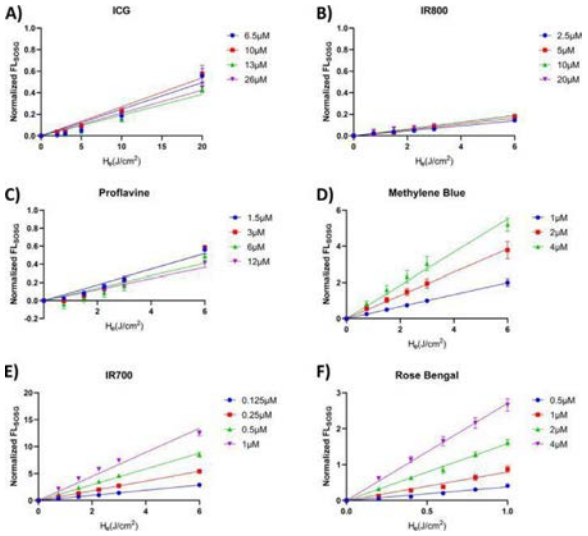


**Figure 4.** Effect of light dose (radiant exposure, wavelength, irradiance, and exposure time) and photosensitizer concentration on the SOSG fluorescence intensity for (A) ICG, (B) IR800, (C) Proflavine, (D) Methylene Blue, (E) IR700 and (F) Rose Bengal

For in-vitro testing, the normalized cell viability curves were obtained from the Neutral-Red Uptake (NRU) assay. A similar trend was observed in the cell viability curves obtained using the bottom-up illumination system due to the associated phototoxicity by the photosensitizer. A similar trend was obtained for the photosensitizers compared to the cell-free assay. However, Proflavine exhibited photocytotoxicity using the NRU assay,despite a lack of singlet oxygen generation with the cell-free assay. This may be due to production of other ROS by proflavine, such as superoxide and hydroxyl radicals which could not be detected with the cell-free SOSG assay.

## 5. Conclusion [133/150 words]

The setups explored in this study feature top-down and bottom-up laser exposure systems suitable for performing high-throughput testing used for PDT experiments. The system provides a 6-well and 4-well illumination field with a variation of 10% with adjustable irradiance up to 10mW/cm$^2$ and can be readily adapted to laser diodes of different wavelengths. In the effectiveness comparison experiments using the cell-free SOSG assay and the in-vitro NRU assay, our results for the two methods appeared consistent and in agreement with the literature. The power limitations of the laser diode used in the custom setup for the study, limit higher irradiance exposure (beyond 10mW/cm$^2$). Ideally the variance and the power output would be optimized in the future through use of more uniform laser diodes or LED arrays for PDT at higher irradiances (upto 400mW/cm$^2$).

## Disclosures if required

*N/A*

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**fDevelopment of Self-Activated Photosensitizers based on the Chemiluminescent System of Marine Coelenterazine**

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**Introduction:** Photodynamic therapy (PDT) of cancer possesses advantageous features, such as a minimally invasive nature and few side-effect [1]. However, its use can be restricted to more surface tumors, due to its dependance on light-irradiation. To overcome this problem, we propose the development of single-molecule photosensitizers (PSs), based on chemiluminescent (CL) marine Coelenterazine (Clz) [2-6], which can be self-activated in the absence of light.

**Approach:** Clz analogs were synthesized via routes optimized by our team [2-6], and their structure was characterized by HR-MS, NMR and FTIR spectroscopy. They were subjected to a through luminometric and photophysical characterization, to determine their CL features and singlet oxygen sensitization [2-6]. Their toxicity was investigated *in vitro* toward different cancer (breast, prostate, neuroblastoma, lung and gastric) and noncancer (breast and keratinocytes) cell lines, via the MTT assay in the absence of light [2-6].

**Results:** The obtained Clz analogs were designed so their CL reaction generate mainly triplet states, instead of singlet excited ones [2-6]. They were shown to indeed be able to sensitize the highly cytotoxic singlet oxygen, without light-irradiation, due to a CL reaction triggered solely by molecule overexpressed in cancer cells (superoxide anion) [2-6]. *In vitro* assays showed that these analogs present cytotoxicity toward all the different studied cancer cell lines [2-6]. Moreover, some of them even showed comparable or even better activity than a reference chemotherapeutic drug [3,4]. Finally, a promising profile of safety was observed in testing with noncancer cells [3].

**Conclusions:** Different single-molecule PSs were developed, which are capable of intracellular self-activation without light-irradiation [2-6]. Namely, they are directly chemiexcited to triplet excited states via a CL reaction triggered by a molecule overexpressed in cancer cells, which generates singlet oxygen without light-irradiation. This new approach was found to induce toxicity toward different cancer cell lines, while possessing a promising profile of safety for noncancer cells. Thus, these PSs can provide a pathway for eliminating the light-related restrictions of PDT, while maintaining its advantages.

**Keywords:** Photosensitizer Systems: Organic, Chemiluminescence, Self-Activated Photosensitizers

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### Erythrosine+KI with Multiple Sessions Green LED-mediated Photodynamic Therapy Potentiate Anticandidiasis Effect

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**Significance:** The concurrent use of double photosensitizers with multiple irradiation sessions is emerging as a promising modality for photodynamic therapy.
**Approach:** *Candida albicans* (ATCC10231) biofilm was treated with either erythrosine 220 $\mu$M, 100 mM KI, or 220 $\mu$M erythrosine+100 mM KI. Control groups were phosphate buffer saline, 1:100,000 U/ml nystatin solution, and Light-only respectively. After 60-secs incubation, experimental groups were irradiated with LED (520-540 nm, 250 mW/cm$^2$, 20 J/cm$^2$/session) for 1 or 3 sessions. The logarithmic transformation of biofilm count was calculated and a comparison at 1, 24 hrs was performed using median±interquartile range. The statistically significant difference was determined using Kruskal Wallis test with post hoc test at $p<0.05$
**Results:** 220 $\mu$M Erythrosine+100 mM KI with 60 J/cm$^2$ (20 J/cm$^2$x3 sessions) exerted the most anticandidal effect that comparable to nystatin effect.
**Conclusions:** Erythrosine 200 $\mu$M+KI 100 mM+3 sessions green LED (total energy 60 J/cm$^2$) effectively reduced *C. albicans* biofilm.

**Keywords:** Erythrosine, KI, green LED, Anti-candidiasis

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### Long-term follow-up results of a pilot study for nodular basal cell carcinoma with PDT using partial home treatment protocol

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**Significance:** Evaluate a photodynamic therapy (PDT) protocol with less time at the hospital and less painful for low-risk basal cell carcinoma (BCC) treatment.
**Approach:** Eight BCCs were selected, debulked, and received 20% methyl aminolevulinate cream. After 3h, the first irradiation was performed at the hospital (20min, 150J/cm$^2$). Then, the cream was reapplied, a portable irradiation prototype was fixed into the lesion. After 1.5h, the patients turned on the prototype for irradiation (2h, 312J/cm$^2$) at home. Disease-free survival rate and pain score during irradiations were evaluated.
**Results:** The clearance at 30 days after PDT was 87.5% by histological analysis. The mean follow-up was 21.5 months and the recurrence-free survival at 22 months was 75%. The pain score was significantly lower at home.
**Conclusions:** A less painful and more comfortable PDT treatment protocol was proved long-term efficient. A randomized clinical trial has been conducted to confirm these results.

**Keywords:** nodular basal cell carcinoma, methyl aminolevulinate, photodynamic therapy, home treatment

## 1. Introduction and Background

Photodynamic therapy (PDT) is a well-established treatment for low-risk basal cell carcinoma (BCC). The standard PDT treatment consists of two sessions performed at one-week interval[1]. A more comfortable and efficient protocol was performed for a single day treatment[2,3]. Although PDT provides an efficient treatment, the pain and the long stay in hospital area during treatment must be improved. So, a new and portable PDT irradiation prototype was developed in order to offer part of PDT treatment at home. A treatment with less irradiation, even for longer time, may reduce the pain felt by the patient.

## 2. Aims

The main objective of this pilot study was to evaluate a new portable irradiation prototype to provide part of the PDT treatment at home, decreasing the time spent at the hospital without compromising the efficacy. The pain score and long-term follow-up recurrence were also evaluated.

## 3. Methods

After a clinical and dermoscopic diagnosis, 8 nodular BCC were submitted to this pilot study. The lesions were debulked and the material was taken to histological confirmation of BCC. Then, a light layer of cream containing 20% methyl aminolevulinate was applied and the area was covered for 3 hours. The first irradiation was performed at the hospital, using a commercial LED device system emitting at 630 nm (LINCE, MMOptics, Brazil). The lesion was irradiated for 20 minutes with 125 mW/cm$^2$ totalizing 150 J/cm$^2$ of fluence. Immediately after the first irradiation, a new layer of cream was applied and the portable irradiation prototype with LED emitting at 630 nm was fixed using a medical adhesive tape. The patient was sent home and advised to turn on the prototype after 1.5 hours, and turn it off after 2 hours of irradiation (totalizing a 312 J/cm$^2$ of fluence). Histological evaluation of the treated area was performed through a punch biopsy 30 days after treatment. The patients were evaluated every 6 months through clinical and dermoscopy evaluation. The recurrence-free survival rate was calculated by the Kaplan-Meier survival curve. The pain during PDT treatment was assessed every 3 minutes during the first irradiation at the hospital and self-reported every 20 minutes during second irradiation at home on a numerical scale from 0 to 10. The median score values were compared between hospital and home irradiations in 7 different moments using the *t-student* test considering significant differences for a *p-value* <0,05.