2-yl	**17** (99)
39 Ar = 4-$CF_3C_6H_4$	**18** (46)
40 Ar = 3-$CF_3C_6H_4$	**19** (46)
41 Ar = 2-$CF_3C_6H_4$	**20** (33)
42 Ar = 4-$NO_2C_6H_4$	**21** (59)
43 Ar = 2-$CH_3CO_2C_6H_4$	**22** (31)
44 Ar = 4-$CH_3COC_6H_4$	**23** (96)

the results were shown in *Figs. 1* and *2*. In *Fig. 1,a*, compound **10** displayed inhibitory activity against topo I at 8.7 and 87 μM and no inhibition of topo I at 0.87 μM concentration. Compound **9** showed inhibition of topo I at 87 μM, and there was no supercoiled DNA observed at 8.7 or 0.87 μM. Moreover, there was complete untwisting of DNA revealed at 0.87, 8.7, or 87 μM of **7** and **8** (data not shown). No supercoiled DNA was observed for **11** and **12** at 8.7 or 87 μM concentration (*Fig. 1,b*). In *Fig. 2,a*, 1-aryldec-3-ene-1,5-diynes **13**–**17** showed no inhibition effects with topo I. 1-[(*Z*)-dec-3-ene-1,5-diynyl)]-4-(trifluoromethyl)benzene (**18**) and 4-[(*Z*)-dec-3-ene-1,5-diynyl)]-acetophenone (**23**) displayed inhibitory activities of topo I on the test concentration as shown in *Fig. 2,b*.

Supercoiled pGEM9zf(−) DNA was treated with 0.025 μg/μl topoisomerase I and compounds **7**–**10**[1]), **11**, and **12**, then analyzed on a 2% agarose gel. *Fig. 1,a*, *Lane 1:* DNA + topo I; *Lane 2:* DNA only; *Lane 3:* DNA + topo I + camptothecin (43.5 μM); *Lanes 4–6:* DNA + topo I + compound **10**; *Lanes 7–9:* DNA + topo I + compound **9**; each group of three lanes contained 0.87, 8.7, and 87 μM of the analogs, respectively. *Fig. 1,b*, *Lane 1:* DNA + topo I; *Lane 2:* DNA only; *Lanes 3* and *4:* DNA + topo I + compound **11**; *Lanes 5* and *6:* DNA + topo I + compound **12**; each group of both lanes contained 8.7 and 87 μM of the analogs, respectively.

Supercoiled pGEM9zf(−) DNA was treated with 0.025 μg/μl topoisomerase I and compounds **13**–**23**, then analyzed on a 2% agarose gel. *Fig. 2,a*, *Lane 1:* DNA only; *Lane 2:* DNA + topo I; *Lanes 3–7.* DNA + topo I + compounds **13**–**17** (87 μM). *Fig. 2,b*, *Lanes 1–6:* DNA + topo I + compounds **18**–**23** (87 μM).

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[1]) The unreported results of compounds **7** and **8** display no topo I inhibition at 8.7 and 87 μM.

Table 2. IC_{50} *Values* [μg/ml] *of Cytotoxicities of Compound* **1**–**23**[a])

Compound	Cells				
	KB	Hela	DLD	NCI	Hepa
1	n.d.	9.97	8.61	–	14.26
2	n.d.	10.62	–	–	14.66
3	n.d.	–	5.03	–	12.49
4	n.d.	6.38	–	–	7.28
5	n.d.	–	–	7.01	1.09
6	14.43	–	–	7.35	–
7	–	–	–	–	–
8	–	–	11.65	–	–
9	–	–	8.98	–	–
10	–	–	16.25	9.22	5.13
11	–	–	–	–	–
12	5.57	–	–	–	–
13	–	9.83	5.22	8.00	–
14	–	–	11.93	–	–
15	–	–	5.15	–	–
16	–	–	6.75	–	–
17	5.93	5.30	3.89	9.43	12.34
18	4.32	5.86	5.71	11.15	17.08
19	6.74	–	7.05	7.73	14.39
20	19.65	2.15	8.91	5.02	12.24
21	–	–	1.66	–	–
22	–	–	7.27	–	–
23	11.19	–	–	13.13	–

[a]) –: $IC_{50} > 20$ μg/ml; the standard of cytotoxicity test is doxorubicin with ED_{50} 0.1 μg/ml. KB: human oral epidermoid carcinoma, Hela: human cervix epithelial carcinoma, DLD (DLD-1): human colon adenocarcinoma, NCI (NCI-H661): human lung large-cell carcinoma, Hepa: human hepatoma.

Cell Cycle Assay of Compound **5**. To obtain more information about the differences of these novel enediynes in affecting whole cells, human hepatoma Hep G2 cell was used, and the growth characteristic of cells following treatment with compounds **5** was measured. As shown in *Fig. 3*, cells were exposed to the vehicle solvent (DMSO) as control, 1 and 10 μM **5** was added to the cells, and, after exposure to the compounds for 72 h, attached cells were analyzed by flow cytometry. The majority (71.5%) of control cells exposed to DMSO were in the G0–G1 phase of cell cycle and only a few cells were detected in either the S phase (22.7%) or G2/M (5.8%) phase. After treatment of compound **5** (1 and 10 μM) for 72 h, cells progressed to the S phase, and the majority of cell population was almost arrested at the S phase. Consistent with this cell-cycle arrest, only 36.6% (1 μM) and 46.9% (10 μM) of the cells were found at the G0–G1 phase, 63.2% (1 μM) and 53.0% (10 μM) in S phase, and 0.2% (1 μM) and 0.1% (10 μM) in G2/M phase. However, the significant blockage of the Hep G2 cell cycle in S phase was observed and induced by both concentrations (1 μM and 10 μM) of compound **5**.

Conclusions. – There are several results arising from this study (*Scheme 5*), and the conclusions are summarized in two parts:

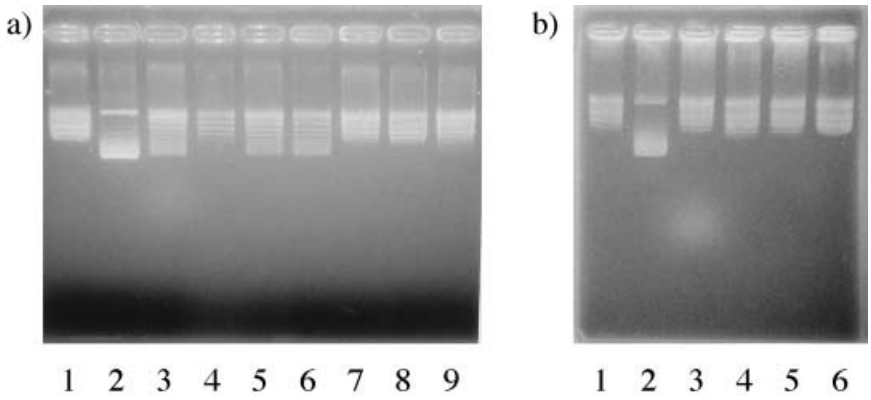


Fig. 1. *Cleavage of supercoiled pGEM9zf(–) DNA by topoisomerase I in the presence of 2-[2-(2-alkynylphenyl)ethynyl)benzonitriles* **7**–**10**,*1,4-bis(dec-3-ene-1,5-diynyl)benzene* (**11**), *and 4,4′-bis(dec-3-ene-1,5-diynyl)-1,1′-biphenyl* (**12**)

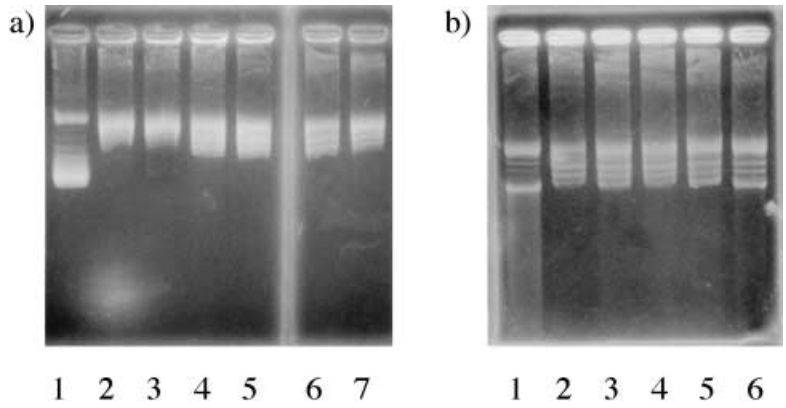


Fig. 2. *Cleavage of supercoiled pGEM9zf(–) DNA by topoisomerase I in the presence of 1-aryldec-3-ene-1,5-diynes* **13**–**23**

I) *For the cytotoxic assay: 1*) New enediynes **1**–**5** have a tendency to exhibit cytotoxic activity with Hepa cells, and the longer alkyl chain, the higher the cytotoxic activity observed. When the substituent at C(6) is a Ph group, it provides the strongest activity against the Hepa cell line. *2*) The series of enediynes **13**–**22** induce the growth inhibition of DLD cells. Among these, compound **21** shows the highest biological activity. Comparison of the results for **15** (Ar = pyridin-2-yl) and **17** (Ar = pyrazin-2-yl) shown in *Table 2* indicates that the increasing number of N-atoms in the aromatic ring leads to a lower IC_{50} value and a wider spectrum of cytotoxicity. Compound **6** and its dimer **12** have similar structures and remain selectively toxic against KB cells.

II) *The inhibitory activity of topo I: 1*) There are two essential features that facilitate the inhibition of topo I by these enediyne derivatives, the enediyne core and CN group. The series of new enediynes **2**–**5** reveal inhibition of topo I [5]. Replacement of the enediyne core with 1,2-diethynylbenzene lowers the inhibition abilities of topo I, which

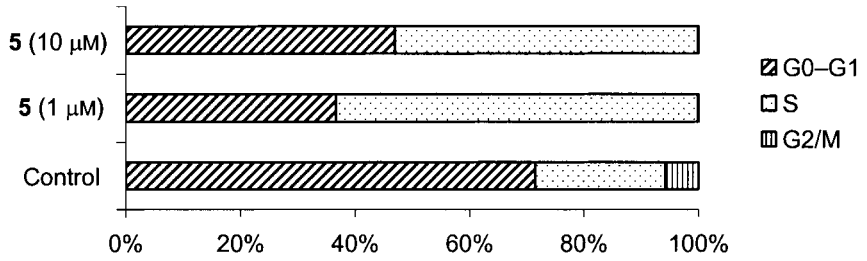


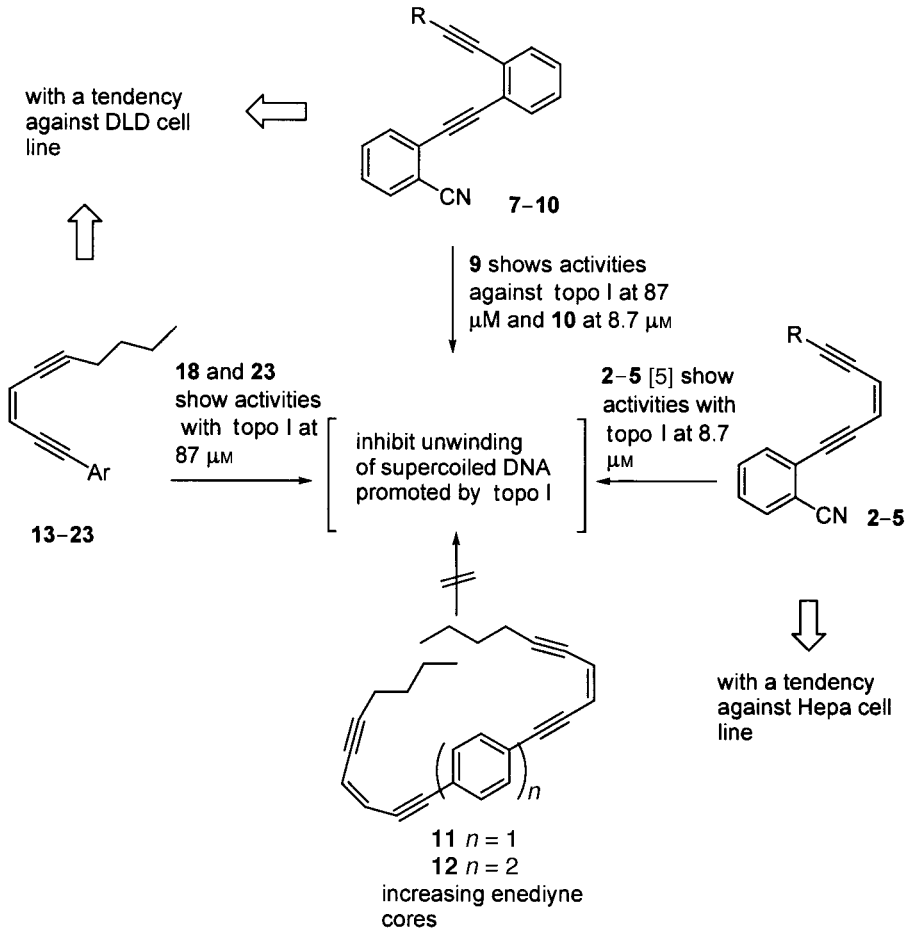
Fig. 3. *Effect of compound* **5** *on cell-cycle distribution* [%] *of human hepatoma Hep G2 cells for 72 h*. Cell-cycle distribution of human hepatoma Hep G2 cells were measured by flow cytometry. Values are expressed as mean for triplicate samples. (Significantly different from control, $p < 0.05$).

may be due to the alteration of conformation of the original enediyne core, or because the ability of 1,2-diethynylbenzene to form a complex with topo I is lower than that of original enediyne core. *2*) All unwinding DNA detected in *Fig. 1*,*b*, indicates that compounds **11** and **12** are not topo I inhibitors. The results clearly show that increasing the quantities of enediyne core cannot enhance the activity against topo I. *3*) Moreover, only compounds **18** and **23** among 1-aryldec-3-ene-1,5-diynes **13**–**23** display topo I inhibitory activity; hence, it is considered that topoisomerase I inhibitors reveal the highly structural selectivity. The observation that **6** and its dimer **12** are not topo I inhibitors but show activities against KB cells indicates that the structures of **12** and **6** interfere with an unknown biological path to cause the cytotoxic activity. In summary, we have synthesized a series of novel enediynes that display selective growth inhibition of various human tumor cell lines, and some of them revealed inhibitory effects with topoisomerase I, although there seems to be no significant relationship between the cytotoxicity and topo I.

On the other hand, it was considered that whole cancer cells had more-complete biological functions than enzymes. Other compensatory pathways would be active when topoisomerase I was inhibited during the periods of DNA replication. The data shown in *Fig. 3* demonstrated that compound **5** induced blockage of the cell-growth cycle in S phase. It was assumed that only inhibiting topoisomerase I would not completely arrest the cell cycle in S phase. Therefore, the results indicate that compound **5** not only targets topo I but also interferes with topo II. Moreover, generally, the cells progressed to a final accrual at G2/M or later phase. The observed small amount of G2/M cells also implied that **5** probably inhibits mitosis or tubulin polymerization, though more evidence is necessary to support this conclusion. However, the significant blockage of the Hep G2 cell cycle in S phase suggested that these novel enediynes probably undergo metabolism by other biological pathways to exhibit cytotoxicity, aside from the inhibitory activity toward topoisomerase I.

Although the picture concerning the cytotoxicities of the compounds is still incomplete, this study has provided several new lead compounds for enediynes, which exhibit activities against various human solid tumors and topo I, and the cell-cycle test of compound **5** indicates that these compounds block the replication of DNA. The above results are helpful in the elucidation of undiscovered biological modes related to the anticancer properties of enediynes.

Scheme 5. *Results of Biological Activities of Three Series of Nonradical Enediynes*



Experimental Part

General Procedure of the Coupling Reaction of 1-Alkynyl-2-iodobenzene with 2-Ethynylbenzonitrile. A degassed soln. of 1-alkynyl-2-iodobenzene (12 mmol) in dry Et_2O (30 ml) containing $Pd(PPh_3)_4$ (0.8 mmol) and CuI (3.2 mmol) was added to a soln. of 2-ethynylbenzonitrile (24 mmol) containing $BuNH_2$ (34 mmol). The resulting soln. was stirred for 6 h at 25°, quenched with sat. aq. NH_4Cl and Na_2CO_3 solns., and extracted with AcOEt. The org. layer was separated and dried ($MgSO_4$). After filtration, the solvent was evaporated *in vacuo*. The residue was purified by flash chromatography (FC) to give the products.

(Z)-1-Chlorooct-1-ene-3-yne (**29**). Yield 65%. Oil. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.28 (*dd*, *J* = 7.3, 0.4, 1 H); 5.84 (*dt*, *J* = 7.3, 2.2, 1 H); 2.38 (*td*, *J* = 7.0, 2.2, 2 H); 1.59–1.42 (*m*, 4 H); 0.92 (*t*, *J* = 7.3, 3 H). EI-MS: 142 (32, M^+), 86 (53), 49 (56), 35 (25). HR-EI-MS: 142.0550 ($C_8H_{11}Cl$; calc. 142.0548).

1-(Trimethylsilyl)dec-3-ene-1,5-diyne (**30**). Yield 53%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.81–8.71 (*m*, 2 H); 2.41 (*t*, *J* = 6.8, 2 H); 1.56–1.44 (*m*, 4 H); 0.92 (*t*, *J* = 6.8, 3 H); 0.19 (*s*, 9 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 121.5; 118.1; 102.2; 101.7; 99.5; 78.1; 30.7; 21.9; 19.4; 13.6; –0.10. EI-MS: 204 (91, M^+), 189 (100), 145 (32), 131 (28). HR-EI-MS: 204.1335 ($C_{13}H_{20}Si^+$; calc. 204.1331).

Dec-3-ene-1,5-diyne (**31**). Compound **30** (3.02 g, 14.80 mmol) was dissolved in dry MeOH (10 ml), and the soln. was stirred with K_2CO_3 (1.0 g) at r.t. for 1.5 h. After the evaporation of MeOH *in vacuo*, the reaction was

quenched with sat. aq. $NaHCO_3$ solns. and the soln. was extracted with AcOEt. The org. layer was separated and dried ($MgSO_4$). After filtration, the solvent was evaporated *in vacuo*. The residue was purified by FC to give **31** (80% yield). Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 5.88–5.75 (*m*, 2 H); 3.28 (*s*, 1 H); 2.41 (*t*, *J* = 7.0, 2 H); 1.58–1.40 (*m*, 4 H); 0.92 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 122.4; 116.9; 99.6; 83.6; 80.9; 77.8; 30.5; 21.8; 19.4; 13.5.

*2-(2-{2-[2-(3,4,5,6-Tetrahydro-2*H*-pyran-2-yl)ethynyl]phenyl}ethynyl)benzonitrile* (**7**). Yield 98%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.84–7.37 (*m*, 8 H); 5.08–5.04 (*m*, 1 H); 4.70 (*d*, *J* = 2.2, 2 H); 4.21–3.93 (*m*, 1 H); 3.69–3.61 (*m*, 1 H); 1.93–1.58 (*m*, 6 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 132.7; 132.6; 132.5; 132.3; 132.2; 128.9; 128.4; 128.2; 127.1; 125.6; 124.9; 117.4; 115.1; 96.8; 94.4; 89.1; 84.0; 61.9; 54.9; 30.3; 25.4; 19.0. HR-MS (EI) calc. for $C_{23}H_{19}NO_2$ 341.1414, found 341.1452.

*2-(2-{2-[5-(3,4,5,6-Tetrahydro-2*H*-pyran-2-yl)pent-1-ynyl]phenyl}ethynyl)benzonitrile* (**8**). Yield 89%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.70–7.51 (*m*, 4 H); 7.47–7.34 (*m*, 2 H); 7.31–7.28 (*m*, 2 H); 4.59–4.56 (*m*, 1 H); 3.95–3.81 (*m*, 1 H); 3.62–3.47 (*m*, 1 H); 2.64 (*t*, *J* = 7.4, 2 H); 1.97–1.46 (*m*, 10 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 132.7; 132.6; 132.5; 132.3; 132.1; 132.0; 128.9; 128.2; 127.4; 127.3; 126.4; 124.3; 117.5; 115.2; 98.8; 94.9; 88.6; 79.3; 66.1; 62.2; 30.7; 28.9; 25.5; 19.5; 16.6. HR-EI-MS: 369.1729 ($C_{25}H_{23}NO_2^+$; calc. 369.1725).

2-{2-[2-(Hex-1-ynyl)phenyl]ethynyl}benzonitrile (**9**). Yield 62%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.71–7.52 (*m*, 4 H); 7.47–7.37 (*m*, 2 H); 7.33–7.25 (*m*, 2 H); 2.52 (*t*, *J* = 7.6, 2 H); 1.66–1.43 (*m*, 4 H); 0.89 (*t*, *J* = 7.6, 3 H). HR-EI-MS: 283.1326 ($C_{21}H_{17}N^+$; calc. 283.1326).

2-{2-[2-(Hept-1-ynyl)phenyl]ethynyl}benzonitrile (**10**). Yield 40%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.70–7.53 (*m*, 4 H); 7.47–7.34 (*m*, 2 H); 7.30–7.25 (*m*, 2 H); 2.51 (*t*, *J* = 7.6, 2 H); 1.69–1.25 (*m*, 6 H); 0.85 (*t*, *J* = 7.6, 3 H). HR-EI-MS: 297.1518 ($C_{21}H_{17}N^+$; calc. 297.1515).

*1,4-Bis[(*Z*)-dec-3-ene-1,5-diynyl]benzene* (**11**). Yield 78%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.41 (*s*, 2 H); 6.02–5.58 (*m*, 4 H); 2.45 (*t*, *J* = 7.2, 2 H); 1.70–1.47 (*m*, 8 H); 0.90 (*t*, *J* = 7.2, 6 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 131.5; 122.2; 120.8; 117.9; 99.9; 95.7; 89.2; 78.4; 30.7; 21.9; 19.6; 13.6. HR-EI-MS: 338.2017 ($C_{26}H_{26}^+$; calc. 338.2035).

*4,4′-Bis[(*Z*)-dec-3-ene-1,5-diynyl]-1,1′-biphenyl* (**12**). Yield 51%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.58–7.51 (*m*, 8 H); 5.98–5.86 (*m*, 4 H); 2.45 (*td*, *J* = 7.2, 2.0, 4 H); 1.65–1.48 (*m*, 8 H); 0.91 (*t*, *J* = 7.4, 6 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 140.1; 132.2; 126.85; 122.6; 120.4; 116.1; 99.6; 95.9; 88.3; 78.5; 31.5; 30.7; 21.9; 14.2. HR-EI-MS: 414.2346 ($C_{32}H_{30}^+$; calc. 414.2349).

*1-Chloro-4-[(*Z*)-dec-3-ene-1,5-diynyl]benzene* (**13**). Yield 31%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.36 (*d*, *J* = 8.8, 2 H); 7.31 (*d*, *J* = 8.6, 2 H); 5.97–5.84 (*m*, 2 H); 2.45 (*t*, *J* = 6.8, 2 H); 1.61–1.45 (*m*, 4 H); 0.9 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 134.4; 132.8; 128.6; 121.7; 120.7; 117.7; 99.7; 94.7; 88.1; 78.3; 30.7; 21.8; 19.5; 13.5. EI-MS: 242 (100, M^+), 201 (16), 199 (40), 192 (78), 165 (63), 164 (45), 163 (52). HR-EI-MS: 242.0863 ($C_{16}H_{15}Cl^+$; calc. 242.0859).

*4-[(*Z*)-Dec-3-ene-1,5-diynyl]benzonitrile* (**14**). Yield 46%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.61 (*d*, *J* = 6.4, 2 H); 7.53 (*d*, *J* = 8.4, 2 H); 5.95 (*d*, *J* = 1.6, 2 H); 2.44 (*t*, *J* = 6.8, 2 H); 1.59–1.44 (*m*, 4 H); 0.9 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 132.0; 131.9; 128.1; 122.2; 118.4; 117.1; 111.5; 100.6; 93.9; 91.3; 78.2; 30.5; 21.8; 19.4; 13.5. EI-MS: 233 (51, M^+), 203 (51), 190 (100), 177 (35), 164 (46), 140 (28). HR-EI-MS: 233.1205 ($C_{17}H_{15}N^+$; calc. 233.1206).

*2-[(*Z*)-Dec-3-ene-1,5-diynyl]pyridine* (**15**). Yield 84%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.58 (*dt*, *J* = 5.0, 1.0, 1 H); 7.63 (*td*, *J* = 7.6, 1.8, 1 H); 7.42 (*td*, *J* = 7.6, 1.0, 1 H); 7.23–7.16 (*m*, 2 H); 5.99–5.93 (*m*, 2 H); 2.43 (*td*, *J* = 7.0, 1.6, 2 H); 1.92–1.43 (*m*, 4 H); 0.87 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 150.5; 143.3; 135.9; 127.2; 122.7; 122.1; 117.3; 100.3; 94.7; 86.8; 78.2; 30.6; 21.8; 19.5; 13.5. EI-MS: 209 (19, M^+), 180 (100), 78 (18), 51 (18). HR-EI-MS: 209.1205 ($C_{15}H_{15}N^+$; calc. 209.1209).

*2-[(*Z*)-Dec-3-ene-1,5-diynyl]thiophene* (**16**). Yield 53%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.29 (*d*, *J* = 5.2, 1 H); 7.22 (*d*, *J* = 2.6, 1 H); 7.00 (*t*, *J* = 5.2, 1 H); 5.94 (*d*, *J* = 10.4, 1 H); 5.86 (*d*, *J* = 10.8, 1 H); 2.45 (*d*, *J* = 6.8, 2 H); 1.63–1.47 (*m*, 4 H); 0.92 (*t*, *J* = 6.8, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 132.1; 127.7; 127.2; 123.3; 120.1; 117.7; 99.8; 91.2; 89.1; 78.4; 30.6; 21.9; 19.5; 13.5. EI-MS: 214 (100, M^+), 184 (41), 171 (80), 165 (53). HR-EI-MS: 214.0817 ($C_{14}H_{14}S^+$; calc. 214.0811).

*2-[(*Z*)-Dec-3-ene-1,5-diynyl]pyrazine* (**17**). Yield 99%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.66 (*d*, *J* = 1.6, 1 H); 8.55 (*s*, 1 H); 8.45 (*d*, *J* = 2.4, 1 H); 5.98 (*m*, 2 H); 2.44 (*t*, *J* = 6.8, 2 H); 1.58–1.43 (*m*, 4 H); 0.88 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 147.8; 144.4; 142.6; 140.3; 123.5; 116.5; 101.3; 91.7; 90.8; 78.1; 30.5; 21.8; 19.5; 13.5. EI-MS: 210 (39, M^+), 181 (100), 168 (14), 127 (17). HR-EI-MS: 210.1158 ($C_{14}H_{14}N_2^+$; calc. 210.1154).

*1-[(*Z*)-Dec-3-ene-1,5-diynyl]-4-(trifluoromethyl)benzene* (**18**). Yield 46%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.57 (*s*, 4 H); 5.95 (*d*, *J* = 1.6, 2 H); 2.46 (*t*, *J* = 6.8, 2 H); 1.58–1.49 (*m*, 4 H); 0.90 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 146.9; 134.6; 131.8; 127.0; 125.3; 121.6; 117.4; 112.1; 100.2; 89.4; 78.3; 30.6; 21.9; 19.5; 13.5. EI-MS: 276 (100, M^+), 233 (59), 207 (31), 192 (24), 165 (47), 49 (43). HR-EI-MS: 276.1174 ($C_{17}H_{15}F_3^+$; calc. 276.1129).

1-[(Z)-Dec-3-ene-1,5-diynyl]-3-(trifluoromethyl)benzene (**19**). Yield 46%. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.72 (*s*, 1 H); 7.61 (*d*, *J* = 7.6, 1 H); 7.55 (*d*, *J* = 8.0, 1 H); 7.45 (*t*, *J* = 7.6, 1 H); 5.97 (*d*, *J* = 10.8, 1 H); 5.92 (*d*, *J* = 10.8, 1 H); 2.46 (*t*, *J* = 6.8, 2 H); 1.62–1.45 (*m*, 4 H); 0.90 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 146.9; 134.6; 128.8; 128.5; 124.8; 124.8; 124.2; 121.5; 117.4; 100.1; 94.2; 88.6; 78.3; 30.6; 21.8; 19.5; 13.4. EI-MS: 276 (100, M^+), 261 (30), 246 (34), 233 (84), 207 (47), 183 (62), 178 (37), 165 (68). HR-EI-MS: 276.1127 ($C_{17}H_{15}F_3^+$; calc. 276.1125).

1-[(Z)-Dec-3-ene-1,5-diynyl]-2-(trifluoromethyl]benzene (**20**). Yield 33%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.64 (*td*, *J* = 7.8, 1.8, 2 H); 7.39–7.37 (*m*, 2 H); 6.02–5.87 (*m*, 2 H); 2.45 (*t*, *J* = 7.0, 2 H); 1.60–1.51 (*m*, 4 H); 0.90 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 146.3; 134.2; 131.2; 128.0; 125.8; 125.7; 121.6; 117.5; 112.1; 100.3; 92.5; 91.5; 78.0; 30.6; 21.9; 19.5; 13.5. EI-MS: 276 (100, M^+), 233 (34), 232 (29), 221 (48), 214 (22), 183 (29), 165 (14). HR-EI-MS: 276.1174 ($C_{17}H_{15}F_3^+$; calc. 276.1174).

1-[(Z)-Dec-3-ene-1,5-diynyl]-4-nitrobenzene (**21**). Yield 59%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.16 (*d*, *J* = 9.0, 2 H); 7.60 (*d*, *J* = 9.0, 2 H); 5.97 (*s*, 2 H); 2.45 (*t*, *J* = 6.4, 2 H); 1.61–1.45 (*m*, 4 H); 0.9 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 147.0; 132.1; 123.5; 122.6; 118.3; 117.0; 100.9; 93.6; 92.2; 78.2; 30.6; 21.8; 19.5; 13.5. EI-MS: 253 (100, M^+), 238 (16), 210 (16), 192 (14), 165 (13), 163 (21). HR-EI-MS: 253.1103 ($C_{16}H_{15}NO_2^+$; calc. 253.1105).

Methyl 2-[(Z)-Dec-3-ene-1,5-diynyl]benzoate (**22**). Yield 31%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.95 (*d*, *J* = 7.8, 1 H); 7.57 (*d*, *J* = 7.4, 1 H); 7.46–7.31 (*m*, 2 H); 6.00 (*d*, *J* = 10.8, 1 H); 5.91 (*d*, *J* = 10.8, 1 H); 3.91 (*s*, 2 H); 2.45 (*t*, *J* = 6.8, 2 H); 1.62–1.42 (*m*, 4 H); 0.87 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 166.5; 134.3; 131.6; 131.5; 130.3; 127.9; 123.7; 120.8; 118.1; 99.6; 94.6; 78.4; 52.0; 30.6; 21.9; 19.5; 13.5. EI-MS: 266 (71, M^+), 237 (75), 224 (51), 223 (70), 209 (82), 191 (21), 181 (49), 176 (24). HR-EI-MS: 266.1307 ($C_{18}H_{18}O_2^+$; calc. 266.1305).

4-[(Z)-Dec-3-ene-1,5-diynyl]acetophenone (**23**). Yield 96%. Oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.92 (*d*, *J* = 8.4, 2 H); 7.54 (*d*, *J* = 8.4, 2 H); 5.99–5.90 (*m*, 2 H); 2.60 (*s*, 3 H); 2.45 (*t*, *J* = 7.2, 2 H); 1.63–1.46 (*m*, 4 H); 0.91 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 197.2; 136.3; 131.7; 128.2; 128.1; 121.6; 117.6; 100.3; 95.0; 90.4; 78.4; 30.7; 26.6; 21.9; 19.5; 13.6. EI-MS: 250 (100, M^+), 235 (37), 165 (29). HR-EI-MS: 250.1358 ($C_{18}H_{18}O^+$; calc. 250.1372).

Evalution of Inhibitory Concentration of Camptothecin for Gel Electrophoresis. Camptothecin, which shows significant topo I inhibition, is widely used as the standard for the comparison of activities of the resp. compounds. To affirm the modest conc. of camptothecin that would still appear in the explicit suppression of topo I, solns. of various conc. were prepared, and the results were obtained from agarose gel electrophresis. It was suggested that camptothecin showed less topo I inhibitory activity when the conc. was 26.1 μM. On the other hand, 43.5 μM camptothecin seemed to inhibit topo I. Therefore, the 43.5 μM concentration was used as the standard conc. of camptothecin, to achieve inhibition of topo I (data not shown).

General Topoisomerase I Assay. All samples were kept in 23 μl total volume; *a*) negative control (DNA alone): contained 10 × topo I buffer (2μl) and pGEM9zf(−) DNA(1 μg/μl), 0.1% BSA (2 μl) and D_2O (18 μl); *b*) positive control (DNA + topo I): contained 10 × topo I buffer (2 μl) and pGEM9zf(−) DNA (1 μg/μl), 1 units/μl of topoisomerase I, 0.1% BSA (2 μl), and D_2O (17 μl); *c*) camptothecin control (DNA + topo I + camptothecin): contained 10 × topo I buffer (2 μl) and pGEM9zf(−) DNA (1 μg/μl), 1 units/μl of topoisomerase I, 0.1% BSA (2 μl), D_2O (15 μl), and camptothecin (2 μl, dissolved in DMSO, final conc. of DMSO was 8.7% (*v*/*v*)); *d*) experiments for compounds **7**–**23** (DNA + topo I + compounds): contained 10 × topo I buffer (2 μl) and pGEM9zf(−) DNA (1 μg/μl), 1 units/μl of topoisomerase I, 0.1% BSA (2 μl), D_2O (15 μl), and compounds (2 μl, dissolved in DMSO, final conc. of DMSO was 8.7% (*v*/*v*)). *a*)–*d*) were well-mixed before incubation. The tubes were incubated in 37° water bath for 30 min.

Gel Electrophoresis. The reaction mixtures were treated with 2% agarose gel in standard TBE buffer (1 ×, 0.06M *Tris*, 0.06M boric acid, and 0.5M EDTA), which had been previously added to 2 μl of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol, 5% SDS, and 0.25% sucrose. The gels were run at 50 V for 1.5 h, and stained with ethidium bromide for 20 min, and then placed into a UV box, and photographic images of the gels were obtained with *Polaroid 665* films.

Cell-Cycle Analysis. Samples of 1 × 10^6 Hep G2 cells were placed on a 6-cm tissue-culture dish, and the cells were allowed to recover for 24 h before any treatment. Then, DMSO or compound **5** (1 μM and 10 μM) was added to the cells for 1 h in complete medium at 37°, washed twice with PBS, and incubated in fresh media. Cells were harvested with trypsin and washed twice with PBS. Samples were fixed in 70% EtOH and stored at 4° for at least 24 h and then washed once with *McIlvaine*'s buffer (0.2M Na_2HPO_4, 0.1M citric acid, pH 7.5) and once with PBS. Samples were stained with PI (propidium iodide) staining soln. (PBS containing 100 μg/ml RNase A and

10 μg/ml PI [sigma]), processed on a *Coulter Elite* flow cytometer, and the data were analyzed with the *Multiplus AV* program.

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