Novel Cyano- and Amidino-Substituted Derivatives of Styryl-2-Benzimidazoles and Benzimidazo[1,2-*a*]quinolines. Synthesis, Photochemical Synthesis, DNA Binding, and Antitumor Evaluation, Part 3

Marijana Hranjec,[†] Marijeta Kralj,*[,‡] Ivo Piantanida,[§] Mirela Sedić,[‡] Lidija Šuman,[‡] Krešimir Pavelić,[‡] and Grace Karminski-Zamola*[,†]

Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, Post Office Box 177, HR-10000 Zagreb, Croatia, Division of Molecular Medicine, "Ruđer Bošković" Institute, Bijenička cesta 54, Post Office Box 180, HR-10000 Zagreb, Croatia, and Division of Organic Chemistry and Biochemistry, "Ruđer Bošković" Institute, Post Office Box 180, HR-10002 Zagreb, Croatia

Received July 20, 2007

Synthesis of novel cyano- and amidino-substituted styryl-2-benzimidazoles and benzimidazo[1,2-*a*]quinolines by condensation reactions and photochemical dehydrocyclization and dehydrohalogenation cyclization is described. Thermal denaturation experiments reveal that cyclic derivatives considerably stabilize DNA double helix, while the effect of their acyclic analogues is negligible. According to the spectroscopic study of the interaction of cyclic derivative **19**, we propose intercalation of benzimidazo[1,2-*a*]quinoline moiety into ct-DNA as a dominant interaction underlying biologically relevant effects of this compound, whereas for its acyclic derivative **11**, we propose binding into the minor groove of DNA. All compounds show noticeable antiproliferative effect. Morpholino- and chloro-substituted compound **9** is the most active among all acyclic derivatives. All cyclic compounds were 2- to 10-fold more potent, which is correlated with their property to intercalate into DNA. The most active imidazolyl-substituted compound **19** inhibits topoisomerase II and induces strong G2/M cell cycle arrest, pointing to the impairment in mitotic progression. Its pronounced selectivity toward colon carcinoma cells encourages further development of this compound as a lead.

Introduction

One of the most important goals in medicinal chemistry is the development of new heterocyclic compounds with antitumor activity. The most used classes of chemotherapeutic agents in cancer therapy comprise molecules that interact with DNA, such as groove binders, DNA alkylating agents, or intercalators. Intercalators in most cases consist of two or more planar fused aromatic or heteroaromatic rings able to insert between adjacent base pairs of a DNA molecule without disturbing the overall stacking pattern.[1,2] This binding mode is characterized by low selectivity toward different DNA or RNA sequences as well as low or nonselective bioactivity.[2] However, the activity of many antitumor drugs is based on intercalation, but selectivity could be improved by introduction of specific substituents on the intercalative aromatic core.[2,3] Several antibiotics are DNA intercalators and are recognized as a new, promising class of antitumor agents.[4]

Substituted benzimidazoles and their azino-fused cyclic derivatives have drawn considerable attention of medicinal and organic chemists due to a wide range of biological activities exerted by this class of compounds.[5−13] Also, the corresponding benzo-annulated analogues, such as benzimidazo[2,1-*a*]isoquinolines, benzimidazo[1,2-*c*]quinazolines, or benzimidazo[3,2-*a*]quinolinium hydrochloride salts showed interesting biological properties. Braña et al. prepared amino- and amido-substituted benzimidazo[1,2-*c*]quinazolines, evaluated their in vitro cytotoxicity against a panel of human and murine cell lines, and showed that the most active compound bound to DNA as an intercalator.[14] The authors also showed intercalation ability of compounds having nearly planar chromophores. Moreover, a series of benzimidazo[2,1-*a*]isoquinolines with carboxamide side chains were prepared to study the biological effects induced by the variation in the side-chain position in this tetracyclic series.[15] It was shown that 6-carboxamides with the side chain attached to one of the central rings were not active, while 1- and 11-carboxamides with the side chain attached to one of the terminal rings of the chromophore short axis exerted reasonable cytotoxicity in vitro and in vivo.

Several methylated derivatives of benzimidazo[1,2-*b*]isoquinolines had the activity similar to ellipticine as mammalian topoisomerase II inhibitors and were also highly active in vitro, whereby they inhibited the growth of several human tumor cell lines.[16] The group of authors prepared a series of benzimidazo-[3,2-*a*]quinolinium hydrochloride salts[17−19] and evaluated their biological activity as well as interaction with DNA and inhibition of topoisomerase II activity.[20,21] All compounds exhibited moderate antitumor activity in vitro, and the results suggested that their primary mechanism of action was inhibition of the enzymes implicated in DNA functionality rather than DNA binding, whereby substitutions in the benzimidazole moiety had striking effects on the biological activity of examined compounds.

Benzimidazo[1,2-*a*]quinolines have remained unexplored for their biological activity most likely because of the lack of general synthetic methods starting from easily accessible precursors. Unsubstituted benzimidazo[1,2-*a*]quinoline was first prepared by the classical method from 2-aminoquinoline and picric acid[22] and later also in reaction of photochemical dehydrocyclization by UV irradiation of methanolic solution of styryl-2-benzimi-

* To whom correspondence should be addressed. Tel.: +385 1 4571 235 (M.K.); ++38514597215 (G.K.-Z.). Fax: +3851 4561 010 (M.K.); ++38514597250 (G.K.-Z.). E-mail: mhorvat@irb.hr, marijeta.kralj@irb.hr (M.K.); gzamola@pierre.fkit.hr (G.K.-Z.).

[†] University of Zagreb.

[‡] Division of Molecular Medicine, "Ruđer Bošković" Institute.

[§] Division of Organic Chemistry and Biochemistry, "Ruđer Bošković" Institute.

10.1021/jm070876h CCC: $37.00
Published on Web 10/13/2007

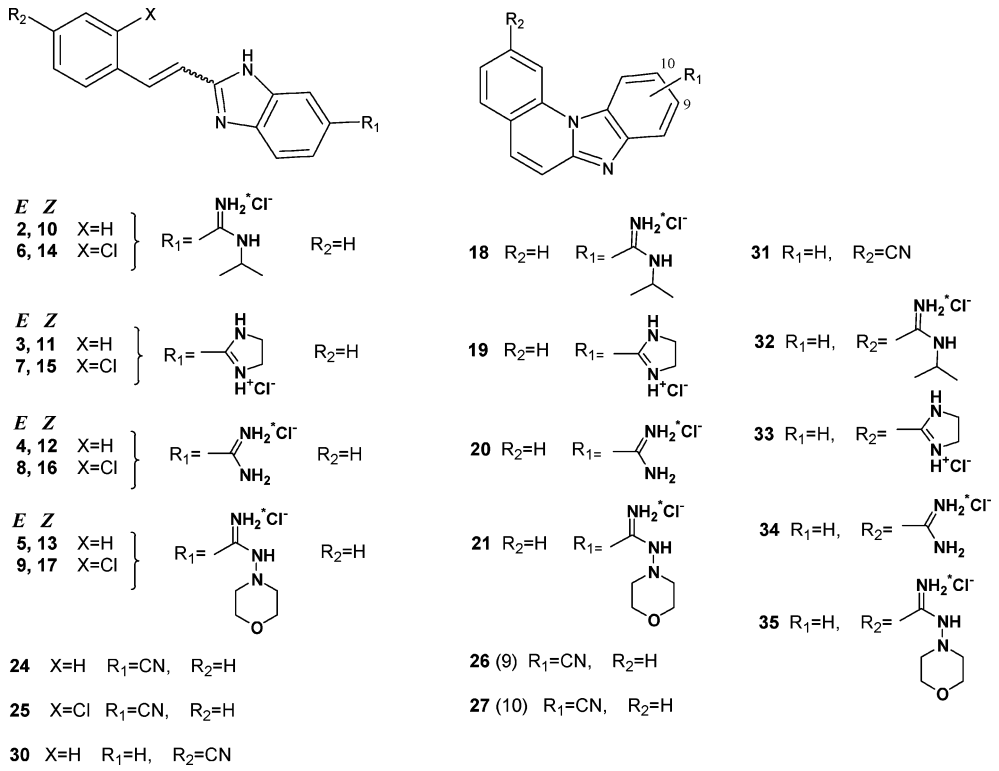


Figure 1. Cyano and amidino-substituted derivatives of 2-styrylbenzimidazoles (**2**−**17**, **24**, **25**, and **30**) and benzimidazo[1,2-*a*]quinolines (**18**−**21**, **26**, **27**, and **31**−**35**).

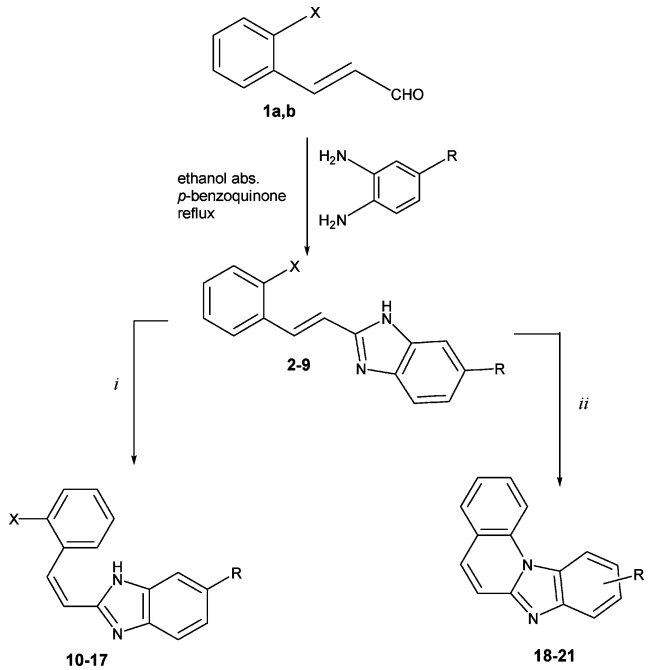
dazole.[23] Recently, Venkatesh has developed a new efficient palladium-catalyzed synthetic method for preparing substituted benzimidazo[1,2-*a*]quinolines starting from phenylamino-substituted benzimidazoles.[24] Other substituted benzimidazo-[1,2-*a*]quinolines were prepared by thermal cyclization of corresponding *o*-substituted 2-styrylbenzimidazoles. Because of their optical properties, they can be used as fluorescent optical whiteners and disperse dyes.[25−27]

These considerations prompted us to explore a new series of cyano- and amidino-substituted styryl-2-benzimidazoles (**2**−**17**, **24**, **25**, **30**) as well as their cylic, fused derivatives, benzimidazo-[1,2-*a*]quinolines (**18**−**21**, **26**, **27**, **31**−**35**) with substituents on different positions of the condensed rings (Figure 1), prepared by reaction of photochemical dehydrocyclization and photochemical dehydrohalogenation cyclization. Full details about the synthesis, evaluation of antitumor activity, and DNA-binding properties are reported herein.

Chemistry

All compounds shown in Figure 1 were prepared according to Schemes 1−3 using different methods for the preparation of benzimidazole nuclei.[28] Novel *N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles **2**−**9** were prepared by the condensation reaction[29−32] of *o*-substituted-3-phenyl-propenales **1a**,**b** with corresponding, earlier-prepared, 4-*N*-amidino-substituted 1,2-phenylenediamines and *p*-benzoquinone in 49−74% yield. 4-*N*-amidino-substituted 1,2-*o*-phenylenediamines were prepared from cyano derivatives in the Pinner reaction using earlier described methods.[33,34] Cyano-substituted *E*-2-styryl-1*H*-benzimidazoles **24** and **25** were prepared from *o*-substituted benzaldehydes and 6-cyano-2-methylbenzimidazole.[35] Novel *N*-amidino-substituted *Z*-2-styryl-1*H*-benzimidazoles **10**−**17** were prepared by photochemical isomerization of ethanolic solution ($c = 1.3 \times 10^{-2}$ mol dm^{-3}) of *N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles **2**−**9**.[23] Reaction of photochemical isomerization was followed by UV/vis spectroscopy according to the Figure 2.

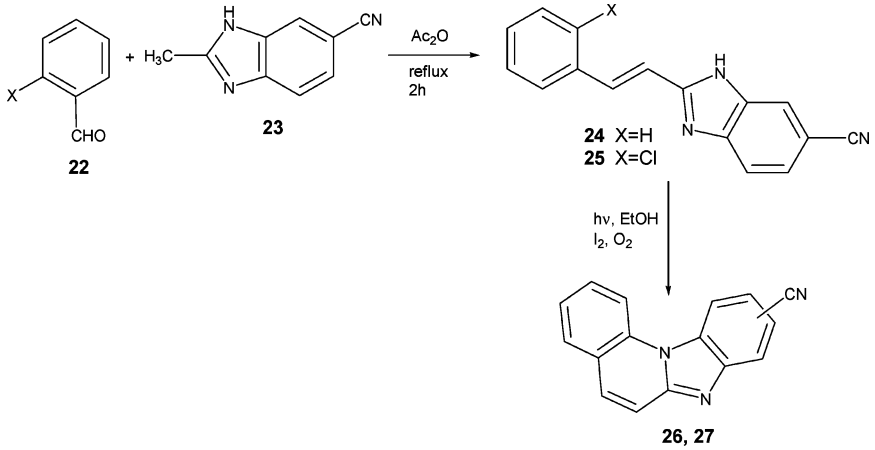
Scheme 1. Synthesis of Amidino-Substituted Derivatives of *E*- and *Z*-Styryl-2-benzimidazoles **2**−**17** and Benzimidazo[1,2-*a*]quinolines **18**−**21**



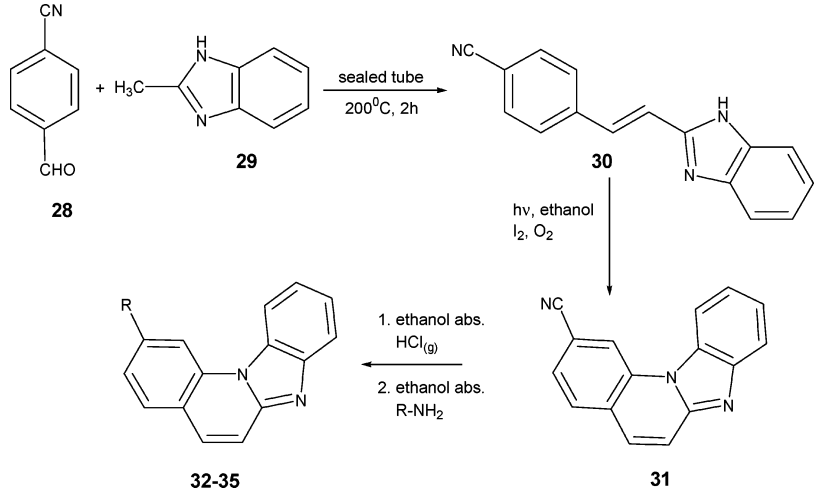
(*i*) in ethanol ($c=1.3\times10^{-2}$ M) by irradiation with 400 W high pressure mercury lamp
(*ii*) in ethanol ($c=1.3\times10^{-3}$ M) by irradiation with 400 W high pressure mercury lamp, with small amount of iodine and air bubbling

All compounds were obtained in 60−78% yield. *N*-amidino-substituted benzimidazo[1,2-*a*] quinolines **18**−**21** and cyano-

Scheme 2. Synthesis of Cyano-Substituted Derivatives of *E*-Styryl-2-benzimidazoles **24**−**25** and Benzimidazo[1,2-*a*]quinolines **26**−**27**



Scheme 3. Cyano- and Amidino-Substituted Benzimidazo[1,2-*a*]quinolines **31**−**35**



substituted benzimidazo[1,2-*a*]quinolines **26**, **27**, and **31** were prepared by photochemical dehydrocyclization, while compounds **18**−**21** were also prepared by photochemical dehydrohalogenation cyclization. There was no significant difference in the time of irradiation, as well as, in the yield of photodehydrocyclization or photodehydrohalogenation reactions according to the Table 1.

These reactions were carried out in ethanolic solution ($c = 1.3 \times 10^{-3}$ mol dm^{-3}) of *N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles **2**−**9** and compounds **18**−**21** were obtained in 31−40% yield as a mixture of two unseparable structure isomers. 2-amidino-benzimidazo[1,2-*a*]quinolines **32**−**35** were prepared by the Pinner reaction from its cyano derivative **31**. The UV/vis electronic absorption data of prepared compounds, recorded at a concentration of $\sim 2 \times 10^{-5}$ mol dm^{-3}, are presented in the Table 2. Cyclization of **2**−**9** into **18**−**21** and **30** into **31**, respectively, resulted in pronounced bathochromic shifts of the absorption maxima of acyclic compounds.

Interaction of Acyclic Derivatives 2−**5; 10**−**13 and their Cyclic Analogues 18**−**21; 32**−**35 with Double-Stranded DNA.** Among all compounds presented in this study, representatives of acyclic derivatives **2**−**5** and **10**−**13** along with their cyclic analogues **18**−**21** and **32**−**35** were chosen for preliminary screening of their noncovalent interactions with ct-DNA. The aim of fast screening was to estimate the probability that biological activity of compounds (addressed in Biological Results and Discussion) is at least partially the consequence of DNA binding.

Taking into account the simplicity and mutual similarity of the studied structures, we decided to perform the fast screening by thermal denaturation experiments (Table 3).

Obtained results reveal that thermal stabilization of ct-DNA by addition of acyclic derivatives **2**−**5** and **10**−**13** is negligible (close to the error of the method), while their cyclic analogues strongly stabilize DNA double helix.

Because studied cyclic compounds **18**−**21** and **32**−**35** are related analogues that stabilize DNA within the same order of magnitude, we chose **19** as a representative for more detailed studies on DNA binding by spectrophotometric titrations and compared it with its acyclic analogue **11**.

Absorbencies of aqueous solutions of **19** and **11** were proportional to their concentrations up to 1×10^{-4} mol dm^{-3}, indicating the lack of significant intermolecular stacking that might give rise to hypochromicity effects. Aqueous solutions of **19** and **11** were stable over longer periods and also under the condition of increased temperature for shorter periods (few hours at up to 100 °C). Compounds **19** and **11** exhibit fluorescence emissions with maxima at about 415 and 383 nm, respectively. Therefore, excitation spectra fit in well with the corresponding absorption spectra. Fluorescence intensities of both compounds were found to be linearly dependent on their concentrations up to 2×10^{-6} mol dm^{-3}.

Addition of ct-DNA to compound **19** ($c = 4.0 \times 10^{-5}$ mol dm^{-3}) resulted in a strong hypochromic effect (39%) and a batochromic shift of maxima for $\Delta\lambda_{max} = +5$ nm, accompanied with pronounced broadening of the UV/vis spectrum, while

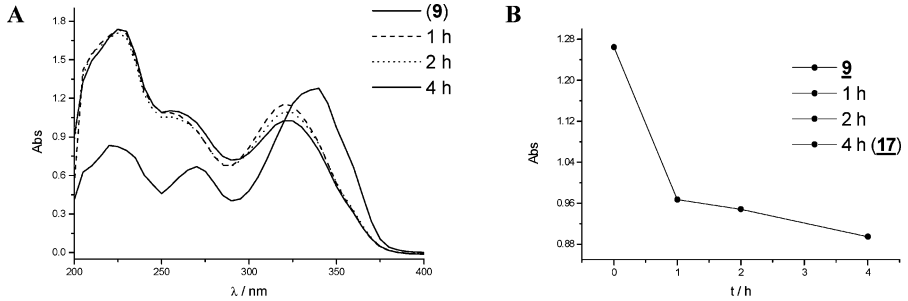


Figure 2. (A) Reaction of photochemical isomerization of compound **9** into compound **17** monitored by UV/vis spectroscopy (ethanol, *c*(sample) = 3.5×10^{-5} mol dm^{-3}); and (B) dependence of absorbance intensity on reaction time.

Table 1. Reaction Times and Yields of Reactions of Photochemical Dehydrocyclization and Dehydrohalogenation Cyclization of Compounds **2**−**9** To Give **18**−**21**

		18	19	20	21
photochemical dehydrocyclization	yield (%)	37	33	40	42
	time (h)	7	6	7	6
photochemical dehydrohalogenation	yield (%)	31	39	40	33
	time (h)	7	7	6	6

Table 2. Electronic Absorption Data of Compounds **2**−**21** and **30**−**35**[a]

	λ_{max} (nm)	$\epsilon \times 10^3$ (dm^3 mol^{-1} cm^{-1})		λ_{max} (nm)	$\epsilon \times 10^3$ (dm^3 mol^{-1} cm^{-1})
2	335	36.4	**15**	313	15.6
	268	17.6		222	29.8
	222	25.0			
3	325	30.8	**16**	315	14.8
	254	25.2		220	29.3
4	334	36.0	**17**	317	14.9
	267	17.8		221	25.9
	223	25.7			
5	335	37.7	**18**	367	7.0
	270	17.4		350	10.1
	220	20.7		331	11.7
				255	30.6
6	334	34.5	**19**	367	7.1
	267	18.2		334	10.1
	219	30.4		261	25.3
7	327	32.4	**20**	367	5.7
	255	21.9		351	7.6
				331	8.8
				257	21.6
8	334	34.0	**21**	368	6.3
	270	18.7		351	8.7
	218	28.7		332	10.0
				258	24.5
9	335	34.5	**30**	351	30.7
	269	17.6			
	220	25.6			
10	316	17.8	**31**	373	9.8
	223	32.7		354	10.7
				267	25.4
				243	28.7
11	312	18.9	**32**	363	8.3
	225	30.9		347	10.1
				264	28.7
				242	32.1
12	317	15.9	**33**	357	6.2
	224	26.6		267	21.5
				242	24.6
13	317	20.0	**34**	347	10.6
	223	36.7		266	30.7
				242	34.9
14	315	14.4	**35**	350	10.0
	221	28.2		266	29.8
				243	32.2

[a] Sodium cacodilate/HCl buffer, $I = 0.05$ mol dm^{-3}, pH = 7.0.

changes in the UV/vis spectrum of **11** ($c = 2.0 \times 10^{-5}$ mol dm^{-3}) upon addition of ct-DNA were much weaker (hypochromic effect 10%, no shift of maximum). However, most of the spectroscopic changes appeared already at excess of studied compounds over ct-DNA, thus hampering the accurate calculation of the binding constant by means of Scatchard equation.[36]

Nevertheless, strong fluorescence of aqueous solutions of **19** and **11** allowed fluorimetric titrations at about 100-times lower concentrations than were used in UV/vis experiments (Figure 3). Addition of ct-DNA strongly quenched emission of **19** (75%) and moderately decreased emission of **11** (37%) and also yielded a hypochromic shift of maximum of compound **11** ($\Delta\lambda_{max} = +8$ nm). Processing of the fluorimetric titration data by means of Scatchard equation gave binding constant log $K_s = 6.2$ and ratio $n_{[\text{bound } \mathbf{19}]/[\text{ct-DNA}]} = 0.19$ for compound **19**, and log $K_s = 6.2$ and ratio $n_{[\text{bound } \mathbf{11}]/[\text{ct-DNA}]} = 0.12$ for compound **11**.

CD Experiments. To further examine the interactions of **19** and **11** with ct-DNA, we carried out CD experiments. Addition of **11** resulted in a marked decrease of the CD bands of ct-DNA (Figure 4C), and in addition, a strong induced CD (ICD) band appeared in the region between $\lambda = 300$ and 400 nm. Because **11** does not have any intrinsic CD spectrum, ICD band must result from the interaction of **11** with ds-DNA, corresponding well with the UV/vis spectrum of **11**/ct-DNA complex collected under the same conditions (Figure 4D).[37] On the other hand, addition of **19** resulted in a decrease of CD bands of ct-DNA at 223 and 245 nm but also yielded an increase of CD band at $\lambda = 275$ nm (Figure 4A). In addition, very weak negative ICD spectrum in the range $\lambda = 300-400$ nm was observed (Figure 4A). Comparison of UV/vis spectrum and CD spectrum of **19**/ct-DNA complex under the same conditions (Figure 4B) pointed that both increase of CD band at $\lambda = 275$ nm as well as weak ICD band at $\lambda = 300-400$ nm correspond well with the UV/vis spectrum of **19**.

In conclusion, although binding constants calculated from fluorimetric titrations for cyclic derivative **19** and its acyclic analogue **11** are comparable, cyclic derivative **19** more significantly stabilized the ct-DNA double helix than the acyclic analogue (Table 3). In addition, changes of the UV/vis spectrum of cyclic derivative **19** upon addition of ct-DNA are significantly stronger than those observed for its acyclic analogue **11**. Aforementioned results obtained for **11** are in good agreement with the strong positive ICD band,[38] thus speaking in favor of the acyclic derivative binding to the ct-DNA minor groove. On the other hand, results obtained for cyclic derivative **19** together with weak negative ICD band[38] point toward intercalative binding mode.

Because structures of other studied cyclic analogues (**18**, **20**, **21**, **32**, **33**, **34**, and **35**) are closely related to **19** and all cyclic analogues show similar thermal stabilization effect (Table 3), we propose intercalation of benzimidazo[1,2-*a*]quinoline moiety of all studied compounds into ct-DNA. Therefore, interaction of cyclic compounds with cellular DNA could be responsible for the observed antiproliferative effects.

Table 3. ΔT_m Values[a] of ct-DNA upon Addition of Acyclic **2**−**5**, **10**−**13**, Cyclic **18**−**21**, and **32**−**35**[b]

cmpd	**2**	**3**	**4**	**5**	**10**	**11**	**12**	**13**	**18**	**19**	**20**	**21**	**32**	**33**	**34**	**35**
ΔT_m/°C	1.1	1.4	0.7	0.1	1.2	1.0	−0.5	1.3	3.7	4.9	3.6	3.2	5.0	3.6	5.2	3.2

[a] Error in ΔT_m: ±0.5 °C. [b] Derivatives at $r_{[compound]/[polynucleotide]} = 0.3$; pH = 7.0; Buffer, sodium cacodilate, $I = 0.05$ mol dm^{-3}.

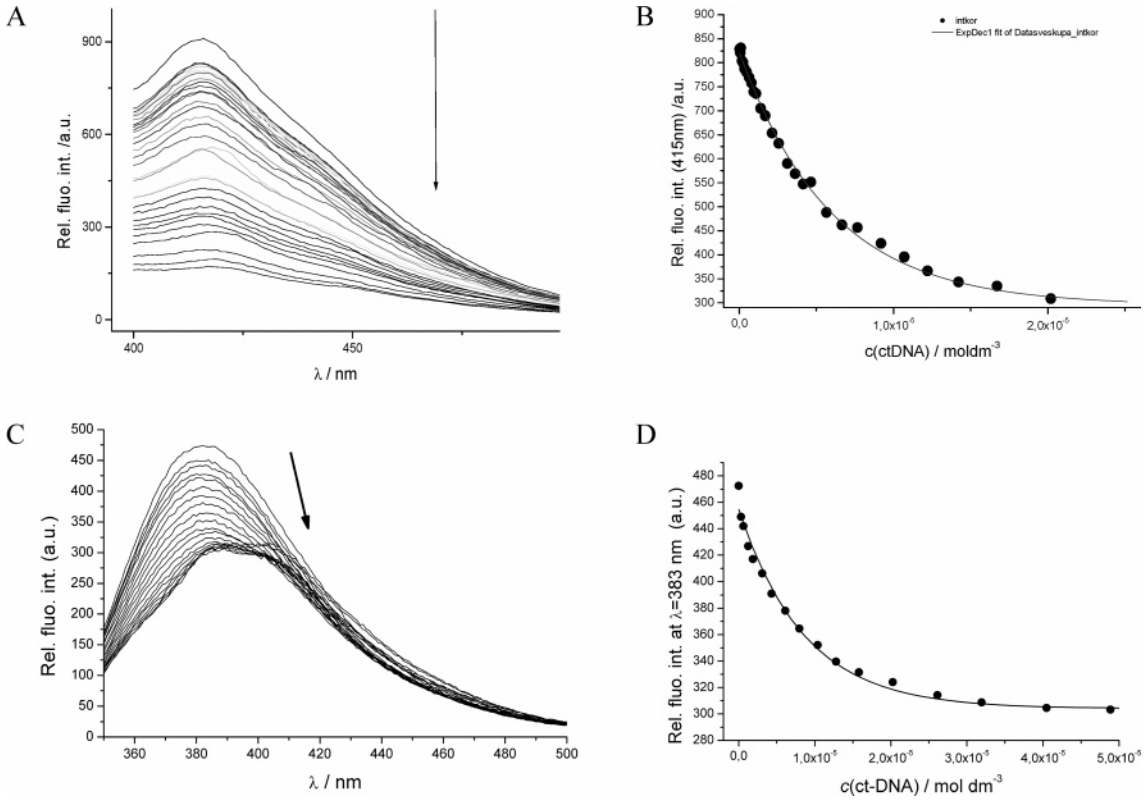


Figure 3. (A) Changes in the fluorescence spectra of **19**; (B) dependence of fluorescence intensity of **19** at $\lambda = 415$ nm on a *c*(ct-DNA); (C) changes in the fluorescence spectra of **11** upon the addition of ct-DNA; and (D) dependence of fluorescence intensity of **11** at $\lambda = 383$ nm on a *c*(ct-DNA). All experiments were done at pH = 7.0 (buffer sodium cacodylate $I = 0.05$ mol dm^{-3}).

Acyclic analogues related to **11** do not stabilize ct-DNA significantly under the conditions used in this fast screening experiment (Table 3). Although these fast screening results do not explicitly prove that all acyclic analogues bind into the minor groove of ct-DNA, like compound **11**, it is most likely that the more potent biological effects of cyclic analogues results from intercalation into cellular DNA, while other modes of interactions possible for acyclic analogues yield lower antiproliferative activity.

Biological Results and Discussion

Compounds **2**−**36** were tested for their potential antiproliferative effects on a panel of seven human cell lines, six of which were derived from different cancer types, including HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma), H460 (lung carcinoma), and one from normal diploid fibroblasts, WI 38.

All tested compounds showed noticeable antiproliferative effect (Table 4). Acyclic *trans*-analogues (**2**−**9**) and their corresponding *cis*-isomers (**10**−**17**) had comparable effects on the cell proliferation. Moreover, all acyclic compounds exerted the most pronounced effect on HeLa and MCF-7 cells. While morpholino-substituted acyclic compounds (**5**, **9, 13** and **17**) had substantial cytostatic effects on normal human fibroblasts (WI 38), other acyclic derivatives did not influence the growth of this cell line. This finding points to the highly differential (selective) effect of the aforementioned compounds. The activity was slightly potentiated by introducing a chloro-substitutent. The most active among all acyclic derivatives proved to be morpholino- and chloro-substituted compound **9**, although it is least selective in regard to normal cells.

From the results presented in Table 4 and Figure 5, it is evident that IC_{50} concentrations of all cyclic compounds (**18**−**21**, **32**−**35**) are 2−10-fold lower than those of their acyclic analogues (**2**−**17**). Moreover, all cyclic compounds showed similar activity toward all tested cell lines, having low micromolar IC_{50} concentrations (1−17 μM), except for **19**, which strongly inhibited the growth of the colon carcinoma cells, SW620 ($IC_{50} = 0.4$ μM). This is opposite to the previously published results, which demonstrated that corresponding amidino-substituted cyclic benzo[b]thieno[2,3-*c*]quinolones[39] inhibited tumor cell growth differently and selectively in comparison with normal cells, although the aromatic surface and the position of amidine substituents are similar to benzimidazo-[1,2-*a*]quinolines presented in this study. This implies the importance of the finely tuned structural and also most likely electronic properties of condensed hetereoaromatic surface with respect to the selectivity between tumor and normal cells.

However, the only exception was compound **20**, which weakly inhibited the growth of normal fibroblasts, thus being the most selective cyclic compound.

Acyclic compounds and the standard chemotheraputic agent etoposide have comparable effects, whereas the cyclic ones have significantly stronger activity than etoposide, but lower activity in comparison with doxorubicin.

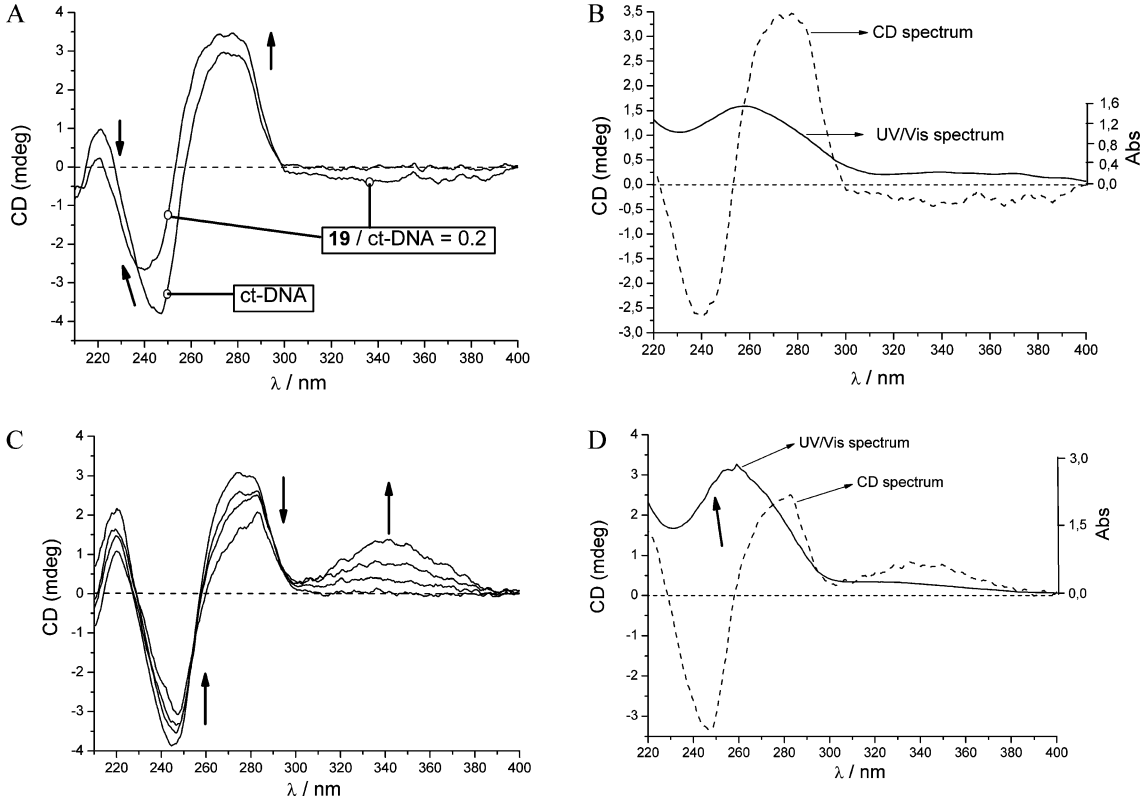


Figure 4. (A) CD spectra of the free ct-DNA (4×10^{-5} mol dm^{-3}) and of **19**/ct-DNA complex at ratio $r_{[\mathbf{19}]/[\text{ct-DNA}]} = 0.2$; (B) comparison of UV/vis spectrum and CD spectrum of **19**/ct-DNA complex at ratio $r_{[\mathbf{19}]/[\text{ct-DNA}]} = 0.2$; (C) CD spectra of the free ct-DNA (4×10^{-5} mol dm^{-3}) and of **11** /ct-DNA complex at ratios $r_{[\mathbf{19}]/[\text{ct-DNA}]} = 0.2$, 0.5, and 1; and (D) comparison of UV/vis spectrum and CD spectrum of **11** /ct-DNA complex at ratio $r_{[\mathbf{19}]/[\text{ct-DNA}]} = 0.5$. All experiments were done at pH = 7 (buffer sodium cacodylate $I = 0.05$ mol dm^{-3}).

Table 4. In Vitro Inhibition of Compounds **2**−**36** on the Growth of Tumor Cells and Normal Human Fibroblasts (WI 38)

	IC_{50}[a] (μM)					
cmpd	Hep-2	HeLa	MiaPaCa-2	SW620	MCF-7	WI 38
2	≥100	11 ± 15	>100	>100	9 ± 8	>100
3	16 ± 2	4 ± 3	19 ± 1	16 ± 2	7 ± 3	21 ± 2
4	11 ± 0.1	2 ± 1	>100	15 ± 5	29 ± 18	>100
5	13 ± 0.5	6 ± 6	16 ± 1	12 ± 1	14 ± 1	20 ± 2
6	70 ± 33	10 ± 2	48 ± 22	66 ± 22	16 ± 2	>100
7	16 ± 0.2	4 ± 1	17 ± 1	14 ± 2	5 ± 0.01	42 ± 13
8	23 ± 5	7.5 ± 3	>100	>100	22 ± 4	>100
9	10 ± 6	3 ± 2	5 ± 1	4 ± 1	4 ± 4	7 ± 9
10	81 ± 9	23 ± 4	84 ± 20	70 ± 1	33 ± 25	>100
11	27 ± 5	35 ± 20	25 ± 3	45 ± 7	17 ± 5	99 ± 35
12	≥100	5.4 ± 4	>100	78 ± 25	36 ± 16	>100
13	61 ± 40	13 ± 2	47 ± 23	22 ± 3	25 ± 9	22 ± 2
14	30 ± 1	19 ± 5	28 ± 2	29 ± 10	35 ± 0.5	>100
15	≥100	11 ± 5	62 ± 23	46 ± 18	23 ± 2	>100
16	29 ± 6	20 ± 2	85 ± 19	>100	38 ± 6	>100
17	9 ± 2	7 ± 4	15 ± 3	15 ± 2	14 ± 0.2	21 ± 2
18	4 ± 0	4 ± 2	3 ± 0.3	3 ± 1	4 ± 2	10 ± 7
19	2 ± 0.02	2 ± 0.2	1 ± 0.2	0.4 ± 0.01	2 ± 1	2 ± 1
20	13 ± 4	5 ± 3	13 ± 1	17 ± 0.2	9 ± 0.4	74 ± 6
21	2 ± 1	3 ± 0.7	2 ± 0.01	2 ± 1	4 ± 0.3	4 ± 0.01
32	1.6 ± 0.06	1.7 ± 0.3	8.1 ± 8	1.6 ± 1.2	2.2 ± 0.2	2.7 ± 0.7
33	2.4 ± 0.6	7 ± 3.6	5.6 ± 0.5	1.4 ± 0.1	5.3 ± 3	7.8 ± 4.7
34	4 ± 1.4	4.9 ± 5.5	4.5 ± 3.6	2.9 ± 0.9	4 ± 1	6.7 ± 7
35	4.7 ± 3	7 ± 5	10 ± 0.3	1.9 ± 1	6.7 ± 4.8	17.7 ± 5
36	15 ± 3	12 ± 0.1	18 ± 6	23 ± 9	16 ± 0.9	14 ± 3
DOX[b]	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.01	0.1 ± 0.01
Eto[b]	N.T.[c]	3 ± 1	15 ± 14	20 ± 3	50 ± 30	N.T.

[a] IC_{50}: the concentration that causes a 50% reduction of the cell growth. [b] DOX, doxorubicin; Eto, etoposide. [c] N.T.: not tested.

Amidino-substituted cyclic compounds showed significantly stronger activity than unsubstituted analogue benzimidazo[1,2-*a*]quinoline **36**[23] (Figure 6). This finding unravels the important role of amidino-group for the growth inhibition activity, whereby the imidazole substituent exerted inhibitory effects particularly toward the colon carcinoma cells (**19** and **33**).

There was no statistical difference between cyclic derivatives bearing an amidino substituent on either the quinoline (**32**−**35**) or the benzimidazole (**18**−**21**) side.

Interestingly, when comparing the benzimidazole compounds bearing a cyano- instead of a amidino- substituent on the benzimidazole side (Table 5), it could be noticed that a cyano

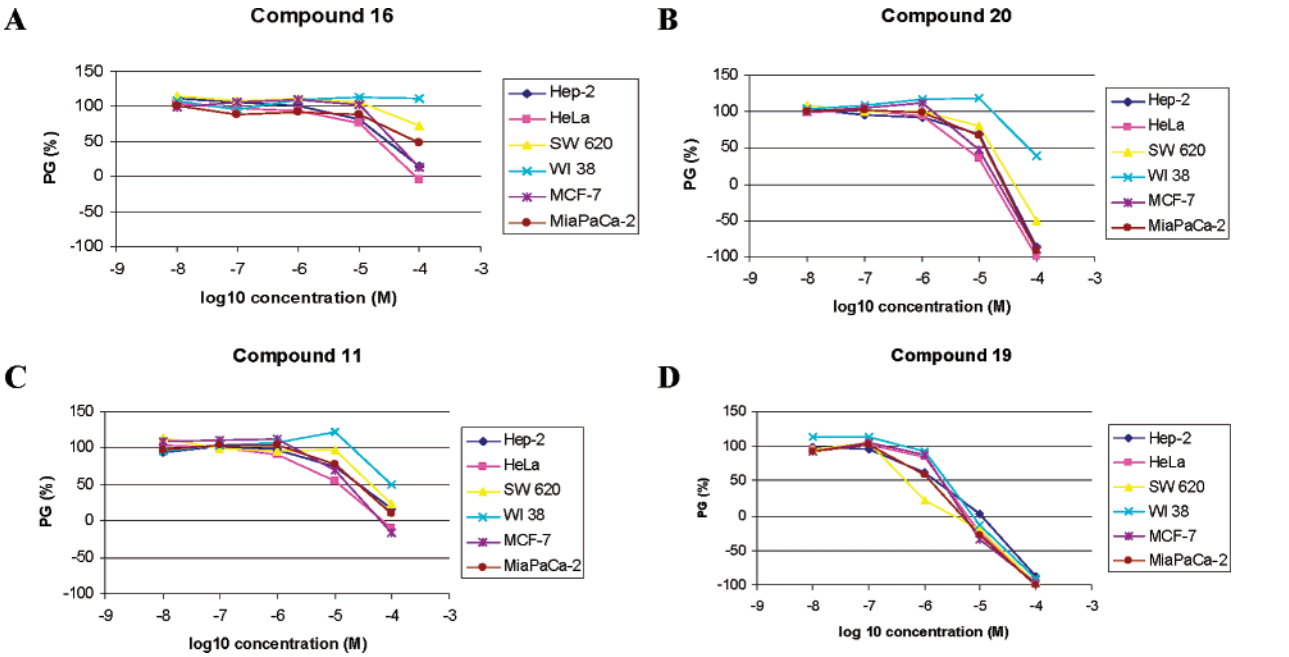
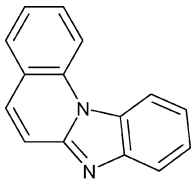


Figure 5. Concentration−response profiles for the representative acyclic (**16** and **11**) and cyclic (**20** and **19**) analogues tested on various human cell lines in vitro. The cells were treated with the compounds at different concentrations and PG was calculated. Each point represents a mean value of four replicates in three individual experiments.



36

Figure 6. Unsubstituted benzimidazo[1,2-*a*]quinoline **36**.

Table 5. In Vitro Inhibition of Cyano-Substituted Compounds on the Growth of Tumor Cells and Normal Human Fibroblasts (WI 38)

	IC_{50}[a] (μM)					
cmpd	H460	HeLa	MiaPaCa-2	SW620	MCF-7	WI 38
24	3 ± 0.7	2 ± 0.2	4 ± 0.9	2.5 ± 0.3	4 ± 2	14 ± 8
26	7 ± 4	4 ± 0.8	7 ± 0.6	2 ± 0.2	21 ± 8	5 ± 1
27	13 ± 1.8	0.05 ± 0.08	15 ± 2	8 ± 2	18 ± 0.6	14 ± 7
30	20 ± 8	19 ± 4	36 ± 3	23 ± 7	14 ± 16	N.T.[b]

[a] IC_{50}: the concentration that causes a 50% reduction of the cell growth. [b] N.T.: not tested.

substituent in general strongly enhances the cytotoxic activity of acyclic compounds, but diminishes the activity of the cyclic ones. The exception to this rule is the extremely pronounced activity of cyclic compound **27** (with cyano group on the benzimidazole side) on the HeLa cell line (IC_{50} = 0.05 μM). Such an extreme selectivity of heterocyclic cyano molecules on HeLa cells was previously observed by our group for carboxanilides bearing a cyano substituent on either the anilide or the benzothiophene part of the molecule,[39] acyclic cyano derivatives of thiophene-2-carboxamides,[40] as well as for methyl-2-cyano-naphtho[2,1-*b*]thiophen-5-carboxylate and 4-ethyl-7-cyano-thieno[2,3-*e*]-benzo[*b*]furan.[41]

All aforementioned HeLa selective molecules fall into two structurally discrete groups: acyclic[39,40] and cyclic.[41] These groups significantly differ in affinity and type of interactions with DNA, but nevertheless, all show HeLa-specific selectivity. Therefore, it seems reasonable to believe that the molecular target that contributes to this biological selectivity is not the DNA itself, but probably a set of proteins specifically expressed

Table 6. Flow Cytometric Analysis of HeLa Cells Treated with **19** and **21**

	HeLa		
cell cycle phase[b]	24 h	48 h	72 h
	Control[a]		
SubG1	3 ± 2	2 ± 0.2	3 ± 0.5
G0/G1	56 ± 1	56 ± 1	54 ± 0.1
S	28 ± 3	29 ± 3	35 ± 1
G2/M	16 ± 4	15 ± 1	12 ± 1
	Treated with **19**[a]		
SubG1	2 ± 0.25	2 ± 0.3	3 ± 0.8
G0/G1	32 ± 0.1[c]	34 ± 1[c]	28 ± 3[c]
S	42 ± 2.4[c]	36 ± 4[c]	32 ± 9
G2/M	26 ± 2[c]	32 ± 9[c]	40 ± 12[c]
	Treated with **21**[a]		
SubG1	3 ± 1	2 ± 0.5	2 ± 0.2
G0/G1	36 ± 0.4[c]	36 ± 1[c]	25 ± 12[c]
S	30 ± 5[c]	37 ± 3	39 ± 21
G2/M	34 ± 5[c]	27 ± 4[c]	35 ± 9[c]

[a] $c = 5\ \mu$M. [b] The results are shown as percentages of cell population in each cell cycle phase. [c] Statistically significant at $p < 0.05$.

in HeLa cells. In addition, published as well as herein presented results point to the exceptionally well-defined binding site of a putative macromolecule, because a number of analogues with a differently positioned cyano group are not equally selective. These results again prove that finely tuned interplay between the general structure of the small molecule and the distribution of interacting substituents precisely control selectivity. Further studies on elucidating this phenomenon are underway.

Cell Cycle Perturbations. To shed more light on the mechanisms underlying the antiproliferative activity of some of the most active compounds, we selected the cyclic compounds **19** and **21** to investigate their influence on the HeLa cell cycle after 24, 48, and 72 hour treatment periods (Table 6). Obtained results clearly showed that, at a concentration of 5×10^{-6} M (~IC_{50}), both compounds induced statistically significant reduction of the G1-phase cells over all three treatment periods. This event was accompanied by an apparent accumulation of HeLa cells in S and G2/M phases of the cell cycle. Interestingly,

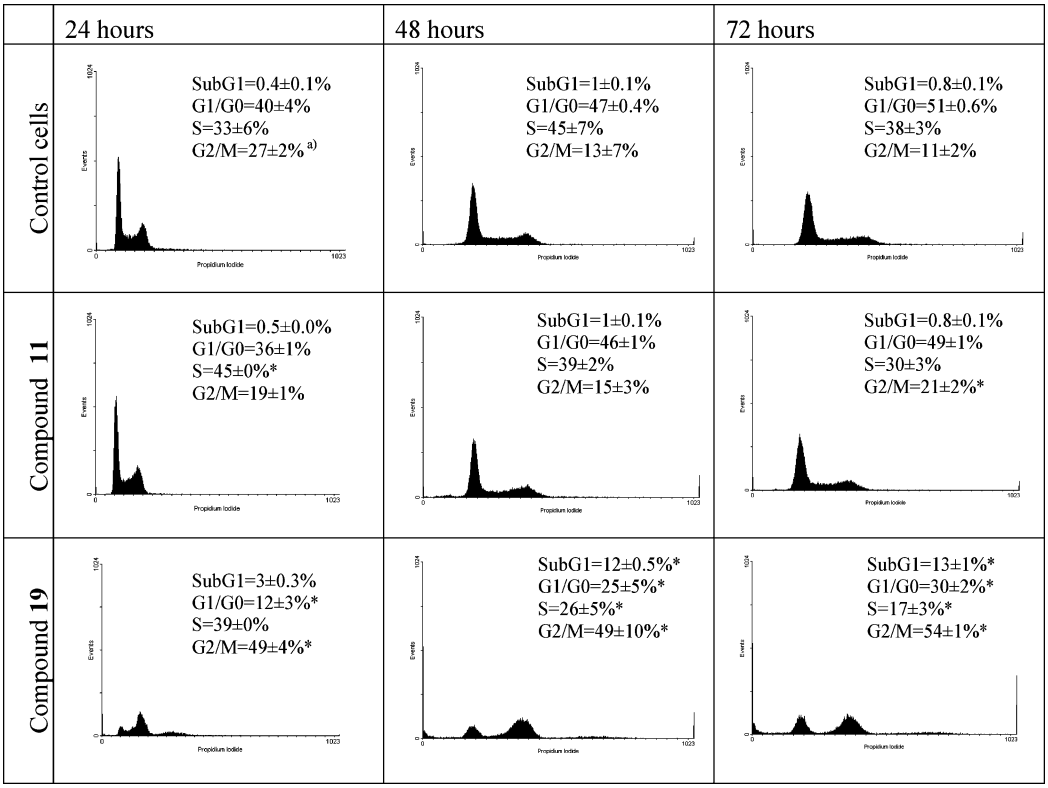


Figure 7. Flow cytometric DNA histograms of SW620 cells treated with compounds **11** and **19** ($c = 5 \times 10^{-6}$ M) after different time points. [a]The results are shown as percentages of cell population in each cell cycle phase. *Statistically significant at $p < 0.05$.

compound **19** has a similar effect on the cell cycle-phase distribution at lower concentration as well (10^{-6} M) throughout all three time points (data not shown). On the other hand, compounds **11** and **17** (acyclic analogues of **19** and **21**) did not have significant influence on the cell cycle distribution of HeLa cells at the tested concentration, which is five times lower than the IC_{50} concentrations for these compounds (data not shown).

Because the compound **19** exerted the most pronounced growth inhibition effect against colon carcinoma cells (SW620), we performed additional cell cycle analyses of this cell line upon treatment with the aforementioned substance (Figure 7). Our results clearly showed that cells treated with this compound at a concentration of 5×10^{-6} M were strongly arrested in G2/M phase after 24 h, which was accompanied by significant reduction in G1 cells. This G2/M delay persisted during the next 48 h, which consequently led to the decrease in the G1 and S-phase cells. Interestingly, lower concentrations of this compound (10^{-6} M and 5×10^{-7} M) also showed a tendency to increase the G2/M population. A marked rise in the sub-G0/G1 population upon treatment with 5×10^{-6} M concentration indicates cell death (apoptosis) induced by the compound **19**. On the other hand, the compound **11**, an acyclic analogue of **19**, had a significant influence on the cell cycle progression only after a 24-hour treatment, particularly at a higher-tested concentration (5×10^{-6} M), at which it induced S-phase arrest and concomitant decrease in G1 and G2/M cells. Interestingly, it seems that this pattern of the cell cycle phase distribution was maintained after 48 h as well, while the cells were apparently arrested in the G2/M phase after 72 h.

Altogether, flow cytometric data showed that compounds **19** and **21** induced G2/M arrest, which points to the impairment of mitotic progression. These findings augmented by the DNA binding results provoked the conclusion that the cyclic compounds presented in this study act as topoisomerase inhibitors.

In Vitro Topoisomerase II Inhibitor Screening. Topoisomerase II plays an essential role in DNA replication, transcription, chromosome formation, and separation of sister chromatids. As this enzyme is particularly active during the G2 and M phases of the cell cycle, we investigated whether our cyclic compounds interfere with mitotic progression via the inhibition of topoisomerase II. For this purpose, we applied an in vitro topo II inhibitor screening system. Figure 8 shows the effects of selected compounds **19** and **11** on the relaxation activity of topoisomerase II, whereby etoposide was used as a control drug. Topo II incubated with pRYG DNA substrate in the assay buffer was used as the positive control (lane 2) and this reaction resulted in the formation of a series of topoisomers (relaxed DNA forms differing in linking number) that could be detected as the ladder between supercoiled and open circular DNA (lane 2). The reaction mixture containing etoposide resulted in the conversion of supercoiled pRYG DNA into relaxed DNA forms and in an increased intensity of the open circular DNA band (cleavage product). This result is in concordance with a well-established role of etoposide in topo II inhibition as a compound that stimulates formation of cleavable complexes (i.e., topo II poison). More interestingly, topo II relaxation products were absent from the reaction mixtures containing test compounds **19** and **11** (lanes 4 and 5, respectively), which implies an antagonizing effect of these substances on topo II action.

Figure 9 shows the influence of test compounds on the formation of the DNA cleavage products. Relaxed DNA bands could be detected in the reaction mixtures containing topoisomerase II incubated without any compound (lane 2) and in the presence of etoposide (lane 3). As expected, etoposide stimulated formation of cleavable complexes, linear DNA species and open circular DNA (lane 3). However, test compounds **19** and **11** (lanes 4 and 5, respectively) did not give

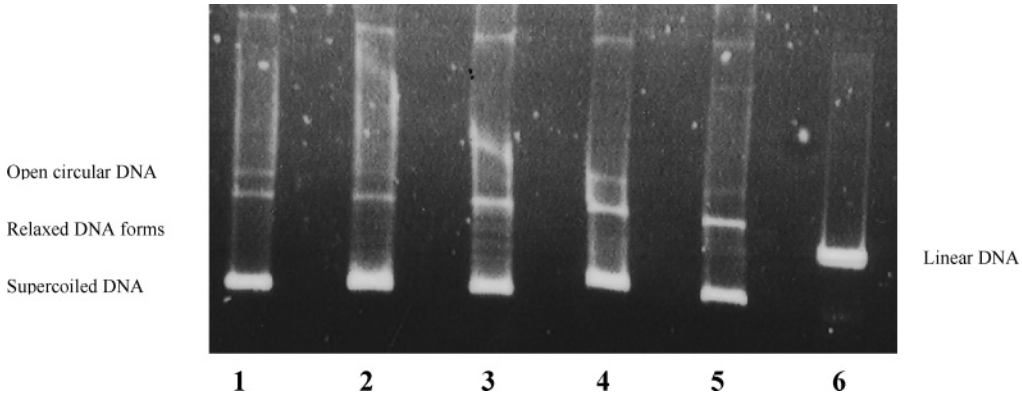


Figure 8. Influence of selected test compounds on topoisomerase II relaxation activity. Topo II was incubated with supercoiled pRYG DNA substrate in the assay buffer alone (lane 2) or in combination with control drug etoposide (lane 3) and selected test compounds, **19** and **11** (lanes 4 and 5, respectively). Supercoiled and linear DNA markers are contained in lanes 1 and 6, respectively.

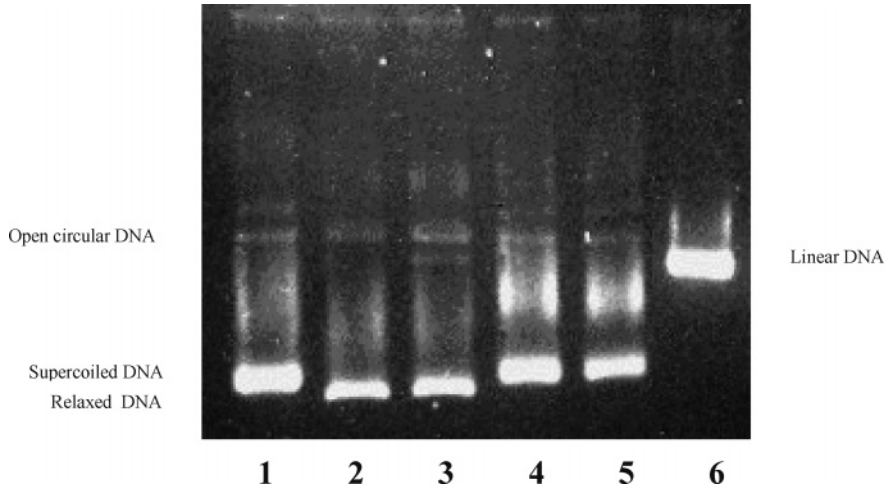


Figure 9. Influence of selected test compounds on the formation of cleavable complexes. Topo II was incubated with supercoiled pRYG DNA substrate in the cleavage buffer alone (lane 2) or in combination with control drug etoposide (lane 3) and selected test compounds, **19** and **11** (lanes 4 and 5, respectively). Supercoiled and linear DNA markers are contained in lanes 1 and 6, respectively.

rise to the formation of linear DNA, but inhibited conversion of supercoiled DNA substrate into relaxed DNA. Altogether, our results suggest that compounds **19** and **11** are catalytic inhibitors of topoisomerase II.

Topo II inhibitors are among the most effective anticancer drugs for many different malignant diseases due to the essential role topoisomerase II plays in the replication and the cell cycle in highly proliferating cancer cells. Unlike topo II poisons (e.g., etoposide and doxorubicin) whose cytotoxicity generally correlates with DNA damage after stabilization of DNA−topo II cleavable complexes, the molecular events by which catalytic inhibitors (e.g., aclarubicin, merbarone and the bisdioxopiperazine derivatives) cause cell death are not fully understood.[42] In spite of an inveterate opinion that catalytic inhibitors of topoisomerase II are non-DNA-damaging agents, Wang et al. showed that two common catalytic inhibitors, namely, merbarone and ICRF-187, induced strong dose-dependent genotoxic effects in cultured cells and observed similar clastogenic effects in vivo in merbarone-treated mice.[43] Our DNA binding study substantiated the studies of Wang et al., as we showed the compound **19** to be the DNA intercalator similarly to aclarubicin, which supports the idea that catalytic inhibitors might damage DNA as well. However, lower antiproliferative activity of the compound **11** in comparison with **19** might be explained by the potential ability of the latter to induce conformational changes leading to additional DNA damage uncoupled from topo II inhibition, and to other mechanistic outcomes (e.g., inhibition of transcription). Observed ability of compound **11** to bind to the DNA minor groove might additionally account for differences in cytostatic effects between these two compounds.

Cells respond to DNA damage by activating a checkpoint that induces G2/M arrest, thus allowing the cells to repair their DNA before entry into mitosis. Strong G2/M arrest observed in HeLa and SW620 cells could thus result from either the cellular response to DNA damage caused by intercalation into DNA or from topo II inhibition, whereby both molecular events prevent the cells from proceeding through mitosis. Our results are in good agreement with other reports, confirming that catalytic inhibitors can induce G2/M arrest. For example, Anderson et al. found that merbarone, IGRF-187, and aclarubicin can induce G2 arrest or significant G2 delay in a dose-dependent manner.[44]

Conclusions

We have shown that cyclic (benzimidazo[1,2-*a*]quinolines), positively charged analogues intercalate into ds DNA, which might account for their significant antiproliferative effects (IC_{50} concentrations are within low micromolar range). On the other hand, the acyclic, either positively charged or neutral molecules, show a different interaction with DNA, correlating well with inferior biological activity (except for **24**). However, their selectivity toward tumor cells in regard to normal cells is more pronounced. Because selectivity is very important for biological

use, this phenomenon should be corroborated in in vivo experiments and, therefore, warrants further evaluation.

Furthermore, our results support the role of tested compounds as catalytic inhibitors of topoisomerase II, at least for two structurally different imidazolyl-substituted analogues **11** and **19**. Strong G2/M cell cycle arrest, particularly observed in SW620 cells treated with imidazolyl-substituted compound **19**, could be either a response to DNA damage produced by its intercalation into DNA or a direct consequence of topo II inhibition, whereby both processes prevent mitotic progression. Its acyclic analogue **11**, which is a minor grove binder, exerts significantly lower cytostatic effect, possibly due to a different DNA interaction mode.

Additionally, one cyano-substituted compound (**27**) showed intriguingly strong and selective antiproliferative activity on HeLa tumor cell line.

Finally, stronger cytostatic activity of amidino-substituted benzimidazo[1,2-*a*]quinolines in comparison with the commercial chemotherapeutic agent etoposide warrants their further optimization as novel intercalators and topoisomerase II inhibitors. In particular, imidazolyl-substituted benzimidazo[1,2-*a*]-quinoline deserves additional consideration as a putative agent against colon carcinoma. Our further studies on the mechanism of action of this compound are underway.

Experimental Section

Chemistry. Melting points were determined on a Koffler hot stage microscope and are uncorrected. IR spectra were recorded on a Nicolet magna 760, a Perkin-Elmer 297, and a Perkin-Elmer Spectrum 1 spectrophotometers with KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 300, Bruker Avance DPX 300 and Bruker Avance DRX 500 spectrometers using TMS as an internal standard in DMSO[a]-d_6. Mass sprectra were recorded on an Agilent 1100 series LC/MSD Trap SL. Elemental analysis for carbon, hydrogen, and nitrogen were performed on a Perkin-Elmer 2400 elemental analyzer and a Perkin-Elmer, Series II, CHNS Analyzer 2400. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. In preparative photochemical experiments, the irradiation was performed at room temperature with a water-cooled immersion well with "Origin Hanau", 400 W, high-pressure, mercury arc lamp using Pyrex glass as a filter. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates.

General Method for the Synthesis of 5(6)-*N*-Amidino-substituted *E*-2-Styryl-1*H*-benzimidazoles (2−5) and 5(6)-*N*-Amidino-substituted *E*-2-[2-(2-Chloro-phenyl)-vinyl]-1*H*-benzimidazoles (6−9). A mixture of corresponding 4-*N*-substituted-1,2-phenylenediamines, 3-phenyl-propenal or 3-(2-chloro-phenyl)-propenal, and *p*-benzoquinone in absolute ethanol (10 mL) was stirred at reflux for 2.5−3 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and diethylether was added. The resulting product was filtered off and washed with diethylether. After recrystallization from ethanol/diethylether or ethanol/acetone, light powders were obtained in very good yields.

***E*-*N*-Isopropyl-2-styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (2).** Compound **2** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-isopropylamidino-1,2-phenylenediamine (0.400 g, 1.75 mmol), 3-phenyl-propenal (0.231 g, 1.75 mmol), and *p*-benzoquinone (0.189 g, 1.75 mmol) in absolute ethanol (10 mL) was refluxed under nitrogen for 2 h and then the reaction mixture was worked up as it is described to give 0.355 g (60%) of gray powder; mp 285−287 °C; IR (KBr, ν_{max}/cm^{-1}) 3423, 2913, 1697, 1613; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.64 (bs, 1H, $NH_{benzimidazole}$), 9.52 (bs, 1H, $NH_{amidine}$), 9.42 (bs, 1H, $NH_{amidine}$), 9.08 (bs, 1H, $NH_{amidine}$), 8.02 (s, 1H, H_{arom}), 7.90 (d, 1H, J = 16.81 Hz, $H_{ethenyl}$), 7.72 (d, 1H, J = 8.32 Hz, H_{arom}), 7.70 (d, 1H, J = 8.21 Hz, H_{arom}), 7.68 (d, 1H, J = 7.20 Hz, H_{arom}), 7.57 (d, 1H, J = 7.2 Hz, H_{arom}), 7.49−7.40 (m, 3H, H_{arom}), 7.30 (d, 1H, J = 16.55 Hz, $H_{ethenyl}$), 4.14 (m, 1H, $CH(CH_3)_2$), 1.31 (d, 6H, J = 5.79 Hz, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.27 (s), 149.65 (s), 143.22 (s), 135.41 (s), 129.14 (d), 128.92 (d, 2C), 127.17 (d, 2C), 122.25 (d), 121.53 (s), 117.07 (d), 115.53 (d, 2C), 111.26 (d), 44.92 (d), 21.25 (q, 2C); MS (*m*/*z*) 305 (M^{+1} (− HCl)); Anal. ($C_{19}H_{21}N_4Cl$) C, H, N.

***E*-2-(2-Styryl-3*H*-benzimidazol-5(6)-yl)-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (3).** Compound **3** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-(2-imidazolinyl)-1,2-phenylenediamine (0.500 g, 2.35 mmol), 3-phenyl-propenal (0.311 g, 2.35 mmol), and *p*-benzoquinone (0.254 g, 2.35 mmol) in absolute ethanol (20 mL) was refluxed under nitrogen for 3 h, and then the reaction mixture was worked up as it is described to give 0.450 g (59%) of light violet powder; mp > 300 °C; IR (KBr, ν_{max}/cm^{-1}) 3381, 3107, 1606, 1514; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.50 (bs, 1H, $NH_{benzimidazole}$), 10.60 (bs, 2H, $NH_{amidine}$), 8.74 (d, 1H, J = 16.40 Hz, $H_{ethenyl}$), 7.97 (d, 1H, J = 8.31 Hz, H_{arom}), 7.92 (s, 1H, H_{arom}), 7.73 (d, 1H, J = 8.25 Hz, H_{arom}), 7.70 (d, 1H, J = 7.20 Hz, H_{arom}), 7.58 (d, 1H, J = 7.10 Hz, H_{arom}), 7.53−7.37 (m, 3H, H_{arom}), 7.30 (d, 1H, J = 16.50 Hz, $H_{ethenyl}$), 4.20 (bs, 4H, $2CH_{2imidazole}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 165.82 (s), 165.78 (s), 135.86 (s), 135.84 (s), 131.24 (d), 130.53 (d), 129.82 (d), 129.69 (s), 129.54 (d), 129.50 (d), 129.31 (d), 128.58 (d), 127.79 (d), 127.52 (d), 117.29 (d), 115.62 (s); MS (*m*/*z*) 289 (M^{+1} (− HCl)); Anal. ($C_{18}H_{17}N_4Cl$) C, H, N.

***E*-2-Styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (4).** Compound **4** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-amidino-1,2-phenylenediamine (0.500 g, 2.68 mmol), 3-phenyl-propenal (0.354 g, 2.68 mmol), and *p*-benzoquinone (0.284 g, 2.68 mmol) in absolute ethanol (15 mL) was refluxed under nitrogen for 2 h, and then the reaction mixture was worked up as it is described to give 0.560 g (70%) of gray powder; mp 289−291 °C; IR (KBr, ν_{max}/cm^{-1}) 3080, 1670, 1640, 1621; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.40 (bs, 2H, $NH_{amidine}$), 9.15 (bs, 2H, $NH_{amidine}$), 8.14 (s, 1H, H_{arom}), 7.90 (d, 1H, J = 16.53 Hz, $H_{ethenyl}$), 7.70 (d, 1H, J = 8.45 Hz, H_{arom}), 7.69 (d, 1H, J = 8.44 Hz, H_{arom}), 7.68 (d, 1H, J = 7.20 Hz, H_{arom}), 7.65 (d, 1H, J = 7.05 Hz, H_{arom}), 7.54−7.36 (m, 3H, H_{arom}), 7.30 (d, 1H, J = 16.5 Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 166.08 (s), 154.04 (s), 136.66 (d), 135.42 (s), 130.06 (d), 129.34 (d), 129.04 (d, 2C), 127.32 (d, 2C), 122.04 (d), 121.10 (s), 116.82 (d), 115.64 (d); MS (*m*/*z*) 263 (M^{+1} (− HCl)); Anal. ($C_{19}H_{20}N_4$-Cl_2·H_2O) C, H, N.

***E*-*N*-Morpholin-4-yl-2-styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (5).** Compound **5** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-morpholinylamidino-1,2-phenylenediamine (0.440 g, 1.62 mmol), 3-phenyl-propenal (0.214 g, 1.62 mmol), and *p*-benzoquinone (0.175 g, 1.62 mmol) in absolute ethanol (10 mL) was refluxed under nitrogen for 2 h, and then the reaction mixture was worked up as it is described to give 0.300 g (49%) of light violet powder; mp 291−293 °C; IR (KBr, ν_{max}/cm^{-1}) 3380, 2926, 1645, 1612; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.60 (bs, 1H, $NH_{benzimidazole}$), 11.23 (bs, 1H, $NH_{amidine}$), 9.77 (s, 1H, $NH_{amidine}$), 9.07 (bs, 1H, $NH_{amidine}$), 8.10 (s, 1H, H_{arom}), 7.87 (d, 1H, J = 16.42 Hz, $H_{ethenyl}$), 7.70 (d, 1H, J = 8.11 Hz, H_{arom}), 7.62 (d, 1H, J = 8.09 Hz, H_{arom}), 7.48 (d, 1H, J = 7.56 Hz, H_{arom}), 7.43 (d, 1H, J = 7.60 Hz, H_{arom}), 7.41−7.38 (m, 3H, H_{arom}), 7.30 (d, 1H, J = 16.54 Hz, $H_{ethenyl}$), 3.78 (bs, 4H, $2CH_{2morpholine}$), 2.96 (bs, 4H, $2CH_{2morphiline}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.31(s), 149.72 (s), 136.49 (d), 135.45 (s), 129.19 (d), 128.95 (d, 2C), 127.21 (d, 2C), 122.08 (d), 119.11 (s), 116.96 (d), 115.59 (d, 2C), 65.52 (t, 2C), 53.86 (t, 2C); MS (*m*/*z*) 348 (M^{+1} (− HCl)); Anal. ($C_{20}H_{22}N_5OCl$·H_2O) C, H, N.

***E*-2-[2-(2-Chloro-phenyl)-vinyl]-*N*-isopropyl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (6).** Compound **6** was

[a] Abbreviations: DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; OD, optical density; PG, percentage of growth; PDT, cell population doubling time.

prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-isopropylamidino-1,2-phenylenediamine (0.618 g, 2.7 mmol), 3-(2-chloro-phenyl)-propenal (0.450 g, 2.7 mmol), and *p*-benzoquinone (0.292 g, 2.7 mmol) in absolute ethanol (10 mL) was refluxed under nitrogen for 2.5 h, and then the reaction mixture was worked up as it is described to give 0.650 g (64%) of gray powder; mp 281−282 °C; IR (KBr, ν_{max}/cm^{-1}) 3423, 3068, 1665, 1613, 1518; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.70 (bs, 1H, $NH_{benzimidazole}$), 9.48 (bs, 1H, $NH_{amidine}$), 9.38 (bs, 1H, $NH_{amidine}$), 9.07 (bs, 1H, $NH_{amidine}$), 8.05 (s, 1H, H_{arom}), 8.03 (d, 1H, J = 15.64 Hz, $H_{ethenyl}$), 7.90 (d, 1H, J = 8.26 Hz, H_{arom}), 7.60 (d, 1H, J = 8.41 Hz, H_{arom}), 7.49 (d, 2H, J = 7.23 Hz, H_{arom}), 7.39−7.34 (m, 2H, H_{arom}), 7.30 (d, 1H, J = 16.20 Hz, $H_{ethenyl}$), 4.10 (m, 1H, CH-$(CH_3)_2$), 1.23 (d, 6H, J = 5.78 Hz, CH$(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.31 (s), 133.33 (s), 132.94 (s), 131.17 (d), 130.58 (d), 129.95 (d, 2C), 127.77 (d, 2C), 127.37 (d, 2C), 122.66 (s), 120.11 (d), 45.03 (d), 21.32 (q, 2C); MS (*m*/*z*) 339 (M^{+1} (− HCl)); Anal. ($C_{19}H_{20}N_4Cl_2 \cdot H_2O$) C, H, N.

***E*-2-{2-[2-(2-Chloro-phenyl)-vinyl]-3*H*-benzimidazo-5(6)-yl}-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (7).** Compound **7** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-(2-imidazolinyl)-1,2-phenylenediamine (0.500 g, 2.35 mmol), 3-(2-chloro-phenyl)-propenal (0.392 g, 2.35 mmol), and *p*-benzoquinone (0.254 g, 2.35 mmol) in absolute ethanol (15 mL) was refluxed under nitrogen for 2.5 h, and then the reaction mixture was worked up as it is described to give 0.630 g (70%) of gray powder; mp 253−255 °C; IR (KBr, ν_{max}/cm^{-1}) 3406, 3130, 1607, 1514; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.70 (bs, 1H, $NH_{benzimidole}$), 10.90 (bs, 2H, $NH_{amidine}$), 8.38 (d, 1H, J = 16.50 Hz, $H_{ethenyl}$), 8.11 (d, 1H, J = 8.40 Hz, H_{arom}), 7.97 (s, 1H, H_{arom}), 7.85 (d, 1H, J = 8.40 Hz, H_{arom}), 7.74 (d, 1H, J = 7.50 Hz, H_{arom}), 7.55 (d, 1H, J = 7.50 Hz, H_{arom}), 7.43−7.40 (m, 2H, H_{arom}), 7.35 (d, 1H, J = 16.70 Hz, $H_{ethenyl}$), 4.35 (bs, 4H, $2CH_{2imidazole}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 165.32 (s), 154.65 (s), 133.24 (s), 133.05 (s), 131.61 (d), 131.21 (d), 130.78 (d, 2C), 127.85 (d, 2C), 127.45 (d, 2C), 119.91 (s), 115.45 (d), 44.24 (t, 2C); MS (*m*/*z*) 323 (M^{+1} (− HCl)); Anal. ($C_{18}H_{16}N_4Cl_2$) C, H, N.

***E*-2-[2-(2-Chloro-phenyl)-vinyl]-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (8).** Compound **8** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-amidino-1,2-phenylenediamine (0.575 g, 3.1 mmol), 3-(2-chloro-phenyl)-propenal (0.513 g, 3.1 mmol), and *p*-benzoquinone (0.335 g, 3.1 mmol) in absolute ethanol (15 mL) was refluxed under nitrogen for 2.5 h, and then the reaction mixture was worked up as it is described to give 0.760 g (74%) of light gray powder; mp 225−227 °C; IR (KBr, ν_{max}/cm^{-1}) 3310, 3183, 1659, 1622; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.42 (s, 2H, $NH_{amidine}$), 9.19 (bs, 2H, $NH_{amidine}$), 8.17 (s, 1H, H_{arom}), 8.13 (d, 1H, J = 16.50 Hz, $H_{ethenyl}$), 7.97 (d, 1H, J = 9.00 Hz, H_{arom}), 7.76 (d, 1H, J = 8.78 Hz, H_{arom}), 7.70 (d, 1H, J = 7.48 Hz, H_{arom}), 7.55 (d, 1H, J = 7.48 Hz, H_{arom}), 7.45−7.39 (m, 2H, H_{arom}), 7.36 (d, 1H, J = 16.50 Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 166.49 (s), 153.85 (s), 133.65 (s), 133.41 (s), 131.78 (d), 131.10 (d), 130.39 (d), 128.21 (d, 2C), 127.81 (d, 2C), 122.61 (d), 121.71 (s), 120.28 (d); MS (*m*/*z*) 297 (M^{+1} (− HCl)); Anal. ($C_{16}H_{14}N_4Cl_2 \cdot H_2O$) C, H, N.

***E*-2-[2-(2-Chloro-phenyl)-vinyl]-*N*-morpholin-4-yl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (9).** Compound **9** was prepared using the general method described for the preparation of **2**−**9**; a mixture of 4-*N*-morpholinylamidino-1,2-phenylenediamine (1.000 g, 3.68 mmol), 3-(2-chloro-phenyl)-propenal (0.613 g, 3.68 mmol), and *p*-benzoquinone (0.397 g, 3.68 mmol) in absolute ethanol (15 mL) was refluxed under nitrogen for 2.5 h, and then the reaction mixture was worked up as it is described to give 1.100 g (72%) of light gray powder; mp 296−298 °C; IR (KBr, ν_{max}/cm^{-1}) 3361, 2922, 1649, 1606; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.73 (bs, 1H, $NH_{benzimidazole}$), 11.22 (bs, 1H, $NH_{amidine}$), 9.77 (s, 1H, $NH_{amidine}$), 9.02 (bs, 1H, $NH_{amidine}$), 8.08 (s, 1H, H_{arom}), 8.06 (d, 1H, J = 16.22 Hz, $H_{ethenyl}$), 7.90 (dd, 1H, J = 9.00 Hz, J = 7.80 Hz, H_{arom}), 7.70 (d, 1H, J = 8.46 Hz, H_{arom}), 7.60 (d, 1H, J = 8.45 Hz, H_{arom}), 7.48 (dd, 1H, J = 9.00 Hz, J = 7.84 Hz, H_{arom}), 7.38−7.34 (m, 2H, H_{arom}), 7.30 (d, 1H, J = 16.20 Hz, $H_{ethenyl}$), 3.71 (bs, 4H, $2CH_{2morpholine}$), 2.90 (bs, 4H, $2CH_{2morpholine}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.27 (s), 133.28 (s), 132.92 (s), 131.34 (d), 130.56 (d), 129.91 (d, 2C), 127.72 (d, 2C), 127.35 (d, 2C), 119.98 (d), 119.34 (s), 65.51 (t, 2C), 53.85 (t, 2C); MS (*m*/*z*) 382 (M^{+1} (− HCl)); Anal. ($C_{20}H_{21}N_5OCl_2 \cdot H_2O$) C, H, N.

General Method for the Synthesis of 5(6)-*N*-Amidino-substituted *Z*-2-Styryl-1*H*-benzimidazoles (10−13) and 5(6)-*N*-Amidino-substituted *Z*-2-[2-(2-Chloro-phenyl)-vinyl]-1*H*-benzimidazoles (14−17). Solutions of the corresponding 5(6)-*N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles **2**−**5** (0.47 mmol) and 5(6)-*N*-amidino-substituted *E*-2-[2-(2-chloro-phenyl)-vinyl]-1*H*-benzimidazoles **6**−**9** in ethanol ($c = 1.3 \times 10^{-2}$ mol dm^{-3}) were irradiated at room temperature with 400 W, high-pressure, mercury lamp using a Pyrex filter for about 4 h, when UV spectra shown that the isomerization is finished. The solutions were concentrated, diethylether was added, and resulting products were filtered off and washed with diethylether. After precipitating from ethanol/diethylether, light powders were obtained.

***Z*-*N*-Isopropyl-2-styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (10).** Compound **10** was prepared using the general method described for the preparation of **10**−**17**; a solution of **2** (0.150 g, 0.44 mmol) in ethanol (35 mL) was irradiated for 4 h and then worked up as it is described to give 0.100 g (67%) of gray powder; mp 190−192 °C; IR (KBr, ν_{max}/cm^{-1}) 3369, 3066, 1665, 1613; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.50 (bs, 1H, $NH_{benzimidazole}$), 9.80 (bs, 1H, $NH_{amidine}$), 9.51 (bs, 1H, $NH_{amidine}$), 9.09 (s, 1H, $NH_{amidine}$), 8.01 (s, 1H, H_{arom}), 7.99 (d, 1H, J = 8.22 Hz, H_{arom}), 7.71 (d, 1H, J = 8.20 Hz, H_{arom}), 7.57 (d, 1H, J = 7.66 Hz, H_{arom}), 7.49 (d, 1H, J = 7.80 Hz, H_{arom}), 7.46−7.37 (m, 3H, H_{arom}), 7.00 (d, 1H, J = 13.22 Hz, $H_{ethenyl}$), 6.70 (d, 1H, J = 13.05 Hz, $H_{ethenyl}$), 3.95−3.82 (m, 1H, CH$(CH_3)_2$), 1.30 (d, 6H, J = 6.00 Hz, CH-$(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.41 (s), 152.16 (s), 137.22 (d), 135.46 (s), 130.09 (d, 2C), 129.09 (d), 128.85 (d), 128.18 (d, 2C), 127.37 (d), 122.71 (s), 117.03 (d), 116.80 (d), 45.03 (d), 21.36 (q, 2C); MS (*m*/*z*) 305 (M^{+1} (− HCl)); Anal. ($C_{19}H_{21}N_4Cl \cdot 2H_2O$) C, H, N.

***Z*-2-(2-Styryl-3*H*-benzimidazol-5(6)-yl)-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (11).** Compound **11** was prepared using the general method described for the preparation of **10**−**17**; a solution of **3** (0.150 g, 0.46 mmol) in ethanol (36 mL) was irradiated for 4 h and then worked up as it is described to give 0.115 g (78%) of light violet powder; mp 220−222 °C; IR (KBr, ν_{max}/cm^{-1}) 3381, 3106, 1605, 1513; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.40 (bs, 1H, $NH_{benzimidazole}$), 10.68 (bs, 2H, $NH_{amidine}$), 8.45 (s, 1H, H_{arom}), 8.10 (d, 1H, J = 8.40 Hz, H_{arom}), 7.99 (d, 1H, J = 8.40 Hz, H_{arom}), 7.72 (d, 1H, J = 7.85 Hz, H_{arom}), 7.52−7.24 (m, 3H, H_{arom}), 7.29 (d, 1H, J = 7.70 Hz, H_{arom}), 7.05 (d, 1H, J = 12.90 Hz, $H_{ethenyl}$), 6.64 (d, 1H, J = 12.90 Hz, $H_{ethenyl}$), 4.10 (bs, 4H, $2CH_{2imidazole}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 165.41 (s), 152.41 (s), 143.36 (s), 137.49 (d), 135.50 (s), 130.19 (d, 2C), 129.63 (d), 129.46 (d), 128.36 (d), 128.23 (d, 2C), 127.44 (d), 116.96 (d), 115.24 (s), 44.63 (t, 2C); MS (*m*/*z*) 289 (M^{+1} (− HCl)); Anal. ($C_{18}H_{17}N_4Cl \cdot H_2O$) C, H, N.

***Z*-2-Styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (12).** Compound **12** was prepared using the general method described for the preparation of **10**−**17**; a solution of **4** (0.140 g, 0.47 mmol) in ethanol (36 mL) was irradiated for 4 h and then worked up as it is described to give 0.098 g (71%) of light violet powder; mp 188−189 °C; IR (KBr, ν_{max}/cm^{-1}) 3084, 1672, 1529; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.30 (bs, 1H, $NH_{benzimidazole}$), 9.31 (bs, 2H, $NH_{amidine}$), 9.10 (bs, 2H, $NH_{amidine}$), 8.08 (s, 1H, H_{arom}), 7.90 (d, 2H, J = 8.44 Hz, H_{arom}), 7.68−7.62 (m, 2H, H_{arom}), 7.35−7.25 (m, 3H, H_{arom}), 6.93 (d, 1H, J = 13.26 Hz, $H_{ethenyl}$), 6.60 (d, 1H, J = 13.28 Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 166.06 (s), 152.25 (s), 137.26 (d), 135.34 (s), 129.93 (d, 2C), 128.95 (d), 128.69 (d), 128.03 (d, 2C), 127.26 (d), 121.93 (d), 121.07 (s), 116.83 (d).

***Z*-*N*-Morpholin-4-yl-2-styryl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (13).** Compound **13** was prepared using the general method described for the preparation of **10**−**17**; a solution of **5** (0.075 g, 0.20 mmol) in ethanol (15 mL) was irradiated for 4 h and then worked up as it is described to give 0.048 g (63%) of light gray powder; mp 197−199 °C; IR (KBr, ν_{max}/cm^{-1}) 3394, 2924, 1652, 1643, 1607; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.33 (bs, 1H, $NH_{benzimidazole}$), 11.18 (bs, 1H, $NH_{amidine}$), 9.74 (s, 1H, $NH_{amidine}$), 9.01 (bs, 1H, $NH_{amidine}$), 7.94 (s, 1H, H_{arom}), 7.67 (d, 1H, J = 8.20 Hz, H_{arom}), 7.59 (d, 1H, J = 8.08 Hz, H_{arom}), 7.40 (t, 1H, J = 7.20 Hz, H_{arom}), 7.32−7.22 (m, 4H, H_{arom}), 6.93 (d, 1H, J = 13.20 Hz, $H_{ethenyl}$), 6.62 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$), 3.71 (bs, 4H, $2CH_{2morpholine}$), 2.90 (bs, 4H, $2CH_{2morpholine}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.25 (s), 137.13 (d), 135.44 (s), 135.40 (s), 130.01 (d, 2C), 129.19 (d), 128.93 (d), 128.70 (d), 128.01 (d, 2C), 127.20 (d), 119.13 (s), 116.93 (d), 65.20 (t, 2C), 53.85 (t, 2C); MS (*m*/*z*) 382 (M^{+1}); Anal. ($C_{20}H_{22}N_5OCl\cdot 2H_2O$) C, H, N.

***Z*-2-[2-(2-Chloro-phenyl)-vinyl]-*N*-isopropyl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (14).** Compound **14** was prepared using the general method described for the preparation of **10**−**17**; a solution of **6** (0.100 g, 0.27 mmol) in ethanol (20 mL) was irradiated for 4 h and then worked up as it is described to give 0.060 g (66%) of light gray powder; mp 191−193 °C; IR (KBr, ν_{max}/cm^{-1}) 3359, 2979, 1667, 1614; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.60 (bs, 1H, $NH_{amidine}$), 9.58 (bs, 1H, $NH_{amidine}$), 9.49 (s, 1H, $NH_{amidine}$), 7.93 (s, 1H, H_{arom}), 7.74 (d, 1H, J = 8.20 Hz, H_{arom}), 7.64 (d, 1H, J = 8.40 Hz, H_{arom}), 7.56 (d, 1H, J = 7.80 Hz, H_{arom}), 7.51 (d, 1H, J = 7.80 Hz, H_{arom}), 7.39 (t, 1H, J = 7.44 Hz, H_{arom}), 7.27 (t, 1H, J = 7.28 Hz, H_{arom}), 7.08 (d, 1H, J = 12.30 Hz, $H_{ethenyl}$), 6.87 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$), 4.16−4.09 (m, 1H, $CH(CH_3)_2$), 1.27 (d, 6H, J = 6.01 Hz, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.21(s), 151.33 (s), 141.22 (s), 134.16 (s), 133.41 (d), 132.72 (s), 131.17 (d), 129.99 (d), 129.11 (d), 127.80 (d), 127.43 (d), 126.62 (d), 125.43 (s), 122.68 (s), 122.26 (d), 119.63 (d), 45.01 (d), 21.30 (q, 2C); MS (*m*/*z*) 339 (M^{+1} (− HCl)); Anal. ($C_{19}H_{20}N_4Cl_2\cdot H_2O$) C, H, N.

***Z*-2-{2-[2-(2-Chloro-phenyl)-vinyl]-3*H*-benzimidazo-5(6)-yl}-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (15).** Compound **15** was prepared using the general method described for the preparation of **10**−**17**; a solution of **7** (0.160 g, 0.45 mmol) in ethanol (35 mL) was irradiated for 4 h and then worked up as it is described to give 0.110 g (66%) of light brown powder; mp 285−287 °C; IR (KBr, ν_{max}/cm^{-1}) 3389, 3105, 1606, 1511; ^{1}H NMR (DMSO-d_6, δ/ppm) 10.61 (bs, 2H, $NH_{amidine}$), 8.25 (s, 1H, H_{arom}), 7.89 (d, 1H, J = 8.40 Hz, H_{arom}), 7.68 (d, 1H, J = 8.40 Hz, H_{arom}), 7.52 (d, 1H, J = 7.80 Hz, H_{arom}), 7.42−7.34 (m, 2H, H_{arom}), 7.27 (d, 1H, J = 7.60 Hz, H_{arom}), 7.11 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$), 6.84 (d, 1H, J = 2.40 Hz $H_{ethenyl}$), 4.00 (t, 4H, J = 6.20 Hz, $2CH_{2imidazole}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 165.28 (s), 151.85 (s), 133.98 (s), 133.76 (d), 133.11 (s), 132.65 (s), 131.73 (d), 130.62 (d), 129.90 (d), 128.99 (d), 127.66 (d), 126.47 (d), 122.40 (d), 119.36 (d), 115.30 (s), 44.13 (t, 2C); MS (*m*/*z*) 323 (M^{+1} (− HCl)); Anal. ($C_{18}H_{16}N_4Cl_2$) C, H, N.

***Z*-2-[2-(2-Chloro-phenyl)-vinyl]-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (16).** Compound **16** was prepared using the general method described for the preparation of **10**−**17**; a solution of **8** (0.200 g, 0.48 mmol) in ethanol (36 mL) was irradiated for 4 h and then worked up as it is described to give 0.110 (60%) of light gray powder; mp 198−200 °C; IR (KBr, ν_{max}/cm^{-1}) 3100, 1670, 1525; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.31 (bs, 2H, $NH_{amidine}$), 9.10 (bs, 2H, $NH_{amidine}$), 8.00 (s, 1H, H_{arom}), 7.63 (d, 1H, J = 7.80 Hz, H_{arom}) 7.59 (bs, 2H, H_{arom}), 7.49 (d, 1H, J = 7.80 Hz, H_{arom}), 7.32 (t, 1H, J = 7.60 Hz, H_{arom}), 7.20 (t, 1H, J = 7.60 Hz, H_{arom}), 7.05 (d, 1H, J = 12.00 Hz, $H_{ethenyl}$), 6.78 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 166.01 (s), 151.49 (s), 140.99 (s), 134.04 (s), 133.68 (d), 132.70 (s), 131.04 (d, 2C), 129.98 (d), 129.08 (d, 2C), 126.60 (d), 125.65 (s), 122.00 (d), 121.19 (s), 119.44 (d); MS (*m*/*z*) 297 (M^{+1} (− HCl)); Anal. ($C_{19}H_{20}N_4Cl_2\cdot H_2O$) C, H, N.

***Z*-2-[2-(2-Chloro-phenyl)-vinyl]-*N*-morpholin-4-yl-3*H*-benzimidazole-5(6)-carboxamidine Hydrochloride (17).** Compound **17** was prepared using the general method described for the preparation of **10**−**17**; a solution of **9** (0.150 g, 0.45 mmol) in ethanol (35 mL) was irradiated for 4 h and then worked up as it is described to give 0.114 (76%) of light violet powder; mp 188−190 °C; ^{1}H NMR (DMSO-d_6, δ/ppm) 11.21 (bs, 1H, $NH_{amidine}$), 9.86 (s, 1H, $NH_{amidine}$), 9.06 (bs, 1H, $NH_{amidine}$), 8.04 (bs, 1H, H_{arom}), 7.73 (d, 1H, J = 8.70 Hz, H_{arom}), 7.65 (d, 1H, J = 8.40 Hz, H_{arom}), 7.58−7.50 (m, 2H, H_{arom}), 7.40 (t, 1H, J = 7.60 Hz, H_{arom}), 7.28 (t, 1H, J = 7.10 Hz, H_{arom}), 7.09 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$), 6.87 (d, 1H, J = 12.60 Hz, $H_{ethenyl}$), 3.91 (bs, 4H, $2CH_{2morpholine}$), 3.00 (bs, 4H, $2CH_{2morpholine}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 163.25 (s), 137.93 (d), 136.45 (s), 135.80 (s), 132.01 (d, 2C), 130.19 (s), 129.13 (d), 128.93 (d), 128.57 (d, 2C), 127.02 (d), 123.44 (d), 119.98 (s), 65.10 (t, 2C), 53.79 (t, 2C); MS (*m*/*z*) 382 (M^{+1} (− HCl)); Anal. ($C_{20}H_{21}N_5OCl_2$) C, H, N.

General Method for the Synthesis of *N*-Amidino-Substituted Benzimidazo[1,2-*a*]quinolines (18−21). A solution of corresponding 5(6)-*N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles (**2**−**5**) and a small amount of iodine (5%), in ethanol ($c = 1.3 \times 10^{-3}$ mol dm^{-3}), were irradiated at room temperature with 400 W, high-pressure, mercury lamp, using a Pyrex filter for about 6−10 h, until the UV spectra showed that the reaction of dehydrocyclization was finished. The air was bubbled through the solution. The solutions were concentrated under reduced pressure, diethylether was added, and the resulting products were filtered off and washed with diethylether. After precipitating from ethanol/diethylether and recrystallization from acetone/water, light powders were obtained in 37−42% yields. Compounds **18**−**21** were prepared as a mixture of 9(10)-structural isomers.

Compounds **18**−**21** were also prepared by reaction of photochemical dehydrohalogenation cyclization of chloro-substituted 5(6)-*N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles **6**−**9**. A solutions of corresponding chloro-substituted 5(6)-*N*-amidino-substituted *E*-2-styryl-1*H*-benzimidazoles (**6**−**9**) in ethanol ($c = 1.2 \times 10^{-3}$ mol dm^{-3}) were irradiated at room temperature with a 400 W, high-pressure, mercury lamp using a Pyrex filter for about 6−10 h, until the UV spectra showed that the reaction of dehydrohalogenation was completed. The solutions were concentrated under reduced pressure, diethyl ether was added and resulting precipitates were filtered off and washed with diethylether. After precipitating from ethanol/diethylether and recrystallization from acetone/water, light powders were obtained in 31−40% yields.

***N*-Isopropyl-benzimidazo[1,2-*a*]quinoline-(9)10-carboxamidine Hydrochloride (18).** Compound **18** was prepared using the general method described for the preparation of **18**−**21**: (a) a solution of **2** (0.100 g, 0.29 mmol) and iodine (8 mg) in ethanol (220 mL) was irradiated for 6 h and then worked up as it is described to give 0.030 g (31%) of yellow powder; (b) a solution of **6** (0.095 g, 0.25mmol) in ethanol (220 mL) was irradiated for 7 h and then worked up as it is described to give 0.035 g (37%) of yellow powder; mp 219−221 °C; IR (KBr, υ/cm^{-1}) 3435, 3105, 1676, 1613, 1537; MS (*m*/*z*) 303 (M^{+1} (− HCl)); Anal. ($C_{19}H_{19}N_4Cl$) C, H, N.

***N*-Isopropyl-benzimidazo[1,2-*a*]quinoline-10-carboxamidine Hydrochloride (60%).** ^{1}H NMR (DMSO-d_6, δ/ppm) 9.76 (bs, 2H, $NH_{amidine}$), 9.26 (bs, 1H, $NH_{amidine}$), 9.09 (s, 1H, H_{arom}), 9.02 (d, 1H, J = 8.46 Hz, H_{arom}), 8.12 (d, 1H, J = 9.75 Hz, $H_{quinoline}$), 8.10 (d, 1H, J = 7.70 Hz, H_{arom}), 8.07 (d, 1H, J = 8.70 Hz, H_{arom}), 7.98−7.86 (m, 2H, H_{arom}), 7.74 (d, 1H, J = 9.48 Hz, $H_{quinoline}$), 7.67 (t, 1H, J = 7.42 Hz, H_{arom}), 4.22−4.18 (m, 1H, $CH(CH_3)_2$), 1.38 (d, 6H, J = 6.00 Hz, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.99 (s), 150.13 (s), 147.42 (s), 134.52 (s), 133.53 (d), 130.75 (d), 129.97 (d), 129.76 (s), 125.19 (d), 124.95 (s), 124.38 (d), 123.09 (s), 120.19 (d), 119.60 (d), 117.05 (d), 115.46 (d), 45.18 (d), 21.30 (q, 2C).

***N*-Isopropyl-benzimidazo[1,2-*a*]quinoline-9-carboxamidine Hydrochloride (40%).** ^{1}H NMR (DMSO-d_6, δ/ppm) 9.80 (bs, 1H, $NH_{amidine}$), 9.62 (bs, 1H, $NH_{amidine}$), 9.22 (bs, 1H, $NH_{amidine}$), 8.95 (d, 1H, J = 8.60 Hz, H_{arom}), 8.90 (d, 1H, J = 8.52 Hz, H_{arom}), 8.38 (s, 1H), 8.09 (d, 1H, J = 7.40 Hz, H_{arom}), 8.05 (d, 1H, J = 9.60 Hz, $H_{quinoline}$), 7.93−7.88 (m, 2H), 7.73 (d, 1H, J = 9.51 Hz,

$H_{quinoline}$), 7.65 (t, 1H, $J = 7.35$ Hz, H_{arom}), 4.22−4.18 (m, 1H, CH-$(CH_3)_2$), 1.36 (d, 6H, $J = 6.00$ Hz, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.94 (s), 149.44 (s), 143.69 (s), 134.58 (s), 133.88 (d), 133.04 (d), 130.70 (d), 129.99 (s), 128.90 (d), 124.60 (s), 122.46 (s), 122.01 (d), 117.17 (d), 116.29 (d), 115.92 (d), 114.99 (d), 45.17 (d), 21.27 (q, 2C).

2-Benzimidazo[1,2-*a*]quinolin-10(9)-yl-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (19). Compound **19** was prepared using the general method described for the preparation of **18**−**21**; (a) a solution of **3** (0.095 g, 0.29 mmol) and iodine (8 mg) in ethanol (220 mL) was irradiated for 6 h and then worked up as it is described to give 0.031 g (33%) of light violet powder; (b) a solution of **7** (0.105 g, 0.29 mmol) in ethanol (220 mL) was irradiated for 7 h and then worked up as it is described to give 0.040 g (39%) of light violet powder; mp > 300 °C; IR (KBr, υ/cm^{-1}) 3400, 3200, 3138, 1634, 1602, 1561; MS (*m*/*z*) 287 (M^{+1} (− HCl)); Anal. ($C_{18}H_{15}N_4Cl$) C, H, N.

2-Benzimidazo[1,2-*a*]quinolin-10-yl-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (60%). ^{1}H NMR (DMSO-d_6, δ/ppm) 11.09 (bs, 2H, $H_{amidine}$), 9.40 (s, 1H, H_{arom}), 9.16 (d, 1H, $J = 8.31$ Hz, H_{arom}), 8.15 (d, 1H, $J = 9.24$ Hz, $H_{quinoline}$), 8.09 (d, 1H, $J = 8.40$ Hz, H_{arom}), 8.04 (d, 1H, $J = 7.70$ Hz, H_{arom}), 8.00−7.91 (m, 2H, H_{arom}), 7.75 (d, 1H, $J = 9.48$ Hz, $H_{quinoline}$), 7.66 (t, 1H, $J = 7.32$ Hz, H_{arom}), 4.08 (bs, 4H, $CH_{2imidaz.}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162. 46 (s),151.28 (s), 148.03 (s), 135.13 (s), 133.98 (d), 133.74 (d), 130.95 (d), 130.16 (d), 129.97 (s), 125.84 (d), 124.24 (d), 123.41 (s), 121.01 (d), 120.13 (d), 119.46 (s), 117.54 (d), 46.03 (t, 2C).

2-Benzimidazo[1,2-*a*]quinolin-9-yl-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (40%). ^{1}H NMR (DMSO-d_6, δ/ppm) 10.83 (bs, 2H, $NH_{amidine}$), 9.00 (d, 1H, $J = 8.91$ Hz, H_{arom}), 8.89 (d, 1H, $J = 8.43$ Hz, H_{arom}), 8.65 (s, 1H), 8.18 (d, 1H, $J = 7.60$ Hz, H_{arom}), 8.09 (d, 1H, $J = 9.52$ Hz, $H_{quinoline}$), 7.97−7.94 (m, 2H), 7.77 (d, 1H, $J = 9$,50 Hz, $H_{quinoline}$), 7.68 (t, 1H, $J = 7$,65 Hz, H_{arom}), 4.06 (bs, 4H, $CH_{2imidaz.}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.999 (s), 150.33 (s), 144.09 (s), 134.87 (s), 134.18 (d), 133.00 (d), 131.25 (d), 130.22 (s), 129.19 (d), 124.98 (s), 123.03 (s), 122.24 (d), 118.00 (d), 116.66 (d), 115.99 (d), 115.09 (d), 45.99 (t), 21.27.

Benzimidazo[1,2-*a*]-9(10)-carboxamidine Hydrochloride (20). Compound **20** was prepared using the general method described for the preparation of **18**−**21**; (a) a solution of **4** (0.085 g, 0.29 mmol) and iodine (7 mg) in ethanol (220 mL) was irradiated for 7 h and then worked up as it is described to give 0.035 g (40%) of light violet powder; (b) a solution of **8** (0.105 g, 0.35mmol) in ethanol (230 mL) was irradiated for 6 h and then worked up as it is described to give 0.041 g (40%) of light violet powder; mp 232−234 °C; IR (KBr, υ/cm^{-1}) 3383, 3122, 1639, 1609, 1534; MS (*m*/*z*) 261 (M^+ (− HCl)); Anal. ($C_{16}H_{13}N_4Cl$) C, H, N.

Benzimidazo[1,2-*a*]quinoline-10-carboxamidine Hydrochloride (60%). ^{1}H NMR (DMSO-d_6, δ/ppm) 9.65 (bs, 2H, $NH_{amidine}$), 9.31 (bs, 2H, $NH_{amidine}$), 9.17 (s, 1H, H_{arom}), 9.06 (d, 1H, $J = 8.49$ Hz, H_{arom}), 8.13 (d, 1H, $J = 9.21$ Hz, $H_{quinoline}$), 8.06 (d, 1H, $J = 8.70$ Hz, H_{arom}), 8.04 (d, 1H, $J = 7.80$ Hz, H_{arom}), 7.99−7.91 (m, 2H, H_{arom}), 7.76 (d, 1H, $J = 9.48$ Hz, $H_{quinoline}$), 7.66 (t, 1H, $J = 7.42$ Hz, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.55 (s), 150.76 (s), 147.45 (s), 135.05 (s), 133.70 (d), 130.98 (d), 130.97 (d), 129.86 (s), 126.99 (d), 126.08 (d), 124.56 (s), 120.86 (d), 119.09 (s), 119.70 (d), 115.99 (d), 115.38 (d).

Benzimidazo[1,2-*a*]quinoline-9-carboxamidine Hydrochloride (40%). ^{1}H NMR (DMSO-d_6, δ/ppm) 9.57 (bs, 2H, $NH_{amidine}$), 9.30 (bs, 2H, $NH_{amidine}$), 8.97 (d, 1H, $J = 8.79$ Hz, H_{arom}), 8.78 (d, 1H, $J = 8.46$ Hz, H_{arom}), 8.49 (s, 1H), 8.08 (d, 1H, $J = 7.50$ Hz, H_{arom}), 8.03 (d, 1H, $J = 9.48$ Hz, $H_{quinoline}$), 7.98−7.96 (m, 2H), 7.73 (d, 1H, $J = 9$,51 Hz, $H_{quinoline}$), 7.64 (t, 1H, $J = 7$,60 Hz, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.33 (s), 149.88 (s), 143.97 (s), 134.02 (s), 133.99 (d), 133.20 (d), 131.09 (d), 130.11 (s), 129.22 (d), 124.33 (s), 121.99 (s), 121.89 (d), 118.03 (d), 117.67 (d), 116.70 (d), 115.10 (d).

***N*-Morpholin-4-yl-benzimidazo[1,2-*a*]quinoline-9(10)-carboxamidine Hydrochloride (21).** Compound **21** was prepared using the general method described for the preparation of **18**−**21**; (a) a solution of **5** (0.110 g, 0.29 mmol) and iodine (9 mg) in ethanol (220 mL) was irradiated for 6 h and then worked up as it is described to give 0.048 g (40%) of yellow powder; (b) a solution of **9** (0.110 g, 0.26mmol) in ethanol (225 mL) was irradiated for 6 h and then worked up as it is described to give 0.035 g (32%) of yellow powder; mp 232−234 °C; IR (KBr, υ/cm^{-1}) 3429, 3177, 1634, 1611; MS (*m*/*z*) 346 (M^{+1} (− HCl)); Anal. ($C_{20}H_{20}N_5OCl$) C, H, N.

***N*-Morpholin-4-yl-benzimidazo[1,2-*a*]quinoline-10-carboxamidine Hydrochloride (60%).** ^{1}H NMR (DMSO-d_6, δ/ppm) 11.44 (s, 1H, $NH_{amidine}$), 10.03 (s, 1H, $NH_{amidine}$), 9.23 (s, 1H, $NH_{amidine}$), 9.18 (s, 1H, H_{arom}), 9.05 (d, 1H, $J = 8.55$ Hz, H_{arom}), 8.16 (d, 1H, $J = 9.57$ Hz, $H_{quinoline}$), 8.14 (d, 1H, $J = 7.44$ Hz, H_{arom}), 8.09 (d, 1H, $J = 8.70$ Hz, H_{arom}), 7.99−7.90 (m, 2H, H_{arom}), 7.77 (d, 1H, $J = 9.51$ Hz, $H_{quinoline}$), 7.66 (t, 1H, $J = 7.46$ Hz, H_{arom}), 3.70 (bs, 4H, $2CH_{2morpholine}$), 3.12 (bs, 4H, $2CH_{2morpholine}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.00 (s), 150.25 (s), 147.63 (s), 134.53 (s), 133.89 (d), 133.35 (d), 130.77 (d), 130.04 (d), 129.83 (s), 125.35 (d), 124.30 (d), 123.12 (s), 120.25 (d), 119.79 (d), 119.26 (s), 117.08 (d), 65.65 (t, 2C), 54.03 (t, 2C).

***N*-Morpholin-4-yl-benzimidazo[1,2-*a*]quinoline-9-carboxamidine Hydrochloride (40%).** ^{1}H NMR (DMSO-d_6, δ/ppm) 11.31 (s, 1H, $NH_{amidine}$), 9.47 (s, 1H, $NH_{amidine}$), 9.22 (s, 1H, $NH_{amidine}$), 9.00 (d, 1H, $J = 8.88$ Hz, H_{arom}), 8.92 (d, 1H, $J = 8.58$ Hz, H_{arom}), 8.45 (s, 1H), 8.15 (d, 1H, $J = 7.80$ Hz, H_{arom}), 8.11 (d, 1H, $J = 9.60$ Hz, $H_{quinoline}$), 7.96−7.92 (m, 2H), 7.75 (d, 1H, $J = 9.57$ Hz, $H_{quinoline}$), 7.65 (t, 1H, $J = 7.58$ Hz, H_{arom}), 3.69 (bs, 2H, $4CH_{2morpholine}$), 3.10 (bs, 4H, $2CH_{2morpholine}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.88 (s), 149.23 (s), 144.09 (s), 134.45 (s), 133.31 (s), 133.00 (d), 130.06 (d), 130.04 (s), 128.85 (d), 123.12 (d), 121.96 (d), 120.26 (s), 118.87 (d), 116.98 (d), 116.32 (d), 115.64 (d), 65.50 (t, 2C), 53.94 (t, 2C).

General Method for the Synthesis of *E*-5(6)-Cyano-substituted-*E*-2-styryl-1*H*-benzimidazole (24) and *E*-5(6)-Cyano-substituted-*E*-2-[2-(2-chloro-phenyl)-vinyl]-1*H*-benzimidazole (25). A mixture of corresponding benzaldehydes **22a**,**b** and 5(6)-cyano-2-methylbenzimidazole **23** in acetic anhydride was stirred at refluxed for 3 h. The reaction mixture was cooled to room temperature and 24 mL of *i*-propanole and a solution of 1.434 g of oxalic acid in 9 mL of *i*-propanole were added, and the resulting product was filtered off and washed with water. Crude product was suspended in water, and aqueous sodium hydroxide was added until pH = 10. The resulting product was filtered off and washed with water. After recrystallization from ethanol, a light yellow powder was obtained.

2-Styryl-3*H*-benzimidazole-6-carbonitrile (24). From 3.6 mL (35.70 mmol) of **22a** and 1.50 g (9.50 mmol) of **23** in 10 mL of acetic anhydride was obtained 1.16 g (48%) of light yellow powder; mp 107−108 °C; IR (KBr, υ/cm^{-1}) 3193, 2224, 1646, 1614, 1518; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.10 (bs, 1H, $NH_{benzimidazole}$), 8.06 (s, 1H, H_{arom}), 7.77 (d, 1H, $J = 16.53$ Hz, $H_{ethenyl}$), 7.71 (d, 2H, $J = 8.19$ Hz, H_{arom}), 7.70 (d, 1H, $J = 8.29$ Hz, H_{arom}), 7.56 (dd, 1H, $J = 8.31$ Hz, $J = 8.34$ Hz, H_{arom}), 7.46−7.39 (m, 3H, H_{arom}), 7.30 (d, 1H, $J = 16.50$ Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.15 (s), 150.71 (s), 139.20 (s), 133.90 (s), 133.26 (s), 130.84 (d), 130.39 (d), 130.19 (d), 128.37 (d, 2C), 127.62 (d, 2C), 123.08 (d), 120.74 (d), 115.43 (d); MS (*m*/*z*) 246 (M^{+1}); Anal. ($C_{16}H_{11}N_3$) C, H, N.

2-[2-(2-Chloro-phenyl)-vinyl]-3*H*-benzimidazole-5(6)-carbonitrile (25). From 3.1 mL (35.72 mmol) of **22b** and 1.50 g (9.50 mmol) of **23** in 10 mL acetic anhydride, to obtained 1.01 g (49%) of gray powder; mp 117−119 °C; IR (KBr, υ/cm^{-1}) 3194, 2222, 1645, 1617, 1520; ^{1}H NMR (DMSO-d_6, δ/ppm) 13.18 (bs, 1H, $NH_{benzimidazole}$), 8.16 (d, 1H, $J = 16.47$ Hz, $H_{ethenyl}$), 7.96 (s, 1H, H_{arom}), 7.87 (dd, 1H, $J = 8.38$ Hz, $J = 8.54$ Hz, H_{arom}), 7.67 (bs, 1H, H_{arom}), 7.60 (dd, 1H, $J = 8.46$ Hz, $J = 8.31$ Hz, H_{arom}), 7.50−7.47 (m, 1H, H_{arom}), 7.40−7.36 (m, 2H, H_{arom}), 7.20 (d, 1H, $J = 16.53$ Hz, $H_{ethenyl}$); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.35 (s), 150.58 (s), 138.23 (s), 135.32 (s), 135.30 (d), 129.04 (d), 128.96 (d, 2C), 127.10 (d, 2C), 122.51 (d), 116.74 (d), 113.85 (d); MS (*m*/*z*) 280 (M^{+1}); Anal. ($C_{16}H_{10}N_3Cl$) C, H, N.

Cyano-Substituted-Benzimidazo[1,2-*a*]quinolines (26−27). A solution of 5(6)-cyano-substituted *E*-2-styryl-1*H*-benzimidazole **24** and a small amount of iodine (5%) in ethanol ($c = 1.3 \times 10^{-3}$ mol

dm^{-3}) and a solution of chloro-substituted *E*-5(6)-cyano-2-styryl-1*H*-benzimidazole **25** in ethanol ($c = 1.3 \times 10^{-3}$ mol dm^{-3}) were irradiated at room temperature with 400 W, high-pressure, mercury lamp using a Pyrex filter for about 6−10 h, until the UV spectra showed that the reactions of photochemical dehydrocyclization and dehydrohalogenation were completed. The air was bubbled through the solution. The solutions were concentrated under reduced pressure, and the mixture of 9(10)-structural isomers was obtained. Isomers **26** and **27** were separated by column chromatography.

Benzimidazo[1,2-*a*]quinoline-10-carbonitrile (26). Yield 0.09 g (20%) of yellow powder after separation by column chromatography (petrol ether/ethyl acetate = 1:1); mp 184−185 °C; IR (KBr, υ/cm^{-1}) 3446, 2224, 1637, 1609, 1595, 1542; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.26 (s, 1H), 8.88 (d, 1H, $J = 8.46$ Hz, H_{arom}), 8.06 (d, 1H, $J = 9.42$ Hz, $H_{quinoline}$), 8.05 (d, 1H, $J = 7.62$ Hz, H_{arom}), 7.98 (d, 1H, $J = 8.44$ Hz, H_{arom}), 7.84 (dd, 1H, $J = 8.40$ Hz, $J = 8.42$ Hz, H_{arom}), 7.80 (t, 1H, $J = 7.20$ Hz, H_{arom}), 7.66 (d, 1H, $J = 9.42$ Hz, $H_{quinoline}$), 7.58 (t, 1H, $J = 7.28$ Hz, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 150.28 (s), 147.05 (s), 134.35 (s), 133.90 (d), 130.90 (d), 129.92 (d), 129.86 (s), 127.59 (d), 125.20 (d), 123.03 (s), 120.59 (d), 119.88 (s), 119.70 (d), 116.90 (d), 116.30 (d), 103.92 (s); MS (*m*/*z*) 244 (M^{+1}); Anal. ($C_{16}H_9N_3$) C, H, N.

Benzimidazo[1,2-*a*]quinoline-9-carbonitrile (27). Yield 0.06 g (12%) of yellow powder after separation by column chromatography (petrol ether/ethyl acetate = 1:1); mp 188−189 °C; IR (KBr, υ/cm^{-1}) 3442, 2220, 1630, 1605, 1595; ^{1}H NMR (DMSO-d_6, δ/ppm) 8.83 (d, 1H, $J = 8.56$ Hz, H_{arom}), 8.45 (d, 1H, $J = 8.42$ Hz, H_{arom}), 8.38 (s, 1H), 8.01 (d, 1H, $J = 7.62$ Hz, H_{arom}), 7.98 (d, 1H, $J = 9.48$ Hz, $H_{quinoline}$), 7.81 (d, 1H, $J = 7.64$ Hz, H_{arom}), 7.79 (t, 1H, $J = 7.40$ Hz, H_{arom}), 7.62 (d, 1H, $J = 9.36$ Hz, $H_{quinoline}$), 7.57 (t, 1H, $J = 7.38$ Hz, H_{arom}); MS (*m*/*z*) 244 (M^{+1}); Anal. ($C_{16}H_9N_3$) C, H, N.

4-[2-(1*H*-Benzimidazol-2-yl]-benzonitrile (30). Heating a mixture of equimolar amounts (37.81 mmol) of 4-cyanobenzaldehyde **28** and 2-methylbenzimidazole **29** in a tube at 200 °C and recrystallization from methanol gave 6.60 g (71%) of yellow powder; mp 220−221 °C; IR (KBr, υ/cm^{-1}) 3311, 2227, 1645; ^{1}H NMR (DMSO-d_6, δ/ppm) 12.77 (s, 1H, $NH_{benzimidazole}$), 7.91 (d, 2H, $J = 7.80$ Hz, H_{arom}), 7.89 (bs, 2H, H_{arom}), 7.72 (d, 1H, $J = 16.44$ Hz, $H_{ethenyl}$), 7.64 (d, 1H, $J = 7.20$ Hz, H_{arom}), 7.52 (d, 1H, $J = 7.10$ Hz, H_{arom}), 7.42 (d, 1H, $J = 16.47$ Hz, $H_{ethenyl}$), 7.22−7.19 (m, 2H, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.43 (s), 150.66 (s), 140.90 (s), 133.28 (d, 2C), 132.82 (d), 131.15 (s), 130.45 (s), 128.17 (d, 2C), 123.42 (d), 122.29 (d), 121.71 (d), 119.38 (d), 119.32 (s), 114.75 (d); MS (*m*/*z*) 246 (M^{+1}); Anal. ($C_{16}H_{11}N_3$) C, H, N.

Benzimidazo[1,2-*a*]quinoline-2-carbonitrile (31). Irradiation of a solution of 0.23 g (0.94 mmol) **30** in ethanol ($c = 1.3 \times 10^{-3}$ mol dm^{-3}) with a 400 W, high-pressure, mercury lamp using a Pyrex filter, at room temperature for 12 h, gave 0.14 g (61%) of yellow powder; mp 210−212 °C; IR (KBr, υ/cm^{-1}) 3438, 2231, 1631, 1609, 1545; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.18 (s, 1H, H_{arom}), 8.90 (d, 1H, $J = 8.55$ Hz, H_{arom}), 8.25 (d, 1H, $J = 8.20$ Hz, H_{arom}), 8.04 (d, 1H, $J = 9.54$ Hz, $H_{quinoline}$), 7.98 (d, 1H, $J = 8.01$ Hz, H_{arom}), 7.95 (d, 1H, $J = 8.80$ Hz, H_{arom}), 7.84 (d, 1H, $J = 9.54$ Hz, $H_{quinoline}$), 7.60−7.54 (m, 2H, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 153.26 (s), 145.59 (s), 132.38 (d), 131.09 (d), 129.43 (d), 127.93 (d), 126.83 (s), 125.20 (d), 123.87 (d), 120.52 (d), 119.68 (d), 119.22 (s), 118.97 (s), 115.52 (d), 112.40 (s), 109.52 (s); MS (*m*/*z*) 244 (M^{+1}); Anal. ($C_{16}H_9N_3$) C, H, N.

General Method for Preparation 2-Amidino-Substituted Benzimidazo[1,2-*a*]quinolines (32−35). A stirred suspension of **31** in absolute ethanol was cooled in an ice−salt bath and was saturated with HCl gas. The flask was then tightly stoppered and the mixture was maintained at room temperature for 3 days until the nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The reaction mixture was purged with N_2 gas and diluted with diethylether (50 mL). The crude imidate was filtered off and was immediately suspended in absolute ethanol (10 mL). The corresponding amine was added, and the mixture was stirred for 1−3 days at room temperature or reflux. The crude product was then filtered off and washed with diethylether.

***N*-Isopropyl-benzimidazo[1,2-*a*]quinoline-2-carboxamidine Hydrochloride (32).** Compound **32** was prepared using the general method described for the preparation of **32**−**35**; from 0.25 g (1.00 mmol) **31** and 0.25 mL (3.07 mmol) of *iso*-propylamine to give 0.16 g (46%) of green powder; mp 261−263 °C; IR (KBr, υ/cm^{-1}) 3400, 3344, 3011, 1670, 1640, 1618, 1543; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.92 (bs, 1H, $NH_{amidine}$), 9.80 (bs, 1H, $NH_{amidine}$), 9.36 (bs, 1H, $NH_{amidine}$), 8.98 (s, 1H, H_{arom}), 8.81 (d, 1H, $J = 9.00$ Hz, H_{arom}), 8.28 (d, 1H, $J = 8.22$ Hz, H_{arom}), 8.07 (d, 1H, $J = 9.57$ Hz, $H_{quinoline}$), 8.00 (d, 1H, $J = 9.0$ Hz, H_{arom}), 7.89 (d, 1H, $J = 8.60$ Hz, H_{arom}), 7.84 (d, 1H, $J = 9.51$ Hz, $H_{quinoline}$), 7.64−7.59 (m, 2H, H_{arom}), 4.20−4.16 (m, 1H, $CH(CH_3)_2$), 1.38 (d, 6H, $J = 6.39$ Hz, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, δ/ppm) 161.90 (s), 147.89 (s), 144.74 (s), 134.67 (s), 131.23 (d), 130.67 (s), 130.43 (s), 130.37 (d), 126.57 (s), 125.31 (d), 124.60 (d), 123.63 (d), 120.57 (d), 120.46 (d), 116.11 (d), 115.66 (d), 45.87 (d), 21.77 (d, 2C); MS (*m*/*z*) 303 (M^{+1} (− HCl)); Anal. ($C_{19}H_{19}N_4Cl$) C, H, N.

2-Benzimidazo[1,2-*a*]quinolin-2-yl-4,5-dihydro-3*H*-imidazol-1-yl Hydrochloride (33). Compound **33** was prepared using the general method described for the preparation of **32**−**35**; from 0.25 g (1.00 mmol) **31** and 0.24 mL (3.50 mmol) of ethylenediamine to give 0.07 g (21%) of yellow powder; mp 225−227 °C; IR (KBr, υ/cm^{-1}) 3434, 3188, 1667, 1621, 1597, 1539; ^{1}H NMR (DMSO-d_6, δ/ppm) 10.46 (bs, 2H, $NH_{amidine}$), 8.96 (s, 1H, H_{arom}), 8.86 (d, 1H, $J = 8.65$ Hz, H_{arom}), 8.26 (d, 1H, $J = 8.25$ Hz, H_{arom}), 8.02 (d, 1H, $J = 9.36$ Hz, $H_{quinoline}$), 8.03−7.95 (m, 2H, H_{arom}), 7.82 (d, 1H, $J = 9.45$ Hz, $H_{quinoline}$), 7.60 (d, 1H, $J = 7.62$ Hz, H_{arom}), 7.58 (d, 1H, $J = 7.47$ Hz, H_{arom}), 4.10 (bs, 2H, CH_2), 3.06 (bs, 2H, CH_2); ^{13}C NMR (DMSO-d_6, δ/ppm) 164.97 (s), 147.80 (s), 144.50 (s), 134.90 (s), 131.24 (d), 131.02 (d), 130.51 (s), 127.65 (s), 127.65 (s), 125.53 (d), 124.22 (d), 123.87 (d), 123.260 (s), 121.03 (d), 120.52 (d), 115.61 (d), 115.56 (d), 45.11 (t), 36.91 (t); MS (*m*/*z*) 287 (M^{+1} (− HCl)); Anal. ($C_{18}H_{15}N_4Cl$) C, H, N.

Benzimidazo[1,2-*a*]quinoline-2-carboxamidine Hydrochloride (34). Compound **34** was prepared using the general method described for the preparation of **32**−**35**; from 0.25 g (1.00 mmol) **31** and $NH_{3(g)}$ to give 0.11 g (36%) of light yellow powder; mp 276−278 °C; IR (KBr, υ/cm^{-1}) 3130, 1691, 1628, 1517; ^{1}H NMR (DMSO-d_6, δ/ppm) 9.20 (bs, 2H, $NH_{amidine}$), 9.16 (bs, 2H, $NH_{amidine}$), 8.93 (s, 1H, H_{arom}), 8.72 (d, 1H, $J = 8.94$ Hz, H_{arom}), 8.19 (d, 1H, $J = 8.10$ Hz, H_{arom}), 7.97 (d, 1H, $J = 9.48$ Hz, $H_{quinoline}$), 7.90 (d, 1H, $J = 8.24$ Hz, H_{arom}), 7.87 (d, 1H, $J = 8.76$ Hz, H_{arom}), 7.84 (d, 1H, $J = 9.42$ Hz, $H_{quinoline}$), 7.53−7.50 (m, 2H, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 164.86 (s), 147.41 (s), 144.27 (s), 134.38 (s), 130.69 (d), 130.21 (s), 130.14 (s), 129.99 (d), 126.27 (s), 124.75 (d), 123.52 (d), 123.07 (d), 120.05 (d), 119.97 (d), 115.13 (d), 115.00 (d); MS (*m*/*z*) 261 (M^{+1} (− HCl)); Anal. ($C_{16}H_{13}N_4Cl$) C, H, N.

***N*-Morpholin-4-yl-benzimidazo[1,2-*a*]quinoline-2-carboxamidine Hydrochloride (35).** Compound **35** was prepared using the general method described for the preparation of **32**−**35**; from 0.25 g (1,00 mmol) **31** and 0.27 mL (2.80 mmol) of 4-*N*-aminomorpholine to give 0.14 g (37%) of light green powder; mp 268−270 °C; IR (KBr, υ/cm^{-1}) 3421, 2866, 1670, 1632, 1619, 1525; ^{1}H NMR (DMSO-d_6, δ/ppm) 11.61 (bs, 1H, $NH_{amidine}$), 9.90 (bs, 1H, $NH_{amidine}$), 9.23 (bs, 1H, $NH_{amidine}$), 9.03 (s, 1H, H_{arom}), 8.85 (d, 1H, $J = 8.92$ Hz, H_{arom}), 8.30 (d, 1H, $J = 8.22$ Hz, H_{arom}), 8.06 (d, 1H, $J = 9.60$ Hz, $H_{quinoline}$), 7.97 (m, 1H, H_{arom}), 7.90 (d, 1H, $J = 8.16$ Hz, H_{arom}), 7.84 (d, 1H, $J = 9.51$ Hz, $H_{quinoline}$), 7.62−7.58 (m, 2H, H_{arom}); ^{13}C NMR (DMSO-d_6, δ/ppm) 162.03 (s), 147.85 (s), 144.70 (s), 134.72 (s), 131.22 (d), 130.64 (s), 130.49 (d), 127.67 (s), 126.99 (s), 125.37 (d), 124.52 (d), 123.63 (d), 120.71 (d), 120.59 (d), 116.27 (d), 115.69 (d); MS (*m*/*z*) 346 (M^{+1} (− HCl)); Anal. ($C_{20}H_{20}N_5OCl$) C, H, N.

Interactions with DNA. The electronic absorption spectra were recorded on Varian Cary 100 Bio spectrometer, CD spectra were recorded on Jasco J815, and fluorescence emission spectra were recorded on Varian Eclipse fluorimeter in all cases using quartz cuvettes (1 cm).

The calf thymus DNA (ct-DNA) was purchased from Aldrich, dissolved in the sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}, pH = 7.0, additionally sonicated and filtered through a 0.45 μm filter, and the concentration of the corresponding solution determined spectroscopically as the concentration of phosphates.[45] The measurements were performed in the aqueous buffer solution (pH = 7.0; sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}). Under the experimental conditions used, the absorbance and fluorescence intensities of studied compounds were proportional to their concentrations, while none of studied compounds showed CD spectrum. In fluorimetric titrations, an excitation wavelength of $\lambda = 355$ nm was used to avoid inner filter effects caused by absorption of excitation light by added polynucleotide and changes of fluorescence emission were monitored at 400 nm. Spectroscopic titrations were performed by adding portions of polynucleotide solution into the solution of the studied compound.

The stability constant (K_s) and [bound compound]/[polynucleotide phosphate] ratio (n) were calculated according to the Scatchard equation by nonlinear least-square fitting,[36] giving excellent correlation coefficients (>0.999) for obtained values for K_s and n.

Thermal melting curves for ct-DNA and its complexes with studied compounds were determined as previously described by following the absorption change at 260 nm as a function of temperature.[45b] The absorbance of the ligand was subtracted from every curve, and the absorbance scale was normalized. Obtained T_m values are the midpoints of the transition curves, determined from the maximum of the first derivative or graphically by a tangent method. Given ΔT_m values were calculated subtracting T_m of the free nucleic acid from T_m of complex. Every ΔT_m value here reported was the average of at least two measurements, the error in ΔT_m is ± 0.5 °C.

Antitumor Activity Assays. Antiproliferative Activity. The HeLa (cervical carcinoma), Hep-2 (laryngeal carcinoma), MCF-7 (breast carcinoma), SW620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), and H460 (lung carcinoma) cells obtained from American Type Culture Collection (ATCC, Rockville, MD) were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The growth inhibition activity was assessed as described previously, according to the slightly modified procedure of the National Cancer Institute, Developmental Therapeutics Program.[39−41,46] The cells were inoculated onto standard 96-well microtiter plates on day 0. The cell concentrations were adjusted according to the cell population doubling time (PDT): 1×10^4/mL for HeLa, Hep-2, H460, MiaPaCa-2, and SW620 cell lines (PDT = 20−24 h) and 2×10^4/mL for MCF-7 cell lines (PDT = 33 h). Test agents were then added in five 10-fold dilutions (10^{-8} to 10^{-4} mol/L) and incubated for an additional 72 h. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The absorbance (OD, optical density) was measured on a microplate reader at 570 nm. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} − mean OD_{tzero}) ≥ 0, then PG = 100 × (mean OD_{test} − mean OD_{tzero})/(mean OD_{ctrl} − mean OD_{tzero}).

If (mean OD_{test} − mean OD_{tzero}) < 0, then PG = 100 × (mean OD_{test} − mean OD_{tzero})/OD_{tzero}, where mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound; mean OD_{test} = the average of optical density measurements after the desired period; and mean OD_{ctrl} = the average of optical density measurements after the desired period with no exposure of cells to the test compound.

Each test was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from concentration−response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e., 50%). If, however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g., PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each result is a mean value from three separate experiments.

Cell Cycle Analysis. Cells (2×10^5) were seeded per well in a 6-well plate. After overnight incubation, tested compounds were added. After the desired length of time, the attached cells were trypsinized, combined with floating cells, washed with phosphate-buffered saline (PBS), and fixed with 70% ethanol. Immediately before the analysis, cells were washed with PBS and incubated with 0.1 μg/μl RNAse A at 37 °C for 15 min. Subsequently, cells were stained with 1 μg/mL of propidium iodide (PI) and analyzed by Becton Dickinson FACScalibur flow cytometer. For each analysis, 20 000 events were measured and obtained results were processed using WinMDI 2.8 (The Scripps Institute, U.S.A.) and Cylchred (Cardiff University, U.K.). Measurements were performed in duplicate for two independent experiments.

In Vitro Topoisomerase II Inhibitor Screening. A topoisomerase II drug screening kit (TopoGEN, U.S.A.) was used to check selected compounds for their inhibitory effect on topo II, whereby two possible modes of inhibition can be detected: stabilization of cleavage complexes that prevents religation of the broken DNA strands and catalytic inhibition (topo II antagonists). For evaluation of the cleavage complex formation, the reaction mixture contained 2 μL 10× cleavage buffer, 1 μL pRYG DNA substrate (supercoiled), 2 μL of 1 mM test compound or control inhibitor (etoposide), 8 units of purified topoisomerase II, and mQ H_2O (variable volume, up to 20 μL). The mixture was incubated at 37 °C for 30 min, and the reaction was terminated by the addition of 2 μL of 10% SDS, followed by proteinase K (50 μg/mL) digestion at 37 °C for 15 min. After extraction with a mixture of chloroform and isoamyl alcohol (24:1), samples were loaded onto 1% agarose gel containing 0.5 μg/mL ethidium bromide along with the marker DNAs (linear and supercoiled pRYG DNAs). Gels were run at 40 V constant voltage in horizontal electrophoresis system (BIO-RAD, U.S.A.). Formation of cleavage products (linear and open circular DNA) was monitored under the UV light at 254 nm (Image Master VDS, Pharmacia Biotech, Sweden). For establishing the effect of test compounds on the topo II relaxation activity, we used the same reaction system and conditions as described above, except that the cleavage buffer was replaced with 10× assay buffer and that ethidium bromide was omitted from the gel. Instead, gels were stained with ethidium bromide (0,5 μg/mL) for 30 min once electrophoresis was completed. Monitoring of the supercoiled DNA conversion into relaxed DNA forms was possible in the assay reaction.

Acknowledgment. Support for this study by the Ministry of Science, Education and Sport of Croatia is gratefully acknowledged (Projects 125-0982464-1356, 098-0982464-2514, 98-0982914-2918, 098-0982464-2393).

Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM070876H