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Structural, vibrational and quantum chemical investigations for 6,7-dichloro-2-methyl-5,8-quinolinedione. Cytotoxic and molecular docking studies

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ABSTRACT

6,7-Dichloro-2-methyl-5,8-quinolinedione was investigated using different experimental methods as well as a variety of various quantum chemical calculations in order to characterize its molecular structure as a potential anticancer active compound. We used X-ray diffraction, IR spectrum analysis supplemented by the density functional theory (DFT) calculations, molecular electrostatic potential (MEP) and molecular orbital (HOMO, LUMO) analyses. The analyzes were carried out with respect to 6,7-dichloro-5,8-quinolinedione. It was found that introduction of the methyl group at the C-2 position distinctly affected the crystal structure, formation of H-bonds and the carbonyl stretching IR bands of the title compound. The molecular electrostatic potential map showed how the electrophilic and nucleophilic regions are located in the molecule. The intra- and intermolecular bonding and interaction between bonds were interpreted using the Natural Bond Orbital (NBO) and Natural Localized Molecular Orbital (NLMO) analysis.

The title compound was tested for its anticancer activity *in vitro* against the several human cancer cell lines. 6,7-Dichloro-2-methyl-5,8-quinolinedione showed a higher cytotoxic activity against cancer cell lines containing a higher level of NQO1 enzyme, like melanoma (C-32) and breast (MCF-7) cancer cell lines. The molecular docking was used to examine the probable interaction between the molecule of the tested compound and the NQO1 enzyme. The analysis showed that the 5,8-quinolinedione moiety of the title compound formed a hydrophobic interaction with phenylalanine (Phe 178), tyrosine (Tyr 126 and Tyr 128) and FAD cofactor.

1. Introduction

5,8-Quinolinedione was obtained in 1884 for the first time, as a product of oxidation of 5-amino-8-hydroxyquinoline [1]. Seventy years later, Urbanski and Krzyzanowski synthesized 6,7-dichloro-5,8-quinolinedione **1** in the three staged reaction (Fig. 1) [2]. Both compounds started to gain interest in 1959, when Rao and Cullen isolated the first 7-amino-5,8-quinolinedione antibiotic, streptonigrin, from bacteria *Streptomyces floccules* [3]. In the next decades, the bioactive compounds containing 5,8-quinolinedione moiety were obtained from different kinds of actinomycetes. All natural 5,8-quinolinedione antibiotics showed high biological activities such as anticancer, antimicrobial, antiviral, anti-inflammatory and anti-malarial activity [4–9]. Unfortunately, they are also toxic for

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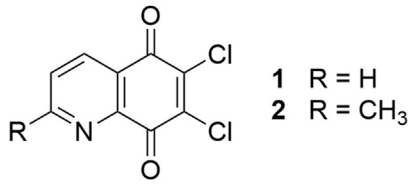

Fig. 1. Chemical structures of the 6,7-dichloro-5,8-quinolinedione **1** and the 6,7-dichloro-2-methyl-5,8-quinolinedione **2**.

many normal cell lines [10,11]. Numerous analyzes of the structure-activity relationship revealed that the most important structural fragment of this group of antibiotics is the 5,8-quinolinedione ring [12–18]. It was found that the 5,8-quinolinedione moiety can interact with nicotinamide quinone oxidoreductase 1 (NQO1, EC 1.6.99.2), resulting in generation of reactive oxygen species, which then can cause mutations of DNA [14,19,20].

The pharmacological activities of natural 5,8-quinolinedione antibiotics encouraged to search for simple analogues, which have a similar mechanism of action, a higher biological effect and a lower toxicity. Generally, the derivatives of 5,8-quinolinedione were synthesized from 6,7-dichloro-5,8-quinolinedione **1** or 6,7-dichloro-2-methyl-5,8-quinolinedione **2** (Fig. 1). According to the literature data [21,22], the introduction of the methyl group at the C-2 position induces changes in the biological activity of the obtained derivatives.

The compound **2** was synthesized for the first time in 1959 as a result of the oxidation of 5,8-diamino-2-methylquinoline [23]. According to the literature data, its X-ray crystal structure has not been described yet. The mechanism of its action with the NQO1 enzyme has not been well known [17,22,24].

In this study, the crystal structure, biological activity and molecular docking of 6,7-dichloro-2-methyl-5,8-quinolinedione **2** to the NQO1 enzyme are presented. For the sake of understanding of the active sites in the compound **2**, the molecular electrostatic potential surface (MEP) was plotted. The HOMO-LUMO orbitals and NBO analysis for the molecule of **2** allowed to elucidate its electronic properties. The IR spectra analysis for **2** supplemented by the density functional theory (DFT) calculations are raported. They are supplemented by the density functional theory (DFT) calculations.

2. Experimental

2.1. Synthesis of 6,7-dichloro-2-metyhyl-5,8-quinolinedione 2

The title compound **2** was prepared according to method described by Mulchin et al. [21]. 2-Methyl-8-hydroxyquinoline (1.00 g; 6.28 mmol) was dissolved in hydrochloric acid (26 mL) and heated to 60 °C, then the solution of sodium chlorate (0.67 g; 6.29 mmol) at portion was added. After 2 h at room temperature, the precipitate was filtered. The crude product was crystallized from ethanol obtaining 0.56 g (yield 56%) of pure 6,7-dichloro-2-metyhyl-5,8-quinolinedione **2**. M.p. 180–181 °C (lit. m.p. 179.1–181.3 °C [21]).

2.2. IR spectroscopy measurement

The infrared spectra (IR) of the compound **2** were recorded in the range 400–3500 cm^{-1} at room temperature using the IRAffinity-1 Shimadzu spectrophotometer. The spectrum resolution was 0.5 cm^{-1}. The KBr pellet method was used for the sample preparation.

2.3. X-ray diffraction analysis

Single crystal was obtained by recrystallization from the dichloromethane. The X-ray diffraction measurement of selected crystal was performed on the Xcalibur kappa diffractometer with the Sapphire3 CCD detector and MoKα radiation. The crystal structure was determined using the SHELXS-2013 and refined using SHELXL-2014/6 program [25]. The hydrogen atoms were found by analysis of the crystal structure using Mercury program. The obtained results were refined by Shelx program. The crystallographic data have been stored in the Cambridge Crystallographic Data Centre (CCDC) as CCDC-1445837. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.

2.4. Cytotoxic activity analysis

The compounds **1**–**2** were tested for cytotoxic activity *in vitro* against four human cancer cell lines: C-32 (human melanoma, ATCC), SNB-19 (human glioblastoma, DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), MDA-MB-231 (human breast adenocarcinoma, ATCC), MCF-7 (human breast adenocarcinoma, ATCC) and normal human fibroblastic cell line (normal human fibroblasts derived from foreskin, ATCC). Cisplatin was used as a reference compound. Details of the cytotoxic activity analysis were previously described in the following literature [22,26]. The tested compounds **1**–**2** with the concentration of 0.1–100 μg/mL DMSO were inducted with the cancer cells for 72 h. The WST-1-formazan (proliferation reagent WST-1 assay, Roche Diagnostics, Mannheim, Germany) was detected using a microplate reader at 450 nm. Results were expressed as a mean value of at least three independent experiments performed in triplicate.

2.5. Computational details

Calculations of the electronic structure for the title compound **2** were performed using the DFT method implemented in the Gaussian 09 program package [27]. Geometry optimization was carried out using the B3LYP exchange–correlation functional with the 6-311G (d.p) basis set. Comparing the calculated results with the X-ray crystallographic data shows good compatibility. The calculated geometries were used to compute electrostatic potential V(r) and the electronic density maps, which define the molecular surfaces [28]. The natural bond orbital analysis was performed by NBO 3.1 program implemented in the Gaussian 09 program [29]. The theoretical wavenumbers were scaled by a factor of 0.9613 [30]. All obtained results were visualized in the GaussView, Version 5 software package [31].

2.6. Hirshfeld surface analysis

The percentage contributions for intermolecular contacts in 6,7-dichloro-2-methyl-5,8-quinolinedione were obtained Hirschfield surface analyses, and 2D fingerprint plots were performed by Crystal-Explorer v.3.1 program [32]. Both *de* and *di* are responsible for the normalized contact distance (d_{norm}).

2.7. Molecular docking study

The crystal structure of human NQO1 enzyme required for molecular docking was collected from the Protein Data Bank (PDB) database with the PDB identifier 1H69 [33]. In the experiment, atomic coordinates of 1H69.pdb dimer named AC composed of two identical monomers (A and C) were used. Ligand's molecule was