Impact of cationic substituents in phenalen-1-one photosensitizers on antimicrobial photodynamic efficacy

Isabelle Tabenski¹, Fabian Cieplik¹, Laura Tabenski¹, Johannes Regensburger², Karl-Anton Hiller¹, Wolfgang Buchalla¹, Tim Maisch²#* and Andreas Späth³#*

¹ Department of Conservative Dentistry and Periodontology, University Medical Center Regensburg, Regensburg, Germany
² Department of Dermatology, University Medical Center Regensburg, Regensburg, Germany
³ Department of Organic Chemistry, University of Regensburg, Regensburg, Germany

* Corresponding authors:
tim.maisch@ukr.de (TM)
andreas.spaeth@chemie.uni-regensburg.de (AS)

# These authors share senior-authorship.

SUPPORTING INFORMATION
Selected Nuclear Magnetic Resonance (NMR) spectra of prepared compounds

Figure S-1: $^1$H-NMR spectrum of compound 2 DMSO-d6

Figure S-2: $^{13}$C-NMR spectrum of compound 2 in DMSO-d6
Figure S-3: $^1$H-NMR spectrum of compound 3 in DMSO-d6
Figure S-4: $^1$H-NMR spectrum of compound 3, in DMSO-d6

Figure S-5: $^1$H-NMR spectrum of compound 4 in DMSO-d6
Figure S-6: $^{13}$C-NMR spectrum of compound 4 in DMSO-d6

Figure S-7: $^1$H-NMR spectra of compound 5 in DMSO-d6
Figure S-8: $^{13}$C-NMR spectrum of compound 5 in DMSO-d6

Figure S-9: $^1$H-NMR spectra of compound 8 in DMSO-d6
Photophysical Characterisation

![Emission Spectrum](image1)

**Figure S-10:** Emission spectrum of compound 5 in H₂O; c = 60 µM

![Absorption Spectra](image2)

**Figure S-11:** Absorption spectra of (5) within a concentration range of 60 – 1000 µM in H₂O; the graphs show no dimerisation in this concentration range.
photostability of SAPYR (1)

absorption [%]

wavelength [nm]

photostability of (2)

absorption [%]

wavelength [nm]
photostability of (3)

photostability of (4)
Figure S-12: Photostability measurements of PN derivatives in a quartz cuvette with an irradiation at 400 nm with 6 J laser energy (10 mW for 10 min); In all cases, the blue spectrum shows the absorption before irradiation, while the red spectrum shows absorption after irradiation. All compounds show no photobleaching under the conditions used.
**Figure S-13:** pH stability of (5) in acidic medium after 20 mins; $c = 60$ µM, in water or dilute aqueous hydrochloric acid, respectively. The derivative is perfectly stable up to pH = 2.

**Figure S-14:** pH stability of (5) in basic medium after 20 mins; $c = 50$ µM, in water or TRIS-buffer 50 mM, respectively. Decomposition begins at pH = 11.
Photodynamic treatment was performed with all 1H-phenalen-1-one derivatives described in this study (1 – 5) using a blue light emitting prototype light source (BlueV, Waldmann, Villingen-Schwenningen, Germany) with an output-intensity of 20 mW/cm². Irradiation of the samples was from below, with direct contact to the bottom of the well plates containing the suspensions, wherefore diffusion of light due to surface tensions in the samples could be excluded. Irradiation was for 60 s, applying a light dose of 1.2 J/cm².

Surviving colonies were counted 24 h (E. faecalis, S. aureus and E. coli) or 48 h (S. mutans, A. naeslundii) later. Relative survival rates were calculated with untreated control groups (L-, 0 μM, without irradiation) being setted as 100%. Horizontal and dashed lines mean reductions of 99.9% (3 log₁₀ steps) and 99.999% (5 log₁₀ steps), respectively.

L+: samples were irradiated; CFU: colony forming units; red column: SAPYR (1); green column: (2); yellow column: (3); blue column: (4); pink column: SAGUA (5) (n=6 experiments: medians including 25% and 75 % quartiles)
A. naeslundii

light, PS conc

S. aureus

light, PS conc

rel. survival (% CFU)
Figure S-15: Photodynamic inactivation of *E. faecalis*, *A. naeslundii*, *S. mutans*, *S. aureus* and *E. coli* by 1H-phenalen-1-one derivatives 1 – 5