ORIGINAL ARTICLE

SPECTRAL AND PHOTOCHEMICAL PROPERTIES OF HYPERBRANCHED NANOSTRUCTURES BASED ON GARDIQUIMOD AND $TPPS_4$

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Abstract

This paper examines certain experimental studies on photophysical (absorption maxima, molar absorption coefficient) and photochemical (singlet oxygen quantum yield) properties of Gardiquimod (GIQ) and its complex with 5,10,15,20-tetra-p-sulphonato-phenyl-porphyrin ($TPPS_4$), as new photosensitizer agent in photodynamic therapy (PDT). The absorption spectra and atomic force microscopy are used, highlighting the complex between both compounds, and its nanometric size.

Rezumat

În această lucrare sunt prezentate studii experimentale ale unor proprietăți fotofizice (maximele de absorbție si coeficienții molari de absorbție) și proprietăți fotochimice (randament cuantic de generare a oxigenului singlet) ale Gardiquimod (GIQ) și complexului său cu 5,10,15,20-tetra-p-sulfonato-fenil-porfirină ($TPPS_4$), ca nou agent fotosensibilizator în terapia fotodinamică. Sunt utilizate spectrele de absorbție și microscopia de forţă atomică, punând în evidență complexul format între cei doi compuși și dimensiunile nanometrice ale acestuia.

Keywords: gardiquimod (GIQ), $TPPS_4$, photodynamic therapy

Introduction

Imiquimod (IQ), known as potential antiviral agent, the first member of the new class of immune response modifiers, is a nucleoside analogue that stimulates the innate immune response via the induction, synthesis and release of specific cytokines [1]. Certain literature reports about its electronic properties, electronic and fluorescence spectra have already been reported [2, 3]. This drug is extensively used in various skin diseases [4-10], although other drugs are good competitors [11].
Gardiquimod (GIQ) [1-(4-amino-2-ethyl-amino-methyl-imidozo[4,5-c] quinolon-1-yl)-2-ethylpropan-2-ol] as IQ analogue is a selective agonist of human and mouse TLR7. This compound induces activation of NF-κB in cells expressing human or mice TLR7 and is more potent than imiquimod [12]. Gardiquimod operates as both an immune system modifier and a reverse transcriptase inhibitor and could be developed as a novel therapeutic agent to block systemic and mucosal transmission of HIV-1 [13].
For improvement of their effectiveness, certain alternative treatment methods have been introduced in clinical practice, photodynamic therapy (PDT) being one example. This is a noninvasive treatment of small and superficial tumours and other non-oncological pathologies, based on topical or systemic administration of a photosensitizer (PS) followed by illumination with light of appropriate wavelength for photoactivation of the PS. The resulting photodynamic reactions yield to cytotoxic singlet oxygen ($^1O_2$) [14, 15].
So far, only imiquimod has been applied in photodynamic therapy, coupled with MAL-PDT for treatment of actinic keratosis and basocellular carcinoma treatment [16, 17].

This paper examines the photophysical properties (absorption maxima, molar absorption coefficient) and photochemical properties (singlet oxygen quantum yields) of Gardiquimod (GIQ) and its complex with 5,10,15,20-tetra-p-sulphonato-phenyl-porphyrin ($TPPS_4$), as new sensitizers in photodynamic therapy (PDT). The complexation with $TPPS_4$ has been experimented in order to improve the efficacy of Gardiquimod in certain dermatologic disease. Their properties have vital significance in the future study and hopefully our data can help in finding some indications for future use in medical applications.

Materials and Methods

Gariquimod, 98% HPLC, Sigma. Its molecular structure is shown in Figure 1.

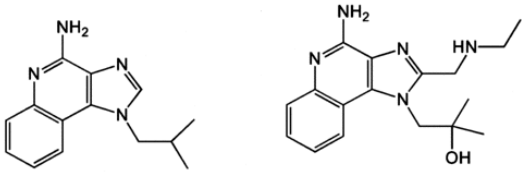

Figure 1.
The structures of imiquimod and gardiquimod

Absorption spectra were registered by means of a SPECORD M400, Carl Zeiss Jena double beam spectrophotometer, provided with a microprocessor. Quartz cuvettes with 0.5-2 cm optical path lengths and containing 1 mL of cell suspension each were used. Molar extinction coefficients at a given wavelength, ε, were obtained using the Beer-Lambert law over the 10^{-4}-10^{-3} mol*dm^{-3} concentration range.

Atomic force microscopy (AFM) investigations were carried out with an Agilent 5500 SPM system, described by PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller from Agilent Technologies, Tempe, AZ, USA (formally Molecular Imaging). The original images for the samples, the 3D topographical images and section analysis over the particles were performed using the PicoView SPM Software, version 1.6.2, Molecular Imaging. Height image data obtained by the AFM is three-dimensional.

The singlet oxygen generation has been determined by the 1,3-diphenylisobenzofuran method (DPBF), after literature method [18].

Measurements were carried out in a quartz cell (1cm x 1cm) at 25°C. Oxygen-saturated solutions (V = 0.2 mL) containing sensitizer and DPBF (c = 0.858 x 10^{-4} M) in DMSO were used. The photolysis cell was placed into the light beam of an UV-Vis spectrophotometer (Carl Zeiss Jena as described above), with DMSO as reference. The decreasing DPBF concentration was followed by a wave program measuring absorbance at 423 nm (ε = 23300 M^{-1}*cm^{-1}) as a function of irradiation time (irradiation cycles, 20 x 20 sec). The rate of DPBF photodegradation in cells was measured according to the following equation:

$$1/\Phi[DPBF] = 1/\Phi^1O_2 + 1/\Phi^1O_2\, k_d/k_a\, 1/[DPBF] \quad (1).$$

The singlet oxygen trap, DPBF, obtained from Fluka (Purum grade) was used as received.

Results and Discussion

GIQ has a relatively complex UV-Vis spectrum, more located in the UV region. Its molar absorption coefficient is rather low, so an enhancer is necessary. At low concentrations (1.13 x 10^{-3} M), the spectrum is dominated by the strong π-π* absorption of the monomer.

Table I
Spectral parameters of GIQ

Compound	Absorption (DMSO)	
	Wavelength λ (nm)	Molar absorption coefficient ε (M^{-1}*cm^{-1})
GIQ	223	3415
	272	1872
	318	1778
	333	1823

As the concentration is increased (1.59 x 10^{-3} M), the monomer band absorption is hypsochromic shifted with 4 nm, this band being assigned to an aggregated form. The appearance of an isosbestic point at 234 nm is consistent with two-component equilibrium in the concentration range depicted in Figure 2.

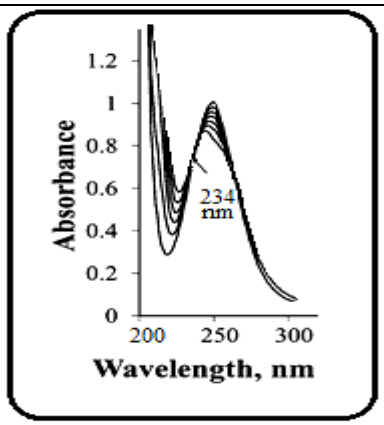

Figure 2.
Changes of UV-Vis spectrum absorption during increase of concentrations (DMSO)

Singlet oxygen generation was performed with DPBF and GIQ that generates ROS upon irradiation. As a ROS quencher, DPBF readily undergoes 1,4-cycloaddition reaction with singlet oxygen to form an endoperoxide that decomposes into the irreversible product (1,2-dibenzoylbenzene) [18]. As shown in the inset of Figure 3, the optical densities of GIQ absorption peak at 427 nm are not actually changed by the irradiation of light in the presence of DPBF, by comparison with the DPBF absorption peak at 423 nm, which indicates generation of ROS from the photo-irradiated surface of the GIQ. Under such circumstances, the quantum yield of $TPPS_4$ is 0.67, for GIQ is 0.23 and for its complex is 0.77, so most approximately as sum of the above compounds.

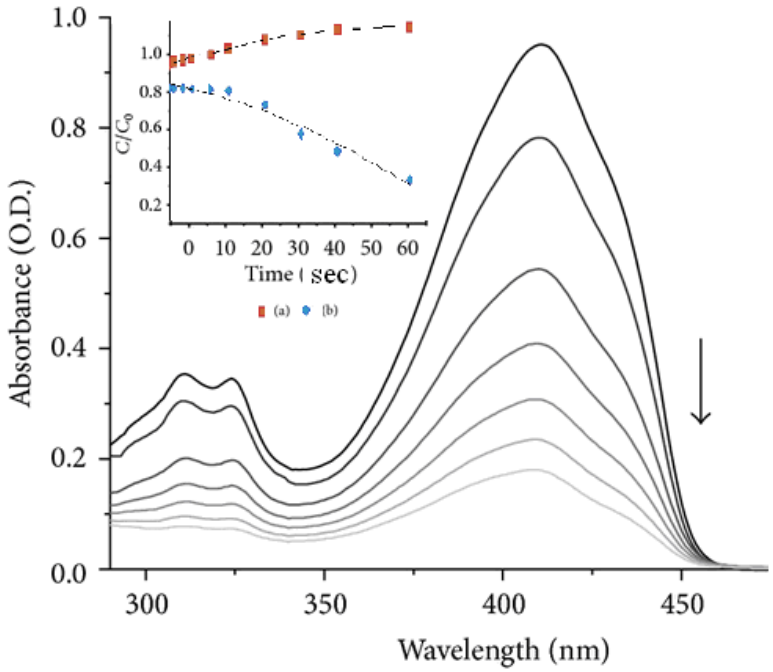

Figure 3.
Reaction time dependent UV-Vis spectra of DPBF in the presence of the GIQ in DMSO solution with polychromatic light irradiation. The inset represents: (a) DPBF in the presence of GIQ during the light irradiation; (b) GIQ in the presence of DPBF during light irradiation.

$TPPS_4$ is an anionic porphyrin, with a very large disk-shape molecule possessing four negative charges sustained by the sulphonate groups from the four corners. The electronic absorption spectrum of $TPPS_4$ as free base is characterised by an intense Soret band at around 420 nm and four Q bands in the 500-700 nm range [19, 20]. By aggregation at concentration higher than 10^{-5} M, new absorption bands (from 490, 707 nm) become dominant, attributed to the aggregated forms (H and J-aggregates) of $TPPS_4$, Figure 4.

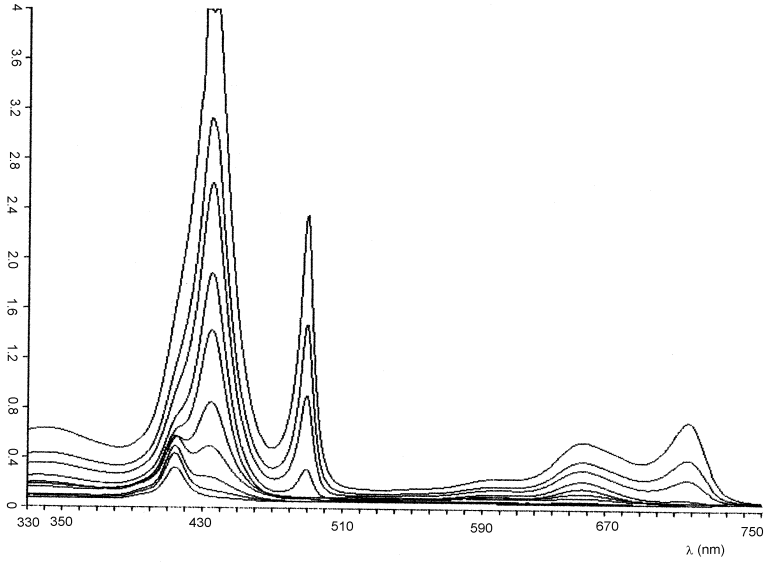

Figure 4.
The absorption spectrum of $TPPS_4$ during aggregation

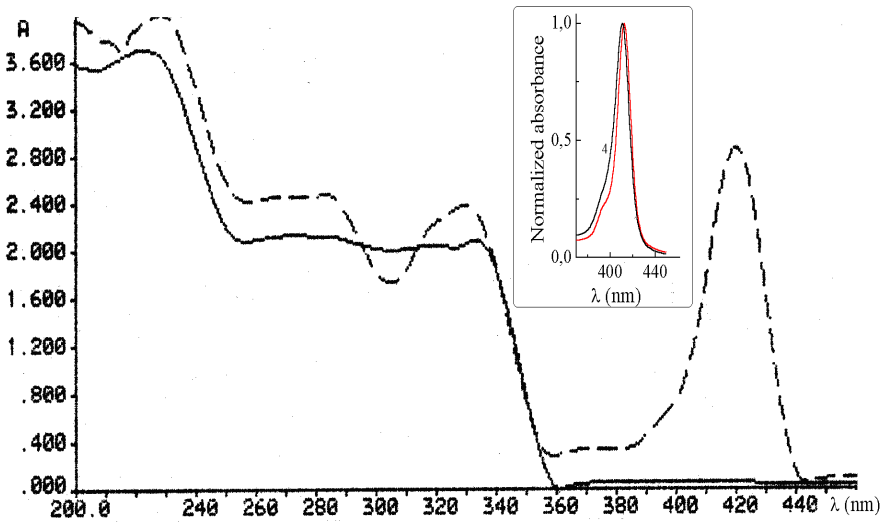

Figure 5.
The complex $TPPS_4$-GIQ (The inset shows the Soret band shift of $TPPS_4$ during complexation with GIQ ($TPPS_4$-GIQ isred).

By interaction of GIQ with $TPPS_4$, a physical type interaction (hydrogen and electrostatic) occurs between the sulphonate groups belonging to porphyrins and GIQ amino groups, Figure 5, highlighted by a strong GIQ batochromic shift f, from 227 to 240 nm. All other GIQ absorption bands f became more resolved and overlapped (see the bands from 318 and 333 nm). Also, the Soret band of $TPPS_4$ is red shifted whereas the Q-bands are blue shifted, as solid proof for complexation between both compounds, in agreement with literature [21, 23]. The posssible structure of this new system is shown in Figure 6.

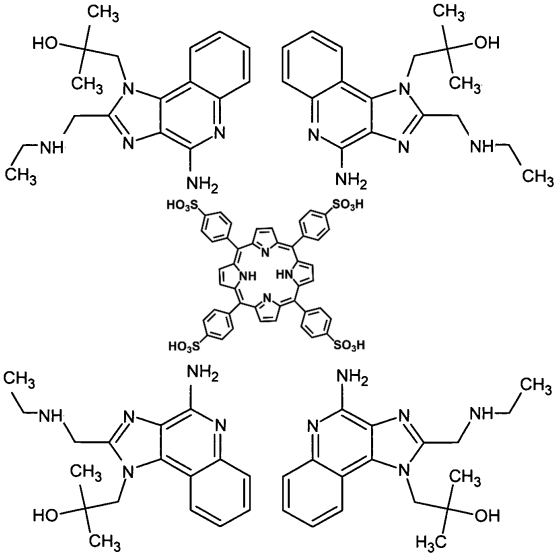

Figure 6.
GIQ –$TPPS_4$ molecular structure

The morphology of thenanostructures GIQ-$TPPS_4$ was characterised using atomic force microscopy (AFM). By complexing with $TPPS_4$, gardiquimod became more homogeneously distributed and assumed a cross shape. $TPPS_4$ is 50 nm in size, with a cylinder shape and long spatial distribution, while the GIQ – $TPPS_4$ is cross shaped and around 40 nm in size. The new GIQ – $TPPS_4$ shape could be explained by spatial redistribution of GIQ around the porphyrin $TPPS_4$ macrocycle, avoiding the aggregation capacity of the porphyrin. The first evidence of the size and shape of GIQ – $TPPS_4$ has been obtained by AFM, Figure 7.

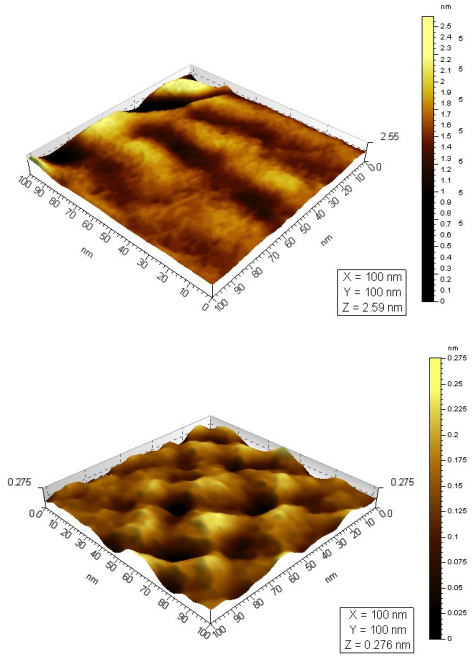

Figure 7.
$TPPS_4$ atomic force microscopy of (above) and its complex with GIQ (below)

This new structure could be more encapsulated into cells, penetrating the cellular membrane more easily [22].

Conclusions

This paper examines the photophysical properties (absorption maxima, molar absorption coefficient) and photochemical properties (singlet oxygen quantum yields) of Gardiquimod (GIQ) and its complex with 5,10,15,20-tetra-p-sulphonato-phenyl-porphyrin ($TPPS_4$),as new sensitizers in photodynamic therapy (PDT). GIQ has a UV-Vis spectrum, located in the UV region, with low molar aggregation coefficients, and is able to aggregate at concentrations higher than 1.59 x 10^{-3} M. GIQ is able to form a complex with $TPPS_4$, with sizes around 40 nm and higher singlet oxygen quantum yield than individual components ($TPPS_4$ - 0.67, GIQ - 0,23, GIQ –$TPPS_4$ - 0.77). The new system has vital significance in the future study and hopefully our data can help in finding certain indications for future use in medical applications.

Acknowledgements

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