# Gallium and functionalised-porphyrins combine to form potential lysosome-specific multi-modal bio-probes

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**ABSTRACT:** A water soluble bimetallic normal ('cold') and radiochemical ('hot') gallium-porphyrin-ruthenium-bipyridine complex (**GaporRu-1**) has been synthesized by microwave methodology in short reaction times with good (>85%) yields. $^{68}$**GaporRu-1** is demonstrated to be a potential multi-modal and functional bioprobe for positron emission tomography (PET), lysosome specific optical imaging and photodynamic therapy.

Positron Emission Tomography (PET) is a promising method to visualise lesions in their early stages with minimal damage.[1] There are only a few lighter elements that have been applied as PET radionuclides e.g. carbon, nitrogen, oxygen and fluorine; these are attractive due to their biocompatibility but the first three suffer from very short half-lives. $^{18}$F-fluorodeoxyglucose (FDG) has been used extensively for tumour location,[2] but the high background FDG uptake by normal tissue can limit its application and development.[3] Furthermore, a cyclotron is required for the production of the above mentioned radionuclides. Therefore, $^{68}$Ga (~$t_{1/2}$ = 68 min) is an attractive radionuclide for PET imaging, due to the fact that $^{68}$Ga is generator-formed. The first generation of biomedical gallium compounds e.g. gallium nitrate, was approved by the FDA in 1994 and used in hypercalcemia, metabolic bone disease, bladder cancer and other microbial infection.[4] We believe that functionalised porphyrins can serve as good ligands for the coordination of $^{68}$Ga. Porphyrins are one of the most promising singlet oxygen ($^1O_2$) generators, that can be applied *in vitro* and *in vivo*.[5] Recently, Fazaeli et al. and Boyle et al. have reported new methodology to complex 'hot' $^{68}$Ga and free-base porphyrin.[6-9] However, further information on solubility, cell permeability, and *in vitro* subcellular localization is urgently needed. The replacement of zinc(II) by gallium(III) facilitates optical and PET imaging for cancer cells, as well as inhibiting cancer cell growth in their early stages, thus creating both a PDT and PET agent.

Herein, a new water-soluble complex, **GaporRu-1** (Figure 1) has been synthesized and fully characterized. The replacement of zinc by gallium lowers the overall acidity of the complexes and exhibits lysosome specific localization in cancer cell lines, compared to mitochondria specificity in the zinc-ruthenium analogue. An impressive singlet oxygen quantum yield has been recorded allowing this to be considered a potential PDT agent. Importantly, we are also able to carry out rapid $^{68}$Ga radiolabelling (~15 min) in the same system using developed microwave reaction methodology, with reaction yields of ~ 85%.

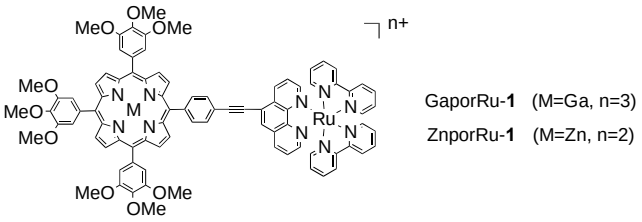


**Figure 1.** Structure of porphyrin-linked ruthenium complexes (**GaporRu-1** and **ZnporRu-1**-control[9]).

The clear yellow solution of **GaporRu-1** was observed in aqueous media (with 0.1% DMSO, Supporting Information, Fig. S6) and the strong Soret band was found at 421 nm (log ε = 5.39). Two Q bands were observed in **GaporRu-1** at 552 and 593 nm.[10] The absorption band observed in the UV-vis region is attributed to the π to π* transition of bipyridine (bpy). The MLCT of Ru moiety is located at ~450 nm, which is merged into the shoulder of intense Soret absorption bands of the porphyrin (~420 nm).

**Table 1. Photophysical properties of the GaporRu-1 and ZnporRu-1**

| Compound | $\lambda_{abs}$/nm (log$\varepsilon$) | Emission/nm ($\lambda_{ex}$=420nm) | $\Phi_{em}$[a]/% | $\Phi_{\Delta}$[b]/% | pKa[c] | Yield% (Microwave) | Yield% (Radiolabelling) | $IC_{50}$[d] |
|---|---|---|---|---|---|---|---|---|
| **GaporRu-1** | 286(4.92), 421(5.39), 552, 593 | 607, 660 | 2.7 | 61.4 | 3.45 | 96% | 85% | 70 μM |
| **ZnporRu-1** | 291(4.86), 432(5.14), 561, 604 | 613, 663 | 2.3 | 19.9 | 4.09 | | | 27 μM |

[a] The emission quantum yields of **GaporRu-1** and **ZnporRu-1** ($\lambda_{ex}$ = 420 nm, in toluene, compared with 5,10,15,20-tetraphenyl-zinc-porphyrin, $\Phi_{em}$ = 0.033). [b] The $^1O_2$ quantum yields of **GaporRu-1** and **ZnporRu-1** ($\lambda_{ex}$ = 420 nm, in $CHCl_3$, compared with the reference standard, 5,10,15,20-tetraphenylporphyrin - $H_2TPP$, $\Phi_{\Delta}$ =0.55). [c] pKa value of compounds **GaporRu-1** and **ZnporRu-1** get from variation of the ratio of the emission intensity at maximum *vs.* pH (295 K, 5% DMSO, $\lambda_{ex}$ = 420nm). [d] $IC_{50}$ of dark toxicity of compounds **GaporRu-1** and **ZnporRu-1** in MCF-7 cell lines.

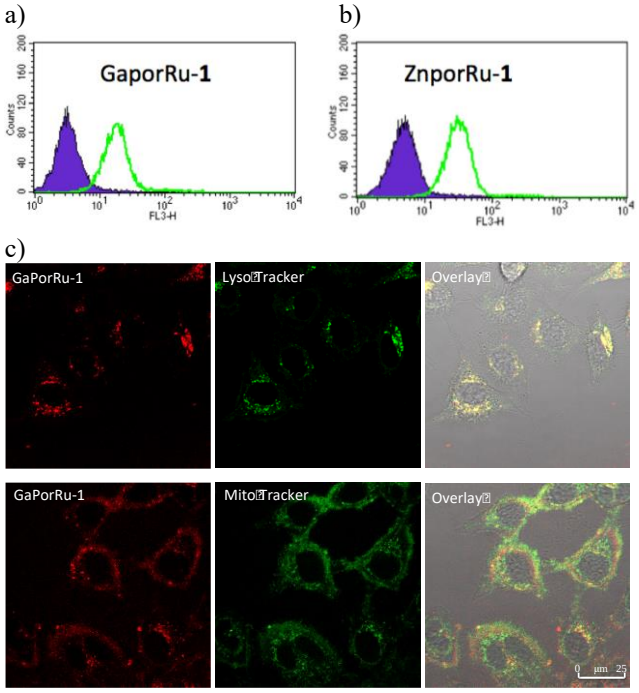


**Figure 2.** Flow cytometry of **GaporRu-1** and **ZnporRu-1** in the HeLa cells (a) and (b), dosed concentration = 10 μM and 6 hours incubation); (c) The confocal *in vitro* images of **GaporRu-1** in HeLa cells (dosed concentration = 2 μM, 6 hours incubation and $\lambda_{ex}$ = 532 nm), and co-staining with lysosome tracker (positive control) and mitochondria tracker (negative control). The merged yellow co-staining emission can only be found with red emission from **GaporRu-1** and green emission from lysotracker.

With the aim of carrying out optical imaging, the visible excitation from the porphyrin was taken at 420 nm due to the lower phototoxicity when compared to UV excitation. Strong red emission bands were found at ~610 nm and ~660 nm for both complexes. For singlet oxygen ($^1O_2$) emission of **GaporRu-1**, an impressive $^1O_2$ quantum yield was recorded e.g. ~ 61.4% in $CHCl_3$, (Table 1). [11,12]

The change from Zn(II) to Ga(III) results in an overall charge increase of the complexes by $1^+$ and enhances the amphiphilic character of **GaporRu-1** compared to our **ZnporRu-1** model compound. It is widely acknowledged that molecules presenting a cationic and amphiphilic nature can possess better cell permeability.[13] The cellular uptake of **GaporRu-1** and **ZnporRu-1** was evaluated by the flow cytometry (Figure 2). The results showed that **GaporRu-1** has 4- to 5- fold better uptake in HeLa cells than **ZnporRu-1** under the same experimental conditions (10 μM **GaporRu-1**/ **ZnporRu-1**, incubation time = 6 hours, Fig. 2a and 2b). Rather than the variation of the cellular uptake rate, the *in vitro* subcellular localization of **GaporRu-1** and **ZnporRu-1** is also different due to the more acidic pKa in **GaporRu-1** (pKa of **GaporRu-1** = 3.45 and **ZnporRu-1** = 4.09). *In vitro* confocal imaging of **GaporRu-1** and **ZnporRu-1** has been studied in HeLa cells and the sub-cellular localization of **GaporRu-1** and **ZnporRu-1** is different, with **ZnporRu-1** residing in the mitochondria,[9] whilst **GaporRu-1** is found in the lysosome. We believe that this is due to the Ga-porphyrin having a unipositive charge whilst the Zn-porphyrin remains neutral resulting in a lysosome specific probe.[9] The co-localization experiments with green lysotracker (positive control) and green mitotracker (negative control) were carried out and only a yellow co-staining emission can be found in red **GaporRu-1** with green lysotracker (Figure 2 c).[14,15] For the development of a PDT-active multimodal imaging probe, the light and dark *in vitro* cytotoxicity of targeted compounds were evaluated. In terms of dark cytotoxicity, **GaporRu-1** is less toxic than **ZnporRu-1** both in HeLa cells and MCF-7 cell lines (Figure S9, S10 and Table 1). In the light cytotoxicity assay, **GaporRu-1** shows an impressive photo-cytotoxicity

effect compared with **ZnporRu-1** (Figure 3). The $IC_{50}$ value of **GaporRu-1** in HeLa cells and MCF-7 cells are 23 μM in HeLa and 18 μM respectively. This can be explained by the different $^1O_2$ quantum yields in **GaporRu-1** ($\Phi_\Delta$ = 61.4%) and **ZnporRu-1** ($\Phi_\Delta$ = 19.9%).

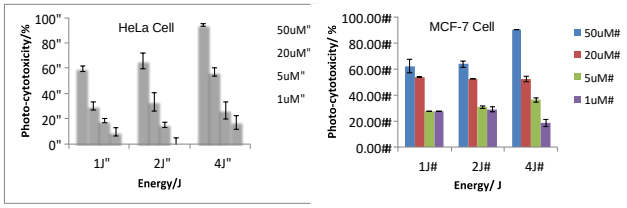


**Figure 3.** Photo-cytotoxicity of **GaporRu-1** with concentration from 1 μM to 50 μM and energy from 1 J to 4 J in assays of cervical cancer cells HeLa and breast cancer cells MCF-7 with 24 hours incubation time.

For the development of PET imaging agents, the labelling with hot gallium is crucial. For the cold analogue **GaporRu-1**, an impressive reaction yield (96 %) can be obtained within 15 minutes using modified microwave reaction methodology. This methodology was also used for the $^{68}$Ga labelling of the porphyrin ligand system, with **$^{68}$GaporRu-1** synthesised with 85% radiolabel incorporation within 15 min (Figure S4). $^{68}$Ga radiolabelling was most effective in acetic acid under microwave heating at 150 $^o$C for 15 minutes (Figure S5). Higher porphyrin concentrations were found to produce better labelling results, however a maximum concentration of 2 mg/ mL was used in order to limit the amount of unlabelled com pound. The reaction yield was determined by radio HPLC analysis, with purity confirmed by comparison with the UV chromatogram of **GaporRu-1** (Figure 4).

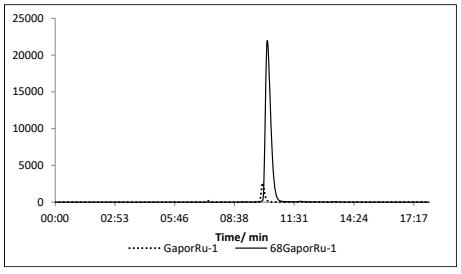


**Figure 4.** Overlay of cold **GaporRu-1** UV HPLC chromatogram and **$^{68}$GaporRu-1** radio chromatogram showing good correlation between the two peaks (NB. UV peak expected at a slightly lower retention time as sample analysed by UV detector prior to the radio detector).

In conclusion, we have rapidly synthesized new water-soluble and emissive bimetallic 'cold' and 'hot' gallium porphyrin-ruthenium complexes by microwave methodology and in good yields (> 85%). The **GaporRu-1** complex exhibits lysosome specific sub-cellular localization with its acidic pKa and a strong singlet oxygen quantum yield, and so can be considered as a novel multi-modal bioprobe for PDT, optical and PET imaging.

## ASSOCIATED CONTENT

*S Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI:10.1021/acs.inorgchem.6b00490.
Experimental details and additional spectroscopic information (PDF)

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### Notes

The authors declare no competing financial interest.

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### Synopsis

An amphiphilic porphyrin conjugated ruthenium complex has been synthesized and successfully labeled with radioactive $^{68}$Ga, which exhibits lysosome specific sub-localization and shows great potential to be a multi-modal bioprobe for photodynamic therapy, optical imaging and positron emission tomography.