Carboranyl-Chlorin e6 as a Potent Antimicrobial Photosensitizer

Elena O. Omarova¹, Pavel A. Nazarov¹, Alexander M. Firsov¹, Marina G. Strakhovskaya²,³, Anastasia Yu. Arkhipova², Mikhail M. Moisenovich², Igor I. Agapov², Valentina A. Ol’shevskaia⁵, Andrey V. Zaitsev⁵, Valery N. Kalinin⁵, Elena A. Kotova¹*, Yuri N. Antonenko¹

¹ Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia, ² Biological Department, Lomonosov Moscow State University, Moscow, Russia, ³ Federal Scientific and Clinical Center for Specialized Medical Service and Medical Technologies, FMBA, Moscow, Russia, ⁴ Shumakov Research Center of Transplantology and Artificial Organs, Moscow, Russia, ⁵ Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia

* kotova@genebee.msu.ru

Abstract

Antimicrobial photodynamic inactivation is currently being widely considered as alternative to antibiotic chemotherapy of infective diseases, attracting much attention to design of novel effective photosensitizers. Carboranyl-chlorin-e₆ (the conjugate of chlorin e₆ with carborane), applied here for the first time for antimicrobial photodynamic inactivation, appeared to be much stronger than chlorin e₆ against Gram-positive bacteria, such as Bacillus subtilis, Staphylococcus aureus and Mycobacterium sp. Confocal fluorescence spectroscopy and membrane leakage experiments indicated that bacteria cell death upon photodynamic treatment with carboranyl-chlorin-e₆ is caused by loss of cell membrane integrity. The enhanced photobactericidal activity was attributed to the increased accumulation of the conjugate by bacterial cells, as evaluated both by centrifugation and fluorescence correlation spectroscopy. Gram-negative bacteria were rather resistant to antimicrobial photodynamic inactivation mediated by carboranyl-chlorin-e₆. Unlike chlorin e₆, the conjugate showed higher (compared to the wild-type strain) dark toxicity with Escherichia coli ΔtolC mutant, deficient in TolC-requiring multidrug efflux transporters.

Introduction

The medicinal chemistry of carba-closo-dodecaboranes (carboranes [1]) has been traditionally centered on their use in boron neutron capture therapy (BNCT) of tumors [2,3]. Based on the known ability of various porphyrin-related photosensitizers to accumulate in tumors and generate cytotoxic reactive oxygen species killing cancer cells, conjugation of carboranes with these compounds was considered as a way to improve both their delivery to tumors and therapeutic efficacy. Actually, such studies resulted in design and synthesis of promising agents for BNCT, photodynamic therapy and fluorescence imaging of tumors [4–12]. On the other hand, in a series of studies carboranes were used as drug pharmacophores [13–15]. So far, no research...
has to our knowledge concerned antimicrobial photodynamic effect of boronated carboranes, although photodynamic inactivation (PDI) of bacteria has long been studied with different photosensitizers [16–32] resulting in a great variety of medicinal applications. Of note, some data on dark bactericidal and fungicidal activity of 1-(aminoalky)-1,2-dicarba-closo-dodecarborane [33] and o-carboranylalanine [34] were earlier reported. The cytotoxic efficacy of derivatives of polyhedric boron complexes could be related to their unique binding [35] and membrane-penetrating [36,37] properties, the latter being associated with delocalization of their charge.

Much attention is attracted now to studying antimicrobial PDI (aPDI), because this therapeutic modality, being effective against drug-resistant infections, might allow for the development of a valuable alternative or supplemental option to the current antibiotic-based treatments [26,38]. The major targets of PDI in bacterial cells are still debated, albeit strong evidence has been obtained in favor of damage to outer cell structures being critical [19,26,30,31,39–46]. In fact, various authors have concluded that although DNA damage occurs, it may not be the primary cause of bacterial cell death [47,48].

Among a great variety of photosensitizers studied as agents in aPDT, derivatives of chlorin [20,23,24,49–53] and bacteriochlorin [54] attracted special attention. In particular, Hamblin and colleagues showed that covalent conjugates of chlorin e₆ with poly-L-lysine [23,24] and polyethyleneimine [51] were efficient photosensitizers (PS) of both gram-positive and gram-negative bacteria, because the polycationic molecular constructs increased binding and penetration of the PS into impermeable gram-negative cells. Chlorin-polyethyleneimine conjugates were also effective in PDI of fungi [54].

Here, we for the first time applied boronated chlorin e₆ amide (BACE, chlorin e₆ 13(1)-N-[2-[N-(1-carba-closo-dodecarboran-1-yl)methyl]aminoethyl]amide-15(2), 17(3)-dimethyl ester) [8,9] for aPDI and found this photosensitizer to show much higher efficacy against Gram-positive than against Gram-negative bacteria.

Materials and Methods

Chemicals

The sodium salt of 13(1)-N-[2-[N-(1-carba-closo-dodecarboran-1-yl)methyl]aminoethyl]amide-15(2),17(3)-dimethyl ester of chlorin e₆ (BACE) was synthesized and described earlier [8,9]. Chlorin e₆ was obtained from Porphyrin Products (Logan, UT). *E. coli* total lipid extract was from Avanti polar lipids (Alabaster, AL). 5(6)-carboxyfluorescein (CF) was from Sigma-Aldrich (St. Louis, MO).

Carboxyfluorescein leakage from liposomes

Dye-loaded liposomes were prepared by evaporation under a stream of nitrogen of a 2% solution of *E. coli* total lipid extract in chloroform followed by hydration with a buffer solution containing 230 mM Tris and 100 mM CF. The mixture was vortexed, passed through a cycle of freezing and thawing, and extruded through 0.1-μm pore size Nucleopore polycarbonate membranes using an Avanti Mini-Extruder. The unbound CF was then removed by passage through a Sephadex G-50 coarse column with a buffer solution containing 10 mM Tris and 100 mM KCl, pH 7.4. To initiate the release of liposome-entrapped CF, the liposomes were incubated in the dark at room temperature with photosensitizers for 5 min and then illuminated with a halogen light source (“NovaFlex”, World Precision Instruments, USA) for 1 min. CF release from liposomes into the bulk solution was monitored by an increase in CF fluorescence resulting from its dequenching upon dilution. Fluorescence of liposomes loaded with 100 mM CF was monitored at 520 nm (excitation at 490 nm) with a Panorama Fluorat 02 spectrofluorimeter.
(Lumex, Russia). The extent of CF efflux was calculated as \((F_t - F_0)/(F_{100} - F_0)\), where \(F_0\) and \(F_t\) represent the initial fluorescence intensity and the fluorescence intensity at the time \(t\), and \(F_{100}\) is the fluorescence intensity after complete disruption of liposomes by addition of the detergent Triton-X100 (final concentration, 0.1% w/w).

### Bacterial strains

Standard laboratory strains *Bacillus subtilis subsp. subtilis* Cohn 1872, strain BR151 (trpC2 lys-3 metB10), and *E. coli* Castellani and Chalmers 1919, strain W3110 (F lambda-IN(rrnD-rrE)1 rph-1) were used in this study. *Staphylococcus aureus* Rosenbach 1884 (entry #144) and *Mycobacterium sp.* (entry #377) were obtained from the Microorganisms Collection of the Moscow State University. The deletion strain JW5503 (ECK3026 in the Keio collection [55], the *E. coli* \(\Delta tolC\) mutant), devoid of the \(tolC\) gene, was kindly provided by Hironori Niki, National Institute of Genetics, Japan [55].

### Bacteria growth

Bacterial cells were grown at 37\(^\circ\)C in LB medium at 140 rpm shaking frequency. Overnight culture was diluted in fresh growth medium and grown to mid-log phase, then washed twice in sodium phosphate buffer (pH 7.4) at 7,000 rpm for 5 min and resuspended to an optical density of about 0.8 at 600 nm, corresponding to approx. \(10^8\) cells/ml. The resulting bacterial suspension was used for further experiments.

### aPDI measured by plating

To measure aPDI, we used the method of serial dilutions. Suspensions of bacteria were incubated in the dark at room temperature for 10 min with 1 nM—10 \(\mu\)M chlorin \(e_6\) or BACE in sterile PBS and illuminated with red light (\(\lambda > 630\) nm) obtained with KS-15 filter from a halogen light source ("NovaFlex", World Precision Instruments, USA). The survival of bacteria was assessed through colony forming unit (CFU) counts. CFU were determined by bacterial plating on Petri dishes of serial dilutions. The fraction of survived cells was calculated as the ratio of CFU of bacteria illuminated in the presence of a certain concentration of a photosensitizer to CFU of the control bacteria (grown in the absence of photosensitizers in the dark).

Alternatively, to avoid serial dilutions, suspensions of bacteria (2–5 \(\times\) \(10^8\) cell/mL) were incubated in the dark at room temperature for 10 min with 1 nM—10 \(\mu\)M chlorin \(e_6\) or BACE in nutrition broth. 0.2 mL of the bacterial suspensions was placed in a 96-well plate and illuminated with red light, as described above. The viable bacteria were assessed through CFU counts. The CFU for bacteria grown in the dark in the absence of photosensitizers was taken as 100% (control).

### Dark toxicity of BACE and chlorin \(e_6\) measured by optical density

Overnight *E. coli* bacterial cells cultures were diluted in fresh LB media. Chlorin \(e_6\) or BACE (100 nM—50 \(\mu\)M) were added to bacterial cultures (1–5 \(\times\) \(10^6\) cells/ml), placed in 96-well plates, Cell density was determined by absorbance at 600 nm using an Multiscan FC multimode reader (Thermo Scientific, USA) after bacteria were allowed to grow in the dark within 21 hours.

### Accumulation of photosensitizers by bacterial cells

Accumulation of photosensitizers by bacterial cells was evaluated from the fluorescence of the pellet obtained after centrifugation at 12000g for 2 min. Cells were incubated for 10 min in the
dark with the indicated amount of a photosensitizer. The pellet obtained after centrifugation was treated with 0.1 M NaOH / 1% SDS. The photosensitizer concentration was determined from its fluorescence by using a calibration curve for the solutions of different concentrations in 0.1 M NaOH / 1% SDS.

Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) measurements were carried out with a homemade FCS setup [56,57] including an Olympus IMT-2 inverted microscope with a 40x, NA 1.2 water immersion objective (Carl Zeiss, Jena, Germany). A Nd:YAG solid state laser was used for excitation of SRB at 532 nm. The fluorescence that passed through an appropriate dichroic beam splitter and a long-pass filter was imaged onto a 50-μm core fiber coupled to an avalanche photodiode (PerkinElmer Optoelectronics, Fremont, CA). The signal from an output was correlated by a correlator card (Correlator.com, Bridgewater, NJ). The data acquisition time was 30 s. The experimental data were obtained under stirring conditions which increased the number of events by about three orders of magnitude thus substantially enhancing the resolution of the method. Concentrations of BACE and chlorin e6 (about 100 nM) were used to produce the count rate of 100 kHz. For peak intensity analysis fluorescence traces with the sampling time of 25 μs were analyzed using WinEDR Strathclyde Electrophysiology Software designed by J. Dempster (University of Strathclyde, UK). The software, originally designed for the single-channel analysis of electrophysiological data, enables one to count the number of peaks \( n(P>F_0) \) of the FCS signal having amplitudes higher than the defined value \( F_0 \) [8,58]. A program of our own design with a similar algorithm (coined Saligat; provided on request) was also used.

Potassium leakage

Potassium concentration was measured with the help of a K⁺-sensitive electrode (NIKO-ANALIT, Moscow, Russia) in the medium of 100 mM choline chloride, 5 mM MOPS, pH 7.4.

Confocal laser scanning microscopy of photosensitizer distribution in bacterial cells

To study photosensitizer distribution in \( B. \) subtilis cells, we used confocal laser scanning microscopy (CLSM). Bacteria cell suspensions were incubated with photosensitizers for 15 min in the minimal volume of PBS on coverslips in a Recon camera of an inverted microscope. Then the mounting medium—a mixture of 20% aqueous gelatin solution with an equal volume of glycerol—was added. Before using, the glycerol-gelatin mixture was heated to 50–60°C for 7 min, then allowed to cool to 37°C and quickly added to a coverslip with bacteria. Digital images were acquired using an Axiovert 200M LSM-510 META microscope (Carl Zeiss AG, Germany). The confocal images were recorded with a Plan-Apochromat 100x/1.4 Oil Ph3 objective. Fluorescence of photosensitizers was excited with a 633 nm He–Ne laser, and emission was detected with a 650–710 nm band pass filter.

Detection of cell membrane integrity

Cell membrane integrity after illumination of bacteria in the presence of a photosensitizer was estimated using propidium iodide (PI). Bacterial cells were incubated for 15 min with 30 nM PI and then examined with a Nikon Eclipse Ti-E confocal laser scanning microscope with a Nikon Eclipse Ti-E A1 laser-scanning confocal system and a Plan Apo 20x/0.75 objective. Images were captured for 25 fields of views for each sample, and the number of cells per field of
view was counted, as determined by PI staining and differential interference contrast microscopy (DIC).

Statistics
All data are presented as means ± standard deviations as a result of 3–5 experiments.

Results and Discussion
Fig 1A displays the dependence of the survival of *B. subtilis* cells after photodynamic treatment on the concentration of photosensitizers, as measured by the conventional colony-counting
method. It is seen that BACE at a concentration of 10 nM was substantially more effective in provoking PDI of *B. subtilis* than chlorin e₆, which correlated with the corresponding difference (about two orders of magnitude) in the accumulation of BACE and chlorin e₆ by *B. subtilis* cells (Fig 2A). It is worth mentioning that here we meant the increased association of the photosensitizer with bacterial cells without indication of its localization on the surface or inside cells. Bearing in mind that BACE and chlorin e₆ were reported to have close values of the quantum yield of generation of singlet oxygen [8], with the latter being the key agent in the BACE photosensitizing activity in model systems [8,58], the increased photodynamic potency of BACE compared to chlorin e₆ could be related to the enhanced accumulation of the boronated photosensitizer by bacterial cells.

In the PDI experiments with the gram-negative bacteria *E. coli*, chlorin e₆ was completely inactive at a concentration of 5 μM (Fig 1B, insert), by contrast to its impact on the gram-
positive species *B. subtilis* (Fig 1A), which was in line with the conclusion made by Zorin and coauthors [20] that the effectiveness of chlorin-mediated photoinactivation of *E. coli* is 100-200-times lower than that of *B. subtilis*. Our observations also agree with the data obtained for chlorin e6 in [24,53]. When applied with the illumination of 4 J/cm², BACE was also ineffective to photosensitize PDI of *E. coli* (Fig 1B). However at 20 J/cm², BACE appeared to effectively suppress the colony-forming activity of *E. coli* beginning from 1 μM (Fig 1B), in contrast to chlorin e6 (Fig 1B, insert). Similar to the case of *B. subtilis*, the enhanced photobactericidal activity of BACE compared to chlorin e6 with *E. coli* could also be attributed to the increased uptake of BACE by the bacterial cells (Fig 2B).

Bright fluorescence of chlorins enabled us to supplement the data on the macroscopic uptake of chlorins by bacterial cells (Fig 2) by FCS measurements of photosensitizer...
accumulation by single bacterial cells (Fig 3). The addition of B. subtilis cells to the solution of BACE resulted in the appearance of high-amplitude peaks in the fluorescence intensity traces recorded with an FCS set-up which reflected uptake of BACE by single bacterial cells (Fig 3A). The fluorescence peaks were much lower with chlorin e6, as compared to BACE. Similar results were obtained with E. coli cells (Fig 3B). Our data on the relationship between the photodynamic efficacy and the bacterial accumulation of BACE are in line with qualitative correlation

Fig 4. A. Membrane integrity of BACE- and chlorin e6-treated B. subtilis cells after illumination. Photosensitizers are depicted in blue color, propidium iodide (PI) in red color. Membrane integrity was assessed by counting PI-positive cells after illumination with red light at 4 J/cm². B. Intracellular localization of the photosensitizers measured by confocal laser microscopy.

doi:10.1371/journal.pone.0141990.g004
Potassium permeability of BACE- and chlorin e6-treated B. subtilis (A) and E. coli (B) cells after illumination measured by a potassium-selective electrode. Cells were incubated with photosensitizers for 10 min prior to illumination (4 J/cm²). Nigericin (1 μM) was added at the end of each recording to induce full potassium efflux.

doi:10.1371/journal.pone.0141990.g005
between PDI of a panel of bacteria species using chlorin e6-polyethyleneimine conjugates [51] and their uptake by bacterial cells.

By applying CLSM to B. subtilis cells incubated with PI, we compared the light-induced effects on cell membrane integrity in the presence of BACE and chlorin e6. As seen from Fig 4A, the percentage of cells permeable to PI was much higher after the photodynamic treatment with BACE, than with chlorin e6, thereby indicating the involvement of membrane damage in the PDI of bacterial cells. This assumption was supported by CLSM images of BACE and chlorin e6 distribution in cells of B. subtilis, which revealed predominant localization of BACE on the surface of bacterial cells (Fig 4B). With E. coli the changes in PI permeability were negligible under these conditions (data not shown).

To further investigate the membrane damage in the course of BACE-mediated aPDI, we measured its impact on the potassium leakage from bacterial cells [59]. As seen from Fig 5A, illumination (4 J/cm²) of B. subtilis cell suspension in the presence of the photosensitizers resulted in significant stimulation of potassium leakage from cells, with BACE being more effective than chlorin e6, whereas noticeable potassium leakage was not observed with E. coli cells under these conditions (Fig 5B).

To compare the photodynamic potencies of the two photosensitizers in a model membrane system mimicking bacterial cell membranes, we examined the ability of BACE and chlorin e6 to sensitize the photodynamic leakage of the fluorescent dye CF from liposomes formed from E. coli total lipid extract with a high content (about 20%) of negatively charged lipids. Fig 6
Fig 7. Phototoxicity and dark toxicity of BACE and chlorin e₆ towards Mycobacterium sp. (A), Staphylococcus aureus (B), and Bacillus subtilis (C) evaluated by CFU counts. Phototoxicity was determined upon illumination with red light at 4 J/cm². The data shown are mean values ± standard deviations.

doi:10.1371/journal.pone.0141990.g007
shows that BACE at a concentration of 30 nM was much more effective than chlorin e₆ in provoking the CF leakage under these conditions. Earlier chlorin e₆-aided photodynamic leakage of CF from liposomes formed of neutral lipids was described in [60–62].

To compare sensitivity of different bacteria to PDI with BACE and chlorin e₆, we determined cell viability for a series of bacteria species growing simultaneously in a 96-well plate under illumination in the presence of different concentrations of photosensitizers by using CFU counts (Fig 7). Remarkably, *Mycobacterium sp.* (Fig 7A) and *Staphylococcus aureus* (Fig 7B) appeared to be highly sensitive to PDI with BACE (and less sensitive to PDI with chlorin e₆), similar to *B. subtilis* (Fig 7C). The most striking difference in sensitivity to BACE and chlorin e₆ was observed with *Mycobacterium sp.*, *S. aureus* and *B. subtilis*, dark toxicity of BACE was about two orders of magnitude lower than its phototoxicity (Fig 7).

The focus of the paper was to compare photodynamic efficacies of BACE and chlorin e₆ under mild illumination conditions, i.e. at 4 J/cm⁻². Under these conditions BACE appeared to be effective with a panel of Gram-positive bacteria and ineffective with Gram-negative bacteria, whereas for killing the latter illumination at 20 J/cm⁻² was required. Therefore, to gain insight in the mechanism of the photobactericidal effect of BACE, we examined its impact on membrane integrity and performed leakage experiments also under mild illumination conditions.
Unlike chlorin e₆, BACE showed increased dark toxicity with the *E. coli* ΔtolC mutant [55], deficient in TolC-requiring multidrug efflux transporters [63], compared to the wild-type strain (Fig 8). Similar difference was found earlier for phenothiazinium antimicrobial photosensitizers [64]. The results obtained with the *E. coli* ΔtolC mutant indicate that BACE is a substrate of *E. coli* multidrug resistance pumps. To support this assumption, we examined dark toxicity of BACE with the *E. coli* ΔacrE mutant, derived by a single deletion of acrE from the same parent strain as the ΔtolC mutant. In *E. coli*, AcrE along with AcrB, AcrD, AcrEF, MdtABC, and MdtEF belong to resistance-nodulation-cell division (RND) family of multidrug efflux transporters that require interaction with TolC to function [65]. In fact, the *E. coli* ΔacrE mutant displayed rather poor sensitivity to BACE (data not shown). Hence, the multifunctional outer membrane channel TolC is required for BACE efflux from *E. coli* cells.

In conclusion, BACE, a photosensitizer of the carboranyl-chlorin type, was shown here for the first time to be highly effective in killing a series of Gram-positive bacteria, with outer cell structures being most likely the major target of the photodynamic action. The enhanced efficacy of BACE as an antimicrobial photosensitizer with respect to chlorin e₆ could be explained by the increased accumulation of the boronated derivative by bacterial cells.

**Acknowledgments**
The work was performed in part at User Facilities Centre of M.V. Lomonosov Moscow State University.

**Author Contributions**
Conceived and designed the experiments: PAN MMM EAK YNA. Performed the experiments: EOO PAN AMF AYA. Analyzed the data: PAN EAK YNA MMM IIA MGS. Contributed reagents/materials/analysis tools: AVZ VAO VNK. Wrote the paper: EAK YNA.

**References**
1. Korbe S, Schreiber PJ, Michl J. Chemistry of the carba-closo-dodecaborate(-) anion, CB(11)H(12)(-). Chem Rev. 2006; 106: 5208–5249. PMID: 17165686
2. Hawthorne MF. The role of chemistry in the development of boron neutron capture therapy of cancer. Angew Chem Int Ed Engl. 1993; 32: 950–984.
3. Valliant JF, Guenther KJ, King AS, Morel P, Schaffer P, Sogbein OO, et al. The medicinal chemistry of carboranes. Coord Chem Rev. 2002; 232: 173–230.
4. Luguya R, Jensen Tj, Smith KM, Vicente MG. Synthesis and cellular studies of a carboranylchlorin for the PDT and BNCT of tumors. Bioorg Med Chem. 2006; 14: 5890–5897. PMID: 16753299
5. Ratajcki M, Osterloh J, Gabel D. Boron-containing chlorins and tetraazaporphyrins: synthesis and cell uptake of boronated pyropheophorbide a derivatives. Anticancer Agents Med Chem. 2006; 6: 159–166. PMID: 16529538
6. Hao E, Friso E, Miotto G, Jori G, Soncin M, Fabris C, et al. Synthesis and biological investigations of tetraakis(p-carboranylthio-tetrafluorophenyl)chlorin (TPFC). Org Biomol Chem. 2008; 6: 3732–3740. doi: 10.1039/b807836j PMID: 18843403
7. O’Shevskaya VA, Nikitina RG, Savchenko AN, Malshakova MV, Vinogradov AM, Golovina GV, et al. Novel boronated chlorin e₆-based photosensitizers: synthesis, binding to albumin and antitumour efficacy. Bioorg Med Chem. 2009; 17: 1297–1306. doi: 10.1016/j.bmc.2008.12.016 PMID: 19121946
8. Moisenovich MM, O’Shevskaya VA, Rokitskaya TI, Ramonova AA, Nikitina RG, Savchenko AN, et al. Novel photosensitizers trigger rapid death of malignant human cells and rodent tumor transplants via lipid photodamage and membrane permeabilization. PLoS One. 2010; 5: e12717. doi: 10.1371/journal.pone.0012717 PMID: 20856679
9. Antonenko YN, Kotova EA, Omarova EO, Rokitskaya TI, O’Shevskaya VA, Kalinin VN, et al. Photodynamic activity of the boronated chlorin e₆ amide in artificial and cellular membranes. Biochim Biophys Acta. 2014; 1838: 793–801. doi: 10.1016/j.bbamem.2013.11.012 PMID: 24287152
10. Efremenko AV, Ignatova AA, Borsheva AA, Grin MA, Bregadze VI, Sivaev IB, et al. Cobalt bis(dicarbollide) versus closo-dodecaborate in boronated chlorin e(6) conjugates: implications for photodynamic and boron-neutron capture therapy. Photochem Photobiol Sci. 2012; 11: 645–652. doi: 10.1039/c2pp05237g PMID: 22262023
11. Efremenko AV, Ignatova AA, Grin MA, Sivaev IB, Mironov AF, Bregadze VI, et al. Chlorin e6 fused with a cobalt-bis(dicarbollide) nanoparticle provides efficient boron delivery and photoinduced cytotoxicity in cancer cells. Photochem Photobiol Sci. 2014; 13: 92–102. doi: 10.1039/c3pp50226k PMID: 24258161
12. Asano R, Nagami A, Fukumoto Y, Miura K, Yazama F, Ito H, et al. Synthesis and biological evaluation of new BSH-conjugated chlorin derivatives as agents for both photodynamic therapy and boron neutron capture therapy of cancer. J Photochem Photobiol B. 2014; 140: 140–149. doi: 10.1016/j.jphotobiol.2014.07.008 PMID: 25123528
13. Endo Y, Iijima T, Yamakoshi Y, Fukasawa H, Miyaura C, Inada M, et al. Potent estrogen agonists based on carborene as a hydrophobic skeletal structure. A new medicinal application of boron clusters. Chem Biol. 2001; 8: 341–355. PMID: 11325590
14. Julius RL, Farha OK, Chiang J, Perry LJ, Hawthorne MF. Synthesis and evaluation of transthyretin amyloidosis inhibitors containing carborene pharmacophores. Proc Natl Acad Sci U S A. 2007; 104: 4808–4813. PMID: 17360344
15. Issa F, Kassiou M, Rendina LM. Boron in drug discovery: carboranes as unique pharmacophores in biologically active compounds. Chem Rev. 2011; 111: 5701–5722. doi: 10.1021/cr2000866 PMID: 21718011
16. Bellin JS,lutwick L, Jonas B. Effects of photodynamic action on E. coli. Arch Biochem Biophys. 1969; 132: 157–164. PMID: 493411
17. Fujita H, Suzuki K. Studies on photodynamic action. Il. The action on Escherichia coli cells. J Radiat Res. 1970; 11: 9–13. PMID: 4914744
18. Malik Z, Hanania J, Nitzan Y. Bactericidal effects of photoactivated porphyrins—an alternative approach to antimicrobial drugs. J Photochem Photobiol B. 1990; 5: 281–293. PMID: 2115912
19. Bertoloni G, Rossi F, Valduga G, Jori G, van Lier J. Photosensitizing activity of water- and lipid-soluble phthalocyanines on Escherichia coli. FEMS Microbiol Lett. 1990; 59: 149–155. PMID: 2125956
20. Fornichev AI, Zorin VP, Zorina TE, Cherenkevich SN. Photodamage of gram-positive and gram-negative bacterial cells in the presence of chlorin e6 derivatives. Mikrobiologija. 1991; 60: 507–512. PMID: 1745143
21. Minnock A, Vernon DI, Schofield J, Griffiths J, Parish JH, Brown ST. Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both gram-negative and gram-positive bacteria. J Photochem Photobiol B. 1996; 32: 159–174. PMID: 8622179
22. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother. 1998; 42: 13–28. PMID: 9700525
23. Soukos NS, Ximenez-Fyvie LA, Hamblin MR, Socransky SS, Hasan T. Targeted antimicrobial phototherapy. Antimicrob Agents Chemother. 1998; 42: 2595–2601. PMID: 9756761
24. Hamblin MR, O'Donnell DA, Murthy N, Rajagopalan K, Michaud N, Sherwood ME, et al. Polycationic photosensitizer conjugates: effects of chain length and Gram classification on the photodynamic inactivation of bacteria. J Antimicrob Chemother. 2002; 49: 941–951. PMID: 12039886
25. Gad F, Zahra T, Hasan T, Hamblin MR. Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria. Antimicrob Agents Chemother. 2004; 48: 2173–2178. PMID: 15155218
26. Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci. 2004; 3: 436–450. PMID: 15122361
27. O'Riordan K, Akilov OE, Hasan T. The potential for photodynamic therapy in the treatment of localized infections. Photodiagnosis Photodyn Ther. 2005; 2: 247–262. doi: 10.1016/S1572-1000(05)00099-2 PMID: 25048867
28. Verma S, Sallum UW, Athar H, Rosenblum L, Foley JW, Hasan T. Antimicrobial photodynamic efficacy of side-chain functionalized benzo[a]phenothiazinium dyes. Photochem Photobiol. 2009; 85: 111–118. doi: 10.1111/j.1751-1097.2008.00403.x PMID: 18657053
29. Strakhovskaya MG, Antonenko YN, Pashkovskaya AA, Kotova EA, Kireev V, Zhukhovitsky VG, et al. Electrostatic binding of substituted metal phthalocyanines to enterobacterial cells: its role in photodynamic inactivation. Biochemistry (Moscow). 2009; 74: 1305–1314.
30. Mikula P, Kalhotka L, Jancula D, Zezulka S, Korinkova R, Cerny J, et al. Evaluation of antibacterial properties of novel phthalocyanines against Escherichia coli—Comparison of analytical methods. J Photochem Photobiol B. 2014; 138: 230–239. doi: 10.1016/j.jphotobiol.2014.04.014 PMID: 24993083
31. Alves E, Faustino MA, Neves MG, Cunha A, Tome J, Almeida A. An insight on bacterial cellular targets of photodynamic inactivation. Future Med Chem. 2014; 6: 141–164. doi: 10.4155/fmc.13.211 PMID: 24467241

32. Simon C, Mohrbacher C, Huttenberger D, Bauer-Marschall I, Krückhahn C, Stachon A, et al. In vitro studies of different irradiation conditions for photodynamic inactivation of Helicobacter pylori. J Photochem Photobiol B. 2014; 141: 113–118. doi: 10.1016/j.jphotochem.2014.09.015 PMID: 25463658

33. Totani T, Aono K, Yamamoto K, Tawara K. Synthesis and in vitro antimicrobial property of o-carborane derivatives. J Med Chem. 1981; 24: 1492–1499. PMID: 7310826

34. Oros G, Ujvary I, Nachman RJ. Antimicrobial activity of o-carboranylalanine. Amino Acids. 1999; 17: 357–368. PMID: 10707765

35. Fanfrlik J, Lepsik M, Horinek D, Havlas Z, Hobza P. Interaction of carboranes with biomolecules: formation of dihydrogen bonds. Chembiosci. 2006; 7: 1100–1105. PMID: 16671116

36. Liberman EA, Topaly VP, Silberstein AY. Charged and neutral ion carriers through bimolecular phospholipid membranes. Biochim Biophys Acta. 1970; 196: 221–234. PMID: 5461129

37. Rokitskaya TI, Zaitsev AV, Ol'shevskaya VA, Kalinin VN, Moisenovich MM, Agapov II, et al. Boronated derivatives of chlorin e6 and fluoride-containing porphyrins as penetrating anions: a study using bilayer lipid membranes. Biochemistry (Moscow). 2012; 77: 975–982.

38. Kishen A, Upadya M, Tegos GP, Hamblin MR. Efflux pump inhibitor potentiates antimicrobial photodynamic inactivation of Enterococcus faecalis biofilm. Photochem Photobiol Photodiag. 2010; 86: 1343–1349. doi: 10.1111/j.1751-1097.2010.00792.x PMID: 20860692

39. Varga N, Alzaatreh J, Capri P, Puri S, Lenhart A, Kister G, et al. Photosensitizer conjugates between polyethyleneimine and chlorin (e6) for broad-spectrum antimicrobial photoinactivation. Future Med Chem. 2014; 6: 141–164. doi: 10.4155/fmc.13.211 PMID: 24467241

40. Embleton ML, Nair SP, Heywood W, Menon DC, Cookson BD, Wilson M. Development of a novel targeting system for lethal photosensitization of antibiotic-resistant strains of Staphylococcus aureus. Antimicrob Agents Chemother. 2005; 49: 3690–3696. PMID: 16127041

41. Demidova TN, Hamblin MR. Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrob Agents Chemother. 2005; 49: 2329–2335. PMID: 15917529

42. Pudziuvyte B, Bakiene E, Bonnett R, Shatunov PA, Magaraggia M, Jori G. Alterations of bacterial structures on the efficiency of photodynamic inactivation by a cationic porphyrinic photosensitizer, lipid oxidation and photoinactivation efficiency in Escherichia coli. J Photochem Photobiol B. 2014; 141: 145–153. doi: 10.1016/j.jphotochem.2014.08.024 PMID: 25463662

43. Pereira MA, Faustino MA, Tome JP, Neves MG, Tome AC, Cavaleiro JA, et al. Influence of external bacterial structures on the efficiency of photodynamic inactivation by a cationic porphyrin. Photochem Photobiol Sci. 2014; 13: 680–690. doi: 10.1039/c3pp05040b PMID: 24549049

44. Flatau P, Meister S, Scheer H. Mechanistic insight of the photodynamic inactivation of Enterococcus faecalis by a tetracationic zinc(II) phthalocyanine derivative. Photodiagnosis Photodyn Ther. 2009; 6: 52–61. doi: 10.1016/j.pdpdt.2009.01.003 PMID: 19447372

45. Demidova TN, Hamblin MR. Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrob Agents Chemother. 2005; 49: 2329–2335. PMID: 15917529

46. Embleton ML, Nair SP, Heywood W, Menon DC, Cookson BD, Wilson M. Development of a novel targeting system for lethal photosensitization of antibiotic-resistant strains of Staphylococcus aureus. Antimicrob Agents Chemother. 2005; 49: 3690–3696. PMID: 16127041

47. Tegos GP, Anbe M, Yang C, Demidova TN, Satti M, Mroz P, et al. Protease-stable polycationic photosensitizer conjugates between polyethyleneimine and chlorin (e6) for broad-spectrum antimicrobial photoinactivation. Antimicrob Agents Chemother. 2006; 50: 1402–1410. PMID: 16569858
52. Fuchs BB, Tegos GP, Hamblin MR, Mylonakis E. Susceptibility of Cryptococcus neoformans to photodynamic inactivation is associated with cell wall integrity. Antimicrob Agents Chemother. 2007; 51: 2929–2936. PMID: 17548495

53. Uliana MP, Pires L, Pratavieira S, Brocksom TJ, de Oliveira KT, Bagnato VS, et al. Photobiological characteristics of chlorophyll a derivatives as microbial PDT agents. Photochem Photobiol Sci. 2014; 13: 1137–1145. doi: 10.1039/c3pp50376c PMID: 24898703

54. Huang L, Huang YY, Mroz P, Tegos GP, Zhiyentayev T, Sharma SK, et al. Stable synthetic cationic bacteriochlorins as selective antimicrobial photosensitizers. Antimicrob Agents Chemother. 2010; 54: 3834–3841. doi: 10.1128/AAC.00125-10 PMID: 20625146

55. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, et al. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol. 2006; 2: 2006.

56. Perevoshchikova IV, Zorov DB, Antonenko YN. Peak intensity analysis as a method for estimation of fluorescent probe binding to artificial and natural nanoparticles: tetramethylrhodamine uptake by isolated mitochondria. Biochim Biophys Acta. 2008; 1778: 2182–2190. doi: 10.1016/j.bbamem.2008.04.008 PMID: 18492484

57. Denisov SS, Kotova EA, Plotnikov EY, Tikhonov AA, Zorshunova GA, Antonenko YN. A mitochondria-targeted protonophoric uncoupler derived from fluorescein. Chem Commun. 2014; 50: 15366–15369.

58. Rokitskaya TI, Kotova EA, Agapov II, Moisovich MM, Antonenko YN. Unsaturated lipids protect the integral membrane peptide gramicidin A from singlet oxygen. FEBS Lett. 2014; 588: 1590–1595. doi: 10.1016/j.febslet.2014.02.046 PMID: 24613917

59. Komagoe K, Kato H, Inoue T, Katsu T. Continuous real-time monitoring of cationic porphyrin-induced photodynamic inactivation of bacterial membrane functions using electrochemical sensors. Photochem Photobiol Sci. 2011; 10: 1181–1188. doi: 10.1039/c0pp00376j PMID: 21472187

60. Mojzisova H, Bonneau S, Maillard P, Berg K, Braut D. Photosensitizing properties of chlorins in solution and in membrane-mimicking systems. Photochem Photobiol Sci. 2009; 8: 778–787. doi: 10.1039/b822269j PMID: 19492105

61. Pushkovskaya A, Kotova E, Zorlu Y, Dumoulin F, Ahsen V, Agapov I, et al. Light-triggered liposomal release: membrane permeabilization by photodynamic action. Langmuir. 2010; 26: 5726–5733. doi: 10.1021/la903867a PMID: 20000430

62. Kotova EA, Kuzevanov AV, Pushkovskaya AA, Antonenko YN. Selective permeabilization of lipid membranes by photodynamic action via formation of hydrophobic defects or pre-pores. Biochim Biophys Acta. 2011; 1808: 2252–2257. doi: 10.1016/j.bbapap.2011.05.018 PMID: 21663731

63. Krishnamoorthy G, Tikhonova EB, Dhamdhere G, Zgurskaya HI. On the role of TolC in multidrug efflux: the function and assembly of AcrAB-TolC tolerate significant depletion of intracellular TolC protein. Mol Microbiol. 2013; 87: 982–997. doi: 10.1111/mmi.12143 PMID: 23331412

64. Tegos GP, Hamblin MR. Phenothiazinium antimicrobial photosensitizers are substrates of bacterial multidrug resistance pumps. Antimicrob Agents Chemother. 2006; 50: 196–203. PMID: 16377686

65. Horiyama T, Nishino K. AcrB, AcrD, and MdtABC multidrug efflux systems are involved in enterobactin export in Escherichia coli. PLoS One. 2014; 9(9): e108642. doi: 10.1371/journal.pone.0108642 PMID: 25259870