Carbonic Anhydrase Inhibitors Featuring a Porphyrin Scaffold: Synthesis, Optical and Biological Properties.

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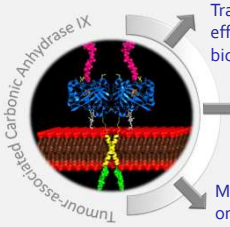
Biological context

Cancer represents one of the most important causes of premature death in the world, responsible for 9.6 million deaths and 18.1 million new cases in 2018.[1] Surgery, chemotherapy and radiotherapy in individual or in combination are commonly used to treat malignant tumors in current clinical practice.[2] Unfortunately, patients undergoing such treatments experienced undesirable severe side effects including infection, inflammation, drug resistance and normal tissue damage. Photodynamic therapy (PDT) has emerged as an interesting alternative to these treatments due to its minimal invasiveness, implementation, low systemic toxicity and high efficiency.[2] PDT uses photosensitizers (PSs), which can be activated by light at specific wavelength to generate reactive oxygen species (ROS) from molecular oxygen and thus, attends to the cancer cells death through apoptosis or necrosis.[4] However, the efficacy of this operational dependence in oxygen in tumor microenvironment can lead to tumor hypoxia, trigger the signaling cascade mediated by hypoxia inducible factor (HIF) and release pro-angiogenic growth factors, which are ultimately responsible for cancer cell survival or tumor regrowth. Human carbonic anhydrase IX (hCA IX, EC 4.2.1.1) has been identified as a crucial downstream gene product in HIF-mediated cascade, regulating both intra- and extracellular pH balance and promoting tumor survival and invasive migration.[5]

Target



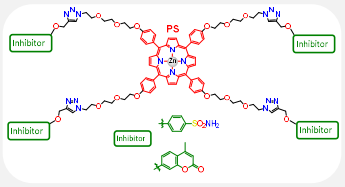
Transmembrane zinc metalloprotein catalyzing efficiently reversible hydration of CO_2 to give bicarbonate and a proton.

Linked to acidosis, connected with the increase of the aggressive / invasive phenotype of tumors, and correlated with therapeutic resistance.

Marker of hypoxia and bad prognostic indicator in oncology

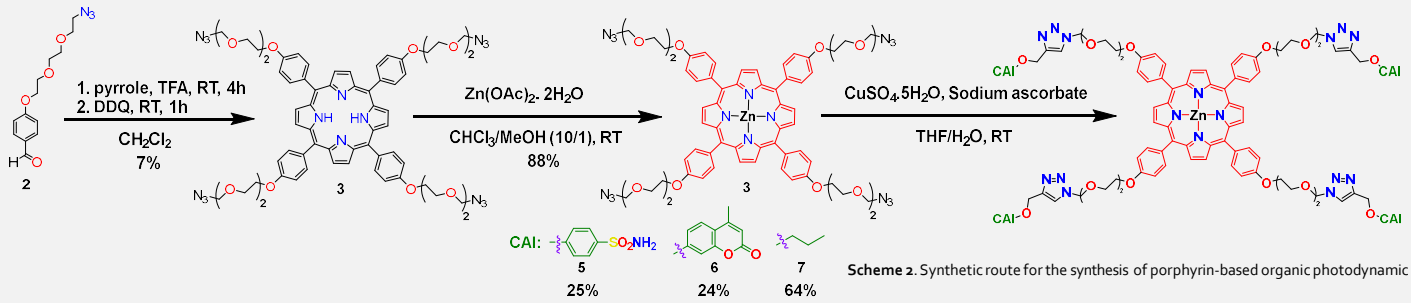
Objectives

- Development of new anticancer agents combining PDT with targeted therapy directed at inhibition of hCA IX
- Synthesis and characterization of conventional photosensitizers (porphyrin) linked with selective hCA IX inhibitors in coumarin or sulfonamide series (Scheme 1).



Scheme 1. Structure of porphyrin-based organic photodynamic inhibitors.

Synthesis



Scheme 2. Synthetic route for the synthesis of porphyrin-based organic photodynamic inhibitors.

Optical properties

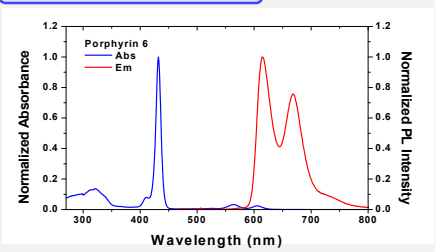


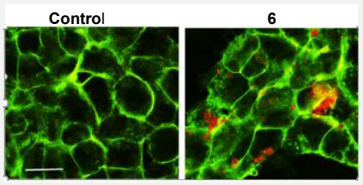
Figure 1. UV-Visible absorption (blue) and emission (red) spectra of porphyrin **6** in DMSO (C = 10^{-6} M, λ_{ex} = 440 nm)

Cpds	λ_{abs} (nm)	λ_{em} (nm)	φ_F (%)
5	431, 561, 604	616, 665	3
6	431, 562, 605	615, 668	3
7	431, 563, 604	615, 667	4

Table 1. Optical data for porphyrins **5** , **6** and **7** in DMSO solution.

Imaging and PDT properties

Fluorescence imaging of Capan-1



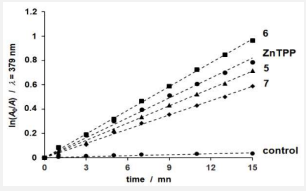
Porphyrin 6 is accumulated at the cell membrane due to the targeting of membranar **hCA IX** and **hCA XII** exploiting the selective inhibitor coumarin groups

Porphyrin 5 is localized on the membranes and inside the cell due to the non-selectivity of sulfonamides groups.

Porphyrin 7 is aggregated in solution.

Figure 3. Fluorescence imaging of pancreas cancer cells (Capan-1) incubated for 24 h with or without (Control) compounds **5-7** at 10 µM

Singlet oxygen ($^1O_2$) generation



All porphyrins are able to generate $^1O_2$. **6** shows better $^1O_2$ generation efficiency than **ZnTPP**, **5** and **7**

Figure 4. Time dependent photodecomposition of DMA photosensitized by **5-7** and **ZnTPP**

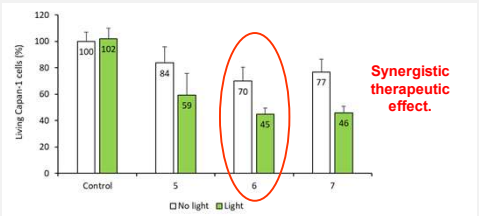


Figure 5. PDT efficiency for Capan-1 cells incubated 24 h with PSs **5-7**

Biological properties

Carbonic anhydrase inhibition assays

Cpds	K_I (nM)[a]			
	hCA I	hCA II	hCA IX	hCA XII
4	>50 000	>50 000	>50 000	>50 000
5	98,7	35,5	48,6	16,9
6	>50 000	>50 000	652,1	92,4
7	>50 000	>50 000	>50 000	>50 000
AAZ	250	12	25	5.7

Table 2. Inhibitory activity of compounds **4-7** against **hCA I, hCA II, hCA IX** and **hCA XII** using acetazolamide **AAZ** as reference drug.

Cytotoxicity assays

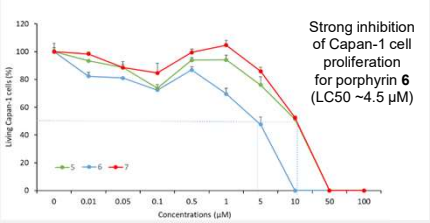


Figure 2. Cytotoxic study of compounds **5-7** incubated with increasing concentrations (from 0.01 to 100 µM) in Capan-1 cells for 72 h.

Conclusion and Outlooks

Zinc (II) porphyrins tetrafunctionalized with carbonic anhydrase inhibitors in sulfonamide or coumarin series were synthesized. We showed the advantageous combination of porphyrin-based **PS** and selective **CAI** without loosing photosensitizing efficiency, in particular, for PS **6** integrating coumarin inhibitor groups. The next step of this study will consist in performing tests on integrated models such as small animals bearing human pancreatic tumors.

References:

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