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Benzoquinazoline derivatives as new agents affecting DNA processing

Giovanni Marzaro [a], Lisa Dalla Via [a], Antonio Toninello [b], Adriano Guiotto [a], Adriana Chilin [a,*]

[a] Department of Pharmaceutical Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy
[b] Department of Biological Chemistry, University of Padova, Via G. Colombo 3, 35121 Padova, Italy

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ABSTRACT

A new series of benzo[*h*]quinazoline and benzo[*f*]quinazoline derivatives was prepared and studied for the biological activity. The compounds carrying a dimethylaminoethyl side chain (**6c**, **8c** and **12**) inhibit cell growth. The ability to form a molecular complex with DNA and to interfere with topoII and topoI relaxation activity was evidenced for the most active **6c** and **8c**, along with the capacity to induce apoptosis on HeLa cells.

1. Introduction

Cancer is still one of the leading cause of death worldwide and any effort to find new and efficient antitumoral agents is helpful and required.

Among the possible approaches to achieve antitumor activity, compounds interacting with DNA play a major role.[1] Many of these drugs exert their antitumor activity by binding to DNA or to enzymes critical for DNA regular functions, thus triggering a cellular response, which eventually leads to cell death.

The class of topoisomerase inhibitors is widely investigated because topoisomerases (both topoI and topoII) represent an important target for successful cancer therapy,[2] and these enzymes are often overexpressed in many tumors.[3]

The antitopo agents show a great variability in their chemical structures. Many potent inhibitors share an acridine skeleton: among them, a lead compound is *m*-amsacrine (**1**) (*m*-AMSA, Fig. 1), a 9-aminoacridine derivative, whose strong antiproliferative activity is due to its interaction with topoII.[4]

Despite its good antitumor activity, especially against leukemia, *m*-AMSA presented problems of drug resistance.[5] A valid alternative to single enzyme inhibitors was found with dual topoI/topoII inhibitors, which target two enzymes working at different points of cell cycle, thus circumventing resistance phenomena. XR5000 (**2**) (DACA, Fig. 1), an acridine-4-carboxamide derivative structurally related to *m*-AMSA, was the first dual inhibitor to enter clinical trial,[6] and it proves to be able to overcome drug resistance.[7] Other structurally different classes of compounds also demonstrated effective dual antitopo activity, delaying the onset of resistance, such as phenazine-1-carboxamide derivatives[8] and 8,9-benzo[*a*]phenazine-11-carboxamide derivatives,[9] among which XR 11576 (**3**, Fig. 1) has entered clinical trials.[10] Finally, similar profile was found for TAS-103 (**4**, Fig. 1), initially identified as dual inhibitor,[11] although it predominantly acts as a topoIIα inhibitor.[12]

All these compounds share some common features, such as a nitrogen heterocyclic ring system and an amino substituent like the methanesulfonamidoaniline or a dimethylaminoethyl group (typical of dual inhibitors). By assembling different but similar nitrogen scaffolds to obtain structurally novel dual topo inhibitors, we designed a new series of benzo[*h*]quinazoline (A) and benzo[*f*]qui-

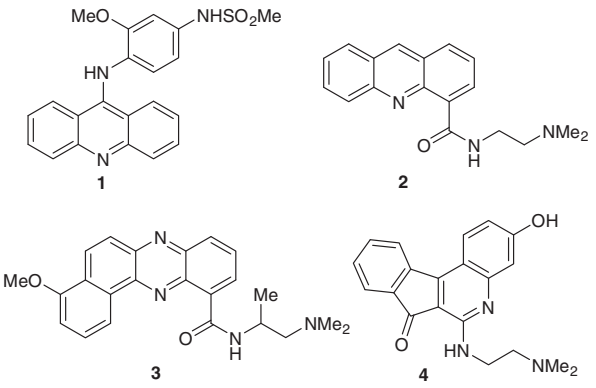

Figure 1. Structures of *m*-AMSA (**1**), DACA (**2**), XR11576 (**3**) and TAS-103 (**4**).

* Corresponding author. Tel.: +39 0498275349; fax: +39 0498275366.
E-mail address: adriana.chilin@unipd.it (A. Chilin).

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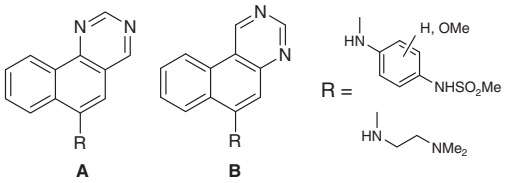


Figure 2. Structures of benzo[*h*]quinazoline (**A**) and benzo[*f*]quinazoline (**B**).

nazoline (B) derivatives bearing the AMSA substituent or the dimethylaminoethyl substituent (Fig. 2).

First of all, molecular modeling studies were performed to evaluate the effect of acridine nucleus replacement with the two isomeric benzoquinazoline scaffolds. Then the synthesized compounds were tested for their antiproliferative effect on human tumor cell lines. Linear flow dichroism experiments (LD) were performed to establish the ability to form a complex with DNA and the ability to interfere with topoI and topoII activities was investigated.

2. Results and discussion

2.1. Molecular modeling studies

Molecular modeling studies were performed on *m*-AMSA and on all the designed compounds, to investigate the binding mode of *m*-AMSA with the DNA and to explore the opportunities to replace acridine nucleus with the two isomeric benzoquinazoline scaffolds. Since no structure of *m*-AMSA intercalated in the DNA was available in the PDB, the nucleic acid template was extracted from a RX complex of another acridine derivative intercalated in a double strand DNA (PDB code: 1G3X). Lamarkian Genetic Algorithm was used to generate 10 independent searches and to assess the binding energy for all the compounds.[13] Intercalation energies are reported in Table 1. In any case the intercalation into the DNA was calculated to be energetically favorable.

Several features of binding mode of *m*-AMSA in the DNA were extracted from the docking studies.

Analysis of the intercalated structure revealed that the nitrogen of the acridine moiety was placed in the middle of the DNA axis, so that it was equidistant (about 4 Å) from all the nitrogen atoms of the bases (Fig. 3).

Moreover, superimposition of the intercalated structure and the isolated *m*-AMSA (Fig. 4), revealed that a large variation on dihedral angle between acridine nucleus and aniline side chain was required to allow interaction with DNA, according to other experimental observations.[14]

On the contrary, for the benzoquinazoline derivatives bearing the same aniline side chain of *m*-AMSA, a lower variation of the dihedral angle between intercalated and isolated structures was observed (data not shown). Thus, in the case of benzoquinazoline derivatives, a lower penalty for dihedral free energy variation was observed with respect to *m*-AMSA.

The good spatial correlation among docked benzoquinazoline structures and *m*-AMSA (see an example in Fig. 5) and the favorable calculated interaction energies suggested that the replacement of acridine with benzoquinazoline could lead to intercalative compounds.

Table 1
Intercalation energy from docking studies

Compound	Average interaction energy (kcal/mol)
m-AMSA	−8.28
6a	−8.14
6b	−8.24
6c	−7.47
8a	−8.17
8b	−8.15
8c	−7.47

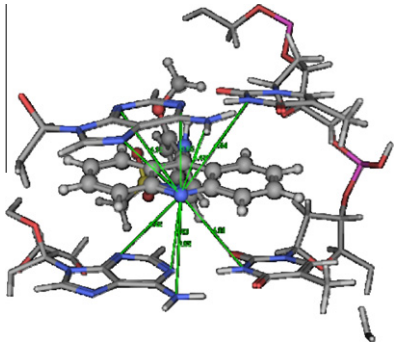


Figure 3. Distance of the acridine nitrogen from the nitrogen atoms of the DNA bases.

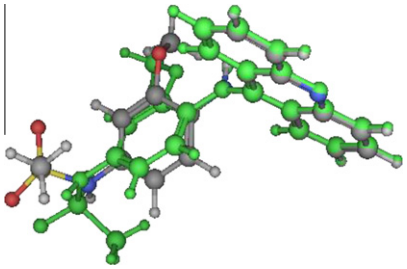


Figure 4. Superimposition of minimized structure for isolated (green) and intercalated *m*-AMSA (colored by atoms).

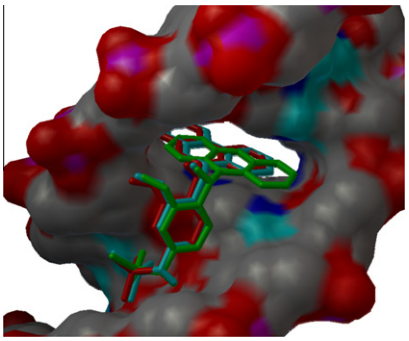
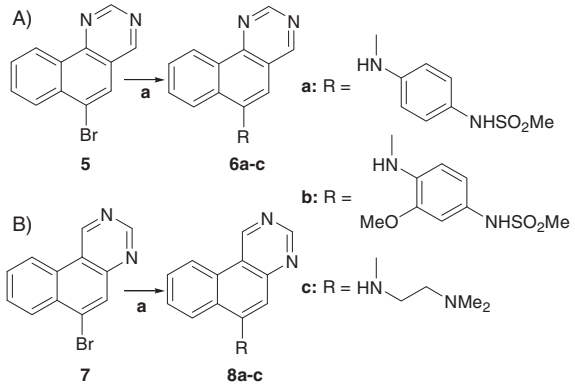


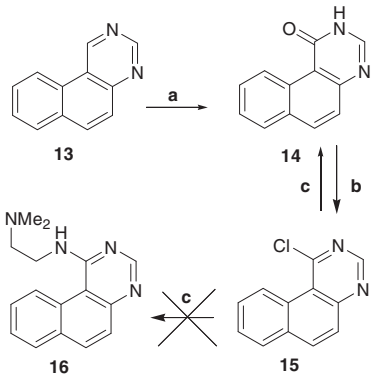
Figure 5. Superimposition of *m*-AMSA (green colored), **6b** and **7b** analogs (red and cyan colored, respectively) into the intercalation site of DNA.

2.2. Chemistry

The starting 6-bromobenzo[*h*]quinazoline (**5**) and 6-bromobenzo[*f*]quinazoline (**7**) were synthesized from the corresponding 4-bromonaphthyl-1-amine and 4-bromonaphthyl-2-amine, as previously described.[15] The bromobenzoquinazolines **5** and **7** were coupled with the 4′-aminomethane-sulfonanilides and with *N*,*N*-dimethylethylendiamine under modified Buchwald–Hartwig conditions using microwave (MW) irradiation to provide compounds **6a–c** and **8a–c** (Scheme 1). The reaction was carried out with palladium acetate, BINAP and cesium carbonate in water instead of classical toluene, which is not only a toxic compound but also a bad solvent for MAOS (microwave assisted organic synthesis) because of its unfavorable dipole moment.



Scheme 1. Reagents and conditions: (a) 4′-aminomethanesulfonanilides or *N*,*N*-dimethylethylenediamine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, H_2O, 150 °C, MW, 20 min.



Scheme 3. Reagents and conditions: (a) CAN, $AcOH/H_2O$, rt, 5 min; (d) CCl_4, Ph_3P, CH_2Cl_2, reflux, 3 h; (c) *N*,*N*-dimethylethylenediamine, *i*PrOH, 80 °C, MW, 15 min.

Besides the 6-substituted derivatives, we synthesized compounds with the side chain in the pirimidine ring of the tricyclic nucleus, in order to verify the importance of other substitution position, considering that DACA, XR11576 and TAS-103 bear the aminoalkyl substituent close to the heterocyclic nitrogen. In this case, the functionalization was carried out only with the dimethylaminoethylamino chain.

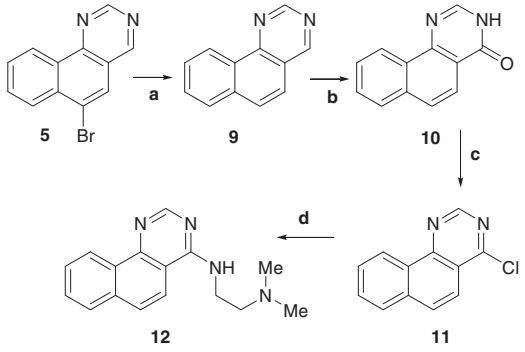
The benzo[*h*]quinazoline (**9**), achievable only by debromination of **5** with sodium ethoxide in abs EtOH under MW irradiation,[15] was selectively oxidized with cerium ammonium nitrate (CAN) in aqueous acetic acid at the 4 position, then submitted to chlorination with $POCl_3$ and Et_3N and finally coupled with *N*,*N*-dimethylethylendiamine in *i*PrOH under MW irradiation, obtaining compound **12** (Scheme 2).

An analogous synthetic route was followed for the benzo[*f*]quinazoline derivative **16**. The starting benzo[*f*]quinazoline (**13**), obtained from naphthyl-2-amine,[15] was selectively oxidized with cerium ammonium nitrate (CAN) in aqueous acetic acid at the 1 position, then submitted to chlorination with CCl_4 and triphenilphosphine in dichloroethane, obtaining the chloro derivative **15** (Scheme 3).

Owing to the known instability of 4-chloro derivatives,[16] compound **15** was immediately submitted to substitution step, but any attempt to carry out the reaction gave only the starting quinazolin-1-one (**14**), due to the ease of hydrolysis of chlorine group which was competitive with the substitution reaction.

2.3. Antiproliferative activity

The ability of the new derivatives to inhibit cell growth was evaluated on HeLa (cervix adenocarcinoma), HL-60 (promyelocytic leukemia) and A431 (human squamous carcinoma) cell lines and expressed as IC_{50} values (Table 2). Ellipticine and *m*-AMSA were used as reference compounds.



Scheme 2. Reagents and conditions: (a) EtONa, abs EtOH, 110 °C, MW, 15 min; (b) CAN, $AcOH/H_2O$, rt, 5 min; (c) $POCl_3$, Et_3N, reflux, 3 h; (d) *N*,*N*-dimethylethylenediamine, *i*PrOH, 80 °C, MW, 15 min.

Table 2
Cell growth inhibition in the presence of the test compounds

Compound	IC_{50}[a] (μM)		
	HeLa	HL-60	A431
6a	>100	26.5 ± 2.9	57.7 ± 4.1
6b	>100	26.9 ± 3.3	>100
6c	15.7 ± 1.7	10.8 ± 1.3	3.6 ± 0.8
8a	>100	>100	>100
8b	>100	30.7 ± 4.5	77.7 ± 4.5
8c	23.2 ± 1.8	4.3 ± 0.4	4.3 ± 0.2
12	>100	61.6 ± 4.9	50.5 ± 2.3
Ellipticine	0.31 ± 0.01	0.64 ± 0.02	0.47±0.03
m-AMSA	0.012 ± 0.002	0.0060 ± 0.0011	0.17 ± 0.02

[a] The results have been expressed as IC_{50} values, that is, the concentration of compound able to induce a 50% reduction in viable cell number relative to control.

The ability of the AMSA-like derivatives **6a–b** and **8a–b** to inhibit cell growth was very weak, nevertheless it must be pointed out that these compounds showed a rather low solubility in aqueous media.

The derivatization of the benzoquinazoline scaffold with a dimethylaminoethylamino chain allowed a significant increase in solubility, and an enhancement in cytotoxicity, but with different extent, depending upon the substitution position. Compounds **6c** and **8c**, bearing the side chain in the central benzene ring of the benzoquinazoline chromophore, showed appreciable antiproliferative activity with IC_{50} values in the micromolar range for all the tested cell lines. On the contrary, compound **12**, bearing the side chain in the pyrimidine ring of the benzoquinazoline nucleus showed a weak cytotoxicity and only in HL-60 and A431 cell lines.

Thus, the introduction of a dimethylaminoethylamino side chain in the benzoquinazoline chromophore gives rise to an antiproliferative effect, which results significantly increased when the side chain is inserted in the central benzene ring (compounds **6c** and **8c**). This could be explained by the better binding mode shown in the modeling studies for both the derivatives with respect to compound **12** (Average Interaction Energy = −7.15 kcal/mol) (Fig. 6).

As previously described for *m*-AMSA, an efficient interaction between ligands and DNA required that the nitrogen atoms of the central aromatic nucleus must be placed in the middle of DNA axis. As a consequence, the scaffold placement drives also the side chain positioning: compound **6c** (Fig. 6A) could place the side chain into the minor groove, thus favouring the intercalation, while compound **12** did not (Fig. 6B).

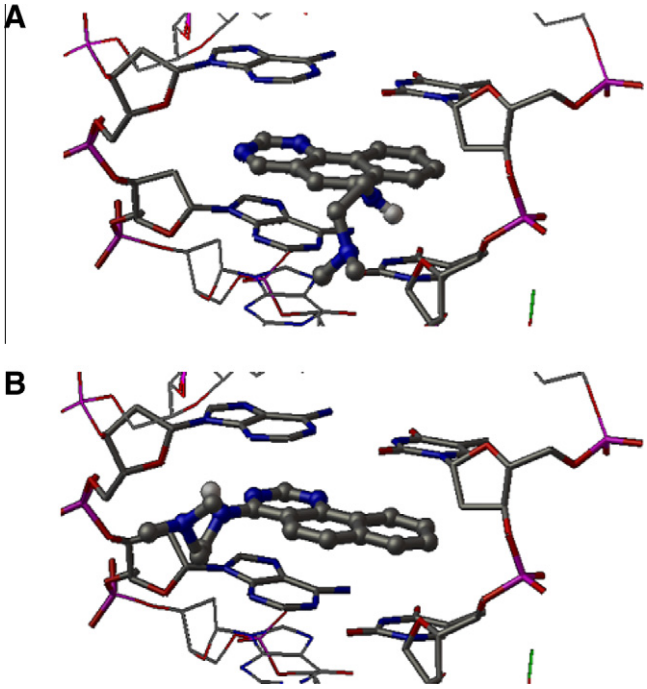


Figure 6. Binding poses predicted for **6c** (A) and **12** (B) into DNA.

2.4. Interaction with DNA

To investigate a possible mechanism of action involved in the cytotoxicity of the most active compounds, linear flow dichroism (LD) experiments were performed in the presence of salmon testes DNA at different [drug]/[DNA] ratios. Indeed, the planarity of the benzoquinazoline chromophore suggests for the new derivatives the ability to form an intercalative complex with DNA.

Figure 7 shows the spectra obtained in the presence of **6c** and **8c** at the same [drug]/[DNA] ratio (Fig. 7A) and the spectra of **12** at different [drug]/[DNA] ratios (Fig. 7B).

The DNA spectrum (traces a) show at 260 nm the typical negative dichroic signal, while in the presence of new benzoquinazolines (traces b and c) a further negative signal at higher wavelengths (300–450 nm) occurs. The occurrence of this latter signal in the presence of test compound (spectra b and c) indicates that the planar benzoquinazoline nucleus forms a molecular complex with DNA and its negative sign means an intercalative geometry of binding.

These results indicate for the tricyclic benzoquinazoline chromophore the ability to insert between base pairs. Nevertheless, this capacity is shared by all the compounds, both the most active **6c** and **8c** and also by **12**. As a consequence, the capacity to form a molecular complex with DNA cannot explain the significant difference in cytotoxicity exerted by **12** with respect to the others two derivatives (Table 2).

2.5. Effect on topos activity

The ability of the new benzoquinazoline derivatives to form an intercalative complex with DNA suggested that their antiproliferative effect could be related to the interference with nuclear enzymes involved in DNA processing, like topos.[17] Figures 8 and 9 show the effect of **6c**, **8c** and **12** on the relaxation of supercoiled plasmid pBR322 DNA catalyzed by topoII.

In detail, both **6c** and **8c** inhibit the relaxation ability of the enzyme in a concentration-dependent manner (lanes c–f and lanes g–j, Fig. 8), and the inhibition becomes evident at 50 μM concentra-

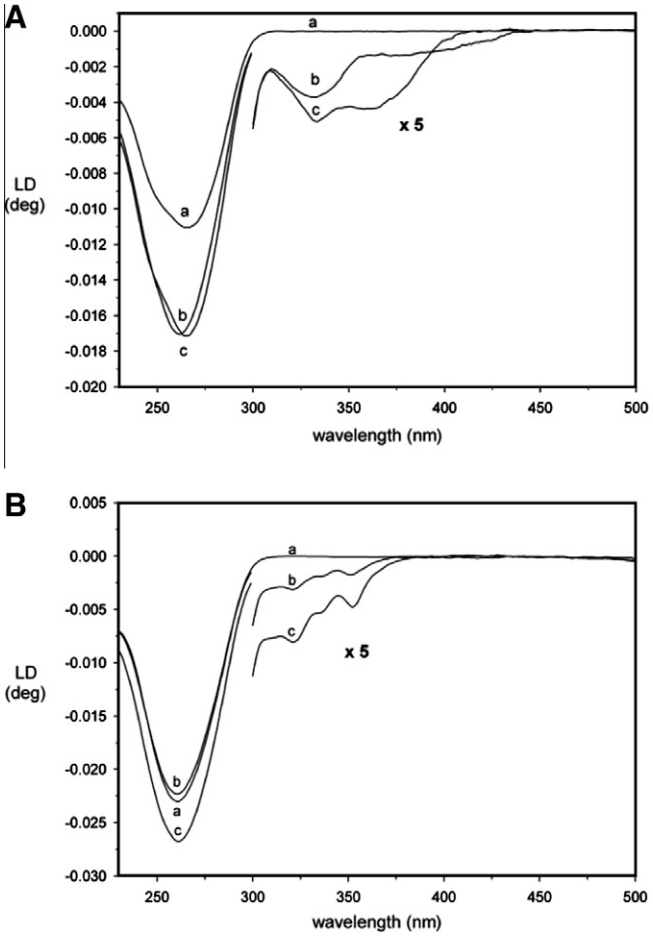


Figure 7. Linear flow dichroism spectra: (A) DNA alone (a) and in the presence of **6c** (b) and **8c** (c) at [drug]/[DNA] = 0.08; (B) DNA alone (a) and in the presence of **12** at [drug]/[DNA] = 0.08 (b) and 0.16 (c).

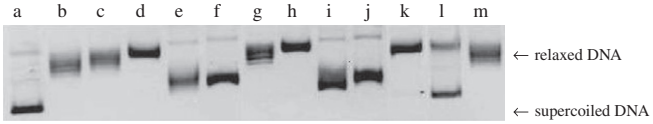


Figure 8. Effect of derivatives **6c**, **8c** and **12** on the relaxation of supercoiled plasmid DNA by human recombinant topoII. Lane a: 0.25 μg pBR322 DNA; lane b: as lane a in the presence of 1 U topoII; lanes c–f: as lane b in the presence of 1, 10, 50, 100 μM **6c**, respectively; lanes g–j: as lane b in the presence of 1, 10, 50, 100 μM **8c**, respectively; lane k: as lane b in the presence of 100 μM **12**; lane l: as lane b in the presence of 8 μM *m*-AMSA; lane m: as lane b in the presence of solvent alone.

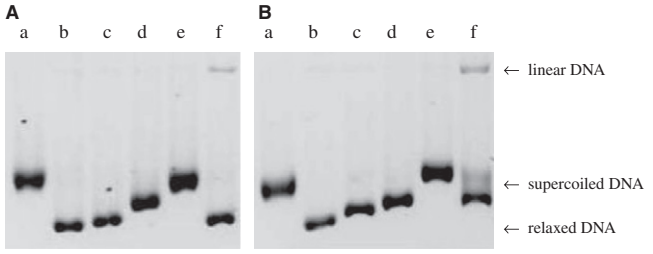


Figure 9. Determination of cleavable complex by human recombinant topoII induced by **6c** (A) and **8c** (B). Lanes a: 0.25 μg pBR322 DNA; lanes b: as lanes a in the presence of 10 U topoII; lanes c–e: as lanes b in the presence of 10, 50, 100 μM test compound, respectively; lanes f: as lanes b in the presence of 8 μM *m*-AMSA.

tions (lanes e and i) as demonstrated by the disappearance of relaxed DNA and the appearance of the supercoiled form. Nevertheless, this latter DNA shows a clear shift in the electrophoretic mobility with respect to the control (lanes a). At higher concentration (100 μM, lanes f and j) the supercoiled DNA and the mobility shift of the band with respect to the control are even more evident and this effect should be attributable to an efficient intercalative capacity of both **6c** and **8c**.

No effect on topoII catalyzed relaxation is exerted by **12**, even at the higher concentration (100 μM, lane k) and this result, in agreement with the data shown in Table 2, suggests a correlation between the topoII inhibitory activity and the cytotoxicity.

Taken together these results suggest that the different position of the aminoalkylamino side chain inside the benzoquinazoline nucleus plays a crucial role for the interaction of the derivatives with DNA that, in the case of **6c** and **8c**, makes the macromolecule less available for the enzymatic processing.

The agents interfering with the catalytic cycle of topoII are divided into two classes: poisons, which stabilize the covalent DNA–topoII complex (also known as the cleavable complex) and catalytic inhibitors, which act on any of the other steps in the catalytic cycle. Many intercalative agents, such as adriamycin, *m*-AMSA and mitoxantrone, are able to stimulate the formation of the cleavable complex, thus acting as poisons.[18–20] The occurrence of the covalent intermediate can be demonstrated experimentally by the enzyme-dependent formation of linear from supercoiled DNA. Figure 9 shows a cleavable complex assay performed in the presence of increasing concentrations of benzoquinazolines **6c** and **8c** and 8 μM *m*-AMSA, taken as reference compound.

As expected, *m*-AMSA, a well-known topoII poison, induces the formation of linear DNA (lanes f). Nevertheless, both benzoquinazoline derivatives are unable to stabilise the formation of double strand breaks (linear DNA) also at higher concentration (100 μM, lanes e). Thus, **6c** and **8c** have to be defined as topoII catalytic inhibitors.

The lack of topoII poisoning effect along with the capacity to form an effective intercalative complex with DNA prompt us to investigate also on the ability to interfere with another DNA-related enzyme such topoI. Figure 10 A and B show the relaxation of supercoiled DNA catalyzed by the enzyme in the absence (lane b) and in the presence of increasing amounts of **6c** and **8c**, respectively (lanes c–g).

The addition of the benzoquinazoline derivatives induces a concentration-dependent inhibition on the relaxation capacity, which appears comparable to that evidenced on topoII (Fig. 8).

2.6. DNA fragmentation

To get further details into the cytotoxic effect of **6c** and **8c**, we investigate on the ability of these derivatives to induce the apoptotic process in HeLa cells. A biochemical hallmark of apoptosis is the cleavage of nuclear DNA following the activation of endonucleases that degrades the high order chromatine into mono and oligonucleosomal DNA fragments. This process gives rise to a 'ladder' of nucleosomal-size multimers.[21] Figure 11 shows the gel electrophoresis of DNA extracted from HeLa cells incubated in the presence of **6c** (lane c), **8c** (lane d) and cisplatin (lane b), taken as reference compound. The results show for both benzoquinazoline derivatives the occurrence of the typical DNA fragmentation, thus suggesting that the cell death provoked by the test compounds occurs through the induction of the apoptotic process.

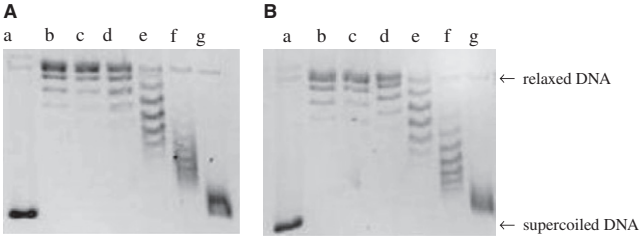


Figure 10. Effect of derivatives **6c** (A) and **8c** (B) on the relaxation of supercoiled plasmid DNA by human recombinant topoI. Lanes a: 0.25 μg pBR322 DNA; lanes b: as lanes a in the presence of 2 U topoI; lanes c–g: as lanes b in the presence of 1, 10, 25, 50, 100 μM test compound, respectively.

Figure 11. Agarose gel electrophoresis of nuclear DNA from HeLa cells. Lane a: untreated control; lane b, 50 μM cisplatin; lane c: 200 μM **6c**; lane d: 200 μM **8c**.

3. Conclusion

The design, synthesis and biological evaluation of a series of benzo[*h*]quinazoline (**6a–c** and **12**) and benzo[*f*]quinazoline (**8a–c**) derivatives are reported. The compounds carrying a dimethylaminoethyl side chain (**6c**, **8c** and **12**) inhibit cell growth: in particular, **6c** and **8c** are more cytotoxic than **12**. Both benzoquinazoline nuclei intercalate between DNA base pairs, and the ability to interfere with topoII and topoI relaxation was evidenced for the most active **6c** and **8c**, carrying the dimethylaminoethyl side chain on the benzene of the quinazoline chromophore.

These results suggest that the mechanism of action responsible for their cytotoxicity lies in the ability to interact with the macromolecule in such a way that the DNA processing enzymes, topoI and topoII, cannot use the macromolecule as substrate for their relaxation activity. This hypothesis is further supported by the fact that both active compounds (**6c** and **8c**) are unable to act as topoII poisons.

The occurrence of nuclear DNA fragmentation after incubation of HeLa cells with **6c** and **8c** suggests that the cytotoxic effect induced by the benzoquinazoline derivatives is the result of the activation of the apoptotic process.

In conclusion, the reported results allow to draw some interesting structure–activity relationships. In particular, the comparison between **6a–c** and **8a–c** evidenced that both the nitrogen nuclei, benzo[*h*]quinazoline or benzo[*f*]quinazoline, induce a similar biological effect. On the contrary, inside each series, the insertion of a protonatable dimethylaminoethylamino side chain seems to be essential for the occurrence of a significant antiproliferative activity. Also the position of this side chain is an important structural requirement, as demonstrated by the different behavior of **6c** and **12**. On the basis of these considerations, the new 6-aminoalkyl substituted benzo[*h*]quinazolines and benzo[*f*]quinazolines can be considered as an interesting model worthy to be further developed and studied with the aim to design more effective drugs.

4. Experimental section

4.1. Computational methodologies

All modeling studies were carried out on a 4 CPU (Intel Core™2 Quad CPU Q9550 2.83 GHz) ACPI ×64 based PC. DNA structure was downloaded from Protein Data Bank (PDB ID: 1G3X) and modified employing Autodock 4.1 software.[22] Minimum energy conformation for each isolated compound was derived using MarvinSketch software[23] and was used as the initial tridimensional structure for the docking. All docking studies were performed with Autodock 4.1 software employing the Lemarkian Genetic Algorithm, generating 10 independent docking pose for each compound. In all the cases, the population size was set to 150 and the maximum number of evaluation was set to 250,000. The dimension and the position of the docking grid were set in a way that the compounds could place their side chains in the minor groove. The DNA was considered as a rigid molecule, while the ligands were considered as flexible molecules. The intercalative energies were calculated as the mean of the docked structures obtained for each compound.

4.2. Chemistry

Melting points were determined on a Gallenkamp MFB-595-010M melting point apparatus and are uncorrected. Analytical TLC was performed on pre-coated 60 F_{254} silica gel plates (0.25 mm; Merck) developing with a $CHCl_3$/MeOH mixture (9:1). Preparative column chromatography was performed using silica gel 60 (0.063–0.100 mm; Merck). The microwave irradiation was performed in a CEM Discover® monomode reactor with the temperature monitored by a built-in infrared sensor. IR spectra were recorded on a Perkin–Elmer 1760 FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Bruker AMX300 spectrometer with TMS as internal standard. Chemical shift values are reported in ppm and coupling constants are reported in Hz. HRMS spectra were obtained using an ESI-TOF Mariner 5220 (Applied Biosystem) mass spectrometer with direct injection of the sample and collecting data in the positive ion mode. Elemental analyses were performed on a Perkin–Elmer 2400 Analyzer and are within ±0.4% of theoretical values. Starting benzoquinazolines **5**, **7** and **13** were prepared according to literature methods.[15]

4.2.1. General procedure for 6-substituted benzo[*h*] quinazolines 6a–c and 6-substituted benzo[*f*]quinazolines 8a–c

A mixture of **5** or **7** (1.0 mmol), *N*-(4-aminophenyl)-methanesulfonamide or *N*-(4-amino-3-methoxyphenyl)methane-sulfonamide[24] or *N*,*N*-dimethylethylenediamine (1.2 mmol), $Pd(OAc)_2$ (0.03 mmol), BINAP (0.04 mmol), Cs_2CO_3 (1.5 mmol) in H_2O (10 mL) was microwave irradiated at 150 °C (power set point 150 W, ramp time 1 min, hold time 20 min). After cooling, the mixture was extracted with EtOAc (3 × 20 mL), and the organic phase was evaporated under reduced pressure. The residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) to give **6** or **8**.

4.2.1.1. *N*-[4-(Benzo[*h*]quinazolin-6′-ylamino)phenyl]methanesulfonamide (6a). Yield 45%; mp: 265 °C; IR (KBr) 3395, 2935, 2865, 1620, 1590, 1510, 1430, 1320, 1220, 1155, 975, 830 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 9.31 (s, 1H, 4′-H), 9.24–9.21 (m, 2H, 2′-He 7′-H or 10′-H), 8.54 (broad s, 1H, *NH*SO_2CH_3), 8.50 (d, *J* = 8.0, 1H, 7′-H or 10′-H), 7.98–7.87 (m, 2H, 8′-H and 9′-H), 7.38 (s, 1H, 5′-H), 7.32–7.23 (m, 4H, 2-H, 3-H, 5-H and 6-H), 2.98 (s, 3H, $NHSO_2CH_3$); ^{13}C NMR (DMSO-d_6): δ = 156.6, 152.5, 145.1, 140.5, 139.3, 132.1, 130.1, 130.0, 129.5, 27.8, 124.4, 124.2, 122.7, 122.4, 121.3, 101.9, 38.8; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{19}H_{17}N_4O_2S$: 365.1067, found 365.0962; Anal. calcd for $C_{19}H_{16}N_4O_2S$: C, 62.62; H, 4.43; N, 15.37; S, 8.80; found C, 62.68; H, 4.41; N, 15.35; S, 8.79.

4.2.1.2. *N*-[4-(Benzo[*h*]quinazolin-6′-ylamino)-3-methoxyphenyl]methanesulfonamide (6b). Yield 62%; mp: 260 °C; IR (KBr) 3360, 2935, 2865, 1655, 1575, 1520, 1435, 1330, 1200, 1150, 980, 770 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 9.22–9.17 (m, 3H, 2′-H, 4′-H and 7′-H or 10′-H), 8.53 (d, *J* = 8.0, 7′-H or 10′-H), 7.97–7.85 (m, 3H, 8′-H, 9′-H and *NH*SO_2CH_3), 7.25 (d, *J* = 8.5, 1H, 5-H), 7.03 (d, *J* = 2.1, 1H, 2-H), 6.89 (dd, *J* = 8.5, *J* = 2.1, 1H, 6-H), 6.74 (s, 1H, 5′-H), 3.29 (s, 3H, O*CH*$_3$), 3.05 (s, 3H, $NHSO_2CH_3$); ^{13}C NMR (DMSO-d_6) δ = 156.2, 153.4, 152.0, 144.6, 141.6, 135.5, 129.9, 128.8, 127.6, 126.4, 125.6, 124.5, 124.4, 122.6, 112.5, 104.9, 100.0, 55.4, 39.0; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{20}H_{19}N_4O_3S$: 395.1172, found 395.1331; Anal. calcd for $C_{20}H_{18}N_4O_3S$: C, 60.90; H, 4.60; N, 14.20; S, 8.13; found C, 60.88; H, 4.62; N, 14.24; S, 8.12.

4.2.1.3. 6-[*N*-(Dimethylamino)ethyl]aminobenzo[*h*]quinazoline (6c). Yield 49%; mp: >300 °C; IR (KBr) 3425, 2940, 2865, 1650, 1575, 1510, 1460, 1200, 1055, 830, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 9.20 (s, 1H, 4-H), 9.13 (s, 1H, 2-H), 9.29 (d, *J* = 7.9, 1H, 7-H or 10-H), 8.00 (d, *J* = 7.9, 1H, 7-H or 10-H), 7.81 (m, 2H, 8-H and 9-H), 6.57 (s, 1H, 5-H), 5.61 (s, 1H, NH), 3.39 (t, *J* = 6.6, 2H, $NHCH_2CH_2N(CH_3)_2$), 2.77 (t, *J* = 6.6, 2H, $NHCH_2CH_2N(CH_3)_2$), 2.33 (s, 6H, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ = 155.5, 151.8, 145.5, 143.3, 130.4, 129.9, 128.4, 127.5, 125.4, 125.4, 120.5, 95.4, 57.2, 45.0, 40.7; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{16}H_{19}N_4$: 267.1610, found 267.1593; Anal. calcd for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04; found C, 72.16; H, 6.75; N, 21.10.

4.2.1.4. *N*-[4-(Benzo[*f*]quinazolin-6′-ylamino)phenyl]methanesulfonamide (8a). Yield 28%; mp: 295 °C; IR (KBr) 3350, 2930, 2865, 1650, 1580, 1510, 1460, 1325, 1225, 1150, 1055, 980, 830, 770 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 9.86 (s, 1H, 1′-H), 8.95 (s, 1H, 3′-H), 8.93 (d, *J* = 7.8, 1H, 7′-H or 10′-H), 8.59 (d, *J* = 7.8, 1H, 7′-H or 10′-H), 7.85–7.76 (m, 2H, 8′-H and 9′-H), 7.03–6.91 (m, 4H, 2-H, 3-H, 5-H and 6-H), 6.74 (s, 1H, 5′-H), 2.59 (s, 3H, $NHSO_2CH_3$); ^{13}C NMR (DMSO-d_6): δ = 156.1, 152.6, 152.5, 147.6, 137.0, 134.4, 129.4, 128.7, 127.8, 124.9, 124.3, 123.1, 122.7, 121.6, 116.9, 101.5, 39.0; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{19}H_{17}N_4O_2S$: 365.1067, found 365.1205; Anal. calcd for $C_{19}H_{16}N_4O_2S$: C, 62.62; H, 4.43; N, 15.37; S, 8.80; found C, 62.62; H, 4.46; N, 15.39; S, 8.85.

4.2.1.5. *N*-[4-(Benzo[*f*]quinazolin-6′-ylamino)-3-methoxyphenyl]methanesulfonamide (8b). Yield 12%; mp: 266 °C; IR (KBr) 3440, 2935, 2865, 1655, 1580, 1515, 1460, 1330, 1150, 975, 830 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 9.93 (s, 1H, 1′-H), 8.99–8.95 (m, 2H, 3′-H e 7′-H or 10′-H), 8.60 (d, *J* = 8.4, 1H, 7′-H or 10′-H), 8.53 (s all, 1H, *NH*SO_2CH_3), 7.88–7.79 (m, 2H, 8′-H and 9′-H), 7.30 (d, *J* = 8.4, 1H, 5-H), 7.04 (d, *J* = 1.9, 1H, 2.H), 6.93 (dd, *J* = 8.4, *J* = 1.9, 1H, 6-H), 6.41 (s, 1H, 5′-H), 3.73 (s, 3H, O*CH*$_3$), 3.06 (s, 3H, $NHSO_2CH_3$); ^{13}C NMR (DMSO-d_6): δ = 156.1, 154.8, 152.7, 152.3, 148.5, 137.3, 129.2, 128.5, 128.4, 127.7, 124.4, 124.1, 123.0, 122.7, 116.4, 111.8, 104.3, 100.5, 55.4; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{20}H_{19}N_4O_3S$: 395.1172, found 395.1261; Anal. calcd for $C_{20}H_{18}N_4O_3S$: C, 60.90; H, 4.60; N, 14.20; S, 8.13; found C, 60.91; H, 4.65; N, 14.18; S, 8.10.

4.2.1.6. 6-[*N*-(Dimethylamino)ethyl]aminobenzo[*f*]quinazoline (8c). Yield 50%; mp: >300 °C; IR (KBr) 3425, 2940, 2865, 1650, 1585, 1545, 1460, 1360, 1250, 1125, 1055, 995, 830, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 10.01 (s, 1H, 1-H), 9.21 (s, 1H, 3-H), 9.49 (s, 1H, NH), 8.96 (d, *J* = 7.5, 1H, 10-H), 8.54 (d, *J* = 7.5, 1H, 7-H), 7.98 (m, 2H, 9-H and 8-H), 7.05 (s, 1H, 5-H), 3.94 (m, 2H, $NHCH_2CH_2N(CH_3)_2$), 3.50 (t, *J* = 6.6, 2H, $NHCH_2CH_2N(CH_3)_2$), 2.91 (s, 6H, $N(CH_3)_2$), 2.35 (s, 9H, 3·CH_3SO_3H); ^{13}C NMR ($CDCl_3$): δ = 156.5, 154.0, 151.7, 149.1, 128.5, 128.4, 128.1, 127.8, 122.5, 122.3, 121.2, 97.5, 56.9, 44.9, 40.2; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{16}H_{19}N_4$: 267.1610, found 267.1599; Anal. calcd for

$C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04; found C, 72.18; H, 6.85; N, 21.06.

4.2.2. Benzo[*h*]quinazoline (9)

A mixture of **5** (1.3 g, 5.0 mmol) and ethanolic EtONa (21% wt, 20 mL) was microwave irradiated at 110 °C (power set point 100 W, ramp time 30 s, hold time 15 min). After cooling, the mixture was diluted with sat. NH_4Cl (200 mL), extracted with $CHCl_3$ (3 × 80 mL), and the organic phase was evaporated under reduced pressure to give **9** (0.88 g, yield 98%); mp: 120 °C; ^{1}H NMR (DMSO-d_6): δ = 9.42 (s, 1H, 4-H), 9.28 (s, 1H, 2-H), 9.21–9.18 (m, 1H, 7-H or 10-H), 7.90–7.87 (m, 1H, 7-H or 10-H), 7.82 (d, *J* = 8.9, 1H, 5-H or 6-H), 7.77–7.73 (m, 2H, 8-H and 9-H), 7.64 (d, *J* = 8.9, 1H, 5-H or 6-H).

4.2.3. Benzo[*h*]quinazolin-4(3*H*)-one (10)

A solution of CAN (4.4 g, 8.0 mmoli) in H_2O (12 ml) was added under stirring at room temperature to a solution of **9** (0.40 g, 2.0 mmol) in AcOH (1 ml). The resulting precipitated was collected by filtration, washed with AcOH, to give **10** (0.4 g, yield 98%); mp: >300 °C; ^{1}H NMR (DMSO-d_6): δ = 8.92 (dd, *J* = 7.2, *J* = 1.6, 1H, 7-H or 10-H), 8.37 (s, 1H, 2-H), 8.09–8.04 (m, 2H, 5-H or 6-H, and 7-H or 10-H), 7.96 (t, *J* = 8.8, 1H, 5-H o 6-H), 7.82–7.71 (m, 2H, 8-H and 9-H).

4.2.4. 4-Chlorobenzo[*h*]quinazoline (11)

A mixture of **10** (0.40 g, 1.9 mmoli), $POCl_3$ (5 ml) and TEA (2 ml) was stirred at reflux for 3 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was suspended in EtOAc (100 mL). The organic phase was washed with sat. $NaHCO_3$ (100 mL), then with H_2O (100 mL) and evaporated under reduced pressure to give **11** (0.25 g, yield 63%); mp: 115 °C; ^{1}H NMR ($CDCl_3$): δ = 9.22 (d, *J* = 7.6, 1H, 7-H or 10-H), 9.16 (s, 1H, 2-H), 8.04 (d, *J* = 9.1, 1H, 5-H or 6-H), 7.97–7.90 (m, 2H, 5-H or 6-H and 7-H or 10-H), 7.87–7.75 (m, 2H, 8-H and 9-H).

4.2.5. 4-[*N*-(Dimethylamino)ethyl]aminobenzo[*h*]quinazoline (12)

A mixture of **11** (80 mg, 0.33 mmoli), *N,N*-dimethylethylenediamine (36 μL, 0.33 mmoli) in *i*PrOH (2 ml) was microwave irradiated at 80 °C (three cycles as follow: power set point 100 W, ramp time 30 s, hold time 15 min). After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) to give **12** (70 mg, yield 80%; mp: >300 °C; IR (KBr) 3435, 2940, 2865, 1650, 1620, 1525, 1360, 1220, 1110, 1055, 830, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 9.17 (m, 1H, 7-H or 10-H), 8.85 (s, 1H, 2-H), 7.91–7.88 (m, 1H, 7-H or 10-H), 7.79 (d, *J* = 8.8, 1H, 5-H or 6-H), 7.73–7.70 (m, 3H, 5-H or 6-H and 8-H and 9-H), 6.91 (s, 1H, NH), 3.79 (t, *J* = 6.3, 2H, NH*CH*$_2$CH$_2$N(CH$_3$)$_2$), 2.78 (t, *J* = 6.3, 2H, NHCH$_2$*CH*$_2$N(CH$_3$)$_2$), 2.41 (s, 6H, N(*CH*$_3$)$_2$); ^{13}C NMR ($CDCl_3$): δ = 155.2, 150.3, 146.8, 146.0, 135.5, 129.2, 128.4, 127.0, 126.7, 124.5, 121.1, 96.4, 57.1, 44.9, 40.4; HRMS-ESI: *m/z* [M+H]$^+$ calcd for $C_{16}H_{19}N_4$: 267.1610, found 267.1588; Anal. calcd for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04; found C, 72.13; H, 6.80; N, 21.10.

4.2.6. Benzo[*f*]quinazolin-1(2*H*)-one (14)

A solution of CAN (4.4 g, 8.0 mmoli) in H_2O (12 ml) was added under stirring at room temperature to a solution of **13** (0.40 g, 2.0 mmol) in AcOH (1 ml). The resulting precipitated was collected by filtration, washed with AcOH, to give **14** (0.40 g, yield 98%); mp: 260 °C; ^{1}H NMR (DMSO-d_6): δ = 12.63 (s, 1H, NH), 9.86 (d, *J* = 8.2, 1H, 7-H or 10-H), 8.30 (d, *J* = 8.2, 1H, 7-H or 10-H), 8.28 (s, 1H, 2-H), 8.07 (d, *J* = 8.4, 1H, 5-H or 6-H), 8.05 (d, *J* = 8.4, 1H, 5-H or 6-H), 7.85 (t, *J* = 8.2, 1H, 8-H o 10-H), 7.60 (t, *J* = 8.2, 1H, 8-H o 10-H).

4.2.7. 1-Chlorobenzo[*f*]quinazoline (15)

A mixture of **14** (0.40 g, 2.0 mmoli), triphenylphosphine (2.1 g, 8.0 mmoli) and CCl_4 (1.9 ml, 20.0 mmoli) in CH_2Cl_2 (5 ml) was stirred at reflux for 3 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3$) to give **15**; (0.21 g, yield 48%); mp: dec on heating; ^{1}H NMR ($CDCl_3$): δ = ^{1}H NMR ($CDCl_3$): δ = 9.75 (d, *J* = 8.4, 1H, 7-H or 10-H), 9.11 (s, 1H, 3-H), 8.23 (d, *J* = 9.0, 1H, 5-H or 6-H), 8.02 (d, *J* = 8.4, 1H, 7-H or 10-H), 7.93 (d, *J* = 9.0, 1H, 5-H or 6-H), 7.87-7.75 (m, 2H, 8-H and 9-H).

4.2.8. Substitution reaction on 15

A mixture of **15** (0.25 g, 1.1 mmoli), *N,N*-dimethyl-ethylenediamine (0.36 ml, 3.3 mmoli) in *i*PrOH (5 ml) was microwave irradiated at 80 °C (three cycles as follow: power set point 100 W, ramp time 30 s, hold time 15 min). After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) to give only **14**.

4.3. Biology

4.3.1. Cell cultures

HL-60 (human myeloid leukemic cells) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15% heat-inactivated fetal calf serum (Biological Industries), HeLa (human cervix adenocarcinoma cells) in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Biological Industries) and A431 (human squamous carcinoma cells) in D-MEM (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Biological Industries). 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.

4.3.2. Inhibition growth assay

HL-60 cells (4×10^4) 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 to the complete medium and incubated for a further 72 h. HeLa 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 concentrations 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, that is, the concentration of the test agent inducing 50% reduction in cell number compared with control cultures.

4.3.3. Nucleic acids

Salmon testes DNA was purchased from Sigma Chemical Company. Its hypochromicity, determined according to Marmur and Doty,[25] was over 35%. pBR322 DNA was purchased from Fermentas Life Sciences.

4.3.4. Linear flow 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:

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

where $A_{//}$ 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[26] at a shear gradient of 500–700 rpm and each spectrum was accumulated four times. A solution of salmon testes DNA (1.9×10^{-3} M) in ETN buffer (containing 10 mM TRIS, 10 mM NaCl, and 1 mM EDTA, pH 7) was used. Spectra were recorded at 25 °C at different [drug]/[DNA] ratios.

4.3.5. DNA topoisomerases relaxation assay

Supercoiled pBR322 plasmid DNA (0.25 μg, Fermentas Life Sciences) was incubated with 1 U topoisomerase II (USB) or 2 U topoisomerase I (USB) and the test compounds as indicated, for 60 min at 37 °C in 20 μL reaction buffer. Reactions were stopped by adding 4 μL stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel at room temperature. The gels were stained with ethidium bromide 1 μg/mL in TAE buffer, transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.3.6. Topoisomerase II cleavage assay

Reaction mixtures (20 μL) containing 10 mM Tris–HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA, 1 mM ATP, 0.25 μg pBR322 plasmid DNA, 10 U topoisomerase II and test compounds were incubated for 60 min at 37 °C. Reactions were stopped by adding 4 μL stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel containing ethidium bromide 0.5 μg/mL at room temperature.

4.3.7. DNA isolation and electrophoresis

HeLa (5×10^5) cells were incubated in standard conditions for 24 h at 37 °C, then the medium was replaced with an equal volume of fresh medium and the test agent was added at the indicated concentration. Control cells were grown under the same conditions with the addition of the solvent alone. After 24 h of incubation, DNA from control and treated cells was extracted according to the procedure described by Sambrook et al.[27] The isolated DNA was dissolved in TE buffer (Tris 10 mM, pH 8, EDTA 1 mM) and analyzed by agarose (1%) gel electrophoresis. The gel was stained with ethidium bromide solution (1 μg/mL), transilluminated by UV light and fluorescence emission visualized using a CCD camera coupled to a Bio-Rad Gel Doc 1000 apparatus.

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