The Impact of Atropisomerism on Redaporfin-PDT Efficacy

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Introduction

Atropisomerism presents an intriguing, but often neglected, source of structural variety in drug development. An awareness of the implications of this diversity in tetrapyrrole structures may enhance photosensitizer development for photodynamic therapy (PDT) and related drug developments. PDT efficacy relies on the delivery of three components which are individually non-toxic, a photosensitizer drug (PS), light at a specific wavelength and oxygen. These combine to generate highly reactive oxygen species which can result in cytotoxicity and vascular damage.[1] We describe below an investigation of the efficacy of the atropisomers of a pre-clinical photosensitizer, Redaporfin (Redp.), for use in PDT. Redaporfin presents suitable parameters for investigating PDT including strong absorption at 750 nm, high ROS yield generated by type I and type II processes and high cure rates associated with anti-tumor immunity *in vivo*.[1,2] Although the photochemical and photophysical properties of the four atropisomers are similar, their therapeutic efficacies are significantly different. Similar results were obtained for bacteriochlorins (Redp.) and related porphyrins (P11).

Redaporfin Atropisomers

Redaporfin's *ipso*-phenyl-macrocyclic residues experience hindered rotation. This results in different spatial distributions of the sulfonamide groups in the meta positions, generating four distinct chemical species in the form of atropisomers.

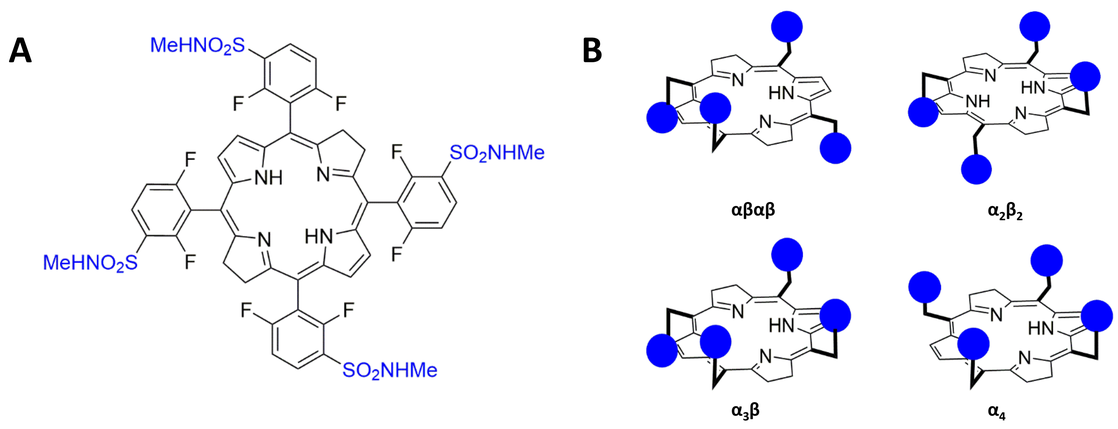


Figure 1. Molecular structure of Redaporfin (A) and Redaporfin atropisomers (B)

Redaporfin atropisomers are separable and stable at room temperature

Separation of Redaporfin atropisomers may be achieved efficiently via flash chromatography. Analytical-HPLC was used to characterize the atropisomer fractions. Analysis of the redaporfin drug mixture demonstrated the relative abundance of atropisomers present; 13.2% αβαβ, 20.9% $\alpha_2\beta_2$, 53% $\alpha_3\beta$ and 12.9% α_4.

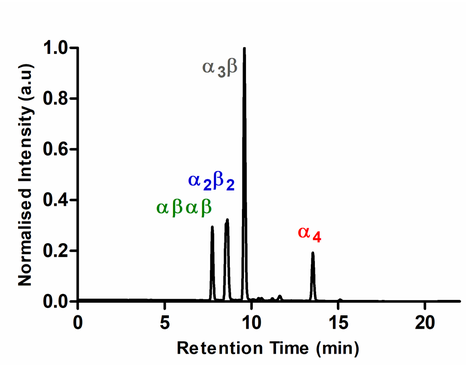


Figure 2. HPLC-chromatogram of Redaporfin drug mixture

Why are the efficacies of redaporfin atropisomers different? *in vitro* studies

Contrasting photo-toxicities were observed for each atropisomer

Photo-toxicities of the atropisomers were evaluated via the resazurin reduction assay. It was revealed that the α_4 atropisomer (represented by the red trend in figure 3) was consistently the most photo-toxic relative to the other atropisomers.

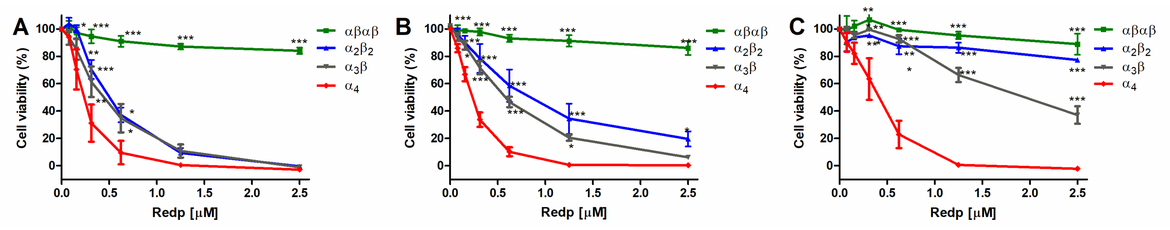


Figure 3. Cell-viability of cell lines (A) U-2 OS, (B) 4-T1, and (C) CT-26 post-incubation with Redp. atropisomers and irradiation at 750nm, 0.2 J/cm^2. Two-way ANOVA (α_4 vs others), * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$

Variation of atropisomer cellular uptake aligned with the different photo-toxicities exhibited by the atropisomers

Enhanced cell-uptake of the α_4 atropisomer was visualized by confocal microscopy (figure 4A) in U-2 OS cells. The relative difference of atropisomer uptake was quantified by flow cytometry following various fixed periods of atropisomer incubation in cells (figure 4B, C & D).

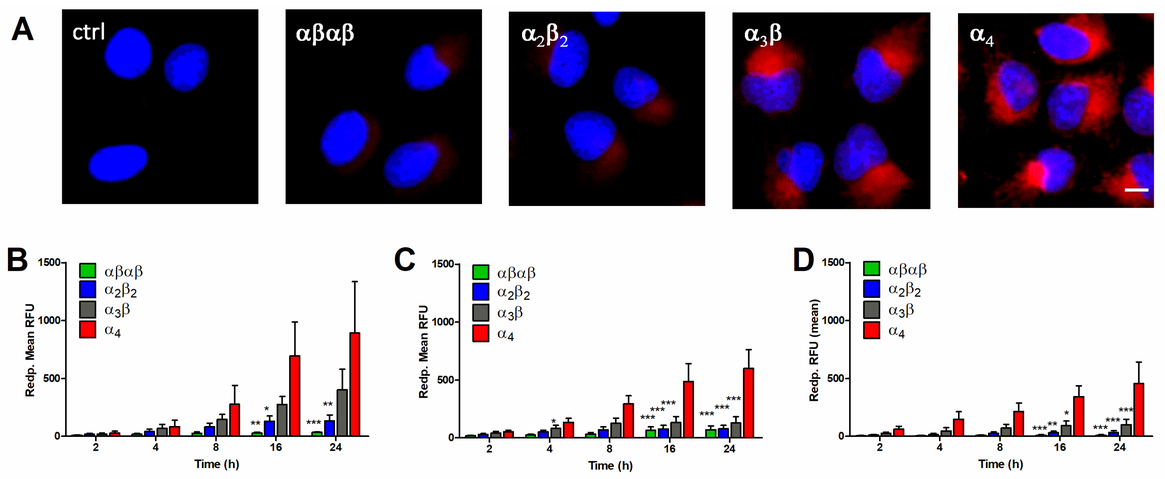


Figure 4. Cellular uptake of Redp. atropisomers assessed via (A) confocal microscopy in U-2 OS cells, (scale bar = 10 μm, Redp. – red, Blue – DAPI) and (B – D) flow cytometry in U-2 OS, 4-T1 and CT26 cells respectively. Two-way ANOVA (α4 vs others)

Variation of atropisomer uptake *in vitro* is a passive process

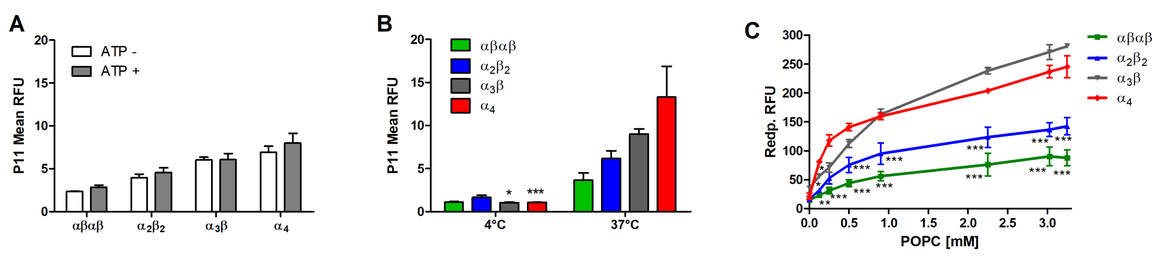


Figure 5. (A) Cellular-uptake of P11 following ATP-depletion and (B) at 4 °C. (C) Redp. atropisomer interaction with POPC liposomes with fluorescence detection by microplate reader

2-deoxy-D-glucose treatment, inducing ATP depletion, did not impact uptake (figure 5A). A decrease in uptake was observed at 4 °C (figure 5B). Cell-uptake of the atropisomers was concluded as a principally passive, temperature-dependent process for each atropisomer. Increased incorporation of α_4 and $\alpha_3\beta$ into POPC liposomes (figure 5C) indicated that variation of cellular-uptake is due to the favourable topological orientation of substituents of these atropisomers which may enhance passive diffusion.

Cellular internalization and PDT- efficacy of atropisomers varies *in vivo*

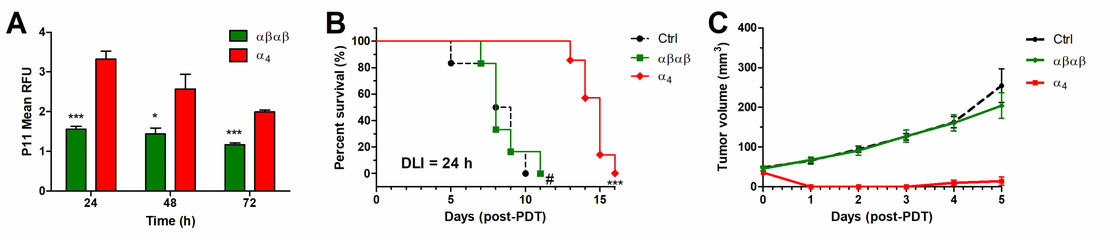
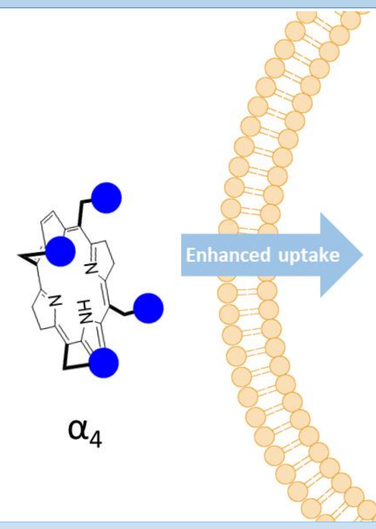


Figure 6. (A) Tumor cell-internalization P11 atropisomers. Mean represented >/= 4 mice per group with SEM, unpaired t-test. (B) Survival curves of BALB.C mice bearing CT26.WT tumors following cellular-PDT (0.35 mg/kg, 60 J/cm^2, DLI = 24 h) (C) Tumor re-growth following cellular-PDT treatment, mean represented >/= 6 mice per group with SEM

Tumor cell-internalization of the P11 atropisomers was demonstrated to vary (figure 6A) in female BALB/C mice bearing CT26.WT tumors. Single cell suspensions of tumors were analyzed by flow-cytometry 24 h post i.v injection. Significantly lower internalization of the αβαβ atropisomer relative to the α_4 atropisomer culminated in lower αβαβ efficacy with a protocol of cellular-PDT (figure 6B). Tumor regrowth in the αβαβ group closely aligned with the Ctrl group (figure 6C).

Conclusion and Outlook

This work demonstrated the strong influence of atropisomerism on the therapeutic outcome of redaporfin-PDT with the α_4 atropisomer displaying enhanced PDT efficacy *in vitro* and *in vivo*. Uniform orientation of the sulfonamide groups in the case of the α_4 form may increase interaction with the cell-membrane and passive diffusion, potentiating PDT-efficacy.



References

1. Rocha, L. B.; Gomes-da-Silva, L. C.; Dąbrowski, J. M.; Arnaut, L. G., *European journal of cancer* 2015, 51 (13), 1822-30.
2. Soares, H. T.; Campos, J. R.; Gomes-da-Silva, L. C.; Schaberle, F. A.; Dabrowski, J. M.; Arnaut, L. G., . *Chembiochem : a European journal of chemical biology* 2016, 17 (9), 836-42.

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