Substituted 2,5′-Bi-1*H*-benzimidazoles: Topoisomerase I Inhibition and Cytotoxicity

Jung Sun Kim,[†] Barbara Gatto,[‡] Chiang Yu,[‡] Angela Liu,[‡] Leroy F. Liu,[‡] and Edmond J. LaVoie*,[†]

Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855, and Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08855

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Several 2′-aryl-5-substituted-2,5′-bi-1*H*-benzimidazole derivatives were synthesized and evaluated as topoisomerase I poisons and for their cytotoxicity toward the human lymphoblast cell line RPMI 8402. This study focused on 18 2,5′-bi-1*H*-benzimidazole derivatives which contained either a 5-cyano, a 5-(aminocarbonyl), or a 5-(4-methylpiperazinyl) group. Among these bibenzimidazoles, the pharmacological activity of 2′-phenyl derivatives and the influence of the different positional isomers of either a 2′-tolyl group or a 2′-naphthyl moiety on cytotoxicity and topoisomerase I inhibitory activity were determined.

Introduction

The chemotherapeutic action of several anticancer agents has been linked to their ability to inhibit nuclear DNA topoisomerases. Topoisomerases are involved in producing the necessary topological and conformational changes in DNA which are critical to many cellular processes such as replication and transcription.[1−3] There are two types of topoisomerases which have been isolated from mammalian cells. These enzymes can be distinguished by whether they function by producing a transient protein-bridged DNA break on one (mammalian topoisomerase I) or both (mammalian topoisomerase II) DNA strands. There have been several reviews on topoisomerases as targets for the development of anticancer agents.[4−6] Several of the anticancer agents in clinical use have been shown to be potent inhibitors of topoisomerase II. Etoposide (VP-16), teniposide (VM-26), mitoxantrone, *m*-AMSA, adriamycin (doxorubicin), and daunomycin have all been demonstrated to possess significant activity as inhibitors of topoisomerase II.[4−6] In comparison to topoisomerase II poisons, however, there are fewer known topoisomerase I inhibitors. Camptothecin is the most extensively studied mammalian topoisomerase I inhibitor. The broad spectrum of potent antineoplastic activity observed for camptothecin[7,8] has resulted in efforts to identify other agents which can poison mammalian topoisomerase I.

Hoechst 33258, 2′-(4-hydroxyphenyl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (NSC-32291, pibenzimol), and Hoechst 33342, 2′-(4-ethoxyphenyl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole, are inhibitors of topoisomerase I.[9−11] These agents are known to bind to the minor groove of DNA and have been shown to bind with A + T specificity. While Hoechst 33258 is cytotoxic, the more lipophilic derivative, Hoechst 33342, exhibits greater cytotoxicity. While studies have been performed to assess the effects of structural variation at the 5-position of 2,5′-bi-1*H*-benzimidazoles on topoisomerase I poisoning,[12] the influence of structural variations at the 2′-position has not been determined. In the present study, several 5-cyano-2′-aryl-2,5′-bi-1*H*-benzimidazole derivatives were synthesized. Topoisomerase I poisoning and cytotoxicity of the 2′-phenyl derivatives as well as all positional isomers of either a 2′-tolyl group or a 2′-naphthyl group were investigated. By the facile conversion of these 5-cyano analogs to their 5-(aminocarbonyl) derivatives, the influence of a functional group capable of hydrogen bonding at the 5-position of these various 2′-aryl-bi-1*H*-benzimidazoles was also assessed. The pharmacological activity of these analogs was compared with similar 2′-aryl-2,5′-bi-1*H*-benzimidazole derivatives containing a 5-(4-methylpiperazinyl) moiety, which at physiological pH is extensively protonated.

Chemistry

The 5-cyano derivatives of several 2′-aryl-2,5′-bi-1*H*-benzimidazoles (**2a**−**f**) were synthesized by coupling of 4-cyano-1,2-phenylenediamine **(1)** with the appropriately substituted 5-formyl-2-arylbenzimidazoles (Scheme 1). The yields of these various 2′-aryl-5-cyano-2,5′-bi-1*H*-benzimidazoles ranged from 63% to 94%. Compounds **2a**−**f** served as convenient intermediates for the synthesis of the 2′-aryl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazoles **3a**−**f**. Treatment of **2a**−**f** with H_2O_2 in the presence of tetrabutylammonium hydrogen sulfate and 5 N NaOH provided the 2′-aryl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazoles **3a**−**f**.

A series of 2-aryl-5-cyano-1*H*-benzimidazoles, **4a**−**f**, was prepared as outlined in Scheme 2 by the coupling of **1** with the appropriately substituted benzaldehyde derivative or naphthylaldehyde. These various benzimidazoles were formed in yields ranging from 51% to 71%. Reduction of **4a**−**f** with Ni−Al in the presence of formic acid followed by hydrolysis provided an effective method for preparation of the 2-aryl-5-formyl-1*H*-benzimidazole intermediates **5a**−**f** required for the formation of the various 2,5′-bi-1*H*-benzimidazole derivatives in this study.

The preparation of the 2′-aryl-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazoles **8a**−**f** was accomplished by coupling of 4-(4-methylpiperazinyl)-1,2-phenylenediamine, **7**, with the appropriate 2-aryl-5-formyl-1*H*-benzimidazole **5a**−**f** as outlined in Scheme 3. Synthesis of 2-nitro-5-(4-methylpiperazinyl)aniline, **6**, was ac-

[†] Rutgers, The State University of New Jersey.
[‡] The University of Medicine and Dentistry of New Jersey.
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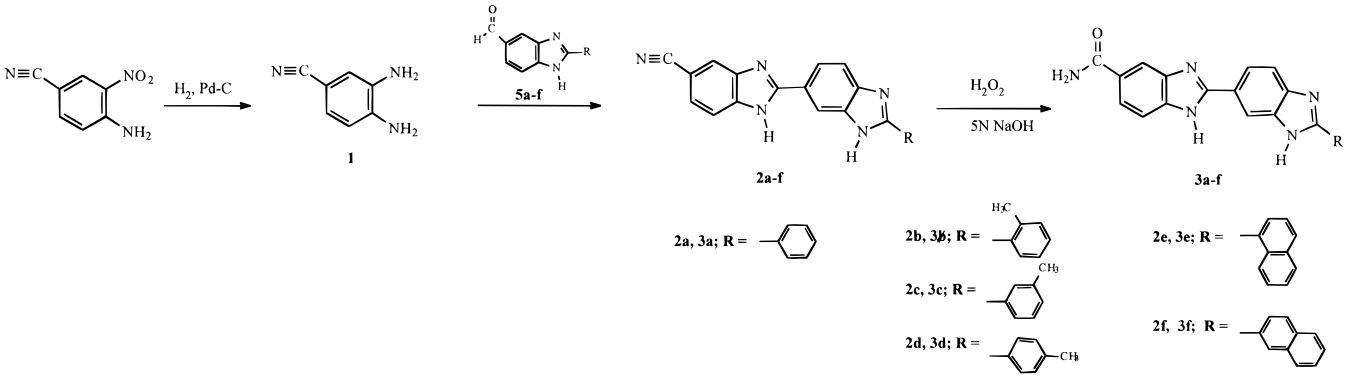
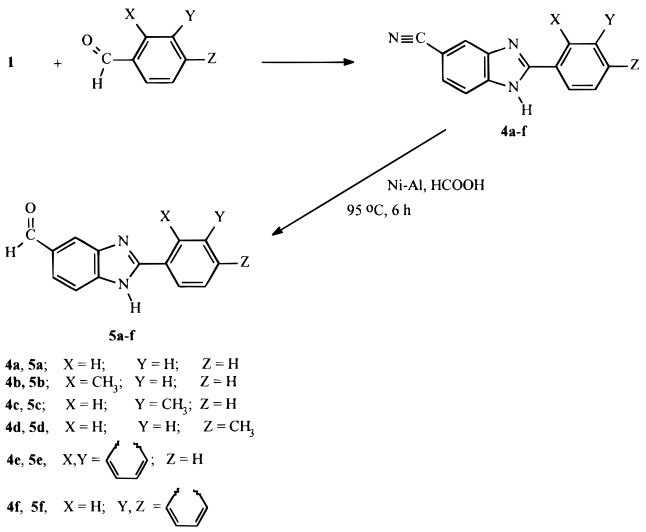
Scheme 1



Scheme 2



complished by reaction of 2-nitro-5-chloroaniline with 1-methylpiperazine. Catalytic hydrogenation of **6** provided **7** in 95% yield.

Results and Discussion

The relative potency of these variously substituted bibenzimidazoles as topoisomerase I poisons was determined by assessing their ability to induce DNA cleavage in the presence of enzyme. The results of these assays are summarized in Table 1. Within the series of compounds **8a**−**f** which possess a 5-(4-methylpiperazinyl) moiety, **8a,c,d** exhibited similar potency to Hoechst 33342 as topoisomerase I poisons. In comparison to **8a** which has a 2′-phenyl substituent, the presence of either an *o*-tolyl group (**8b**) or a naphthyl moiety (**8e,f**) at the 2′-position was associated with a reduction in their relative potency as topoisomerase poisons by ca. 1 order of magnitude. This study does indicate that within this series of 5-(4-methylpiperazinyl)-substituted-2,5′-bi-1*H*-benzimidazoles, substituents with varied steric effects can be accommodated at the 2′-position with retention of topoisomerase I activity. In general, individual compounds within this series, compounds **8a**−**f**, were among the more cytotoxic derivatives against the human lymphoblastoma cell line RPMI 8402. These results are consistent with those previously observed for other 2,5′-bi-1*H*-benzimidazoles where the presence of a 5-(4-methylpiperazinyl) or 5-oxy(4-methylpiperidinyl) substituent was associated with enhanced cytotoxicity.[12] With the exception of the 2′-(2-naphthyl) derivative **8f**, the other derivatives within this series of compounds were more than 1 order of magnitude less cytotoxic than Hoechst 33342.

It has been recently demonstrated that within a series of terbenzimidazole derivatives, similar activity as topoisomerase I poisons and cytotoxicity to Hoechst 33342 can be achieved with compounds which do not have a basic tertiary amine attached to the 5-position.[13] Earlier studies have demonstrated that 5-cyano-2′-(*p*-methoxyphenyl)-2,5′-bi-1*H*-benzimidazole has ca. one-third the potency of Hoechst 33342 as an inhibitor of topoisomerase I.[13] In this series, the only 5-cyano analog evaluated that had similar potency to Hoechst 33342 as a topoisomerase I poison was the 2′-(4-tolyl) derivative **2d**. 2′-Phenyl-5-cyano-2,5′-bi-1*H*-benzimidazole, **2a**, as well as the other isomeric tolyl derivatives **2b,c**, was significantly less potent. Both isomers of 5-cyano-2′-naphthyl-2,5′-bi-1*H*-benzimidazole, **2e,f**, had ca. one-tenth the potency of Hoechst 33342 as topoisomerase I poisons. The absence of a clear correlation between cytotoxicity and potency as topoisomerase I inhibitors was most evident within this series of 5-cyano-2′-substituted-2,5′-bi-1*H*-benzimidazoles. While differences in cellular absorption may be responsible for the absence of such a correlation, it cannot be assumed from these data that the mechanism associated with the cytotoxicity of these various analogs is exclusively mediated through their potential as topoisomerase I poisons.

The 5-(aminocarbonyl)-2′-substituted-2,5′-bi-1*H*-benzimidazole derivatives **3a**−**f** were all less active than their corresponding 5-cyano-2,5′-bi-1*H*-benzimidazoles, with the exception of **2a**. Compounds **2a** and **3a** were among the weaker topoisomerase I poisons and among the least cytotoxic of the derivatives evaluated. None of the 5-(aminocarbonyl)-2′-substituted-2,5′-bi-1*H*-benzimidazole derivatives **3a**−**f** exhibited greater potency than their corresponding 5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole derivatives as either topoisomerase I inhibitors or cytotoxic agents.

Evaluation of the data associated with each series of compounds (the 5-cyano, 5-(aminocarbonyl), and 5-piperazinyl series) did reveal similarities. It was observed, for example, that within all three series of 5-substituted-bi-1*H*-benzimidazoles, the 2′-(2-naphthyl) and 2′-(1-naphthyl) derivatives are among the more cytotoxic analogs. The increased lipophilicity which would be expected with these naphthyl derivatives appears to correlate with their greater cytotoxic activity. Similar comparisons of the relative activity of these agents as

Scheme 3

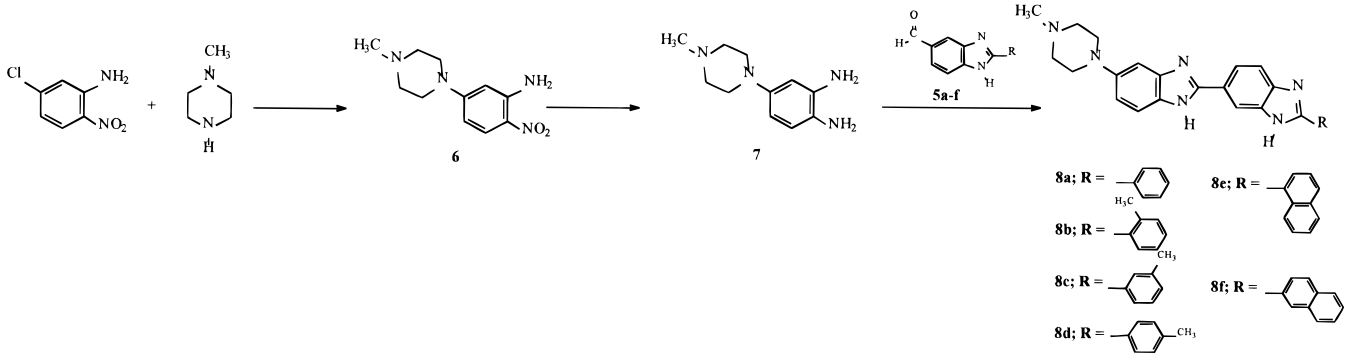
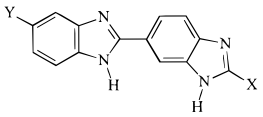


Table 1. Cytotoxicity and Topoisomerase I-Mediated Cleavage of DNA Induced by 2,5′-Bi-1*H*-benzimidazoles



compound	X	Y	topo I-mediated cleavage[a]	cytotoxicity IC_{50} (μM)[b]
Hoechst 33342	4-ethoxyphenyl	4-methylpiperazinyl	1.0	0.03
2a	phenyl	-CN	>100	>25
2b	2-tolyl	-CN	≫100	1.2
2c	3-tolyl	-CN	100	0.86
2d	4-tolyl	-CN	1	0.57
2e	1-naphthyl	-CN	10	0.65
2f	2-naphthyl	-CN	10	0.13
3a	phenyl	$-CONH_2$	10	>25
3b	2-tolyl	$-CONH_2$	100	8.2
3c	3-tolyl	$-CONH_2$	100	8.2
3d	4-tolyl	$-CONH_2$	10	>25
3e	1-naphthyl	$-CONH_2$	20	3.7
3f	2-naphthyl	$-CONH_2$	100	0.74
8a	phenyl	4-methylpiperazinyl	1	0.49
8b	2-tolyl	4-methylpiperazinyl	10	0.59
8c	3-tolyl	4-methylpiperazinyl	1	0.36
8d	4-tolyl	4-methylpiperazinyl	1	0.83
8e	1-naphthyl	4-methylpiperazinyl	20	0.33
8f	2-naphthyl	4-methylpiperazinyl	10	0.11

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to that of 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. [b] IC_{50} has been calculated after 4 days of continuous drug exposure. No indication of cytotoxicity was considered indicative for IC_{50} values substantially greater than the highest dose assayed, 25 μM.

topoisomerase I poisons indicate that those analogs with a 2′-(4-tolyl) group are among the potent inhibitors of topoisomerase I. These data suggest that the presence of a lipophilic substituent at the *para* position within this various series of 2,5′-bi-1*H*-benzimidazoles may enhance topoisomerase I inhibitory activity. There is, however, no similar consistency observed for these tolyl derivatives with regard to the results obtained on their relative cytotoxicity. Further studies are needed to more fully assess those structural features of 2,5′-bi-1*H*-benzimidazoles which are associated with optimal activity as topoisomerase I poisons and cytotoxic activity.

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. Chromatotron 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 ^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. Mass spectra were obtained from Midwest Center for Mass Spectrometry within the Department of Chemistry at the University of Nebraska-Lincoln. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and are within ±0.4% of the theoretical value.

3,4-Diaminobenzonitrile (1). 4-Amino-3-nitrobenzonitrile (2.02 g, 12.4 mmol) in 100 mL of EtOAc was reduced by hydrogenation using 45 psi of H_2 and 10% Pd−C (500 mg) for 1.5 h.[14,15] After passing through a bed of Celite, solvent was removed *in vacuo* and 1.56 g (94.5%) of white solid was obtained: mp 143−145 °C (lit.[15] mp 146 °C); ^1H NMR δ 3.39 (brs, 2H), 3.82 (brs, 2H), 6.68 (d, 1H, J = 8.0), 6.95 (d, 1H, J = 1.8), 7.05 (dd, 1H, J = 8.0, 1.8); ^{13}C NMR δ 99.8, 116.2, 118.7, 124.1, 125.3, 137.7, 143.2. The crude diamine obtained from hydrogenation was typically used directly without further purification.

General Procedure for the Preparation of 5-Cyano-2,5′-bi-1*H*-benzimidazoles: 2′-Phenyl-5-cyano-2,5′-bi-1*H*-benzimidazole (2a). A stirred solution of **5a** (218 mg, 0.98

mmol) and **1** (131 mg, 0.98 mmol) in 3 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 removed the nitrobenzene. Product was obtained by employing a gradient from 100% EtOAc to 5% MeOH/EtOAc. Concentration *in vacuo* provided 249 mg (76%) of a white solid: mp 240 °C dec; IR (KBr) 3149, 2222, 1442, 1291; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.75−7.85 (m, 4H), 7.97 (d, 1H, J = 8.7), 8.13 (d, 1H, J = 8.8), 8.25−8.30 (m, 2H), 8.36−8.41 (m, 2H), 8.66 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 106.6, 112.3, 114.2, 115.6, 115.7, 119.2, 120.1, 122.1, 125.3, 128.1, 128.4, 129.9, 132.1, 133.4, 133.7, 135.6, 137.9, 152.3, 152.8.

2′-*o*-Tolyl-5-cyano-2,5′-bi-1*H*-benzimidazole (2b). A solution of **5b** (262 mg, 1.11 mmol) and **1** (148 mg, 1.11 mmol) was treated as described for **2a**. Column chromatography using 40−100% EtOAc/hexanes provided 295 mg (76%) of crude product. A portion of the crude product (57 mg) was further purified by chromatotron chromatography using 40% EtOAc/hexane to provide 49.3 mg of a white solid: mp >280 °C; IR (KBr) 3199, 2223, 1441, 1294; ^{1}H NMR (CD_3OD) δ 2.51 (s, 3H), 7.26−7.44 (m, 4H), 7.57−7.69 (m, 3H), 7.83 (s, 1H), 7.94 (dd, 1H, J = 8.5, 1.7), 8.27 (s, 1H); ^{13}C NMR (CD_3OD) δ 20.9, 106.7, 121.0, 127.5, 131.2, 131.7, 132.6, 139.0.

2′-*m*-Tolyl-5-cyano-2,5′-bi-1*H*-benzimidazole (2c). A solution of **5c** (253 mg, 1.07 mmol) and **1** (142 mg, 1.07 mmol) was treated as described for **2a**. Column chromatography using 0−100% EtOAc/hexanes and then 0−50% MeOH/EtOAc provided 236 mg (63%) of a white solid: mp 245 °C dec; IR (KBr) 3142, 2220, 1437; ^{1}H NMR (CD_3OD) δ 2.40 (s, 3H), 7.24−7.43 (m, 3H), 7.59 (d, 2H, J = 8.1), 7.78−7.88 (m, 4H), 8.14 (s, 1H); ^{13}C NMR (CD_3OD) δ 21.8, 106.4, 110.1, 121.0, 123.2, 123.2, 124.6, 125.4, 127.3, 128.7, 130.3, 130.5, 132.7, 140.4.

2′-*p*-Tolyl-5-cyano-2,5′-bi-1*H*-benzimidazole (2d). A solution of **5d** (235 mg, 0.99 mmol) and **1** (132 mg, 0.99 mmol) was treated as described for **2a**. Column chromatography using 50−100% EtOAc and then 0−10% MeOH/EtOAc provided 258 mg (78%) of a white solid: mp 229 °C dec; IR (KBr) 3164, 2222, 1439, 1296; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 2.47 (s, 3H), 7.56 (d, 2H, J = 8.1), 7.70 (dd, 1H, J = 8.4, 1.4), 7.85 (d, 1H, J = 8.4), 8.00 (d, 1H, J = 8.5), 8.16 (d, 2H, J = 8.1), 8.24 (s, 1H), 8.35 (dd, 1H, J = 8.5, 1.4), 8.57 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 21.5, 105.1, 113.3, 115.3, 115.9, 120.0, 120.6, 122.1, 124.4, 124.9, 126.7, 128.1, 130.4, 134.5, 136.0, 138.6, 140.7, 143.7, 152.0, 153.9.

2′-(Naphth-1-yl)-5-cyano-2,5′-bi-1*H*-benzimidazole (2e). A solution of **5e** (96 mg, 0.35 mmol) and **1** (47 mg, 0.35 mmol) was treated as described for **2a**. Column chromatography using 20−50% EtOAc/hexanes provided 127 mg (94%) of a white solid: mp >280 °C; IR (KBr) 3060, 2222, 1548, 1439, 1138; ^{1}H NMR (DMSO-d_6) δ 7.59−7.78 (m, 6H), 8.1−8.2 (m, 5H), 8.6 (s, 1H), 9.09 (dd, 1H, J = 8.4, 2.2); ^{13}C NMR (DMSO-d_6) δ 104.0, 120.4, 125.6, 125.9, 126.4, 126.7, 127.4, 127.5, 128.5, 128.7, 130.7, 130.9, 133.9.

2′-(Naphth-2-yl)-5-cyano-2,5′-bi-1*H*-benzimidazole (2f). A solution of **5f** (149 mg, 0.55 mmol) and **1** (73 mg, 0.55 mmol) was treated as described for **2a**. Column chromatography using 50−100% EtOAc/hexanes provide 167 mg (80%) of a white solid: mp 254 °C dec; IR (KBr) 3119, 2220, 1436, 1293; ^{1}H NMR (CD_3Cl_3 + 3 drops of CD_3OD) δ 7.29−7.34 (m, 3H), 7.49 (d, 1H, J = 9.0), 7.56−7.80 (m, 6H), 7.85 (d, 1H, J = 9.1), 8.08 (s, 1H), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 105.0, 115.7, 120.3, 122.2, 123.4, 123.6, 126.2, 126.4, 127.0, 127.1, 127.7, 128.0, 128.8, 129.0, 133.3, 134.4, 154.3, 156.1.

General Procedure for the Preparation of 2-(Benzimidazol-5-yl)-5-(aminocarbonyl)benzimidazoles: 2′-Phenyl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3a). A solution of **2a** (170 mg, 0.51 mmol) in 3 mL of 30% MeOH/CH_2Cl_2 was cooled in an ice bath. To this solution were added 1.0 mL of 30% H_2O_2, 50 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH. The reaction mixture was allowed to stir overnight at room temperature. The CH_2Cl_2 layer was removed, and the H_2O layer was extracted with EtOAc (3 × 3 mL). The organic layers were combined, washed with brine and water, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography using a gradient from 100% EtOAc to 25% MeOH/EtOAc afforded 126 mg (70.4%) of a white solid: mp 242 °C dec; IR (KBr) 3364, 3169, 1651, 1621, 1441, 1390, 1287; ^{1}H NMR (CD_3OD) δ 7.49−7.52 (m, 3H), 7.59 (d, 1H, J = 8.4), 7.68 (d, 1H, J = 8.4), 7.78 (dd, 1H, J = 8.4, 1.8), 7.97 (dd, 1H, J = 8.5, 1.6), 8.05−8.09 (m, 2H), 8.16 (d, 1H, J = 1.3), 8.30 (d, 1H, J = 1.2); ^{13}C NMR (CD_3OD) δ 115.1, 115.2, 115.5, 116.3, 116.8, 123.3, 123.7, 125.4, 128.3, 129.7, 130.5, 130.8, 132.0, 155.7, 156.5, 173.2; HRMS (FAB) calcd for $C_{21}H_{16}N_5O$ (M^+ + 1) 354.1356, found 354.1361.

2′-*o*-Tolyl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3b). A biphasic reaction mixture consisting of a solution of **2b** (238 mg, 0.68 mmol) in 30% MeOH/CH_2Cl_2 in the presence of 1.0 mL of 30% H_2O_2, 50 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH was performed as outlined for **3a**. Column chromatography using a gradient from 100% EtOAc to 5% MeOH/EtOAc afforded 39 mg (16%) of a white solid: mp >290 °C; IR (KBr) 3389, 3180, 1648, 1387, 1287; ^{1}H NMR (CD_3OD) δ 2.53 (s, 3H), 7.28−7.46 (m, 3H), 7.63 (d, 2H, J = 7.7), 7.74 (d, 1H, J = 8.4), 7.81 (dd, 1H, J = 8.4, 1.6), 8.05 (dd, 1H, J = 8.5, 1.4), 8.19 (s, 1H), 8.38 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.3, 118.4, 119.5, 126.3, 129.8, 129.9, 131.6, 137.5, 168.7; HRMS (FAB) calcd for $C_{22}H_{18}N_5O$ (M^+ + 1) 368.1512, found 368.1502.

2′-*m*-Tolyl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3c). A biphasic reaction mixture consisting of a solution of **2c** (183 mg, 0.53 mmol) in 30% MeOH/CH_2Cl_2 in the presence of 1.0 mL of 30% H_2O_2, 38 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH was performed as outlined for **3a**. Column chromatography using a gradient from 0% to 10% MeOH/EtOAc afforded 22 mg (12%) of a white solid: mp 250 °C dec; IR (KBr) 3393, 3186, 1652, 1600, 1438, 1386; ^{1}H NMR (DMSO-d_6) δ 2.46 (s, 3H), 7.36−7.85 (m, 4H), 8.02−8.27 (m, 4H), 8.37 (s, 1H), 8.50 (d, 1H, J = 3.24); ^{13}C NMR (DMSO-d_6) δ 21.3, 124.1, 127.5, 129.2, 130.0, 131.2, 131.7, 138.6, 154.0, 168.7; HRMS (FAB) calcd for $C_{22}H_{18}N_5O$ (M^+ + 1) 368.1512, found 368.1517.

2′-*p*-Tolyl-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3d). A biphasic reaction mixture consisting of a solution of **2d** (200 mg, 0.57 mmol) in 30% MeOH/CH_2Cl_2 in the presence of 1.1 mL of 30% H_2O_2, 42 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH was performed as outlined for **3a**. Column chromatography using a gradient from 0% to 10% MeOH/EtOAc afforded 109 mg (52%) of a white solid: mp 245 °C dec; IR (KBr) 3374, 3180, 1659, 1609, 1393, 1296; ^{1}H NMR (CD_3OD) δ 2.43 (s, 3H), 7.38 (d, 2H, J = 8.0), 7.65 (d, 1H, J = 8.7), 7.74 (d, 1H, J = 8.4), 7.83 (dd, 1H, J = 8.2, 1.7), 8.02 (d, 2H, J = 8.2), 8.03 (dd, 1H, J = 7.0, 1.7), 8.19 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (CD_3OD) δ 21.8, 123.3, 123.7, 125.4, 128.0, 128.3, 129.7, 131.1, 131.2, 142.8, 156.0, 173.2; HRMS (FAB) calcd for $C_{22}H_{18}N_5O$ (M^+ + 1) 368.1512, found 368.1504.

2′-(Naphth-1-yl)-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3e). A biphasic reaction mixture consisting of a solution of **2e** (78 mg, 0.20 mmol) in 30% MeOH/CH_2Cl_2 in the presence of 0.3 mL of 30% H_2O_2, 14 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH was performed as outlined for **3a**. Column chromatography using a gradient from 0% to 10% MeOH/EtOAc afforded 63 mg (77%) of a white solid: mp >288 °C; IR (KBr) 3378, 3190, 1653, 1387; ^{1}H NMR (CD_3OD) δ 7.56−7.66 (m, 4H), 7.78−7.90 (m, 3H), 7.95−8.11 (m, 3H), 8.19 (d, 1H, J = 1.1), 8.43 (s, 1H), 8.49−8.53 (m, 1H); ^{13}C NMR (CD_3OD) δ 115.4, 115.5, 115.6, 116.4, 116.9, 123.4, 123.7, 125.6, 126.5, 126.9, 127.9, 128.7, 128.9, 129.7, 129.8, 129.9, 132.3, 132.7, 135.7, 155.7, 156.6, 173.2; HRMS (FAB) calcd for $C_{25}H_{18}N_5O$ (M^+ + 1) 404.1512, found 404.1523.

2′-(Naphth-2-yl)-5-(aminocarbonyl)-2,5′-bi-1*H*-benzimidazole (3f). A biphasic reaction mixture consisting of a solution of **2f** (122 mg, 0.32 mmol) in 30% MeOH/CH_2Cl_2 in the presence of 0.3 mL of 30% H_2O_2, 22 mg of tetrabutylammonium hydrogen sulfate, and 3 mL of 5 N NaOH was performed as outlined for **3a**. Column chromatography using a gradient from 0% to 10% MeOH/EtOAc afforded 85 mg (66%) of a white solid: mp 270 °C dec; IR (KBr) 3410, 3180, 1643, 1543, 1429, 1392, 1287; ^{1}H NMR (CD_3OD) δ 7.37−7.42 (m, 2H),

7.48 (d, 1H, J = 8.7), 7.57 (d, 1H, J = 8.5), 7.69 (dd, 2H, J = 9.1, 1.5), 7.77−7.85 (m, 3H), 7.99 (dd, 1H, J = 8.6, 1.7), 8.06 (s, 1H), 8.13 (s, 1H), 8.34 (s, 1H); ^{13}C NMR (CD_3OD) δ 114.9, 115.4, 116.3, 123.2, 123.6, 124.8, 125.2, 127.7, 128.0, 128.1, 128.6, 129.0, 129.5, 129.9, 130.1, 134.6, 135.8, 155.4, 156.2, 173.2; HRMS (FAB) calcd for $C_{25}H_{18}N_5O$ (M^+ + 1) 404.1512, found 404.1501.

General Procedure for the Preparation of 5-Cyanobenzimidazoles: 2-Phenyl-5-cyanobenzimidazole (4a). A solution of **1** (1.32 g, 9.9 mmol) and benzaldehyde (1.05 g, 9.9 mmol) in 20 mL of nitrobenzene was heated at 145 °C overnight under N_2 as described for **2a.** Column chromatography using 100% hexane to remove nitrobenzene and a gradient elution from 10% to 40% EtOAc/hexanes provided 1.17 g (54%) of a pale yellow solid. Recrystallization from EtOAc and hexanes provided white crystals (mp 210−212 °C) as the hydrochloride: mp 270−271 °C (lit.[16] mp 232−235 °C); IR (KBr) 3259, 3046, 2230, 1615, 1538, 1459, 1305; ^{1}H NMR ($CDCl_3$ + 2 drops of CD_3OD) δ 7.39−7.47 (m, 4H), 7.56 (d, 1H, J = 9.0), 7.85 (s, 1H), 8.01−8.06 (m, 2H); ^{13}C NMR ($CDCl_3$ + 2 drops of CD_3OD) δ 105.7, 120.0, 126.6, 127.4, 129.3, 129.6, 131.5. Due to hydration with H_2O ($C_{14}H_9N_3 \cdot {}^1/_3H_2O$), this compound failed to give satisfactory microanalysis for $C_{14}H_9N_3$. Anal. ($C_{14}H_9N_3 \cdot {}^1/_3H_2O$) C, H, N.

2-*o*-Tolyl-5-cyanobenzimidazole (4b). A solution of **1** (1.34 g, 10.1 mmol) and *o*-tolylaldehyde (1.21 g, 10.1 mmol) in 20 mL of nitrobenzene was treated as described for **4a** and provided 1.43 g (61%) of a pale yellow solid. Recrystallization from hexanes and EtOAc gave white needles: mp 185−187 °C; IR (KBr) 3067, 2790, 2667, 2216, 1453, 1282; ^{1}H NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 2.50 (s, 3H), 7.20−7.43 (m, 5H), 7.55 (d, 1H, J = 7.5), 7.62 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 21.0, 105.6, 120.4, 126.5, 126.7, 129.6, 130.2, 131.0, 131.9, 138.0, 156.0. Anal. ($C_{15}H_{11}N_3$) C, H, N.

2-*m*-Tolyl-5-cyanobenzimidazole (4c). A solution of **1** (1.29 g, 9.7 mmol) and *m*-tolylaldehyde (1.16 g, 9.7 mmol) in 20 mL of nitrobenzene was treated as described for **4a** and provided 1.54 g (69%) of a pale yellow solid. Recrystallization from hexanes and EtOAc gave pale orange flakes: mp 180−181 °C; IR (KBr) 3272, 3016, 2216, 1451, 1287; ^{1}H NMR ($CDCl_3$ + 5 drops of CD_3OD) δ 2.33 (s, 3H), 7.18−7.32 (m, 2H), 7.37 (dd, 1H, J = 8.4, 1.5), 7.54 (d, 1H, J = 8.4), 7.75−7.81 (m, 3H); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD) δ 21.7, 105.3, 120.6, 124.5, 126.2, 128.1, 129.1, 129.4, 132.2, 139.8, 157.9. Anal. ($C_{15}H_{11}N_3$) C, H, N.

2-*p*-Tolyl-5-cyanobenzimidazole (4d). A solution of **1** (1.27 g, 9.5 mmol) and *p*-tolylaldehyde (1.14 g, 9.5 mmol) in 20 mL of nitrobenzene was treated as described for **4a** and provided 1.43 g (64%) of a pale yellow solid. Recrystallization from hexanes and EtOAc gave white flakes: mp 243−244 °C; IR (KBr) 3259, 3026, 2227, 1619, 1551, 1449, 1374, 1304, 1234, 1188; ^{1}H NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 2.29 (s, 3H), 7.18 (d, 2H, J = 8.1), 7.35 (dd, 1H, J = 8.3, 1.5), 7.52 (d, 1H, J = 8.3), 7.79 (s, 1H), 7.83 (d, 2H, J = 8.1); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 21.7, 105.2, 120.4, 126.4, 127.3, 130.2, 141.8. Anal. ($C_{15}H_{11}N_3$) C, H, N.

2-(Naphth-1-yl)-5-cyanobenzimidazole (4e). A solution of **1** (1.12 g, 8.4 mmol) and 1-naphthylaldehyde (1.31 g, 8.4 mmol) in 20 mL of nitrobenzene was treated as described for **4a** and provided 1.46 g (64%) of a pale yellow solid. Recrystallization from hexanes and EtOAc gave pale orange crystals: mp 217−218 °C; IR (KBr) 3046, 2780, 1677, 2215, 1405, 1287; ^{1}H NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 7.41−7.52 (m, 5H), 7.73 (dd, 1H, J = 7.2, 1.2), 7.75−7.88 (m, 2H), 7.91 (d, 1H, J = 8.3), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 105.6, 120.5, 125.4, 125.8, 126.5, 127.0, 127.3, 127.9, 128.7, 129.0, 131.3, 131.6, 134.3. Anal. ($C_{18}H_{11}N_3$) C, H, N.

2-(Naphth-2-yl)-5-cyanobenzimidazole (4f). A solution of **1** (1.18 g, 8.9 mmol) and 2-naphthylaldehyde (1.39 g, 8.9 mmol) in 20 mL of nitrobenzene was treated as described for **4a** and provided 1.44 g (60%) of a pale yellow solid. Recrystallization from hexanes and EtOAc gave pale yellow needles: mp 237−238 °C; IR (KBr) 3261, 2232, 1535, 1444, 1286; ^{1}H NMR (DMSO-d_6) δ 7.62−7.66 (m, 4H), 7.80 (d, 1H, J = 9.0), 8.0−8.1 (m, 2H), 8.12 (d, 1H, J = 9.1), 8.34 (dd, 1H, J = 8.6, 1.6), 8.82 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 104.3, 120.3, 124.2, 126.9, 127.1, 127.4, 127.9, 128.1, 128.9, 129.0, 133.0, 134.1. Anal. ($C_{18}H_{11}N_3$) C, H, N.

General Method for the Conversion of 5-Cyanobenzimidazoles to 5-Formylbenzimidazoles: 2-Phenyl-5-formylbenzimidazole (5a). Using a similar procedure to that described previously,[17] Ni−Al (3.93 g) was added to a solution of **4a** (820 mg, 3.74 mmol) in formic acid (59.6 mL) and H_2O (19.7 mL). The reaction mixture was heated at 95 °C for 6 h. The hot mixture was filtered through a bed of Celite, and the reaction flask and Celite bed were rinsed with water. The aqueous solution was concentrated to dryness. After addition of H_2O to this residue, a white precipitate formed. The pH of this suspension was adjusted to 9 by the dropwise addition of 2 N NaOH. The product was obtained by extraction with EtOAc. The EtOAc extract was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography using a gradient of 0−50% EtOAc/hexanes to give 499 mg (60%) of a gum. Recrystallization with hexanes provided a white solid: mp 173−174 °C; IR (KBr) 3259, 3057, 2831, 1662, 1608, 1460, 1294; ^{1}H NMR ($CDCl_3$ + 3 drops of CD_3OD) 7.36−7.90 (m, 3H), 7.60 (d, 1H, J = 9.0), 7.71 (dd, 1H, J = 8.4, 1.5), 8.01−8.06 (m, 3H), 9.93 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD) δ 124.8, 127.4, 129.4, 129.5, 131.3, 132.0, 158.0, 193.1. Anal. ($C_{14}H_{10}N_2O$) C, H, N.

2-*o*-Tolyl-5-formylbenzimidazole (5b). A reaction mixture of Ni−Al (3.58 g) and **4b** (896 mg, 3.84 mmol) in formic acid (54.4 mL) and H_2O (17.9 mL) was treated as **5a**. Purification by column chromatography using a gradient of 30−40% EtOAc/hexanes gave 647 mg (71%) of a gum. Recrystallization with hexanes provided a white solid: mp 139−140 °C; IR (KBr) 3045, 2926, 2737, 1692, 1609, 1437, 1283, 1146; ^{1}H NMR δ 2.59 (s, 3H), 7.23−7.45 (m, 4H), 7.64 (d, 1H, J = 7.7), 7.81 (dd, 1H, J = 8.34, 1.34), 7.97 (s, 1H), 9.99 (s, 1H); ^{13}C NMR δ 21.3, 124.4, 124.5, 126.7, 129.8, 130.2, 130.9, 132.0, 132.3, 138.0, 171.8, 192.6. Anal. ($C_{15}H_{12}N_2O$) C, H, N.

2-*m*-Tolyl-5-formylbenzimidazole (5c). A reaction mixture of Ni−Al (4.62 g) and **4c** (1.03 g, 4.41 mmol) in formic acid (70.4 mL) and H_2O (23.2 mL) was treated as **5a**. Purification by column chromatography using 40% EtOAc/hexanes gave 535 mg (51%) of a gum. Recrystallization with hexanes provided a white solid: mp 163−165 °C; IR (KBr) 3045, 2926, 1686, 1615, 1419, 1276; ^{1}H NMR δ 2.20 (s, 3H), 7.20−7.32 (m, 2H), 7.65 (d, 1H, J = 8.3), 7.79 (d, 1H, J = 8.4), 7.96 (d, 1H, J = 9.8), 7.99 (s, 1H), 8.01 (s, 1H), 9.98 (s, 1H); ^{13}C NMR δ 21.7, 124.7, 125.1, 128.4, 129.4, 129.6, 132.2, 132.3, 139.6, 156.3, 193.0. Anal. ($C_{15}H_{12}N_2O$) C, H, N.

2-*p*-Tolyl-5-formylbenzimidazole (5d). A reaction mixture of Ni−Al (3.70 g) and **4d** (821 mg, 3.52 mmol) in formic acid (56.1 mL) and H_2O (18.5 mL) was treated as **5a**. Purification by column chromatography using a gradient of 30−40% EtOAc/hexanes gave 535 mg (64%) of a gum. Recrystallization with hexanes provided a white solid: mp 184−185 °C; IR (KBr) 3045, 2960, 1688, 1610, 1559, 1436, 1369, 1282, 1113; ^{1}H NMR δ 2.20 (s, 3H), 7.21 (d, 2H, J = 8.1), 7.64 (d, 1H, J = 8.2), 7.77 (dd, 1H, J = 8.4, 1.2), 8.06 (d, 2H, J = 8.1), 8.11 (s, 1H), 9.99 (s, 1H); ^{13}C NMR δ 21.9, 126.7, 127.5, 130.5, 132.1, 142.0, 193.0. Anal. ($C_{15}H_{12}N_2O$) C, H, N.

2-(Naphth-1-yl)-5-formylbenzimidazole (5e). A mixture of Ni−Al (1.60 g) and **4f** (454 mg, 1.69 mmol) in formic acid (25 mL) and H_2O (8 mL) was treated as **5a**. Purification by column chromatography using a gradient of 20−40% EtOAc/hexanes gave 293 mg (64%) of a gum. Recrystallization with hexanes provided a white solid: mp 198 °C; IR (KBr) 3049, 2955, 2720, 1678, 1614, 1531, 1367, 1273; ^{1}H NMR δ 7.20 (d, 1H, J = 7.5), 7.26−7.45 (m, 3H), 7.57 (d, 1H, J = 7.3), 7.64 (s, 1H), 7.66 (d, 1H, J = 8.4), 7.74−7.80 (m, 2H), 8.40 (d, 1H, J = 8.4), 9.79 (s, 1H); ^{13}C NMR δ 124.1, 125.3, 125.8, 127.0, 127.5, 127.8, 128.7, 128.9, 131.4, 131.5, 132.1, 134.2, 155.4, 192.6. Anal. ($C_{18}H_{12}N_2O$) C, H, N.

2-(Naphth-2-yl)-5-formylbenzimidazole (5f). A mixture of Ni−Al (2.56 g) and **4e** (731 mg, 2.71 mmol) in formic acid (38.4 mL) and H_2O (12.8 mL) was treated as **5a**. Purification by column chromatography using 40% EtOAc/hexanes gave 443 mg (60%) of a gum. Recrystallization with hexanes provided a white solid: mp 190−191 °C; IR (KBr) 3294, 1668, 1609, 1383, 1300, 1128; ^{1}H NMR δ 7.40−7.54 (m, 2H), 7.69−

7.86 (m, 5H), 8.12 (s, 1H), 8.18 (dd, 1H, J = 8.6, 1.8), 8.57 (s, 1H), 9.93 (s, 1H); ^{13}C NMR δ 124.0, 125.4, 126.6, 127.5, 127.6, 128.2, 128.3, 129.1, 129.6, 132.4, 133.5, 134.8, 155.6, 192.8. Anal. ($C_{18}H_{12}N_2O$) C, H, N.

5-(1-Methyl-4-piperazinyl)-2-nitroaniline (6). 5-Chloro-2-nitroaniline (2.01 g, 11.59 mmol), 1-methylpiperazine (2.58 mL, 23.18 mmol), and K_2CO_3 (1.6 g, mmol) in 20 mL of DMF was heated at 120 °C for 18 h. Purification on a silica gel column with 20% MeOH/EtOAc as eluant gave 2.14 g (78%) of a bright yellow solid: mp 153−4 °C (lit.[18] mp 155 °C); ^{1}H NMR δ 2.33 (s, 3H), 2.51 (t, 4H, J = 5.3), 3.37 (t, 4H, J = 5.1), 5.95 (d, 1H, J = 2.7), 6.16 (brs, 2H), 6.27 (dd, 1H, J = 9.7, 2.6), 8.00 (d, 1H, J = 9.7); ^{13}C NMR δ 46.8, 47.3, 55.1, 98.8, 106.2, 125.2, 128.7, 147.6, 156.0.

5-(1-Methyl-4-piperazinyl)-1,2-diaminobenzene (7). Compound **6** (464 mg, 1.96 mmol) was shaken with 45 psi of H_2 on 10% Pd−C (107 mg) in EtOAc for 6 h. Passing the reaction mixture through a bed of Celite and concentrating the solvent *in vacuo* gave 383 mg (95%) of an orange fluffy solid: mp 85 °C; ^{1}H NMR δ 2.30 (s, 3H), 2.52 (t, 4H, J = 5.3), 3.02 (t, 4H, J = 5.2), 3.29 (brs, 4H), 6.29 (dd, 1H, J = 8.1, 2.3), 6.31 (d, 1H, J = 2.2), 6.58 (d, 1H, J = 8.4); ^{13}C NMR δ 46.6, 51.0, 55.8, 106.5, 108.9, 118.6, 128.1, 136.8, 146.7. This phenylenediamine[18] was typically used directly after hydrogenation without purification.

Preparation of 5-(1-Methyl-4-piperazinyl)-2,5′-bi-1*H*-benzimidazoles. These bibenzimidazole derivatives were prepared as previously outlined for compounds **2a** and **4a**.

2′-Phenyl-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8a). A solution of **7** (130 mg, 0.63 mmol) and **5a** (140 mg, 0.63 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using a gradient from 0% to 50% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−30% MeOH/EtOAc; 80 mg (31%) was obtained: mp 224 °C (lit.[18] mp 190 °C); ^{1}H NMR (CD_3OD) δ 2.73 (s, 3H), 2.66 (t, 4H, J = 4.7), 3.18 (t, 4H, J = 4.5), 6.90 (dd, 1H, J = 8.4, 1.7), 7.40 (d, 1H, J = 2.0), 7.45−7.53 (m, 4H), 7.68 (d, 1H, J = 8.7), 7.93 (dd, 1H, J = 8.5, 1.7), 8.06−8.11 (m, 2H), 8.24 (s, 1H); ^{13}C NMR (CD_3OD) δ 46.2, 51.9, 56.3, 102.5, 116.7, 116.9, 123.0, 126.2, 128.2, 130.5, 130.9, 132.0, 149.8, 153.9, 155.4; HRMS (EI) calcd for $C_{25}H_{24}N_6$ 408.2062, found 408.2062.

2′-*o*-Tolyl-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8b). A solution of **7** (149 mg, 0.72 mmol) and **5b** (170 mg, 0.72 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using a gradient from 0% to 40% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−30% MeOH/EtOAc to provide 172 mg (57%) of a pale yellow solid: mp 248 °C; IR (KBr) 3067, 2933, 2800, 1437, 1280; ^{1}H NMR (CD_3OD) δ 2.38 (s, 3H), 2.55 (s, 3H), 2.68 (t, 4H, J = 4.9), 3.23 (t, 4H, J = 4.9), 7.06 (dd, 1H, J = 8.8, 2.2), 7.15 (s, 1H), 7.34−7.46 (m, 3H), 7.52 (d, 1H, J = 9.0), 7.66 (d, 1H, J = 8.0), 7.76 (d, 1H, J = 8.6), 8.02 (dd, 1H, J = 8.0, 1.1), 8.33 (s, 1H); ^{13}C NMR (CD_3OD) δ 20.9, 46.4, 52.1, 56.5, 116.7, 122.4, 126.2, 127.4, 131.2, 131.4, 131.5, 132.5, 138.9, 149.9; HRMS (FAB) calcd for $C_{26}H_{27}N_6$ (M^+ + 1) 423.2298, found 423.2296.

2′-*m*-Tolyl-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8c). A solution of **7** (209 mg, 1.02 mmol) and **5c** (240 mg, 1.02 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using a gradient from 0% to 20% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−20% MeOH/EtOAc. An orange solid, 370 mg (86%), was obtained: mp 235 °C dec (lit.[18] mp 236 °C); ^{1}H NMR (CD_3OD) δ 2.37 (s, 3H), 2.46 (s, 3H), 2.66 (t, 4H, J = 3.9), 3.20 (t, 4H, J = 4.0), 7.03 (dd, 1H, J = 8.8, 2.1), 7.12 (s, 1H), 7.35−7.52 (m, 3H), 7.70 (dd, 1H, J = 7.4, 2.3), 7.88−7.98 (m, 3H), 8.27 (s, 1H); ^{13}C NMR (CD_3OD) δ 21.8, 46.4, 52.1, 56.4, 116.7, 122.9, 123.0, 123.1, 125.4, 126.3, 128.8, 130.4, 130.8, 132.7, 140.5, 149.9.

2′-*p*-Tolyl-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8d). A solution of **7** (166 mg, 0.8 mmol) and **5d** (190 mg, 0.8 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using a gradient from 0% to 30% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−30% MeOH/EtOAc to provide 287 mg (85%) of a greenish white solid: mp 240 °C dec (lit.[18] mp >200 °C); ^{1}H NMR (CD_3OD) δ 2.36 (s, 3H), 2.40 (s, 3H), 2.65 (t, 4H, J = 4.7), 3.20 (t, 4H, J = 4.8), 7.03 (dd, 1H, J = 8.8, 2.2), 7.12 (s, 1H), 7.34 (d, 2H, J = 8.2), 7.50 (d, 1H, J = 8.7), 7.68 (d, 1H, J = 8.4), 7.92 (d, 1H, J = 1.7), 7.98 (d, 2H, J = 8.3), 8.25 (s, 1H); ^{13}C NMR (CD_3OD) δ 21.7, 46.4, 52.1, 56.4, 116.7, 122.9, 126.2, 128.2, 131.1, 142.6, 149.9.

2′-(Naphth-1-yl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8e). A solution of **7** (109 mg, 0.5 mmol) and **5e** (144 mg, 0.5 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using a gradient from 0% to 20% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−20% MeOH/EtOAc to provide 133.6 mg (55%) of an orange solid: mp 251−252 °C; IR (KBr) 3415, 3043, 2926, 2809, 1438, 1386, 1282; ^{1}H NMR (CD_3OD) δ 2.34 (s, 3H), 2.63 (t, 4H, J = 4.7), 3.19 (t, 4H, J = 4.3), 7.02 (dd, 1H, J = 8.8), 7.12 (s, 1H), 7.48−7.64 (m, 4H), 7.78 (d, 1H, J = 8.5), 7.88 (d, 1H, J = 7.2), 7.95−8.06 (m, 3H), 8.36 (s, 1H), 8.53 (m, 1H); ^{13}C NMR (CD_3OD) δ 46.4, 52.0, 56.4, 116.7, 123.1, 126.4, 126.5, 126.9, 127.9, 128.6, 129.0, 129.7, 129.9, 132.2, 132.7, 135.7, 149.9, 153.9, 155.4; HRMS (FAB) calcd for $C_{29}H_{27}N_6$ (M^+ + 1) 459.2298, found 459.2282.

2′-(Naphth-2-yl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (8f). A solution of **7** (87 mg, 0.4 mmol) and **5f** (115 mg, 0.4 mmol) in 3 mL of nitrobenzene was heated under N_2 at 145 °C overnight. Column chromatography using 50% MeOH/EtOAc gave the crude product which was further purified by chromatotron chromatography using 0−10% MeOH/EtOAc to provide 36 mg (18%) of an orange solid: mp 245−246 °C (lit.[19] mp 245 °C); ^{1}H NMR (CD_3OD) δ 2.7 (s, 3H), 3.14 (t, 4H, J = 4.68), 3.17 (t, 4H, J = 4.04), 6.91 (dd, 1H, J = 8.8, 2.2), 6.98 (s, 1H), 7.38−7.46 (m, 3H), 7.58 (d, 1H, J = 8.4), 7.79−7.86 (m, 4H), 8.04 (dd, 1H, J = 8.0, 1.7), 8.12 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (CD_3OD) δ 45.7, 51.4, 56.1, 102.6, 116.6, 116.7, 116.8, 122.9, 124.9, 125.9, 127.9, 128.0, 128.1, 128.7, 129.1, 129.9, 130.1, 134.7, 135.8, 140.3, 149.4, 153.8.

Topoisomerase I-Mediated DNA Cleavage Assays. DNA topoisomerase I was purified from calf thymus gland as reported previously.[20,21] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[22] The end-labeling of the plasmid was accomplished as previously described.[23] The cleavage assays were performed as previously reported.[9] The drug and DNA in the presence of topoisomerase I were 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).[24−26] 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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