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New benzoquinolizin-5-one derivatives as furocoumarin analogs: DNA-interactions and molecular modeling studies

Giorgia Miolo *, Stefano Moro, Daniela Vedaldi, Sergio Caffieri, Adriano Guiotto, Francesco Dall'Acqua

Department of Pharmaceutical Sciences, University of Padua, Via Marzolo 5, 35131 Padua, Italy

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Abstract

Three derivatives of 1*H*,5*H* and 3*H*,5*H*-benzo[ij]quinolizin-5-one (BQZ [1]), previously prepared by chemical synthesis with the aim of obtaining furocoumarin analogs, have been studied. These are able to intercalate inside DNA and by subsequent irradiation with UVA light, to photoreact with DNA. Compound **I** (10-methoxy-7-methyl-1*H*,5*H*-benzo[ij]quinolizin-5-one) has a potentially photoreactive 2,3 double bond because of its conjugation with the pyridine ring of quinolinone, while compounds **II** (10-acetoxy-7-methyl-3*H*,5*H*-benzo[ij]quinolizin-5-one) and **III** (10-methoxy-7-methyl-3*H*,5*H*-benzo[ij]quinolizin-5-one) have a potentially photoreactive 1,2 double bond conjugated with the benzene ring of quinolinone. Compounds **I** and **III**, having a tricyclic planar structure, intercalate inside the DNA, while compound **II** cannot intercalate efficiently because of the steric hindrance of the acetoxy group in 10, lying outside the plane of the molecule and rotated by an angle of 77.6° with respect to the tricyclic plane. The photoreaction of BQZ with DNA structure, as already known for psoralen and angelicin derivatives, consists of a [2 + 2] photocycloaddition reaction with the pyrimidine bases. The main photoadduct between the 2,3 double bond of **I** and the 5,6 double bond of thymine has been isolated and characterized by NMR, showing a *cis–anti* structure. Theoretical calculations, using AM1 Hamiltonian, have been carried out to describe the photocycloaddition reaction mechanism better. From a theoretical point of view, in the case of BQZ both the 1,2 or 2,3 double bonds and the 6,7 double bond may be involved in the [2 + 2] photocycloaddition. Spin densities and molecular orbital symmetries of compound **I**, in its triplet state, suggest that the 2,3 double bond interacts favorably with the 5,6 double bond of thymine moiety. On the contrary, the acetoxy substituent in position 10 of **II** seems to play a negative role in the DNA intercalation process.

Keywords: Furocoumarin analogs; Benzoquinolizinones; DNA-intercalation; DNA-photobinding; Molecular modeling

1. Introduction

It is well known that some psoralens are used, in combination with UVA, for the photochemotherapeutic treatment of some skin diseases, e.g. psoriasis and vitiligo [1] and some autoimmune disorders [2]. However, since their efficacy is at times limited by side effects [1], over the last years new furocoumarins and analogs have been synthesized and studied with the aim of obtaining new photochemotherapeutic agents.

The tricyclic molecule of furocoumarin is characterized by a divinyl benzene chromophore, which can photoreact with DNA under the appropriate conditions, engaging one or both the vinyl groups to give C_4-cycloaddition reactions, among others.

Recently, some derivatives of benzoquinolizinone (BQZ) have been prepared by chemical synthesis [3]. When designing these new compounds, maintaining the divinylbenzene group inside a tricyclic flat and compact skeleton which should undergo intercalation inside DNA has been considered. Compounds **II** and **III** include the divinylbenzene moiety; they are formed by a quinolinone nucleus condensed with a dihydropyridine moiety. Compound **I** is an isomer, in which the 1,2-double bond of compounds **II** and **III** is located in the 2,3 position, and therefore the divinylbenzene moiety is not present, even if the 2,3 vinyl bond is at least partially conjugated with the chromophore (Fig. 1).

* Corresponding author. Fax: + 39-049-827 5366.

E-mail address: miolo@purple.dsfarm.unipd.it (G. Miolo)

[1] BQZ, benzoquinolizinone; 8-MOP, 8-methoxypsoralen; NOE, nuclear Overhauser effect.

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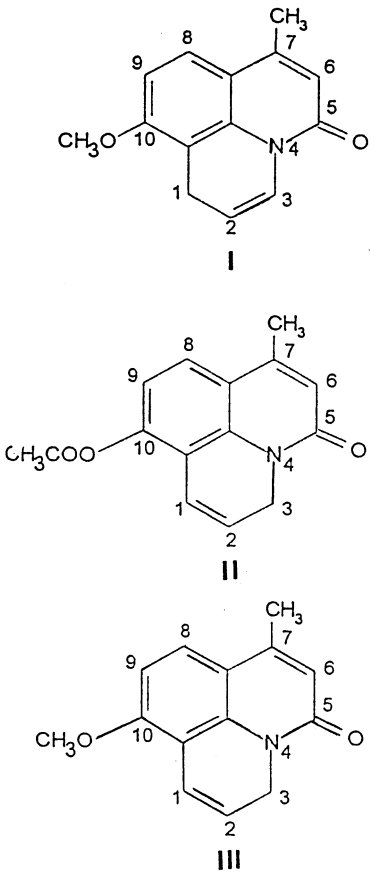

Fig. 1. Molecular formula of (10-methoxy-7-methyl-1*H*,5*H*-benzo[ij]quinolizin-5-one) (**I**), (10-acetoxy-7-methyl-3*H*,5*H*-benzo[ij]-quinolizin-5-one) (**II**), and (10-methoxy-7-methyl-3*H*,5*H*-benzo[ij]-quinolizin-5-one) (**III**).

The aim was to obtain flat molecules, able to intercalate inside DNA and, by subsequent irradiation, to photoreact with DNA. The main target of these molecules, therefore, should be DNA, even if they may produce other photochemical events.

In this paper we describe the interactions between the new molecules with DNA in the ground state, evaluating both intercalation and stability of the complexes. Subsequently, we have studied DNA photobinding and the cross-linking ability of the new compounds. For compound **I**, the cycloadduct formed with thymine has been isolated and characterized.

The different DNA photobinding properties exhibited by the three compounds has been considered in terms of molecular modeling.

2. Experimental

2.1. Chemicals

BQZs were synthesized as described in Ref. [3].

Compound **I** was submitted to a labeling procedure with tritium by the Radiochemical Centre of Amersham International plc (UK) and purified by TLC Silica Gel preparative plates (Cat.5717), E. Merck, Darmstadt, Germany, developing with chloroform; the specific radioactivity was 7.28 Ci/mol. 8-MOP specific activity was 2.1 Ci/mol.

A MINAXI β TRI-CARB 4000 Series Liquid Scintillation Counter was used for radioactivity measurements. Emulsifier scintilllator 299™ was purchased from Packard (Downers Grove, IL, USA).

Calf thymus DNA (hypochromicity higher than 40%), salmon testes DNA, and bacterial DNA from *Clostridium perfringens*, *Micrococcus lysodeikticus* and *Escherichia coli* were supplied by Sigma-Aldrich Co., St. Louis, MO, USA.

Thin-layer chromatography (TLC) 60 F_{254} Silica Gel plates were purchased from E. Merck. Hydroxylapatite Bio-Gel type was supplied by Bio-Rad Laboratories, Richmond, CA, USA.

Linear flow dichroism experiments were performed on a Jasco IF 500 spectrometer. Irradiation was carried out by means of two Philips HPW 125 lamps emitting almost completely at 365 nm; irradiation intensity, determined, by a Cole Parmer radiometer (mod. 9750300, Cole-Parmer Instrument Company, Niles, IL) equipped with a 365 nm light sensor, was 0.26 J/cm^2/min.

2.2. Complexes in the ground state

2.2.1. Fluorimetric titrations

Fluorimetric titrations for aqueous solutions ($1.1–1.2 \times 10^{-5}$ M) of compounds in the presence of increasing amounts of calf thymus DNA (final concentration 4×10^{-3} M) were made on quartz cuvettes (1 cm optical path). A Perkin–Elmer spectrofluorimeter, Mod. LS-50 connected to a Data Station, Mod. 3600 was used.

2.2.2. Computation of interaction parameters

The method of computation of the binding parameters of the complexes, i.e. K (association constant to an isolated site), n (number of nucleotides occluded by a bound compound) and $1/n$ (frequency of the binding sites), involved an iterative procedure designed to satisfy the McGhee and von Hippel equation [4], from the experimentally determined values of r (ligand molecules bound per nucleotide) and c (free ligand, mol/l). A program based on the least-squares method of the Taylor series expansion of the above equation was recycled until K and n changed by less than 1% and finally gave the values of K and n with a calculated binding isotherm at 5% saturation increments.

2.2.3. Linear flow dichroism measurements

Linear flow dichroism of each compound (1.6×10^{-4} M) in the presence of 3.8×10^{-3} M calf thymus DNA aqueous solutions containing 2×10^{-3} M NaCl and 1×10^{-3} M EDTA was measured on a Jasco J 500 spectrophotometer equipped with the flow dichroism attachment. In this device the usual cell chamber is replaced by a quartz cylindrical cell containing a quartz rotating cylinder. A calcite prism polarizes the monochromatic light parallel or perpendicular to the flow line. Velocity gradient of the laminar flow under our experimental conditions was 2900 s^{-1}. Calculations were made according to Wada [5] and Wada and Kozawa [6].

2.3. Interaction with DNA upon irradiation

2.3.1. DNA photobinding 'in vitro'

An ethanol solution (3×10^{-5} M) of each compound was added to an aqueous solution of calf thymus DNA (2.3×10^{-3} M) containing 2×10^{-3} M NaCl and 1×10^{-3} M EDTA. The solutions were gently shaken for 15 min in the dark and irradiated with UVA light for increasing periods of time in thermostated test tubes. After irradiation, a part (part A) of each solution was poured in two volumes of ethanol in the presence of 2 M NaCl, and the precipitated DNA was centrifuged (7000 rpm), washed with 80% ethanol and redissolved in water. Each DNA sample was then hydrolyzed in 1 N HCl at 100°C for 60 min. After neutralization, phosphate buffer (0.5 M, pH 6.98) was added and each solution of DNA was analyzed by spectrofluorimeter.

For compound **I**, DNA photobinding experiments were also performed with bacterial DNA from *C. Perfringens*, *M. Lysodeikticus* and *E. coli*.

2.3.2. Determination of cross-links

The second part (part B) of the solutions irradiated with increasing UVA doses were analyzed for the formation of DNA cross-links. Each solution was heated for 10 min in a boiling water bath, immersed in ice for 15 min and chromatographed on a column (0.7×4 cm) of hydroxylapatite, using a linear gradient of 0.05–0.3 M phosphate buffer, pH 6.98 and a flow rate of 15 drops/min. Fractions of 3.5 ml were collected and their absorbance at 260 nm was determined. The presence of cross-links (renatured double-stranded DNA) was evaluated and quantified according to Lawley and Brookes [7].

2.3.3. Isolation of photoadducts

Isolation of photoadducts was accomplished according to Ref. [8]. Briefly, aqueous DNA solution (1 mg/ml) was irradiated in the presence of compounds at saturation, precipitated with ethanol, washed to eliminate unbound molecules, hydrolized and chromatographed on TLC silica plates to separate photoadducts. A mixture of 80:20 ethyl acetate:*n*-exane was used to separate photoproducts for compound **I** and 100% ethyl acetate for compound **II**. Bands were visualized by irradiating the plates with 254 nm light and 365 nm light emitted by a UV lamp VL-4LC, Vilber Lourmat (Marne la Vallee, France). Bands of interest were scraped off, photoadducts extracted with chloroform and ethanol, concentrated and analyzed by UV absorption measurements and NMR analysis.

2.3.4. Photoadduct analysis

Photosplitting experiments (254 nm) were performed for a preliminary detection of photoadducts. TLC eluted photoproducts were irradiated with 254 nm light. This irradiation is able to reconvert furocoumarin photoadducts to parent compounds (furocoumarin and pyrimidine base). After irradiation, a drop of this sample was loaded onto a TLC plate together with: (a) unsplit sample, (b) pure compound and (c,d) both pyrimidine bases as control samples. Photoadducts were identified by the presence of parent compound and one of the two pyrimidine bases formed after photosplitting.

Spectrophotometric measurements were also determined on the isolated photoproducts solutions after increasing irradiation times with 254 nm light.

2.4. Computational methods

All molecular modeling calculations were performed on an IBM RISC System 6000 model 250 Unix workstation. All ground state geometries were fully optimized without geometry constraints using RHF/AM1 semi-empirical calculations [9]. Lowest triplet state geometries were fully optimized without geometry constraints using UHF/AM1 semi-empirical calculations [9]. Vibrational frequency analysis was used to characterize the minimum stationary points (zero imaginary frequencies). The Spartan 4.x software package was utilized for all quantum mechanical calculations [10].

3. Results

3.1. Interaction with DNA in the dark

3.1.1. Formation of a molecular complex with DNA: binding parameters

Planar polycyclic molecules are known to be able to intercalate inside the lipophilic portion of DNA. Furocoumarins, facilitated by their planar conjugated structure, intercalate into DNA between two pairs of bases [11]. Our compounds possess the quinolinonic nucleus

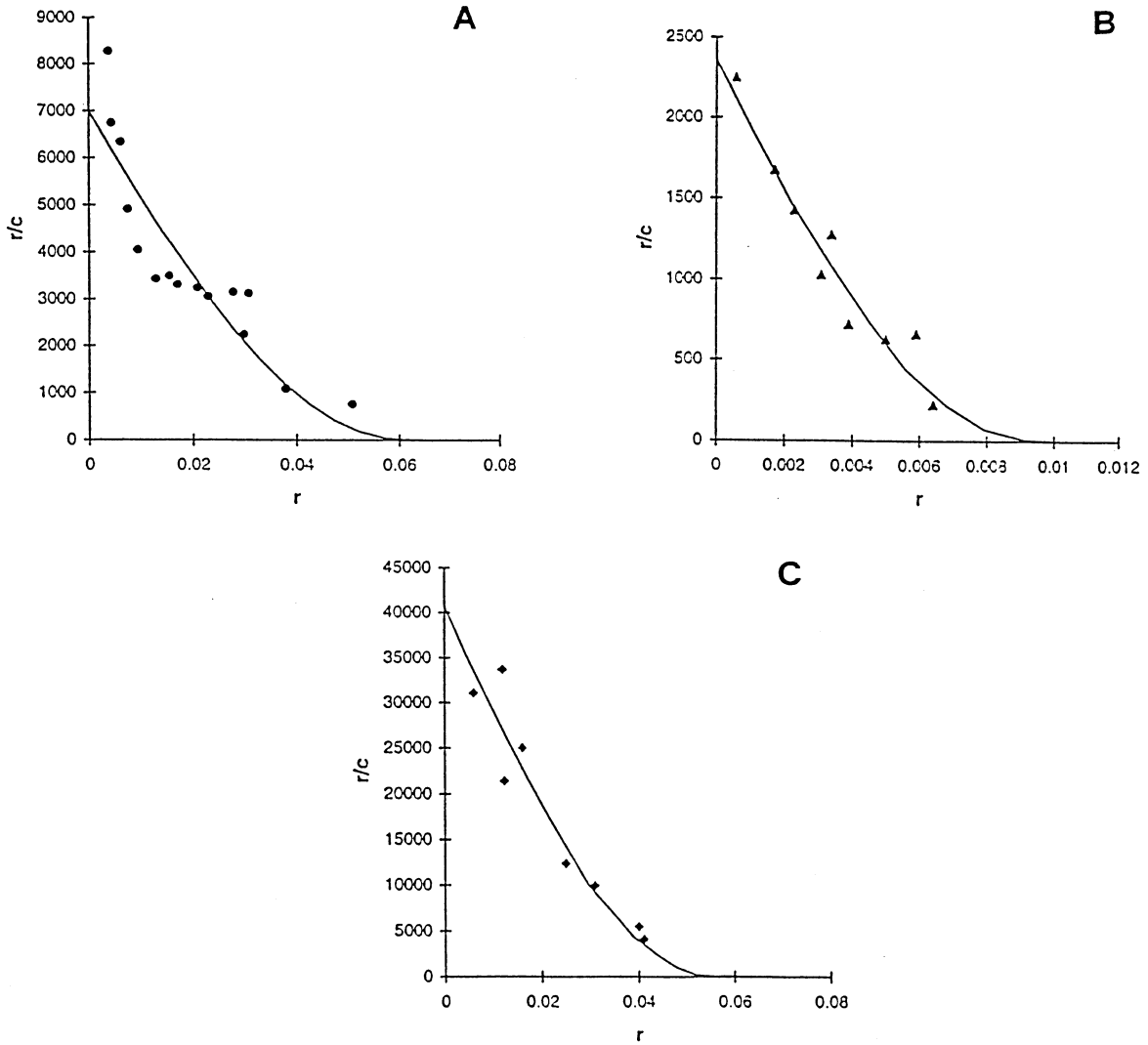

Fig. 2. Scatchard plots for binding in the ground state of BQZ **I** (A), **II** (B) and **III** (C) to calf thymus DNA. Curves were calculated by computer according to the McGhee and von Hippel method [5] on the basis of experimental values of r and c reported in the figure.

and the third dihydropyridinic ring not completely conjugated. Nevertheless, some analogs, pyranocoumarins, having a moiety with an incomplete conjugation of the tricyclic system, have demonstrated a good intercalation capacity [12].

The formation of a non-covalent complex in the dark between the BQZ derivatives in the ground state and DNA was determined. From a qualitative point of view, this was obtained by fluorescence spectra, where a quenching of fluorescence of the ligands was observed in the presence of DNA.

The binding parameters of the complexes were determined by following the binding process between the compound and the macromolecule by fluorescence titration experiments.

The values of r (number of molecules of ligand bound per nucleotide) and c (number of ligands free in the experimental conditions, in mol/l) were calculated from the binding data according to Peacocke and Skerrett [13]. These data are reported according to the classic Scatchard plot [14], i.e. plotting r/c against r (Fig. 2).

The isotherms reported were calculated according to the McGhee and von Hippel method [5] on the basis of the experimental values of r and c. The binding parameters K (association constant to an isolated site), n (number of nucleotides occluded by a bound ligand) and $1/n$ (frequency of binding sites, i.e. the number of molecules of ligand bound to every nucleotide) were also calculated by the same method (Table 1).

All three compounds are able to form a complex with DNA in the dark, but compound **III** demonstrated the strongest affinity towards DNA, higher than that of compound **I** (having the same substituents but with the olefinic bond in position 2,3 of the dihydropyridinic ring not conjugated with the benzene ring), and much higher than that of compound **II** (having the acetoxy instead of methoxy group in position 10).

Table 1
Binding parameters of complexes between BQZ and DNA

Comp.	K values	n [a]	$1/n$ [b]
I	7000 ± 1000	14.04	7.12×10^{-2}
II	2360 ± 195	87.83	1.14×10^{-2}
III	40700 ± 3780	15.28	6.54×10^{-2}

[a] According to McGhee and von Hippel [4], n is defined as the number of nucleotides occluded by one molecule of compound.

[b] According to McGhee and von Hippel [4], $1/n$ defines the frequencies of binding sites.

3.1.2. Flow dichroism studies

Intercalation is known to be a crucial step for compounds able to covalently bind DNA under irradiation, i.e. furocoumarins [11].

The linear flow dichroism of aqueous solutions of BQZ with DNA was studied to determine whether they intercalate inside duplex DNA during complexation.

By this technique, the long and stiff molecule of DNA is oriented in flow, resulting in a peculiar negative dichroism of the macromolecule, with a minimum at 260 nm [6]. When a small planar ligand undergoes intercalation between two base pairs, it assumes an ordered position similar to that of purines and pyrimidines. Therefore, a negative dichroism in the correspondence of the chromophore of the ligand can be seen when its transition moment is polarized in parallel with the planar chromophore, as for DNA bases.

The negative linear dichroism was observed in the range between 320 and 350 nm, where $\pi-\pi^*$ transitions, which are polarized in molecule plane [15], are responsible for the strongest absorption of the considered compounds. In Fig. 3 it can be seen, according to complexation data, that compound **III** is the best intercalator, while compound **II** cannot intercalate, or intercalates very slightly, inside the macromolecule. This behavior can be explained by the steric hindrance of the acetoxy group, as can be seen by the tridimensional structure of the molecule in which this group lies out of the plane, increasing its thickness (see later).

3.2. Interaction with DNA upon UVA irradiation

3.2.1. Covalent DNA photobinding

It is well known that furocoumarins undergo covalent photocycloaddition with pyrimidine bases of DNA under UVA irradiation. Fluorescent furan-side monoadducts are the main photoproducts formed together with lower amounts of pyrone-side non-fluorescent monoadducts [16].

In the case of BQZ, both 1,2 or 2,3 double bond and 6,7 double bond can be involved in C_4-cycloaddition; therefore, these molecules can behave as both monofunctional and bifunctional agents.

A first approach to detect covalent photoaddition to DNA was made by measuring the fluorescence acquired by the macromolecule, under increasing UVA doses. Before fluorimetric determinations, photomodified DNA was precipitated, washed and hydrolyzed to eliminate fluorescence quenching, due to intercalated ligand.

Only compound **I** was able to form an appreciable quantity of fluorescent products with DNA (Fig. 4). This fact supports that formation of fluorescent photoproducts takes place between BQZ **I** and DNA.

Of the three compounds studied, compound **I** was tritiated, thus it was possible to determine its photoreactivity towards DNA quantitatively.

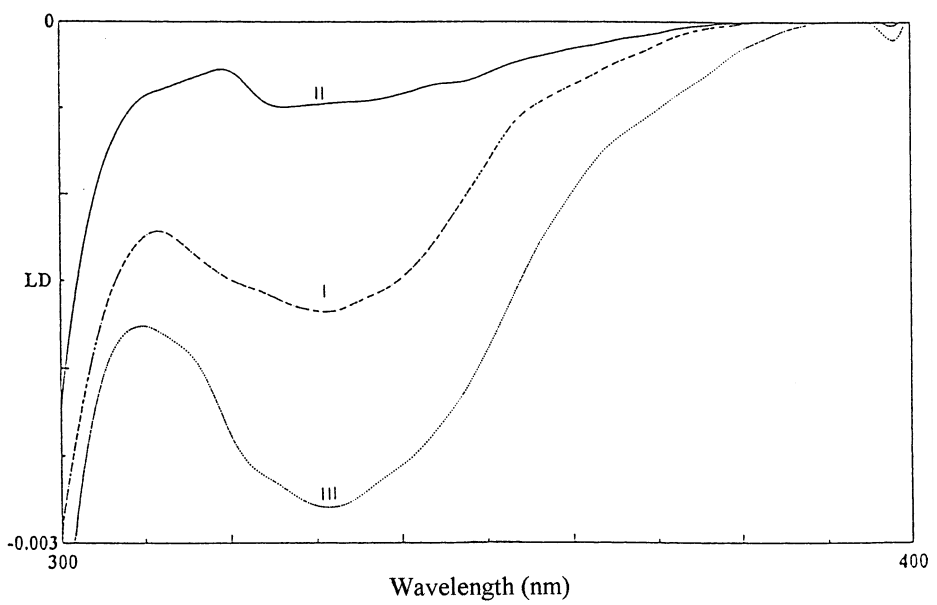

Fig. 3. Linear flow dichroism spectrum of BQZ–DNA complexes formed in the dark. Optical cells having a 1 mm optical path were used.

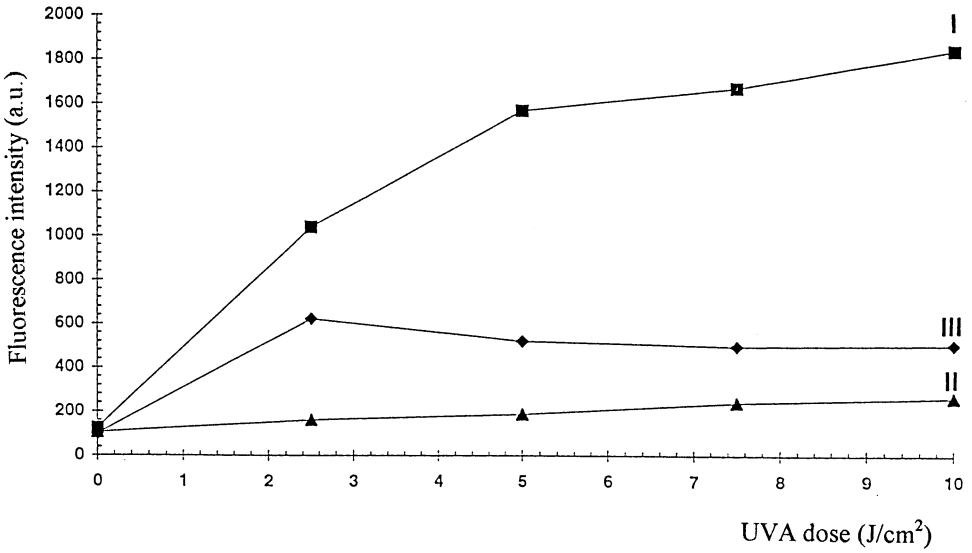

Fig. 4. Fluorescence intensity acquired by DNA irradiated in the presence of BQZ **I** ($\lambda_{ex} = 328$; $\lambda_{fl} = 425$), **II** ($\lambda_{ex} = 340$; $\lambda_{fl} = 400$) and **III** ($\lambda_{ex} = 283$; $\lambda_{fl} = 433$) at increasing UVA doses.

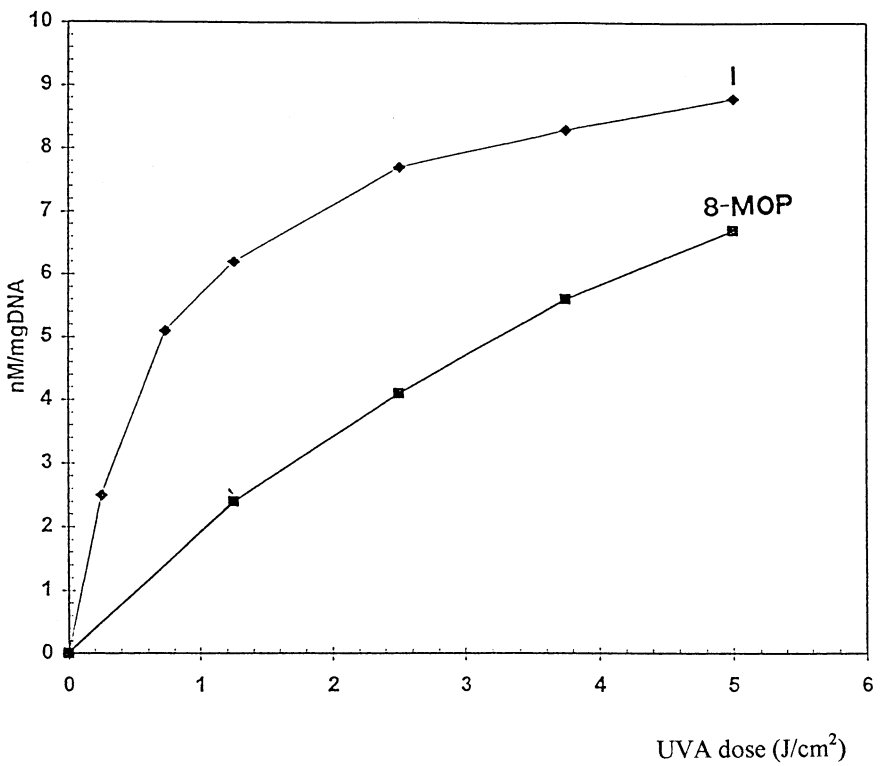

Fig. 5. Photobinding of BQZ **I** and 8-MOP (as reference compound) to calf thymus DNA under increasing UVA doses.

The amount of BQZ **I** covalently photobound to salmon testes DNA, as a function of irradiation dose (365 nm), is reported in Fig. 5, in terms of nmol of ligand bound/mg of DNA. 8-MOP, the compound most widely used in photochemotherapy, was used as the reference compound.

Table 2 reports the rate constant value of this photoreaction. It can be seen that BQZ **I** is more photoreactive than 8-MOP.

Table 2
Rate constant of photoreaction of BQZ **I** and 8-MOP with DNA

Comp.	Rate constant (min^{-1})
BQZ **I**	6.91×10^{-2}
8-MOP	3.13×10^{-2}

Table 3
BQZ **I** photobound to bacterial DNAs (nmol/mg of DNA) with increasing UVA doses

Bacterial DNA	5 J/cm^2	7.5 J/cm^2
C. perfringens	45.3	48
E. coli	39.9	41
M. lysodeikticus	22.7	27.9

Further photoreaction experiments with bacterial DNA with different bases composition (69% A-T, *C. perfringens*; 50% A-T, *E. coli*; 28% A-T, *M. lysodeikticus*) have demonstrated that compound **I** prefers A-T rich sequences (Table 3), as previously demonstrated for other furocoumarins [17]. Indeed, when alternated, AT sequences are flexible and facilitate intercalation of bulky compounds.

3.2.2. Determination of cross-links

For all three BQZs no evidence of cross-link formation was detected in the experimental conditions applied.

3.2.3. Isolation and characterization of cycloadducts

A large scale photoreaction between the three compounds and DNA was made in order to isolate and characterize the photoadducts formed with DNA. TLC was used to isolate photoadducts after acid hydrolysis of photomodified DNA. The presence of photoadducts was revealed by irradiating the plate with both 254 and 365 nm light. As expected from fluorescence measurements, compound **I** was able to form a main fluorescent photoproduct ($R_f = 0.37$) whereas for compounds **II** and **III** fluorescent photoproducts were not detected in these conditions. For all three BQZ no non-fluorescent photoproducts were observed at all.

The only photoproduct observed for compound **I** was eluted from the plate and analyzed. Photosplitting experiments suggested that it is a photocycloadduct between the compound and thymine. Indeed, its irradiation in ethanol solution with UVC induced photosplitting to BQZ **I** and thymine, as has been observed by TLC and UV spectra.

NMR analysis revealed that this adduct corresponds to a C_4-cycloadduct between the 2,3-double bond of BQZ **I** and the 5,6-double bond of thymine. Saturation of the 2,3 double bond is clearly shown by the upfield shift of the corresponding protons (in the parent compound H_2 and H_3 resonances were at 5.5 and 7.6 ppm, respectively). H_1 protons, which are benzylic and allylic at the same time in BQZ **I** (3.5 ppm) lost the latter feature and resonate at 2.7 ppm in the adduct.

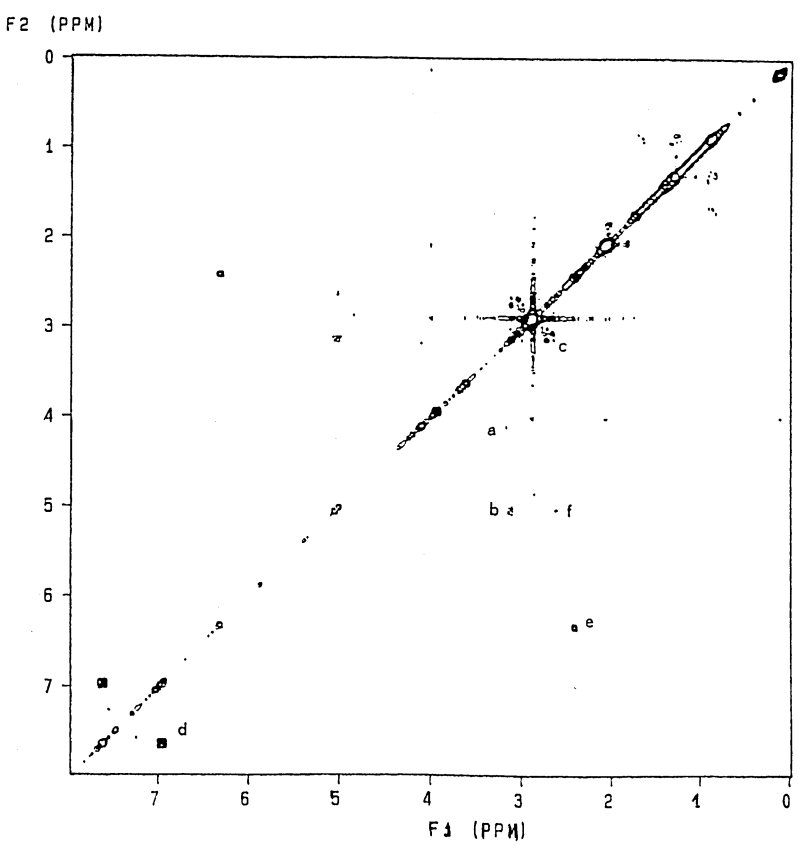

Fig. 6. 2D-NMR spectrum of photoadduct between BQZ **I** and thymine.

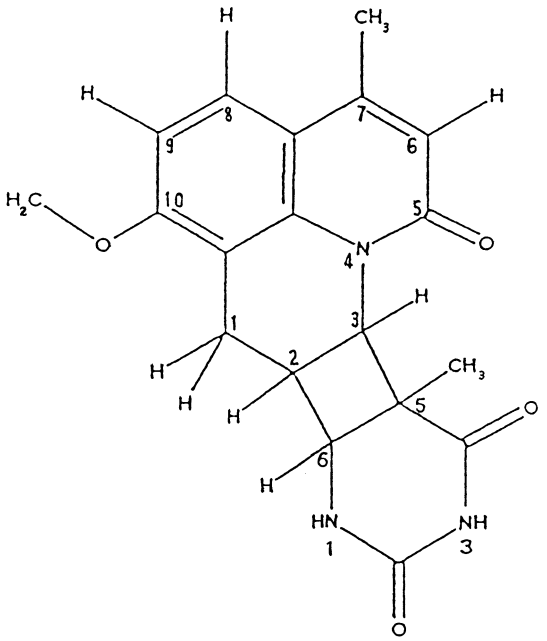

Fig. 7. Proposed structure of the photoadduct between BQZ **I** and thymine (*cis–anti*).

^{1}H NMR (200 MHz, acetone-d_6), δ (ppm): 8.7 (broad, 1H, N_{3T}), 7.64 (d, 1H, $J = 8.8$ Hz, H_8), 7.03 (apparent s, 1H, H_{1T}), 6.95 (d, 1H, $J = 8.8$ Hz, H_9), 6.31 (q, 1H, $J = 1.2$ Hz, H_6), 5.03 (dd, 1H, $J = 9.2$ and 1.5 Hz, H_3), 4.10 (ddd, 1H, $J = 6.8$, 2.5 and 1.5 Hz, H_{6T}), 3.93 (s, 3H, OMe), 3.1 (m, 1H, H_2), 2.7 (m, 2H, H_1), 2.42 (d, 3H, Me_7), 1.73 (s, 3H, Me_{5T}).

The regiochemistry of the adduct was studied by homonuclear decoupling and 2D experiments (Fig. 6). Saturation of H_{1T} simplifies the signal at 4.1 ppm, which was then assigned to H_{6T}. Cross peak 'a' shows that H_{6T} is coupled with the proton at ca. 3.1 ppm which, in turn, is coupled with protons resonating at 5.03 ('b') and 2.7 ppm ('c'). As the former signal must be assigned to H_3, because of its deshielding by nitrogen, C_{6T} is linked to C_2: the adduct therefore possesses the *anti* regiochemistry. Its stereochemistry could not be determined experimentally, as NOE experiments were unsuccessful. However, considering that all cycloadducts so far isolated from DNA have shown *cis* configuration, such an arrangement may be preferred over the *trans* one on the basis of the following observations: (1) the coupling constant between H_{6T} and H_2 is 6.8 Hz, analogous to that found in other cycloadducts in which the *cis* arrangement was demonstrated; and (2) a small but detectable coupling (1.5 Hz) exists between two diagonal protons of the cyclobutane ring (H_{6T} and H_3). Fig. 7 shows the structure proposed for the adduct.

In conclusion, compound **I** is able to engage its 2,3 double bond in the photoreaction with thymine after DNA intercalation. Usually furocoumarins involve their olefinic bonds (in furan and pyrone rings) during photoreaction with DNA. The conjugation of those electrons in the mesomery of the benzene ring seemed to be necessary for the photoreactivity of the olefinic bonds. However, the 2,3 double bond of compound **I**, not conjugated with the benzene ring, could be involved in that mesomery through the pair of electrons on nitrogen.

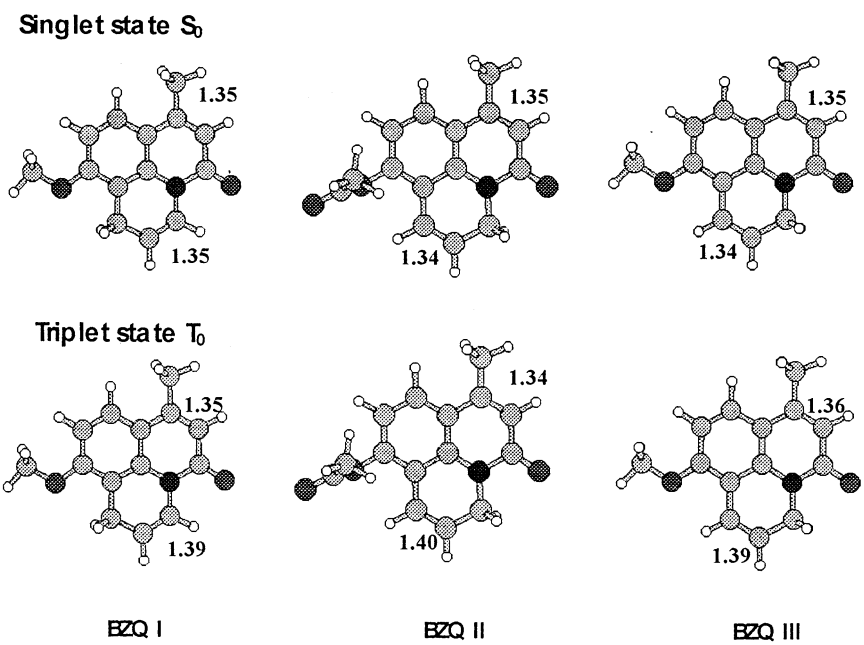

Fig. 8. BQZ optimized structures of both lowest singlet (S_0; RHF/AM1) and triplet state (T_0; UHF/AM1).

Table 4
Calculated bond orders of both S_0 (RHF/AM1) and T_0 (UHF/AM1) states for two double bonds present in the BQZ derivatives

Comp.	Singlet state (S_0)		Triplet state (T_0)	
	Bond 1,2 [a]	Bond 6,7	Bond 1,2 [a]	Bond 6,7
BZQ **I**	1.86	1.75	1.45	1.60
BZQ **II**	1.87	1.75	1.51	1.65
BZQ **III**	1.87	1.75	1.63	1.56

[a] BZQ **I** presents the double bond in 2,3 position instead of 1,2 position.

Table 5
Calculated atomic spin densities (UHF/AM1) for the four carbons of two double bonds present in the BQZ derivatives in their T_0 states

Comp.	Triplet state (T_0)		Triplet state (T_0)	
	Atom 1 [a]	Atom 2 [a]	Atom 6	Atom 7
BZQ **I**	0.59	−0.39	−0.38	0.45
BZQ **II**	0.51	−0.89	−0.26	0.33
BZQ **III**	0.45	−0.35	−0.40	0.48

[a] BZQ **I** presents the double bond in 2,3 position instead of 1,2 position.

3.3. Quantum mechanics calculations

As already described in detail for psoralen and angelicin derivatives, the photoreactivity of BQZs with the DNA structure can also be described as [2 + 2]-photocycloaddition reaction with the pyrimidine bases [18,19]. To elucidate the mechanism of the photocycloaddition reaction, AM1 semiempirical calculations were performed to obtain the minimum energy geometries and the electronic structures of all BQZs studied. Thymine, which was found experimentally to be the principal counterpart for the photochemical process, has also been studied using AM1 semiempirical calculations.

From the Frontier Molecular Orbitals (FMO) theory [20], it is well known that, when irradiated with UV light, BQZs can absorb a quantum of radiation which causes the promotion of one electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). BQZ moiety in its excited single state (S_1) can undergo an intersystem crossing (ISC) to the lowest triplet state (T_0). As described previously, it is plausible to think that one of the two singly occupied molecular orbitals of the lowest triplet state T_1, generally named α-HOMO and α-LUMO, can interact with one of the thymine molecular orbitals, with appropriate symmetry, during the photocycloaddition reaction. In this particular case, either 1,2 (or 2,3) and 6,7 double bonds can be involved in C_4-cycloaddition. To predict the regiochemistry of the [2 + 2]-photocycloaddition reaction between BQZ and thymine moieties, we have analyzed bond lengths and bond orders of both 1,2 (or 2,3) and 6,7 double bonds of BQZ structures. Optimized geometry structures of both the lowest singlet S_0 and its triplet states T_0 are shown in Fig. 8. Bond distances of both potentially reactive double bonds are also included. Taking into account the increase in the two double bond lengths (1,2 or 2,3) of all three T_0 structures, we can speculate that both these double bonds seem to be engaged in the photocycloaddition reaction. Accordingly, the calculated 1,2 and 2,3 bond orders decrease when passing from S_0 to T_0 states. These data are shown in Table 4. From a structural point of view, it is interesting to observe that the acetoxy substituent in position 10 of **II** is rotated with respect to the tricyclic plane (C9–10–O–CO) by an angle of 77.6°. This arrangement could affect the DNA intercalation process, decreasing (as has been shown experimentally) the formation of a non-covalent complex in the dark phase between the BQZ derivative in the ground state and DNA. To better understand the regiochemistry of the photocycloaddition reaction we have also calculated the atomic spin densities for the four carbons of two double bonds present in the BQZ derivatives in those T_0 states. As reported in Table 5, carbon atoms in 1,2 and 2,3 present higher spin density compared with the carbons in 6,7 positions. Only for derivative **III** did we find almost the same spin density localized on 1,2 and 6,7 double bonds. This fact might be the reason for the lower photoreactivity presented by this compound.

To rationalize the stereochemistry of the [2 + 2]-photocycloaddition reaction, we have analyzed molecular orbital energies and symmetries of both BQZ and thymine moieties. In particular, we have focused our attention on derivative **I**, which is the only one able to form a cyclobutane derivative. As reported in Fig. 9, AM1 calculations showed that there is an energetically favored interaction between the α-LUMO ($E_{\alpha\text{-HOMO}} = -1.36$ eV) of **I** and the LUMO ($E_{\text{HOMO}} = -0.28$ eV) of the thymine moiety. Considering the α-LUMO and LUMO coefficients, this interaction is related to the 5,6 double bond of the pyrimidine base and 2,3 double bond of the derivative **I**. In fact, stereochemistry of the photocycloaddition can be predicted on the basis of maximum frontier orbital overlap [21]. As shown in Fig. 9, taking the molecular orbital interactions into account, a preference for the *endo* product (*cis–anti* adduct) was explained as due to secondary overlap between the atomic orbitals of thymine and those of derivative **I**. No such interactions were possible in the *exo* interaction mode. These theoretical data support the experimental evidence obtained by NMR spectroscopy that during the [2 + 2]-photocycloaddition reaction the *cis–anti* adduct was formed.

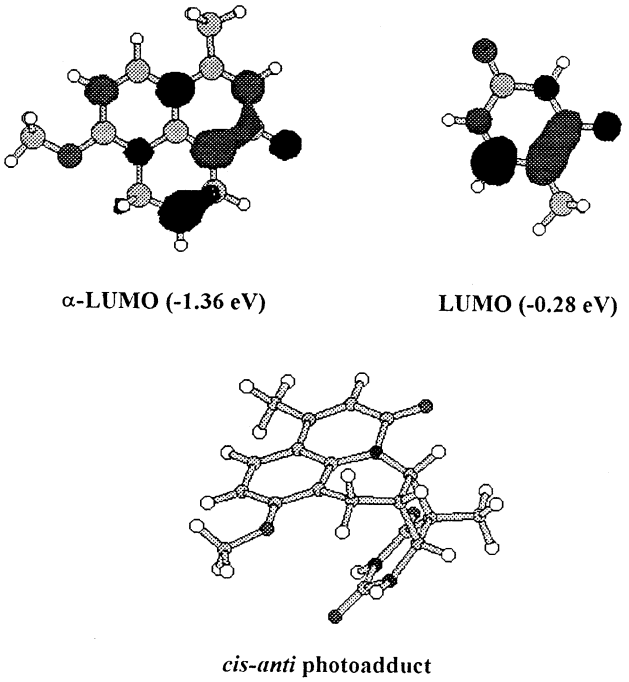

Fig. 9. (Top) Frontier orbital energies (eV) and a possible HOMO–LUMO combination for the reaction of BZQ **I** with thymine. (Bottom) Optimized structure (RHF/AM1) of the *cis–anti* photoadduct.

4. Conclusions

The interactions between three derivatives of *1H,5H* and *3H,5H*-benzo[ij]quinolizin-5-one and DNA have been studied in both ground and excited states. Theoretical calculations were also performed to predict the behavior of the new compounds in both the intercalation step and in the subsequent [2 + 2] photocycloaddition to DNA bases.

Compounds **I** and **III** are planar tricyclic molecules able to intercalate inside a duplex macromolecule. On the contrary, compound **II** does not undergo intercalation inside DNA. This fact may be explained by the steric hindrance of the acetoxy substituent in 10, which increases the thickness of the molecule, as can be seen by its tridimensional structure model.

By irradiating compound **I** with UVA light, a covalent binding through the 2,3 double bond takes place with thymine. The C_4-cycloaddition product has been isolated and characterized: showing a *cis–anti* structure. Moreover, compound **I** has shown a higher photoreactivity towards DNA than 8-MOP and preferential photobinding to A-T rich sequences.

Compounds **II** and **III** showed a very poor ability to photoreact with the macromolecule, although their double bonds (1,2 and 6,7) are potentially photoreactive. While for compound **II** this can be explained by its lack of intercalation, for compound **III** semiempirical calculations and computer aided studies were useful. Indeed, minimum energy geometries and electronic structures for both ground and excited states (lowest energy singlet and lowest energy triplet) were calculated. The influence of substituent in position 10 accounted for the different abilities of the compounds to photoreact with DNA. In particular electron donor substituents in 10, i.e. methoxy group for compound **III**, reduces the stability of the (π–π^*) triplet excited state on 1,2 double bond, thus reducing its photoreactivity.

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