Synthesis and Evaluation of Terbenzimidazoles as Topoisomerase I Inhibitors

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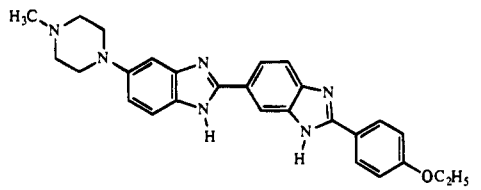
The synthesis and pharmacological activity of a series of terbenzimidazoles are described. The ability of these derivatives to induce DNA cleavage in the presence of topoisomerase I was evaluated *in vitro*. These analogs were also assayed for their cytotoxicity in RPMI 8402 cells and the camptothecin-resistant CPT-K5 cells. In addition the potential for these compounds to serve as substrates for MDR1 was also determined. Several terbenzimidazoles exhibited similar cytotoxicity against variants of human tumor cells that either overexpress MDR1 or are camptothecin-resistant.

Introduction

DNA topoisomerases are nuclear enzymes that control and modify the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands.[1-3] Topoisomerase II enzymes alter the topological state of DNA by means of a double-strand break in the DNA. Topoisomerase II represents an effective pharmacological target for the development of cancer chemotherapeutics.[4-6] Among the clinical agents in use which are recognized as topoisomerase II inhibitors are etoposide (VP-16), teniposide (VM-26), mitoxantrone, *m*-AMSA, adriamycin (doxorubicin), ellipticine, and daunomycin. In comparison to topoisomerase II poisons, there are comparatively few topoisomerase I inhibitors. Camptothecin represents the most extensively studied mammalian topoisomerase I inhibitor. The broad spectrum of potent antineoplastic activity observed for camptothecin[7,8] has prompted further efforts to identify other agents which can effectively poison mammalian topoisomerase I. Among the agents which have been identified as topoisomerase I inhibitors are the alkaloids fagaronine[9] and nitidine as well as derivatives of chelerythrine[10] and berberine[11] and the fungal metabolites bulgarein[12] and saintopin.[13] Indolocarbazoles related to the anitbiotic K252a are also topoisomerase I poisons which induce cleavable complex formation with DNA.[14] It has recently been demonstrated that Hoechst 33342 (**1**), 2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole, is an inhibitor of topoisomerase I. This agent, which binds to the minor groove of DNA, traps the reversible cleavable complex derived from DNA and topoisomerase I and produces a limited number of highly specific single-strand DNA breaks.[15,16] A limitation of Hoechst 33342 as an anticancer agent is the previously reported observation that it is not effective against tumor cell lines which overexpress MDR1. While KB3-1 cells are known to be quite sensitive to Hoechst 33342, with an IC_{50} of ca. 9 nM, this compound is ca. 130-fold less cytotoxic to KBV-1 cells, which are known to overexpress MDR1. Recently, several analogs of this bibenzimidazole have been synthesized (**2**) to further investigate the structure–activity relationships associated with their potency as topoisomerase I inhibitors and the related cytotoxicity.[17]



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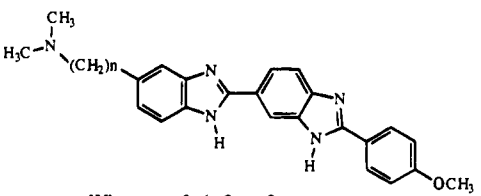

Where n = 0, 1, 2, or 3

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The present study details the synthesis of several terbenzimidazoles and evaluates their potential to induce DNA cleavage in the presence of mammalian topoisomerase I. The results associated with the cytotoxicity observed for these analogs in various tumor cell lines, including camptothecin-resistant cells and cells which overexpress human MDR1, are also presented.

Chemistry

The preparation of various substituted terbenzimidazoles is outlined in Scheme 1. With the exception of phenylenediamine which was commercially available, the appropriately substituted phenylenediamines were synthesized by catalytic hydrogenation of the respective *o*-nitroaniline derivatives. These phenylenediamines were then coupled with 5-formyl-2-(benzimidazol-5′-yl)-benzimidazole, **9**, by heating in nitrobenzene at 150 °C to provide the various terbenzimidazoles **10**–**16** in yields ranging from 43% to 96%.[18,19]

The requisite nitroanilines, as outlined in Scheme 1, with the exception of **3** which was commercially available, were synthesized from 4-bromo-2-nitroaniline, **17**. Compound **17** was prepared from *o*-nitroaniline in good yield, 94%, using 2,4,4,6-tetrabromo-2,5-cyclohexadienone as the bromination reagent.[20] While allyltributyltin and phenyltributyltin were commercially available, the pyridyltributyltin derivatives were prepared from tributyltin chloride and 2-, 3-, and 4-bromopyri-

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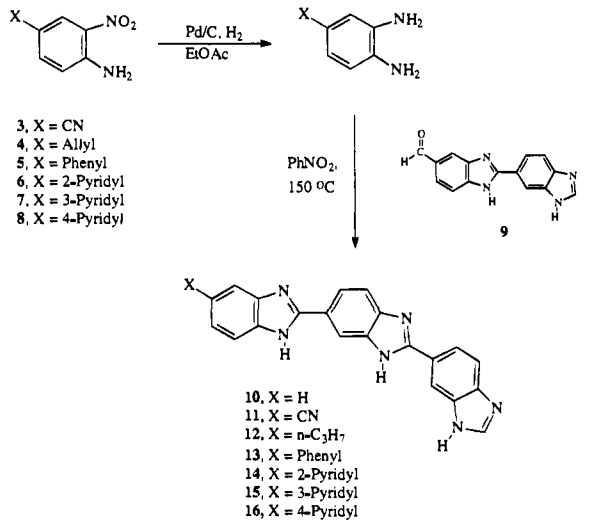
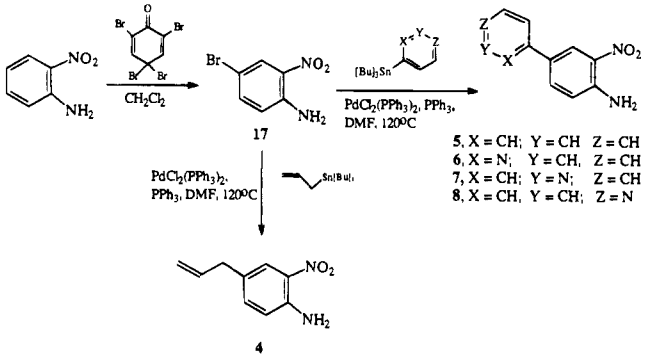
Scheme 1



Scheme 2



dine, respectively.[21] These tributyltin derivatives were then coupled with 4-bromo-2-nitroaniline using $PdCl_2(PPh_3)_2$ as the catalyst in DMF as outlined in Scheme 2 to provide compounds **4**–**8**, respectively.[22]

The preparation of 5-formyl-2-(benzimidazol-5′-yl)benzimidazole, **9**, was accomplished as outlined in Scheme 3. Reduction of 5-benzimidazolecarboxylic acid to 5-(hydroxymethyl)benzimidazole was accomplished using $LiAlH_4$. Oxidation of the resulting crude benzylic alcohol with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide provided in two steps the desired 5-formylbenzimidazole in 32% overall yield.[23] Coupling of 5-formylbenzimidazole with 4-cyano-1,2-phenylenediamine provided 5-cyano-2-(benzimidazol-5′-yl)benzimidazole, **19**, which when treated with Ni–Al catalyst in the presence of aqueous formic acid gave 5-formyl-2-(benzimidazol-5′-yl)benzimidazole, **9**, in 65% yield.[24]

The synthetic methods used in the preparation of the bibenzimidazole 5-cyano-2-[2′-(*p*-methoxyphenyl)benzimidazol-5′-yl]benzimidazole, **22**, and the terbenzimidazole 5-cyano-2-[2′-[[2″-(*p*-methoxyphenyl)benzimidazol-5″-yl]benzimidazol-5′-yl]benzimidazole, **24**, are outlined in Scheme 4. Coupling of *p*-methoxybenzaldehyde with

Table 1. Topoisomerase I-mediated DNA Cleavage and Cytotoxicity of Bi- and Terbenzimidazoles

compd	topo I-mediated DNA cleavage[b]	cytotoxicity IC_{50}[a] (μM), cell lines			
		RPMI	CPT-K5	KB3-1	KBV-1
Hoechst 33342	1	0.03	0.9	0.01	1.2
10	1.1	14	28	ND	ND
11	1	>25[c]	>25[c]	ND	ND
12	100	7.6	20	ND	ND
13	2	0.09	0.58	0.58	0.35
14	3.3	0.16	5.8	0.05	0.09
15	2	0.035	2.5	0.02	0.02
16	2	0.035	2.5	0.02	0.01
19	1000	>25[c]	ND	ND	ND
22	3.3	27	ND	ND	ND
24	1000	>20[c]	ND	ND	ND

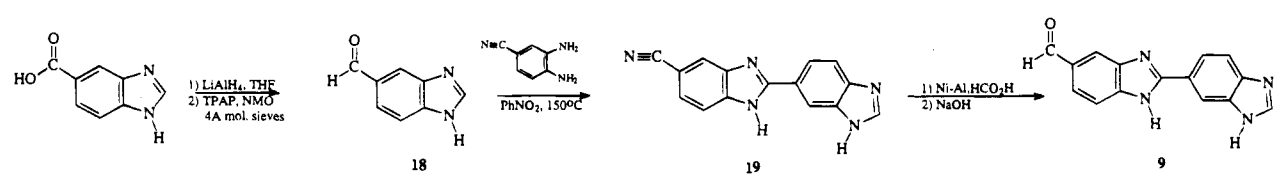
[a] IC_{50} was calculated after 4 days of continuous drug exposure. ND = not determined. [b] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to Hoechst 33342, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase I. Cleavage is calculated from the intensity of the strongest Hoechst specific band. [c] No indication of cytotoxicity was considered indicative of IC_{50} values substantially greater than the highest doses asayed.

4-cyano-1,2-phenylenediamine gave 5-cyano-2-(*p*-methoxyphenyl)benzimidazole, **20**, in 72%. Conversion of **20** to 5-formyl-2-(*p*-methoxyphenyl)benzimidazole, **21**, was accomplished with Ni–Al catalyst in the presence of aqueous formic acid. Condensation of this aldehyde a second time with 4-cyano-1,2-phenylenediamine gave the bibenzimidazole derivative **22**. Conversion of **22** to the aldehyde **23** was accomplished as previously described in the preparation of **21**. The preparation of the terbenzimidazole analog **24** was prepared by condensation of **23** with 4-cyano-1,2-phenylenediamine.

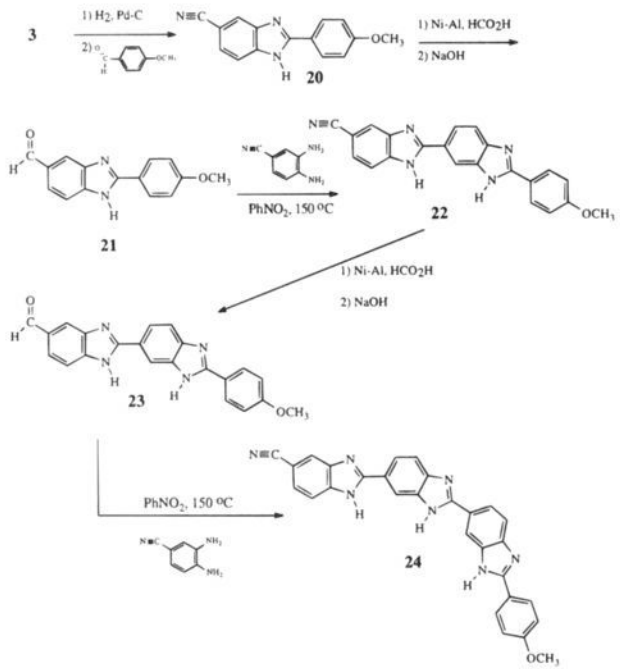
Results and Discussion

Comparison of compounds **10**–**16** with Hoechst 33342 (**1**) as inhibitors of topoisomerase I demonstrated that several of these terbenzimidazoles had similar potency, Table 1. While **10** and **11** exhibited similar potency in their inhibition of topoisomerase I as observed with Hoechst 33342 (Figure 1), both of these compounds failed to exhibit significant cytotoxicity toward the human lymphoblast cell line RPMI 8402. Our initial hypothesis for the lack of cytotoxicity observed with **11** was that this agent was, for reasons unknown, not being incorporated into these cells. This hypothesis was validated by studies performed with fluorescence microscopy which indicated that **11**, in contrast to Hoechst 33342, did not enhance chromatin fluorescence unless the cell membrane was disrupted by detergent-induced lysis (unpublished result). The 5-phenyl-substituted terbenzimidazole **13** was approximately one-half as potent as Hoechst 33342 as a topoisomerase I inhibitor. In contrast to **10** and **11**, however, it had significant cytotoxicity toward RPMI 8402 cells. As observed with Hoechst 33342, **13** was also effective against camptothecin-resistant CPT-K5 cells.[25] The relative resistance

Scheme 3



Scheme 4



of Hoechst 33342 and **13**, expressed as the ratio of the IC_{50} values of the resistant verses the drug sensitive cell line, is ca. 30-fold as compared to the relative resistance of camptothecin which is 2500-fold.[15] A similar effect was observed in another pair of cell lines; **13** has an IC_{50} of 0.015 μg/mL in the human ovarian tumor cell line A2780 relative to an IC_{50} of 0.03 μg/mL in CPT-2000, a varient of A2780 selected for camptothecin resistance and known to contain a mutant camptothecin-resistant topoisomerase I (J. Hwang, personal communication). The 5-*n*-propyl terbenzimidazole derivative **12** was much less active than either **10**, **11**, or **13** as an inhibitor of topoisomerase I. Its weak activity as a topoisomerase I inhibitor correlated with its weak cytotoxicity. The activity of several of these compounds was also evaluated using recombinant human topoisomerase I. As in indicated in Figure 2, several of these analogs induced similar DNA cleavage in the presence of human topoisomerase I as compared to that observed with topoisomerase I isolated from calf thymus.

The cytotoxic activity of Hoechst 33342 and **13** was also evaluated against KB3-1 and KBV-1 cells.[26,27] The primary difference between these cell lines is in the degree to which human MDR1 (P-glycoprotein) is expressed. Recent studies have demonstrated that antineoplastic agents which are cationic at physiological pH are more likely to serve as substrates for MDR1 and, therefore, are likely to be less effective against cells that overexpress P-glycoprotein. In view of the fact that Hoechst 33342 is extensively protonated at physiological pH, it is not surprising that the IC_{50} differs by ca. 2 orders of magnitude for KB3-1 as compared to KBV-1 cells.[28] In contrast to Hoechst 33342, there is little difference between the IC_{50} values observed for **13** in these two cell lines. Thus, **13** appears not to be a substrate for human MDR1. These data indicate that these terbenzimidazole derivatives may have significant chemotherapeutic advantages as compared to Hoechst 33342 or pibenzimol (Hoechst 33258), 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole.

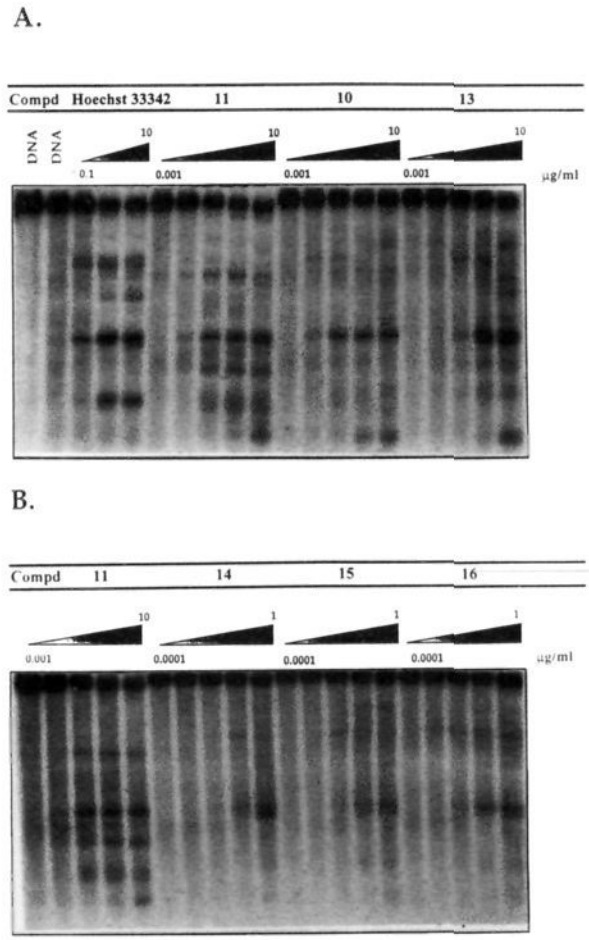


Figure 1. Stimulation of enzyme-mediated DNA cleavage by terbenzimidazoles using calf thymus DNA topoisomerase I. Enzyme-mediated DNA cleavage using calf thymus topoisomerase I was performed as described in the Experimental Section. The left-most lane in each panel is the DNA control without topoisomerase I. The second lane from the left in each panel is the control with topoisomerase alone. The rest of the lanes are with topoisomerase I and serially (10-fold each) *diluted compound from 0.1 to 10 μM for Hoechst 33342 or from* 0.001 to 10 μM for the rest. The faster migrating bands represent DNA fragments resulting from topoisomerase I poisoning.

These data indicated that substitution of these terbenzimidazoles with a 5-aryl substituent was associated with derivatives which are active as topoisomerase I inhibitors and cytotoxic to tumor cells. Terbenzimidazoles substituted at the 5-position with either a 2-, 3-, or 4-pyridyl group, **14**–**16**, were evaluated for their potency as topoisomerase I inhibitors and for cytotoxicity as summarized in Table 1. These analogs, similar to **13**, have activity as topoisomerase I inhibitors. The 3- and 4-pyridyl analogs **15** and **16** are somewhat more active than the 2-pyridyl derivative **14** as topoisomerase I inhibitors as well as cytotoxic agents. As was observed with **13**, these pyridyl-substituted terbenzimidazoles had similar cytotoxicity to KB3-1 cells as well as to KBV-1 cells which overexpress MDR1. A principal advantage of these aryl-substituted terbenzimidazoles as compared to Hoechst 33342 is their efficacy against cell lines which express MDR1.

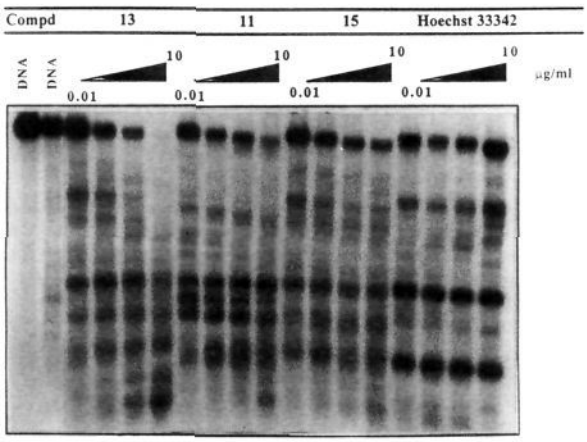


Figure 2. Stimulation of enzyme-mediated DNA cleavage by terbenzimidazoles using human DNA topoisomerase I. The assays were performed as described in Figure 1 except that recombinant human topoisomerase I was used.

Evaluation of the topoisomerase I inhibition and cytotoxicity of **19**, **22**, and **24** provided additional insight into the structure–activity relationships associated with these benzimidazole derivatives. The activity observed for **19** as a topoisomerase I inhibitor in comparison to **11** suggests that the 2′-(benzimidazol-5″-yl) moiety is critical to the activity observed for the terbenzimidazoles evaluated in this study. As observed with **22**, there appears to be a modest decrease in potency in topoisomerase I inhibition observed for analogs of **13** where the 2′-(benzimidazol-5″-yl) moiety is replaced by a *p*-methoxyphenyl group.

The lack of significant DNA cleavage observed with **24** in the presence of topoisomerase I suggests that the addition of a 2″-(*p*-methoxyphenyl) substituent to terbenzimidazoles structurally related to **13** interferes with their potential as topoisomerase I inhibitors. These data suggest that steric factors associated with substituents at this position may substantially influence the activity of similar terbenzimidazoles.

Experimental Section

General. Melting points were determined with a Thomas-Hoover unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in $CDCl_3$ (unless otherwise noted) with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). A few drops of CF_3COOH improved ^{13}C NMR spectra by allowing for increased solubility and formation of the protonated form of these benzimidazoles, thereby eliminating tautomeric differences among carbon atoms. Mass spectra were obtained from the Midwest Center for Mass Spectrometry within the Department of Chemistry at the University of Nebraska-Lincoln. Compound homogeneity was determined by analytical reverse-phase HPLC for all compounds for which HRMS data are provided. Coumpounds were analyzed using at least two of the following conditions: (method A) a Microsorb C-8 column (Rainin Instrument Co., Inc.) using methanol:H_2O (65:35) with a flow rate of 1 mL/min; (method B) a VYDAC C-18 column (The Separations Group) using methanol:0.1 M potassium phosphate buffer (pH 7.0) (70:30) with a flow rate of 1 mL/min; or (method C) a VYDAC C-18 column and methanol:H_2O:THF (70:20:10) as eluent and a flow rate of 0.5 mL /min. HPLC analyses were performed with a Hewlett-Packard 1090 liquid chromatograph equipped with diode array UV detection. On the basis of these analyses, purity ranged from >98.3% to >99.9% for all compounds analyzed, with the exception of **13** which was 97.6% pure as determined by HPLC. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and are within ±0.4%. THF was freshly distilled form sodium and benzophenone prior to use. Allyltributyltin and phenyltributyltin were purchased from Aldrich Chemical Co.

General Procedure for $PdCl_2(PPh_3)_2$-Catalyzed Coupling Reaction of 4-Bromo-2-nitroaniline (13) with Tin Compounds. 4-Allyl-2-nitroaniline (4): prepared from 4-bromo-2-nitroaniline (**17**; 1.70 g, 7.84 mmol) and allyltributyltin (3.38 g, 10.2 mmol) as a yellow solid in 96% yield as described above for **5**; mp 28–29 °C; IR (KBr) 3490, 3374, 1638, 1518, 1341, 1253; ^1H NMR δ 7.90 (1H, d, J = 2.0), 7.19 (1H, dd, J = 8.5, 2.0), 6.77 (1H, d, J = 8.5), 6.05 (NH, brs), 6.00–5.80 (1H, m), 5.11 (1H, dd, J = 1.4, 1.4), 5.04 (1H, ddd, J = 6.6, 3.0, 1.5), 3.28 (1H, d, J = 6.6); ^{13}C NMR δ 143.81, 137.13, 129.34, 125.59, 119.49, 116.95, 39.18; HRMS (EI) calcd for $C_9H_{10}N_2O_2$ 178.0742, found 178.0746.

4-Phenyl-2-nitroaniline (5). A solution of 4-bromo-2-nitroaniline (**17**; 1.0 g, 4.67 mmol), tributylphenyltin (2.2 g, 6.07 mmol), bis(triphenylphosphine)palladium(II) chloride (164 mg, 0.234 mmol), and triphenylphosphine (613 mg, 2.34 mmol) in DMF (15 mL) was heated under N_2 at 120 °C overnight. After the solution was cooled to room temperature, the reaction mixture was directly chromatographed on silica gel eluting with 2–5% EtOAc/hexane to give 752 mg (75%) of **5** as a yellow solid: mp 169–171 °C; IR ($CHCl_3$) 3517, 3398, 3022, 1635, 1525, 1250; ^1H NMR δ 8.38 (1H, d, J = 2.2), 7.66 (1H, dd, J = 8.7, 2.2), 7.59–7.54 (2H, m), 7.49–7.34 (3H, m), 6.90 (1H, d, J = 8.8), 6.13 (NH, brs); ^{13}C NMR δ 144.2, 139.3, 135.0, 130.9, 129.5, 127.8, 126.8, 124.4, 119.8, 112.8. Anal. Calcd ($C_{12}H_{10}N_2O_2$) C, H, N.

4-(2-Pyridyl)-2-nitroaniline (6): prepared from 4-bromo-2-nitroaniline (**17**; 597 mg, 2.75 mmol) and 2-(tributylstannyl)-pyridine (1.01 g, 2.75 mmol) as a yellow solid in 52% yield as described above for **5**; mp 146–148 °C; IR ($CHCl_3$) 3516, 3397, 3020, 1634, 1524, 1341, 1250; ^1H NMR δ 8.74 (1H, d, J = 2.2), 8.63 (1H, dd, J = 4.9, 1.5), 8.13 (1H, dd, J = 8.8, 2.1), 7.78–7.66 (2H, m), 7.20 (1H, ddd, J = 4.8, 4.7, 1.9), 6.92 (1H, d, J = 8.8), 6.37 (NH, brs); ^{13}C NMR δ 155.6, 150.1, 145.6, 137.4, 134.5, 129.1, 124.7, 122.4, 119.8, 119.7. Anal. ($C_{11}H_9N_3O_2$) C, H, N.

4-(3-Pyridyl)-2-nitroaniline (7): prepared from 4-bromo-2-nitroaniline (**17**; 1.42 g, 6.53 mmol) and 3-(tributylstannyl)-pyridine (3.60 g, 9.79 mmol) as a yellow solid in 32% yield as described above for **5**; mp 177–179 °C; IR ($CHCl_3$) 3515, 3399, 3052, 2983, 1638, 1524, 1341, 1259; ^1H NMR δ 8.68 (1H, d, J = 1.7), 8.42 (1H, dd, J = 4.8, 1.5), 8.22 (1H, d, J = 2.2), 7.74 (1H, ddd, J = 7.9, 2.4, 1.6), 7.50 (1H, dd, J = 8.7, 2.2), 7.23 (1H, ddd, J = 8.0, 4.8, 0.8), 6.92 (1H, d, J = 8.8), 6.56 (NH, brs); ^{13}C NMR δ 148.7, 147.8, 145.4, 135.0, 134.4, 133.8, 126.5, 124.4, 124.0, 120.4. Anal. ($C_{11}H_9N_3O_2$) C, H, N.

4-(4-Pyridyl)-2-nitroaniline (8): prepared from 4-bromo-2-nitroaniline (**17**; 165 mg, 0.76 mmol) and 4-(tributylstannyl)-pyridine (280 mg, 0.76 mmol) as a yellow solid in 25% yield as described above for **5**; mp 230–232 °C; IR ($CHCl_3$) 3518, 3398, 3032, 1636, 1528, 1344; ^1H NMR (CD_3OD) δ 8.55 (2H, d, J = 6.3), 8.52 (1H, d, J = 2.3), 7.84 (1H, dd, J = 8.9, 2.3), 7.71 (2H, d, J = 6.4), 7.13 (1H, d, J= 8.9); ^{13}C NMR (DMSO) δ 154.3, 148.4, 141.9, 134.2, 131.0, 126.7, 122.3, 120.7, 120.4; HRMS (EI) calcd for $C_{11}H_9N_3O_2$ 215.0695, found 215.0698.

5-Formyl-2-(benzimidazol-5′-yl)benzimidazole (9). A mixture of 5-cyano-2-(benzimidazol-5′-yl)benzimidazole (**19**; 148 mg, 0.57 mmol), Ni–Al catalyst (500 mg), formic acid (7 mL), and water (3 mL) was heated at reflux under N_2 for 4 h. The hot reaction mixture was immediately filtered through a plug of Celite and evaporated to give a yellow solid. The yellow solid was then dissolved in hot water (5 mL), and the solution was neutralized to pH 9 by 2 N NaOH. The solid precipitated was collected by suction filtration and further purified by flash chromatography on silica gel (15% MeOH/EtOAc) to give 142 mg (95%) of **9** as a white solid: mp >275 °C; IR (KBr) 3106, 2835, 1685, 1618, 1432, 1293; ^1H NMR (CD_3OD) δ 10.01 (1H,

s), 8.39 (1H, s), 8.35 (1H, s), 8.13 (1H, s), 8.06 (1H, dd, J = 8.6, 1.6), 7.83 (1H, dd, J = 8.4, 1.4), 7.77 (1H, d, J = 8.5), 7.71 (1H, d, J = 8.3); HRMS (FAB) calcd for $C_{15}H_{11}N_4O$ 263.0933, found 263.0932.

General Procedures for Preparing 5-Substituted Terbenzimidazoles. 2-[2′-(Benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (10). A mixture of 5-formyl-2-(benzimidazol-5′-yl)benzimidazole (**9**; 121 mg, 0.46 mmol) and phenylenediamine (60 mg, 0.55 mmol) in nitrobenzene (8 mL) was heated at 150 °C under N_2 overnight. The mixture was cooled to room temperature and chromatographed on silica gel (0–20% MeOH/EtOAc) to afford 155 mg (96%) of **10** as a solid: mp >275 °C; IR (KBr) 3400, 3157, 1630, 1542, 1438, 1294; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 9.71 (1H, s), 8.75 (1H, s), 8.65 (1H, d, J = 1.1), 8.48 (1H, dd, J = 8.7, 1.5), 8.21 (1H, dd, J = 8.6, 1.6), 8.14 (1H, d, J = 8.8), 8.08 (1H, d, J = 8.7), 7.90 (2H, dd, J = 6.2, 3.1), 7.61 (2H, dd, J = 6.1, 3.1); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 153.3, 149.7, 140.3, 137.9, 133.2, 132.0, 131.7, 126.2, 125.4, 125.1, 123.6, 118.1, 116.2, 115.8, 114.2, 114.13, 114.09; HRMS (FAB) calcd for $C_{21}H_{15}N_6$ 351.1358, found 351.1367.

5-Cyano-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (11). Hydrogenation of **3** (70 mg, 0.43 mmol) was accomplished at 40 psi of H_2 at room temperature for 1 h using 10% Pd–C (30 mg) in EtOAc (10 mL). The reaction mixture was filtered and concentrated *in vacuo* to afford a solid. The solution of this solid and **9** (87 mg, 0.33 mmol) in nitrobenzene (5 mL) was heated at 150 °C under N_2 overnight. The mixture was cooled to room temperature and chromatographed directly on silica gel (0–10% MeOH/EtOAc) to give 107 mg (86%) of 11 as a solid: mp >280 °C; IR (KBr) 3416, 3148, 2222, 1626, 1553, 1441, 1292; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 8.50 (1H, s), 8.46 (1H, s), 8.40 (1H, s), 8.18–8.11 (3H, m), 7.81–7.75 (3H, m), 7.62 (1H, dd, J = 8.3, 1.5); HRMS (FAB) calcd for $C_{22}H_{13}N_7$ 376.1310, found 376.1309.

5-Propyl-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (12): prepared from 4-allyl-2-nitroaniline (**4**; 312 mg, 1.75 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole (**9**; 121 mg, 0.46 mmol) in 79% yield as described above for 11; solid; mp >270 °C; IR (KBr) 3421, 3068, 2957, 1434; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 9.66 (1H, s), 8.73 (1H, s), 8.59 (1H, s), 8.48 (1H, dd, J = 8.7, 1.5), 8.13 (1H, dd, J = 8.7, 1.4), 8.11 (1H, d, J = 8.7), 8.02 (1H, d, J = 8.5), 7.79 (1H, d, J = 8.4), 7.66 (1H, s), 7.45 (1H, dd, J = 8.5, 1.3), 2.80 (2H, t, J = 7.0), 1.70 (2H, m), 0.96 (3H, t, J = 7.2); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 153.84, 149.74, 141.64, 141.01, 139.37, 133.10, 132.26, 131.99, 130.34, 127.08, 126.26, 125.14, 122.91, 117.52, 116.32, 116.06, 115.76, 113.78, 112.99, 37.45, 24.73, 13.74; HRMS (FAB) calcd for $C_{24}H_{21}N_6$ 393.1827, found 393.1827.

5-Phenyl-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (13): prepared from 4-phenyl-2-nitroaniline (**5**; 247 mg, 1.15 mmol) and 5-formyl-2-(benzimidazol-5′-yl)-benzimidazole (**9**; 201 mg, 0.77 mmol) in 89% yield as described for 11; solid; mp 262–264 °C dec; IR (KBr) 3402, 3104, 1627, 1552, 1442, 1290; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 9.66 (1H, s), 8.74 (1H, s), 8.65 (1H, s), 8.50 (1H, dd, J = 8.8, 1.1), 8.21 (1H, dd, J = 8.7, 1.4), 8.12 (1H, d, J = 8.8), 8.06 (1H, s), 8.05 (1H, d, J = 8.4), 7.97 (1H, d, J = 8.7), 7.89 (1H, dd, J = 8.7, 1.5), 7.80 (2H, d, J = 7.0), 7.61–7.47 (3H, m); HRMS (FAB) calcd for $C_{27}H_{19}N_6$ 427.1671, found 427.1666.

5-(2-Pyridyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (14): prepared from 4-(2-pyridyl)-2-nitroaniline (**6**; 110 mg, 0.50 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole (**9**; 51 mg, 0.25 mmol) in 84% yield as described above for 11; solid; mp >275 °C; IR (KBr) 3411, 3157, 1630, 1593, 1432; ^{1}H NMR (CD_3OD) δ 8.59 (1H, d, J = 4.8), 8.35 (1H, s), 8.31–8.25 (2H, m), 8.10 (1H, s), 8.04–7.94 (2H, m), 7.85-7.77 (3H, m), 7.72 (1H, d, J = 8.6), 7.68 (1H, d, J = 8.7), 7.64 (1H, d, J = 8.7), 7.30 (1H, m); HRMS (FAB) calcd for $C_{26}H_{18}N_7$ 428.1624, found 428.1611.

5-(3-Pyridyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (15): prepared from 4-(3-pyridyl)-2-nitroaniline (**7**; 183 mg, 0.85 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole (**9**) in 46% yield as described above for 11; solid; mp >275 °C; IR (KBr) 3400, 3070, 2836, 1438, 1289; ^{1}H NMR (CD_3OD) δ 8.83 (1H, d, J = 1.6), 8.49 (1H, dd, J = 4.9, 1.5), 8.38 (1H, d, J = 1.1), 8.31 (1H, d, J = 1.1), 8.29 (1H, s), 8.11 (1H, ddd, J = 8.0, 2.3, 1.6), 8.05 (1H, dd, J = 8.5, 1.6), 8.00 (1H, dd, J = 8.5, 1.6), 7.81 (1H, d, J = 1.1), 7.77–7.68 (3H, m), 7.55–7.47 (2H, m); HRMS (FAB) calcd for $C_{26}H_{18}N_7$ 428.1624, found 428.1612.

5-(4-Pyridyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (16): prepared from 4-(4-pyridyl)-2-nitroaniline (**8**; 35 mg, 0.16 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole (**9**; 50 mg, 0.19 mmol) in 43% yield as described above for 11; solid; mp >280 °C; IR (KBr) 3411, 3118, 1600, 1552, 1439, 1290; ^{1}H NMR (CD_3OD) δ 8.51 (2H, d, J = 6.2), 8.33 (1H, d, J = 1.1), 8.27 (1H, s), 8.25 (1H, d, J = 1.1), 8.01 (1H, dd, J = 8.6, 1.7), 7.96 (1H, dd, J = 8.9, 2.0), 7.87 (1H, d, J = 1.0), 7.74–7.56 (6H, m); HRMS (FAB) calcd for $C_{26}H_{18}N_7$ 428.1624, found 428.1625.

4-Bromo-2-nitroaniline (17). A solution of 2-nitroaniline (5 g, 36.2 mmol) in CH_2Cl_2 (100 mL) was cooled to −10 °C and treated with 90% 2,4,4,6-tetrabromo-2,5-cyclohexadienone (19.8 g, 43.5 mmol) in five portions. The mixture was stirred at −10–0 °C for 1 h. After being warmed to room temperature, the reaction mixture was washed by 2 N NaOH (60 mL) and brine (50 mL), dried over Na_2SO_4, and evaporated. Flash chromatography on silica gel (5% EtOAc/hexane) gave 7.40 g (94%) of **17** as a yellow solid: mp 109–110 (lit.[20b] mp 109–110 °C); ^{1}H NMR δ 8.27 (1H, d, J = 2.3), 7.43 (1H, dd, J = 8.9, 2.4), 6.73 (1H, d, J = 8.8), 6.09 (NH, brs).

5-Formylbenzimidazole (18). A suspension of 5-benzimidazolecarboxylic acid (1.57 g, 9.7 mmol) in dry THF (50 mL) was cooled to −78 °C under N_2 and treated with $LiAlH_4$ (736 mg, 19.4 mmol). After the addition, the mixture was allowed to warm slowly to room temperature and then stirred at room temperature overnight. The reaction was quenched by MeOH and H_2O cautiously, and the mixture was passed through a short silica gel column eluting with 10% MeOH/EtOAc. The eluate was concentrated to give 876 mg of crude alcohol as a solid. The crude alcohol (876 mg) was dissolved in a mixture of DMF (3 mL), THF (10 mL), and CH_2Cl_2 (40 mL). 4-Methylmorpholine *N*-oxide (2.25 g, 19.2 mmol), 4 Å molecular sieves (5 g), and TPAP (169 mg, 0.48 mmol) were subsequently added to the crude alcohol solution. The mixture was stirred at room temperature overnight and filtered through a pad of silica gel eluting with 10% MeOH/EtOAc. The eluate was concentrated and further purified by flash chromatography on silica gel eluting with 0–10% MeOH/EtOAc to give 452 mg (32%, two steps) of **18** as a white solid: mp 164–166 °C; IR (KBr) 3087, 2818, 1690, 1292; ^{1}H NMR (CD_3OD) δ 9.95 (1H, s), 8.34 (1H, s), 8.08 (1H, d, J = 1.5), 7.74 (1H, dd, J = 8.4, 1.5), 7.63 (1H, d, J = 8.4); ^{13}C NMR (CD_3OD) δ 194.2, 146.0, 143.0, 139.8, 133.6, 124.9, 120.7, 116.6. Anal. Calcd for $C_8H_6N_2O$: C, 65.75; H, 4.14; N, 19.17. Found: C, 65.60; H, 4.17; N, 19.08.

5-Cyano-2-(benzimidazol-5′-yl)benzimidazole (19). A mixture of 5-formylbenzimidazole (**18**; 211 mg, 1.44 mmol) and 4-cyano-1,2-phenylenediamine (230 mg, 1.73 mmol) in nitrobenzene (10 mL) was heated at 150 °C under N_2 overnight. The mixture was cooled to room temperature and directly chromatographed on silica gel eluting with 0–15% MeOH/EtOAc to give 244 mg (65%) of **19** as a solid: mp >270 °C; IR (KBr) 3110, 2826, 2224, 1627, 1426, 1294; ^{1}H NMR (CD_3OD) δ 8.41 (1H, s), 8.33 (1H, s), 8.07 (1H, dd, J = 8.6, 1.5), 7.98 (1H, s), 7.78 (1H, d, J = 8.4), 7.73 (1H, d, J = 8.4), 7.56 (1H, dd, J = 8.4, 1.5); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 153.4, 140.4, 138.3, 132.9, 131.6, 127.0, 125.8, 125.3, 120.8, 119.8, 116.0, 115.8, 113.9, 105.5; HRMS (FAB) calcd for $C_{15}H_{10}N_5$ 260.0936, found 260.0935.

5-Cyano-2-(*p*-methoxyphenyl)benzimidazole (20): prepared from 4-cyano-1,2-phenylenediamine (2.7 g, 20 mmol) and *p*-anisaldehyde (2.7 g, 20 mmol) in 72% yield as described above for **19**; white solid; mp 221–223 °C; IR (KBr) 3425, 2222, 1616, 1434, 1255; ^{1}H NMR (CD_3OD) δ 7.92 (2H, d, J = 8.9), 7.80 (1H, s), 7.57 (1H, d, J = 8.4), 7.42 (1H, dd, J = 8.4, 1.5), 6.99 (2H, d, J = 8.9), 3.80 (3H, s); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 163.1, 153.1, 137.7, 135.1, 130.0, 127.9, 119.5, 119.4, 117.5, 115.4, 115.3, 106.4, 55.9; HRMS (EI) calcd for $C_{15}H_{11}N_3O$ 249.0902, found 249.0903.

5-Formyl-2-(*p*-methoxyphenyl)benzimidazole (21): prepared from 5-cyano-2-(*p*-methoxyphenyl)benzimidazole (**20**; 263 mg, 1.06 mmol) in 65% yield as described above for **9**; white solid; mp 68–70 °C; IR (KBr) 2959, 2837, 1688, 1610, 1452, 1297, 1251; ^{1}H NMR (CD_3OD) δ 9.97 (1H, s), 8.06 (1H, s), 8.01 (2H, d, J = 9.1), 7.78 (1H, dd, J = 8.4, 1.5), 7.64 (1H, d, J = 8.4), 7.05 (2H, d, J = 9.0), 3.85 (3H, s); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 192.3, 163.9, 152.1, 136.4, 133.8, 132.7, 130.5, 126.1, 116.5, 115.5, 115.3, 114.7, 56.0; HRMS (EI) calcd for $C_{15}H_{12}N_2O_2$ 252.0899, found 252.0894.

5-Cyano-2-[2′-(*p*-methoxyphenyl)benzimidazol-5′-yl]-benzimidazole (22): prepared from 4-cyano-1,2-phenylenediamine (165 mg, 1.24 mmol) and 5-formyl-2-(*p*-methoxyphenyl)-benzimidazole (**21**; 313 mg, 1.24 mmol) in 72% yield as described above for **19**; white solid; mp 200 °C dec; IR (KBr) 3421, 3200, 2223, 1615, 1439, 1256; ^{1}H NMR (DMSO-d_6) δ 8.67 (1H, s), 8.51 (2H, d, J = 8.8), 8.42 (1H, dd, J = 8.7, 1.3), 8.13 (1H, s), 7.94 (1H, d, J = 8.6), 7.80 (1H, d, J = 8.3), 7.63 (1H, dd, J = 8.4, 1.3), 7.22 (2H, d, J = 8.8), 3.88 (3H, s); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 163.6, 153.6, 151.5, 140.4, 138.3, 134.7, 133.2, 130.4, 126.9, 125.0, 124.9, 120.6, 119.9, 116.0, 115.7, 115.5, 115.0, 112.9, 105.3, 56.1; HRMS (FAB) calcd for $C_{22}H_{15}N_5O$ 366.1355, found 366.1357.

5-Formyl-2-[2′-(*p*-methoxyphenyl)benzimidazol-5′-yl]-benzimidazole (23): prepared from 5-cyano-2-[2′-(*p*-methoxyphenyl)benzimidazol-5′-yl]benzimidazole (**22**; 81 mg, 0.22 mmol) in 65% yield as described above for **9**; solid; mp >270 °C; IR (KBr) 3426, 3210, 1610, 1435, 1251; ^{1}H NMR (CD_3OD) δ 9.83 (1H, s), 8.01 (1H, s), 7.91 (1H, s), 7.82 (2H, d, J = 8.9), 7.76 (1H, dd, J = 8.5, 1.5), 7.64 (1H, dd, J = 8.4, 1.3), 7.53 (1H, d, J = 8.4), 7.48 (1H, d, J = 8.5), 6.88 (2H, d, J = 9.0), 3.74 (3H, s); ^{13}C NMR (CD_3OD) δ 193.99, 163.33, 157.28, 155.53, 145.10, 142.43, 140.60, 140.42, 133.22, 129.73, 125.26, 124.43, 122.88, 122.79, 118.84, 116.33, 116.14, 115.62, 114.58, 56.10; HRMS (EI) calcd for $C_{22}H_{16}N_4O_2$ 368.1273, found 368.1279.

5-Cyano-2-[2′-[2″-(*p*-methoxyphenyl)benzimidazol-5″-yl]benzimidazol-5′-yl]benzimidazole (24): prepared from 4-cyano-1,2-phenylenediamine (14 mg, 0.1 mmol) and 5-formyl-2-[2′-(*p*-methoxyphenyl)benzimidazol-5′-yl]benzimidazole (**23**; 29 mg, 0.08 mmol) in 55% yield as described above for **19**; solid; mp >270 °C; IR (KBr) 3425, 2222, 1617, 1437, 1255; ^{1}H NMR (DMSO-d_6) δ 8.52 (1H, s), 8.37 (1H, s), 8.22–8.04 (5H, m), 7.87–7.59 (4H, m), 7.17 (2H, d, J = 8.8), 3.89 (3H, s); HRMS (FAB) calcd for $C_{29}H_{20}N_7O$ 482.1729, found 482.1732.

Topoisomerase I-Mediated DNA Cleavage Assays. DNA topoisomerase I was purified from calf thymus gland as reported previously.[29] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[30] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[31] The cleavage assays were performed as previously reported.[15] Human topoisomerase was isolated as a recombinant fusion protein using a T7 expression system (unpublished results).

Cytotoxicity Assay. The cytotoxicity was determined using the MTT microtiter plate tetrazolium cytotoxicity assay (MTA).[32-34] The human lymphoblast RPMI 8402 and its camotothecin-resistant variant cell line CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Insitute, Nagoya, Japan).[25] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50}, cells were exposed continuously with varying concentrations of drug concentrations, and MTT assays were performed at the end of the fourth day. The drug sensitive human epidermoid carcinoma KB3-1 cell line and its vinblastine-selected multidrug-resistant variant KBV-1 cells[26,27] were provided by Dr. Michael Gottesmann (National Cancer Institute, Bethesda, ML). These cells were grown as monolayer cultures at in 5% CO_2 and maintained by regular passage in Dulbecco's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum. KBV-1 cells were similarly maintained except they were grown in the presence of 1 μg/mL vinblastine.

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References

(1) Wang, J. C. DNA topoisomerases. *Annu. Rev. Biochem.* **1985**, *54*, 665–697.
(2) Liu, L. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* **1989**, *58*, 351–375.
(3) D'Arpa, P.; Liu, L. Topoisomerase-targeting antitumor drugs. *Biochim. Biophys. Acta* **1989**, *989*, 163–177.
(4) Bodley, A. L.; Liu, L. F. Topoisomerases as novel targets for cancer chemotherapy. *Biotechnology* **1988**, *6*, 1315–1318.
(5) Schneider, E.; Hsiang, Y.-H.; Liu, L. F. DNA topoisomerase as anticancer drug targets. *Adv. Pharmacol.* **1990**, *21*, 149–183.
(6) Chen, A. Y.; Liu, L. F. DNA topoisomerases: Essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191–218.
(7) Gallo, R. C.; Whang-Peng, J.; Adamson, R. A. H. Studies on the antitumor activity, mechanism of action, and cell cycle effects of camptothecin. *J. Natl. Cancer Inst.* **1971**, *46*, 789–795.
(8) Giovanella, B. C.; Hinz, H. R.; Kozielski, A. J.; Stehlin, A. J., Jr.; Silber, R.; Potmesil, M. Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-(S)-camptothecin. *Cancer Res.* **1991**, *51*, 3052–3055.
(9) Larsen, A. K.; Grondard, L.; Couprie, J.; Desoize, B.; Comoe, L.; Jardillier, J.-C.; Riou, J.-F. The antileukemic alkaloid fagaronine is an inhibitor of DNA topoisomerase I and II. *Biochem. Pharmacol.* **1993**, *46*, 1403–1412.
(10) Fang, S.-D.; Wang, L.-K.; Hecht, S. M. Inhibitors of DNA topoisomerase I isolated from the roots of Zanthoxylum nitidum. *J. Org. Chem.* **1993**, *58*, 5025–5027.
(11) Makhey, D.; Gatto, B.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E., Protoberberine alkaloids and related compounds as dual inhibitors of mammalian topoisomerase I and II. *Med. Chem. Res.* **1994**, *5*, 1–12.
(12) Fujii, N.; Yamashita, Y.; Saitoh, Y.; Nakano, H. Induction of mammalian DNA topoisomerase I-mediated DNA cleavage and DNA unwinding by Bulgarein. *J. Biol. Chem.* **1993**, *268*, 13160–13165.
(13) Yamashita, Y.; Kawada, S.-z.; Fujii, N.; Nakano, H. Induction of mammalian topoisomerase I and II mediated DNA cleavage by Saintopin, a new antitumor agent from fungus. *Biochemistry* **1991**, *30*, 5838–5845.
(14) Yamashita, Y.; Fujii, N.; Murakaya, C.; Ashizawa, T.; Okabe, M.; Nakano, H. Induction of mammalian DNA topoisomerase mediated DNA cleavage by antitumor indolocarbazole derivatives. *Biochemistry* **1992**, *31*, 12069–12075.
(15) Chen, A. Y.; Yu, C.; Bodley, A. L.; Peng, L. F.; Liu, L. F. A new mammalian DNA topoisomerase I poison Hoechst 33342: Cytotoxicity and drug resistance in human cell cultures. *Cancer Res.* **1993**, *53*, 1332–1335.
(16) Chen, A.; Yu; C.; Gatto, B.; Liu, L. F. DNA minor groove-binding ligands: A different class of mammalian DNA toposiomerase I inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8131–8135.
(17) Sun, Q.; Gatto, B.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E. J. Structure activity of topoisomerase I poisons related to Hoechst 33342. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2871–2876.
(18) Singh, M. P.; Joseph, T.; Kumar, S.; Bathini, Y.; Lown, J. W. Synthesis and sequence -specific DNA binding of a topoisomerase inhibitory analog of Hoechst 33258 designed for altered base and sequence recognotion. *Chem. Res. Toxicol.* **1992**, *5*, 597–607.
(19) Bathini, Y.; Lown, J. W. Convenient routes to substituted benzimidazoleas and imadazolo[4,5-b]pyridines using nitrobenzene as oxidant. *Syn. Commun.* **1990**, *20*, 955–963.
(20) (a) Fox, G. J.; Hallas, G.; Hepworth, J. D.; Paskins, K. N. para-Bromination of aromatic amines: 4-Bromo-N,N,-dimethyl-3-(trifluoromethyl)aniline. *Org. Syn.* **1973**, *55*, 20–23. (b) Heppolette, R. L.; Miller, J. The S_N mechanism in aromatic compounds. V. Halogen substituents. *J. Am. Chem. Soc.* **1953**, *75*, 4265–4268.
(21) Peters, D.; Hornfeldt, A.-B.; Gronowitz, S. Synthesis of various 5-substituted uracils. *J. Heterocycl. Chem.* **1990**, *27*, 2165–2173.

(22) Iwao, M.; Takehara, H.; Furukawa, S.; Wantanabe, M. A regiospecific synthesis of carbazoles via consecutive palladium-catalyzed cross-coupling and aryne-mediated cyclization. *Heterocycles* **1993**, *36*, 1483–1492.
(23) Cherif, A.; Farquhar, D. N-(5,5-Diacetoxypent-1-yl)doxorubicin: A new intensely potent doxorubicin analogue. *J. Med. Chem.* **1992**, *35*, 3208–3114.
(24) Pipier, J. R.; McCaleb, G. S.; Montogomery, J. A.; Kisliuk, R. L.; Glaumont, Y.; Thorndike, J.; Sirotnak, F. M. Synthesis and antifolate activity of 5-methyl-5,10-dideaza analogues of aminopterin and folic acid and an alternative synthesis of 5,10-dideazatetrahydrofolic acid, a potent inhibitor of glycinamide ribonucleotide formyltransferase. *J. Med. Chem.* **1988**, *31*, 2164–2169.
(25) Andoh, T.; Okada, K. Drug resistance mechanisms of topoisomerase I drugs. *Adv. Pharmacol.* **1994**, *29B*, 93–103.
(26) Aliyama, S.; Fojo, A.; Hanover, J. A.; Pastan; I.; Gottesman, M. M. Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somatic Cell Mol. Genet.* **1985**, *11*, 117–126.
(27) Shen, D.-W.; Fojo, A.; Chin, J. E.; Roninson, I. B.; Richert; N.; Paston, I.; Gottesman, M. M. Human multi-drug resistant cell lines; increased mdr1 expression can precede gene amplification. *Science* **1986**, *232*, 643–645.
(28) Chen, A. Y.; Liu, L. F. Design of topoisomerase inhibitors to overcome MDR1-mediated drug resistance. *Adv. Pharmacol.* **1994**, *29B*, 245–256.
(29) Halligan, B. D.; Edwards, K. A.; Liu, L. F. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. *J. Biol. Chem.* **1985**, *260*, 2475–2482.
(30) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, a Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1982; pp 149–185.
(31) Liu, L. F.; Rowe, T. C.; Yang, L.; Tewey, K. M.; Chen, G. L. Cleavage of DNA by mammalian topoisomerase II in cultured human cells. *J. Biol. Chem.* **1983**, *258*, 15365–15370.
(32) Mosmann, T. J. Rapid colorimetric assay for cellular growth and survivcal: application to proliferation and cytotoxicity assays. *Immunol. Methods* **1983**, *65*, 55–63.
(33) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942.
(34) Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1986**, *89*, 271–277.

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