The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Chemiluminescent self-activating photosensitizers for a selective anticancer therapy

Chaired by DR. ALFREDO BERZAL-HERRANZ;
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pharmaceuticals MDPI

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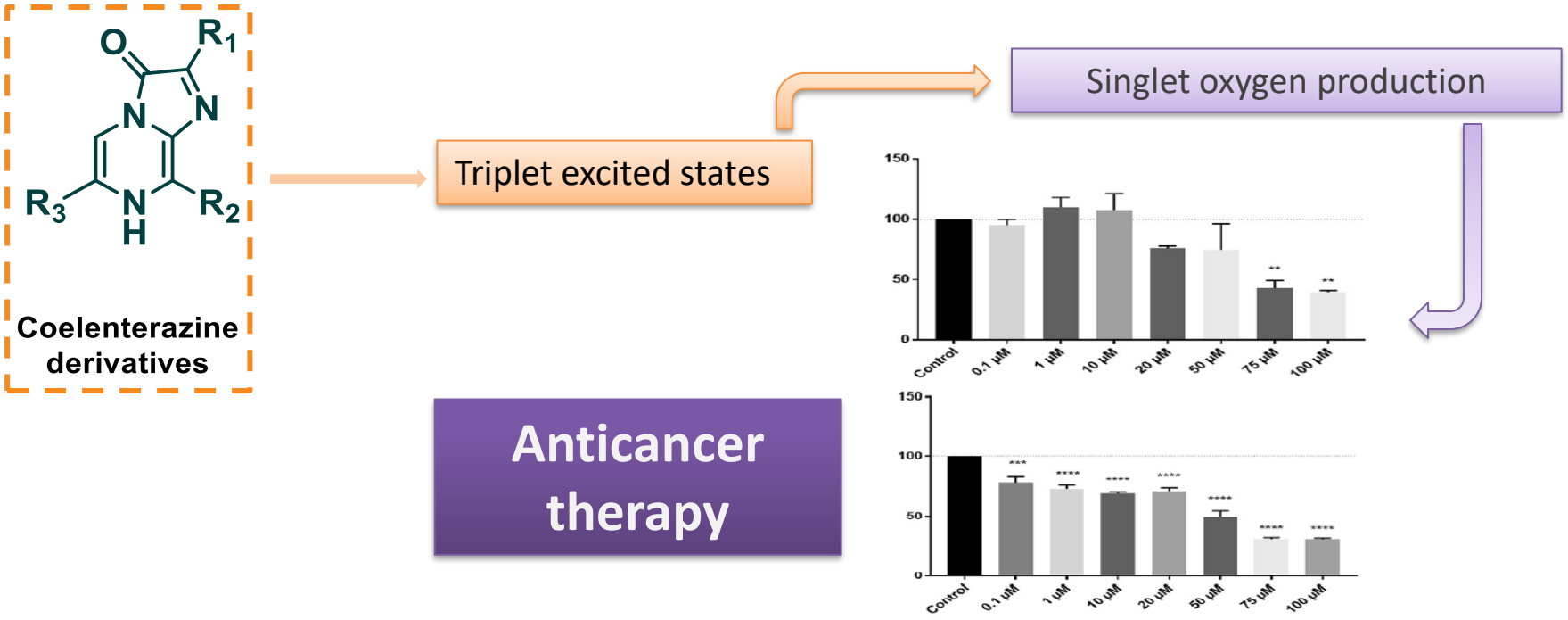
Chemiluminescent self-activating photosensitizers for a selective anticancer therapy

Graphical Abstract



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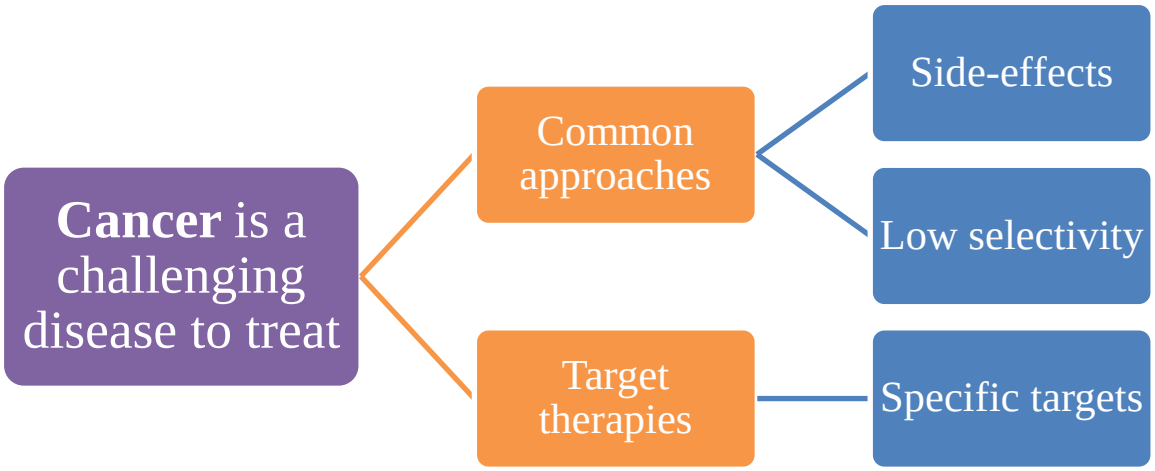
Abstract: Cancer is a challenging disease to treat, regarding treatment efficiency and side-effects. To overcome these problems, extensive studies are exploring therapies with reduced side-effects, such as photodynamic therapy (PDT). PDT has advantages over conventional therapies, however its dependence on light limits it to treating tumors under the skin/ outer lining of organs. We have developed new photosensitizers self-activated intracellularly with tumor-selectivity based on chemiluminescent reactions involving a cancer marker. The photosensitizer is directly chemiexcited to a triplet excited state generating singlet oxygen, without an external light source. Thus, we aimed to develop self-activating photosensitizers which can be used for light-free photodynamic therapy, eliminating its light-related restrictions. Cytotoxicity assays with breast and prostate cell lines showed that the novel photosensitizers possess significant toxicity toward tumor cells, while not affecting normal cells. Besides we compared the activity of these compounds with standard treatments, finding higher cytotoxicity.

Keywords: Anticancer; Chemiluminescence; Self-illuminating; Tumor-selective.

Introduction

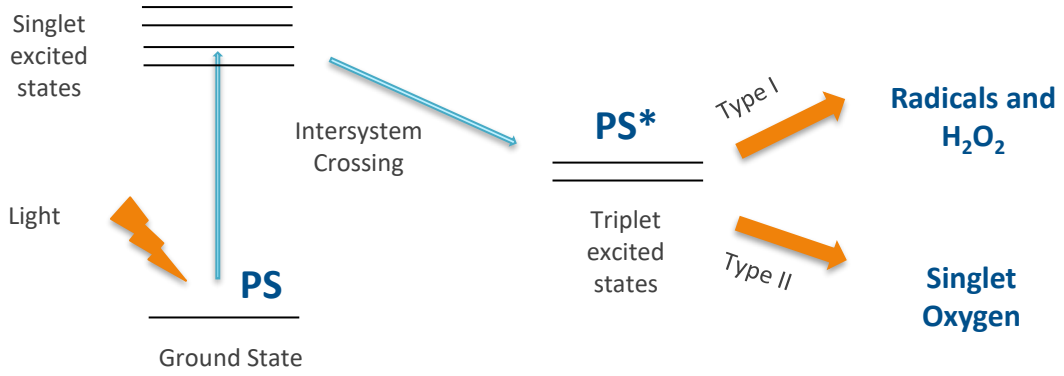
Cancer was responsible for almost 10 million deaths in 2020 *(GLOBOCAN)*



Photodynamic therapy (PDT)

Introduction

Photodynamic therapy (PDT)



Scheme 1. Schematic representation of the mechanisms of action of PDT.

Advantages

- High selectivity
- Reduced side-effects

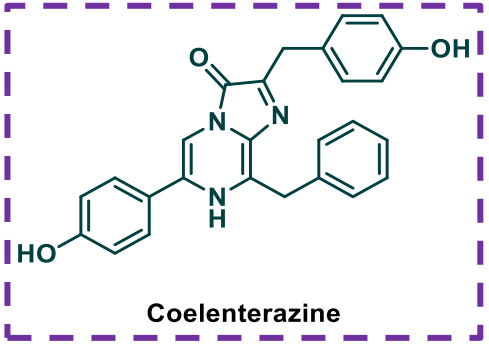
Disadvantages

- Depth penetration <1 cm
- Tumors located on the lining of internal organs/cavities and directly under the skin
- **Unsuccessful against metastatic tumours**

Design PSs that can be activated intracellularly without the need for external light sources

Introduction

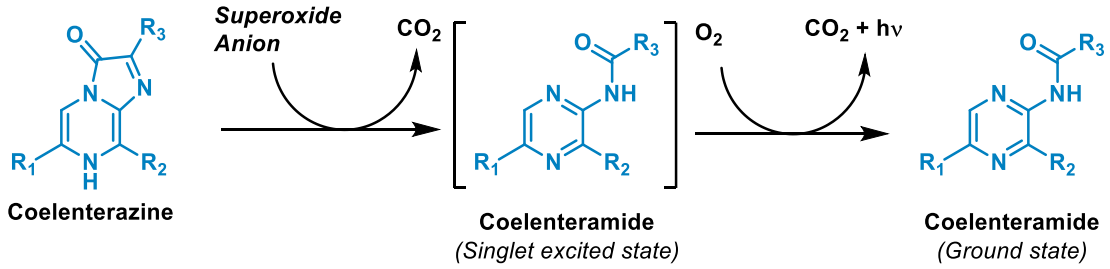
Objectives



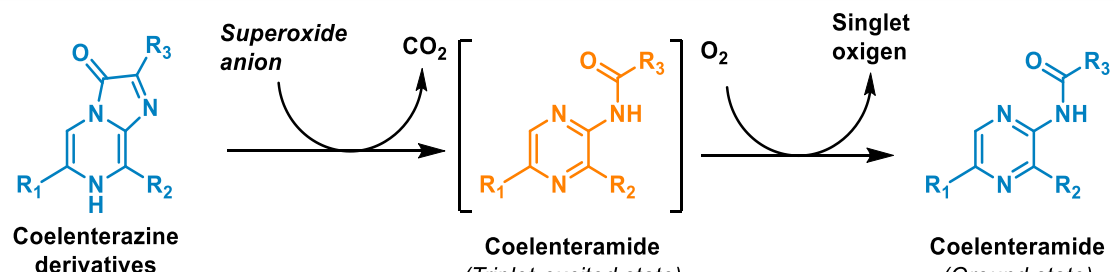
Coelenterazine

Chemiluminescence:
Conversion of thermal energy into excitation energy

Coelenterazine chemiluminescent system

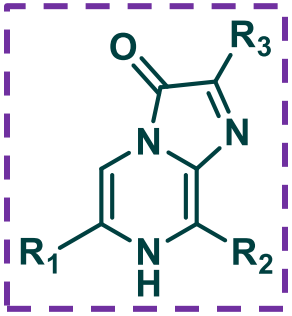


Proposed chemiluminescent system

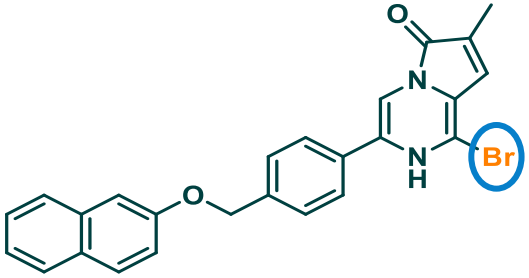


Introduction

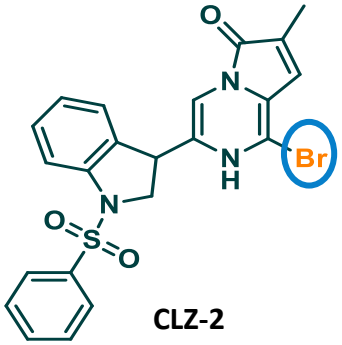
Objectives



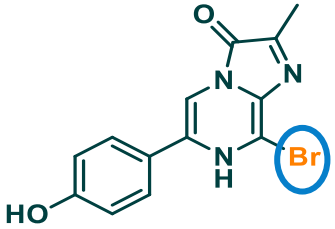
Heavy atom effect



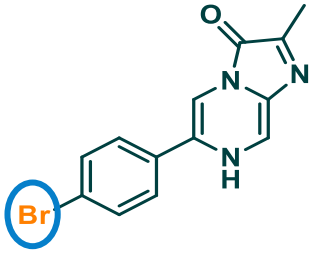
CLZ-1



CLZ-2



CLZ-3



CLZ-4

Patent PCT/IB2019/053642 (Pending)

Scheme 2. Chemical structures of synthetized Coelenterazine analogues.

Results and discussion

Chemiluminescent/Fluorescent reaction characterization

Chemiluminescent reaction triggered by superoxide anion

Singlet oxygen production

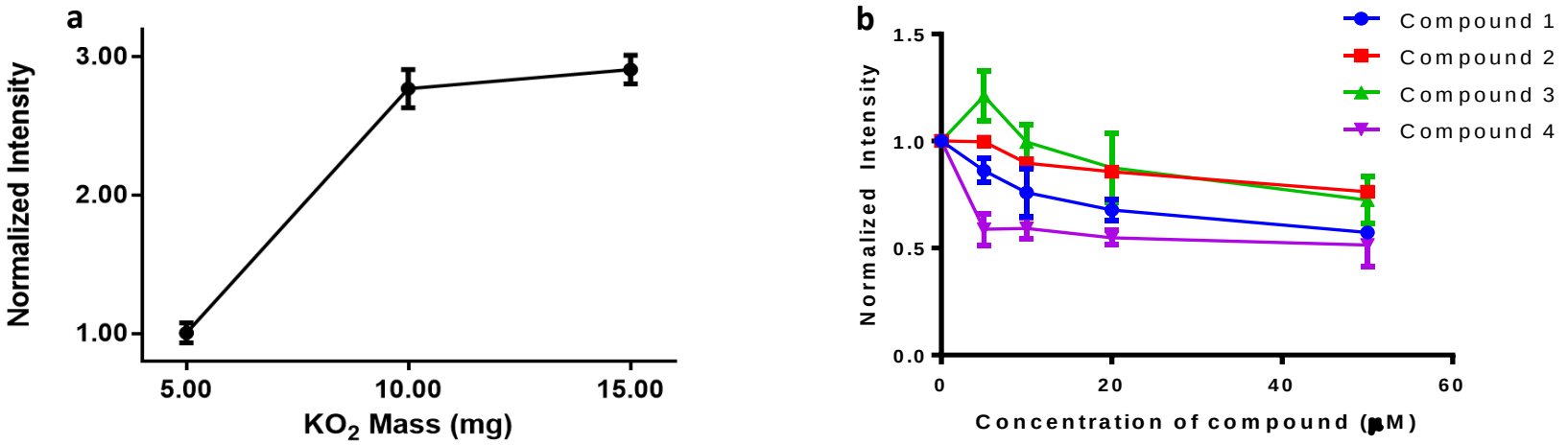


Figure 1. Chemiluminescence output of CLZ-4 as a function of KO_2 in methanol (**a**). Effect exerted on the fluorescence (I/I_0) of the singlet oxygen sensor, ABDA, as a function of the concentration of compounds (**b**).

ABDA: Fluorescence probe for singlet oxygen production
9,10-Anthracenediyl-bis(methylene)dimalonic acid

Results and discussion

In vitro cytotoxic potential

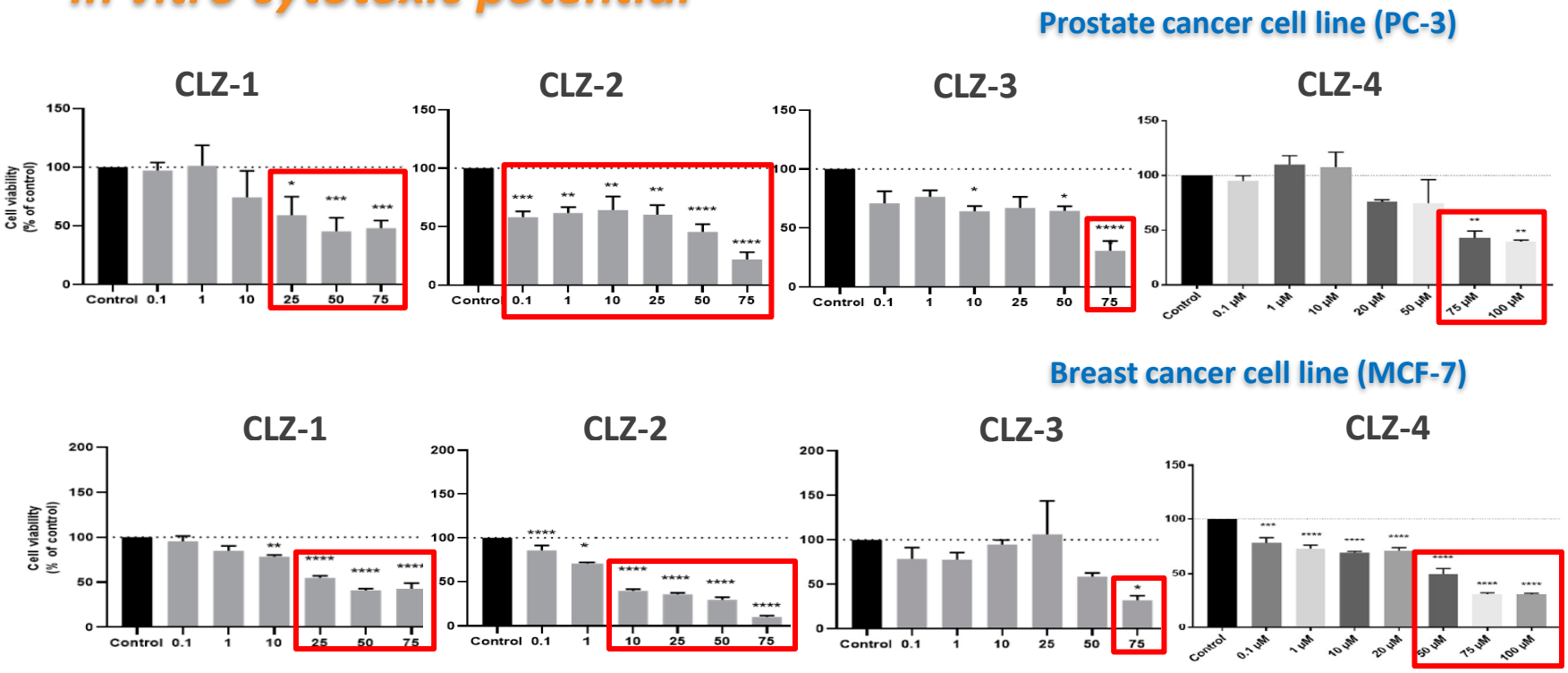
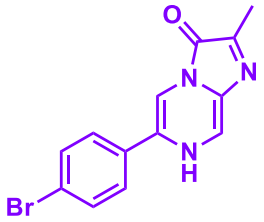


Figure 2. Percent viability of **PC-3** and **MCF-7** cell line measured by MTT reduction assays for **CLZ 1-3** (72 h) and **CLZ-4** (24h), at increasing concentrations. Percent viability was obtained by comparing with control cell viability, considered as 100%.

Results and discussion

In vitro selectivity evaluation in healthy breast cell line (MCF-10A)

Selectivity to cancer cells



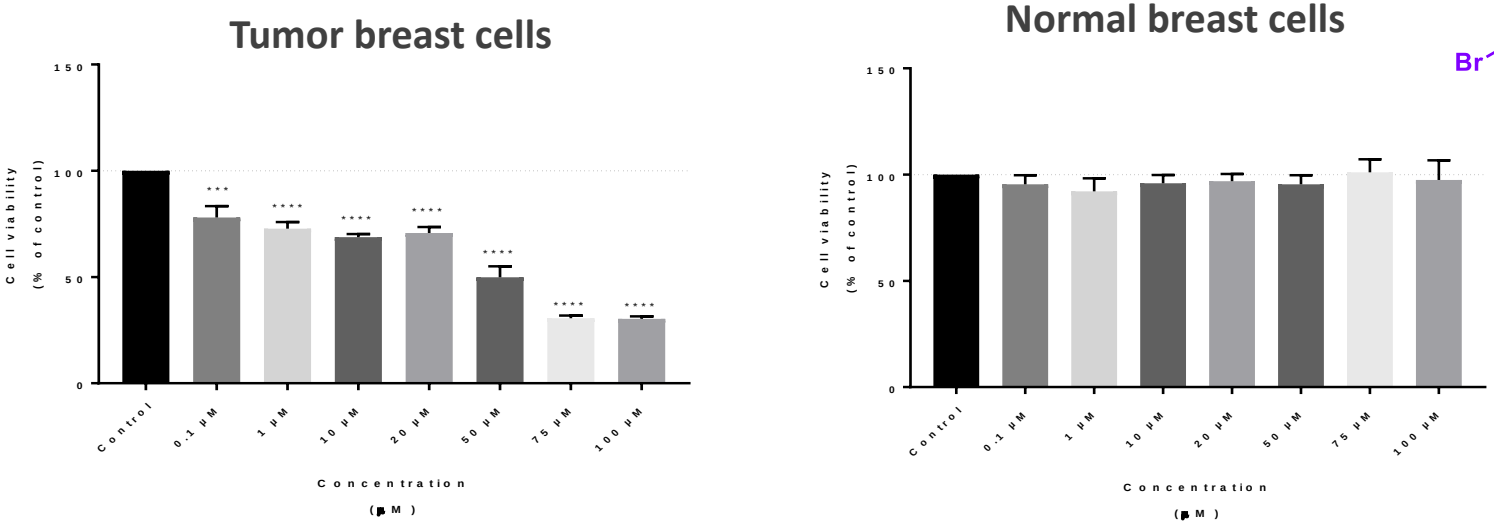
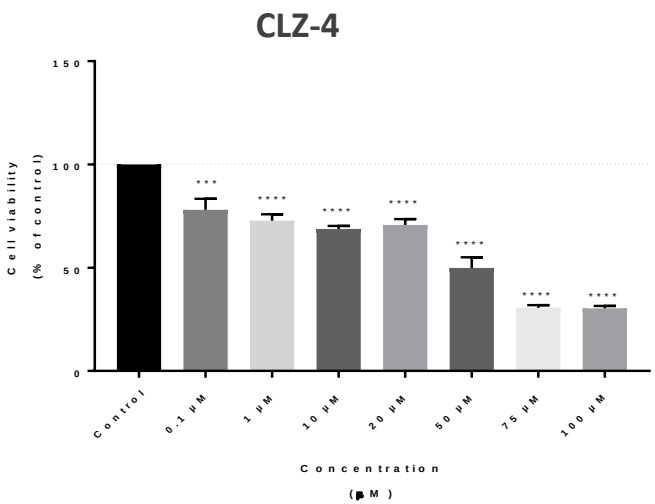
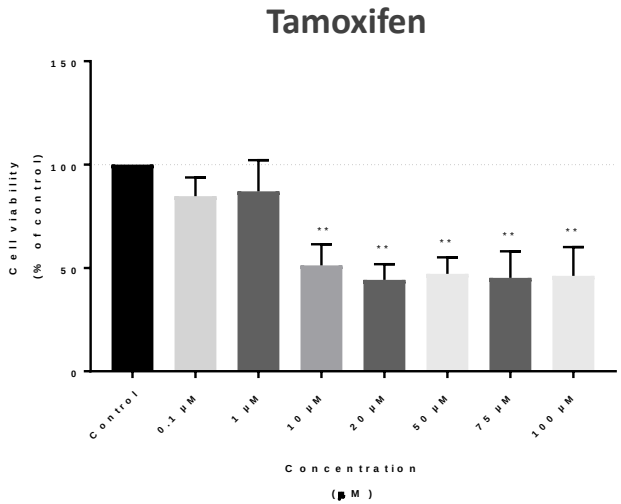


Figure 3. Relative viabilities of **MCF-7** and **MCF-10A** cells after 24 h incubation with increasing concentrations of **CLZ-4**. Percent viability was obtained by comparing with control cell viability, considered as 100%.

Results and discussion

In vitro comparison with standard treatment for breast cancer





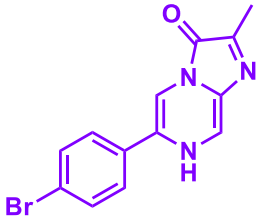
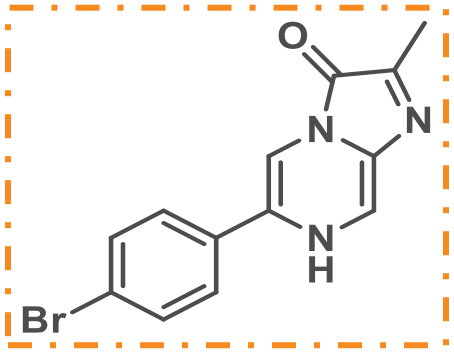
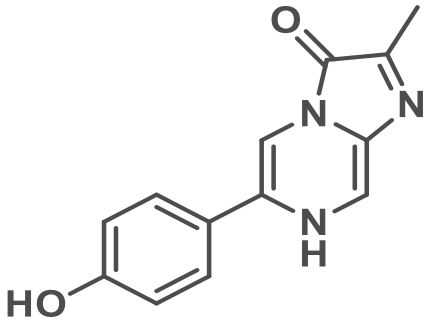


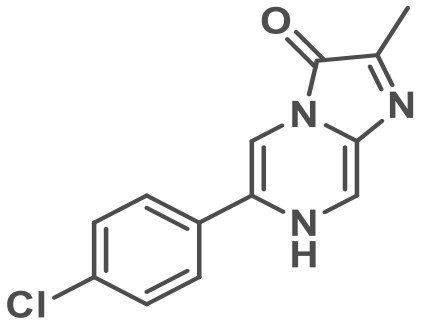
Figure 4. Percent viability measured by MTT reduction assays for **CLZ-4** (24 h) and **tamoxifen**, at increasing concentrations. Percent viability was obtained by comparing with control cell viability, considered as 100%.

Results and discussion



Halogen effect





Results and discussion

Chemiluminescent reaction characterization

Higher triplet excited states production

Chemiluminescent reaction triggered by superoxide anion

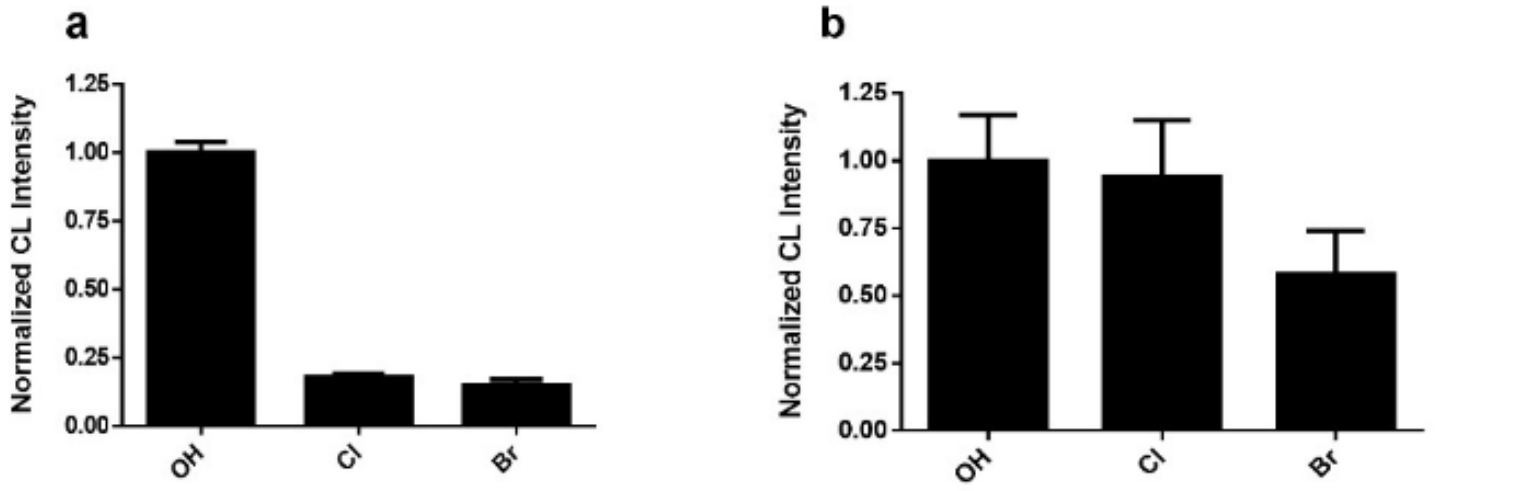


Figure 5. Normalized chemiluminescence output of OH-, Cl-, and Br-Cla in DMF-acetate buffer pH 5.14 (0.68%) (**a**). Chemiluminescence intensity of OH-, Cl-, and Br-Cla in the presence of 20 mg of KO_2 in metanol (**b**) .

Results and discussion

In vitro cytotoxic potential

Colon cancer cell line (HT-29)

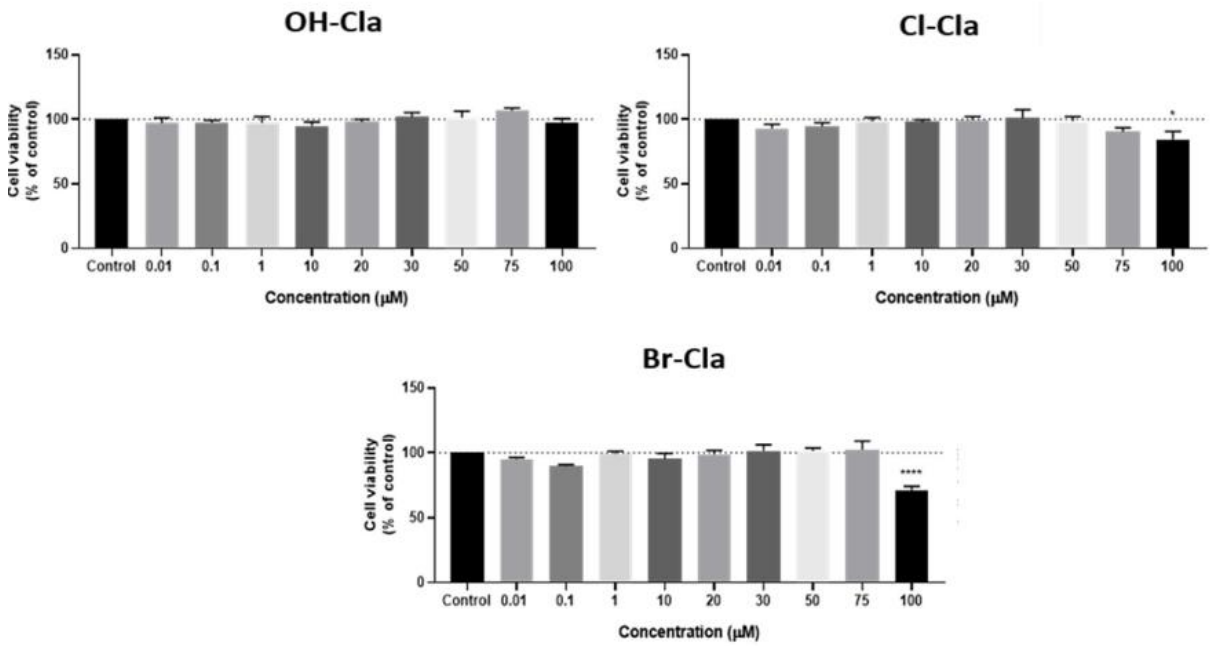


Figure 6. Effect of OH-, Cl-, and Br-Cla on HT-29 cell viability. Cells were cultured in the presence of increasing concentrations of each compound. After 48 h, an MTT assay as performed to measured cellular viability. Results are presented as mean ± SEM. * Statistically significant vs. control at $p < 0.05$; **** statistically significant vs. control at $p < 0.0001$.

Results and discussion

In vitro cytotoxic potential

Neuroblastoma cell line (SH-SY5Y)

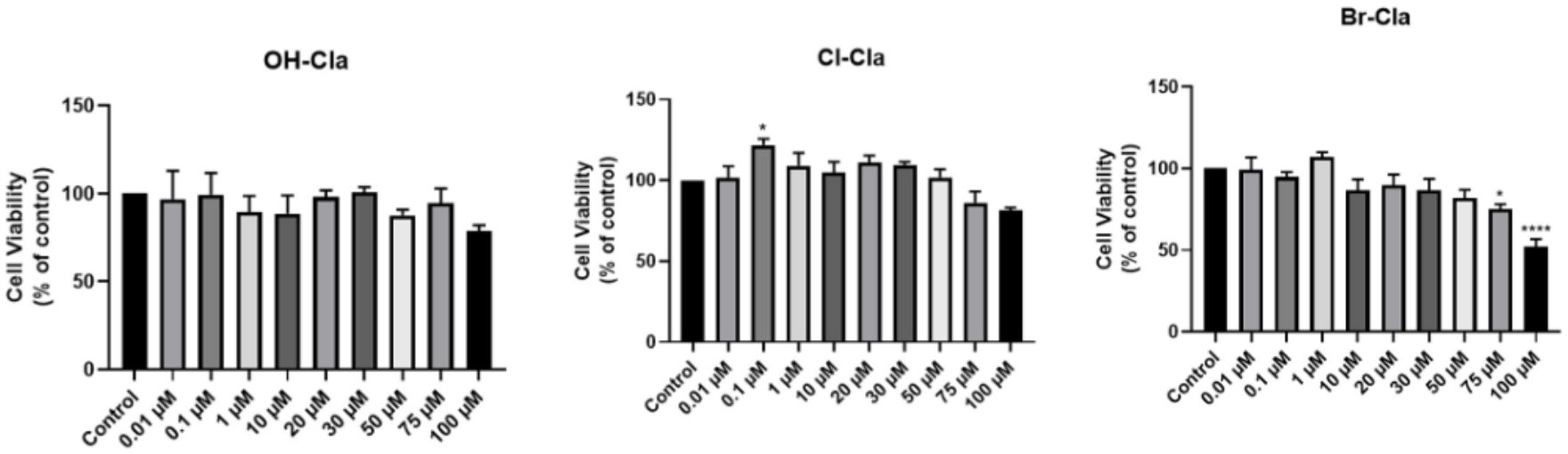


Figure 7. Effects of OH-, Cl-, and Br-Cla on SH-SY5Y cellular viability. Cells were cultivated in the presence of increasing concentrations of each compound. After 48 h, an MTT assay was performed to measure cellular viability. Results are presented as mean ± SEM. * Statistically significant vs. control at $p < 0.05$; **** statistically significant vs. control at $p < 0.0001$.

Results and discussion

In vitro cytotoxic potential

Neuroblastoma cell line (SH-SY5Y)

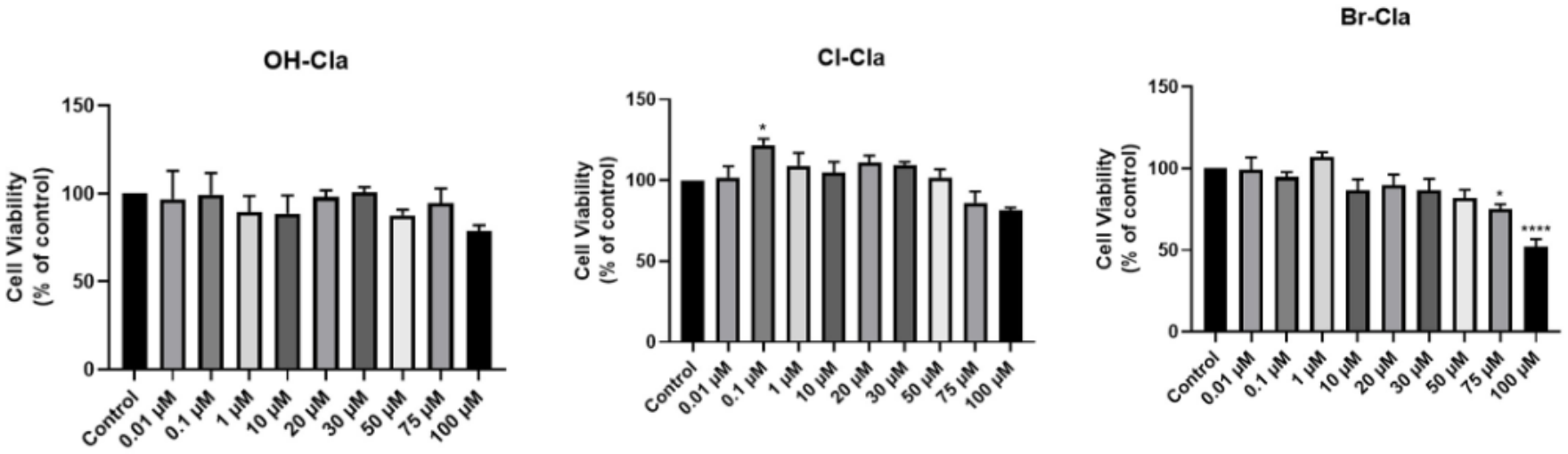
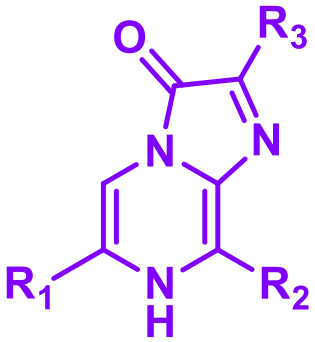


Figure 7. Effects of OH-, Cl-, and Br-Cla on SH-SY5Y cellular viability. Cells were cultivated in the presence of increasing concentrations of each compound. After 48 h, an MTT assay was performed to measure cellular viability. Results are presented as mean ± SEM. * Statistically significant vs. control at $p < 0.05$; **** statistically significant vs. control at $p < 0.0001$.

Conclusions

- New Coelenterazine derivatives (heavy atom effect) were designed and synthesized for improving PDT
- Intracellular self-activation (superoxide anion), in the absence of light
- Selectivity to cancer cells, inducing significant cytotoxicity in different tumor cell lines, even higher than reference drugs
- Pathway for eliminating the light related restrictions that PDT presents.



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