Bioorganic & Medicinal Chemistry 19 (2011) 6329–6339

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel biologically active nitro and amino substituted benzimidazo[1,2-*a*]quinolines

Nataša Perin [a], Lidija Uzelac [b], Ivo Piantanida [c], Grace Karminski-Zamola [a], Marijeta Kralj [b,*], Marijana Hranjec [a,*]

[a] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev Trg 20, PO Box 177, HR-10000 Zagreb, Croatia
[b] Division of Molecular Medicine, Laboratory of Experimental Therapy, Ruđer Bošković Institute, Bijenička cesta 54, PO Box 180, HR-10000 Zagreb, Croatia
[c] Division of Organic Chemistry and Biochemistry, Laboratory for Study of Interactions of Biomacromolecules, Ruđer Bošković Institute, Bijenička Cesta 54, PO Box 180, HR-10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 13 July 2011
Revised 1 September 2011
Accepted 2 September 2011
Available online 10 September 2011

Keywords:
Benzimidazoles
Benzimidazo[1,2-*a*]quinolines
Antiproliferative activity
Interaction with ct-DNA
Cytometry analysis

ABSTRACT

This manuscript described the synthesis and biological activity of novel nitro substituted *E*-2-styryl-benzimidazoles and *E*-2-(2-benzimidazolyl)-3-phenylacrylonitriles and nitro and amino substituted benzimidazo[1,2-*a*]quinolines (**4**–**5**, **6**–**11**, **17**–**20**, and **21**–**32**). All of the compounds showed significant growth inhibitory effect towards five tumor cell lines, whereby the IC_{50} concentrations of **11**, **20**, **28**, **29**, **30**, **32** are in the low micromolar range (IC_{50} = 2–19 μM). The DNA binding experiments did not show significant affinity of two selected compounds towards ct-DNA. The flow cytometry analysis of potential cell cycle perturbations after the treatment with compounds **9**, **11**, **25**, and **29** demonstrated that all of the compounds (5 μM ≈ IC_{50}) significantly delayed the progression through G1 phase, as demonstrated by the accumulation of cells in G1 phase, accompanied with the reduction of the cell number in the cells in S phase, which does not point to DNA damage as the main mechanism of action. Also, fluorescence microscopy study showed cytoplasmic distribution of the compounds, demonstrating that DNA is not the primary target of compounds. Thus, considerable antiproliferative effects of studied compounds are due to interactions with other biological targets within cells.

1. Introduction

Since classical chemotherapy, which uses small molecules or bioactive natural products, is still the mainstay of cancer treatment, there has been a tremendous growth in recent years in the number and types of new heterocyclic anticancer agents with an emphasis on creating new DNA active drugs. DNA molecule is one of the principal targets for anticancer agents designed to block the proliferation of cancer cells, since DNA synthesis and replication are important processes for cellular growth.[1] Understanding of the molecular basis for cytotoxicity by anticancer agents is very important for the development of novel, more selective and efficient agents with greater specificity of action.[2–4] The benzimidazole unit which has a structural similarity with purine, is also the key building block for a variety of derivatives that are known to play crucial roles in the functions of a number of biologically important molecules. Besides, benzimidazoles represent the major backbone of numerous of synthetic medicinal and biochemical agents possessing different chemical and pharmacological features which impart them diverse biological properties like anticancer,[5–7] antiviral,[8,9] antibacterial,[10,11] antifungal,[12] antihistaminic[13] and anticonvulsant activity.[14]

As a part of our continuing search for potential anticancer agents related to benzimidazole derivatives, we have recently reported on the synthesis, cytostatic evaluation, DNA/RNA interaction study and proteomic profiling of a series of amidino-substituted heterocyclic benzimidazoles and benzimidazo[1,2-*a*]quinolines.[15,16] Biological study confirmed the anticancer potential of this class of compounds, especially that of positively charged analogs of benzimidazo[1,2-*a*]quinolines which intercalate into ds-DNA or RNA. Thus, the most promising 2-imidazolinyl amidino substituted compounds inhibited tumor growth, caused severe disturbance of the cell cycle, impairment in mitotic progression, and inhibited topoisomerases, which was related to their high DNA binding capacity. Recent publications also showed that nitro substituted benzazolo[3,2-*a*]quinolinium derivatives are potential antineoplastic agents that interacts with DNA by intercalation and that topoisomerase II is an important target for their biological action.[17,18] It was also concluded that the cytotoxic properties of these compounds appeared to be more dependent on their ability to inhibit topoisomerase II than on DNA binding affinity or intercalation.[19] Substituted, earlier

* Corresponding authors. Tel.: +385 14597245; fax: +385 14597250 (M.H.); tel.: +385 1 4571 235; fax: +385 1 4561 010 (M.K.).
E-mail addresses: mhorvat@irb.hr, marijeta.kralj@irb.hr (M. Kralj), mhranjec@fkit.hr (M. Hranjec).

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doi:10.1016/j.bmc.2011.09.002

prepared benzimidazo[1,2-*a*]quinoline-6-carbonitriles exerted pronounced antiproliferative activity with the cyano moiety important for the activity but not the selectivity of tested compounds.

Some of above mentioned compounds also showed significant interaction with ct-DNA, supporting the fact that their antitumor activity could partially be the consequence of DNA-binding.[20]

Prompted by the results from the previously reported studies, we set out to explore and synthesize novel acyclic nitro **4**, **5**, **17**–**20** and cyclic nitro **6**, **7**, and **21**–**24** and amino **8**–**9** and **25**–**28** substituted benzimidazole[1,2-*a*]quinolines with the substituents on the different positions of the condensed tetracyclic ring. The hydrochloride salts **10**–**11** and **29**–**32** were prepared to improve their solubility in water (Fig. 1). Newly synthesized compounds were tested for their antiproliferative activity on the panel of several human tumor cell lines while study of interaction with *calf thymus* DNA (ct-DNA) was performed for compounds **11** and **25**.

2. Results and discussion

2.1. Chemistry

All newly prepared compounds (Fig. 1) were synthesized according to the two main procedures shown in Scheme 1, by the conventional methods of organic synthesis for the preparation of similar heterocyclic compounds starting from the corresponding *o*-phenylenediamines **1**–**2**. In the cyclocondensation reaction between the *E*-3-(2-chloro-5-nitrophenyl)acrylic acid **3** with *o*-phenylenediamines **1**–**2** in polyphosphoric acid (PPA), *E*-2 -styryl-benzimidazoles **4**–**5** were prepared. In the reaction between *o*-phenylenediamines **1**–**2** with 2-cyanoacetamide corresponding substituted 2-cyanomethylbenzimidazoles **12–14** were prepared. They gave in the reaction of aldol condensation with aromatic aldehydes **15**–**16** substituted *E*-2-(2-benzimidazolyl)-3-phenylacrylonitriles **17**–**20**.

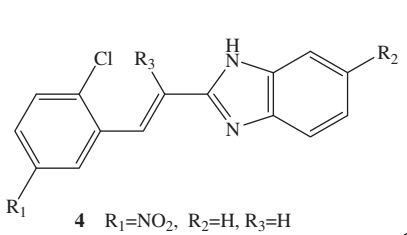
Cyclic nitro substituted derivatives of benzimidazo[1,2-*a*]quinolines **6**–**7** and benzimidazo[1,2-*a*]quinolines-6-carbonitriles **21**–**24** were prepared by the thermal reaction using sulfolane for dehydrohalogenation cyclization at 280 °C followed by UV/Vis spectroscopy.[21] Acyclic derivatives **5** and **18**–**20** with substituents on the benzimidazole nuclei gave in the cyclization reaction two inseparable regioisomers. Amino substituted cyclic derivatives **8**–**9** and **25**–**28** were prepared from nitro substituted compounds by reduction with $SnCl_2 \times 2H_2O$ in MeOH and concentrated HCl. The hydrochloride salts of amino substituted benzimidazo [1,2-*a*]quinolines **10**–**11** and **29**–**32** were prepared with HCl gas in order to ensure better solubility.

All structures of novel *E*-2-styryl-benzimidazoles **4**–**5**, *E*-2 -(2-benzimidazolyl)-3-phenylacrylonitriles **17**–**20**, benzimidazo[1,2-*a*]quinolines **6**–**11** and benzimidazo[1,2-*a*]quinolines-6-carbonitriles **21**–**32** were determined by the NMR analysis based on the analysis of H–H coupling constants as well as chemical shifts. In ^{1}H NMR spectra of *E*-2-styryl-benzimidazoles **4**–**5** besides all other aromatic protons, two doublets for *trans*-ethylenic protons with coupling constants of 16.5 Hz can be observed. Singlet of the NH group of benzimidazole nuclei is placed around 13 ppm. The photocyclization reaction leads to a downfield shift of the signals of the ethylenic protons and most other aromatic protons as well as disappearance of the NH group at the benzimidazole nuclei, thus confirming the cyclic structure formation. In ^{1}H NMR spectra doublets for two protons on quinoline ring, with coupling constants 9.5 Hz, can be observed and these values are characteristic for this type of fused compounds.

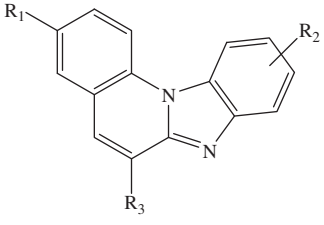
2.2. Interaction of compounds 11 and 25 with ct-DNA in an aqueous medium

2.2.1. UV/Vis, fluorimetric and CD titrations

Since application of spectrophotometric methods is necessary for DNA binding studies of compounds **11** and **25**, their aqueous buffered solutions were characterized by means of electronic absorption (UV/Vis) and fluorescence emission spectroscopy (Table 1). Stock solution of compound **11** was prepared in water (c(**11**) = 1.05×10^{-3} mol dm^{-3}) while stock solution of compound **25** was prepared, due to the poor solubility in water, in DMSO (c(**25**) = 1.68×10^{-3} mol dm^{-3}). Small aliquots of DMSO stock solution of compound **25** were added into the aqueous medium, provided that DMSO content in experiments was less than 1%. Stock solutions were stable over prolonged periods of time. Absorbencies of aqueous buffered solutions are proportional to their concentrations up to 1.52×10^{-5} mol dm^{-3} (**11**) and 2.5×10^{-5} mol dm^{-3} (**25**) indicating that there is no significant intermolecular stacking which should give rise to hypochromic effects. Studied compounds **11** and **25** exhibited strong fluorescence emission with maximum at 521 nm and 562 nm. Fluorescence emissions of **11** and **25** were proportional to their concentration in the range from 4×10^{-8} mol dm^{-3} to 2×10^{-6} mol dm^{-3}, and their excitation spectra were in concordance with the corresponding absorption spectra. Absorption maxima, corresponding molar extinction coefficients (ε) and fluorescence emission maxima are presented in Table 1.



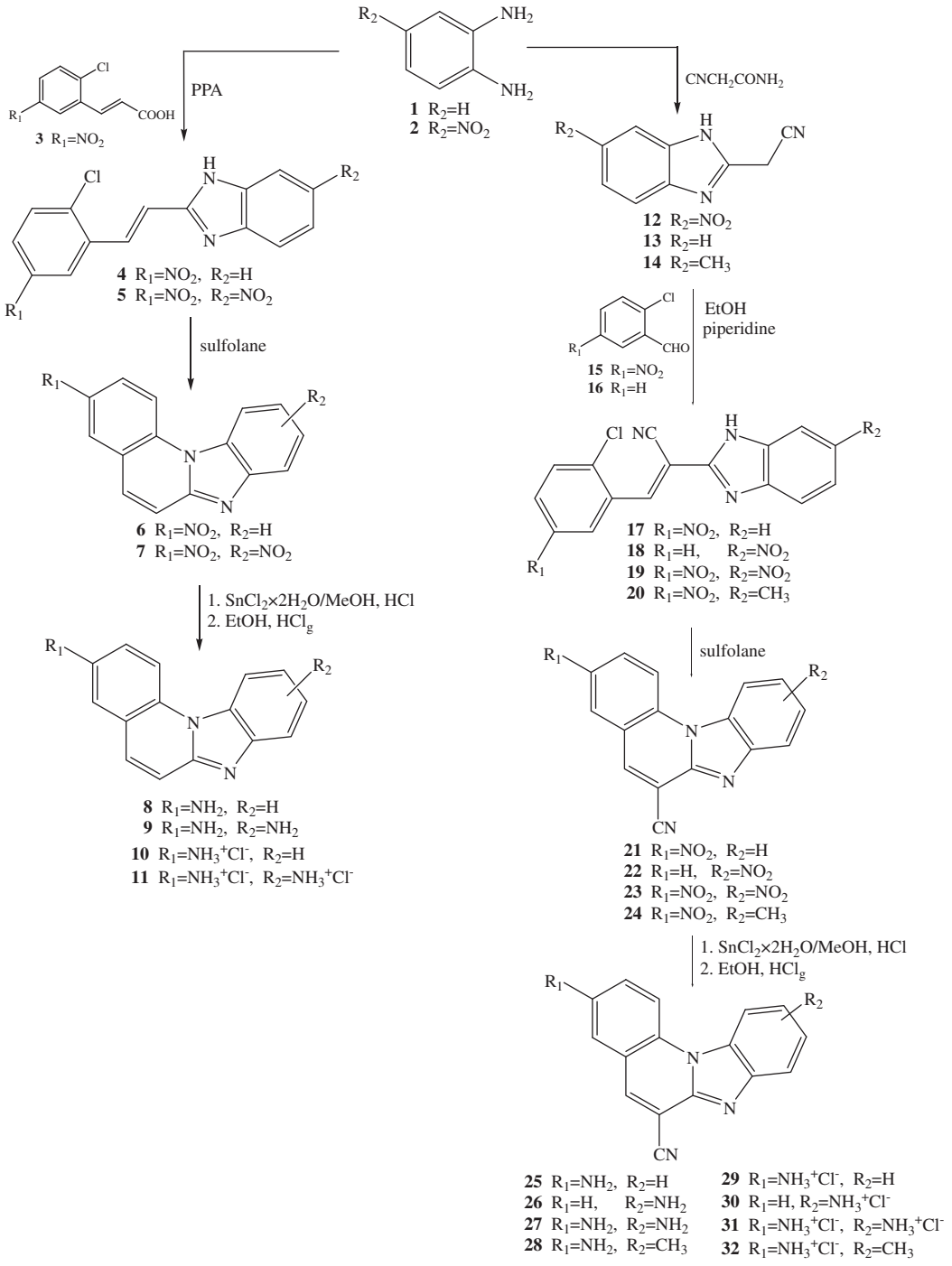
4 R_1=NO_2, R_2=H, R_3=H
5 R_1=NO_2, R_2=NO_2, R_3=H
17 R_1=NO_2, R_2=H, R_3=CN
18 R_1=H, R_2=NO_2, R_3=CN
19 R_1=NO_2, R_2=NO_2, R_3=CN
20 R_1=NO_2, R_2=CH_3, R_3=CN



6 R_1=NO_2, R_2=H, R_3=H
7 R_1=NO_2, R_2=NO_2, R_3=H
8 R_1=NH_2, R_2=H, R_3=H
9 R_1=NH_2, R_2=NH_2, R_3=H
10 R_1=$NH_3^+Cl^-$, R_2=H, R_3=H
11 R_1=$NH_3^+Cl^-$, R_2=$NH_3^+Cl^-$, R_3=H
21 R_1=NO_2, R_2=H, R_3=CN
22 R_1=H, R_2=NO_2, R_3=CN
23 R_1=NO_2, R_2=NO_2, R_3=CN

24 R_1=NO_2, R_2=CH_3, R_3=CN
25 R_1=NH_2, R_2=H, R_3=CN
26 R_1=H, R_2=NH_2, R_3=CN
27 R_1=NH_2, R_2=NH_2, R_3=CN
28 R_1=NH_2, R_2=CH_3, R_3=CN
29 R_1=$NH_3^+Cl^-$, R_2=H, R_3=CN
30 R_1=H, R_2=$NH_3^+Cl^-$, R_3=CN
31 R_1=$NH_3^+Cl^-$, R_2=$NH_3^+Cl^-$, R_3=CN
32 R_1=$NH_3^+Cl^-$, R_2=CH_3, R_3=CN

Figure 1. Prepared *E*-2-styryl-benzimidazoles **4**–**5**, *E*-2-(2-benzimidazolyl)-3-phenylacrylonitriles **17**–**20** and benzimidazo[1,2-*a*]quinolines **6**–**11** and **21**–**32**.



Scheme 1.

Table 1
Electronic absorption maxima, molar extinction coefficients and fluorescence emission maxima of studied compounds in sodium cacodylate buffer (I = 0.05 mol dm^{-3}, pH 7)

Compd	Absorption maxima		Fluorescence emission (λ_{max}/nm)
	λ_{max}/nm	$\varepsilon \times 10^3$/dm^3 mol^{-1} cm^{-1}	
11	353	23.14	521
	261	20.93	
	251	23.13	
25	347	10.18	562
	273	37.81	

Addition of ct-DNA to a buffered solution of compounds **11** and **25** resulted in bathochromic and hypochromic effects of UV/Vis spectra of studied compounds (Fig. 2). as follows: H (**11**, 352 nm) = 44%, $\Delta\lambda$ (**11**, 352 nm) = 20 nm and H (**25**, 347 nm) = 28%, $\Delta\lambda$ (**25**, 347 nm) = 13 nm, H (**25**, 362 nm) = 17%, $\Delta\lambda$ (**25**, 362 nm) = 14 nm (H (hypochromic effect) = (Abs (compound) − Abs (complex))/Abs (compound)) × 100; values Abs (complex) estimated by non-linear extrapolation; $\Delta\lambda$ (shift of the absorbance maximum) = λ (complex) − λ (compound)).

Addition of ct-DNA to a buffered compounds solution strongly quenched fluorescence intensity of **11** while enhanced fluorescence intensity of **25** (Fig. 3).

Affinity of compounds toward ct-DNA and density of binding was calculated by processing of UV/Vis and fluorescence titration data according to Scatchard equation (Table 2).[22]

Since CD spectroscopy is a highly sensitive method toward conformational changes in the secondary structure of polynucleotides, we have chosen it to get insight into the changes of polynucleotide properties induced by small molecule binding.[23,24] Studied compounds are achiral and therefore do not possess intrinsic CD spectrum. The addition of studied compounds **11** and **25** to the ct-DNA resulted in small increase of CD band at 275 nm, which suggested small changes in the helical structure of DNA upon binding (Supplementary data, Fig. S1). The absence of the measurable induced (ICD) band at >300 nm pointed toward non-homogenous orientation of bound molecules of **11** and **25** in respect to DNA helical axis.[24]

2.2.2. Thermal denaturation experiments and viscometry

In the thermal denaturation experiments addition of compound **11** did not yield any measurable effect on the T_m value of ct-DNA while addition of **25** yielded weak but measurable stabilization of the ct-DNA double helix (Table 3).

Viscometry experiments (Supplementary data) yielded values of α(**11**) = 0.88 and α(**25**) = 0.40, former value differing from the value obtained for ethidium bromide (α(**EB**) = 0.90) by the error of the method ($\alpha \pm 0.1$). Obtained results support intercalation of only charged derivative **11**, while its neutral analog **25** most likely agglomerates along DNA double helix.

2.2.3. Discussion of interaction of compounds 11 and 25 with ct-DNA

Viscometry results showed that compound **11** ($\alpha = 0.88 \pm 0.1$) elongated ct-DNA similarly to referent intercalator (EB, $\alpha = 0.90 \pm 0.1$), while addition of **25** yielded only negligible DNA elongation ($\alpha = 0.4 \pm 0.1$) accompanied by strongly non-linear effect of the viscometry increase. Obtained results support intercalation of only charged derivative **11**, while its neutral analog **25** most likely agglomerates along DNA double helix. However, **11** does not show any impact on ct-DNA thermal denaturation ($\Delta T_m = 0$), which is not common for intercalators. Possible explanation is that bulky amino substituents hamper complete insertion of the large condensed aromatic moiety between DNA basepairs, whereby partial intercalation does elongate DNA similar to EB (viscometry) but yields very weak ICD effect and binding constant ($\log K_s = 5.1$) an order of magnitude lower than one could expect from the aromatic surface (for instance under the same experimental conditions fluorimetric titration of EB with ct-DNA gave $\log K_s = 6$).

2.3. Biological results and discussion

All of the compounds showed significant growth inhibitory effect towards five tumor cell lines, four of which were derived from solid tumors and one leukemia cell line (Table 4). Compounds **4**, **5**, **23**, **24** exerted moderate, while **11**, **20**, **28**, **29**, **30**, and **32** strong antiproliferative activity (in the low micromolar range). There is in general no significant difference in sensitivity between cell lines, except compound **4**, which showed interesting selectivity towards

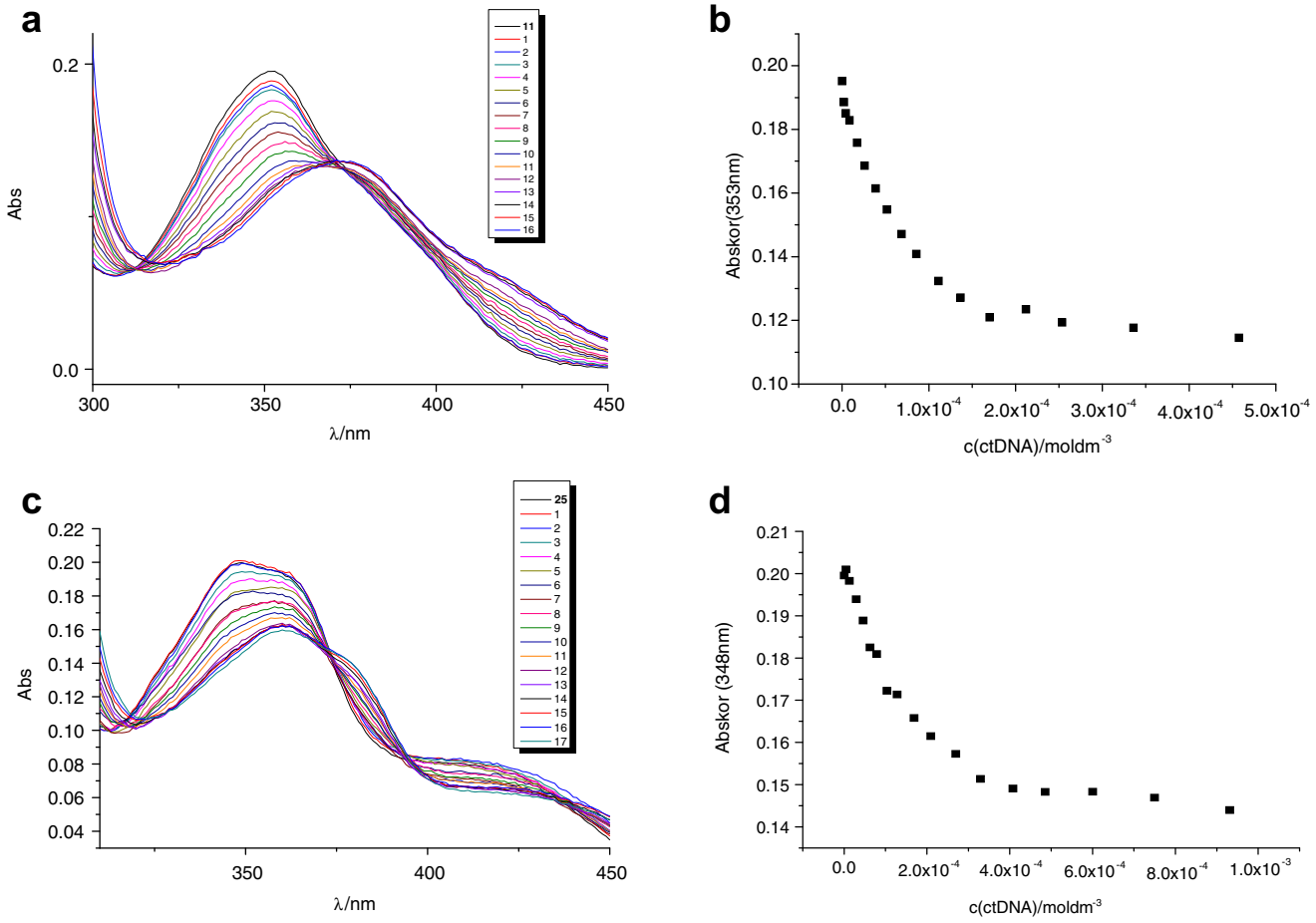


Figure 2. (a) The UV/Vis titration of **11** ($c = 2.0 \times 10^{-5}$ mol dm^{-3}) with ct-DNA; b) spectroscopic changes at $\lambda_{max} = 353$ nm as a function of ct-DNA concentration; c) The UV/Vis titration of **25** ($c = 1.9 \times 10^{-5}$ mol dm^{-3}) with ct-DNA; d) spectroscopic changes at $\lambda_{max} = 348$ nm as a function of ct-DNA concentration.

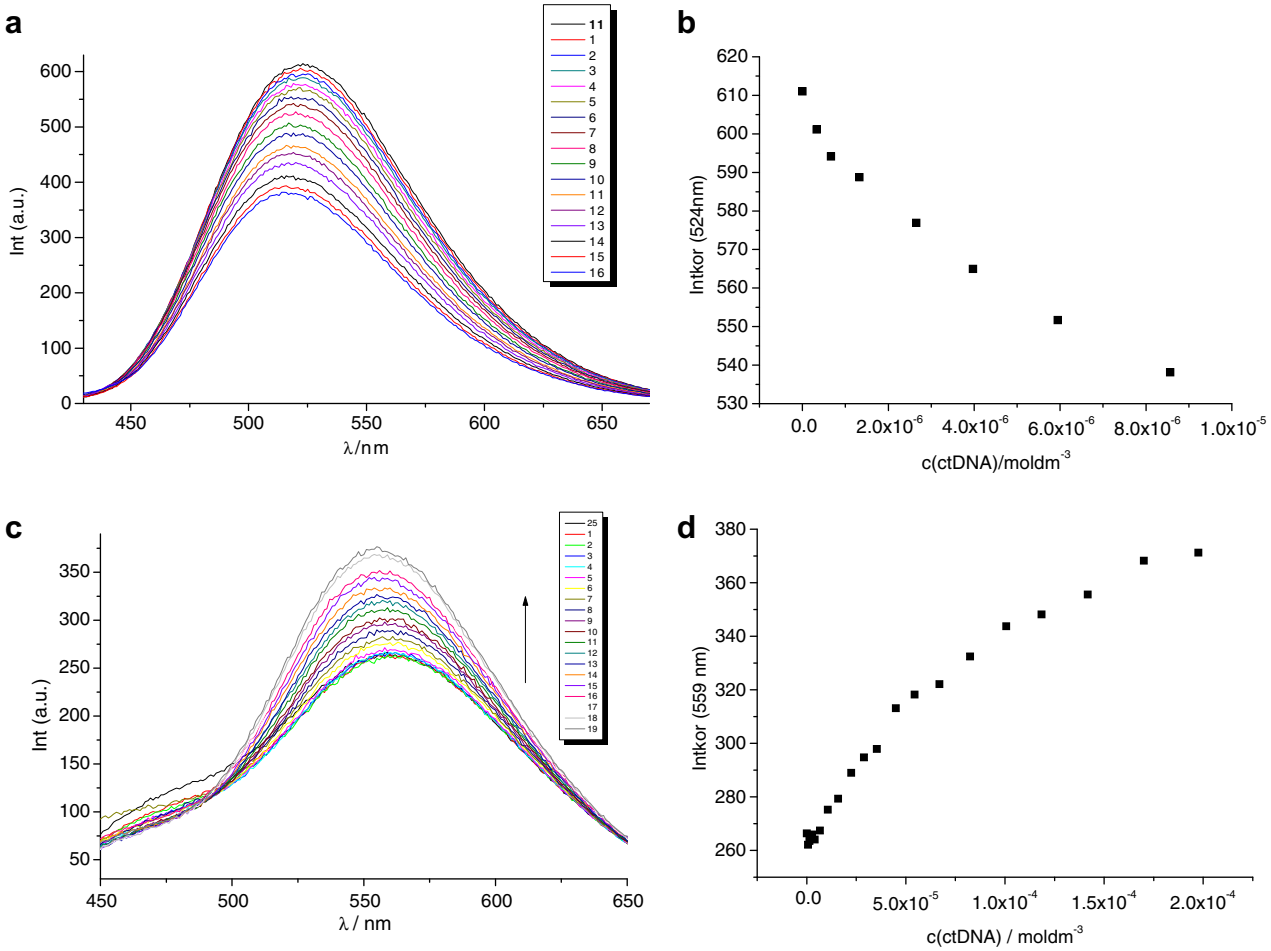


Figure 3. a) Changes in fluorescence spectrum of **11** ($c = 3.3 \times 10^{-7}$ mol dm^{-3}) upon titration with ct-DNA ($c = 1.00 \times 10^{-3}$ mol dm^{-3}); b) Dependence of **11** emission at $\lambda = 524$ nm on c(ct-DNA); c) Changes in fluorescence spectrum of **25** ($c = 1.12 \times 10^{-6}$ mol dm^{-3}) upon titration with ct-DNA ($c = 2.00 \times 10^{-3}$ mol dm^{-3}); d) Dependence of **25** emission at $\lambda = 559$ nm on c(ct-DNA); All experiments were carried out at pH 7.0, (buffer sodium cacodylate $I = 0.05$ mol dm^{-3}).

Table 2
Binding constants (log K_s)[a] calculated from the UV/Vis and fluorimetric titrations with ct-DNA at pH 7 (buffer sodium cacodylate, $I = 0.05$ mol dm^{-3})

Compound	Fluorescence log K_s	UV/Vis log K_s
11	4.8	4.6
25	5.5	5.1

[a] Processing of titration data by Scatchard equation gave Scatchard ratios $n_{[\text{bound compound}]/[\text{polynucleotide}]} = 0.1–0.2$, for better comparison binding constants were re-calculated for fixed ratio $n_{[\text{bound compound}]/[\text{polynucleotide}]} = 0.2$.

Table 3
ΔT_m values[a] (°C) of ct-DNA upon addition of different ratios r[b] of **11** and **25** at pH 7.0 (buffer sodium cacodylate, $I = 0.05$ mol dm^{-3})

r[b]	**11**	**25**
0.1	0	0.9
0.2	0	1.9
0.3	0	0.2

[a] Error in ΔT_m: ± 0.5 °C.
[b] r = [compound]/[polynucleotide].

HCT 116 cells, while being modestly active towards other cell lines). It should be noted though, that compounds **4**–**6**, **20**, **23**–**25**, **29**, and **30** precipitated at the maximal tested concentration, which might hamper their full effect. Since there is in general no significant difference in the IC_{50} concentrations between the activity of tested compounds, and the DNA binding experiments did not show significant affinity of two selected compounds towards ds-DNA (diamino-substituted derivative **11** as dihydrochloride salt and amino–cyano substituted derivative **25**), we attempted the flow cytometry analysis of potential cell cycle perturbations after the treatment with diamino-substituted derivative **9** and its dihydrochloride analog **11** and their mono-substituted analogs (**25** and **29**, respectively) (Table 5). The results demonstrated that all of the compounds (5 μM ≈ IC_{50}) significantly delayed the progression through G1 phase, as demonstrated by the accumulation of cells in G1 phase, accompanied with the reduction of the cell number in the cells in S phase, 24 h after the addition of the compounds. Even more drastic S phase reduction was obtained after the treatment with higher concentration (10 μM, data not shown). This effect diminished after 48 h; predominantly in the cells treated with **25** and **29**, while the most pronounced effect was obtained with the charged dihydrochloride derivative **11**. These results again suggest that the DNA is not the main target of the tested compounds, although some affinity of the charged compounds towards DNA was demonstrated. Namely, contrary to here-presented results, intercalators induce strong G2/M phase delay/arrest, as also demonstrated in our previously-published studies.[15,16] We therefore performed fluorescence microscopy study with the compounds **11** and **29** to check the intracellular distribution of the compounds in the tumor cells. The results confirm that

Table 4
IC_{50} values (in μM)[a,b]

Compound	Cell lines				
	MOLT-4	HCT 116	SW 620	MCF-7	H 460
4	>100	4 ± 0.2	28 ± 2	32 ± 19	>100
5	5 ± 5	21 ± 13	16 ± 2	19 ± 2	13 ± 1
6	3 ± 1	6 ± 3	6 ± 1	4 ± 1	8 ± 0.2
9	11 ± 2	4 ± 3	3 ± 2	8 ± 0.3	3 ± 1
11	5 ± 1	4 ± 3	3 ± 1	4 ± 2	3 ± 1
18	6 ± 5	16 ± 4	14 ± 1	10 ± 0.2	17 ± 0.3
19	3 ± 1	5 ± 0.2	20 ± 1	19 ± 9	15 ± 3
20	5 ± 2	3 ± 1	4 ± 3	3 ± 2	19 ± 5
22	24 ± 22	5 ± 1	7 ± 1	8 ± 2	3 ± 1
23	4 ± 2	4 ± 2	38 ± 14	10 ± 0.2	11 ± 1
24	43 ± 25	6 ± 3	7 ± 4	⩾100	5 ± 0.1
25[c]	6±3	4 ± 1	4 ± 1	4 ± 3	3 ± 0.1
26[c]	⩾10	4 ± 2	5 ± 3	⩾10	4 ± 0.03
27	11 ± 0.4	6 ± 1	9 ± 2	3 ± 1	5 ± 2
28	17 ± 12	11 ± 3	10 ± 6	11 ± 0.2	9 ± 2
29	2 ± 0.1	3 ± 0.04	6 ± 2	4 ± 2	2 ± 0.2
30	3 ± 2	4 ± 1	4 ± 0.1	4 ± 2	2 ± 0.3
31	5 ± 4	5 ± 3	4 ± 2	3 ± 2	4 ± 3
32	8 ± 5	4 ± 3	4 ± 1	6 ± 3	3 ± 0.1

[a] IC_{50}: the concentration that causes 50% growth inhibition.
[b] Compounds were dissolved in DMSO to a stock solutions concentration of $1–4 \times 10^{-2}$ M. However, the precipitation in the cell culture medium at 10^{-4} M after 72 h was observed for compounds **4**–**6**, **20**, **23**–**25**, **29** and **30**.
[c] The highest tested concentration was 10 μM.

Table 5
The effects of **25**, **29**, **9**, and **11** at **5** μM on the cell cycle distribution of HCT 116 cells after the 24- and 48-hours treatments. The numbers represent the percentages of cells in respective cell cycle phase (G1, S, and G2/M), along with the percentage of cells in the subG1 (dead cells) obtained by flow cytometry

Treatment		Percentage of cells (%)			
		SubG0/G1	G0/G1	S	G2/M
24 h	Control	3	40	39	21
	25	6	62	20	18
	29	4	55	20	25
	9	1	49	33	18
	11	1	65	14	21
48 h	Control	1	40	35	25
	25	3	47	25	28
	29	4	45	31	24
	9	2	54	25	21
	11	2	60	23	17

the compounds are not localized in the nucleus, but are distributed throughout the cytoplasm both after 2 and 24 h (Fig. 4 and data not shown). Certain differences, however, can be observed between the localization of the two compounds; compound **29** is localized more distinctively near nucleus, pointing to possible colocalization with endoplasmatic reticulum, while compound **11** is evenly distributed, forming small cytoplasmic aggregates, which could suggest their localization in the lysosomes, or endosomes.

3. Conclusions

In this work we have presented the synthesis of novel acyclic nitro substituted *E*-2-styryl-benzimidazoles **4**–**5**, *E*-2-(2-benzimidazolyl)-3-phenylacrylonitriles **17**–**20** and cyclic substituted benzimidazole[1,2-*a*]quinolines **6**–**11** and **21**–**32**. Detailed studies of two chosen compounds (**11** and **25**) revealed that they only weakly interact with ct-DNA, whereby **11** partially intercalates and **25** just agglomerates along DNA double helix. Accordingly, biological studies (impact on the cell cycle and fluorescence microscopy) also showed that DNA is not the primary target of compounds. Thus, considerable antiproliferative effects of studied compounds are due to interactions with other biological targets within cells.

4. Experimental

4.1. Chemistry

4.1.1. General methods

All chemicals and solvents were purchased from commercial suppliers. Melting points were recorded on an Original Keller Mikroheiztisch apparatus (Reichert, Wien) and Büchi 535. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or Varian Gemini 600 NMR spectrometers at 300, 600, 150 and 75 MHz, respectively. All NMR spectra were measured in DMSO-d_6 solutions using TMS as an internal standard. Chemical shifts are reported in ppm (δ) relative to TMS. IR spectra were recorded on FTIR-ATR and Perkin-Elmer Spectrum 1 spectrophotometers. 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. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates.

4.1.2. General method for preparation of compounds 4–5

A mixtures of equimolar amounts of corresponding 3-(2-chloro-5-nitrophenyl)acrylic acid **3** and 1,2-phenylenediamines **1–2** together with PPA (10 g) were heated at 180 °C for 3 h. The cooled mixture was poured into ice-water (200 ml) and the resulting solution was treated with 25% $NH_3 \times H_2O$ to pH 9. The suspension was filtered off and the crude product was washed with water to pH 7 and recrystallized from ethanol to obtain *E*-2-styryl-benzimidazoles **4**–**5**.

4.1.2.1. *E*-{2-[2-(2-Chloro-5-nitrophenyl)-ethenyl]}benzimidazole 4. Compound **4** was prepared using above described method, from 3-(2-chloro-5-nitrophenyl)acrylic acid **3** (1.00 g, 4.40 mmol) and 1,2-phenylenediamine **1** (0.48 g, 4.40 mmol) to obtain a light brown powder (0.67 g, 51%); mp 133–136 °C; IR (diamond) ν/cm^{-1}: 3074, 1570, 1520; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.85 (br s, $NH_{arom.}$, 1H), 8.70 (d, J = 2.7 Hz, $H_{arom.}$, 1H), 8.18 (dd, J_1 = 2.7 Hz, J_2 = 8.8 Hz, 1H), 7.97 (d, J = 16.4 Hz, $H_{arom.}$, 1H), 7.85 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 7.66 (d, J = 7.8 Hz, $H_{arom.}$, 1H), 7.55 (br s, $H_{arom.}$, 1H), 7.52 (d, J = 16.3 Hz, $H_{arom.}$, 1H), 7.22 (d, J = 8.3 Hz, $H_{arom.}$, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 150.23 (s), 147.42 (s), 139.29 (s), 135.61 (s, 2C), 131.80 (d, 2C), 127.71 (d, 2C), 124.54 (d), 123.94 (d, 2C), 122.20 (d, 2C); elemental analysis calcd (%) for $C_{15}H_{10}ClN_3O_2$: C, 60.11; H, 3.36; N, 14.02; found: C, 60.33; H, 3.50; N, 14.09.

4.1.2.2. *E*-{2-[2-(2-Chloro-5-nitrophenyl)-ethenyl]}5(6)-nitrobenzimidazole 5. Compound **5** was prepared using above described method, from 3-(2-chloro-5-nitrophenyl)acrylic acid **3** (1.00 g, 4.40 mmol) and 4-nitro-1,2-phenylenediamine **2** (0.67 g, 4.40 mmol) to obtain a brown powder (0.42 g, 27%); mp >300 °C; IR (diamond) ν/cm^{-1}: 3358, 1613, 1520, 1338; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.74 (d, J = 2.6 Hz, $H_{arom.}$, 1H), 8.50 (d, J = 2.1 Hz, $H_{arom.}$, 1H), 8.21 (dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, $H_{arom.}$, 1H), 8.13 (dd, J_1 = 2.3 Hz, J_2 = 9.0 Hz, $H_{arom.}$, 1H), 8.08 (d, J = 16.5 Hz, $H_{arom.}$, 1H), 7.86 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 7.77 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 7.60 (d, J = 16.3 Hz, $H_{arom.}$, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 154.75 (s), 149.41 (s), 147.47 (s), 147.27 (s), 143.18 (s), 139.60 (s), 135.00 (s), 131.80 (d), 130.67 (d), 124.95 (d), 122.93 (d), 122.84 (d), 122.34 (d), 122.17 (d), 118.57 (d); elemental analysis calcd (%) for $C_{15}H_9ClN_4O_4$: C, 52.26; H, 2.63; N, 16.25; found: C, 52.44; H, 2.89; N, 16.43.

4.1.3. General method for preparation of compounds 17–20

Solution of equimolar amounts of 2-cyanomethylbenzimidazole derivatives **12–14**, corresponding heteroaromatic aldehydes and

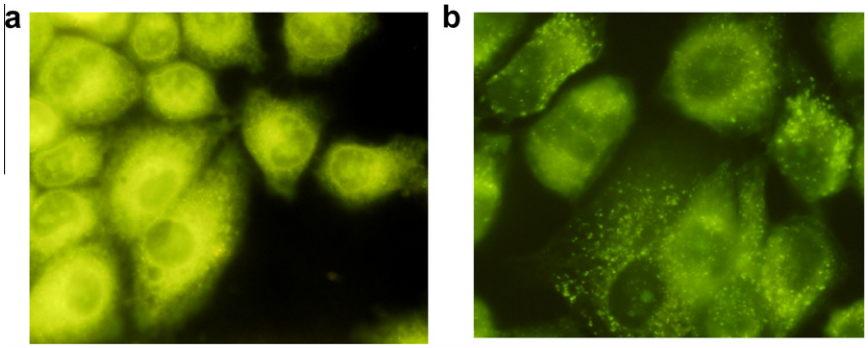


Figure 4. Fluorescent microscopy images of H 460 cells treated with compound **29** a) 100 μM for 2 h; and compound **11** b) 100 μM for 2 h; both showing predominately cytoplasmic distribution of the compounds.

few drops of piperidine in absolute ethanol, were refluxed for 2–3 h. After reaction mixture was cooled to the room temperature, the crude product was filtered off and recrystallized from ethanol.

4.1.3.1. 2-(2-Benzimidazolyl)-3-(2-chloro-5-nitrophenyl)acrylonitrile 17. Compound **17** was prepared from **13** (0.69 g, 4.4 mmol) and 2-chloro-5-nitro-benzaldehyde **15** (0.81 g, 4.4 mmol) in absolute ethanol (10 ml) after refluxing for 2.5 h and recrystallization from ethanol to yield 1.16 g (66%) of brown crystals; mp >300 °C; IR (diamond) ν/cm^{-1}: 3271, 2235, 1603, 1514; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.41 (s, 1H, NH$_{benzim.}$), 8.99 (d, 1H, J = 2.7 Hz), 8.50 (s, 1H, H$_{arom.}$), 8.38 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.9 Hz), 7.98 (d, 1H, J = 8.9 Hz, H$_{arom.}$), 7.76 (d, 1H, J = 7.6 Hz, H$_{benzim.}$), 7.60 (d, 1H, J = 7.8 Hz, H$_{benzim.}$), 7.35–7.27 (m, 2H, H$_{benzim.}$); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 147.20 (s), 147.17 (s), 146.93 (s), 141.15 (s), 139.59 (d), 133.25 (s), 132.33 (d, 2C), 132.17 (s), 132.09 (d), 127.37 (d, 2C), 125.12 (d, 2C), 115.68 (s), 109.72 (s); elemental analysis calcd (%) for $C_{16}H_9ClN_4O_2$: C, 59.18; H, 2.79; N, 17.25; found: C, 59.33; H, 2.93; N, 17.45.

4.1.3.2. 2-[2-(5(6)-Nitro)benzimidazolyl)-3-(2-chlorophenyl) acrylonitrile 18. Compound **18** was prepared from **12** (2.00 g, 9.9 mmol) and 2-chlorobenzaldehyde **16** (1.40 g, 9.9 mmol) in absolute ethanol (10 ml) after refluxing for 2 h and recrystallization from ethanol to yield 2.52 g (78%) of brown crystal; mp 209–213 °C; IR (diamond) ν/cm^{-1}: 3228, 2239, 1508, 1336, 1054; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.95 (br s, NH$_{arom.}$, 1H), 8.64 (s, H$_{arom.}$, 1H), 8.56 (s, H$_{arom.}$, 1H), 8.19 (dd, J_1 = 8.9 Hz, J_2 = 2.24 Hz, H$_{arom.}$, 1H), 7.96 (d, J = 8.6 Hz, H$_{arom.}$, 1H), 7.83 (d, J = 8.9 Hz, H$_{arom.}$, 1H), 7.71 (dd, J_1 = 7.7 Hz, J_2 = 2.2 Hz, H$_{arom.}$, 1H), 7.64–7.56 (m, H$_{arom.}$, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 155.75 (d), 151.48 (s), 144.04 (d), 143.78 (s), 136.38 (s), 134.56 (s), 133.66 (d), 133.10 (s), 131.31 (s), 130.62 (d), 130.26 (d), 128.31 (d), 119.31 (d), 115.53 (s), 113.70 (d), 106.35 (s); elemental analysis calcd (%) for $C_{16}H_9ClN_4O_2$: C, 59.18; H, 2.79; N, 17.25; found: C, 59.34; H, 2.96; N, 17.01.

4.1.3.3. 2-[2-(5(6)-Nitro)benzimidazolyl)-3-(2-chloro-5-nitrophenyl)acrylonitrile 19. Compound **19** was prepared from **12** (0.92 g, 4.9 mmol) and 2-chloro-*5*-nitro-benzaldehyde **15** (1.00 g, 4.9 mmol) in absolute ethanol (10 ml) after refluxing for 2 h and recrystallization from ethanol to yield 0.30 g (22%) of brown powder.; mp >300 °C; IR (diamond) ν/cm^{-1}: 2230, 1606, 1527, 1341; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.01 (d, J = 2.6 Hz, H$_{arom.}$, 1H), 8.62 (s, H$_{arom.}$, 1H), 8.57 (d, J = 2.1 Hz, H$_{arom.}$, 1H), 8,42 (dd, J_1 = 2.7 Hz, J_2 = 8.9 Hz, H$_{arom.}$, 1H), 8.18 (dd, J_1 = 2.2 Hz, J_2 = 8.9 Hz, H$_{arom.}$, 1H), 8.00 (d, J = 8.9 Hz, H$_{arom.}$, 1H), 7.83 (d, J = 8.9 Hz, H$_{arom.}$, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 151.38 (s), 146.80 (s), 141.17 (d), 143.70 (s), 143.12 (s), 139.39 (s), 132.53 (s), 132.10 (d), 127.39 (d), 124.74 (d), 119.26 (d), 115.63 (d), 115.13 (s), 113.61 (d), 108.80 (s); elemental analysis calcd (%) for $C_{16}H_8ClN_5O_4$: C, 51.98; H, 2.18; N, 18.94; found: C, 52.28; H, 2.22; N, 19.06.

4.1.3.4. 2-[2-(5(6)-Methyl)benzimidazolyl)-3-(2-chloro-5-nitrophenyl)acrylonitrile 20. Compound **20** was prepared from **14** (0.50 g, 2.9 mmol) and 2-chloro-5-nitro-benzaldehyde (0.54 g, 2.9 mmol) in absolute ethanol (20 ml) after refluxing for 1 h and recrystallization from ethanol to yield 0.39 g (40%) of yellow powder; mp 200–203 °C; IR (diamond) ν/cm^{-1}: 2225, 1633, 1524, 1345; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.95 (br s, NH, 1H), 8.96 (d, J = 2.6 Hz, H$_{arom.}$, 1H), 8.45 (s, H$_{arom.}$, 1H), 8.37 (dd, J_1 = 2.6 Hz, J_2 = 8.9 Hz, H$_{arom.}$, 1H), 7.98 (d, J = 8.9 Hz, H$_{arom.}$, 1H), 7.55 (d, J = 8.3 Hz, H$_{arom.}$, 1H), 7.44 (s, H$_{arom.}$, 1H), 7.12 (d, J = 8.4 Hz, H$_{arom.}$, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 146.84 (s), 140.80 (s), 138.64 (d), 134.60 (s), 132.99 (s), 131.99 (d), 131.97 (s), 126.95 (d), 124.94 (d), 124.77 (d), 119.65 (d), 115.41 (s), 112.98 (s), 111.86 (d), 109.47 (s), 21.97 (d); elemental analysis calcd (%) for $C_{17}H_{11}ClN_4O_2$: C, 60.28; H, 3.27; N, 16.54; found: C, 60.44; H, 3.56; N, 16.77.

4.1.4. General method for preparation of compounds 6–7 and 21–24

Compounds **4–5** and **17–20** were dissolved in 1–4 ml of sulfolane and reaction mixture was heated for 0.5–1.5 h at 280 °C. The cooled mixture was poured into water (25 ml) and the resulting product was filtered off and recrystallizated from ethanol to obtain benzimidazo[1,2-*a*]quinolines **4–5** and **17–20**.

4.1.4.1. 3-Nitro-benzimidazo[1,2-*a*]quinoline 6. Compound **6** was prepared using above described method from compound **4** (0.50 g, 1.16 mmol) in sulfolane (3 ml) after 1.5 h to obtain a brown powder (0.42 g, 95%); mp 276–278 °C; IR (diamond) ν/cm^{-1}: 1609, 1545, 1506; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.05 (d, J = 2.6 Hz, H$_{arom.}$, 1H), 9.01 (d, J = 9.3 Hz, H$_{arom.}$, 1H), 8.73 (d, J = 8.6 Hz, H$_{arom.}$, 1H), 8.56 (dd, J_1 = 2.7 Hz, J_2 = 9.2 Hz, H$_{arom.}$, 1H), 8.19 (d, J = 9.5 Hz, H$_{arom.}$, 1H), 7.99 (dd, J_1 = 1.7 Hz, J_2 = 7.1 Hz, H$_{arom.}$, 1H), 7.83 (d, J = 9.5 Hz, H$_{kinol.}$, 1H), 7.62 (dt, J_1 = 1.8 Hz, J_2 = 7.4 Hz, H$_{arom.}$, 1H), 7.59 (dt, J_1 = 1.5 Hz, J_2 = 7.5 Hz, H$_{arom.}$, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 148.14 (s), 144.87 (s), 143.45 (s), 138.86 (s), 131.69 (d), 130.67 (s), 125.68 (d), 125.59 (d), 124.79 (d), 124.00 (d), 123.64 (s), 120.76 (d), 119.91 (d), 117.29 (d), 115.20 (d); elemental analysis calcd (%) for $C_{15}H_9N_3O_2$: C, 68.44; H, 3.45; N, 15.96; found: C, 68.66; H, 3.70; N, 16.23.

4.1.4.2. 3,8(9)-Dinitro-benzimidazo[1,2-*a*]quinoline7. Compound **7** was prepared using above described method from compound **5** (0.35 g, 1.02 mmol) in sulfolane (1 ml) after 45 min to obtain a brown powder (0.05 g, 15%); mp >300 °C; IR (diamond) ν/cm^{-1}: 3065, 1614,

1513,1335; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.42 (s, $H_{arom.}$, 1H), 9.07 (dd, J_1 = 2.4 Hz, J_2 = 8.3 Hz, $H_{arom.}$, 2H), 8.98 (dd, J_1 = 3.0 Hz, J_2 = 9.6 Hz, $H_{arom.}$, 2H), 8.89 (d, J = 9.2 Hz, $H_{arom.}$, 1H), 8.74 (s, $H_{arom.}$, 1H), 8.64 (dd, J_1 = 2.5 Hz, J_2 = 9.3 Hz, $H_{arom.}$, 1H), 8.55 (dd, J_1 = 2.3 Hz, J_2 = 8.9 Hz, $H_{arom.}$, 1H), 8.45 (dd, J_1 = 1.6 Hz, J_2 = 9.1 Hz, $H_{arom.}$, 1H), 8.35 (d, J = 9.8 Hz, $H_{arom.}$, 2H), 8.26 (d, J = 9.7 Hz, $H_{arom.}$, 1H), 8.10 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 7.90 (d, J = 9.9 Hz, $H_{arom.}$, 1H), 7.86 (d, J = 9.8 Hz, $H_{arom.}$, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): not enough soluble; elemental analysis calcd (%) for $C_{15}H_8N_4O_4$: C, 58.45; H, 2.62; N, 18.18; found: C, 58.67; H, 2.89; N, 18.43.

4.1.4.3. 3-Nitro-6-cyano-benzimidazo[1,2-*a*]quinoline 21. Compound **21** was prepared using above described method from compound **17** (0.50 g, 1.54 mmol) in sulfolane (2.5 ml) after 1.5 h to obtain a brown powder (0.43 g, 97%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2239, 1510, 1334; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.08 (d, J = 2.7 Hz, $H_{arom.}$, 1H), 8.98 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.96 (s, $H_{arom.}$, 1H), 8.72 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.63 (dd, J_1 = 2.7 Hz, J_2 = 9.0 Hz, $H_{arom.}$, 1H), 8.08–8.05 (m, $H_{arom.}$, 1H), 7.67–7.64 (m, $H_{arom.}$, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 144.52 (s), 143.79 (s), 143.23 (s), 140.04 (d), 139.09 (s), 130.41 (s), 127.12 (d), 126.47 (d), 125.90 (d), 124.48 (d), 121.34 (s), 120.69 (d), 117.23 (d), 114.85 (d), 114.82 (s), 103.42 (s); elemental analysis calcd (%) for $C_{16}H_8N_4O_2$: C, 66.67; H, 2.80; N, 19.44; found: C, 66.89; H, 2.94; N, 19.67.

4.1.4.4. 8(9)-Nitro-6-cyano-benzimidazo[1,2-*a*]quinoline 22. Compound **22** was prepared using above described method from compound **18** (1.50 g, 4.62 mmol) in sulfolane (4 ml) after 1 h to obtain a brown powder (0.28 g, 31%); mp 240–245 °C; IR (diamond) ν/cm^{-1}: 3060, 2233, 1517, 1338; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.97 (s, $NH_{arom.}$, 1H), 8.93 (s, $H_{arom.}$, 1H), 8.93 (s, $H_{arom.}$, 1H), 8.85 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.84 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.81 (d, J = 2.3 Hz, $H_{arom.}$, 1H), 8.34 (dd, J_1 = 9.2 Hz, J_2 = 2.3 Hz, $H_{arom.}$, 1H), 8.18 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.17 (dd, J_1 = 7.8 Hz, J_2 = 1.3 Hz, $H_{arom.}$, 2H), 8.04 (td, J_1 = 7.4 Hz, J_2 = 1.4 Hz, $H_{arom.}$, 1H), 8.03 (td, J_1 = 7.5 Hz, J_2 = 1.4 Hz, $H_{arom.}$, 1H), 7.75–7.70 (m, $H_{arom.}$, 2H), 7.62–7.56 (m, $H_{arom.}$, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 147.36 (s), 144.39 (s), 143.13 (s), 142.49 (d), 137.05 (d), 134.23 (s), 134.01 (d), 132.16 (d), 132.02 (d), 131.49 (d), 130.86 (s), 130.81 (s), 130.46 (d), 130.13 (d), 129.75 (d), 127.82 (d), 127.58 (d), 126.84 (s), 126.07 (d), 121.45 (s), 117.93 (d), 116.11 (d), 115.64 (d), 115.51 (d), 114.88 (s), 101.05 (s); elemental analysis calcd (%) for $C_{16}H_8N_4O_2$: C, 66.67; H, 2.80; N, 19.44; found: C, 66.90; H, 2.88; N, 19.78.

4.1.4.5. 3,8(9)-Dinitro-6-cyano-benzimidazo[1,2-*a*]quinoline 23. Compound **23** was prepared using above described method from compound **19** (0.25 g, 0.68 mmol) in sulfolane (1 ml) after 45 min to obtain a brown powder (0.03 g, 15%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2250, 1611, 1519, 1441, 1346; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.42 (d, J = 1.8 Hz, $H_{arom.}$, 1H), 9.13–9.09 (m, $H_{arom.}$, 3H), 9.06 (s, $H_{arom.}$, 1H), 9.03 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 9.00 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 8.90 (d, J = 9.3 Hz, $H_{arom.}$, 1H), 8.86 (d, J = 2.3 Hz, $H_{arom.}$, 1H), 8.72 (dd, J_1 = 2.3 Hz, J_2 = 9.4 Hz, $H_{arom.}$, 1H), 8.65 (dd, J_1 = 2.60 Hz, J_2 = 9.3 Hz, $H_{arom.}$, 1H), 8.49 (dd, J_1 = 1.8 Hz, J_2 = 9.0 Hz, $H_{arom.}$, 1H), 8.40 (dd, J_1 = 2.3 Hz, J_2 = 9.2 Hz, $H_{arom.}$, 1H), 8.22 (d, J = 9.0 Hz, $H_{arom.}$, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 159.29 (s), 148.30 (s), 147.71 (s), 144.31 (s) 144.27 (s), 143.83 (s), 143.69 (s), 142.80 (d), 142.21 (d), 138.90 (s), 138.80 (s), 134.50 (s), 131.15 (s), 129.96 (s), 128.37 (d), 127.68 (d), 127.11 (d), 127.01 (d), 125.39 (s), 122.13 (s), 122.00 (s), 121.61 (d), 121.33 (d), 119.25 (d), 118.28 (d), 117.99 (d), 116.55 (d), 116.01 (d), 114.75 (s), 111.85 (d), 103.68 (s); elemental analysis calcd (%) for $C_{16}H_7N_5O_4$: C, 57.66; H, 2.12; N, 21.01; found: C, 57.86; H, 2.33; N, 21.28.

4.1.4.6. 3-Nitro-8(9)-methyl-6-cyano-benzimidazo[1,2-*a*]quinoline 24. Compound **24** was prepared using above described method from compound **20** (0.25 g, 0.74 mmol) in sulfolane (1 ml) after 50 min to obtain a green powder (0.13 g, 56%); mp 250–254 °C; IR (diamond) ν/cm^{-1}: 2236, 1611, 1521, 1387; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.90 (d, J = 2.4 Hz, $H_{arom.}$, 1H), 8.88 (d, J = 2.7 Hz, $H_{arom.}$, 1H), 8.74 (s, $H_{arom.}$, 1H), 8.69 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 8.86 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.66 (d, J = 8.3 Hz, $H_{arom.}$, 1H), 8.47–8.41 (m, $H_{arom.}$, 2H), 8.31 (d, J = 8.4 Hz, $H_{arom.}$, 1H), 8.24 (s, $H_{arom.}$, 1H), 7.78 (d, J = 8.3 Hz, $H_{arom.}$, 1H), 7.67 (d, J = 2.6 Hz, $H_{arom.}$, 1H), 7.36 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 7.30 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 2.56 (s, CH_3, 3H), 2.51 (s, CH_3, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 144.17 (s), 143.97 (s), 143.69 (s), 142.69 (s), 142.88 (s), 142.48 (s), 139.32 (d), 139.06 (d), 138.64 (s), 138.48 (s), 135.54 (s), 134.45 (s), 130.21 (s), 130.16 (s), 128.09 (s), 127.26 (d), 126.79 (d), 126.66 (d), 126.21 (d), 126.14 (d), 125.78 (d), 121.04 (s), 120.89 (s), 120.03 (d), 119.98 (d), 116.83 (d), 116.74 (d), 114.64 (s), 114.19 (d), 114.02 (d), 103.23 (s), 103.07 (s), 21.55 (d), 21.07 (d); elemental analysis calcd (%) for $C_{17}H_{10}N_4O_2$: C, 67.55; H, 3.33; N, 18.53; found: C, 67.89; H, 3.56; N, 18.83.

4.1.5. General method for the synthesis of amino-benzimidazo[1,2-*a*]quinoline derivatives 8–9 and 25–28

Corresponding nitro-benzimidazo[1,2-*a*]quinoline derivatives **6–7** and **21–24** and solution of $SnCl_2 \times 2H_2O$ in MeOH and concentrated HCl were refluxed for 0.5–1.5 h. After cooling, the reaction mixture was evaporated under vacuum and dissolved in water (50 ml). The resulting solution was treated with 20% NaOH to pH 14. Resulting product was filtered off and washed with water to obtain amino-benzimidazo[1,2-*a*]quinolines.

4.1.5.1. 3-Amino-benzimidazo[1,2-*a*]quinoline 8. Compound **8** was prepared using above described method from compound **6** (0.40 g, 1.52 mmol), $SnCl_2 \times 2H_2O$ (2.90 g, 12.62 mmol), HCl_{concd} (5.3 ml) and H_2O (5.3 ml) to obtain a green powder (0.25 g, 71%); mp 240–243 °C; IR (diamond) ν/cm^{-1}: 3192, 1612, 1562; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.59 (d, J = 7.5 Hz, $H_{arom.}$, 1H), 8.52 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 7.85 (d, J = 7.4 Hz, $H_{arom.}$, 1H), 7.73 (d, J = 9.5 Hz, $H_{arom.}$ 1H), 7.73 (d, J = 9.5 Hz, $H_{arom.}$, 1H), 7.47–7.40 (m, $H_{arom.}$, 2H), 7.13 (dd, J_1 = 2.5 Hz, J_2 = 8.9 Hz, $H_{arom.}$, 1H), 7.07(d, J = 2.5 Hz, $H_{arom.}$,1H), 5.45 (br s, NH_2, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 147.44 (s), 146.18 (s), 144.59 (s), 131.89 (d), 130.57 (s), 126.78 (s), 124.71 (s), 124.09 (d), 122.37 (d), 120.02 (d), 118.22 (d), 117.36 (d), 116.78 (d), 114.72 (d), 111.90 (d); elemental analysis calcd (%) for $C_{15}H_{11}N_3$: C, 72.56; H, 4.87; N, 22.57; found: C, 72.87; H, 4.98; N, 22.66.

4.1.5.2. 3,8(9)-Diamino-benzimidazo[1,2-*a*]quinoline 9. Compound **9** was prepared using above described method from compound **7** (0.14 g, 0.45 mmol), $SnCl_2 \times 2H_2O$ (1.70 g, 7.54 mmol), HCl_{concd} (3.1 ml) and MeOH (3.1 ml) to obtain a brown powder (0.03 g, 28%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2917, 1633, 1631, 1438; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.35 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.22 (d, Hz, J = 8.8 Hz, $H_{arom.}$, 2H), 7.68 (d, J = 1.5 Hz, $H_{arom.}$, 1H), 7.60 (d, J = 9.5 Hz, $H_{arom.}$, 1H), 7.53 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.49 (d, J = 9.6 Hz, $H_{arom.}$, 1H), 7.38 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.35 (d, J = 9.7 Hz, $H_{arom.}$, 1H), 7.11 (dd, J_1 = 2.7 Hz, J_2 = 9.1 Hz, $H_{arom.}$, 1H), 7.07 (dd, J_1 = 3.0 Hz, J_2 = 9.4 Hz, $H_{arom.}$, 1H), 7.03–7.00 (m, $H_{arom.}$, 2H), 6.91 (d, J = 2.0 Hz, $H_{arom.}$, 1H), 6.83 (dd, J_1 = 1.7 Hz, J_2 = 8.7 Hz, $H_{arom.}$, 1H), 6.77 (dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, $H_{arom.}$, 1H), 5.33 (br s, NH_2, 8H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 146.50 (s), 146.21 (s), 145.89 (s), 145.78 (s), 145.55 (s), 145.26 (s), 144.81 (s), 136.45 (s), 131.56 (s), 130.99 (d), 129.45 (d), 127.01 (s), 126.53 (s), 124.91 (s), 124.60 (s), 122–27 (s), 120.12 (d), 118.63 (d), 117.60 (d), 117.19 (d), 116.49 (d), 116.16 (d), 116.96 (d), 114.99 (d), 114.16 (d),

112.72 (d), 112.01 (d), 111.53 (d), 101.24 (d), 97.56 (d); elemental analysis calcd (%) for $C_{15}H_{12}N_4$: C, 77.23; H, 4.75; N, 18.01; found: C, 77.51; H, 4.98; N, 18.32.

4.1.5.3. 3-Amino-6-cyano-benzimidazo[1,2-*a*]quinoline 25. Compound **25** was prepared using above described method from compound **21** (0.20 g, 0.69 mmol), $SnCl_2 \times 2H_2O$ (1.30 g, 5.76 mmol), HCl_{concd} (2.0 ml) and MeOH (2.0 ml) to obtain a yellow powder (0.12 g, 67%); mp >300 °C; IR (diamond) ν/cm^{-1}: 3335, 2239, 1568; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.67 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 8.64 (s, $H_{arom.}$, 1H), 8.61 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 7.97 (d, J = 7.5 Hz, $H_{arom.}$, 1H), 7.59–7.49 (m, $H_{arom.}$, 2H), 7.31 (dd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, $H_{arom.}$, 1H), 7.16 (d, J = 2.6 Hz, $H_{arom.}$, 1H), 5.70 (ss, NH_2, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 146.32 (s), 143.78 (s), 143.59 (s), 140.42 (d), 130.25 (s), 127.06 (s), 124.51 (d), 122.94 (d), 122.56 (s), 121.11 (d), 120.00 (d), 116.70 (d), 115.72 (s), 114.54 (d), 100.67 (s); elemental analysis calcd (%) for $C_{16}H_{10}N_4$: C, 74.40; H, 3.90; N, 21.69; found: C, 74.64; H, 4.11; N, 21.76.

4.1.5.4. 8(9)-Amino-6-cyano-benzimidazo[1,2-*a*]quinoline 26. Compound **26** was prepared using above described method from compound **22** (0.25 g, 0.87 mmol), $SnCl_2 \times 2H_2O$ (1.63 g, 7.20 mmol), HCl_{concd} (3.0 ml) and MeOH (3.0 ml) to obtain a brown powder (0.21 g, 89%); mp 195–199 °C; IR (diamond) ν/cm^{-1}: 3211, 2229, 1504, 1448; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.72 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.69 (s, $H_{arom.}$, 1H), 8.57 (s, $H_{arom.}$, 1H), 8.54 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.39 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 8.10 (t, J = 7.8 Hz, $H_{arom.}$, 1H), 8.09 (t, J = 7.9 Hz, $H_{arom.}$, 1H), 7.96 (t, J = 8.4 Hz, $H_{arom.}$, 1H), 7.95 (t, J = 8.4 Hz, $H_{arom.}$, 1H), 7.94 (s, $H_{arom.}$, 1H), 7.70 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.62 (t, J = 7.5 Hz, $H_{arom.}$, 1H), 7.60 (t, J = 7.5 Hz, $H_{arom.}$, 1H), 7.03 (d, J = 2.0 Hz, $H_{arom.}$, 1H), 6.97 (dd, J_1 = 8.7 Hz, J_2 = 1.7 Hz, $H_{arom.}$, 1H), 6.92 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, $H_{arom.}$, 1H), 5.47 (br s, NH_2, 2H), 5.39 (br s, NH_2, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 150.67 (s), 147.62 (s), 147.20 (s), 146.26 (s), 144.57 (s), 139.14 (d), 138.14 (d), 138.10 (d), 136.52 (s), 136.19 (s), 135.82 (s), 133.81 (d), 133.16 (d), 132.26 (s), 131.46 (d), 131.38 (d), 125.14 (d), 124.96 (d), 123.07 (s), 121.91 (s), 121.34 (s), 121.10 (d), 116.20 (s), 116.09 (s), 115.94 (d), 115.48 (d), 115.38 (d), 113.96 (d), 102.08 (s), 101.92 (d), 101.05 (s), 97.30 (d); elemental analysis calcd (%) for $C_{16}H_{10}N_4$: C, 74.40; H, 3.90; N, 21.69; found: C, 74.58; H, 4.14; N, 21.92.

4.1.5.5. 3,8(9)-Diamino-6-cyano-benzimidazo[1,2-*a*]quinoline 27. Compound **27** was prepared using above described method from compound **23** (0.09 g, 0.27 mmol), $SnCl_2 \times 2H_2O$ (1.01 g, 4.49 mmol), HCl_{concd} (1.9 ml) and MeOH (1.9 ml) to obtain a brown powder (0.08 g, 81%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2236, 1633, 1564, 1446, 1360; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.46 (s, $H_{arom.}$, 1H), 8.44 (d, J = 9.1 Hz, $H_{arom.}$, 1H), 8.36 (s, $H_{arom.}$, 1H), 8.29 (dd, J_1 = 2.6 Hz, J_2 = 8.9 Hz, $H_{arom.}$, 2H), 7.70 (s, $H_{arom.}$, 1H), 7.63 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.26 (dd, J_1 = 2.5 Hz, J_2 = 9.2 Hz, $H_{arom.}$, 1H), 7.22 (dd, J_1 = 2.7 Hz, J_2 = 8.9 Hz, $H_{arom.}$, 1H), 7.11 (s, $H_{arom.}$, 1H), 7.10 (s, $H_{arom.}$, 1H), 6.98 (s, $H_{arom.}$, 1H), 6.91 (dd, J_1 = 1.6 Hz, J_2 = 8.8 Hz, $H_{arom.}$, 1H), 6.86 (dd, J_1 = 2.2 Hz, J_2 = 9.0 Hz, $H_{arom.}$, 1H), 5.56 (br s, NH_2, 4H), 5.29 (br s, NH_2, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 147.11 (s), 146.61 (s), 146.40 (s), 146.30 (s), 146.05 (s), 143.84 (s), 141.92 (s), 138.67 (d), 138.01 (d), 136.23 (s), 131.91 (s), 127.72 (s), 127.47 (s), 123.12 (s), 128.09 (s), 122.70 (s), 121.75 (d), 120.76 (d), 120.44 (d), 116.70 (d), 116.54 (s), 116.41 (s), 116.28 (d), 115.04 (d), 113.56 (d), 112.58 (d), 112.02 (d), 101.70 (d), 101.53 (s), 101.41 (d), 100.50 (s), 97.01 (d); elemental analysis calcd (%) for $C_{16}H_{101}N_5$: C, 70.32; H, 4.06; N, 25.63; found: C, 70.56; H, 4.32; N, 25.87.

4.1.5.6. 3-Amino-8(9)-methyl-6-cyano-benzimidazo[1,2-*a*]quinoline 28. Compound **28** was prepared using above described method from compound **24** (0.09 g, 0.30 mmol), $SnCl_2 \times 2H_2O$ (0.56 g, 2.49 mmol), HCl_{concd} (1.0 ml) and MeOH (1.0 ml) to obtain a green powder (0.07 g, 85%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2236, 1632, 1567, 1531, 1448; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.63 (s, $H_{arom.}$, 1H), 8.60 (s, $H_{arom.}$, 1H), 8.57 (d, J = 8.5 Hz, $H_{arom.}$, 1H), 8.52 (s, $H_{arom.}$, 1H), 8.48 (d, J = 8.5 Hz, $H_{arom.}$, 2H), 8.23 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 8.05 (s, $H_{arom.}$, 1H), 7.82 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 7.36 (s, $H_{arom.}$, 1H), 7.39–7.32 (m, $H_{arom.}$, 3H), 7.15 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 5.66 (s, NH_2, 2H), 5.55 (s, NH_2, 2H), 2.62 (s, CH_3, 3H), 2.53 (s, CH_3, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 163.69 (s), 147.12 (s), 146.27 (s), 146.03 (s), 144.02 (s), 143.03 (s), 140.75 (s), 139.85 (d), 134.53 (d), 134.40 (d), 133.90 (s), 132.84 (s), 132.84 (s), 132.42 (s), 130.47 (s), 128.02 (s), 127.06 (s), 126.13 (d), 125.94 (d), 124.51 (d), 124.18 (d), 123.09 (s), 122.89 (s), 121.21 (d), 120.08 (d), 119.95 (d), 119.82 (s), 119.55 (d), 119.03 (d), 116.78 (d), 116.28 (d), 115.82 (s), 21.56 (d), 21.13 (d); elemental analysis calcd (%) for $C_{17}H_{12}N_4$: C, 74.98; H, 4.44; N, 20.58; found: C, 75.32; H, 4.67; N, 20.83.

4.1.6. General method for the synthesis of amino-benzimidazo [1,2-*a*]quinoline hydrochlorides 10–11 and 29–32

A stirred suspension of compounds **8–9** and **25–28** in absolute ethanol (5–10 ml) was saturated with $HCl_{(g)}$. After 24 h of stirring small amount of diethylether was added, resulting product was filtered off and washed with diethylether to obtain amino-benzimidazo[1,2-*a*]quinoline hydrochlorides.

4.1.6.1. 3-Amino-benzimidazo[1,2-*a*]quinoline hydrochloride 10. Compound **10** was prepared using above described method from compound **8** (0.12 g, 0.52 mmol) to obtain a green powder (0.11 g, 80%); mp 285–288 °C; IR (diamond) ν/cm^{-1}: 3026, 2716, 2544, 2492, 1612, 1572; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.97 (d, J = 9.4 Hz, $H_{arom.}$, 1H), 8.92 (d, J = 9.8 Hz, $H_{arom.}$, 1H), 8.46 (d, J = 9.5 Hz, $H_{arom.}$, 1H), 8.04 (d, J = 8.0 Hz, $H_{arom.}$, 1H), 7.92 (d, J = 9.4 Hz, $H_{arom.}$, 1H), 7.83–7.72 (m, $H_{arom.}$, 2H), 7.58 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 7.56 (br s, $H_{arom.}$, 1H), 5.35 (br s, NH_3^+, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 113.18 (s), 138.48 (d), 132.88 (s), 128.85 (s), 128.80 (s), 128.11 (d), 125.68 (d), 125.56 (s), 124.38 (d), 124.36 (d), 124.34 (d), 118.85 (d), 116.84 (d), 115.34 (d), 111.91 (d); elemental analysis calcd (%) for $C_{15}H_{12}ClN_3$: C, 66.79; H, 4.48; N, 15.58; found: C, 66.92; H, 4.70; N, 15.81.

4.1.6.2. 3,8(9)-Diamino-benzimidazo[1,2-*a*]quinoline hydrochloride 11. Compound **11** was prepared using above described method from compound **9** (0.10 g, 0.33 mmol) to obtain a light brown powder (0.11 g, 86%); mp 270–273 °C; IR (diamond) ν/cm^{-1}: 2818, 2577, 1630, 1564, 1503; ^1H NMR (300 MHz, DMSO-d_6): δ = 9.00 (s, $H_{arom.}$, 1H), 8.91 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.82 (d, J = 9.1 Hz, $H_{arom.}$, 1H), 8.65 (d, J = 9.1 Hz, $H_{arom.}$, 1H), 8.42 (d, J = 9.5 Hz, $H_{arom.}$, 1H), 8.37 (d, J = 9.6 Hz, $H_{arom.}$, 2H), 8.01 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.87 (d, J = 9.3 Hz, $H_{arom.}$, 1H), 7.86 (s, $H_{arom.}$, 1H), 7.84 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.81 (d, J = 9.3 Hz, $H_{arom.}$, 1H), 7.78 (d, J = 1.7 Hz, $H_{arom.}$, 1H), 7.65 (d, J = 8.6 Hz, $H_{arom.}$, 1H), 7.64 (s, $H_{arom.}$, 1H), 7.42 (dd, J_1 = 1.7 Hz, J_2 = 8.6 Hz, $H_{arom.}$, 1H), 7.79 (br s, NH_3^+, 12H); ^{13}C NMR (150 MHz, DMSO-d_6): δ =144.17 (s), 143.44 (s), 136.48 (d), 136.40 (d), 134.98 (s), 129.73 (s), 129.70 (s), 129.63 (s), 129.60 (s), 129.58 (s), 128.48 (s), 125.02 (d), 124.97 (d), 124.80 (s), 124.71 (s), 124.67 (s), 124.56 (s), 124.26 (d), 121.39 (d), 119.77 (d), 119.73 (d), 118.07 (d), 117.73 (d), 117.03 (d), 116.85 (d), 116.62 (d), 112.94 (d), 112.32 (d), 108.84 (d); elemental analysis calcd (%) for $C_{15}H_{14}Cl_2N_4$: C, 56.09; H, 4.39; N, 17.44; found: C, 56.33; H, 4.52; N, 17.48.

4.1.6.3. 3-Amino-6-cyano-benzimidazo[1,2-*a*]quinoline hydrochloride 29. Compound **29** was prepared using above described method from compound **25** (0.10 g, 0.39 mmol) to obtain a yellow powder (0.05 g, 44%); mp >288 °C; IR (diamond) ν/cm^{-1}: 3321, 3142, 3045, 2812, 2582, 2050, 1664, 1579; ^1H NMR (300 MHz, DMSO-d_6): δ = 9.16 (s, $H_{arom.}$, 1H), 9.02 (d, J = 8.6 Hz, $H_{arom.}$, 1H), 8.99 (s, $H_{arom.}$, 1H), 8.91 (d, J = 8.4 Hz, $H_{arom.}$, 1H), 8.12 (d, J = 7.7 Hz, $H_{arom.}$, 1H), 7.76 (d, J = 7.6 Hz, $H_{arom.}$, 1H), 7.66–7.57 (m, $H_{arom.}$, 2H), 5.50 (s, NH_3^+, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.67 (s), 163.19 (s), 147.59 (s), 142.17 (d), 139.81 (s), 132.61 (s), 129.12 (s), 128.44 (d), 128.24 (s), 126.99 (d), 126.02 (d), 124.61 (d), 118.68 (d), 118.11 (d), 116.09 (d), 112.97 (s); elemental analysis calcd (%) for $C_{16}H_{11}ClN_4$: C, 65.20; H, 3.76; N, 19.01; found: C, 65.43; H, 3.94; N, 19.32.

4.1.6.4. 8(9)-Amino-6-cyano-benzimidazo[1,2-*a*]quinoline hydrochloride 30. Compound **30** was prepared using above described method from compound **26** (0.05 g, 0.19 mmol) to obtain a light brown powder (0.05 g, 99%); mp 271–275 °C; IR (diamond) ν/cm^{-1}: 2786, 2564, 1976, 1675, 1502; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.96 (s, $H_{arom.}$, 1H), 8.94 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 8.89 (s, $H_{arom.}$, 1H), 8.83 (d, J = 9.1 Hz, $H_{arom.}$, 1H), 8.78 (s, $H_{arom.}$, 1H), 8.68 (d, J = 8.5 Hz, $H_{arom.}$, 1H), 8.31 (t, J = 7.8 Hz, $H_{arom.}$, 1H), 8.80 (t, J = 7.7 Hz, $H_{arom.}$, 1H), 8.20 (s, $H_{arom.}$, 1H), 8.07 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.04 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.76 (d, J = 8.1 Hz, $H_{arom.}$, 1H), 7.74 (d, J = 8.0 Hz, $H_{arom.}$, 1H), 7.53 (d, J=8.7 Hz, $H_{arom.}$, 1H), 7.12 (d, J = 7.7 Hz, $H_{arom.}$, 1H), 7.36 (d, J = 7.9 Hz, $H_{arom.}$, 1H), 3.92 (br s, NH_3^+, 3H), 3.90 (br s, NH_3^+, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 163.76 (s), 163.46 (s), 151.70 (s), 149.61 (s), 145.70 (s), 145.44 (s), 144.93 (s), 143.15 (s), 138.57 (s), 136.68 (d), 136.46 (d), 134.88 (s), 134.53 (s), 133.31 (d), 132.87 (d), 131.87 (d), 131.68 (d), 129.29 (s), 126.18 (d), 126.15 (d), 125.90 (d), 122.18 (s), 122.16 (s), 120.52 (d), 119.41 (d), 119.15 (s), 117.89 (d), 117.89 (d), 116.44 (d), 116.20 (d), 115.16 (d); elemental analysis calcd (%) for $C_{16}H_{11}ClN_4$: C, 65.20; H, 3.76; N, 19.01; found: C, 65.43; H, 3.89; N, 19.25.

4.1.6.5. 3,8(9)-Diamino-6-cyano-benzimidazo[1,2-*a*]quinoline hydrochloride 31. Compound **31** was prepared using above described method from compound **27** (0.05 g, 0.18 mmol) to obtain a brown powder (0.04 g, 73%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2577, 2242, 1626, 1566, 1449; ^1H NMR (300 MHz, DMSO-d_6): δ = 8.94 (s, $H_{arom.}$, 1H), 8.85 (s, $H_{arom.}$, 1H), 8.79 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 8.78 (d, J = 8.9 Hz, $H_{arom.}$, 1H), 8.48 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 8.17 (s, $H_{arom.}$, 1H), 8.03 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.99 (s, $H_{arom.}$, 1H), 7.84 (d, J = 8.1 Hz, $H_{arom.}$, 1H), 7.81 (d, J = 8.8 Hz, $H_{arom.}$, 2H), 7.79 (d, J = 8.0 Hz, $H_{arom.}$, 1H), 7.62 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 7.57 (d, J = 8.8 Hz, $H_{arom.}$, 1H), 5.48 (br s, NH_3^+, 12H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 145.70 (s), 145.43 (s), 143.98 (s), 142.76 (s), 141.36 (d), 141.11 (d), 134.51 (s), 132.18 (s), 131.91 (s), 130.27 (s), 130.20 (s), 130.16 (s), 129.74 (s), 129.71 (s), 128.75 (s), 126.58 (d), 125.94 (d), 125.91 (d), 122.75 (s), 122.62 (s), 121.31 (d), 120.86 (d), 119.02 (d), 117.69 (d), 116.71 (d), 116.19 (d), 115.57 (s), 114.39 (d), 109.93 (d), 102.20 (s), 101.94 (s); elemental analysis calcd (%) for $C_{16}H_{13}Cl_2N_5$: C, 55.51; H, 3.78; N, 20.23; found: C, 55.76; H, 3.93; N, 20.44.

4.1.6.6. 3-Amino-8(9)-methyl-6-cyano-benzimidazo[1,2-*a*]quinoline hydrochloride 32. Compound **32** was prepared using above described method from compound **28** (0.04 g, 0.15 mmol) to obtain a green powder (0.03 g, 74%); mp >300 °C; IR (diamond) ν/cm^{-1}: 2846, 2582, 2340, 1677, 1625; ^1H NMR (300 MHz, DMSO-d_6): δ = 9.18 (s, $H_{arom.}$, 1H), 9.07 (d, J = 8.4 Hz, $H_{arom.}$, 1H), 8.96 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 8.91 (d, J = 9.0 Hz, $H_{arom.}$, 1H), 8.76 (d, J = 8.6 Hz, $H_{arom.}$, 1H), 8.71 (s, $H_{arom.}$, 1H), 8.21 (s, $H_{arom.}$, 1H), 7.96 (d, J = 8.3 Hz, $H_{arom.}$, 1H), 7.87 (s, $H_{arom.}$, 1H), 7.76–7.70 (m, $H_{arom.}$, 3H), 7.56 (d, J = 8.7 Hz, $H_{arom.}$, 1H), 7.50 (d, J = 8.2 Hz, $H_{arom.}$, 1H), 5.01 (br s, NH_3^+, 6H), 2.65 (s, CH_3, 3H), 2.57 (s, CH_3, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 164.18 (s), 164.14 (s), 162.71 (s), 162.38 (s), 137.12 (s), 137.06 (s), 136.71 (d), 136.12 (s), 135.15 (s), 135.10 (s), 132.16 (s), 129.94 (s), 129.36 (d), 128.45 (s), 128.34 (d), 127.79 (s), 127.11 (d), 126.35 (d), 126.31 (s), 125.62 (d), 123.90 (s), 123.88 (s), 123.45 (s), 123.13 (s), 118.36 (d), 118.17 (d), 117.95 (d), 117.63 (d), 115.79 (d), 115.60 (d), 115.24 (d), 115.13 (d), 21.43 (d), 21.21 (d); elemental analysis calcd (%) for $C_{17}H_{13}ClN_4$: C, 66.13; H, 4.24; N, 18.15; found C, 66.37; H, 4.36; N, 18.42.

4.2. Spectroscopy

The electronic absorption spectra were recorded on Varian Cary 50 and Varian Cary 100 Bio spectrometer, CD spectra on Jasco J815, in all cases using quartz cuvettes (1 cm). Fluorescence emission spectra were recorded on Varian Eclipse fluorimeter (quartz cuvettes, 1 cm), from 350 to 600 nm. The sample concentration in fluorescence measurements had an optical absorbance below 0.05 at the excitation wavelength. 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. The measurements were performed in the aqueous buffer solution (pH 7.0; sodium cacodylate buffer, I = 0.05 mol dm^{-3}).

4.3. Interactions with DNA

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 corresponding solution determined spectroscopically as the concentration of phosphates.[25] The measurements were performed in the aqueous buffer solution (pH 7.0; sodium cacodylate buffer, I = 0.05 mol dm^{-3}). 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 non-linear least-square fitting, a giving excellent correlation coefficients (>0.999) for obtained values for K_s and n. Thermal denaturation 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. 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.

4.4. Antitumor evaluation assay

The experiments were carried out on five human cell lines, which are derived from four cancer types. The following cell lines were used: SW 620 and HCT 116 (colon carcinoma), H 460 (lung carcinoma), MCF-7 (breast carcinoma) and MOLT-4 (T-lymphoblast leukemia). MCF-7, SW 620, HCT 116 and H 460 cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) while MOLT-4 cells were cultured in suspension in RPMI medium, both 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.[7,8,15,16,20] The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1×10^4 to 3×10^4 cells/mL, depending on the doubling times of a specific cell line. Test agents were then added in ten-fold dilutions (10^{-8} to 10^{-4} M) and incubated for further 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 dehydrogenise activity in viable cells. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$If\,(meanA_{test} - meanA_{tzero}) \geq 0, then PG = 100 \times (meanA_{test} - meanA_{tzero})/(meanA_{ctrl} - meanA_{tzero})$$

$$If\,(meanA_{test} - meanA_{tzero}) < 0, then: PG = 100 \times (meanA_{test} - meanA_{tzero})/A_{tzero},$$

where the mean A_{tzero} is the average of optical density measurements before exposure of cells to the test compound, the mean A_{test} is the average of optical density measurements after the desired period of time and the mean A_{ctrl} is the average of optical density measurements after the desired period of time with no exposure of cells to the test compound. 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 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 test was performed in quadruplicate in at least two individual experiments.

4.5. Cell cycle analysis

Tumor cells (2×10^5) were seeded per well into a 6-well plate. After 24 h the tested compounds were added at various concentrations (as shown in the Results section). After the desired length of time the attached cells were trypsinized, combined with floating cells, washed with phosphate buffer saline (PBS), fixed with 70% ethanol and stored at -20 °C. Immediately before the analysis, the cells were washed with PBS and stained with 50 μg/ml of propidium iodide (PI) with the addition of 0.2 μg/μl of RNAse A. The stained cells were then analyzed with Becton Dickinson FACScalibur (Becton Dickenson) flow cytometer (20,000 counts were measured). The percentage of the cells in each cell cycle phase was determined using the ModFit LT™ software (Verity Software House) based on the DNA histograms. The tests were performed in duplicates and repeated at least twice.

4.6. Intracellular distribution of compounds 11 and 29

H 460 cells were seeded on round microscopic cover slips placed in 24-well-plates (100,000 cells/well) and grown at 37 °C for 24 h in DMEM, as described above. Cells were then incubated with compounds **11** and **29** both in 100 μM final concentrations for 120 min and 20 μM final concentrations for 24 h. Cover slips were rinsed twice with PBS, placed on the microscopic slides and immediately analyzed. The uptake and intracellular distribution of tested chemicals were analyzed under the fluorescence microscope (Olympus BX51) and recorded with Olympus DP70 Digital Camera.

Acknowledgment

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

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.002.

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