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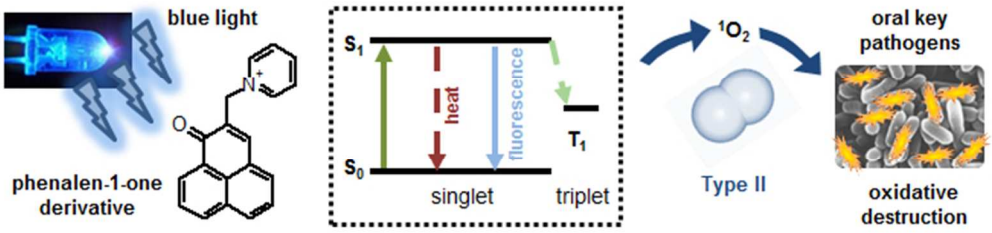
blue light
phenalen-1-one
derivative
S1
S0
heat
fluorescence
T1
singlet
triplet
1O2
Type II
oral key
pathogens
oxidative
destruction

Improving photodynamic inactivation of bacteria in dentistry: Highly effective and fast killing of oral key pathogens with novel tooth-colored type-II photosensitizers

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KEYWORDS: singlet oxygen, photodynamic inactivation, PIB, 7-perinaphthenone, phenalen-1-one, antimicrobial, photosensitizer, type-II, oral key pathogen, dental, tooth-colored

ABSTRACT: Increasing antibiotic resistances in microorganisms involve serious problems in public health. This demands alternative approaches for killing pathogens to supplement standard treatment methods. Photodynamic inactivation of bacteria (PIB) uses light activated photosensitizers (PS) to generate reactive oxygen species immediately upon illumination,

inducing lethal phototoxicity. Positively charged phenalen-1-one derivatives are a new generation of photosensitizers for light-mediated killing of pathogens with outstanding singlet oxygen quantum yield Φ_Δ of > 97%. Upon irradiation with a standard photopolymerizer light (bluephase® C8, 1260 ± 50 mW/cm^2) the photosensitizers showed high activity against the oral key pathogens *Enterococcus faecalis*, *Actinomyces naeslundii*, *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*. At a concentration of 10 µM a maximum efficacy of more than 6 $\log_{10}$ steps (≥ 99.9999 %) of bacteria killing is reached in less than one minute (light dose 50 J/cm^2) after one single treatment. The pyridinium substituent as positively charged moiety is especially advantageous for antimicrobial action.

INTRODUCTION: Increasingly stringent hygiene standards and the worldwide spread of infectious nosocomial diseases[1] have led to a high interest in compounds, methods and applications, which prevent the growth of multiresistant microbes. The search for alternatives to antibiotic treatment is of enormous significance for the treatment of pathogen infections, especially in the wake of the appearance of the first strains of multiresistant bacteria (MRSA) and arising vancomycin resistant strains (VRSA) in 2002.[2]

Although the need for novel antimicrobial active agents exists, nearly no new antibiotics are being developed, due to exploding development costs and the loss of profitability in this marketing sector.[3,4] Even worse, half-life times until resistances develop have gotten shorter when compared to the situation 10 years ago.[4] Furthermore in March 2013 the Nature News Blog highlighted the importance of immediate international action to combat drug-resistant bacteria which will otherwise pose a "catastrophic threat".[5]

One alternative treatment is photodynamic inactivation of bacteria (PIB),[6] which uses chromophors, called photosensitizers (PS), which can transfer either charge (type-I) or energy (type-II) to oxygen to generate reactive oxygen species (ROS) after being illuminated with

visible light. In case of a type-II reaction the highly reactive singlet oxygen is generated which kills bacteria via oxidative burst.[7]

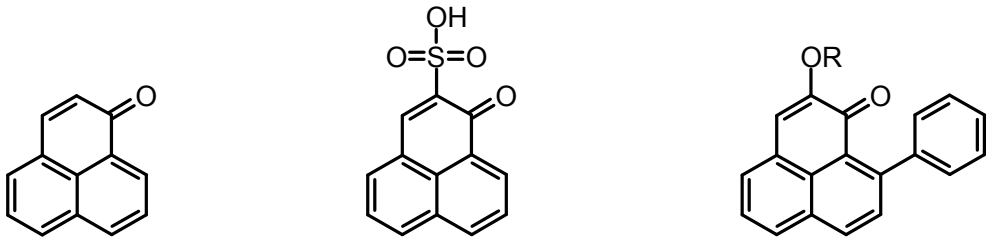
Most commonly used photosensitizers are phenothiazines like methylene blue[8], which predominantly act via type-I mechanism of action, or porphyrines like TMPyP[9] and their derivatives or analogs.[10] In any case, nearly no novel classes of photosensitizers have been developed in the last years except fullerenes[11].

ROS like superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) or the free hydroxyl radical ($HO^{\bullet}$) are ubiquitous in bacterial life.[12] Bacteria express superoxide dismutases (SOD), catalases and peroxidases as a defense mechanism against this kind of oxidative stress. These enzymes are able to catalyze the reaction of ROS to H_2O and molecular oxygen,[13] resulting in a defense mechanism effective against the active agents generated by type-I mechanism of the photodynamic processes. Signs of this exist, such as the fact as expression of SOD increased in *Staphylococcus aureus* after treatment with oxidative stress-generating agents[14] and SOD-activity of *S. aureus* was increased after photodynamic treatment with Protoporphyrin IX in PIB-susceptible strains.[15] A strain-dependent phenomenon of PIB-treated *S. aureus* isolates became apparent: In 40 methicilin-resistant *S. aureus* (MRSA) and 40 methicillin-sensitive *S. aureus* (MSSA) strains were found four strains being resistant to PIB by protoporphyrine diarginate.[16] In contrast, no defense mechanism against singlet oxygen is known to date. This fact emphasizes the benefit of new photosensitizer classes acting only via type-II mechanism (generation of singlet oxygen) to fight the rising numbers of multiresistant bacteria efficiently and avoid any development of resistance against PIB derived oxidative stress induced by oxygen radicals (type-I mechanism of action).

In the search for a new lead-structure for active compounds for medical treatments, nature can serve as a source of inspiration. Plants defend themselves from pathogenic infections by inducing biosynthesis of antimicrobial secondary metabolites, termed phytoalexins.[17] One class are phenalen-1-ones and related compounds biosynthesized by higher plants and also

fungi. Some rare examples are active by drug action alone, i.e. by inhibition of succinate dehydrogenase. Some plant extracts were found to be enriched in antifungal phytoalexins, in which the most potent active substances are phenyl-phenalen-1-one derivatives. Anigorufone (**3a**) and its natural analogue 2-methoxy-9-phenyl-phenalen-1-one (**3b**) show high leishmanicidal activity ($EC_{50} < 60$ µg/mL).[18]

But the occurrence of phenalen-1-one chromophors in plants also suggests that they respond to attacks of pathogens by biosynthesizing photosensitizers, which are able to use solar energy for defense using singlet oxygen via illumination.[19] A wide range of naturally occurring phenalen-1-ones and related compounds can be found.[20]



(1) **(2)** **(3a)** R = H **(3b)** R = Me

Figure 1: Phenalen-1-one (**1**), its derivatives phenalen-1-one-2-sulfonic acid (**2**), the phytoalexin anigorufone (**3a**) and its analog 2-methoxy-9-phenyl-phenalen-1-one (**3b**)

From the abundance of the phenalen-1-one chromophore in nature, a low dark toxicity of this structure can be expected. It has already been shown that phenalen-1-one does not contribute significantly to the mutagenicity or carcinogenicity of combustion emission extracts.[21]

Phenalen-1-one or 7-perinapthenone (PN, **1**), is a sensitizer of singlet oxygen with a quantum yield close to one in solvents of different polarities. It has established itself as universal standard for the quantitative determination of singlet oxygen in solution.[22] For the first time Nonell et al. presented a water soluble derivative, 1H-Phenalen-1-one-2-sulfonic acid (**2**). Sulfonation of PN strongly stabilized the chromophor against photobleaching and did not influence its effectiveness as an extremely efficient singlet molecular oxygen sensitizer (phi

delta Φ_Δ = 97 %).[23] But its negative charge prevents its use as an antimicrobial photosensitizer in aqueous surroundings, since positive charges are essential for adhering to or penetration through the cell walls of pathogens[24].

Following these findings, we have made it our mission to utilize the excellent properties of phenalen-1-ones for antimicrobial photodynamic therapy by attaching a positive charge to the system and examining it for various applications. With its almost neutral color, the photosensitizer is especially suitable for use in dentistry.

We present a series of phenalen-1-one (7-perinaphthenone, PN) derivatives with excellent solubility in water, high photostability in the therapeutic time window and a positive charge for good adherence to the cell walls of pathogens. This new generation of photosensitizers for photodynamic inactivation of bacteria shows a singlet oxygen quantum yield Φ_Δ of > 97%.

PIB is used in dentistry as a supportive tool in periodontology and for endodontic treatments. However, the most widely used PS in clinical practice at this time are phenothiazine dyes like methylene blue (MB) and toluidine blue (TBO)[25], which are unpopular with the patients´ esthetic feeling due to their strong blue color.[26] In periodontal application this may lead to a temporary staining of the oral soft tissue for at least a few hours.[27] However, when PIB with phenothiazines is done in endodontics, this may lead to persistent blue staining of dental structure via diffusion of the PS into dentinal tubules with a consequential need for further treatment of decoloration with appropriate chemical compounds.[28] In contrast, our novel PN-derivatives (**5** - **9**, see scheme 1) have a tooth-like color with no esthetic limitations for their use as PS in the oral cavity.

In this study, PIB with the new generation of PN-derivatives (**5** - **9**) was tested against oral key pathogens: Gram-positive *Enterococcus faecalis (EF)*, *Actinomyces naeslundii (AN)*, *Streptococcus mutans (SM),* and Gram-negative *Aggregatibacter actinomycetemcomitans (AA)*.

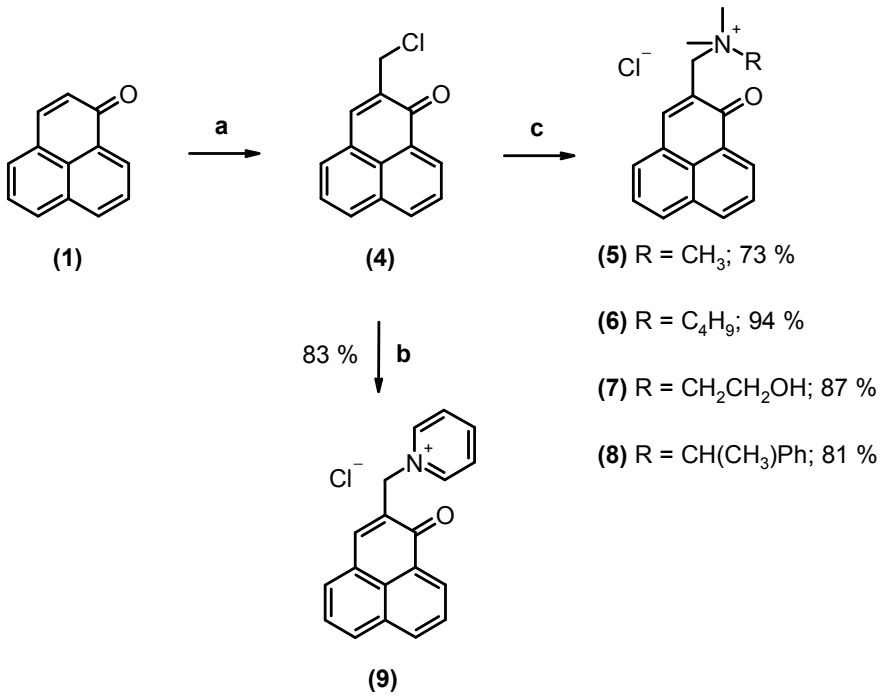
Enterococcus faecalis is the prevalent species associated with secondary root canal infections after failed endodontic treatment.[29] Moreover, *Enterococci* are known for their resistances to diverse antimicrobial agents.[30] *Actinomyces naeslundii* and *Streptococcus mutans* are referred to as early colonizers in the formation of dental plaque.[31] *Streptococcus mutans* is also well-known as key species in the pathogenesis of dental caries.[32] *Aggregatibacter actinomycetemcomitans*is is known to be a cause of for severe forms of periodontitis, the so-called localized aggressive periodontitis (LAP).[33]

The aim of this study is (a) to investigate the properties of the novel photosensitizers for PIB in dentistry, and (b) to find a effectiveness-relationship of the different derivative structures based on 7-perinaphthenone in order to select a lead candidate for further development of the novel photosensitizer class.

RESULTS AND DISCUSSION

Preparation of photosensitizers and investigation of their properties

As a simple and reliable approach to install the positive charge in phenalen-1-on (7-perinaphthenone, **1**), chloromethylation of the structure leading to (**4**), followed by alkylation of a tertiary amine or a pyridine with this compound, is very suitable. Substitution in this manner either with the pyridinium moiety (**9**, *SAPYR*) or with trialkylammonium salts (**5** to **8**) gives five new photosensitizers:



Scheme 1: Synthesis of phenalen-1-one derivatives (**4 - 9**); *conditions: a) $HCl_{(aq)}$, HOAc, H_3PO_4, HCHO, 120°C, over night, 36 %; b) pyridine, DMF, RT, over night, then 50°C, 5 h, 83 %; c) tertiary amine, DMF, RT, over night, then 50°C, 2 – 10 h, 73 – 94 %*

The products are obtained by conversion of (**4**) in methanol, ethanol, acetone or N,N-dimethylformamide (DMF) with excess amine at slightly elevated temperature in good to excellent yields (73 – 94 %). The purification is only in need of a short column/plug filtration of (**4**). Purification of the PN salts was achieved by precipitation with diethylether and re-precipitation from ethanol with diethylether, until the UV/Vis spectra showed constant absorption. *SAPYR* (**9**) was additionally washed with ice-cold dichloromethane. The purity of all compounds was checked by NMR and HPLC-MS and was ≥ 98 %. This represents a straight forward and easy synthesis and purification protocol for the preparation of a variety of positive charged 7-perinapthenone derivatives in larger scale.

Analysis of the structures clearly showed the substituent being located at the α-position to the carbonyl. The proton signal for this position disappeared in comparison to the proton NMR

spectrum of the starting material phenalen-1-one (**1**). We also detected a weak long range coupling of the substituent´s methylene group to the proton in β-position to the carbonyl in the COSY spectra of compounds **5** and **9** in D_2O. In addition, the coupling pattern of the aromatic naphthalene subsystem is not affected in any of the prepared compounds after substitution (all spectra can be found in the supporting information).

Photophysics, Singlet-Oxygen Generation Ability and Aggregation

Compounds **5** to **9** were investigated for their photophysical stability and absorption characteristics, singlet-oxygen generation ability and aggregation behavior. All measurments were conducted in Millipore water (18 MΩ). The molar extinction coefficients for compounds **5** to **9** are similar and match the value of $9800 \pm 400\ L \cdot mol^{-1} \cdot cm^{-1}$ within the error margins (Fig. 2).

The light source for photodynamic treatment is commercially available. A hand-held light-curing unit for polymerization of dental resins was used (bluephase® C8 IvoclarVivadent). However, it has to be considered, that there is only a partial overlap of the emission spectrum of this blue-light emitting lamp and the absorption spectra of the respective PN-derivatives used for the present study (approx. 5 % of applied energy will be absorbed) (Fig. 2). Though, this light source was selected, as it is widely applied in dental practice. Furthermore, it does not emit radiation in wavelengths of the UV range that might cause mutagenicity against bacterial cultures or surrounding tissues. Nevertheless, development of a light source that obtains a better overlap with PN-derivatives has to be considered for further studies, in order to advance PIB-efficacy.

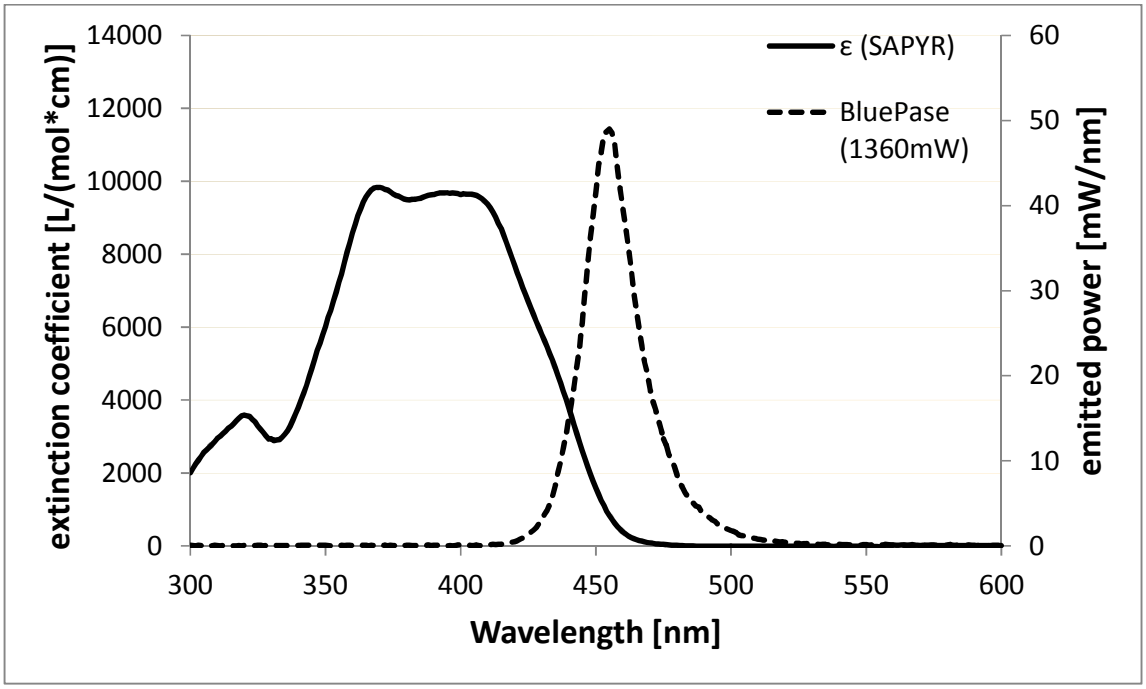


Figure 2: Emission spectrum of bluephase® C8 IvoclarVivadent in comparison to absorption of *SAPYR* (**9**)

Nevertheless, all compounds investigated in this study are excellent singlet oxygen generators with quantum yields $\Phi_\Delta > 97$ %. Singlet oxygen could be clearly identified by its life time at an emission wavelength of 1270 ± 10 nm and the typical finger print signal recorded at different wavelengths around 1270 nm (Fig. 3).

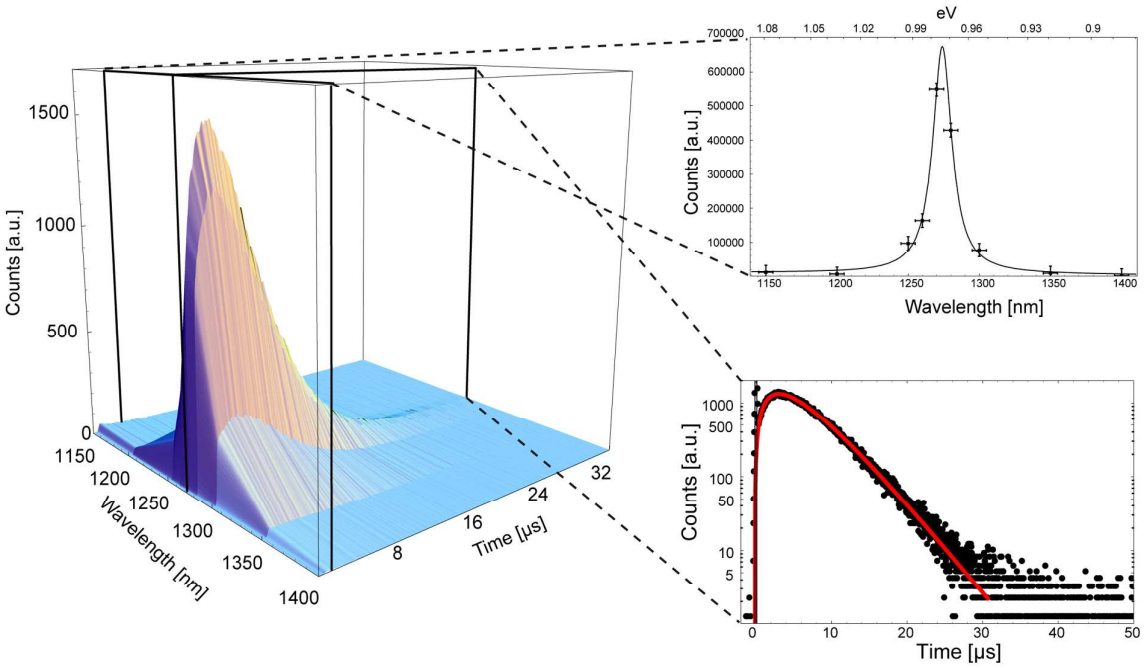
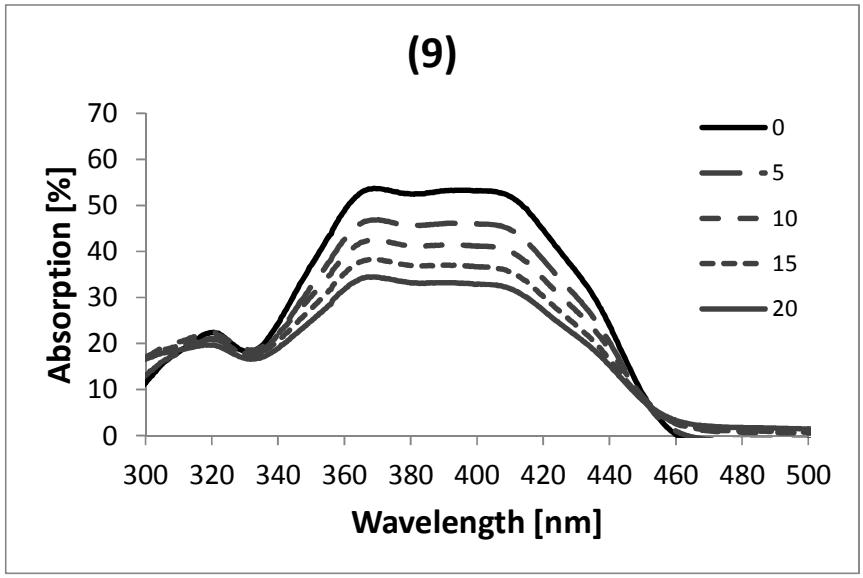


Figure 3: 3D-Plot of the time- and spectrally resolved singlet oxygen luminescence ("finger print" of $^1O_2$) generated by PN-derivative **5** (left) and typical singlet oxygen luminescence signal upon excitation at 405 nm (right bottom). All measurements were conducted in air saturated H_2O at 25°C. Singlet oxygen is generated after excitation and detected in the range of 1150 to 1400 nm with a decay time ≈ 3.5 µs for all derivatives and the measured wavelengths.



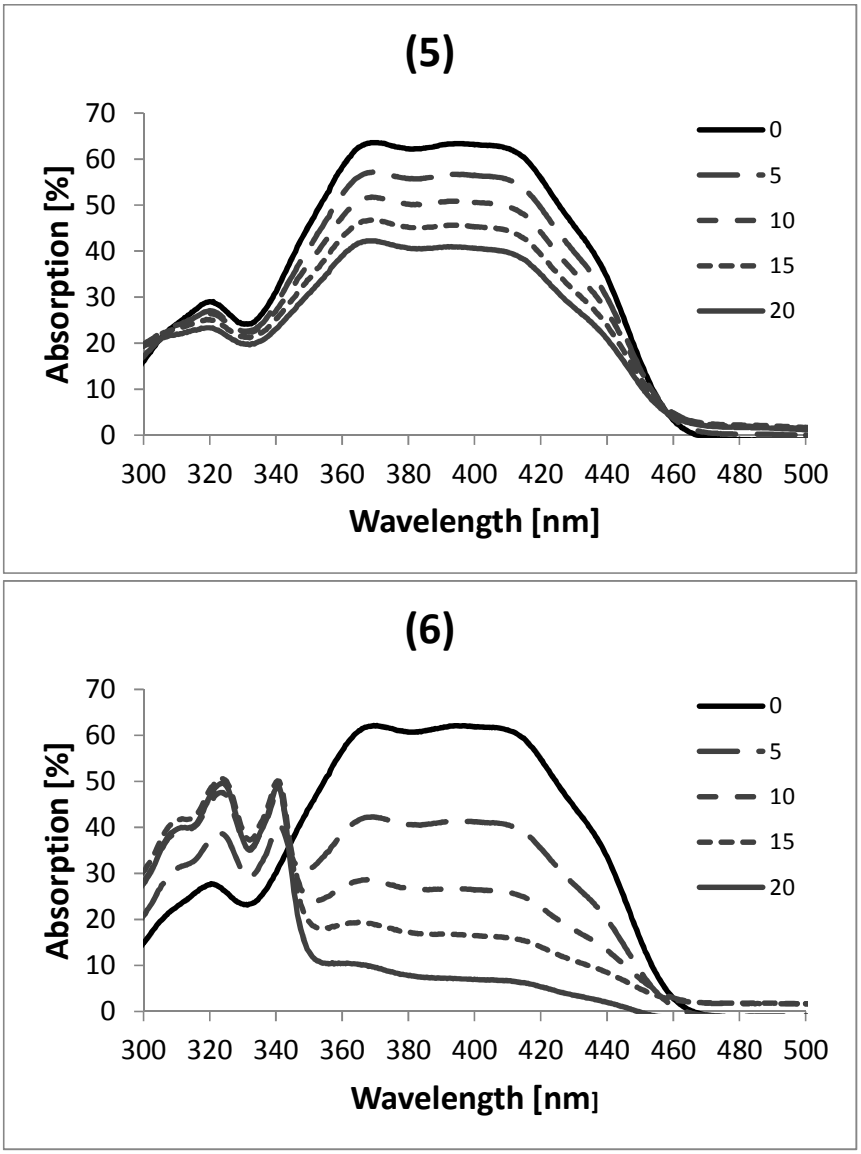


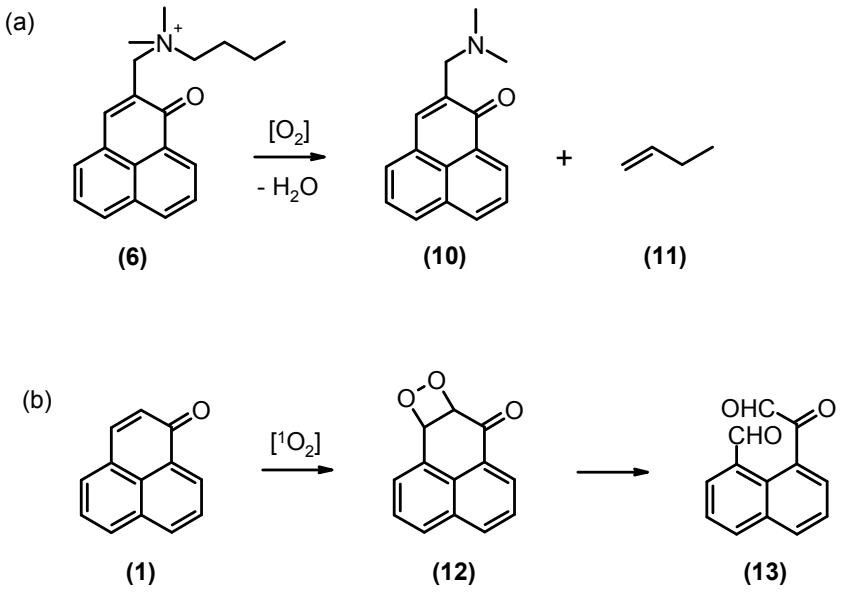
Figure 4: Photostability measurements of (**5**), (**6**) and (**9**) in a quartz cuvette with an irradiation at 405 nm with 125 ± 3 J laser energy; time values in the graphs legend are given in minutes

All compounds show photobleaching, which is approx. 10 % for most of the examples upon irradiation with laser energy at 405 nm for 5 minutes, equals 32 J (Fig. 4). These are quite stringent conditions in comparison to the irradiation with the BluePhase C8 lamp for 20 s, 40 s and 120 s. In comparison to the laser beam irradiation, the bleaching of the

photosensitizers can be neglected when using the lamp. Due to the overall short illumination time of 120 s photobleaching seems not a relevant factor for antimicrobial testing.

The potential pathway for the photo-degradation of the phenalen-1-one chromophor with involvement of $^1O_2$ for these chemical structures may be explained as follows: Degradation of the molecules **5** to **9** is expected to start with an elimination of the positively charged moiety. We assume, this takes place by an analog mechanism to Hofmann elimination forming a tertiary amine and an alkene.[34] Following this mechanism, the alkyl-dimethylammonium group is more easily eliminated (Scheme 2, (a)). The elimination of trimethylammonium group and the pyridinium moiety is more difficult, because no corresponding alkene can be formed. This explains the different photostabilities of the novel PS (Fig. 4).

We hypothesize the process is followed by a degradation of the chromophor starting from the keton-bearing ring. By comparison of aromatic systems known from the literature concerning their reactivity with oxygen species, a high stability of the two condensed aromatic rings in the phenalen-1-one structure can be derived.[35] This part of the molecule can be seen as stable versus reactions like epoxidation or peroxidation by singlet oxygen under the given conditions. The more reactive ("*vulnerable*") part is certainly the α,β-unsaturated Michael system. As long as the PS´s (**5** – **9**) double bond is blocked by their charged substituent located at the α-position, attack of the electrophilic singlet oxygen in a 2+2 cycloaddition reaction[36] is hampered by the altered electronics and the higher steric hindrance (scheme 2). This is in good accordance to the finding, that substitution of (**1**) stabilizes the system.[23] Attack of the Michael systems double bond in the not substituted chromophor phenalen-1-one (**1**), or the products mentioned above, can take place more easily. Tertiary amines (for example **10**) are also accessible to oxidation. After the double bond has been substituted by singlet oxygen (**12**), the resulting C-C single bond is quickly cleaved forming two aldehyde groups (**13**).[37] This process finally leads to breakdown and bleaching of the chromophor (Scheme 2, (b)).



Scheme 2: Initial steps in the assumed breakdown pathway for the photosensitizer structures; (a) elimination of an alkene in (**6**) under oxidative conditions and warming (Hofmann elimination); (b) substitution of phenalen-1-one (**1**) by singlet oxygen and oxidative bond cleavage

Aggregation of the PN photosensitizers was studied over a wide concentration range via UV/Vis spectroscopy (Fig. 5) and by temperature dependent NMR studies (see Fig. 6). The beginning of aggregation was observed in the millimolar concentration range.

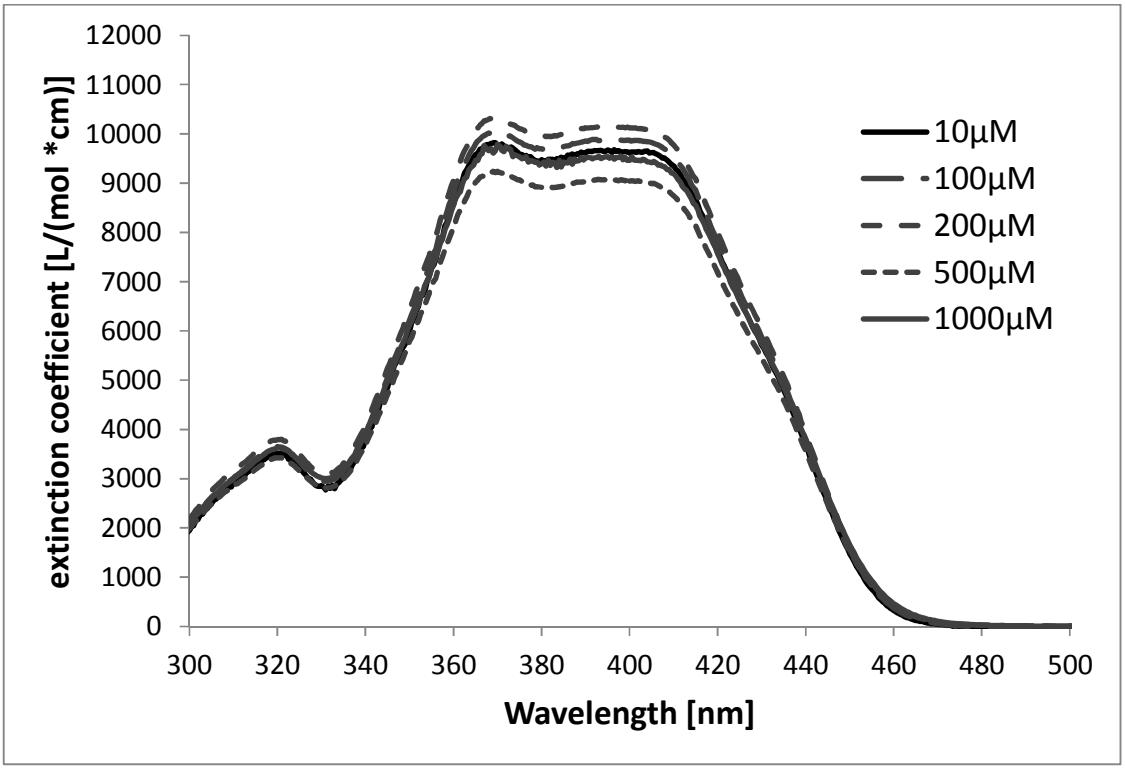


Figure 5: Absorption spectra of (**9**) within a concentration range of 100 – 1000 µM in H_2O; within the error margins no dimerization can be observed in the concentration range covered.

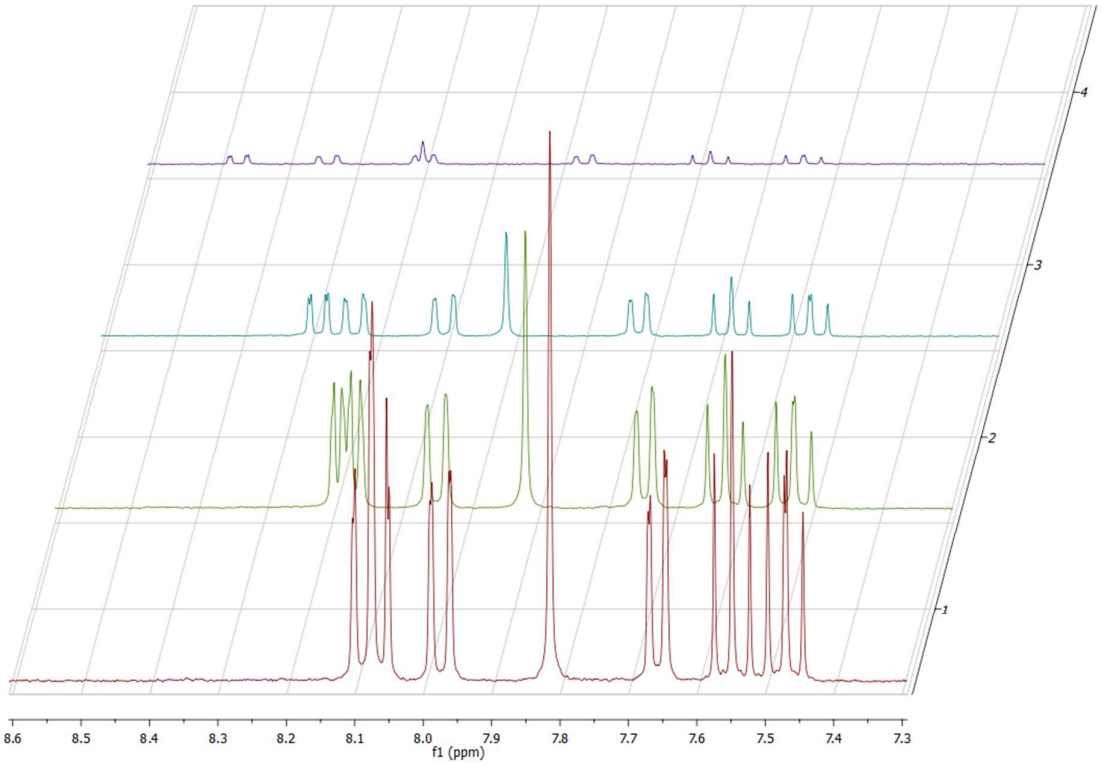


Figure 6: Aromatic region of the proton NMR spectra of (**5**) in concentration of 1 mM (4), 5 mM (3), 10 mM (2) and 20 mM (1) in D_2O; x-values (f1) in ppm relative to external standard TMS, 1 ppm = 400 Hz. The spectra show dimerisation beginning in the millimolar concentration range.

Aggregation can be neglected below the millimolar range (< 1 mM) and is thus no critical factor for the biological studies. Typical therapeutic concentrations in PIB match the micromolar range (1 to 500 μM).

Polarity and Stability

The polarity of the novel photosensitizers was estimated by measuring the octanol-water coefficient. Distribution of $1*10^{-4}$ mol of each PN salt between both phases was measured by UV/Vis spectroscopy after 10 minutes of stirring at room temperature. Table 1 summarizes the results and gives data for the photophysical measurements as described above:

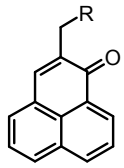
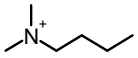
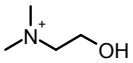
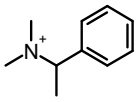
Photosensitizer 		λ_{max} absorption [nm][(c)]	λ_{max} emission [nm]	Singlet oxygen quantum yield Φ_Δ[(d)]	Loss in absorption [%] after 5 / 20 mins[(e)]	octanol / water coefficient log P
Residue R =						
Cl	(**4**) (a)	356 - 410	487 ± 5	n.d.	n.d.	+ 1.3
	(**5**) (b)	360 - 417	488 ± 5	1.03 ± 0.05	10 / 36	- 1.4
	(**6**) (b)	364 - 413	489 ± 5	1.03 ± 0.05	33 / 89	- 0.8
	(**7**) (b)	360 - 418	491 ± 5	1.06 ± 0.05	66 / 74	- 1.3
	(**8**) (b)	362 - 418	492 ± 5	1.05 ± 0.05	16 / 53	- 0.1
	(**9**) (b)	363 - 410	489 ± 5	1.02 ± 0.05	12 / 38	- 1.3

Table 1: Crucial physical parameters of the phenalen-1-one derivatives; conditions: at 25 °C, (a) in EtOH, (b) in Millipore water, (c) all compounds show a broad absorption maximum in this region, which cannot be resolved in distinct maxima, (d) 7-perinaphthenone-2-sulfonic acid (PNS) with a $\Phi_\Delta = 1.03 \pm 0.10$ served as reference PS, (e) excitation with 125 ± 3 J of laser light at 405 nm

pH stability was determined by recording UV-Vis spectra of compounds **5** and **9** in buffer solutions at different pH values after incubation for 5 and 30 minutes. The novel derivatives

show excellent stability in acidic medium (down to pH = 2), but decompose slowly in alkaline solutions with pH > 9 (see supporting information for spectra).

The temperature stability of compounds **5** and **9** was investigated by temperature dependent NMR studies in D_2O (Fig. 7). No change appeared in the spectrum after heating to 90 °C for more than 10 minutes. The compounds are perfectly temperature stable in solution in the therapeutic time window:

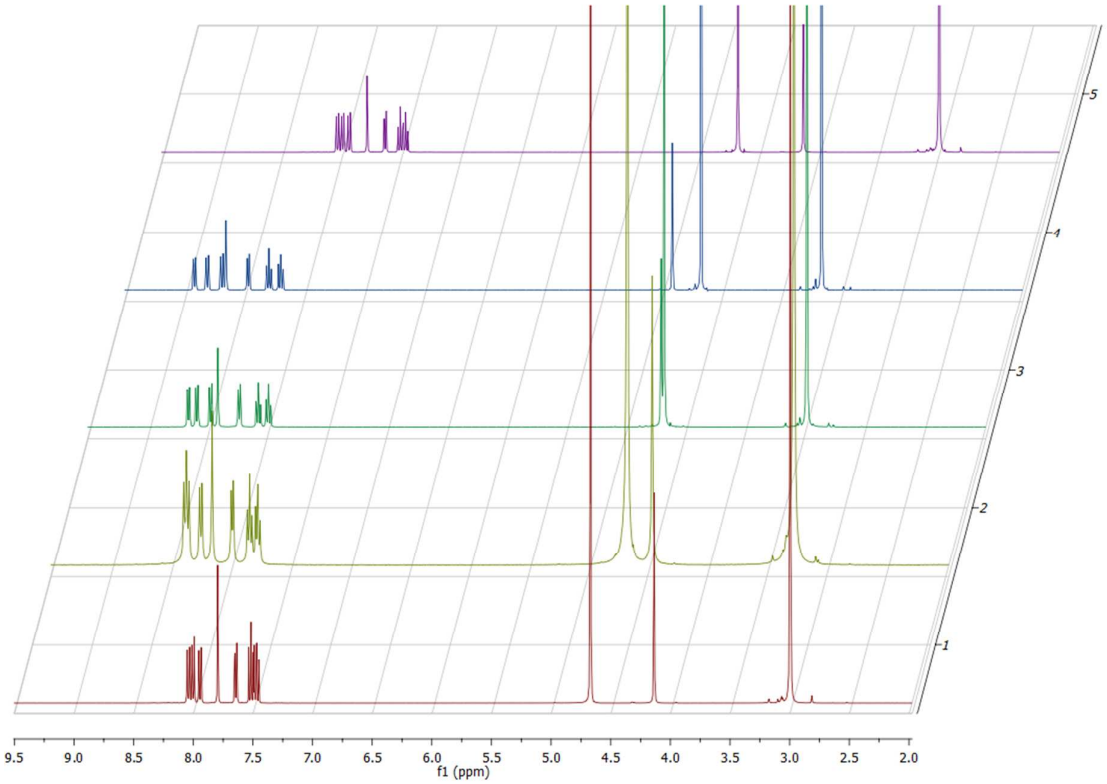


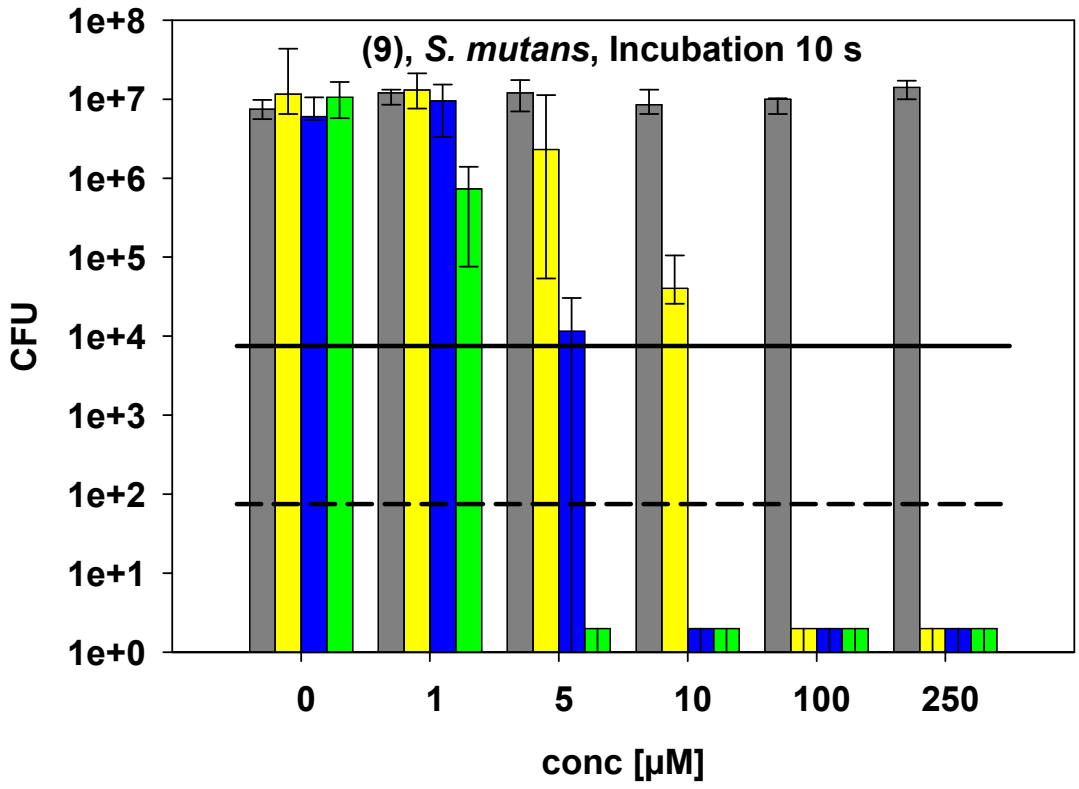
Figure 7: Temperature stability of **5** by NMR; temperature steps: room temperature (1), 50 °C (2), 70 °C (3) and 90 °C (4), then cooling to room temperature (5), c = 70 mM; x-values (f1) in ppm relative to external standard TMS, 1 ppm = 400 Hz.

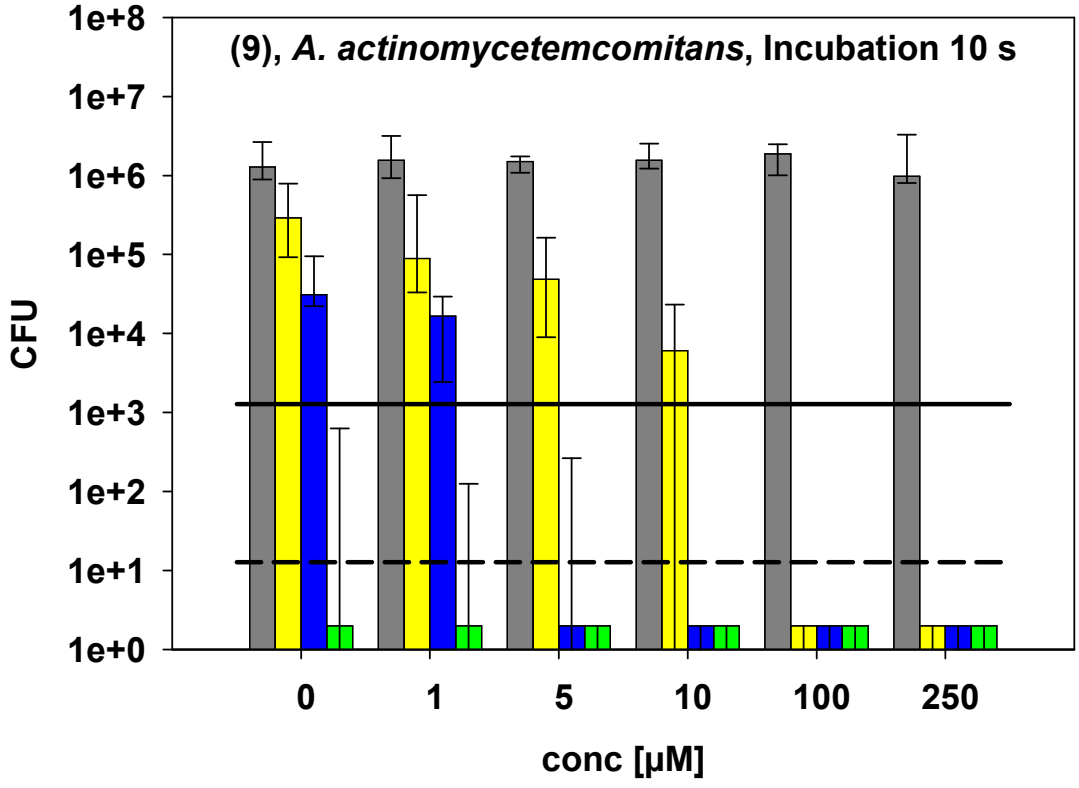
The shift of the spectrum in the aromatic region of more than 0.5 ppm again indicates aggregation in the millimolar range (c = 70 mM).

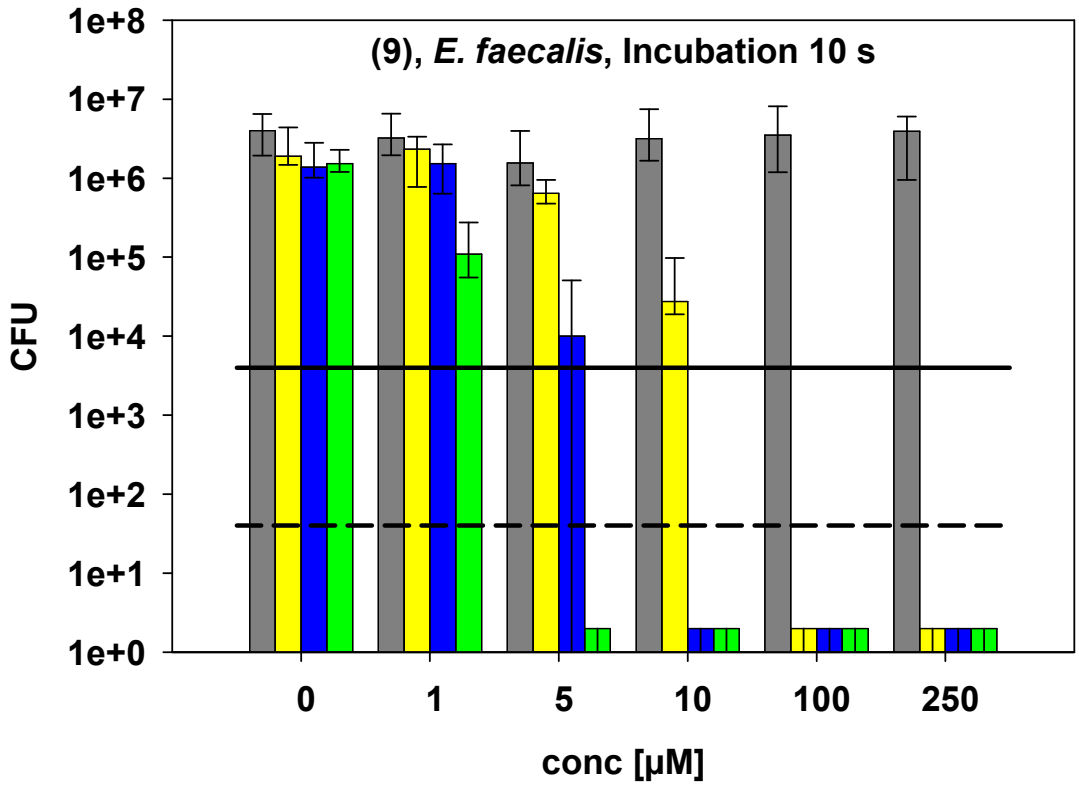
Photodynamic Inactivation of Bacteria (PIB):

PIB was performed against oral key pathogens in suspension using different concentrations of each PN-derivative (0 μM, 1 μM, 5 μM, 10 μM, 100 μM, 250 μM) and obtaining irradiation periods of 20 s, 40 s and 120 s. Hereby all PN-derivatives were evaluated against Gram-positive *EF*, whereas (**9**) and (**7**) were also tested against Gram-positive *SM* and *AN* and Gram-negative *AA*. Results were evaluated by the method of Miles, Misra and Irwin.[42]

In all cases, illumination alone or treatment with PN-derivative only (dark control) resulted in no decrease of the colony forming units (CFU) compared to untreated control groups. Figure 8 shows the results of the PIB efficacy using derivative **9** against all oral pathogens in suspension. Incubation of *EF*, *SM*, *AA* and *AN* with different concentrations (0 - 250 μM) of (**9**) caused a decrease in viability upon illumination (Fig. 8). (**9**) at 5 μM exhibited already a disinfection efficacy of ≥ 99.999 % (corresponding to ≥ 5 $\log_{10}$ reduction of CFU) upon illumination for 120 s. Higher concentrations of (**9**) did not increase further the antibacterial efficacy of PIB. Furthermore PIB efficacy was as well dependent from the applied light of 25 J/cm^2, 50 J/cm^2 or 150 J/cm^2 in samples of *SM*, *AN* and *EF*. For example, (**9**) concentration of 5 μM achieved a killing efficacy of between 3 and 5 $\log_{10}$ using a light doses of 50 J/cm^2 and > 6 $\log_{10}$ using a light dose of 150 J/cm^2 Light alone does not have any effect on the viability of *SM*, *AN* and *EF*. In contrast, samples of *AA* exhibited a strong light-toxicity without any (**9**). There was a reduction up to 1, 2 and 6 $\log_{10}$ steps of CFU of AA after irradiating for 20 s, 40 s and 120 s, respectively (Fig. 8). Killing efficacy against AA was not enhanced by PIB using (**9**) concentrations up to 250 μM.


(9), S. mutans, Incubation 10 s
CFU
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
0
1
5
10
100
250
conc [µM]


(9), A. actinomycetemcomitans, Incubation 10 s
CFU
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
0
1
5
10
100
250
conc [µM]


(9), E. faecalis, Incubation 10 s
CFU
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
0
1
5
10
100
250
conc [µM]

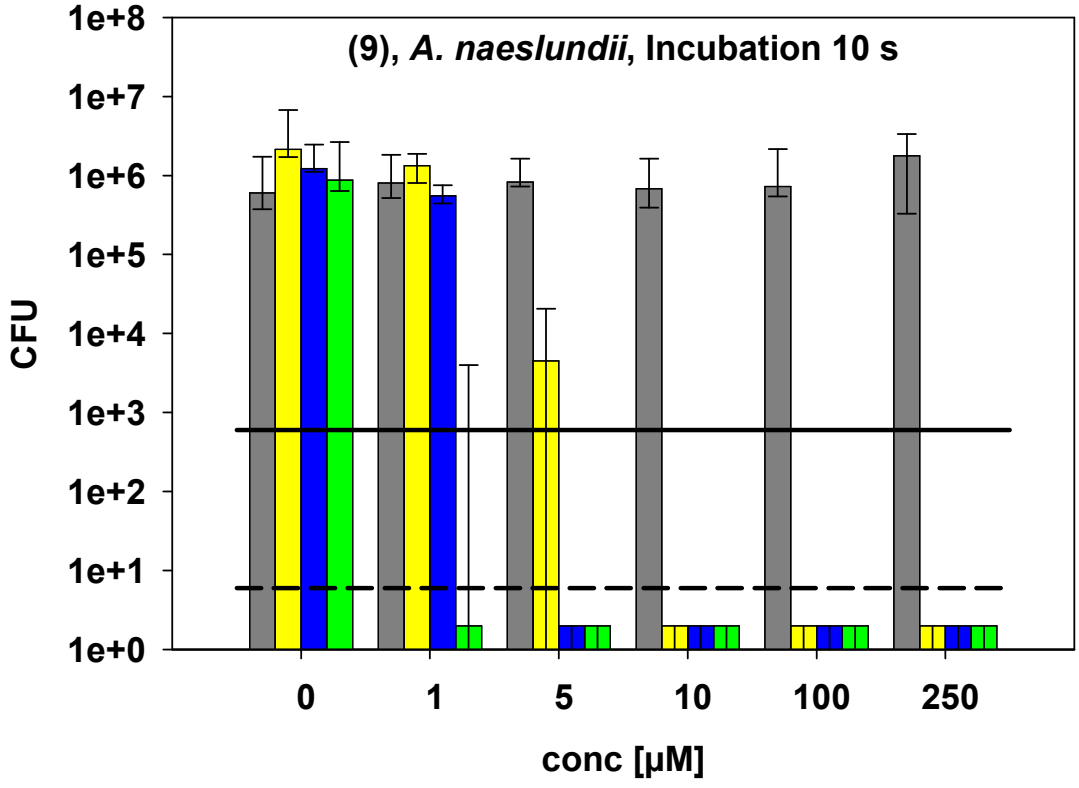

(9), A. naeslundii, Incubation 10 s
CFU
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
0
1
5
10
100
250
conc [µM]

Figure 8: Results of photodynamic inactivation of four different bacteria by (**9**) using the hand-held dental light-curing unit (bluephase® C8, IvoclarVivadent, Schaan, Liechtenstein); *Output-intensity of 1260 ± 50 mW/cm², irradiation for 20 s, 40 s or 120 s, corresponding to applied energies of 25 J/cm², 50 J/cm² and 150 J/cm², respectively. Surviving colonies were counted 24 h later. Grey column: controls without illumination. Coloured columns: **(9)** + light activation, yellow 20 s, blue 40 s and green 120 s illumination, respectively. Black solid line corresponds to a reduction of 3 log_{10} steps (99.9 % killing efficacy), black dashed line to a reduction of 5 log_{10} steps in bacterial numbers (99.999 % killing efficacy) related to controls without illumination and without **(9)**. (n = 3 independent experiments: bars represent the median including 25 – 75 % quartiles)*

PIB-results with all PN-derivatives against *EF* are summarized in Table 2.

Enterococcus faecalis

		0 µM	1 µM	5 µM	10 µM	100 µM
(9)	20 s	-	-	-	<3	>6
	40 s	-	-	<3	>6	>6
	120 s	-	<3	>6	>6	>6
(5)	20 s	-	-	-	-	>6
	40 s	-	-	<3	<3	>6
	120 s	-	<3	>6	>6	>6
(6)	20 s	-	-	-	<3	>6
	40 s	-	-	<3	<3	>6
	120 s	-	<3	≈3	>6	>6
(7)	20 s	-	-	-	<3	>6
	40 s	-	-	-	<3	>6
	120 s	-	-	<3	>6	>6
(8)	20 s	-	-	<3	<3	>6
	40 s	-	-	<3	<3	>6
	120 s	-	<3	>6	>6	>6

Table 2: PIB-results with all PN-derivatives against *EF*. *(-) means < 1 log_{10} step reduction of*

the number of bacteria. This was defined as virtually no efficacy. Threshold for the evaluation of effectiveness was 3 log_{10} steps (99.9 % inactivation).

Overall, derivative **9** showed the best killing efficacy compared to the other PN-derivatives, whereas (**7**) was the least effective photosensitizer. PIB with (**9**) or (**5**) caused a pronounced decrease of CFU against all tested species, whereby (**9**) was more effective at lower concentrations and illumination periods (Fig. 8). (**9**) as the most potent PS reached more than 6 log_{10} steps of bacteria reduction up to total eradication in only 40 seconds. At concentrations of 10 μM sterilization conditions can be realized.

Depending on the tested species, (**9**) revealed an efficacy of PIB-killing of ≥ 5 log_{10} steps CFU (≥ 99.999 % = disinfection) at concentrations effective from 5 μM and illumination time of either 120 s or even 40 s (*AN*, *AA*), whereas higher concentrations of (**5**) or (**8**) or longer irradiation times were necessary to obtain similar results.

An overview of PIB-killing rates against all four species using (**9**) and (**7**) can be found in table 3.

Enterococcus faecalis						
		0 µM	1 µM	5 µM	10 µM	100 µM
(9)	20 s	-	-	-	<3	>6
	40 s	-	-	<3	>6	>6
	120 s	-	<3	>6	>6	>6
(7)	20 s	-	-	-	<3	>6
	40 s	-	-	-	<3	>6
	120 s	-	-	<3	>6	>6

Streptococcus mutans						
		0 µM	1 µM	5 µM	10 µM	100 µM
(9)	20 s	-	-	<3	<3	>6
	40 s	-	-	<3	>6	>6
	120 s	-	<3	>6	>6	>6
(7)	20 s	-	-	-	-	>6
	40 s	-	-	-	<3	>6
	120 s	-	-	<3	>6	>6

Actinomyces naeslundii						
		0 µM	1 µM	5 µM	10 µM	100 µM
(9)	20 s	-	-	<3	>5	>5
	40 s	-	-	>5	>5	>5
	120 s	-	>5	>5	>5	>5
(7)	20 s	-	-	-	<3	>5
	40 s	-	-	<3	>5	>5
	120 s	-	-	>5	>5	>5

Aggregatibacter actinomycetemcomitans						
		0 µM	1 µM	5 µM	10 µM	100 µM
(9)	20 s	<3	<3	<3	>6	>6
	40 s	<3	<3	>6	>6	>6
	120 s	>6	>6	>6	>6	>6
(7)	20 s	-	-	<3	<3	>3
	40 s	<3	<3	<3	>3	>6
	120 s	>3	>6	>6	>6	>6

Table 3: PIB-killing rates against all four species by compound **9** and **7**. *(-) means < 1 log_{10} step reduction of the number of bacteria. This was defined as virtually no efficacy. Threshold for the evaluation of effectiveness was 3 log_{10} steps (99.9 % inactivation).*

The photo-stability of (**7**) was the lowest in all compounds. Due to the short treatment time

employed in this study, we assume that small antimicrobial activity of (**7**) might be partially caused by decomposition. However, the polarity caused by the additional polar hydroxyl-group should play the key role.

Although light activated reduction of *AA* by the novel photosensitizers was efficient and the dominant process for the bacteria killing, *AA* was the only species, where treatment with light alone led to a reduction of CFU. This light-toxicity can be explained due to the presence of endogenous photosensitizers in *AA*-cells.[38]

CONCLUSION:

Equipping the singlet oxygen standard 7-perinaphthenone with the ability to bind to the surface of pathogens led to novel antimicrobial active and very effective photosensitizers with outstanding single oxygen quantum yield, which can be used to improve PIB in dental practice. This was accompanied with the successful development of a straight forward and simplified synthesis and purification protocol for the novel compounds. The preparation of a variety of positive charged 7-perinapthenone derivatives is now possible in a larger scale with good to excellent yields (73 – 95 %). All derivatives showed high stability over a wide range of changing pH (2 – 9), good temperature-stability and photostability in the therapeutic time window for typical PIB applications.

The light activation of the new generation of PN-derivatives **5** to **9** achieved antibacterial efficacy against oral key pathogens of 99.999% (*AN*) or even 99.9999 % (*AA*, *EF*, *SM*) at concentrations of at least 5 µM of the respective PS and an illumination period of 120 s or 40 s (5 µM: *AA*, *AN*) for the most efficient derivate (**9**, "*SAPYR"*).

A polar substituent seems to cause a decrease in effectiveness, whereas a pyridinium moiety is advantageous for antimicrobial action. A suboptimal balance between charge and lipophilicity can be an explanation for a weaker and slower attachment to the cell wall. In addition,

derivatives with a substituent based on a quaternary dimethylammonium moiety tend to lower photostability. The trimethylammonium and the pyridinium substituent are more stable, due to reduced elimination ability of these residues. With compound **9**, or „*SAPYR*“, it was recently demonstrated, that this type-II photosensitizer exhibits a high killing efficacy (reduction of > 5 $\log_{10}$ CFU) against monospecies and polyspecies biofilms.[39] Compound **9** may serve as lead candidate for further studies.

Even though the absorption spectra of the PN derivatives only accounted for a 5% overlap with the emission spectrum of the light source used, PIB with the novel class of PS was already able to compete with the technological standard (> 5 $\log_{10}$ pathogen reduction) in a fast and efficient process, elucidating the enormous capability of this new generation of photosensitizers. Nevertheless, further studies with more appropriate light sources are necessary to optimize PIB efficiency using these new photosensitizers.

Ongoing studies in our lab extending our investigations into this interesting molecule class towards other key pathogens while also investigating a library of substitution patterns, will show the scope and limitations of a pure singlet oxygen generator for antimicrobial photodynamic therapy.

EXPERIMENTAL SECTION

General materials and methods

Commercial reagents and starting materials were purchased from Acros Organics, Alpha-Aesar, TCI Europe, Fluka, Merck, Frontier Scientific or Sigma-Aldrich and used without further purification. The dry solvents acetone, dichloromethane, dimethylsulfoxide and

dimethylformamide were purchased from Roth (RotiDry Sept) or Sigma-Aldrich (puriss., absolute), stored over molecular sieves under nitrogen and were used as received.

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with 0.2 mm layer thickness and detection via UV light at 254 nm / 366 nm or through staining with ninhydrin in ethanol. Flash column chromatography was performed on Merck silica gel (Si 60 40-63 μm) either manually or on a Biotage® solera™ flash purification system. Column chromatography was performed on silica gel (70–230 mesh) from Merck.

Melting points were measured on a SRS melting point apparatus (MPA100 Opti Melt) and are uncorrected.

NMR spectra were recorded on Bruker Avance 300 (^{1}H 300.13 MHz, ^{13}C 75.47 MHz, T = 300 K), Bruker Avance 400 (^{1}H 400.13 MHz, ^{13}C 100.61 MHz, T = 300 K), Bruker Avance 600 (^{1}H 600.13 MHz, ^{13}C 150.92 MHz, T = 300 K) and Bruker Avance III 600 Kryo (^{1}H 600.25 MHz, ^{13}C 150.95 MHz, T = 300 K) instruments. The chemical shifts are reported in δ[ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants *J* are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = doublet of triplets, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in ^{13}C-spectra was determined with 2D-spectroscopy (COSY, HSQC and HMBC) or DEPT technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift 0.01 ppm (^{1}H NMR) and 0.1 ppm (^{13}C NMR), coupling constant *J* 0.1 Hz. The solvents used for the measurements are reported for each spectrum.

IR spectra were recorded with a Bio-Rad FT-IR-FTS 155 spectrometer. Fluorescence spectra were recorded on a 'Cary Eclipse' fluorescence spectrophotometer and absorption spectra on a "Cary BIO 50" UV/VIS/NIR spectrometer from Varian. All measurements were performed in 1 cm quartz cuvettes (Hellma) and UV-grade solvents (Baker or Merck) at 25 °C.

Mass spectra were recorded on Varian CH-5 (EI), Finnigan MAT95 (EI-, CI- and FAB-MS), Agilent Q-TOF 6540 UHD (ESI-MS, APCI-MS), Finnigan MAT SSQ 710 A (EI-MS, CI-MS) or Thermo Quest Finnigan TSQ 7000 (ES-MS, APCI-MS) spectrometer. Xenon serves as the ionization gas for FAB.

Synthesis and Purification of the compounds

The purity of all synthesized compounds was determined by NMR spectroscopic methods (Bruker Avance 300, DMSO-d6) and HPLC-MS confirming a purity of > 98%.

Phenalen-1-one **(1)**[40]

β-Naphthole (10.4 g, 72 mmol), glycerine (20.0 mL, 270 mmol) and sodium 3-nitrobenzenesulphonate (12.4 g, 65 mmol) in 36 mL conc. sulfuric acid and 24 mL water was heated to 140 °C for 1 h. The resulting black tar was poured onto ice and diluted with water. After crystallization overnight in the fridge, the supernatant was discarded and the product was extracted with warm toluene (60°C, 10x 50 mL). The pure product crystallized as yellow-brown solid while evaporating the solvent under reduced pressure to give 4.12 g (22.8 mmol, 32 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ[ppm] = 8.62 (dd, *J* = 1.0 Hz, *J* = 7.4 Hz, 1H), 8.19 (dd, *J* = 1.2 Hz, 8.1 Hz, 1H), 8.02 (dd, *J* = 1.1.Hz, *J* = 8.3 Hz, 1H), 7.75 (m, 3H), 7.58 (m, 1H), 6.73 (d, *J* = 9.8 Hz, 1H). - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ[ppm] = 185.8 (q), 141.9 (+), 135.1 (+), 132.4 (q), 132.1 (+), 131.6 (+), 130.6 (+), 129.7 (q), 129.4 (+), 128.0 (q), 127.7 (q), 127.3 (+), 126.8 (+); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 181.1 (100, MH^+); - **MF:** $C_{13}H_8O$; - **MW:** 180.2 g/mol

2-Chlormethyl-1H-phenalen-1-one **(2)**

Phenalen-1-one **1** (5.40 g, 30 mmol) and paraformaldehyde (16.50 g, 72.0 mmol) in 120 mL acetic acid and 75 mL phosphoric acid (85 %) was heated to 110 °C. Hydrochloric acid (36 % w/w, 80 mL) was added over a period of 30 min. The brown solution was heated for further 8 h. After cooling to room temperature, 250 mL of ice cold distilled water were added. The reaction mixture was carefully neutralized with a saturated solution of K_2CO_3. The product was extracted with dichloromethane (3x 100 mL). Further purification was carried out by flash chromatography (silica gel, dichloromethane/petroleum ether 1:1, R_f = 0.3) and gave 2.37 g of bright yellow, fine powder (10.41 mmol, 36 %).

M.p.: 133 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ[ppm] = 8.58 – 8.53 (dd, *J* = 1.2 Hz, *J* = 7.3 Hz, 1H), 8.14 - 8.08 (dd, *J* = 1.0 Hz, *J* = 8.1 Hz, 1H), 7.96 – 7.91 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.81 (s, 1H), 7.74 – 7.65 (m, 2H), 7.56 – 7.47 (dd, *J* = 7.0 Hz; *J* = 1.2 Hz; 1H), 4.62 (s, 2H, -C*H2*-); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ[ppm] = 183.4 (q), 140.3 (+), 135.4 (q), 135.2 (+), 132.2 (+),132.0 (q), 131.9 (+), 130.8 (+), 128.8 (q), 127.2 (+), 127.1 (q), 127.0 (q), 126.8 (+), 41.5 (-); - **IR** (neat): ν[cm^{-1}] = 3040 (w), 2969 (w), 2843 (w), 1635 (s), 1622 (s), 1594 (s), 1568 (s), 1507 (m),1422 (m), 937 (m), 789 (s), 767 (s); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 229.0 (100, MH^+); - **HR-MS** (ESI): m/z calcd. for $C_{14}H_{10}ClO$ (MH+): 229.0415. Found: 229.0414 (MH+); - **MF**: $C_{14}H_9OCl$; - **MW:** 228.7 g/mol;

General procedure I: Synthesis of positively charged phenalen-1-one derivatives

2-(Chloromethyl)-1H-phenalen-1-one (**4**) (230 mg, 1 mmol) was dissolved in DMF (2 mL). The appropriate tertiary amine or pyridine (10 mmol) in DMF (2 mL) was added drop wise

via a syringe. The solution was stirred for 24 h at room temperature in the dark. Stirring was continued at 50 °C for 3 h. After cooling to room temperature, diethylether (30 mL) was added to precipitate the product. The product was settled with the aid of a centrifuge and the supernatant was discarded. The precipitate was re-suspended in diethylether (15 mL), filtered off by suction and washed several times with diethylether. The yellow solid was dried at the air in the dark. If necessary, the product can be further purified by column chromatography (silica gel, $CHCl_3$/MeOH 6:1→ 5:1).

*1-((1-oxo-1H-phenalen-2-yl)methyl)pyridinium chloride (**9**)*

Pyridine (790 mg, 0.8 mL, 10 mmol) was reacted according to general procedure I. The product was additionally washed with icecold dichloromethane. The product is a yellow powder (255 mg, 0.83 mmol, 83 %).

M.p.: 147°C; - **^{1}H-NMR** (600 MHz, D_2O): δ[ppm] = 8.86 (d, *J* = 6.1 Hz, 2H), 8.46 (t, *J* = 7.9 Hz, 1H), 7.99 (t, *J* = 7.2 Hz, 2H), 7.73 (s, 1H), 7.68 (d, *J* = 8 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 7.1 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 5.32 (s, 2H); - **^{13}C-NMR** (150 MHz, D_2O): δ[ppm] = 184.2 (q), 146.2 (q), 145.5 (+), 144.7 (+), 136.7 (+), 135.1 (+), 134.4 (+), 130.9 (+), 130.8 (q), 129.4 (+), 128.1 (+), 128.0 (q), 127.1 (+), 127.0 (+), 126.6 (+), 125.5 (q), 124.9 (q), 60.5 (-); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 272.1 (100, M^+); - **HR-MS** (ESI): m/z calcd. for $C_{19}H_{14}NO$: 272.1070 (M+). Found 272.1078 (M+); - **UV** (H_2O): λ/ [nm] (ε / [$M^{-1}cm^{-1}$]) = 256 (22100), 322 (3300), 360 - 420 (9600); - **Elem.** calcd. for $C_{19}H_{14}NOCl*H_2O$: C 70.04 %, H 4.95 %, N 4.30 %. Found: C 68.45 %, H 5.06 %, N 4.27 %. – **MW** = 308.24 g/mol; - **MF** = $C_{19}H_{14}NOCl$;

*N,N-dimethyl-N-((1-oxo-1H-phenalen-2-yl)methyl)butan-1-aminium chloride (**6**)*

N,N-Dimethylbutylamine (1.02 g, 1.42 mL, 10 mmol) was reacted according to general procedure I to give pale yellow powder (310 mg, 0.94 mmol, 94 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ[ppm] = 8.88 (s, 1H), 8.56 (dd, *J* = 1.2 Hz, *J* = 7.4 Hz, 1H), 8.22 (dd, *J* = 1 Hz, *J* = 8.1 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 8.03 (d, *J* = 7.4 Hz, 1H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.61 (t, *J* = 7.2 Hz, 1H), 5.02 (s, 2H), 3.55 (m, 2H), 3.30 (s, 6H), 1.83 (m, 2H), 1.38 (m, app hex, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.5 Hz, 3H).- **^{13}C-NMR** (75 MHz, $CDCl_3$): δ[ppm] = 184.3 (q), 151.3 (+), 136.1 (+), 135.0 (+), 134.1 (+), 131.9 (q), 131.7 (+), 128.6 (q), 127.5 (+), 127.3 (+), 126.6 (q), 126.2 (q), 65.5 (-), 61.1 (-), 50.3 (+), 25.0 (-), 19.8 (-), 13.8 (+). - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 294.1 (100, M^+); - **HR-MS** (ESI): m/z calcd. for $C_{20}H_{24}NO$: 294.1859 (M+). Found 294.1873 (M+); - **UV** (H_2O): λ/ [nm] (ε / [$M^{-1}cm^{-1}$]) = 255 (21200), 320 (3200), 360 - 420 (9800); - **MW** = 329.87 g/mol; - **MF** = $C_{20}H_{24}NOCl$;

*2-hydroxy-N,N-dimethyl-N-((1-oxo-1H-phenalen-2-yl)methyl)ethanaminium chloride (**7**)*

N,N-Dimethylethanolamine (890 mg, 1.02 mL, 10 mmol) was reacted according to general procedure I to yield pale yellow powder (277 mg, 0.87 mmol, 87 %).

^{1}H-NMR (300 MHz, DMSO-d6): δ[ppm] = 8.58 - 8.54 (dd, *J* = 1 Hz, *J* = 7.3 Hz, 1H), 8.55 - 8.50 (dd, *J* = 1.2 Hz, *J* = 7.4 Hz, 1H), 8.52 (s, 1H), 8.37 (dd, *J* = 1 Hz, *J* = 7.8 Hz, 1H), 8.18 (dd, *J* = 0.9 Hz, *J* = 7.1 Hz, 1H), 7.95 (t, *J* = 7.7 Hz, 1H), 7.84 – 7.78 (m, 1H), 5.62 (t, *J* = 5.1 Hz, 1H), 4.61 (s, 2H), 3.99 – 3.88 (m, 2H), 3.59 – 3.51 (m, 2H), 3.15 (s, 6H).- **^{13}C-NMR** (75 MHz, DMSO-d6): δ[ppm] = 183.3 (q), 149.3 (+), 135.8 (+), 134.2 (+), 133.6 (+), 131.6 (q), 130.8 (+), 128.3 (q), 127.6 (+), 127.3 (+), 126.8 (q), 126.0 (q), 65.6 (-), 61.5 (-), 55.0 (-), 50.4

(+). - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 282.1 (100, M^+); - **HR-MS** (ESI): m/z calcd. for $C_{18}H_{20}NO_2$: 282.1494 (M+). Found 282.1495 (M+); - **UV** (H_2O): λ/ [nm] (ε / [$M^{-1}cm^{-1}$]) = 256 (20900), 322 (3100), 360 - 420 (9700); - **MW** = 317.82 g/mol; - **MF** = $C_{18}H_{20}NO_2Cl$;

*N,N-dimethyl-N-((1-oxo-1H-phenalen-2-yl)methyl)-1-phenylethanaminium chloride (**8**)*

(S)-(-)-N,N-Dimethyl-1-phenethylamine (1.49 g, 1.66 mL, 10 mmol) was reacted according to general procedure I. Compound **8** is a bright yellow powder (277 mg, 0.81 mmol, 81 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ[ppm] = 8.94 (s, 1H), 8.51 (dd, *J* = 1.1 Hz, *J* = 7.3 Hz, 1H), 8.20 (m, app. d, *J* = 8.6 Hz,1H), 8.04 (m, app. t, *J* = 8.6 Hz, 2H), 7.74 (t, *J* = 7.9 Hz, 2H), 7.68 (m, app. bs, 1H), 7.58 (m, app. t, *J* = 8.6 Hz, 1H), 7.44 – 7.37 (m, 3H), 5.72 (q, *J* = 6.9 Hz, 1H), 5.17 – 4.91 (m, 2H), 3.08 (s, 3H), 3.05 (s, 3H), 1.95 (d, *J* = 6.9 Hz, 3H).- **^{13}C-NMR** (75 MHz, $CDCl_3$): δ[ppm] = 184.4 (q), 151.7 (+), 136.1 (+), 135.1 (+), 134.1 (+), 133.0 (q), 131.9 (q), 131.6 (+), 130.7 (+), 129.3 (+), 128.6 (q), 127.5 (+), 127.3 (+), 126.9 (q), 126.3 (q), 74.0 (+), 59.4 (-), 48.1 (+), 46.1 (+), 15.6 (+). - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 342.1 (100, M^+); - **HR-MS** (ESI): m/z calcd. for $C_{24}H_{24}NO$: 342.1858 (M+). Found 342.1867 (M+); - **UV** (H_2O): λ/ [nm] (ε / [$M^{-1}cm^{-1}$]) = 256 (21100), 320 (3300), 360 - 420 (9800); - **MW** = 377.91 g/mol; - **MF** = $C_{24}H_{24}NOCl$;

*N,N,N-trimethyl-1-(1-oxo-1H-phenalen-2-yl)methanaminium chloride (**5**)*

In Schlenck-flask 2-(chloromethyl)-1H-phenalen-1-one (**4**) (230 mg, 1 mmol) was dissolved in ethanol (60 mL). Trimethylamine in ethanol (5 mL, 5.6 M, 23 mmol) was added via the septum by a syringe. The solution was stirred over night at room temperature. Stirring was

continued at 50°C for 30 h. The solvent was evaporated down to approx. 3 mL. Diethylether (50 mL) was added to precipitate the product. The product was settled with the aid of a centrifuge and the supernatant was discarded. The precipitate was re-suspended in diethylether (15 mL), settled again and the supernatant was decanted off. This washing step was repeated twice. Finally, the product was washed with 2 mL of icecold dichloromethane. The solid residue was dried at reduced pressure to give a bright yellow powder (210 mg, 0.73 mmol, 73 %).

^{1}H-NMR (600 MHz, D_2O): δ[ppm] = 8.02 (d,*J* = 8 Hz, 1H), 7.97 (d,*J* = 6.3 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.77 (s, 1H), 7.62 (d, *J* = 7 Hz, 1H), 7.50 (t,*J* = 7.8 Hz, 1H) 7.45 (t, *J* = 7.8 Hz, 1H), 4.12 (s, 2H), 2.98 (s, 9H).- **^{13}C-NMR** (150 MHz, D_2O): δ[ppm] = 184.4 (q), 150.4 (+), 137.0 (+), 135.6 (+), 135.0 (+), 131.7(q), 131.2 (+), 127.4 (+), 127.2 (+), 127.1 (q), 126.0 (q), 125.0 (q), 124.9 (q), 62.9 (-), 52.8 (+). - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): e/z (%) = 252.1 (100, M^+); - **HR-MS** (ESI): m/z calcd. for $C_{17}H_{18}NO$: 252.1388 (M+). Found 252.1393 (M+); - **UV** (H_2O): λ/ [nm] (ε / [$M^{-1}cm^{-1}$]) = 254 (21000), 321 (3200), 360 - 420 (9700); - **MW** = 287.79 g/mol; - **MF** = $C_{17}H_{18}NOCl$;

Determination of singlet oxygen quantum yields and photophysics

All PNs were solved in pure water with concentrations from 1 µM to 5 mM. The extinction coefficient ε was calculated with all absorption spectra from 1 µM to 5 mM. Therefore a photospectrometer (Beckmann DU 640) was used and the path of light was adapted to the concentration by quartz cuvettes with different thickness.

For the estimation of the photostability all PNs were irradiated with energy between 122 and 128 J in a time span of 20 minutes. Irradiation occurred by a tunable laser system (NT242-

SH/SFG, Ekspla, Lithania) with a wavelength of 405 nm. Absorption spectra were recorded in distinct time intervals.

The oxygen consumption of the solutions was measured by an oxygen microsensor (Presens, Regensburg, Germany) with a starting concentration of oxygen in water of 270 µM (air saturated).

Singlet oxygen signals were recorded with a high sensitive NIR-system described by *Bäumler et al*.[41] and a tunable laser system. The singlet oxygen signal of 20 seconds of all PNs were recorded over 20 minutes to estimate the stability of singlet oxygen generation depending on absorption of radiation and the energy transfer from the PS to oxygen in the ground state.

The $^1O_2$ quantum yield Φ_Δ of the PNs was determined using the water-soluble perinaphthenone sulfonic acid (PNS) with a $\Phi_\Delta = 1.03 \pm 0.10$ as a reference PS (synthesized with a purity of 99 % according the protocol by Nonell *et al.*[23] in the Department of Organic Chemistry, University of Regensburg, Germany).

Therefore the absorbed energy of a phenalen-1-one (**1**) solution and the PNS (**2**) solution (both 10 µM) were compared to their emitted singlet oxygen luminescence at 1270 nm as described in literature.[41] Both PS were excited at 405 nm generated by a tuneable laser system (NT 242-SH/SFG, Ekspla, Vilnius, Lithuania) with different excitation powers of 10 mW, 30 mW, 52 mW and 70mW for 50 seconds.

Absorption spectra were recorded on a Varian Cary BIO 50 UV/VIS/NIR spectrometer with temperature control using 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck, Baker or Acros) or Millipore water (18 MΩ). Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Light source

Illumination of all samples incubated with PN-derivatives was with a hand-held dental light-curing unit (bluephase® C8, IvoclarVivadent, Schaan, Liechtenstein) with an output-intensity of 1360 ± 30 mW/cm^2 in "high power"-mode, measured with a thermal low power sensor (Nova 30A-SH, Ophir-Spiricon, North Logan, UT). Lighting was from below, with direct contact to the bottom of the well containing the sample, wherefore diffusion of light due to surface tensions in the samples could be excluded (see Fig. 2 for spectrum of the irradiation device). Consequently, the intensity at level of the samples was diminished to 1260 ± 50 mW/cm^2. Irradiation was for 20 s, 40 s or 120 s applying light doses of 25 J/cm^2, 50 J/cm^2 or 150 J/cm^2, respectively.

Bacterial culture

Four reference strains, *Enterococcus faecalis* (*EF*; ATCC 29212), *Streptococcus mutans* (*SM*; ATCC 25175), *Actinomyces naeslundii* (*AN*; T14V) and *Aggregatibacter actinomycetemcomitans* (*AA*; ATCC43718) were used in this. All strains were provided by the Institute of Medical Microbiology and Hygiene, University Hospital Regensburg, Germany.

The culture medium for all strains was Brain Heart Infusion broth (BHI broth; Sigma-Aldrich, St. Louis, MO). Incubation was at 37 °C on a shaker as overnight-cultures, whereby *EF*, *SM* and *AN* were grown aerobe and *AA* was grown anaerobe. When the cultures reached the static growth-phase, bacteria were harvested by centrifugation (3000 rpm, 5 min; Megafuge 1.0, HeraeusSepatech, Osterode, Germany), re-suspended in sterile PBS (Sigma-Aldrich, St. Louis, MO) and diluted to yield an optical density corresponding to 10^7-10^8 bacteria/mL, measured at 600nm with a photosprectrometer (DU® 640, Beckman-Coulter, Krefeld, Germany). These suspensions were used for PIB-experiments.

Photodynamic Inactivation of Bacteria (PIB)

Bacterial cell suspensions were mixed in 96-well microtiter plates (Corning Costar®, Corning, NY) one-to-one either with 100 µL H_2O or with 100 µL PS obtaining different final concentrations the PN-derivatives of 0 µM, 1 µM, 5 µM, 10 µM, 100 µM, and 250 µM. The bacteria were incubated with each PN-derivative for 10 sec (see Fig. 8), 7.5 min and 15 min (see supporting information). Immediately afterwards samples were illuminated (20 s, 40 s, 120 s) or maintained in the dark during the same period. Subsequently serial tenfold dilutions (10^{-2} to 10^{-7}) were prepared in BHI-broth and aliquots (3 x 20 µL) were plated on agar-plates, as described elsewhere.[42] Hereby Mueller-Hinton-Agar was used for *EF*, BHI-agar for *AA* and *SM,* and bloodagar for *AN* (provided by the Institute of Medical Microbiology and Hygiene, University Hospital Regensburg, Germany). Incubation of *EF*, *SM,* and *AN* was aerobically at 37 °C for 24 h (*EF*) or 48 h (*SM*, *AN*). In contrast, *AA* was incubated anaerobically at 37 °C using a box for anaerobes containing anaer-generators (bioMérieux, Marcy-l'Etoile, France) for 48 h. Afterwards CFU were counted.

Data analysis

All PIB results are shown as medians, including 25 % and 75 % quartiles, which were calculated using SPSS for Windows, ver. 20.0 (SPSS Inc., Chicago, IL) from the values of three independent experiments, each performed in triplicate. Experimental results were referred to untreated controls, which were neither incubated with PN-derivatives nor illuminated. In addition all experiments were compared to samples only incubated with PN-derivatives and not illuminated, so-called dark control.[43] A reduction of at least three magnitudes of $\log_{10}$ of viable median numbers of bacteria was considered as biologically relevant, whereas a reduction of at least five magnitudes of $\log_{10}$ of viable median numbers of bacteria was considered as disinfection efficacy with regard to the guidelines of hand hygiene.[44]

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ABBREVIATIONS: *Enterococcus faecalis (EF)*, *Actinomyces naeslundii (AN)*, *Streptococcus mutans (SM), Aggregatibacter actinomycetemcomitans (AA)*, 7-perinaphthenone (PN), photodynamic inactivation of bacteria (PIB), vancomycin resistant staphylococcus aureus (VRSA), methicillin-resistant staphylococcus aureus (MRSA), methicillin-sensitive staphylococcus aureus (MSSA), colony forming unit (CFU), reactive oxygen species (ROS), localized aggressive periodontitis (LAP)

ASSOCIATED CONTENT: For experimental details, materials and methods relating to synthesis and characterization, selected NMR spectra, as well as all singlet-oxygen measurements, UV-Vis data concerning aggregation and stability please see the **Supporting Information**. "This material is available free of charge via the Internet at http://pubs.acs.org."

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