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6-Nitroquinolones in dimethylsulfoxide: Spectroscopic characterization and photoactivation of molecular oxygen

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ABSTRACT

The synthesized 6-nitroquinolone derivatives were characterized by UV/vis, NMR spectroscopy and the EPR spin trapping technique was applied to monitor the photoinduced generation of non-persistent radical species. The NMR spectra of ethyl 1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylate (**1**) and 1,4-dihydro-6-nitro-4-oxoquinoline-3-carboxylic acid (**3**) confirmed the dominance of the oxo-tautomer in dimethylsulfoxide (DMSO) solutions. The electronic absorption spectra of 6-nitroquinolones **1** and **3** with the amino hydrogen at nitrogen of the enaminone system (N–C=C–C=O) revealed the interaction of >NH with the aprotic DMSO solvent, which leads to the formation of the corresponding *N*-deprotonated species. The photoinduced reactions of 6-nitroquinolones in the aerated DMSO solutions were investigated by monitoring the electronic spectra upon a steady-state irradiation. While the ethyl 1,4-dihydro-1-ethyl-6-nitro-4-oxoquinoline-3-carboxylate (**2**) possessing an ethyl group at the nitrogen of the 4-pyridone moiety exhibits a rather low stability upon UVA photoexcitation, the changes in the electronic spectra observed for the derivatives **1** and **3** were attributed to the alterations in the concentration of their N^-/NH forms caused by the water absorption in the hygroscopic DMSO, with no evidence of photodecomposition. The photoexcitation (365 nm or 400 nm monochromatic radiation) of 6-nitroquinolones in aerated DMSO solutions containing spin trapping agents revealed the generation of superoxide radical anion spin-adducts, along with further spin-adducts of carbon- and oxygen-centered radicals produced by the interaction of photoexcited nitroquinolone molecules with the solvent. In the scope of various biological activities of quinolone-like compounds, a cytotoxic effect of ethyl 1,4-dihydro-1-ethyl-6-nitro-4-oxoquinoline-3-carboxylate (**2**) on cancer human (HeLa) and murine (B16) and non-cancer (NIH-3T3) cell lines was evaluated, demonstrating a rather low cytotoxicity towards studied cell lines.

1. Introduction

Nitrogen-containing heterocyclic compounds are abundant structural motifs occurring in a variety of bioactive natural substances, synthetic drugs, and pharmaceuticals with widespread applications [1–8]. Molecules with this heterocyclic pattern have been a subject of a great multidisciplinary research for decades as they exhibit a wide range of biological activities, e.g., antibacterial, antifungal, antitubercular, antimalarial and anticancer [9–12]. Among the various *N*-heterocyclic scaffolds, 4-quinolone (1,4-dihydro-4-oxoquinoline) derivatives have attracted

Abbreviations: ATCC, American type culture collection; DMEM, Dulbecco's modified eagle medium; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DMSO, dimethylsulfoxide; EMPO, 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide; EPR, electron paramagnetic resonance; hfcc, hyperfine coupling constants; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ND, nitrosodurene; PBS, phosphate-buffered saline; POBN, α-(4-pyridyl *N*-oxide)-*N*-*tert*-butylnitrone; ROS, reactive oxygen species; SOD, superoxide dismutase; SW, magnetic field sweep width; TEA, triethylamine; Tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidine *N*-oxyl; UV/vis, ultraviolet/visible.

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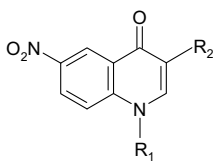
significant attention due to their numerous therapeutic effects and relatively simple synthetic routes [10–13]. Four generations of quinolones have been developed so far [14–16], each bringing new range of activity; *i.e.*, enhanced gram-negative activity and systemic distribution of the second-generation, expanded activity against gram-positive bacteria and atypical pathogens of the third-generation and an additional activity against pneumococci and anaerobes of the fourth-generation quinolones [14]. The preparation of new generation of quinolones with broad-spectrum applications in medicinal practice, is mainly based on the modifications of the substitution pattern at the quinolone structure [3,14]. Consequently, an intensive effort is still oriented on the synthesis of quinolone derivatives with improved antibacterial activities and pharmacokinetic parameters [14], employing the target structural modifications of the quinolone derivatives in order to control their therapeutic properties [17–19]. The combination of the quinolone bacteriostatic potential with the antimycobacterial impact of nitro pharmacophore may result in the enhanced biological activities of the synthesized nitroquinolone derivatives [12,20–26]. Additionally, the presence of electron-withdrawing NO_2 chromophore causes an intense light absorption of nitroquinolones in UVA and visible regions, which motivates a progressive research of their photoinduced processes [27,28].

The quinolone-based drugs, generally considered as well tolerated, may exhibit several side effects [14,15] and among these the phototoxic properties represent a serious problem [29–31], which stimulated the photophysical, photochemical and photobiological studies on quinolones [32–38]. Since the photoinduced reactions of quinolone derivatives can involve different processes (e.g., decarboxylation, defluorization, oxidation of substituents, singlet oxygen and superoxide radical anion generation) [32–41] the detailed knowledge on the UV/vis radiation activity/sensitivity of quinolone derivatives is an important point to be considered for their potential applications in the biological systems.

The present study brings a complex spectroscopic characterization of selected 6-nitroquinolone derivatives (Table 1) using UV/vis, NMR and EPR spectroscopy. The role of amino hydrogen at nitrogen of the enaminone system (N–C=C–C=O) on the behavior of 6-nitroquinolone in aprotic dimethylsulfoxide (DMSO) solvent was evaluated from the sets of electronic spectra. The ability of 6-nitroquinolones to generate paramagnetic intermediates upon UVA photoexcitation (λ_{max} = 365 and 400 nm) was studied by means of in situ EPR spectroscopy using the spin trapping technique. The cytotoxic effects of 6-nitroquinolone **2** on cancer human (HeLa) and murine (B16) and non-cancer (NIH-3T3) cell lines was also evaluated.

Table 1
Overview of 6-nitroquinolones investigated.



R_1	R_2	6-Nitroquinolone
H	$COOCH_2CH_3$	**1**
CH_3CH_2	$COOCH_2CH_3$	**2**
H	COOH	**3**

2. Material and methods

2.1. Chemicals and reagents

The investigated 6-nitroquinolones were prepared as described in Ref. [42]. An overview of their structure, substituents characterization and numeration is summarized in Table 1.

The stock solutions of 6-nitroquinolones (c = 0.5–1 mM) were freshly prepared for each series of experiments in anhydrous DMSO (Merck, SeccoSolv, max. 0.05% H_2O). Due to their limited solubility in mixed solvent DMSO/water (50%, vol.) or deionized water, the solutions were prepared at lower concentrations. The spin trapping agent 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO; Sigma-Aldrich) was distilled before application. Other spin trapping agents, i.e., 2,3,5,6-tetramethylnitrosobenzene (nitrosodurene, ND; Sigma-Aldrich), α-(4-pyridyl *N*-oxide)-*N*-*tert*-butylnitrone (POBN; Sigma-Aldrich) and 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide (EMPO; Enzo Life Sciences) were used without purification. All spin trapping agents were stored at −18 °C. The stock solutions of the spin traps were prepared in DMSO. Since the solubility of ND in DMSO is limited, a saturated solution of ND in the presence of 6-nitroquinolone was prepared directly before measurement. The deuterated solvent d_6-DMSO was purchased from Merck and triethylamine (TEA) from Sigma-Aldrich. Superoxide dismutase (SOD; Sigma-Aldrich, from bovine erythrocyte, specific activity 4470 units/mg) was used for the target termination of superoxide radical anions. Other chemicals used, *i.e.*, NaOH, H_3PO_4, were obtained from Mikrochem (Slovak Republic).

2.2. Cell lines

Cancer cell lines HeLa (human cervical cancer cells) and B16 (murine melanoma cancer cells) and non-cancer cell line NIH-3T3 (murine fibroblast cells) were obtained from American Type Culture Collection (ATCC; Rockville, MD, USA). These cells were grown at 37 °C in DMEM medium in humidified 5% CO_2 and 95% air atmosphere. The medium was enriched with 10% (vol.) heat-inactivated fetal calf serum, penicillin G (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}). Before a uniform monolayer of HeLa, B16 and NIH-3T3 cells was formed, the cells were freed from the surface of the culture dish by a 0.25% solution of trypsin, and subcultivated two times a week. The cells were plated on the Petri dishes (diameter 60 mm) at a density of 4×10^4 (HeLa), 1×10^5 (B16) and 7×10^4 (NIH-3T3) cells per mL of medium and incubated for 24 h prior to the experiments. Cell viability was determined by a Trypan blue exclusion test. All culture medium compounds were obtained from Sigma-Aldrich, fetal calf serum was purchased from BIOCOM Company (Slovak Republic).

2.3. Experimental methods and apparatus

2.3.1. UV/vis spectroscopy

The UV/visible spectra of 6-nitroquinolones in DMSO were recorded at 25 °C using a UV-3600 UV/vis/NIR spectrophotometer (Shimadzu, Japan). A thermoelectrically temperature controlled cell holder (TCC-240A, Shimadzu) was used to control the temperature.

The sets of steady-state experiments were performed to monitor the changes in the electronic spectra of 6-nitroquinolones upon discontinuous irradiation in DMSO solutions or in mixed solvent (DMSO/water, 50%, vol.). The freshly prepared solutions were irradiated under air in a 1 cm quartz cell using a monochromatic LED source (λ_{max} = 365 nm or λ_{max} = 400 nm; Bluepoint LED, Hönle UV Technology). For the UV LED_{365} (λ_{max} = 365 nm) the irradiance value (13 mW cm^{-2}) was determined using