

STUDY OF THE OPTICAL PROPERTIES OF 2-THIOHYDANTOIN DERIVATIVES

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Abstract. This study presents the optical properties of a new hydantoin derivative, generically called SZ-2. A stability assay using UV/VIS/NIR spectra was performed up to 250 days, as well as FTIR spectroscopic characterization. Liquid samples of SZ-2 in DMSO in bulk were exposed to 355 nm pulsed laser radiation emitted by a Nd:YAG laser for different time intervals. The behavior of SZ-2 molecules under laser beam influence was highlighted based on their absorption spectra before and after exposure to coherent light. Also, the ability to generate singlet oxygen of SZ-2 was investigated through a photochemical method using 355 nm Nd:YAG laser beam irradiation.

Key words: electronic spectra, FTIR spectroscopy, hydantoin derivatives, multidrug resistance, Nd:YAG, singlet oxygen.

1. INTRODUCTION

To-date it is a challenge to identify ways to combat the failure of treatment of infectious diseases and cancer, due to the multidrug resistance acquired by bacteria and/or malignant tumors.

In cancer patients, the major mechanism of multidrug resistance is the elevated expression of ATP-dependent drug efflux pumps, which reduces the accumulation of anticancer agents [1]. In this respect, there is a need to find new drugs or to manipulate the existing ones in order to break through multidrug resistance.

A group of chemicals whose anticancer properties were recently studied is constituted by the hydantoins class [2, 3]. Hydantoin (2,4 – imidazolidinedione) is a heterocyclic organic compound which was obtained initially through the

reduction of allantoin, a constituent of urine. Since its synthesis in 1861 by Baeyer, a huge number of derivatives have been prepared [4]. Among these, several 5,5-disubstituted hydantoin derivatives have medical applications as hypnotics and, in the treatment of epilepsy, as anticonvulsants. Depending on the nature of substitution on the hydantoin ring, these derivatives possess a wide range of other pharmaceutical properties, including antitumor, anti-inflammatory, anti-HIV, hypolipidemic, anti-arrhythmic, and anti-hypertensive effects. More than these, recently, the cytotoxic properties of spirohydantoin derivatives were tested in ovarian and breast cancer cells [2]. Also, anti-cancer effect of Palladium (II) complex of thiohydantoin was studied on the human colon carcinoma cell lines [3]. Withal, hydantoin, thiohydantoin, and their substitution products have many other uses in agriculture or industry fields [5].

In this study, the optical properties of 5-(3-chlorobenzylidene)-2-thioxoimidazolidin-4-one ($C_{10}H_7ClN_2OS$, $M = 238.69$ g/mol), generically called SZ-2 are presented. A stability assay using UV/VIS/NIR spectra was performed up to 250 days, as well as FTIR spectroscopic characterization.

Liquid samples of SZ-2 in dimethyl sulfoxide (DMSO) in bulk were exposed to the third harmonic of a pulsed Nd:YAG laser for different time intervals. The behavior of SZ-2 molecules under laser beam influence was highlighted based on their absorption spectra before and after exposure to coherent light.

Also, samples of SZ-2 were tested as possible photosensitive agents for photodynamic therapy through the photochemical method using 355 nm Nd:YAG laser beam irradiation.

2. MATERIALS AND METHODS

5-(3-chlorobenzylidene)-2-thioxoimidazolidin-4-one ($C_{10}H_7ClN_2OS$, $M = 238.69$ g/mol), generically named SZ-2 (Fig. 1) was synthesized at Jagiellonian University, Cracow, Poland [6, 7].

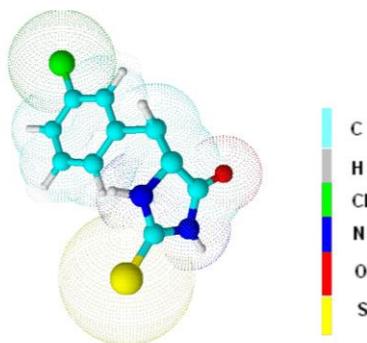


Fig. 1 – The 3D chemical structure of SZ-2.

The compound is presented under the form of light yellow crystals, keeping the appearance of thiohydantoin [8]. It was dissolved into DMSO (purchased from Merck, purity $\geq 99\%$) in a concentration range between $10^{-5}M$ and $10^{-3}M$. There were three groups of solutions that were kept in the following conditions: at $4^{\circ}C$ in dark, at $22^{\circ}C$ in dark, and at $22^{\circ}C$ in ambient light.

Solutions of SZ-2 were characterized by FTIR spectroscopy using the Nicolet IS50 FTIR spectrophotometer (Thermo Scientific, USA). The apparatus operates with Omnic 9 Standard software and the utilized experimental spectral resolution was 0.09 cm^{-1} .

Stability study of SZ-2 solutions in DMSO was performed up to 250 days in quartz cells of 0.1 cm optical path using an UV/VIS/NIR spectrophotometer (Lambda 950, Perkin Elmer, USA). The used experimental spectral resolution was 0.05 nm for UV/VIS domain and 0.2 nm for NIR spectral range. The cumulated measuring errors limit is $\pm 1.045\%$, which contains the measuring error limit of the spectrophotometer, and the cells positioning error [9].

An 1 cm^3 volume of SZ-2 solution at $10^{-4}M$ in DMSO was irradiated with a laser beam emitted at 355 nm by a pulsed Nd:YAG laser (Surelite II, Excel Technology, Continuum, USA) up to 30 min. The output laser beam was directed to the sample quartz cell through an UG5 optical filter in order to cut-off the residual 532 nm radiation. 10% of emitted beam was splitted to a powermeter (QE25 Gentec, Canada) for monitoring the beam energy on the sample cell. The laser radiation had the following characteristics: 10 pps – pulse repetition rate, 5 ns – FTWHM, and the beam energy on the sample – 38 mJ.

Singlet oxygen was generated via the excitation of SZ-2 molecules at 355 nm using the third harmonic generation (THG) of a pulsed Nd:YAG laser (Continuum Minilite II, USA, frequency 10 Hz, energy 3.8 mJ). The singlet oxygen phosphorescence (at 1270 nm) was detected by a NIR photomultiplier (Hamamatsu H-10330, Japan) and registered with a digital scope (Tektronix DPO 7254, USA). The phosphorescence signals were averaged over 1000 laser pulses. The experimental set-up was described in detail elsewhere [10]. The lifetime of singlet oxygen is given based on the time constant of the mono-exponential fitting curve for the phosphorescence signal.

The quantum yield for singlet oxygen generation by SZ-2 was measured relative to the reference ZnPc in DMSO [11]. The samples were measured under the same experimental conditions, in air equilibrated solutions. The quantum yield is given by the formula [12]:

$$\Phi = \Phi_{ref} \frac{I}{I_{ref}} \frac{A_{ref}}{A} . \quad (1)$$

In equation (1) Φ is the quantum yield of singlet oxygen generation, I is the phosphorescence intensity of singlet oxygen extrapolated at $t=0$, and A is the absorbance at 355 nm. Subscript *ref* corresponds to ZnPc in DMSO. The

phosphorescence intensity at $t = 0$ is obtained by extrapolation to $t = 0$ of the fitting curve for the phosphorescence signals.

3. RESULTS AND DISCUSSIONS

The FTIR spectrum of SZ-2 solution in DMSO reveals typical maximum for C=O (bending) and C=S (in plane stretching) vibrations at 517 cm^{-1} . Regarding the vibrational modes of C-H coupling, although the position of its stretching and bending frequencies remains nearly constant in hydrocarbons, the attachment of CH_3 or CH_2 to atoms other than carbon, or to a carbonyl group or aromatic ring may result in a significant shift of the C-H stretching and bending frequencies [13, 14]. So, absorption arising from C-H stretching may be observed in $(2900\text{--}3200)\text{ cm}^{-1}$, while the asymmetrical bending vibration occurs near 1450 cm^{-1} . The out-of-plane C-H bending vibration between $(1000\text{--}650)\text{ cm}^{-1}$ is assigned to alkene ring, and some absorption peaks in $(1563\text{--}700)\text{ cm}^{-1}$ belong to vibrations involving interaction between C=S stretching and C-N stretching. The peaks at 690 cm^{-1} and 704 cm^{-1} may be assigned to C-Cl in plane stretching vibrations, C=S out-of-plane deformations, and torsion of the aromatic ring.

At 1660 cm^{-1} occurs the absorption fingerprint of cyclo-alkene due to the C=C stretch vibration coupled with C-C stretching of the adjacent bond. It is shifted compared to the unconjugated alkene typical absorption ($\sim 1640\text{ cm}^{-1}$) probably because the angles between C-C and C=C become smaller and the interaction of these two bounds decreases. Also, between 1870 and 1540 cm^{-1} it can be observed the C=O stretching absorption band. Within its given range the position of C=O is determined by the following factors: the physical state, electronic and mass effects of neighboring substitutes, conjugation, hydrogen bonding and ring strain. The resonance effect of -NH increases the C=O bond length and reduces the frequency of absorption comparing to the typical ketone fingerprint [13–15].

This thiohydantoin derivative has two N-H bonds in its molecule. One is adjacent to a carbonyl radical, and other is surrounded by both carbonyl and thiocarbonyl radicals. Since the two N-H bonds are not equivalent, they show different acid-base properties. Also, the addition of DMSO as solvent pointed out different acid properties of the two N-H bonds [16]. The absorption peaks located in $(3500\text{--}3400)\text{ cm}^{-1}$ interval could be assigned to the N-H stretching vibration occurred in dilute solutions. In the meantime, the absorption in $(900\text{--}700)\text{ cm}^{-1}$ domain arises from N-H wagging of secondary amine [13]. The two N-H bonds of SZ-2 solution in DMSO give two N-H stretching signals, at 3448 cm^{-1} and 3483 cm^{-1} . One may suppose that the higher wavenumber band corresponds to the N-H stretching vibration of the N-H bond adjacent to carbonyl radical and the lower wavenumber band to the N-H bond surrounded by the carbonyl and thiocarbonyl radicals [17].

As a result of light absorption, SZ-2 molecules pass from the ground state to a higher energy electronic level. The most probable transition occurs from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), with energy differences between electronic levels which vary from 125 to 650 kJ/mole [18].

UV/VIS spectra of all the imidazolidines, whose structures are very close to uracil framework exhibit absorption peaks in (200–250) nm and (300–400) nm spectral regions. The first absorption band is attributed to $\pi - \pi^*$ transition of N-C=O chromophore and the second one to the corresponding transition of carbonyl group [19]. The difference in peak positions is due to the effect of attached substituents. These spectral characteristics are due most likely to $\pi - \pi^*$ transitions of the carbonyl group of imidazolidine ring rather than $n - \pi^*$ transitions of it. This is because of the benzene ring connected to the hydantoin frame, which causes extensive delocalization in this structure and is thus responsible for $\pi - \pi^*$ transitions [20–23].

The UV/VIS absorption spectrum of SZ-2 solution in DMSO (Fig. 2) is characterized by broad bands due to simultaneous electronic and vibrational-rotational excitation. The absorption peaks are located at 254 nm, 293 nm, 364 nm, 379 nm and 445 nm. It could be observed the higher magnitude of maximum located at 364 nm and 379 nm, which are assigned to carbonyl chromophore in position 4 of the imidazolidine ring due to greater delocalization [20].

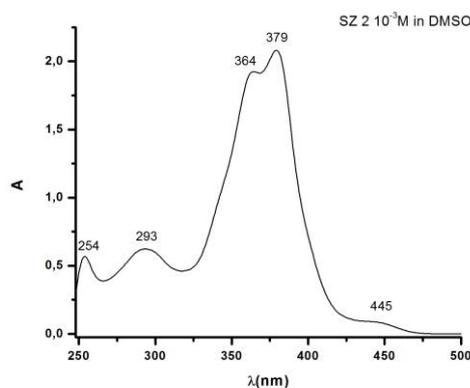


Fig. 2 – UV/VIS absorption spectrum of SZ-2 in DMSO.

We have to mention that the solution pH is increasing with its concentration. This behavior is accompanied by 8 nm bathochromic shift of the first absorption peak and a very slight color change from colorless solution at lower pH to light yellow in strong basic media. This color change for $10^{-3} M$ SZ-2 solution in DMSO corresponds to the appearance of the 445 nm absorption maxima, which indicates that the solution begins to absorb violet light thus appearing yellow in basic environment [24, 25].

Stability studies of SZ-2 solutions in DMSO were performed in three light/temperature conditions for solutions concentrations between $10^{-5}M$ and $10^{-3}M$, as it was specified above. All the monitored solutions have not preserved the absorption characteristics, considering the cumulated measuring errors [9] (Fig. 3).

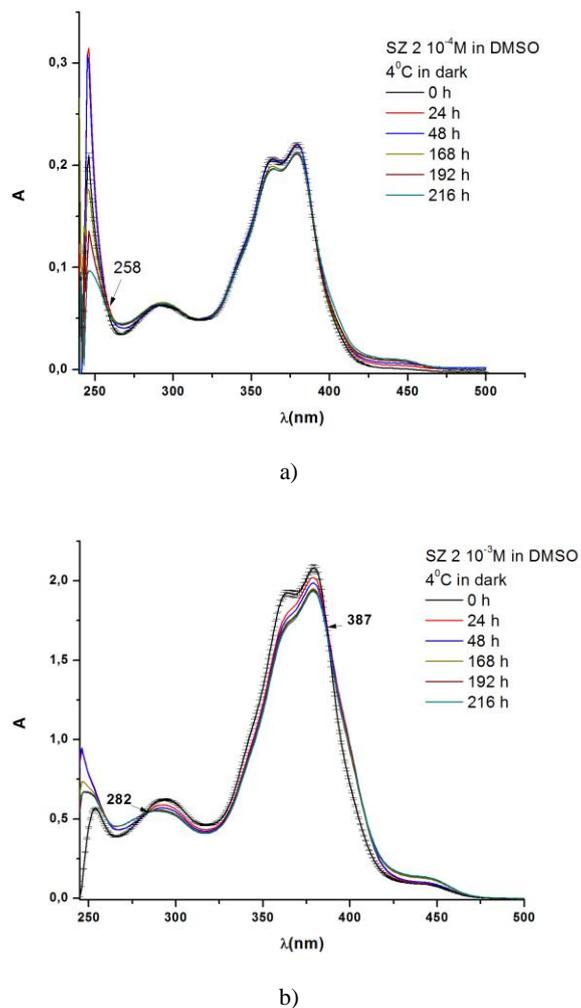


Fig. 3 – The absorption spectra of SZ-2 solutions in DMSO kept in dark at 4°C:
a) $10^{-4}M$ and b) $10^{-3}M$.

As an example, Fig. 3 presents the absorption spectra of SZ-2 solution in DMSO kept in dark at 4°C up to 9 days (216 h), including the experimental error bar, for two concentrations: $10^{-4}M$ and $10^{-3}M$. These pictures show slight decrease

in absorbance, that exceeds the experimental errors limits along with isosbestic points as follows: at 282 nm and 387 nm for solutions at $10^{-3} M$ concentration, and at 258 nm for $10^{-4} M$ concentration when solutions kept in dark at both 4 °C and 22 °C.

During the time interval extended for studies, for solutions at concentration $10^{-3} M$ kept in dark at 4 °C we pointed out an 8 nm blue shift of 254 nm peak and a 5 nm blue shift of 294 nm absorption peak; this behavior is highlighted for both $10^{-4} M$ and $10^{-3} M$ concentrations in ambient temperature conditions (22 °C). More than this, for solutions kept at 22 °C in ambient light conditions, it was observed the appearance of new absorption peaks in NIR at 1456 nm and 1557 nm, whose intensities increase in time. Also, a 4 nm hypsochromic shift of 1940 nm maximum was noticed (Fig. 4).

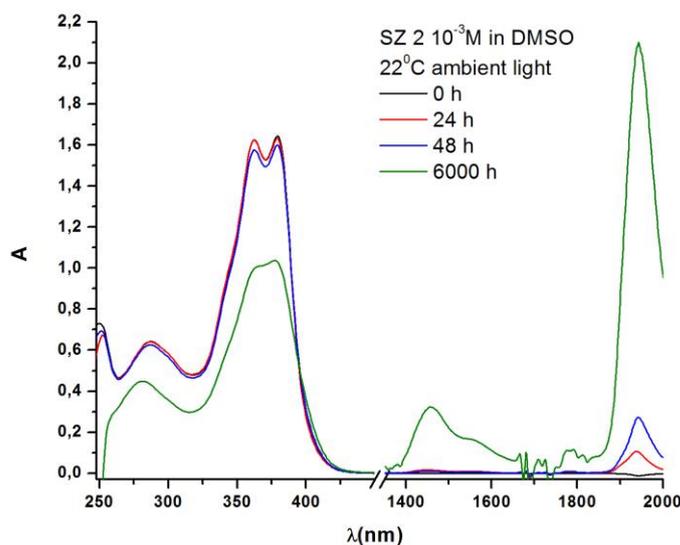


Fig. 4 – The absorption spectrum of SZ-2 solution in DMSO kept at 22°C in ambient light conditions.

SZ-2 solution samples were exposed to pulsed Nd:YAG laser radiation emitted at 355 nm up to 30 min. After each irradiation session the absorption spectra were acquired in order to evaluate molecular modifications induced by laser beam exposure, eventually (Fig. 5).

We observed that the spectral fingerprint in UV disappeared after 10 min of pulsed laser irradiation. In the meantime, the 246 nm peak increases with the duration of laser beam exposure.

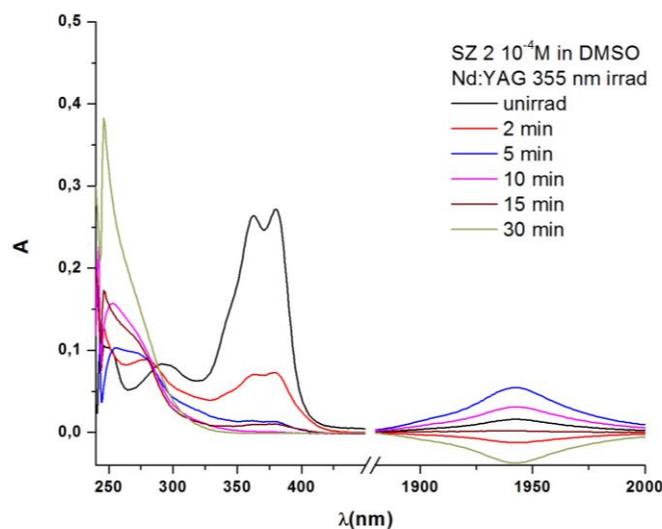


Fig. 5 – The absorption spectra of SZ-2 $10^{-3} M$ solution in DMSO.

In Fig. 6 the absorption spectra for studied solutions prepared in DMSO for SZ-2 at $10^{-4} M$ concentration and the standard ZnPc at $5 \times 10^{-5} M$ are shown.

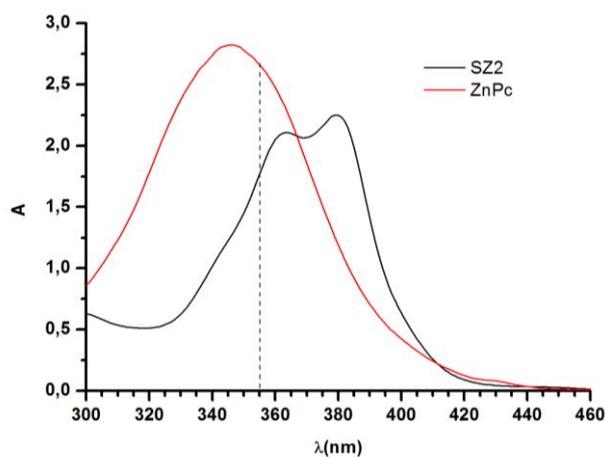


Fig. 6 – Absorption spectra of SZ-2 $10^{-4} M$ and of standard ZnPc $5 \times 10^{-5} M$ solutions in DMSO.

The phosphorescence of the singlet oxygen generated by SZ-2 sample is given in Fig. 7. A typical singlet oxygen lifetime of about $6 \mu s$ is obtained when DMSO is used as solvent [10].

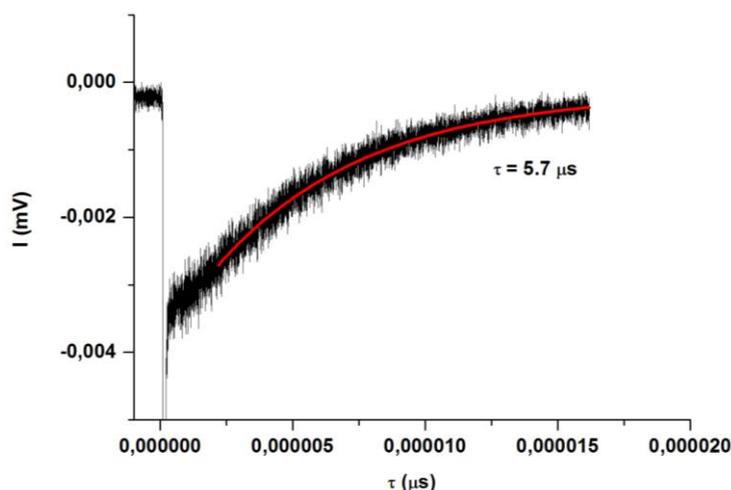


Fig. 7 – The phosphorescence signal for singlet oxygen generated by SZ-2 sample.

Using equation (1) and the signals measured in the same conditions for SZ-2 and ZnPc samples, a quantum yield value of 0.12 for SZ-2 singlet oxygen generation was obtained.

4. CONCLUSIONS

The reported results show that the new compound 5-(3-chlorobenzylidene)-2-thioxoimidazolidin-4-one ($C_{10}H_7ClN_2OS$), generically called SZ-2, which is member of Hydantoin class of medicines is not stable if dissolved in DMSO when it is exposed to UV laser beams or to the ambient light, nor kept in dark. Also, the solutions explored are sensitive to storage temperature conditions.

This is important from the following point of view: the medicine may be modified in view of applications for which the parent compound was not produced, by exposure to UV laser radiation in controlled conditions. In this case, besides the molecular modifications evidenced by UV-VIS absorption spectroscopy we have demonstrated that singlet oxygen is produced. This could recommend the compound for photodynamic therapy applications or, at least, for use at a larger scale, namely using the effects against bacteria that acquired multiple resistance at treatment with antibiotics.

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