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Abstract: This manuscript describes the synthesis, 3D-derived QSAR, antiproliferative activity and DNA binding properties of 2-amino, 5-amino and 2,5-diaminosubstituted benzimidazo[1,2-a]quinolines prepared by microwave assisted amination. Their antiproliferative effect was assessed on three tumor cell lines against colon, lung and breast carcinoma cell lines in vitro. The activities tested ranged from moderate to very strong. 2-amino-subsituted analogues demonstrated stronger antiproliferative activity compared to 5-amino, or 2,5-diamino substituted derivatives, while N-methyl or 3,5-dimethylpiperazinyl substituted analogues were the most active ones. Their DNA binding abilities and mode of interaction were evaluated using spectroscopic (melting temperature studies, UV/Visible and fluorescence spectra analyses, circular dichroism) and biochemical experiments (topoisomerase I-mediated DNA relaxation and DNase I footprinting experiments). From this series, only two compounds (36 and 37) evidenced strong DNA binding properties. Both 36 and its iodide salt 37 intercalate between adjacent base pairs of the DNA helix but the 37 did not show any effect on tumor cells. Only compound 33 presented a very weak topoisomerase I poisoning activity. 3D-QSAR analysis identified hydrogen bonding properties, hydrophobicity, molecular flexibility, and distribution of hydrophobic regions as the molecular properties with the highest influence on the antiproliferative activity against all three studied cell lines.

Zagreb, 16th December 2015.

COVER LETTER

Dear Prof. H. Galons,

Enclosed please find our manuscript entitled **„Antiproliferative potency of 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines: 3D QSAR study and DNA binding properties“** (Authors: Nataša Perin, Raja Nhili, Maja Cindrić, Branimir Bertoša, Darko Vušak, Irena Martin-Kleiner, William Laine, Grace Karminski-Zamola, Marijeta Kralj, Marie-Hélène David-Cordonnier and Marijana Hranjec*).

Neither this manuscript nor one with substantially similar content under our authorship has been published or submitted for publication elsewhere.

In the present paper we report describes the synthesis, 3D-derived QSAR, antiproliferative activity and DNA binding properties of 2-amino, 5-amino and 2,5-diaminosubstituted benzimidazo[1,2-*a*]quinolines. Biological evaluation of antiproliferative activities *in vitro* revealed that 2-amino-subsituted analogues demonstrated stronger antiproliferative activity compared to 5-amino or 2,5-diamino substituted derivatives while some selective compounds showed strong effects on tumour cell lines. The DNA binding abilities results revealed that two compounds evidenced strong DNA binding properties. The molecular properties with the highest influence on the antiproliferative activity against all studied cell lines were identified by 3D-QSAR analysis.

We hope that you will find the results obtained in this study interesting in terms of their possible use in improving the current therapeutic strategies in treating cancer, and that you will accept this manuscript for publication in European Journal of Medicinal Chemistry.

Sincerely yours,

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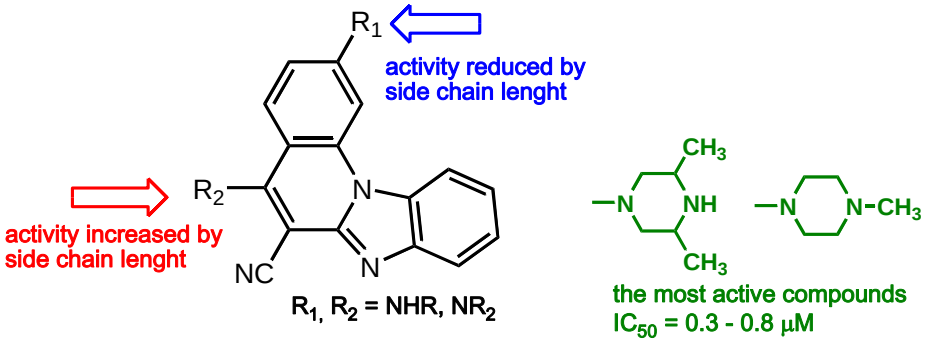
Graphical Abstract

Antiproliferative potency of 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines: 3D QSAR study and DNA binding properties

Nataša Perin[a], Raja Nhili[b], Maja Cindrić[a], Branimir Bertoša[c], Darko Vušak[c], Irena Martin-Kleiner[d], William Laine[b], Grace Karminski-Zamola[a], Marijeta Kralj[d], Marie-Hélène David-Cordonnier[b] and Marijana Hranjec[a*]



Research Highlights

- Amino substituted benzimidazo[1,2-*a*]quinolines with antiproliferative potency
- Amino side chains on different positios on tetracyclic skeleton
- Molecular properties identified by 3D-QSAR analysis
- 2-Amino subsituted analogues showed the strongest antiproliferative activity
- Compounds **36** and **37** evidenced strong DNA binding properties

Antiproliferative potency of 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines: 3D QSAR study and DNA binding properties

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Abstract

This manuscript describes the synthesis, 3D-derived QSAR, antiproliferative activity and DNA binding properties of 2-amino, 5-amino and 2,5-diaminosubstituted benzimidazo[1,2-*a*]quinolines prepared by microwave assisted amination. Their antiproliferative effect was assessed on three tumor cell lines against colon, lung and breast carcinoma cell lines *in vitro.* The activities tested ranged from moderate to very strong. 2-amino-subsituted analogues demonstrated stronger antiproliferative activity compared to 5-amino, or 2,5-diamino substituted derivatives, while *N*-methyl or 3,5-dimethylpiperazinyl substituted analogues were the most active ones. Their DNA binding abilities and mode of interaction were evaluated using spectroscopic (melting temperature studies, UV/Visible and fluorescence spectra analyses, circular dichroism) and biochemical experiments (topoisomerase I-mediated DNA relaxation and DNase I footprinting experiments). From this series, only two compounds (**36** and **37**) evidenced strong DNA binding properties. Both **36** and its iodide salt **37** intercalate between adjacent base pairs of the DNA helix but the **37** did not show any effect on tumor cells. Only compound **33** presented a very weak topoisomerase I poisoning activity. 3D-QSAR analysis identified hydrogen bonding properties, hydrophobicity, molecular flexibility, and distribution of hydrophobic regions as the molecular properties with the highest influence on the antiproliferative activity against all three studied cell lines.

Key words: benzimidazoles, benzimidazo[1,2-*a*]quinolines, 3D-QSAR, antiproliferative activity, DNA binding properties

1. Introduction

Benzimidazole scaffold represents one of the most important structural motifs widely existing in a variety of bioactive natural products and numerous of synthetic medical and biochemical agents possessing different chemical and pharmacological features [1–5]. Importantly, due to the fact that benzimidazoles are structural isosters with naturally occurring nucleotides, their derivatives play a crucial role in the function of many biologically important molecules and can easily interact with biomolecules like DNA, RNA or different proteins of the living systems. DNA molecule, which plays a central role in life processes, is still one of the principal targets in anticancer drug development strategies [6]. Thus, the understanding of the molecular basis for cytotoxicity by anticancer agents is very important for the rational development of novel, more selective and efficient agents with greater specificity of action [7]. The most used classes of chemotherapeutic agents comprise molecules that interact with DNA like intercalators, alkylating agents or groove binders [8]. Organic intercalators are a class of polyaromatic compounds which usually consist of planar and fused aromatic or heteroaromatic rings that can insert or intercalate between two adjacent base pairs of duplex DNA and inhibit nucleic acid synthesis [9]. The incorporation of the benzimidazole nuclei with another heteroaromatic skeleton is an important synthetic strategy in drug discovery and can lead to highly conjugated, planar benzannulated benzimidazoles, which have the ability to intercalate between adjacent DNA base pairs. High fluorescence intensity and possibility of interaction with important biomacromolecules of the living systems offer also their potential use as a fluorescent probes for detection of biological important molecules as DNA or different proteins in biomedicinal diagnostics [10, 11]. Moreover, quinolines as a one of the most important groups of nitrogen heterocycles, are important structural units widely existing in natural alkaloids, therapeutics and synthetic analogues with wide range of biological activities and have a great importance in the natural, medicinal and environmental sciences.

Quinoline-fused benzimidazoles, recently prepared and published in our research group, showed to be a very promising class of tetracyclic intercalators while biological activity studies comprising cytostatic evaluation, DNA/RNA interaction study, inhibition of topoisomerase I and II and proteomic profiling confirmed the anticancer potential of this class of compounds. Positively charged amidino substituted benzimidazo[1,2-*a*]quinolines intercalate into double-stranded DNA or RNA with the pronounced selectivity towards colon carcinoma cells, inhibited topoisomerase II and induced strong G2/M cell cycle arrest [12]. Very recently we have reported on the biological activity of 2-aminobenzimidazo[1,2-

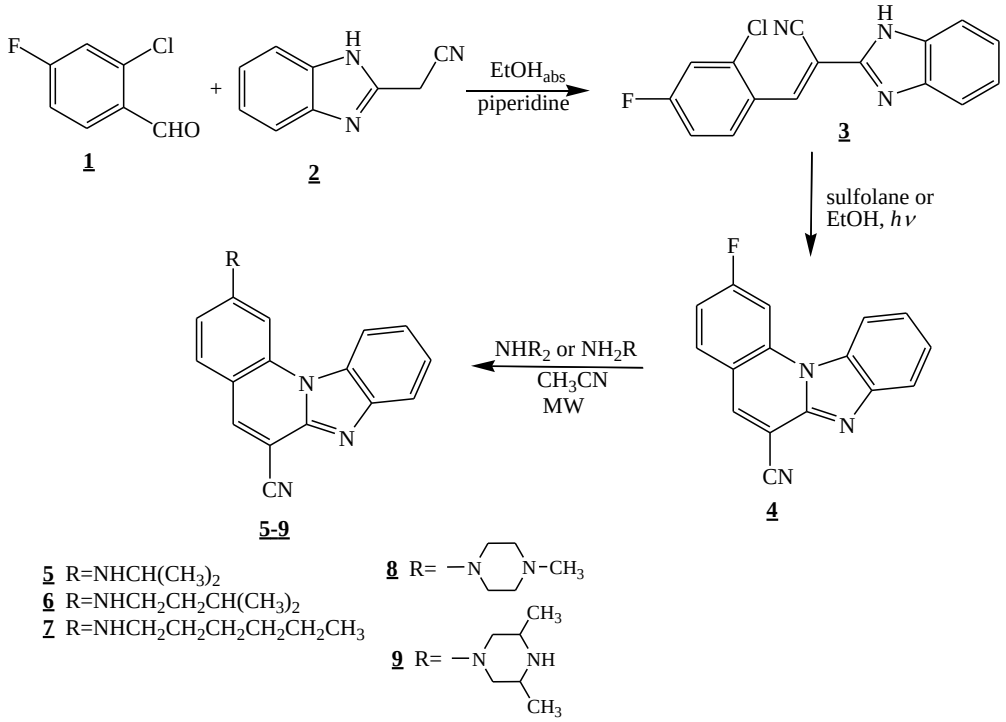
a]quinoline-6-carbonitriles with different lengths of the secondary or tertiary amino chains linked to the tetracyclic skeleton which significantly influenced the antiproliferative activity in submicromolar range of concentrations [13]. To study the influence of position change of amino substituents on the tetracyclic skeleton, we have also published the synthesis, antiproliferative activity and DNA binding properties of 5-aminobenzimidazo[1,2-*a*]quinoline-6-carbonitriles which showed moderate antiproliferative effect toward human cancer cell lines [14].

As a continuation of further development of these type of compounds and consideration of anticancer potential of benzimidazo[1,2-*a*]quinoline skeleton as a promising lead structure in the search for more selective and efficient chemotherapeutics, within this manuscript we have described the synthesis, antiproliferative effect and DNA binding properties of 2-amino, 5-amino and mostly 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines. All newly synthesized compounds were tested for their antiproliferative activity on the panel of three human tumour cell lines and for each cell line 3D-QSAR models were obtained.

2. Results and discussion

2.1. Chemistry

All newly prepared compounds were synthesized according to the two main procedures shown in Scheme 1 and Scheme 2, by the conventional methods of organic synthesis for the preparation of similar benzimidazole derivatives, starting from 4-fluorobenzaldehyde **1** or benzoylchlorides **10**–**11** and 2-cyanomethylbenzimidazole **2** which gave in the aldol reaction, in absolute ethanol using piperidine as a base, acyclic benzimidazole derivatives **3** and **12**–**13** in good yields (75%, 44%, 49%). 2-Fluorobenzimidazo[1,2-*a*]quinoline **4** (72%) as a main precursor for the synthesis of 2-aminobenzimidazo[1,2-*a*]quinolines was prepared by thermic cyclization in sulfolane from acyclic precursor **3**. Fused keto substituted benzimidazo[1,2-*a*]quinolines **14** and **15** were prepared by thermic cyclization in DMF using *t*-KOBu as a base from acyclic precursors **12** and **13** in good yields (57% and 96%). The main precursors for the synthesis of 5-amino and 2,5-diaminobenzimidazo[1,2-*a*]quinolines, chloro substituted benzimidazo[1,2-*a*]quinolines **16** (70%) and **17** (96%), were prepared in the reaction of compounds **14** and **15** with the $POCl_3$ and PCl_5.

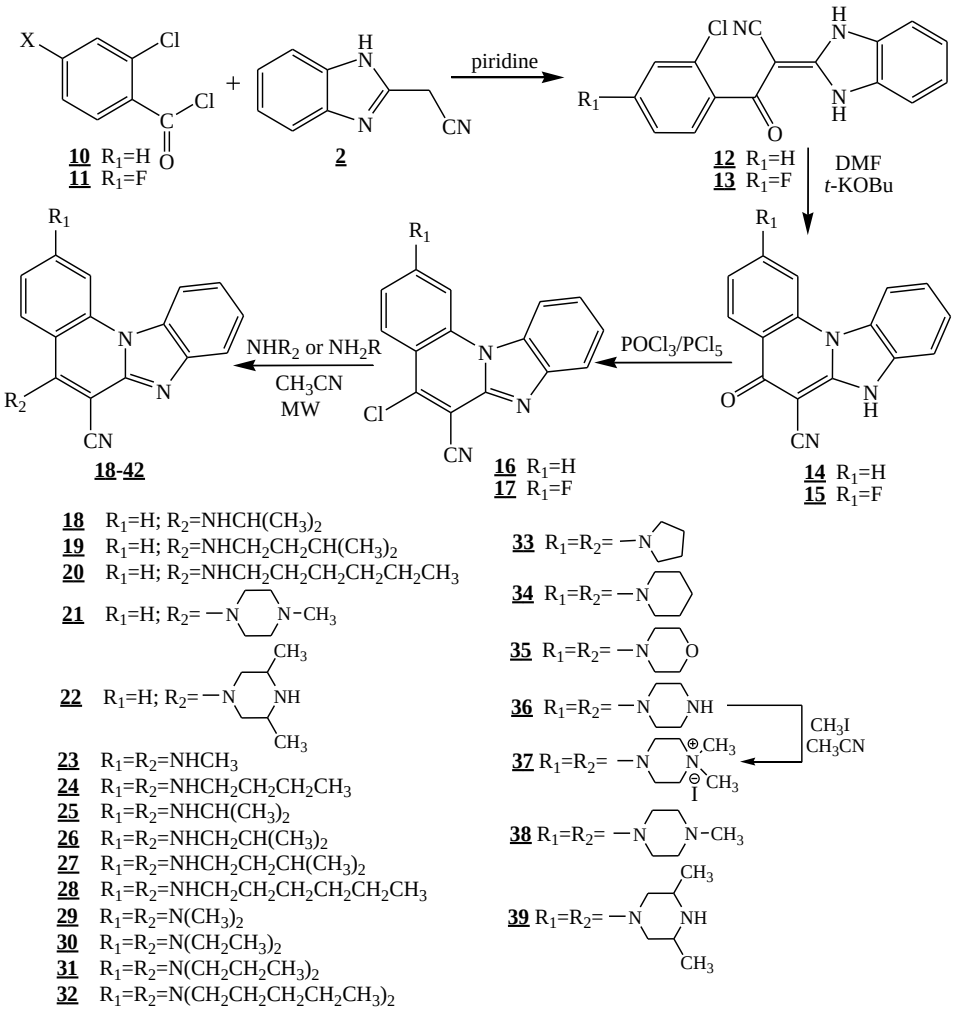


Scheme 1.

Based on the series of experiments which were undertaken in order to optimize the reaction times and yields and the fact that microwave synthesis provided shorter reaction time, highly increased yield as well as simple product isolation procedure, targeted amino and diamino substituted benzimidazo[1,2-*a*]quinolines **5**–**9** and **18**–**39** were finally prepared by using uncatalyzed microwave assisted amination in low to moderate yields (15% to 74%). This reaction was performed in acetonitrile with five to sevenfold excess of the corresponding amine by using 800 W power, 170 °C and 40 bar. *N*,*N*-dimethylated piperazinyl substituted compound **37** as a iodide salt was prepared from derivative **36** with an excess of methyl-iodide in 47% yield.

The structures of all prepared compounds were determined by the NMR analysis based on the analysis of H-H coupling constants as well as chemical shifts and by mass spectroscopy. The reaction of cyclization of acyclic precursors 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. Generally, ^{1}H NMR spectra of all amino substituted benzimidazo[1,2-*a*]quinolines **5**–**9** and **18**–**39** showed a downfield shift of the aromatic protons in comparison to halogeno substituted precursor **4** and **16** and **17**. Also, it can be observed the appearance of protons related to amino substituents in the aliphatic part both in ^{1}H and ^{13}C NMR.

^{1}H NMR spectra of tertiary amino substituted derivatives **21**–**22** and **33**–**39** showed a downfield shift of the H-1, H-3, H-4 and H-5 protons of quinoline part of molecule.



Scheme 2.

2.2. 3D-QSAR analysis

In order to explore physical and chemical properties with the highest influence on antiproliferative activities of investigated benzimidazo[1,2-*a*]quinolines, 3D-QSAR analysis was performed. Using antiproliferative activities of the compounds presented in this paper and similar compounds whose antiproliferative activities were previously measured in the same laboratory [13, 14], 3D-QSAR models were derived (Table 1, Figure 1). 3D-QSAR models **1**, **2**, and **3** were derived using antiproliferative activity data against H460, HCT 116 and MCF-7 cell lines, respectively.

Table 1. Statistical properties of 3D-QSAR models.

Cell line	Model	*nO* [a]	*LV* [b]	R^2	Q^{2} [c]	*SDEC*[d]	*SDEP*[e]
H460	**1**	49	5	0.817	0.489	0.345	0.577
HCT 116	**2**	50	5	0.790	0.427	0.370	0.611
MCF-7	**3**	50	5	0,857	0.614	0.266	0.437

[a] Number of objects used to build the model. [b] Number of latent variables. [c] Q^2 is the cross-validated predictive performance and is given by $Q^2 = 1 - \frac{\sum_{n=1}^{n}\left(y_{\exp(i)} - y_{pred(i)}\right)^2}{\sum_{n=1}^{n}\left(y_{\exp(i)} - \langle y_{\exp}\rangle\right)^2}$; where $y_{pred(i)}$ corresponds to the predicted and $y_{exp(i)}$ to the experimentally determined inhibition, pIC_{50} for the compound *i*, respectively. [d]*SDEC* is standard deviation of error of calculation. [e]*SDEP* is the standard deviation in cross-validated prediction and is given by $SDEP = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{\exp(i)} - y_{pred(i)}\right)^2}{n}}$.

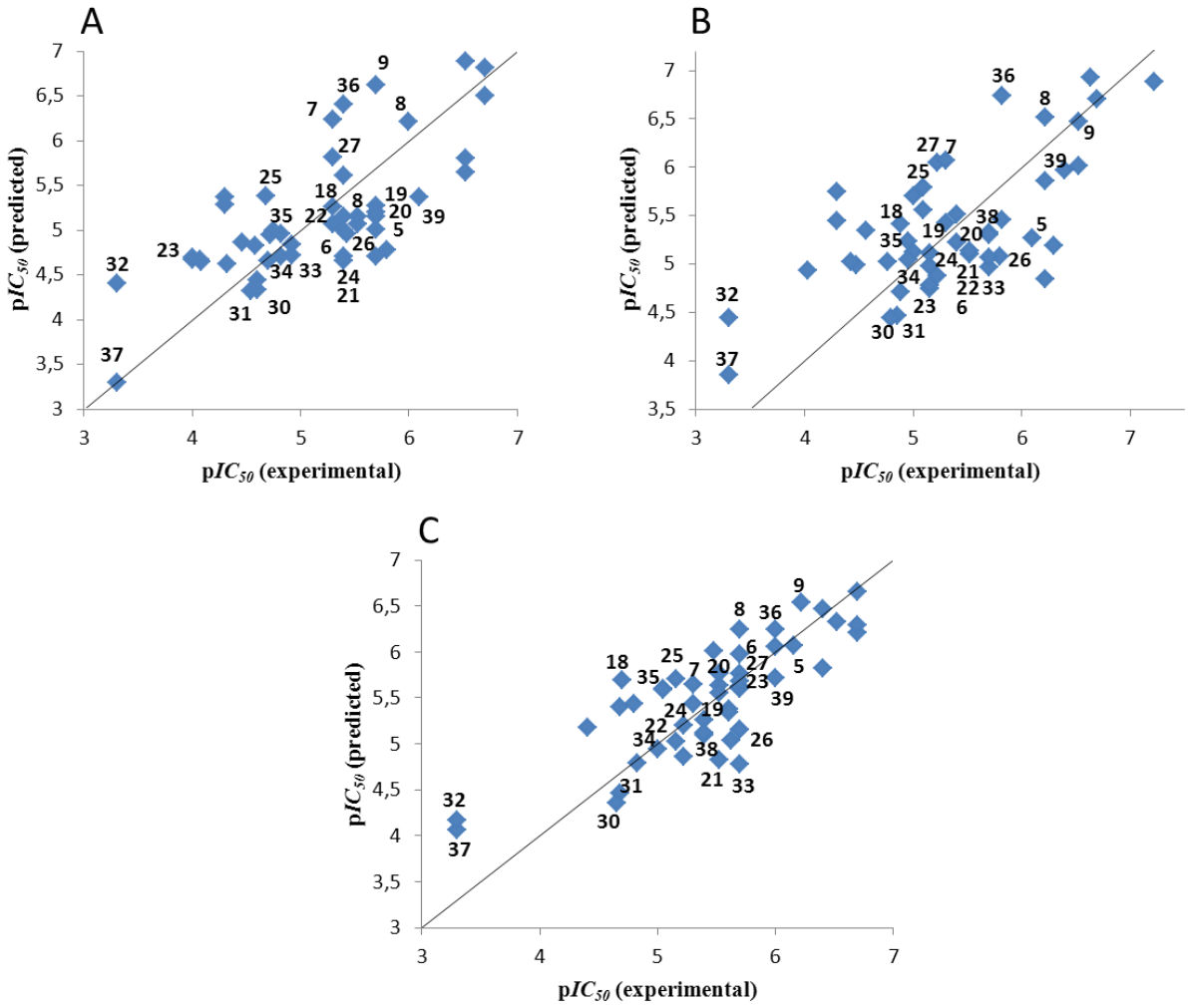


Figure 1. Predicted *vs* experimental antiproliferative activity (expressed as pIC_{50} – negative logarithmic value of concentration that causes 50% growth inhibition of the tumor cell lines of: A) model **1** (H460), B) model **2** (HCT 116), and C) model **3** (MCF-7).

QSAR analysis of the obtained models identified the molecular properties with the highest influence on antiproliferative activities against studied cell lines (Figure 2A). In case of model **1** (H460 cell line), the descriptors with the highest positive influence on antiproliferative activity are: molecular flexibility (FLEX_RB), descriptors related to H-bond properties (WN5, WN6, WO1-4, DRDRDO, DRACAC, DRACDO, ACACDO, ACACAC), protein binding (PB) and hydrophobicity (D3-7, ID2-4, CD3-6). The descriptors with the highest negative influence on antiproliferative activity against H460 cell line are: percentage of unionized species at different pH values (%FU4, %FU5), amphiphilic moment (A- vector that connects the center of hydrophobic and center of hydrophilic regions), and ratio of molecular volume and molecular surface (R). Similar descriptors are found as important for the activity against cell line HCT 116 cell line (Figure 2B). The highest positive influence have the descriptors related to H-bonding properties (WN6, WO1-WO5, DRDRDO, DRACAC, DRACDO, ACACDO, ACACAC), molecular flexibility (FLEX_RB), hydrophobic regions (D3-D8, ID3, ID4, CD3, CD4, DD1-DD4), and protein binding (PB), while the highest negative influence have the percentage of unionized species at different pH values (%FU4, %FU5), amphiphilic moment (A), and integy moment (ID1 - vector that connects the center of a molecule and the center of hydrophobic regions). In the case of the MCF-7 cell line (Figure 2C), again the descriptors related to H-bonds properties (WN6, WO1-WO5, DRDRDO, DRACAC, DRACDO, ACACDO, ACACAC), molecular flexibility (FLEX_RB), hydrophobicity (D3-D8, ID3, ID4, CD3, CD4, DD1-DD4), and protein binding (PB) have the highest positive influence, while the descriptors related to the unbalance between the centre of mass of a molecule and the barycenter of its hydrophobic regions (A – amphiphilic moment, ID1 – integy moment), partition coefficient of cyclohexane/water (LOGP c-Hex), and ratio of molecular volume and molecular surface (R) have the highest negative influence on the antiproliferative activity. Therefore, increase of H-bonding properties, hydrophobicity and flexibility, as well as decrease of unbalance in distribution of hydrophobic and hydrophilic regions in the molecule, should lead to increase in compound’s antiproliferative activity against all three studied cell lines.

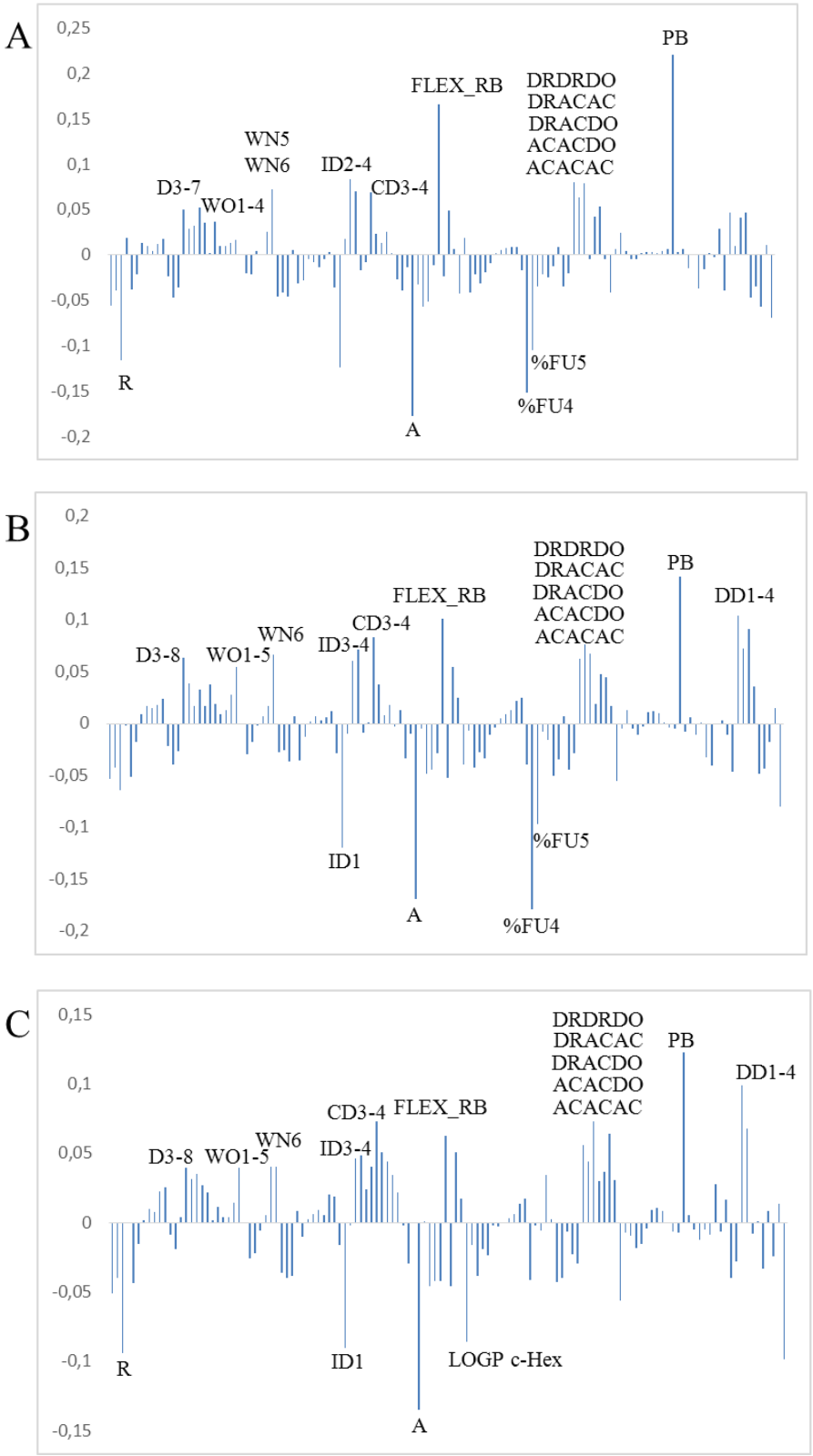


Figure 2. PLS coefficients of 3D-derived QSAR model: A) model **1** (H460), B) model **2** (HCT 116) and C) model **3** (MCF-7). Descriptors with the highest impact on the activity are labeled; list and description of all 128 VolSurf+ descriptors is given in the VolSurf+ manual [15].

2.3. Biological Results and Discussion

The antiproliferative activities of new 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines were tested on human colon, breast and lung tumor cell lines (Table 1). Among 2-amino substituted benzimidazo[1,2-*a*]quinolines (**5**–**9**) three compounds strongly inhibited the growth of all cell lines. The most cytotoxic (IC_{50} < 1 μM) were *N*-methylpiperazinyl and dimethyl-piperazinyl substituted compounds **8** and **9**. Comparing the activity of compounds with different lengths of amino side chains (**5**–**7**), it can be seen that the activity is reduced by the side chain length, possibly because the longer side chains increase the amphiphilic moment (A) and increase the unbalance in distribution of hydrophobic regions in the molecule. Since these properties are found by QSAR analysis as negatively correlated with the activities, their increase leads to decrease of antiproliferative activities. In case of compounds **18, 19,** and **20** the opposite effect of the side chain length on the activities was noticed because the position of the chain is different and in this case the longer aliphatic chain decreases the unbalance in distribution of hydrophobic regions in the molecule and has the opposite effect on the activity. Therefore, the activities of compounds **19** and **20** are comparable to compounds **6** and **7** that bear the same side chains, while the activity of compound **5** is much higher than the activity of the compound **20**. Among the most abundant group of 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines, piperazinyl-substituted compounds (**36**, **38**, **39**) showed the most pronounced antiproliferative activity, whereby dimethyl-piperazinyl substituted benzimidazo[1,2-*a*]quinoline (**39**) was the most active one. The most pronounced antiproliferative activity of above mentioned compounds could be due the presence of another N heteroatom which contributes to the additional interactions with potential biological targets. Interestingly, *N*,*N*-dimethyl substituted quaternary iodide salt **37** was completely inactive, similarly to previously published *N*,*N*-dimethyl substituted quaternary iodide salt with amino substituent at position 5 on benzimidazo[1,2-*a*]quinoline skeleton. This is in agreement with QSAR analysis that identified the percentage of unionised species as negatively correlated descriptor with the activities against H460 and HCT 116 cell lines. Piperidinyl and morpholinyl-substituted analogues **34**, **35** showed moderate activity (IC_{50} 10–20 μM), while pyrrolydinyl-substituted analogue **33** showed pronounced activity on HCT 116 and MCF-7 cell lines. 2,5-diamino substituted derivatives with secondary amino side chains **23**–**27** showed moderate antiproliferative activity while disubstituted derivatives **30**–**32** with tertiary amino side chains showed decrease of antiproliferative activity which lead to the fact that secondary amine side

chain is generally slightly preferred over tertiary amine side chain. Also, disubstituted derivatives **28** and **32** with the longest amino side chains did not show antiproliferative effect towards tested cell lines. In general, there was no significant difference in the sensitivity towards the tested compounds between the cell lines, which may indicate common mechanisms of action of new compounds. The only exception is compound **23**, which showed less pronounced activity toward H460 cell line.

Table 1. IC_{50} values (in μM) [a]

	Cell lines		
Compounds	**HCT116**	**MCF-7**	**H460**
5	0.8±0.2	0.7±0.4	2±0.8
6	3±0.3	2±0.09	4±0.3
7	5±0.3	5±0.6	5±2
8	0.6±0.3	2±1	1±0.8
9	0.3±0.08	0.6±0.2	0.5±0.04
18	13±0.4	20±3	5±2
19	5±1	4±0.5	2±0.5
20	4±1	3±0.6	2±0.3
21	3±0.2	3±0.3	4±0.6
22	3±0.2	6±2	4±0.3
23	7±3	2±1	≥100
24	7±1	5±0.8	4±1
25[b]	≥10	7±1	21±2
26	2±0.2	2±0.6	3±0.2
27	6±0.3	2±0.05	5±0.2
28	>100	>100	>100
30	16±4	22±3	25±2
31	14±5	21±6	29±0.2
32	>100	>100	>100
33	2±0.5	2±0.6	12±1
34	11±3	10±3	15±2
35	11±5	9±1	18±8
36	1.5±0.3	1±0.001	4±0.6
37	>100	>100	>100
38	2±0.5	4±1	3±0.5
39	0.4±0.09	1±0.2	0.8±0.3

[a] IC_{50}; the concentration that causes 50% growth inhibition
[b] The highest tested concentration was 10 μM.

2.4. DNA Binding Properties

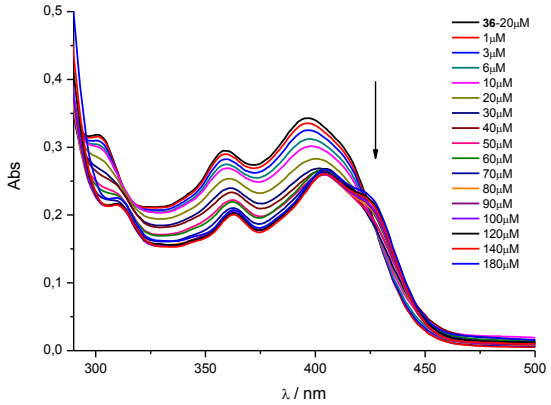
As a first approach to evaluate drug/DNA binding, we challenged compounds **23**, **24**, **26** and **30** to **37** for double strand DNA stabilization of calf thymus-DNA (CT-DNA) using DNA melting temperature experiments. As part of their potential DNA interaction, the temperature for which half of the double strand DNA is melted to single stand DNA (Tm) may increase upon DNA binding of the tested compound (positive ΔTm) suggesting stabilization of the DNA helix, or, rarely decrease, evidencing DNA denaturation by the evaluated compound (negative ΔTm).

From this series, only compounds **36** and **37** presented potent DNA helix stabilization whereas none of the other tested compounds changed the intrinsic melting temperature of CT-DNA. For compound **36**, the ΔTm values were 15.9 °C and 20.1 °C at 1:2 and 1:4 drug/DNA ratios, respectively. By contrast, compound **37** induced-stabilization of the DNA helix required more than 24 °C at 1:4 drug/DNA ratio to achieve denaturation of half of the DNA (Tm), a value that could not be determined using higher drug/DNA ratios since less than half of the DNA denaturation could be achieved using temperature as high as 100 °C, suggesting very strong DNA stabilization upon binding of **37** to DNA. Those two DNA binding compounds were further evaluated using spectrophotometric analysis.

First, DNA binding propensity of **36** and **37** was underlined using UV/Visible spectra measurement evidencing the modification of the absorption spectra of **36** (Figure 3A) or **37** in the presence of increasing concentrations of CT-DNA with hypochromic and bathochromic effects, a spectral modification frequently associated with DNA intercalation.

However, spectral changes did not revealed any isosbestic point (typically arguing for single binding mode), suggesting that the mode of binding of the compounds to the DNA helix is rather a complex binding. In parallel, the intrinsic fluorescent properties of the compounds were also evaluated in the presence of increasing CT-DNA/compound ratios. Fluorescent measurement evidenced an increase in the fluorescent properties of compound **36** (Figure 3B).

A



B

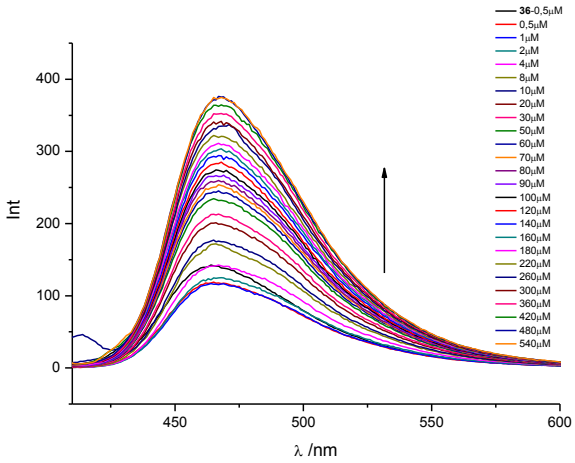


Figure 3. A) UV/Vis titration of compound **36** with CT-DNA, B) Fluorimetric titration of compound **36** with CT-DNA

In order to get an insight in the binding mode of **36** and **37**, two approaches were used: circular dichroism spectra analysis and topoisomerase I-induced DNA relaxation. The first approach is based on the difference on the absorbance between right and left orientated light at a wavelength from 230 to 530 nm. The absence of intrinsic CD of **36** and **37** was validated using the highest evaluated dose (60 µM, Figure 4, dashed lines). Upon binding of either **36** or **37** on CT-DNA, the intrinsic CD of CT-DNA (50 µM, pointed lines) was changed with (i) a decrease of the intensity of the positive intrinsic CD of CT-DNA at 275 nm (suggesting modifications in the base stacking and/or the ellipticity of the DNA helix) and (ii) the appearance of a new negative induced circular dichroic (ICD) signal from 290 to 440 nm corresponding to the absorption bands of either compounds **36** or **37** (suggesting the intercalation of the planar chromophore between adjacent base pairs of the DNA, thus

creating a new dichroic signal). Both changes are dependent on the compounds concentration (top to bottom).

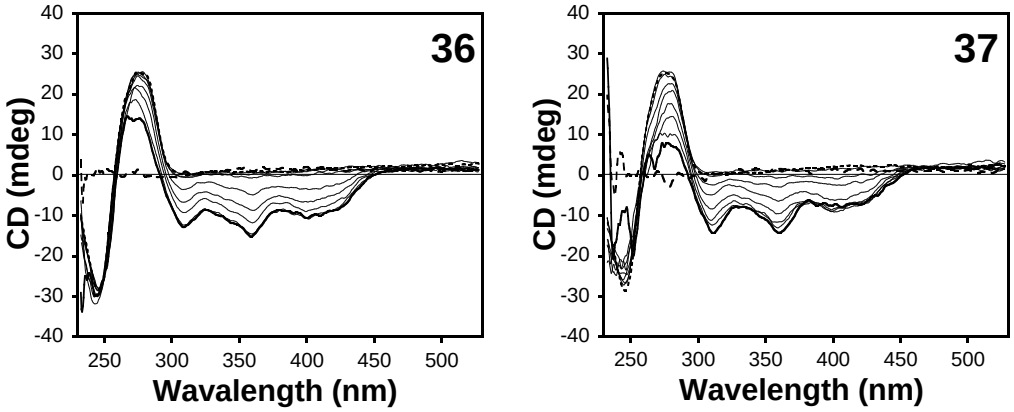


Figure 4. Circular dichroism spectra. CT-DNA (50 µM of base pairs) was incubated alone (dashed bold lines) with increasing concentrations of **36** or **37** from 1 to 60 (full bold lines) µM (top to bottom). Pointed bold lines correspond to the CD spectra of the compound alone (60 µM).

In order to comfort the DNA intercalation profile of **36** and **37** in comparison with other derivatives, we used the property of the DNA topoisomerase I enzyme to resolve the constraints of a negatively supercoiled circular DNA and to generate positively supercoiled circular DNA generated upon intercalation between adjacent base pairs [16]. Intercalation between adjacent base pairs lengthens the DNA helix, diminishes the angle of rotation between each base pair and thus induces major constraints to the DNA structure. Topoisomerase I enzyme resolves the constraints that are generated upon negative supercoiling of the plasmid DNA when produced in bacteria (*Sc*) (Figure 5). Topoisomerase I thus generates different DNA topoisomers (*Topo*) corresponding to different number of released supercoils in the circular plasmid and up to fully relaxed circular DNA (*Rel*). Blockade of topoisomerase I re-ligation step by poison drugs such as camptothecin (CPT) results in the generation of nicked plasmid (*Nck*) that is only opened on one strand whereas DNA intercalation is associated with the formation of progressive positive supercoiled plasmid with each topoisomers being more difficult to assign individually due to binding of the compounds that reduces DNA migration within the agarose gel, as seen with **36** and **37** in Figure 5. This experiment confirms the DNA intercalation propensity of **36** and **37** but not the

other evaluated non DNA binders. This DNA intercalation is not associated with topoisomerase I poisoning effect of **36** and **37** as demonstrated by the lack of increase in the intensity of the nicked form (*Nck*) of the DNA. Such intercalation process is not associated with sequence selective binding as evaluated using DNase I footprinting experiments of increasing concentrations of either **36** or **37** (data not shown).

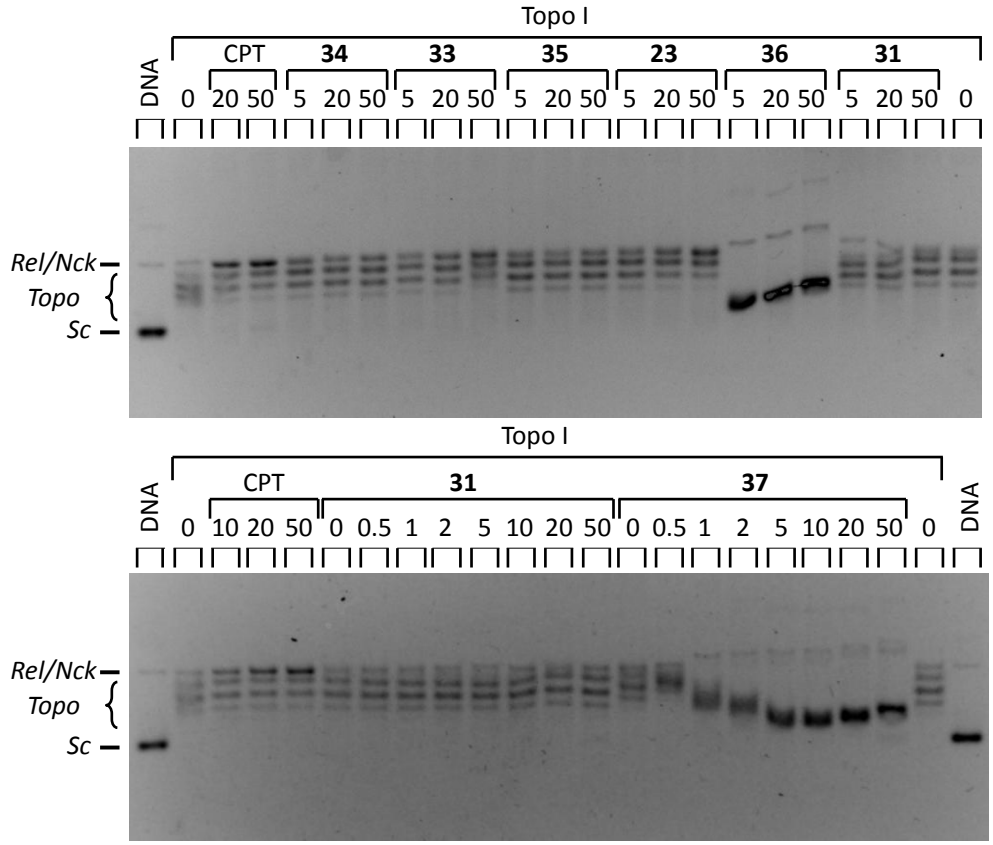


Figure 5. Topoisomerase I-induced DNA relaxation. The pUC19 supercoiled plasmid ("DNA") was incubated with topoisomerase I ("0") alone or in the presence of the indicated concentrations (μM) of compounds as specified on the top of the lanes. *Rel*, relaxed DNA; *Nck*, nicked DNA; *Topo*, topoisomers; *Sc*, supercoiled DNA.

Compounds **33** and **23** presented a small increase of the band corresponding to the relaxed/nicked form. However, only compound **33** presented a very weak Topoisomerase I poisoning activity as confirmed upon migration in ethidium bromide containing agarose gels (see the weak increase in the nicked form using 100 μM as the highest drug concentration) whereas no poisoning activity was evidenced using compound **23** (Figure 6).

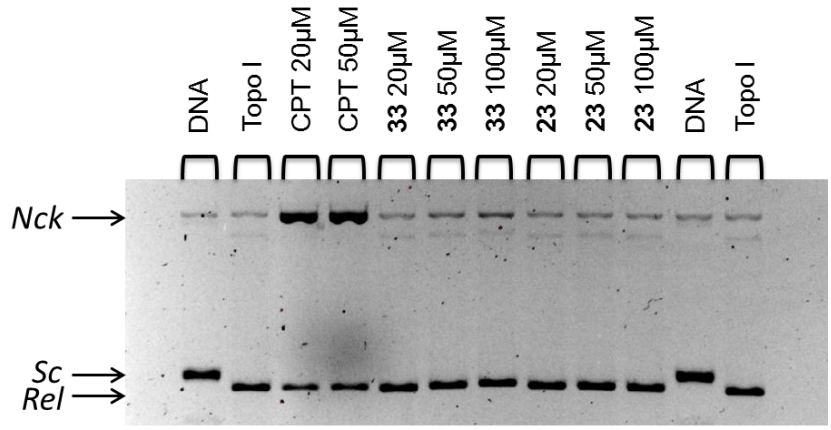


Figure 6. Topoisomerase I poisoning effects of compounds 33 and 23. Native supercoiled pUC19 plasmid DNA (lanes "DNA") was incubated with topoisomerase I in the absence (lanes "Topo I") or presence of increasing concentrations of compounds **33** and **23** and the reference drugs camptothecin (CPT). DNA samples were separated under electrophoresis on an ethidium bromide containing 1% agarose gel. The gels were photographed under UV light. *Sc*, supercoiled; *Rel*, relaxed; *Nck*; nicked; *Lin*, linear.

3. Conclusion

Herein we present the synthesis of 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines with different type and length of amino side chains placed on either one or two positions on quinoline nuclei with potential chemotherapeutic activities. Amino-substituted compounds were synthesized by uncatalyzed microwave assisted amination from corresponding chloro or fluoro substituted precursors. The antiproliferative activities tested on human colon, breast and lung tumor cell lines ranged from moderate to very strong. 2-amino-subsituted analogues demonstrated stronger antiproliferative activity compared to 5-amino, or 2,5-diamino substituted derivatives. In general, *N*-methyl or 3,5-dimethylpiperazinyl substituted analogues were the most active ones (submicromolar IC_{50} concentrations), while derivatives with long and/or branched side chains were less active (mono-substituted analogues) or completely inactive (di-substituted analogues). 3D-QSAR analysis showed that the molecular properties with the highest positive and the highest negative influence on antiproliferative activity are common for all three studied cell lines. The increase compound's H-bonding properties, hydrophobicity and flexibility, as well as the

decrease of unbalance in distribution of hydrophobic and hydrophilic regions in the molecule, should increase the compound's antiproliferative activity against all three studied cell lines. By evaluating some compounds in both series for their DNA binding propensities, we evidenced that only two of those selected compounds were potent DNA binding agents, associated with strong DNA helix stabilization (as evidenced using melting temperature studies) and intercalative mode of DNA interaction (Figures 4 and 5). This DNA binding is not associated with sequence selective binding (data not shown). The DNA intercalation process does not correlate with their cytotoxic effects evaluated on both colon (HCT116), breast (MCF-7) and lung (H460) carcinoma since if the piperazinyl compound **36** as a potent cytotoxic effect in all cell lines (1.5, 1 and 4 µM, respectively), the *N,N*-dimethyl substituted quaternary iodide salt **37** is totally inactive (>100µM) in the three cellular models which is in agreement with the QSAR analysis.

4. Experimental part

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 and 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 of 2-amino substituted benzimidazo[1,2-*a*]quinolines

4.2.1. 2-(2-Benzimidazolyl)-3-(2-chloro-4-fluorophenyl)acrylonitrile ***3.*** Compound **3** was prepared from **2** (1.00 g, 6.37 mmol) and 2-chloro-4-fluorobenzaldehyde **1** (1.00 g, 6.31 mmol) in absolute ethanol (7 ml) after refluxing for 2 h and recrystallization from ethanol to yield 1.41 g (75%) of slightly yellow crystals; m.p. 234–237 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 13.31 (s, 1H, $NH_{benzim.}$), 8.46 (s, 1H, $H_{arom.}$), 8.21 (dd, 1H, J_1=6.22 Hz, J_2=9.07 Hz, $H_{arom.}$), 7.74 (dd, 1H, J_1=2.69 Hz, J_2=8.82 Hz, $H_{arom.}$), 7.66 (bs, 2H, $H_{benzim.}$), 7.51 (dt, 1H, J_1=2.81 Hz, J_2=8.07 Hz, $H_{benzim.}$), 7.30 (bs, 1H, $H_{benzim.}$); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 163.77 (s, J_{CF}=251.09 Hz), 147.05 (s), 143.73 (s), 140.69 (d), 135.62 (s, J_{CF}=7.20 Hz), 135.32 (s), 131.93 (d, J_{CF}=9.64 Hz), 128. 41 (s, J_{CF}=3.52 Hz), 124.54 (d), 122.97 (d), 119.96 (d), 119.17 (d, J_{CF}=25.36 Hz), 115.80 (s), 115.81 (d, J_{CF}=21.71 Hz), 112.23 (d), 107.16 (s); Found: C, 64.81; H, 3.04; N, 6.40. Calc. for $C_{17}H_{12}N_4$: C, 64.55; H, 3.05; N, 6.38%; MS (ESI): *m/z*= 298.1 ($[M+1]^+$).

4.2.2. 2-Fluorobenzimidazo[1,2-a]quinoline-6-carbonitrile ***4.*** Compound **3** (0.50 g, 1.68 mmol) was dissolved in 4 ml of sulfolane and reaction mixture was heated for 25 minutes at 280 °C. The cooled mixture was poured into water (15 ml) and the resulting product was filtered off and recrystallizated from ethanol (450 ml) to obtain a yellow powder (0.32 g, 72%); m.p. 250–254 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.77 (s, 1H, $H_{arom.}$), 8.71 (d, 1H, J=8.25 Hz, $H_{arom.}$), 8.52 (dd, 1H, J_1=2.10 Hz, J_2=10.89 Hz, $H_{arom.}$), 8.20 (dd, 1H, J_1=6.44 Hz, J_2=8.42 Hz, $H_{arom.}$), 7.99 (d, 1H, J=8.21 Hz, $H_{arom.}$), 7.61 (t, 1H, J=6.82 Hz, $H_{arom.}$), 7.58–7.51 (m, 2H, $H_{arom.}$); ^{13}C NMR (DMSO-d_6): δ/ppm= 164.67 (s, J_{CF}=251.84 Hz), 144.35 (s), 143.63 (s), 140.01 (d), 136.85 (s, J_{CF}=12.50 Hz), 133.75 (d, J_{CF}=11.01 Hz), 130.22 (s), 125.28 (d), 123.70 (d), 120.13 (d), 118.14 (s, J_{CF}=2.28 Hz), 115.26 (s), 114.83 (d), 113.82 (d, J_{CF}=23.37 Hz), 103.08 (d, J_{CF}=27.68 Hz), 100.35 (s); Found: C, 73.27; H, 3.10; N, 16.05. Calc. for $C_{17}H_{12}N_4$: C, 73.56; H, 3.09; N, 16.00%; MS (ESI): *m/z*= 262.1 ($[M+1]^+$).

4.2.3. General method for preparation of compounds ***5–9***

Compounds **5–9** were prepared using microwave irradiation, at optimized power and reaction time, from compound **4** 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 dichlormethane/methanol as eluent.

4.2.3.1. 2-N-i-propylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **5**. Compound **5** was prepared using above described method from **4** (100 mg, 0.38 mmol) and *i*-propylamine (0.15 mL, 1.76 mmol) after 6 h of irradiation to yield 46 mg (43%) of yellow crystals; m.p. 230–233 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.51 (d, 1H, J=8.10 Hz, $H_{arom.}$), 8.46 (s, 1H, $H_{arom.}$), 7.94 (dd, 1H, J_1=1.28 Hz, J_2=7.50 Hz, $H_{arom.}$), 7.81 (d, 1H, J=8.85 Hz, $H_{arom.}$), 7.77 (s, 1H, $H_{arom.}$), 7.58 (dt, 1H, J_1=1.28 Hz, J_2=7.30 Hz, $H_{arom.}$), 7.52 (dt, 1H, J_1=1.28 Hz, J_2=7.30 Hz, $H_{arom.}$), 7.27 (d, 1H, J=7.56 Hz, NH), 6.91 (dd, 1H, J_1=1.75 Hz, J_2=8.85 Hz, $H_{arom.}$), 3.98–3.87 (m, 1H, CH), 1.28 (d, 6H, J=6.59 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 152.97 (s), 145.90 (s), 144.18 (d), 140.12 (d), 138.11 (s), 132.93 (d), 130.40 (s), 124.70 (d), 122.40 (d, 2C), 119.56 (d), 116.76 (s), 114.33 (d, 2C), 111.04 (s), 91.92 (s), 43.36 (d), 22.24 (q, 2C); Found: C, 75.73; H, 5,52; N, 18,75. Calc. for $C_{19}H_{16}N_4$: C, 75.98; H, 5.37; N, 18.65%; MS (ESI): m/z= 301.2 ($[M+1]^+$).

4.2.3.2. 2-N-i-pentylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **6**. Compound **6** was prepared using above described method from **4** (80 mg, 0.30 mmol) and isopentylamine (0.25 mL, 2.15 mmol) after 5 h of irradiation to yield 29 mg (29%) of yellow crystals; m.p. 200–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.50 (d, 1H, J=8.36 Hz, $H_{arom.}$), 8.46 (s, 1H, $H_{arom.}$), 7.94 (dd, 1H, J_1=1.40 Hz, J_2=7.77 Hz, $H_{arom.}$), 7.80 (d, 1H, J=8.93, $H_{arom.}$), 7.74 (s, 1H, $H_{arom.}$), 7.57 (dt, 1H, J_1=1.00 Hz, J_2=7.39 Hz, $H_{arom.}$), 7.51 (dt, 1H, J_1=1.28 Hz, J_2=7.52 Hz, $H_{arom.}$), 7.31 (t, 1H, J=5.17 Hz, NH), 6.90 (dd, 1H, J_1=1.70 Hz, J_2=8.85 Hz, $H_{arom.}$), 3.32 (q, 2H, J=7.08 Hz, CH_2), 1.80–1.75 (m, 1H, CH), 1.57 (q, 2H, J=6.85 Hz, CH_2), 0.98 (d, 6H, J=6.54 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 153.84 (s), 145.92 (s), 144.21 (s), 140.10 (d), 138.11 (s), 132.81 (d), 130.40 (s), 124.34 (d), 122.34 (d), 119.56 (d), 116.73 (s), 114.32 (d), 111.16 (s), 92.00 (s), 40.60 (t), 37.28 (t), 25.23 (d), 22.37 (q, 2C); Found: C, 76.93; H, 5.99; N, 17.08. Calc. for $C_{21}H_{20}N_4$: C, 76.80; H, 6.14; N, 17.06%; MS (ESI): m/z= 329.3 ($[M+1]^+$).

4.2.3.3. 2-N-hexylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **7**. Compound **7** was prepared using above described method from **4** (90 mg, 0.34 mmol) and hexylamine (0.24 mL, 1.20 mmol) after 3 h of irradiation to yield 60 mg (54%) of yellow crystals; m.p. 97–104 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm= 8.48 (d, 1H, J=8.28 Hz, $H_{arom.}$), 8.43 (s, 1H, $H_{arom.}$), 7.92 (d, 1H, J=7.62 Hz, $H_{arom.}$), 7.78 (d, 1H, J=8.76 Hz, $H_{arom.}$), 7.70 (s, 1H, $H_{arom.}$), 7.56 (t, 1H, J=7.62 Hz, $H_{arom.}$), 7.50 (dt, 1H, J_1=1.06 Hz, J_2=7.72 Hz, $H_{arom.}$), 7.31 (t, 1H, J=5.10 Hz, NH), 6.88 (dd, 1H, J_1=1.74 Hz, J_2=8.76 Hz, $H_{arom.}$), 1.66 (p, 2H, J=7.26 Hz, CH_2),

1.44 (p, 2H, *J*=7.36 Hz, CH_2), 1.36–1.31 (m, 4H, CH_2), 0.88 (t, 3H, *J*=7.08 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 153.87 (s), 145.85 (s), 144.00 (s), 140.14 (d), 130.34 (s), 124.73 (d), 122.38 (d, 2C), 119.45 (d), 116.72 (s), 114.34 (d, 2C), 111.14 (s), 91.61 (s), 42.40 (t), 31.06 (t), 28.36 (t), 26.27 (t), 22.68 (t), 13.90 (q); Found: C, 76.98; H, 6.28; N, 16.74. Calc. for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36%; MS (ESI): *m/z*= 343.2 ($[M+1]^+$).

4.2.3.4. 2-[N-(4-N-methylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **8**. Compound **8** was prepared using above described method from **4** (90 mg, 0.34 mmol) and 4-*N*-methylpiperazine (0.10 mL, 0.90 mmol) after 3 h of irradiation to yield 65 mg (56%) of yellow crystals; m.p. 267–273 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.56 (s, 1H, $H_{arom.}$), 8.55 (d, 1H, *J*=7.68 Hz, $H_{arom.}$), 7.94 (dd, 1H, J_1=1.36 Hz, J_2=7.48 Hz, $H_{arom.}$), 7.91 (d, 1H, *J*=9.15 Hz, $H_{arom.}$), 7.80 (d, 1H, *J*=1.05 Hz, $H_{arom.}$), 7.59 (t, 1H, *J*=6.78 Hz, $H_{arom.}$), 7.54 (dt, 1H, J_1=1.42 Hz, J_2=7.34 Hz, $H_{arom.}$), 7.33 (dd, 1H, J_1=1.72 Hz, J_2=9.25 Hz, $H_{arom.}$), 3.61 (t, 4H, *J*=4.80 Hz, CH_2), 2.54 (t, 4H, *J*=5.09 Hz, CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 154.61 (s), 146.15 (s), 144.57 (s), 140.34 (d), 138.60 (s), 132.83 (d), 130.78 (s), 125.38 (d), 123.46 (d), 120.17 (d), 116.87 (s), 115.35 (d), 113.02 (d), 112.84 (s), 98.13 (d), 94.88 (s), 54.79 (t, 2C), 47.14 (t, 2C), 46.16 (t, 2C); Found: C, 73.98; H, 5.71; N, 20.31. Calc. for $C_{21}H_{19}N_5$: C, 73.88; H, 5.61; N, 20.51%; MS (ESI): *m/z*= 342.2 ($[M+1]^+$).

4.2.3.5. 2-[N-(3,5-dimethylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **9**. Compound **9** was prepared using above described method from **4** (90 mg, 0.34 mmol) and 2,6-dimethylpiperazine (0.150 g, 1.30 mmol) after 6 h of irradiation to yield 39 mg (33%) of yellow powder; m.p. 263–268 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.47 (s, 1H, $H_{arom.}$), 8.43 (dd, 1H, J_1=1.19Hz, J_2=7.13 Hz, $H_{arom.}$), 7.94 (dd, 1H, J_1=1.41 Hz, J_2=7.56 Hz, $H_{arom.}$), 7.84 (d, 1H, *J*=9.12 Hz, $H_{arom.}$), 7.68 (s, 1H, $H_{arom.}$), 7.58 (dt, 1H, J_1=1.21, J_2=6.59 Hz, $H_{arom.}$), 7.53 (dt, 1H, J_1=1.28 Hz, J_2=6.69 Hz, $H_{arom.}$), 7.27 (dd, 1H, J_1=1.66 Hz , J_2=9.13 Hz, $H_{arom.}$), 4.01 (d, 2H, *J*=10.53 Hz, CH_2), 2.95–2,90 (m, 2H, CH), 2.55 (d, 2H, *J*=11.70 Hz, CH_2), 1.13 (d, 6H, *J*=6.21 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 154.27 (s), 146.15 (s), 144.56 (s), 140.18 (d), 138.57 (s), 132.74 (d), 130.72 (s), 125.29 (d), 123.38 (d), 120.13 (d), 116.92 (s), 115.14 (d), 112.93 (d), 112.53 (s), 97.85 (d), 94.51 (s), 53.67 (t, 2C), 50.61 (d, 2C), 19.56 (q, 2C); Found: C, 74.40; H, 6.08; N, 19.52. Calc. for $C_{22}H_{21}N_5$: C, 74.34; H, 5.96; N, 19.70%; MS (ESI): *m/z*= 356.3 ($[M+1]^+$).

4.3. Synthesis of 5-amino substituted benzimidazo[1,2-*a*]quinolines

4.3.1. 2-(2-benzimidazolyl)-3-keto-(2-chlorophenyl)acrylonitrile ***12*** [14].

A solution of 1.57 g (10.00 mmol) 2-cyanomethylbenzimidazole **2** and 1.33 mL (10.50 mmol) 2-chloro-benzoylchloride **10** 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 (300 MHz, DMSO-d_6): 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 (75 MHz, DMSO-d_6): 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).

4.3.2. 5-ketobenzimidazo[1,2-a]quinoline-6-carbonitrile ***14*** [14].

A solution of 0.50 g (1.69 mmol) 2-(2-benzimidazolyl)-3-keto-(2-chlorophenyl)acrylonitrile **12** 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 (300 MHz, DMSO-d_6): 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 (150 MHz, DMSO-d_6): 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).

4.3.3. 5-chlorobenzimidazo[1,2-a]quinoline-6-carbonitrile ***16*** [14].

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 (300 MHz, DMSO-d_6): 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 (150 MHz, DMSO-d_6): 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).

4.3.4. General method for preparation of compounds 18–22

Compounds **18**–**22** were prepared using microwave irradiation, at optimized reaction time with power 800 W and 40 bar pressure, from compound **16** 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.

*4.3.4.1. 5-N-i-propylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **18**.*

Compound **18** was prepared using above described method from **16** (100 mg, 0.38 mmol) and *i*-propylamine (0.10 mL, 1.12 mmol) after 2 h of irradiation to yield 42 mg (39%) of white powder; m.p. 260–263 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.66 (d, 1H, J=8.08 Hz, $H_{arom.}$), 8.55 (dd, 1H, J_1=0.92 Hz, J_2=7.76 Hz, $H_{arom.}$), 8.43 (d, 1H, J=8.08 Hz, $H_{arom.}$), 7.93 (dt, 1H, J_1=0.92 Hz, J_2=8.12 Hz, $H_{arom.}$), 7.75 (dd, 1H, J_1=0.60 Hz, J_2=7.86 Hz, $H_{arom.}$), 7.59 (t, 1H, J=7.60 Hz, $H_{arom.}$), 7.55 (t, 1H, J=6.81 Hz, NH), 7.42 (t, 1H, J=7.50 Hz, $H_{arom.}$), 7.33 (dt, 1H, J_1=1.17 Hz, J_2=7.40 Hz, $H_{arom.}$), 4.90–4.79 (m, 1H, CH), 5.99 (d, 6H, J=6.68 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 149.15 (s), 148.99 (s), 144.67 (s), 135.05 (s), 132.96 (d), 130.79 (s), 124.78 (d), 124.13 (d), 123.88 (d), 121.28 (d), 118.36 (d), 117.53 (s), 116.54 (s), 116.14 (d), 113.62 (d), 71.84 (s), 45.87 (d), 23.00 (q, 2C); Found: C, 75.77; H, 5.69; N, 18.54. Calc. for $C_{19}H_{16}N_4$: C, 75.98; H, 5.37; N, 18.65%; MS (ESI): *m/z*= 301.2 ($[M+1]^+$).

*4.3.4.2. 5-N-i-pentylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **19**.*

Compound **19** was prepared using above described method from **16** (50 mg, 0.18 mmol) and *i*-pentylamine (0.11 mL, 0.90 mmol) after 2 h of irradiation to yield 9 mg (15%) of white powder; m.p. 105–108 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.67 (d, 1H, J=8.65 Hz, $H_{arom.}$), 8.49 (d, 1H, J=8.35 Hz, $H_{arom.}$), 8.43 (d, 1H, J=8.35 Hz, $H_{arom.}$), 8.11 (t, 1H, J=5.68 Hz, NH), 7.93 (t, 1H, J=7.46 Hz, $H_{arom.}$), 7.74 (d, 1H, J=7.76 Hz, $H_{arom.}$), 7.59 (t, 1H, J=7.76 Hz, $H_{arom.}$), 7.41 (t, 1H, J=7.46 Hz, $H_{arom.}$), 7.33 (t, 1H, J=7.61 Hz, $H_{arom.}$), 3.91 (q, 2H, J=6.86 Hz, CH_2), 1.67–1.58 (m, 1H, CH), 1.43 (q, 2H, J=7.16 Hz, CH_2), 0.88 (q, 6H, J=6.27 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 149.95 (s), 149.03 (s), 144.67 (s), 135.02 (s), 132.95 (d), 130.81 (s), 124.40 (d), 124.28 (d), 123.88 (d), 121.27 (d), 118.34 (d), 117.60 (s), 116.57 (s), 116.25 (d), 113.62 (d), 71.46 (s), 42.59 (t), 37.21 (t), 22.39 (d), 22.09 (q, 2C); Found: C, 77.05; H, 6.02; N, 16.93. Calc. for $C_{21}H_{20}N_4$: C, 76.80; H, 6.14; N, 17.06%; MS (ESI): *m/z*= 329.3 ($[M+1]^+$).

*4.3.4.3. 5-N-hexylaminobenzimidazo[1,2-a]quinoline-6-carbonitrile **20**.*

Compound **20** was prepared using above described method from **16** (100 mg, 0.36 mmol) and hexylamine (0.20 mL, 1.52 mmol) after 3 h of irradiation to yield 56 mg (48%) of yellow powder; m.p. 121–125 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm= 8.65 (d, 1H, *J*=8.34 Hz, $H_{arom.}$), 8.44 (dd, 1H, J_1=0.84 Hz, J_2=8.37 Hz, $H_{arom.}$), 8.41 (d, 1H, *J*=8.27 Hz, $H_{arom.}$), 8.07 (t, 1H, *J*=5.58 Hz, NH), 7.90 (dt, 1H, J_1=0.87 Hz, J_2=7.73 Hz, $H_{arom.}$), 7.73 (d, 1H, *J*=7.80 Hz, $H_{arom.}$), 7.57 (t, 1H, *J*=7.59 Hz, $H_{arom.}$), 7.40 (t, 1H, *J*=7.47 Hz, $H_{arom.}$), 7.32 (dt, 1H, J_1=0.93 Hz, J_2=7.73 Hz, $H_{arom.}$), 3.86 (q, 2H, *J*=6.58 Hz, CH_2), 1.78 (p, 2H, *J*=7.19 Hz, CH_2), 1.42 (p, 2H, *J*=7.28 Hz, CH_2), 1.31 (m, 4H, CH_2), 0.86 (t, 3H, *J*=7.08 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 149.88 (s), 149.00 (s), 144.65 (s), 135.00 (s), 132.11 (d), 130.79 (s), 124.31 (d), 124.25(d), 123.86 (d), 121.25 (d), 118.32 (d), 117.54 (s), 116.51 (s), 116.22 (d), 113.59 (d), 71.42(s), 44.05 (t), 30.92 (t), 29.37 (t), 25.56 (t), 21.96 (t), 13.60 (q); Found: C, 77.36; H, 6.18; N, 16.46. Calc. for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36%; MS (ESI): *m/z*= 343.2 ($[M+1]^+$).

*4.3.4.4. 5-[N-(4-N-methylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **21**.*

Compound **21** was prepared using above described method from **16** (100 mg, 0.36 mmol) and 4-*N*-methylpiperazine (0.10 mL, 0.90 mmol) after 3 h of irradiation to yield 53 mg (43%) of yellow powder; m.p. 253–257 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.77 (d, 1H, *J*=8.46 Hz, $H_{arom.}$), 8.59 (d, 1H, *J*=7.86 Hz, $H_{arom.}$), 8.17 (dd, 1H, J_1=0.78 Hz, J_2=8.04 Hz, $H_{arom.}$), 7.95 (dt, 1H, J_1=0.95 Hz, J_2=7.38 Hz, $H_{arom.}$), 7.89 (dd, 1H, J_1=1.08 Hz, J_2=7.44 Hz, $H_{arom.}$), 7.63 (t, 1H, *J*=7.70 Hz, $H_{arom.}$), 7.52 (t, 1H, *J*=7.20 Hz, $H_{arom.}$), 7.46 (dt, 1H, J_1=1.06 Hz, J_2=7.69 Hz, $H_{arom.}$), 3.63 (t, 4H, *J*=4.58 Hz, CH_2), 2.66 (t, 4H, *J*=4.51 Hz, CH_2), 2.33 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 157.28 (s), 146.94 (s), 144.31 (s), 136.10 (s), 133.18 (d), 130.63 (d), 127.53 (d), 124.65 (d), 124.60 (d), 122.58 (d), 119.46 (s), 119.41 (d), 116.33 (d), 115.91 (s), 114.26 (d), 89.09 (s), 54.88 (t, 2C), 52.10 (t, 2C), 45.77 (q); Found: C, 73.76; H, 5.81; N, 20.40. Calc. for $C_{21}H_{19}N_5$: C, 73.88; H, 5.61; N, 20.51%; MS (ESI): *m/z*= 342.2 ($[M+1]^+$).

*4.3.4.5. 5-[N-(3,5-dimethylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **22**.*

Compound **22** was prepared using above described method from **16** (100 mg, 0.36 mmol) and 2,6-dimethylpiperazine (0.100 g, 0.87 mmol) after 3 h of irradiation to yield 60 mg (47%) of yellow powder; m.p. 247–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.73 (d, 1H, *J*=8.46 Hz, $H_{arom.}$), 8.56 (d, 1H, *J*=7.86 Hz, $H_{arom.}$), 8.14 (d, 1H, *J*=8.19 Hz, $H_{arom.}$), 7.93 (t,

1H, *J*=7.88 Hz, $H_{arom.}$), 7.87 (d, 1H, *J*=7.86 Hz, $H_{arom.}$), 7.61 (t, 1H, *J*=7.53 Hz, $H_{arom.}$), 7.51 (t, 1H, *J*=7.45 Hz, $H_{arom.}$), 7.44 (t, 1H, *J*=7.63 Hz, $H_{arom.}$), 3.60 (d, 2H, *J*=11.16 Hz, CH_2), 3.10 (m, 2H, CH), 3.04 (d, 2H, *J*=11.11 Hz, CH_2), 1.05 (d, 6H, *J*=5.97 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 157.06 (s), 147.13 (s), 144.36 (s), 136.14 (s), 133.08 (d), 130.54 (s), 127.53 (d), 127.68 (d), 124.60 (d), 124.45 (d), 122.47 (d), 119.49 (s), 119.41 (s), 119.33 (d), 116.30 (d), 115.99 (s), 114.19 (d), 100.41 (s), 59.01 (t, 2C), 50.85 (d, 2C), 39.24 (q, 2C); Found: C, 74.18; H, 6.16; N, 19.60. Calc. for $C_{22}H_{21}N_5$: C, 74.34; H, 5.96; N, 19.70%; MS (ESI): *m/z*= 356.3 ($[M+1]^+$).

4.4. Synthesis of 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines

*4.4.1. 2-(2-benzimidazolyl)-3-keto-(4-fluoro-2-chlorophenyl)acrylonitrile **13***.

A solution of 1.63 g (10.40 mmol) 2-cyanomethylbenzimidazole **2** and 2,00 g (10.40 mmol) 4-fluoro-2-chlorobenzoylchloride **11** in pyridine (13 mL) was refluxed for 1.5 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 (1.36 g, 49%); m.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm=13.10 (bs, 2H, NH), 7.59–7.51 (m, 4H, $H_{arom.}$), 7.36–7,30 (m, 3H, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 184.75 (s), 162.40 (s, J_{CF}=247.17 Hz), 150.84 (s), 137.56 (s, J_{CF}=3.50 Hz), 131.24 (s, J_{CF}=10.75 Hz), 130.86 (s), 130.65 (d, J_{CF}=9.17 Hz), 124.14 (d, 2C), 120.30 (s), 117.46 (d, J_{CF}=25.01 Hz), 114.83 (d, J_{CF}=21.13 Hz), 112.74 (d, 2C), 67.77 (s).

*4.4.2. 2-fluoro-5-ketobenzimidazo[1,2-a]quinoline-6-carbonitrile **15***.

A solution of 1.00 g (3.37 mmol) 2-(2-benzimidazolyl)-3-keto-(4-fluoro-2-chlorophenyl)acrylonitrile **13** and 0.88 g *t*-KOBu in DMF (12 mL) was refluxed for 2 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 (0.85 g, 96%); m.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.29 (dd, 1H, J_1=6.99 Hz, J_2=8.79 Hz, $H_{arom.}$), 8.19 (d, 1H, *J*=8.10 Hz, $H_{arom.}$), 8.13 (dd, 1H, J_1=2.30 Hz, J_2=11.06 Hz, $H_{arom.}$), 7.48 (dd, 1H, J_1=0.84 Hz, J_2=7.86 Hz, $H_{arom.}$), 7.27 (dd, 1H, J_1=0.95 Hz, J_2=6.20 Hz, $H_{arom.}$), 7.23 (dt, 1H, J_1=2.19 Hz, J_2=8.53 Hz, $H_{arom.}$), 7.11(dt, 1H, J_1=1.10 Hz, J_2=7.87 Hz, $H_{arom.}$); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 171.64 (s), 163.59 (s, J_{CF}=245.44 Hz), 154.08 (s), 137.13 (s, J_{CF}=5.85 Hz), 130.90 (s), 128.02 (d, J_{CF}=10.45 Hz), 122.99 (d), 121.30 (s), 120.27 (s), 119.06 (d), 116.35 (d), 112.31 (d), 110.33 (d, J_{CF}=21.68 Hz), 101.83 (d, J_{CF}=27.06 Hz), 73.59 (s).

*4.4.3. 5-chloro-2-fluorobenzimidazo[1,2-a]quinoline-6-carbonitrile **17**.*

A solution of 0.60 g (2.15 mmol) 2-fluoro-5-ketobenzimidazo[1,2-*a*]quinoline-6-carbonitrile and 0.24 g (1.15 mmol) PCl_5 in $POCl_3$ (12 mL) was refluxed for 1.5 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 (0.62 g, 96%); m.p. 250–257 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.71 (d, 1H, J=8.49 Hz, $H_{arom.}$), 8.58 (dd, 1H, J_1=2.27 Hz, J_2=10.37 Hz, $H_{arom.}$), 8.41 (dd, 1H, J_1=6.06 Hz, J_2=9.12 Hz, $H_{arom.}$), 8.00 (dd, 1H, J_1=1.32 Hz, J_2=7.67 Hz, $H_{arom.}$), 7.67–7.60 (m, 2H, $H_{arom.}$), 7.57 (dt, 1H, J_1=1.34 Hz, J_2=7.61 Hz, $H_{arom.}$); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 165.29 (s, J_{CF}=253.77 Hz), 144.34 (s), 143.82 (s), 142.18 (s), 136.82 (s, J_{CF}=12.18 Hz), 130.91 (d, J_{CF}=10.95 Hz), 130.21 (s), 125.66 (d), 124.13 (d), 120.27 (d), 116.32 (s, J_{CF}=1.61 Hz), 114.99 (d), 114.01 (d, J_{CF}=23.40 Hz), 113.32 (s), 103.43 (d, J_{CF}=27.53 Hz), 101.64 (s).

4.4.4. General method for preparation of compounds 23–39

Compounds **23**–**39** were prepared using microwave irradiation, at optimized reaction time with power 800 W and 40 bar pressure, from compound **17** 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.

*4.4.4.1. 2,5-di-(N-methylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **23**.*

Compound **23** was prepared using above described method from **17** (50 mg, 0.50 mmol) and a 33% solution of methylamine in ethanol (0.26 mL, 1.90 mmol) after 12 h of irradiation to yield 35 mg (57%) of light brown powder; m.p. >280 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.29 (d, 1H, J=8.07 Hz, $H_{arom.}$), 8.08 (d, 1H, J=9.21 Hz, $H_{arom.}$), 7.89 (d, 1H, J=5.16 Hz, NH), 7.69 (dd, 1H, J_1=0.78 Hz, J_2=7.89 Hz, $H_{arom.}$), 7.55 (d, 1H, J=1.62 Hz, $H_{arom.}$), 7.41 (t, 1H, J=7.52 Hz, $H_{arom.}$), 7.33 (dt, 1H, J_1=1.03 Hz, J_2=7.73 Hz, $H_{arom.}$), 7.06 (d, 1H, J=4.62 Hz, NH), 6.78 (dd, 1H, J_1=1.86 Hz, J_2=9.18 Hz, $H_{arom.}$), 3.39 (d, 3H, J=4.98 Hz, CH_3), 2.93 (d, 3H, J=4.59 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 153.99 (s, 2C), 152.41 (s), 137.26 (s), 131.06 (s), 126.07 (d), 124.61 (d), 121.41 (d), 118.93 (s), 117.96 (d), 113.94 (d), 109.72 (d), 104.98 (s), 96.56 (d), 94.79 (s), 82.64 (s), 32.41 (q), 29.74 (q); Found: C, 71.60; H, 5.09; N, 23.31. Calc. for $C_{18}H_{15}N_5$: C, 71.74; H, 5.02; N, 23.24%; MS (ESI): *m/z*= 302.4 ($[M+1]^+$).

*4.4.4.2. 2,5-di-(N-butylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **24**.*

Compound **24** was prepared using above described method from **17** (100 mg, 0.34 mmol) and *n*-butylamine (0.18 mL, 1.80 mmol) after 4 h of irradiation to yield 75 mg (57%) of light yellow powder; m.p. 150–153 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm= 8.26 (d, 1H, *J*=8.22 Hz, $H_{arom.}$), 8.16 (d, 1H, *J*=9.18 Hz, $H_{arom.}$), 7.67 (d, 1H, *J*=7.86 Hz, $H_{arom.}$), 7.64 (t, 1H, *J*=6.09 Hz, NH), 7.61 (s, 1H, $H_{arom.}$), 7.37 (t, 1H, *J*=7.67 Hz, $H_{arom.}$), 7.28 (dt, 1H, J_1=1.08 Hz, J_2=7.76 Hz, $H_{arom.}$), 6.94 (t, 1H, *J*=5.22 Hz, NH), 6.77 (dd, 1H, J_1=1.98 Hz, J_2=9.18 Hz, $H_{arom.}$), 3.81 (q, 2H, *J*=6.88 Hz, CH_2), 3.25 (q, 2H, *J*=6.36 Hz, CH_2), 1.74 (m, 1H, CH_2), 1.63 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 0.96 (t, 3H, *J*=7.38 Hz, CH_3), 0.94 (t, 3H, *J*=7.38 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 153.19 (s), 151.18 (s), 150.61 (s), 145.43 (s), 137.37 (s), 131.28 (s), 126.40 (d), 124.08 (d), 120.98 (d), 119.06 (s), 118.42 (d), 113.74 (d), 109.01 (d), 105.08 (s), 97.17 (d), 68.35 (s), 44.09 (t), 42.51 (t), 32.27 (t), 31.09 (t), 20.25 (t), 19.72 (t), 14.24 (q, 2C); Found: C, 74.75; H, 7.00; N, 18.25. Calc. for $C_{24}H_{27}N_5$: C, 74.72; H, 7.06; N, 18.17%; MS (ESI): *m/z*= 386.5 ($[M+1]^+$).

*4.4.4.3. 2,5-di-(N-i-propylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **25**.*

Compound **25** was prepared using above described method from **17** (100 mg, 0.34 mmol) and *i*-propylamine (0.40 mL, 2.35 mmol) after 7 h of irradiation to yield 31 mg (26%) of white powder; m.p. 240–245 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.27 (d, 1H, *J*=8.19 Hz, $H_{arom.}$), 8.21 (d, 1H, *J*=9.24 Hz, $H_{arom.}$), 7.68 (d, 1H, *J*=7.80 Hz, $H_{arom.}$), 7.62 (d, 1H, *J*=1.30 Hz, $H_{arom.}$), 7.38 (t, 1H, *J*=7.56 Hz, $H_{arom.}$), 7.29 (t, 1H, *J*=7.40 Hz, $H_{arom.}$), 7.11 (d, 1H, *J*=8.55 Hz, NH), 6.90 (d, 1H, *J*=7.68 Hz, NH), 6.78 (dd, 1H, J_1=1.29 Hz, J_2=9.03 Hz, $H_{arom.}$), 4.83–4.72 (m, 1H, CH), 3.91–3.80 (m, 1H, CH), 1.38 (t, 6H, *J*=6.30 Hz, CH_3), 1.25 (t, 6H, *J*=6.27 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 152.37 (s, 2C), 152.33 (s), 150.53 (s), 145.44 (s), 137.49 (s), 131.28 (s), 126.93 (d), 124.10 (d), 121.02 (d), 118.93 (s), 118.45 (d), 113.73 (d), 109.14 (d), 105.06 (s), 97.56 (d), 68.90 (s), 46.12 (d), 43.66 (d), 23.67 (q, 2C), 22.78 (q, 2C); Found: C, 73.84; H, 6.53; N, 19.63. Calc. for $C_{22}H_{23}N_5$: C, 73.92; H, 6.49; N, 19.59%; MS (ESI): *m/z*= 358.2 ($[M+1]^+$).

*4.4.4.4. 2,5-di-(N-i-butylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **26**.*

Compound **26** was prepared using above described method from **17** (100 mg, 0.34 mmol) and *i*-butylamine (0.50 mL, 5.04 mmol) after 6 h of irradiation to yield 25 mg (19%) of yellow crystals; m.p. 128–133 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.29 (d, 1H, *J*=8.10 Hz, $H_{arom.}$), 8.19 (d, 1H, *J*=9.24 Hz, $H_{arom.}$), 7.76 (t, 1H, *J*=6.16 Hz, NH), 7.68 (d, 1H, *J*=7.86 Hz,

$H_{arom.}$), 7.67 (s, 1H, $H_{arom.}$), 7.37 (t, 1H, *J*=7.37 Hz, $H_{arom.}$), 7.30 (dt, 1H, J_1=1.05 Hz, J_2=7.67 Hz, $H_{arom.}$), 7.04 (t, 1H, *J*=5.48 Hz, NH), 6.81 (dd, 1H, J_1=1.64 Hz, J_2=9.17 Hz, $H_{arom.}$), 3.62 (t, 2H, *J*=6.63 Hz, CH_2), 3.10 (t, 2H, *J*=6.11 Hz, CH_2), 2.20–2.11 (m, 1H, CH), 1.99–1.91 (m, 1H, CH), 1.03 (d, 6H, *J*=6.66 Hz, CH_3), 0.98 (d, 6H, *J*=6.63 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 153.39 (s), 151.25 (s), 150.60 (s), 145.44 (s), 137.39 (s), 131.29 (s), 126.41 (d), 124.08 (d), 120.97 (d), 118.97 (s), 118.43 (d, 2C), 113.73 (d, 2C), 105.11 (s), 51.38 (t), 50.60 (t), 28.72 (d), 28.13 (d), 20.83 (q, 2C), 20.04 (q, 2C); Found: C, 74.25; H, 7.10; N, 18.56. Calc. for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06; N, 18.17%; MS (ESI): *m/z*= 386.3 ($[M+1]^+$).

*4.4.4.5. 2,5-di-(N-i-pentylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **27**.*

Compound **27** was prepared using above described method from **17** (200 mg, 0.72 mmol) and *i*-pentylamine (0.17 mL, 1.50 mmol) after 6 h of irradiation to yield 34 mg (28%) of white powder; m.p. 111–113 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.27 (d, 1H, *J*=8.19 Hz, $H_{arom.}$), 8.13 (d, 1H, *J*=9.26 Hz, $H_{arom.}$), 7.68 (d, 1H, *J*=7.48 Hz, $H_{arom.}$), 7.62 (s, 1H, $H_{arom.}$), 7.61 (t, 1H, *J*=5.68 Hz, NH), 7.38 (t, 1H, *J*=7.66 Hz, $H_{arom.}$), 7.28 (t, 1H, *J*=7.83 Hz, $H_{arom.}$), 6.93 (t, 1H, *J*=4.63 Hz, NH), 6.78 (d, 1H, *J*=9.26 Hz, $H_{arom.}$), 3.85 (q, 2H, *J*=6.88 Hz, CH_2), 3.26 (q, 2H, *J*=7.48 Hz, CH_2), 1.81–1.69 (m, 2H, CH), 1.66 (q, 2H, *J*=7.12 Hz, CH_2), 1.55 (q, 2H, *J*=6.77 Hz, CH_2), 0.97 (d, 6H, *J*=5.26 Hz, CH_3), 0.94 (d, 6H, *J*=5.33 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 157.72 (s), 150.90 (s), 150.13 (s), 145.06 (s), 136.99 (s), 130.86 (s), 130.52 (s), 125.91 (d), 123.55 (d), 120.92 (d), 118.34 (s), 117.97(d), 113.18 (d), 108.11 (d), 104.86 (s), 96.85 (d), 40.65 (t), 40.16 (t), 38.47 (t), 37.51 (t), 25.39 (d), 25.28 (d), 22.40 (q, 4C); Found: C, 75.37; H, 7.54; N, 17.09. Calc. for $C_{26}H_{31}N_5$: C, 75.51; H, 7.56; N, 16.93%; MS (ESI): *m/z*= 414.3 ($[M+1]^+$).

*4.4.4.6. 2,5-di-(N-hexylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **28**.*

Compound **28** was prepared using above described method from **17** (100 mg, 0.36 mmol) and hexylamine (0.27 mL, 3.90 mmol) after 6 h of irradiation to yield 58 mg (41%) of light yellow powder; m.p. 156–160 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm= 8.26 (d, 1H, *J*=8.13 Hz, $H_{arom.}$), 8.13 (d, 1H, *J*=9.15 Hz, $H_{arom.}$), 7.69 (s, 1H, $H_{arom.}$), 7.68 (d, 1H, *J*=7.42 Hz, $H_{arom.}$), 7.61 (s, 1H, NH), 7.37 (t, 1H, *J*=7.44 Hz, $H_{arom.}$), 7.28 (dt, 1H, J_1=0.99 Hz, J_2=7.68 Hz, $H_{arom.}$), 6.97 (t, 1H, *J*=5.13 Hz, NH), 6.76 (dd, 2H, J_1=1.53 Hz, J_2=9.09 Hz, $H_{arom.}$), 3.80 (t, 2H, *J*=6.45 Hz, CH_2), 3.24 (q, 2H, *J*=6.29 Hz, CH_2), 1.74 (p, 2H, *J*=7.29 Hz, CH_2), 1.64 (p, 2H, *J*=7.14 Hz, CH_2), 1.49–1.37 (m, 4H, CH_2), 1.37–1.28 (m, 8H, CH_2), 0.89

(t, 3H, *J*=6.75 Hz, CH_3), 0.87 (t, 3H, *J*=6.81 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 153.17 (s), 151.16 (s), 150.62 (s), 145.45 (s), 137.39 (s), 131.29 (s), 126.39 (d), 124.06 (d, 2C), 120.24 (d, 2C), 118.96 (s), 118.41 (d), 113.72 (d), 105.10 (s), 44.37 (t), 42.88 (t), 31.69 (t), 31.44 (t), 30.11 (t), 28.97 (t), 26.79 (t), 26.08 (t), 22.58 (t), 22.49 (t), 14.39 (q), 14.31 (q); Found: C, 76.19; H, 7.96; N, 15.85. Calc. for $C_{28}H_{35}N_5$: C, 76.15; H, 7.99; N, 15.86%; MS (ESI): *m/z*= 442.6 ($[M+1]^+$).

4.4.4.7. 2,5-di-(N,N-dimethylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile ***29***.

Compound **29** was prepared using above described method from **17** (260 mg, 0.88 mmol) and dimethylamine (0.90 mL, 13.60 mmol) after 4 h of irradiation to yield 152 mg (52%) of light brown powder; m.p. 235–238 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.25 (d, 1H, *J*=8.52 Hz, $H_{arom.}$), 7.89 (d, 1H, *J*=9.30 Hz, $H_{arom.}$), 7.79 (d, 1H, *J*=7.65 Hz, $H_{arom.}$), 7.45 (t, 1H, *J*=7.46 Hz, $H_{arom.}$), 7.40 (t, 1H, *J*=8.25 Hz, $H_{arom.}$), 7.37 (s, 1H, $H_{arom.}$), 6.92 (d, 1H, *J*=8.19 Hz, $H_{arom.}$), 3.29 (s, 6H, CH_3), 3.19 (s, 6H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 158.74 (s), 152.65 (s), 148.57 (s), 144.76 (s), 138.11 (s), 130.41 (s), 129.49 (d), 124.04 (d), 121.59 (d), 118.68 (d), 117.18 (s), 113.77 (d), 109.29 (d), 107.69 (s), 95.52 (d), 81.69 (s), 44.57 (q, 2C), 39.81 (q, 2C); Found: C, 72.89; H, 5.83; N, 21.28. Calc. for $C_{20}H_{19}N_5$: C, 72.93; H, 5.81; N, 21.26%; MS (ESI): *m/z*= 330.4 ($[M+1]^+$).

4.4.4.8. 2,5-di-(N,N-diethylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile ***30***.

Compound **30** was prepared using above described method from **17** (80 mg, 0.27 mmol) and diethylamine (0.12 mL, 1.10 mmol) after 5 h of irradiation to yield 36 mg (34%) of yellow crystals; m.p. 211–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.27 (d, 1H, *J*=7.65 Hz, $H_{arom.}$), 7.95 (d, 1H, *J*=9.39 Hz, $H_{arom.}$), 7.86 (dd, 1H, J_1=1.32 Hz, J_2=7.56 Hz, $H_{arom.}$), 7.50 (s, 1H, $H_{arom.}$), 7.48 (t, 1H, *J*=7.60 Hz, $H_{arom.}$), 7.44 (dt, 1H, J_1=1.29 Hz, J_2=7.70 Hz, $H_{arom.}$), 7.01 (dd, 1H, J_1=2.18 Hz, J_2=9.41 Hz, $H_{arom.}$), 3.64 (q, 4H, *J*=7.03 Hz, CH_2), 3.60 (q, 4H, *J*=7.02 Hz, CH_2), 1.27 (t, 6H, *J*=7.00 Hz, CH_3), 1.16 (t, 6H, *J*=7.05 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 158.28 (s), 151.20 (s), 148.51 (s), 145.18 (s), 139.16 (s), 130.93 (s), 129.83 (d), 124.78 (d), 122.50 (d), 119.56 (d), 117.16 (s), 114.04 (d), 110.24 (d), 109.93 (s), 95.58 (d), 87.94 (s), 47.53 (t, 2C), 44.93 (t, 2C), 13.56 (q, 2C), 12.66 (q, 2C); Found.: C, 74.29; H, 7.16; N, 18.55. Calc. for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06; N, 18.17%; MS (ESI): *m/z*= 386.5 ($[M+1]^+$).

*4.4.4.9. 2,5-di-(N,N-dipropylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **31**.*

Compound **31** was prepared using above described method from **17** (100 mg, 0.34 mmol) and dipropylamine (0.50 mL, 5.90 mmol) after 4 h of irradiation to yield 35 mg (23%) of yellow powder; m.p. 223–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.23 (d, 1H, J=7.92 Hz, $H_{arom.}$), 7.95 (d, 1H, J=9.39 Hz, $H_{arom.}$), 7.85 (dd, 1H, J_1=1.07 Hz, J_2=8.11 Hz, $H_{arom.}$), 7.50 (t, 1H, J=7.23 Hz, $H_{arom.}$), 7.50 (s, 1H), 7.44 (dt, 1H, J_1=1.04 Hz, J_2=7.31 Hz, $H_{arom.}$), 7.02 (dd, 1H, J_1=1.88 Hz, J_2=9.35 Hz, $H_{arom.}$), 3.62–3.52 (m, 8H, CH_2), 1.78–1.59 (m, 8H, CH_2), 1.01 (t, 6H, J=7.34 Hz, CH_3), 0.88 (t, 6H, J=7.31 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 159.10 (s), 151.52 (s), 148.71 (s), 145.27 (s), 139.14 (s), 130.91 (s), 129.94 (d), 124.77 (d), 122.24 (d), 119.55 (d), 117.41 (s), 113.85 (d), 110.27 (d), 109.23 (s), 95.85 (d), 86.46 (s), 55.21 (t, 2C), 52.73 (t, 2C), 21.22 (t, 2C), 20.33 (t, 2C), 11.75 (q, 2C), 11.68 (q, 2C); Found: C, 76.14; H, 8.06; N, 15.80. Calc. for $C_{28}H_{35}N_5$: C, 76.15; H, 7.99; N, 15.86%; MS (ESI): m/z= 442.6 ($[M+1]^+$).

*4.4.4.10. 2,5-di-(N,N-dipentylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **32**.*

Compound **32** was prepared using above described method from **17** (150 mg, 0.50 mmol) and dipentylamine (0.80 mL, 3.90 mmol) after 4 h of irradiation to yield 75 mg (27%) of yellow powder; m.p. 126–128 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.23 (d, 1H, J=8.40 Hz, $H_{arom.}$), 7.92 (d, 1H, J=9.36 Hz, $H_{arom.}$), 7.84 (d, 1H, J=7.83 Hz, $H_{arom.}$), 7.50 (t, 1H, J=7.20 Hz, $H_{arom.}$), 7.48 (s, 1H, J=1.95 Hz, $H_{arom.}$), 7.40 (t, 1H, J=7.23 Hz, $H_{arom.}$), 6.93 (dd, 1H, J_1=1.82 Hz, J_2=9.36 Hz Hz, $H_{arom.}$), 3.61–3.52 (m, 8H, CH_2), 1.72–1.65 (m, 4H, CH_2), 1.64–1.60 (m, 4H, CH_2), 1.44–1.63 (m, 8H, CH_2), 1.29–1.21 (m, 8H, CH_2), 0.92 (t, 6H, J=6.89 Hz, CH_3), 0.82 (t, 6H, J=6.89 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 158.45 (s), 150.98 (s), 148.21 (s), 144.79 (s), 138.61 (s), 130.40 (s), 129.37 (d), 124.30 (d), 121.52 (d), 119.06 (d), 116.80 (s), 113.34 (d), 109.70 (d), 108.83 (s), 95.36 (d), 86.15 (s), 52.95 (t, 2C), 50.62 (t, 2C), 28.64 (t, 2C), 28.44 (t, 2C), 27.07 (t, 2C), 26.22 (t, 2C), 21.99 (t, 2C), 21.77 (t, 2C), 13.93 (q, 2C), 13.78 (q, 2C); Found: C, 78.06; H, 9.24; N, 12.70. Calc. for $C_{36}H_{51}N_5$: C, 78.07; H, 9.28; N, 12.65%; MS (ESI): m/z= 554.8 ($[M+1]^+$).

*4.2.4.11. 2,5-di-(N-pyrrolidinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **33**.*

Compound **33** was prepared using above described method from **17** (210 mg, 0.70 mmol) and pyrrolidine (0.10 mL, 1.22 mmol) after 5 h of irradiation to yield 58 mg (22%) of yellow powder; m.p. 265–270 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.16 (d, 1H, J=8.22 Hz, $H_{arom.}$), 7.97 (d, 1H, J=9.24 Hz, $H_{arom.}$), 7.71 (d, 1H, J=7.68 Hz, $H_{arom.}$), 7.39 (t, 1H, J=7.56

Hz, $H_{arom.}$), 7.31 (t, 1H, *J*=7.71 Hz, $H_{arom.}$), 7.19 (d, 1H, *J*=1.86 Hz, $H_{arom.}$), 6.63 (dd, 1H, J_1=1.98 Hz, J_2=9.27 Hz, $H_{arom.}$), 3.93 (t, 4H, *J*=6.54 Hz, CH_2), 3.44 (t, 4H, *J*=6.24 Hz, CH_2), 2.05–2.03 (m, 4H, CH_2), 1.99–1.96 (m, 4H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 154.67 (s), 150.24 (s), 149.63 (s), 145.06 (s), 137.52 (s), 130.55 (s), 129.81 (d), 123.76 (d), 120.85 (d), 118.77 (s), 118.07 (d), 113.42 (d), 108.60 (d), 106.61 (s), 95.38 (d), 73.78 (s), 54.03 (t, 2C), 47.47 (t, 2C), 25.33 (t, 2C), 24.94 (t, 2C); Found: C, 75.36; H, 6.13; N, 18.51. Calc. for $C_{24}H_{23}N_5$: C, 75.56; H, 6.08; N, 18.36%; MS (ESI): *m/z*= 382.5 ($[M+1]^+$).

*4.4.4.12. 2,5-di-(N-piperidinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **34**.*

Compound **34** was prepared using above described method from **17** (420 mg, 1.42 mmol) and piperidine (0.15 mL, 1.50 mmol) after 10 h of irradiation to yield 150 mg (43%) of yellow powder; m.p. 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.33 (d, 1H, *J*=8.34 Hz, $H_{arom.}$), 7.89 (d, 1H, *J*=2.34 Hz, $H_{arom.}$), 7.82 (dd, 1H, J_1=7.92 Hz, J_2=0.90 Hz, $H_{arom.}$), 7.70 (d, 1H, *J*=2.34 Hz, $H_{arom.}$), 7.48 (dt, 1H, J_1=0.90 Hz, J_2=7.80 Hz, $H_{arom.}$), 7.43 (dt, 1H, J_1=1.35 Hz, J_2=7.17 Hz, $H_{arom.}$), 7.23 (dd, 1H, J_1=2.40 Hz, J_2=9.42 Hz, $H_{arom.}$), 3.59 (m, 4H, CH_2), 3.54 (t, 4H, *J*=5.38 Hz, CH_2), 1.80 (m, 4H, CH_2), 1.70 (m, 8H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 158.32 (s), 153.41 (s), 148.29 (s), 144.62 (s), 138.33 (s), 130.34 (s), 128.70 (d), 124.21 (d), 121.95 (d), 118.89 (d), 116.91 (s), 113.93 (d), 111.72 (d), 109.15 (s), 97.65 (d), 83.63 (s), 53.56 (t, 2C), 47.92 (t, 2C), 26.13 (t, 2C), 24.90 (t, 2C), 23.80 (t), 23.68 (t); Found: C, 76.05; H, 6.75; N, 17.20. Calc. for $C_{26}H_{27}N_5$: C, 76.25; H, 6.65; N, 17.10%; MS (ESI): *m/z*= 410.5 ($[M+1]^+$).

*4.4.4.13. 2,5-di-(N-morpholinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **35**.*

Compound **35** was prepared using above described method from **17** (70 mg, 0.24 mmol) and morpholine (0.20 mL, 2.31 mmol) after 5 h of irradiation to yield 73 mg (74%) of yellow powder; m.p. >300°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.39 (d, 1H, *J*=8.16 Hz, $H_{arom.}$), 7.98 (d, 1H, *J*=9.24 Hz, $H_{arom.}$), 7.85 (dd, 1H, J_1=0.84 Hz, J_2=7.89 Hz, $H_{arom.}$), 7.73 (d, 1H, *J*=1.71 Hz, $H_{arom.}$), 7.50 (t, 1H, *J*=7.23 Hz, $H_{arom.}$), 7.43 (dt, 1H, J_1=0.93 Hz, J_2=7.70 Hz, $H_{arom.}$), 7.23 (dd, 1H, J_1=1.83 Hz, J_2=9.34 Hz, $H_{arom.}$), 3.89 (t, 4H, *J*=4.38 Hz, CH_2), 3.84 (t, 4H, *J*=5.19 Hz, CH_2), 3.59 (t, 4H, *J*=4.35 Hz, CH_2), 3.53 (t, 4H, *J*=4.69 Hz, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 157.19 (s), 153.81 (s), 147.92 (s), 144.55 (s), 138.19 (s), 130.33 (s), 128.85 (d), 124.41 (d), 122.18 (d), 119.06 (d), 116.57 (s), 114.26 (d), 111.49 (d), 109.88 (s), 98.03 (d), 84.90 (s), 66.54 (t, 2C), 65.85 (t, 2C), 52.50 (t, 2C), 46.74 (t, 2C);

Found: C, 69.62; H, 5.65; N, 16.97. Calc. for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 16.94%; MS (ESI): *m/z*= 414.5 ($[M+1]^+$).

*4.4.4.14. 2,5-di-(N-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **36***.

Compound **36** was prepared using the above method from **17** (50 mg, 0.17 mmol) and piperazine (0.100 g, 1.20 mmol) after 1 h of irradiation to yield 50 mg (72%) of yellow crystals; m.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.39 (d, 1H, *J*=8.01 Hz, $H_{arom.}$), 7.97 (d, 1H, *J*=9.33 Hz, $H_{arom.}$), 7.85 (dd, 1H, J_1=1.20 Hz, J_2=7.86 Hz, $H_{arom.}$), 7.73 (d, 1H, *J*=1.92 Hz, $H_{arom.}$), 7.50 (dt, 1H, J_1=0.97 Hz, J_2=7.57 Hz, $H_{arom.}$), 7.44 (dt, 1H, J_1=1.45 Hz, J_2=7.71 Hz, $H_{arom.}$), 7.25 (dd, 1H, J_1=2.03 Hz, J_2=9.40 Hz, $H_{arom.}$), 3.50 (m, 8H, CH_2), 2.99 (t, 4H, *J*=4.68 Hz, CH_2), 2.93 (t, 4H, *J*=4.89 Hz, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 158.36 (s), 154.39 (s), 145.06 (s), 138.75 (s), 130.83 (s), 129.30 (d), 124.80 (d), 122.57 (d), 119.44 (d), 117.33 (s), 114.44 (d), 114.64 (d), 112.04 (d), 109.91 (s), 100.53 (s), 98.24 (d), 84.42 (s), 54.09 (t, 2C), 48.13 (t, 2C), 46.47 (t, 2C), 45.82 (t, 2C); Found: C, 70.12; H, 6.00; N, 23.88. Calc. for $C_{24}H_{25}N_7$: C, 70.05; H, 6.12; N, 23.83%; MS (ESI): *m/z*= 412.2 ($[M+1]^+$).

*4.4.4.15. 2,5-di-[N-(4-N-methylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **38***.

Compound **38** was prepared using above described method from **17** (100 mg, 0.36 mmol) and 4-*N*-methylpiperazine (0.10 mL, 0.90 mmol) after 3 h of irradiation to yield 50 mg (34%) of yellow powder; m.p. 250–256 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm= 8.38 (d, 1H, *J*=8.28 Hz, $H_{arom.}$), 7.94 (d, 1H, *J*=9.36 Hz, $H_{arom.}$), 7.83 (dd, 1H, J_1=0.96 Hz, J_2=8.22 Hz, $H_{arom.}$), 7.73 (d, 1H, *J*=2.16 Hz, $H_{arom.}$), 7.49 (dt, 1H, J_1=0.62 Hz, J_2=7.50 Hz, $H_{arom.}$), 7.43 (d t, 1H, J_1=1.00 Hz, J_2=7.73 Hz, $H_{arom.}$), 7.25 (dd, 1H, J_1=2.22 Hz, J_2=9.36 Hz, $H_{arom.}$), 3.58 (t, 4H, *J*=4.80 Hz, CH_2), 3.56 (t, 4H, *J*=5.10 Hz, CH_2), 2.62 (bs, 4H, CH_2), 2.54 (t, 4H, *J*=4.92 Hz, CH_2), 2.31 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 157.46 (s), 153.55 (s), 148.06 (s), 144.55 (s), 138.22 (s), 130.33 (s), 128.74 (d), 124.34 (d), 122.13 (d), 118.98 (d), 116.69 (s), 114.19 (d), 111.74 (d), 109.56 (s), 98.02 (d), 54.99 (t, 2C), 54.25 (t, 2C), 52.02 (t, 2C), 45.55 (t, 2C), 45.78 (q), 45.66 (q); Found: C, 71.10; H, 6.73; N, 22.17. Calc. for $C_{26}H_{29}N_7$: C, 71.04; H, 6.65; N, 22.31%; MS (ESI): *m/z*= 440.2 ($[M+1]^+$).

*4.4.4.16. 2,5-di-[N-(3,5-dimethylpiperazinyl)]benzimidazo[1,2-a]quinoline-6-carbonitrile **39***.

Compound **39** was prepared using above described method from **17** (100 mg, 0.36 mmol) and 2,6-dimethylpiperazine (0.100 g, 0.90 mmol) after 3 h of irradiation to yield 51 mg (32%) of yellow powder; m.p. 233–236 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.27 (d, 1H,

J=7.95 Hz, $H_{arom.}$), 7.86 (d, 1H, *J*=9.36 Hz, $H_{arom.}$), 7.81 (d, 1H, *J*=7.56 Hz, $H_{arom.}$), 7.61 (s, 1H, $H_{arom.}$), 7.47 (t, 1H, *J*=7.68 Hz, $H_{arom.}$), 7.41 (t, 1H, *J*=7.29 Hz, $H_{arom.}$), 7.23 (dd, 1H, J_1=1.36 Hz, J_2=9.68 Hz, $H_{arom.}$), 3.95 (d, 2H, *J*=10.74 Hz, CH_2), 3.54 (d, 2H, *J*=11.01 Hz, CH_2), 3.07 (m, 2H, CH), 3.00 (d, 2H, *J*=11.25 Hz, CH_2), 2.89 (m, 2H, CH), 2.46 (d, 2H, *J*=9.54 Hz, CH_2), 1.11 (d, 6H, *J*=6.18 Hz CH_3), 1.05 (d, 6H, *J*=6.18 Hz CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm= 157.71 (s), 153.68 (s), 148.73 (s), 145.09 (s), 138.77 (s), 130.60 (s), 129.31 (d), 124.71 (d), 122.45 (d), 119.38 (d), 117.29 (s), 114.44 (d), 112.07 (d), 109.65 (s), 101.52 (s), 98.15(d), 59.34 (t, 2C), 53.65 (t, 2C), 51.43 (d, 2C), 50.56 (d, 2C), 19.65 (q, 2C), 19.24 (q, 2C); Found: C, 71.72; H, 7.15; N, 21.13. Calc. for $C_{28}H_{33}N_7$: C, 71.92; H, 7.11; N, 20.97%; MS (ESI): *m/z*= 468.2 ($[M+1]^+$).

*4.4.5. 2,5-di-[N-(4-N,N-dimethylpiperazin-1-yl])benzimidazo[1,2-a]quinoline-6-carbonitrile diiodide **37**.*

A mixture of compound **36** (50 mg, 0.12 mmol) and anhydrous potassium carbonate (0.017 g, 0.11 mmol) was refluxed in acetonitrile (30 mL) with methyl jodide (0.063 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 **37** as yellow powder (42 mg, 47%); m.p. >300 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm= 8.55 (d, 1H, *J*=7.95 Hz, $H_{arom.}$), 8.10 (d, 1H, *J*=9.30 Hz, $H_{arom.}$), 7.91 (dd, 1H, J_1=0.93 Hz, J_2=7.92 Hz, $H_{arom.}$), 7.88 (d, 1H, *J*=1.62 Hz, $H_{arom.}$), 7.57 (t, 1H, *J*=7.27 HZ, $H_{arom.}$), 7.50 (dt, 1H, J_1=1.05 Hz, J_2=7.89 Hz, $H_{arom.}$), 7.32 (dd, 1H, J_1=1.56 Hz, J_2=9.33 Hz, $H_{arom.}$), 3.97 (t, 8H, *J*=4.41 Hz, CH_2), 3.78 (t, 4H, *J*=4.34 Hz, CH_2), 3.69 (t, 4H, *J*=4.74 Hz, CH_2), 3.34 (s, 6H, CH_3), 3.30 (s, 6H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm= 155.95 (s), 152.79 (s), 148.37 (s), 144.50 (s), 138.08 (s), 130.24 (s), 129.18 (d), 124.75 (d), 122.53 (d), 119.35 (d), 116.21 (s), 114.52 (d), 112.20 (d), 110.37 (s), 99.09 (d), 86.82 (s), 60.72 (t, 2C), 59.96 (t, 2C), 50.45 (q, 4C), 45.62 (q), 45.66 (t, 2C), 40.95 (t, 2C); Found: C, 46.39; H, 4.78; N, 13.70. Calc. for $C_{28}H_{35}I_2N_7$: C, 46.49; H, 4.88; N, 13.55%; MS (ESI): *m/z*= 234.8 ($[M+1]^+$).

4.3. 3D-QSAR modelling

3D-QSAR models were derived using antiproliferative activity data against against H460, HCT 116, MCF-7 of the compounds presented in this paper and similar compounds whose antiproliferative activities have been measured in the same laboratory and published previously [13, 14]. Altogether 51 compounds were used (Table S1 in the supplement).

Negative logarithmic values of concentrations that cause 50% growth inhibition of the cell lines (pIC_{50}) were used as measure of biological activity for generating 3D- QSAR models. For the poorly active compounds whose IC_{50} values were not explicitly measured, but just estimated as "≥10", ">10", "≥100", "> 100", pIC_{50} was set to 5.000, 4.301, 4.000, 3.301, respectively.

3D structure of each compound was generated from the SMILES code using VolSurf+ 3D structure generator. Molecular descriptors for each compound, based on its 3D structure, were generated by the VolSurf+ program [15]. Series of 128 descriptors that refer to molecular size and shape, to hydrophilic and hydrophobic regions and to the balance between them, to the "charge state" descriptors, to lipophilicity, to molecular diffusion, log*P*, log*D*, to the presence/distribution of pharmacophoric descriptors, to molecular flexibility, to H-bond interaction, and to descriptors on some other relevant ADME properties. The definition of all 128 VolSurf+ descriptors is given in the VolSurf+ manual [15].

Using Partial Least Square (PLS) analysis, the relationship between the 3D structure-based molecular descriptors and biological activities was studied. Autoscaling pretreatment, by which every variable is the mean centered and scaled to give unit variance, was applied. For each cell line, different 3D-QSAR models were generated (models labeled **1**, **2**, and **3**, for the cell lines H460, HCT 116, and MCF-7, respectively). The number of significant latent variables (nLV) and quality of the models were determined using the leave-one-out (LOO) cross-validation procedure. Standard deviation of error of calculation (SDEC) and standard deviation of error of prediction (SDEP) were calculated for each model. The PLS coefficients of the obtained models were analyzed in order to investigate influence of each descriptor on compounds' antiproliferative activity.

4.4. 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 [13, 14]. 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:

If (mean A_{test} – mean A_{tzero}) ≥ 0, then PG = 100 × (mean A_{test} – mean A_{tzero}) / (mean A_{ctrl} – meanA_{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.

4.5. 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.

4.5.1. 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 to 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 = $Tm_{[compound + DNA]}$ - $Tm_{[DNA\ alone]}$).

4.5.2. UV/Visible spectroscopy

The UV/Visible spectra were obtained in a quartz cuvette of 10 mm pathlength containing compounds **36** or **37** (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.

4.5.3. Fluorescence spectroscopy

Fluorescence spectra were recorded from 400 to 700 nm essentially as described [16]. The fluorescent drugs (10 µM) were diluted in 1 mL of BPE buffer in the presence or absence of increasing concentrations of CT-DNA. 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.

4.5.4. Circular dichroism

For circular dichroism, CT-DNA (50 µM) was incubated with or without (control) increasing concentrations of compounds **36** or **37** (1, 5, 10, 20, 30, 40, 50, 60 µM) in BPE. The absence of intriniseque CD was validated using the highest concentration of compound (60 µM). The CD spectra were collected in a quartz cell of 10 mm path length 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 [16].

4.5.5. Topoisomerase I – mediated DNA relaxation and poisoning activities

DNA intercalation and topoisomerase I poisoning activities were evaluated using pUC19 supercoiled plasmid DNA and human topoisomerase I (Topogen, USA) as previously described [16, 17].

4.5.6. DNase I footprinting

DNase I footprinting experiments were conducted essentially as described [18]. The gels were exposed to storage screen for the appropriated delay at room temperature. The results were collected using a Pharos-PMI equipment (BioRad).

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