

**Supplementary Materials**

**Screening of photosensitizers-ATP binding cassette (ABC) transporter interactions *in-vitro***

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Petteri Uusimaa<sup>3</sup>, Huang Chiao Huang<sup>1#</sup>**

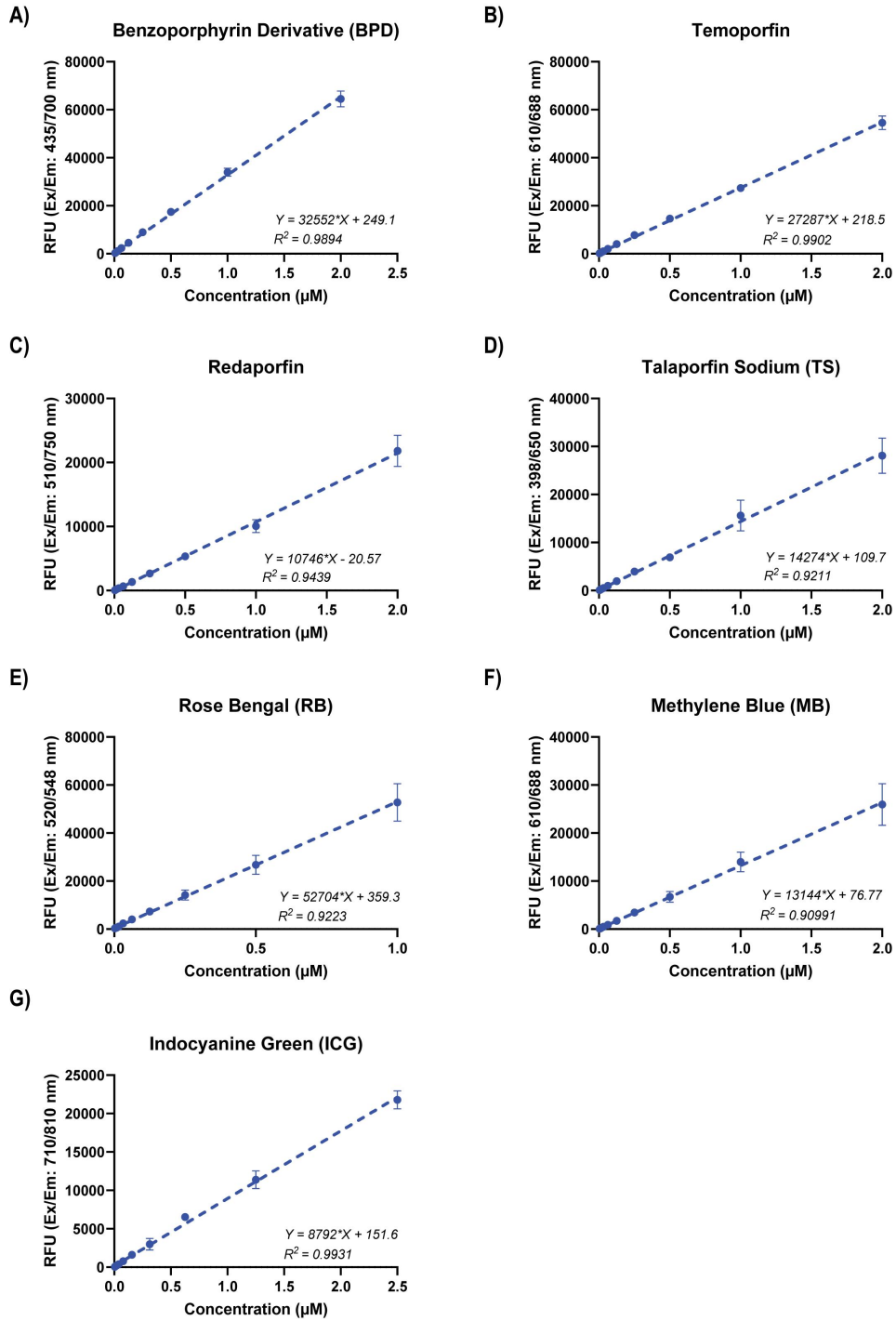
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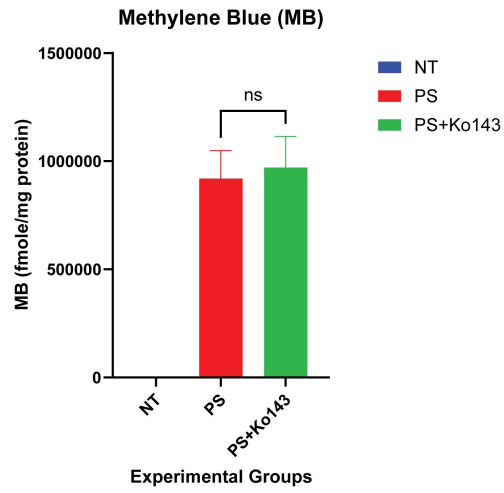
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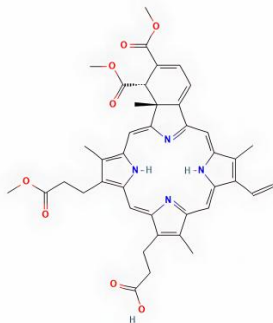
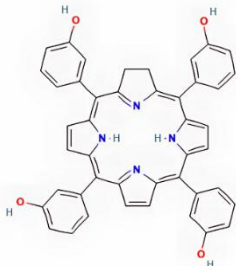

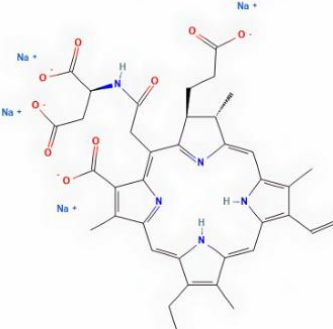


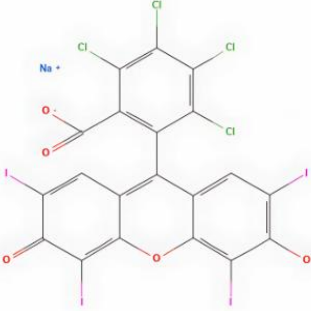
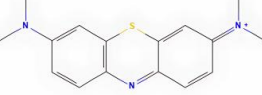
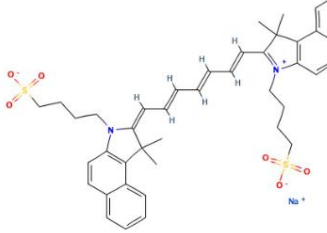
**Supplementary Figure 1. Fluorescence intensity standard curves for tested photosensitizers.** The tested photosensitizers were excited at specific excitation and emission wavelengths photosensitizer [BPD: Ex/Em: 430/700; ICG: 710/810 nm; MB: Ex/Em: 610/688 nm; RB: Ex/Em: 520/575; Temoporfin: Ex/Em 422/660 nm: 430/700; Redaporfin: Ex/Em: 510/750 nm; and Talaporfin sodium: Ex/Em: 398/650 nm]. The figure plots show the mean  $\pm$  standard error fluorescence intensity obtained at known concentration of the photosensitizer. The unknown fluorescence intensity for photosensitizer obtained from cell lysates was extrapolated using the standard curve linear equation to obtain the intracellular concentration of the photosensitizer. The standard curves show a consistent linear trend at the plotted concentrations.



**Supplementary Figure 2. Intracellular accumulation of Methylene Blue in MCF-7 MX100 cells (ABCG2) in presence of ABCG2 inhibitor Ko143.** The intracellular accumulation of Methylene Blue was quantified using the extraction method stated in the method section using Ko143 inhibitor. No significant difference in intracellular accumulation of Methylene Blue was observed between the PS and PS+Ko143 group.

Supplementary Table 1. Summary of selected clinically used photosensitizers and their applications

Name	Alternative names	MW (g/mol)	Structure and chemical formula	Approval	Status of use
Benzoporphyrin Derivative (BPD)	Verteporfin, Visudyne®, BPD-MA	718.8	 $C_{41}H_{42}N_4O_8$ <sup>[1]</sup>	Approved by the FDA in 2000 for sub foveal choroidal neovascularization due to age related macular degeneration (AMD), pathologic myopia, or presumed ocular histoplasmosis	NCT04429139, NCT03033225, NCT06306638, NCT02872064, NCT02464761, NCT00049959, NCT02702700, NCT03067051, NCT02939274, NCT00002647, NCT06381154, NCT00007969)
Temoporfin	Foscan, meso-Tetrahydroxyphenylchlorin, m-THPC, Foslip, 5,10,15,20-tetra(m-hydroxyphenyl)chlorin	680.7	 $C_{44}H_{32}N_4O_4$ <sup>[2]</sup>	Approved by European Medical Agency (EMA) for advanced head and neck cancer in 2001, Phase I/II clinical trials in the US for bile duct carcinoma and lung cancer	NCT03003065, NCT01854684, NCT01415986, NCT01637376, NCT01718223, NCT01086488, NCT00003856
Redaporfin	LUZ11, 5,10,15,20-tetrakis(2,6-difluoro-3-N-methylsulfamoylphenyl)bacteriochlorin	1,135.1	 $C_{48}H_{38}F_8N_8O_8S_4$ <sup>[3]</sup>	Received an orphan drug designation from the EMA in 2016, Phase I/II trials for head and neck cancer	NCT02070432
Talaporfin Sodium	Laserphyrin, NPe6, LS11, mono-L-aspartyl chlorin e6	799.7	 $C_{38}H_{37}N_5Na_4O_9$ <sup>[4]</sup>	Approved in Japan in 2004 for early stage lung cancer, Phase III trials for hepatocellular carcinoma, Phase II trials for refractory colorectal liver metastases	NCT00122876, NCT00716469, NCT00068068, NCT00083785, NCT00028405)

Rose Bengal	Robengatope (brand name), Rose Bengal sodium I-131, PV-10 sodium	1,017.6	 <p>The structure shows a central xanthone core with two carbonyl groups at the 9 and 10 positions. At the 2 and 7 positions, there are two chlorine atoms and one iodine atom. At the 4 and 5 positions, there are two chlorine atoms and one iodine atom. The sodium counterion is shown as Na+.</p> <p><math>C_{20}H_2Cl_4I_4Na_2O_5^{[5]}</math></p>	Phase I/II studies for treatment of neuroendocrine tumors and melanoma	NCT02693067, NCT01760499, NCT00237354, NCT00521053
Methylene Blue	Methylthioninium chloride, basic blue-9, urolene blue, provayblue	319.9	 <p>The structure shows a phenothiazine ring system with two dimethylamino groups at the 10 and 10a positions. The counterion is a chloride ion (Cl-).</p> <p><math>C_{16}H_{18}ClN_3S^{[6]}</math></p>	Approved by the FDA for the treatment of pediatric and adult patients with acquired methemoglobinemia	NCT00784849, NCT01520324, NCT04842968, NCT02982148
Indocyanine Green	ICG, IC-Green, cardiogreen, foxgreen, (Green, Indocyanine), Ujoveridin, Vofaverdin, Vophaverdin, Wofaverdin, Cardio-Green, Cardio Green, Cardiogreen	775.0	 <p>The structure shows a complex polycyclic system with a central nitrogen atom coordinated to two sulfonate groups (SO3- Na+). The molecule is highly conjugated and contains multiple methyl groups.</p> <p><math>C_{43}H_{47}N_2NaO_6S_2^{[7]}</math></p>	FDA approved for cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography	NCT0125375

**Supplementary Table 2. The molar extinction coefficients(MEC) of the panel of photosensitizers**

Photosensitizer	MEC (@wavelength)	Solvent	Ref.
Benzoporphyrin derivative	34,895 M <sup>-1</sup> ·cm <sup>-1</sup> (687 nm)	DMSO	[8]
Temoporfin	26,776 ± 1,085.51 M <sup>-1</sup> ·cm <sup>-1</sup> (651 nm)	DMSO	Calculated
Redaporfin	67,537.3 ± 2,771.2 M <sup>-1</sup> ·cm <sup>-1</sup> (743 nm)	DMSO	Calculated
Talaporfin sodium	23,458 M <sup>-1</sup> ·cm <sup>-1</sup> (651 nm)	PBS	Calculated
Rose bengal	90,400 M <sup>-1</sup> ·cm <sup>-1</sup> (559 nm)	Water	[9]
Methylene blue	7.41 × 10 <sup>4</sup> M <sup>-1</sup> ·cm <sup>-1</sup> (665 nm)	Water	[10]
Indocyanine Green (ICG)	262,100 M <sup>-1</sup> ·cm <sup>-1</sup> (780 nm)	Water	[11]

The table shows the MEC values of the panel of photosensitizers tested in this study. The molar extinction coefficient of photosensitizers were calculated for Temoporfin, Redaporfin and Talaporfin sodium and specific wavelengths. The MEC for BPD, RB, MB and ICG was obtained from the literature.

**Supplementary Table 3. Normalized fluorescence intensity of panel of tested photosensitizers**

Photosensitizers	ABCg2	ABCg2+FTC	P-gp	P-gp+Valspodar	MRP1	MRP-1+MK571
Rose bengal	0.78 ± 0.18	2.42 ± 0.19	0.93 ± 0.32	1.48 ± 0.52	1.52 ± 0.41	4.45 ± 0.03
Benzoporphyrin derivative (BPD)	0.45 ± 0.17	1.00 ± 0.06	0.22 ± 0.03	0.86 ± 0.07	1.15 ± 0.31	0.78 ± 0.24
Temoporfin/Foscan®	0.96 ± 0.20	1.03 ± 0.24	0.79 ± 0.17	0.55 ± 0.04	1.37 ± 0.20	0.70 ± 0.08
Methylene blue	1.42 ± 0.64	1.04 ± 0.11	0.93 ± 0.25	0.98 ± 0.32	1.16 ± 0.31	1.22 ± 0.28
Redaporfin	0.89 ± 0.42	0.82 ± 0.49	0.20 ± 0.05	<b>0.57 ± 0.18*</b>	0.83 ± 0.30	0.73 ± 0.34
Talaporfin sodium	1.59 ± 0.01	1.59 ± 0.11	1.03 ± 0.04	1.20 ± 0.13	0.97 ± 0.30	0.84 ± 0.18
Indocyanine green	1.28 ± 0.44	1.68 ± 0.54	0.72 ± 0.68	0.99 ± 0.90	0.89 ± 0.11	1.14 ± 0.20

The table shows the average normalized fluorescence intensity values for tested agents using flow cytometry. The fluorescence intensity was normalized to signal from the control parental cell line.

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