Pergamon

*Bioorganic & Medicinal Chemistry*, Vol. 4, No. 4, pp 621–630, 1996

0968-0896/96 $15.00 + 0.00

S0968-0896(96)00047-8

# Structure–Activity Relationships of Benzimidazoles and Related Heterocycles as Topoisomerase I Poisons

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**Abstract**—A series of substituted 2-(4-methoxyphenyl)-1*H*-benzimidazoles were synthesized and evaluated as inhibitors of topoisomerase I. The presence of a 5-formyl-, 5-(aminocarbonyl)-, or 5-nitro group (i.e., substituents capable of acting as hydrogen bond acceptors) correlated with the potential of select derivatives to inhibit topoisomerase I. In contrast to bi- and terbenzimidazoles, the substituted benzimidazoles that were active as topoisomerase I poisons exhibited minimum or no DNA binding affinity. 5-Nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole exhibited the highest activity and was significantly more active than the 4-nitro positional isomer. The 5- and 6-nitro derivatives of 2-(4-methoxyphenyl)benzoxazole, 2-(4-methoxyphenyl)benzothiazole, and 2-(4-methoxyphenyl)indole were synthesized and their relative activity as topoisomerase I inhibitors determined. None of these heterocyclic analogues were effective in significantly inhibiting cleavable-complex formation in the presence of DNA and topoisomerase I, suggesting a high degree of structural specificity associated with the interaction of these substituted benzimidazoles with the enzyme or the enzyme–DNA complex. In evaluating their cytotoxicity, these new topoisomerase I poisons also exhibited no significant cross-resistance against cell lines that express camptothecin-resistant topoisomerase I. Copyright © 1996 Elsevier Science Ltd

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## Introduction

DNA topoisomerases represent a unique class of nuclear enzymes that alter the topological state of DNA by breaking and rejoining the phosphodiester backbone of DNA.[1–3] Topoisomerase inhibitors as a class of pharmacological agents have the potential to exhibit antineoplastic activity as well as selective antibacterial activity.[2] Mammalian topoisomerase I is capable of altering the topology of DNA by transiently breaking one DNA strand while topoisomerase II enzymes alter the topological state of DNA by means of a double strand break in the DNA. Inhibitors of topoisomerase that interfere with the breaking and rejoining reactions of these enzymes by trapping an abortive enzyme–DNA cleavable complex have been termed topoisomerase poisons.[2] These agents differ in the mechanism of action from the classical view of an enzyme inhibitor. It is the stabilization of the cleavable complex that is responsible for DNA fragmentation and cytotoxicity.[2] Mammalian topoisomerase II is well known as an effective pharmacological target for the development of cancer chemotherapeutics.[4–6] Etoposide (VP-16), teniposide (VM-26), mitoxantrone, *m*-AMSA, adriamycin (doxorubicin), ellipticine, and daunomycin are among the cancer chemotherapeutic agents in clinical use which are established topoisomerase II inhibitors.

Topoisomerase I poisoning has recently been recognized as an attractive pharmacological target for the development of novel chemotherapeutics.[7–10] Comparatively few topoisomerase I poisons, relative to topoisomerase II poisons, have been identified. Camptothecin and its structurally related analogues are among the more extensively investigated topoisomerase I poisons. Although derivatives of camptothecin such as CPT-11 and topotecan (NSC-609699) are likely to be among the first mammalian topoisomerase I poisons to be made available for general clinical use, these compounds contain a labile lactone moiety which is critical for cytotoxicity. This labile pharmacophore is responsible for the short half life in vivo and is thus one of the less desirable chemical properties associated with several of the camptothecin analogues. The broad spectrum of potent antineoplastic activity observed for camptothecin[11,12] has prompted further efforts to identify other agents which can effectively poison mammalian topoisomerase I. Among the agents which have been identified as topoisomerase I poisons are the alkaloids fagaronine,[13] nitidine as well as derivatives of chelerythrine[14] and berberine,[15] and the fungal metabolites, bulgarein[16] and saintopin.[17] Indolocarbazoles related to the antibiotic K252a are also topoisomerase I poisons which induce cleavable complex formation with DNA.[18]

It has recently been demonstrated that Hoechst 33342, 2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole, is a topoisomerase I poison.[19,20] One limitation of Hoechst 33342 as an anticancer agent is that it is significantly less cytotoxic toward tumor cell

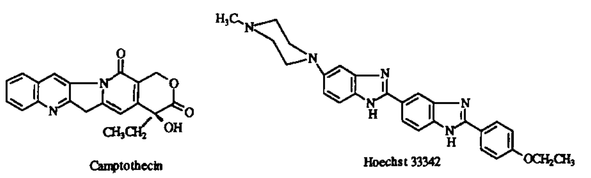
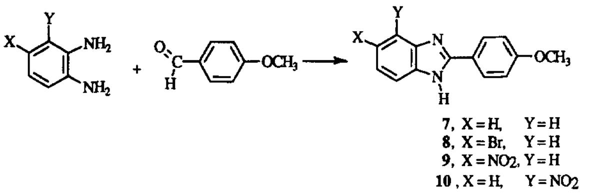


Chart 1. Structure of the topoisomerase I inhibitors, camptothecin and Hoechst 33342.



Scheme 2.

lines which overexpress MDR1. The structure–activity relationships observed for various bibenzimidazoles as they relate to topoisomerase I poisoning and cytotoxicity have been recently reported.[21] It has also been recently observed that specific terbenzimidazole derivatives exhibit similar activity as topoisomerase I poisons and, in contrast to Hoechst 33342, are not substrates for MDR1.[22]

Several of the synthetic intermediates required for the preparation of these bi- and terbenzimidazoles were evaluated and revealed that select derivatives did possess activity as topoisomerase I inhibitors. In the present study the structure–activity associated with 2,5-substituted benzimidazoles as topoisomerase I poisons and their relative cytotoxic activity against human lymphoblastoma, RPMI 8402 cells, and its camptothecin-resistant variant CPT-K5 were investigated. The potential of similarly substituted benzoxazoles, benzothiazoles, and indoles to exhibit activity as topoisomerase I inhibitors was also evaluated.

## Chemistry

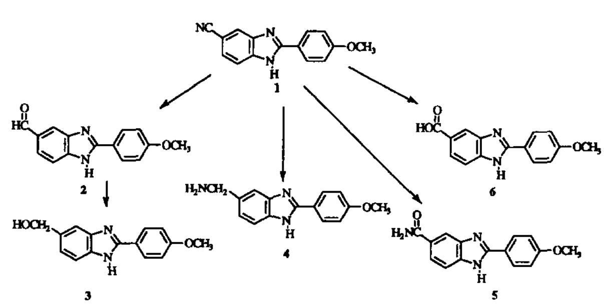
The 5-cyano-2-(4-methoxyphenyl)-1*H*-benzimidazole (**1**) was synthesized and converted to 5-formyl-2-(4-methoxyphenyl)-1*H*-benzimidazole (**2**) as previously described.[22] As outlined in Scheme 1, the cyano derivative **1** served as a versatile intermediate for the formation of the various 5-substituted benzimidazoles (**3**–**6**). Reduction of **2** with Na(CN)$BH_3$ provided the benzyl alcohol derivative **3**. The procedures employed for conversion of **1** to the benzylamine,[23] the amide,[24] and the acid[25] derivatives were adopted from literature procedures employed with similar compounds.

The influence of structure on the ability of these benzimidazoles to inhibit topoisomerase I was further explored by the preparation of compounds **7**–**10** as outlined in Scheme 2. These benzimidazoles were synthesized in yields ranging from 48 to 89% by reaction of the appropriate phenylenediamine with *p*-methoxybenzaldehyde in nitrobenzene at 150 °C.

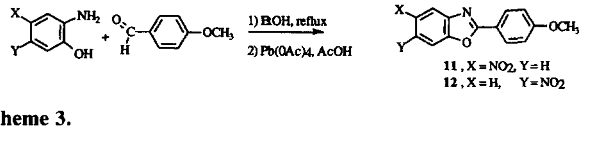
The benzoxazole analogues related to 5-nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole (**9**) were synthesized as outlined in Scheme 3 using an approach previously reported for the preparation of similar benzoxazoles.[26] In contrast to **9** where tautomerization of the benzimidazole makes 5- and 6-nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole indistinguishable, there exists two distinct isomers in the structurally related benzoxazole analogues. Thus, 5-nitrobenzoxazole (**11**) and 6-nitrobenzoxazole (**12**) were each synthesized by this unambiguous approach. The yields obtained for the preparation of **11** and **12**, were 68 and 80%, respectively.

The preparation of the benzothiazole analogues related to **9** was performed by methods similar to those previously reported.[27] The synthetic approach which was used for these analogues is outlined in Scheme 4. Either the 4- or 5-nitro derivative of *o*-chloroaniline was reacted with *p*-methoxybenzoylchloride to yield the *p*-methoxybenzamides **13** and **14** in 82 and 88% yields, respectively. These intermediates were converted to their thiobenzamides, **15** and **16**, in 42 and 47% yields, respectively, by reaction with $P_2S_5$ in toluene. Cyclization of these thiobenzamides was accomplished by heating **15** or **16** in 2-pyrrolidone at 100 °C in the presence of NaOH to provide the benzothiazole derivatives, **17** and **18**, in yields of 77 and 60%, respectively.

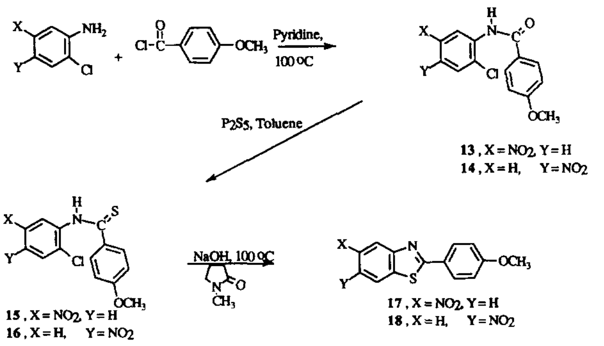
The preparation of 5-nitro-2-(4-methoxyphenyl)indole and 6-nitro(4-methoxyphenyl)indole was accomplished as outlined in Scheme 5. The Heck reaction[28] was employed to couple 4-vinylanisole to either 4-nitro-2-bromoaniline (**19**) or 5-nitro-2-bromoaniline (**20**). The resulting nitrostilbene derivatives (**21** and **22**) were treated with tosyl chloride in pyridine to form the tosyl derivatives (**23** and **24**). Cyclization of **23** and **24** to their indole derivatives, **25** and **26**, respectively, was accomplished using palladium acetate as catalyst and *p*-benzoquinone as a reoxidant.[29] Because of the



Scheme 1.



Scheme 3.

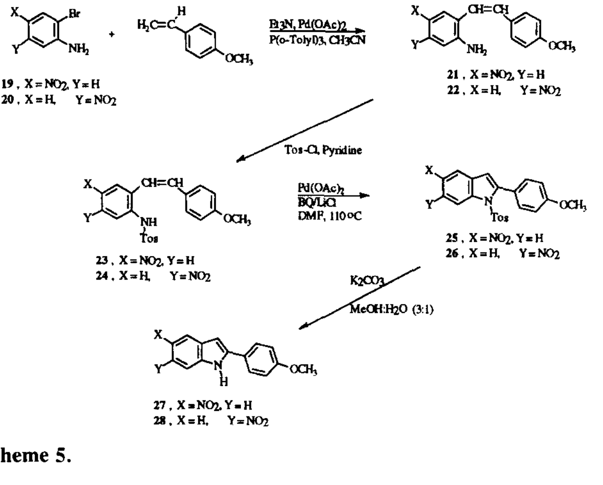


Scheme 4.

instability of the tosyl group upon cyclization to the indole, **25** and **26** were not characterized, but directly hydrolyzed with $K_2CO_3$ to provide the desired indole derivatives, **27** and **28**.

## Results and Discussion

The relative potencies of compounds **1–10** with regard to induction of topoisomerase I-mediated DNA cleavage are provided in Table 1. In a comparison of the topoisomerase I activity of those compounds illustrated in Scheme 1, it was evident that compounds **2** and **5** had significant activity as topoisomerase I poisons. Only very marginal activity was observed for compound **3**, while compounds **1**, **4**, and **6** were essentially inactive. These results suggest that the presence of a substituent that can act exclusively as a hydrogen acceptor at the 5-position of these 2-(4-methoxyphenyl)-1*H*-benzimidazoles is associated with an increase in potency as a topoisomerase I poison. Those analogues with 5-substituents that either possess a formal charge at physiological pH or are capable of acting as hydrogen donors were, in general, less active. Greater selectivity in the mode of interaction with the enzyme–DNA complex of those derivatives which are exclusively hydrogen acceptors may be associated with their increased potency as topoisomerase I poisons. Additional confirmation of this structure–activity relationship was provided in an analysis of the relative topoisomerase I activity of **7–9**. While both **7** and **8** were devoid of any significant activity, 5-nitro-(4-methoxyphenyl)-1*H*-benzimidazole, **9**, was active as a topoisomerase I poison. In a comparison of the topoisomerase I poisoning ability of the isomeric nitrobenzimidazole **9** and **10** as topoisomerase I poisons, it was evident that the presence of the nitro substituent at the 5(6)-position was critically linked to activity. It is possible that the intramolecular hydrogen bonding which could occur with 4(7)-nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole (**10**) could be responsible for the diminished activity observed for this positional isomer. The induced DNA cleavage which occurred in the presence of topoisomerase I with various concentrations of **2**, **5**, **9**, and **10** is illustrated in Figure 1.



Scheme 5.

Hoechst 33342 is a bibenzimidazole derivative which has been demonstrated to be a poison of mammalian topoisomerase I.[21] Recently, terbenzimidazole derivatives have also been reported to be potent topoisomerase I poisons.[22] In contrast to these terbenzimidazoles which can bind to DNA, no DNA binding affinity was observed with **9**. Using ethidium bromide displacement as an index of relative DNA binding, **9** possessed less than 0.001% of the relative binding affinity of Hoechst 33342 (unpublished results). The DNA cleavage pattern which was observed for the substituted benzimidazoles in the current study was clearly different from the DNA fragmentation pattern previously observed with either Hoechst 33342 or certain terbenzimidazole derivatives. The gel provided

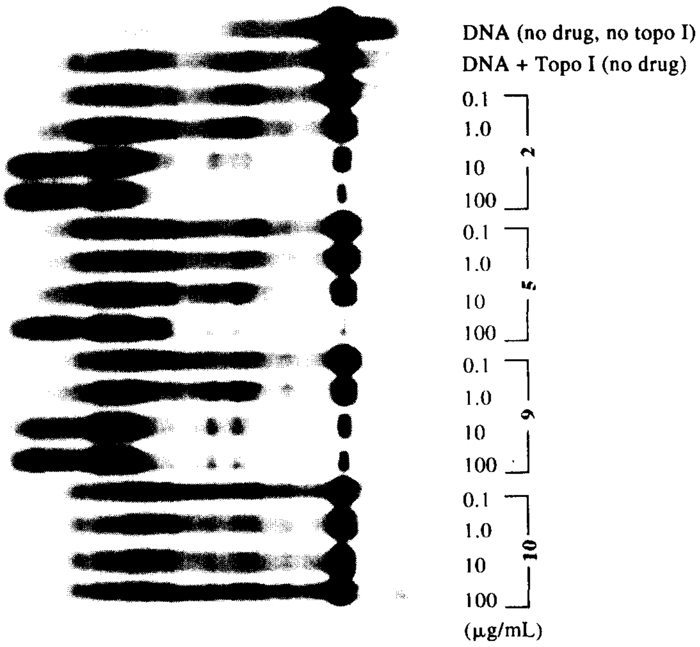


**Figure 1.** Stimulation of enzyme-mediated DNA cleavage by substituted 2-(4-methoxyphenyl)benzimidazoles using calf thymus topoisomerase I. The top lane is the DNA control without topoisomerase I. The second lane from the left in each panel is the control with topoisomerase alone. The rest of the lanes are the topoisomerase I and serially (10-fold each) diluted compound from 0.1 to 100 µg/mL for compounds **2**, **5**, **9**, and **10**.

**Table 1.** Topoisomerase I-mediated DNA cleavage and cytotoxicity of 4- and 5-substituted benzimidazoles

| Compound | Topo I-mediated DNA cleavage[b] | Cytotoxicity $IC_{50}$ ($\mu$M)[a] | |
|---|---|---|---|
| | | RPMI 8402 | CPT-K5 |
| **1** | ≥1000 | 16 | 16 |
| **2** | 1 | 36 | 50 |
| **3** | 100 | 98 | 98 |
| **4** | ≥1000 | 119 | 59 |
| **5** | 10 | 66 | 75 |
| **6** | ≥1000 | >150[c] | >150[c] |
| **7** | ≥1000 | 45 | 36 |
| **8** | ≥1000 | 2.3 | 1.5 |
| **9** | 1 | 28 | 46 |
| **10** | ≥1000 | 11 | 3.7 |
| **11** | inactive | >150[c] | >150[c] |
| **12** | inactive | >150[c] | >150[c] |
| **17** | inactive | >150[c] | >150[c] |
| **18** | inactive | >150[c] | >150[c] |
| **27** | inactive | 130 | 131 |
| **28** | inactive | 93 | 93 |

[a] $IC_{50}$ has been calculated after 4 days of continuous drug exposure. $IC_{50}$ of camptothecin and Hoechst 33342 in RPMI 8402 cells is 0.006 and 0.03 $\mu$M and in CPT-K5 cells is >60 and 0.9 $\mu$M, respectively.

[b] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e. concentrations relative to compound **9**, 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. Compound **9** has 0.2% of the potency of camptothecin and approximately 0.3% of the potency of Hoechst 33342 as a topoisomerase I poison.

[c] No indication of cytotoxicity was considered indicative of $IC_{50}$ values substantially greater than the highest doses assayed.

in Figure 2 clearly illustrates the differences in the DNA fragmentation pattern obtained with either **9**, Hoechst 33342, the terbenzimidazole, 5-phenyl-2-[benzimidazo-2′-(benzimidazo-5″-yl)-5′-yl]benzimidazole (QS-II-48), or camptothecin (CPT) in the presence of topoisomerase I. The DNA fragmentation pattern observed with these substituted 2-(4-methoxyphenyl)benzimidazoles more closely resembles that observed with camptothecin. Comparisons of relative extents of DNA fragmentation observed with these substituted 2-(4-methoxyphenyl)benzimidazoles, therefore, can be more acccurately determined using camptothecin as the positive control. Compound **9** possesses approximately 0.2% of activity of camptothecin as a topoisomerase I poison. The fact that the cleavage pattern of these select benzimidazole derivatives is distinct from that of Hoechst 33342, but identical to that of camptothecin is consistent with the absence of significant DNA binding affinity.

The structure–activity associated with these substituted 2-(4-methoxyphenyl)benzimidazoles as topoisomerase I poisons was extended into an investigation as to whether a benzoxazole, benzothiazole, or indole ring could be substituted for the benzimidazole moiety. The tautomerization that occurs in the case of 5-substituted benzimidazole derivatives such as 5-nitro-(4-methoxyphenyl)-1*H*-benzimidazole makes it indistinguishable for its 6-nitro positional isomer. This is not the case with the various heterocyclic ring systems which were

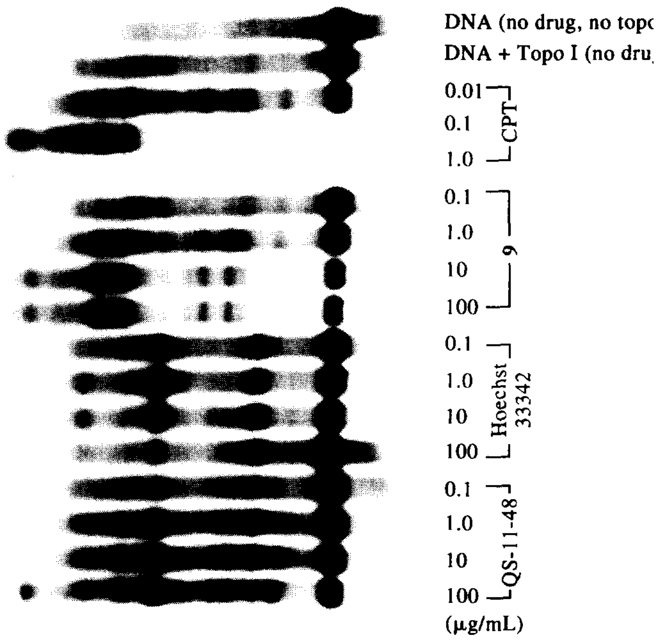


**Figure 2.** Stimulation of enzyme-mediated DNA cleavage by **9**, camptothecin (CPT), bi- and terbenzimidazoles using calf thymus DNA topoisomerase I. The assays were performed as described in Figure 1 for the comparison of the DNA fragmentation pattern of **9** with Hoechst 33342, QS-II-48, and CPT. The relative doses employed ranged from 0.01 to 1.0 µg/mL for CPT, 0.1 to 100 µg/mL for **9**, Hoechst 33342, and QS-II-48.

investigated in this study. As compound **9** represents the most potent topoisomerase I poison among the benzimidazoles evaluated in this study, 5- and 6-nitro-2-(4-methoxyphenyl)benzoxazole, 5- and 6-nitro-2-(4-methoxyphenyl)benzothiazole, and 5- and 6-nitro-2-(4-methoxyphenyl)indole analogues were synthesized and evaluated as topoisomerase I poisons. Assays performed to assess the poisoning of topoisomerase I demonstrated that both 5- and 6-nitro-2-(4-methoxyphenyl)benzoxazole, **11** and **12**, were inactive. These data suggest that the NH of 5(6)-nitro-2-(4-methoxyphenyl)benzimidazole may be critically linked to its pharmacological activity. This is consistent with the lack of activity observed with either 5- or 6-nitro-2-(4-methoxyphenyl)benzothiazole, **17** and **18**. The absence of any activity observed with 5- and 6-nitro-2-(4-methoxyphenyl)indole, **27** and **28**, further suggests that the benzimidazole moiety with both of its N-heteroatoms is critical for the retention of activity within this series of topoisomerase I poisons.

The cytotoxicity of these various 2-(4-methoxyphenyl)-1*H*-benzimidazoles and their structurally related benzoxazoles, benzothiazoles, and indole analogues was determined in the human lymphoblast cell line, RPMI 8402, and in its camptothecin resistant variant cell line, CPT-K5. The absence of any topoisomerase I inhibition by the benzoxazole, benzothiazole, and indole analogues evaluated in this study is consistent with their lack of appreciable cytotoxicity. The $IC_{50}$ values for the benzoxazole analogues, **11** and **12**, and the benzothiazole derivatives, **17** and **18**, were >150 μM for both RPMI 8402 and CPT-K5 cells while those for **9** were 28 and 46 μM, respectively. The indole derivatives were only slightly more cytotoxic with $IC_{50}$ values in RPMI 8402 cells of 130 and 93 μM for **27** and **28**, respectively. Similar $IC_{50}$ values were obtained with **27** and **28** in CPT-K5 cells.

One of the interesting developments from these studies was the observation that most of these compounds exhibited less cross-resistance to the CPT-K5 cell line. $IC_{50}$ in RPMI 8402 and CPT-K5 for **9** was 28 and 46 μM, for **2** was 36 and 50 μM and those of **5** were 66 and 75 μM, respectively. The cytotoxic potential of some of the substituted benzimidazoles, however, did not correlate with their potency as topoisomerase I poisons. This is clearly evident in the case of the 5-bromo derivative, **8**, which has very weak activity as a topoisomerase I inhibitor, but is the most cytotoxic of the benzimidazole derivatives in this series with an $IC_{50}$ of 2.3 μM in RPMI 8402 and 1.5 μM in CPT-K5 cells. In addition, 4-nitro-2-(4-methoxyphenyl)benzimidazole, **10**, which is also several orders of magnitude less potent as a topoisomerase I inhibitor than **9**, exhibits comparable cytotoxic activity. These data indicate that mechanisms other than inhibition of topoisomerase may be associated with the cytotoxicity of some of these benzimidazoles. Alternatively, there exists the possibility that differences in the ability of some of these analogues to cross cell membranes could obscure the detection of a correlation between cytotoxicity and relative potency as a topoisomerase I poison.

These data indicate that there are well defined structure–activity requirements associated with the ability of 2-(4-methoxyphenyl)benzimidazoles and related compounds to inhibit topoisomerase I. The critical importance of these simple substituted benzimidazoles is that they represent a new lead for developing novel topoisomerase I drugs. They do not contain the labile lactone ring which has been linked to the rapid inactivation of camptothecin in vivo. Moreover, they could complement camptothecin-type antitumor drugs as no cross-resistance was observed with most of these analogues. Nonetheless, significant improvements in overall potency are required if related compounds are to be considered as effective agents for this pharmacological target. Studies are in progress to examine whether further structural modifications, especially with regard to substituents at the 2-position, can result in enhancement of the topoisomerase I inhibition and cytotoxicity of 5-nitrobenzimidazoles.

## Experimental

### General

Melting points were determined with a Thomas–Hoover Unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin–Elmer 1600 Fourier transform spectrophotometer and are reported in $cm^{-1}$. $^1H$ and $^{13}C$ NMR 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. A few drops of $CF_3COOH$ improved $^{13}C$ spectra by allowing for increased solubility and formation of the protonated form of these benzimidazoles, thereby eliminating tautomeric differences among carbon atoms. Mass spectra were obtained from Midwest Center for Mass Spectrometry within the Department of Chemistry at the University of Nebraska-Lincoln. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm, (ICN Biomedicals, Eschwegge), using the solvent systems indicated. Chromatotron chromatography refers to the use of a Model 8924 chromatotron (Harrison Research, CA). Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within ±0.4%.

**5-Hydroxymethyl-2-(4-methoxyphenyl)-1*H*-benzimidazole (3).** 5-Formyl-2-(4-methoxyphenyl)-1*H*-benzimidazole (**2**, 279 mg, 1.11 mmol) and a trace of Bromocresol Green were dissolved in methanol (5 mL), and $Na(CN)BH_3$ (45 mg, 0.72 mmol) was added. The soln immediately turned deep blue, and 1 N HCl in methanol was added dropwise to restore the yellow color. The solution was stirred for 3 h with the occasional addition of the HCl:methanol soln to maintain the yellow color. The reaction mixture was concd in vacuo and the residue dissolved in ethyl acetate, washed with 10% sodium bicarbonate and brine, then dried ($MgSO_4$). The ethyl acetate solution was filtered and concd in vacuo. The residue was chromatographed eluting with 0–3% methanol:ethyl acetate to give 201 mg (71%) of **3** as a white solid; mp 221–222 °C; IR (KBr) 3413, 3079, 2926, 1612, 1490, 1430, 1254, 1180, 1011; $^1H$ NMR (DMSO-$d_6$+3 drops of $CF_3COOH$): δ 3.92 (s, 3H), 4.70 (s, 2H), 7.32 (d, 2H, $J=9$), 7.50 (d, 1H, $J=9$), 7.75–7.80 (m, 2H), 8.20 (d, 2H, $J=9$); $^{13}C$ NMR (DMSO-$d_6$+3 drops $CF_3COOH$) δ 56.1, 62.8, 111.1, 113.5, 115.2, 115.5, 124.8, 130.1, 130.7, 131.9, 141.4, 149.1, 163.5; Anal. calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N 11.02; found: C, 70.93; H, 5.62; N, 10.98.

**5-(Aminomethyl)-2-(4-methoxyphenyl)-1*H*-benzimidazole (4).** Hydrogenation of **1** (45 mg, 0.18 mmol) was accomplished at 40 psi $H_2$ at room temperature overnight using $PtO_2$ (33 mg) in EtOH:$CHCl_3$ (25:1). The reaction mixture was filtered and concd in vacuo to afford crude product. The crude product was chromatographed directly on silica gel using 40% methanol:ethyl acetate as eluent to give 39 mg (86%) of **4** as a white solid; mp 280 °C (dec); IR (KBr) 3428, 2933, 1611, 1503, 1266, 1189, 1092; $^1H$ NMR (DMSO-$d_6$+3 drops of $CF_3COOH$): δ 3.96 (s, 3H), 4.36 (s, 2H), 7.29 (d, 2H, $J=9$), 7.71 (dd, 1H, $J=9$), 7.88 (d, 1H, $J=9$), 7.99 (s, 1H), 8.17 (d, 2H, $J=9$); $^{13}C$ NMR (DMSO-$d_6$+3 drops of $CF_3COOH$): δ 44.4, 56.8, 115.7, 115.8, 116.0, 117.0, 128.8, 131.5, 133.3, 133.6, 152.5, 166.3; HRMS (EI) calcd for $C_{15}H_{15}N_3O$ *m*/*z* 253.1215, found *m*/*z* 253.1205.

**5-(Aminocarbonyl)-2-(4-methoxyphenyl)-1*H*-benzimidazole (5).** A solution of **1** ( 51 mg, 0.21 mmol) in 2 mL of 30% methanol : methylene chloride was cooled in an ice bath. To this solution was added 1.0 mL of 30% $H_2O_2$, 35 mg of tetrabutylammonmium hydrogen sulfate and 2 mL of 5 N NaOH. The reaction mixture was allowed to stir overnight at room temperature. The methylene chloride layer was sepd and the $H_2O$ layer extracted with ethyl acetate (2 × 5 mL). The organic layers were combined, washed with water and brine, then dried ($Na_2SO_4$). The ethyl acetate solution was filtered and concd in vacuo. Column chromatography using 20–40% ethyl acetate : hexanes afforded 33 mg (60%) of **5** as a white solid: mp 195 °C (dec); IR (KBr) 3457, 3164, 1661, 1611, 1391, 1253; $^1H$ NMR (DMSO-$d_6$ + 3 drops of $CF_3COOH$): δ 3.93 (s, 3H), 7.34 (d, 2H, $J = 9$), 7.87 (d, 1H, $J = 9$), 8.09 (dd, 1H, $J = 9$, 1.5), 8.23–8.27 (m, 3H); $^{13}C$ NMR (DMSO-$d_6$ + 3 drops of $CF_3COOH$): δ 56.1, 113.4, 113.6, 115.0, 115.6, 125.5, 130.5, 131.7, 132.2, 133.7, 150.9, 163.8, 167.2; HRMS calcd for $C_{15}H_{13}N_3O_2$ *m/z* 267.1008, found *m/z* 267.1002.

**5-Carboxy-2-(4-methoxyphenyl)-1*H*-benzimidazole (6).** A solution of **1** (170 mg, 0.68 mmol) in 10 mL of ethanol and 7 mL of 6 N NaOH was refluxed overnight. The reaction mixture was cooled to room temperature and acidified to pH ≈4–5 by addition of 1 N HCl to give a light yellow precipitate. After filtration, the crude product was chromatographed using 20% methanol : ethyl acetate to give 151 mg (83%) of **6**; a white solid; mp >275 °C ; IR (KBr) 3418, 1690, 1611, 1505, 1086, 943; $^1H$ NMR (DMSO-$d_6$ + 3 drops $CF_3COOH$): δ 3.93 (s, 3H), 7.34 (d, 2H, $J = 9$), 7.90 (d, 1H, $J = 9$), 8.14 (dd, 1H, $J = 9$, 1.5), 8.23 (d, 2H, $J = 9$), 8.32 (s, 1H); HRMS (EI) calcd for $C_{15}H_{12}N_2O_2$ *m/z* 252.0899, found *m/z* 252.089.

**2-(4-Methoxyphenyl)-1*H*-benzimidazole (7).** 1,2-Phenylenediamine (488 mg, 4.51 mmol) and *p*-anisaldehyde (542 mg, 4.51 mmol) in 3 mL nitrobenzene at 145 °C gave 900 mg of white solid (89%) after column chromatography using 10–30% ethyl acetate : hexanes. Recrystallization from ethyl acetate provided white needles; mp 229–230 °C (lit.[30] mp 231 °C); IR (KBr) 3056, 2935, 1610, 1443, 1255; $^1H$ NMR (DMSO-$d_6$): δ 3.85 (s, 3H), 7.13 (d, 2H, $J = 9$), 7.16–7.21 (m, 2H), 7.53–7.60 (m, 2H), 8.14 (d, 2H, $J = 9$); $^{13}C$ NMR (DMSO-$d_6$): δ 56.1, 114.0, 115.2, 115.6, 126.0, 130.2, 131.9, 149.3, 163.6.

**5-Bromo-2-(4-methoxyphenyl)-1*H*-benzimidazole (8).** A mixture of *p*-anisaldehyde (178 mg, 1.31 mmol) and 5-bromophenylenediamine (243 mg, 1.31 mmol) in nitrobenzene was heated at 150 °C under $N_2$ overnight. The mixture was cooled to room temperature and chromatographed using 10–30% ethyl acetate : hexanes to afford 241 mg (61%) of **8**; white solid; mp 191.5–193 °C (dec); IR (KBr) 3426, 3071, 2959, 1614, 1493, 1438, 1257; $^1H$ NMR (DMSO-$d_6$ + 3 drops of $CF_3COOH$): δ 3.92 (s, 3H), 7.30 (d, 2H, $J = 9$), 7.68 (dd, 1H, $J = 9$, 1.5), 7.78 (d, 1H, $J = 9$), 8.02 (d, 1H, $J = 1.5$), 8.19 (d, 2H, $J = 9$); $^{13}C$ NMR (DMSO-$d_6$ + 3 drops $CF_3COOH$): δ 56.0, 112.6, 115.0, 115.5, 115.8, 116.6, 118.0, 118.3, 128.9, 130.3, 131.3, 133.4, 150.4, 163.8; Anal. calcd for $C_{14}H_{11}BrN_2O$: C, 55.47; H, 3.66; N, 9.24; found: C, 55.28; H, 3.76; N, 9.12.

**5-Nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole (9).** Using the procedures detailed for the preparation of **8**, a mixture of *p*-anisaldehyde (224 mg, 1.65 mmol) and 5-nitrophenylenediamine (253 mg, 1.65 mmol) were reacted to provide 212 mg (48%) of **9**; white solid; mp 237–238 °C; IR (KBr) 3296, 2931, 1612, 1500, 1448, 1290, 1180; $^1H$ NMR (DMSO-$d_6$ + 3 drops of $CF_3COOH$): δ 3.90 (s, 3H), 7.27 (d, 2H, $J = 9$), 7.91 (d, 1H, $J = 9$), 8.20 (d, 2H, $J = 9$), 8.30 (d, 1H, $J = 9$), 8.55 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$ + 3 drops $CF_3COOH$): δ 55.9, 110.7, 114.7, 115.4, 116.4, 120.4, 130.3, 133.9, 138.2, 144.5, 154.1, 163.6; Anal. calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61; found: C, 62.42; H, 4.15; N, 15.53.

**4-Nitro-2-(4-methoxyphenyl)-1*H*-benzimidazole (10).** Using the procedures detailed for the preparation of **8**, a mixture of *p*-anisaldehyde (445 mg, 3.27 mmol) and 4-nitrophenylenediamine (500 mg, 3.27 mmol) were reacted to provide 693 mg (79%) of **10** as a white solid: mp 232–233.5 °C; IR (KBr) 3406, 1612, 1492, 1340, 1254; $^1H$ NMR (DMSO-$d_6$ + 3 drops of $CF_3COOH$): δ 3.89 (s, 3H), 7.20 (d, 2H, $J = 9$), 7.59 (t, 2H, $J = 8$), 8.16 (d, 1H, $J = 8$), 8.23–8.32 (m, 3H); $^{13}C$ NMR (DMSO-$d_6$ + 3 drops $CF_3COOH$): δ 55.8, 114.8, 116.9, 120.6, 122.4, 124.2, 128.1, 131.1, 134.6, 138.3, 153.9, 163.2; Anal. calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61; found: C, 62.43; H, 4.16; N, 15.53.

**2-(4-Methoxyphenyl)-5-nitrobenzoxazole (11).** The synthetic approach for this compound was adopted from the literature.[26] 2-Amino-4-nitrophenol (300 mg, 1.95 mmol) and *p*-anisaldehyde (265 mg, 1.95 mmol) were refluxed in 10 mL ethanol for 3 h. After the reaction mixture was cooled, ethanol was removed in vacuo. The resulting Schiff base was dissolved in 12 mL of acetic acid and lead tetraacetate (864 mg, 1.95 mmol) was added and stirred at room temperature for 1 h. Reaction was then diluted with 30 mL $H_2O$ and extracted with ethyl acetate. Product combined with some residual *p*-anisaldehyde was obtained after column chromatography using 0–10% ethyl acetate : hexanes. The aldehyde was removed by trituration with hexane to provide 360 mg (68% yield) of the desired benzoxazole, **11**; reddish white solid; mp 180–181 °C (lit.[31] 182–184 °C); IR (KBr) 1610, 1503, 1339, 1246, 1169, 1015; $^1H$ NMR: δ 3.91 (s, 3H), 7.05 (d, 2H, $J = 9$), 7.63 (d, 1H, $J = 9$), 8.27 (d, 2H, $J = 9$), 8.26 (dd, 1H, $J = 9$, 2), 8.59 (d, 1H, $J = 2$); $^{13}C$ NMR δ 56.1, 110.9, 115.1, 116.3, 118.9, 121.2, 130.4, 144.9, 148.2, 150.5, 164.1, 168.1; HRMS (EI) calcd for $C_{14}H_{10}N_2O_4$ *m/z* 270.0640, found *m/z* 270.0641.

**2-(4-Methoxyphenyl)-6-nitrobenzoxazole (12).** The title compound was prepared following the procedure described for **11**. The reaction of 2-amino-5-nitrophenol (339 mg, 2.20 mmol) and *p*-anisaldehyde (299 mg, 2.20 mmol) gave 475 mg (80%) of **12**; reddish white solid; mp 214–215 °C (lit.[32] 216–217 °C); IR

(KBr) 1606, 1505, 1342, 1261, 1175, 1119, 1012; $^1$H NMR: δ 3.92 (s, 3H), 7.06 (d, 2H, $J=9$), 7.79 (d, 1H, $J=9$), 8.23 (d, 2H, $J=9$), 8.31 (dd, 1H, $J=9$, 2), 8.46 (d, 1H, $J=2$); $^{13}$C NMR: δ 56.1, 107.4, 115.5, 118.8, 119.7, 121.3, 130.5, 145.9, 148.3, 150.3, 163.9, 168.1; HRMS (EI) calcd for $C_{14}H_{10}N_2O_4$ *m/z* 270.0640, found *m/z* 270.0643.

**4-Methoxy-2′-chloro-5′-nitrobenzanilide (13).** The synthetic approach was adopted from the literature.[27] *p*-Anisoyl chloride (1.26 g, 7.39 mmol) was added dropwise with cooling to a stirred solution of 2-chloro-5-nitroaniline (1.27 g, 7.47 mmol) in pyridine. After addition, the mixture was heated at 100 °C for 30 min and then cooled to room temperature. Water was added and the precipitate was collected and washed with water. Purification on a silica gel column eluting with 10% ethyl acetate: hexanes gave 2.01 g of a pale yellow solid (88%). Recrystallization from methylene chloride and hexanes gave pale yellow needles; mp 193–194 °C; IR (Nujol) 3267, 1687, 1604, 1519, 1458, 1343; $^1$H NMR: δ 3.90 (s, 3H), 7.03 (d, 2H, $J=9$), 7.58 (d, 1H, $J=9$), 7.90 (d, 2H, $J=9$), 7.94 (dd, 1H, $J=9$, 2.5), 8.48 (br s, NH), 9.52 (d, 1H, $J=2.5$); $^{13}$C NMR: δ 56.1, 114.6, 116.6, 119.3, 126.2, 129.5, 129.2, 129.9, 136.4, 147.8, 163.7, 163.2; Anal. calcd for $C_{14}H_{11}ClN_2O_4$: C, 54.83; H, 3.62; N, 9.13; found: C, 54.70; H, 3.66; N, 9.23.

**4-Methoxy-2′-chloro-4′-nitrobenzanilide (14).** The title compound was obtained by the procedure described for **13**. *p*-Anisoyl chloride (1.98 g, 11.6 mmol) and 2-chloro-4-nitroaniline (2 g, 11.6 mmol) gave 2.9 g of a pale yellow solid (82%). Recrystallization from methylene chloride and hexanes gave pale yellow needles: mp 158–159 °C; IR ($CHCl_3$) 3413, 3036, 1684, 1604, 1598, 1586, 1345; $^1$H NMR: δ 3.90 (s, 3H), 7.02 (d, 2H, $J=9$), 7.89 (d, 2H, $J=9$), 8.20 (dd, 1H, $J=9$, 2), 8.32 (d, 1H, $J=2$), 8.63 (br s, NH), 8.84 (d, 1H, $J=9$); $^{13}$C NMR: δ 56.1, 114.9, 120.5, 122.9, 124.2, 125.2, 126.1, 129.7, 141.7, 142.0, 163.9, 165.2; Anal. calcd for $C_{14}H_{11}ClN_2O_4$: C, 54.83; H, 3.62; N, 9.13; found: C, 54.60; H, 3.53; N, 9.02.

**4-Methoxy-2′-chloro-5′-nitrothiobenzanilide (15).** Phosphorus pentasulfide (714 mg, 3.24 mmol) was added to a solution of **13** (660 mg, 2.2 mmol) in toluene (20 mL) and the mixture heated at 70 °C for 20 h. After the reaction was cooled and evapd to dryness, the residual solid was chromatographed on silica gel with 50–60% chloroform: hexanes as eluent to afford 292 mg (42%) of product as white needles after recrystallization from methylene chloride and hexanes: mp 161–162 °C; IR (KBr) 3355, 1599, 1512, 1347, 1248, 1178; $^1$H NMR: δ 3.90 (s, 3H), 6.97 (d, 2H, $J=9$), 7.66 (d, 1H, $J=9$), 7.93 (d, 2H, $J=9$), 8.08 (dd, 1H, $J=9$, 2.5), 9.30 (br s, NH), 9.89 (d, 1H, $J=2.5$); $^{13}$C NMR: δ 56.0, 114.4, 119.9, 121.7, 129.1, 130.4, 133.3, 135.3, 137.3, 146.9, 163.5, 198.1; Anal. calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10; H, 3.43; N, 8.68; found: C, 51.96; H, 3.44; N, 8.58.

**4-Methoxy-2′-chloro-4′-nitrothiobenzanilide (16).** Phosphorus pentasulfide (1.35 g, 6.05 mmol) was added to a solution of **14** (1.23 g, 4.03 mmol) in toluene (20 mL) and the mixture was refluxed for 20 h. After the reaction was cooled and evapd to dryness, the residual solid was chromatographed on silica gel with 10% ethyl acetate: hexanes as eluent to afford 617 mg (48%) of product as white needles after recrystallization from methylene chloride and hexanes; mp 153–154 °C; IR ($CHCl_3$) 3365, 2975, 2829, 1598, 1580, 1544, 1507, 1345; $^1$H NMR: δ 3.90 (s, 3H), 7.00 (d, 2H, $J=7$), 7.92 (d, 2H, $J=7$), 8.22 (dd, 1H, $J=9$, 2.5), 8.39 (d, 1H, $J=2.5$), 9.30 (d, 1H, $J=9$), 9.50 (br s, NH); $^{13}$C NMR: δ 56.1, 114.6, 123.1, 123.4, 125.4, 126.1, 129.4, 135.7, 141.8, 144.6, 163.9, 198.0; Anal. calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10; H, 3.44; N, 8.68; found: C, 51.92; H, 3.51; N, 8.58.

**2-(4-Methoxyphenyl)-5-nitrobenzothiazole (17).** To a solution of **15** (262 mg, 0.81 mmol) in *N*-methyl-2-pyrrolidone (10 mL) was added sodium methoxide (66 mg, 1.23 mmol) and the mixture was heated at 110 °C for 3.5 h. The cooled reaction mixture was poured into water and the resulting precipitate was collected and dried. Separation on a silica gel chromatotron plate with 50% hexanes: chloroform gave 180 mg (77%) of yellow solid; mp 213–214 °C; IR (KBr) 1604, 1512, 1489, 1344, 1259, 1179; $^1$H NMR: δ 3.90 (s, 3H), 7.03 (d, 2H, $J=7$), 7.99 (d, 1H, $J=9$), 8.05 (d, 2H, $J=7$), 8.23 (dd, 1H, $J=9$, 2), 8.85 (d, 1H, $J=2$); $^{13}$C NMR: δ 56.1, 115.1, 118.6, 119.7, 122.3, 125.9, 129.9, 142.0, 147.4, 154.6, 163.2, 171.7; HRMS (EI) calcd for $C_{14}H_{10}N_2O_3S$ *m/z* 286.0412, found *m/z* 286.0411.

**2-(4-Methoxyphenyl)-6-nitrobenzothiazole (18).** The title compound was prepared following the procedure described for **17**. Compound **16** (227 mg, 0.71 mmol) in *N*-methyl-2-pyrrolidone (10 mL) and sodium methoxide (57 mg, 1.06 mmol) gave 120 mg (60%) of yellow solid; mp 196–197 °C (lit.[33] 188–190 °C); IR (KBr) 1595, 1472, 1340, 1246; $^1$H NMR: δ 3.91 (s, 3H), 7.03 (d, 2H, $J=9$), 8.07 (d, 3H, $J=9$), 8.35 (dd, 1H, $J=9$, 2), 8.80 (d, 1H, $J=2$); $^{13}$C NMR: δ 56.1, 115.1, 118.6, 123.4, 123.2, 126.0, 130.2, 135.6, 142.6, 158.6, 163.4; HRMS (EI) calcd for $C_{14}H_{10}N_2O_3S$ *m/z* 286.0412, found *m/z* 286.0410.

**2-Bromo-4-nitroaniline (19).** 4-Nitroaniline (1.08 g, 7.8 mmol) was dissolved in 100 mL methylene chloride in a salt/ice bath (−10 °C) and stirred for 3 h after the addition of 2,4,4,6-tetrabromo-2,5-cyclohexadienone (3.20 g, 7.8 mmol). The reaction was washed with NaOH and $H_2O$. Methylene chloride was concd in vacuo and the resulting mixture was separated on a silica gel column with 10% ethyl acetate: hexanes to give 1.08 g (64%) of yellow solid; mp 105 °C (lit.[34] 104.5 °C); IR (KBr) 3481, 3372, 1623, 1581, 1485, 1311, 1118; $^1$H NMR: δ 4.85 (br s, 2H), 6.75 (d, 1H, $J=9$), 8.03 (dd, 1H, $J=9$, 2.5), 8.37 (d, 1H, $J=2.5$); $^{13}$C NMR: δ 107.5, 113.9 125.4, 129.7, 139.4, 150.3.

**2-Amino-5-nitro-4′-methoxystilbene (21).** Heck reaction[28] was carried out with *p*-vinylanisole (830 mg, 6.19 mmol) and **19** (1.07 g, 4.95 mmol) in 15 mL DMF in a sealed tube after addition of triethylamine (1.7 mL, 12.4 mmol), tri(*o*-tolyl)phosphine (30.12 mg, 0.099 mmol) and $Pd(OAc)_2$ (11.12 mg, 0.05 mmol) for 3 days at 120 °C. Reaction was neutralized with 10% HCl and extracted with ether and dried in $Na_2SO_4$. Purification on a silica gel column eluting with 10% ethyl acetate: hexanes provided 1.32 g (99%) of yellow solid which was recrystallized in methylene chloride and hexanes to give bright yellow needles; mp 141–142 °C; IR (KBr) 3440, 3342, 1631, 1576 1487, 1293, 1172; $^1$H NMR ($CDCl_3$+3 drops $CD_3OD$): δ 3.84 (s, 3H), 4.59 (br s, 2H), 6.66 (d, 1H, *J*=9), 6.85 (d, 1H, *J*=16), 6.91 (d, 2H, *J*=9), 7.04 (d, 1H, *J*=16), 7.45 (d, 2H, *J*=9), 7.97 (dd, 1H, *J*=9, 2.5), 8.27 (d, 1H, *J*=2.5); $^{13}$C NMR: δ 55.8, 112.3, 114.7 114.9, 119.8, 123.5, 123.9, 125.0, 128.5, 129.8, 133.2, 150.4, 160.0; Anal. calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36; found: C, 66.55; H, 5.28; N, 10.26.

**2-Amino-4-nitro-4′-methoxystilbene (22).** Heck reaction[28] was carried out with *p*-vinylanisole (1.16 g, 8.65 mmol) and 2-bromo-5-nitroaniline (1.5 g, 6.9 mmol) in 15 mL DMF in a sealed tube after addition of triethylamine (2.4 mL, 17.3 mmol), tri(*o*-tolyl)phosphine (42 mg, 0.138 mmol) and $Pd(OAc)_2$ (15.5 mg, 0.07 mmol) for 24 h at 120 °C. Reaction was neutralized with 10% HCl and extracted with ether and dried in $Na_2SO_4$. Purification on a silica gel column eluting with 10% ethyl acetate:hexanes provided 1.48 g (79%) of dark orange solid which was recrystallized in methylene chloride and hexanes to give red needles; mp 182–183 °C; IR (KBr) 3478, 3379, 1510, 1338, 1240; $^1$H NMR (DMSO-$d_6$): δ 3.80 (s, 3H), 6.03 (br s, 2H), 6.99 (d, 2H, *J*=9), 7.20 (d, 1H, *J*=16), 7.32 (d, 1H, *J* = 16), 7.37 (dd, 1H, *J*=9, 2.5), 7.53 (d, 1H, *J*=2.5), 7.66 (d, 2H, *J*=9), 7.67 (d, 1H, *J*=9); $^{13}$C NMR (DMSO-$d_6$): δ 55.2, 109.2, 110.9 114.4, 120.3, 126.0, 128.2, 128.7, 130.1, 131.3, 147.1, 159.6; Anal. calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36; found: C, 66.71; H, 5.27; N, 10.28.

**2-Tosylamino-5-nitro-4′-methoxystilbene (23).** Compound **21** (690 mg, 2.56 mmol) was dissolved in 13 mL of pyridine and stirred at room temperature overnight after addition of toluenesulfonyl chloride (586 mg, 3.07 mmol). Pyridine was removed in vacuo and 605 mg (55%) product was obtained by column chromatography of the residue using 0–10% ethyl acetate: hexanes. Recyrstallization with methylene chloride and hexanes gave yellow needles; mp 170–172 °C; IR (KBr) 3245, 1603, 1517, 1340, 1242, 1166; $^1$H NMR: δ 2.37 (s, 3H), 3.86 (s, 3H), 6.65 (d, 1H, *J*=15), 6.91 (d, 1H, *J*=15), 6.92 (d, 2H, *J*=9), 7.01 (br s, NH), 7.26 (d, 2H, *J*=8.0), 7.37 (d, 2H, *J*=9), 7.60 (d, 1H, *J*=9.0), 7.71 (d, 2H, *J*=8), 8.05 (dd, 1H, *J*=9, 2.5), 8.28 (d, 1H, *J*=2.5); $^{13}$C NMR: δ 22.1, 55.9, 114.0, 118.0, 122.6, 123.0, 123.5, 127.7, 128.9, 130.5, 131.9, 136.2, 136.4, 139.6, 145.3, 145.4, 146.3, 160.8; Anal. calcd for $C_{22}H_{20}N_2O_5S$: C, 62.25; H, 4.75; N, 6.60; found: C, 62.35; H, 4.76; N, 6.63.

**2-Tosylamino-4-nitro-4′-methoxystilbene (24).** The title compound was prepared following the procedure described for **23**. Compound **22** (1.2 g, 4.52 mmol) in 26 mL of pyridine and toluenesulfonyl chloride (1.03 mg, 5.4 mmol) gave 1.3 g of yellow solid (80%) which was recrystallized in methylene chloride and hexanes and gave yellow needles; mp 203–205 °C; IR (KBr) 3250, 1595, 1513 1336, 1254, 1166; $^1$H NMR (DMSO-$d_6$): δ 2.19 (s, 3H), 3.83 (s, 3H), 6.97 (d, 1H, *J*=16), 7.01 (d, 2H, *J*=9), 7.16 (s, 1H), 7.25 (d, 2H, *J*=8), 7.42 (d, 2H, *J*=9), 7.56 (d, 2H, *J*=8), 7.93 (d, 1H, *J*=9), 8.00 (d, 1H, *J*=2), 8.07 (dd, 1H, *J*=9, 2), 10.28 (br s, NH); $^{13}$C NMR (DMSO-$d_6$): δ 20.6, 55.4, 107.4, 114.4, 119.3, 121.7, 122.8, 126.4, 126.8, 129.0, 129.2, 130.1, 134.2, 134.3, 136.9, 140.5, 143.8, 145.9; Anal. calcd for $C_{22}H_{20}N_2O_5S$: C, 62.25; H, 4.75; N, 6.60; found: C, 62.19; H, 4.80; N, 6.67.

**1-Tosyl-2-methoxyphenyl-5-nitroindole (25).** The synthetic procedure was adopted from the literature.[29] Compound **23** (480 mg, 1.13 mmol) was dissolved in 20 mL DMF and after addition of $Pd(OAc)_2$ (25.4 mg, 0.113 mmol), *p*-benzoquinone (244 mg, 2.26 mmol) and LiCl (479 mg, 11.3 mmol) the reaction mixture was heated at 110 °C overnight. Due to the instability of the product, a quick short column with methylene chloride was done to remove the tarry impurities and without further purification was hydrolyzed in the next step. Crude yield was 85%; mp 138–140 °C; IR (KBr) 1607, 1511, 1447, 1347, 1252, 1176; $^1$H NMR: δ 2.32 (s, 3H), 3.90 (s, 3H), 6.60 (s, 1H), 6.97 (d, 2H, *J*=9), 7.09 (d, 2H, *J*=8), 7.26 (d, 2H, *J*=8), 7.38 (d, 2H, *J*=9), 8.22 (dd, 1H, *J*=9, 2), 8.36 (d, 1H, *J*=2), 8.44 (d, 1H, *J*=9);$^{13}$C NMR: δ 22.1, 55.9, 112.6, 112.8, 113.6, 116.9, 117.0, 120.0, 123.7, 127.3, 130.0, 130.4, 132.4, 135.1, 141.5, 145.4, 145.9, 161.0.

**1-Tosyl-2-methoxyphenyl-6-nitroindole (26).** The title compound was prepared following the procedure described for **25**. Compound **24** (1.08 g, 2.54 mmol) in 43 mL DMF, $Pd(OAc)_2$ (57 mg, 0.254 mmol), *p*-benzoquinone (549 mg, 5.08 mmol) and LiCl (1.08 g, 25.4 mmol) gave 1.02 g (95% crude yield); mp 195–197 °C; IR (KBr) 1604, 1521, 1373, 1340, 1250, 1174; $^1$H NMR: δ 2.32 (s, 3H), 3.91 (s, 3H), 6.57 (s, 1H), 6.98 (d, 2H, *J*=9), 7.09 (d, 2H, *J*=8), 7.28 (d, 2H, *J*=8), 7.42 (d, 2H, *J*=9), 7.52(d, 1H, *J*=8), 8.18 (dd, 1H, *J*=9, 2), 9.24 (d, 1H, *J*=2); $^{13}$C NMR: δ 22.0, 55.2, 112.3, 113.4, 113.7, 120.1, 120.9, 123.8, 127.4, 130.1, 132.4, 134.9, 135.8, 137.3, 145.2, 145.8, 147.7, 161.2.

**2-Methoxyphenyl-5-nitroindole (27).** Compound **25** (190 mg, 0.45 mmol) was dissolved in methanol:$H_2O$ (3:1) (35 mL) and after addition of $K_2CO_3$ (308 mg, 2.25 mmol) was refluxed for 6 h. Quantitative yield of a dark scarlet solid was obtained; mp 220–221 °C; IR (KBr) 3353, 1616, 1503, 1464, 1318, 1255; $^1$H NMR (DMSO-$d_6$): δ 3.84 (s, 3H), 7.10 (s, 1H), 7.11 (d, 2H, *J*=9), 7.54 (d, 1H, *J*=9), 7.87 (d, 2H, *J*=9), 8.00 (dd, 1H, $J=9$, 2), 8.51 (d, 1H, $J=2$), 12.22 (br s, NH); $^{13}$C NMR (DMSO-$d_6$): δ 55.5, 99.7, 111.6, 114.8, 116.7, 116.8, 123.9, 127.2, 128.5, 140.5, 141.2, 141.9, 159.4; HRMS (EI) calcd for $C_{15}H_{12}N_2O_3$ *m/z* 268.0848, found *m/z* 268.0848.

**2-Methoxyphenyl-6-nitroindole (28).** Compound **26** (200 mg, 0.47 mmol) was dissolved in methanol:$H_2O$ (3:1) (40 mL) and after addition of $K_2CO_3$ (325 mg, 2.35 mmol) was refluxed for 6 h. An orange solid (102 mg, 81%) was obtained; mp 233–234 °C; IR (KBr) 3417, 1605, 1490, 1371, 1282, 1255; $^1$H NMR (DMSO-$d_6$): δ 3.85 (s, 3H), 7.05 (s, 1H), 7.12 (d, 2H, $J=9$), 7.68 (d, 1H, $J=9$), 7.90 (d, 2H, $J=9$), 7.91 (dd, 1H, $J=9$, 2), 8.27 (d, 1H, $J=2$), 12.23 (br s, NH); $^{13}$C NMR (DMSO-$d_6$): δ 55.8, 99.0, 107.8, 114.9, 115.1, 119.9, 123.7, 127.6, 134.3, 135.6, 141.7, 144.8, 160.2; HRMS (EI) calcd for $C_{15}H_{12}N_2O_3$ *m/z* 268.0848, found *m/z* 268.0844.

### Topoisomerase I-mediated DNA cleavage assays

DNA topoisomerase I was purified from calf thymus gland as reported previously.[35] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[36] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-fill in with Klenow polymerase as previously described.[37] The cleavage assays were performed as previously reported.[19]

### Cytotoxicity assay

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[38–41] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya). The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% $CO_2$ and maintained by regular passage in RPMI medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of $IC_{50}$, cells were exposed continuously with varying drug concentrations and MTT assays were performed at the end of the fourth day.

## Acknowledgments

We are grateful to the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln for providing mass spectral data and for the partial support of this facility by the National Science Foundation, Biology Division (Grant No. DIR9017262). This study was supported by Grant CA 39662 from the National Cancer Institute (L.F.L.) and a fellowship grant from the Johnson & Johnson Discovery Research Fund (E.J.L.).

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(*Received in U.S.A.* 27 *October* 1995; *accepted* 22 *January* 1996)