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Photochemical and photobiological studies on furoquinazolines as new psoralen analogs

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ABSTRACT

Linear (**L**) and angular (**A**) 4′,5′-dimethylfuroquinazolines (FQZs) were synthesized and studied as furocoumarin analogs. These molecules proved to be photounstable with a photodegradation extent correlated to UVA light doses. Both compounds did intercalate inside the DNA double helix, but were not able to photobind DNA bases under UVA irradiation. This behavior was further rationalized through docking studies. The photosensitizing effects of these compounds were evaluated on Jurkat tumor cells and NCTC-2544 human keratinocytes, with and without antioxidants, to demonstrate the involvement of a photodynamic mechanism. Indeed, significant amounts of singlet oxygen and superoxide anion were generated in the presence of both compounds, that account for the oxidative damage induced to some isolated biological substrates (DNA, amino acids, proteins and lipids).

Photophysical studies by use of a flash photolysis set up showed detectable triplet population and production of singlet reactive oxygen species for linear furoquinazoline, which can be responsible for the oxidation of biological substrates, and therefore can affect the cell proliferation.

1. Introduction

Furocoumarins are a family of natural and synthetic compounds used for the photochemotherapeutic treatment of skin diseases, lymphomas and other autoimmune disorders [1,2]. In particular, 8-methoxypsoralen (8-MOP) is the best known and used furocoumarin derivative. PUVA therapy (8-MOP and UVA) demonstrated to be very effective for the treatment of psoriasis, as a result of photoinduced selective lesions to DNA. Psoralens can localize in mitochondria and, upon PUVA treatment, they are able to damage these organelles, leading to cell death by apoptosis [3].

Photopheresis, *i.e.* the extracorporeal irradiation of 8-MOP containing blood, has shown clinical efficacy in variants of cutaneous T-cell lymphomas, graft-versus-host disease, systemic sclerosis, solid organ transplant rejection and Crohn's disease [4]. Linear furocoumarins (psoralens) have also been used for sterilising blood components under UV irradiation [5]. Moreover, since psoralens are able to block potassium channels, they are potentially useful as drugs for the treatment of multiple sclerosis [6]. More recently, some psoralen derivatives have proved to inhibit NF-kB/DNA interactions, demonstrating potential anti-inflammatory profile useful for cystic fibrosis treatment [7,8].

Angular furocoumarins (angelicins) have proved to regulate the expression of human γ-globin genes without irradiation, suggesting their potential use in the treatment of hematological disorders, including beta-thalassemia and sickle cell anemia [9,10]. Although PUVA therapy is very effective in treating some diseases, such as psoriasis and mycosis fungoides, some severe long term side-effects have been observed, *e.g.* risk of skin cancer, genotoxicity and skin phototoxicity [11–13]. Therefore, the research in the field of new photoactive analogs of furocoumarins, with reduced side-effects, is efficient and worthwhile.

In this study, we evaluated the *in vitro* photosensitizing properties of two newly synthesized psoralen analogs, namely a linear and an angular furoquinazolines (FQZs) (Fig. 1), as new photoactive agents.

In these new derivatives, the pyrone nucleus of furocoumarins was substituted with a pyrimidine ring. The furan system was maintained for two reasons: (i) to have a planar polycyclic structure sufficiently "large" to allow a stable complexation with DNA (bicycle molecules move in and out too quickly from DNA) and (ii) to have at least one potential site of photoreaction for the cycloaddition with DNA bases, typical of furocoumarins exposed to UVA

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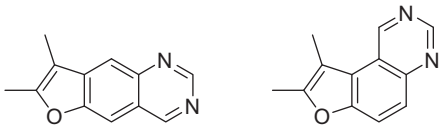

Fig. 1. Structures of the furoquinazolines (FQZs).

irradiation, even though the double bond in the 3,4-position of the pyrimidine ring could have enough vinyl character to give cycloaddition reaction.

2. Materials and method

2.1. Chemicals

The Linear and Angular FQZs were synthesized as reported in Supplementary Electronic Information. All commercial chemicals and solvents used for synthetic procedures were analytical grade and were used without further purification. DNA from salmon testes, BSA, amino acids, thiobarbituric acid and agarose were purchased from Sigma–Aldrich, Milano, Italy. DABCO, BHA, DMTU, sodium azide, alpha-tocopherol, were purchased from Bio Rad Laboratories (Segrate, Milano, Italy). The column used for HPLC analyses was a Phenomenex Gemini C18 5 μm, 250 × 4.6 mm. HPLC grade solvents and thin-layer chromatography (TLC; 60 F254 silica gel plates) were purchased from Merck, Darmstadt, Germany.

2.2. Fluorimetric characterization

Fluorescence spectra and quantum yields were measured by a Spex Fluorolog-2 F112AI spectrofluorimeter (mean deviation of two independent experiments, ca. 10% for Φ_F) in air-equilibrated solutions (absorbance < 0.1 at the excitation wavelength, λ_{exc}) using 9,10-diphenylanthracene in cyclohexane as fluorimetric standard (Φ_F = 0.73 in aerated solvent) [14] and neutral density filters (transmittance = 25%) on the excitation line.

Fluorescence lifetimes, τ_F, were measured by use of an Edinburgh Instrument 199S spectrofluorimeter equipped with a LED source (λ_{exc} = 370 nm) using the single photon counting method.

2.3. Irradiation procedure

For UVA irradiation of FQZ solutions alone (for photolysis experiments) or in the presence of biological targets (for photosensitization experiments), a Philips HPW 125 lamps were used. These latter mainly emit at 365 nm, though a small fraction of the energy pertains to the UVB region (0.0005 J/cm^2/min at 312 nm). The total energy hitting the sample was monitored by means of a radiometer (Mod. 97503, Cole-Parmer Instrument Company, Niles, IL, USA), equipped with a 365-CX sensor. The irradiance was about 0.52 J/cm^2/min. The samples were maintained at room temperature during irradiation.

2.4. Photolysis experiments

Solutions of the two compounds (4×10^{-5} M in methanol) were irradiated in 1 cm quartz cuvettes at controlled temperature (25 °C) with increasing UVA radiant exposures, up to 30 J/cm^2. Photolysis was evaluated by UV spectrophotometry (UV–Vis Varian Cary 50 Spectrophotometer) analyzing the change in the original spectrum upon irradiation, and by HPLC analysis measuring the decrease (%) of the area under the peak corresponding to the intact compound. The results are the mean of at least three experiments.

2.4.1. HPLC analysis

In order to determine the photostability of the two FQZs and to measure the yield of photolysis, irradiated samples were filtered (0.22 μm cellulose acetate membranes) and analyzed by an HPLC (PerkinElmer Series 200 instrument) equipped with a diode array detector set at 245 nm and 330 nm, using a reverse phase column (Phenomenex Gemini C18 5 μm, 250 × 4.6 mm). The mobile phase consisted of a mixture of acetonitrile and water (50% for 5 min, then linearly increased up to 100% acetonitrile within 15 min) at a flow rate of 1 mL/min.

2.5. Isolation and characterization of photoproducts

2.5.1. TLC separation

The isolation of photolysis products of FQZs was made by TLC-silica gel plates (60 F_{254}, 20 × 20 cm, 0.2 mm, E. Merck, Darmstadt, Germany), eluting with a $CHCl_3$/MeOH 99:1 mixture. Bands of interest, visualized with a Mineral Light Lamp VL-4LC VILBERT-LOURMAT, were scraped off and the expected photoproducts extracted with chloroform and ethanol and then analyzed by UV absorption, MS and NMR.

2.5.2. NMR analysis

^{1}H NMR were performed in deuterated acetone, deuterated DMSO or $CDCl_3$ using a Bruker AMX300 spectrometer (300 MHz).

2.6. Cellular phototoxicity

Human T-cell leukemia cells (Jurkat) were grown in RPMI-1640 medium and human keratinocytes (NCTC-2544) in DMEM medium (Dulbecco's modified Eagle's medium). All cellular media were supplemented with 115 U/mL of penicillin G, 115 μg/mL streptomycin and 10% fetal bovine serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 μL of complete medium containing 8×10^3 Jurkat cells or 5×10^3 NCTC-2544 cells. Plates were incubated at 37 °C in a humidified 5% CO_2 incubator for 18 h before starting the experiments. Drugs were dissolved in DMSO and then diluted with Hank's Balanced Salt Solution (HBSS, pH 7.2) for phototoxicity test. DMSO never exceeded 1% in the final solutions. After removal of the medium, 100 μL of the drug solution (concentration range: 10–200 μM) were added to each well, incubated at 37 °C for 30 min and then irradiated (1.25, 2.5 or 3.75 J/cm^2). After irradiation, drug solutions were replaced with medium and plates were incubated for 72 h. Cell viability was assayed by MTT test, as previously described [15]. The same experiments were performed on Jurkat cells in the presence of the hydroxyl radical scavengers dimethylthiourea (DMTU, 1 mM), sodium azide (NaN_3, 1 mM) or Vitamin E (60 μM) with FQZs (100 μM) upon 1.25 J/cm^2 of UVA irradiation.

2.7. Interaction with DNA in the dark

2.7.1. Linear flow dichroism measurements

The linear flow dichroism spectrum ($LD(\lambda) = A\|(\lambda) - A\perp(\lambda)$) of each compound in the presence of salmon testes DNA dissolved in ETN 0.01 M (TRIS 10 mM, EDTA 1 mM, NaCl 10 mM) was measured on a Jasco J-500 circular dichroism spectropolarimeter converted for LD and equipped with an IBM PC and a Jasco J interface. The measured solutions were prepared by additions of FQZs solution (5×10^{-3} M in ethanol) to DNA solution (1.9×10^{-3} M in ETN, OD_{260} = 12.5) to obtain a ligand/DNA ratio of 0.08 (R = 12.5). The measuring device was designed by Wada and Kozawa [16].

A constant share gradient of 1000 s^{-1} was used to record the LD spectra, and the baseline was taken at 0 gradient. From the LD