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Some fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors

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Abstract

Our previously synthesized 37 compounds, which are 2,5,6-substituted benzoxazole, benzimidazole, benzothiazole, and oxazolo(4,5-*b*)pyridine derivatives, were tested for their eukaryotic DNA topoisomerase II inhibitory activity in cell free system and 28 were found to inhibit the topoisomerase II at an initial concentration of 100 μg/ml. After further testing at a lower range of concentrations, 12 derivatives, which were considered as positive topoisomerase inhibitors, exhibited IC_{50} values between 11.4 and 46.8 μM. Etoposide was used as the standard reference drug to compare the inhibitor activity. Among these compounds, 2-phenoxymethylbenzothiazole (**3f**), 6-nitro-2-(2-methoxyphenyl)benzoxazole (**1a**), 5-methylcarboxylate-2-phenylthiomethylbenzimidazole (**3c**), and 6-methyl-2-(2-nitrophenyl)benzoxazole **(1c)** were found to be more active than the reference drug etoposide. Present results point out that, besides the very well-known bi- and ter-benzimidazoles, compounds with single bicycle fused ring systems in their structure such as benzimidazole, benzoxazole, benzothiazole, and/or oxazolopyridine derivatives also exhibit significant topoisomerase II inhibitory activity.

Keywords: Topoisomerase II; Inhibitory activity; Benzoxazoles; Benzimidazoles; Benzothiazoles; Oxazolopyridines

DNA topoisomerases are ubiquitous enzymes that control and modify the topological states of DNA. They can catalyze several interconversions between topological isomers of DNA by transiently breaking single or double strands, and resealing them after reorganization of the topology. Topoisomerase I (Topo I) breaks a single DNA stand, while topoisomerase II (Topo II) breaks both strands and requires ATP for full activity [1–4]. Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies [3,5–9]. In recent years, detailed investigations of bi- and ter-benzimidazole derivatives revealed that these compounds constitute a new class of Topo I and II inhibitors [7,8, 10–12]. Work on such compounds indicates that a fused ring system in the structure is critical for the activity.

We synthesized several derivatives of benzoxazoles, benzimidazoles, and related fused heterocyclic compounds, which exhibited significant in vitro antimicrobial and antiviral activities [13–20]. The aim of this study was to investigate the inhibitory activity of our previously synthesized fused heterocyclic compounds, which are given in Table 1, on eukaryotic DNA Topo II in cell-free systems. As a result of this study, predictions that could be revealed from the structure activity relationships of these tested compounds possibly will lead to design more active new Topo II inhibitors.

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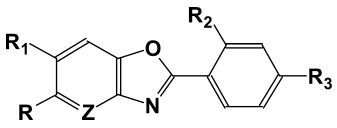
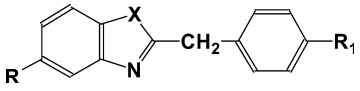
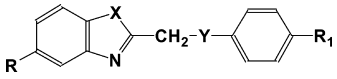
Table 1
Training set of compounds tested for eukaryotic DNA topoisomerase II inhibitory activity

Compound	R	R_1	R_2	R_3	Z
					
1a	—H	—NO_2	—OCH_3	—H	—CH=
1b	—H	—CH_3	—F	—H	—CH=
1c	—H	—CH_3	—NO_2	—H	—CH=
1d	—NH_2	—H	—H	—C_2H_5	—CH=
1e	—CH_3	—H	—CH_3	—CH_3	—CH=
1f	—Cl	—H	—H	—C_2H_5	—CH=
1g	—CH_3	—H	—OCH_3	—H	—CH=
1h	—NO_2	—H	—H	—H	—CH=
1i	—Cl	—H	—H	—Cl	—CH=
1j	—CH_3	—H	—H	—$NHCH_3$	—CH=
1k	—NO_2	—H	—H	—OC_2H_5	—CH=
1l	—H	—H	—H	—C_2H_5	—N=
1m	—H	—H	—H	—Cl	—N=
1n	—H	—H	—H	—$C(CH_3)_3$	—N=
1p	—H	—H	—H	—CH_3	—N=

Compound	R	R_1	X
			
2a	—NO_2	—Br	—O—
2b	—H	—OCH_3	—O—
2c	—CH_3	—NO_2	—NH—
2d	—CH_3	—CH_3	—NH—
2e	—CH_3	—NH_2	—NH—

Compound	R	R_1	X	Y
				
3a	—H	—Cl	—S—	—O—
3b	—CH_3	—H	—NH—	—S—
3c	—$COOCH_3$	—H	—NH—	—S—
3d	—H	—H	—NH—	—CH_2—
3e	—NO_2	—H	—NH—	—O—
3f	—H	—H	—S—	—O—

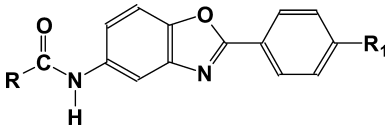
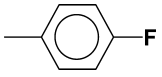
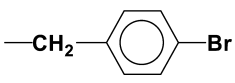
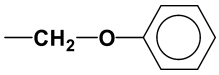
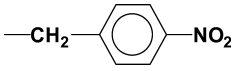
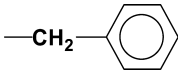
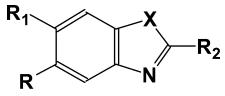
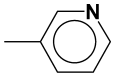
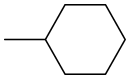
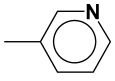
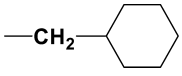
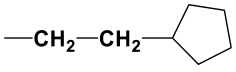
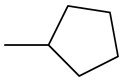
Compound	R	R_1
		
4a		—H
4b		—C_2H_5

Table 1 (*continued*)

Compound	R	R_1
4c		—F
4d		—H
4e		—F

Compound	R	R_1	R_2	X
				
5a	—H	$-CH_3$		—O—
5b	—Cl	$-NO_2$		—O—
5c	—H	—H		—O—
5d	—Cl	—H		—NH—
5e	—H	—H		—NH—
5f	—Cl	—H		—NH—

Materials and methods

Materials. DNA topoisomerase II from *Drosophila melanogaster* embryos was purchased from United States Biochemical. pBluescript plasmid was isolated from *Escherichia coli* XL1-blue strain culture by alkaline lysis technique [21]. Plasmid concentration was determined spectrophotometrically. Fused heterocyclic test compounds were synthesized as previously described and the structures of all the derivatives were supported by spectral data [13–20]. Solutions of the tested compounds in 20% dimethyl sulfoxide (DMSO) were freshly prepared. All the other chemicals were of analytical grade.

Measurement of relaxation activity. Relaxation activity of DNA topoisomerase II was determined by measuring the conversion of supercoiled pBluescript plasmid DNA to its relaxed form [22]. The reaction mixture contained 10 mM Tris–HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/ml bovine serum albumin, 1 mM ATP, 2 μg/ml pBluescript plasmid, 0.01% DMSO, 1–2 U of enzyme, and different concentrations of drugs in a total volume of 20 μl. The mixture was incubated for 16 h at 26 °C. After incubation period, 6 μl of loading buffer containing 2 mM orange G and 55 % glycerol in electrophoresis buffer (60 mM Tris, 30 mM acetic acid, and 1.5 mM EDTA, pH 8.0) was added and mixture was subjected to electrophoresis on 0.8% agarose, at 95 V for 2 h. After the electrophoresis, gels were stained with ethidium bromide (1 μg/ml) and photographed under UV light. Band distribution was analyzed with GDS 8000 Complete Gel Documentation and Analysis System (Gel Works 1D Intermediate, version 2.5; Ultra Violet Products). The rate of formation of the newly formed bands was used as a measure of the enzyme activity. Inhibitory activities were presented as micromolar test compounds that caused 50% inhibition per unit of enzyme, under the assay conditions. Etoposide was used as the reference drug.

Results and discussion

Eukaryotic DNA Topo II activity in cell-free system was evaluated by relaxation assay. The relaxation assay utilises supercoiled plasmid as substrate and has been used by many investigators to study the catalytic activity of Topo I and II types. The supercoiled substrate and its

relaxed product can easily be distinguished in ethidium bromide stained gels since relaxed isomers migrate more slowly than the supercoiled isomer [23]. The change in the molecular shape without a change in the molecular weight can be differentiated, since more compact molecules move faster as compared to their more relaxed counterparts. If the molecules were completely relaxed, a single band closest to the application point would be obtained after the electrophoresis. On the other hand, if all the molecules were in supercoiled form, a fastest moving single band would be obtained. Actually, since there is equilibrium between the different topological forms of the DNA molecules, at the end of the electrophoresis, several bands were obtained. A typical electrophoresis pattern is seen in Fig. 1.

The rate of formation of the newly formed bands during the incubation period was used as a measure of the enzyme activity. The enzyme was incubated with three different concentrations of the test compounds. Decrease in the percentage of the activity as compared to the activity obtained without any agent was used as the inhibitory activity of the compounds. Inhibitory activities were presented as micromolar concentrations of the compounds that cause 50% inhibition per unit of enzyme (IC_{50}), under the assay conditions. From the plots obtained with three different concentrations of the drugs, IC_{50} values were obtained and the results are the averages of two to three estimations. If no inhibition was obtained at 100 μM, the drug was assumed to have no inhibitory activity on eukaryotic DNA Topo II.

When we tested the training set of 37 compounds of benzoxazole, benzimidazole, benzothiazole, and oxazolo(4,5-*b*)pyridine derivatives shown in Table 1 by using the relaxation assay, 28 compounds were found to be able to inhibit the eukaryotic DNA Topo II in cell-free system at an initial concentration of 100 μg/ml. These 28

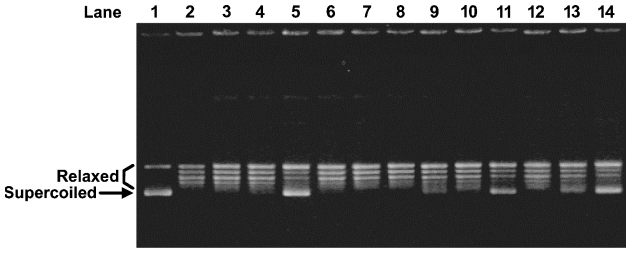


Fig. 1. Electrophoregram showing the inhibitory effects of some tested compounds (**1m**, **4a**, and **3c**) and reference drug etoposide on eukaryotic DNA Topo II. Lane 1, incubation mixture without enzyme. Lane 2, incubation mixture with 2 U of enzyme. Lanes 3–5, incubation mixture with 2 U of enzyme and a known Topo II inhibitor (etoposide) at concentrations of 10, 20, and 100 μM, respectively. Lanes 6–8, compound **1m** at concentrations 10, 20, and 100 μM, respectively, with the incubation mixture and 2 U of enzyme. Lanes 9–11, compound **4a** at concentrations 10, 20, and 100 μM, respectively, with the incubation mixture and 2 U of enzyme. Lanes 12–14, compound **3c** at concentrations 10, 20, and 100 μM, respectively, with the incubation mixture and 2 U of enzyme. The relaxation assay in cell-free system was performed as described in the Materials and methods.

compounds were further tested at a lower range of concentrations to define their inhibitory activity and etoposide was used as the standard drug in order to compare their activity. Table 2 shows the Topo II IC_{50} results of the tested compounds and the standard drug etoposide. Of these 28 compounds, 12 derivatives had IC_{50} values between 11.4 and 46.8 μM range and they were considered as positive Topo II inhibitors.

The most potent eukaryotic Topo II inhibitors (in order of potency with the IC_{50} values in μM shown in parentheses) were **3f** (11.4), **1a** (17.0), **3c** (17.0), **1c** (18.8), **1k** (22.4), **4a** (24.1), **3b** (27.4), and **3e** (28.4). Among these compounds, 2-phenoxymethylbenzothiazole (**3f**), 6-nitro-2-(2-methoxyphenyl)benzoxazole (**1a**), 5-methylcarboxylate-2-phenylthiomethylbenzimidazole (**3c**), and 6-methyl-2-(2-nitrophenyl)benzoxazole **(1c)** were found to be more active than the reference drug etoposide.

Table 2
Eukaryotic DNA topoisomerase II 50% inhibitory activity of the tested compounds and the reference drug etoposide as the micromolar (μM) concentration of IC_{50} values

Compound	IC_{50} (μM)
1a	17.0
1b	433.2
1c	18.8
1d	115.5
1e	44.4
1f	NE
1g	433.0
1h	32.4
1i	NE
1j	128.4
1k	22.4
1l	45.6
1m	119.5
1n	108.3
1p	91.2
2a	NE
2b	86.6
2c	NE
2d	101.9
2e	46.8
3a	NE
3b	27.4
3c	17.0
3d	NE
3e	28.4
3f	11.4
4a	24.1
4b	315.1
4c	206.9
4d	420.1
4e	420.1
5a	NE
5b	101.9
5c	NE
5d	308.1
5e	216.6
5f	NE
Etoposide	21.8

NE, not effected.

Moreover, 5-nitro-2-(4-ethoxyphenyl)benzoxazole **(1k)**, 5-(4-fluorophenylcarboxyamide)-2-phenylbenzoxazole **(4a)**, 5-methyl-2-phenylthiomethylbenzimidazole **(3b)**, and 5-nitro-2-phenoxymethylbenzimidazole **(3e)** had Topo II inhibitory activities comparable to etoposide. Additionally, 5-nitrobenzoxazole **(1h)**, 5-methyl-2-(2,4-dimethylphenyl)benzoxazole **(1e)**, 2-(4-ethylphenyl)oxazolo(4,5-*b*)pyridine **(1l)**, and 5-methyl-2-(4-aminobenzyl) benzimidazole **(2e)** were found to be significantly active, having IC_{50} values between 32.4 and 46.8 μM. On the other hand, compounds with IC_{50} values above the 46.8 μM range (**1b**, **1d, 1g, 1j, 1m–p, 2b, 2d, 4b–e, 5b, 5d,** and **5e**) were assumed as non-active and not taken into consideration.

The results shown in Table 2 indicate that either having sterically bulky substituents such as phenylacetamide or phenoxyacetamide groups at position 5 (compounds **4b–e**) or holding a non-aromatic moiety as cyclohexyl or cyclopentyl rings and/or a pyridine ring at position 2 (compounds **5a–f**) of the fused heterocyclic nucleus causes a severely reduced or lack of activity. On the other hand, different fused heterocyclic nuclei in the structures of the most potent Topo II inhibitors (**1a, 1c, 3c**, and **3f)** are indicating bioisosteric properties for the enzyme inhibitory activity.

In conclusion, the results of this study point out that in addition to the very well-known bi- and ter-benzimidazoles [7,10–12], compounds with single bicycle fused ring systems in their structure such as benzimidazole, benzoxazole, benzothiazole, and/or oxazolopyridine derivatives also exhibit significant Topo II inhibitory activity. Since DNA topoisomerases are considered as important targets for cancer chemotherapy, present findings may provide advanced opportunities to design and develop new chemotherapeutic agents.

Acknowledgment

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