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Topoisomerase I Inhibition and Cytotoxicity of 5-Bromo- and 5-Phenylterbenzimidazoles

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Abstract—Topoisomerase I is an enzyme that is essential for maintaining the three-dimensional structure of DNA during the processes of transcription, translation and mitosis. With the introduction of new clinical agents that are effective in poisoning topoisomerase I, this enzyme has proved to be an attractive molecular target in the development of anticancer drugs. Several terbenzimidazoles have been identified as potent topoisomerase I poisons. Structure–activity data on various terbenzimidazoles have revealed that the presence of lipophilic substituents at the 5-position of various terbenzimidazoles correlates with enhanced cytotoxicity. While the effect of having substituents at both the 5- and 6-positions had not been evaluated, previous studies did indicate that the presence of a fused benzo-ring at the 5,6-position results in a significant decrease in topoisomerase I poisoning activity and cytotoxicity. In the present study we investigated whether substituents at both the 5- and 6-positions of varied terbenzimidazoles would allow for retention of topo I poisoning activity. The 6-bromo, 6-methoxy, or 6-phenyl derivatives of both 5-bromo- and 5-phenylterbenzimidazole were synthesized and evaluated for topo I poisoning activity, as well as their cytotoxicity toward human lymphoblastoma cells. The data indicate that such derivatives do retain similar topo I poisoning activity and possess cytotoxicity equivalent to either 5-bromo- or 5-phenylterbenzimidazole. Significant enhancement in the topoisomerase I poisoning activity and cytotoxicity of 5-phenylterbenzimidazole is observed when the 2″-position is substituted with either a chloro or trifluoromethyl substituent. The influence of such substituents on the biological activity of 5,6-dibromoterbenzimidazole (**6a**) was also explored. In the case of either 2″-chloro-5,6-dibromoterbenzimidazole (**6b**) or 2″-trifluoromethyl-5,6-dibromoterbenzimidazole (**6c**), topoisomerase I poisoning was not enhanced relative to **6a**. While cytotoxicity toward RPMI 8402 was also not significantly affected, comparative studies performed against several solid human tumor cell lines did reveal a significant increase in cytotoxicity observed for **6c** as compared to **6a**.

Introduction

DNA topoisomerases I and II are nuclear enzymes necessary for maintaining the three-dimensional structure of DNA, especially during the processes of transcription, translation and mitosis.[1–4] Topoisomerase I (topo I) causes transient single-strand breaks, relieving torsional tension along the fork of replicating DNA. Topoisomerase poisons produce their antitumor effects by stabilizing the cleavable ternary complex consisting of topo I enzyme, DNA, and drug.

Several bibenzimidazoles and terbenzimidazoles have been synthesized and identified as topo I poisons.[5–10] Extensive studies have been performed on the structure–activity of terbenzimidazoles. Chart 1 lists several terbenzimidazoles that have potent activity as topo I poisons when evaluated in a subcellular assay. Structure–activity data on these various terbenzimidazoles revealed that the presence of lipophilic substituents at the 5-position of various terbenzimidazoles did correlate with enhanced cytotoxicity.[8–10] Consequently, **1** and **2** were significantly less cytotoxic than **3a–c** and **4a** in the human lymphoblastoma cell line, RPMI 8402. These differences in cytotoxic activities have been attributed to differences in cellular penetration/uptake. Comparison of the relative cytotoxic activities of 4-phenylterbenzimidazole and 5-phenylterbenzimidazole also demonstrated that the

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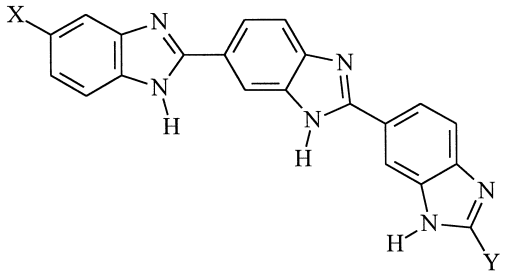


1 X = H; Y = H
2 X = CN; Y = H
3a X = Phenyl; Y = H
3b X = Phenyl; Y = Cl
3c X = Phenyl; Y = CF_3
4a X = Br; Y = H

Chart 1.

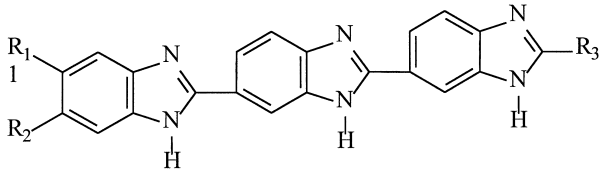
5-substituted analogue was significantly more potent.[9] While the effect of having substituents at both the 5- and 6-position had not been evaluated, previous studies did indicate that the presence of a fused benzo-ring at the 5,6-position was associated with a significant decrease in topoisomerase I poisoning activity and cytotoxicity.[9]

The objective of the present study was primarily focused on determining whether substituents at both the 5- and 6-position of varied terbenzimidazoles would allow for retention of their activity as topo I poisons. Specifically, the influences of 6-bromo, 6-methoxy, or 6-phenyl substituents attached to either 5-phenyl- or 5-bromoterbenzimidazole on topo I poisoning activity, as well as their cytotoxicity toward human lymphoblastoma cells, were examined. Several 2″-substituted derivatives of **3a** were recently synthesized and evaluated as topo I poisons and for cytotoxic activity.[11] Enhanced cytotoxicity and topo I poisoning activity were observed when a chloro or trifluoromethyl substituent was at the 2″-position, as in **3b** and **3c**. In the present study, we also examined whether, in the case of 5,6-dibromoterbenzimidazole, the presence of a chloro or trifluoromethyl substituent at the 2″-position would enhance its topoisomerase I poisoning activity and cytotoxicity.

Chemistry

Comparative studies were performed on several 5-phenyl- and 5-bromoterbenzimidazole derivatives. Table 1 summarizes the various terbenzimidazoles that were evaluated in this study. Methods for the preparation of **3a–c** and **4a** have been previously described.[8–11] Compounds **4b**, **4c**, **5** and **6b** were prepared as shown in Scheme 1. Intermediates **10a–c** were prepared by reduction of the requisite nitroaniline. A literature procedure was employed for the preparation of **10c**.[12] The *o*-phenylenediamines **10a–c** were condensed with the 3,4-dinitrobenzaldehyde, **11**, to give **12a–c**.[13] 3,4-Dinitrobenzaldehyde was prepared from 3,4-dinitrobenzoic acid by reduction to the alcohol with borane/THF and subsequent oxidation of the alcohol with pyridinium chlorochromate to the aldehyde.[14] Reduction of the dinitro intermediates was carried out using Pd/C in the presence of H_2 in case of **12b** and Raney Nickel in ethanol in case of **12a** and **12c** to provide the diamines **13a–c**. Compound **13a** was reacted with **14** and **15** to give products **4b** and **4c**, respectively. Compound **13b** was condensed with **16** to give **5.** Reaction of **13c** with **17** provided **6b**. Aldehydes **14–16** were prepared from their corresponding nitriles by HCOOH/Ni/Al reduction. The requisite nitrile intermediates were obtained by reacting 4-cyano-1,2-phenylenediamine with urea,[15] propionaldehyde[16] or trifluoroacetic acid.[16] Aldehyde **17** was prepared as previously described.[11]

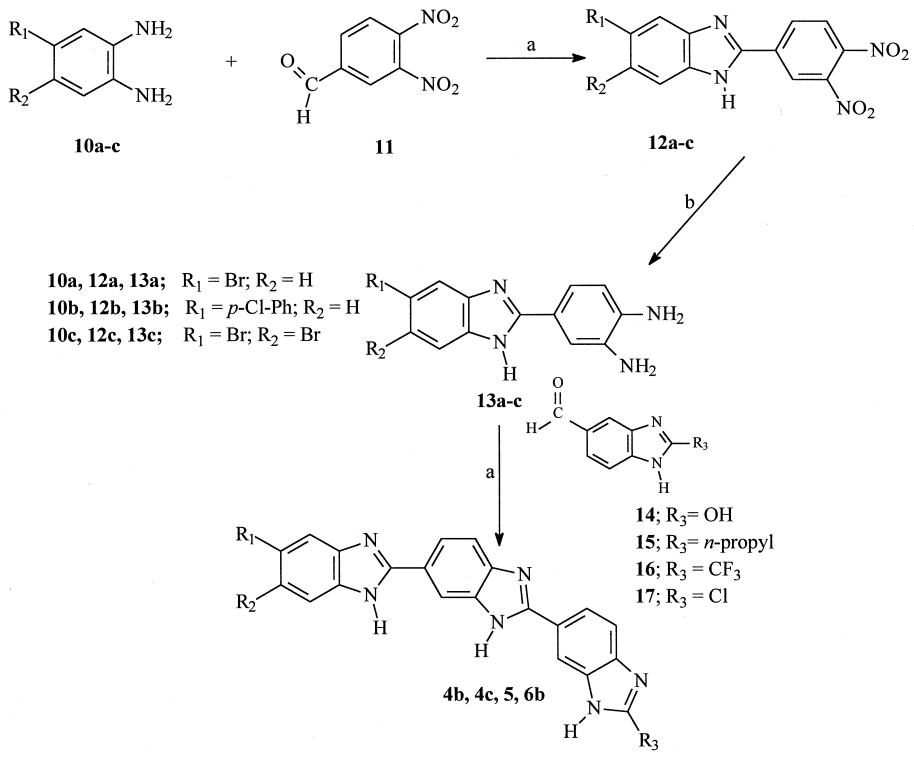
Table 1. 5-Phenylterbenzimidazoles and 5-bromoterbenzimidazoles evaluated for topoisomerase I poisoning activity and cytotoxicity



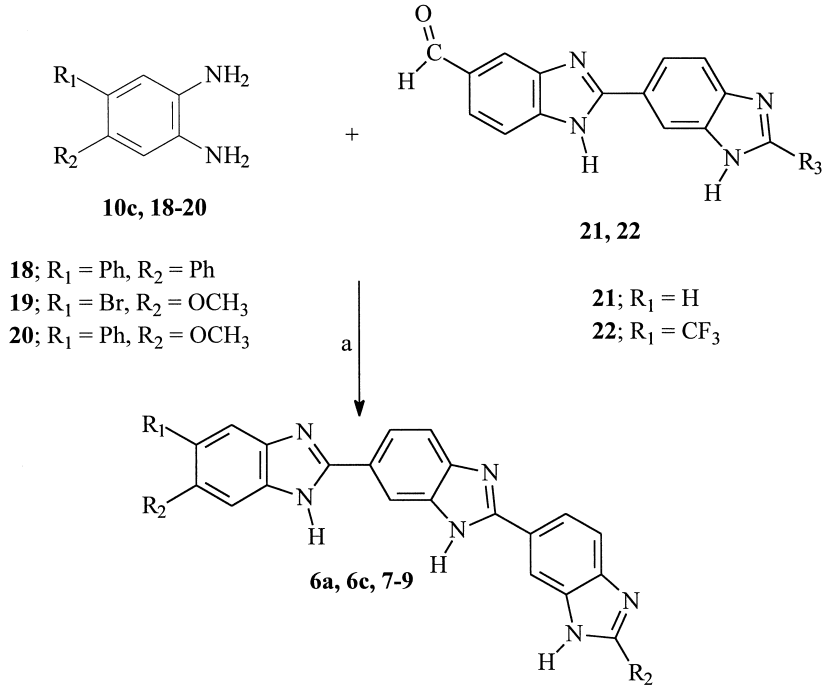
Compound	R_1	R_2	R_3
3a	Phenyl	H	H
3b	Phenyl	H	Cl
3c	Phenyl	H	CF_3
4a	Br	H	H
4b	Br	H	OH
4c	Br	H	$CH_2CH_2CH_3$
5	*p*-Cl-Phenyl	H	CF_3
6a	Br	Br	H
6b	Br	Br	Cl
6c	Br	Br	CF_3
7	Phenyl	Phenyl	H
8	Br	OCH_3	H
9	Phenyl	OCH_3	H

The methods used for the synthesis of compounds **6a**, **6c** and **7–9** are outlined in Scheme 2. The phenylenediamines, **18–20**, were prepared by reduction of their specific nitroaniline precursors. The nitroaniline precursor for **18** was prepared by Suzuki coupling of 3,4-dibromo-6-nitroaniline using phenylboronic acid, wherein both of the bromine atoms were replaced by phenyl groups using Pd° as the catalyst.[17] 3-Bromo-4-methoxy-6-nitroaniline was commercially available and was reduced with Raney Nickel to give **19**. Suzuki coupling of 3-bromo-4-methoxy-6-nitroaniline provided 4-methoxy-2-nitro-5-phenylaniline, which was converted to **20** by hydrogenation using Pd/C as catalyst. The *o*-phenylenediamines **10c**, **18**, **19** and **20** were condensed with **21** at 145 °C to yield terbenzimidazoles **6a**, **7**, **8** and **9**, respectively. Reaction of **10c** with **22** provided **6c**.

Aldehyde **21** was prepared as described in the literature.[8] Compound **22** was synthesized as outlined in Scheme 3. The 4-cyano-*o*-phenylenediamine (**23**) was refluxed in trifluoroacetic acid for 6 h to give the 5-cyano-2-trifluoromethylbenzimidazole.[11,18] The resulting nitrile was reduced to the formyl derivative, **24**, with Ni/Al and formic acid in 41% yield. Compound **24** was condensed with **23** and reduced to give **22** in 40% yield.[11]



Scheme 1. (a) $PhNO_2$, 145 °C; (b) Pd/C; H_2 or NiAl (Ra-Ni); EtOH.

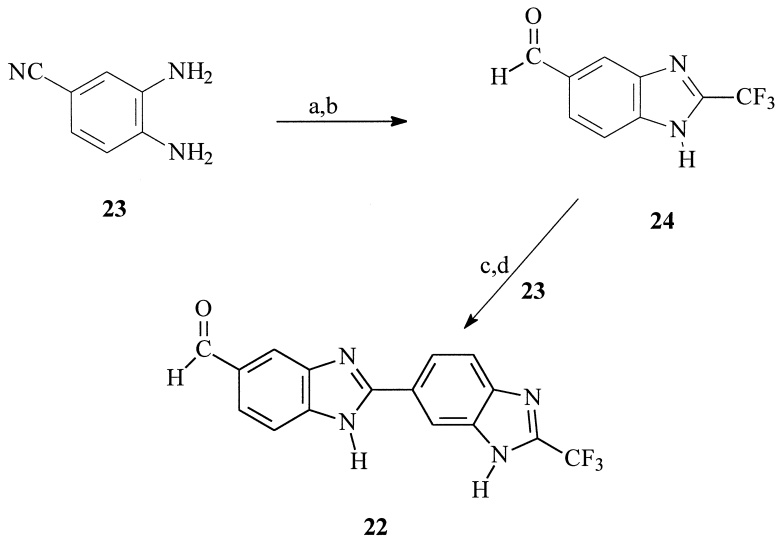


Scheme 2. (a) $PhNO_2$, 145 °C.

Results and Discussion

Topoisomerase I poisoning activity

Major differences were not observed in the topo I poisoning activities of **3a–c**, **4a–c**, **5**, **6a–c**, and **7–9** (Table 2). Previous studies indicated that variation of the 2″-substituents within a series of 5-phenylterbenzimidazole derivatives did significantly influence topo I poisoning activity. In a comparison of the relative topo I poisoning activity of various 5-bromoterbenzimidazoles, however, the presence of either a 2″-hydroxyl group (**4b**) or a 2″-propyl group (**4c**) did not influence topo I poisoning activity relative to the 2″-unsubstituted analogue, **4a**. Compound **5**, wherein the phenyl moiety of 5-phenylterbenzimidazole is replaced with a *p*-chlorophenyl group, had similar topo I poisoning activity and cytotoxicity to



Scheme 3. (a) CF_3COOH, 90 °C; (b) HCOOH, NiAl (R); (c) $PhNO_2$, 145 °C; (d) HCOOH, NiAl (Ra-Ni).

Table 2. Relative topoiosmerase I poisoning activities of cytotoxicities of 5-phenylterbenzimidazoles and 5-bromoterbenzimidazoles

Compound	Relative topo I-mediated DNA cleavage[a]	Cytotoxicity[b] RPMI-8402 IC_{50} (μM)	Cytotoxicity[b] CPT-K5 IC_{50} (μM)
3a	1.0	0.09	0.39
3b	0.1	0.07	6.0
3c	0.5	0.04	>10
4a	1.0	1.6	2.0
4b	1.0	6.5	>10
4c	1.0	0.21	>10
5	1.0	0.33	1.4
6a	1.0	0.26	2.0
6b	1.0	0.11	0.09
6c	1.0	0.25	8.8
7	1.0	1.6	>10
8	2.0	1.6	>10
9	0.5	0.35	>10

[a]Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e. concentrations relative to compound **3a**, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.
[b]IC_{50} values were calculated after 4 days of continuous drug exposure. The methods employed for these assays are detailed in Experimental.

3a. While studies did indicate that steric bulk could be tolerated at the 5-position, the 5,6-benzofused analogue of terbenzimidazole, 2-[2′-(benzimidazol-5″-yl)benzimidazol-5-yl]-1*H*-naphth[2,3-d]imidazole, did not exhibit significant cytotoxic activity.[9] The weak minor groove DNA binding affinity of this naphthimidazole derivative relative to terbenzimidazole and 5-phenylterbenzimidazole has been correlated with its lack of activity as a topo I poison.[19] The strong potential for this naphthimidazole derivative to self associate, rather than steric properties incompatible with retention of topo I poisoning activity, offers an alternative explanation for the lack of activity observed with this terbenzimidazole derivative.

We were uncertain prior to the present study whether 5,6-disubstituted terbenzimidazole derivatives would retain activity as topo I poisons. The data listed in Table 2 clearly indicate that 5,6-dibromoterbenzimidazole (**6a**) and 5,6-diphenylterbenzimidazole (**7**) exhibit similar activity to 5-phenylterbenzimidazole (**3a**). Among the other 5,6-disubstituted terbenzimidazoles that were evaluated, 5-bromo-6-methoxyterbenzimidazole (**8**) was only slightly less potent than **6a** as a topo I poison. 5-Phenyl-6-methoxyterbenzimidazole (**9**), however, did appear somewhat more potent than **3a** as a topo I poison. These data demonstrate that 5,6-disubstituted terbenzimidazoles can have activity as topo I poisons. While there was some minor variation in activity, for the derivatives evaluated in this study it would appear that such substituents did not exert a major impact on topo 1 poisoning activity.

The presence of electron-withdrawing substituents at the 2″-position in the case of 5-phenylterbenzimidazoles was observed to increase their relative activity as topo I poisons. We investigated whether a similar effect could be seen in the case of derivatives of 5,6-dibromoterbenzimidazole (**6a**). As shown in Table 2, the presence of either a 2″-chloro (**6b**) or a 2″-trifluoromethyl group (**6c**) did not significantly influence topo 1 poisoning activity within this subset of terbenzimidazole derivatives.

Cytotoxicity

The relative cytotoxic activities of **3a–c**, **4a–c**, **5**, **6a–c**, and **7–9** against the human lymphoblastoma cell line, RPMI 8402, and its camptothecin-resistant variant, CPT-K5, are listed in Table 2. Previous studies on terbenzimidazoles revealed that presence of a lipophilic substituent at either the 5- or 6-position was an important factor associated with cytotoxic activity.[8–10]

The effects of a 2″-substituent on cytotoxicity was also examined for several 5-phenylterbenzimidazole derivatives.[11] No similar studies were performed with derivatives of 5-bromoterbenzimidazole, **4a**. The presence of a 2″-hydroxyl group as in **4b** resulted in a decrease in cytotoxicity. This was especially apparent in the camptothecin-

resistant cell line, CPT-K5. As observed with 5-phenylterbezimidazoles, the presence of a 2″-*n*-propyl group as in **4c** enhanced cytotoxicity relative to **4a** in RPMI 8402 cells. As previously observed with 5-phenylterbenzimidazole,[11] the presence of the 2″-propyl group, however, resulted in cross-resistance against CPT-K5 cells that was not apparent in the 2″-unsubstituted analogue, **4a**. Compound **5**, which differs from 5-phenylterbenzimidazole with respect to the presence of a *p*-chloro substituent on the phenyl moiety and the 2″-trifluoromethyl group, exhibited only slightly less cytotoxicity relative to **3a** in both of these cell lines. When compared with compound **3c**, where the only difference was the *p*-chloro group on the 5-phenyl moiety, slightly lower toxicity was observed for **5** in RPMI 8402 cells. Both **3c** and **5** exhibited cross-resistance to CPT-K5 cells.

The 5,6-benzofused analogue of terbenzimidazole, 2-[(2′-(benzimidazol-5″-yl)benzimidazol-5-yl]-1*H*-naphtho [2,3-d]imidazole, in earlier studies did not exhibit significant cytotoxic activity, despite the fact that previous studies did indicate that substantial steric bulk could be tolerated at the 5-position.[9] While these data are consistent with the lack of topo I poisoning activity observed with this derivative, the strong potential observed for this naphthimidazole derivative to self associate could explain the lack of cytotoxicity observed with this terbenzimidazole derivative. In either event, it remained uncertain as to the relative cytotoxic activity that one would observe with 5,6-disubstituted terbenzimidazoles. The 5,6-disubstituted terbenzimidazoles that we targeted for synthesis incorporated within their structure at least one lipophilic moiety, either a phenyl or bromo group at the 5-position. All three 5,6-dibromo compounds, **6a–c**, exhibited cytotoxicity comparable to 5-phenylterbenzimidazole in RPMI 8402 cells. As was the case with 5-phenylterbenzimidazole derivative, no cross-resistance to CPT-K5 cells was observed with 5,6-dibromoterbenzimidazole, **6a,** or 2″-chloro derivative, **6b**. As is the case with **3c**, however, the presence of a trifluoromethyl substituent at the 2″-position (**6c**) was associated with significant cross-resistance.

It was anticipated that electron-withdrawing substituents at the 2″-position of 5,6-dibromoterbenzimidazole would exhibit significantly increased cytotoxicity. Neither the 2″-chloro nor the 2″-trifluoromethyl derivative, **6b** and **6c**, were more cytotoxic than **6a** toward RPMI 8402 cells. In an extended evaluation of cytotoxicity, which included several different human tumor lines, however, significantly enhanced cytotoxic activity was observed for **6c** relative to **6a** (Table 3). In the highly differentiated mammary tumor cell line, MDA-MB-435, and the ovarian tumor cell line OVCAR-3, **6c** was five to six times more cytotoxic than **6a**. Much greater differences in cytotoxicity were seen with the ovarian tumor cell line, SK-OV-3, and the prostate tumor cell lines PC-3 and DU-145. However, in these human tumor cell lines, **6c** was consistently less cytotoxic than **3c**, the 2″-trifluoromethyl derivative of 5-phenylterbenzimidazole.

Other 5,6-disubstituted terbenzimidazoles evaluated in this study were the 6-methoxy derivatives of 5-bromoterbenzimidazole and 5-phenylterbenzimidazole and the 6-phenyl derivative of 5-phenylterbenzimidazole. 5,6-Diphenylterbenzimidazole (**7**) and 5-phenyl-6-methoxyterbenzimidazole (**9**) were less cytotoxic than **3a**. In addition, both of these substituted 5-phenylterbenzimidazoles were cross-resistant to CPT-K5. While 5-bromo-6-methoxyterbenzimidazole (**8**) had similar cytotoxic activity to **6a** in RPMI 8402 cells, it was also cross-resistant to CPT-K5 cells.

Table 3. Cytotoxicity of **3c**, **6a** and **6c** in various solid human tumor cell lines

	IC_{50} values (μM)[a]				
Compound	MDA-MB-435	OVCAR-3	SK-OV-3	PC-3	DU-145
3c	0.08	0.26	0.55	0.06	0.59
6a	0.80	> 30	> 30	> 30	> 30
6c	0.15	> 5.0	1.4	0.23	2.0

[a]IC_{50} values were calculated after 4 days of continuous drug exposure. The methods employed for these assays are similar to those detailed in Experimental. These cytotoxicity assays were performed under contract by Southern Research Institute, Birmingham, AL, USA.

There are two structural modifications that appear to have a pronounced effect on the cytotoxicity of terbenzimidazole derivatives. The presence of lipophilic substituents at either 5- and/or 6-position consistently appears to favor cytotoxicity. While a 5,6-benzo-fused ring does cause a major loss in cytotoxic activity, the presence of functionality at both the 5- and 6-position does not necessarily result in a reduction in cytotoxic activity. The presence of a chloro or 2-trifluoromethyl substituent at the 2″-position does not necessarily alter activity against RPMI 8402 cells. Analogues with a 2″-trifluoromethyl group, however, do tend to exhibit significant cross-resistance to its camptothecin-resistant variant, CPT-K5. In comparative studies in other solid tumor cell lines, however, the 2″-trifluoromethyl derivative of 5,6-dibromoterbenzimidazole (**6c**) proved to be significantly more cytotoxic than the 2″-unsubstituted analogue (**6a**). While strong electron-withdrawing substituents on the 2″-position can significantly alter the pK_a of hydrogen attached to N1″, the influence of this specific modification of the physicochemical properties of terbenzimidazoles on biological activity has not been determined. Further studies are needed to elucidate the impact of such modifications on cell penetration/uptake as well as on the interaction with the components associated with the pharmacological target, the cleaved complex comprised of topo I and DNA.

Experimental

Melting points were determined with a Thomas-Hoover Unimelt capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm (ICN Biomedicals, Eschwegge, Germany) using the solvent systems indicated. Radial chromatography refers to the use of a Model 8924 chromatotron (Harrison Research, CA). 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 spectra were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated 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 the terbenzimidazoles, thereby eliminating tautomeric differences among carbon atoms. Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO. The purity of all compounds for which HRMS data are provided was determined by analytical reverse-phase HPLC. Compounds were analyzed using both of the following conditions: (method A) a Vydac C-18 column (The Separations Group) using methanol:H_2O (87:13) with a flow rate of 1 mL/min; (method B) a Microsorb C-8 column (Rainin Instrument Co., Inc.) using methanol:0.1 M potassium phosphate buffer (pH 7.0) (70:30) with a flow rate of 1 mL/min. HPLC analyses were performed with a Hewlett–Packard 1090 liquid chromatograph equipped with a diode array UV detection monitoring at 254 and 335 nm. The % purity of these compounds was calculated from the peak area assuming that the extinction coefficient of the compound of interest and the impurity are the same. On the basis of these analyses, all the compounds were found to be 98.0–99% pure in these systems. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within ± 0.4% of the theoretical value. The syntheses of **3a–c** and **4a** have been detailed in the literature.[8–11]

5-Bromo-2-[2′-(2″-hydroxybenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (4b). 5-Bromo-2-(3,4-diaminophenyl)benzimidazole (33.3 mg, 0.11 mmol) and 5-formyl-2-hydroxybenzimidazole[11] (17.7 mg, 0.11 mmol) were heated at 145 °C in nitrobenzene (3 mL) overnight. Nitrobenzene was removed with a Kugelrohr and the compound was purified by flash column chromatography. Elution with 5–15% methanol/ethyl acetate gave 23.5 mg (0.05 mmol) of yellow colored compound in 48% yield: mp > 260 °C; IR (KBr) 3409, 3211, 1698, 1558, 1482, 1384, 1279; UV (MeOH) 335, 240 nm (log ε = 4.73, 4.69); ^1H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.30 (d, 1H, J = 8.3), 7.69 (dd, 1H, J = 1.8, 8.7), 7.82 (s, 1H), 7.86–7.99 (m, 2H), 8.09–8.13 (m, 2H), 8.32 (dd, 1H, J = 1.4, 8.7), 8.61 (s, 1H), 11.38 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 108.0, 109.6, 113.9, 115.2, 115.3, 116.3, 117.2, 117.9, 121.1, 122.4, 125.2, 128.7, 130.8, 132.7, 133.2, 134.9, 134.9, 135.6, 150.4, 153.1, 155.7; HRMS (FAB) calculated for $C_{21}H_{14}BrN_6O$ (MH^+) 445.0412, found 445.0408.

5-Bromo-2-[2′-(2″-*n*-propylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (4c). 5-Bromo-2-(3,4-diaminophenyl)benzimidazole (75 mg, 0.25 mmol) and 5-formyl-2-*n*-propylbenzimidazole[11] (46.5 mg, 0.25 mmol) were refluxed in nitrobenzene (2 mL) overnight. Nitrobenzene was removed with a Kugelrohr and the compound purified by flash column chromatography. Elution with 5–15% methanol/ethyl acetate gave 41% (47.5 mg, 0.1 mmol) of pure yellow compound: mp > 260 °C; IR (KBr): 3246, 2965, 2873, 1627, 1548, 1438, 1284; UV (MeOH) 330, 235 nm (log ε = 4.75, 4.73); ^1H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 0.95 (t, 3H), 1.88–1.97 (m, 2H), 3.18 (t, 2H), 7.7 (dd, 1H, J = 1.7, 8.8), 7.83 (d, 1H, J = 8.6), 8.07–8.18 (m, 3H), 8.22–8.26 (m, 1H), 8.42 (d, 1H, J = 8.1), 8.65 (d, 2H, J = 3.5); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 13.4, 20.3, 28.4, 113.7, 115.2, 115.6, 116.1, 116.9, 119.0, 123.7, 124.2, 125.2, 128.9, 131.7, 131.9, 133.6, 134.0, 136.8, 139.2, 150.8, 153.1, 157.3; HRMS (FAB) calculated for $C_{24}H_{20}BrN_6$ (MH^+) 471.0933, found 471.0923.

5-(*p*-Chlorophenyl)-2-[2′-(2″-trifluoromethylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (5). 5-(*p*-Chlorophenyl)-2-(3,4-diaminophenyl)benzimidazole (65 mg, 0.19 mmol) and 5-formyl-2-trifluoromethylbenzimidazole[11] were heated together in nitrobenzene (4 mL) at 150 °C. The nitrobenzene was removed with a Kugelrohr, and the crude mixture was loaded on a column. Elution with 90:10 ethyl acetate:hexanes gave the product (32 mg, 0.06 mmol) in 30% yield: mp > 260 °C; IR (KBr) 3153, 2965, 1546, 1444, 1162, 810; UV (MeOH) 335, 240 nm (log ε = 4.45, 4.40); ^1H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.56–7.64 (m, 2H), 7.73–8.06 (m, 6H), 8.17–8.25 (m, 2H), 8.33 (dd, 1H, J = 1.5, 8.8), 8.67 (d, 1H, J = 1.4); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 114.8, 115.5, 116.0, 117.3, 117.5, 123.3, 123.9, 124.1, 125.1, 125.2, 127.45, 127.49, 129.3, 129.4, 129.4, 132.5, 133.1, 133.5, 136.9, 137.0, 138.2, 138.6, 140.6, 150.6, 150.8, 154.4; HRMS (FAB) calculated for $C_{28}H_{17}ClF_3N_6$ (MH^+) 529.1155, found 529.1156.

5,6-Dibromo-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (6a). 4,5-Dibromo-1,2-phenylenediamine (128 mg, 0.48 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole[8] (126 mg, 0.48 mmol) were heated in nitrobenzene (6 mL) at 145 °C overnight. The nitrobenzene was removed with a Kugelrohr and the mixture loaded on a column for purification. Elution with 1–10% methanol/ethyl acetate gave 0.1 g (41%) of pure compound: mp > 260 °C; IR (KBr) 3405, 3198, 1626, 1544, 1385, 1292; ^1H (DMSO-d_6 + 3 drops CF_3COOH) δ 8.03–8.13 (m, 2H), 8.17 (s, 2H), 8.25 (d,1H, J = 9.2), 8.42 (d, 1H, J = 8.6), 8.59 (s, 1H), 8.74 (s, 1H), 9.75 (s, 1H); ^{13}C (DMSO-d_6 + 3 drops CF_3COOH) δ 114.7, 114.9, 115.8, 116.0, 118.9, 119.6, 120.7, 123.3, 124.5, 125.6, 131.5, 133.5, 134.8, 135.8, 138.0, 151.9, 152.3; HRMS (FAB) calculated for $C_{21}H_{13}Br_2N_6$ (MH^+) 506.9568, found 506.9574.

5,6-Dibromo-2-[2′-(2″-chlorobenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (6b). 5,6-Dibromo-2-(3,4-diaminophenyl)benzimidazole (95 mg, 0.25 mmol) and 5-formyl-2-chlorobenzimidazole[11] (45 mg, 2.5 mmol) were refluxed in nitrobenzene (2 mL) overnight. Nitrobenzene was removed with a Kugelrohr and the compound purified by flash column chromatography. Elution with 1–20% methanol/ethyl acetate gave 51.9% (70 mg,

0.13 mmol) pure yellow compound: mp > 270 °C; IR (KBr) 3259, 2961, 1708, 1629, 1542, 1440, 1289; ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 8.01–8.02 (m, 2H), 8.08–8.09 (m, 2H), 8.14-8.19 (m, 1H), 8.28–8.32 (m, 1H), 8.50 (s, 1H), 8.55 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 112.7, 113.1, 115.2,117.2, 118.5, 119.5, 122.6, 124.0, 129.7, 134.9, 139.1, 152.6, 153.3; HRMS (FAB) calculated for $C_{21}N_6Br_2H_{12}Cl$ (MH^+) 540.9178, found 540.9180.

5,6-Dibromo-2-[2′-(2″-trifluoromethylbenzimidazol-5″-yl) benzimidazol-5′-yl]benzimidazole (6c). 4,5-Dibromo-1,2-phenylenediamine (60 mg, 0.22 mmol) and 73 mg (0.22 mmol) of 5-formyl-2-(2′-trifluoromethylbenzimidazol-5′-yl)benzimidazole[11] were heated in nitrobenzene (10 mL) overnight at 150 °C. The nitrobenzene was removed with a Kugelrohr and the mixture loaded on a column. Elution with 90:10 ethyl acetate:*n*-hexanes yielded 25 mg (20% yield, 0.05 mmol) of compound: mp > 260 °C; IR (KBr) 3047, 2931, 1720, 1544, 1441, 1290, 1155, 981; UV (MeOH) 335, 265 nm (log ε = 4.45, 4.48); ^{1}H NMR (DMSO + 3 drops CF_3 COOH) δ 8.02–8.09 (m, 2H), 8.14 (s, 2H), 8.56–8.29 (m, 2H), 8.57 (s, 1H), 8.69 (s, 1H); ^{13}C NMR (DMSO + 3 drops CF_3 COOH) δ 113.6, 115.4, 118.2, 119.4, 120.8, 124.0, 124.1, 124.4, 135.0, 136.6, 137.8, 152.6, 152.9; HRMS (FAB) calcd. for $C_{22}H_{12}BrF_3N_6$ (MH^+) 574.9441, found 574.9431.

5,6-Diphenyl-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl] benzimidazole (7). 4,5-Diphenyl-1,2-phenylenediamine (151 mg, 0.58 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole[8] (152 mg, 0.58 mmol) were heated in nitrobenzene (4 mL) overnight at 145 °C under nitrogen. The nitrobenzene was removed with a Kugelrohr. Compound was purified by flash column chromatography. Elution with 2–10% methanol/ethyl acetate gave 108 mg (0.22 mmol) of pure yellow compound in 37% yield: mp > 260 °C; IR (KBr) 3399, 3059, 1629, 1551, 1441, 1292; UV (MeOH) 345, 245 (log ε 4.50, 4.43); ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.17–7.33 (m, 10H), 7.84 (s, 2H), 8.06–8.25 (m, 3H), 8.49 (dd, 1H, J = 1.2, 8.9), 8.66 (s, 1H), 8.75 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 113.9, 115.2, 115.8, 116.2, 116.3, 117.9, 123.3, 126.3, 126.7, 127.9, 128.4, 128.4, 128.5, 130.1, 131.8, 131.9, 133.0, 138.9, 139.0, 140.6, 141.2, 153.7, 161.0; HRMS (FAB) calculated for $C_{33}H_{23}N_6$ (MH^+) 503.1984, found 503.1989.

5-Bromo-6-methoxy-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (8). 4-Bromo-5-methoxy-1,2-phenylenediamine (123 mg, 0.57 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole[8] (150 mg, 0.57 mmol) in nitrobenzene (4 mL) were heated at 145 °C overnight under nitrogen. Nitrobenzene was removed with a Kugelrohr. Chromatographic separation with 1–10% methanol/ethyl acetate afforded 104 mg (40%) of pure compound: mp > 260 °C; IR (KBr) 3456, 1631, 1385, 1195, 1159, 810; UV (MeOH) 335, 225 (log ε = 4.5, 4.63); ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 3.91 (s, 3H), 7.17–7.29 (m, 1H), 7.78 (d, 1H, J = 8.8), 8.00–8.13 (m, 3H), 8.49–8.53 (m, 1H), 8.66 (s, 1H), 8.79 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 56.2, 96.4, 114.1, 115.0, 115.6, 115.8, 115.9, 116.4, 118.0, 118.4, 123.1, 125.3, 125.6, 126.3, 127.4, 131.9, 133.1, 133.1, 138.4, 140.8, 149.0, 153.4; HRMS (FAB) calculated for $C_{22}H_{16}BrN_6O$ (MH^+) 459.0569, found 459.0573.

5-Phenyl-6-methoxy-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (9). 4-Methoxy-5-phenyl-1,2-phenylenediamine (139 mg, 0.65 mmol) and 5-formyl-2-(benzimidazol-5′-yl)benzimidazole[8] (170 mg, 0.65 mmol) were heated together in nitrobenzene (5 mL) overnight at 145 °C. Nitrobenzene was removed with a Kugelrohr and the compound was loaded onto a column. (1–10%) Methanol/ethyl acetate gave 120 mg (41%) of pure product: mp > 260 °C; IR (KBr) 3298, 3050, 2987, 1630, 1541, 1438, 1283; UV (MeOH) 335, 265 (log ε = 4.4, 4.36); ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 3.92 (s, 3H), 7.39–7.57 (m, 6H), 7.72 (s, 1H), 8.11–8.15 (m, 1H), 8.19–8.27 (m, 2H), 8.47 (dd, 1H, J = 1.46, 8.07), 8.64 (s, 1H), 8.76 (s, 1H), 9.75 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 56.4, 95.7, 114.6, 115.2, 116.0, 116.2, 119.0, 119.1, 125.6, 126.3, 127.6, 127.8, 128.3, 129.8, 130.7, 131.5, 132.4, 133.4, 137.6, 148.8, 152.8, 156.8; HRMS (FAB) calculated for $C_{28}H_{20}N_6O$ (MH^+) 457.1777, found 457.1770.

4-Bromo-1,2-phenylenediamine (10a). 4-Bromo-2-nitroaniline (600 mg, 2.76 mmol) was dissolved in 25 mL absolute ethanol and 2.72 g (14 mmol) $SnCl_2$ was added. The mixture was refluxed overnight. Ethanol was removed in vacuo and the mixture basified with 2N NaOH to pH 11. Ether extraction, drying the ether layer over anhydrous Na_2SO_4 and concentration in vacuo afforded 486 mg (2.6 mmol, 94% yield) of the crude diamine which was used for the next step without characterization.

4-(*p*-Chlorophenyl)-1,2-phenylenediamine (10b). 4-(*p*-Chlorophenyl)-2-nitroaniline (0.79 g, 3.2 mmol) in a mixture of methanol/ethyl acetate in equal parts (40 mL) was reduced using 115 mg of 10% Pd/C. Hydrogenation was carried out at 40 psi pressure for 10 h. The mixture was filtered through Celite and the bed washed with methanol. The methanol layer was concentrated in vacuo to yield 0.71 g (3.2 mmol) of the crude diamine. The yield was quantitative. The crude diamine was used as such without purification: ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.09 (d, 1H, J = 8.3), 7.24 (dd, 1H, J = 8.3, 2.12), 7.32 (d, 1H, J = 2.1), 7.47–7.60 (m, 4H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 118.9, 120.9, 121.9, 126.3, 127.4, 127.9, 129.2, 132.1, 133.3, 138.5, 139.7.

4-(*p*-Chlorophenyl)-2-nitroaniline. 4-Bromo-2-nitroaniline (1 g, 4.6 mmol) was dissolved in DME (13 mL). Tetrakis(triphenylphosphine)palladium (266 mg, 0.23 mmol), *p*-chlorophenylboronic acid (1.14 g, 6.9 mmol), and 2 M Na_2CO_3 (5 mL) were added to the reaction mixture and refluxed at 90 °C overnight. The reaction mixture was concentrated in vacuo and loaded onto a column. Flash column chromatography using 1–3% ethyl acetate/*n*-hexanes afforded 0.79 g (0.32 mmol) of compound in 69% yield: mp 172–173 °C; IR (KBr) 3479, 3358, 1639,

1487, 1414, 1344, 1240, 1093, 822; ^{1}H NMR ($CDCl_3$) δ 6.14 (brs, 2H), 6.90 (d, 1H, $J=8.7$), 7.37–7.51 (m, 4H), 7.61 (dd, 1H, $J=2.2$, 8.7), 8.35 (d, 1H, $J=2.1$); ^{13}C NMR ($CDCl_3$) 119.9, 124.4, 128.0, 128.1, 129.6, 132.9, 133.9, 134.7, 134.7, 134.8, 137.7, 144.4; HRMS (EI) calculated for $C_{12}H_9ClN_2O_2$ m/z 248.0352, found 248.0353.

4,5-Dibromo-1,2-phenylenediamine (10c). 3,4-Dibromo-6-nitroaniline (189 mg, 0.64 mmol) was dissolved in anhydrous ethanol (8 mL) to which was added Raney Nickel catalyst (400 mg).[12] The hydrogenation apparatus was at a hydrogen pressure of 50 psi. After 45 min the deep yellow color originally present was completely discharged, indicating complete reduction of the nitro into the amino groups. The reaction mixture was filtered through Celite and the bed was washed with methanol. The methanol was concentrated in vacuo to give 135 mg of the crude diamine in 82% yield. The crude diamine was used as such without purification: ^{1}H NMR ($CDCl_3$) δ 3.37 (br, 4H), 6.93 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 114.0, 121.0, 135.8.

5-Bromo-2-(3,4-dinitrophenyl)benzimidazole (12a). 4-Bromo-1,2-phenylenediamine (275 mg, 1.5 mmol) and 3,4-dinitrobenzaldehyde (300 mg, 1.5 mmol) in 2 mL nitrobenzene were heated at 145 °C overnight. The nitrobenzene was removed with a Kugelrohr. Column purification (1–10% ethyl acetate/hexanes) afforded 209 mg (0.57 mmol, 39% yield) of the pure product: mp 79–80 °C; IR (KBr) 3133, 1613, 1544, 1436, 1359, 1086, 832; ^{1}H NMR (DMSO + 3 drops CF_3COOH) δ 7.48 (dd, 1H, $J=1.8$, 8.8), 7.70 (d, 1H, $J=8.8$), 7.96 (d, 1H, $J=1.46$), 8.45 (d, 1H, $J=8.5$), 8.68 (dd, 1H, $J=1.8$, 8.5), 8.93 (d, 1H, $J=1.8$); ^{13}C NMR (DMSO + 3 drops CF_3COOH) δ 116.4, 117.5, 118.7, 123.7, 127.1, 132.1, 134.5, 137.6, 140.2, 142.4, 142.8, 148.5; HRMS (EI) calculated for $C_{13}H_7BrN_4O_4$ m/z 361.9650, found m/z 361.9648.

2-(3,4-Dinitrophenyl)-5-(*p*-chlorophenyl)benzimidazole (12b). A stirred solution of 4-(*p*-chlorophenyl)-1,2-diaminobenzene (0.7 g, 3.2 mmol) and 3,4-dinitrobenzaldehyde (0.63 g, 3.2 mmol) in nitrobenzene (13 mL) was heated at 150 °C under N_2 overnight. Nitrobenzene was removed with a Kugelrohr and the crude mixture was purified by flash column chromatography. Elution with 10% ethyl acetate/hexanes provided 0.55 g (1.39 mmol) of a bright yellow–red compound in 44% yield: mp 75–76 °C; IR (KBr) 3392, 2927, 1542, 1359, 1093, 845, 809; ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.55 (d, 2H, $J=8.3$), 7.65 (d, 1H, $J=8.4$), 7.77–7.84 (m, 3H), 7.96 (s, 1H), 8.47 (d, 1H, $J=8.8$), 8.68–8.73 (m, 1H), 8.95 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 113.7, 116.6, 123.4, 123.4, 123.5, 127.2, 127.3, 129.0, 129.2, 131.8, 132.4, 135.1, 135.2, 139.0, 139.6, 139.7, 142.1, 142.9, 148.3; HRMS (EI) calculated for $C_{19}H_{11}ClN_4O_4$ m/z 394.0463, found 394.0463.

5,6-Dibromo-2-(3,4-dinitropheny)benzimidazole (12c). 4,5-Dibromo-1,2-phenylenediamine (250 mg, 0.94 mmol) and 3,4-dinitrobenzaldehyde (188 mg, 0.94 mmol) in 3 mL nitrobenzene were heated at 145 °C overnight. The nitrobenzene was removed with a Kugelrohr. Column purification (5–20% ethyl acetate/hexanes) afforded 230 mg (0.51 mmol, 54.7% yield) of the pure product: mp 95–97 °C; IR (KBr) 3313, 3054, 1604, 1551, 1437, 1362, 1238; ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 7.48 (dd, 1H, $J=2.0$, 8.6), 7.71 (d, 1H, $J=8.6$), 7.97 (d, 1H, $J=1.6$), 8.25 (s, 1H), 8.43 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 116.7, 117.9, 118.3, 123.6, 126.8, 131.1, 134.5, 137.9, 140.2, 142.4, 142.9, 148.5.

5-Bromo-2-(3,4-diaminophenyl)benzimidazole (13a). 5-Bromo-2-(3,4-dinitrophenyl)-benzimidazole (140 mg, 0.38 mmol) was dissolved in absolute ethanol (8 mL). $SnCl_2$ (0.8 g, 4.2 mmol) was added and the mixture refluxed overnight. Ethanol was removed in vacuo and the mixture basified with 2N NaOH to pH 11. Repeated extraction with ether, drying the ether layer over anhydrous Na_2SO_4 and concentration in vacuo yielded 0.11 g (0.37 mmol) of the crude diamine in 98% yield. The diamine was used without further purification: ^{1}H NMR (DMSO) δ 6.63 (d, 1H, $J=8.1$), 7.26 (m, 2H), 7.39 (m, 2H), 6.65 (d, 1H, $J=1.8$).

2-(3,4-Diaminophenyl)-5-(*p*-chlorophenyl)benzimidazole (13b). 2-(3,4-Dinitrophenyl)-5-(*p*-chlorophenyl)benzimidazole (125 mg, 0.32 mmol) was dissolved in ethyl acetate (25 mL) and reduced by hydrogenation using 10% Pd/C (25 mg) as the catalyst overnight. The catalyst was removed by Celite filtration. The ethyl acetate was concentrated in vacuo and the residue was dried to yield 0.11 g (0.33 mmol) of crude solid. The yield was quantitative. This crude solid was further used without purification: ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 6.92 (d, 1H, $J=8.42$), 7.57–7.62 (m, 4H), 7.65–7.81 (m, 4H), 7.92 (s, 1H); ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 110.2, 111.3, 114.1, 115.3, 117.7, 123.8, 124.7, 129.18, 129.22, 129.33, 129.34, 131.9, 132.9, 133.0, 136.7, 138.7, 143.7, 150.8.

5,6-Dibromo-2-(3,4-diaminophenyl)benzimidazole (13c). 5,6-Dibromo-2-(3,4-dinitrophenyl)benzimidazole (0.2 g, 0.45 mmol) was dissolved in 20 mL anhydrous ethanol to which was added about 0.4 g Raney Nickel catalyst. Hydrogenation was carried out at a hydrogen pressure of 50 psi. After 2 h, the deep yellow color originally present was completely discharged, indicating complete reduction of the nitro into the amine group. The reaction mixture was filtered through Celite and the bed was washed with ethanol. The ethanol layer was concentrated in vacuo to give 155 mg (0.41 mmol) of the crude diamine in 91.5% yield. The crude diamine was used as such without purification: ^{1}H NMR (DMSO-d_6 + 3 drops CF_3COOH) δ 3.7 (brs, 4H), 7.45 (dd, 1H, $J=8.4$), 7.75 (d, 1H, $J=8.4$), 7.95 (d, 1H, $J=1.6$), 8.18 (s, 1H), 8.51 (s, 1H).

5-Formyl-2-propylbenzimidazole (15). 5-Cyano-2-propylbenzimidazole (0.33 g, 1.83 mmol) was dissolved in HCOOH (26 mL). Water (2 mL) and Ni/Al catalyst (1.8 g) were added and the mixture was refluxed at 110 °C for 6 h. The hot reaction mixture was filtered through a bed of Celite and the filtrate concentrated in vacuo. pH was

adjusted to 9 with 2N NaOH and the mixture extracted with ethyl acetate. Purification was done with flash column chromatography. Elution with 30:70 ethyl acetate: *n*-hexanes gave 0.32 g (1.7 mmol, 95% yield) of pure product: mp 79–80 °C; IR (KBr) 2935, 2850, 1690, 1420, 1283; ^{1}H NMR (DMSO + 3 drops CF_3 COOH) δ 0.99 (t, 3H), 1.91 (sextet, 2H), 3.13 (t, 2H), 8.03 (m, 2H), 8.36 (s, 1H), 10.17 (s, 1H); ^{13}C NMR (DMSO + 3 drops CF_3 COOH) δ 13.5, 20.2, 28.4, 114.9, 116.7, 125.9, 131.5, 133.8, 135.1, 157.6, 192.5; HRMS (EI) calculated for $C_{11}H_{12}N_2O$ *m*/*z* 189.1031, found 189.1029.

5-Cyano-2-propylbenzimidazole. 3,4-Diaminobenzonitrile (0.5 g, 3.8 mmol) and butyraldehyde (0.4 mL) were heated in 5 mL nitrobenzene overnight in a sealed tube. Nitrobenzene was removed with a Kugelrohr and the mixture loaded on a column for chromatographic purification. Elution with 30:70 ethyl acetate:*n*-hexanes provided 0.34 g (1.8 mmol, 49% yield) of the pure compound: mp 160–161 °C; IR (KBr) 2965, 2874, 2223, 1625, 1543, 1412, 1295, 825; ^{1}H NMR (DMSO + 3 drops CF_3 COOH) δ 0.98 (t, 3H), 1.86 (sextet, 2H), 3.12 (t, 2H), 7.93–7.96 (m, 2H), 8.42 (s, 1H); ^{13}C NMR (DMSO + 3 drops CF_3 COOH) δ 13.3, 20.1, 28.3, 106.7, 115.5, 118.6, 119.3, 129.0, 131.1, 134.1; HRMS (EI) calculated for $C_{11}H_{11}N_3$ *m*/*z* 187.1109, found 187.1104.

4,5-Diphenyl-1,2-phenylenediamine (18). 4,5-Diphenyl-2-nitroaniline (200 mg, 0.69 mmol) in ethanol (50 mL) was reduced using 40 mg 10% Pd/C. Hydrogenation was carried out at 40 psi pressure for 10 h. The mixture was filtered through Celite and the bed washed with methanol. The methanol layer was concentrated in vacuo to yield 151 mg of the crude diamine in 84% yield. The crude diamine was used as such without purification: ^{1}H NMR (CD_3OD) δ 6.78 (s, 2H), 7.01–7.16 (m, 10H); ^{13}C (CD_3OD) δ 118.1, 121.2, 127.2, 127.7, 130.4, 131.8, 138.3.

4,5-Diphenyl-2-nitroaniline. 3,4-Dibromo-6-nitroaniline (332 mg, 1.12 mmol) was dissolved in DME (20 mL). Tetrakis (triphenylphosphine) palladium (65 mg, 0.06 mmol), phenyl boronic acid (200 mg, 1.64 mmol), and 2 M Na_2CO_3 (10 mL) were added to the reaction mixture and refluxed at 90 °C overnight. The reaction mixture was concentrated in vacuo and loaded onto a column. 1–3% Ethyl acetate/*n*-hexanes afforded 259 mg of pure yellow compound in 80% yield: mp 139–141 °C; IR (KBr) 3476, 3363, 2924, 1621, 1476, 1263, 1089; ^{1}H NMR ($CDCl_3$) δ 6.11 (brs, 2H), 6.86 (s, 1H), 7.04–7.09 (m, 5H), 7.15–7.26 (m, 5H), 8.21 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 120.6, 127.2, 127.7, 128.2, 128.3, 128.5, 128.5, 129.3, 129.4, 129.5, 129.8, 130.1, 130.9, 131.8, 139.9, 139.9, 144.1, 149.0; HRMS (EI) calculated for $C_{18}H_{14}N_2O_2$ *m*/*z* 290.1059, found 290.1055.

4-Bromo-5-methoxy-1,2-phenylenediamine (19). 3-Bromo-4-methoxy-6-nitroaniline (150 mg, 0.61 mmol) was dissolved in ethanol (10 mL) and hydrogenation was carried out using 350 mg Raney Nickel for 30 min. The reaction mixture was filtered through Celite bed and washed with methanol. The methanolic layer was dried in vacuo to give 127 mg of the crude diamine in 97% yield, which was used as such without purification: ^{1}H NMR ($CDCl_3$) δ 3.20 (br, 2H), 3.39 (br, 2H), 3.8 (s, 3H), 8.36 (s, 1H), 6.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.4, 100.4, 102.3, 122.5, 128.7, 136.7, 150.9.

4-Methoxy-5-phenyl-1,2 phenylenediamine (20). 4-Methoxy-2-nitro-5-phenylaniline (165 mg, 0.68 mmol) in ethyl acetate (20 mL) was reduced in the presence of H_2 at atmospheric pressure using 20 mg of 10% Pd/C as the catalyst, overnight. The mixture was filtered through Celite bed and the bed was washed with methanol. All the washings were concentrated in vacuo to yield 139 mg (96% yield) of the crude diamine, which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 3.49 (brs, 4H), 3.71 (s, 3H), 6.42 (s, 1H), 6.74 (s, 1H), 7.26–7.52 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 56.8, 101.8, 120.9, 127.5, 128.4, 128.5, 128.5, 129.8, 130.0, 136.6, 139.2, 142.4, 153.7.

4-Methoxy-2-nitro-5-phenylaniline. 3-Bromo-4-methoxy-6-nitroaniline (400 mg, 1.62 mmol) was dissolved in DME (20 mL). Tetrakispalladium triphenyl phosphine (94 mg, 0.08 mmol) served as the catalyst. To this mixture were added phenyl boronic acid (300 mg, 1.01 mmol) and 2 M Na_2CO_3 (1.8 mL) and the mixture refluxed at 90 °C overnight. The reaction mixture was concentrated in vacuo and purified by flash column chromatography. 1–10% Ethyl acetate/*n*-hexanes gave 280 mg (1.14 mmol) of pure product in 71% yield: mp 105–106 °C; IR (KBr) 3435, 3328, 2933, 1570, 1480, 1227, 1029, 692; ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H), 5.94 (br, 2H), 6.79 (s, 1H), 7.39–7.64 (m, 5H), 7.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.6, 106.8, 121.3, 128.7, 128.7, 128.8, 128.8, 128.9, 129.7, 136.7, 140.2, 141.4, 148.7; HRMS (EI) calculated for $C_{13}H_{12}N_2O_3$ *m*/*z* 244.0848, found *m*/*z* 244.0847.

Topoisomerase I-mediated DNA cleavage assays. Human topo I was expressed in *Escherichia coli* and isolated as a recombinant protein using a T7 expression system as previously described.[20] DNA topoisomerase I was purified from calf thymus gland as reported previously.[21] Plasmid YepG was purified using the QIAGEN Plasmid MAXI kit. DNA end labeling was performed by digesting YEpG with B*am*H I, followed by end-filling using Klenow polymerase. The topo I-mediated cleavage assay was performed by incubating topo I with labeled DNA in the presence of different concentrations of drugs or solvent. Reaction was carried out at 23 °C for 15 min and subsequently stopped by addition of SDS–proteinase K, followed by 37 °C incubation for 1 h. Samples were added with loading dye containing 200 mM NaOH to denature the double stranded DNA. DNA fragments were separated on an agarose gel followed by autoradiography. Topo I-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, i.e. concentrations relative to **3a**, whose value is arbitrarily assumed as 1.0. Compound **3a** has approximately 50% of the potency of Hoechst 33342 as a topo I poison. Cleavage was calculated on the intensity of the strongest specific band. The DNA cleavage pattern for camptothecin is distinctly different from terbenzimidazoles, making comparisons of relative potency difficult. An estimation of the relative potency of camptothecin was made by comparing the doses of

compound **3a** and camptothecin that induced fragmentation of approximately 10% of the labeled DNA.

MTT-microtiter plate tetrazolinium cytotoxicity assay. The cytotoxicity was determined by the MTT assay.[22–24] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5, were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan). Cells were maintained in suspension at 37 °C with 5% CO_2 in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). The cytotoxicity assay was performed using 96-well microtiter plates with 2000 cells/well, in 200 μL of growth medium. Cells were exposed continuously for 4 days to different drug concentrations, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in 4 replicate wells.

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