Supporting Information
for

Triarylphosphonium-conjugated Sn(IV)-porphyrins for Antimicrobial Photodynamic Therapy: Impact of Substituents on Lipophilicity, Aggregation, and Photoantibacterial Activity

Gayathri MP,[a] Nandhakumar Muruganandham,[b] Sutharsan Govindarajan,*[b] Balaji Babu*[a]

[a]*Department of Chemistry, SRM University-AP, India;* [b]*Department of Biological Sciences, SRM University-AP, India.*

**Corresponding authors: sutharsan.g@srmap.edu.in; balaji.b@srmap.edu.in*

Equipment

UV-visible absorption spectra were measured on a Shimadzu UV−2550 spectrophotometer, the fluorescence in Spark Tecan. ^{1}H, ^{19}F, ^{31}P NMR were measured using 400MHz NMR spectrometer (JNM-ECZL400R). Kessil PR160L-427nm is used for the light treatment. All microscopy images were processed using NIS Elements AR software (Nikon). For plate images Bio-Rad ChemiDoc imaging system was used and to process the image Fiji is used.

Lipophilicity

The lipophilicity ($\log P_{o/w}$) values of Sn(IV)-porphyrins (**SnP1**-**SnP3**) were measured by shake-flask method using UV-Visible spectroscopy.[1]

Singlet oxygen quantum yield studies

The singlet oxygen quantum yields of Sn(IV)-porphyrins (**SnP1**-**SnP3**) were determined by comparative method in DMF using 9,10-dimethylanthracene (DMA) as a chemical scavenger and zinc tetraphenylporphyrin (**ZnTPP**), as the reference standard.[2] The slope of irradiation time vs absorbance of DMA were used to calculate the singlet oxygen quantum yields according to the standard relative method.[3]

$$\Phi_{\Delta}^{samp} = (\phi_{\Delta}^{ref}) \times (m_{samp}/m_{std}) \times (F_{std}/F_{samp})$$

The superscript 'samp' and 'std' refers to Sn(IV)-porphyrins and standard dye respectively. The parameter 'm' represents the slope obtained from the plot of the change in absorbance (ΔA) vs light irradiation time to DMA. The absorption correction factor (F) was calculated using $F = 1-10^{-OD}$, where OD corresponds to the optical density of wavelength used for irradiation.

Antibacterial assay

Bacterial cultures (*E. coli* and MRSA) were initially grown overnight in 5ml LB broth at 37°C overnight in an incubator, then diluted 1:100 in fresh LB and incubated at the temperature of 37°C until it reached the OD of around 0.6. 1 ml of bacteria was taken for each concentration, then it was centrifuged to collect the pellet and the supernatant was discarded. To the tube 1 ml of 1XPBS buffer was added to resuspend the pellet. The Sn(IV)-porphyrins (**SnP1**-**SnP3**) were added at appropriate concentrations, and the bacterial suspensions were further incubated at 37°C for 1 h. Following 1h of incubation, the samples were centrifuged, the supernatant was removed and the pellet was resuspended in 1ml of 1X PBS buffer. Then it was equally divided (500 µL+500 µL), in which one-half of the samples were irradiated with a 427 nm LED light for 30 min (22 $mWcm^{-2}$, 40 Jcm^{-2}), while a parallel set was kept in the dark for the same time period. Post-irradiation, the samples were subjected to serial dilution up to 10^{-6} and approximately 3 µl aliquots from each dilution was carefully spotted onto LB agar plates. The plates were subsequently incubated at 37°C overnight and colony-forming units per millilitre (CFU/ml) values were calculated for dark and light treated samples using the following formula:

$$CFU/ml = [(No.\ of\ colonies \times dilution\ factor)]/[volume\ spotted\ (in\ ml)]$$

All experiments were carried out in triplicate, and the average values were plotted using GraphPad Prism.

Fluorescence microscopy

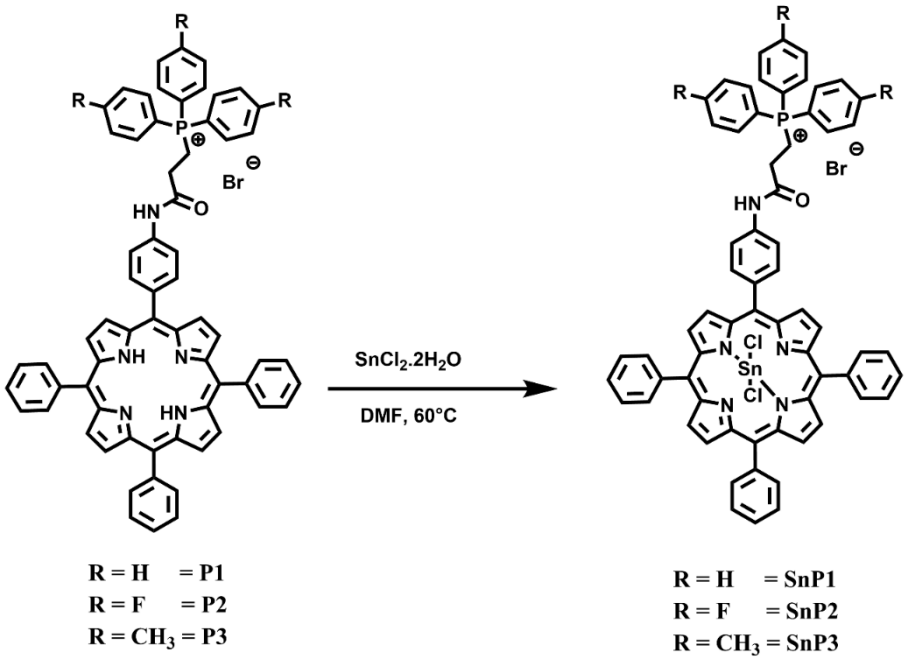
For imaging experiments, agarose pads were made consisting of 1% agarose, prepared in 1X PBS buffer solution. The melted agarose was transferred to the microscopic slide and covered with cove slip. Overnight culture was raised of the respective bacteria (MRSA and *E. coli*) in 5ml LB broth and kept for incubation at 37°C overnight. The overnight culture was then diluted at 1:100 with fresh LB and at the temperature of 37°C until it reached the OD of around 0.6. The cells were treated with Sn(IV)-porphyrins (**SnP1**-**SnP3**) as detailed in the previous section. The cells were treated with 4′,6-diamidino-2-phenylindole (DAPI) (10 µg/ml) for about 10 min for DNA staining. The cells were thoroughly washed with 1XPBS to remove excess DAPI and subsequently re-suspended in 100 µL of 1XPBS. Microscopy was performed using a Nikon Eclipse Ti2-E system fitted with a 100×CFI Plan Apochromat oil immersion objective and a DSQi-2 Monochrome Camera (Nikon) and images were processed using NIS Elements AR software (Nikon).

DCFDA-ROS assay

The 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assay was used to provide evidence for production of reactive oxygen species (ROS) by synthesized **SnP2**. Same procedure as antibacterial assay was followed but before light treatment the *E. coli* and MRSA cells were pre-incubated with SnP3 (10µM and 20nm respectively) for 1 h in the dark. DCFDA (10 µM, final concentration) was then added and incubation continued for 30 min in the dark. After washing with PBS (x3), the cells were irradiated with 427 nm LED for 15 min and subsequently imaged using microscopy.

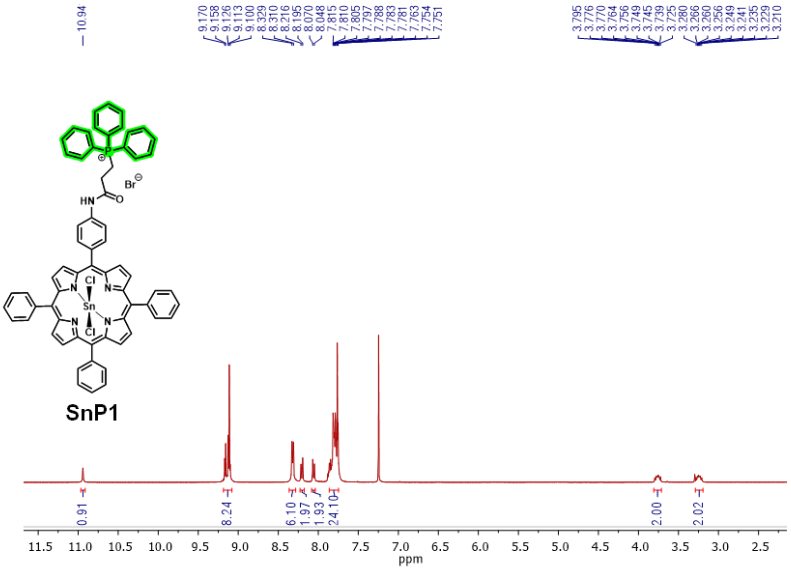
References

1. J. X. Soares, Á. Santos, C. Fernandes and M. M. M. Pinto, *Chemosensors*, 2022, **10 (8)**, 340
2. R. Bresolí-Obach, J. Torra, R. P. Zanocco, A. L. Zanocco and S. Nonell, *Methods Mol. Biol.*, 2021, **2202**, 165–188.
3. W. Li , L. Li , H. Xiao , R. Qi , Y. Huang , Z. Xie , X. Jing and H. Zhang, *RSC Adv.*, 2013, **3**, 13417-13421.

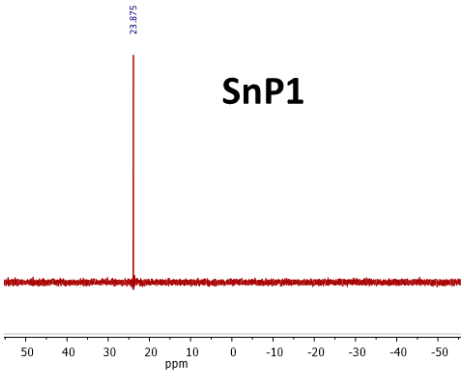


Scheme S1. Synthetic scheme for the Sn(IV)-porphyrins (**SnP1- SnP3).**

(a) ^{1}H NMR



(b) ^{31}P -NMR



(c) ^{31}P -NMR

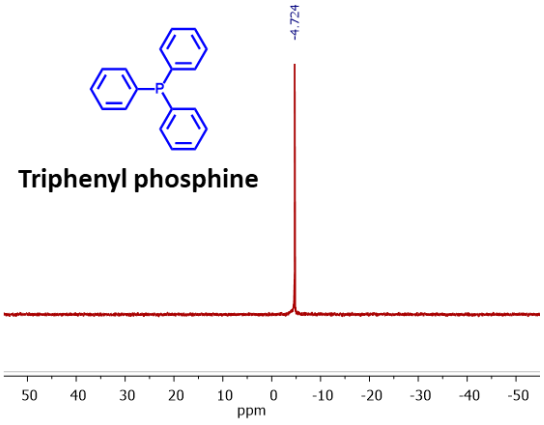
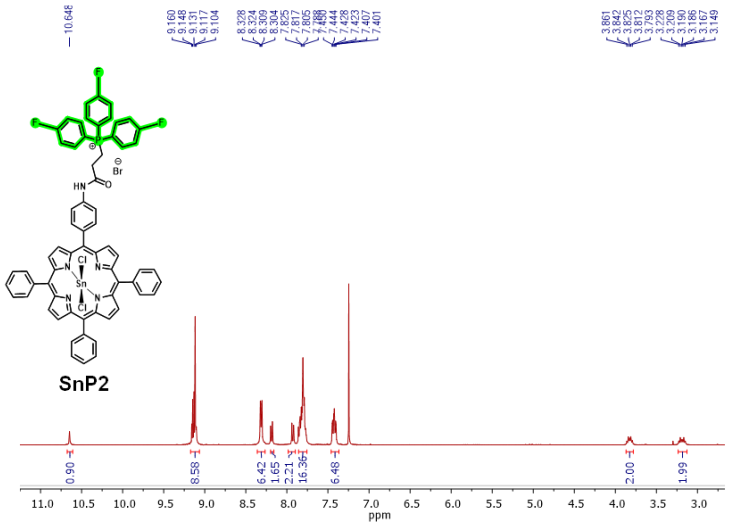
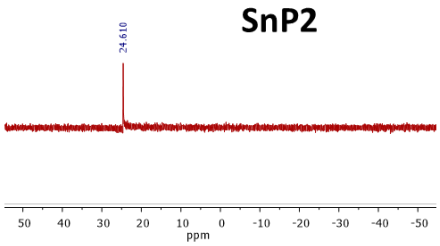


Figure S1. (a) ^{1}H NMR and (b) ^{31}P NMR of **SnP1** and (c) ^{31}P NMR of free triphenylphosphine. In $CDCl_3$, 400 MHz.

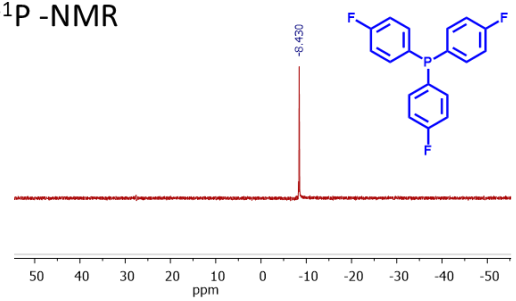
(a) ^{1}H NMR



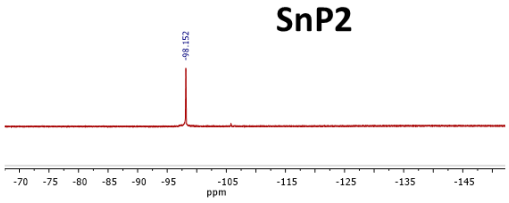
(b) ^{31}P -NMR



(c) ^{31}P -NMR



(d) ^{19}F -NMR



(e) ^{19}F -NMR

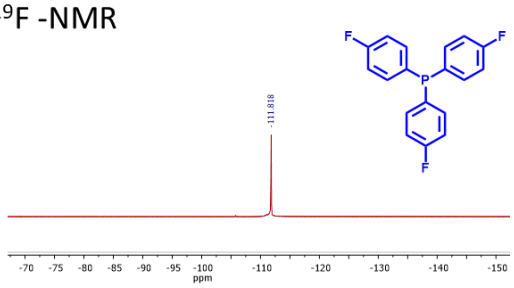


Figure S2. (a) ^{1}H NMR and (b) ^{31}P NMR (d) ^{19}F NMR of **SnP2;** (c) **^{31}**P NMR and (e) ^{19}F NMR of free Tris (4-fluorophenyl) phosphine. In $CDCl_3$, 400 MHz.

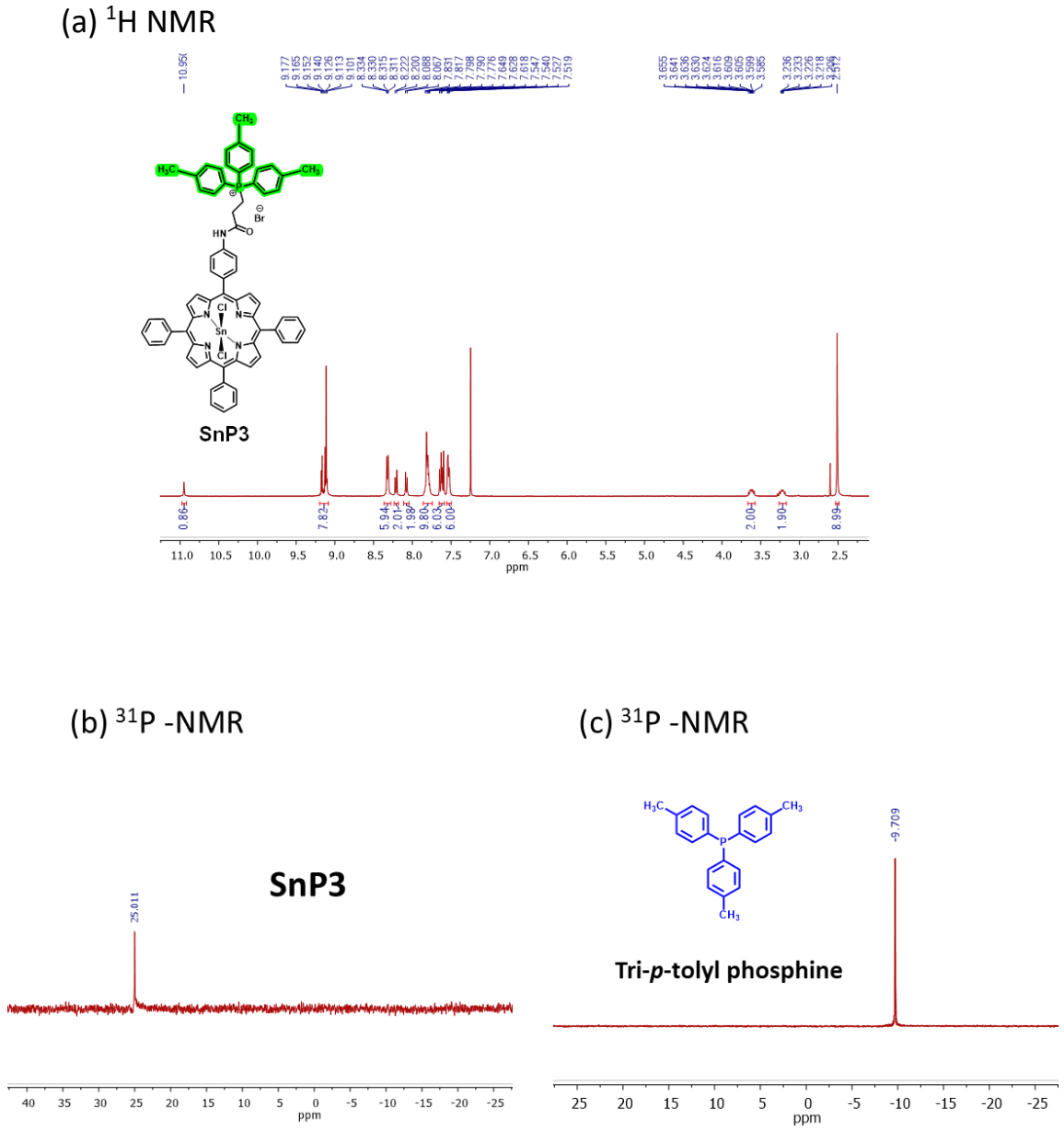


Figure S3. (a) ^{1}H NMR and (b) ^{31}P NMR of **SnP3** and (c) ^{31}P NMR of free tri-*p*-tolylphosphine. In $CDCl_3$, 400 MHz.

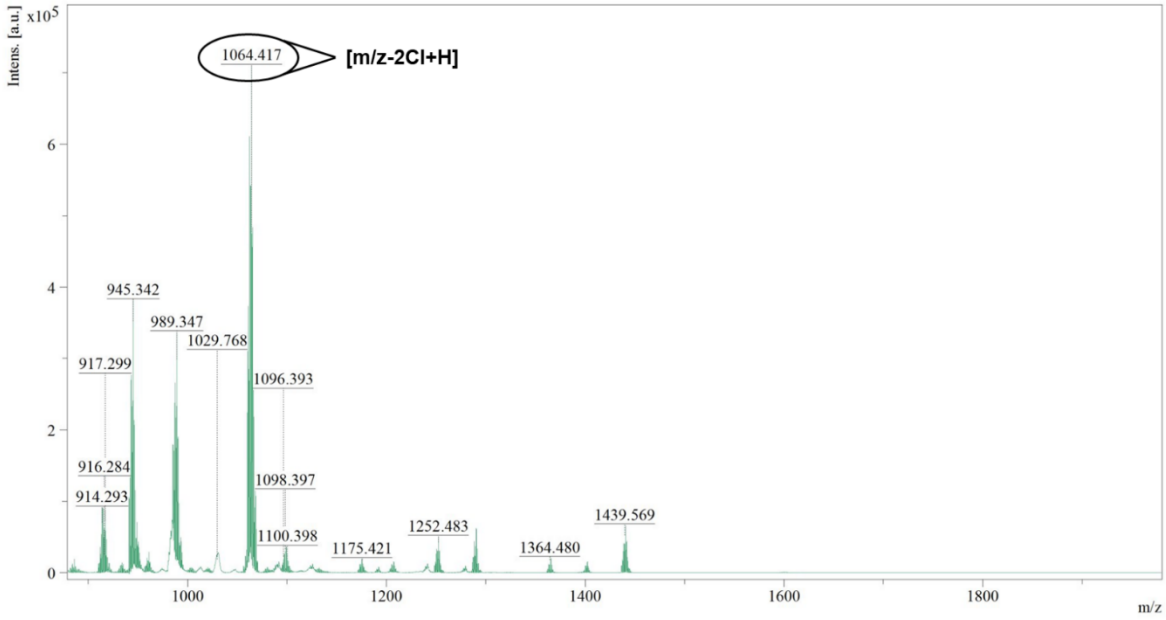


Figure S4. HRMS of **SnP1**.

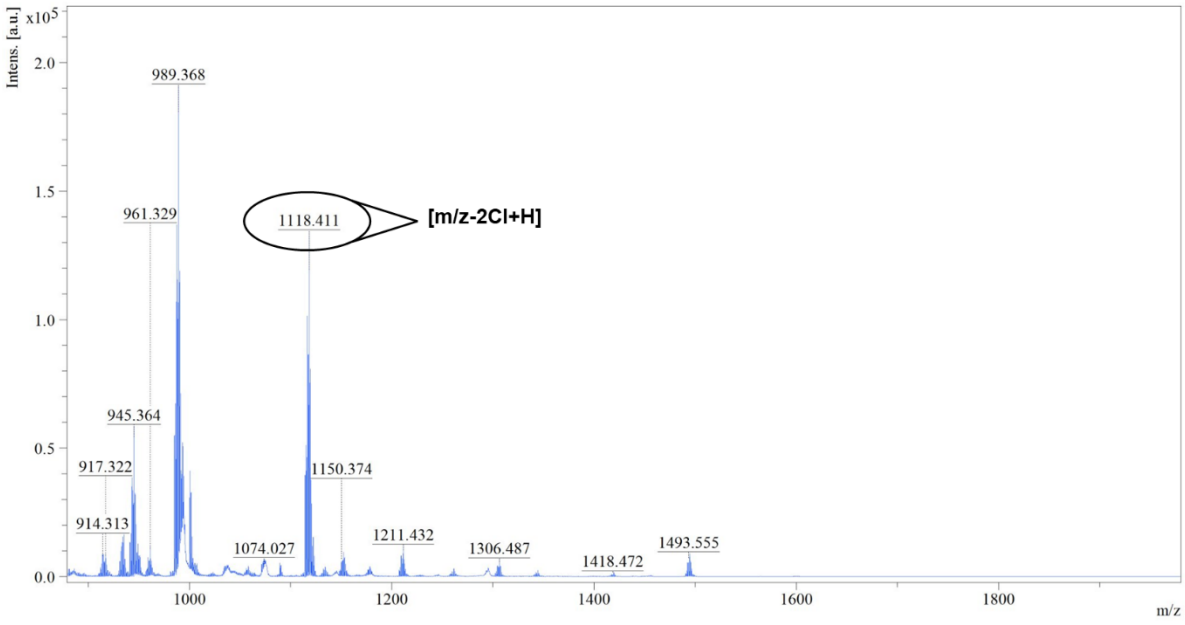


Figure S5. HRMS of **SnP2**.

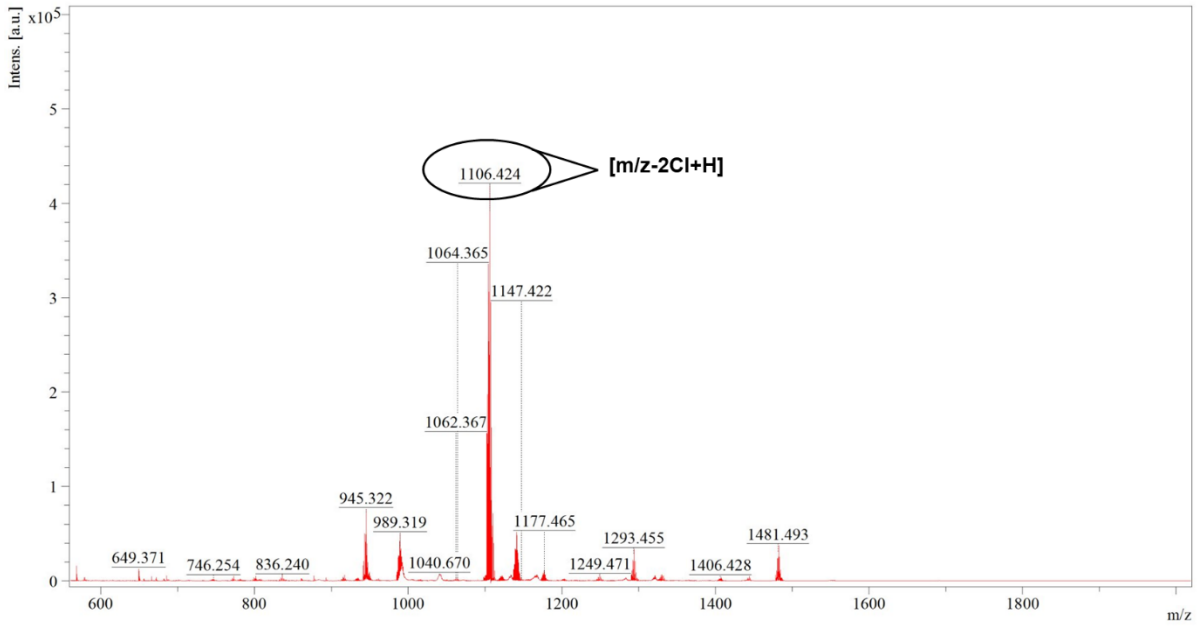


Figure S6. HRMS of **SnP3**.

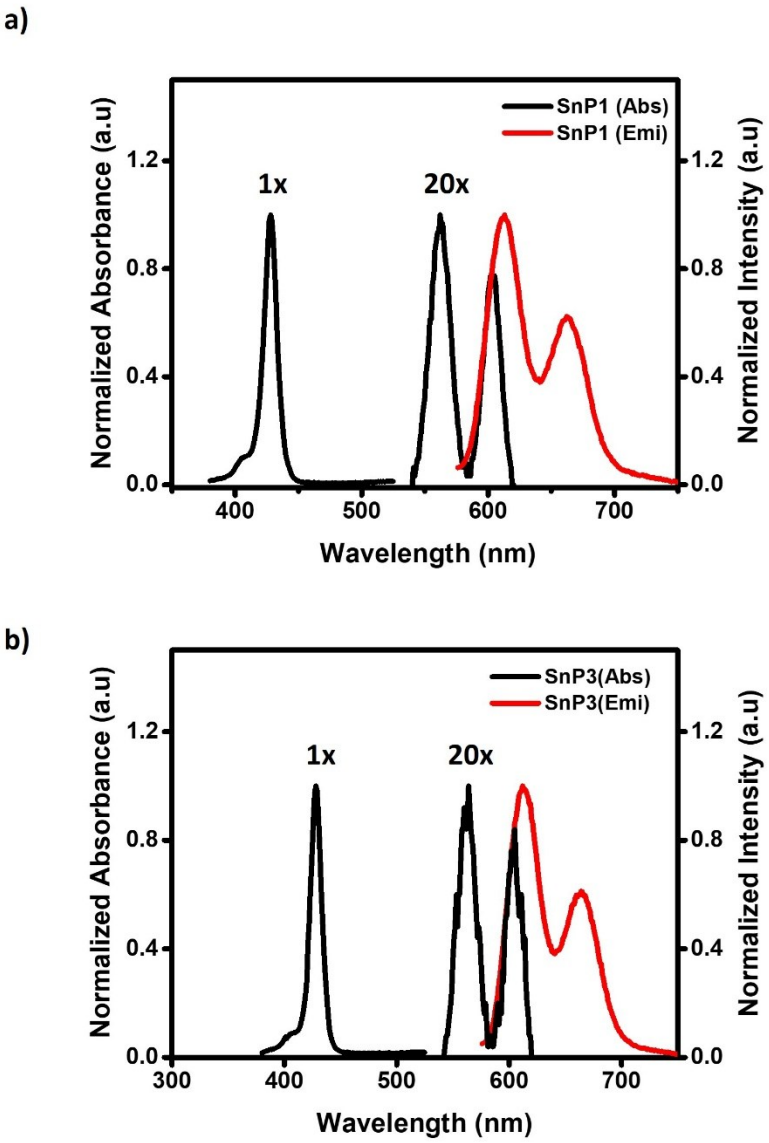


Figure S7. Absorption (black) and Emission (red) spectra of (a) **SnP1** (b) **SnP3** in DMF.

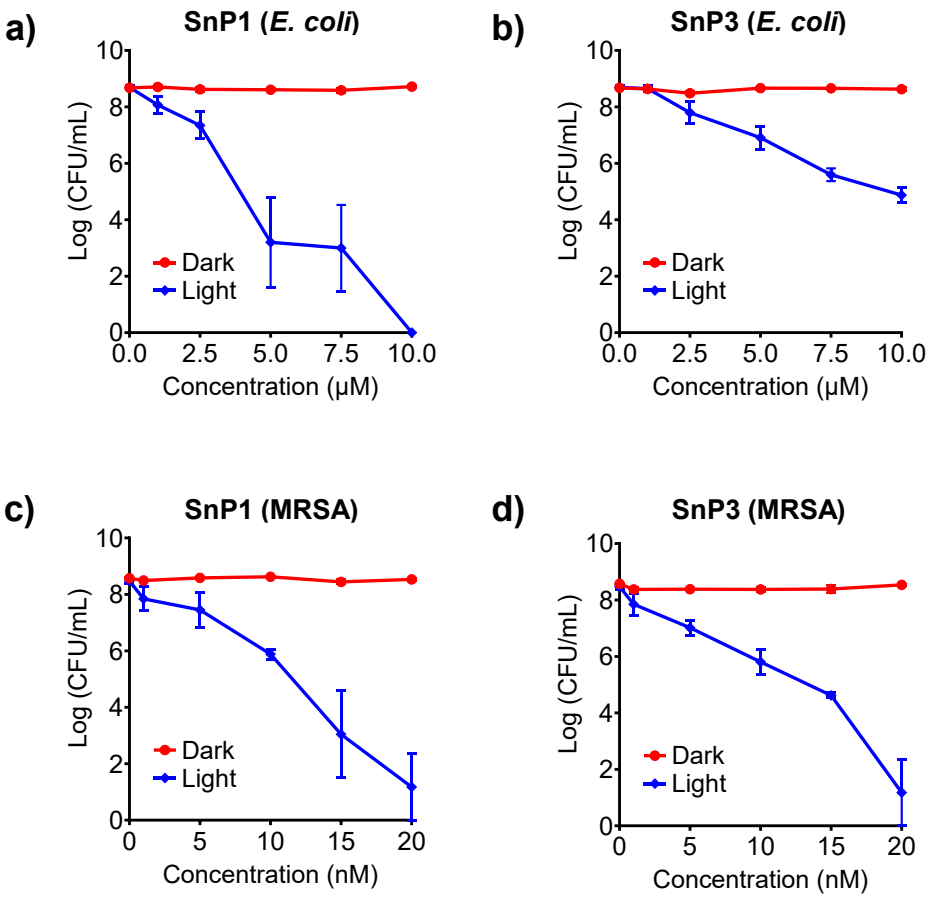


Figure S8. Log reduction graph of a) **SnP1** b) **SnP3** against *E. coli* and c) **SnP1** d) **SnP3** against MRSA in the dark and after light irradiation with a 427 nm LED (22 mW cm^{-2}, 40 J cm^{-2}) for 30 min, with increasing concentrations.

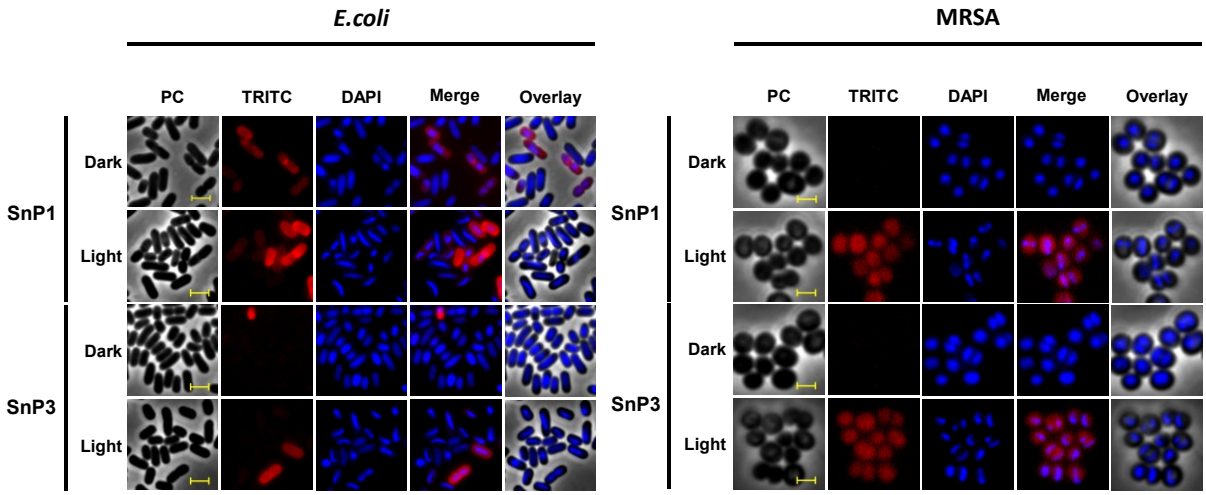


Figure S9. Fluorescence microscopy images of **SnP1** & **SnP3**. *E. coli* and MRSA is treated with 10 μM and 20nM respectively, of **SnP1** & **SnP3** in the dark and after light exposure (427 nm LED, 22 mW cm^{-2}). Localization of the **SnP1** & **SnP3** compounds was observed in the TRITC filter of fluorescence microscopy, and the cells were observed with phase contrast (PC). PC images (grey), DAPI (blue) and the compound fluorescence (Red) are shown in individual panels. The scale bar corresponds to 2 μm.