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Short communication

Synthesis and biological activity evaluation of 1*H*-benzimidazoles via mammalian DNA topoisomerase I and cytostaticity assays

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Abstract

Benzimidazoles are important compounds because of their antibacterial, antifungal, antimicrobial, antiprotozoal and antihelmintic activities. Some benzimidazole derivatives also interfere with the reactions of DNA topoisomerases, enzymes functioning at almost all stages of the cell cycle. In this study, nine 1*H*-benzimidazole derivatives with substituents at positions 2 and 5 were synthesized and the structure of the compounds was elucidated by instrumental methods. The characterized compounds were screened to identify if they interfered with mammalian type I DNA topoisomerase activity via in vitro supercoil relaxation assays. Selected compounds were subjected to cytostatic assays using HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) cells. Our results showed that 5-chloro-2-(2-hydroxyphenyl)-1*H*-benzimidazole exerted the most profound topoisomerase I inhibition and cytotoxicity.

Keywords: 1*H*-Benzimidazole derivatives; Synthesis; Type I DNA topoisomerase; MTT assay; Plasmid Supercoil relaxation assays

1. Introduction

Benzimidazoles are important agents found in many bioactive compounds from natural and synthetic sources. These compounds have been intensively studied over last years because of their antibacterial [1], antifungal [2], antimicrobial [3], antiprotozoal [4] and antihelmintic [5] activities. Some benzimidazole derivatives are also active as type I DNA topoisomerase inhibitors [6,7]. DNA topoisomerases are essential enzymes that regulate conformational changes in DNA topology by transient breakage of nucleic acid backbone during many genetic processes, including DNA replication, transcription, recombination and transposition [8,9]. These enzymes are classified as type I (topo I) and type II (topo II) topoisomerases according to the reaction mechanisms; type I topoisomerases catalyze topological changes in duplex -DNA by reversibly nicking one strand, whereas type II enzymes catalyze the transient breakage of both strands simultaneously [9]. DNA topoisomerases were shown to be important targets for several chemotherapeutic agents [7,10–14]. In this study, nine 1*H*-benzimidazole derivatives with substituents at positions 2 and 5 were synthesized, namely: 2-(1*H*-benzimidazol-2-yl) phenol (I), 2-[2-(2-morpholin-4-ylethoxy)phenyl]-1*H*-benzimidazole (II), 2-[2-(2-piperidin-1-ylethoxy)phenyl]-1*H*-benzimidazole (III), 2-[2-(2-pyrrolidin-1-ylethoxy)phenyl]-1*H*-benzimidazole (IV), 5-chloro-2-[2-(2-morpholin-4-ylethoxy)phenyl]-1*H*-benzimidazole (V), 5-chloro-2-[2-(2-piperidin-1-ylethoxy)-phenyl]-1*H*-benzimidazole (VI), 5-chloro-2-[2-(2-pyrrolidin-1-ylethoxy)phenyl]-1*H*-benzimidazole (VII), 2-[2-(5-chloro-1*H*-benzimidazol-2-yl)phenoxy]-*N*,*N*-diethylethanamine (VIII)

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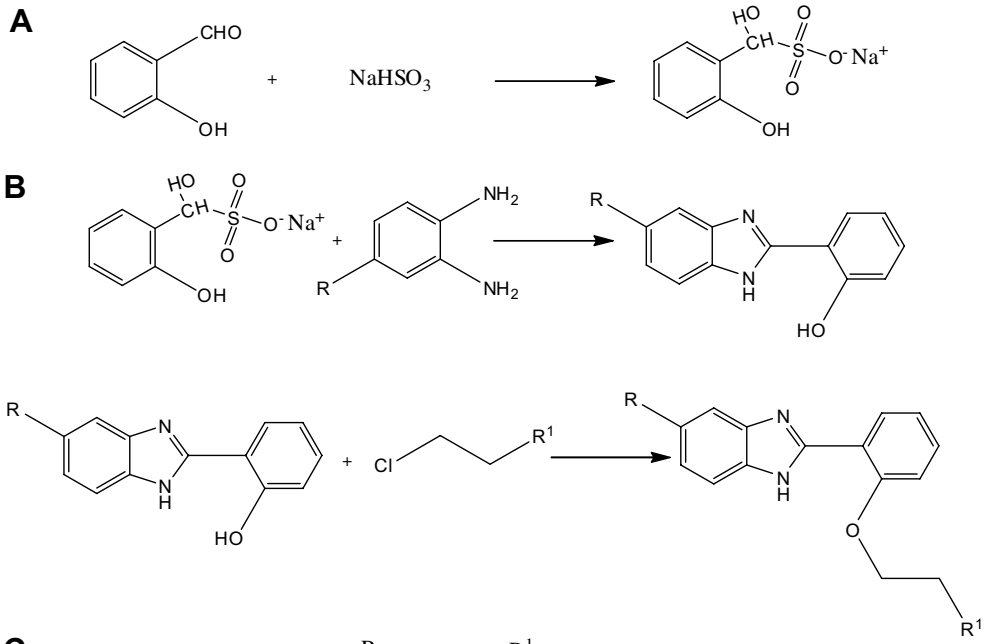

C

	R	R^1
I	H	-
II	H	-N O
III	H	-N
IV	H	-N
V	Cl	-N O
VI	Cl	-N
VII	Cl	-N
VIII	Cl	-N(C_2H_5)2
IX	Cl	-

Fig. 1. The synthesis pathway and the structures of the compounds.

and 2-(5-chloro-1*H*-benzimidazol-2-yl)phenol (IX) (Figs. 1 and 2). Following characterization of a number of parameters including melting points, UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data, the compounds were evaluated for their interference with topo I reactions. Selected derivatives were subjected to the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay in vitro against three human tumoral cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma).

2. Experimental

2.1. Synthesis of the benzimidazole derivatives

The preparation of the substituted benzimidazoles is outlined in Fig. 1 [15,16]. Briefly, 0.04 mol of 2-hydroxybenzaldehyde and 0.04 mol of sodium bisulfide, dissolved in 20 mL of ethanol and water, respectively, were mixed and stirred for 10 min at room temperature. The mixture was filtered and sodium hydroxy (2-hydroxyphenyl)methane sulfonate salt (86%) was obtained from the crude extract (Fig. 1, panel A). The crude salt (0.01 mol) and *o*-phenylenediamine (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. 2-(2-Hydroxyphenyl)-1*H*-benzimidazole (53.5%) was filtered and recrystallized from methanol/water (Fig. 1, panel B) [15]. 5-Chloro-2-(2-hydroxyphenyl)-1*H*-benzimidazole (43.73%) was synthesized with 4-chloro-*o*-phenylenediamine as described and recrystallized from methanol/water [16]. After reacting 2-(2-hydroxyphenyl)-1*H*-benzimidazole (0.005 mol) and 4-(2-chloroethly) morpholine (0.01 mol) with sodium hydroxide (0.015 mol) in 10 mL ethanol, the mixture was refluxed at 95 °C for 2 h in an oil bath. Ethanol was evaporated and the residue was extracted with ether. The compounds **II** (7.7%) and **V** (8.14%) were obtained by column chromatography and crystallization with

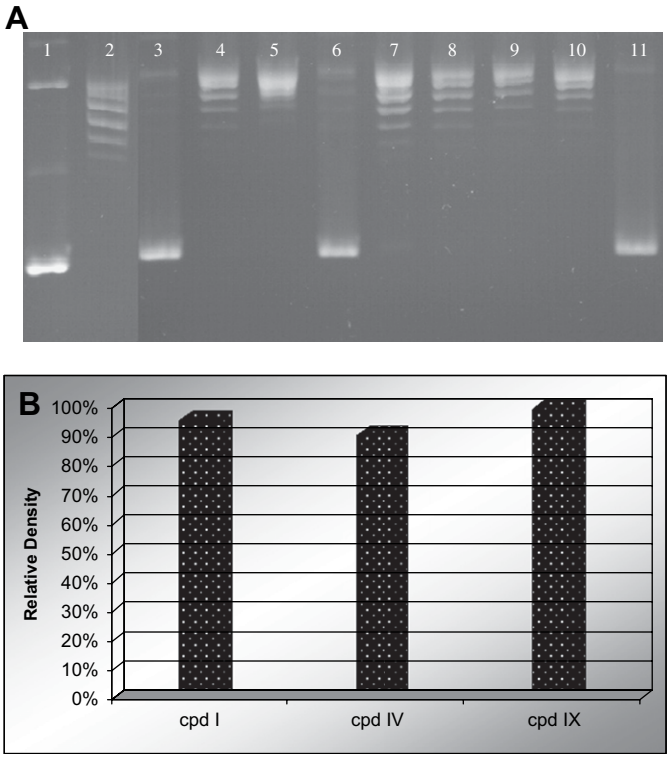

Fig. 2. (A) A representative assay using 1 μg/μL concentration of the test compounds in topo I reactions, lane 1, pBR322 DNA without enzyme; lane 2, supercoil relaxation with 1 unit of DNA topoisomerase I; lanes 3–11, same as lane 2 in the presence of the test compounds from **I** to **IX**, respectively. (B) Quantitative assessment of the inhibition obtained with the compounds (see Section 2 for the details).

methanol/water. 4-(2-Chloroethly) morpholine was replaced with 1-(2-chloroethly) piperidine for the synthesis of compounds **III** (10.29%) and **VI** (7.45%), 1-(2-chloroethly)pyrrolidine for the synthesis of compounds **IV** (6.84%) and **VII** (9.70%), and 2-chloro-*N*,*N*-diethlyethanamine for the synthesis of compound **VIII** (11.18%) (Fig. 1, panel C).

2.2. Characterization of the benzimidazole derivatives

Melting points were determined with a capillary melting point apparatus (Buchi 510, BUCHI, Flawil, Switzerland). The IR spectra of compounds were monitored as potassium bromide pellets (FT/IR-430, JASCO, Tokyo, Japan). The NMR spectra (400 MHz for ^{1}H and 100 MHz for ^{13}C) were recorded in CD_3OD and $CDCl_3$ (AS 400 Mercury Plus NMR Varian, Palo Alto, USA). Chemical shifts were measured as parts per million (δ). The J values, given in Hz. Mass spectra (CI MS) were measured in methanol solution (HP 6890 Series GC System Mass spectrometer and HP 6890 Mass Selective Detector, Hewlett Packard, Palo Alto, USA). UV spectra were taken on a spectrometer in methanol solution (UV-160, Shimadzu, Kyoto, Japan). 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.

2.3. Plasmid supercoil relaxation assays

Plasmid supercoil relaxation assays were carried out as described [17]. Briefly, 20 μL of reaction mixture contained 1 unit of calf thymus topoisomerase I, 0.5 mg of supercoiled (sc) pBR322 (Takara, Otsu-Shiga, Japan), in the presence or absence of the test compounds in 35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, and 0.1% bovine serum albumin. A stock solution of 10 mg/mL CPT in DMSO was serially diluted for comparison purposes. The relaxation products were analyzed on 1% agarose gels in TBE buffer (45 mM Tris Borate and 1 mM EDTA, pH 8.0) in a horizontal electrophoresis apparatus (5 V/cm) (Thermo EC250) and photographed under UV light after staining in ethidium bromide (EtdBr) solution (0.5 μg/mL). The relationship between the binding of EtdBr and the amount of fluorescence given by sc and relaxed DNA (rlx DNA) under UV light was carried out as described [11]. DNA bands were quantified from gel photo images using BioRad Multianalyst (version 1.1). One unit of the enzyme activity was defined as the activity removing the supercoils from 500 ng of sc plasmid substrate at 37 °C.

2.4. Cytostatic assays

Antiproliferative effects of the test compounds were measured in vitro on three human tumoral cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma), by using the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay [18]. Briefly, cancer cells (5000/well) were seeded onto a 96-well microplate and attached to the bottom of the well overnight. On the second day, 200 μL of new medium containing the test substances was added. After incubation for 72 h, the living cells were assayed by the addition of 20 μL of 5 mg/mL MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The medium was then removed, and the precipitated crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO) during a 60 min period of shaking. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls. All in vitro experiments were carried out on two microplates with at least five parallel wells. Doxorubicin was used as positive control. Stock solutions of the tested substances (30 mM) were prepared with DMSO. The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Their antiproliferative effects were determined in the concentration range 0.1–30 mM, the dose–response curves were fitted by means of the computer program GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA) and the IC_{50} values were calculated.

3. Results and discussion

The results of chemical analyses of the synthesized 1*H*-benzimidazole derivatives are summarized in Tables 1 and 2. Table 1 contains the data for the melting point, UV spectra,

Table 1
Results of the chemical analyses of the compounds **I–IX**

Compound	m.p. (°C)	UV λ_{max} (nm)	IR ν_{max} (cm^{-1})	CI MS $[M+1]^+$
I	237	329, 318, 292, 213	3325, 2924, 1602, 1490, 1452, 961	211
II	157	311, 293, 211	3302, 2961, 2817, 1604, 1584, 1470, 1450, 955	325
III	130	301, 211	3291, 2933, 2852, 1583, 1462, 1443, 942	323
IV	115	302, 211	3065, 2965, 2820, 1603, 1581, 1459, 1440, 960	309
V	180	315, 215	3314, 2963, 2823, 1603, 1579, 1461, 1422, 948	359
VI	144	301, 212	3276, 2938, 2824, 1602, 1582, 1459, 1422, 955	357
VII	141	308, 212	3220, 2963, 2815, 1602, 1581, 1459, 1420, 965	343
VIII	126	305, 212	3071, 2964, 2822, 1602, 1582, 1460, 1420, 951	345
IX	277	332, 321, 216	3325, 2923, 1600, 1584, 1489, 1420, 964	245

IR spectra and mass spectroscopy. Our data in Table 1 show that the ethylenic bands at 205–210 nm and benzenoid bands at 250–350 nm are in total agreement with the literature data [19–21]. The IR spectra obtained in solid phase at 2400–3200 cm^{-1} are characteristic for benzimidazole derivatives (Table 1) [22]. ^1H NMR results are given in Table 2. The aromatic proton signals of *o*-hydroxy phenyl substituent at position 2 were observed within prospective chemical shift values and divisions while the hydrogen atoms at positions 4 and 7 were not detected at the prospective divisions [23]. The

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

Compound	NMR
I	^1H NMR 7.00 (1H, td, $J=1.2$, 7.4 Hz, H-5′), 7.02 (1H, d, $J=8.4$ Hz, H-3′), 7.27 (2H, m, H-5, H-6), 7.37 (1H, td, $J=1.6$, 7.8 Hz, H-4′), 7.59 (1H, d, $J=7.6$ Hz, H-7), 7.70 (1H, d, $J=7.6$ Hz, H-4), 8.04 (1H, dd, $J=1.6$, 8 Hz, H-6′), 13.15–13.12 (1H, bs, N–H) ^{13}C NMR 113.3 (C-1′), 117.9 (C-5′), 119.8 (C-3′), 123.5 (C-5, C-6), 126.9 (C-6′), 132.4 (C-4′), 152.4 (C-2), 158.7 (C-2′)
II	^1H NMR 2.66 (4H, t, $J=4.8$ Hz, H-3‴, H-5‴), 2.94 (2H, t, $J=5.6$ Hz, H-2″), 3.84 (4H, q, $J=4.8$ Hz, H-2‴, H-6‴), 4.34 (2H, t, $J=5.6$ Hz, H-1″), 7.02 (1H, dd, $J=0.8$, 7.6 Hz, H-3′), 7.16 (1H, td, $J=1.2$, 7.6 Hz, H-5′), 7.26 (1H, t, $J=5.6$ Hz, H-6*), 7.28 (1H, t, $J=6.4$ Hz, H-5*), 7.41 (1H, td, $J=2$, 7.8 Hz, H-4′), 7.67 (2H, bs, H-4, H-7), 8.59 (1H, dd, $J=2$, 7.8 Hz, H-6′) ^{13}C NMR 53.5 (C-2″), 57.6 (C-3‴, C-5‴), 64.1(C-1″), 67.0 (C-2‴, C-6‴), 112.9 (C-4, C-7, C-3′), 119.0 (C-1′), 122.3 (C-5′), 122.7 (C-5, C-6), 130.7 (C-6′), 131.1 (C-3a, C-7a, C-4′), 150.1 (C-2), 156.3 (C-2′)
III	^1H NMR 1.55 (2H, m, H-4‴), 1.72 (4H, m, H-3‴, H-5‴), 2.60 (4H, bs, H-2‴, H-6‴), 2.87 (2H, t, $J=5.6$ Hz, H-2″), 4.33 (2H, t, $J=5.6$ Hz, H-1″), 7.02 (1H, d, $J=8.4$ Hz, H-3′), 7.15 (1H, t, $J=7.6$ Hz, H-5′), 7.25 (1H, t, $J=6.6$ Hz, H-6*), 7.26 (1H, t, $J=6.4$ Hz, H-5*), 7.40 (1H, td, $J=2$, 7.8 Hz, H-4′), 7.66 (1H, bs, H-7), 7.68 (1H, bs, H-4), 8.54 (1H, dd, $J=1.6$, 7.8 Hz, H-6′) ^{13}C NMR 11.2 (C-4‴), 46.8 (C-3‴, C-5‴), 54.4 (C-2‴, C-6‴), 57.7 (C-2″), 64.7 (C-1″), 113.1 (C-4, C-7, C-3′), 119.7 (C-1′), 122.2 (C-5′), 122.4 (C-5, C-6), 130.7 (C-6′), 131.1 (C-4′, C-3a, C-7a), 150.3 (C-2), 156.4 (C-2′)
IV	^1H NMR 1.93 (4H, m, H-2‴, H-5‴), 2.78 (4H, m, H-3‴, H-4‴), 3.04 (2H, t, $J=5.4$ Hz, H-2″), 4.33 (2H, t, $J=5.2$ Hz, H-1″), 7.02 (1H, d, $J=8.4$ Hz, H-3′), 7.15 (1H, td, $J=1.2$, 7.5 Hz, H-5′), 7.23 (1H, t, $J=6.6$ Hz, H-6*), 7.24 (1H, t, $J=7.2$ Hz, H-5*), 7.39 (1H, td, $J=2$, 7.7 Hz, H-4′), 7.60 (2H, bs, H-4, H-7), 8.50 (1H, dd, $J=1.6$, 8 Hz, H-6′) ^{13}C NMR 24.0 (C-3‴, C-4‴), 53.6 (C-2‴, C-5‴), 55.3 (C-2″), 67.3 (C-1″), 113.9 (C-4, C-7, C-3′), 120.2 (C-1′), 122.3 (C-5, C-6, C-5′), 130.7 (C-6′), 130.9 (C-3a, C-7a, C-4′), 150.4 (C-2), 156.7 (C-2′)
V	^1H NMR 2.67 (4H, t, $J=4.7$ Hz, H-3‴, H-5‴), 2.96 (2H, t, $J=5.4$ Hz, H-2″), 3.83 (4H, t, $J=4.7$ Hz, H-2‴, H-6‴), 4.35 (2H, t, $J=5.5$ Hz, H-1″), 7.03 (1H, d, $J=8$ Hz, H-3′), 7.18 (1H, dd, $J=0.8$, 7.6 Hz, H-5′), 7.23 (1H, t, $J=1.6$, 8.8 Hz, H-6), 7.42 (1H, td, $J=1.6$, 7.8 Hz, H-4′), 7.52 (1H, bs, H-4), 7.58 (1H, bs, H-7), 8.53 (1H, dd, $J=1.6$, 8 Hz, H-6′) ^{13}C NMR 53.5 (C-2″), 57.5 (C-3‴, C-5‴), 64.0 (C-1″), 66.9 (C-2‴, C-6‴), 112.9 (C-4, C-7, C-3′), 118.8 (C-1′), 122.4 (C-5′), 123.2 (C-6), 128.2 (C-5), 130.7 (C-6′), 131.6 (C-3a, C-7a, C-4′), 151.2 (C-2), 156.2 (C-2′)
VI	^1H NMR 1.56 (2H, m, H-4‴), 1.70 (4H, m, H-3‴, H-5‴), 2.59 (4H, bs, H-2‴ H-6‴), 2.86 (2H, t, $J=5.2$ Hz, H-2″), 4.32 (2H, t, $J=5.2$ Hz, H-1″), 6.99 (1H, d, $J=8$ Hz, H-3′), 7.14 (1H, t, $J=7.8$ Hz, H-5′), 7.19 (1H, dd, $J=1.6$, 8.4 Hz, H-6), 7.38 (1H, td, $J=1.6$, 7.8 Hz, H-4′), 7.70 (1H, bs, H-7), 7.75 (1H, bs, H-4), 8.49 (1H, dd, $J=1.6$, 7.8 Hz, H-6′), 12.03 (1H, bs, NH) ^{13}C NMR 24.5 (C-4‴), 25.9 (C-3‴, C-5‴), 54.4 (C-2″), 57.7 (C-2‴, C-6‴), 64.7 (C-1″), 113.0 (C-4, C-7, C-3′), 119.4 (C-1′), 122.2 (C-6), 123.0 (C-5′), 130.8 (C-6′), 131.3 (C-3a, C-7a, C-4′), 151.5 (C-2), 156.4 (C-2′)
VII	^1H NMR 1.91 (4H, m, H-3‴, H-4‴), 2.78 (4H, bs, H-2‴, H-5‴), 3.04 (2H, t, $J=5.2$ Hz, H-2″), 4.33 (2H, t, $J=5.2$ Hz, H-1″), 7.02 (1H, d, $J=8.8$ Hz, H-3′), 7.15 (1H, t, $J=7.4$ Hz, H-5′), 7.19 (1H, dd, $J=1.2$, 8.6 Hz, H-6), 7.40 (1H, td, $J=2$, 7.8 Hz, H-4′), 7.80–7.56 (2H, 2× bs, H-4, H-7), 8.55 (1H, dd, $J=1.6$, 7.8 Hz, H-6′) ^{13}C NMR 24.0 (C-3‴, C-4‴), 53.6 (C-2‴, C-5‴), 55.3 (C-2″), 67.3 (C-1″), 114.0 (C-4, C-7, C-3′), 119.9 (C-1′), 122.4 (C-5′), 122.8 (C-6), 130.7 (C-6′), 131.3 (C-3a, C-7a, C-4′), 151.5 (C-2), 156.7 (C-2′)
VIII	^1H NMR 1.15 (6H, t, $J=7.6$ Hz, H-2‴), 2.81 (4H, q, $J=7.6$ Hz, H-1‴), 2.96 (2H, t, $J=5.2$ Hz, H-2″), 4.31 (2H, t, $J=5.2$ Hz, H-1″), 7.05 (1H, d, $J=8$ Hz, H-3′), 7.15 (1H, t, $J=7.6$ Hz, H-5′), 7.20 (1H, dd, $J=1.6$, 7.6 Hz, H-6), 7.40 (1H, td, $J=2$, 7.6 Hz, H-4′), 7.70 (1H, bs, H-4), 7.78 (1H, bs, H-7), 8.50 (1H, dd, $J=1.6$, 7.6 Hz, H-6′), 12.85 (1H, bs, N–H) ^{13}C NMR 11.1 (C-2‴), 46.7 (C-1‴), 51.6 (C-2″), 65.8 (C-1″), 114.0 (C-4, C-7, C-3′), 119.8 (C-1′), 122.5 (C-5′), 122.9 (C-6), 130.6 (C-6′), 131.3 (C-3a, C-7a, C-4′), 151.2 (C-2), 156.5 (C-2′)
IX	^1H NMR 7.01 (1H, td, $J=1.2$, 7.8 Hz, H-3′), 7.02 (1H, dd, $J=1.2$, 7.4 Hz, H-5′), 7.28 (1H, d, $J=6.8$ Hz, H-6), 7.38 (1H, td, $J=1.2$, 7.8 Hz, H-4′), 7.78–7.61 (2H, 2× bs, H-4, H-7), 8.04 (1H, dd, $J=1.6$, 7.8 Hz, H-6′), 13.25–12.71 (1H, br s, N–H) ^{13}C NMR 113.3 (C-1′), 117.9 (C-5′), 120 (C-3′), 123.7 (C-6), 127.3 (C-6′), 132.8 (C-4′), 153.5 (C-2), 158.5 (C-2′)

compounds substituted with the terminal of ethoxy chain at position 2 of phenyl group gave rise to similar chemical shift values and divisions at the ring of the benzimidazole nucleus as **I** and **IX** (Table 2). The lack of the signal belonging to **3a**, **4**, **7** and **7a** carbon in ^{13}C NMR is noteworthy in that it may suggest a proton exchange due to 1,3-tautomerization (Table 2) [23].

We employed plasmid supercoil relaxation assays to identify if the synthesized derivatives interfere with topo I reactions. Our assay uses sc plasmid DNA and relies on the ability of topoisomerase I to rlx DNA, which can be separated as discrete bands using gel electrophoresis. An inhibition of the relaxation activity due to the presence of a particular interfering agent is monitored in the form of an accumulated faster-migrating sc DNA (form I). A representative gel analysis of supercoil relaxation activity of DNA topoisomerase I in the presence of 1 μg/μL of the compounds **I**–**IX** is given in Fig. 2. As seen in Fig. 2A, the sc DNA (Fig. 2A, lane 1) was fully relaxed by the enzyme (Fig. 2A, lane 2). Relaxation of sc plasmid substrate, pBR322, was inhibited upon incubation with the compounds **I**, **IV** and **IX** (Fig. 2A, lanes 3, 6 and 11, respectively) while the other compounds, **II**, **III**, **V**, **VI**, **VII** and **VIII** (Fig. 2A, lanes 4, 5,and 7–9, respectively) did not interfere with the enzyme's ability to relax the supercoils. We previously reported that the enzyme's relaxation activity was not affected by DMSO [7]. We quantified the percent values of the sc vs. rlx DNA bands using the gel photo images for these three compounds (Fig. 2B). The average quantitative values showed that the strongest inhibition was obtained with **I**. The percent inhibition for **I**, **IV** and **IX** was found to be 95.4%, 90.2% and 99%, respectively (Fig. 2B). When we made a serial comparison of topo I reactions using decreased concentrations of the test compounds, the inhibition diminished gradually, which shows that the effects were concentration-dependent (data not shown).

Many reports in recent years indicated that interference with normal DNA topoisomerase functions coincide with cytotoxic properties for several naturally occurring and synthetic compounds [10,14]. We therefore subjected **I**, **IV** and **IX** to MTT assay in HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) cells to identify a possible correlation, if any. The results of cytostatic analyses are summarized in Table 3. We also plotted the percent effect for A431 and MCF7 cell lines by calculating the exact concentrations at which the cell proliferation is inhibited by 50% (Fig. 3). The most effective compounds in this set of compounds were **IX** as it exhibited relatively low

Table 3
The results of the cytostaticity assays for the compounds **I**, **IV** and **IX**

Calculated IC_{50} values (μM)			
Compound	A431	HeLa	MCF7
I	>30	26.45	16.03
IV	>30	19.82	>30
IX	6.16	6.04	6.94
Doxorubicin	0.19	0.16	0.31

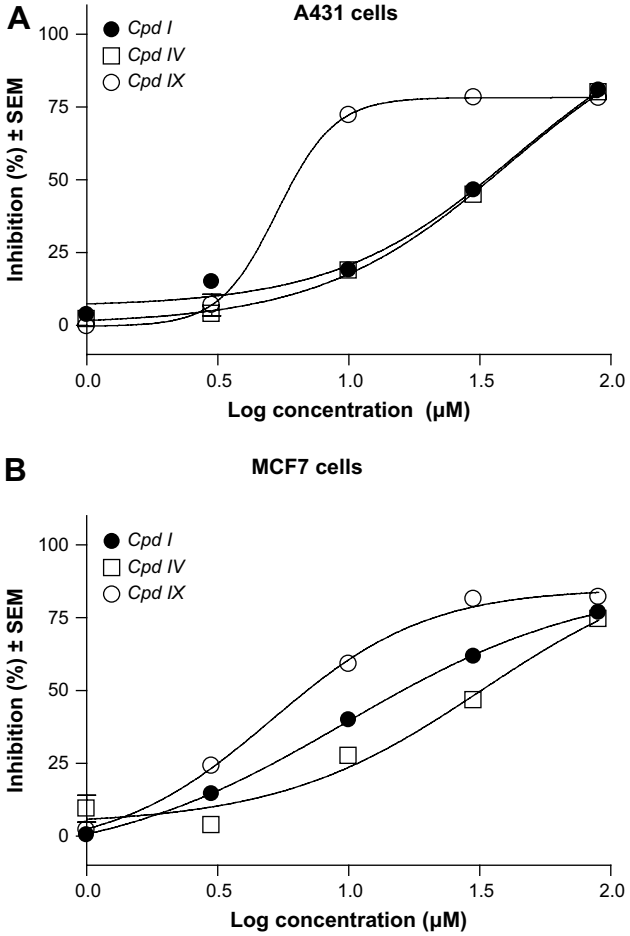

Fig. 3. The graphical representation of the cytostaticity obtained by the compounds **I**, **IV** and **IX**. (A) The plots are given as the inhibitory percent vs. the concentration for the test compounds against A431 cell line using the calculated exact concentrations at which the cell proliferation is inhibited by 50%. (B) The same parameter plotted for the results obtained with MCF7 cell line.

calculated IC_{50} values on all three cell lines (Table 3 and Fig. 3). The compound **I** was a strong inhibitor in topo I relaxation but it gave rise to a high IC_{50} (Fig. 3). A slight change in the inhibitory activity was manifested upon substitution with chlorine at the 5-position of benzimidazole nucleus (data not shown). On the other hand, **IV**, which also gave a high degree of interference in topo I reactions, was not active as a cytostatic agent (Table 3 and Fig. 3). This differs from **II**, **III**, **V** and **VI** by the presence of either a 5C ring within the amine structure at the terminal of ethoxy chain or chlorine atom (Fig. 1). Because of the solubility concern, **VIII** was not differentiated with the same parameters.

The compounds interfering with DNA topoisomerases, in general, are not structurally related to each other. This is most probably due to the presence of multiple mechanisms in the course of topoisomerase inhibition. Our results showed that the topo-inhibition obtained by the three compounds in topo I reactions was not correlated to their cytostatic effects. Therefore, the interpretation of the effects of **I**, **IV** and **IX** in both topo reactions and cytotoxicity requires further kinetic analyses.

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