RESEARCH ARTICLE

# REVISED Photophysical study and *in vitro* approach against *Leishmania panamensis* of dicloro-5,10,15,20-tetrakis(4-bromophenyl)porphyrinato Sn(IV) [version 3; peer review: 2 approved]

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## Abstract

**Background:** Photodynamic therapy activity against different biological systems has been reported for porphyrins. Porphyrin modifications through peripheral groups and/or by metal insertion inside the ring are main alternatives for the improvement of its photophysical properties. In this study, we synthesized and characterized 5,10,15,20-tetrakis(4-bromophenyl)porphyrin and the dicloro-5,10,15,20-tetrakis(4-bromophenyl)porphyrinato Sn(IV).
**Methods:** Metal-free porphyrin was synthesized using the Alder method, while the Sn(IV)-porphyrin complex was prepared by combining metal-free porphyrin with stannous chloride in DMF; the reaction yields were 47% and 64% respectively. Metal-free porphyrin was characterized by UV-Vis, FT-IR, ESI-mass spectrometry and $^{13}$C-NMR. Additionally, the Sn(IV) -porphyrin complex was characterized using UV-Vis and FT-IR. Cyclic voltammetry tests in four different solvents. The fluorescence quantum yield ($\Phi_f$) was measured using fluorescein as a standard, the singlet oxygen quantum yield ($\Phi_D$) was estimated using the standard 5,10,15,20-(tetraphenyl)porphyrin (H2TPP) and the quencher of singlet oxygen 1,3-diphenylisobenzofuran (DPBF).
**Results:** UV-Vis assay showed typical Q and Soret bands for porphyrin

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Any reports and responses or comments on the

and its metallo-porphyrin complex. Compounds showed photoluminescence at the visible range of electromagnetic spectrum. The inclusion of the metal in the porphyrin core changed the $\Phi_f$ from 0.15 to 0.05 and the $\Phi_D$ increased from 0.55 to 0.59. Finally, the effect of the compounds on the viability of *L. panamensis* was evaluated by means of the MTT test. The results showed that both compounds decreased the viability of the parasite; this inhibitory activity was greater under light irradiation; the porphyrin compound had $IC_{50}$ of 16.5 μM and the Sn(IV)-porphyrin complex had $IC_{50}$ of 19.2 μM. **Conclusion:** The compounds were synthesized efficiently, their characterization was carried out by different spectroscopy techniques and their own signals were evidenced for both structures, both compounds decreased the cell viability of *L. panamensis*.

**Keywords**
Photodynamic therapy, porphyrin, Leishmania panamensis, Photophysical study, in vitro, porphyrinato

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**REVISED** **Amendments from Version 2**

We have made improvements to this version of the article thanks to the suggestions of reviewer 2, the changes realized are described below. We thank reviewer 2 for the suggested corrections, all were very accurate and solicited valuable information that improved this version of the article.

1. Methodology: (a) Added punctual information on the parasite inoculum used: inoculum of 5 × $10^6$ cells/mL (promastigote). (b) Information was provided on the type of microplates used: 96-well microplates. (c) Relevant information was provided on incubation and irradiation times of the parasite cultures versus treatments: the assays were irradiated for 24, 24, and 72 hours.

2. Results: (a) We have corrected the range of photoactivation of the compounds; by mistake we had included 400-7000 nm, being correct 400-700 nm. (b) We have changed reference 53, for a more appropriate one from Valery Tuchin's. (c) In Figure 5b, we provide details on the label about points I, II, and III and the solvent used. (d) The exact *p*-values obtained from the statistical comparisons are included. (e) We have included an item on the results of leishmanicidal activity of compounds 1 and 2, in addition, the statistical comparisons obtained where it is shown that the activities are similar.

3. Bibliographies: We changed reference 53, for a more appropriate one from Valery Tuchin's.

**Any further responses from the reviewers can be found at the end of the article**

## Introduction

Porphyrins and metalloporphyrins are versatile macrocyclic organic compounds, from the structural viewpoint; the porphyrin main skeleton consists of four pyrrole rings bound through their alpha carbons (α-C) with four aldehydes.[1,2] These structural characteristics confer on the porphyrins a variety of properties such as high conjugation, symmetry, and planarity. Additionally, they acquire the ability to complex with a large number of metals in their interior through the coordination of the four pyrrolic nitrogen atoms submerged in the molecule.[3,4] The conjugated and aromatic structure of porphyrins allows interactions between π electrons and different metals, facilitating the binding to their coordination centers.[5] Porphyrins have absorption and intense electronic emission at wavelengths greater than 400 nm, small energy for HOMO-LUMO transitions and the ability to adjust their optical redox properties.[6-10] All these properties make porphyrins relevant macromolecules in several chemistry fields (e.g. material science, optics, catalysis, transformation and storage of energy, medicine, pharmacology).[11-17] In recent years, porphyrins have emerged as important promising photosensitizers in photodynamic therapy (PDT); porphyrin and its derivatives demonstrate great efficacy as both antibacterial and antiviral agents against different species due to its exceptional photodynamic properties.[18,19] Porphyrins have potential applications in biological sciences, therefore, it is pertinent to develop simple synthetic pathways that lead to compounds with unique physical and chemical characteristics.

Nowadays different strategies to improve porphyrin photodynamic properties have been applied: (a) structural modification of the base ring with the addition of a variety of substituents at *messo* position, and (b) the inclusion of metals into the porphyrin core.[20-22] The photophysical properties of porphyrins and their metallic-derivatives are affected for both the peripherally and/or axial substituents and the central metal into porphyrin core.[23] The porphyrins and their metallic-derivatives act efficiently as sensitizing agents and they have presented phototoxic activity against different pathogens, such as bacteria, fungi, viruses and parasites.[24-27] Different porphyrinic photosensitizers have reported anti-leishmanicidal activity against *Leishmania tarentolae* in promastigote stage (ethyl and diethyl carbaporphyrin), against amastigotes of *L. panamensis*, and promastigotes of *L. major* and *L. braziliensis* (β-substituted porphyrinic systems).[28-32] Improved pharmacological responses have been found when incorporating metals in the macrocycle, as reported by Gomes *et al.* when evaluating the activity against *L. amazonensis* against derivatives metalated with Bi (III) and Sb (IV) (IC50 of 93.8 μM and 52.4 μM respective).[33] Another Zn (II) metalated porphyrin derivative evaluated against promastigotes of *L. braziliensis* reduced parasite viability with greater efficiency than the metal-free derivative.[34] This type of modification alters the steric and electronic nature of porphyrins giving rise to new molecules that have specific and unique properties, and it also presents a promising alternative for modifying photophysical properties of the compounds: (a) quantum singlet oxygen performance, (b) the range of the therapeutic window, (c) photostability and (d) lipophilicity could be improved.[35]

Although, *5,10,15,20-tetrakis(4-bromophenyl)-porphyrin* (**compound 1**) and dicloro-5,10,15,20-tetrakis(4-bromophenyl) porphyrinato Sn (IV) (**compound 2**) are commercially available, the reports about of its application as sensitizers in PDT are few. Therefore, in this study, we analyzed the photophysical behavior of (**1**) and (**2**) as regards their potential use in PDT against *Leishmania panamensis.*

## Methods

### Preparation and identification of compounds

All reagents and solvents were purchased from Sigma Aldrich. We prepare the porphyrin 5,10,15,20-tetrakis(4-bromophenyl) porphyrin (**1**) based on Adler's method,[36] introducing a small modification that consisted of leaving the reaction for 8 hours at room temperature and stirring in an open container, using the oxidative power of oxygen to convert more of the chlorin by-product into porphyrin. In summary, equimolar amounts of pyrrole and 4-bromobenzaldehyde were mixed in propionic acid for 8 hours at ambient temperature under an atmosphere of air. Dicloro-5,10,15,20-tetrakis(4-bromophenyl) porphyrinato Sn (IV) (**2**) was synthesized by porphyrin precipitation in metal chloride solicitation (Figure 1). The formation of the final products was followed by thin layer chromatography on aluminum foil UV254 TLC, the mobile phase was petroleum ether-ethyl acetate (2:1). The compounds were characterized using $^{13}C$ NMR spectra (Bruker AC-400 spectrometer); the $^{13}C$ NMR chemical shifts are reported as ppm (δ), relative to $CDCl_3$ (signal located at 7.29 ppm). Infrared spectrum was measured on the equipment ECO-ART alpha Bruker FTIR spectrometer. To perform UV-Vis spectrum (using a UV-2401PC UV-Vis spectrophotometer), we dissolved $2.0\times10^{-5}$ g of each compound in ethyl acetate, and finally, we obtained the mass spectrum by dissolving the compound in methanol (using ESI-LC-MS/MS ion Trap amaZon, Bruker spectrometer). Furthermore, we measured fluorescence quantum yield (using a PTI um 40 fluorimeter) and singlet oxygen quantum yield,[37] and electrochemical characterization was performed in four different dissolvents (Dimethylformamide-DMF, dichloromethane -DCM, dimethylsulfoxide-DMSO, tetrahydrofuran-THF) containing $1.0\times10^{-1}$ M tetrabutylammonium perchlorate ($((C_4H_9)_4N(ClO_4)$, Aldrich 98% purity) as a supporting electrolyte for all electrochemical measurements.

Compound (**1**) was prepared by mixing 10 mmol pyrrole and 10 mmol 4-bromobenzaldehyde in 80 ml propionic acid for 8 hours at ambient temperature in an open container. The product was extracted from the reaction medium by adding 60 mL cold methanol and filtering by gravity, the filtrate was dried at room temperature, obtaining 1.4136 g of a purple solid that was finally purified using column chromatography (mobile phase; ether-ethyl acetate 20:2). Yield: 47.28%; melting point > 300°C; UV-Vis (ethyl acetate) 414, 512, 546, 591, 645; FT-IR ($cm^{-1}$): N-H (3350), C=C (1470.28), C=N (1088.22), C-N (964.28); $^{13}C$ NMR ($CDCl_3$, 400 MHz): 118.86 (Ar), δ = 122.92 (Ar), δ = 130.22 (β-py), δ = 131.95 (Ar), δ = 135.95 ($Ar_{ipso}$), δ = 140.91 (Ar-Br); (M+H) m/z = 930.9. These results concur with previous reports for this compound.[37,38]

Compound (**2**) was prepared by mixing 0.5478 mmol of (**1**) with 2.2164 mmol $SnCl_2.2H_2O$ in DMF (80 mL) for 4 hours at ambient temperature, and by stirring. After that, we added cold water and TBrPP-Sn (IV) precipitated; this solid was washed and dried at ambient temperature. The compound was purified by column chromatography (mobile phase; petroleum ether-ethyl acetate 5:1). Yield: 64.4%; melting point > 300°C; UV-Vis: 426, 560, 600. These results concur with previous reports for this compound.[37,39]

### Photophysical properties

Fluorescence quantum yield ($\phi_f$) was determined by the comparative method in a PTI um 40 fluorimeter. Using as a standard fluorescein dissolved in water, porphyrin (**1**) and metalloporphyrin (**2**) was dissolved in ethyl acetate. All fluorescences were determined taking as excitation wavelength the maximum of the Soret band, using a 2 nm slit and a 420-750 nm scan. The fluorescence quantum yield was calculated with the following *eq. 1*.[37,40,41]

$$\phi_x = \phi f_{est} * \frac{F_x * A_{est} * \eta_{est}^2}{F_{est} * A_x * \eta_x^2} \quad \text{(Eq.1)}$$

$F_x$ and $F_{est}$ are the areas under the fluorescence emission curve of compounds (**1**), (**2**) and the standard. A x and A are sample absorbances and standard at the excitation wavelength. η x and η are the respective refractive indices of the solvents (ethyl acetate; η = 1.3724, water; η = 1.33336).

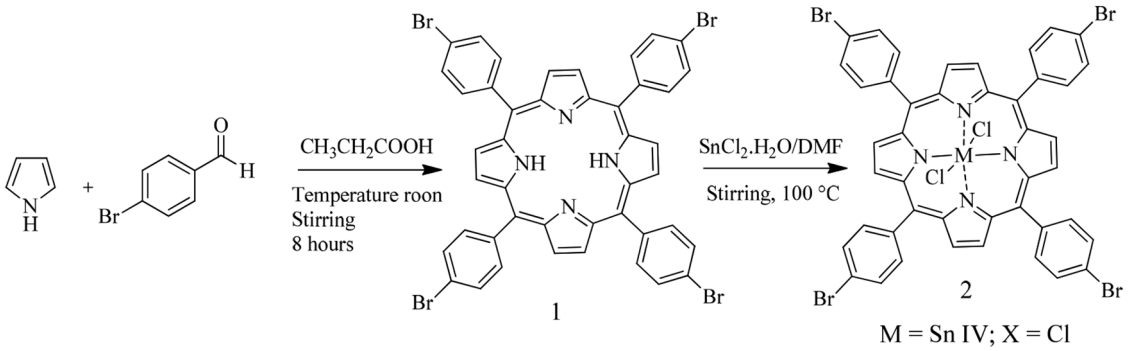


**Figure 1. Synthesis route of compounds (1) and (2).**

Singlet oxygen quantum yield ($\Phi_\Delta$) of (**1**) and (**2**) was performed by the graphical method, using 1,3-diphenylisobenzofuran (DPDF) as singlet oxygen scavenger, and singlet oxygen generator standard 5,1,15,20-(tetraphenyl) porphyrin (H2TPP). The tests were carried out by preparing a 1×10-9 M solution of each compound in DMF in triplicate and calculated with *eq. 2*.[37,40,41]

$$\phi\Delta = \phi\Delta_{\text{standar}} * \frac{W}{W^{\text{standar}}} \quad \text{(Eq.2)}$$

Where: ΦΔstandard is the singlet oxygen quantum yield of the H2TPP standard in DMF (0.64). W and $W_{standard}$ are the slopes of the degradation curves of the DPDF.

### Electrochemical characterization

Cyclic voltammetry (CV) data were recorded using a single-compartment electrochemical cell with a maximum electrolyte volume of 10 mL. A CH Instruments (Model 600E) Electrochemical Analyzer was used for the electrochemical measurements. The working electrode was a glassy carbon 3 mm in diameter on Teflon R (CH Instruments). The reference electrode used was an $Ag^+/Ag$ electrode on Teflon R; we used a solution $1.0\times10^{-3}$ M $AgNO_3$ in electrolyte support; the auxiliary electrode was a platinum of 99.99% from CH Instruments. The electrochemical characterization was carried out using cyclic voltammetry and linear voltammetry. The peak current intensity $i_p[A]$, in cyclic voltammetry, is given by the Randles-Sevcik *Eq.3*:[42]

$$\mathrm{i_p} = \left(2.69\times10^5\right)\mathrm{n}^{\frac{3}{2}}\mathrm{AD}^{\frac{1}{2}}\mathrm{C}v^{\frac{1}{2}} \quad \text{(Eq.3)}$$

where $n$ is the number of electrons in the redox reaction, $A[cm^2]$ is the area of the working electrode, $D[cm^2s^{-1}]$ is the diffusion coefficient for the electroactive species, $v[Vs^{-1}]$ is the scan rate, and $C\,[mol\,cm^{-3}]$ is the concentration of the electroactive species in the electrode. The anodic and cathodic peak currents are equal, and the ratio $i_{p,a}/i_{p,c}$ is 1.0. The half-wave potential, $E_{1/2}$, is midway between the anodic and cathodic peak potentials, *Eq. 4*.

$$\mathrm{E_{1/2}} = \frac{\mathrm{E_{p,a}} + \mathrm{E_{p,c}}}{2} \quad \text{(Eq.4)}$$

### Biological assay

*Leishmania panamensis* (UA140) was used in *in vitro* tests for the evaluation of the leishmanicidal potential of the compounds (**1**) and (**2**). Leishmanicidal activity was determined as the ability of the compounds to decrease the viability of the parasite, for this the MTT method is widely used in the literature (MTT Assay Protocols, Thermo Fisher Scientific). Conditions were previously standardized by our working group.[37,43-45]

### Parasite culture and viability using MTT assay

*Leishmania panamensis* (UA140) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 1% glutamine and 1% antibiotics (200 U penicillin/200 µg Amikacin) under incubation conditions 5% $CO_2$.[37,45] The metacyclic promastigotes in the infectious stage were isolated from stationary cultures. The parasite viability was estimated by MTT assay.[37,43-45] Anti-leishmanicidal activity was evaluated at different concentrations (1, 10, 50, 100 and 200 µM) of compound and positive control (Glucantime), against a parasitic inoculum of $5 \times 10^6$ cells/mL (promastigote). Test compounds and positive control were dissolved in dimethyl sulfoxide (DMSO), working concentrations were obtained by adding 10 µL of compound in a final volume of 200 µL to each well of the 96-well microplate, the treatments were maintained under visible light irradiation, with incubation times of 24, 48 and 72 hours. The irradiation source was Omnilux lamps (EL10000AG), with a range λ emission lamp = 420 nm–450 nm, and an incident photon flow per unit volume $I_o$ was $5.7 \times 10^{-7}$ Einstein*$L^{-1}s^{-1}$. Each trial was performed in triplicate. Plates were analyzed using SkanIt software. We applied an ANOVA test to determine the differences or similarities between treatments and positive control. In addition, a post hoc analysis was performed using Tukey statistics. Finally, differences were considered to be significant when $p < 0.05$.

## Results and discussion

### UV-Vis assay

The UV-Vis spectrum of (**1**) (Figure 2), shows a band of maximum absorption located at 414 nm (Soret band), generated by $a_{1u}(\pi)$-$e_g^*(\pi)$ transitions and four lower absorption Q band located at 515nm, 547nm, 588nm and 645 nm, which corresponds to $a_{2u}(\pi)$-$e_g^*(\pi)$ transitions.[46,47] The UV-Vis spectrum of compound (**2**) shows one Soret band and only two Q bands. When the Sn (IV) ion coordinates nitrogen atoms inside the porphyrin ring, the porphyrin symmetry increases. Furthermore, the reduction in the number of Q bands indicates that the metal effectively entered the macrocycle.[48] The intensity of the Q bands is correlated with the relative stability of the metalloporphyrin: when the signals are of low

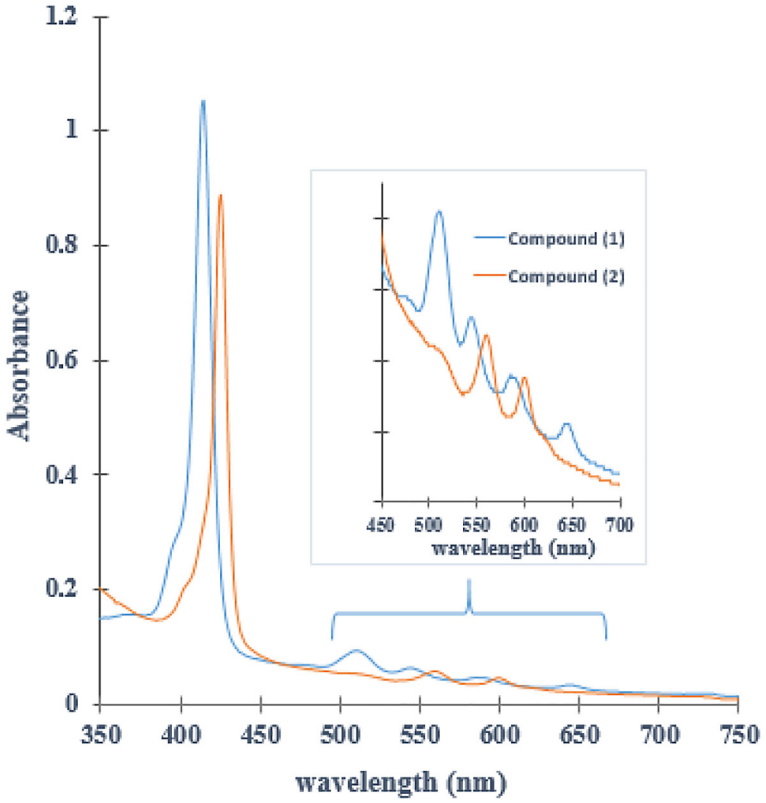


**Figure 2. UV-Vis spectrum (1) and (2).** The bands α and β represent the Q band in the porphyrin Sn (IV) complex (**2**).

intensity, the metallocomposites are highly stable and their atoms are located in the square plane.[49,50] Ohsaki *et al.* reported a similar change in the UV-Vis spectrum after tin (IV)-insertion into the porphyrin core synthesized in water at ambient temperature.[51] Moreover, the Soret band for (**2**) had red shift from 414 nm to 425nm (near to 11 nm). The direct coordination between Sn (IV) ion and porphyrin core could extend conjugation from porphyrin to metal ion; in this case, the electronic excitation will require lower energy absorption due to increasing conjugation–this process requires longer wavelength than pure porphyrin.[10,52] Figure 2 shows that (**1**) and (**2**) have photo-activity inside window 400 to 700 nm. Although the compounds do not have a considered absorption in the red rank of the visible light spectrum from 600 nm to 800 nm (this radiation can reach a penetration depth of 8.0 mm inside tissue), they absorb radiation inside range 500-600 nm. This radiation penetrates approximately 4.0 mm, and such penetration capacity is suitable for potential application in cutaneous treatments.[53]

## Photophysical properties

*Deamination of $\Phi_f$*

Information related to the efficiency of fluorescence emission is important to explain the inactivation pathway related to PDT.[54] Figure 3 shows fluorescence emission for (**1**) and (**2**); both show photoluminescence at a visible range in the electromagnetic spectrum. As shown in Figure 3, fluorescence emission wavelength was located at 651 nm for both compounds, the energy transition did not change after the Sn (IV) ion insertion; however, fluorescence emission intensity was more high compound (**1**). This effect is due to the Sn (IV) ions insertion inside the porphyrin core decreases significantly fluorescence effect. As shown in Table 1, the Φf of (**1**) was three times greater than (**2**); the Sn (IV) ions inside the porphyrin core could increase the non-radiative decay of the excited singlet state of porphyrin,[37] Sn (IV) ions within the porphyrin nucleus could increase disintegration by inter-system crossover (ISC), and this pathway is governed by orbital spin coupling at the central atoms; in this case, the insertion of Sn (IV) reduces the fluorescence emission.[9,55,56] A similar effect was reported for cupper insertion inside *meso*-porphyrinic complexes.[56,57]

*Deamination of $\Phi_\Delta$*

The generation of singlet oxygen produced by (**1**) and (**2**) was quantified by chemical entrapment using DPBF. To estimate the $\Phi_\Delta$, the degradation of DPBF was measured at 415 nm over time (Figure 4), the lower the absorbance, the

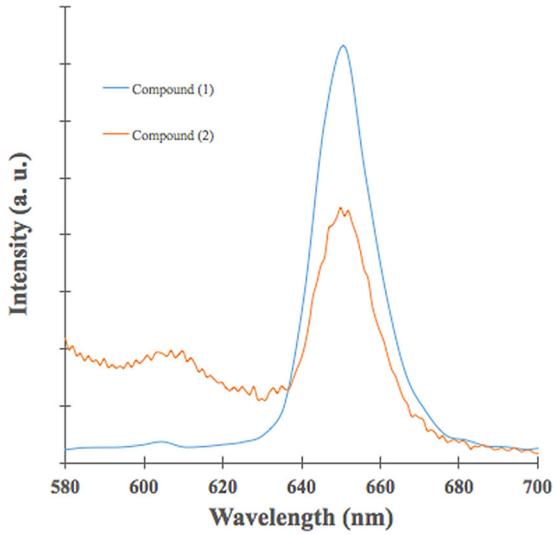


**Figure 3. Emission spectra of (1) and (2).**

**Table 1. Photophysical properties ($\Phi_f$ y $\Phi_\Delta$) of (1) and (2).**

| Compound | $\lambda_{abs}$ | | | | | $\lambda_{em}$ | $\Phi_f$ | $\Phi_\Delta$ |
|---|---|---|---|---|---|---|---|---|
| | Soret Band | Q Bands | | | | | | |
| **(1)** | 414 | 512 | 546 | 591 | 645 | 651 | 0.15 ± 0.01 | 0.55 ± 0.03 |
| **(2)** | 426 | 560 | 594 | --- | --- | 651 | 0.05 ± 0.01 | 0.59 ± 0.04 |

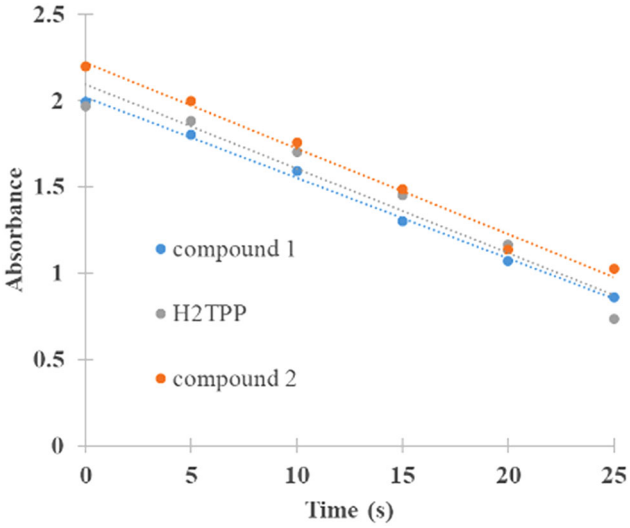


**Figure 4. Absorbance and degradation of DPBF at 415 nm as a function of reaction time with (1), (2) and standard. Linear fit is also shown.**

greater the degradation of DPBF mediated by singlet oxygen. The complex Sn (IV)-porphyrin (**2**) presented higher $\Phi_\Delta$ compared to (**1**) (Table 1), the difference in $\Phi_\Delta$ between the compounds is 7%, and this difference is directly attributed to the insertion of the metal ion Sn (IV) inside the macrocycle. Sn (IV) would be generating greater stability of the triplet state of the molecule and improving the interaction with molecular oxygen, which is reflected as a greater translocation of the molecular oxygen spin and its subsequent conversion into singlet oxygen.[4,59-62] These sensitizers show promise in PDT for its $\Phi_\Delta$ values, and could in the future be candidates in clinical trials like its counterpart Lutetium Texaphyrin, which has a $\Phi_\Delta$ as low as 0.11.[63]

## Electrochemical characterization

The biological systems present microheterogeneity, caused by the coexistence of microphases such as aqueous polar and highly hydrophobic lipid.[64,65] Therefore it is relevant to study the physicochemical properties of sensitizers at different media. We have studied the electrochemical behavior of (**2**) using cyclic voltammograms (CV) in four different solvents. Figure 5 shows CVs and Table 2 lists electrochemical parameters. Porphyrin had irreversible one-electron oxidation at $E_{pa}$, which varies between 0.55 V for DMF and 1.0 V for THF, in addition to one quasi-reversible reduction peak between −1.01 V for THF and −1.41 for DMF (Figure 5a). The latter is clearer when DMF and DMSO are solvents. CVs (in detail) in DMSO, presenting three redox processes: (**a**) oxidation processes (I and II) related to the formation of monocationic and dicationic porphyrin spaces, (**b**) (a) oxidation processes (I and II) related to the formation of

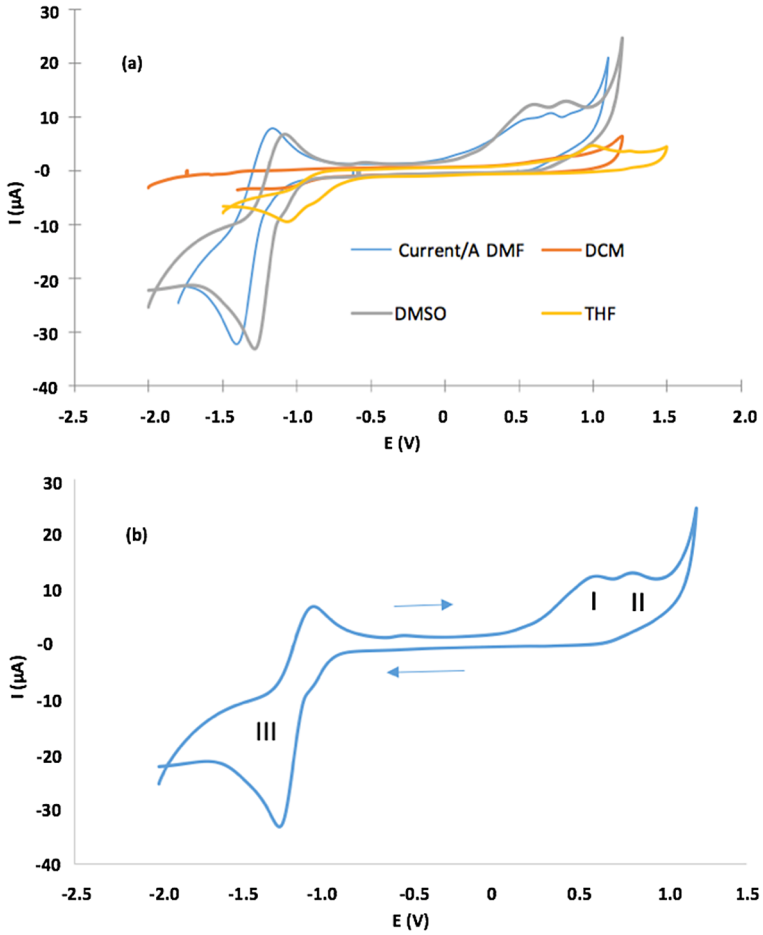


**Figure 5. a) CVs of the Sn (IV)-porphyrin complex in DMF, DCM, DMSO and THF (0.1 M of $TBAClO_4$. Scan rate = 0.1 V/s). b) CVs (in detail) for oxidation and reduction of the Sn (IV)-porphyrin complex in DMSO.** In the figure 5b, I and II correspond to oxidation processes that form mono- and di-cationic porphyrin species, and III corresponds to reduction processes that form anionic porphyrin species in DMSO as solvent.

**Table 2. Determination of half-wave potentials for four different solvents.**

| Solvent | Oxidation | | Reduction First | $\Delta E_{1/2}$ |
|---|---|---|---|---|
| | Second | First | | |
| DMF | 0.71 | 0.54 | −1.41 | 0.98 |
| DCM | – | 0.83 | −1.11 | 0.97 |
| DMSO | 0.81 | 0.59 | −1.27 | 0.93 |
| THF | 1.25 | 1.00 | −1.01 | 1.01 |

monocationic and dicationic porphyrin spaces, (b) reduction process (III) that results in the formation of the anionic porphyrin species[66] (Figure 5b).

The conformational and structural changes observed in reversible electron transfer reactions can be examined through the potential differences between the first and second oxidation states.[46,67] Table 2 shows the oxidation and reduction potentials. The couple III presented an almost reversible behavior accompanied by a separation of anodic-cathodic peaks ΔEp> 60 mV, in addition, it presented an anodic-cathodic peak potential ratio of approximately unity (1.0). This result is characteristic of a nearly reversible monoelectronic process. The electron-attractant character of the bromine substituents could be significantly influencing the electrochemical properties of the derivatives (**1**) and (**2**).[68,69] Likewise, Table 2, shows a slight effect on $\Delta E_{1/2}$ by change of dissolution solvent. THF had the highest $\Delta E_{1/2}$ due to lower dielectric constant value (ε), and this solvent had smaller dielectric constant value ($\varepsilon_{DMSO}$ = 46.70, $\varepsilon_{DMF}$ = 36.70, $\varepsilon_{DCM}$ = 8,93 and $\varepsilon_{THF}$ = 7.58) between all aprotic dipolar solvents under study herein. Our data on the potentials for oxidation and reduction of TBrPP-Sn (IV) (Table 2) are consistent with previous reports published in the literature on metalloporphyrin-like compounds.[70,71]

We studied the scanning speed effect on the current response of CVs for each solvent to determine if the redox process was controlled by diffusion or by adsorption. Figure 6a shows Cvs for (**2**) at different scanning speeds in DMF. Figure 6a shows an anode peak for all scan rates in the range 0.8-1.3 V for all solvents used. The relationship found between the scanning speed and the peak current was directly proportional with linear increase, and the peak potential anodically shifted; additionally, when the scanning speed was increased, the peak became broader. Figure 6b shows that the peak current correlated with the square root of the scan rate for each solvent studied. Table 3 shows $R^2$ and linear equation fitting for each test. The fitting results indicated that the process is controlled by diffusion, then hydrodynamics of media (e.g. polarity, density, viscosity) determines the redox process rate.[66-68] Furthermore, the linear fit of the line plot of Ip versus $v^{1/2}$ indirectly indicates a relationship between the diffusion coefficient and DMSO; and DMF had the highest slope value, suggesting that the diffusion coefficient for these solvents was greater than the diffusion coefficient for THF and DCM. This result is associated with the value of the dielectric constant, as discussed.[67,68]

Figure 7 shows dissolvent effect on electrochemical band gap value (**2**) for solvents studied in this work. The data obtained for the redox potentials (pH = 7.0 and room temperature) of the water separation reaction and the carbon dioxide reduction reactions to produce methane and methanol: Potential per redox couple; E (H2O/O2) = −5.26 eV; E (H +/H2) = −4.03 eV; E (CO2 / CH4) = −3.79 eV; E (CO2/CH3OH) = −3.65 eV.[72] It is evident that the value of the electrochemical band gap depends on the polarity of the solvent, (**2**) had the smallest defective gap in THF. A requirement for the photosensitizer in PDT is the band gap; electrochemical characterization indicates that it is appropriate and suitable. Finally, the potentials described in Table 2 corresponding to the redox process of (**2**) are in agreement with other reports for processes based on rings in porphyrin complexes.[72,73]

## Biological assay

A wide variety of molecules have been evaluated as possible therapy against *Leishmania* spp in recent years: (i) aluminum and zinc phthalocyanines (ii) methylene blue, 5-aminolevulinic acid and porphyrin. The field of research on new substances with challenging properties in medicine and pharmacology is an important topic, which has become more

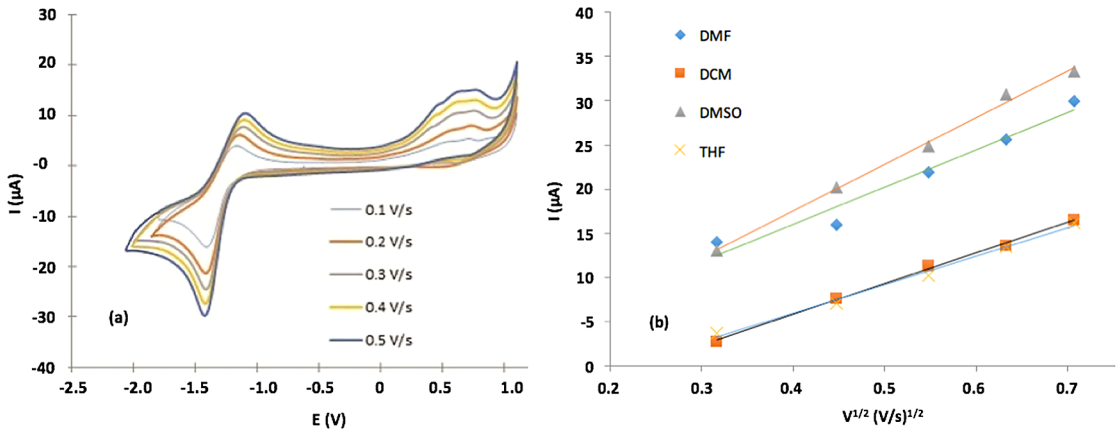


**Figure 6. a) CV measured at scanning speeds of 0.1-0.5 $Vs^{-1}$ for a $1\times10^{-3}$ M solution of the Sn (IV) -porphyrin complex in DMF solvent (0.1 M TBAC104). b) Estimation of the relationship between the maximum current and the square root of the scanning speed.**

**Table 3. Fitting parameters for $I_p$ versus $v^{1/2}$ plot to each solvent.**

| Solvent | Linear equation | $R^2$ | slope ($\mu AV^{-1/2}s^{1/2}$) |
|---|---|---|---|
| **DMF** | $y=4.22\times10^{-5}x-8.1\times10^{-7}$ | 0.956 | 4.22 |
| **DCM** | $y=3.47\times10^{-5}x-8.1\times10^{-6}$ | 0.997 | 34.7 |
| **DMSO** | $y=5.29\times10^{-5}x-3.6\times10^{-6}$ | 0.995 | 52.9 |
| **THF** | $y=3.21\times10^{-5}x-6.9\times10^{-6}$ | 0.992 | 32.1 |

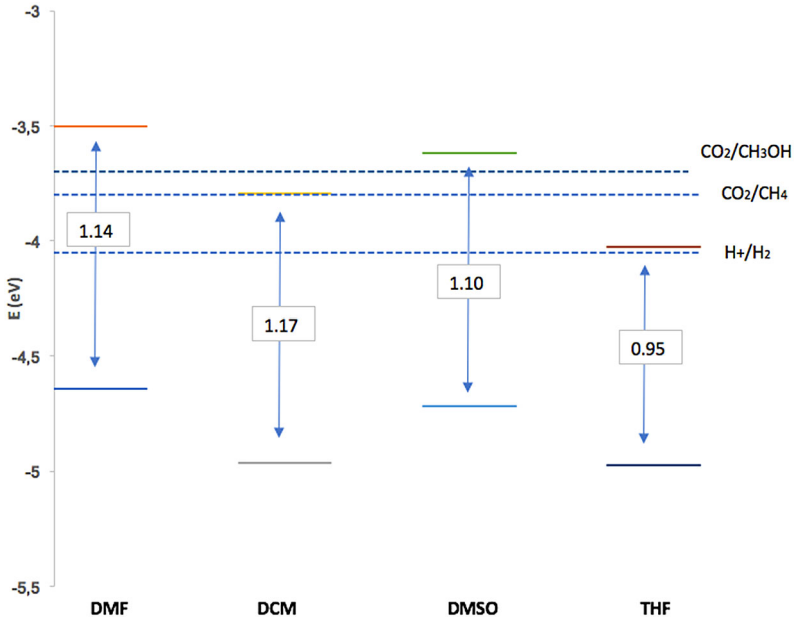


**Figure 7. Band gap and band edge positions to Sn-Porphyrin derivative for dissolvent used in this work.** Y-axis energy levels for the reduction and oxidation potentials of the dissociation reactions. pH = 7 y room temperature.

relevant due to the appearance of resistant and emerging microorganisms.[74-76] The compounds (**1**) and (**2**) have been used to evaluate their effects on the viability of *L. panamensis* using the MTT method. The results are presented as cell viability of *L. panamensis* after exposure to different concentrations of (**1**) and (**2**) during incubation periods of 24, 48 and 72 hours, in darkness and under irradiation. The same procedure was done for the positive control (Glucantime).

The results show that (**1**) and (**2**) presented inhibitory activities on parasite viability (Figure 8). The decrease in the viability of *L. panamensis* was observed to a greater degree on the irradiated tests, this is due to the increased interaction capacity of the test compounds with oxygen, which induced the production of singlet oxygen.[77,78]

The contrary effect was evidenced for the reference standard, which reached better inhibitory activities on parasite viability in darkness. The highest effect leishmanicide was observed when the parasite was exposed to light for 24 hours and with concentrations of the compounds higher than 100 μM. Furthermore, the irradiation time (48–72) had no significant effect on viability (%) results compared to samples irradiated for 24 hours. The $IC_{50}$ (Table 4) was determined using data found in the 24-hour test. Under irradiation, the $IC_{50}$ of both compounds was lower compared to the positive control (Glucantime), with values of 16.5μM ($p$ = 0.02) and 19.5 μM ($p$ = 0.03) respectively. Additionally, we found slight differences between the activity of compound (**1**) and (**2**); compound (**1**) presented an $IC_{50}$ = 16.5 μM vs $IC_{50}$ = 19.2 μM of compound (**2**), however, these differences were statistically non-significant ($p$ = 0.74). Therefore, these two compounds have similar behaviors in terms of parasite inhibition, which correlates with their $\Phi_\Delta$ values ($\Phi_\Delta$ compound **1** = 0.55 and $\Phi_\Delta$ compound **2** = 0.59). The activation of sensitizers by light action ensures lower $IC_{50}$ values. Besides, in the absence of light, the response was lower, thus being in line with findings of other reports.[78] Our results also show that (**1**) and (**2**) cause some damage to the parasite, decreasing its survival rate by 1.5-fold compared to the standard in the presence of irradiation, which could induce a reduction in the healing time of a lesion.

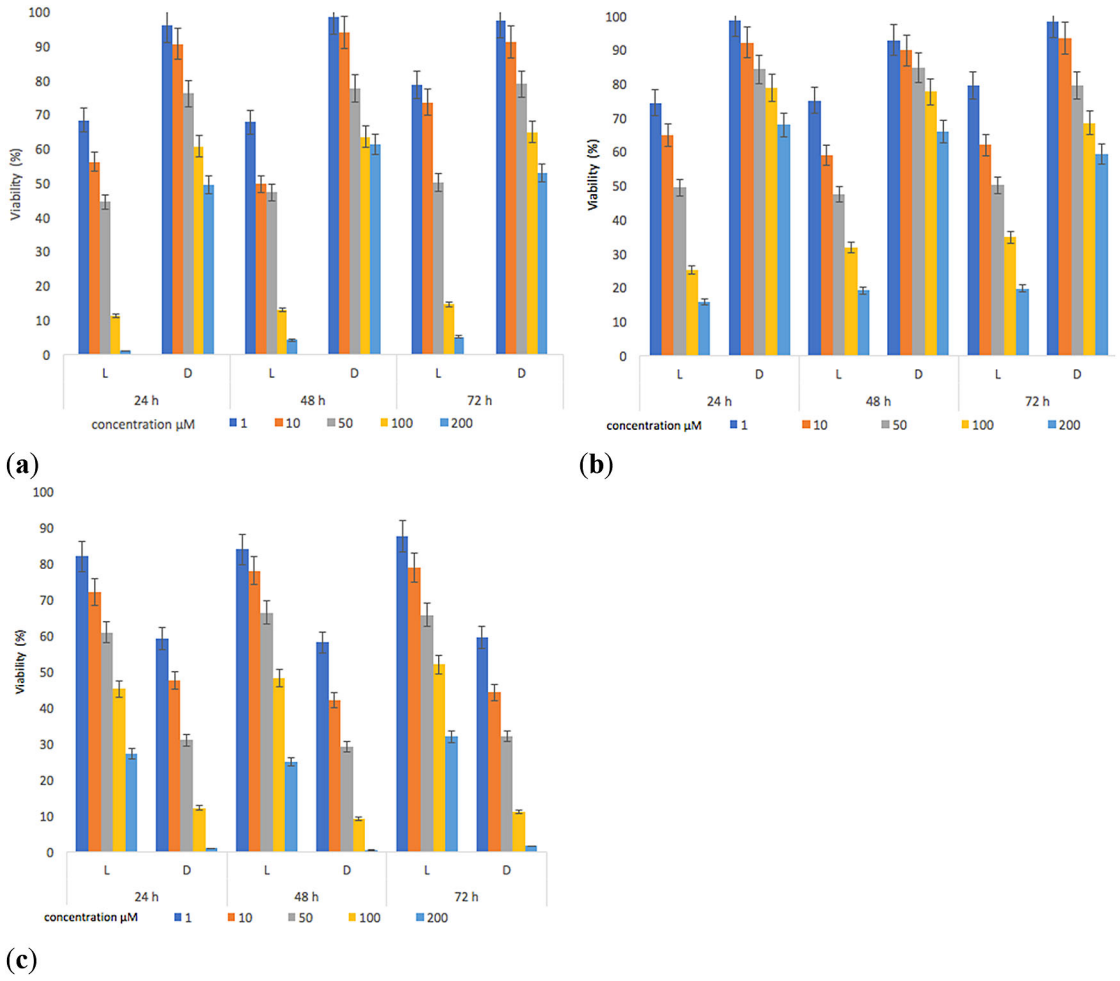


**Figure 8. Parasite viability percentage results for *L. panamensis* against: (a) compounds (1), (b) compounds (2) and (c) positive control drug with incubation periods of 24, 48 and 72 hours.**

**Table 4. $IC_{50}$ values for compounds TBrPP, TBrPP-Sn (IV) and positive control against *L. panamensis,* 24-hour incubation treatments.**

| compound | $CI_{50}$ (µM) ± SD | |
|---|---|---|
| | Light | Darkness |
| **(1)** | 16.5±0.1 | 105.0±0.3 |
| **(2)** | 19.0±0.1 | > 200 |
| **Gluc** | 56.1±0.1 | 8.8±0.1 |

## Conclusions

In the present study, we synthesized and characterized (**1**) and (**2**). Compound structures were confirmed by spectroscopic techniques (UV-Vis, FT-IR, $^{13}$C- NMR and ESI-mass). Sn (IV) ion insertion inside in the porphyrin core reduced significantly $\Phi_f$ 0.15 to 0.05. Furthermore, $\Phi_\Delta$ increased from 0.55 to 0.59 after metal insertion inside the porphyrin core. Electrochemical results showed that electrochemical properties were affected by the solvent dielectric constant, where THF had the highest $\Delta E_{1/2}$ due to a lower dielectric constant value. Moreover, the electrochemical assay showed a quasi-reversible reduction peak between −1.01 V in THF and −1.41 in DMF. The inhibitory results shown that (**1**) and (**2**) presented inhibitory activities on parasite viability. The highest inhibitory activity on the parasite was observed when the treatments were irradiated for 24 hours and with concentrations of the compounds of 200 µM, strengthening the hypothesis that parasite mortality is mediated by reactive oxygen species (especially singlet oxygen). These compounds

were synthesized with low-cost methods and with acceptable synthesis yield, a fundamental aspect in the search for sensitizer candidates. The results of biological activity suggest these compounds could be applied in future applications of *in vivo* models as potential sensitizers of photodynamic therapy.

## Data availability

### Underlying data

Mendeley Data: Complementary material, http://dx.doi.org/10.17632/h2vmrdz4sg.1.[79]

This project contains the following underlying data:

- 1. UV-Vis compound (**1**) and (**2**).xlsx [UV-Vis spectrum of compound 1 and 2]
- 2. Gráficos, RENDIMIENTO CUÁNTICO DE FLUORESCENCIA.xlsx [Graphics, Quantum fluorescence yield of compound 1 and 2]
- 2.1 Calculo de Qf.xlsx [Calculation of Quantum fluorescence yield (Qf) of compound 1 and 2]
- 3. 1. Compound 1 (DMSO). Rendimiento Cuántico Oxígeno Singulete – copia.xlsx [Calculation of Quantum Yield Oxygen Single of Compound 1 in DMSO]
- 3.2. Compound 2 (DMSO). Rendimiento Cuántico Oxígeno Singulete.xlsx [Calculation of Quantum Yield Oxygen Single of Compound 2 in DMSO]
- 4. ESI-MS, Compound 1.jpg [Mass spectrum of Compound 1 in methanol]
- 5. FT-IR, Compound 1.pdf [FT-IR spectrum of Compound 1]
- 6. RMN 13C, Compound 1.mnova [RMN 13C spectrum of Compound 1 in CDCl3]
- 7. Assay Biologoly_Compound 1 and 2_Luz vs L. Panamensis_Promastigote.xlsx [Assay Biological of Compound 1 and 2 in the presence of light against L. Panamensis promastigote]



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# Open Peer Review

## Current Peer Review Status: ✔ ✔

**Version 3**

Reviewer Report 10 November 2021

https://doi.org/10.5256/f1000research.78968.r99462



✔ **Martha Simões Ribeiro**
Center for Lasers and Applications, Nuclear and Energy Research Institute (IPEN-CNEN), São Paulo, Brazil

No further comments. The authors revised the manuscript properly.

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** Optical therapy

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Version 2**

Reviewer Report 22 September 2021

https://doi.org/10.5256/f1000research.57235.r93076



? **Martha Simões Ribeiro**
Center for Lasers and Applications, Nuclear and Energy Research Institute (IPEN-CNEN), São Paulo, Brazil

This work aimed to verify the photodynamic activity of 5,10,15,20-tetrakis(4-bromophenyl)-porphyrin and dicloro-5,10,15,20-tetrakis(4-bromophenyl) porphyrinato Sn (IV) on promastigotes

of *Leishmania panamensis* after porphyrin synthesis and characterization.

Some issues should be addressed by the authors:

- Please, report the initial inoculum of parasites and the type of microwell plate.
- Please, report the emission of the light source. I am not sure what it means by visible light.
- Please, provide the light dose. Literature commonly uses J/cm^2.
- I am not sure if parasites were irradiated by 24, 48 and 72 h or if they were incubated by those times. Please, clarify.
- Authors report that figure 2 shows the photoactivity of the compounds between 400 and 7000 nm. Please, change 7000 for 700 nm.
- Please add another reference for information regarding light penetration in the skin. Ref 53 is not appropriated. My suggestion is to look for Steven Jacques or Valery Tuchin's papers.
- Please, add information regarding I, II, and III in the legend of figure 5 b.
- The irradiation impaired the glucantime antileishmanial effect. Do authors have a hypothesis for this finding?
- Authors should compare parasite metabolic activity following compound 1 and compound 2-mediated photodynamic treatment. Statistical analysis is necessary. It seems to me that compound 1 is significantly better than compound 2. However, compound 2 presented a higher singlet oxygen quantum yield. The authors should discuss this issue.
- I think the last sentence of the manuscript before conclusions is not supported by the authors' findings. Indeed, the authors assumed parasite damage since they do not show any image. However, MTT measures metabolic activity and its sensitivity is not high. Besides, glucantime in the dark promoted similar inactivation. Please, rephrase.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
No

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** Optical therapy

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 15 July 2021

https://doi.org/10.5256/f1000research.57235.r88766



**John Mack**
Institute for Nanotechnology Innovation, Department of Chemistry, Rhodes University, Makhanda, South Africa

The revisions appear to be acceptable.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** I carry out research that is relevant to all aspects of this study.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Version 1**

Reviewer Report 24 May 2021

https://doi.org/10.5256/f1000research.55710.r85271



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**John Mack**
Institute for Nanotechnology Innovation, Department of Chemistry, Rhodes University, Makhanda, South Africa

The manuscript describes the synthesis and further characterization of a Sn(IV) tetraarylporphyrin complex including its photosensitizer properties *and in vitro* studies of its activity against *L. panamensis.* The work has been carried out competently for the most part. The results are very much in line with expectations but are of sufficient interest in the context of the effect of the porphyrins on the viability of *L. panamensis* to merit publication on a platform like F1000. I recommend approval after minor revisions. I urge the authors to carefully consider the following points while preparing their revised manuscript:

(a) The references should be brought up to date where appropriate. They appear to stop at around 2018 or so.

(b) Were DMSO stock solutions used to solubilize the dyes for the *in vitro* studies? If so, the details must be provided.

(c) Were filters used for the fluorescence measurements in Figure 3 and was a solvent baseline subtracted. The baseline should not rise like it does for compound 2. If not, the fluorescence quantum yields will have to be recalculated.

(d) Can Figure 1 be redrafted in Chemdraw?

(e) Decimal points should be used in the y-axis labels of Figure 2.

(f) There is no need for "(u.a.)" in the y-axis title of Figure 4. Having absorbance values > 2 is problematic in this context in terms of the linear range of the spectrometer used, but if reasonable linearity was obtained there is probably no need to repeat the measurements.

(g) The phrase "have photo-activity inside window 400 to 7000 nm" needs to be fixed.

(h) The $\lambda_{abs}$ values for the Q bands should also be provided in Table 1. $\lambda_{em}$ would normally be preferred rather than $\lambda_{emi}$.

(i) The authors should carefully re-evaluate whether their use of three significant figures in Table 2 is justified.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** I carry out research that is relevant to all aspects of this study.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**