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Heterocyclic Bibenzimidazole Derivatives as Topoisomerase I Inhibitors

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Abstract—A series of 2′-heterocyclic derivatives of 5-phenyl-2,5′-1*H*-bibenzimidazoles were evaluated for topoisomerase I poisoning activity and cytotoxicity. Topo I poisoning activity was associated with 2′-derivatives that possessed a hydrogen atom capable of hydrogen bond formation, suggesting that the interatomic distances between such hydrogen atoms and the heteroatoms on the adjacent benzimidazole influence activity. © 2000 Elsevier Science Ltd. All rights reserved.

Several benzimidazole derivatives are active as human topoisomerase I (topo I) poisons.[1–3] Hoechst 33258, 2′-(4-hydroxyphenyl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole (**1a**, NSC 32291, pibenzimol) and Hoechst 33342, 2′-(4-ethoxyphenyl)-5-(4-methylpiperazinyl)-2,5′-bi-1*H*-benzimidazole, **1b**, are representative members of this structurally-unique class of topo I poisons (Chart 1). More recently, terbenzimidazoles have been identified as topo I poisons.[4] These agents bind to the minor groove of DNA with AT + topo I specificity.[5,6] 5-Phenylterbenzimidazole **2**, and its derivatives are among the more extensively studied terbenzimidazoles. Recent studies with 5-phenyl-2″-substituted terbenzimidazoles revealed that modifications at the 2″-position influenced topo I poisoning activity as well as cytotoxicity.[7,8]

The addition of a methyl substituent at the 1″-position of 5-phenylterbenzimidazole results in a loss of topo I poisoning activity and cytotoxicity.[9] These data suggest that the hydrogen atom on one of the heteroatoms of the 2′-benzimidazole may be critical to the topo I poisoning activity of terbenzimidazoles. Alternatively, the *N*-methyl substituent on the 2′-benzimidazole could introduce a steric interaction unfavorable to stabilization of the cleavable complex consisting of topo I enzyme, DNA and drug. The present study examines the relative biological activity of 5-phenylbibenzimidazoles with a benzotriazole, quinoxaline, quinoxalinedione, or indole attached to their 2′-position. The biological activities of these varied heterocyclic bibenzimidazoles are discussed as they relate to their structure and physicochemical properties.

The synthetic approach used for the preparation of 2′-(benzotriazo-5-yl), 2′-(quinoxalin-6-yl), and 2′-(quinoxalin-2,3-dione-6-yl) derivatives of 5-phenylbibenzimidazole is outlined in Scheme 1. The common intermediate for the preparation of **4**–**6** was 5-phenyl-2-[2′-(3,4-phenylenediamine)]benzimidazol-5-yl)benzimidazole (**3**). This *o*-phenylenediamine was prepared as previously described by reduction of its dinitro analogue.[7,8] Compound **4** was prepared by the addition of $NaNO_2$ in H_2O to a solution of **3** in 0.1 N HCl as used in the preparation of benzotriazoles.[10] Compound **4** had to be protected from light to prevent decomposition. Compound **5** was synthesized by adding an aqueous solution of glyoxal to an aqueous suspension **3** at 70 °C.[11] Compound **6** was prepared by refluxing the *o*-phenylenediamine, **3**, together with oxalic acid in 4 N HCl.[12]

The pharmacological activities of these compounds are summarized in Table 1. The methods used in this study to evaluate relative topo I poisoning activity and cytotoxicty have been described.[7,8] The benzotriazole analogue (**4**), showed comparable activity to **1** both as a

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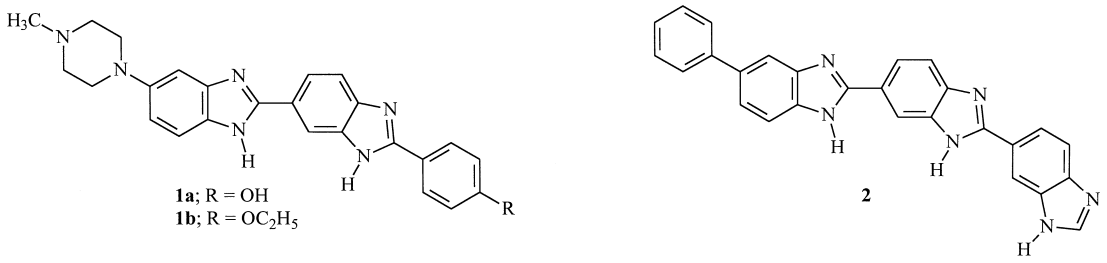
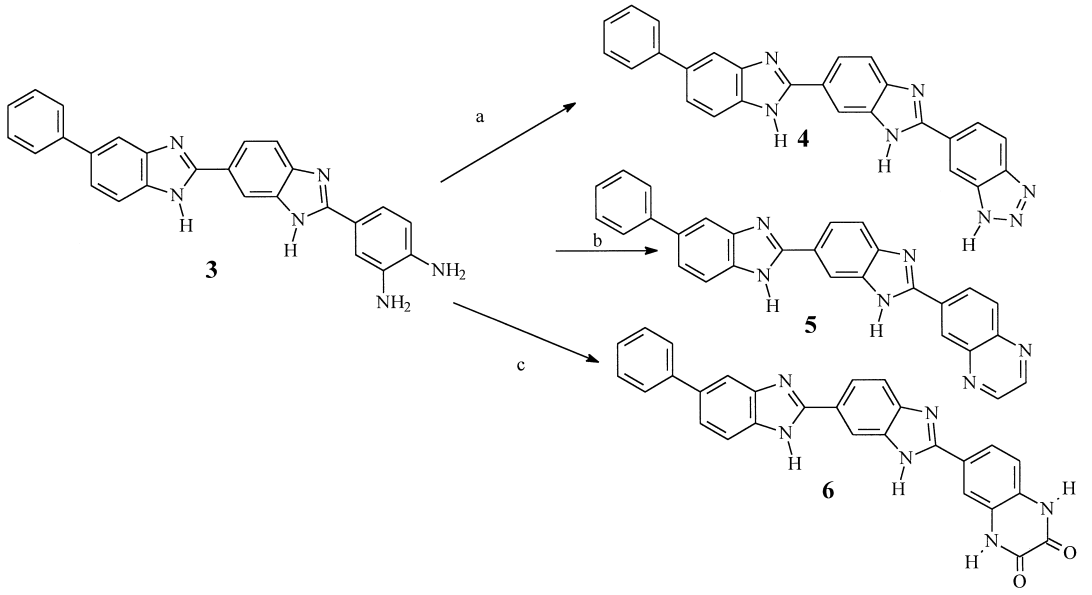


Chart 1.



Scheme 1. (a) $NaNO_2$, 0.1 N HCl, 10 °C; (b) HCOCOH/$NaHSO_3$; (c) Oxalic acid, 4 N HCl, reflux.

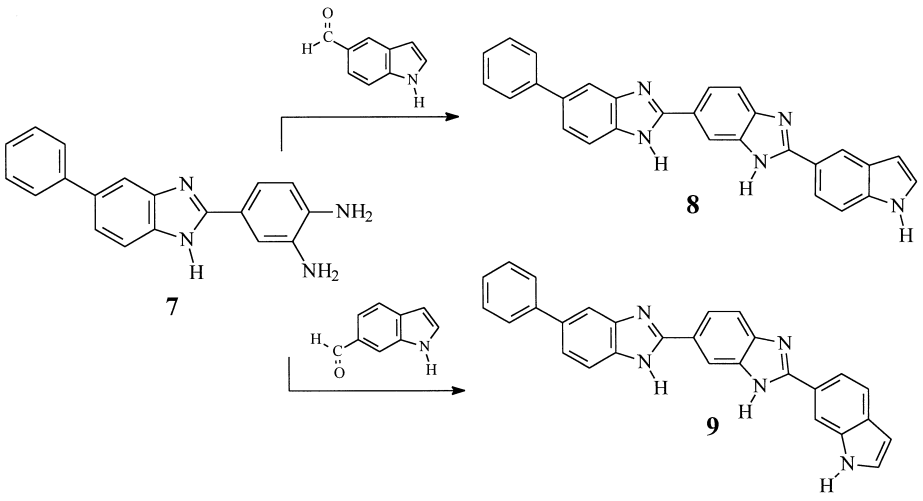
Table 1. Topoisomerase I activity, DNA binding affinity and cytotoxicity

Compound	Topo-I[a]	K_{Tm} (M^{-1})[b]	RPMI 8402 (IC_{50})[d]	CPT-K5 (IC_{50})[d]
2	1	2.8×10^8	0.09	0.70
4	1	n.d.[c]	0.47	0.47
5	>100	n.d.[c]	20	>20
6	1	n.d.[c]	2.3	21
8	50	3.5×10^7	0.28	0.38
9	5	1.9×10^8	0.015	0.2

[a]The values assigned reflect the relative effective concentrations of drug that are able to produce the same degree of cleavage on the plasmid DNA in the presence of human topoisomerase I. Assays were performed as previously described (ref 8).
[b]K_{Tm} denotes the drug-poly(dA)·poly(dT) association constant at the melting temperatures (Tm) of the drug–DNA complex and was determined as described previously (ref 8). The Tm values are as follows: 81.5, 73.4, and 80.0 °C for the poly(dA)·poly(dT) complexes with **2**, **8** and **9**, respectively.
[c]n.d. = not determined.
[d]RPMI 8402 is a human lymphoblast tumor cell line; CPT-K5 is a camptothecin-resistant variant cell line derived from RPMI 8402.

topo I poison and as a cytotoxic agent. No significant cross-resistance ($\Delta IC_{50}>10x$) was observed in the camptothecin-variant cell line, CPT-K5. The quinoxaline-2,3-dione analogue (**6**), was also as active as **1** as a topoisomerase I poison, but did not exhibit similar cytotoxicity toward RPMI 8402 cells. A significant decrease in overall activity was observed for the quinoxaline analogue, **5.** These data suggest for these 5-phenylbibenzimidazoles that 2′-heterocyclics with a hydrogen atom attached to a heteroatom have increased topo I poisoning activity and cytotoxicity. To assess more fully the possible spatial requirements associated with such hydrogen-donating functionality, the 2′-(indolo-5-yl) and 2′-(indolo-6-yl) derivatives of 5-phenylbibenzimidazole, **8** and **9**, were synthesized. The synthetic approach employed for the preparation of these indole derivatives is outlined in Scheme 2. The synthesis of the common intermediate **7** has been previously described.[7,8] Literature procedures were employed for the formation of 5-formylindole and 6-formylindole.[13] As can be seen in Table 1, there was a major difference in both topo I poisoning activity and cytotoxicity between **8** and **9**. Studies were performed to determine if differences in DNA binding affinity were associated with the differences observed for these isomers with regard to topo-I poisoning activity. The results of our DNA binding studies, as shown in Table 1, reveal that **9** does have a greater DNA binding affinity, which correlates with its greater potency relative to **8** as a topo I poison.

Molecular modeling was performed with **2**, **6**, **8** and **9** to determine the interatomic distance between either the

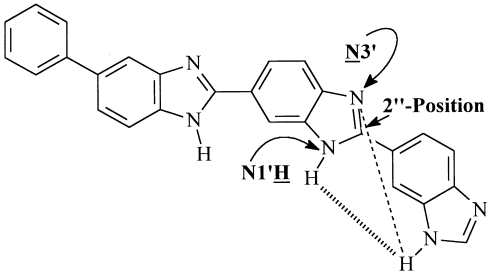


Scheme 2.

N3′ or the NH1′ atoms of the bibenzimidazole moiety and NH atoms incorporated within the various 2′-substituents.[14] Data were obtained from the more energetically favored conformations associated with rotation about the bond extending from the 2′-position to the attached heterocycle. These data are provided in Table 2. In the case of **2**, the benzimidazole extending from the 2′-position can be viewed as being attached at either its 5- or 6-position, in view of its ability to tautomerize. The two tautomers associated with this benzimidazole moiety are referred to as tautomer A or B depending upon whether the bibenzimidazole moiety is viewed as being attached to its 5- or 6-position, respectively. The increased interatomic distance between hydrogen acceptor and a hydrogen donor group or between hydrogen donor groups determined for **8** extends beyond the range observed for either of the tautomeric forms of **2**. This may be related to the decreased relative topo I poisoning activity for **8**. In contrast the interatomic distances between such functionality in the case of **6** and **9** is within the range observed for the tautomeric forms of **2** and may be associated with their retention of activity as topo I poisons.

These results suggest that several 2′-heterocyclic derivatives of 5-phenyl-2,5′-1*H*-bibenzimidazoles can be envisioned that would retain activity as topo I poisons. Heterocyclic derivatives that have incorporated within their structure a hydrogen atom capable of participating in hydrogen bond formation, can have topo I poisoning activity comparable to terbenzimidazole analogues. These data also indicate that DNA binding affinity could explain the differences in biological activity between the positional isomers **8** and **9**. In addition, the results of molecular modeling suggest that for retention of topo I poisoning activity, the distance between the NH1′ and N3′-position of the 2,5′-1*H*-bibenzimidazole portion of the molecule and a hydrogen donating

Table 2. Estimation of interatomic distances by molecular modeling



Compound	Reference atoms[a]	Distance (Å)[b]	Reference atoms[c]	Distance (Å)[b]
2	N3′-NH1″	5.7–6.6	NH1′-NH1″	5.2–6.9
6	N3′-NH1″; N3′-NH4″	5.2–7.2	NH1′-NH1″; NH1′-NH4″	4.7–7.1
8	N3′-NH1″	7.2–7.3	NH1′-NH1″	7.0–7.2
9	N3′-NH1″	5.6–6.5	NH1′-NH1″	5.1–6.8

[a]Reference atoms include the N3′ atom, which can act as a hydrogen acceptor and one or more NH substituents associated with the 2′-heterocycle that are capable of acting as hydrogen donors in the formation of a hydrogen bonding interaction.
[b]Interatomic distances were calculated by molecular modeling using the AM1 semi-empirical method.[14] The range obtained reflects the values for four of the lower energy conformations.
[c]Reference atoms include the NH1′ atom, which can act as a hydrogen donor, and one or more NH substituents associated with the 2′-heterocycle that are capable of acting as hydrogen donors in the formation of a hydrogen bonding interaction.

substituent attached at the 2′position should be < 7.0 Å and < 7.2 Å, respectively.

Experimental

5-Phenyl-2-(2′-(benzotriazol-5-yl)benzimidazol-5′-yl)benzimidazole (4). The *o*-phenylenediamine **3** (58 mg, 0.14 mmol) was dissolved in 0.1 N HCl. This solution was placed in an ice bath and while maintaining a reaction temperature below 10 °C, $NaNO_2$ (10.2 mg) in 5 mL water was added dropwise. As described in literature,[10] the reaction was stirred for 15 min and neutralized with 0.1 N KOH. Extraction with ethyl acetate followed by chromatographic separation with 10% methanol/ethyl acetate gave 42 mg (71%) of a dark-brown solid which had to be immediately stored in an amber vial because of its light sensitivity. Compound **4** had: mp >280 °C; IR (KBr) 3385, 3128, 3056, 1626, 1431, 1287; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.47–7.61 (m, 3H), 7.79–8.07 (m, 6H), 8.15–8.19 (m, 2H), 8.40 (d, 1H, J = 9.0 Hz), 8.63 (s, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 107.4, 111.7, 114.1, 114.6, 115.9, 116.3, 117.8, 122.3, 123.2, 125.5, 125.6, 126.6, 128.0, 129.2, 129.5, 131.9, 133.2, 134.7, 138.7, 139.8, 141.4, 147.1, 150.7, 154.3; HRMS (FAB) calcd for $C_{26}H_{17}N_7$ (MH^+) 428.1624, found 428.1622.

5-Phenyl-2-(2′-(quinoxalin-6-yl)benzimidazol-5′-yl)benzimidazole (5). The *o*-phenylenediamine **3** (55 mg, 0.13 mmol) was dissolved in water (4 mL) and heated to 70 °C as described by Johns and McLaughlin.[11] Glyoxal·2$NaHSO_3$ (50 mg, 0.13 mmol) was dissolved in hot water (80 °C, 3 mL) and added to the diamine slowly. After 15 min, the reaction was cooled to room temperature and Na_2CO_3 was added. Extraction with ether followed by chromatographic separation with 10% methanol/ethyl acetate gave 38 mg (67%) of yellow solid. Compound **5** had: mp 235 °C; IR (KBr) 3385, 3169, 1624, 1554, 1431, 1297; ^{1}H NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 7.46–7.61 (m, 3H), 7.80 (d, 2H, J = 8.0 Hz), 7.89–8.26 (m, 5H), 8.36 (d, 1H, J = 9.0), 8.69–8.78 (m, 2H), 9.04–9.10 (m, 3H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 111.7, 114.6, 116.5, 116.6, 117.9, 123.5, 123.9, 125.6, 127.5, 128.1, 128.2, 128.3, 128.6, 130.6, 131.6, 132.9, 138.9, 139.1, 139.7, 142.5, 143.7, 143.8, 147.3, 150.5, 153.1; HRMS (FAB) calcd for $C_{28}H_{19}N_6$ (MH^+) 439.1671, found 439.1677.

5-Phenyl-2-(2′-(quinoxalin-2,3-dione-6-yl)benzimidazol-5′-yl)benzimidazole (6). The *o*-phenylenediamine **3** (40 mg, 0.096 mmol) and oxalic acid (20 mg, 0.22 mmol) in 4 N HCl were refluxed overnight similar to procedure detailed in the literature.[12] The product precipitated out as a brownish solid to give 15 mg (33%). Compound **6** had: mp >280 °C; IR (KBr) 3339, 3217, 2845, 1623, 1578, 1506, 1469, 1272; ^{1}H NMR (DMSO-d_6) δ 6.96 (d, 1H, J = 9.0 Hz), 7.41–7.60 (m, 4H), 7.77–8.00 (m, 7H), 8.32 (d, 1H, J = 9.0 Hz), 8.57 (s, 1H); ^{13}C NMR (DMSO-d_6 + 3 drops of CF_3COOH) δ 106.5, 107.4, 111.7, 114.1, 114.7, 115.2, 116.7, 119.5, 122.3, 124.7, 125.7, 127.5, 128.2, 129.4, 131.9, 133.2, 138.8, 139.7, 139.8, 149.7, 152.7, 158.2; HRMS (FAB) calcd for $C_{28}H_{19}N_6O_2$ (MH^+) 471.1569, found 471.1584.

5-Phenyl-2-(2′-(indole-5-yl)benzimidazol-5′-yl)benzimidazole (8). 2-(3,4-Diaminophenyl)-5-phenyl-benzimidazole (220 mg, 0.73 mmol) and 5-formylindole (95 mg, 0.65 mmol) in nitrobenzene (5 mL) were heated at 145 °C overnight. Nitrobenzene was removed using a Kugelrohr and the compound purified by flash column chromatography. Chromatographic separation with 80% ethyl acetate/hexanes provided 116 mg (37%) of pure yellow solid: mp >270 °C; IR (KBr) 3422, 1624, 1545, 1443; ^{1}H NMR (CD_3OD + 3 drops CF_3COOH) δ 6.56 (m, 1H), 7.21–7.34 (m, 4H), 7.34–7.55 (m, 4H), 7.59–7.72 (m, 4H), 7.89 (m, 2H), 8.20 (m, 1H); ^{13}C NMR (CD_3OD + 3 drops CF_3COOH) δ 103.8, 113.2, 113.9, 114.3, 116.3, 121.2, 121.79, 122.81, 123.5, 123.7, 125.4, 127.9, 128.2, 128.6, 130.09, 130.14, 137.9, 139.4, 140.9, 143.4, 155.3, 157.8. HRMS (FAB) calcd for $C_{28}N_5H_{19}$ (MH^+) 426.1719, found 426.1722.

5-Phenyl-2-(2′-(indole-6-yl)benzimidazol-5′-yl)benzimidazole (9). 2-(3,4-Diaminophenyl)-5-phenylbenzimidazole (200 mg, 0.69 mmol) and 6-formylindole (100 mg, 0.69 mmol) in nitrobezene (5 mL) were heated at 145 °C overnight. Nitrobenzene was removed using a Kugelrohr and the compound purified by flash column chromatography. Chromatographic separation using 80% ethyl acetate/hexanes provided 117 mg (40%) of a pure yellow solid: mp >270 °C; IR (KBr) 3417, 1625, 1550, 1444; ^{1}H NMR (CD_3OD + 3 drops CF_3COOH) δ 6.47–6.49 (m, 2H), 7.33–7.36 (m, 4H), 7.42–7.65 (m, 4H) 7.89–7.92 (m, 4H), 8.15–8.24 (m, 2H); ^{13}C NMR (CD_3OD + 3 drops CF_3COOH) δ 103.19, 111.83, 113.89, 116.35, 119.3, 122.12, 122.86, 123.63, 125.45, 128.12, 128.52, 128.81, 130.10, 131.92, 137.88, 140.88, 143.37, 155.22, 157.49. HRMS (FAB) calcd for $C_{28}N_5H_{19}$ (MH^+) 426.1719, found 426.1714.

Acknowledgements

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9. A mixture of 2[2′-(1″-methylbenzimidazol-5″-yl)benzimidazol-5′-yl]benzimidazole and 2[2′-(1″-methylbenzimidazol-6″-yl)benzimidazol-5′-yl]benzimidazole were prepared by the coupling of intermediate **7** with a mixture of 1-methyl-5-formyl- and 1-methyl-6-formylbenzimidazole. Bioassays were performed with the resulting mixture of products.
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14. Molecular modeling was performed with PC Spartan *Pro*, Wavefunction, Inc. (Irvine, CA). Four of the lower energy conformers associated with rotation about the 2′-bond between the bibenzenzimidazole and the attached heterocycle were selected and interatomic bond lengths between the atoms specified in Table 2 were determined.