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Some new bi- and ter-benzimidazole derivatives as topoisomerase I inhibitors

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Abstract

The discovery of DNA topoisomerases has added a new dimension to the study of anticancer drugs. In the last years detailed investigation of bi- and ter-benzimidazole derivatives revealed that these compounds are a new class of topoisomerase I inhibitors that poisons mammalian topoisomerase I. In this context a survey about topoisomerase I poisoning activity and cytotoxicity of bi- and ter-benzimidazoles is given. Moreover some recent results about new derivatives, some structure–activity relationships and comparison of activity of various functional groups are discussed.

Keywords: Topoisomerase I; Benzimidazoles; Inhibitors

1. Introduction

DNA topoisomerase I and II are important enzymes for both anticancer and antimicrobial activity. DNA topoisomerase was first discovered by Wong in 1971. The enzyme catalyzes relaxation of negatively supercoiled DNA in the absence of an energy cofactor. It was proposed that this enzyme also catalyzes transient nicking of the DNA double helix and possesses both DNAse and ligase activity in one polypeptide [1]. Since the discovery of *E. coli* topoisomerase I, investigators have isolated many other DNA topoisomerases from both prokaryotes and eukaryotes [2–6].

The role of mammalian DNA topoisomerases as molecular targets for anticancer drugs was not recognized until 1984 [7–9]. Investigators have since carried out extensive studies of the mechanism of action of topoisomerase targeting drugs [10–18].

Topoisomerases have a significant role in DNA metabolism and chromosome structure; therefore, they are important in almost all stages of the cell cycle. Topoisomerases bind to DNA and form a 'cleavable complex'; their enzymatic activities enable catenation, decatenation and relaxation of DNA. Topoisomerase I is ATP independent and introduces transient single-strand breaks; it is therefore required during transcription, especially elongation, but also for chromatin condensation. Conversely, topoisomerase II requires ATP and introduces DNA double strand breaks, which enable catenation and decatenation of DNA. Topoisomerase II plays an important role in chromosome assembly, condensation and segregation of chromosomes in anaphase, as well as in the completion of transcription [19–23].

Camptothecin was the first topoisomerase I drug, isolated from *Camptotheca acuminata* [24]. The ring-open form of camptothecin is inactive against topoisomerase I. The activity of camptothecin sodium salt could result from the pH dependent conversion into the lactone form [25] (Chart 1).

Since the identification of topoisomerase I as the primary molecular target of camptothecin, several new topoisomerase I poisons have been reported which many of them are DNA minor groove binding mode [26–33]. Some of these DNA minor groove binders, Hoechst 33258 and 33342 [19–23](Chart 1), which are topoisomerase I poisons, possess benzimidazole ring systems. These drugs appear to interrupt the breakage-reunion cycle of topoisomerase I by stabilizing a reversible topoisomerase I cleavable complex [29,34–38].

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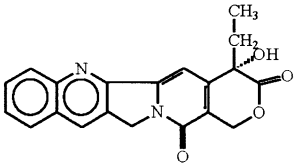
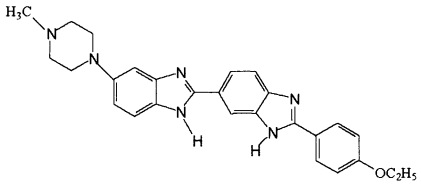
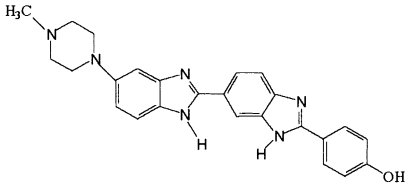


Camptothecin



Hoechst 33342



Hoechst 33258

Some new bi- and ter- benzimidazole derivatives as topoisomerase I inhibitors

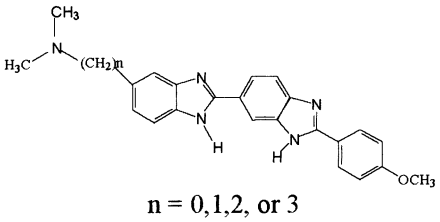
Chart 1.

Also topoisomerase II is the molecular target of many anticancer drugs, including some of the most important anticancer drugs such as adriamycin and etoposide [11,12,39–44].

2. Some new bi- and ter-benzimidazole derivatives as topoisomerase I inhibitors

2′-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1H-benzimidazole (Hoechst 33342) is an important inhibitor of topoisomerase I and it is shown to be an inhibitor of topoisomerase II [19–23]. This agent, which binds to the minor groove of DNA, traps the reversible cleavable complex derived from DNA and topoisomerase I and produces a limited number of highly specific single-strand DNA breaks.

Recently, several analogs of Hoechst 33342 have been synthesized to investigate the structure–activity relationship between topoisomerase I inhibition and cytotoxicity [45] (Fig. 1).



n = 0,1,2, or 3

Fig. 1.

Moreover, the related terbenzimidazole derivatives have been synthesized and investigated for their topoisomerase I inhibitor activity (Table 1).

Comparison of compounds **1**–**7** and **8**, **9**, **10** with Hoechst 33342 (Chart 2) as inhibitors of topoisomerase I demonstrated that several of these terbenzimidazoles had similar potency (Table 1) [46]. The activity observed for **9** as a topoisomerase I inhibitor in comparison to **2** suggest that the 2′-(benzimidazol-5″-yl) moiety is critical to the activity observed for terbenzimidazoles evaluated. For compound **10**, there appears to be a modest decrease in potency in topoisomerase I inhibition observed for analog **4** where the 2′-(benzimidazol-5″-yl) moiety is replaced by a *p*-methoxyphenyl group.

Evaluation of the topoisomerase I inhibition and cytotoxicity of **8**, **9**, and **10** provided additional insight into the structure–activity relationships associated with these benzimidazole derivatives.

The lack of significant DNA cleavage observed with **8** in the presence of topoisomerase I suggests that the addition of a 2″-(*p*-methoxyphenyl) substituent to terbenzimidazoles, structurally related to **4**, interferes with their potential as topoisomerase I inhibitors. These data suggest that steric factors associated with substituents at this position may substantially influence the activity of similar terbenzimidazoles.

Among the terbenzimidazoles evaluated, **1** and **2** were active as topoisomerase poisons, but did not exhibit significant cytotoxicity. The basis for this lack of cytotoxicity was ascribed to their poor penetration into cells. In contrast, the presence of a 5-phenyl or 5-pyridyl substituent on these terbenzimidazoles as in **4**

Table 1
Topoisomerase I mediated DNA cleavage and cytotoxicity of bi- and ter-benzimidazoles

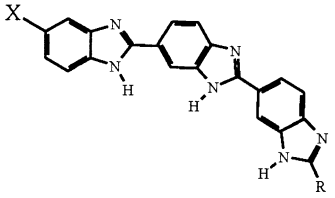
Comp.	Topoisomerase I-mediated DNA cleavage [a]	Cytotoxicity IC_{50} [b] (μM), cell lines			
		RPMI	CPT-K5	KB3-1	KBV-1
Hoechst 33342	1	0.03	0.9	0.01	1.2
1	1.1	14	28	ND	ND
2	1	>25[c]	>25[c]	ND	ND
3	100	7.6	20	ND	ND
4	2	0.09	0.58	0.58	0.35
5	3.3	0.16	5.8	0.05	0.09
6	2	0.035	2.5	0.02	0.02
7	2	0.035	2.5	0.02	0.01
8	1000	>20[c]	ND	ND	ND
9	1000	>25[c]	ND	ND	ND
10	3.3	27	ND	ND	ND

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to Hoechst 33342.
[b] IC_{50} was calculated after 4 days of continuous drug exposure. ND, not determined.
[c] No indication of cytotoxicity was considered indicative of IC_{50} values substantially greater than the highest doses assayed.

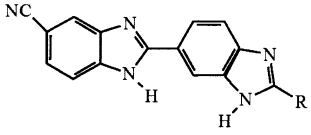
and **5** resulted in retention of activity as topoisomerase I poisons, as well as significant cytotoxicity against several tumor cell lines.

In another study, the relative activity of 4,5- and 5,6-benzo fused terbenzimidazoles and 4-phenyl and 5-naphtylbenzimidazoles as topoisomerase I poisons and cytotoxic agents was compared to the 5-phenylterbenzimidazole (Chart 3) [47].

The cytotoxicity of the naphthylterbenzimidazoles and 5-(*p*-methoxyphenyl)-terbenzimidazole **7** and 5-(*p*-chlorophenyl)terbenzimidazole **8** against the human lymphoblastoma cell line, RPMI 8402 and their relative activity as topoisomerase I poisons were determined. While the 5-(2-naphthyl)terbenzimidazole, **6**, had similar activity to 5-phenylterbenzimidazole, **1**, 5-(1-naphthyl)terbenzimidazole, **5**, was less cytotoxic. While **7** was slightly more potent as a topoisomerase I poison than either **1** or **8**, all of these 5-phenylterbenzimidazoles had similar cytotoxic activity. These data indicate that para-substituents on the 5-phenyl ring retain activity. The pharmacological activity observed for **6**, in which there is a 2-naphthyl substituent at the 5-position of these terbenzimidazoles suggest that *meta*-substituents on a 5-phenyl moiety could also be well tolerated. The loss of antitumor activity in the case of the 1-naphthyl analog **5** suggests that 5-(*ortho*-substituted phenyl)terbenzimidazoles may exhibit diminished activity. The relative activity of 4-phenyl-terbenzimidazole, **4**, and the 4,5-benzo-fused and 5,6-benzo-fused terbenzimidazoles **2** and **3** as topoisomerase I poisons was also determined. In contrast to **1** and **5**, **3** was not a topoisomerase I poison and did not possess cytotoxic activity. Its lack of activity relative to **1** as a topoisomerase I poison provides a useful probe for further comparison and



1, X= H ,R=H
2, X= CN, R=H
3, X= n-C_3H_7, R=H
4, X= Phenyl, R=H
5, X= 2- Pyridyl, R=H
6, X= 3- Pyridyl, R=H
7, X= 4- Pyridyl, R=H
8, X= CN, R= p-methoxyphenyl



(9, R=H)
(10, R= p- methoxyphenyl)

Chart 2.

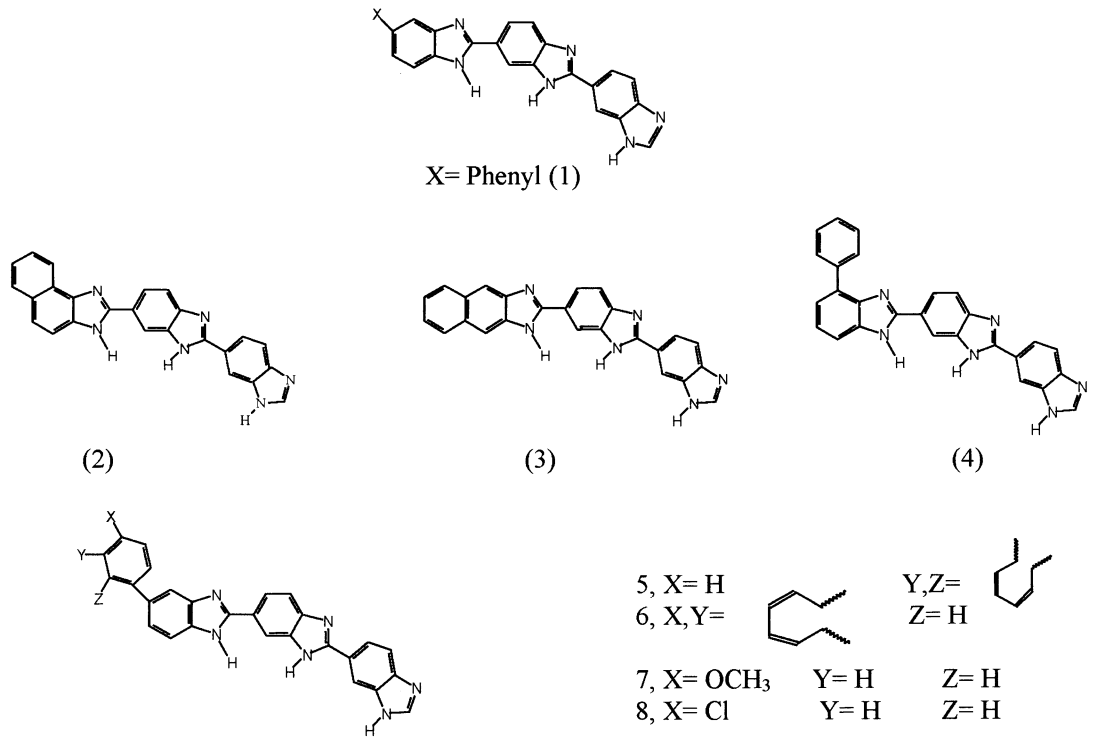
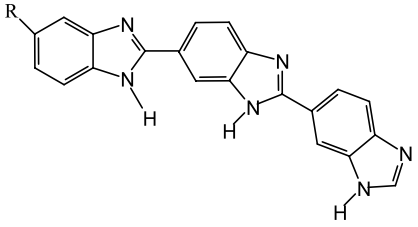


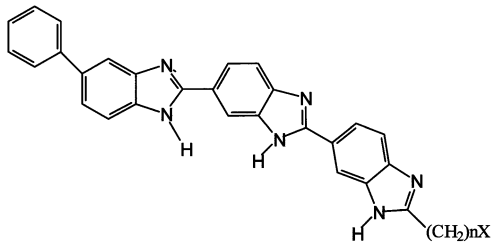
Chart 3.

Table 2
Physicochemical parameters and biological results associated with 5-substituted terbenzimidazoles



Code	R	π [a]	σ_p	σ_m	Topo [b]	Cytotoxicity [c]
1	1-naphthyl	3.19 [d]	NA [e]	0.06	10	1.26
2	2-naphthyl	3.19 [d]	NA [e]	0.06	10	0.16
3	Phenyl	1.96	−0.01	0.06	1	0.19
4	Propyl	1.45	−0.17	−0.06	0.5	15.31
5	Br	0.86	0.23	0.39	1	1.63
6	Piperidine	0.85	−0.57	NA [e]	0.5	0.63
7	Cl	0.71	0.23	0.37	1	1.30
8	F	0.14	0.06	0.34	0.05	1.70
9	H	0.00	0.00	0.00	1	5.00
10	OCH_3	−0.02	−0.27	0.12	0.05	0.79
11	NO_2	−0.28	0.78	0.71	0.5	113.9
12	CN	−0.57	0.66	0.56	0.1	133.4
13	OH	−0.67	−0.37	0.12	0.5	150.3
14	NH_2	−1.23	−0.66	−0.16	1	61.64

[a] Hansch π value was used except where otherwise noted.
[b] Values provided reflect relative effective concentrations needed to produce a similar degree of DNA fragmentation.
[c] Values represent the IC_{50} values (μM).
[d] Calculated value using Rekker's method.
[e] NA indicates that values were not available in the literature.



Compound	n	X
1	0	H
2	0	OH
3	0	SH
4	0	NH
5	0	Cl
6	0	CF_3
7	1	H
8	1	OH
9	1	$NHCOC_6H_5$
10	2	H
11	2	OCH_3
12	2	OH
13	2	NH_2
14	2	$NHCOCH_3$
15	2	CH_3

Chart 4.

examination of the biophysical interactions associated with DNA and topoisomerase which are critically linked to the topoisomerase poisoning activity of terbenzimidazoles.

The QSAR study was prepared on a group which was selected 14 compounds possessing terbenzimidazole ring and π, σ_p and σ_m as physicochemical parameters (Table 2) [48].

As a result, a one variable simple linear equation, best defines the relationship between these physicochemical properties of these terbenzimidazoles and their cytotoxic activity.

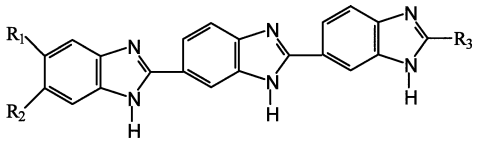
$$\log 1/IC_{50} = 0.996\pi - 0.949$$

$$n = 10, \quad r = 0.84, \quad s = 0.232, \quad F = 18.44$$

These data indicated that the critical parameter associated with the cytotoxicity is their π values. Electronic parameters (5-*para* and 5-*meta*) did not contribute to the cytotoxicity and to the topoisomerase I activity of these compounds whereas lipophilicity (π) did significantly correlate with the cytotoxicity of these 5-substituted terbenzimidazoles.

Several 5-phenyl-2″-substituted terbenzimidazoles with varied physicochemical properties were investigated in another study (Chart 4) [49].

As a result, terbenzimidazoles represent a structurally unique class of topoisomerase I poisons. Comparative biological and biophysical data have suggested that formation of a ternary enzyme-DNA-terbenzimidazole complex may involve alignment of terbenzimidazole molecule such that its 2″-end is in close proximity to the enzyme. Analogues of 5-phenyl-terbenzimidazole with various 2″-substituents that may potentially interact with the enzyme and further stabilize the cleavable form of the ternary complex were synthesized. Several of the 5-phenylterbenzimidazole derivatives that were among the more potent topoisomerase I poisons did exhibit greater cytotoxicity toward RPMI 8402 cells.



Compound	R_1	R_2	R_3
1a	Br	H	H
1b	Br	H	OH
1c	Br	H	$CH_2CH_2CH_3$
2	p-Cl-Phenyl	H	CF_3
3a	Br	Br	H
3b	Br	Br	Cl
3c	Br	Br	CF_3
4	Phenyl	Phenyl	H
5	Br	OCH_3	H
6	Phenyl	OCH_3	H

Chart 5.

Table 3
Relative topoisomerase I poisoning activities and cytotoxicity of 5-phenyl terbenzimidazoles and 5-bromoterbenzimidazoles

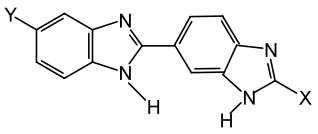
Comp.	Relative topoisomerase I-mediated DNA cleavage	Cytotoxicity	
		RPMI-8402 IC_{50} (μM)	CPT-K5 IC_{50} (μM)
1a	1.0	1.6	2.0
1b	1.0	6.5	>10
1c	1.0	0.21	>10
2	1.0	0.33	1.4
3a	1.0	0.26	2.0
3b	1.0	0.11	0.09
3c	1.0	0.25	8.8
4	1.0	1.6	>10
5	2.0	1.6	>10
6	0.5	0.35	>10

The more potent topoisomerase I poisons **5**, **6** and **15** were among the most cytotoxic derivatives.

These studies confirm that substituents of the 2″-end can influence both topoisomerase I poisoning activity and cytotoxicity of terbenzimidazoles. These data also suggest that the presence of electronegative substituents at the 2″-position is associated with enhanced topoisomerase I poisoning activity and cytotoxicity. There was no evidence to suggest that this enhanced activity was related to an increased interaction with the enzyme. The increase in potency for such substituted terbenzimidazoles may be related, in part, to an enhanced binding to DNA that could stabilize the cleavable ternary complex formed by enzyme, drug, and DNA.

In another study, it was examined whether in the case of 5,6-dibromo-terbenzimidazole, the presence of a

Table 4
Cytotoxicity and topoisomerase I-mediated cleavage of DNA induced by 2,5′-bi-1H-benzimidazoles



Comp.	X	Y	Topoisomerase I-mediated cleavage [a]	Cytotoxicity IC_{50} (μM) [b]
Hoechst 33342	4-ethoxyphenyl	4-methylpiperazinyl	1.0	0.03
1a	phenyl	–CN	>100	>25
1b	2-tolyl	–CN	≫100	1.2
1c	3-tolyl	–CN	100	0.86
1d	4-tolyl	–CN	1	0.57
1e	1-naphthyl	–CN	10	0.65
1f	2-naphthyl	–CN	10	0.13
2a	phenyl	$-CONH_2$	10	>25
2b	2-tolyl	$-CONH_2$	100	8.2
2c	3-tolyl	$-CONH_2$	100	8.2
2d	4-tolyl	$-CONH_2$	10	>25
2e	1-naphthyl	$-CONH_2$	20	3.7
2f	2-naphthyl	$-CONH_2$	100	0.74
3a	phenyl	4-methylpiperazinyl	1	0.49
3b	2-tolyl	4-methylpiperazinyl	10	0.59
3c	3-tolyl	4-methylpiperazinyl	1	0.36
3d	4-tolyl	4-methylpiperazinyl	1	0.83
3e	1-naphthyl	4-methylpiperazinyl	20	0.33
3f	2-naphthyl	4-methylpiperazinyl	10	0.11

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration i.e., concentrations relative to that of Hoechst 33342, whose value is arbitrarily assumed as 1, that are able to produce the some cleavage on the plasmid DNA in the presence of a thymus topoisomerase I. Cleavage is calculated from the intensity of the strongest Hoechst specific band.

[b] IC_{50} has been calculated after 4 days of continuous drug exposure. No indication of cytotoxicity was considered indicative for IC_{50} values substantially greater than the highest dose assayed, 25 μM.

chloro or trifluoromethyl substituent at the 2″-position would enhance its topoisomerase I poisoning activity and cytotoxicity (Chart 5) [50].

The data listed in Table 3 demonstrate that 5,6-disubstituted terbenzimidazoles have activity as topoisomerase I poisons. 5,6-Disubstituted terbenzimidazoles that were evaluated, 5-bromo-6-methoxyterbenzimidazole **5** was only slightly less potent than **3a** as a topoisomerase I poison. While there was some minor variation in the activity, for the derivatives evaluated it would appear that such substituents did not exert a major impact on topoisomerase I poisoning activity. The presence of electron-withdrawing substituents at the 2″-position in the case of 5-phenylterbenzimidazoles was observed to increase their relative activity as topoisomerase I poisons. It was investigated whether a similar effect could be seen in the case of derivatives of 5,6-dibromoterbenzimidazole **3a**. The presence of either a 2″-chloro **3b** or a 2″-trifluoromethyl group **3c** did not significantly influence topoisomerase I poisoning activity within this subset of terbenzimidazole derivatives.

There are two structural modifications that appear to have a pronounced effect on the cytotoxicity of terbenzimidazole derivatives. One of them is the presence of lipophilic substituents at either 5- and/or 6-position consistently appears to favor cytotoxicity. The other modification is, having a 5,6-benzo-fused ring that cause a major loss in cytotoxic activity. However, while the presence of functionality at both the 5- and 6-position does not necessarily result in a reduction in cytotoxic activity. Whereas the presence of a chloro or 2-trifluoromethyl substituent at 2″-position does not alter the activity against RPMI 8402 cells.

Table 5
Topoisomerase I activity, DNA binding affinity and cytotoxicity

Comp.	Topoisomerase-I [a]	K_{Tm} (M^{-1}) [b]	RPMI 8402 (IC_{50}) [c]	CPT-K5 (IC_{50}) [c]
1	1	2.8×10^8	0.09	0.70
2	1	n.d. [d]	0.47	0.47
3	>100	n.d. [d]	20	>20
4	1	n.d. [d]	2.3	21
5	50	3.5×10^7	0.28	0.38
6	5	1.9×10^8	0.015	0.2

[a] The values assigned reflect the relative effective concentrations of drug that are able to produce the same degree of cleavage on the plasmid DNA in the presence of human topoisomerase I.

[b] K_{Tm} denotes the drug-poly (dT)·poly (dT) association constant at the melting temperatures (T_m) of the drug–DNA complex. The T_m values are as follows: 81.5, 73.4, and 80.0 °C for the poly (dA)·poly (dT) complexes with 2, 8, and 9, respectively.

[c] RPMI8402 is a human lymphoblast tumor cell line; CPT-K5 is a camptothecin-resistant variant cell line derived from RPMI 8402.

[d] n.d., not determined.

While Hoechst 33258 is cytotoxic, the more lipophilic derivative Hoechst 33342 exhibits greater cytotoxicity [51,52]. Whereas studies were performed to assess the effects of structural variation at the 5-position of 2,5′-bi-1H-benzimidazoles on topoisomerase I poisoning, the influence of structural variations at the 2′-position was not been determined.

In another study, several 5-cyano-2′-aryl-2,5′-bi-1H-benzimidazole derivatives were synthesized. Topoisome-

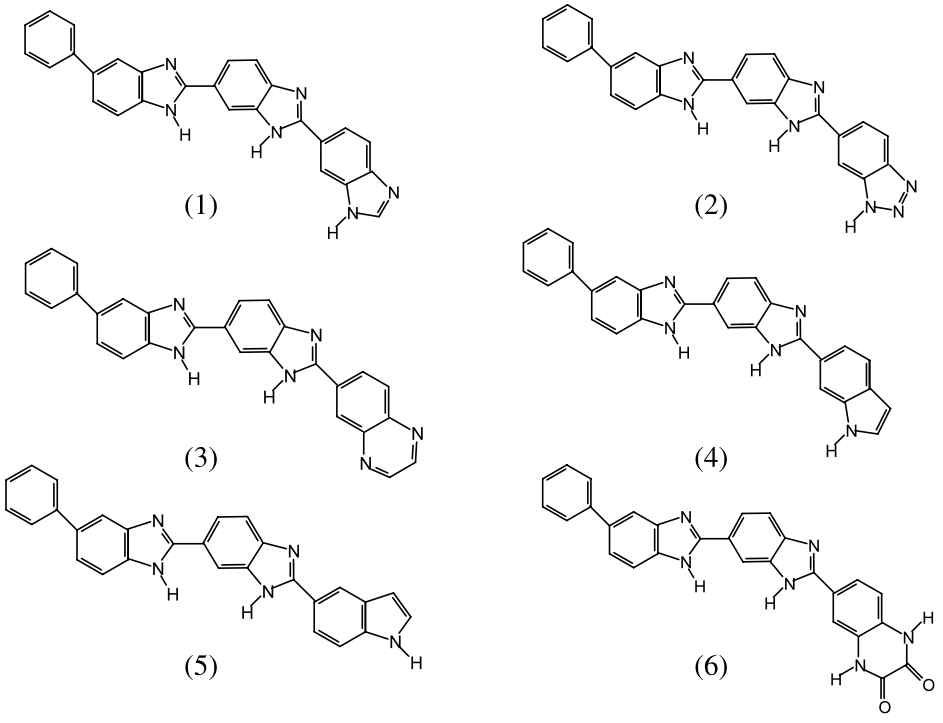


Chart 6.

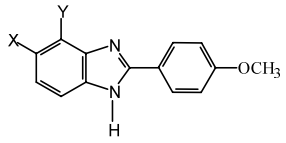
rase I poisoning and cytotoxicity of the 2′-phenyl derivatives as well as all positional isomers of either a 2′-tolyl group or a 2′-naphthyl groups were investigated (Table 4) [52].

Within the series of compounds **3a**–**f** which possess a 5-(4-methylpiperazinyl) moiety, **3a**, **c**, **d** exhibited similar potency to Hoechst 33342 as topoisomerase I poisons. In general, individual compounds within this series, compounds **3a**–**f**, were among the more cytotoxic derivatives against the human lymphoblastoma cell line RPMI 8402.

The 5-(aminocarbonyl)-2′-substituted-2,5′-bi-1H-benzimidazole derivatives **2a**–**f** were all less active than their corresponding 5-cyano-2,5′-bi-1H-benzimidazoles, with the exception of **1a**. Compounds **1a** and **2a** were among the weaker topoisomerase I poisons and among the least cytotoxic of the derivatives evaluated. None of the 5-(aminocarbonyl)-2′-substituted-2,5′-bi-1H-benzimidazole derivatives **2a**–**f** exhibited greater potency than their corresponding 5-(4-methylpiperazinyl)-2,5′-bi-1H-benzimidazole derivatives as either topoisomerase I inhibitors or cytotoxic agents.

Evaluation of the data associated with each series of compounds (the 5-cyano, 5-aminocarbonyl, and 5-piperazinyl series) did reveal similarities. It was observed, for example, that within all three series of 5-substituted-bi-1H-benzimidazoles, the 2′-(2-naphthyl) and 2′-(1-naphthyl) derivatives were among the more cytotoxic analogs. The increased lipophilicity, which would be expected with these naphthyl derivatives, appears to correlate with their greater cytotoxic activity.

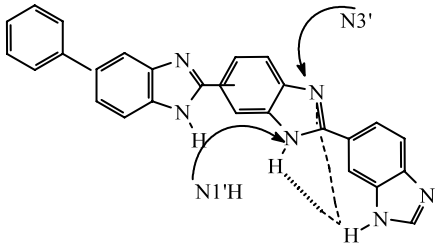
Table 7
Derivative examined for topoisomerase I inhibition activity



NO	X	Y
1	CN	H
2	COH	H
3	CH_2OH	H
4	CH_2NH_2	H
5	$CONH_2$	H
6	COOH	H
7	H	H
8	Br	H
9	NO_2	H
10	H	NO_2

Similar comparisons of the relative activity of these agents as topoisomerase I poisons indicated that analogs with a 2′-(4-tolyl) group were among the most potent inhibitors of topoisomerase I. These data suggest that the presence of a lipophilic substituent at the para position within the various series of 2,5′-bi-1H-benzimidazoles may enhance topoisomerase I inhibitory activity. There is, however, no similar consistency observed

Table 6
Estimation of interatomic distances by molecular modeling



Comp.	Reference atoms [a]	Distance (Å)	Reference atoms [b]	Distance (Å)
1	N3′–NH1″	5.7–6.6	NH1′–NH1″	5.2–6.9
6	N3′–NH1″; N3′–NH4″	5.2–7.2	NH1′–NH1″; NH1′–NH4″	4.7–7.1
5	N3′–NH1″	7.2–7.3	NH1′–NH1″	7.0–7.2
4	N3′–NH1″	5.6–6.5	NH1′–NH1″	5.1–6.8

[a] Reference atoms include the N3′ atom, which can act as a hydrogen acceptor and one or more NH substituents associated with the 2′-heterocycle that are capable of acting as hydrogen donors in the formation of a hydrogen bonding interaction.

[b] Reference atoms include the NH1′ atom, which can act as a hydrogen donor and one or more NH substituents associated with the 2′-heterocycle that are capable of acting as hydrogen donors in the formation of a hydrogen bonding interaction.

for the tolyl derivatives with regard to the results obtained for their relative cytotoxicity.

In another study, the relative biological activity of 5-phenyl bibenzimidazoles with a benzotriazole, quinoxaline, quinoxalinedione or indole attached to their 2′-position was examined (Chart 6) [53].

According to the Table 5, the benzotriazole analogue **2** showed comparable activity to Hoechst 33342 both as a topoisomerase I poison and as a cytotoxic agent. No significant cross-resistance ($\Delta IC_{50} > 10_x$) was observed in the camptothecin-variant cell line, CPT-K5. The quinoxaline-2,3-dione analogue **6** was also as active as Hoechst 33342, which is a topoisomerase I poison, but did not exhibit similar cytotoxicity toward RPMI 8402 cells. A significant decrease in overall activity was observed for the quinoxaline analogue, **3**. These data suggest for these 5-phenylbibenzimidazoles that 2′-heterocyclics with a hydrogen atom attached to a heteroatom have increased topoisomerase I poisoning activity and cytotoxicity. The result of DNA binding studies, reveal that **6** does have a greater DNA binding affinity, which correlates with its greater potency relative to **5** as a topoisomerase I poison. Molecular modeling was performed with **1**, **4**, **5**, and **6** to determine the interatomic distance between either the N3′ or the NH1′ atoms of the bibenzimidazole moiety and NH atoms incorporated within the various 2′-substituents. Data were obtained from the more energetically favored conformations associated with rotation about the bond extending from the 2′-position to the attached heterocycle.

The results suggest that several 2′-heterocyclic derivatives of 5-phenyl-2,5′-1H-bibenzimidazoles can be envisioned that they would retain activity as topoisomerase I poisons. Heterocyclic derivatives that have incorporated within their structure a hydrogen atom capable of participating in hydrogen bond formation can have topoisomerase I poisoning activity comparable to terbenzimidazole analogues. These data also indicate that DNA binding affinity could explain the differences in biological activity between the positional isomers **4** and **5**. The results of molecular modeling suggest that for retention of topoisomerase I poisoning activity, the distance between the NH1′ and N3′-position of the 2,5′-1H-bibenzimidazole portion of the molecule and a hydrogen donating substituent attached at the 2′ position should be < 7.0 and < 7.2 Å, respectively (Table 6).

In another study, some benzimidazole derivatives were synthesized and examined for the topoisomerase I inhibition activity (Table 7) [54]. As a result, compounds **2** and **5** had significant activity as topoisomerase I poisons. Only very marginal activity was observed for compounds **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-methoxy-phenyl)-1H-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-methoxy-phenyl)-1H-benzimidazole, **9**, was active as a topoisomerase I poison. In a comparison of the topoisomerase I poisoning ability of the isomeric nitro-benzimidazole **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 intermolecular hydrogen bonding which could occur with 7-nitro-2-(4-methoxyphenyl)-1H-benzimidazole **10** could be responsible for the diminished activity observed for this positional isomer.

3. Conclusion

DNA topoisomerases have an intrinsic involvement in biological functions of cell for presenting a target of successful chemotherapy of certain cancers. While among the clinically used drugs of the classical topoisomerase II inhibitor series have selectivity and multidrug resistance problems, topoisomerase I inhibitors have a good selectivity and their resistance problem is under control. The topoisomerase I inhibitors represent a new class of promising antitumor active agents for chemotherapy. Further investigation is needed to identify and design topoisomerase I inhibitors with little or no toxicity.

In this study, a new class, which includes some bi- and ter-benzimidazole derivatives is, described as topoisomerase I inhibitors. In general 5-position of terbenzimidazoles is an important position for their activity. The presence of phenyl, naphthyl or pyridyl groups at this position influence cytotoxicity. In addition to this, para substituents at 5-position needed for the activity as topoisomerase I poisoning. For terbenzimidazoles 2′-position is the critical position to be a topoisomerase I inhibitor. 2″-Position is also important for both topoisomerase I poisoning activity and cytotoxicity. It is proved that electronegative substituents at 2″-position also increase topoisomerase I poisoning activity. The researches for 5-phenyl-2,5′-bi-1H-benzimidazoles indicate that these derivatives also have topoisomerase I

poisoning activity as well as terbenzimidazoles. Among these bibenzimidazole 2′-naphthyl derivatives have more potent cytotoxic activity. A lipophilic substituent at *p*-position of these compounds increase topoisomerase I inhibition activity. It is obvious that a heterocyclic ring at 2′-position also enhances the activity.

We previously synthesized some benzimidazoles and its analogues as benzoxazoles, benzothiazoles and determined their antimicrobial activity as MIC values between 6.25 and 200 μg/ml against some Gram positive and Gram negative bacteria and the fungus *Candida albicans*. We newly started to determine the DNA topoisomerase activity of our compounds in order to interpret the mechanism of the antimicrobial activity [55–58].

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