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Biological activity of bis-benzimidazole derivatives on DNA topoisomerase I and HeLa, MCF7 and A431 cells

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Abstract

Benzimidazoles of both natural and synthetic sources are the key components of many bio-active compounds. Several reports have shown antifungal, antiviral, H_2 receptor blocker and antitumor activities for benzimidazoles and their derivatives. In this study, we synthesized twelve bis-benzimidazole derivatives by selecting di(1*H*-benzo[*d*]imidazol-2-yl)methane as the main compound. The numbers of carbons at 2 positions of bis-benzimidazole derivatives were changed from 1 to 4, and derivatives were synthesized with methyl substitutions at 5- and/or 6- positions. The compounds were screened via *in vitro* plasmid superciol relaxation assays using mammalian DNA topoisomerase I and cytostatic assays were carried out against HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) cells for selected derivatives. Our results suggest that the malonic acid derivatives of bis-benzimidazoles, namely, bis(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)methane and bis(5,6-dimethyl-1*H*-benzo[d]imidazol-2-yl)methane, were remarkably active compounds in interfering with DNA topoisomerase I and the former compound was also found to be cytotoxic against MCF7 and A431 cells. The inhibitory effects obtained with these derivatives are significant as these compounds can be potential sources of anticancer agents.

Keywords: *Bis-benzimidazoles, DNA topoisomerase I, Cytotostaticity*

Introduction

Benzimidazoles and their derivatives are important compounds because of their pharmacologic properties. The diverse substitutions of benzimidazole compounds give rise to antihelmintic [1,2], antifungal [3–5], antitumor [6], antiviral [3,4], H_2 receptor blocker and proton pump inhibitor activities [7]. Several benzimidazole derivatives are also active as inhibitors of DNA topoisomerases [8–11]. DNA topoisomerases are essential enzymes that 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 [12]. Intermediates in the strand passage reaction involve either single- or double-stranded breaks, defining type I and type II enzymes, respectively. Type I topoisomerases (topo I) 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 [12,13]. Over the past years, DNA topoisomerases have been recognized as an effective approach for the development of chemotherapeutics [14–20]. As part of our work in searching for biologically active compounds, we synthesized twelve bis-benzimidazole derivatives of which five of them were original with different substitutions at position 5- (Table I). The structures

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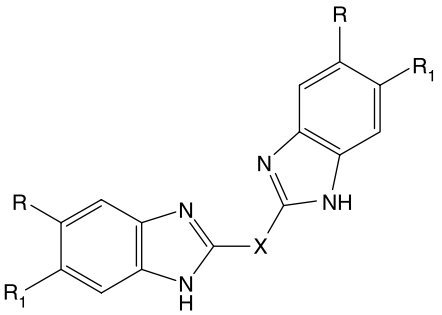
Table I. Physical constants of the synthesized compounds.



Compound	R	R_1	X	m.p. (°C)	Yield (%)	Mol. formula
1	H	H	CH_2	295–298	25.6	$C_{15}H_{12}N_4$
2	CH_3	H	CH_2	283–286	7.5	$C_{17}H_{16}N_4$
3	CH_3	CH_3	CH_2	278–283	7.1	$C_{19}H_{20}N_4$
4	H	H	$(CH_2)_2$	344–345	69.0	$C_{16}H_{14}N_4$
5	CH_3	H	$(CH_2)_2$	271	22.0	$C_{18}H_{18}N_4$
6	CH_3	CH_3	$(CH_2)_2$	275–276	19.0	$C_{20}H_{22}N_4$
7	H	H	$(CH_2)_3$	267	37.2	$C_{17}H_{16}N_4$
8	CH_3	H	$(CH_2)_3$	120	16.8	$C_{19}H_{20}N_4$
9	CH_3	CH_3	$(CH_2)_3$	296	21.7	$C_{21}H_{24}N_4$
10	H	H	$(CH_2)_4$	331	93.3	$C_{18}H_{18}N_4$
11	CH_3	H	$(CH_2)_4$	230	17.6	$C_{20}H_{22}N_4$
12	CH_3	CH_3	$(CH_2)_4$	142	23.0	$C_{22}H_{26}N_4$

of the synthesized compounds were elucidated by melting point, IR, ^{1}H NMR and LC-MS analyses. The compounds were screened for their interference with topo I activity via *in vitro* supercoil relaxation assays. Selected derivatives were subjected to the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide]) assay *in vitro* on three human cell lines; HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) [21].

Experimental

Chemistry

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) were recorded in CD_3OD. Chemical shifts were measured in parts per million (δ). *J* values are given in Hz. Mass spectra (LC-MS) were performed on a Waters 2695 Alliance Micromass ZQ LC-MS Spectrometer Electrosprey Ionization (ESI). 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. All chemical drawings and calculations were performed by using the ChemDraw Ultra 9.0

General procedure for the synthesis of the bis-benzimidazole derivatives. The bis-benzimidazoles were synthesized by the method of acid-catalyzed condensation of a substituted *o*-phenylenediamine with carboxylic acid [2]. All of the bis-benzimidazoles were symmetrically substituted using the same subtituent on both benzimidazole moieties. The 12 bis-benzimidazoles with various substitutions covered in this study are given in Table I. Briefly, 0.005 moles of carboxylic acid and 0.01 moles of substituted *o*-phenylenediamine in 10 mL of 4*N* HCl were refluxed for 6 h–13 h at 135°C in an oil bath. The mixture was cooled and neutralized with 25% NH_4OH. The precipitate was filtered and recrystallized from ethanol/water.

The yield percentages and the melting points of the synthesized compounds are listed in Table I and spectral data obtained are as follows;

*Di(1H-benzo[d]imidazol-2-yl)methane (**1**)*. IR (KBr) cm^{-1}: 3060, 2834, 1621, 1542, 1430, 1033. ^{1}H-NMR (CD_3OD) δ: 4.56 (2H, s, —CH_2—), 7.21 (4H, dd, J = 3.2, 5.6 Hz, H-5, H-5′, H-6, H-6′), 7.52 (4H, dd, J = 3.2, 6.4 Hz, H-4, H-4′,H-7, H-7′). ES-MS m/z Calculate (M^+): 248.28, Found ($M^+ + 1$): 249.31.

*Bis(5-methyl-1H-benzo[d]imidazol-2-yl)methane (**2**)*. IR (KBr) cm^{-1}: 3378, 2919, 1627, 1542, 1448, 1031. ^{1}H-NMR (CD_3OD) δ: 2.42 (6H, s, 2xAr—CH_3), 4,50 (2H, s, —CH_2—), 7.04 (2H, dd, J = 1.6, 8 Hz, H-6, H-6′), 7.31 (2H, brs, H-4, H-4′), 7.39 (2H, d, J = 8.4 Hz, H-7, H-7′). ES-MS m/z Calculate (M^+): 276.34, Found ($M^+ + 1$): 277.40.

*Bis(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)methane (**3**)*. IR (KBr) cm^{-1}: 3384, 2921, 1629, 1533, 1448, 1022. ^{1}H-NMR (CD_3OD) δ: 2.33 (12H, s, 4xAr—CH_3), 4.46 (2H, s, —CH_2—), 7.27 (4H, s, H-4, H-4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 304.39, Found ($M^+ + 1$): 305.44.

*1,2-di(1H-benzo[d]imidazol-2-yl)ethane (**4**)*. IR (KBr) cm^{-1}: 3432, 2931, 1627, 1569, 1454, 1014. ^{1}H-NMR (CD_3OD) δ: 3.93 (4H, s, —CH_2CH_2—), 7.62 (4H, dd, J = 3.2, 6.4 Hz, H-5, H-5′, H-6, H-6′), 7.81 (4H, dd, J = 3.2, 6.4 Hz, H-4, H-4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 262.31, Found ($M^+ + 1$):263.16.

*1,2-bis(5-methyl-1H-benzo[d]imidazol-2-yl)ethane (**5**)*. IR (KBr) cm^{-1}: 3023, 2917, 1631, 1540, 1452, 1022. ^{1}H-NMR (CD_3OD) δ: 2.42 (6H, s, 2xAr—CH_3), 3.36 (4H, s, —CH_2CH_2—), 7.01 (2H, dd, J = 0.8, 8.2 Hz, H-6, H-6′), 7.27 (2H, brs, H-4, H-4′), 7.36 (2H, d, J = 8.4 Hz, H-7, H-7′). ES-MS m/z Calculate (M^+): 290.36, Found ($M^+ + 1$): 291.38.

*1,2-bis(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)-ethane (**6**)*. IR (KBr) cm^{-1}: 3363, 2842, 1627, 1565, 1465, 1006. ^{1}H-NMR (CD_3OD) δ: 2.43 (12H, s, 4xAr—CH_3), 3.85 (4H, s, —CH_2CH_2—), 7.54 (4H, s, H-4, H4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 318.42, Found ($M^+ + 1$): 319.17.

*1,3-di(1H-benzo[d]imidazol-2-yl)propane (**7**)*. IR (KBr) cm^{-1}: 3357, 2917, 1627, 1562, 1459, 1006. ^{1}H-NMR (CD_3OD) δ: 2.36 (2H, quin., J = 7.6, —CH_2*CH_2*CH_2—), 2.98 (4H, t, J = 7.4, —*CH_2*-CH_2*CH_2—), 7.18 (4H, dd, J = 3.6, 6 Hz, H-5, H-5′, H-6, H-6′), 7.48 (4H, m., H-4, H4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 276.34, Found ($M^+ + 1$): 277.39.

*1,3-bis(5-methyl-1H-benzo[d]imidazol-2-yl)propane (**8**)*. IR (KBr) cm^{-1}: 3357, 2917, 1627, 1562, 1459, 1006. ^{1}H-NMR (CD_3OD) δ: 2.34 (2H, quin, J = 7.2, —CH_2*CH_2*CH_2—), 2.42 (6H, s, 2xAr—CH_3), 2.96 (4H, t, J = 7.2 Hz, —*CH_2*CH_2*CH_2*—), 7.03 (2H, dd, J = 0.8, 8.4 Hz, H-6, H-6′), 7.27 (2H, dd, J = 0.8, 2.4 Hz, H-4, H-4′), 7.36 (2H, d, J = 8 Hz, H-7, H-7′). ES-MS m/z Calculate (M^+): 304.39, Found ($M^+ + 1$): 305.45.

*1,3-bis(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)propane (**9**)*. IR (KBr) cm^{-1}: 3097, 2933, 1631, 1538, 1454, 998. ^{1}H-NMR (CD_3OD) δ: 2.30 (2H, m., —CH_2*CH_2*CH_2—), 2.32 (12H, s, 4xAr—CH_3), 2.91 (4H, t, J = 7.6, —*CH_2*CH_2*CH_2*—), 7.23 (4H, s, H-4, H4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 332.44, Found ($M^+ + 1$): 333.47.

*1,4-di(1H-benzo[d]imidazol-2-yl)butane (**10**)*. IR (KBr) cm^{-1}: 3434, 3046, 2956, 1619, 1548, 1438, 1039. ^{1}H-NMR (CD_3OD) δ: 2.11 (4H, quin., J = 3.2 Hz, —CH_2*CH_2*CH_2*CH_2), 3.35 (4H, m., —*CH_2*CH_2CH_2*CH_2*—), 7.57 (4H, dd, J = 3.2, 6.4 Hz, H-5, H-5′, H-6, H-6′), 7.76 (4H, dd, J = 3.2, 6 Hz, H-4, H4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 290.36, Found ($M^+ + 1$): 291.43.

*1,4-bis(5-methyl-1H-benzo[d]imidazol-2-yl)butane (**11**)*. IR (KBr) cm^{-1}: 3397, 3046, 2921, 1631, 1540, 1452, 1045. ^{1}H-NMR (CD_3OD) δ: 1.89 (4H, m, —CH_2*CH_2*CH_2*CH_2), 2.41 (6H, s, 2xAr—CH_3), 2.90 (4H, m., —*CH_2*CH_2CH_2*CH_2*—), 6.99 (2H, dd, J = 1.2, 8.4 Hz, H-6, H-6′), 7.25 (2H, brs, H-4, H-4′), 7.33 (2H, d, J = 8 Hz, H-7, H-7′). ES-MS m/z Calculate (M^+): 318.42, Found ($M^+ + 1$): 319.49.

*1,4-bis(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)butane (**12**)*. IR (KBr) cm^{-1}: 3376, 3203, 2931, 1635, 1542, 1452, 1043. ^{1}H-NMR (CD_3OD) δ: 1.91 (4H, m, —CH_2*CH_2*CH_2*CH_2), 2.35 (12H, s, 4xAr—CH_3), 2.96 (4H, m., —*CH_2*CH_2CH_2*CH_2*—), 7.28 (4H, s, H-4, H4′, H-7, H-7′). ES-MS m/z Calculate (M^+): 346.47, Found ($M^+ + 1$): 347.53 ($M^+ + 1$).

Plasmid supercoil relaxation assays

Plasmid supercoil relaxation assays were carried out as described [22]. Briefly, 20 μL of reaction mixture contained one 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 dithio erythrole (DTT), 5 mM spermidine, and 0.1% bovine serum albumin. A stock solution of 10 mg/mL CPT in dimethl sulfoxide (DMSO) was serially diluted for comparisons. The relaxation products were analyzed on 1% agarose gels in Tris-borate EDTA (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 [23]. DNA bands were quantified from gel photographs using BioRad Multianalyst (ver. 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.

Cytostatic assays

Antiproliferative effects of the test compounds were measured *in vitro* on three human cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma), by using the MTT assay [21]. 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 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 GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA) and the IC_{50} values were calculated.

Results and discussion

Chemistry

The ^1H NMR spectrum of non-substituted compounds (**1**, **4**, **7** and **10**) having symmetrical structures, contain two 1,2-disubstituted systems. The proton signals of alkyl chains combining benzimidazol rings were observed at the expected form/region [24]. Protons of benzimidazoles were seen in a defined form of ABX systems in compounds having methyl groups at 5- position (**2**, **5**, **8** and **11**) while they were assigned as four hydrogen singlets at 4- and 7- positions of the benzimidazole rings for the derivatives bearing methyl groups at both 5- and 6- positions (**3**, **6**, **9** and **12**).

Biological activity assays

The most frequently employed method for monitoring DNA topoisomerase activity utilizes an *in vitro* supercoil relaxation assay using plasmid substrates [11,13]. We employed this method to identify if the synthesized derivatives interfere with topo I reactions. Our assay employs supercoiled plasmid DNA (sc DNA) and relies on the ability of the enzyme to relax sc DNA, which can be separated as discrete bands using gel electrophoresis. An inhibition of relaxation activity due to the presence of a particular inhibitor is monitored in the form of an accumulated faster-migrating sc DNA (Form I). A representative assay using 1 μg/μL concentration of the test compounds in topo I reactions is given in Figure 1. The sc DNA (Figure 1A, lane 1) was fully relaxed (Form II) (Figure 1A, lane 2) and the presence of the organic solvent DMSO did not interfere with the topo I activity (Figure 1A, lane 3). Relaxation of sc DNA substrate was inhibited upon incubation with some of the compounds to different extends (Figure 1, lanes 4-15). The **2** and **3** were found to exert strongest interference (Figure 1A, lanes 8 and 12, respectively), while the **7** and **5** showed an intermediate effect in supercoil relaxation activity of the enzyme (Figure 1A, lanes 6 and 9, respectively). The other derivatives gave rise **to** either neglectable (Figure 1A. lanes; 4, 11, 13 and 15 for **1**, **11**, **6** and **12**, respectively) or no interference with topo I reaction (Figure 1A. lanes; 5, 7, 10 and 14 for the **4**, **10**, **8** and **9**, respectively). We then quantified sc and rlx DNA band intensities and the average values were summarized in Figure 1B. The most effective derivatives **2** and **3** had average interference percentages of 98% and 87%, respectively, as revealed with the remaining sc band intensity

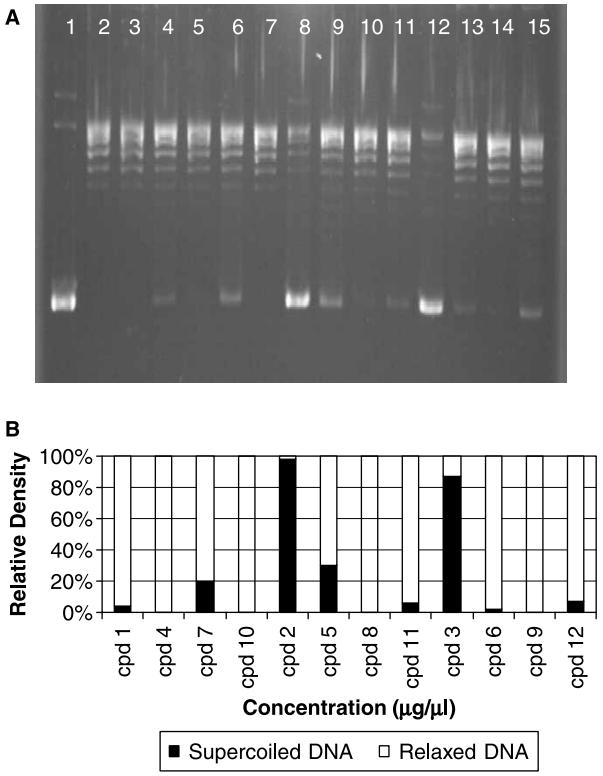


Figure 1. Inhibitory activity of compounds **1-12** on DNA topoisomerase I. **A**. A representative assay using 1 μg/μL concentration of the test compounds in topo I reactions., lane 1, pBR322 DNA without enzyme; lane 2, pBR322 with 1 unit of DNA topoisomerase I; lane 3, same as lane 2 in DMSO; lanes 4-15, same as lane 2 in the presence of the test compounds from 1 to 12, respectively. **B**. Quantitative assessment of the inhibition obtained with the compounds.

with reference to sc pBR322 while this value varied between 2%–30% for **6**, **7**, **11** and **12** (Figure 1B). We next made a serial comparison of topo I reactions using decreasing concentrations of **1**, **2** and **4** as well as the well-known anti-tumor drug, Camptothecin (CPT). The inhibition obtained with the test compounds decreased upon dilutions, which showed the detected sc DNA bands were concentration-dependent (data not shown).

Many reports in recent years have shown that DNA topoisomerase inhibition by numerous natural and synthetic compounds is closely associated with the cytotoxic potentials of the inhibitory compounds [14,16,18,20]. A representative number of compounds (4/12) were selected to carry out MTT assays *in vitro* on HeLa, MCF7 and A431 cells to identify if their effects on topo I reactions coincided with their cytotoxicity. The selected compounds were the **3**, **5**, **6** and **9**. The **3** and **5** represented high (87%) and intermediate (30%) interference in topo I results, respectively. The other two cpds represented either neglectable (**6**; 2%) or derivatives with no interference (**9**; 0%). Among the four cpds, only **3** manifested a considerable cytotoxicity with relatively low calculated IC_{50} values of 3.74 μM and 3.58 μM in A431 and MCF7 cells, respectively, while this value was 13.98 μM when HeLa cells were used (Table II). These values are higher than those of the reference agent doxorubicin reflecting a lower cytotoxic potency but a compound with IC_{50} of 5–10 μM can be considered as a lead for design and synthesis of further analogues. The other compounds did not give a considerable cytotoxicity in MTT tests (data not shown).

The characteristic of **3** is to have the shortest alkyl group and to bear two methyl groups, which are electron donors at both rings (Table I). It has a comparable structure to **2** as well, the other remarkably effective compound on topo I. Considering the resemblance of **2** and **3** to each other and their difference from the other tested compounds, the length of alkyl chain and methyl substitutions could be regarded as the main determinants of the biological activity results. On the other hand, given the complex mechanism of topo I reaction, which involves the enzyme binding to DNA, the breakage of DNA strands, and the religation of the DNA following the strand-passing step, our data do not accommodate a complete mechanistic interpretation of the structure-related activity. Nevertheless, the inhibitory effects obtained with some of bis-benzimidazole derivatives are a significant result as these compounds can be potential sources of anticancer agents.

Table II. Calculated IC_{50} values in MTT tests.

	Calculated IC_{50} values (μM)		
Test Compound	A431	HeLa	MCF7
Cpd.**3**	3.74	13.98	3.58
Doxorubicin	0.19	0.16	0.31

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Notes

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References

[1] Matthews CJ, Broughton V, Bernardinelli G, Melich X, Brand G, Wills AC, Williams AF. Molecular bricklaying: The protonated benzimidazole moiety as a synthon for crystal engineering. New J Chem 2003;27:354–358.
[2] Roderick WR, Nordeen Jr. CW, Von Esch AM, Appell RN. Bisbenzimidazoles. Potent inhibitors of rhinoviruses. J Med Chem 1972;15:655–658.
[3] Agh-Atabay NM, Dulger B, Gucin F. Synthesis and investigation of antimicrobial activity of some bisbenzimidazole-derived chelating agents. Eur J Med Chem 2003;38:875–881.
[4] Arjmand F, Mohani B, Ahmad S. Synthesis, antibacterial, antifungal activity and interaction of CT-DNA with a new benzimidazole derived Cu(II) complex. Eur J Med Chem 2005;40:1103–1110.
[5] Gunes HS, Cosar G. Synthesis of some hydroxamic acid derivatives of benzimidazole and their antibacterial and antifungal activities. Arzneim-Forsch/Drug Res 1992;42:1045–1048.
[6] Kim JS, Sun Q, Yu C, Liu A, Liu LF, LaVoie EJ. Quantative structure-activity relationships on 5-substituted terbenzimidazoles as topoisomerase I poisons and antitumor agents. Bioorg Med Chem 1998;6:163–172.
[7] Malaty H, El-Zimaity HMT, Gentra RM, Cole RA, Graham DY. High-dose proton pump inhibitor plus amoxycillin for the treatment or retreatment of *Helicobacter pylori* infection. Aliment Pharm Therap 1996;10(6):1001–1004.
[8] Sun Q, Gatto B, Yu C, Liu A, Leroy LF, La Voie EJ. Structure activity of topisomerase I poisons related to Hoechst 33342. Bioorg Med Chem 1994;4(24):2871–2876.
[9] Jin S, Kim JS, Sim S, Liu A, Pilch DS, Liu LF, La Voie EJ. Heterocyclic bibenzimidazole derivatives as topoisomerase I inhibitors. Bioorg Med Chem Lett 2000;10:719–723.
[10] Seaton A, Higgins C, Mann J, Baron A, Bailly C, Neidle S, Van den Berg H. Mechanistic and anti-proliferative studies of two novel, biologically active bis-benzimidazoles. Eur J Cancer 2003;39:2548–2555.
[11] Alpan AS, Gunes HS, Topcu Z. 1*H*-Benzimidazole derivatives as mammalian DNA topoisomerase I inhibitors. Acta Biochim Pol 2007;54(3):561–565.
[12] Wang JC. DNA topoisomeases. Ann Rev Biochem 1996;65:635–692.
[13] Bjornsti MA, Osheroff N. DNA topology and enzymes. Totowa, New Jersey: Humana Press; 1999.

[14] Kim JS, Yu C, Liu A, Liu LF, La Voie EJ. Terbenzimidazoles: Influence of 2″-, 4- and 5-substituents on cytotoxicity and relative potency as topoisomerase I poisons. J Med Chem 1997;40:2818–2824.

[15] Topcu Z. DNA topoisomerases as targets for anticancer drugs. J Clin Pharm Ther 2001;26:405–416.

[16] Martín-Cordero C, López-Lázaro M, Gálvez M, Ayuso MJ. Curcumin as a DNA topoisomerase II poison. J Enz Inhib Med Chem 2003;18:505–509.

[17] Thomas CJ, Rahier NJ, Hecht SM. Camptothecin: Current perspectives. Bioorg Med Chem 2004;12(7):1585–1604.

[18] Gálvez M, Martín-Cordero C, Ayuso MJ. Iridoids as DNA topoisomerase I poisons. J Enz Inhib Med Chem 2005;20: 389–392.

[19] Esteves-Souza A, Pissinate K, Nascimento MG, Grynberg NF. Synthesis, cytotoxicity and DNA-topoisomerase inhibitory activity of new asymmetric ureas and thioureas. Bioorg Med Chem 2006;14(2):492–499.

[20] Ishar MPS, Singh G, Singh S, Sreenivasan KK, Singh G. Design, synthesis, and evluation of novel 6-chloro-/fluoro-chromone derivatives as potential topoisomerase inhibitor anticancer agents. Bioorg Med Chem Lett 2006;16: 1366–1370.

[21] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.

[22] Topcu Z, Castora FJ. Mammalian mitochondrial DNA topoisomerase I preferentially relaxes supercoils in plasmids containing specific mitochondrial DNA sequences. Biochim Biophys Acta 1995;1264:377–387.

[23] Topcu Z. Densitometric quantification of DNA topoisomers in ethidium bromide-stained agarose gels and chemiluminescence-detected X ray films. Acta Biochim Pol 2000;47: 835–839.

[24] Hesse M, Meier H, Zeeh B. Spectroscopic methods in organic chemistry. In: Enders D, Noyori R, Trost BM, editors. New York: Georg Thieme Verlag Stuttgart; 1997.