Assessing amphiphilic ABAB Zn(II) phthalocyanines with enhanced photosensitization abilities in \textit{in vitro} photodynamic therapy studies against cancer

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SUPPORTING INFORMATION

1. RELEVANT FIGURES MENTIONED IN THE TEXT

\textit{Scheme S 1. Synthesis of ftalonitrile A-1 from A-2.}
Figure S1. UV-vis spectra for concentration-dependent studies of ABAB-1, A_{1,2}B-1 and A_{1,4} in toluene. Linear regression between maxima intensity and concentration.
Figure S2. UV-vis spectra for concentration-dependent studies of ABAB-1, A_A-1 and A_4-1 in THF. Linear regression between maxima intensity and concentration.

Figure S3. Normalized fluorescence spectra of ABAB-1, A_2-1 and A_4-1 in toluene and THF solutions.
Figure S4. Calculation of fluorescence quantum yields in toluene and THF solutions of ABAB-1, A3B-1 and A4-1.

Figure S5. Time-resolved fluorescence spectra of ABAB-1, A3B-1 and A4-1 in toluene and THF.
Figure S6. a-c) Absorbance decrease of DPBF over time due to $^1$O$_2$ photoinduced generation by ABAB-1, A$_3$B-1 and A$_4$-1 in DMSO. d) Representation of the relative $^1$O$_2$ efficiency of ABAB-1 (93%), A$_3$B-1 (85%) and A$_4$-1 (52%).

Figure S7. $^1$O$_2$ production of ABAB-1, A$_3$B-1 and A$_4$-1 with regard to the reference phenalenone (PN) in toluene and THF.

Figure S8. Fluorescence spectra of ABAB-1, A$_3$B-1 and A$_4$-1 in different DMSO/water ratios.
Figure S9. UV-vis spectra of the octanol (O) and water (W) phases (three consecutive experiments) in n-octanol/water partition experiments with ABAB-1, A3B-1 and A4-1.

Figure S10. Phototoxicity induced by ABAB-1, A3B-1 and A4-1 in SCC-13 and HeLa cells, which were incubated with concentrations of 1·10^{-6} or 1·10^{-7} M for 5 h and then irradiated with red light at variable doses. The response was dependent, on both cells line, of the concentration of Pcs and the light dose. Each value corresponds to the mean obtained from three independent experiments ± SD. (* p < 0.05; ** p<0.01; *** p < 0.001).
Figure S11. ROS production detected by the DHF-DA fluorescent probe after PDT with Zn(II)Pcs ABAB-1, A3B-1 and A4-1 and red light. Cells were incubated with 1·10^{-6} M Pcs for 5 h and in the last hour of incubation DHF-DA was added, to a final concentration of 6 μM. Cells were exposed to red light (3 and 6 J/cm²). The fluorescence signal was observed by fluorescence microscopy (λexc = 436 nm). Representative photographs of cells subjected to ABAB-PDT. Intracellular fluorescence intensity of all Zn(II)Pcs was measured by ImageJ. ***P<0.001.
2. NMR AND MASS SPECTROMETRY CHARACTERIZATION

4,5-bis-(4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenoxy)phthalonitrile (A-1):

MS and HR-MS:
$^1$H NMR in CDCl$_3$:
\[13^C \text{NMR in CDCl}_3:\]

\[\text{A3B ZnPc 2:}\]
MS and HR-MS:
$^1$H NMR in DMSO-$d_6$:

$^{13}$C NMR in THF-$d_8$:
ABAB Zn(II)Pc 1:

MS and HR-MS:
$^{13}$C NMR in CDCl₃:

$\begin{array}{cccccccccc}
58.76 & 67.63 & 69.68 & 70.23 & 70.40 & 70.68 & 71.76 & 77.16 \\
114.51 & 115.54 & 118.72 & 121.51 & 122.56 & 124.73 & 130.85 & 131.20 & 131.46 & 134.77 & 136.85 & 142.60 & 151.09 & 151.93 & 152.43 & 153.44 & 154.65 & 155.95 & 157.69
\end{array}$
A:B Zn(II)Pc 1:

MS and HR-MS:
$^{13}$C NMR in THF-d$_8$: 

![NMR spectrum graph]
As \( \text{Zn(II)} \text{Pc} \):
$^{13}$C NMR in DMSO-$d_6$: 

[Image of a 13C NMR spectrum with peaks labeled]