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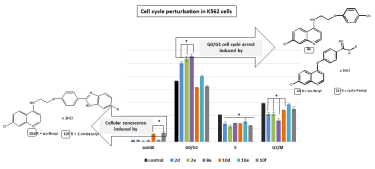

New Quinoline-Arylamidine Hybrids: Synthesis, DNA/RNA Binding and Antitumor Activity

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Abstract

Four series of new hybrid molecules with 7-chloroquinoline and arylamidine moieties joined through the rigid -O- (groups **I (2a-g)** and **II** (**5a-g)**) or flexible -NH-CH_2-CH_2-O- (groups **III (8a-g)** and **IV (10a-g)**) linker were synthesized, and their DNA/RNA binding properties and cytotoxic activity were tested, against several human cancer lines. The compounds and their interaction with DNA and RNA were studied by UV-Vis and CD spectroscopy. The obtained results showed that the binding affinity of the investigated compounds increases proportionally with the increase of the length and number of groups able to form hydrogen bonds with ds-polynucleotides. Improvement of binding was additionally achieved by reduction of the structural rigidity of the investigated compounds, new hybrid compounds preferentially bind to ctDNA. For most of them the DNA/RNA grooves are dominant binding sites, except for the compounds from group **II** for which intercalation in polyA-polyU was the dominant binding mode. The antiproliferative effects were tested by the MTT test on normal (MDCK1), carcinoma (HeLa and CaCo2) and leukemia cell lines (Raji and K462). The GI_{50} values for all investigated compounds ranged from 5 to more than 100 × 10^{-6} mol dm^{-3}. Carcinoma cells were more resistant to the investigated compounds than leukemia cells. The most effective compounds against leukemia cell lines were from group **IV (10a-g)**, with GI_{50} values ranging from of 5 and 35 × 10^{-6} mol dm^{-3}. The cell cycle arrest was investigated by flow cytometry and the obtained results indicate that the selected compounds, **2d**, **2e**, **8a**, **10d**, **10e**, and **10f,** induce changes in the cell cycle of treated cells, but the cycle phase distribution varies between them. A significant decrease in the number of cells in S phase ($p < 0.001$) was

observed in all treated cells, but only **10d** and **10f** induce cell cycle arrest at G0/G1 phase, dominantly.

Key words: Hybrid molecule, 7-Chloroquinoline, Aromatic amidine, DNA/RNA binding, Antitumor activity

1. **Introduction**

The rising burden of cancer has a huge cost, enormous human potential is lost and the treatment of cancer has an escalating economic impact. Although cancer therapies have improved significantly in recent years, the clinical use of chemotherapeutics is often limited due to chemo-resistance and/or the undesirable toxic effects caused by non-specific cell killing at an effective dose of drugs [1, 2].

To overcome these problems, new drug discovery and development strategies are focusing on molecules that act simultaneously on multiple targets, either by a combination of two (or more) drugs or by combining two (or more) active pharmacophores covalently connected in a single-hybrid molecule with dual activity [3, 4]. The clinical development of an optimal combinational therapy is costly, and drug-drug interaction may result in additive toxic side effects. On the other hand, dual-drugs are designed to interact with multiple targets, induce synergistic effects and improve the efficacy and safety of drugs in the treatment of complex diseases [5, 6]. Due to the complex chain of events that causes cancer, it is important to address combinational modalities in order to provide a better response to cancer therapy.

An additional advantage of this method may arise from the fact that chemotherapy usually causes immunosuppression in patients, and thus makes them susceptible to various infectious diseases. As a result, the development of a single drug for treatment of more than one disease would make an important contribution to the patient's therapy.

This approach has stimulated us to design, synthesize and evaluate a new class of hybrid molecules, by joining two pharmacophores which are present in a number of natural and synthetic agents: 7-chloroquinoline and phenyl- or phenylbenzimidazolyl-amidine (arylamidine).

Chloroquine (CQ), 7-chloro-4-aminoquinoline, has been the mainstay of malaria chemotherapy for much of the past seven decades. The drug has several advantages, including limited host toxicity, ease of use, low cost, and effective synthesis. More recently, CQ and its analogs have been the subject of intense interest in the development of safe and effective anticancer drugs, due to the fact that parasites and cancer cells share basic characteristics related to the metabolic requirements associated with their high proliferation rate [7]. Several

4-aminoquinoline based derivatives have been reported for their significant activity against different cancer cell lines [8-11]. Among the 7-chloro-4-amino(oxo)quinoline derivatives, derivatives with N-heterocycles in the side chain have an important place. These pyrimidine [12], triazine [13] or pyrazoline [14] based hybrids showed a wide range of activity. The mechanism of action for their anticancer activity is not clearly understood, but data from physiochemical studies suggest that CQ forms a complex with DNA, resulting in defects in DNA synthesis and repair. It was been concluded that the ability to form a H-bond between the N-diethylamino lateral side chain of CQ and a DNA base may contribute to the DNA intercalative activity of the quinoline nucleus [15, 16].

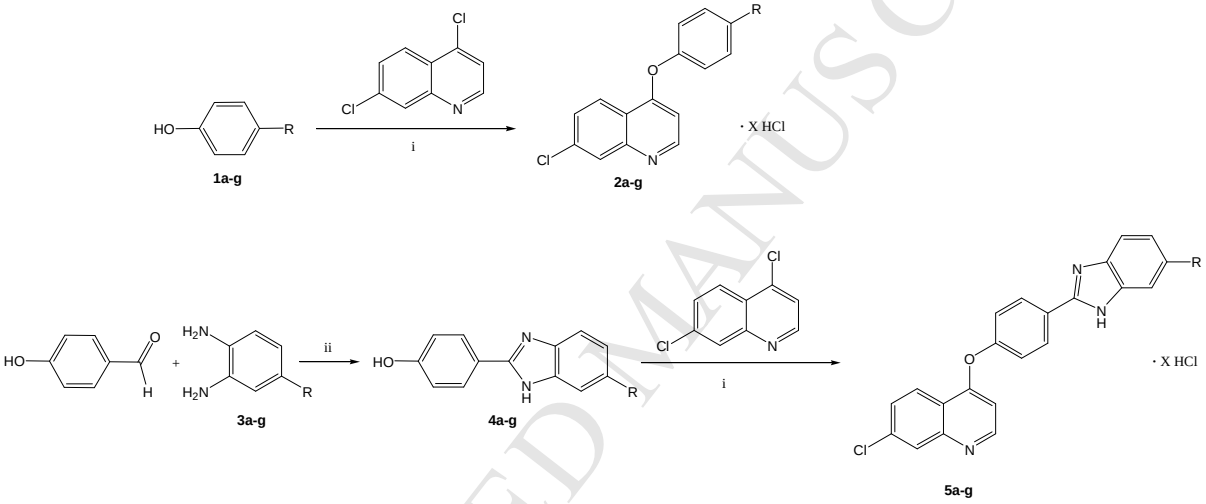
On the other hand, aromatic amidines, DNA-affinic and highly water soluble moieties, have exhibited a wide range of outstanding therapeutic applications, for instance as ACIS inhibitors [17], botulinum neurotoxin A inhibitors [18], and anticancer [19], antiparasitic [20], and antibacterial [21,22] agents. These compounds bind to the DNA minor groove by a combination of ionic, hydrophobic and hydrogen bonding interactions [23]. Although the mechanism of action of aromatic amidines is not fully elucidated, it has been proven that their bioactivity is the result of DNA binding and the subsequent inhibition of DNA-dependent enzymes (topoisomerases, polymerases, and nucleases), or possibly by direct inhibition of transcription [24]. The generally accepted view is that the (un)symmetrical diamidines, with two cationic terminal groups bind more tightly to the DNA and show better biological activity, but recently published studies have shown that non-symmetrical mono-arylamidines may be a new lead in the treatment of cancer and infectious diseases [25,26].

Although 7-chloroquinoline and arylamidines are present in many compounds with broad pharmacological activities, their combination in a single hybrid molecule has only been described in a few papers [27,28]. Aromatic amidine, as a pharmacophore, was chosen because numerous benzimidazolyl- and phenyl-amidines previously synthesized and evaluated in our group showed potent anticancer [29-31], antibacterial [22] and antiparasitic activities [32]. Parallel studies showed a correlation between the biological activity and DNA binding affinity of the investigated molecules. Compounds with stronger binding to DNA showed more potent activity. These studies have indicated that the structure of the terminal cationic groups of the tested compounds has a significant contribution to the strength of binding to DNA, and biological activity. Therefore, we synthesized and evaluated the antitumor activity of a series of compounds with a wide variety of amidine moieties: unsubstituted amidine, alkyl substituted amidines and cyclic amidines.

Since amidines show stronger interaction with DNA than 7-chloroquinoline, we decided to keep 7-CQ as the unchanged standard in all the newly synthesized hybrid molecules, and modify the second pharmacophore as well, as a link between them to study the effect of these modifications on the compound's DNA/RNA affinity and selectivity, and biological activity. The results discussed in the present paper reflect our efforts to discover new, potentially anticancer chemotherapeutic agents.

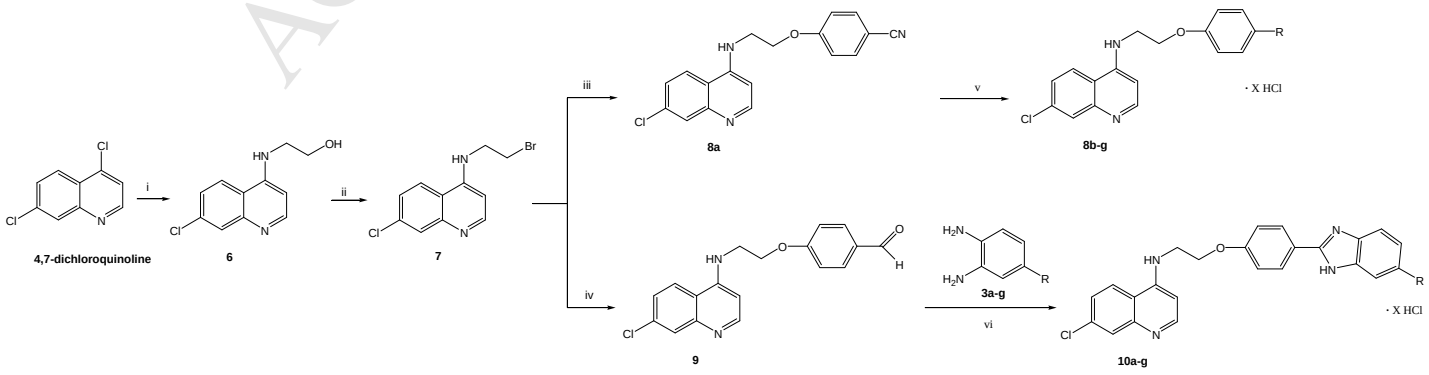
2. Results and discussion

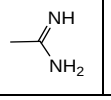
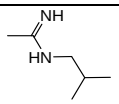
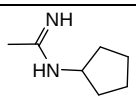
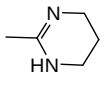
2.1. Chemistry



Compounds **1-5**	**a**	**b**	**c**	**d**	**e**	**f**	**g**
R	—CN	NH, NH_2	NH, HN	NH, HN	NH, HN	N, N, H	N, HN

Scheme 1: Reagents and conditions: (i) (a) K_2CO_3, DMF, reflux; (b) EtOH/HCl(g); (ii) (a) benzoquinone, MeOH; (b) EtOH/HCl(g).



Compounds **3,8,10**	**a**	**b**	**c**	**d**	**e**	**f**	**g**
R	—CN						

Scheme 2: Reagents and conditions: (i) 1-ethanolamine, 130 °C, 5h, 90%; (ii) HBr, H_2SO_4, 170 °C, 3.5h 58%; (iii) 4-hydroxybenzonitrile, DMF, 70 °C, 66%; (iv) 4-hydroxybenzaldehyde, DMF, 70 °C, 62%; (v) (a)HCl(g), MeOH; (b) appropriate amine, MeOH, reflux or ambient temperature; (c) EtOH/HCl(g); (vi) (a)$NaHSO_3$, EtOH, reflux; (b) EtOH/HCl(g).

Schemes 1 and 2 outline our approach to the synthesis of the target hybrid molecules containing two pharmacophores, 7-chloroquinoline and aromatic amidine, joined through a short, rigid (-O-) or a long, more flexible (-NH-CH_2-CH_2-O-) linker. The phenol- and phenol-benzimidazole amidines attached with a short linker to 7-CQ were synthesized according to the procedure outlined in Scheme 1. 4,7-Dichloroquinoline was allowed to react with substituted phenol (**1a-g**) or 2-(4-hydroxyphenyl)-1*H*-benzimidazole derivatives (**4a-g**) in the presence of potassium carbonate in dry dimethylformamide (DMF) to give the expected products as a free base. Stirring amidine derivatives with ethanolic HCl produced the corresponding water soluble di(tri)hydrochloride salts **2b-g** and **5b-g**. This reaction path proved to be better than the alternative route that would imply synthesis of nitrile intermediates **2a** or **5a** and their conversion to amidines. Phenol amidines **1b-g**, widespread building blocks, were synthesized according to the Pinner method [31] from 4-hydroxybenzonitrile (**1a**), dry methanol saturated with HCl(g) and the appropriate amine. The synthesis and physical properties of precursors **4a**,**b**,**f** and **g** from the phenol-benzimidazole series have been described previously [33]. The remaining compounds from this series (**4c**,**d**,**e**) were newly synthesized and a description of the synthesis, as well as the physical properties of these compounds, is given in the present paper. All spectroscopic and analytical data of earlier synthesized compounds correspond with information previously given, and will not be shown in this paper.

Scheme 2 illustrates the synthetic route for the synthesis of compounds with an aminoethoxy linker. The required intermediates **8a** and **9** were prepared by the typical aromatic substitution reaction using the method reported by Guantai *et al*. [34]. 4,7-Dichloroquinoline was heated with excess ethanolamine to yield 2-(7-chloroquinolin-4-ylamino)-ethanol (**6**). The latter compound was converted to (2-bromoethyl)-(7-chloroquinolin-4-yl)-amine (**7**) by gentle reflux in a mixture of hydrobromic and sulfuric acid

[35]. Bromide (**7**) reacted with 4-hydroxybenzonitrile (**1a**) in the presence of potassium carbonate in dry dimethylformamide (DMF) to give 4-(2-(7-chloroquinolin-4-ylamino)ethoxy)benzonitrile (**8a**) in moderate yield. This nitrile was then converted to targeted amidine hydrochloride salts (**8b-g**) applying the Pinner reaction.

Synthesis of phenoxy-benzimidazol amidine trihydrochlorides **10b-g** was performed in two steps. The bromide **7** was first allowed to react with commercially available 4-hydroxybenzaldehyde in the presence of potassium carbonate, to afford benzaldehyde intermediate (**9**) [36]. The benzimidazoles **10a-g** were prepared in a good yield by employing a condensation reaction between aldehyde **9** and 3,4-diaminobenzonitrile (**3a**) or 3,4-diaminobenzamidine derivatives **3b-g**, by boiling in the presence of $NaHSO_3$.

2.2. Spectroscopy

2.2.1. Spectroscopic characterization of compounds

Based on the structure, the compounds were divided into four groups: group **I** (**2a-g**, Scheme 1), group **II** (**5a-g**, Scheme 1), group **III** (**8a-g**, Scheme 2), group **IV** (**10a-g**, Scheme 2). All compounds, except the nitrile derivatives, were dissolved in water, $c = 1 \times 10^{-3}$ mol dm^{-3}. Nitriles were dissolved in DMSO ($c = 1 \times 10^{-3}$ mol dm^{-3}). The aqueous solutions of the studied compounds were stable for several weeks. The absorbance of all solutions, measured in the range of 220-900 nm, was proportional to their concentrations up to 3.5×10^{-5} mol dm^{-3}, indicating that there was no significant intermolecular stacking that could give rise to hypochromicity effects. The UV-Vis spectra of compounds revealed negligible temperature dependent changes (25–90 °C) and excellent reproducibility upon cooling to 25 °C. Absorption maxima and the corresponding molar extinction coefficients (ε) are given in Table S1.

2.2.2. Spectrophotometric titrations of compounds with ds-polynucleotides in aqueous medium

UV-Vis titrations were performed in a buffered medium (sodium cacodylate buffer, I = 0.05 mol dm^{-3}, pH 7.0) at room temperature, Table 1. Positions of absorption maxima in UV-Vis spectra of compounds **2a-g** (<300 nm) inhibit use of UV-Vis titrations for determination of the affinity and binding constants of investigated compounds toward polynucleotides (*ct*DNA and polyA-polyU) due to the overlapping of the signals of compounds and ds-polynucleotides. UV-Vis titrations with ds-polynucleotides were carried out for compounds **5a-g** by adding increasing aliquots of ds-polynucleotide solutions to a

fixed concentration of each compound. The decrease in the absorption maxima (25-80%) accompanied by a bathochromic shift (λ = 4-7 nm) indicates the formation of a new DNA-ligand species, except for nitrile **5a**. The absorption spectra were recorded until saturation was observed. The binding constants for compounds **5b-g** were determined in the range of 5×10^5 – 7×10^7 mol dm^{-3}. To assess the sequence selectivity of these compounds, the experiment was repeated with polyA-polyU. A bathochromic shift (λ = 2-6 nm) was observed along with the decrease in new bands (7-70%), indicating the formation of new compound-polyA-polyU complexes. The UV-Vis titration spectra revealed that alkylation of amidine **5b** and its replacement with cyclic amidine moieties increased affinity toward *ct*DNA and polyA-polyU. Among amidines **5b-g**, the isopropyl derivative **5c** showed the highest affinity toward ctDNA, while **5g** showed the highest affinity toward polyA-polyU.

The change of linker between quinoline and aryl amidine, by substitution of oxygen with 2-aminoethanol produced the next set of compounds, **8a-g and 10a-g**. The UV-Vis spectra of **8a-g** showed minimal changes during titration, with ds-polynucleotides indicating that those compounds interact only though a very weak electrostatic external mode, and as a result, no further studies were conducted with them. Introduction of the benzimidazole group between the phenyl and amidine moieties in the previous series resulted in a new group of compounds, **10a-g**. Titration of compounds from this series with *ct*DNA (58 % AT base pairs) resulted in pronounced hypochromism accompanied by a bathochromic shift ($\Delta\lambda$ = 6–11 nm), showing the formation of a new DNA ligand species. Addition of *ct*DNA to the solution of compound **10f**, until r = 0.15, yielded a decrease in absorption maxima accompanied by bathochromic shifts ($\Delta\lambda$ = 7 nm). Further addition of the mentioned polynucleotides ($r \leq 0.15$) increased the position of maxima which produced sufficient data for accurate processing using the Scatchard equation. To assess the sequence selectivity of the investigated compounds, the experiment was repeated with ds-RNA polynucleotides (polyA-polyU). Changes in the UV-Vis spectra of **10a**-**10c** and **10g** after the addition of polyA-polyU were minor, indicating an external, electrostatic binding mode. The addition of polyA-polyU to the solutions of compounds **10d-f** resulted in hypochromic (24-50 %) and bathochromic (3 -7 nm) changes as a result of the formation of complexes. The density of the binding sites (Table 1, ratio n) and the absence of isosbestic points in the UV-Vis spectra obtained by titration with both polynucleotides indicates several simultaneous binding modes. In general, high n values suggest DNA/RNA groove binding as the dominant binding mode, possibly accompanied by agglomeration along the DNA/RNA double helix, but this cannot exclude intercalation accompanied by agglomeration or groove binding. The binding constants K_s and ratios n

obtained by processing UV-Vis titration data with the Scatchard equation [37] are summarized in Table 1.

Table 1. The hypochromic effects (H/%)[a], binding constants (log*Ks*) and ratios *n* ([compound]/[polynucleotide phosphate]) calculated from the UV/Vis titrations of compounds with ds-polynucleotides (sodium cacodilate buffer, I = 0.05 M, pH = 7).

	ctDNA			**pApU**		
	H/%	log K_s	*n*	H/%	log K_s	*n*
5b	79.8	6.83	0.69	6.9	6.43	0.44
5c	60.0	7.87	0.42	26.8	5.36	0.36
5d	60.2	5.88	0.47	17.9	6.05	0.37 fix
5e	36.7	6.23	0.56	22.8	6.31	0.45 fix
5f	68.5	5.76	0.34	69.7[b]	-[e]	> 1
5g	25.5	6.96	0.39	15.5	7.29	0.33
8a	26.2[b]	-[e]	0.58[b]	-[c]	-[c]	-[c]
10b	17.7	6.72	0.58	-[c]	-[c]	-[c]
10c	23.7	6.63	0.45	-[c]	-[c]	-[c]
10d	32.2	6.51	0.35	26.0	7.02	0.46
10e	24.4	6.29	0.48	49.7	6.81	0.75
10f	26.0, 21.6[b]	-[d]	-[d]	37.7	6.71	0.57
10g	31.0	6.52	0.56 fix	-[c]	-[c]	-[c]

[a] Titration data were processed using the Scatchard equation [37]; accuracy of the result obtained $n \pm 10 - 30\%$, consequently log K_s values vary in the same order of magnitude.
[b] determine from experimental data
[c] too small changes
[d] mixed binding modes
[e] cannot be determined

2.2.3. Thermal melting studies

Thermal melting enables the rapid qualitative evaluation of the relative binding affinities of compounds to ds-polynucleotides. Changes in the melting point (T_m) of *ct*DNA and polyA-polyU upon the addition of compounds were measured in the range of *r* [compound] / [polynucleotide] = 0.1–0.5 (Table 2). Compounds from group **I** (**2b-g**) showed moderate stabilization of *ct*DNA. Toward polyA-polyU only the cyclic derivatives **2f** and **2g** produced stabilization (r = 0.3 (**2f**) and r = 0.1 (**2g**)). In general, the data for compounds **5b-g**

show that as the aromatic stacking surface was extended the *ct*DNA binding affinity increased. On examining the data for *ct*DNA it can be seen that the compounds from group **II** have greater affinity than compounds from groups **I**, Table 2. The ΔT_m was also found to increase as a function of the length and the flexibility of the compounds. Compounds from group **IV** (**10a-g**) showed significantly higher stabilization of *ct*DNA in comparison with compounds from other groups.

Table 2. ΔT_m values (°C)[a] of studied ds-polynucleotides upon addition of compounds at a different ratio r[b] (pH = 7, sodium cacodilate buffer, I = 0.05 M).

	ctDNA			**PolyA-polyU**		
Compd	0.1	0.3	0.5	0.1	0.3	0.5
2b	1.76	2.17	2.07	0	-0.16	-0.26
2c	2.01	2.28	2.28	0.36	-0.36	-[d]
2d	1.91	1.97	1.85	-0.71	-0.80	-0.44
2e	1.35	1.43	1.51	0.64	0.65	0.28
2f	2.65	2.67	2.73	1.18	2.18	1.49
2g	2.08	1.86	1.96	1.62	1.22	1.32
5b	2.07	2.84	2.90	2.98	0.72	0.21
5c	1.07	1.75	2.24	-0.44	1.32	0.43
5d	1.07	1.41	2.08	1.64	0.78	0.25
5e	1.41	2,91	3,07	0.18	4.06	6.82
5f	1.60	3.20	4.08	-0.16	0.78	1.12
5g	3.30	3.31	3,89	2.84	1.98	1.82
10a	3.96	2.58	2.60	-	-	-
10b	4.84	8.40	8.40	-	-	-
10c	5.83	9.05	9.83	-	-	-
10d	4.31	8.15	8.67	1.24	0.75	-[d]
10e	3.71	7.80	7.80	3.02	1.14	4.67/ 35.35
10f	5.75	-[d]	-[d]	4.62	5.16	8.86/ 33.48
10g	6.33	9.76	10.77	-	-	-

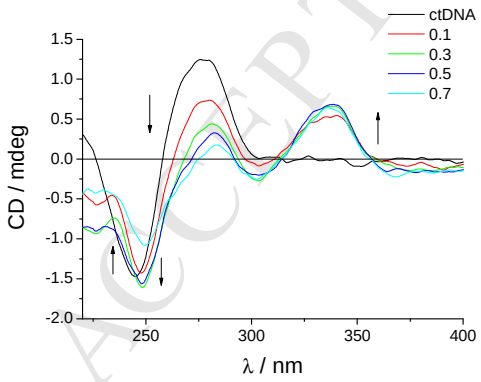
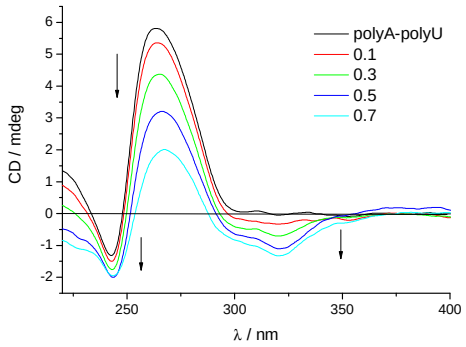
[a] Error in ΔT_m: ±0.5 °C
[b] r = [compound]/[polynucleotide]
[c] - = not recorded
[d] cannot be determine

Addition of extended compounds strongly stabilized the double helix of *ct*DNA, where the obtained ΔT_m values suggested saturation of binding sites at about r = 0.3–0.5. Biphasic curves observed for the interactions of compounds **10e** and **10f** with polyA-polyU at higher ratios r may indicate agglomeration of compounds along the polynucleotide [38]. Furthermore, the stronger thermal stabilization of DNA in comparison to the RNA analogue (poly A–poly U) may be attributed to the much narrower and hydrophobic minor groove of the ds-DNA with respect to ds-RNA [39].

2.2.4. Circular dichroism (CD) experiments

In order to gain an insight into the changes to polynucleotide properties induced by small molecule binding, we chose CD spectroscopy as a method, highly sensitive toward conformational changes in the secondary structure of polynucleotides. The investigated compounds do not possess intrinsic CD spectra, so induced CD spectra (ICD) upon the binding of compounds to polynucleotides, will provide us useful information about binding modes.

Interpretation of the CD spectra in the region of DNA absorbance is difficult due to contributions arising from both changes in DNA conformation due to accommodating the ligand, and changes in transitions of the ligand due to the new environment. For this reason, we focused on the region of the spectrum >300 nm.

5f		

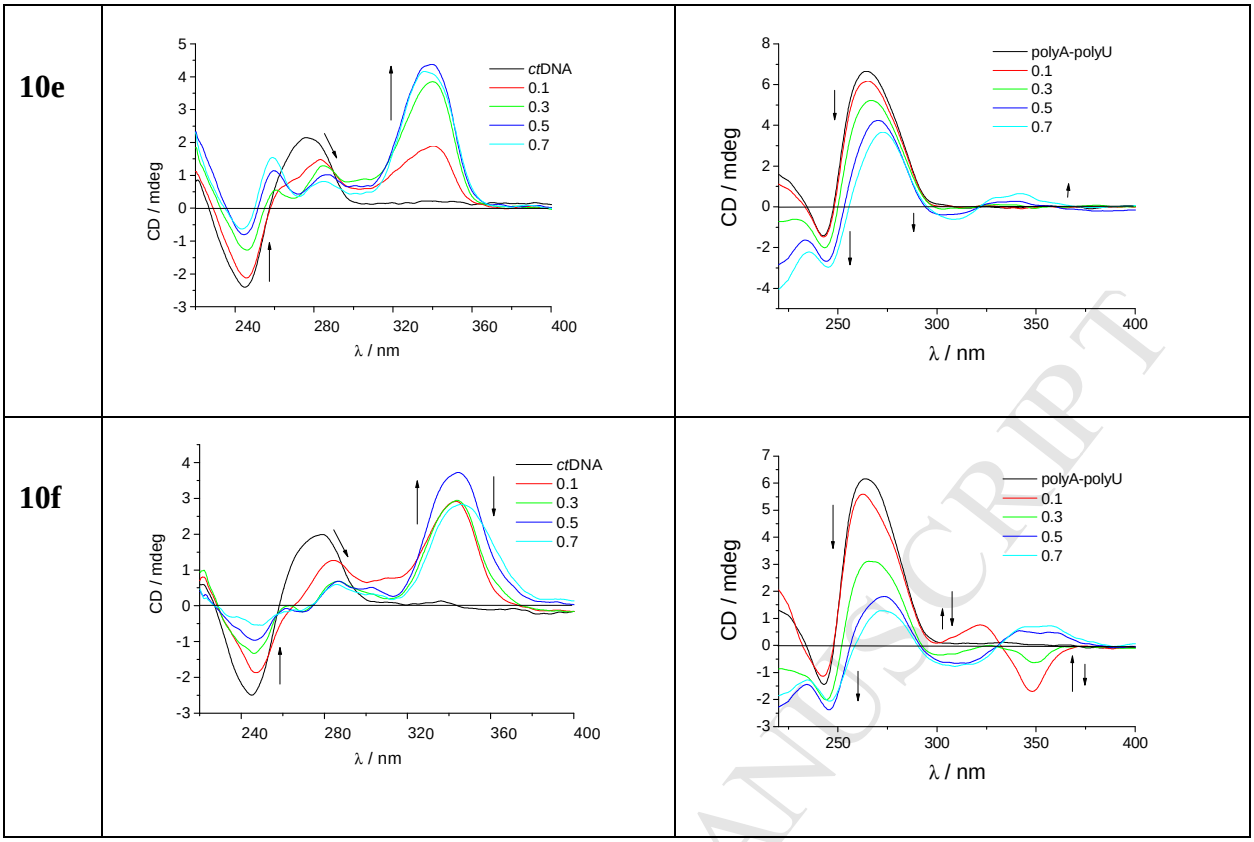


Figure 1. CD titrations of ct-DNA (c = 2.0 ×10^{-5} mol dm^{-3}) and polyA-polyU (c = 2.0 ×10^{-5} mol dm^{-3}) with **5f**, **10e** and **10f** (ratio, r = [compound]/[polynucleotide] = 0.1, 0.3, 0.5 and 0.7).

Depending on the compound's relative orientation and distance towards the helical axis, different changes in the CD spectra are obtained. The concentration dependent CD signature of compounds from group **II** was determined first. As could be seen from the ICD spectra, the changes to the ds-polynucleotides CD signals are directly proportional to the concentration (Supporting Information, Figure S1). After the addition of compounds to the solution of ctDNA a positive ICD band arises >300 nm, and increases again with increasing amount of compound until the point of saturation. Meanwhile, the CD signal of polynucleotide (around 280 nm) decreases without dramatic changes in shape, indicating its binding into grooves but without any major disruption of the DNA helix. The addition of **10a-g** of group **IV** to the *ct*DNA solution resulted in a decrease in CD signal 280 nm and appearance of a new positive maximum in the range of 300-400 nm (Fig. 1). In contrast to the ICD spectra of compounds from the group **II**, addition of larger compounds induced drastic changes to the CD signal of *ct*DNA, indicating significant disruption of the DNA helix. In addition, the absence of isoelliptic points pointed toward the formation of several types of

compound/DNA complexes, depending on the concentration ratio. This is in agreement with the results obtained from UV-Vis titrations. The strongly pronounced non-linear dependence of changes in the CD spectra on the ratio *r* indicates saturation of dominant binding sites at about *r* = 0.3-0.5 (Fig. 2). These *r* values are again in good agreement with the experimental and calculated data obtained from UV-Vis titrations (Table 1) and thermal melting experiments.

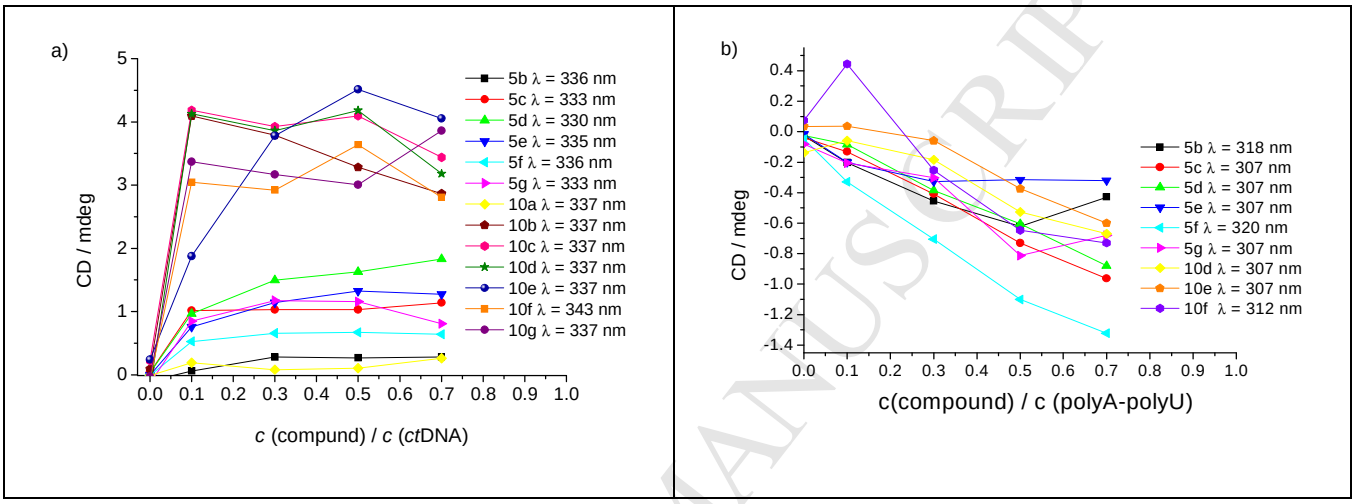


Figure 2. Dependence of CD signal on ratio *r*. (*r* = [compound]/[polynucleotide] = 0.1, 0.3, 0.5 and 0.7).

With regard to polyA-polyU, the appearance of a negative signal (> 300 nm) in the ICD spectra of compounds **5b-g** showed intercalation as the dominant binding mode. The changes in the ICD spectra showed no saturation of binding sites at $n \approx 0.25$ which may be assumed according to the dominate binding mode. This result, together with the lack of isoelliptic points in the ICD spectra, points to the possibility of several simultaneous binding modes. Addition of the compounds **10d-f** to ds-RNA (poly A–poly U) resulted in bisignate ICD bands (> 300 nm) with a positive and negative part localized on either side of the ligand´s absorption maximum. Such bisignate ICD bands could be attributed to the dimer formation [31], most likely within the ds-RNA major groove, since the shallow and very wide RNA minor groove is not convenient for the binding of small molecules. Due to the large size of the binding site, dimeric aggregates are easily accommodated, and therefore the secondary structure of the double helix is not significantly disturbed (Figure 1). In the ICD spectra of compound **10f** the increasing concentration of compound ($r \geq 0.3$) generates an ICD signal of the opposite sign, suggesting the presence of several different forms of compound aggregates along the ds-RNA [40].

2.3. Biological activity

2.3.1. Antiproliferative effects of the investigated compounds

Numbers of recent studies conducted on tumor cell lines have shown that chloroquinoline derivatives and aromatic amidines individually are cytotoxic against tumor cells [41-43]. Since the results of the current study obtained from hybrid molecules in cell-free testing suggest their various modes of interaction with nucleic acids (Tables 1 and 2; Figures 1, 2 and S1), it was interesting to examine whether there are also differences in their activity *in vitro* on the growth of different tumor cell lines.

The newly synthesized compounds were tested for their effects on the growth of normal cells (MDCKI) and human tumor cell lines of different histological origin, CaCo2, HeLa, K562 and Raji. The obtained results, presented as the concentration achieving 50% of cell growth inhibition (GI_{50} value), show that the investigated compounds differentially influenced tumor cell growth, depending on the cell line, as well as on the dose applied. As shown in Table 3, GI_{50} values for all investigated compounds ranged from 5 to more than 100 $\times 10^{-6}$ mol dm^{-3}. Carcinoma cell lines were more resistant to the investigated compounds than leukemia cells. Most of the tested compounds with a short linker, group **I** (**2a-g**, Scheme 1) and group **II** (**5a-g**, Scheme 1), exhibited strong inhibitory potential, but without significant distinction between the normal and investigated tumor cell lines (GI_{50} values ranging from 30 to >100 $\times 10^{-6}$ mol dm^{-3}). Compounds **2d** and **2e** with GI_{50} ranging from 16 to 29 $\times 10^{-6}$ mol dm^{-3} displayed the best effects on the leukemia cells. Unsubstituted amidine **5b** possesses poor inhibitory activity compared to other compounds from group **II**. That is in agreement with its lower activity toward *ct*DNA. Alkyl substituted amidines (**5c**-**e**) and cyclic amidines (**5f,g**) showed improved binding affinity as well as inhibitory effects on the treated cells' growth (Table 2 and Table 3).

Furthermore, the phenol- and phenol-benzimidazole amidines attached with a short linker to 7-CQ had less effect on the growth of Raji and K562 cells compared to the compounds with a 2-aminoethanol linker (compounds **8a-g** and **10a-g)**. The obtained results prove that antiproliferative effects can be improved with the extension of the molecules' central linker. This extension enhances the flexibility and adaptability of the molecular conformation, causing variations in the DNA binding mode [44]. Unlike other compounds from group **III** that had weak cytotoxicity on treated cell lines (GI_{50} values ranging 50 to >100 $\times 10^{-6}$ mol dm^{-3}), compound **8a** showed significant inhibitory potential against leukemia cells, with GI_{50} values between 6 and 7 $\times 10^{-6}$ mol dm^{-3}. Although the tested phenoxy-benzimidazol

amidines (**10a-g**) showed significantly higher stabilization of *ct*DNA in comparison with compounds from other groups (Fig. 1, Table 2), these compounds (**10b-g)** showed very weak antiproliferative capacity on the normal cells and carcinoma cell lines. Unlike other investigated groups of compounds, all compounds from group **IV** (**10a-g)** significantly influenced the growth of leukemia cells with GI_{50} values between 5 and 27 × 10^{-6} mol dm^{-3}.

Table 3. Sensitivity of human tumor and normal cells to investigated compounds.

GI_{50} (x10^{-6} mol dm^{-3})					
Cell lines	**MDCK1**	**HeLa**	**CaCo-2**	**K562**	**Raji**
Group I					
2a	88.5 ± 1.0	>100	>100	86.7 ± 1.8	91.4 ± 10.8
2b	52.8 ± 4.7	59.1 ± 9.4	57.6 ± 3.1	62.5 ± 15.9	53.0 ± 4.4
2c	57.7 ± 6.6	56.6 ± 11.2	47.7 ± 6.0	82.9 ± 4.3	51.8 ± 6.2
2d	30.3 ± 4.8	52.3 ± 15.1	71.9 ± 17.6	16.9 ± 3.1	25.5 ± 11.1
2e	35.0 ± 7.9	48.6 ± 16.4	13.5 ± 8.0	18.8 ± 0.9	19.7 ± 10.6
2f	56.6 ± 3.3	57.7 ± 14.0	64.0 ± 15.6	48.7 ± 12.3	40.8 ± 6.8
2g	37.5 ±1 0.2	57.0 ± 13.1	53.3 ± 11.9	62.0 ± 4.0	50.8 ± 5.2
Group II					
5a	93.1 ±17.1	>100	>100	>100	>100
5b	>100	>100	82.9 ± 15.9	>100	111.4 ± 33.1
5c	61.8 ± 3.1	51.3 ± 0.6	53.3 ± 5.6	69.0 ± 19.1	48.2 ± 17.9
5d	53.5 ± 4.8	57.0 ± 18.8	43.2 ± 2.8	59.7 ± 7.4	46.7 ± 7.2
5e	55.5 ± 5.5	61.9 ± 11.1	47.4 ± 3.7	54.6 ± 13.2	41.5 ± 17.3
5f	77.1 ± 18.2	>100	36.8 ± 18.3	27.6 ± 8.9	40.6 ± 8.6
5g	67.1 ± 13.5	64.4 ± 5.6	34.5 ± 4.2	21.6 ± 11.0	50.5 ± 6.7
Group III					
8a	>100	84.4 ± 20.3	95.3 ± 35.0	7.2 ± 1.5	6.2 ± 0.9
8b	74.4 ± 4.7	74.0 ± 8.0	68.6 ± 6.2	49.5 ± 4.6	58.9 ± 5.4
8c	>100	>100	>100	>100	>100
8d	>100	>100	92.6 ± 28.5	77.3 ± 15.2	86.2 ± 26.0
8e	>100	>100	84.5 ± 15.2	76.9 ± 8.6	96.9 ± 14.4
8f	78.4 ± 5.6	60.8 ± 5.3	50.7 ± 5.9	51.9 ± 3.7	51.5 ± 7.2
8g	>100	>100	>100	>100	>100
Group IV					
10a	22.1 ± 8.1	56.6 ± 22.1	28.3 ± 11.3	7.8 ± 3.3	5.2 ± 1.1
10b	71.6 ± 2.7	>100	>100	34.7 ± 4.5	29.3 ± 8.3
10c	>100	>100	>100	17.2 ± 0.4	19.6 ± 9.8
10d	94.1 ± 19.5	>100	>100	15.1 ± 0.8	28.5 ± 1.2
10e	99.2 ± 28.3	>100	>100	11.4 ± 2.8	17.1 ± 13.6
10f	>100	>100	117.4 ± 29.1	8.6 ± 1.9	27.5 ± 1.4
10g	100.5 ± 18.0	>100	>100	13.3 ± 1.6	19.0 ± 2.3

GI_{50} – Drug concentration that inhibited cell growth by 50%. Data represent mean GI_{50} (x10^{-6} mol dm^{-3}) values ± standard deviation (SD) of the three independent experiments.

Exponentially growing cells were treated with compounds during a 72-hr period. Growth inhibition was analyzed using MTT survival assay.

2.3.2. Cell cycle distribution

The cell cycle involves a complex series of molecular and biochemical signaling pathways. Cell proliferation is governed by the cell cycle, which is a complex and stepwise process, and uncontrolled cell proliferation is a hallmark of tumor cells [45]. Suppression of cancer cell growth by many anticancer therapeutics correlates with perturbations in the cell cycle progression [46]. To gain insights into the mechanism of suppression of cancer cell proliferation, we examined the influence of selected compounds on the cell cycle distribution. Six compounds (**2d, 2e, 8a, 10d, 10e, and 10f**), were selected for cell cycle analysis on K562 cells, based on their cytotoxicity results. Representative flow histograms, depicting cell cycle distribution in K562 cells, following 24 hour exposure to the tested compounds are shown in Figure 3. Tested compounds induce changes in the cell cycle of treated cells but the cycle phase distribution varies between them. Cells treated with compounds from group **I** (**2d** and **2e**) and the group **III** (**8a**) have a similar pattern of effect. Compared to the control, untreated cells, the treated cells showed an enrichment of the G_0-G_1 fraction (Fig. 3). This was accompanied by a decrease in both S phase and G_2-M phase cells. DNA damage during the G1 phase and antiproliferative signals induced a G1 phase arrest prior to entry into the S phase. A SubG1 peak is indicative of DNA fragmentation, and that cells are dying, most probably through apoptosis [47,48].

Although all the tested compounds showed a significant decrease in the number of cells in the S phase ($p < 0.001$) only cells treated by two compounds (**10d** and **10f)** from group **IV**, had a significantly larger cell fraction in the subG0 phase than the control cells, which means that these cells went into senescence. Compound **10d** is the most effective, affecting significantly both S and G2/M phases. Compounds **10e** and **10f** showed significant changes in the S phase as well. Increased levels of p53 in normal cells and cells with a functional p53 gene are responsible for the cell cycle arrests in the G1 and G2 phases *in vitro*, and for signaling apoptosis in response to excessive or persistent damage [47]. In the case of K562, which is a p53 null cell line, the drug may cause its effect through other alternate pathways, which need to be investigated.

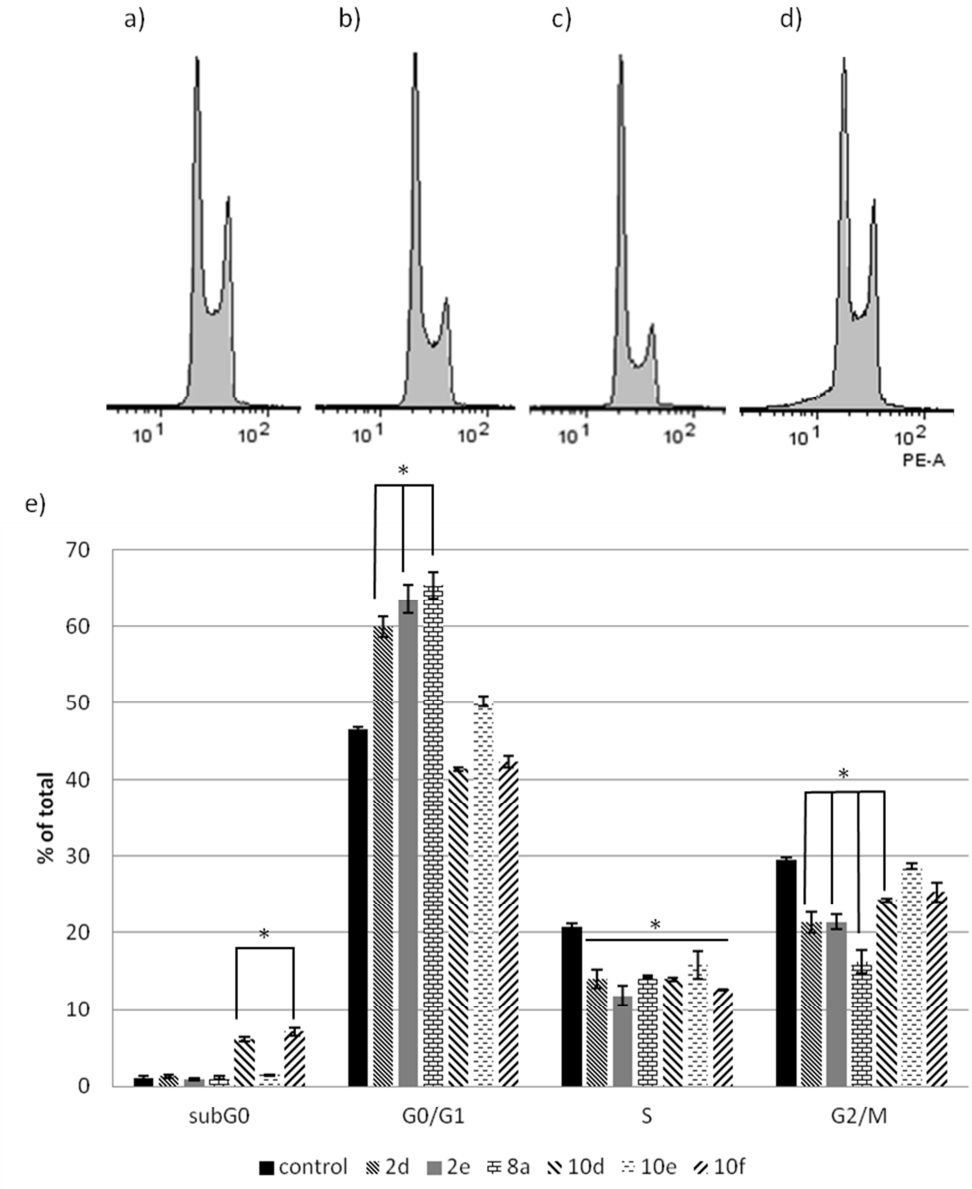


Figure 3. Cell cycle distribution.

The K562 cells were treated with 10^{-5} mol dm^{-3} of tested compounds for 24 hrs, stained with PI and analyzed by flow cytometry. PI stain histograms show a difference between the **control** (a), **2e** (b), **8a** (c) and **10f** (d). The chart (e) shows the numerical values for phase distribution. An asterisk (*) denotes values statistically significantly different when compared to the control ($p < 0.001$)

4. Conclusion

This study reports the synthesis of four series of new molecular hybrids with two moieties, 7-chloroquinoline and arylamidine, using various synthetic methods. All the novel molecular hybrids contain a quinoline moiety, whereas the linkers between quinoline and arylamidine, aryl core, and terminal amidine moiety were modified.

The potential inhibitory capacity of the newly synthesized compounds on the human tumor cell lines of different histological origin, as well as their interaction with DNA and RNA was determined. It was shown that the binding affinity of the investigated compounds increases proportionally to the increase in the length and number of groups able to form hydrogen bonds with ds-polynucleotides. Improvement of binding was additionally achieved by reduction of the structural rigidity of the investigated compounds

All the compounds bound preferentially to ctDNA, precisely in the DNA grooves. Compounds from group **IV** showed the highest affinity to ds-polynucleotides. According to their pronounced positive ICD bands, as well as moderate affinity and thermal stabilization effects, compounds from groups **II** and **IV** preferentially bound into the minor groove of *ct*DNA. The ICD spectra of compounds from group **II** with polyA-polyU showed intercalation as the dominant binding mode, while compounds from group **IV** preferentially bound into polyA-polyU grooves.

Antitumor selectivity was clearly seen in the effects on carcinoma and leukemia cells, with the higher efficacy of the investigated compounds against leukemia cell lines. Unlike the other groups of tested compounds, compounds from group **IV** significantly influenced the growth of leukemia cells, with GI_{50} values between 5 and 27 × 10^{-6} mol dm^{-3}. The highest selectivity towards leukemia cells, and low cytotoxicity against normal cells, was obtained for the compound **8a**.

Based on these results regarding the antiproliferative capacity of tested compounds, further research may be carried out to investigate the mechanisms of action of the compounds from the group **IV** in different types of leukaemia cells in more detail. Compounds from this series will serve as a template for the future design of new analogues with improved antitumor activities.

4. Experimental section

4.1. Chemistry

The compounds **1a**-**g**, **3a**-**g** [29] and **4a**,**b**,**f**,**g** [33] are already known. Procedures for the preparation of all final products are presented below. All solvents and reagents were used without purification from commercial sources. The progress of the chemical reaction was monitored by TLC on silica-gel 60 F_{254} DC-plastikfolienC16H and detected under UV light. Melting points were determined on a Büchi 510 melting point apparatus and were uncorrected. IR spectra (ν_{max}/cm^{-1}) were obtained on a Bruker Vertex 70 spectrophotometer.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer and chemical shifts (δ/ppm) were referred to TMS as internal standard. Theelectrospray ionization (ESI) Q-ToF was used for the high-resolution mass spectra measurements. Elemental analyses were performed by the Applied Laboratory Research department at INA d.d., Research and Development Sector, Central Analytical Laboratory.

*4.1.1. General method for the synthesis the compounds from group **I** (**2a-g**)*

A solution of 4,7-dichloroquinoline (1.2 mmol), compounds **1a-g** (1 mmol) and K_2CO_3 (0.03 mol) in dry DMF (50 mL) was refluxed with stirring under nitrogen for 8 hrs. The reaction mixture was cooled at room temperature, diethyl ether was added, and the resulting solid was filtered off. The products were dissolved in EtOH/HCl(g) and precipitated with mixture acetone:diethyl ether (4:1), filtered off, and dried. It was repeated a few times until the product were analytically pure.

*4.1.1.1 4-(7-Chloroquinolin-4-yloxy)benzonitrile (**2a**).*

Yield 0.1502 g (86.2 %). mp 152–154 °C; IR (ν_{max}/cm^{-1}): 3428, 2991, 2232, 1701, 1607, 1431, 1232, 1108, 785, 713, 462; ^{1}H NMR (DMSO-d_6) δ/ppm: 8.81 (d, 1H, J = 5.06 Hz, CH), 8.25 (d, 1H, J = 9.48 Hz, CH), 8.14 (d, 1H, J = 2.05 Hz, CH), 8.01 (d, 2H, J = 8.55 Hz, CH_2), 7.71 (dd, 1H, J_1 = 8.77 Hz, J_2 = 1.79 Hz, CH), 7.50 (d, 2H, J = 8.93 Hz, CH_2), 6.90 (d, 1H, J = 4.46 Hz, CH). ^{13}C NMR (DMSO-d_6) δ/ppm: 159.9, 158.4, 153.5, 150.3, 135.6, 135.5, 128.1, 127.9, 124.2, 121.8, 120.0, 118.8, 108.6, 107.4. HRMS calcd. for $C_{16}H_{10}ClN_2O$ $(M + H)^+$: 281.0482; found: 281.0482. Anal. calcd. for $C_{16}H_9ClN_2O \times 0.25H_2O$ (M_r = 285.22): C 67.38, H 3.36, N 9.82; found: C 67.55, H 3.36, N 9.51.

*4.1.1.2. 4-(7-Chloroquinolin-4-yloxy)benzimidamide dihydrochloride (**2b**).*

Yield 75.5%; mp 251–252 °C; IR (ν_{max}/cm^{-1}): 3085, 2387, 1683, 1629, 1587, 1483, 1421, 1288, 1200, 1080, 984, 804, 680, 600, 522, 459; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.48 (s, 2H, NH_2), 9.26 (s, 2H, NH_2), 8.96 (d, 1H, J = 5.21 Hz, CH), 8.39 (d, 1H, J = 9.22 Hz, CH), 8.28 (d, 1H, J = 1.96 Hz, CH), 8.05 (d, 2H, J = 8.97 Hz, CH2), 8.35 (dd, 1H, J1 = 9.29 Hz, J2 = 2.52 Hz, CH), 7.61 (d, 2H, J = 8.90 Hz, CH2), 6.92 (d, 1H, J = 5.49 Hz, CH). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.7, 163.7, 157.2, 150.0, 144.1, 137.8, 131.1, 128.9, 125.9, 124.7, 123.0, 121.2, 119.4, 106.0. HRMS calcd. for $C_{16}H_{13}ClN_3O$ $(M + H)^+$: 298.0747; found: 298.0749. Anal. calcd. for $C_{16}H_{12}ClN_3O \times 2HCl$ (M_r = 370.66): C 51.85, H 3.81, N 11.34; found: C 51.71, H 3.92, N 11.12.

*4.1.1.3. 4-(7-Chloroquinolin-4-yloxy)-N-isopropylbenzimidamide trihydrochloride (**2c**).*

Yield 45.2%; mp 198–199 °C; IR (ν_{max}/cm^{-1}): 3439, 2977, 2746, 1704, 1648, 1578, 1508, 1427, 1369, 1312, 1207, 1182, 1128, 1086, 858, 810, 623, 518, 458; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.69 (d, 2H, J = 8.59, NH_2), 9.55 (s, 1H, NH), 9.22 (S, 1H, CH), 9.00 (d, 1H, J = 5.66 Hz, CH), 8.43 (d, 1H, J = 8.98 Hz, CH), 8.32 (d, 1H, J = 1.86 Hz, CH2), 7.95 (d, 2H, J = 8.72 Hz, CH), 7.86 (dd, 1H, J1 = 9.15 Hz, J2 = 2.01 Hz, CH), 7.61 (d, 2H, J = 8.90 Hz, CH), 6.84 (d, 1H, J = 5.67 Hz, CH), 4.10 (m, 1H, CH), 1.29 (d, 6H, J = 6.36 Hz, CH3) ^{13}C NMR (DMSO-d_6) δ/ppm: 160.9, 156.7, 150.3, 137.5, 131.3, 128.8, 127.2, 124.5, 121.0, 119.4, 105.8, 45.2, 21.2. HRMS calcd. for $C_{19}H_{19}ClN_3O$ $(M + H)^+$: 340.1212; found: 340.1218. Anal. calcd. for $C_{19}H_{18}ClN_3O \times 3HCl \times 0.75H_2O$ (M_r = 462.72): C 49.32, H 4.90, N 9.08; found: C 49.33, H 5.15, N 8.95.

*4.1.1.4. 4-(7-Chloroquinolin-4-yloxy)-N-isobutylbenzimidamide trihydrochloride (**2d**).*

Yield 54.2%; mp 196–197 °C; IR (ν_{max}/cm^{-1}): 3057, 2965, 2710, 1670, 1590, 1474, 1439, 1364, 1301, 1207, 1081, 1011, 850, 700, 468; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.01 (s, 1H, NH), 9.64 (s, 1H, NH), 9.29 (s, 1H, NH), 9.05 (d, 1H, J = 5.84 Hz, CH), 8.47 (d, 1H, J = 8.99 Hz, CH), 8.38 (d, 1H, J = 1.66 Hz, CH), 8.00 (d, 2H, J = 8.65 Hz, CH), 7.90 (dd, 1H, J1 = 8.99 Hz, J2 = 1.75 Hz, CH), 7.63 (d, 2H, J = 8.62 Hz, CH), 6.89 (d, 1H, J = 5.85 Hz, CH), 3.30 (m, 2H, CH), 2.04 (m, 1H, CH_2), 0.98 (d, 6H, J = 6.61 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.2, 162.5, 157.1, 150.6, 138.2, 131.7, 129.4, 127.5, 125.1, 123.6, 121.6, 119.9, 106.2, 50.1, 27.5, 20.4. HRMS calcd. for $C_{20}H_{21}ClN_3O$ $(M + H)^+$: 354.1373; found: 354.1372. Anal. calcd. for $C_{20}H_{20}ClN_3O \times 3HCl \times 1.5H_2O$ (M_r = 490.26): C 49.00, H 5.35, N 8.57; found: C 49.18, H 5.34, N 8.53.

*4.1.1.5. 4-(7-Chloroquinolin-4-yloxy)-N-cyclopentylbenzimidamide trihydrochloride (**2e**).*

Yield 45.2%; mp 198–199 °C; IR (ν_{max}/cm^{-1}): 2942, 2688, 1670, 1616, 1570, 1411, 1293, 1204, 1175, 1062, 842, 731, 446; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.77 (d, 1H, J = 7.00 Hz, NH), 9.56 (s, 1H, NH), 9.19 (s, 1H, NH), 8.99 (d, 1H, J = 5.58 Hz, ArH), 8.41 8d, 1H, J = 8.91 Hz, ArH), 8.31 (d, 1H, J = 1.57 Hz, ArH), 7.94 (d, 2H, J = 8.71 Hz, ArH), 7.84 (dd, 1H, J_1 = 8,95 Hz, J_2 = 1.96 Hz, ArH), 7.59 (d, 2H, J = 8.73 Hz, ArH), 6.84 (d, 1H, J = 5.63 Hz,ArH), 4.20 (m, 1H, CH), 2.07 (m, 2H, CH_2), 1.74 (m, 4H, CH_2), 1.59 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 164.0, 161.5, 156.5, 149.9, 137.8, 131.5, 128.9, 127.2, 124.7, 122.9, 121.0, 119.4, 105.6, 54.4, 31.3, 23.7. HRMS calcd. for $C_{21}H_{21}ClN_3O$ $(M + H)^+$: 366.1373; found: 366.1377. Anal. calcd. for $C_{21}H_{20}ClN_3O \times 3HCl$ (M_r = 475.24): C 53.07, H 4.88, N 8.84; found: C 53.18, H 4.98, N 8.51.

*4.1.1.6. 7-Chloro-4-(4-(4,5-dihydro-1H-imidazol-2-yl)phenoxy)quinoline dihydrochloride (**2f**).*

Yield 74.4%; mp 201–202 °C; IR (ν_{max}/cm^{-1}): 2965, 1579, 1417, 1297, 1214, 1181, 1080, 856, 830, 806, 631, 515; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.86 (s, 2H, NH), 8.96 (d, 1H, J = 5.53 Hz, CH), 8.37 (d, 1H, J = 8.22 Hz, CH), 8.28 (d, 1H, J = 2.09 Hz, CH), 8.24 (d, 2H, J = 8.79 Hz, CH), 7.83 (dd, 1H, J_1 = 7.21 Hz, J_2 = 2.00 Hz, CH), 7.65 (d, 2H, J = 8.76 Hz, CH), 7.00 (d, 1H, J = 5.54 Hz, CH), 4.02 (s, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 163.6, 163.1, 157.8, 150.3, 137.6, 131.8, 128.8, 124.5, 123.4, 121.3, 120.0, 119.5, 106.5, 44.3; HRMS calcd. for $C_{18}H_{15}ClN_3O$ $(M + H)^+$: 324.0904; found: 324.0907. Anal. calcd. for $C_{18}H_{14}ClN_3O \times 2HCl \times 1H_2O$ (M_r = 414.72): C 52.13, H 4.37, N 10.13; found: C 52.21, H 4.76, N 9.99.

*4.1.1.7. 7-Chloro-4-(4-(1,4,5,6-tetrahydropyrimidin-2-yl)phenoxy)quinoline dihydrochloride (**2g**).*

Yield 68.9%; mp 198–199 °C; IR (ν_{max}/cm^{-1}): 2329, 1639, 1588, 1417, 1297, 1209, 1084, 856, 825, 737, 696, 594, 543, 511; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.09 (s, 2H, NH), 8.91 (d, 1H, J = 1.06 Hz, CH), 8.35 (d, 1H, J = 8.95 Hz, CH), 8.23 (d, 1H, J = 1.68 Hz, CH), 8.01 (d, 1H, J = 8.76 Hz, CH), 7.92 (d, 2H, J = 8.76 Hz, CH), 7.78 (dd, 1H, J_1 = 8.92 Hz, J_2 = 1.99 Hz, CH), 7.57 (d, 2H, J = 8.74 Hz, CH), 6.86 (d, 1H, J = 5.46 Hz, CH), 3.50 (s, 4H, CH_2) 1.98 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 163.3, 161.9, 158.1, 156.8, 150.4, 137.5, 131.3, 130.6, 128.7, 126.7, 126.3, 124.5, 123.6, 121.1, 121.0 119.4, 105.9, 38.8, 17.6; HRMS calcd. for $C_{19}H_{17}ClN_3O$ $(M + H)^+$: 338.1060; found: 338.1064. Anal. calcd. for $C_{19}H_{16}ClN_3O \times 2HCl \times 1.5H_2O$ (M_r = 437.75): C 52.13, H 4.84, N 9.60; found: C 52.06, H 4.75, N 9.60.

*4.1.2. General method for the synthesis of hydrochloride derivatives of 2-(4-hydroxyphenyl)-1H-benz[d]imidazole-6-carboximidamide (**4c,d,e**).*

A solution of 4-hydroxybenzaldehyde (1.2 mmol), 3,4-diaminobenzamidine (**3c,d,e**) (1.0 mmol) and benzoquinone (1.2 mmol mol) in dry methanole (40 mL) was refluxed with stirring under nitrogen for 8 hrs. The reaction mixture was cooled to room temperature, diethyl ether was added, and the resulting solid was filtered off. The products were dissolved in EtOH/HCl(g) and perticipitated with mixture acetone:diethyl ether (5:1), filtered off, and dried. It was repeated a few times until the product were analytically pure.

*4.1.2.1. 2-(4-Hydroxyphenyl)-N-isopropyl-1H-benzo[d]imidazole-6-carboximidamide dihydrochloride (**4c**).*

Yield 49.1%; mp > 290 °C; IR (ν_{max}/cm^{-1}): 3081, 2652, 1676, 1601, 1500, 1385, 1283, 1186, 843, 816, 700, 520, 566; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.50 (brs, 1H, OH), 9.64 (d, 1H, J =

8.93 Hz, NH), 9.49 (s, 1H, NH), 9.06 (s, 1H, NH), 8.25 (d, 2H, *J* = 8.72 Hz, CH), 8.05 (d, 1H, *J* = 1.55 Hz, CH), 7.85 (d, 1H, *J* = 8.12 Hz, CH), 7.67 (dd, 1H, J_1 = 8.91 Hz, J_2 = 1.79 Hz, CH), 7.05 (d, 2H, *J* = 8.77 Hz, CH), 4.09 (m, 1H, CH), 1.31 (d, 6H, *J* = 6.38 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 162.67, 162.21, 152,70, 130.84, 125,51, 125.01, 124.99, 116.84, 115.08, 114.37, 45.65, 21.73. HRMS calcd. for $C_{17}H_{19}N_4O$ $(M + H)^+$: 295.1559; found: 295.1562. Anal. calcd. for $C_{17}H_{18}N_4O \times 2HCl \times 1.5H_2O$ (M_r = 394.30): C 51.78, H 5.88, N 14,21; found: C 51.89, H 5.67, N 14.34.

*4.1.2.2. 2-(4-Hydroxyphenyl)-N-isobutyl-1H-benzo[d]imidazole-6-carboximidamide dihydrochloride (**4d**).*

Yield 72.1%; mp > 290 °C; IR (ν_{max}/cm^{-1}): 3069, 2664, 1676, 1607, 1505, 1454, 1389, 1352, 1292, 1218, 1181, 862, 821, 705, 649, 566, 538, 515; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.70 (brs, 1H, OH), 9.91 (s, 1H, NH), 9.58 (s, 1H, NH), 9.13 (s, 1H, NH), 8.30 (d, 2H, *J* = 8.72 Hz, CH), 8.10 (s, 1H, CH), 7.89 (d, 1H, *J* = 8.49 Hz, CH), 7.73 (dd, 1H, J_1 = 8.53 Hz, J_2 = 1.35 Hz, CH), 7.06 (d, 2H, *J* = 8.77 Hz, CH), 3.29 (t, 2H, J_1 = 6.97 Hz, J_2 = 7.87 Hz, CH_2), 2.05 (m, 1H, CH), 1.00 (d, 6H, *J* = 6.63 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 163.0, 162.4, 152.1, 130.5, 125.0, 124.5, 116.4, 114.4, 114.0, 49.8, 27.0, 19.9. HRMS calcd. for $C_{18}H_{21}N_4O$ $(M + H)^+$: 309.1712; found: 309.1720. Anal. calcd. for $C_{18}H_{20}N_4O \times 2HCl \times 2.5\ H_2O$ (M_r = 426.34): C 50.71, H 6.38, N 13.14; found: C 50.43, H 6.59, N 13.23.

*4.1.2.3. 2-(4-Hydroxyphenyl)-N-cyclopentyl-1H-benzo[d]imidazole-6-carboximidamide dihydrochloride (**4e**).*

Yield 73.6%; mp > 290 °C; IR (ν_{max}/cm^{-1}): 3081, 2664, 2364, 1670, 1601, 1497, 1289, 1197, 850, 700, 538; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.53 (brs, 1H, OH), 9.72 (d, 1H, *J* = 7.35 Hz, NH), 9.49 (s, 1H, NH), 8.99 (s, 1H, NH), 8.23 (d, 2H, *J* = 8.70 Hz, ArH), 8.04 (s, 1H, ArH), 7.83 (d, 1H, *J* = 8.49 Hz, ArH), 7.65 (dd, 1H, J_1 = 8.51 Hz, J_2 = 1.25 Hz, ArH), 7.04 (d, 2H, *J* = 8.77 Hz, ArH), 4.17 (m, 1H, CH), 2.07 (m, 2H, CH_2), 1.66 (m, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 162.9, 162.8 152.4, 136.5, 133.2, 131.1, 125.6, 125.4, 116.9, 115.1, 114.7, 114.3, 54.9, 31.9, 24.2. HRMS calcd. for $C_{19}H_{21}N_4O$ $(M + H)^+$: 321.1715; found: 321.1717. Anal. calcd. for $C_{19}H_{20}N_4O \times 2HCl \times 3\ H_2O$ (M_r = 447.36): C 51.01, H 6.31, N 12.52; found: C 51.34, H 6.13, N 12.70.

*4.1.3. General method for the synthesis the compounds from group **II** (**5a-g**)*

A solution of 4,7-dichloroquinoline (1.2 mmol), compounds **4a-g** (1 mmol) and K_2CO_3 (0.03 mol) in dry DMF (50 mL) was refluxed with stirring under nitrogen for 2d. The reaction

mixture was cooled at room temperature, diethyl ether was added, and the resulting solid was filtered off. The products were dissolved in EtOH/HCl(g) and precipitated with mixture acetone:diethyl ether (4:1), solid was filtered off, and dried. It was repeated few times until the product were analytically pure.

*4.1.3.1. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-1H-benzo[d]imidazole-6-carbonitrile (**5a**).*

Yield 57.6 %; mp 178–179 °C; IR (ν_{max}/cm^{-1}): 3423, 2225, 1616, 1569, 1496, 1421, 1215, 1095, 858, 819; ^{1}H NMR (DMSO-d_6) δ/ppm: 8.75 (d, 1H, J = 5.09 Hz, CH), 8.34 (d, 2H, J = 7.72 Hz, CH), 8.31 (d, 1H, J = 8.55 Hz, CH), 8.11 (d, 1H, J = 2.29 Hz, CH), 7.71 (d, 1H, J = 2.60 Hz, CH), 7.69 (d, 1H, J = 1.86 Hz, CH), 7.90 (d, 1H, J = 1.49 Hz, CH_2), 7.54 (d, 1H, J = 1.67 Hz, CH), 7.50 (d, 2H, J = 9.11 Hz, CH), 6.85 (d, 1H, J = 5.02 Hz, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 160.9, 155.7, 153.5, 152.6, 148.8, 135.5, 129.3, 127.4, 126.8, 126.7, 125.8, 123.9, 121.3, 119.9, 119.5, 105.9, 104.1; HRMS calcd. for $C_{23}H_{14}ClN_4O$ $(M + H)^+$: 397.0856; found: 397.0849.

*4.1.3.2. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**5b**).*

Yield 49.1%; mp > 250 °C; IR (ν_{max}/cm^{-1}): 3262, 3095, 2645, 1690, 1633, 1585, 1436, 1287, 1181, 1089, 859; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.39 (s, 2H, NH), 9.04 (s, 2H, NH), 8.99 (d, 1H, J = 5.82 Hz, CH), 8.51 (d, 2H, J = 8.70 Hz, CH), 8.50 (d, 1H, J = 9.15 Hz, CH), 8.32 (d, 1H, J = 1.81 Hz, CH), 8.21 (s, 1H, CH), 7.90 (dd, 1H, J_1 = 9.04 Hz, J_2 = 1.96 Hz, CH), 7.86 (d, 1H, J = 8.98 Hz CH), 7.74 (dd, 1H, J_1 = 8.59 Hz, J_2 = 1.45 Hz, CH), 7.65 (d, 2H, J = 8.77 Hz, CH), 7.04 (d, 1H, J = 5.83 Hz, CH); ^{13}C NMR (DMSO-d_6) δ/ppm: 166.1, 155.5, 153.2, 151.8, 136.2, 129.6, 127.9, 127.0, 124.2, 124.0, 122.8, 121.6, 121.4, 119.5, 117.6, 116.1, 108.8, 105.9; HRMS calcd. for $C_{23}H_{17}ClN_5O$ $(M + H)^+$: 414.1122; found: 414.1128. Anal. calcd. for $C_{23}H_{16}ClN_5O \times 3HCl \times 3H_2O$ (M_r = 577.29): C 47.85, H 4.36, N 12.13; found: C 48.16, H 4.34, N 12.12.

*4.1.3.3. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-N-isopropyl-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**5c**).*

Yield 40.0%; mp > 250 °C; IR (ν_{max}/cm^{-1}): 3062, 2592, 2353, 1583, 1416, 1295, 1211, 1170, 1079, 859; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.60 (d, 1H, J = 9.07 Hz, NH), 9.47 (s, 1H, NH), 9.06 (s, 1H, NH), 8.97 (d, 1H, J = 7.06 Hz, CH), 8.56 (d, 2H, J = 10.67 Hz, CH), 8.49 (d, 1H, J = 9.70 Hz, CH), 8.30 (s, 1H, CH), 8.09 (s, 1H, CH), 7.88 (dd, 1H, J_1 = 8.94 Hz, J_2 = 2.54

Hz, CH), 7.85 (d, 1H, *J* = 9.09 Hz, CH), 7.64 (d, 2H, *J* = 9.73 Hz, CH), 7.60 (s, 1H, CH), 7.01 (d, 1H, *J* = 6.26 Hz, CH), 4.11 (m, 1H, CH), 1.32 (d, 6H, *J* = 6.41 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 165.4, 161.8, 155.6, 151.4, 149.0, 142.1, 138.7, 138.2, 130.7, 129.5, 125.1, 124.9, 124.7, 124.3, 122.0, 121.44, 121.40, 119,3, 115.7, 114.6, 105.8, 45.2, 21.3. HRMS calcd. for $C_{26}H_{23}ClN_5O$ $(M + H)^+$: 456.1591; found: 456.1595. Anal. calcd. for $C_{26}H_{22}ClN_5O \times 3HCl \times 3H_2O$ (M_r = 619.37): C 50.42, H 5.04, N 11.31; found: C 50.23, H 4.65, N 11.04.

*4.1.3.4. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-N-isobutyl-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**5d**).*

Yield 39.1%; mp 223–224 °C; IR (ν_{max}/cm^{-1}): 3062, 2645, 1681, 1609, 1425, 1296, 1192, 1109, 859, 821; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.87 (t, 1H, *J* = 5.47 Hz, NH), 9.54 (s, 1H, NH), 9.11 (s, 1H, NH), 9.03 (d, 1H, *J* = 6.02 Hz, CH), 8.61 (d, 2H, *J* = 8.81 Hz, CH), 8.54 (d, 1H, *J* = 9.00 Hz, CH), 8.40 (d, 1H, *J* = 1.99, CH), 8.14 (d, 1H, *J* = 1.27, CH), 7.93 (dd, 1H, J_1 = 8.85 Hz, J_2 = 1.98 Hz, CH), 7.89 (d, 1H, *J* = 8.66 Hz, CH), 7.70 (dd, 2H, J_1 = 8.66 Hz, J_2 = 1.89 Hz, CH), 7.69 (d, 2H, *J* = 8.85 Hz, CH), 7.08 (d, 1H, *J* = 6.00 Hz, CH), 3.30 (t, 2H, *J* = 6.65 Hz, CH), 2.06 (m, 1H, CH), 1.00 (d, 6H, *J* = 6.65 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 163.9, 163.5, 155.9, 152.9, 150.7, 144.9, 138.1, 130.5, 129.3, 126.6, 125.1, 124.1, 123.8, 123.6, 122.2, 119.9, 116.9, 106.3, 50.2, 27.5, 20.4. HRMS calcd. for $C_{27}H_{25}ClN_5O$ $(M + H)^+$: 470.1748; found: 470.1753. Anal. calcd. for $C_{27}H_{24}ClN_5O \times 3HCl \times 2H_2O$ (M_r = 615.39): C 52.70, H 5.08, N 11.38; found: C 52.51, H 5.08, N 11.06.

*4.1.3.5. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-N-cyclopentyl-1H-benzo[d]imidazole-6-carboximidamide dihydrochloride (**5e**).*

Yield 23.8%; mp 228–229 °C; IR (ν_{max}/cm^{-1}): 3069, 2954, 1607, 1571, 1420, 1291, 1205, 1169, 1083, 861, 818; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.63 (d, 1H, NH), 9.41 (s, 1H, NH), 8.94 (s, 1H, NH), 8.88 (d, 1H, *J* = 5.41 Hz, CH), 8.44 (d, 2H, *J* = 8.66 Hz, CH), 8.41 (d, 1H, *J* = 8.91 Hz, CH), 8.21 (d, 1H, *J* = 1.82 Hz, CH), 8.06 (s, 1H, CH), 7.82 (d, 1H, *J* = 8.81 Hz, CH), 7.79 (dd, 1H, J_1 = 9.04 Hz, J_2 = 1.86 Hz, CH), 7.59 (dd, 1H, J_1 = 8.77 Hz, J_2 = 1.85 Hz, CH), 7.58 (d, 2H, *J* = 8.70 Hz, CH), 6.93 (d, 1H, *J* = 5.43 Hz, CH), 4.17 (m, 1H, CH), 2.07 (m, 2H, CH_2), 1.75 (m, 4H, CH_2), 1.69 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 164.7, 162.7, 155.5, 151.8, 149.5, 138.2, 130.7, 130.4, 129.1, 126, 125.3, 124.8, 124.2, 123.8, 122.3, 121.8, 119.3, 116.5, 105.8, 54.3, 31.4, 23.7. HRMS calcd. for $C_{28}H_{25}ClN_5O$ $(M + H)^+$: 482.1748;

found: 482.1750. Anal. calcd. for $C_{28}H_{24}ClN_5O \times 2HCl \times 1.5H_2O$ (M_r = 581.93): C 57.79, H 5.02, N 12.03; found: C 57.59, H 5.20, N 11.72.

*4.1.3.6. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-6-(4,5-dihydro-1H-imidazol-2-yl)-1H-benzo[d]imidazole dihydrochloride (**5f**).*

Yield 57.6 %; mp 227–228 °C; IR (ν_{max}/cm^{-1}): 3035, 2953, 2353, 1583, 1494, 1417, 1297, 1218, 1177, 1084, 858, 821; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.58 (s, 2H, NH), 8.92 (d, 1H, J = 5.71 Hz, CH), 8.46 (d, 1H, J = 8.67 Hz, CH), 8.44 (d, 1H, J = 9.16 Hz, CH), 8.37 (s, 1H, CH), 8.25 (d, 1H, J = 2.29 Hz, CH), 7.85 (s, 1H, CH), 7.82 (d, 2H, J = 2.21 Hz, CH), 7.58 (d, 2H, J = 8.89 Hz, CH), 6.97 (d, 1H, J = 6.16 Hz, CH_2), 4.01 (s, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 165.7, 163.7, 155.8, 154.0, 151.3, 146.0, 137.5, 130.3, 128.9, 127.3, 124.9, 124.6, 123.4, 122.0, 119.9, 116.7, 116.3, 106.2, 44. HRMS calcd. for $C_{25}H_{19}ClN_5O$ $(M + H)^+$: 440.1278; found: 440.1279. Anal. calcd. for $C_{25}H_{18}ClN_5O \times 2HCl \times 2.5H_2O$ (M_r = 557.86): C 53.83, H 4.52, N 12.55; found: C 54.17, H 4.54, N 12.21.

*4.1.3.7. 2-[4-(7-Chloroquinolin-4-yloxy)phenyl]-6-(1,4,5,6-tetrahydropyrimidin-2-yl)-1H-benzo[d]imidazole dihydrochloride (**5g**).*

Yield 74.4%; mp 137–138 °C; IR (ν_{max}/cm^{-1}): 3044, 2655, 1767, 1672, 1610, 1570, 1494, 1385, 1297, 1037, 814; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.99 (s, 2H, NH), 8.95 (d, 1H, J = 5.54 Hz, CH), 8.49 (d, 2H, J = 9.28 Hz, CH), 8.45 (s, 1H, CH), 8.28 (d, 1H, J = 2.97 Hz, CH), 8.10 (d, 1H, J = 1.54 Hz, CH), 7.85 (d, 2H, J = 8.07 Hz, CH), 7.63 (d, 2H, J = 9.63 Hz, CH), 7.62 (d, 1H, J = 8.85 Hz, CH), 3.53 (t, 4H, J_1 = 6.85 Hz, J_2 = 5.71 Hz, CH_2), 2.02 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 164.6, 159.3, 155.4, 151.9, 149.6, 138.1, 130.4, 129.1, 124.8, 123.6, 123.0, 122.3, 121.8, 119.3, 105.8, 38.8, 17.8. HRMS calcd. for $C_{26}H_{21}ClN_5O$ $(M + H)^+$: 454.1435; found: 454.1438. Anal. calcd. for $C_{26}H_{20}ClN_5O \times 2HCl \times 3.2H_2O$ (M_r = 584.50): C 53.43, H 4.90, N 11.98; found: C 53.31, H 4.67, N 12.02.

*4.1.4.1. 4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]benzonitrile (**8a**).*

A suspension of (2-bromoethyl)-(7-chloroquinolin-4-yl)-amine (**7**) (1.52 g, 5.35 mmol), 4-hydroxybenzonitrile (0.764 g, 6.41 mmol) and potassium carbonate (2.21 g, 16 mmol) in dimethylformamide (40 mL) was heated at 60 °C for 24 h under nitrogen. After cooling to room temperature, the reaction was diluted with 150 mL of dichloromethane and washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The oil obtained, was diluted with methanol (5 mL),

and the product precipitates with addition of water as white powder (1.15 g, 66%); mp 175 °C. IR (ν_{max}/cm^{-1}): 3434, 3273, 2223, 1608, 1578, 1510, 1257, 1172, 1146, 1052, 890, 844, 797, 549. ^{1}H NMR (DMSO-d_6) δ/ppm: 8.43 (d, J = 5.4 Hz, 1H, ArH), 8.27 (d, J = 9.1 Hz, 1H, ArH), 7.80 (d, J = 2.2 Hz, 1H, ArH), 7.76 (d, J = 8.8 Hz, 2H, ArH), 7.46 (dd, J = 8.9, 2.2 Hz, 2H, NH + ArH), 7.13 (d, J = 8.8 Hz, 2H, ArH), 6.61 (d, J = 5.4 Hz, 1H, ArH), 4.35 (t, J = 5.4 Hz, 2H, CH_2), 3.71 (q, J = 5.3 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 161.89, 151.91, 149.95, 149.05, 134.15, 133.41, 127.54, 124.16, 124.01, 119.05, 117.40, 115.57, 102.92, 98.93, 66.11, 41.71. HRMS calcd. for $C_{18}H_{15}ClN_3O$ $(M + H)^+$: 324.0903; found: 324.0901. Anal. calcd. for $C_{18}H_{14}ClN_3O \times 0.5H_2O$ (M_r = 332.79): C 64.97, H 4.54, N 12.63; found: C 64.89, H 4.42, N 12.51.

*4.1.5. General method for the synthesis the compounds from group **III (8b-g)***

A solution of nitrile **8a** (600 mg, 1.85 mmol) in anhydrous methanol (50 mL) was cooled in an ice bath and saturated with dry HCl gas. The reaction mixture was then sealed, and stirred at room temperature until the starting nitrile was no longer detectable with IR. The reaction mixture was evaporated to dryness and dried over KOH. The crude imidate ester hydrochloride was suspended in anhydrous MeOH (30 mL) and the ammonia or appropriate amine was added. The desired product was isolated with column cromatography (CH_2Cl_2/MeOH 5:1; MeOH/NH_4OH 3:1) and converted to hydrochloride salt using anhydrous ethanol saturated with HCl(g).

*4.1.5.1. 4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]benzimidamide trihydrochloride (**8b**).*

The crude imidate ester hydrochloride was dissolved in dry MeOH and saturated with dry ammonia gas. A solution was stirred at room temperature and the progress of the reaction was monitored by TLC. After three days the desired product was isolated using column cromatography as white solid (207 mg, 23%); mp 143 °C. IR (ν_{max}/cm^{-1}): 1610, 1583, 186, 1457, 1269, 1211, 1187, 1171, 1087, 1045, 841, 814. ^{1}H NMR (DMSO-d_6) δ/ppm: 9.99 (t, J = 5.2 Hz, 1H, NH), 9.11 (s, 2H, NH), 8.68 (s, 2H, NH), 8.46 (d, J = 5.6 Hz, 1H, ArH), 8.32 (d, J = 9.0 Hz, 1H, ArH), 7.82 (s, 1H, ArH), 7.81 (d, J = 8.9 Hz, 2H, ArH), 7.52 (dd, J = 9.0, 2.2 Hz, 1H, ArH), 7.18 (d, J = 8.9 Hz, 2H, ArH), 6.68 (d, J = 5.7 Hz, 1H, ArH), 4.38 (t, J = 5.3 Hz, 2H, CH_2), 3.78 (q, J = 5.2 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.27, 163.37, 162.72, 130.20, 129.35, 124.57, 119.68, 119.55, 114.85, 113.97, 110.58, 98.96, 66.18, 41.92, 40.20. HRMS calcd. for $C_{18}H_{18}ClN_4O$ $(M + H)^+$: 341.1169; found: 341.1168. Anal. calcd. for

$C_{18}H_{17}ClN_4O \times 3HCl \times 2H_2O$ (M_r = 486.22): C 44.46, H 4.98, N 11.52; found: C 44.64, H 4.71, N 11.15.

*4.1.5.2. 4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]-N-isopropylbenzimidamide trihydrochloride (**8c**).*

A solution of crude imidate and isopropyl amine (438 mg, 7.41 mmol) in dry MeOH was stirred at room temperature. The progress of the reaction was monitored by TLC. After three days the desired product was isolated using column cromatography as white solid (274 mg, 29%); mp 78 °C. IR (ν_{max}/cm^{-1}): 3240, 3093, 2973, 1612, 1580, 1515, 1457, 1264, 1124, 1083, 1050, 838, 808, 743. ^{1}H NMR (DMSO-d_6) δ/ppm: 9.54 (s, 1H, NH), 9.38 (d, J = 8.1 Hz, 1H, NH), 9.26 (s, 1H, NH), 8.88 (s, 1H, NH), 8.67 (d, J = 9.2 Hz, 1H, ArH), 8.59 (d, J = 6.9 Hz, 1H, ArH), 8.05 (d, J = 1.9 Hz, 1H, ArH), 7.76 (dd, J = 9.1, 1.9 Hz, 1H, ArH), 7.70 (d, J = 8.8 Hz, 2H, ArH), 7.15 (d, J = 8.8 Hz, 2H, ArH), 7.01 (d, J = 7.0 Hz, 1H, ArH), 4.42 (t, J = 4.9 Hz, 1H, CH_2), 4.1 – 3.9 (m, 3H, CH +CH_2), 1.26 (d, J = 6.3Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 161.88, 161.77 151.94, 150.13, 149.07, 133.46, 130.00 , 127.49, 124.26, 124.19 121.82, 117.48, 114.59, 98.90, 66.10, 41.75, 29.02, 21.53. HRMS calcd. for $C_{21}H_{24}ClN_4O$ (M + H)$^+$: 383.1638; found: 383.1641. Anal. calcd. for $C_{21}H_{23}ClN_4O \times 3HCl \times 1H_2O$ (M_r = 510.29): C 49.43, H 5.53, N 10.98; found: C 49.09, H 5.46, N 10.67.

*4.1.5.3. 4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]-N-isobutylbenzimidamide trihydrochloride (**8d**).*

A solution of crude imidate and isobutylamine (542 mg, 7.41 mmol) in dry MeOH was stirred at room temperature. The progress of the reaction was monitored by TLC. After three days the desired product was isolated using column cromatography as white solid (233 mg, 24%); mp 80 °C. IR (ν_{max}/cm^{-1}): 3441, 1688, 1612, 1579, 1512, 1456, 1384, 1261, 1190, 1143, 1049, 844, 813, 657. ^{1}H NMR (DMSO-d_6) δ/ppm: 9.72 (t, J = 4.8 Hz, 1H, NH), 9.38 (s, 1H, NH), 9.26 (s, 1H, NH), 9.03 (s, 1H, NH), 8.70 (d, J = 9.2 Hz, 1H, ArH), 8.52 (d, J = 6.5 Hz, 1H, ArH), 8.06 (d, J = 2.1 Hz, 1H, ArH), 7.77 (d, J = 8.9 Hz, 2H, ArH), 7.64 (dd, J = 9.1, 2.0 Hz, 1H, ArH), 7.15 (d, J = 8.8 Hz, 2H, ArH), 6.88 (d, J = 6.6 Hz, 1H, ArH), 4.42 (t, J = 5.3 Hz, 2H, CH_2), 3.91 (q, J = 4.7 Hz, 2H, CH_2), 3.29 – 3.20 (m, 2H), 2.00 (m, 1H, CH), 0.94 (d, J = 6.6 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 162.39, 162.13, 154.99, 144.31, 137.38, 130.13, 126.57, 125.52, 121.04, 120.41, 115.74, 114.76, 99.08, 66.14, 49.34, 42.48, 26.96, 19.87. HRMS calcd. for $C_{22}H_{26}ClN_4O$ (M + H)$^+$: 397.1795; found: 397.1803. Anal. calcd. for

$C_{22}H_{25}ClN_4O \times 3HCl \times 1H_2O$ (M_r = 524.31): C 50.40, H 5.77, N 10.69; found: C 50.49, H 5.65, N 10.58.

*4.1.5.4. 4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]-N-cyclopentylbenzimidamide dihydrochloride (**8e**).*

A solution of crude imidate and cyclopentylamine (630 mg, 7.41 mmol) in dry MeOH was stirred at room temperature. The progress of the reaction was monitored by TLC. After three days the desired product was isolated using column cromatography as white solid (246 mg, 26%); mp 141–142 °C. IR(ν_{max}/cm^{-1}): 3353, 1607, 1589, 1513, 1462, 1412, 1368, 1292, 1260, 1166, 1141, 1044. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.43 (s, 1H, NH), 9.79 (s, 1H, NH), 9.48 (d, J = 7.2 Hz, 1H, NH), 9.29 (s, 1H, NH), 8.87 (s, 1H, NH), 8.73 (d, J = 9.2 Hz, 1H, ArH), 8.60 (d, J = 7.1 Hz, 1H, ArH), 8.10 (d, J = 2.0 Hz, 1H, ArH), 7.78 (dd, J = 9.1, 2.1 Hz, 1H, ArH), 7.71 (d, J = 8.9 Hz, 2H, ArH), 7.14 (d, J = 8.9 Hz, 2H, ArH), 7.04 (d, J = 7.1 Hz, 1H, ArH), 4.43 (t, J = 5.2 Hz, 2H, CH_2), 4.18 – 4.05 (m, 1H, CH), 4.00 (q, J = 5.2 Hz, 2H, CH_2), 2.04 (dd, J = 14.0, 8.7 Hz, 2H, CH_2), 1.78 – 1.48 (m, 6H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 161.97, 161.71, 155.75, 142.91, 138.75, 137.84, 130.36, 126.76, 125.96, 121.05, 119.17, 115.49, 114.56, 99.02, 66.17, 54.09, 42.46, 31.32, 23.64. HRMS calcd. for $C_{23}H_{26}ClN_4O$ $(M + H)^+$: 409.1795; found: 409.1801. Anal. calcd. for $C_{23}H_{25}ClN_4O \times 2HCl \times 1.75H_2O$ (M_r = 513.37): C 53.81, H 5.99, N 10.91; found: C 53.86, H 5.83, N 10.73.

*4.1.5.5. 7-Chloro-N-{2-[4-(4,5-dihydro-1H-imidazol-2-yl)phenoxy]ethyl}quinolin-4-amine dihydrochloride (**8f**).*

A solution of crude imidate and ethane-1,2-diamine (277 mg, 4.63 mmol) in dry MeOH was refluxed. The progress of the reaction was monitored by TLC. After 24 hours the desired product was isolated using column chromatography as the white solid (265 mg, 29%); mp 144–145 °C. IR (ν_{max}/cm^{-1}): 3407, 3122, 3094, 1618, 1510, 1403, 1267, 1191, 1140, 1092, 1042, 842, 740. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.55 (s, 1H, NH), 10.61 (s, 2H, NH), 9.82 (t, J = 5.2 Hz, 1H, NH), 8.75 (d, J = 9.2 Hz, 1H, ArH), 8.59 (d, J = 7.1 Hz, 1H, ArH), 8.12 (d, J = 2.1 Hz, 1H, ArH), 8.04 (d, J = 8.9 Hz, 2H, ArH), 7.77 (dd, J = 9.1, 2.1 Hz, 1H, ArH), 7.21 (d, J = 8.9 Hz, 2H, ArH), 7.03 (d, J = 7.2 Hz, 1H, ArH), 4.47 (t, J = 5.0 Hz, 2H, CH_2), 4.01 (q, J = 5.1 Hz, 2H, CH_2), 3.95 (s, 4H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 163.96, 162.90, 155.85, 142.88, 138.63, 137.94, 130.88, 66.26, 44.09, 42.49. HRMS calcd. for $C_{20}H_{20}ClN_4O$ $(M + H)^+$: 367.1326; found: 367.1315. Anal. calcd. for $C_{20}H_{19}ClN_4O \times 2HCl \times 3H_2O$ (M_r = 493.81): C 48.65, H 5.51, N 11.35; found: C 48.84, H 5.26, N 11.10.

*4.1.5.6. 7-Chloro-N-{2-[4-(1,4,5,6-tetrahydropyrimidin-2-yl)phenoxy]ethyl}quinolin-4-amine dihydrochloride (**8g**).*

A solution of crude imidate and propane-1,2-diamine (343 mg, 4.63 mmol) in dry MeOH was refluxed. The progress of the reaction was monitored by TLC. After 24 hours the desired product was isolated using column cromatography as white solid (229 mg, 22%); mp 144–145 °C. IR (ν_{max}/cm^{-1}): 3407, 3204, 3008, 2932, 1634, 1609, 1582, 1452, 1374, 1316, 1260, 1196, 1092, 1142, 1091, 1050, 814, 704. ^{1}H NMR (DMSO-d_6) δ/ppm: 10.01 (s, 2H, NH), 8.50 (m, 3H, NH + ArH), 7.94 (d, J = 2.0 Hz, 1H, ArH), 7.78 (d, J = 8.8 Hz, 2H), 7.56 (dd, J = 9.0, 2.0 Hz, 1H, ArH), 7.16 (d, J = 8.8 Hz, 2H, ArH), 6.77 (d, J = 6.1 Hz, 1H, ArH), , 4.40 (t, J = 5.2 Hz, 2H, CH_2), 3.83 (q, J = 5.1 Hz, 2H, CH_2), 3.46 (s, 4H, CH_2), 2.05 – 1.87 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 162.49, 158.60, 153.31, 147.98, 144.56, 136.00, 129.97, 125.86, 125.65, 123.93, , 120.83, 117.01, 115.26, 99.44, 66.64, 42.51, 18.31. HRMS calcd. for $C_{21}H_{22}ClN_4O$ (M + H)$^+$: 381.1482; found: 381.1477. Anal. calcd. for $C_{21}H_{21}ClN_4O \times 2HCl \times 1.75H_2O$ (M_r = 566.24): C 44.55, H 4.90, N 9.89; found: C 44.64, H 4.75, N 9.58.

*4.1.6.1. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-1H-benzo[d]imidazole-6-carbonitrile (**10a**).*

The solution of compound **9** (300 mg, 0.92 mmol), nitrile **3a** (122 mg, 0.92 mmol) and water solution of $NaHSO_3$ (40%, 5 mL) in ethanol (50 mL) was refluxed for 6h. The hot solution was filtered, filtrate was evaporated to dryness, and the residue was suspended in 30 mL of water and stirred overnight. The solid was collected and dissolved in methanol, with addition of water product precipitates as a brown powder (150 mg, 41%); mp 274 °C. IR (ν_{max}/cm^{-1}): 3421, 2920, 2850, 2368, 2217, 1610, 1581, 1490, 1450, 1425, 1249, 1180, 1141, 1053, 806. ^{1}H NMR (DMSO-d_6) δ/ppm 13.24 (s, 1H, NH), 8.47 (d, J = 5.6 Hz, 1H, ArH), 8.33 (d, J = 9.0 Hz, 1H, ArH), 8.14 (d, J = 6.8 Hz, 2H, ArH), 7.97 (brs, 1H), 7.82 (d, J = 2.1 Hz, 1H, ArH), 7.75 (s, 1H, ArH), 7.66 (s, 1H, ArH), 7.56 (d, J = 7.0 Hz, 1H, ArH), 7.51 (dd, J = 9.0, 2.1 Hz, 1H, ArH), 7.17 (d, J = 8.8 Hz, 2H, ArH), 6.70 (d, J = 5.7 Hz, 1H, ArH), 4.38 (t, J = 5.4 Hz, 2H, CH_2), 3.79 (d, J = 5.0 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm 160.42, 151.00, 150.51, 134.19, 128.57, 126.19, 124.61, 124.24, 121.84, 120.02, 117.13, 115.08, 103.64, 98.99, 65.92, 42.04. HRMS calcd. for $C_{25}H_{19}ClN_5O$ (M + H)$^+$: 440.1278; found: 440.1291.

*4.1.7. General method for the synthesis of the amidines from group **IV** (**10b-g**).*

The solution of compound **9** (300 mg, 0.92 mmol) appropriate 3,4-diaminobenzamidine **3b-g** and water solution of $NaHSO_3$ (40%, 5 mL) in ethanol (50 mL) was refluxed for 6h. The hot

solution was filtered, filtrate was evaporated to dryness, and the residue was suspended in 30 mL of water and stirred overnight. Solid was collected with filtration and suspended in methanol saturated with hydrogen chloride, addition of diethyl ether resulted in precipitation of product.

*4.1.7.1. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**10b**).*

Prepared from amidine **3b** (171 mg, 0.92 mmol) and compound **9**, as brown product (266 mg, 49%); mp 249 °C decomp. IR (ν_{max}/cm^{-1}): 3410, 3230, 3103, 2914, 2848, 1679, 1612, 1507, 1464, 1383, 1306, 1263, 1228, 1190, 1045, 835, 740, 650. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.43 (s, 1H, NH), 9.78 (t, J = 5.3 Hz, 1H, NH), 9.45 (s, 2H, NH), 9.13 (s, 2H, NH), 8.73 (d, J = 9.2 Hz, 1H, ArH), 8.61 (d, J = 7.0 Hz, 1H, ArH), 8.35 (d, J = 8.8 Hz, 2H, ArH), 8.18 (d, J = 1.0 Hz, 1H, ArH), 8.10 (d, J = 2.1 Hz, 1H, ArH), 7.93 – 7.72 (m, 3H, ArH), 7.23 (d, J = 8.9 Hz, 2H, ArH), 7.07 (d, J = 7.2 Hz, 1H,), 4.47 (t, J = 5.0 Hz, 2H, CH_2), 4.03 (q, J = 5.1 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 165.81, 161.04, 155.92, 153.12, 142.80, 138.53, 137.98, 129.49, 126.91, 125.88, 123.08, 122.40, 119.05, 115.47, 115.28, 99.11, 66.05, 42.62. HRMS calcd. for $C_{25}H_{22}ClN_6O$ $(M + H)^+$: 457.1543; found: 457.1554. Anal. calcd. for $C_{25}H_{21}ClN_6O \times 3HCl \times 1.5H_2O$ (M_r = 593.33): C 50.61, H 4.59, N 14.16; found: C 50.49, H 4.57, N 14.29.

*4.1.7.2. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-N-isopropyl-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**10c**).*

Prepared from amidine **3c** (210 mg, 0.92 mmol) and compound **9**, as brown product (266 mg, 45%); mp 204 °C. IR (ν_{max}/cm^{-1}): 3416, 3239, 3090, 2916, 1673, 1495, 1452, 1382, 1308, 1265, 1187, 1136, 842, 581. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.78 (s, 1H, NH), 9.99 (t, J = 5.6 Hz, 1H, NH), 9.71 (d, J = 7.7 Hz, 1H, NH), 9.56 (s, 1H, NH), 9.21 (s, 1H, NH), 8.83 (d, J = 9.2 Hz, 1H, ArH), 8.59 (d, J = 6.3 Hz, 1H, ArH), 8.46 (d, J = 8.8 Hz, 2H, ArH), 8.19 (d, J = 2.1 Hz, 1H, ArH), 8.09 (d, J = 1.0 Hz, 1H, ArH), 7.86 (d, J = 8.5 Hz, 1H, ArH), 7.76 (dd, J = 9.1, 2.1 Hz, 1H, ArH), 7.70 (dd, J = 8.5, 1.4 Hz, 1H, ArH), 7.24 (d, J = 9.0 Hz, 2H, ArH), 7.05 (d, J = 7.2 Hz, 1H, ArH), 4.50 (t, J = 5.1 Hz, 2H, CH_2), 4.23 – 4.08 (m, 1H, CH), 4.03 (q, J = 5.0 Hz, 2H, CH_2), 1.31 (d, J = 6.4 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 161.84, 161.45, 155.89, 152.18, 142.73, 138.49, 137.93, 129.86, 126.84, 125.87, 124.50, 124.04, 118.99, 115.44, 115.38, 99.07, 66.13, 45.10, 42.56, 28.98, 21.21. HRMS calcd. for $C_{28}H_{28}ClN_6O$ $(M + H)^+$: 499.2013; found: 499.2019. Anal. calcd. for $C_{28}H_{27}ClN_6O \times 3HCl \times 3.25H_2O$ (M_r = 666.946): C 50.43, H 5.52, N 12.60; found: C 50.37, H 5.25, N 12.45.

*4.1.7.3. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-N-isobutyl-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**10d**).*

Prepared from amidine **3d** (223 mg, 0.92 mmol) and compound **9**, as brown product (286 mg, 47%); mp 266 °C decomp. IR (ν_{max}/cm^{-1}): 3426, 3221, 3103, 3061, 2957, 2923, 1675, 1630, 1603, 1583, 1499, 1454, 1382, 1302, 1264, 1192, 840, 809, 760. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.48 (s, 1H, NH), 9.89 (t, J = 5.4 Hz, 1H, NH), 9.81 (t, J = 5.5 Hz, 1H, NH), 9.56 (s, 1H, NH), 9.12 (s, 1H, NH), 8.72 (d, J = 9.2 Hz, 1H, ArH), 8.58 (d, J = 6.4 Hz, 1H, ArH), 8.38 (d, J = 8.8 Hz, 2H, ArH), 8.08 (d, J = 2.1 Hz, 2H, ArH), 7.85 (d, J = 8.5 Hz, 1H, ArH), 7.76 (dd, J = 9.1, 2.1 Hz, 1H), 7.69 (m, dd, J = 8.6, 1.4 Hz, 1H, ArH), 7.22 (d, J = 9.0 Hz, 2H, ArH), 7.04 (d, J = 7.2 Hz, 1H, ArH), 4.46 (t, J = 4.9 Hz, 2H, CH_2), 4.00 (q, J = 5.1 Hz, 2H, CH_2), 3.26 (t, J = 6.5 Hz, 2H, CH_2), 2.02 (m, 1H, CH), 0.97 (d, J = 6.6 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6) δ/ppm: 163.51, 162.23, 156.37, 152.33, 143.21, 139.00, 138.41, 130.60, 127.33, 126.43, 125.22, 124.77, 119.47, 115.96, 115.93, 115.29, 114.68, 99.57, 66.68, 50.19, 43.03, 27.46, 20.39. HRMS calcd. for $C_{29}H_{30}ClN_6O$ $(M + H)^+$: 513.2170; found: 513.2178. Anal. calcd. for $C_{29}H_{29}ClN_6O \times 3HCl \times 2.25H_2O$ (M_r = 662.95): C 52.54, H 5.55, N 12.68; found: C 52.53, H 5.32, N 12.36.

*4.1.7.4. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-N-cyclopentyl-1H-benzo[d]imidazole-6-carboximidamide trihydrochloride (**10e**).*

Prepared from amidine **3e** (234 mg, 0.92 mmol) and compound **9**, as brown product (261 mg, 43%); mp 249 °C decomp. IR (ν_{max}/cm^{-1}): 3412, 3230, 3104, 3063, 2958, 2916,1603, 1496, 1447, 1380, 1353, 1262, 1195, 1046, 840, 654. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.53 (s, 1H, NH), 9.85 (t, J = 7.5 Hz, 2H, NH), 9.60 (s, 1H, NH), 9.14 (s, 1H, NH), 8.76 (d, J = 9.2 Hz, 1H, ArH), 8.61 (d, J = 8.5 Hz, 1H, ArH), 8.46 (d, J = 8.8 Hz, 2H, ArH), 8.13 – 8.05 (m, 2H, ArH), 7.89 (d, J = 8.5 Hz, 1H, ArH), 7.78 (dd, J = 9.1, 2.1 Hz, 1H, ArH), 7.73 (dd, J = 8.5, 1.5 Hz, 1H, ArH), 7.27 (d, J = 9.0 Hz, 2H, ArH), 7.07 (d, J = 7.2 Hz, 1H, ArH), 4.50 (t, J = 4.9 Hz, 2H, CH_2), 4.26 – 4.13 (m, 1H, CH), 4.04 (q, J = 5.0 Hz, 2H, CH_2), 2.17 – 1.99 (m, 2H, CH_2), 1.90 – 1.48 (m, 6H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 162.94, 162.13, 156.38, 152.42, 143.25, 139.00, 138.42, 130.51, 127.34, 126.41, 125.11, 124.88, 119.48, 115.96, 115.92, 115.54, 114.57, 99.58, 66.66, 54.85, 43.04, 31.86, 24.18. HRMS calcd. for $C_{30}H_{30}ClN_6O$ $(M + H)^+$: 525.2169; found: 525.2177. Anal. calcd. for $C_{30}H_{29}ClN_6O \times 3HCl \times 1.5H_2O$ (M_r = 661.45): C 54.48, H 5.33, N 12.71; found: C 54.59, H 5.27, N 12.98.

*4.1.7.5. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-6-(4,5-dihydro-1H-imidazol-2-yl)-1H-benzo[d]imidazole trihydrochloride (**10f**).*

Prepared from amidine **3f** (195 mg, 0.92 mmol) and compound **9**, as brown product (293 mg, 50%); mp 260–262 °C. IR (ν_{max}/cm^{-1}): 3434, 3220, 3093, 2919, 1609, 1495, 1455, 1378, 1268, 1201, 1034, 821, 693. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.54 (s, 1H, NH), 10.84 (s, 2H, NH), 9.84 (t, J = 5.4 Hz, 1H, NH), 8.75 (d, J = 9.2 Hz, 1H, ArH), 8.60 (d, J = 6.6 Hz, 1H, ArH), 8.43 (s, 1H, ArH), 8.37 (d, J = 8.6 Hz, 2H, ArH), 8.12 (d, J = 2.0 Hz, 1H, ArH), 7.97 (d, J = 8.4 Hz, 1H, ArH), 7.87 (d, J = 8.5 Hz, 1H, ArH), 7.77 (dd, J = 9.1, 2.0 Hz, 1H, ArH), 7.22 (d, J = 8.8 Hz, 2H, ArH), 7.06 (d, J = 7.2 Hz, 1H, ArH), 4.47 (t, J = 5.1 Hz, 2H, CH_2), 4.02 (s, 6H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 164.76, 161.29, 155.89, 142.52, 138.59, 137.80, 129.85, 126.69, 126.23, 118.90, 115.51, 115.28, 99.03, 66.15, 44.23, 42.25. HRMS calcd. for $C_{27}H_{24}ClN_6O$ $(M + H)^+$: 483.1700; found: 483.1701. Anal. calcd. for $C_{27}H_{23}ClN_6O \times 3HCl \times 2.5H_2O$ (M_r = 637.39): C 50.88, H 4.90, N 13.19; found: C 50.75, H 4.74, N 12.83.

*4.1.7.6. 2-{4-[2-(7-Chloroquinolin-4-ylamino)ethoxy]phenyl}-6-(1,4,5,6-tetrahydropyrimidin-2-yl)-1H-benzo[d]imidazole trihydrochloride (**10g**).*

Prepared from amidine **3g** (208 mg, 0.92 mmol) and compound **9**, as brown product (260 mg, 45%); mp 263–265 °C; IR (ν_{max}/cm^{-1}): 3420, 2919, 1606, 1502, 1452, 1378, 1304, 1267, 1187, 1041, 981, 821, 573. ^{1}H NMR (DMSO-d_6) δ/ppm: 14.35 (s, 1H, NH), 10.06 (s, 2H, NH), 9.75 (t, J = 5.3 Hz, 1H, NH), 8.71 (d, J = 9.2 Hz, 1H, ArH), 8.61 (d, J = 5.4 Hz, 1H, ArH), 8.34 (d, J = 8.2 Hz, 2H, ArH), 8.08 (d, J = 1.8 Hz, 2H, ArH), 7.85 (d, J = 8.4 Hz, 1H, ArH), 7.80 (dd, J = 9.1, 2.0 Hz, 1H, ArH), 7.66 (d, J = 8.3 Hz, 1H, ArH), 7.23 (d, J = 8.7 Hz, 2H, ArH), 7.07 (d, J = 7.2 Hz, 1H, ArH), 4.47 (t, J = 5.1 Hz, 2H, CH_2), 4.03 (d, J = 5.2 Hz, 2H, CH_2), 3.58 (s, 4H, CH_2), 2.08 – 1.97 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ/ppm: 159.11, 155.89, 142.57, 138.58, 137.82, 129.83, 126.72, 126.17, 118.92, 115.50, 115.31, 99.04, 66.15, 42.48, 38.80, 17.77. HRMS calcd. for $C_{28}H_{26}ClN_6O$ $(M + H)^+$: 497.1857; found: 497.1857. Anal. calcd. for $C_{28}H_{25}ClN_6O \times 3HCl \times 1.25H_2O$ (M_r = 628.84): C 53.48, H 4.89, N 13.36; found: C 53.48, H 4.86, N 13.07.

4.2. Spectroscopic studies

Polynucleotides were purchased as noted: polyA-polyU (Sigma), calf thymus *ct*DNA (Aldrich). Polynucleotides were dissolved in sodium cacodylate buffer (I = 0.05 mol dm^{-3}, pH 7.0). Calf thymus *ct*-DNA was additionally sonicated and filtered through a 0.45 mm filter. Polynucleotide concentration was determined spectroscopically as a concentration of

phosphates. Electronic absorption spectra were obtained on the PerkinElmer Lambda 25 spectrometer and CD spectra were collected on a Jasco J-810 spectrometer in quartz cuvettes (1 cm). The spectroscopic studies were performed in an aqueous buffer solution (pH 7.0; Na-cacodylate buffer, I = 0.05 mol dm^{-3}). UV-Vis titrations were performed at room temperature by adding portions of polynucleotide solution into the solution of the studied compound. Obtained data were corrected for dilution. The binding constant (K_s) and [bound compound]/[polynucleotide phosphate] ratio (n) were calculated according to the Scatchard equation [36]. The values for Ks and n given in Table 1 all have satisfactory correlation coefficients (>0.99) [31]. Thermal melting curves for *ct*-DNA and polyA-polyU and their complexes with studied compounds were determined as previously described [43] by following the absorption change at 260 nm as a function of temperature. Absorbance of the ligands was subtracted from every curve, and the absorbance scale was normalized. Melting temperature (T_m) values are the midpoints of the transition curves, determined from the maximum of the first derivative and checked graphically by the tangent method. Given ΔT_m values were calculated subtracting T_m of the free nucleic acid from T_m of the complex. Every ΔT_m value here reported was the average of at least two measurements, the error in ΔT_m is ± 0.5 °C.

4.3. Antiproliferative effects

Effects on the normal and tumors cells' growth were determined using the colorimetric methyltetrazolium (MTT) assay. Experiments were carried out on four tumor human cell lines (HeLa, CaCo-2, K562 and Raji) and on one normal canine cell line (MDCK I).. The adherent cells, MDCK1, HeLa, and $CaCO_2$, were seeded in 96 micro-well plates at concentration of 2×10^4 cells/cm^3 and allowed to attach overnight in the CO_2 incubator (IGO 150 CELLlife^{TM}, JOUAN, Thermo Fisher Scientific**,** Waltham, MA, USA). After 72 hrs of the exposure to tested compounds, medium was replaced with 5 mg/cm^3 MTT solution and the resulting formazane crystals were dissolved in DMSO. Leukemia cells (1x10^5 cells ⁄cm^3), were plated onto 96 micro-well plates and after 72 hrs of incubation, 5mg/cm^3 MTT solution was added to each well and incubated 4 hrs in the CO_2 incubator. To each well, 10% SDS with 0.01 mol dm^{-3} HCl was added to dissolve water-insoluble MTT-formazane crystals.. The elisa microplate reader (iMark, BIO RAD, Hercules, CA, USA) was used for measurement of the absorbance at 595nm. All experiments were performed at least three times in triplicates. The percentage of cell growth (PG) was calculated using the following equation:

$$PG= (A_{compound} - A_{background} / A_{control} - A_{background}) X100$$

where $A_{background}$ at the adherent cells is the absorbance of MTT solution and DMSO; $A_{background}$ at the suspension cells is the absorbance of the medium without cells, but containing MTT and 10%SDS with 0.01 mol dm^{-3} HCl; and $A_{control}$ is the absorbance of cell suspension grown without tested compounds.

4.4. Cell cycle distribution

To analyze the effect of the selected compounds on cell cycle, cells were seeded in 6-well plates ($5x10^5$ cells per well) and treated for 24 hrs (10^{-5} mol dm^{-3}). Cells were then collected and fixed in ethanol. Fixed samples were stained with propidium iodide (PI) and analyzed by flow cytometry (FACS Canto II, BD Biosciences). Results were analyzed using FlowLogic software. Statistical analysis (ANOVA with Bonferroni correction) was done using XLSTAT software.

4.5. Statistical analysis

Calculation of GI_{50} value curves and QC analysis is performed by using the Excel tools and GraphPadPrism software (La Jolla, CA), v. 5.03. Briefly, individual concentration effect curves are generated by plotting the logarithm of the concentration of tested compounds(X) vs corresponding percent inhibition values (Y) using least squares fit.The best fit GI_{50} values are calculated using Log (inhibitor) versus normalized response - Variable slope equation, where Y Ľ 100/(1 ţ 10 ((LogIC_{50} _ X) * HillSlope)). QC criteria parameters (Z0, S:B, R2, HillSlope) were checked for every GI_{50} curve.

Conflict of interests

The authors declare no conflict of interest.

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Research highlights:

- Four series of novel 7-chloroquinoline-arylamidine hybrids were synthesized
- All compounds bind preferentially to ctDNA
- Antiproliferative activity of the compounds was evaluated in four cancer cell lines
- **8a** showed strongest inhibitory activity with IG_{50} = 6.2 µM against Raji cell line
- Compounds **2a**,**e**, **8a**, **10d**,**e**,**f** induced changes in cell cycle of treated cells