Terbenzimidazoles: Influence of 2″-, 4-, and 5-Substituents on Cytotoxicity and Relative Potency as Topoisomerase I Poisons

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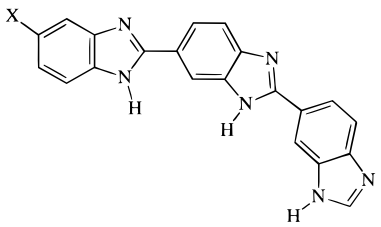
Terbenzimidazoles poison the nuclear enzyme topoisomerase I and possess significant cytotoxic activity against several human tumor cell lines. The relative pharmacological activity of 4,5- and 5,6-benzoterbenzimidazoles was compared to that of 5-phenylterbenzimidazole (**3**). 5,6-Benzoterbenzimidazole is inactive as a topoisomerase I poison and did not exhibit significant cytotoxic activity. In contrast, 4,5-benzoterbenzimidazole retained activity as a topoisomerase I poison but exhibited weak cytotoxic activity relative to **3**. While 5-(1-naphthyl)terbenzimidazole is less potent than **3** as a topoisomerase I poison and cytotoxic agent, 5-(2-naphthyl)-terbenzimidazole has comparable activity to **3**. The presence of a *p*-methoxy or *p*-chloro substituent on the phenyl moiety did not dramatically alter the pharmacological activity of **3**. Several analogs of **3** were synthesized wherein the 2″-substituent varied from methyl, ethyl, propyl, isopropyl, phenyl to *p*-methoxyphenyl. Evaluation of the intrinsic activity of these analogs as topoisomerase I poisons indicates that topoisomerase I poisoning was not diminished by the presence of a methyl, ethyl, propyl, and isopropyl substituent at the 2″-position. Among the various 2″-substituted analogs evaluated, only in the case of 2″-(*p*-methoxyphenyl)-5-phenylterbenzimidazole was a significant decrease in cytotoxicity observed.

Introduction

DNA topoisomerases are nuclear enzymes that are involved in generating the necessary topological and conformational changes in DNA critical to many cellular processes such as replication and transcription.[1−3] Poisoning of mammalian topoisomerase has been recognized as an effective approach for the development of cancer chemotherapeutics.[4−7] Recently, several bibenzimidazoles and terbenzimidazoles have been identified as topoisomerase I poisons.[8−13] Among the terbenzimidazoles evaluated, **1** and **2** (Chart 1) were active as topoisomerase poisons but did not exhibit significant cytotoxicity. The basis for this lack of cytotoxicity was ascribed to their poor penetration into cells. In contrast, the presence of a 5-phenyl or 5-pyridyl substituent on these terbenzimidazoles, as in **3** and **4**, resulted in retention of activity as topoisomerase I poisons, as well as significant cytotoxicity against several tumor cell lines.[12]

The cytotoxic activity observed for **3** prompted further studies on the effect of various aryl substituents at the 4- and 5-positions of these terbenzimidazoles on their pharmacological activity. In the present study, the relative activity of 4,5- and 5,6-benzo-fused terbenzimidazoles and 4-phenyl- and 5-naphthylbenzimidazoles as topoisomerase I poisons and cytotoxic agents was compared to the 5-phenylterbenzimidazole, **3**. Addition of a 2″-(*p*-methoxyphenyl) substituent to **2** resulted in a loss of topoisomerase I poisoning activity.[12] The influence of steric factors associated with various substituents at the 2″-position of **3** was also assessed with regard to their influence on intrinsic activity as topoisomerase I poisons and on cytotoxicity.

Chart 1



1, X = H
2, X = CN

3,

4,

Chemistry

The method used for the preparation of 4,5-benzoterbenzimidazole (**9**) and 5,6-benzoterbenzimidazole (**10**) is outlined in Scheme 1. Condensation of either 1,2-diaminonaphthalene (**6**) (prepared from 1-nitro-2-(acetylamino)naphthalene[14]) or 2,3-diaminonaphthalene (**7**) with 5-formyl-2-(benzimidazol-5′-yl)benzimidazole[12] (**5**) at 145 °C in nitrobenzene provided **9** and **10** in yields of 67% and 53%, respectively. The preparation of the 4-phenylterbenzimidazole, **11**, was accomplished under similar reaction conditions by reaction of 3-phenyl-1,2-phenylenediamine[15] (**8**) with **5** as outlined in Scheme 1.

The 5-naphthylterbenzimidazoles **17** and **18**, as well as 5-(*p*-methoxyphenyl)- and 5-(*p*-chlorophenyl)terbenzimidazoles **19** and **20**, were similarly prepared by condensation of the appropriate 1,2-phenylenediamine with **5**. The variously substituted 1,2-phenylenediamine intermediates required for the formation of these terbenzimidazoles were prepared from their respective

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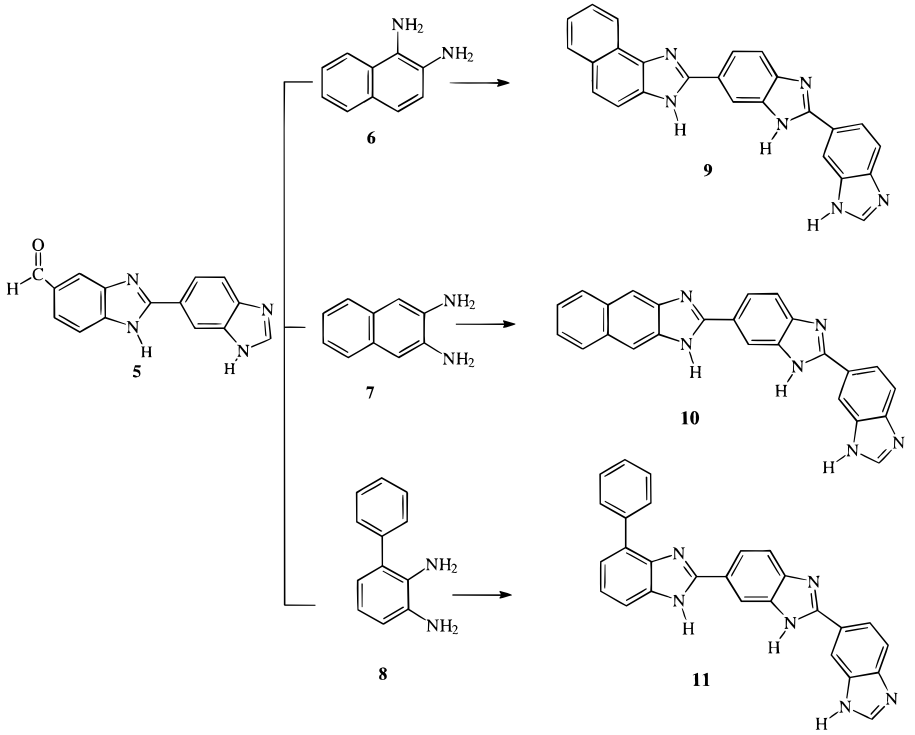
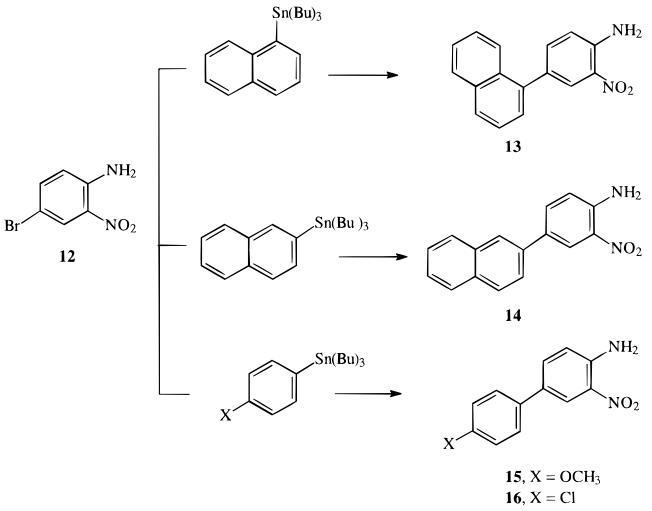
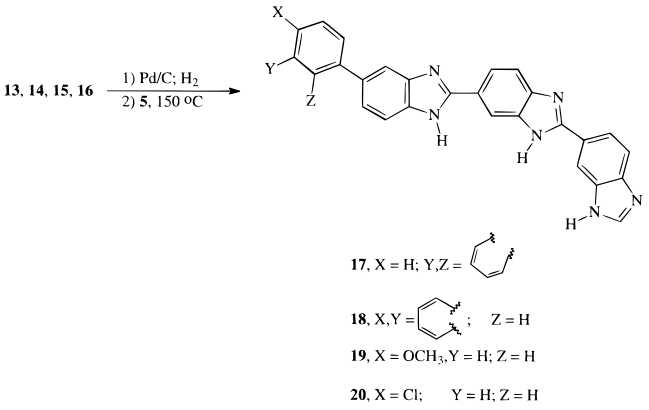
Scheme 1



Scheme 2



Scheme 3



2-nitroaniline precursors, which were synthesized as outlined in Scheme 2. Reaction of the tributyltin derivatives formed from 1-bromonaphthalene, 2-bromonaphthalene, 4-bromoanisole, or 4-chlorobromobenzene with **12** provided the 2-nitroaniline derivatives **13**−**16**. Compounds **13**−**16** were hydrogenated in the presence of Pd/C to generate their 1,2-phenylenediamine derivatives which were used without further purification.

Condensation of the various 1,2-phenylenediamines generated from **13**−**16** with **5** as outlined in Scheme 3 provided the 5-naphthyl- and phenyl-substituted terbenzimidazoles **17**−**20**.

The synthetic approach used for the preparation of 2″-substituted derivatives of 5-phenylterbenzimidazole is outlined in Scheme 4. Reaction of 4-phenyl-1,2-phenylenediamine[12] (**21**) with 3,4-dinitrobenzaldehyde[16] (**22**) resulted in the formation of 2-(3,4-dinitrophenyl)-5-phenylbenzimidazole (**23**) in 66% yield. Catalytic hydrogenation of **23** followed by reaction of the resulting crude diamine with **22** provided 5-phenyl-2-[2′-(3,4-dinitrophenyl)benzimidazol-5′-yl]benzimidazole (**24**) in 94% yield. Compound **24** was used as the general intermediate for the preparation of a series of 2″-substituted 5-phenylterbenzimidazoles. The crude diamine formed from the catalytic hydrogenation of **24** was reacted with acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, benzaldehyde, and *p*-methoxybenzaldehyde in yields which ranged from 28% to 89%.

Results and Discussion

The cytotoxicity of the naphthylterbenzimidazoles **17** and **18**, 5-(*p*-methoxyphenyl)terbenzimidazole (**19**), and 5-(*p*-chlorophenyl)terbenzimidazole (**20**) against the human lymphoblastoma cell line, RPMI 8402, and their relative activity as topoisomerase I poisons are listed in Table 1. While the 5-(2-naphthyl)terbenzimidazole, **18**, had similar activity to 5-phenylterbenzimidazole, **3**, 5-(1-naphthyl)terbenzimidazole, **17**, was less cytotoxic. While **19** was slightly more potent as a topoisomerase I poison than either **3** or **20** (Table 1), all of these 5-phenylterbenzimidazoles had similar cytotoxic activ-

Scheme 4

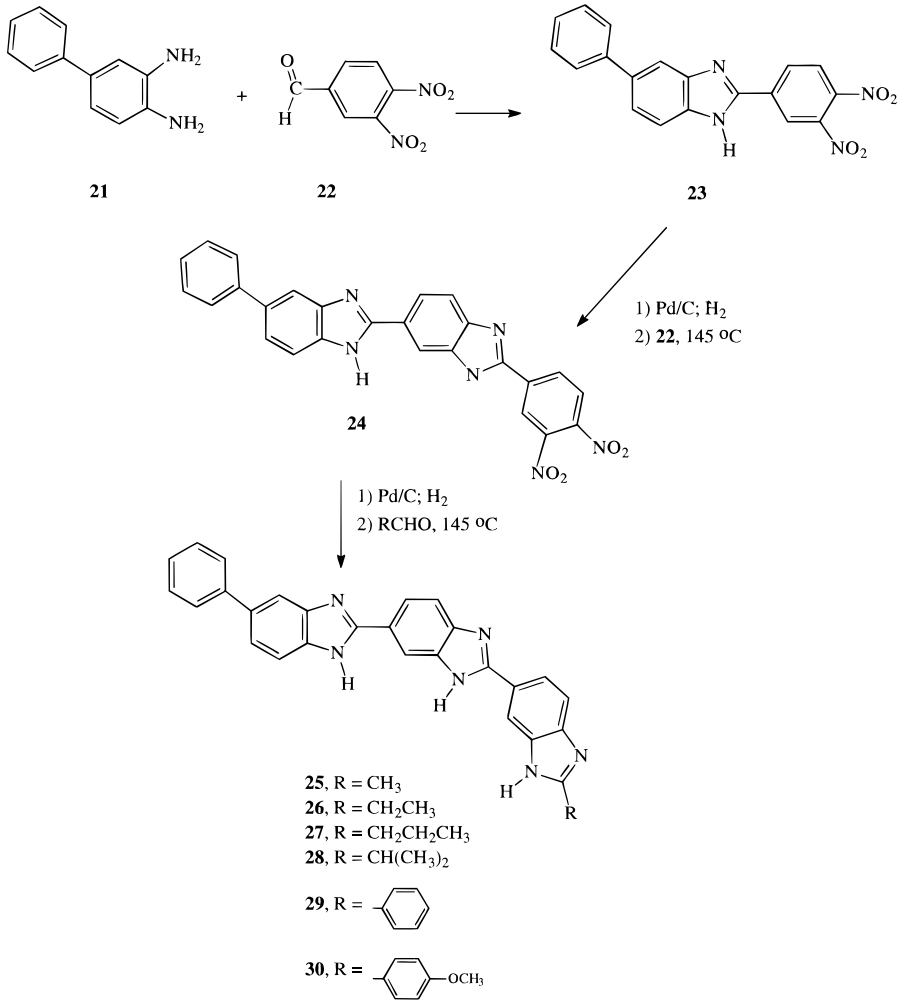


Table 1. Topoisomerase I-Mediated DNA Cleavage and Cytotoxicity of 4,5- and 5,6-Benzoterbenzimidazoles, 4- and 5-Phenylterbenzimidazoles, and 2″-Substituted 5-Phenylterbenzimidazoles

compd	Topo I-mediated DNA cleavage[b]	cytotoxicity[a] IC_{50} (μM)
3	1	0.09
9	1[c]	25
10	>1000	>100[d]
11	5[c]	7.0
17	2	2.1
18	1	0.08
19	0.5	0.09
20	2	0.09
25	2	0.11
26	1	0.11
27	0.5	0.06
28	0.5	0.06
29	20	0.06
30	20	0.38

[a] IC_{50} was calculated after 4 days of continuous exposure of RPMI 8402 cells to drug. [b] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to **3**, whose value is arbitrarily assumed as 1, that produce the same extent of cleavage on the plasmid DNA in the presence of recombinant human topoisomerase I. Cleavage is calculated from the intensity of the strongest specific band. [c] The altered DNA fragmentation pattern associated with these substituted terbenzimidazoles limits the accuracy of comparative data on relative RECs as compared to **3**. [d] No indication of cytotoxicity was considered indicative of IC_{50} values substantially greater than the highest doses assayed.

ity. These data indicate that *para*-substituents on the 5-phenyl ring retain activity. The pharmacological activity observed for **18**, in which there is a 2-naphthyl substituent at the 5-position of these terbenzimidazoles, suggests that *meta*-substituents on a 5-phenyl moiety could also be well tolerated. The loss of antitumor activity in the case of the 1-naphthyl analog **17** suggests that 5-(*ortho*-substituted phenyl)terbenzimidazoles may exhibit diminished activity.

The relative activity of 4-phenylterbenzimidazole, **11**, and the 4,5-benzo-fused and 5,6-benzo-fused terbenzimidazoles **9** and **10** as topoisomerase I poisons is also listed in Table 1. In contrast to **3** and **17**, **10** was not a topoisomerase I poison (Figures 1 and 2) and did not possess cytotoxic activity. Its lack of activity relative to **3** as a topoisomerase I poison provides a useful probe for further comparison and examination of the biophysical interactions associated with DNA and topoisomerase which are critically linked to the topoisomerase I poisoning activity of terbenzimidazoles. Studies have demonstrated that the principal interaction of **3** with DNA occurs by binding to its minor groove.[17] Recently, the X-ray structure of the Hoechst 33258-related trisbenzimidazole−oligonucleotide complex has been identified by Clark et al.[18] Comparative studies are in progress to assess the biophysical basis for the differences in pharmacological activity between **3** and this benzo-fused terbenzimidazole. An altered DNA fragmentation pattern was obtained for **9** and **11** when assayed as topoisomerase I poisons (Figures 1 and 2).

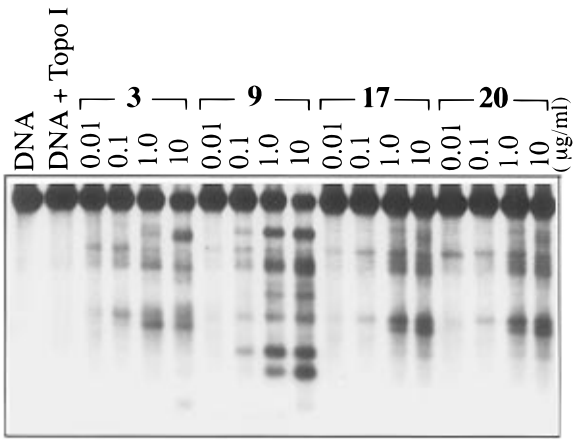


Figure 1. Stimulation of enzyme-mediated DNA cleavage by 4- or 5-substituted terbenzimidazoles using recombinant human topoisomerase I. Enzyme-mediated DNA cleavage using human topoisomerase I was performed as described in the Experimental Section. The left-most lane is the DNA control without topoisomerase I. The second lane from the left is the DNA control with topoisomerase I. The rest of the lanes are DNA with topoisomerase I and serially (10-fold each) diluted compounds from 0.01 to 10 μg/mL for **3**, **9**, **17**, and **20**.

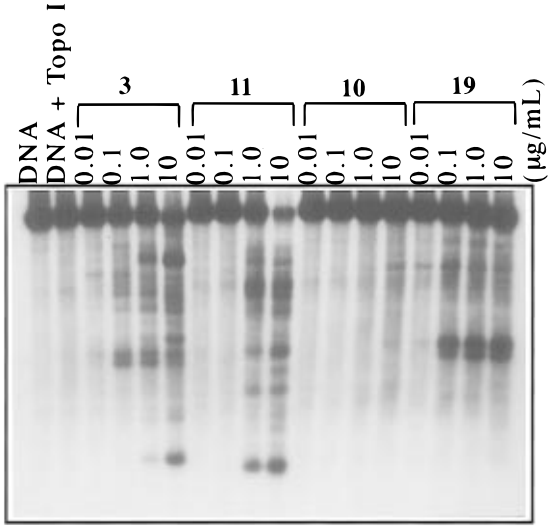


Figure 2. Stimulation of enzyme-mediated DNA cleavage by 4- or 5-substituted terbenzimidazoles using recombinant human topoisomerase I. The assays were performed as described for Figure 1. The left-most lane is the DNA control without topoisomerase I. The relative doses employed ranged from 0.01 to 10 μg/mL for **3**, **11**, **10**, and **19**.

This altered DNA fragmentation pattern hampers efforts to provide an accurate assessment of their potency relative to **3**. Both **9** and **11** clearly exhibited less cytotoxic activity. These data indicate that the presence of substituents at the 4-position appear to be associated with reduced cytotoxic activity.

Table 1 lists the relative activity of the 2″-substituted 5-phenylterbenzimidazoles **25**−**30**. Previous studies on the relative activity of terbenzimidazoles suggested that the presence of a 2″-(*p*-methoxyphenyl) substituent, as in the case of 5-cyanoterbenzimidazole,[12] would result in a loss of topoisomerase I poisoning activity. The results in Table 1 indicate that alkyl analogs **25**−**28** of 5-phenylterbenzimidazole, which range from methyl, ethyl, propyl, to isopropyl, retain activity as topoisomerase I poisons and possess similar cytotoxic activity. Only modest differences in their activity as either topoisomerase I poisons or cytotoxic agents were observed relative to **3** (Figure 3). Introduction of a 2″-

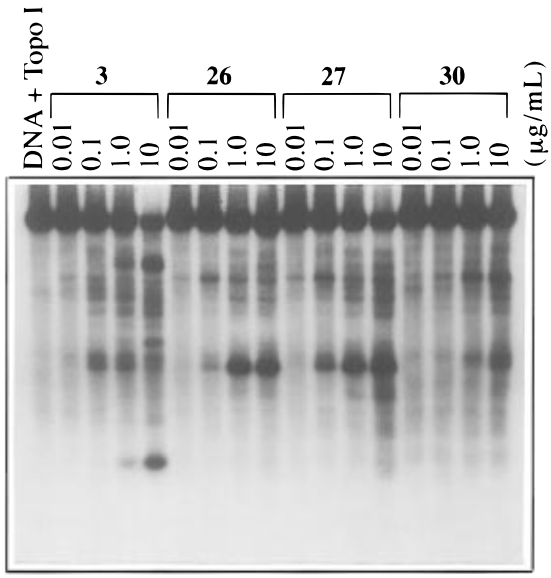


Figure 3. Stimulation of enzyme-mediated DNA cleavage by **3** and 2″-substituted terbenzimidazoles using recombinant human topoisomerase I. The assays were performed as described for Figure 1. The left-most lane is the DNA control with topoisomerase I. The relative doses employed ranged from 0.01 to 10 μg/mL for **3**, **26**, **27**, and **30**.

phenyl substituent did result in a significant loss of activity as a topoisomerase I poison for **29** and **30** (Figure 3). While **29** did retain significant cytotoxic activity, **30** was less active than the other 2″-substituted 5-phenylterbenzimidazoles. These data indicate that in the case of human topoisomerase I there exists greater structural tolerance at the 2″-position of these 5-phenylterbenzimidazoles than previously anticipated. Studies are in progress to evaluate these various analogs for their relative activity as poisons toward various camptothecin-resistant mutant forms of topoisomerase I.

Experimental Section

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 were recorded on a Varian Gemini-200 Fourier transform spectrometer. NMR spectra (200 MHz for ^1H and 50 MHz for ^{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) (95:5) 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 detector monitoring at 254 and 335 nm. The percent 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 values.

1,2-Diaminonaphthalene (**6**) was prepared from 1-nitro-2-(acetylamino)naphthalene[14] which was hydrolyzed by refluxing with 1.2 N HCl to 1-amino-2-nitronaphthalene. This reaction mixture was made basic and extracted with EtOAc, and the resulting crude 1-amino-2-nitronaphthalene was hydrogenated using 10% Pd/C in EtOAc. 2,3-Diaminonaphthalene (**7**) was commercially available (Aldrich Chemical Co., Milwaukee, WI). The syntheses of **5**,[12] **8**,[15] **12**,[12] **21**,[12] and **22**[16] have been detailed in the literature.

General Procedure for Preparing 4,5- and 5,6-Benzo-Fused Terbenzimidazoles and 4-Phenylterbenzimidazoles: 2-[2′-(Benzimidazol-5″-yl)benzimidazol-5′-yl]-naphth[1,2-*d*]imidazole (9). A stirred solution of **5** (119 mg, 0.45 mmol) and 1,2-naphthalenediamine[14] (144 mg, 0.90 mmol) in 10 mL of nitrobenzene was heated at 145 °C under N_2 overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 0−20% MeOH/EtOAc provided 110 mg (61%) of a brownish white solid: mp >280 °C; IR (KBr) 3058, 1435, 1383; ^{1}H NMR (DMSO-d_6) δ 7.46−7.54 (m, 1H), 7.61−7.87 (m, 5H), 8.03 (d, 1H, J = 8.1), 8.12−8.23 (m, 2H), 8.38−8.44 (m, 2H), 8.52−8.60 (m, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 113.3, 113.8, 115.7, 115.8, 115.9, 116.3, 118.4, 121.4, 122.1, 123.0, 124.9, 126.0, 126.8, 127.3 128.1, 128.5, 129.5, 129.9, 131.0, 132.4, 133.5, 139.2, 141.3, 148.5, 153.7; HRMS (FAB) calcd for $C_{25}H_{17}N_6$ (MH^+) 401.1515, found 401.1508.

2-[2′-(Benzimidazol-5″-yl)benzimidazol-5′-yl]naphth-[2,3-*d*]imidazole (10): prepared from **5** (102 mg, 0.39 mmol) and 2,3-diaminonaphthalene (124 mg, 0.78 mmol) in 10 mL of nitrobenzene; elution with 10% MeOH/EtOAc provided 125 mg (80%) of a brownish white solid; mp >280 °C; IR (KBr) 3043, 1626, 1441, 1389, 1271; ^{1}H NMR (DMSO-d_6) δ 7.37−7.42 (m, 2H), 7.80 (d, 2H, J = 8.5), 8.01−8.06 (m, 2H), 8.11−8.24 (m, 4H), 8.40 (s, 1H), 8.51 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 110.6, 110.7, 113.7, 115.7, 116.3, 116.9, 117.3, 123.4, 125.9, 126.4, 128.4, 131.3, 131.9, 132.1, 133.2, 139.8, 142.5, 154.0, 154.2; HRMS (FAB) calcd for $C_{25}H_{17}N_6$ (MH^+) 401.1515, found 401.1499.

4-Phenyl-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]-benzimidazole (11): prepared from **5** (128 mg, 0.49 mmol) and 3-phenyl-1,2-phenylenediamine[15] (100 mg, 0.49 mmol) in 6 mL of nitrobenzene to provide 107 mg (51%) of a brownish white solid; mp >280 °C; IR (KBr) 3043, 1626, 1441, 1389, 1271; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.55−7.68 (m, 5H), 7.84−7.90 (m, 3H), 8.00 (d, 1H, J = 8.5), 8.12 (d, 1H, J = 8.5), 8.23 (dd, 1H, J = 8.5, 1.5), 8.46 (d, 1H, J = 9.0), 8.66 (s, 1H), 8.72 (s, 1H), 9.63 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 112.9, 114.0, 115.7, 115.8, 116.5, 116.6, 118.9, 124.1, 125.1, 125.3, 125.6, 125.7, 126.1, 128.6, 129.0, 129.2, 131.6, 132.1, 133.3, 133.4, 136.5, 138.4, 140.6, 151.4, 153.3; HRMS (EI) calcd for $C_{27}H_{18}N_6$ (M^+) 426.1593, found 426.1605.

General Procedure for the Synthesis of 4-Substituted 2-Nitroaniline Derivatives: 4-(1-Naphthyl)-2-nitroaniline (13). 1-Bromonaphthalene (5 g, 24 mmol) was dissolved in freshly distilled THF (40 mL); 1.1 equiv of 1.6 M *n*-BuLi (16.6 mL) was added dropwise at −78 °C. After 30 min of stirring, tributyltin chloride (6.51 mL, 24 mmol) was added and the reaction mixture was brought to room temperature overnight. The reaction was quenched by stirring in open air. THF was removed *in vacuo*, and the mixture was quickly passed through a short silica gel column eluting with 100% hexanes. Without further purification, the crude 1-(tributylstannyl)naphthalene (9.4 g, 23 mmol), together with 4-bromo-2-nitroaniline (3.3 g, 15 mmol), $Pd(PPh_3)_2Cl_2$ (528 mg, 0.75 mmol), and PPh_3 (1.97 g, 7.5 mmol) in DMF (70 mL), was heated under N_2 at 120 °C overnight. After the reaction mixture had cooled, DMF was removed *in vacuo* and the crude mixture purified on a silica gel column eluting with 5−20% EtOAc/hexanes to provide 395 mg (10%) of a scarlet solid. Recrystallization in CH_2Cl_2 and hexanes provided orange crystals: mp 189 °C; IR (KBr) 3467, 3351, 3172, 1629, 1524, 1408, 1344, 1244; ^{1}H NMR ($CDCl_3$) δ 6.29 (brs, 2H), 6.94 (d, 1H, J = 9.0), 7.39−7.59 (m, 6H), 7.88 (d, 2H, J = 9.0), 8.29 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6) δ 120.6, 122.8, 124.2, 124.8, 126.4, 126.9, 127.5, 128.0, 128.9, 132.5, 134.1, 134.3, 135.5, 136.1, 140.7. Anal. ($C_{16}H_{12}N_2O_2$) C,H,N.

4-(2-Naphthyl)-2-nitroaniline (14): prepared from 2-(tributylstannyl)naphthalene (5.17 g, 12.4 mmol), which was prepared from 2-bromonaphthalene (5 g, 24 mmol), 1.6 M *n*-BuLi (16.6 mL), and tributyltin chloride (6.51 mL, 24 mmol), with 4-bromo-2-nitroaniline (3.3 g, 15 mmol) in DMF (70 mL) using $Pd(PPh_3)_2Cl_2$ (528 mg, 0.75 mmol) and PPh_3 (1.97 g, 7.5 mmol) to provide 766 mg (35%) of orange crystals; mp 187 °C; IR (KBr) 3467, 3351, 3172, 1629, 1524, 1408, 1344, 1244; ^{1}H NMR (DMSO-d_6) δ 7.20 (d, 1H, J = 9.0), 7.50−7.59 (m, 2H), 7.62 (brs, 2H), 7.84 (dd, 1H, J = 8.5, 2.0), 7.91−8.02 (m, 4H), 8.21 (s, 1H), 8.40 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6) δ 120.4, 122.9, 124.2, 124.5, 126.2, 126.7, 127.4, 127.7, 128.3, 128.8, 130.3, 132.3, 133.7, 134.7, 135.8, 145.9. Anal. ($C_{16}H_{12}N_2O_2$) C,H,N.

4-(*p*-Methoxyphenyl)-2-nitroaniline (15): prepared from (*p*-methoxyphenyl)tributyltin (1.92 g, 4.83 mmol), which was prepared from *p*-bromoanisole (1.28 g, 6.86 mmol), 1.6 M *n*-BuLi (4.75 mL), and tributyltin chloride (2.83 mL, 10 mmol), with 4-bromo-2-nitroaniline (700 mg, 3.22 mmol) in DMF (18 mL) using $Pd(PPh_3)_2Cl_2$ (111 mg, 0.16 mmol) and PPh_3 (414 mg, 1.6 mmol) to provide 300 mg (38%) of orange crystals; mp 170−171 °C; IR (KBr) 3479, 3367, 1648, 1502, 1350, 1235; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H), 7.01 (d, 2H, J = 9.0), 7.12 (d, 1H, J = 9.0), 7.51 (brs, 2H), 7.58 (d, 2H, J = 9.0), 7.75 (dd, 1H, J = 9.0, 2.0), 8.15 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6) δ 55.5, 114.7, 120.2, 121.8, 127.2, 127.5, 130.7, 131.9, 134.4, 145.4, 158.8. Anal. ($C_{13}H_{12}N_2O_3$) C,H,N.

4-(*p*-Chlorophenyl)-2-nitroaniline (16): prepared from (*p*-chlorophenyl)tributyltin (2.02 g, 5.04 mmol), which was prepared from *p*-chlorobromobenzene (1.0 g, 5.16 mmol), 1.6 M *n*-BuLi (3.54 mL), and tributyltin chloride (2.10 mL, 7.76 mmol), with 4-bromo-2-nitroaniline (730 mg, 3.36 mmol) in DMF (18 mL) using $Pd(PPh_3)_2Cl_2$ (118 mg, 0.17 mmol) and PPh_3 (440 mg, 1.70 mmol) to provide 270 mg (32%) of scarlet needles; mp 170 °C; IR (KBr) 3467, 3351, 1634, 1509, 1487, 1339, 1238; ^{1}H NMR (DMSO-d_6) δ 7.14 (d, 1H, J = 9.0), 7.49 (d, 2H, J = 8.5), 7.61 (brs, 2H), 7.68 (d, 2H, J = 8.5), 7.80 (dd, 1H, J = 9.0, 2.0), 8.23 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6) δ 120.4, 122.0, 126.2, 127.7, 128.2, 130.7, 132.0, 134.3, 137.4, 146.0. Anal. ($C_{12}H_9N_2O_2Cl$), C,H,N.

General Procedure for Preparing 5-Substituted Terbenzimidazoles: 5-(1-Naphthyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (17). Hydrogenation of **13** (166 mg, 0.63 mmol) was accomplished at 45 psi of H_2 at room temperature for 32 h using 10% Pd/C (33 mg) in EtOAc (100 mL). The crude diamine (132 mg, 0.56 mmol), obtained after passage through a bed of Celite and removal of EtOAc *in vacuo*, was dissolved in 10 mL of nitrobenzene containing **5** (74 mg, 0.28 mmol). This reaction mixture was heated at 145 °C under N_2 overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 10−20% MeOH/EtOAc provided a 67% yield of a brownish white solid; mp >280 °C; IR (KBr) 3048, 2925, 1624, 1436, 1385, 1290; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.59−7.73 (m, 4H), 7.78−7.83 (m, 2H), 7.95 (s, 1H), 8.02−8.21 (m, 6H), 8.50 (d, 1H, J = 9.0), 8.68 (s, 1H), 8.75 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 113.9, 114.1, 114.9, 115.8, 116.1, 116.4, 117.8, 123.2, 124.0, 125.3, 125.8, 125.9, 126.4, 127.0, 127.5, 127.7, 128.3, 128.4, 129.4, 131.2, 131.7, 131.8, 132.5, 133.0, 133.7, 138.1, 138.8, 141.5, 150.6, 153.8, 158.1; HRMS (FAB) calcd for $C_{31}H_{21}N_6$ (MH^+) 477.1828, found 477.1813.

5-(2-Naphthyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (18). The crude diamine (107 mg, 0.45 mmol), which was obtained by hydrogenation of **14** (123 mg, 0.45 mmol) in EtOAc (100 mL) using 10% Pd/C (33 mg) for 9 h, with **5** (119 mg, 0.45 mmol) provided 114 mg (53%) of a brownish white solid; mp >280 °C; IR (KBr) 3046, 2954, 2923, 2862, 1441, 1287; ^{1}H NMR (DMSO-d_6) δ 7.52−7.59 (m, 2H), 7.66−7.81 (m, 4H), 7.93−8.07 (m, 5H), 8.12−8.19 (m, 2H), 8.29

(s, 1H), 8.40 (s, 1H), 8.45 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 112.0, 113.9, 114.7, 115.8, 116.1, 116.3, 117.8, 123.2, 125.3, 125.7, 125.8, 126.3, 126.7, 126.8, 126.9, 127.8, 128.6, 129.0, 131.0, 132.7, 133.0, 133.1, 133.6, 134.5, 135.5, 137.0, 138.6, 139.1, 141.4, 150.7, 153.7; HRMS (FAB) calcd for $C_{31}H_{21}N_6$ (MH^+) 477.1828, found 477.1828.

5-(4-Methoxyphenyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (19). The crude diamine (148 mg, 0.69 mmol), which was obtained by hydrogenation of **15** (205 mg, 0.84 mmol) in EtOAc (100 mL) using 10% Pd/C (40 mg) for 4 h, with **5** (165 mg, 0.63 mmol) provided a 54% yield of a brownish white solid; mp 275 °C; IR (KBr) 3395, 3064, 2821, 1610, 1517, 1437, 1243; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 7.07 (d, 2H, J = 8.5), 7.48 (dd, 1H, J = 8.5, 1.5), 7.64−7.81 (m, 6H), 8.10−8.28 (m, 2H), 8.40 (s, 1H), 8.42 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 55.5, 107.4, 110.9, 113.8, 114.5, 114.7, 114.8, 115.8, 116.1, 116.3, 117.6, 123.1, 125.2, 126.2, 128.6, 131.2, 131.9, 132.0, 133.0, 138.5, 139.2, 141.5, 150.3, 154.0, 159.8; HRMS (FAB) calcd for $C_{28}H_{21}N_6O$ (MH^+) 457.1777, found 457.1784.

5-(4-Chlorophenyl)-2-[2′-(benzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (20). The crude diamine, which was obtained by hydrogenation of **16** (177 mg, 0.66 mmol) in EtOAc (100 mL) using 10% Pd/C (40 mg) for 4 h, with **5** (99 mg, 0.38 mmol) in 5 mL of nitrobenzene provided a 49% yield of a brownish white solid; mp >280 °C; IR (KBr) 3046, 2820, 1621, 1549, 1426, 1282; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3-COOH) δ 7.56 (d, 2H, J = 8.5), 7.82 (d, 2H, J = 8.5), 7.88−8.12 (m, 6H), 8.48 (d, 1H, J = 8.5), 8.63 (s, 1H), 8.72 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 111.8, 113.8, 114.7, 115.8, 116.1, 117.7, 123.0, 124.1, 125.1, 125.2, 125.3, 125.4, 129.2, 129.3, 129.4, 131.9, 132.1, 133.0, 133.1, 137.2, 138.5, 139.3, 141.6, 150.8, 153.8; HRMS (FAB) calcd for $C_{27}H_{18}N_6Cl$ (MH^+) 461.1281, found 461.1273.

2-(3,4-Dinitrophenyl)-5-phenylbenzimidazole (23). A stirred solution of 4-phenyl-1,2-phenylenediamine[12] (340 mg, 1.9 mmol) and **22** (374 mg, 1.9 mmol) in 10 mL of nitrobenzene was heated at 145 °C under N_2 overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 10% EtOAc/hexanes provided 455 mg (66%) of a yellow solid; mp 146 °C; IR (KBr) 3097, 2971, 1608, 1540, 1445, 1365, 1286; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.37−7.55 (m, 3H), 7.67−8.79 (m, 3H), 7.85 (d, 1H, J = 8.5), 7.97 (d, 1H, J = 1.0), 8.50 (d, 1H, J = 8.5), 8.71 (dd, 1H, J = 8.5, 2.0), 8.97 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 113.2, 116.3, 123.8, 124.4, 127.2, 127.2, 127.7, 129.4, 132.2, 133.7, 137.3, 137.4, 138.1, 140.5, 142.5, 142.8, 147.6; HRMS (FAB) calcd for $C_{19}H_{13}N_4O_4$ (MH^+) 361.0937, found 361.0935.

5-Phenyl-2-[2′-(3,4-dinitrophenyl)benzimidazol-5′-yl]-benzimidazole (24). Compound **23** (370 mg, 1.03 mmol) was dissolved in EtOAc (100 mL) and reduced by hydrogenation using 10% Pd/C (80 mg) for 1.5 h. After passing through a bed of Celite, the EtOAc was removed *in vacuo*. The crude diamine (220 mg, 0.67 mmol) and **22** (131 mg, 0.67 mmol) in 10 mL of nitrobenzene were heated at 145 °C under N_2 overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 0−10% MeOH/EtOAc provided 300 mg (94%) of a yellow solid; mp >280 °C; IR (KBr) 3065, 2960, 1603, 1540, 1439, 1360, 1280; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.46−7.60 (m, 3H), 7.80 (d, 2H, J = 7.0), 7.87−8.00 (m, 2H), 8.05−8.10 (m, 2H), 8.19 (dd, 1H, J = 8.5, 2.0), 8.50 (d, 1H, J = 8.5), 8.66 (s, 1H), 8.77 (dd, 1H, J = 8.5, 2.0), 9.00 (d, 1H, J = 2.0); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 111.6, 114.5, 116.0, 117.0, 117.3, 123.3, 125.6, 127.1, 127.5, 128.2, 129.4, 131.5, 132.3, 132.7, 134.8, 138.8, 139.6, 140.4, 142.3, 142.5, 142.8, 150.5, 150.9; HRMS (FAB) calcd for $C_{26}H_{17}N_6O_4$ (MH^+) 477.1311, found 477.1307.

General Procedure for Preparing 2″-Substituted Terbenzimidazoles: 5-Phenyl-2-[2′-(2″-methylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (25). In EtOAc (50 mL) was dissolved **24** (75 mg, 0.16 mmol), which was reduced by hydrogenation using 10% Pd/C (15 mg) for 1.5 h. After passing through a bed of Celite and removal of the EtOAc *in vacuo*, the crude diamine (63 mg, 0.13 mmol) and acetaldehyde (5.7 mg, 0.13 mmol) in 3 mL of nitrobenzene were heated at 145 °C in a sealed tube overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 10% MeOH/EtOAc provided 26 mg (45%) of a brownish white solid; mp >280 °C; IR (KBr) 3033, 2968, 2932, 1551, 1443; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 2.88 (s, 3H), 7.47−7.61 (m, 3H), 7.80 (d, 2H, J = 8.5), 7.88−8.01 (m, 2H), 8.04−8.29 (m, 4H), 8.44 (d, 1H, J = 8.5), 8.65 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 14.3, 111.7, 114.6, 114.9, 116.2, 116.4, 117.5, 117.6, 123.0, 123.1, 124.8, 125.5, 126.0, 126.2, 127.5, 128.2, 129.5, 131.8, 133.0, 133.1, 134.9, 138.7, 139.7, 150.7, 153.8, 153.9; HRMS (FAB) calcd for $C_{28}H_{21}N_6$ (MH^+) 441.1828, found 441.1846.

5-Phenyl-2-[2′-(2″-ethylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (26): prepared using the crude diamine derived from **24** (75 mg, 0.16 mmol) and propionaldehyde (7.3 mg, 0.13 mmol) using 3 mL of nitrobenzene in a sealed tube overnight to provide a 28% yield of a brownish white solid; mp >280 °C; IR (KBr) 3019, 2961, 1624, 1436, 1261; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 1.48 (t, 3H), 3.23 (q, 2H), 7.46−7.61 (m, 3H), 7.79−8.01 (m, 4H), 8.06−8.28 (m, 4H), 8.45 (dd, 1H, J = 9.0, 1.0), 8.66 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 11.6, 20.3, 111.7, 113.3, 114.6, 115.1, 116.2, 123.6, 125.1, 125.6, 127.5, 128.2, 129.3, 129.4, 131.7, 132.9, 133.3, 133.5, 138.9, 139.7, 140.4, 150.4, 153.5, 158.9; HRMS (FAB) calcd for $C_{29}H_{23}N_6$ (MH^+) 455.1985, found 455.1974.

5-Phenyl-2-[2′-(2″-propylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (27). This was prepared from the crude diamine derived from **24** (113 mg, 0.24 mmol) and butyraldehyde (13.8 mg, 0.24 mmol) using 3 mL of nitrobenzene in a sealed tube overnight. The cooled reaction mixture was then purified directly by column chromatography. Elution with 0−20% MeOH/EtOAc provided 45 mg (40%) of a brownish white solid; mp >280 °C; IR (KBr) 3100, 2961, 2925, 2874, 1436, 1283; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 1.01 (t, 3H), 2.77 (q, 2H), 2.87 (t, 2H), 7.45−7.60 (m, 3H), 7.80 (d, 2H, J = 7.0), 7.82−8.00 (m, 2H), 8.06−8.14 (m, 3H), 8.25 (d, 1H, J = 7.0), 8.46 (d, 1H, J = 9.0), 8.69 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 13.5, 20.3, 28.4, 111.7, 113.5, 115.1, 115.8, 115.9, 116.2, 116.6, 123.9, 124.5, 125.2, 125.6, 127.5, 128.2, 129.4, 131.7, 131.9, 132.9, 133.4, 137.6, 138.9, 139.6, 150.3, 153.4, 154.5, 157.1; HRMS (FAB) calcd for $C_{30}H_{25}N_6$ (MH^+) 469.2141, found 469.2132.

5-Phenyl-2-[2′-(2″-isopropylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (28): prepared from **24** (113 mg, 0.24 mmol) and isobutyraldehyde (13.6 mg, 0.19 mmol) in 5 mL of nitrobenzene at 145 °C in a sealed tube overnight in 51% yield, brownish white solid; mp >280 °C; IR (KBr) 3056, 2961, 2925, 2867, 1443, 1283; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 1.53 (d, 6H, J = 7.0), 3.54 (q, 1H, J = 7.0), 7.46−7.61 (m, 3H), 7.79 (d, 2H, J = 7.0), 7.87−8.00 (m, 2H), 8.04−8.08 (m, 3H), 8.21 (d, 1H, J = 9.0), 8.46 (d, 1H, J = 9.0), 8.66 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 20.2, 27.6, 111.7, 113.1, 114.6, 115.1, 116.1, 116.2, 116.3, 123.2, 123.3, 125.0, 127.5, 128.2, 129.4, 131.7, 131.8, 133.0, 133.2, 138.8, 139.7, 141.2, 149.2, 150.7, 153.8, 161.2; HRMS (FAB) calcd for $C_{30}H_{25}N_6$ (MH^+) 469.2141, found, 469.2154.

5-Phenyl-2-[2′-(2″-phenylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole (29): prepared from **24** (80 mg, 0.17 mmol) and benzaldehyde (15 mg, 0.14 mmol) using 3 mL of nitrobenzene at 145 °C under N_2 overnight to provide a 57% yield of a brownish white solid; mp >280 °C; IR (KBr) 3056, 2925, 1624, 1436, 1283; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3-COOH) δ 7.44−7.61 (m, 4H), 7.72−8.13 (m, 9H), 8.20−8.41 (m, 4H), 8.66 (s, 2H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3-COOH) δ 107.4, 111.8, 111.9, 114.1, 114.7, 115.6, 115.7, 116.1, 116.2, 123.5, 124.4, 127.5, 128.2, 129.5, 129.8, 130.2, 132.0, 133.2, 137.1, 138.7, 139.8, 140.4, 143.1, 150.5, 150.8, 152.5, 154.0, 160.7; HRMS (FAB) calcd for $C_{33}H_{23}N_6O$ (MH^+) 503.1984, found 503.1982.

5-Phenyl-2-[2′-[2″-(*p*-methoxyphenyl)benzimidazol-5″-yl]benzimidazol-5′-yl]benzimidazole (30): prepared from the crude diamine derived from **24** (80 mg, 0.17 mmol) and *p*-anisaldehyde (22.9 mg, 0.17 mmol) using 3 mL of nitrobenzene at 145 °C under N_2 overnight to provide a 89% yield of a brownish white solid; mp >280 °C; IR (KBr) 3056, 2933, 1611,

1437, 1255; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 3.94 (s, 3H), 7.33−7.60 (m, 5H), 7.80 (d, 2H, J = 8.0), 7.88−8.01 (m, 2H), 8.07−8.12 (m, 3H), 8.24−8.30 (m, 3H), 8.44 (d, 1H, J = 9.0), 8.66 (d, 2H, J = 2.0); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 56.0, 111.7, 113.3, 114.6, 115.1, 115.6, 115.7, 116.2, 121.5, 122.8, 124.3, 125.2, 125.6, 127.5, 127.6, 129.4, 130.5, 130.8, 131.7, 132.9, 133.2, 134.9, 138.8, 139.7, 140.2, 150.3, 151.7, 153.6, 163.8; HRMS (FAB) calcd for $C_{34}H_{25}N_6O$ (MH^+) 533.2089, found 533.2072.

Topoisomerase I-Mediated DNA Cleavage Assays. Human topoisomerase I was expressed in *Escherichia coli* and purified as previously described.[19] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[20,21] The end-labeling of the plasmid was accomplished as previously described.[21] The cleavage assays were performed as previously reported.[8] The drug and DNA in the presence of topoisomerase I was incubated for 30 min at 37 °C. After development of the gels, 24 h exposure was typically used to obtain autoradiographs outlining the extent of DNA fragmentation.

Cytotoxicity Assay. The cytotoxicity as listed for several of the compounds which were prepared as part of this study was determined using human lymphoblast RPMI 8402 cells and the MTT microtiter plate tetrazolium cytotoxicity assay (MTA).[22−24] The cytotoxicity assay was performed using 2000 cells/well, in 200 μL of growth medium, which 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). The cells were exposed continuously for 4 days to different drug concentrations and assayed at the end of the fourth day. Each assay was performed with a control which did not contain any drug. All assays were performed at least twice in six replica wells.

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