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## Graphical Abstract

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

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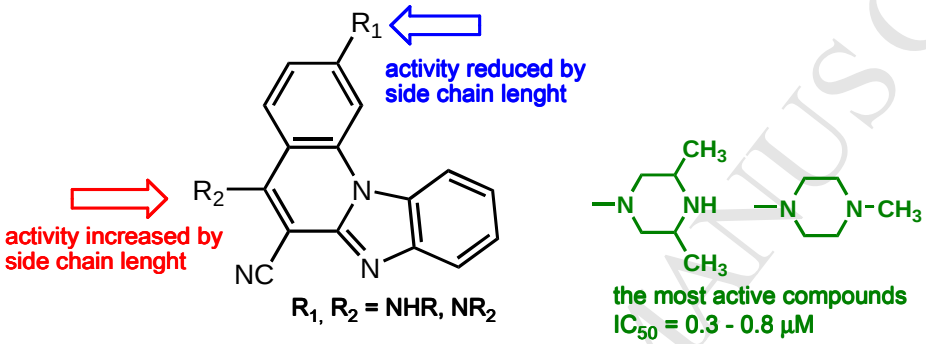

# Amino substituted benzimidazo[1,2-*a*]quinolines: Antiproliferative potency, 3D QSAR study and DNA binding properties

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**Abstract**

We describe the synthesis, 3D-derived quantitative structure-activity relationship (QSAR), antiproliferative activity and DNA binding properties of a series of 2-amino, 5-amino and 2,5-diamino substituted benzimidazo[1,2-*a*]quinolines prepared by environmentally friendly uncatalyzed microwave assisted amination. The antiproliferative activities were assessed *in vitro* against colon, lung and breast carcinoma cell lines; activities ranged from submicromolar to micromolar. The strongest antiproliferative activity was demonstrated by 2-amino-substituted analogues, whereas 5-amino and or 2,5-diamino substituted derivatives resulted in much less activity. Derivatives bearing 4-methyl- or 3,5-dimethyl-1-piperazinyl substitutents emerged as the most active. DNA binding properties and the mode of interaction of chosen substituted benzimidazo[1,2-*a*]quinolines prepared herein were studied using melting temperature studies, a series of spectroscopic studies (UV/Visible, fluorescence, and circular dichroism), and biochemical experiments (topoisomerase I-mediated DNA relaxation and DNase I footprinting experiments). Both compound **36** and its bis-quaternary iodide salt **37** intercalate between adjacent base pairs of the DNA helix while compound **33** presented a very weak topoisomerase I poisoning activity. A 3D-QSAR analysis was performed to identify hydrogen bonding properties, hydrophobicity, molecular flexibility and distribution of hydrophobic regions as these molecular properties had the highest impact on the antiproliferative activity against the three cell lines.

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

## 1. Introduction

The benzimidazole moiety is one of the most important organic structural motifs found in a variety of bioactive natural products as well as numerous synthetic medical and biochemical agents, which possess different chemical and pharmacological features [1–5]. Importantly, due to the structural similarity with naturally occurring nucleotides, benzimidazole derivatives can easily interact with biomolecules like DNA, RNA or proteins in living systems, thus playing a crucial role in their function. The DNA molecule is still one of the principal targets in anticancer drug development strategies since it has a central role in many life processes [6]. Understanding the molecular basis for cytotoxicity by anticancer agents is critical for the rational development of novel, more selective and efficient agents with greater specificity of action [7]. The most used classes of chemotherapeutic agents are comprised of molecules that interact with DNA such as intercalators, alkylating agents or groove binders [8]. Organic intercalators are a class of polycyclic aromatic compounds that 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 combination of the benzimidazole moiety with other heteroaromatic groups can lead to highly conjugated, planar benzannulated benzimidazoles with improved biological properties. Their high fluorescence intensity and possibility of interaction with biomacromolecules suggest the potential use of benzannulated benzimidazoles as fluorescent probes for detection of biologically important molecules such as DNA or proteins in biomedicinal diagnostics [10, 11]. Moreover, the quinoline ring system, which widely exists in a large number of alkaloids and synthetic analogues with various biological actions, is also of great importance in medicinal and natural product chemistry [12, 13].

Quinoline-fused benzimidazoles, recently prepared and published in our research group, have been shown to be a very promising class of tetracyclic intercalators; studies of their biological activity comprising cytostatic evaluation, DNA/RNA interaction, inhibition of topoisomerase I and II, and proteomic profiling have confirmed the anticancer potential of this class of compounds [14, 15]. Positively charged amidino-substituted benzimidazo[1,2-*a*]quinolines intercalate into double-stranded DNA or RNA with the pronounced selectivity towards colon carcinoma cells [14].

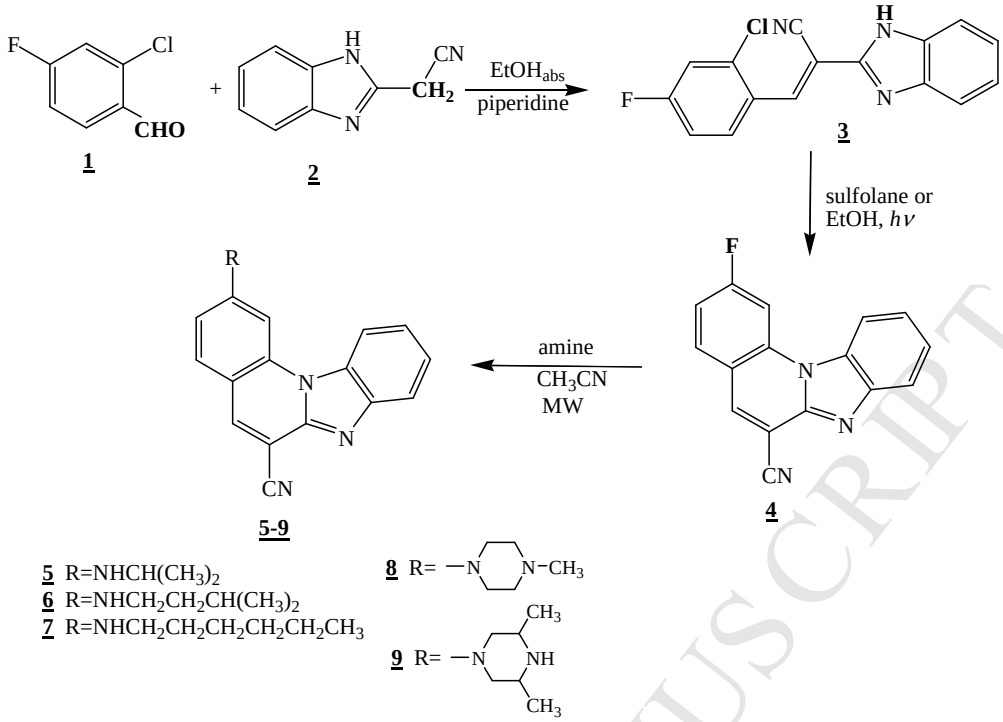
In addition, we have reported on the biological action of 2-aminobenzimidazo[1,2-a]quinoline-6-carbonitriles as antiproliferative agents [15]. The obtained biological activity demonstrated that the structural features and length of the amino side chains influence the

antiproliferative potency of these compounds. Some of the examined amino side chains yielded analogues with activities in the sub-micromolar range. In a more recent paper, we reported the synthesis, antiproliferative activity and DNA binding properties of a series of 5-aminobenzimidazo[1,2-*a*]quinoline-6-carbonitriles and showed that the displacement of the amino side chain to position 5 of the quinoline-fused benzimidazole moiety leads to compounds with significantly decreased potency [16]. Based on previous knowledge and in consideration of the promising anticancer potential of the benzimidazo[1,2-*a*]quinoline scaffold, herein we described the design and synthesis of novel 2-amino, 5-amino and 2,5-diamino substituted analogues, aimed at improving antiproliferative activity and selectivity. The newly synthesized compounds were tested *in vitro* for their antitumor properties using three human tumor cell lines, and for each cell line 3D-QSAR models were obtained. Moreover, we present the DNA binding ability results of selected compounds.

## 2. Methods

### *2.1 Chemistry*

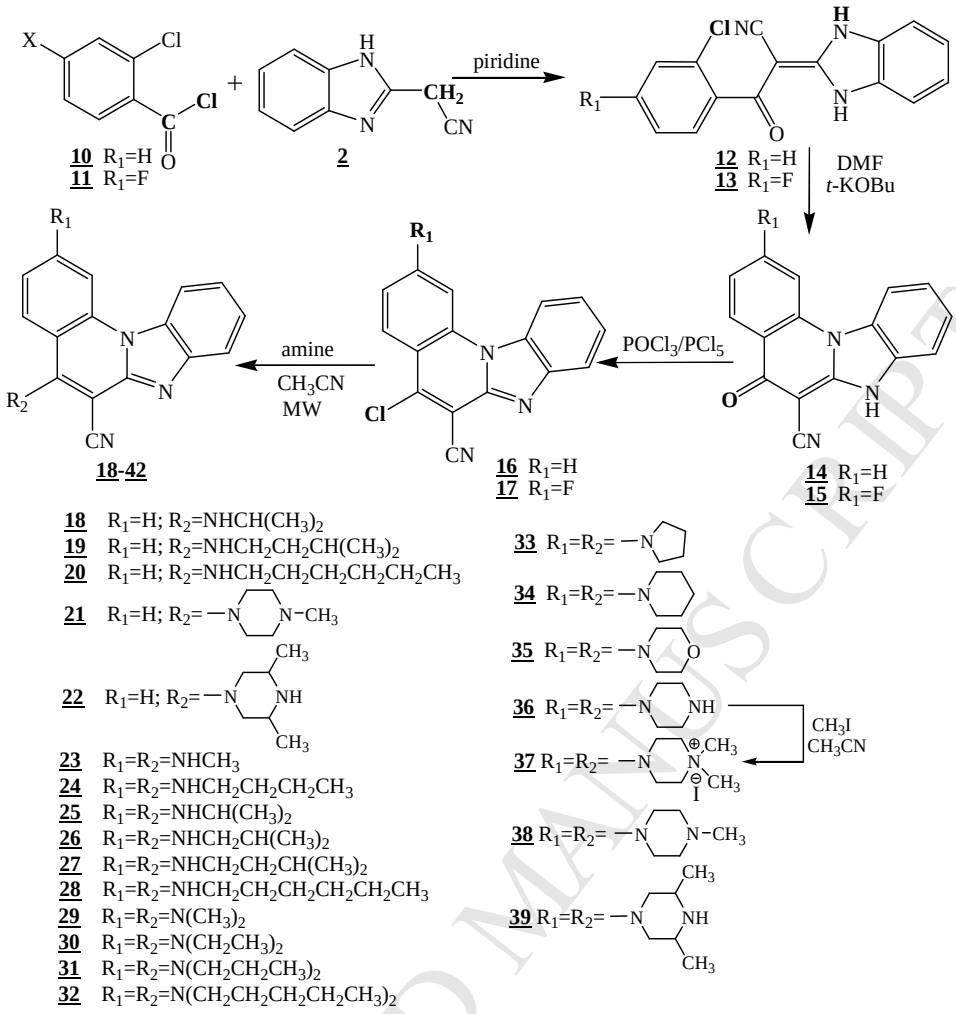
The new target compounds **5**–**9** and **18**–**39** were synthesized according to the procedures shown in Schemes 1 and 2, respectively, by employment of conventional methods for construction of structurally related fused benzimidazole derivatives. Thus, 4-fluorobenzaldehyde **1** (Scheme 1) or benzoyl chlorides (Scheme 2) were condensed with 2-(1*H*-benzimidazol-2-yl)acetonitrile **2** in the presence of piperidine in absolute ethanol to provide the corresponding benzimidazol substituted nitriles **3**, **12** and **15** in suitable yields (75 %, 44 %, and 49 %, respectively) according to previously published procedures [15, 16]. As shown in Scheme 1, compound **3** underwent a thermal cyclization in sulfolane to afford 2-fluorobenzimidazo[1,2-*a*]quinoline-6-carbonitrile **4** (72 %), which was the common precursor for the preparation of the desired compounds **5**–**9**. On the other hand, the acyclic intermediates **12** and **13** were subjected to thermal cyclization reaction in DMF under basic conditions (potassium *tert*-butoxide) to give the corresponding 5-oxo-5,7-dihydrobenzimidazo[1,2-*a*]quinoline-6-carbonitriles **14** and **15** in 57 % and 96 % yields, respectively (Scheme 2). Subsequent treatment of these intermediates with $POCl_3$ and $POCl_5$ gave the respective 5-chloro substituted benzimidazo[1,2-*a*]quinoline-6-carbonitriles **16** (70 % yield) and **17** (96 % yield), which were the precursors for the preparation of the final products **18**–**39**.




**Scheme 1.** Synthesis of 2-amino substituted derivatives **5**–**9**

The halogen compounds **4**, **16** and **17** were converted to the targeted amino and diamino analogues **5**–**9** and **18**–**39**, respectively in 15–74 % yields upon reaction with five- to seven-fold excess of the appropriate amine in acetonitrile under microwave irradiation. After determining the optimal yields in the shortest reaction time, this microwave assisted amination was conducted by using 800 W power, 170 °C and 40 bar pressure. The *bis*-quaternary iodide salt **37** was obtained in 47 % yield by treating piperazine analogue **36** with an excess of methyl iodide in the presence of anhydrous potassium carbonate in acetonitrile.

The structures of all prepared compounds were determined by NMR spectroscopy ($^{1}$H and $^{13}$C NMR), mass spectrometry, and elemental analysis. $^{1}$H NMR spectra of the cyclization reaction products showed a downfield shift of the aromatic protons and absence of the benzimidazol NH proton in comparison with the respective acyclic intermediates. These $^{1}$H NMR spectroscopic differences confirmed the formation of the benzimidazo[1,2-*a*]quinoline heterocycle.




**Scheme 2.** Synthesis of 5-amino (**18**–**22**) and 2,5-diamino (**23**–**39**) substituted derivatives

### *2.2 3D-QSAR analysis*

3D-QSAR models were derived using antiproliferative activity data 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 [15, 16]. Altogether, 51 compounds were used (Table S1 in the Supplement).

The 3D structure of each compound was generated from the SMILES code using VolSurf+ 3D structure generator. Molecular descriptors for each compound were generated based on its 3D structure using the VolSurf+ program [17]. A 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 of some other relevant ADME properties were considered. The definition of all 128 VolSurf+ descriptors is given in the VolSurf+ manual [17, 18].

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.

3D-QSAR analysis was used to explore the physical and chemical properties having the highest influence on the antiproliferative activities of investigated benzimidazo[1,2-*a*]quinoline-6-carbonitriles. 3D-QSAR models **1**, **2**, and **3** (Table 1, Figure 1) were derived using the antiproliferative activities of the herein presented compounds together with the antiproliferative activities of similar compounds [15, 16] 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, p$IC_{50}$ 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 $SDEP = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{\exp(i)} - y_{pred(i)}\right)^2}{n}}$.

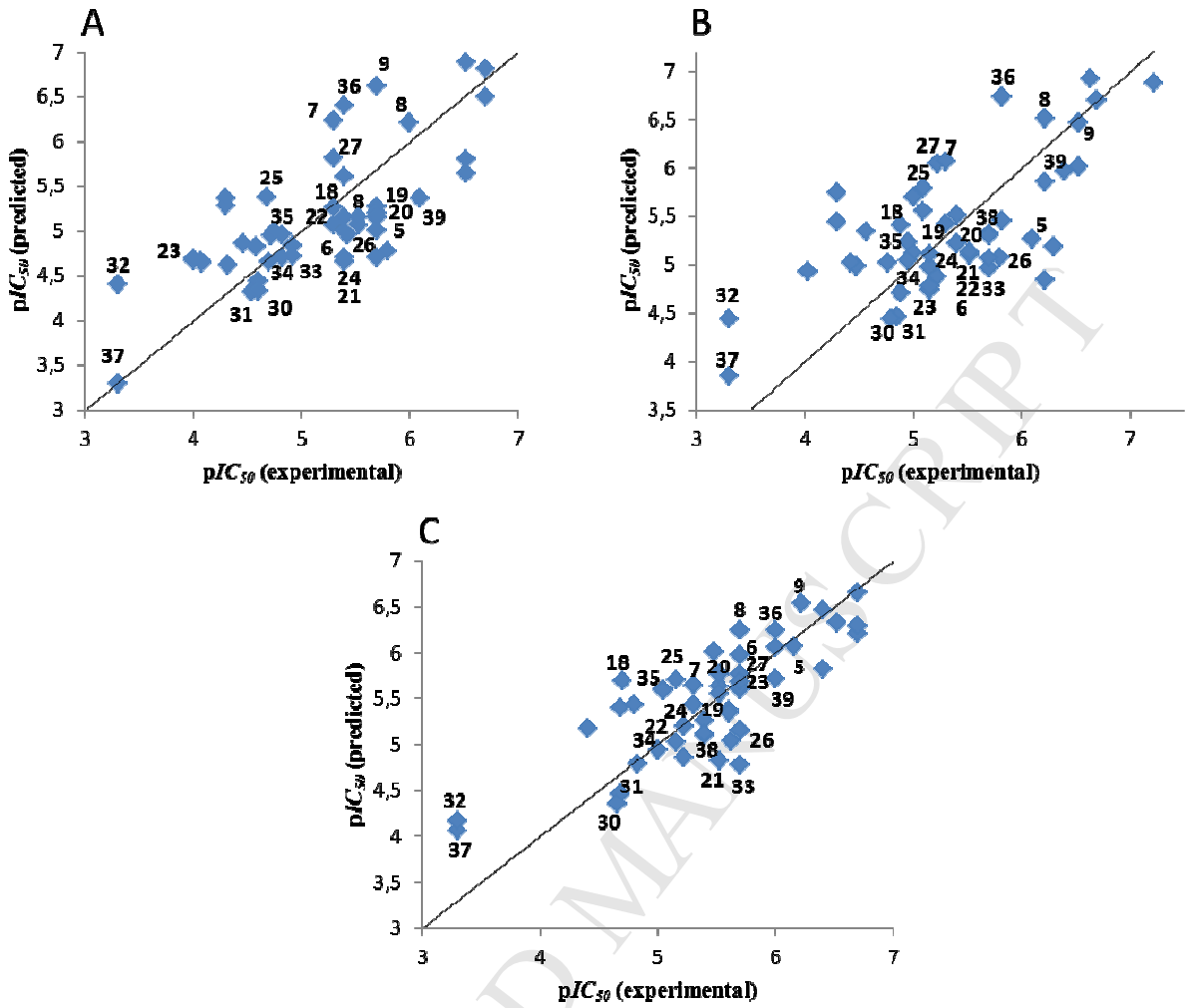



**Figure 1.** Predicted *vs* experimental antiproliferative activity (expressed as p$IC_{50}$ – negative logarithmic value of concentration that causes 50 % growth inhibition of the tumor cell lines of: A) model **1** (H460), B) model **2** (HCT 116) and C) model **3** (MCF-7).

## 3. Results and Discussion

### *3.1 Antiproliferative activity*

#### *3.1.1. Antiproliferative activity in human cell lines*

The antiproliferative activities of new benzimidazo[1,2-*a*]quinoline-6-carbonitriles bearing different amino side chains were tested on human colon, breast and lung tumor cell lines (Table 2) [15, 16, 18]. The most active and/or structurally interesting compounds were also tested on immortalized human mammary epithelial cells to test their potential selectivity

towards tumor cells. The obtained results were compared with known antitumor agents doxorubicin and etoposide.

**Table 2.** $IC_{50}$ values (in μM)[a]

| | Cell lines | | | |
|---|---|---|---|---|
| Compounds | **HCT 116** | **MCF-7** | **H460** | **HMLE** |
| **5** | 0.8±0.2 | 0.7±0.4 | 2±0.8 | 1±0.1 |
| **6** | 3±0.3 | 2±0.09 | 4±0.3 | 3±0.4 |
| **7** | 5±0.3 | 5±0.6 | 5±2 | N.T.[c] |
| **8** | 0.6±0.3 | 2±1 | 1±0.8 | 0.5±0.4 |
| **9** | 0.3±0.08 | 0.6±0.2 | 0.5±0.04 | 0.2±0.03 |
| **18** | 13±0.4 | 20±3 | 5±2 | N.T. |
| **19** | 5±1 | 4±0.5 | 2±0.5 | N.T. |
| **20** | 4±1 | 3±0.6 | 2±0.3 | N.T. |
| **21** | 3±0.2 | 3±0.3 | 4±0.6 | N.T. |
| **22** | 3±0.2 | 6±2 | 4±0.3 | 3±0.4 |
| **23** | 7±3 | 2±1 | ≥100 | N.T. |
| **24** | 7±1 | 5±0.8 | 4±1 | N.T. |
| **25**[b] | ≥10 | 7±1 | 21±2 | N.T. |
| **26** | 2±0.2 | 2±0.6 | 3±0.2 | N.T. |
| **27** | 6±0.3 | 2±0.05 | 5±0.2 | 6±0.8 |
| **28** | >100 | >100 | >100 | N.T. |
| **30** | 16±4 | 22±3 | 25±2 | N.T. |
| **31** | 14±5 | 21±6 | 29±0.2 | N.T. |
| **32** | >100 | >100 | >100 | N.T. |
| **33** | 2±0.5 | 2±0.6 | 12±1 | N.T. |
| **34** | 11±3 | 10±3 | 15±2 | N.T. |
| **35** | 11±5 | 9±1 | 18±8 | N.T. |
| **36** | 1.5±0.3 | 1±0.001 | 4±0.6 | 2±0.8 |
| **37** | >100 | >100 | >100 | N.T. |
| **38** | 2±0.5 | 4±1 | 3±0.5 | N.T. |
| **39** | 0.4±0.09 | 1±0.2 | 0.8±0.3 | 0.2±0.01 |
| **doxorubicin** | 0.07±0.02 | 0.02±0.01 | 0.03±0.01 | 0.01±0.002 |
| **etoposide** | 5±2 | 1±0.7 | 0.1±0.04 | 0.4±0.09 |

[a] $IC_{50}$; the concentration that causes 50 % growth inhibition
[b] The highest tested concentration was 10 μM.
[c] N.T.; not tested

Among the 2-amino substituted derivatives (**5**–**9**), three compounds strongly inhibited the growth of all cell lines. The most cytotoxic ($IC_{50}$ < 1 μM) were the 4-methyl-1-piperazinyl and 3,5-dimethyl-1-piperazinyl substituted compounds **8** and **9**.

The comparison of antiproliferative activity with different amino side chains lengths (**5**–**7**) displayed that the side chain length decreased the activity, probably due to the increase of the amphiphilic moment (A) and the unbalanced distribution of hydrophobic regions in the molecule (see Section 3.1.2). On the other hand, the results obtained for compounds **18, 19** and **20** revealed the opposite effect of the side chain length on the activities; for these compounds, the amino side chain is displaced to the 5- position and since the aliphatic chain does not decrease the unbalanced distribution of hydrophobic regions in the molecule, an increase in antiproliferative activity results. Therefore, the activities of compounds **19** and **20** are comparable to **6** and **7** bearing the 2-methylbutyl and 1-hexyl amino side chains. The activity of compound **5**, bearing the 2-methylethyl side chain, is more pronounced in comparison to the activity of compound **18**. Among the most abundant group of 2,5-diamino substituted derivatives, 1-piperazinyl substituted compounds **36**, **38** and **39** displayed the most pronounced antiproliferative activity, whereby 3,5-dimethyl-1-piperazinyl substituted derivative **39** was the most active one. The high antiproliferative activity of these compounds can be explained by the presence of an additional N heteroatom, which contributes to additional interactions with potential biological targets. Interestingly, the substituted quaternary iodide salt **37** was completely inactive, which is comparable to the previously obtained antiproliferative activity of a similar derivative with the substituent placed at the position 5 [16], and is captured by QSAR analysis of the results regarding the percentage of unionized species (see Section 3.1.2). 1-Piperidinyl and 4-morpholinyl substituted analogues **34** and **35** showed moderate activity ($IC_{50}$ 10–20 μM), while 1-pyrrolidinyl 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 in comparison to the less active derivatives **30**–**32** with tertiary amino side chains.

The lower antiproliferative activity displayed by derivatives with tertiary, rather than secondary, amino side chains confirmed that the secondary amine side chain is generally slightly preferred for antiproliferative activity, which is in agreement with previously published results [15, 16]. Moreover, disubstituted derivatives **28** and **32** bearing the longest amino side chains did not show any antiproliferative effect towards the 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 the new compounds. The exception was compound **23**, with less pronounced activity toward H460 cell line. Furthermore, although the selected compounds similarly affected the proliferation of healthy (non-tumor) and tumor cells, it is evident that the control anti-tumor compounds - doxorubicin and etoposide were more cytotoxic towards the non-tumor cells.

Within this scientific study we have additionally confirmed that the antiproliferative activity is highly dependent both on the type and length as well as the position of the amino substituent on the tetracyclic skeleton (Figure 2). The obtained results indicated that the most promising antiproliferative agents are those bearing the amino substituent at the position 2, and displacement of the amino side chain from position 2 to position 5 significantly decreased the activity. Introduction of two amino substituents at the positions 2 and 5, in comparison to the 2-substituted derivatives, slightly decreased the antiproliferative activity.

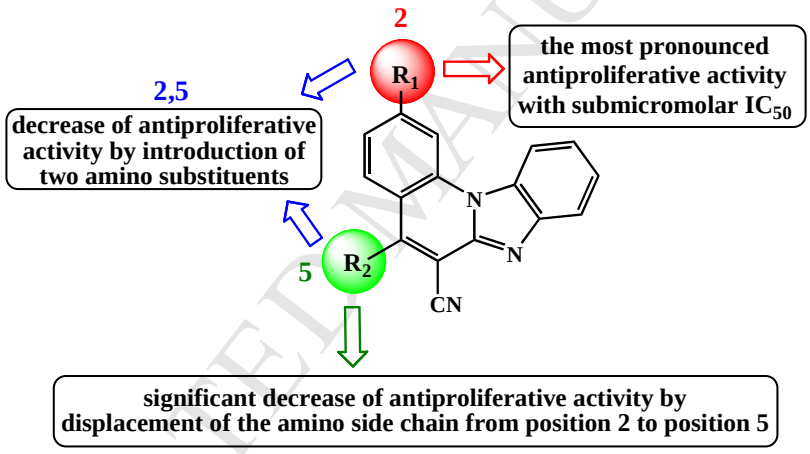



**Figure 3.** SAR of amino substituted benzimidazo[1,2-*a*]quinoline-6-carbonitriles

Since previously published results clearly showed that the most active compounds are those bearing the 2-methylpropylamino and 1-piperazinyl side chains at position 2 [15], we designed and prepared the derivatives with the isoalkylamine and substituted 1-piperazinyl side chains. 2-methylethyl and 2-methylbutylamino side chains were used to evaluate the influence of the isoalkylamine side chain length on the antiproliferative activity. The obtained results confirmed that the 2-methylpropylamino substituted derivative displayed the strongest activity, in sub-micromolar range. Furthermore, by choosing the 4-methyl-1-piperazinyl and 3,5-dimethyl-1-piperazinyl side chains, our intention was to confirm the assumption that the presence of another N heteroatom contributes to the interactions with biological targets probably due to hydrogen bonding. The obtained results indicated that the antiproliferative

activity decreased in comparison to the 1-piperazinyl substituted derivatives bearing the side chain at 2-, 5- and 2,5-positions. The lowest activity among the 1-piperazinyl substituted derivatives was observed for compounds bearing 4-methyl-1-piperazinyl side chains, which do not posses a N atom accessible for interactions with biological targets. Derivatives bearing the 3,5-dimethyl-1-piperazinyl side chains showed decreased activity due, presumably, to the steric hindrance of methyl groups which reduce the interactions of N heteroatom with potential biological targets. Benzimidazo[1,2-*a*]quinoline-6-carbonitriles, especially those bearing amino side chains at position 2 of the tetracyclic skeleton, should be further optimized as promising compounds for anticancer therapy. Of particular interest would be the synthesis of derivatives with different types and lengths of amino side chains at different positions by combining the amino side chains that showed the greatest impact on the antiproliferative activity herein.

*3.1.2 QSAR analysis of antiproliferative activities*

QSAR analysis of the obtained models identified the molecular properties with the highest influence on the antiproliferative activities against the studied cell lines (Figure 3A). Analysis of model **1** (H460 cell line) revealed that 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) have the largest positive influence on the antiproliferative activity. Also, the percentage of unionized species at different pH values (%FU4, %FU5), amphiphilic moment (A; the vector that connects the center of hydrophobic and center of hydrophilic regions) and ratio of molecular volume to molecular surface (R) were identified as the descriptors with the greatest negative influence on antiproliferative activity against H460 cell line.

Similar descriptors are found to be important for the activity against cell line HCT 116 (Figure 3B). The descriptors having the highest positive influence on antiproliferative activity are those 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). The percentage of unionized species at different pH values (%FU4, %FU5), amphiphilic moment (A) and integy moment (ID1; the vector that connects the center of a molecule and the center of hydrophobic regions) had the largest negative influence on antiproliferative activity on the HCT 116 cell line. In the case of the MCF-7 cell line (Figure 3C), the same descriptors as for cell line HCT 116 had the greatest positive influence on antiproliferative activity.

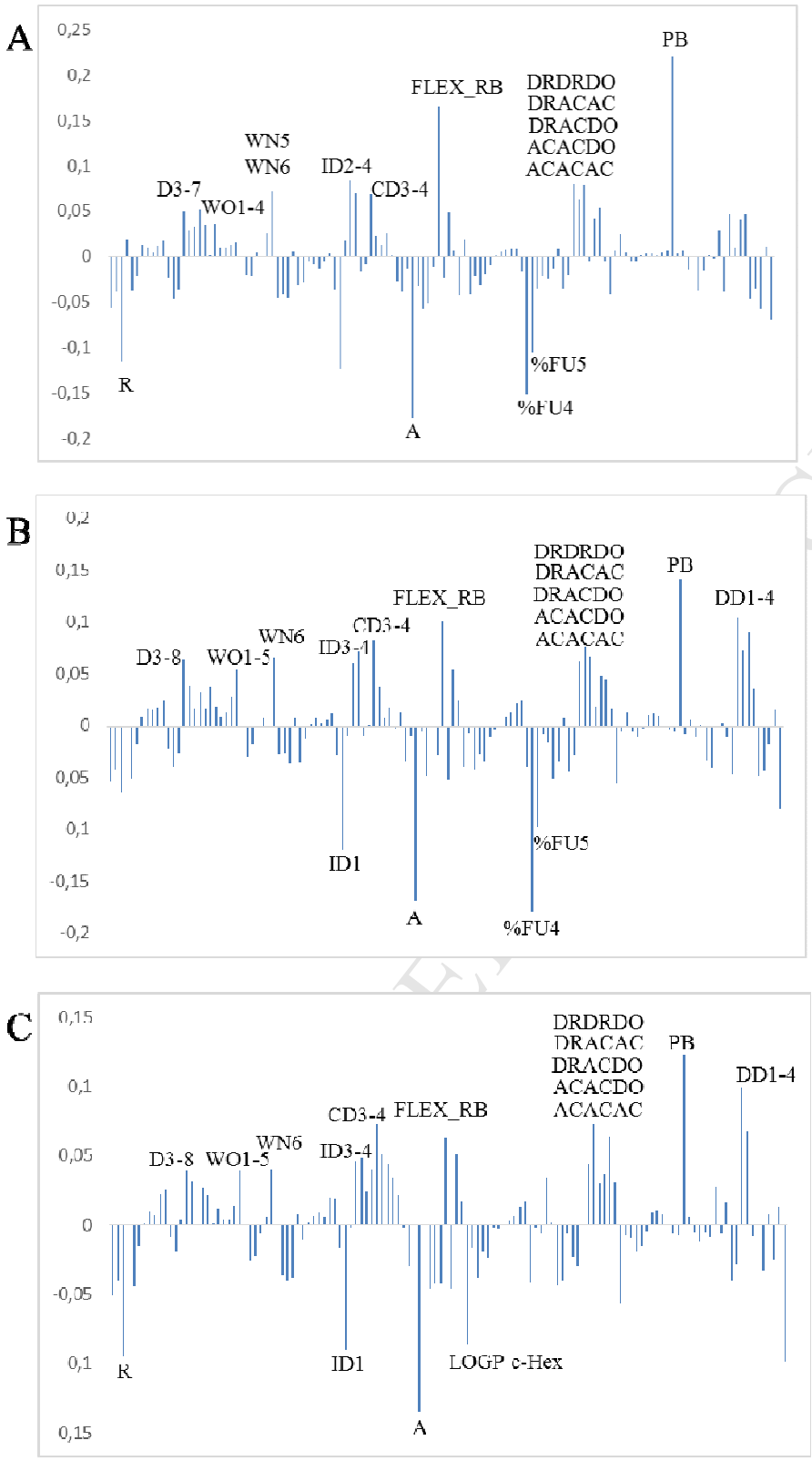



**Figure 3.** PLS coefficients of 3D-derived QSAR model: A) model **1** (H460), B) model **2** (HCT 116) and C) model **3** (MCF-7). Descriptors with the highest impact on the activity are labeled; list and description of all 128 VolSurf+ descriptors is given in the VolSurf+ manual [17].

Furthermore, the descriptors related to the unbalance between the centre of mass of the molecule and the barycentre of its hydrophobic regions (amphiphilic moment, A, and integy moment, ID1), the partition coefficient of cyclohexane/water (LOGP c-Hex) and the ratio of molecular volume and molecular surface (R) showed the highest negative influence on the antiproliferative activity.

Therefore, an increase of H-bonding properties, hydrophobicity and flexibility, as well as decrease of the uneven distribution of hydrophobic and hydrophilic regions in the molecule should lead to an increase of antiproliferative activity against all three studied cell lines.

### *3.2. DNA Binding Properties*

Considering the simplicity and mutual similarity of the studied structures, fast screening by thermal denaturation experiments were carried out. The temperature at which half of the double stranded DNA is degenerated to single stand DNA ($T_m$) may increase upon binding of the tested compound to DNA (positive $\Delta T_m$), thus suggesting the stabilization of the DNA helix; rarely a decrease is observed upon DNA binding, evidencing DNA denaturation by the bound compound (negative $\Delta T_m$). Evaluation of the DNA degeneration temperature values obtained after binding of the chosen compounds **9**, **23**, **24**, **26** and **30** to **37** to circulating tumor DNA (ct-DNA) revealed compounds **36** and **37** as strong DNA binders (Table 3).

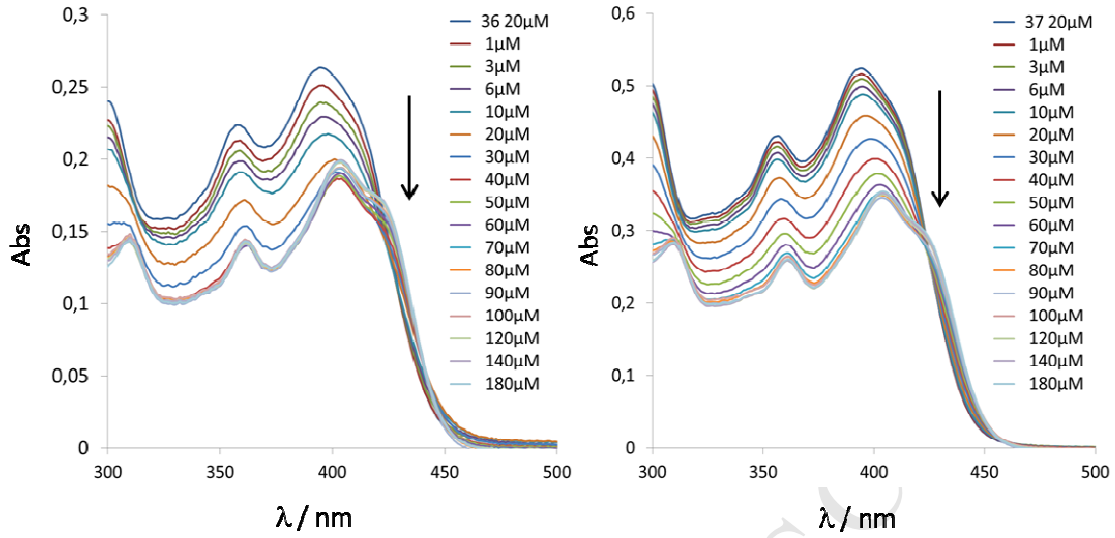
**Table 3.** DNA melting temperature studies. The measurement were performed at the indicated drug/DNA ratios (R); nd = not determined.

| **Compounds** | **ΔTm (°C)** | | |
|---|---|---|---|
| | **R=0.5** | **R=0.25** | **R=0.1** |
| **9** | 0 | nd | nd |
| **23** | 1.2 | nd | nd |
| **31** | 0 | nd | nd |
| **33** | 0.6 | nd | nd |
| **34** | 0.7 | nd | nd |
| **35** | 0.5 | nd | nd |
| **36** | 20.1 | 15.9 | 5.1 |
| | **R=0.25** | **R=0.1** | **R=0.05** |
| **37** | nd | 20.7 | 2.1 |

Compounds bearing amino substituents on position 2 which showed significant antiproliferative activity were not chosen since the DNA binding of similar compounds was already studied [16]. Exception was compound **9** with 3,5-dimethylpiperazin-1-yl substituent. Interestingly, only compounds **36** and **37** presented potent DNA helix stabilization whereas none of the other tested compounds changed the intrinsic degeneration temperature of ct-DNA. Compound **36** revealed $\Delta T_m$ values 15.9 °C and 20.1 °C at 1:2 and 1:4 compound/DNA ratios, respectively. $\Delta T_m$ values using higher compound/DNA ratios could not be determined since less than half of the DNA denaturation could be achieved using temperature as high as 100 °C. This observation suggested very strong and significant DNA stabilization due to the binding of **37** to DNA.

Based on the aforementioned considerations, compounds **36** and **37** were chosen for further evaluation by using spectrophotometric analysis. The DNA binding properties of **36** and **37** were underlined firstly by using UV/Visible spectroscopy. UV/Visible measurements were performed using a fixed concentration of compounds incubated with increasing concentrations of ct-DNA. Obtained results showed the absorption spectra modification for both studied compounds **36** and **37** (Figure 4A); hypsochromic and bathochromic shifts were observed, which are frequently associated with DNA intercalation. However, spectral changes did not reveal any isosbestic point (typically observed for a single binding mode), suggesting that the mode of binding of the compounds to the DNA helix is rather a complex. In addition, the intrinsic fluorescence properties of compounds **36** and **37** were studied by using fluorescence spectroscopy in the presence of increasing ct-DNA/compound ratios (Figure 4B) and evidenced an increase of the fluorescence intensity of compounds **36** or **37** upon binding to ct-DNA.

A)




B)

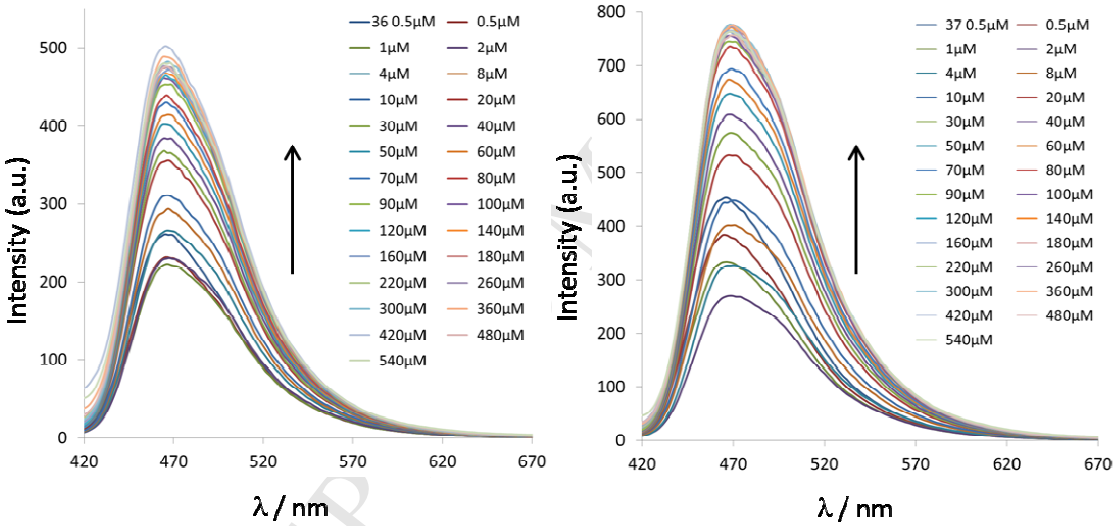



**Figure 4.** A) UV/Vis titration of compound **36** with ct-DNA, B) Fluorimetric titration of compound **36** with ct-DNA

To gain insight into the binding mode of compounds **36** and **37**, circular dichroism measurements and topoisomerase I-induced DNA relaxation were performed. By using circular dichroism, the orientations of the molecules relative to the DNA helix (intercalation, groove binding) were investigated. The absence of intrinsic CD of **36** and **37** was validated using the highest evaluated dose (60 µM, Figure 5, dashed lines).

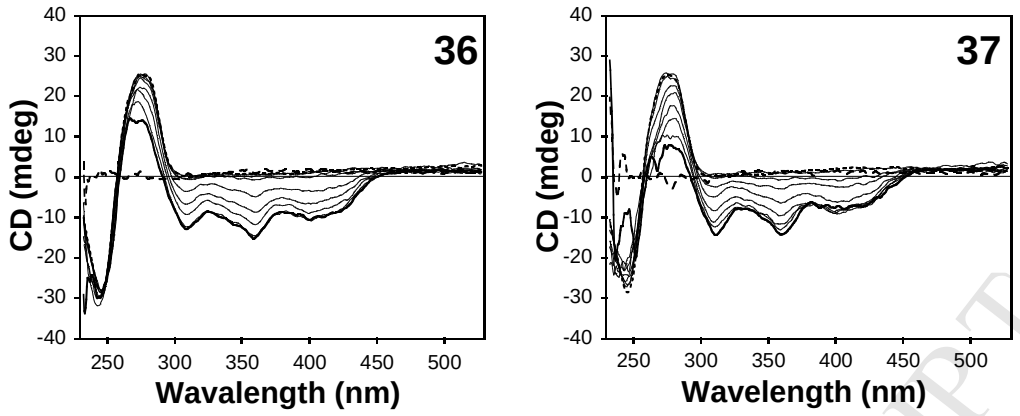



**Figure 5. Circular dichroism spectra.** ct-DNA (50 µM of base pairs) was incubated alone (dashed bold lines) with increasing concentrations of **36** or **37** from 1 to 60 (full bold lines) µM (top to bottom). Pointed bold lines correspond to the CD spectra of the compound alone (60 µM).

Binding of either compound **36** or **37** on ct-DNA decreased the intensity of the positive intrinsic CD of ct-DNA at 275 nm, thus suggesting modifications in the base stacking and/or the ellipticity of the DNA helix. Furthermore, binding of the compounds to DNA induced 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 compound/chromophore between adjacent base pairs of the DNA and creation of a new dichroic signal. Both changes were dependent on the compounds concentration.

In order to compare the DNA intercalation profile of **36** and **37** with other derivatives, the DNA topoisomerase I enzyme was used to dissipate the constraints of a negatively supercoiled circular DNA and to generate positively supercoiled circular DNA upon intercalation between adjacent base pairs [18]. 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. The topoisomerase I enzyme resolves the constraints that are generated upon negative supercoiling of the plasmid DNA when produced in bacteria (*Sc*) (Figure 6) and 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*). Blockage of the topoisomerase I re-ligation step by poison drugs such as camptothecin (CPT) results in the generation of a 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 reduce DNA migration within the agarose gel, as seen with **36** and **37** in Figure 5. Within this experiment we confirmed the DNA intercalation propensity of **36** and **37** but not the other evaluated compounds that were not found to bind to DNA.

Observed DNA intercalation is not associated with the 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 an intercalation process is not associated with sequence selective binding as evaluated using DNase I footprinting experiments with increasing concentrations of either **36** or **37** (data shown in Fig. S35).

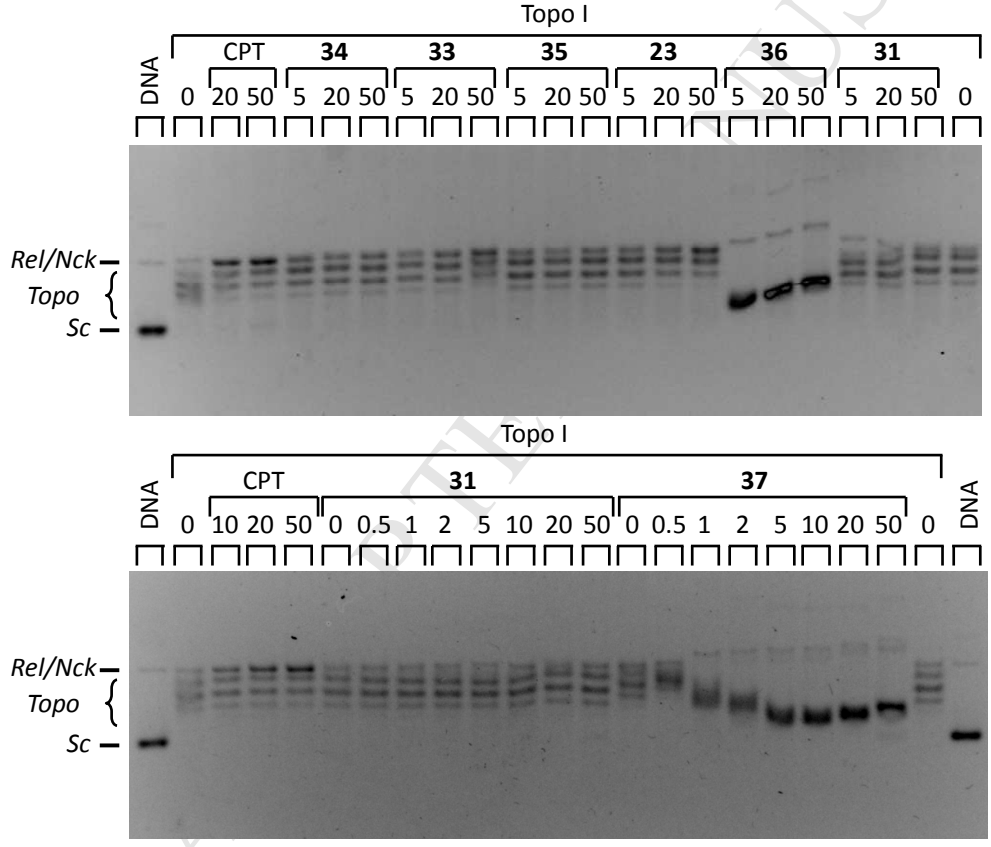



**Figure 6.** Topoisomerase I-induced DNA relaxation. The pUC19 supercoiled plasmid ("DNA") was incubated with topoisomerase I ("0") alone or in the presence of the indicated concentrations (µM) of compounds as specified on the top of the lanes. *Rel*, relaxed DNA; *Nck*, nicked DNA; *Topo*, topoisomers; *Sc*, supercoiled DNA.

Compounds **33** and **23** displayed 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 7).

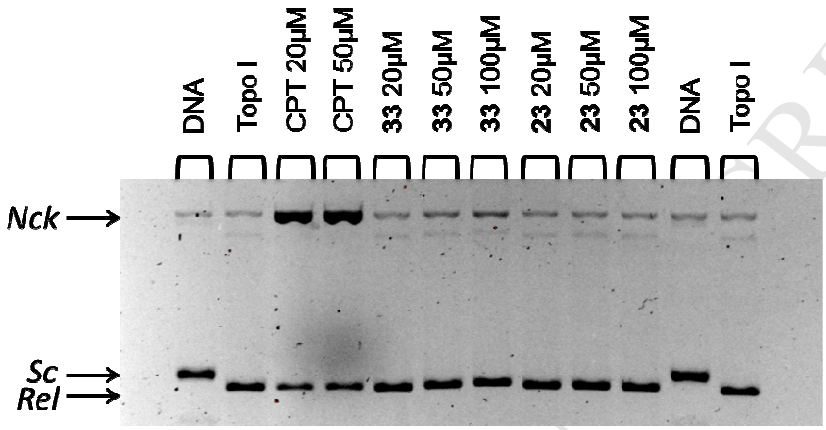



**Figure 7. Topoisomerase I poisoning effects of compounds 33 and 23.** Native supercoiled pUC19 plasmid DNA (lanes "DNA") was incubated with topoisomerase I in the absence (lanes "Topo I") or presence of increasing concentrations of compounds **33** and **23** and the reference drugs camptothecin (CPT). 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.

## 4. Conclusions

Herein we present the synthesis of amino substituted benzimidazo[1,2-*a*]quinoline-6-carbonitriles with different structural features and length of amino side chains incorporated at either one or two positions on the tetracyclic skeleton. The new compounds have been designed and synthesized by uncatalyzed microwave-assisted amination and explored with 3D-QSAR analysis, and their antiproliferative activities and DNA binding abilities were evaluated. The antiproliferative activities have been evindenced on human colon, breast and lung tumor cell lines. 2-amino substituted analogues **5**–**9** demonstrated a more pronounced antiproliferative activity when compared to 5- (**18**–**22**) and 2,5-diamino substituted derivatives **23**–**39**.

The results obtained from antiproliferative activity examination were in good agreement with previously published results. The antiproliferative activity results obtained for the synthesized compounds, in particular those with the most pronounced activity of the 2-substituted derivatives, were in good agreement with our previously published results [15]. Among the 2-substituted derivatives, 4-methyl-1-piperazinyl **8** or 3,5-dimethyl-1-piperazinyl **9** substituted analogues were the most active. Moreover, derivatives with long and/or branched side chains were less active (mono-substituted analogues) or completely inactive (di-substituted analogues).

In general, 3D-QSAR analysis revealed the same molecular properties having the largest positive and the largest negative influence on antiproliferative activity on all three studied cell lines. The increase of antiproliferative activity against all three studied cell lines could be explained by increased H-bonding properties, hydrophobicity and flexibility, as well as the decrease of unbalance in distribution of hydrophobic and hydrophilic regions in the molecule.

Compounds **36** and **37** were to be the most potent DNA binders of the compounds studied and was associated with strong DNA helix stabilization evidenced by using melting temperature studies and intercalative mode of DNA interaction (Figures 5 and 6). Additional experiments with topoisomerase I-induced DNA relaxation confirmed that DNA binding is not associated with sequence selective binding. The DNA intercalation process of the studied compounds does not correlate with the cytotoxic effects evaluated on colon (HCT 116), breast (MCF-7) and lung (H460) carcinoma cells.

1-Piperazinyl substituted compound **36** displayed a potent cytotoxic effect in all cell lines (1.5, 1 and 4 μM, respectively), while the quaternary iodide salt **37** was totally inactive ($IC_{50}$ > 100 μM). The new series of derivatives, bearing the amino substituent at position 2, demonstrated a high potential for expansion and optimization for their antiproliferative activity, in particular when either 2-methylpropyl or 1-piperazinyl amino substituents were used.

## 5. Experimental part

### 5.1 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 (at 300 and 75 MHz) or Varian Gemini 600 (at 600 and 150 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. Analyses are indicated by the symbols of the elements, and the results obtained were within ±0,4 % of the theoretical values.

### 5.2 Synthesis of 2-amino substituted benzimidazo[1,2-*a*]quinolines

*5.2.1 General method for preparation of compounds* ***5–9***

Compounds **5**–**9** were prepared by reacting 4 with excess of the appropriate amine in acetonitrile (10 mL) under microwave irradiation (800 W power) at 170 °C and 40 bar pressure for the required reaction time. After cooling, the reaction mixture was filtered off and and the crude solid was purified by column chromatography on $SiO_2$ using dichloromethane/methanol 100:1 as eluent.

*5.2.1.1 2-[(1-Methylethyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile* **5**. Following the above general method, compound **4** (100 mg, 0.38 mmol) reacted with isopropylamine (0.15 ml, 1.76 mmol) after 6 h of irradiation to yield 46 mg (43 %) of **5** as 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 ($[M+1]^+$).

*5.2.1.2 2-[(3-Methylbutyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile* **6**. Following the above general method, compound **4** (80 mg, 0.30 mmol) reacted with isopentylamine

(0.25 ml, 2.15 mmol) after 5 h of irradiation to yield 29 mg (29 %) of **6** as 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 ($[M+1]^+$).

*5.2.1.3 2-(Hexylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* **7**. Following the above general method, compound **4** (90 mg, 0.34 mmol) reacted with hexylamine (0.24 ml, 1.20 mmol) after 3 h of irradiation to yield 60 mg (54 %) of **7** as 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, $H_{arom.}$), 3.35-3.28 (m, 2H, $CH_2$), 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 ($[M+1]^+$).

*5.2.1.4 2-(4-Methyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile* **8**. Following the above general method, compound **4** (90 mg, 0.34 mmol) reacted with 1-methylpiperazine (0.10 ml, 0.90 mmol) after 3 h of irradiation to yield 65 mg (56 %) of **8** as 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 ($[M+1]^+$).

*5.2.1.5 2-(3,5-Dimethyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile* **9**.

Following the above general method, compound **4** (90 mg, 0.34 mmol) reacted with 2,6-dimethylpiperazine (0.150 g, 1.30 mmol) after 6 h of irradiation to yield 39 mg (33 %) of **9** as 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 ($[M+1]^+$).

### 5.3 Synthesis of 5-amino substituted benzimidazo[1,2-*a*]quinolines

*5.3.1 General method for preparation of compounds **18–22***

Compounds **18**–**22** were prepared by reacting **16** with excess of the appropriate amine in acetonitrile (10 mL) under microwave irradiation (800 W power) at 170 °C and 40 bar pressure for the required reaction time. After cooling, the reaction mixture was filtered off and and the crude solid was purified by column chromatography on $SiO_2$ using dichloromethane/methanol 100:1 as eluent.

*5.3.3.1 5-[(1-Methylethyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile **18***. Following the above general method, compound **16** (100 mg, 0.38 mmol) reacted with isopropylamine (0.10 ml, 1.12 mmol) after 2 h of irradiation to yield 42 mg (39 %) of **18** as 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 ($[M+1]^+$).

*5.3.3.2 5-[(3-Methylbutyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile* ***19***. Following the above general method, compound **16** (50 mg, 0.18 mmol) reacted with *i*-pentylamine (0.11 ml, 0.90 mmol) after 2 h of irradiation to yield 9 mg (15 %) of **19** as 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 ($[M+1]^+$).

*5.3.3.3 5-(Hexylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***20***. Following the above general method, compound **16** (100 mg, 0.36 mmol) reacted with hexylamine (0.20 ml, 1.52 mmol) after 3 h of irradiation to yield 56 mg (48 %) of **20** as 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 ($[M+1]^+$).

*5.3.3.4 5-(4-Methyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **21***. Following the above general method, compound **16** (100 mg, 0.36 mmol) reacted with 1-methylpiperazine (0.10 ml, 0.90 mmol) after 3 h of irradiation to yield 53 mg (43 %) of **21** as 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]$^+$).

*5.3.3.5 5-(3,5-Dimethyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **22***.
Following the above general method, compound **16** (100 mg, 0.36 mmol) reacted with 2,6-dimethylpiperazine (0.100 g, 0.87 mmol) after 3 h of irradiation to yield 60 mg (47 %) of **22** as 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]$^+$).

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

*5.4.1 α-(1H-benzimidazol-(3H)-idene)-2-chloro-4-fluoro-γ-oxobenzenepropanenitrile **13***. A solution of (1*H*-benzimidazol-2-yl)acetonitrile **2** (1.63 g, 10.40 mmol) and 2-chloro-4-fluorobenzoyl chloride **11** (2.00 g, 10.40 mmol) in pyridine (13 ml) was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into water (100 ml) and the precipitated solid was filtered off and recrystallized from ethanol to obtain **13** as 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).

*5.4.2 2-Fluoro-5-oxo-5,7-dyhidrobenzimidazo[1,2-a]quinoline-6-carbonitrile* ***15***. A solution of compound **13** (1.00 g, 3.37 mmol) and t-BuOK (0.88 g, 7.84 mmol) in DMF (12 ml) was refluxed for 2 h. After cooling, the reaction mixture was evaporated under vacuum, and the residue was triturated with water (50 ml). The resulting solid was filtered off and recrystallized from ethanol to obtain **15** as 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).

*5.4.3 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 the residue was triturated with water (10 mL). The resulting solid was filtered off and washed with water to obtain 17 as 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).

***5.4.4 General method for preparation of compounds 23–39***

Compounds **23**–**39** were prepared by reacting **17** with excess of the appropriate amine in acetonitrile (10 mL) under microwave irradiation (800 W power) at 170 °C and 40 bar pressure for the required reaction time. After cooling, the reaction mixture was filtered off and and the crude solid was purified by column chromatography on $SiO_2$ using dichloromethane/methanol 100:1 as eluent.

*5.4.4.1 2,5-Bis(methylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***23***. Following the above general method, compound **17** (50 mg, 0.50 mmol) reacted with 33 % solution of methylamine in ethanol (0.26 ml, 1.90 mmol) after 12 h of irradiation to yield 35 mg (57 %) of **23** as 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 ($[M+1]^+$).

*5.4.4.2 2,5-Bis(butylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***24***. Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with *n*-butylamine (0.18 ml, 1.80 mmol) after 4 h of irradiation to yield 75 mg (57 %) of **24** as 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 ($[M+1]^+$).

*5.4.4.3 2,5-Bis[(1-methylethyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile **25***.

Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with isopropylamine (0.40 ml, 2.35 mmol) after 7 h of irradiation to yield 31 mg (26 %) of **25** as 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 ($[M+1]^+$).

*5.4.4.4 2,5-Bis[(2-methylpropyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile **26***.

Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with isobutylamine (0.50 ml, 5.04 mmol) after 6 h of irradiation to yield 25 mg (19 %) of **26** as 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 ($[M+1]^+$).

*5.4.4.5 2,5-Bis[(3-methylbutyl)amino]benzimidazo[1,2-a]quinoline-6-carbonitrile **27***.

Following the above general method, compound **17** (200 mg, 0.72 mmol) reacted with isopentylamine (0.17 ml, 1.50 mmol) after 6 h of irradiation to yield 34 mg (28 %) of **27** as 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 ($[M+1]^+$).

*5.4.4.6 2,5-Bis(hexylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***28***. Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with hexylamine (0.27 ml, 3.90 mmol) after 6 h of irradiation to yield 58 mg (41 %) of **28** as 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 ($[M+1]^+$).

*5.4.4.7 2,5-Bis(dimethylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***29***. Following the above general method, compound **17** (260 mg, 0.88 mmol) reacted with dimethylamine (0.90 ml, 13.60 mmol) after 4 h of irradiation to yield 152 mg (52 %) of **29** as 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 ($[M+1]^+$).

*5.4.4.8 2,5-Bis(diethylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **30***. Following the above general method, compound **17** (80 mg, 0.27 mmol) reacted with diethylamine (0.12 ml, 1.10 mmol) after 5 h of irradiation to yield 36 mg (34 %) of **30** as 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 ($[M+1]^+$).

*5.4.4.9 2,5-Bis(dipropylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **31***. Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with dipropylamine (0.50 ml, 5.90 mmol) after 4 h of irradiation to yield 35 mg (23 %) of **31** as 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 ($[M+1]^+$).

*5.4.4.10 2,5-Bis(dipentylamino)benzimidazo[1,2-a]quinoline-6-carbonitrile **32***. Following the above general method, compound **17** (150 mg, 0.50 mmol) reacted with dipentylamine (0.80 ml, 3.90 mmol) after 4 h of irradiation to yield 75 mg (27 %) of **32** as 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 ($[M+1]^+$).

*5.2.4.11 2,5-Di-(1-pyrrolidinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **33***. Following the above general method, compound **17** (210 mg, 0.70 mmol) reacted with pyrrolidine (0.10 ml, 1.22 mmol) after 5 h of irradiation to yield 58 mg (22 %) of **33** as 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 ($[M+1]^+$).

*5.2.4.12 2,5-Di-(1-piperidinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **34***. Following the above general method, compound **17** (420 mg, 1.42 mmol) reacted with piperidine (0.15 ml, 1.50 mmol) after 10 h of irradiation to yield 150 mg (43 %) of **34** as 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 ($[M+1]^+$).

*5.4.4.13 2,5-Di-(4-morpholinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **35***. Following the above general method, compound **17** (70 mg, 0.24 mmol) reacted with morpholine (0.20 ml, 2.31 mmol) after 5 h of irradiation to yield 73 mg (74 %) of **35** as 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 ($[M+1]^+$).

*5.4.4.14 2,5-Di-(1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **36***. Following the above general method, compound **17** (50 mg, 0.17 mmol) reacted with piperazine (0.100 g, 1.20 mmol) after 1 h of irradiation to yield 50 mg (72 %) of **36** as 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 ($[M+1]^+$).

*5.4.4.15 2,5-Di(4-methyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile **38***. Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with 1-methylpiperazine (0.10 ml, 0.90 mmol) after 3 h of irradiation to yield 50 mg (34 %) of **38** as 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 ($[M+1]^+$).

*5.4.4.16 2,5-Di(3,5-dimethyl-1-piperazinyl)benzimidazo[1,2-a]quinoline-6-carbonitrile* ***39***. Following the above general method, compound **17** (100 mg, 0.34 mmol) reacted with 2,6-dimethylpiperazine (0.100 g, 0.90 mmol) after 3 h of irradiation to yield 51 mg (32 %) of **39** as 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 4,4'-(6-cyanobenzimidazo[1,2-a]auinoline-2,5-diyl)-bis(1,1-dimethylpiperazinium)-jodide* ***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 ($[M+1]^+$).

### 5.3 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 [15, 16]. Altogether 51 compounds were used (Table S1 in the Supplement). Negative logarithmic values of concentrations that cause 50 % growth inhibition of the cell lines (p$IC_{50}$) 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 [17]. 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 were considered. The definition of all 128 VolSurf+ descriptors is given in the VolSurf+ manual [17, 18].

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.

### 5.4 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) and one non-tumor cell line (HMLE-shGFP; immortalized human mammary epithelial cells (kindly provided by Prof. R. Weinberg, MIT). The tumor 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, while HMLE-shGFP cells were maintained in a 1:1 mixture of DMEM + 10 % FBS, insulin, hydrocortisone, and HuMEC medium (Thermo Scientific) in a humidified atmosphere with 5 % $CO_2$ at 37 °C.

The growth inhibition activity was assessed as described previously [15, 16, 18]. The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at $3\times10^4$ cells/mL (HCT 116, H 460) to $5\times10^4$ 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 $A_{ctrl}$ – mean$A_{tzero}$).

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.

**5.5 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.

*5.5.1 DNA melting temperature*

The DNA melting temperature analysis was performed as described in [19, 20]. Briefly, 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 = $Tm_{[compound + DNA]}$ - $Tm_{[DNA\ alone]}$).

*5.5.2 UV/Visible spectroscopy*

The UV/Visible spectra were obtained essentially as described [20, 21]. 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.

*5.5.3 Fluorescence spectroscopy*

Fluorescence spectra were recorded from 400 to 700 nm essentially as described [15, 16, 22]. 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.

*5.5.4 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 [19].

*5.5.5 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 [19, 20, 23, 24].

*5.5.6 DNase I footprinting*

DNase I footprinting experiments were conducted essentially as described [25, 26]. 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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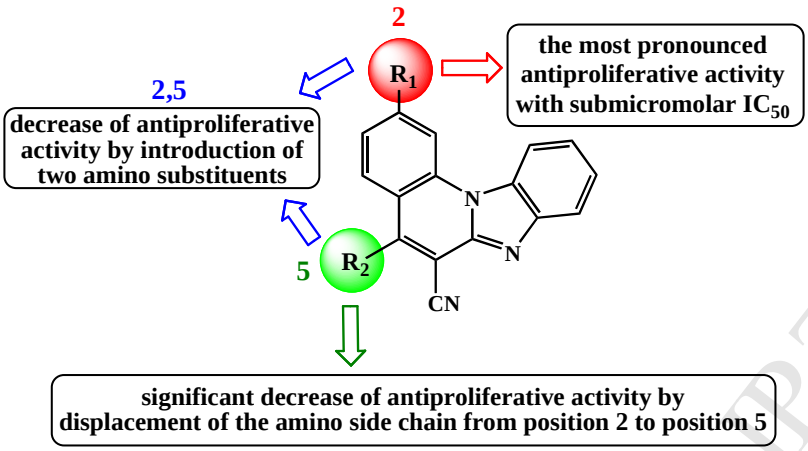


**Figure 2.** SAR of amino substituted benzimidazo[1,2-*a*]quinoline-6-carbonitriles

**Research Highlights**

- Amino substituted benzimidazo[1,2-*a*]quinoline-6-carbonitriles with antiproliferative activity
- Molecular properties identified by 3D-QSAR analysis
- 2-Amino subsituted analogues showed the most potent antiproliferative activity
- Compounds **36** and **37** evidenced strong DNA binding properties