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Synthesis, in vitro antiproliferative activity and DNA-interaction of benzimidazoquinazoline derivatives as potential anti-tumor agents

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Abstract

The synthesis of benzimidazoquinazoline derivatives bearing different alkylamino side chains is reported. All new compounds tested by means of an in vitro assay exhibit antiproliferative activity toward human tumor cell lines. The cytotoxic effect depends on the type of side chain inserted in the planar nucleus and in some cases it is comparable to that of the well-known drug ellipticine. In order to understand the mechanism of action of these compounds, the interaction with DNA has been investigated. Linear flow dichroism measurements allowed us to verify the formation of a molecular complex with DNA and the corresponding geometry of interaction. Intrinsic binding constants have also been evaluated by performing fluorimetric titrations.

Keywords: Benzimidazoquinazoline; Antiproliferative activity; DNA binding

1. Introduction

Most anti-tumor drugs bind to DNA suggesting a direct relationship between their interaction with the macromolecule and the therapeutic effect. In this connection the DNA has been considered by far the preferred target for the test of new compounds with possible anti-tumor activity.

Among the DNA binding compounds one of the most interesting groups is the intercalators, characterized by the presence of a planar aromatic chromophore, which following the interaction with the nucleic acid, locates between two consecutive base pairs of the double helix.

The complex formation with the macromolecule is usually considered reversible and van der Waals contact with the base pairs, hydrophobic interactions and charge transfer forces were hypothesized to contribute to bonding energy. The binding of DNA intercalators induces unwinding, lengthening and stiffening of the double helix, thus introducing structural changes, which affect both the condensation of chromatin and its interaction with DNA-associated enzymes [1].

In the broad class of intercalating anti-cancer drugs, the planar heterocyclic moieties play an important role. Among them, benzimidazole can be considered an interesting tool for the development of compounds exerting biological properties. Many chemical structures carrying a benzimidazole group indeed, show anti-tumor activity [2–4].

Furthermore, notwithstanding the undoubted importance of the planar aromatic ring system, interestingly the addition of groups or side chains which project from one or both the grooves of the DNA double helix and which interact like external binder moiety, appears also to be crucial for drug activity. Indeed, a large number of anti-tumor compounds have been modified by linking to a DNA intercalator a suitable side chain, thus modulating the biological properties and increasing solubility under physiological conditions [5]. In this connection it is worth noting that the optimization of

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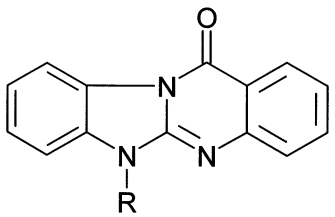


1: R=$(CH_2)_2N(CH_3)_2$

2: R=$(CH_2)_3N(CH_3)_2$

3: R=$(CH_2)_2N(C_2H_5)_2$

4: R=$(CH_2)_3N(C_2H_5)_2$

5: R=$(CH_2)_2NH(CH_2)_2OH$

Fig. 1. Chemical structures of the benzimidazoquinazoline derivatives.

the charged side chains of the derivatives of anthracenedione, designed as analogues of the anthracycline antibiotics, led to the well-known anti-tumor agents ametantrone and mitoxantrone [6,7].

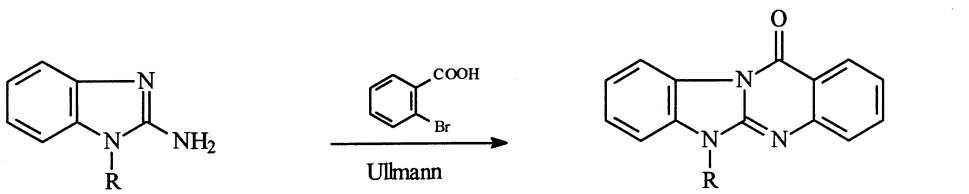
In this paper we report the synthesis of new derivatives **1**–**5** characterized by the presence of a benzimidazoquinazoline nucleus carrying different alkylamino-substituted side chains. In detail, in the 6 position of the planar moiety, dialkylamino alkyl side chains have been inserted in **1**–**4**, and a hydroxyethylaminoethyl side chain has been inserted in **5** (Fig. 1).

An evaluation of their antiproliferative activity in vitro was carried out using human tumor cell lines. Moreover, using spectroscopic techniques the DNA binding process has been investigated. Linear flow dichroism measurements were performed to establish the geometry of the complex formed between the macromolecule and the new derivatives. The binding parameters have been obtained by fluorimetric titrations.

2. Chemistry

The new target compounds **1**–**4** were conveniently prepared from the appropriate 1-alkyl-2-aminobenzimidazoles **6**–**9** by reaction with 2-bromobenzoic acid, in Ullmann conditions (Scheme 1). The starting alkylated derivatives **7**–**9** have already been described [8], and the new 1-dimethylaminoethyl-2-aminobenzimidazole (**6**) was synthesized following a similar experimental procedure. All products, **1**–**4**, were purified by recrystallization and their structures were confirmed by analytical and spectral data (Table 1).

The synthesis of the desired ethanolamino derivative **5** was carried out using as starting material the heterocyclic compound **10** (Scheme 2), which was obtained following a literature method [9]. The heterocycle **10** was converted into the intermediate chloroethyl derivative **11** by reaction with 1-bromo-2-chloroethane in anhydrous DMF solution, in the presence of sodium hydride. Then, compound **5** was conveniently obtained by reaction of **11** with ethanolamine in ethanol refluxing solution. IR, ^{1}H NMR and mass spectral data were consistent with the proposed 6-alkyl substituted structure. In the ^{1}H NMR spectrum, the triplets at 4.35 and 2.99 ppm ($J = 6.20$ Hz) assigned, respectively, to the methylene bound to the 6-position of the benzimidazoquinazoline nucleus and to its adjacent methylene group, showed chemical shift values quite similar to those of the corresponding methylene groups in the spectra of derivatives **1**–**4**. Moreover, the assignment of the position of the side alkyl chain was further confirmed by the considerations emerging from the IR spectra, which, for compound **5**, showed a strong C=O absorption band at $\nu = 1690$ cm^{-1}, superimposable

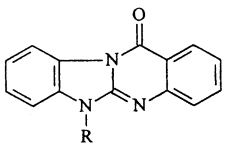


6-9 **1-4**

1: R= $CH_2CH_2N(CH_3)_2$
2: R= $CH_2CH_2CH_2N(CH_3)_2$
3: R= $CH_2CH_2N(CH_2CH_3)_2$
4: R= $CH_2CH_2CH_2N(CH_2CH_3)_2$

Scheme 1.

Table 1
Physical and spectral data of compounds **1–5**



No.	R	Yield (%)	M.p. (°C)	^{1}H-NMR DMSO-d$_6$ (δ ppm)	MS m/z (M^+)	Formula
1	$(CH_2)_2N(CH_3)_2$	55	160–165	2.2 (s, 6H, $N(CH_3)_2$), 2.73 (t, 2H, $CH_2CH_2N(CH_3)_2$), 4.37 (t, 2H, $CH_2CH_2N(CH_3)_2$), 7.29–8.41 (m, 8H, ArH).	306	$C_{18}H_{18}N_4O$
2	$(CH_2)_3N(CH_3)_2$	29	163–165	1.97 (m, 2H, $CH_2CH_2CH_2N(CH_3)_2$), 2.13 (s, 6H, $N(CH_3)_2$), 2.23 (t, 2H, $CH_2CH_2CH_2$ $N(CH_3)_2$), 4.31 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$), 7.31–8.42 (m, 8H, ArH).	320	$C_{19}H_{20}N_4O$
3	$(CH_2)_2N(CH_2CH_3)_2$	46	140–145	0.83 (t, 6H, $N(CH_2CH_3)_2$), 2.4 (q, 4H, $N(CH_2CH_3)_2$), 2.87 (t, 2H, $CH_2CH_2N(C_2H_5)_2$), 4.37 (t, 2H, $CH_2CH_2N(C_2H_5)_2$), 7.35–8.45 (m, 8H, ArH).	334	$C_{20}H_{22}N_4O$
4	$(CH_2)_3N(CH_2CH_3)_2$	49	130–135	0.89 (t, 6H, $N(CH_2CH_3)_2$), 2.02 (m, 2H, $CH_2CH_2CH_2N(C_2H_5)_2$), 2.29 (q, 4H, $N(CH_2CH_3)_2$), 2.71 (t, 2H, $CH_2CH_2CH_2N(CH_2CH_3)_2$), 4.31 (t, 2H, $CH_2CH_2CH_2N(C_2H_5)_2$), 7.20–8.50 (m, 8H, ArH).	348	$C_{21}H_{24}N_4O$
5	$(CH_2)_2NHCH_2CH_2OH$	72	180–183	2.61 (t, 2H, CH_2CH_2OH), 2.99 (t, 2H, CH_2CH_2NH), 3.36 (t, 2H, CH_2CH_2OH), 4.35 (t, 2H, CH_2CH_2NH), 4.4 (br.t, 1H, OH exch.), 7.21–7.78 (m, 6H, ArH), 8.18–8.50 (m, 2H, ArH) [a]	322	$C_{18}H_{18}N_4O_2$

[a] Recorded on a Varian Gemini 200 (200-MHz) spectrometer.

with that of compounds **1**–**4**, similar to that reported in literature for 6-methylbenzimidazo[2,1-*b*]quinazolin-12(6*H*)-one ($\nu = 1684$ cm^{-1}) but different from that of the 5-methyl isomer ($\nu = 1724$ cm^{-1}) [9].

3. Experimental

3.1. Chemistry

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. IR spectra were obtained on a PYE/UNICAM Model PU 9561 spectrophotometer as Nujol mulls. ^{1}H NMR spectra were recorded, if not otherwise indicated, on a Varian CFT-20 (80 MHz) spectrometer, in DMSO-d_6 solution, using TMS as the internal standard. Mass spectra were obtained on a Hewlett–Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Magnesium sulfate was always used as the drying agent. Evaporations were performed in vacuo (rotating evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254). Elemental analyses (C, H, N) were performed and agreed with the theoretical values within $\pm 0.4\%$.

3.1.1. 1-(2-Dimethylaminoethyl)-2-aminobenzimidazole (6)

1-Dimethylamino-2-chloroethane hydrochloride (1.17 g, 8.14 mmol) was added to a stirred solution of 2-aminobenzimidazole (1.0 g, 7.5 mmol) and potassium hydroxide (1.0 g, 18.8 mmol) in ethanol. The mixture was stirred at room temperature for 5 h and then the inorganic material (KCl) was filtered off. The solution was evaporated to dryness and the residue obtained was purified by recrystallization from acetone to give 0.446 g (30% yield) of pure **6**; m.p. 140–142°C; ^{1}H NMR: δ 2.21 (s, 6H, $N(CH_3)_2$), 2.51 (t, 2H, $CH_2CH_2N(CH_3)_2$), 4.03 (t, 2H, $CH_2CH_2N(CH_3)_2$), 6.39 (s, 2H, NH_2 exch.), 6.39–7.16 (m, 4H, ArH); MS: m/z 204 (M^+).

3.1.2. 6-(Dialkylaminoalkyl)benzimidazo[2,1-b]-quinazolin-12(6H)-ones (1–4)

General procedure: A suspension of the appropriate 1-(dialkylaminoalkyl)-2-aminobenzimidazole (**6**–**9**) (7.5 mmol), 2-bromobenzoic acid (1.51 g, 7.5 mmol), anhydrous potassium carbonate (1.24 g, 9 mmol), copper-bronze (0.08 g) and a catalytic amount of KI in 10 ml of anhydrous DMF was heated at 170°C until the disappearance of the starting materials (5–7 h, TLC analysis, chloroform/methanol 7:3 as eluant). After cooling, the reaction mixture was diluted with water

and left to stand at 4°C overnight. The precipitated crude products were collected and purified by recrystallization from ethanol (Table 1).

3.1.3. 6-(2-Chloroethyl)benzimidazo[2,1-b]quinazolin-12(6H)-one (**11**)

Sodium hydride (50% dispersion in mineral oil, 0.14 g, 2.8 mmol) was added in small portions to a stirred suspension of 0.60 g (2.56 mmol) of compound **10** in 15 ml of anhydrous DMF, under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h, then supplemented dropwise with 0.3 ml (1 mmol) of 1-bromo-2-chloroethane and left at room temperature with stirring for 16 h. Compound **11** was recovered from the reaction mixture as insoluble material, washed with water and purified by recrystallization from DMF, obtaining 0.54 g (71% yield) of pure derivative **11**; m.p. 237–238°C; ^{1}H NMR: δ 4.12 (t, 2H, N–CH_2), 4.64 (t, 2H, CH_2–Cl), 7.33–7.76 (m, 6H, ArH); 8.17–8.50 (m, 2H, ArH); MS: m/z 297 (M^+).

3.1.4. 6-(β-Ethanolaminoethyl)benzimidazo[2,1-b]-quinazolin-12(6H)-one (**5**)

0.16 ml (2.70 mmol) of ethanolamine was added to a suspension of compound **11** (0.20 g, 0.67 mmol) in 30 ml of ethanol. The reaction mixture was refluxed for 40 h and during this time, two amounts of 0.08 ml of ethanolamine were further added. The resulting suspension was filtered to recover the unreacted starting product **11** (0.029 g). The solution was evaporated to dryness under reduced pressure and the resulting crude residue was purified by recrystallization from ethanol to give pure compound **5** (0.156 g) (Table 1).

3.2. Biological activity

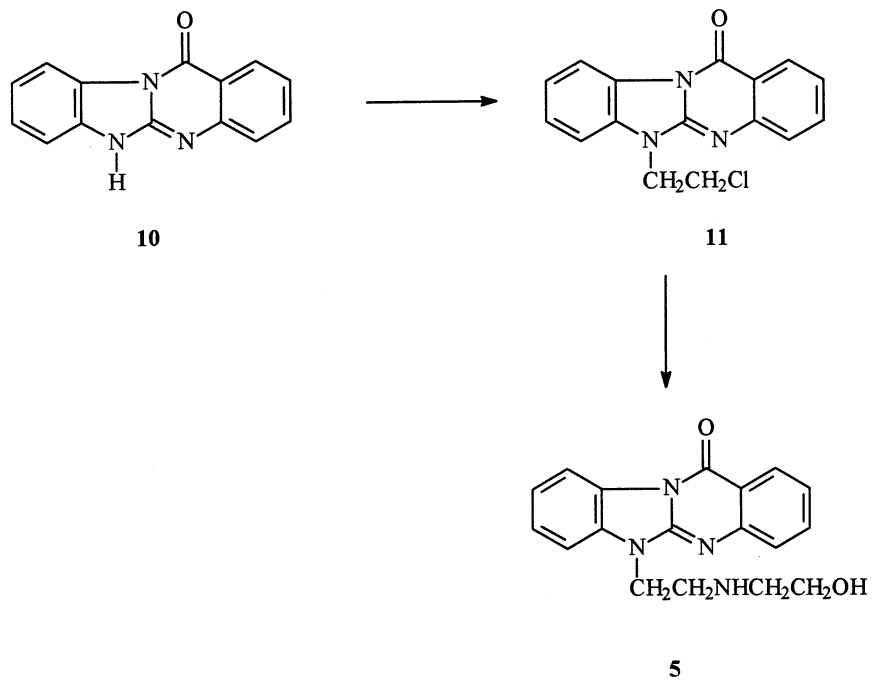
3.2.1. Cell cultures

HL-60 (human myeloid leukaemic cells) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15% heat-inactivated fetal calf serum (Seromed), HeLa (human cervix adenocarcinoma cells) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Seromed) and A431 (human squamous carcinoma cells) were grown in D-MEM (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Seromed). 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Sigma Chemical Co.) were added to the media. The cells were cultured at 37°C in a moist atmosphere of 5% carbon dioxide in air.

3.2.2. Inhibition growth assay

HL-60 cells (3×10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added in complete medium and incubated for a further 72 h.

HeLa (3×10^4) and A431 (4×10^4) cells were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, the medium was replaced with an equal volume of fresh medium and various concentra-



Scheme 2.

Table 2
Growth cell inhibition of test compounds **1–5** and ellipticine as reference drug

Compounds	IC_{50} (μM)		
	HL-60	HeLa	A-431
1	0.5 ± 0.05	2.1 ± 0.3	0.8 ± 0.2
2	1.4 ± 0.2	4.3 ± 0.7	1.2 ± 0.1
3	1.7 ± 0.2	8.6 ± 0.5	3.5 ± 0.1
4	2.2 ± 0.4	9.6 ± 0.9	3.6 ± 0.3
5	0.6 ± 0.1	1.90 ± 0.07	0.50 ± 0.09
Ellipticine	0.64 ± 0.02	0.31 ± 0.01	0.47 ± 0.03

tions of the test agents were added. The cells were then incubated in standard conditions for a further 72 h.

A trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as IC_{50} values, i.e. the concentrations of the test agent inducing 50% reduction in cell numbers compared with the control cultures.

3.3. Interaction with DNA

3.3.1. Nucleic acid

Salmon testes DNA was purchased from Sigma Chemical Co. Aqueous solutions of nucleic acid containing Tris 10 mM, EDTA 1 mM (pH 7.0) and NaCl 0.01 M or 0.5 M depending on the desired ionic strength were used (ETN buffer).

3.3.2. Spectrophotometric determinations

UV absorption spectra were recorded at room temperature with a Perkin–Elmer model Lambda 5 spectrometer. DNA and test compound concentrations were determined by absorption measurements, using the following extinction coefficients: $\varepsilon = 6600\ M^{-1}$/cm at 260 nm for DNA, $\varepsilon = 21\,520\ M^{-1}$/cm at 297 nm for **1**, $\varepsilon = 21\,940\ M^{-1}$/cm at 299 nm for **2**, $\varepsilon = 21\,680\ M^{-1}$/cm at 297 nm for **3**, $\varepsilon = 23\,930\ M^{-1}$/cm at 299 nm for **4**, $\varepsilon = 23820\ M^{-1}$/cm at 300 nm for **5**.

3.3.3. Flow linear dichroism

Linear dichroism (LD) measurements were performed on a Jasco J500A circular dichroism spectropolarimeter converted for LD and equipped with an IBM PC and a Jasco J interface.

Linear dichroism is defined as:

$$LD_{(\lambda)} = A_{\parallel(\lambda)} - A_{\perp(\lambda)}$$

where $A_{\parallel}$ and $A_{\perp}$ correspond to the absorbances of the sample when polarized light is oriented parallel or perpendicular to the flow direction, respectively. The orientation is produced by a device designed by Wada and Kozawa [10] at a shear gradient of 500–700 rpm.

The reduced linear dichroism is defined as:

$$LD_{(r)} = LD_{(\lambda)}/A_{iso(\lambda)}$$

where $A_{iso(\lambda)}$ is the absorbance of the sample in the absence of flow. This quantity may be related to an orientation factor (S) and the angle between the active transition moment in the chromophore and the DNA helix axis, α [11,12]:

$$LD_r = 3/2\ (3\cos^2\alpha - 1)S$$

Assuming a value of $\alpha = 90°$ for the DNA base-pair chromophore with respect to a local helix axis, it is possible to evaluate α_L for a given ligand:

$$\alpha_L = \arccos[1/3\text{-}(LD_r)_L/3(LD_r)_{DNA}]^{1/2}$$

where $(LD_r)_L$ is the reduced linear dichroism for the ligand, $(LD_r)_{DNA}$ is the reduced LD for DNA and α_L defines the ligand–DNA relative orientation. For the intercalated system, $(LD_r)_L \approx (LD_r)_{DNA}$ and $\alpha_L \cong 90°$.

A solution of the salmon testes DNA (1.6×10^{-3}M) in the ETN buffer containing 10 mM NaCl was used. Spectra were recorded at 25°C at different [DNA]/[drug] ratios.

3.3.4. Fluorimetric determinations

Fluorescence spectra were recorded on a Perkin–Elmer LS50B luminescence spectrometer at $\lambda_{ex} = 340$ nm. The measurements were carried out at room temperature in ETN buffer in the presence of 0.01 or 0.5 M NaCl as indicated in Fig. 3.

Titration was performed by addition of 0.5×10^{-6} M test compound to samples containing different concentrations of DNA. The buffer background was subtracted from intensities for binding calculations. The amounts of free and bound ligand were determined as previously indicated [8].

Experimental binding data were plotted according to the method of Scatchard [13] and analysed by using the approach of McGhee and von Hippel [14] to obtain the intrinsic binding constant (K_i).

4. Results and discussion

4.1. Antiproliferative activity

The antiproliferative activity of compounds **1**–**5** has been evaluated by means of an in vitro test carried out on three human tumor cell lines to determine the concentration (μM) that cause the death of 50% of the cells in comparison with the control cultures. The results of the experiments, performed as indicated in Section 3, are reported in Table 2. The well-known drug ellipticine was used as reference compound.

The results obtained indicate that independent of the cell line taken into account, a general behavior in cytotoxic ability can be used as evidence. Indeed, in all

cases the compounds exerting the higher activity are **5** and **1**, whilst the lesser active appears to be compound **4**. In particular, it is to be noted that in HL-60 and A-431 cells the derivatives **1** and **5** exhibited IC_{50} values comparable to that of ellipticine.

Due to the fact that all the new derivatives possess the same chromophore moiety, the differences in behavior above underlined have to be attributed to the different aminoalkyl side chains (Fig. 1). From the comparison among the antiproliferative activities of compounds **1**–**4** appears that both the presence of three methylene units linked to the nitrogen belonging to the benzimidazole nucleus of the planar moiety (derivatives **2** and **4**) and the presence of ethyl substituents in the basic nitrogen of the side chain (derivatives **3** and **4**) concur to decrease the cytotoxic effect. This indicates that the distance of the basic nitrogen from the planar chromophore and the steric hindrance around the same atom play a crucial role in the interactions between the compounds and the biological target(s) accountable for the antiproliferative effect. Indeed, the optimal chemical structure appears to be that of **1**, whose side chain shows the minimal length beside the minimal steric hindrance around the basic nitrogen. Interestingly, this behavior is in agreement with a previous work where the antiproliferative activity of purinoquinazoline derivatives carrying the aminoalkyl side chains of **1**–**4** had also been investigated [8]. Altogether these results underline the capacity of the side chains to modulate the cellular consequences of a planar aromatic nucleus.

The rationale which drove to the synthesis of compound **5** was based on the properties of the well-known drug mitoxantrone. In particular, a hydroxyethylaminoethyl group has been inserted in the position 6 of the planar chromophore. The results obtained confirm the important role played by this type of side chain, indeed the antiproliferative effect exerted by **5** is significantly high and comparable to that of compound **1** in all the cell lines taken into account.

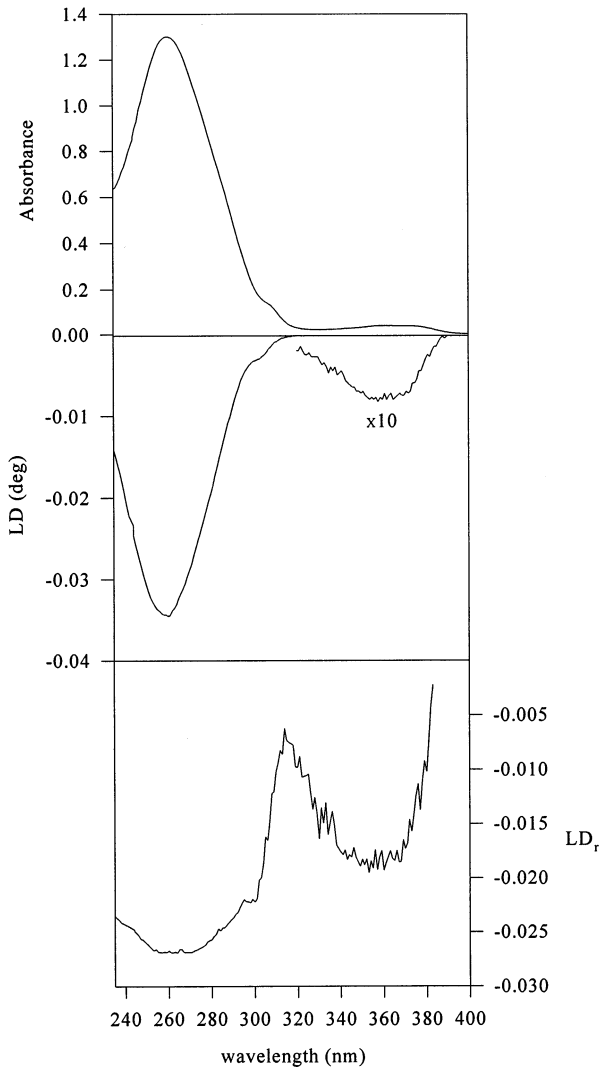


Fig. 2. Absorbance (upper panel), linear dichroism (LD, middle panel) and reduced linear dichroism (LD_r, lower panel) spectra of compound **5** in the presence of DNA (1.6×10^{-3} M) at [DNA]/[drug] = 12.5.

4.2. Flow linear dichroism measurements

Biochemical evidence suggests that deoxyribonucleic acid is among the principal cell targets for many antitumor agents and that the biological activities of these drugs appear to be related to their specific affinities and to their particular mode of binding [15].

To investigate the interaction of compounds **1**–**5** with DNA, flow linear dichroism (LD) spectra were performed as reported in Section 3. In Fig. 2 the flow LD of **5** for a [DNA]/[drug] = 12.5 was reported as representative spectrum (middle panel). The upper panel and the lower panel depicted the light absorption and the reduced linear dichroism (LD_r) spectra, respectively, evaluated for the same sample.

The LD spectra of DNA solutions containing the test compounds display a strong negative signal at 260 nm, as expected for this macromolecule. Furthermore, a significant negative signal appears in the ligand absorption region (320–380 nm). Since these small molecules cannot themselves become oriented in the flow field, the appearance of a dichroic signal in this region is attributable to the induced orientation of the ligand chromophore upon interaction with the macromolecule, so indicating the formation of a molecular complex with DNA. Moreover, the negative sign of the spectrum in this region can indicate an orientation of the molecular plane of the ligand chromophore preferentially parallel to the plane of the DNA bases [8].

More specific information about the geometry of the complexation between **1**–**5** and DNA can be obtained by (LD_r) estimation and the subsequent calculations of the values of the average orientation angle α_L by means

Table 3
Calculated values of the average orientation angle α_L for the test compounds **1–5** ([DNA] = 1.6×10^{-3} M, [DNA]/[drug] = 12.5.)

Compounds	α_L (°)
1	90
2	80
3	82
4	74
5	73

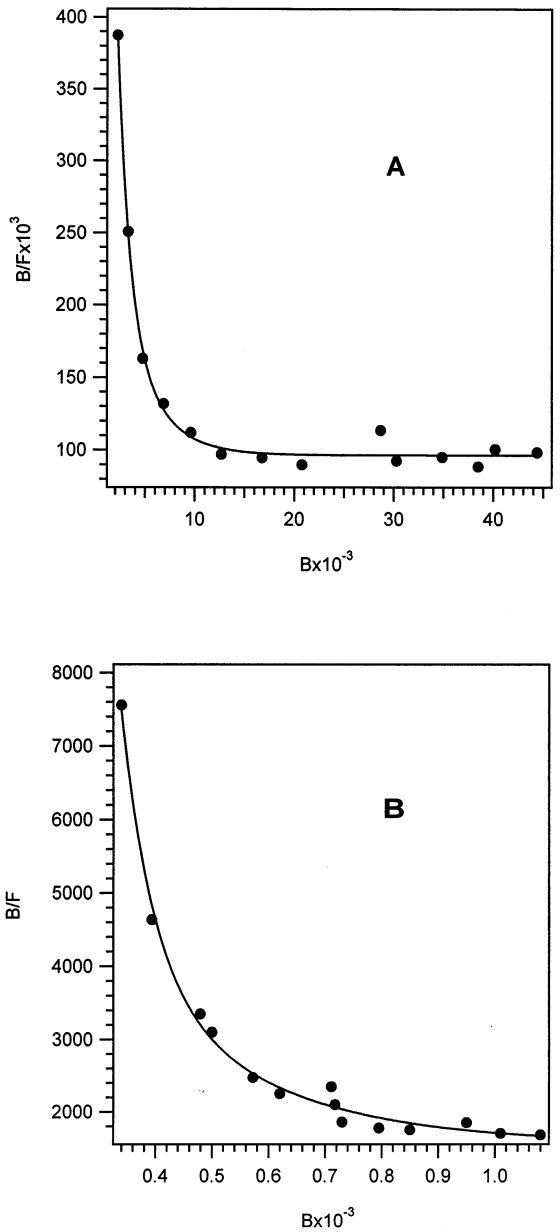


Fig. 3. Scatchard plots for the interaction of compound **1** with DNA in the presence of 0.01 M (A) and 0.5 M (B) ionic strength. *B* is the ratio between bound drug and moles of DNA base pairs and *F* is the free drug concentration.

of the equations reported in Section 3. In Table 3 the calculated values of α_L for the test compounds are reported.

The α_L values calculated for compounds **1**, **2** and **3** are 90, 80 and 82°, respectively. Large values of α_L (80–90°) are retained consistent with an intercalative mode of binding [16], and this means that in the complex between the above indicated compounds and DNA the planar aromatic moiety belonging to the drugs assumes a perpendicular geometry with respect to the helix axis of the macromolecule.

With regard to compounds **4** and **5**, the calculated α_L values, i.e. 73 and 74°, respectively (see Table 3), are lower in comparison with previous ones. An α_L value smaller than 80–90° obtained for the ligand absorption band indicates that the corresponding ligand transition deviates from co-planarity with the DNA base plane. For example, if the drug was bound in the minor groove an α_L value around 45° would be expected [17]. According to previous results achieved for the interaction with DNA of analogues of ellipticine having heterocyclic ring system with three rings and a dimethylaminoethyl side chain [18], the α_L values observed for **4** and **5** can be retained indicative for the occurrence of more than one type of binding mode. In particular, the resulting α_L values suggest a binding mode where both intercalation (for which $\alpha_L = 90°$ is expected) and groove binding (for which α_L should be 45°) take place.

By analysing the chemical structures of **1–5** with the calculated α_L values, it is conceivable to ascribe to the different side chains a crucial role in determining the geometry of complexation with the double helix of DNA. In detail, comparing the behavior shown by **4** with that of its congeners **1–3**, the increase of the lengthening beside the presence of steric hindrance in the terminal part of the side chain seem to promote the capacity to give rise to external binding which, at least in part, competes with the intercalation ability of the planar moiety. In the case of **5** which is characterized by having a hydroxyethylaminoethylamino side chain, it is reasonable to state that external interactions occur due to its particular side chain which is probably arranged in an elongated manner with respect to the planar moiety so that interaction (e.g. hydrogen bonds) between the terminal hydroxyl group and DNA base atoms located externally to the double helix can take place.

4.3. Binding parameters

The new derivatives **1–5** show a significant fluorescence signal which is dramatically quenched upon addition of the macromolecule. These properties allow us to determine the thermodynamic parameters for the DNA binding process by using fluorimetric titration and the

methodological approach reported in Section 3. The binding parameters obtained for compound **1** and represented as Scatchard plots, are reported in Fig. 3 as an example. The ionic strengths of 0.01 M (Fig. 3A) and 0.5 M (Fig. 3B) have been taken into account.

The intrinsic binding constant (K_i) obtained in the above mentioned conditions for compounds **1–5** are reported in Table 4. The higher values obtained are those corresponding to **1** and **5**, while lower and comparable values have been found for **2–4**. Taking into account that **1** and **5** are the most active compounds in inducing cytotoxicity (Table 2), it appears that for these derivatives a high affinity in the formation of a molecular complex with the macromolecule could be fundamental to promote biological events which lead to antiproliferative effects.

Furthermore, it is interesting to observe that for all compounds the increase in ionic strength induces a clear decrease in the binding affinity, so confirming the involvement of charged groups in the complex formation with DNA.

5. Conclusions

The new benzimidazoquinazoline derivatives **1–5** show significant antiproliferative activity toward human tumor cell lines and in particular **1** and **5** exert an effect comparable to that of the well-known drug ellipticine in HL-60 and A-431 cells.

Linear flow dichroism studies reveal that these structures are able to form a complex with DNA mainly by intercalation. From the calculation of α_L it can be hypothesized that for compound **4** and **5** beside the intercalation a concurrent external mode of binding can take place. It seems reasonable that the presence in the side chain of the basic amino nitrogen at a suitable distance from the planar moiety (compound **4**) or the presence of a terminal hydroxy group (compound **5**) results in the possibility to give rise to an external binding ascribable to electrostatic interactions or hydrogen-bond formation.

Comparing complexation geometry with the antiproliferative activity, it is interesting to note that a relationship seems to exist (see Tables 2 and 3). Actually, the cytotoxicity exhibited by **1–4** appears to be correlated with the capacity to form an intercalative complex being lower for the compound **4** for which another mode of binding occurs, at least in part. Interestingly, unlike the behavior shown by **4**, for **5** the ability to form an intercalative complex with the macromolecule does not correlate with the cytotoxic capacity. Indeed, cytotoxicity appears to be comparable to that observed for **1**, which behaves as an intercalator, and is clearly higher than that of **4**, which has a similar value of α_L. For compound **5** it is thus reasonable to assume that the external interactions due to the hydroxyethylaminoethylamino side chain play an important role in modulating cytotoxic activity promoting the formation of a complex with DNA able to induce marked antiproliferative effects.

Moreover, higher values of intrinsic binding constants toward the macromolecule accompany higher cytotoxic activity confirming that the ability to interact with the macromolecule is fundamental to exert the cellular effect.

These results allow us to confirm the importance of the intercalation in the cytotoxic ability, but also to affirm the crucial role carried out by the presence of suitable side chains and in this framework these structures can constitute an useful tool for the development of rationales devoted to the synthesis of new drugs endowed with better specificity of action towards DNA.

Table 4
Intrinsic binding constants (K_i) of complexes between test compounds **1–5** and DNA

Comp.	$K_i \times 10^{-5}$ (M^{-1}) (ionic strength 0.01 M)	$K_i \times 10^{-5}$ (M^{-1}) (ionic strength 0.5 M)
1	2.3 ± 0.3	0.085 ± 0.013
2	1.09 ± 0.14	0.20 ± 0.03
3	1.11 ± 0.09	0.14 ± 0.02
4	1.14 ± 0.12	0.18 ± 0.02
5	2.5 ± 0.3	0.079 ± 0.012

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