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1*H*-Benzimidazole derivatives as mammalian DNA topoisomerase I inhibitors

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Benzimidazole is one of the most important heterocyclic groups manifesting various biological properties, such as antibacterial, antifungal, antimicrobial, antiprotozoal and antihelmintic activities. Several benzimidazole derivatives are also active as inhibitors of type I DNA topoisomerases. In this study, three 1*H*-benzimidazole derivatives with different electronic characteristics at position 5-, namely 5-chloro-4-(1*H*-benzimidazole-2-yl)phenol (Cpd I), 5-methyl-4-(1*H*-benzimidazole-2-yl)phenol (Cpd II) and 4-(1*H*-benzimidazole-2-yl)phenol (Cpd III), were synthesized and evaluated for their effects on mammalian type I DNA topoisomerase activity using quantitative *in vitro* plasmid supercoil relaxation assays. For the structure elucidation of the compounds, melting points, UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analyses were interpreted. Among the compounds, 5-methyl-4-(1*H*-benzimidazole-2-yl)phenol (Cpd II) manifested relatively potent topoisomerase I inhibition.

Keywords: 1*H*-benzimidazole derivatives, type I DNA topoisomerase, plasmid supercoil relaxation assays

INTRODUCTION

Compounds containing benzimidazole rings, which are formed *via* the fusion of imidazole and benzene rings, have been used extensively for pharmaceutical purposes since 1960 (Buu-Hoi *et al.*, 1963). 1*H*-Benzimidazole rings, which exhibit remarkable basic characteristics due to their nitrogen content, comprise the active substances of several drugs. A number of biological activities, such as antibacterial, antifungal, antimicrobial, antiprotozoal and antihelmintic activities have been attributed to these compounds (Gunes & Cosar, 1992; Göker *et al.*, 2001; Valdez *et al.*, 2002; Aguirre *et al.*, 2004). Several benzimidazole derivatives are also active as inhibitors of type I DNA topoisomerases (topo I) (Jin *et al.*, 2000). Topoisomerases are ubiquitous enzymes, found in prokaryotes, eukaryotes, viruses and organelles, such as mitochondria and chloroplast (Wang, 1996). These enzymes regulate conformational changes in DNA topology by catalyzing concerted breakage and rejoining of DNA strands during many genetic processes, including DNA replication, transcription, recombination and transposition. Topoisomerases are classified on the basis of the reaction mechanism; type I topoisomerases make a single-stranded break in a DNA duplex, mediate passage of the intact strand through the break, and then reseal it. Type II topoisomerases (topo II), on the other hand, create transient breaks in both strands of a duplex, pass an intact DNA segment through the break and then reseal the cleavage site. Over the last years, topoisomerase poisoning has been recognized as an effective approach for the development of chemotherapeutics, as these enzymes are cellular targets for an ever-increasing number of antibacterial and anticancer agents (Kim *et al.*, 1997; Topcu, 2001). In this study, because of the known importance of the relationship between the biological activity and the chemical structure, we synthesized three 1*H*-benzimidazole derivatives with different electronic characteristics at position 5- and evaluated the corresponding functional change in the molecules. The compounds synthesized in this study are 5-chloro-

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Abbreviations: CPT, camptothecin; DMF, dimethylflormamide; DMSO, dimethylsulfoxide.

4-(1*H*-benzimidazole-2-yl)phenol (Cpd I), 5-methyl-4-(1*H*-benzimidazole-2-yl)phenol (Cpd II) and 4-(1*H*-benzimidazole-2-yl)phenol (Cpd III) (Fig. 1). The synthesized compounds were screened for their interference with mammalian topo I activity *via in vitro* supercoil relaxation assays and the chemical structures of the synthesized compounds were elucidated by spectroscopic (UV, IR, ^{1}H NMR, ^{13}C NMR and mass) and elemental analyses.

MATERIALS AND METHODS

Synthesis and characterization of the benzimidazole derivatives. The preparation of various substituted benzimidazoles is outlined in Fig. 1. Briefly, 0.04 moles of 4-hydroxybenzaldehyde and 0.04 moles of sodium bisulfide, dissolved in 20 mL of ethanol and water, respectively, were mixed and stirred for 1 h at room temp. The mixture was filtered and sodium hydroxyl(4-hydroxyphenyl)methansulfonate salt (71%) was obtained from the crude extract. The crude salt (0.01 mol) and 4-chloro-2-aminophenyleneamine (0.01 mol) in 30 mL of dimethyl formamide (DMF) were refluxed for 2 h at 150°C in an oil bath and the mixture was poured in an ice bath. 5-chloro-4-(1*H*-benzimidazole-2-yl)phenol (90.4%) was filtered and recrystallized from methanol/water. 4-Chloro-2-aminophenyleneamine was replaced with 4-methyl-2-aminophenylamine and *o*-phenylenediamine for the synthesis of compounds II and III, respectively.

Melting points were determined with a Buchi 510 capillary melting point apparatus. The IR spectra of compounds were monitored as potassium bromide pellets on a Jasco FT/IR-430 spectrometer. The NMR spectra were recorded on a Varian AS 400 Mercury Plus NMR spectrometer. NMR spectra (400 MHz for ^{1}H and 100 MHz for ^{13}C) were recorded in CD_3OD. Chemical shifts were measured in parts per million (δ). *J* values are given in Hz. Mass spectra (CI MS), were measured on a Hewlett Packard HP 6890 Series GC System Mass spectrometer with an HP 6890 Mass Selective Detector. UV spectra were taken on a Shimadzu UV-160 spectrophotometer in methanol solution. Elemental analyses (C, H and N) were determined on CHNS-932 Elemental Analysis Instrument. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60F_{254}) with detection by UV light. All starting materials and reagents were high-grade commercial products purchased from Aldrich, Fluka or Merck.

Plasmid supercoil relaxation assays. Plasmid supercoil relaxation assays were carried out as described (Topcu & Castora, 1995). Briefly, 20 μL of reaction mixture contained one unit of calf thymus topoisomerase I, 0.5 μg of supercoiled (sc) pBR322 (TAKARA, Otsu-Shiga, JA, Japan), in the presence or absence of the compounds. One unit of the enzyme activity was defined as the activity removing supercoils from 500 ng of supercoiled plasmid substrate at 37°C in 30 min.

A stock solution of 10 μg/mL camptothecin (CPT) in dimethylsulfoxide (DMSO) was serially diluted for comparisons. The relaxation products were analyzed on 1% agarose gels and photographed under UV light. DNA bands were quantified from gel photographs using BioRad Multianalysist (Ver: 1.1) (Topcu, 2000). Each assay was performed at least twice. All reactions were carried out in DNase-free 1.5 mL microcentrifuge tubes.

RESULTS AND DISCUSSION

The results of chemical identification of the compounds are summarized in Tables 1 and 2. Table 1 contains the data for the melting point, UV spectra, IR spectra, mass spectroscopy and elemental analyses. Because the ethylenic and benzeneoid bands are expected at 205–210 nm and 250–350 nm, respectively, the spectral results in Table 1 are in total agreement with the literature data (DeSelms, 1962a; 1962b; Güneş, 1993). The IR spectra, obtained in solid phase at 2400–3200 cm^{-1}, are based on N-H--N type hydrogen bonds, which are characteristic for benzimidazole derivatives (Preaston *et al.*, 1981). Moreover, the N–H strengthening at 3390–3460 cm^{-1}, C=C and C=N strengthening at 1650–1500 cm^{-1} and 1500–1400 cm^{-1} corresponding to benzenoid ring at 1000 cm^{-1} and 960 cm^{-1} (Table 1).

^{1}H NMR results for the non-substituted compound showed a 1,2-disubstituted benzene system at the A ring of the benzimidazole nucleus and a 1,4-disubstituted benzene system due to the *p*-hydroxy phenyl substituent at position

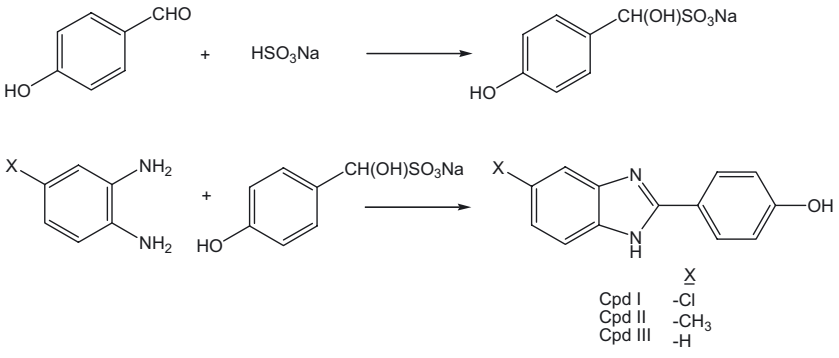

Figure 1. Synthesis scheme of the benzimidazole derivatives

Table 1. Chemical analyses of compounds I–III.

Compound	m.p. (°C)	UV λ_{max} (nm)	IR ν_{max} (cm^{-1})	CI MS $[M+1]^+$	Elemental analyses
Cpd I ($C_{13}H_9ClN_2O$)	257	311, 253, 217	3631, 3201, 1610, 1465, 805	246	Calc.:C,63.81;H,3.71;N,11.45 Found: C,63.61;H,3.52;N,11.60
Cpd II ($C_{14}H_{12}N_2O$)	270	311, 255, 216	3244, 3031, 1609, 1455, 799	225	Calc.:C,74.98;H,5.39;N,12.49 Found: C,73.47;H,5.49;N,12.67
Cpd III ($C_{13}H_{10}N_2O$)	271 (Nagai *et al.*, 1973)	305, 251, 217	3311, 3056, 1610, 1500, 836	211	Calc.:C,74.27;H,4.79;N,13.33 Found: C,74.36;H,4.84;N,13.29

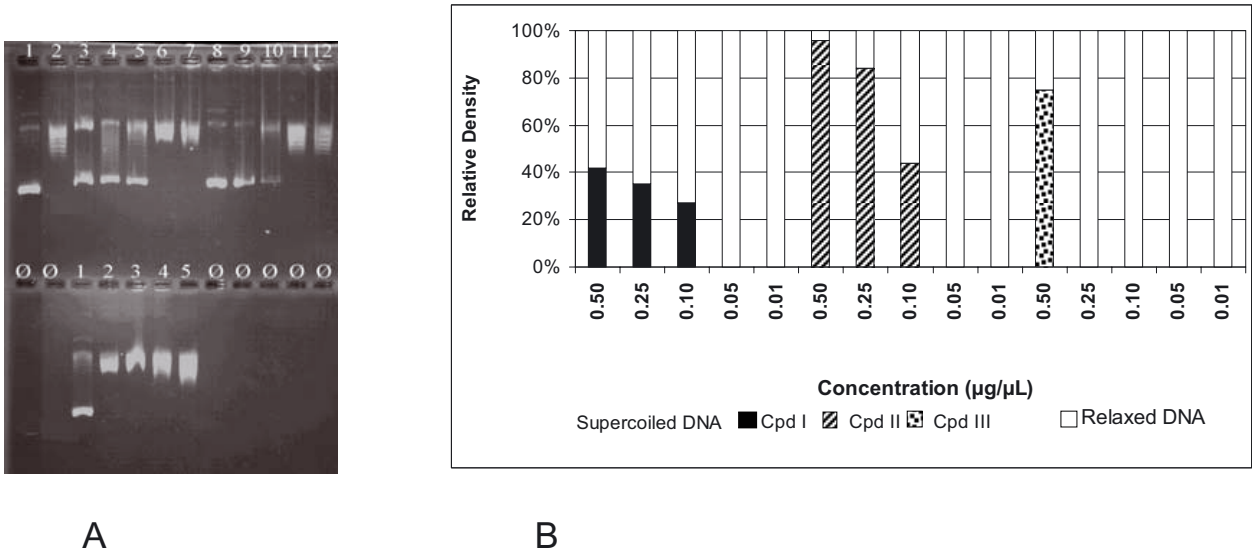

Figure 2. Inhibitory activity of compounds I–III on DNA topoisomerase I.
A. Agarose gel analysis of plasmid supercoil relaxation in the presence of varying concentrations of compounds I–III. B. Quantitative assessment of the inhibition obtained with the compounds. Bars show the percentage of supercoil *vs*. relaxed DNA.

2- (Table 2). The chlorinated derivative Cpd I and the methylated derivative the Cpd II gave different results when compared to the non-substituted compound (Table 2). Three protons of the methyl group at position 5- were detected at δ 2.43 in the methylated spectrum (Table 2). Unlike the methylated compounds, the protons were not detected due to the presence of Cl at the same position among the chlorinated compounds. The lack of the signal belonging to 3a, 4, 7 and 7a C in ^{13}C NMR is noteworthy that may suggest a proton exchange due to 1, 3-tautomerization (Sridharan *et al.*, 2005).

The supercoil relaxation activity of DNA topoisomerase I in the presence of varying concentrations

Table 2. ^1H and ^{13}C NMR results for the compounds I–III.

NMR	
Cpd I	**^1H NMR**: 6.93 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 7.18 (1H, dd, *J* = 2, 8.6 Hz, H-6), 7.48 (1H, d, *J* = 8.6 Hz, H-7), 7.52 (1H, d, *J* = 1.6 Hz, H-4), 7.91 (2H, d, *J* = 8.6 Hz, H-2′, H-6′) **^{13}C NMR**: 115.6 (C-3′, C-5′), 120.6 (C-1′), 122.6 (C-6), 127.8 (C-5), 128.4 (C-2′, C-6′), 154.1 (C-2), 160.1 (C-4′)
Cpd II	**^1H NMR**: 2.43 (3H, s, Ar-CH_3), 6.91 (2H, d, *J* = 9 Hz, H-3′, H-5′), 7.02 (1H, dd, *J* = 0.8, 8.2 Hz, H-6), 7.32 (1H, brs, H-4), 7.40 (1H, d, *J* = 8.2 Hz, H-7), 7.89 (2H, d, *J* = 9 Hz, H-2′, H-6′) **^{13}C NMR**: 20.8 (Ar-CH_3), 114.0 (C-4*), 114.4 (C-7*), 115.9 (C-3′, C-5′), 121.4 (C-1′), 124.0 (C-6), 128.4 (C-2′, C-6′), 132.5 (C-5), 152.6 (C-2), 159.9 (C-4′)
Cpd III	**^1H NMR**: 6.93 (2H, d, *J* = 9 Hz, H-3′, H-5′), 7.21 (2H, dd, *J* = 3.1, 6.2 Hz, H-5, H-6), 7.55 (2H, dd, *J* = 3.1, 6.3 Hz, H-4, H-7), 7.93 (2H, d, *J* = 9 Hz, H-2′, H-6′) **^{13}C NMR**: 115.7 (C-3′, C-5′), 120.9 (C-1′), 122.4 (C-5, C-6), 128.3 (C-2′, C-6′), 138.9 (C-3a and/or C-7a), 152.7 (C-2), 159.8 (C-4′)

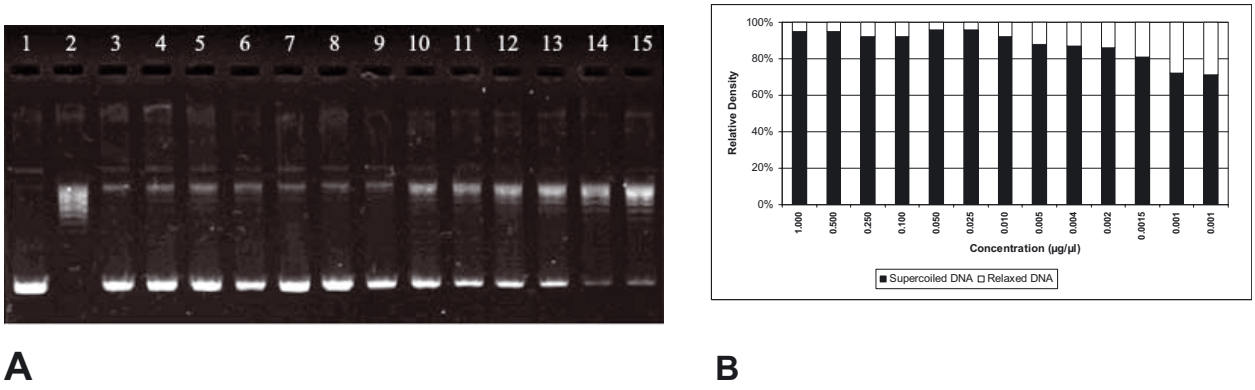

Figure 3. Inhibitory activity of CPT on DNA topoisomerase I.
A. Agarose gel analysis of plasmid supercoil relaxation in the presence of varying concentrations of CPT. Lane 1, pBR322 DNA without enzyme; lane 2, supercoil relaxation with 1 unit of DNA topoisomerase I; lane 3–11, same as lane 2 in the presence of decreasing concentrations of CPT (1.0 μg/μL to 0.001 μg/μL). B. Quantitative assessment of the inhibition obtained with the compounds. Bars show the percentage of supercoil *vs*. relaxed DNA.

of compounds I through III is given in Fig. 2. The supercoiled DNA (Fig. 2A, upper lane 1) was fully relaxed by the enzyme in the presence of the organic solvent, DMSO, without any inhibition of the DNA topoisomerase I activity (Fig. 2A, upper lane 2). Relaxation was inhibited upon incubation with Cpd I (Fig. 2A upper lanes 3 to 7) or Cpd II (Fig. 2A, upper lanes 8 to 12) to varying extents in a concentration-dependent manner. Cpd I and II at concentrations below 0.1 μg/μL showed no inhibitory activity (Fig. 2A, upper lanes 6, 7, 11 and 12). On the other hand, compound III gave an inhibition at the highest concentration only (0.5 μg/μL) (Fig. 2A, lower lane 1). Unlike Cpds I and II, Cpd III was not effective at lower concentrations (Fig. 2A, lower lanes 2–5). The inhibitory effect exerted by these three compounds was quantified by densitometry and plotted in Fig. 2B. In support of Fig. 2A, the compound III was the weakest in its interference with topoisomerase I activity. Among the other two Cpds, the inhibition by Cpd I extended up to the concentration of 0.25 μg/μL while this concentration resulted transformation of a remarkable percentage of supercoiled pBR322 to relaxed pBR322 for Cpd II (Fig. 2B). However, the latter compound was more effective at high concentration (compare the upper lanes of 3 and 8 in Fig. 2A).

Inhibitors of DNA topoisomerases may act at any of the three major steps in the mechanism of action of the enzymes; the enzyme binding to DNA, the breakage of DNA strands, and the religation of the DNA following the strand-passing step. We next compared the inhibitory activities of the 1*H*-benzimidazole derivatives with a known DNA topoisomerase I inhibitor, CPT (Fig. 3). Pure CPT gave a much stronger inhibition at concentrations similar to those used for compounds I–III in comparable (Fig. 3A, lanes 3–6) as well as at lower ones (Fig. 3A, lanes 7–15). Figure 3B shows quantification of the results from Fig. 3A. Although, the inhibition at the higher CPT concentrations (1.00 to 0.05 μg/μL) was abundant, it was less precise when compared to lower CPT concentrations (0.01 to 0.001 μg/μL) (Fig. 3B).

Taken together, the inhibitory effects of 1*H*-benzimidazole derivatives are a significant result as these compounds can be potential sources of anticancer agents. The mechanism of the effects exerted by the compounds covered in our study is subject to further investigation.

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