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Original article

Synthesis, antiproliferative activity and DNA binding properties of novel 5-Aminobenzimidazo[1,2-*a*]quinoline-6-carbonitriles

Nataša Perin [a], Raja Nhili [b], Katja Ester [c], William Laine [b], Grace Karminski-Zamola [a], Marijeta Kralj [c], Marie-Hélène David-Cordonnier [b,*], Marijana Hranjec [a,*]

[a] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, P. O. Box 177, HR-10000 Zagreb, Croatia
[b] INSERM U837, Jean-Pierre Aubert Research Centre (JPARC), Team "Molecular and Cellular Targeting for Cancer Treatment", Université Lille 2, IMPRT-IFR-114, Institut pour la Recherche sur le Cancer de Lille, Place de Verdun, F-59045 Lille cedex, France
[c] Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička cesta 54, P. O. Box 180, HR-10000 Zagreb, Croatia

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ABSTRACT

The synthesis of 5-amino substituted benzimidazo[1,2-*a*]quinolines prepared by microwave assisted amination from halogeno substituted precursor was described. The majority of compounds were active at micromolar concentrations against colon, lung and breast carcinoma cell lines *in vitro*. The *N,N*-dimethylaminopropyl **9** and piperazinyl substituted derivative **19** showed the most pronounced activity towards all of the three tested tumor cell lines, which could be correlated to the presence of another N heteroatom and its potential interactions with biological targets. The DNA binding studies, consisting of UV/Visible absorbency, melting temperature studies, and fluorescence and circular dichroism titrations, revealed that compounds **9**, **19** and **20** bind to DNA as strong intercalators. The cellular distribution analysis, based on compounds' intrinsic fluorescence, showed that compound **20** does not enter the cell, while compounds **9** and **19** do, which is in agreement with their cytotoxic effects. Compound **9** efficiently targets the nucleus whereas **19**, which also showed DNA intercalating properties *in vitro*, was mostly localised in the cytoplasm suggesting that the antitumor mechanism of action is DNA-independent.

1. Introduction

Benzimidazoles are important and fundamental building skeletons of various essential synthetic and natural pharmacological compounds [1–3]. Heterocycles bearing a benzimidazole nucleus possess a broad spectrum of pharmacological features like anticancer [4], antibacterial [5], antifungal [6], antiviral [7], antihistaminic [8] *etc*. In recent years, there is a permanent and growing interest in the synthesis of fused, benzannulated benzimidazole derivatives as a direct consequence of their great importance in the natural, medicinal and environmental sciences. Usually, cyclic benzimidazole derivatives have a highly conjugated planar chromophore bearing excellent spectroscopic properties important for their application in several other areas like optoelectronics or chemosensors, with side chain substituents designed to enable additional interactions with potential biological targets [9–11]. High fluorescence intensity and possibility of interaction with important biomacromolecules of the living systems offer their potential use as fluorescent probes for detection of important molecules as DNA or different proteins in biomedical diagnostics [12].

A group of authors prepared amino- and amido-substituted benzimidazo[1,2-*c*]quinazolines with different length of side chains, evaluated *in vitro* cytotoxicity against a panel of human and murine cell lines and showed that the most active compound was shown to be an intercalator [13]. Moreover, some benzimidazo[2,1-*a*]isoquinolines with carboxamide side chains were used for studying biological effects induced by the variation in the side-chain position in this tetracyclic series [14]. Lamazzi et al. have described the antitumor potency of benzimidazo[1,2-*c*]quinazoline-6-carbonitriles which block significantly the growth of L1210 cells [15]. Volovenko and co-workers [16] have published the synthesis of benzimidazo[1,2-*a*]quinoline-6-carbonitriles which were tested by another group of authors as potential fluorescent probes for protein structure investigation including albumin,

* Corresponding authors.
E-mail addresses: marie-helene.david@inserm.fr (M.-H. David-Cordonnier), mhranjec@fkit.hr (M. Hranjec).

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lysozyme and papain [17]. Babichev [18] has published the synthesis of amino substituted benzimidazo[1,2-*c*]quinazoline-6-carbonitriles which were tested for their antimicrobial activity against common bacterial, yeast and fungal pathogens. 6-dialkylaminoalkylbenzimidazo[1,2-*c*]quinazolines were synthesized as potential interferon inducers, antiviral and DNA binding agents [19]. Recently, we have prepared various benzimidazo[1,2-*a*]quinoline-6-carbonitriles and their heteroaromatic fluorene analogues which exerted pronounced antiproliferative activity with the cyano moiety important for the activity but not the selectivity of tested compounds [20]. Some of the tested compounds significantly enhanced fluorescence emission upon addition of CT-DNA and thus offer the potential application as DNA-specific fluorescent probes. Furthermore, fluorescence microscopy study of some amino and positively charged diamino substituted benzimidazo[1,2-*a*]quinolines demonstrated that they are weak intercalators, confirming that DNA is not the primary biological target of this compounds [21]. Very recently, we have reported on the biological activity of 2-aminobenzimidazo[1,2-*a*]quinoline-6-carbonitriles with different lengths of the secondary or tertiary amino chains linked to the tetracyclic skeleton which significantly influenced the antiproliferative activity [22].

Motivated by all above-mentioned considerations and as an extension of our previous scientific research related to benzimidazo[1,2-*a*]quinolines as potential anticancer agents, we report within this manuscript the synthesis of 5-aminobenzimidazo[1,2-*a*]quinoline-6-carbonitriles, their antiproliferative activity *in vitro* and the DNA binding studies of some of the most active compounds.

2. Results and discussion

2.1. Chemistry

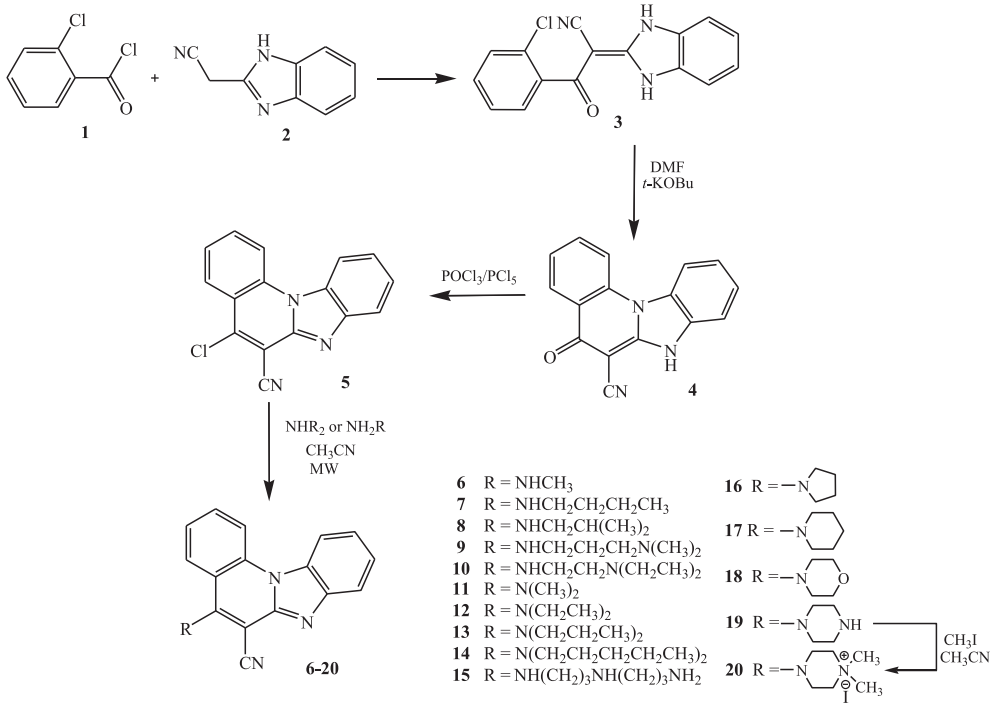
All compounds were prepared according to the main experimental procedures shown in Scheme 1 by conventional methods of organic synthesis. Acyclic precursor **3** was prepared in the reaction of aldol condensation of 2-chlorobenzoylchloride **1** with 2-cyanomethylbenzimidazole **2** in absolute ethanol using piperidine as a base in moderate yield (44%). Fused keto derivative **4** was prepared by previously described termic cyclization reaction in DMF using *t*-KOBu as a base. The main precursor for the synthesis of targeted compounds, chloro substituted benzimidazo[1,2-*a*]quinoline **5** was prepared in the reaction of compound **4** with the $POCl_3$ and PCl_5 with yield of 70% [16,18].

Based on the series of experiments which were undertaken in order to optimize the reaction times and yields including thermal reactions with an access of corresponding amine in dioxane, catalyzed or microwave assisted aminations, finally the amination reactions were conducted by microwave assisted synthesis by using 800 W power, 170 °C and 40 bar in acetonitrile in order to obtain the highest yields in the shortest reaction time. The results lead us to conclude that the microwave assisted amination provided shorter reaction time, highly increased yield as well as simple product isolation procedure [11a,22].

Thus, amino-substituted benzimidazo[1,2-*a*]quinolines were prepared from chloro substituted benzimidazo[1,2-*a*]quinoline **5** by uncatalyzed microwave assisted nucleophilic aromatic substitution using five or six-fold excess of the corresponding amine.

All compounds were obtained in low to moderate yield (30–74%) except the *N,N*-diamino substituted derivatives **12**, **13** and **14** which were obtained in a very low yield 12–24% despite the optimization of the reaction conditions. *N,N*-dimethylated piperazinyl substituted compound **20** as a iodide salt was prepared from derivative **19** with an excess of methyl-iodide.

The structures of all prepared derivatives **6**–**20** were determined by using ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. NMR analysis was based on the values of H–H coupling constants and chemical shifts in the ^{1}H and ^{13}C NMR spectra. The reaction of cyclization of acyclic precursor **3** provides a downfield shift of the aromatic protons and disappearance of one proton of the NH group on the benzimidazole nuclei which confirmed the formation of the benzimidazo[1,2-*a*]quinoline skeleton. Halogenation of the fused



Scheme 1. Synthesis of amino substituted benzimidazo[1,2-*a*]quinolines.

derivative **4** leads also to a downfield shift of the all aromatic protons as well as reaction of amination. In the NMR spectra of amino-substituted derivatives it can be observed the appearance of protons related to amino substituents in the aliphatic part both in ^{1}H and ^{13}C NMR spectra (Table S1).

2.2. Biological results and discussion

All tested compounds showed antiproliferative effect on the tested cell lines. In general, they exert modest to prominent effect on HCT 116 and MCF-7 cell lines, with IC_{50} concentrations in a micromolar range, and modest effect on H 460 cell line (Table 1). Compounds **9** and **19** showed the most pronounced activity towards all three tested cell lines. Compounds **10** and **16** were not tested due to significant precipitation in aqueous media.

We studied the influence of amino side chains with different type of nitrogen atom, as well as different chain lengths on the biological activity. Compounds **6**–**8** bearing secondary amino side chains showed selectivity towards MCF-7 cell lines, but without any effect of the chain length. The introduction of additional nitrogen heteroatom in the structure of compound **9** through the *N,N*-dimethylaminopropyl side chain increased the activity towards HTC 116 and H 460 cell lines in comparison to compounds **6**–**8**. Introduction of another nitrogen heteroatom in the structure of compound **15** with 3,3′-diaminodipropylamino substituent decreased antiproliferative effect towards all cell lines. The length of *N,N*-dialkylamino side chains influenced the activity of compounds **11**–**14**, whereby compounds with longest side chains (**13** and **14**) exerted the most pronounced activity. Compound **17** with cyclic amino piperidine substituent showed better activity towards MCF-7 cells while the introduction of another oxygen heteroatom in the structure of morpholinyl substituted compound **18** decreased the antiproliferative effects. The most active compound with cyclic amino substituent was piperazinyl substituted derivative **19** which showed strong antiproliferative effect towards all tested tumor cell lines. The most pronounced antiproliferative activity of compound **19** could be explained by the presence of another N heteroatom which could contribute to the interactions with potential biological targets. Iodide salt of *N,N*-dimethylpiperazinyl substituted compound **20** showed the weakest antiproliferative activity among all tested compounds. Quaternization of piperazine *N* heteroatom was performed to evaluate the effect of positive permanent charges on the antiproliferative activity. The weakest antiproliferative activity of compound **20** could be due to the steric hindrance of two attached methyl groups which reduce the interaction of N heteroatom with potential biological targets. From the structure–activity relationship (SAR) studies it could be concluded that secondary amine side chain is generally slightly preferred over tertiary amine or cyclic amine side chain except for piperazine substituent.

Table 1
IC_{50} values (in μM ± sd).[a]

IC_{50}[a] (μM)			
Compound	Cell lines		
	HTC 116	MCF-7	H 460
6	7 ± 1	3 ± 0.4	84 ± 3
7	17 ± 2	3 ± 1	15 ± 2
8	33 ± 9	3 ± 0.7	35 ± 6
9	1.5 ± 0.3	2 ± 0.4	4 ± 0.6
11	27 ± 8	21 ± 5	48 ± 0.4
12	13 ± 0.3	7 ± 3	25 ± 8
13	6 ± 1	4 ± 1	20 ± 2
14	7 ± 1	6 ± 3	26 ± 7
15	8 ± 2	9 ± 3	12 ± 0.1
17	10 ± 6	2.5 ± 0.6	19 ± 7
18	37 ± 2	16 ± 3	83 ± 4
19	2 ± 0.2	2 ± 0.4	4 ± 0.6
20	93 ± 6	15 ± 7	≥100
Doxorubicin	0.07 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
Etoposide	5 ± 2	1 ± 0.7	0.1 ± 0.04

[a] IC_{50}; the concentration that causes 50% growth inhibition.

2.3. DNA binding properties

From this new series, compounds **6**–**9**, **11**–**14** and **17**–**20** were evaluated for their DNA binding propensities. Measurements of the stabilization of the DNA double helix (Δ*Tm*) were performed as a first screening. Each compound was incubated with CT-DNA at a drug/bp ratio of 0.5 prior to be subjected to heat denaturation and measurement of the absorbance of the DNA at 260 nm. The hyperchromicity at 260 nm correlates with the release of single-stranded DNA. The melting point was deduced from the midpoint of the hyperchromicity and corresponds to the temperature for which half of the DNA is in a single strand form and the other stays in the double strand form. From those 12 compounds, only compounds **9**, **19** and **20** are able to stabilize the DNA helix with an increase of 6.3, 2.9 and 7.3 °C, respectively to achieve their melting point from comparison with CT-DNA alone. Those compounds were further analyzed at $R = 1$, leading to an increase of the Δ*Tm* values (Table 2).

Those three compounds were selected for further spectroscopic analysis. UV/Visible spectroscopy of those compounds evidences bathochromic and/or hypochromic effects upon binding to increasing concentrations of CT-DNA (Fig. 1, upper panels). Only UV/Visible spectroscopy performed with compound **20** evidences a clear isosbestic point at 410 nm. The hypochromic effect was quantified at the various CT-DNA concentrations relatively to the absorbency of the drug alone at the indicated maximum emission peaks (Fig. 1, bottom panels).

Both compounds were then evaluated in fluorescence spectroscopy upon binding to DNA (Fig. 2, upper panels) and plotted accordingly to Stern–Volmer equation (Fig. 2, bottom panels). A more complex binding is observed using **9** with a small quenching effect seen using the highest drug/DNA ratios, followed by a strong enhancement of fluorescence. The fluorescence spectra only evidenced an enhancement of fluorescence for **19** and **20**. Therefore, the enhancing constant K_{SV} deduced from Stern–Volmer plots were only calculated for those two compounds.

The orientation of the molecules relatively to the DNA helix (intercalation, groove binding) was investigated using circular dichroism spectrometry. Of the three compounds that efficiently binds DNA, only **19** and **20** presented a small change of the CD spectra of CT-DNA (Fig. 3). The deduced induced CD (ICD, embedded panels) for compounds **19** and **20** evidenced small negative spectra at the drug absorption wavelength that are characteristic for DNA intercalation between adjacent base pairs.

Such intercalation process is confirmed using topoisomerase I-induced DNA relaxation experiments (Fig. 4). Compounds **19** and

Table 2
Variation of the melting temperature of CT-DNA upon binding with the various compounds. Only the active compounds are presented at the indicated drug/DNA (bp) ratios (*R*).

Compound	Δ*Tm* (°C)	
	$R = 0.5$	$R = 1$
9	6.3	10.2
19	2.9	4.4
20	7.3	10.7

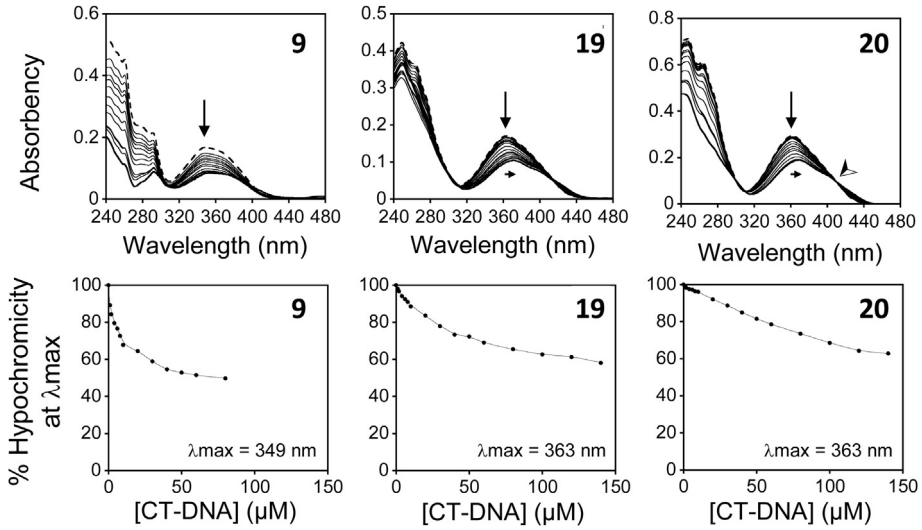


Fig. 1. UV/Visible absorbency (upper panels) and percentage of hypochromicity (bottom panels) for compounds **9**, **19** and **20** (20 μM) upon binding to increasing concentrations of CT-DNA from 1 to 80 (**7**) or 140 μM (**19**, **20**). Full arrows correspond to hypochromic (↓) and bathochromic (→) effects, respectively. Arrow head localized the isosbestic point for compound **20**.

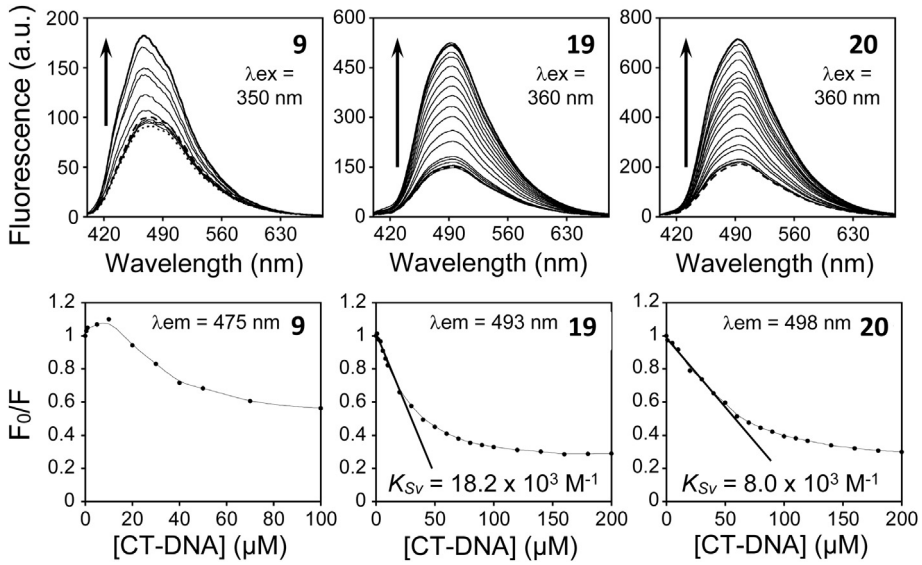


Fig. 2. Fluorescence titration for drug/DNA binding. Upper panels: the fluorescence spectra were performed using 10 μM of the indicated compounds incubated alone (dashed lanes) or with increasing concentrations (bottom to top) of CT-DNA from 0.1 to 100 (compound **9**) or 200 (bold lanes) μM (compounds **19** and **20**) at the indicated excitation wavelengths. Bottom panels: Stern–Volmer plots at the indicated maximum emission wavelengths. Linear fitting (plain lane) of the initial points were using for compounds **19** and **18** to deduced the enhancement constant K_{SV}.

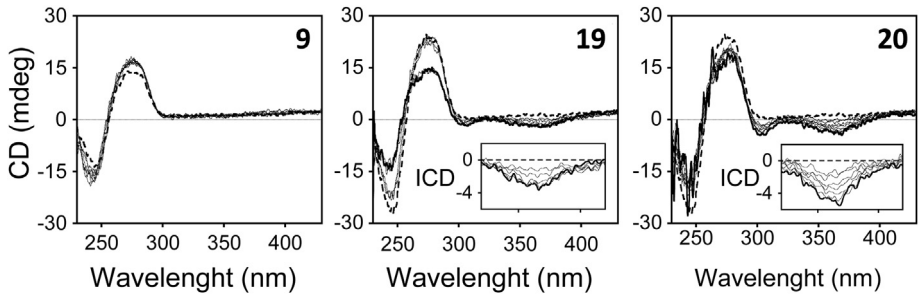


Fig. 3. Circular dichroism. CD spectra are presented for UV/Visible wavelengths from 230 to 430 nm using CT-DNA alone (dashed lanes) or incubated with increasing concentrations of **9**, **19** or **20** (from 1 to 70 μM). Embedded panels for **19** and **20** correspond to the induced circular dichroism spectra (ICD = $CD_{[CT\text{-}DNA+compound]}$-$CD_{[CT\text{-}DNA]}$) using increasing concentrations of compounds (top to bottom).

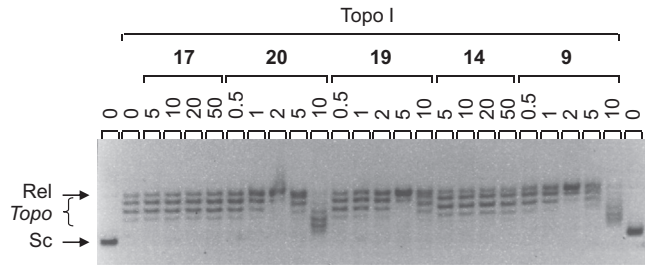


Fig. 4. Topoisomerase I – induced DNA relaxation. Supercoiled circular plasmid DNA (Sc) is treated with topoisomerase I (Topo I) in the presence of increasing concentrations (μM) of the indicated compounds to evidence the presence of different topoisomers (*Topo*) or the relaxed circular DNA form (Rel) that were generated in each DNA samples.

20, but not **17** and **14** used here as controls, facilitate the relaxation of supercoiled (Sc) circular plasmid DNA as evidenced by the formation of fully relaxed plasmid DNA forms (Rel) using 5 and 2 μM of **19** and **20**, respectively, followed by the formation of positively supercoiled plasmid DNA at higher concentrations. Such profiles are typical for DNA intercalation as for ethidium bromide [23]. Interestingly, this functional experiment also reveals DNA intercalation using compound **9**. Such intercalation propensity was not evidenced using CD titration (Fig. 3) which is a strong method for identifying DNA intercalation as a single mode of binding but less sensitive and informative in the case of more complex binding to the DNA helix. This is in agreement with fluorescence titration of compound **9** evidencing a two phase binding to CT-DNA (Fig. 2). Functionally, none of the compounds evidences topoisomerase I-poisoning effect as part of their mechanism of action.

We then looked at potential sequence selective binding of the five compounds to DNA but as expected from many classic DNA intercalators, DNase I footprinting did not revealed any sequence-specific DNA binding (data not shown).

Finally, we looked at cell entry and distribution of the various compounds in HT-29 colon carcinoma cell line, based on their fluorescence properties. After 16 h of treatment with 5 μM of compounds, cells were labeled with MitoRed for cytoplasm localization and fixed using paraformaldehyde 2% prior to be analyzed using an apotome fluorescence microscope (Fig. 5). Interestingly, compound **9** shows nuclear localization whereas **14**, **17** and **19** are cytoplasmic, contrasting with the less cytotoxic compound **20** that failed to enter the cells. This results strongly suggest that i) compound **9** targets the nucleus through intercalation in the DNA (associated with an increase in the fluorescence intensity upon binding to DNA as evidenced *in vitro*, Fig. 2); ii) compound **20**, that strongly binds DNA *in vitro*, lacks to enter the cell in agreement with its poor cytotoxic activity; iii) compounds **14**, **17** and **19** seems not to target the DNA in cells as part of their mechanism of action even if compounds as **17** and **19** share *in vitro* DNA binding potency. For those last 3 compounds, the mechanism of action in the cytoplasm needs to be further evaluated.

3. Conclusions

In this manuscript the synthesis of 5-amino substituted benzimidazo[1,2-*a*]quinolines **6**–**20** as potential antiproliferative and DNA binding agents is described. In general secondary amine side chain is associated with more pronounced antiproliferative activity on three tumor cell lines than does tertiary amine or cyclic amine side chain, except for piperazine substituent **19**. The most active compounds of this new series were the *N,N*-dimethylaminopropyl side chain-bearing compound **9** and piperazinyl substituted derivative **19**. These two compounds presented strong DNA binding

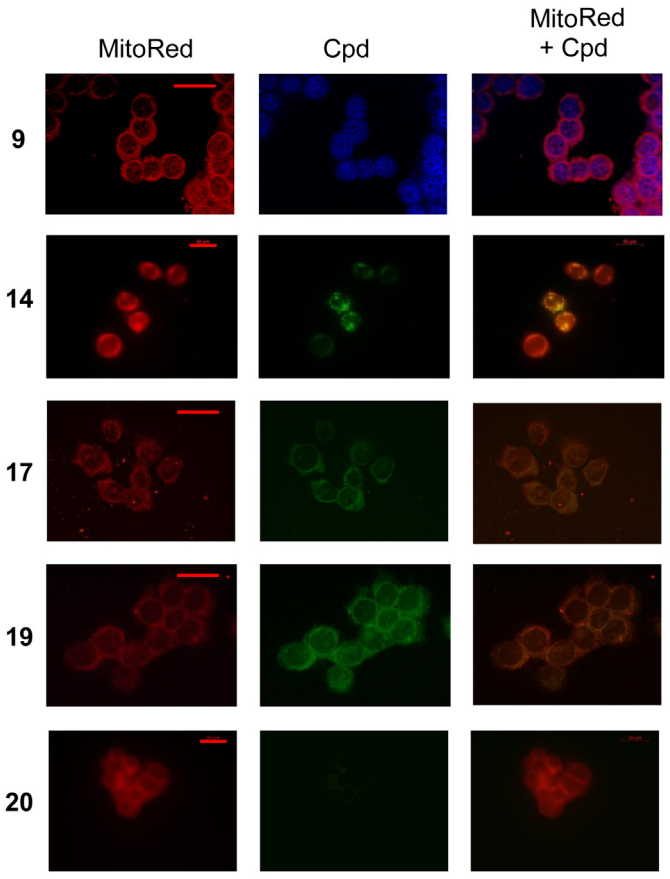


Fig. 5. Cellular distribution in HT-29 colon carcinoma cells. Plated cells were treated with 5 μM of the various indicated compounds for 16 h prior to be labeled with MitoRed dye as a cytoplasmic marker and fixed using paraformaldehyde. Cells were analyzed using an apotome-equipped fluorescence microscope (Zeiss) at 63× (**9, 17, 19**) or 40× (**14, 20**) enlargement. Scales are indicated as red lines (20 μm) in the first panels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

properties and intercalates in the DNA helix but compound **9** was the only one that entered the cell and localized in the nucleus, whereas compound **19** enters the cell with its main localization in the cytoplasm, suggesting that DNA is not its functional target. Interestingly, the dimethyl-*N*-piperazinyl compound **20**, which has similar *in vitro* DNA binding properties to **9** and **19**, is poorly active on cell proliferation, which is in agreement with its inability to enter the cell. The fluorescent compounds **14** and **17** are cytotoxic to the evaluated cell lines and localized in the cytoplasm as compound **19**. It would be of interest in the future to determine which cytoplasmic target is implicated in the cellular effect of compounds **14**, **17**, **19** and the other compounds of this series.

4. Experimental section

4.1. General methods

All chemicals and solvents were purchased from commercial suppliers Aldrich and Acros. Melting points were recorded on SMP11 Bibby and Büchi 535 apparatus. All NMR spectra were measured in DMSO-d_6 solutions using TMS as an internal standard. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or Varian Gemini 600 at 300, 600, 150 and 75 MHz, respectively. Chemical shifts are reported in ppm (δ) relative to TMS. All compounds were routinely checked by TLC with Merck silica gel 60F-

254 glass plates. Microwave-assisted synthesis was performed in a Milestone start S microwave oven using quartz cuvettes under the pressure of 40 bar. Mass spectra were recorded on an Agilent 1200 series LC/6410 QQQ instrument. The electronic absorption spectra were recorded on Varian Cary 50 spectrometer using quartz cuvette (1 cm). Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin–Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value.

4.2. Synthesis

4.2.1. 2-(2-Benzimidazolyl)-3-keto-(2-chloro-phenyl)acrylonitrile **3** [16]

A solution of 1.57 g (10.00 mmol) 2-cyanomethylbenzimidazole and 1.33 mL (10.50 mmol) 2-chlorobenzoylchloride in pyridine (10 mL) was refluxed for 2 h. The cooled mixture was poured into water (100 mL) and the resulting product was filtered off and recrystallized from ethanol to obtain a brown powder (1.31 g, 44%); m.p. >300 °C.

^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm = 13.09 (bs, 2H, NH), 7.59–7.50 (m, 3H, $H_{arom.}$), 7.48–7.42 (m, 3H, $H_{arom.}$), 7.32–7.28 (m, 2H, $H_{arom.}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 185.01 (s), 151.04 (s), 140.93 (s), 130.88 (s, 2C), 130.80 (d), 130.02 (s), 129.91 (d), 128.91 (d), 127.51 (d), 124.09 (d, 2C), 120.29 (s), 112.71 (d, 2C), 67.62 (s).

4.2.2. 5-Ketobenzimidazo[1,2-a]quinoline-6-carbonitrile **4** [16]

A solution of 0.50 g (1.69 mmol) 2-(2-benzimidazolyl)-3-keto-(2-chloro-phenyl)acrylonitrile and 0.44 g *t*-KOBu in DMF (6 mL) was refluxed for 3 h. After cooling, the reaction mixture was evaporated under vacuum and dissolved in water (50 mL). Resulting product was filtered off and recrystallized from ethanol to obtain a white powder (0.25 g, 57%); m.p. >300 °C.

^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm = 8.42 (d, 1H, J = 8.37 Hz, $H_{arom.}$), 8.25 (dd, 1H, J_1 = 1.52 Hz, J_2 = 7.82 Hz, $H_{arom.}$), 8.20 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 7.71 (dt, 1H, J_1 = 1.68 Hz, J_2 = 7.95 Hz, $H_{arom.}$), 7.47 (d, 1H, J = 7.83 Hz, $H_{arom.}$), 7.38 (t, 1H, J = 7.49 Hz, $H_{arom.}$), 7.23 (t, 1H, J = 7.56 Hz, $H_{arom.}$), 7.10 (t, 1H, J = 7.56 Hz, $H_{arom.}$); ^{13}C NMR (DMSO-d_6, 150 MHz): δ/ppm = 172.34 (s), 153.85 (s), 145.83 (s), 136.33 (s), 131.21 (s), 130.97 (d), 126.39 (d), 124.61 (s), 122.83 (d), 122.56 (d), 120.55 (s), 118.73 (d), 116.36 (d), 114.85 (d), 112.21 (d).

4.2.3. 5-Chlorobenzimidazo[1,2-a]quinoline-6-carbonitrile **5** [16]

A solution of 0.20 g (0.77 mmol) 5-ketobenzimidazo[1,2-*a*] quinoline-6-carbonitrile and 0.08 g (0.39 mmol) PCl_5 in $POCl_3$ (4 mL) was refluxed for 2 h. After cooling, the reaction mixture was evaporated under vacuum and dissolved in water (10 ml). Resulting product was filtered off and washed with water to obtain a yellow powder (0.15 g, 70%); m.p. >300 °C.

^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm = 8.96 (d, 1H, J = 8.37 Hz, $H_{arom.}$), 8.79 (dd, 1H, J_1 = 2.16 Hz, J_2 = 6.46 Hz, $H_{arom.}$), 8.42 (dd, 1H, J_1 = 1.39 Hz, J_2 = 8.20 Hz, $H_{arom.}$), 8.13 (dt, 1H, J_1 = 1.52 Hz, J_2 = 7.89 Hz, $H_{arom.}$), 8.06 (dd, 1H, J_1 = 2.18 Hz, J_2 = 6.10 Hz, $H_{arom.}$), 7.81 (t, 1H, J = 7.54 Hz, $H_{arom.}$), 7.69–7.58 (m, 2H, $H_{arom.}$); ^{13}C NMR (DMSO-d_6, 150 MHz): δ/ppm = 144.64 (s), 142.98 (s), 136.17 (s), 135.04 (d), 131.08 (s), 128.32 (d), 126.18 (d), 125.86 (d), 124.50 (d), 120.91 (d), 119.84 (s), 116.79 (d), 115.28 (d), 113.59 (s), 103.11 (s).

4.2.4. General method for preparation of compounds **6**–**19**

Compounds **6**–**19** were prepared using microwave irradiation, at optimized reaction time at 170 °C with power 800 W and 40 bar pressure, from compound **5** in acetonitrile (10 mL) with excess of added corresponding amine. After cooling, the reaction mixture was filtered off and resulting product was separated by column chromatography on SiO_2 using dichloromethane/methanol as eluent.

*4.2.4.1. 5-N-methylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **6**.* Compound **6** was prepared using above described method from **5** (0.140 g, 0.50 mmol) and methylamine (0.26 mL, 2.50 mmol) after 1 h of irradiation to yield 0.100 g (73%) of yellow powder; m.p. >153–156 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.63 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.39 (d, 1H, J = 8.22 Hz, $H_{arom.}$), 8.35 (d, 1H, J = 8.17 Hz, $H_{arom.}$), 8.17 (q, 1H, J = 4.74 Hz, NH), 7.89 (t, 1H, J = 7.56 Hz, $H_{arom.}$), 7.73 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.56 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.40 (t, 1H, J = 7.54 Hz, $H_{arom.}$), 7.32 (t, 1H, J = 7.56 Hz, H_{arom}), 3.44 (d, 3H, J = 4.98 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 158.28 (s), 151.64 (s), 149.57 (s), 145.22 (s), 135.44 (s), 133.33 (d), 131.33 (s), 124.74 (d), 124.61 (d), 124.33 (d), 121.76 (d), 118.87 (d), 118.16 (s), 116.96 (s), 116.67 (d), 114.05 (d), 32.54 (q); Found: C, 74.93; H, 4.53; N, 20.54. Calc. for $C_{17}H_{12}N_4$: C, 74.98; H, 4.44; N, 20.58%.

*4.2.4.2. 5-N-butylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **7**.* Compound **7** was prepared using above described method from **5** (0.100 g, 0.36 mmol) and butylamine (0.18 mL, 1.80 mmol) after 2 h of irradiation to yield 0.070 g (62%) of white powder; m.p. 217–220 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.64 (d, 1H, J = 8.83 Hz, $H_{arom.}$), 8.49 (dd, 1H, J_1 = 0.75, Hz, J_2 = 8.94 Hz, $H_{arom.}$), 8.40 (d, 1H, J = 8.38 Hz, $H_{arom.}$), 8.17 (t, 1H, J = 4.89 Hz, NH), 7.90 (dt, 1H, J_1 = 1.12 Hz, J_2 = 7.20 Hz, $H_{arom.}$), 7.72 (d, 1H, J = 8.16 Hz, $H_{arom.}$), 7.57 (t, 1H, J = 7.71 Hz, $H_{arom.}$), 7.39 (t, 1H, J = 7.15 Hz, $H_{arom.}$), 7.30 (dt, 1H, J_1 = 1.11 Hz, J_2 = 7.20 Hz, $H_{arom.}$), 3.86 (q, 2H, J = 6.82 Hz, CH_2), 1.79–1.74 (m, 2H, CH_2), 1.46–1.40 (m, 2H, CH_2), 0.94 (t, 3H, J = 7.41 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): 150.41 (s), 149.54 (s), 145.14 (s), 135.48 (s), 133.41 (d), 131.29 (s), 124.99 (d), 124.74 (d), 124.35 (d), 121.74 (d), 118.79 (d), 118.12 (s), 117.03 (s), 116.69 (d), 114.10 (d), 71.78 (s), 44.20 (t), 32.03 (t), 29.47 (t), 14.21 (q); Found: C, 76.36; H, 5.71; N, 17.93. Calc. for $C_{20}H_{18}N_4$: C, 76.41; H, 5.77; N, 17.82%.

*4.2.4.3. 5-N-i-butylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **8**.* Compound **8** was prepared using above described method from **5** (0.140 g, 0.50 mmol) and isobutylamine (0.25 mL, 2.50 mmol) after 2 h of irradiation to yield 0.092 g (58%) of yellow crystals; m.p. 232–234 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.64 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.50 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.40 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.14 (t, 1H, J = 5.94 Hz, NH), 7.91 (t, 1H, J = 7.74 Hz, $H_{arom.}$), 7.73 (d, 1H, J = 7.98 Hz, $H_{arom.}$), 7.58 (t, 1H, J = 7.68 Hz, $H_{arom.}$), 7.40 (t, 1H, J = 7.56 Hz, $H_{arom.}$), 7.32 (t, 1H, J = 7.71 Hz, H_{arom}), 3.68 (t, 2H, J = 6.63 Hz, CH_2), 2.23–2.15 (m, 1H, CH), 0.99 (d, 6H, J = 6.60 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 150.58 (s), 149.49 (s), 145.24 (s), 135.59 (s), 134.81 (s), 133.40 (d), 131.34 (s), 124.84 (d), 124.76 (d), 124.35 (d), 121.76 (d), 118.87 (d), 117.96 (s), 117.13 (s), 116.70 (d), 114.06 (d), 72.44 (s), 51.63 (t), 28.59 (d). 20.04 (q, 2C); Found: C, 76.45; H, 5.65; N, 17.90. Calc. for $C_{20}H_{18}N_4$: C, 76.41; H, 5.77; N, 17.82%.

*4.2.4.4. 5-[N-(N,N-dimethylaminopropyl-1-amino)]benzimidazo[1,2-a]quinoline-6-carbonitrile **9** [18].* Compound **9** was prepared using above described method from **5** (0.100 g, 0.36 mmol) and *N,N*-dimethylamino-propyl-1-amine (0.23 mL, 1.80 mmol) after 2 h of irradiation to yield 0.050 g (40%) of yellow powder; m.p. 193–195 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.65 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.41 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.37 (d, 1H, J = 8.97 Hz, $H_{arom.}$), 8.32 (t, 1H, J = 6.24 Hz, NH), 7.91 (t, 1H, J = 7.58 Hz, $H_{arom.}$), 7.73 (d, 1H, J = 7.58 Hz, $H_{arom.}$), 7.59 (t, 1H, J = 7.59 Hz, $H_{arom.}$), 7.41 (t,

1H, J = 7.59 Hz, $H_{arom.}$), 7.33 (t, 1H, J = 7.59 Hz, $H_{arom.}$), 3.94 (q, 2H, J = 6.21 Hz, CH_2), 2.94 (t, 2H, J = 6.21 Hz, CH_2), 2.60 (s, 6H, CH_3), 2.09 (qvin, 2H, J = 6.90 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 150.50 (s), 149.36 (s), 145.10 (s), 135.48 (s), 133.48 (d), 131.26 (s), 124.85 (d), 124.70 (d), 124.41 (d), 121.85 (d), 118.86 (d), 118.17 (s), 116.97 (s), 116.75 (d), 114.13 (d), 72.14 (s), 56.05 (t), 44.24 (q), 42.87 (t), 25.94 (t); Found: C, 73.62; H, 6.10; N, 20.28. Calc. for $C_{21}H_{21}N_5$: C, 73.44; H, 6.16; N, 20.39%.

4.2.4.5. 5-[N-(N,N-diethylethylenediamino)]benzimidazo[1,2-a]quinoline-6-carbonitrile ***10*** *[18].* Compound **10** was prepared using above described method from **5** (0.070 g, 0.25 mmol) and *N,N*-diethylethylenediamine (0.18 mL, 1.30 mmol) after 2 h of irradiation to yield 0.042 g (47%) of yellow powder; m.p. 240–242 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.66 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.41 (d, 1H, J = 8.22 Hz, $H_{arom.}$), 8.32 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 7.91 (t, 1H, J = 7.65 Hz, $H_{arom.}$), 7.84 (s, 1H, NH), 7.74 (d, 1H, J = 7.92 Hz, $H_{arom.}$), 7.59 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.41 (t, 1H, J = 7.53 Hz, $H_{arom.}$), 7.33 (t, 1H, J = 7.68 Hz, H_{arom}), 3.95 (t, 2H, J = 7.18 Hz, CH_2), 2.82 (t, 2H, J = 6.60 Hz, CH_2), 2.57 (q, 4H, J = 7.02 Hz, CH_2), 0.97 (t, 6H, J = 7.05 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 150.75 (s), 149.33 (s), 145.22 (s), 135.57 (s), 133.43 (d), 131.33 (s), 124.82 (d), 124.47 (d), 124.36 (d), 121.81 (d), 118.90 (d), 117.89 (s), 117.07 (s), 116.77 (d), 114.08 (d), 111.81 (s), 52.24 (t), 47.08 (t, 2C), 42.96 (t), 12.48 (q, 2C); Found: C, 74.02; H, 6.57; N, 19.41. Calc. for $C_{22}H_{23}N_5$: C, 73.92; H, 6.49; N, 19.59%.

4.2.4.6. 5-N,N-dimethylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile ***11****.* Compound **11** was prepared using above described method from **5** (0.210 g, 0.75 mmol) and dimethylamine (0.76 mL, 3.80 mmol) after 4 h of irradiation to yield 0.090 g (41%) of yellow powder; m.p. 185–187 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.69 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.50 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.18 (dd, 1H, J_1 = 1.11 Hz, J_2 = 8.19 Hz, $H_{arom.}$), 7.90 (dt, 1H, J_1 = 1.23 Hz, J_2 = 7.49 Hz, $H_{arom.}$), 7.83 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.58 (t, 1H, J = 7.40 Hz, $H_{arom.}$), 7.47 (t, 1H, J = 7.41 Hz, $H_{arom.}$), 7.41 (dt, 1H, J_1 = 1.01 Hz, J_2 = 7.73 Hz, $H_{arom.}$), 3.34 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 158.83 (s), 147.83 (s), 144.97 (s), 136.59 (s), 133.44 (d), 131.09 (s), 128.88 (d), 124.81 (d), 124.70 (d), 122.75 (d), 119.96 (s), 119.69 (d), 116.64 (d), 116.61 (s), 114.53 (d), 87.83 (s), 44.96 (q, 2C); Found: C, 75.70; H, 4.82; N, 19.48. Calc. for $C_{18}H_{14}N_4$: C, 75.50; H, 4.93; N, 19.57%.

4.2.4.7. 5-N,N-diethylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile ***12****.* Compound **12** was prepared using above described method from **5** (0.210 g, 0.75 mmol) and diethylamine (0.39 mL, 3.80 mmol) after 3 h of irradiation to yield 0.029 g (12%) of yellow powder; m.p. 160–162 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.77 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.60 (d, 1H, J = 8.22 Hz, $H_{arom.}$), 8.20 (d, 1H, J = 8.04 Hz, $H_{arom.}$), 7.95 (t, 1H, J = 7.68 Hz, $H_{arom.}$), 7.90 (d, 1H, J = 7.98 Hz, $H_{arom.}$), 7.61 (t, 1H, J = 7.59 Hz, $H_{arom.}$), 7.52 (t, 1H, J = 7.53 Hz, $H_{arom.}$), 7.47 (t, 1H, J = 7.65 Hz, $H_{arom.}$), 3.67 (q, 4H, J = 7.02 Hz, CH_2), 1.19 (t, 6H, J = 6.96 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 157.94 (s), 148.21 (s), 144.85 (s), 136.75 (s), 133.58 (d), 131.13 (s), 128.28 (d), 125.12 (d), 125.03 (d), 123.16 (d), 121.49 (s), 120.00 (d), 116.72 (d), 116.08 (s), 114.75 (d), 113.72 (s), 47.52 (t, 2C), 13.47 (q, 2C); Found: C, 76.50; H, 5.68; N, 17.82. Calc. for $C_{20}H_{18}N_4$: C, 76.41; H, 5.77; N, 17.82%.

4.2.4.8. 5-N,N-dipropylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile ***13****.* Compound **13** was prepared using above described method from **5** (0.140 g, 0.50 mmol) and dipropylamine (0.35 mL, 2.50 mmol) after 4 h of irradiation to yield 0.027 g (16%) of yellow powder; m.p. 185–187 °C.

^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm = 8.77 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.59 (d, 1H, J = 7.80 Hz, $H_{arom.}$), 8.20 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 7.90 (t, 1H, J = 7.71 Hz, $H_{arom.}$), 7.88 (d, 1H, J = 7.68 Hz, $H_{arom.}$), 7.62 (t, 1H, J = 7.61 Hz, $H_{arom.}$), 7.51 (t, 1H, J = 7.683 Hz, $H_{arom.}$), 7.45 (t, 1H, J = 7.83 Hz, H_{arom}), 3.61 (t, 4H, J = 6.99 Hz, CH_2), 1.72 (m, 4H, CH_2), 0.85 (t, 6H, J = 7.26 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 158.56 (s), 147.30 (s), 144.82 (s), 136.80 (s), 133.62 (d), 131.08 (s), 128.47 (d), 125.08 (d), 125.03 (d), 123.07 (d), 120.93 (s), 119.87 (d), 116.93 (d), 116.93 (d), 116.34 (s), 114.75 (d), 92.54 (s), 55.25 (t, 2C), 21.14 (t, 2C), 11.70 (q, 2C); Found: C, 77.37; H, 6.56; N, 16.07. Calc. for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36%.

4.2.4.9. 5-N,N-dipentylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile ***14****.* Compound **14** was prepared using above described method from **5** (0.140 g, 0.50 mmol) and dipentylamine (0.52 mL, 2.50 mmol) after 3 h of irradiation to yield 0.048 g (24%) of yellow powder; m.p. 149–151 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.79 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.59 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.19 (d, 1H, J_1 = 0.87 Hz, J_2 = 8.49 Hz, $H_{arom.}$), 7.95 (t, 1H, J_1 = 0.96 Hz, J_2 = 7.73 Hz, $H_{arom.}$), 7.89 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.63 (t, 1H, J = 7.59 Hz, $H_{arom.}$), 7.52 (t, 1H, J = 7.44 Hz, $H_{arom.}$), 7.46 (dt, 1H, J_1 = 0.95 Hz, J_2 = 7.72 Hz, H_{arom}), 3.64 (t, 4H, J = 7.14 Hz, CH_2), 1.64 (qvin, 4H, J = 6.92 Hz, CH_2), 1.29–1.20 (m, 8H), 0.79 (t, 6H, J = 6.93 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 158.39 (s), 147.27 (s), 144.91 (s), 144.37 (s), 136.82 (s), 133.58 (d), 131.15 (s), 128.36 (d), 125.02 (d, 2C), 123.08 (d), 121.10 (s), 119.93 (d), 116.87 (d), 116.17 (s), 114.73 (d), 93.52 (s), 53.61 (t, 2C), 28.96 (t, 2C), 27.53 (t, 2C), 22.21 (t, 2C), 14.86 (q, 2C); Found: C, 78.49; H, 7.63; N, 13.88. Calc. for $C_{26}H_{30}N_4$: C, 78.35; H, 7.59; N, 14.06%.

4.2.4.10. 5-N-(3,3′-diaminodipropylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile ***15****.* Compound **15** was prepared using above described method from **5** (0.200 g, 0.72 mmol) and 3,3′-diaminodipropylamine (0.31 mL, 2.20 mmol) after 3 h of irradiation to yield 0.200 g (74%) of yellow powder; m.p. 210–214 °C.

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm = 8.68 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.54 (d, 1H, J = 8.25 Hz, $H_{arom.}$), 8.43 (d, 1H, J = 8.19 Hz $H_{arom.}$), 7.93 (t, 1H, J = 7.79 Hz, $H_{arom.}$), 7.74 (d, 1H, J = 7.71 Hz, $H_{arom.}$), 7.61 (t, 1H, J = 7.70 Hz, $H_{arom.}$), 7.41 (t, 1H, J = 7.55 Hz, $H_{arom.}$), 7.33 (dt, 1H, J = 7.65 Hz, H_{arom}), 4.00 (t, 1H, J = 6.45 Hz, NH), 2.85 (q, 4H, J = 7.02 Hz, CH_2), 2.78 (t, 2H, J = 6.60 Hz, CH_2), 2.71 (t, 2H, J = 6.76 Hz, CH_2), 2.62 (m, 1H, NH), 2.00 (t, 2H, J = 6.60 Hz, NH_2), 1.81–1.67 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ/ppm = 157.49 (s), 150.15 (s), 149.04 (s), 144.77 (s), 135.06 (s), 132.87 (d), 130.86 (s), 124.69 (d), 124.28 (d), 123.84 (d), 121.26 (s), 118.35 (d), 117.59 (d), 116.71 (s), 116.13 (d), 113.54 (d), 46.12 (t), 46.03 (t), 42.56 (t), 37.66 (t), 28.40 (t), 26.83 (t); Found: C, 70.84; H, 6.54; N, 22.62. Calc. for $C_{22}H_{22}IN_5$: C, 70.94; H, 6.44; N, 22.56%.

4.2.4.11. 5-N-pyrrolydinylbenzimidazo[1,2-a]quinoline-6-carbonitrile ***16****.* Compound **16** was prepared using above described method from **5** (0.100 g, 0.36 mmol) and pyrrolidine (0.18 mL, 1.80 mmol) after 2 h of irradiation to yield 0.070 g (63%) of yellow powder; m.p. 230–231 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.60 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.40 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 8.30 (d, 1H, J = 8.010 Hz, $H_{arom.}$), 7.87 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.75 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.51 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.42 (t, 1H, J = 7.50 Hz, $H_{arom.}$), 7.35 (t, 1H, J = 7.26 Hz, $H_{arom.}$), 4.02 (t, 4H, J = 5.62 Hz, CH_2), 2.00 (t, 4H, J = 6.37 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ/ppm = 154.80 (s), 149.82 (s), 145.47 (s), 135.93 (s), 132.88 (d), 131.13 (s), 128.96 (d), 124.44 (d), 123.93 (d), 121.87 (d), 119.22 (s), 119.06

(d), 118.13 (s), 116.16 (d), 114.08 (d), 78.47 (s), 54.67 (t, 2C), 25.72 (t, 2C); Found: C, 77.05; H, 5.30; N, 17.65. Calc. for $C_{20}H_{16}N_4$: C, 76.90; H, 5.16; N, 17.94%.

4.2.4.12. 5-N-piperydinylbenzimidazo[1,2-a]quinoline-6-carbonitrile ***17*** *[16].* Compound **17** was prepared using above described method from **5** (0.200 g, 0.72 mmol) and piperidine (0.36 mL, 3.60 mmol) after 2 h of irradiation to yield 0.070 g (30%) of yellow powder; m.p. 238–240 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.73 (d, 1H, J = 8.49 Hz, $H_{arom.}$), 8.55 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.13 (dd, 1H, J_1 = 1.44 Hz, J_2 = 8.22 Hz, $H_{arom.}$), 7.93 (dt, 1H, J_1 = 1.44 Hz, J_2 = 7.14 Hz, $H_{arom.}$), 7.86 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 7.62 (dt, 1H, J_1 = 0.84 Hz, J_2 = 8.05 Hz, $H_{arom.}$), 7.50 (dt, 1H, J_1 = 0.90 Hz, J_2 = 8.04 Hz, $H_{arom.}$), 7.44 (dt, 1H, J_1 = 1.29 Hz, J_2 = 8.25 Hz, $H_{arom.}$), 3.59 (t, 4H, J = 5.28 Hz, CH_2), 1.83 (m, 4H, CH_2), 1.73 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): (δ/ppm) = 158.02 (s), 147.06 (s), 144.29 (s), 136.01 (s), 133.02 (d), 130.49 (s), 127.38 (d), 124.54 (d), 124.90 (d), 122.44 (d), 119.61 (s), 119.29 (d), 116.23 (d), 116.03 (s), 114.16 (d), 88.52 (s), 53.61 (s, 2C), 26.01 (s, 2C), 23.55 (s); Found: C, 77.45; H, 5.58; N, 16.97. Calc. for $C_{21}H_{18}N_4$: C, 77.28; H, 5.56; N, 17.17%.

4.2.4.13. 5-N-morpholinylbenzimidazo[1,2-a]quinoline-6-carbonitrile ***18****.* Compound **18** was prepared using above described method from **5** (0.050 g, 0.18 mmol) and morpholine (0.08 mL, 0.90 mmol) after 3 h of irradiation to yield 0.041 g (69%) of yellow crystals; m.p. >300 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.79 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.61 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 8.24 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 7.97 (t, 1H, J = 7.77 Hz, $H_{arom.}$), 7.91 (d, 1H, J = 8.04 Hz, $H_{arom.}$), 7.64 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.53 (t, 1H, J = 7.32 Hz, $H_{arom.}$), 7.48 (t, 1H, J = 7.29 Hz, $H_{arom.}$), 3.92 (t, 4H, J = 4.32 Hz, CH_2), 3.65 (t, 4H, J = 4.35 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 157.60 (s), 151.07 (s), 144.90 (s), 136.53 (s), 133.85 (d), 131.16 (s), 128.16 (d), 125.19 (d), 125.04 (d), 123.17 (d), 120.00 (d), 119.94 (s), 116.82 (d), 116.29 (s), 114.75 (d), 93.51 (s), 67.00 (t, 2C), 53.13 (t, 2C); Found: C, 73.34; H, 4.93; N, 16.84. Calc. for $C_{20}H_{16}N_4O$ (326.3): C, 73.15; H, 4.91; N, 17.06%.

4.2.4.14. 5-N-piperazinylbenzimidazo[1,2-a]quinoline-6-carbonitrile ***19****.* Compound **19** was prepared using the above method from **5** (0.140 g, 0.50 mmol) and piperazine (0.260 g, 3.00 mmol) after 8 h of irradiation to yield 0.044 g (27%) of yellow powder; m.p. 243–245 °C.

^{1}H NMR (DMSO-d_6, 600 MHz): δ/ppm = 8.76 (d, 1H, J = 8.34 Hz, $H_{arom.}$), 8.59 (d, 1H, J = 8.16 Hz, $H_{arom.}$), 8.20 (d, 1H, J = 8.04 Hz, $H_{arom.}$), 7.96 (t, 1H, J = 7.54 Hz, $H_{arom.}$), 7.90 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.63 (t, 1H, J = 7.53 Hz, $H_{arom.}$), 7.52 (t, 1H, J = 7.41 Hz, $H_{arom.}$), 7.47 (t, 1H, J = 7.50 Hz, $H_{arom.}$), 3.66 (t, 4H, J = 4.35 Hz, CH_2), 3.22 (t, 4H, J = 4.55 Hz, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 160.13 (s), 157.71 (s), 144.88 (s), 136.68 (s), 133.79 (d), 131.09 (s), 128.04 (d), 125.24 (d), 125.05 (d), 123.23 (d), 120.12 (d), 116.75 (d), 116.15 (s), 114.73 (d), 90.97 (s), 52.06 (t, 2C), 45.42 (t, 2C); Found: C, 73.28; H, 5.16; N, 21.56. Calc. for $C_{20}H_{19}N_5$: C, 73.37; H, 5.23; N, 21.34%.

4.2.5. 5-N-(4-N,N-dimethylpiperazin-1-yl)benzimidazo[1,2-a] quinoline-6-carbonitrile iodide ***20***

A mixture of compound **19** (0.077 g, 0.23 mmol) and anhydrous potassium carbonate (0.033 g, 0.23 mmol) was refluxed in acetonitrile (25 mL) with methyl iodide (0.060 mL, 0.96 mmol) for 2 h. The reaction mixture was concentrated under reduced pressure to a volume of 5 mL and filtered off to yield pure compound **20** as yellow powder (0.063 g, 55%); m.p. >300 °C.

^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm = 8.84 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 8.65 (d, 1H, J = 7.31 Hz, $H_{arom.}$), 8.26 (dd, 1H, J_1 = 0.98 Hz, J_2 = 8.15 Hz, $H_{arom.}$), 8.03 (dt, 1H, J_1 = 1.02 Hz, J_2 = 7.85 Hz, $H_{arom.}$), 7.95 (dd, 1H, J_1 = 1.25 Hz, J_2 = 7.85 Hz, $H_{arom.}$), 7.68 (t, 1H, J = 7.67 Hz, $H_{arom.}$), 7.57 (dt, 1H, J_1 = 0.92 Hz, J_2 = 7.35 Hz, $H_{arom.}$), 7.52 (dt, 1H, J_1 = 1.34 Hz, J_2 = 7.49 Hz, $H_{arom.}$), 4.00 (t, 4H, J = 4.27 Hz, CH_2), 3.80 (t, 4H, J = 4.35 Hz, CH_2), 3.32 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 156.43 (s), 146.81 (s), 144.85 (s), 136.71 (s), 134.04 (d), 131.06 (s), 128.22 (d), 125.31 (d), 125.24 (d), 123.49 (d), 120.18 (d), 119.72 (s), 116.83 (d), 115.95 (s), 114.87 (d), 61.46 (t, 2C), 51.67 (q, 2C), 46.08 (t, 2C); Found: C, 54.55; H, 4.68; N, 14.52. Calc. for $C_{22}H_{22}IN_5$: C, 54.67; H, 4.59; N, 14.49%.

4.3. Antiproliferative evaluation assay

The experiments were carried out on three human cell lines, which are derived from three cancer types: HCT 116 (colon carcinoma), H 460 (lung carcinoma) and MCF-7 (breast carcinoma). The cells were cultured as monolayers and maintained in Dulbecco's modified Eagle 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 [21]. The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 3×10^4 cells/mL (HCT 116, H 460) to 5×10^4 cells/mL (MCF-7), 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 dehydrogenase 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:

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

If (mean A_{test} − mean A_{tzero}) < 0, then: PG = 100 × (mean A_{test} − mean A_{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. Minimum two individual experiments were carried out and each test point was performed in quadruplicate.

4.4. DNA binding experiments

The tested compounds were dissolved in DMSO as 5 or 10 mM stock solutions. CT-DNA (Sigma Aldrich, France) was prepared in water and dialyzed overnight. Both were aliquoted and stored at −20 °C to then be freshly diluted in the appropriate aqueous buffer.

4.4.1. DNA melting temperature

CT-DNA (20 μM) was incubated or not with 10 or 20 μM of the various tested compounds (R = drug/base pair ratio of 0.5 or 1) in 1 mL BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1). The absorbency at 260 nm was measured in quartz cells using an Uvikon XL spectrophotometer thermostated with a peltier cryostat every min over a range of 20–100 °C with an increment of 1 °C per min. The Tm values were deduced from the midpoint of the hyperchromic transition obtained from first-derivative plots. The variation of melting temperature (ΔTm) were obtained by subtracting the melting temperature measurement of CT-DNA alone (control Tm) to that obtained with DNA incubated with the compounds (ΔTm values = $Tm_{[\text{compound+DNA}]} - Tm_{[\text{DNA alone}]}$).

4.4.2. UV/Visible spectroscopy

The UV/Visible spectra were obtained in a quartz cuvette of 10 mm pathlength containing compounds **9**, **19** and **20** (20 μM) diluted in 1 mL of BPE buffer in the absence or presence of increasing concentrations of CT-DNA (1, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 80, 100, 120, 140 μM). Due to precipitation at higher DNA/drug ratio, spectra were only measured up to 80 μM of **9**. Each spectrum was recorded from 240 nm to 480 nm using an Uvikon XL spectrophotometer and referenced against a cuvette containing DNA at identical concentration.

4.4.3. Fluorescence spectroscopy

Fluorescence spectra were recorded from 400 to 700 nm essentially as described [23]. The fluorescent drugs (10 μM) were diluted in 1 mL of BPE buffer in the presence or absence of increasing concentrations of CT-DNA (from 0.5 to 80 μM for **9** and 1–200 μM for **19** and **20**). Excitation wavelengths were 350 nm for compound **9** and 360 nm for compounds **19** and **20**. The quenching constant K_{sv} was deduced from Stern–Volmer method where the ratio of fluorescence of the compound alone (F_0) over the fluorescence of the compound in the presence of CT-DNA (F) is presented as a function of CT-DNA concentration. In this configuration, $F_0/F = 1 + K_{sv}$[CT-DNA]. The slope K_{sv} is considered as an equilibrium constant for the static quenching process.

4.4.4. Circular dichroism

For circular dichroism, the various drugs (50 μM) were incubated with or without (control) a fixed or increasing concentrations of CT-DNA (from 0.1 to 100 μM) in BPE. The CD spectra were collected in a quartz cell of 10 mm pathlength from 480 to 230 nm using a J-810 Jasco spectropolarimeter at a controlled temperature of 20 °C fixed by a PTC-424S/L peltier type cell changer (Jasco) as described previously [23].

4.4.5. Topoisomerase I – mediated DNA relaxation

DNA intercalation evaluated by using pUC19 supercoiled plasmid DNA and human topoisomerase I (Topogen, USA) as previously described [24].

4.4.6. DNase I footprinting

DNase I footprinting experiments were conducted essentially as described [25]. The gels were exposed to storage screen for the appropriated delay at room temperature. The results were collected using a Pharos-PMI equipment (BioRad).

4.5. Cellular distribution

HT-29 colon carcinoma cells (30 000 cells) were plated on Lab-Tek glass culture chambers (Nunc) for 24 h prior to the addition of the various tested compounds (5 μM) for 16 h. Cells were then carefully washed twice with PBS prior to the addition of 250 nM of MitoFluor Red 588 (Mito-Red, Molecular Probes, Invitrogen) as a cytoplasmic marker for 30 min at 37 °C. After three washing of the cells using 500 μl of PBS, 2% of paraformalydehyde was added in PBS for 15 min and cells were washed again twice and dried. The slide was mounted on Vectashield (CliniSciences) and analyzed with an apotome-equipped fluorescence microscope (Zeiss) with 40× or 63× immersion lens. Images were collected using AxioVizion software.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.049.

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