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Novel metal complexes of boronated chlorin e_6 for photodynamic therapy

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ABSTRACT

Various structural modifications of chlorins are aimed at optimization of biomedical characteristics of these plant-derived tetrapyrrolic compounds. In particular, conjugation with boron polyhedra improves the efficacy of chlorin e_6 derivatives as antitumor photosensitizers. To obtain the compounds that may possess several clinically favorable characteristics, we synthesized a series of metal chlorin e_6 conjugates with 1-carba-*closo*-dodecaborate anion that contain Pd(II), Sn(IV) or Zn(II) in the coordination sphere of the chlorin macrocycle. The compounds were synthesized by alkylation of amino group in chlorin e_6 metal complexes with 1-trifluoromethanesulfonylmethyl-1-carba-*closo*-dodecaborate cesium. The water soluble Pd(II) complex of chlorin e_6 13(1)-*N*-{2-[*N*-(1-carba-*closo*-dodecaboran-1-yl)methyl]aminoethyl}amide-15(2), 17(3)-dimethyl ester (compound **6**) evoked low dark cytotoxicity; in striking contrast, **6** potently sensitized human tumor cells to illumination with monochromatic red light. Confocal microscopic studies demonstrated that photoactivation of **6** rapidly (within minutes) changed the patterns of intracellular drug distribution from diffuse cytoplasmic to clustered perinuclear. Co-localization experiments revealed that **6** associated with lysosomes in illuminated cells. These events were paralleled by alteration of mitochondrial shape, a decrease of mitochondrial transmembrane electric potential and the loss of plasma membrane impermeability for propidium iodide, the latter being a hallmark of cell necrosis. Similar mechanisms of cell photodamage were found for structurally close Pd(II) complex of chlorin with neutral carborane and for Sn(IV) chlorin conjugated with the anionic carborane. Thus, metal complexes of carboranylchlorins are efficient photosensitizers capable of triggering rapid necrosis. These compounds are promising for further development as multipotent agents in which each moiety, i.e., metal, the chlorin macrocycle and the boron substituent, as well as the entire complex, can be useful in cancer diagnostics and treatment.

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1. Introduction

Chlorins, a class of plant-derived tetrapyrrols, emerge as perspective photosensitisers for photodynamic therapy (PDT). This therapeutic modality is based on activation by light of the drug accumulated in the tumor [1,2]. The photodynamically active species is singlet oxygen $^1O_2$ generated in situ by energy transfer from an excited drug (photosensitizer) to oxygen molecule [3]. Chlorins demonstrate a remarkably strong light absorption in the red visible region and high efficiency in generating reactive oxygen species [4]. These characteristics of chlorins allow for deeper penetration of tissue photodamage that is particularly essential in cancer treatment.

Chlorins, as well as other pyrrole containing compounds, are suitable for a variety of chemical modifications aimed at optimization of their therapeutic potential. Among these modifications, the conjugation of boron polyhedra to the chlorin macrocycle has been used to obtain the drugs for boron neutron capture therapy, thereby proving the concept of dual advantage of these conjugates for binary anticancer strategies [5–8]. Furthermore, other authors [9–11] and we [12,13] demonstrated that boronated porphyrins and chlorins are more efficient in PDT alone than their parental boron-free counterparts. These studies strongly support a critical role of structural diversity in search for clinically applicable chlorin-based photosensitizers.

The photosensitizing properties of the conjugate can be improved by introduction of metal ions into the coordination sphere of chlorins. Indeed, metals change the photophysical and spectroscopic characteristics of chlorins, thereby modulating their

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stability, hydrophobicity, the ability to form aggregates in solutions, and intracellular distribution [14,15]. Thermodynamic stability of metal chlorins depends on the electronegativity χ, the oxidation number Z and the effective ionic radius r_i of a central metal ion. Consequently, the metal complexes of high χ and Z values and small r_i such as Ru(III), Sn(IV) are more stable than those of low χ and Z and big r_i (Mg(II), Cd(II)). The nature of the central metal influences both the ground and the excited states of the complexes due to strong charge transfer and electrostatic interaction. It is especially notable for the metal ions with not fully occupied d orbitals (open shell) where d electrons can significantly integrate with π and π^* orbitals of the macrocycles [14,16,17], thereby affecting the number and the position of bands in UV–Vis absorption spectra and the fluorescent properties of compounds. Therefore, the diamagnetic complexes with the central transition-metal ions of the second and third raw (such as Ru(III), Pt(II) and Pd(II)) show intense phosphorescence. A lower degree of aggregation of the tetrapyrrole containing compounds, which is clinically advantageous, can be achieved by introducing a closed-shell metal ion possessing octahedral d^2sp^3 hybrid orbitals (e.g., Zn(II)). This modification would result in steric hindrance caused by axial ligands.

Pre-clinical and clinical trials demonstrated the therapeutic potential of metal chlorins. The Pd (II) complex of bacteriochlorin (Tookad) showed high efficacy in PDT of experimental tumors and in clinical settings [18]. Water-soluble Gd(III) and Lu(III) texaphyrin complexes have been tested as radio- and photosensitizers [19]. These complexes, in various formulations, have been proved efficient in treatment of patients with brain metastases, breast cancer and atherosclerosis. Tin containing compounds (e.g., ethyl ethiopurpurin, Purlytin™) have been tested as agents in therapy of age-related macular degeneration and Kaposi's sarcoma [5,20–22]. Furthermore, metal complexes of chlorins can be used in combined therapeutic regimens such as PDT and radiotherapy, PDT and chemotherapy, etc. Thus, one may anticipate that the complexes of chlorin, carborane and metal would be therapeutically valuable.

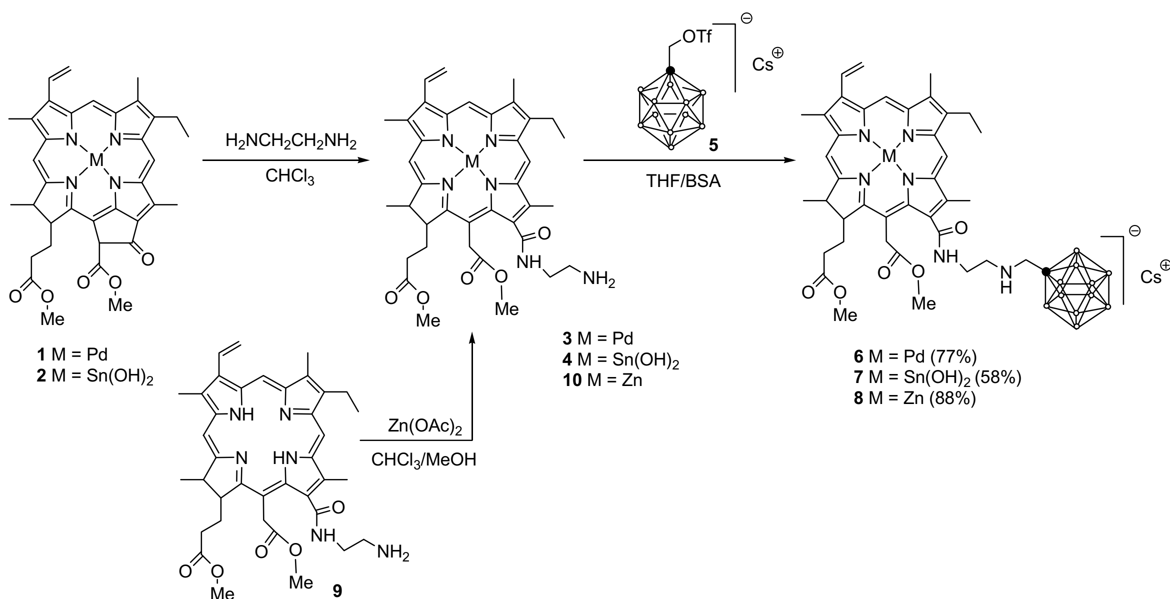
Recently, we reported the synthesis of novel metal containing conjugates of chlorin e_6 with the neutral carborane [23]. In the present study we synthesized a series of metal containing derivatives of chlorin e_6 that contain Zn(II), Pd(II) or Sn(IV) in the coordination sphere of the chlorin e_6 macrocycle. These compounds differ from previously reported compounds in the structure of boron polyhedron, namely, they contain 1-carba-*closo*-dodecaborate anion, $CB_{11}H_{12}^-$ as boron component. We report here the dark cytotoxicity and photosensitizing properties of novel compounds. In particular, Pd(II) complex of chlorin e_6 13(1)-*N*-{2-[*N*-(1-carba-*closo*-dodecaboran-1-yl)methyl]aminoethyl}amide-15(2), 17(3)-dimethyl ester demonstrated low dark cytotoxicity and was capable of inducing rapid necrosis of cultured human tumor cells after photoactivation.

2. Results and discussion

2.1. Chemistry

Synthesis of new boronated Pd(II) and Sn(IV) containing carboranylchlorins proceeded in two step transformations starting from the corresponding complexes of Pd(II) (**1**) or Sn(IV) (**2**) of methylpheophorbide-*a* [23]. Further transformation of exo-ring V by cleavage of C13(1)–C13(2) bond in compounds **1** and **2** with ethylene diamine resulted in corresponding amino derivatives **3** and **4**. Alkylation of amino group in **3** and **4** with 1-trifluoromethanesulfonylmethyl-1-carba-*closo*-dodecaborate cesium **5** in THF in the presence of N,O-bis(trimethylsilyl)acetamide (BSA) led to green-colored boronated derivatives **6** and **7** of chlorin e_6 isolated in 77% and 58% yields, respectively (Scheme 1). An attempt to convert Zn(II) complex of methylpheophorbide-*a* [24,25] using ethylene diamine failed to give the corresponding Zn(II) complex of amino chlorin e_6. Preparation of boronated Zn(II) complex **8** included the metallation of free base amino chlorin **9** [26] with the excess of zinc acetate [27], resulting in compound **10**. Subsequent treatment of **10** with triflate **5** in refluxing THF using BSA as a base afforded boronated Zn(II) chlorin e_6 derivative **8** in 88% yield (Scheme 1). The structure of each novel compound was examined by NMR, IR, UV–Vis spectroscopy and mass spectrometry.

The ^{1}H NMR spectra of **6–8** showed three typical signals of pyrrolyl protons of chlorin system between δ = 10.09–8.73 ppm. We also observed the doublet signals of nonequivalent protons of the methylene group in the 15(1)-position at 5.23–5.61 ppm that resulted from the opening of the exo-ring. The signals of protons of amide groups exhibited themselves as singlets at δ = 2.87–



Scheme 1. Synthesis of chlorin e_6 metal complexes with anionic boron polyhedron.

2.94 ppm. The protons of the BH group of the carborane anion in **6–8** were observed in the high-field region within δ = 1.4–3.0 ppm. The protons of the methylene group bonded to the carborane anion manifested themselves as singlets at δ = 2.66–2.83 ppm. The ^{11}B NMR spectra of **6–8** contained three signals with a ratio of 1:5:5 typical for 1-carba-*closo*-dodecaborate polyhedron. In the IR spectra of **6–8** the absorption bands of CO groups at 1721–1723 cm^{-1} (ester) and the carborane BH groups at 2534 cm^{-1} were observed. We detected the amide I and amide II absorption bands at 1658 (**7**), 1631 (**6**), 1628 (**8**) and 1542 (**8**), 1520(**6**), 1518(**7**) cm^{-1}, respectively. A broad band at 3437 cm^{-1} confirmed the presence of OH group in **7**. The UV–Vis spectra of **6–8** in $CHCl_3$ solution displayed four bands resulting from $\pi \rightarrow \pi^*$ transition of the aromatic macrocycle, whereas in metal complexes of methylpheophorbide-*a* **1, 2** five bands were observed. All Q bands in **6** were blue-shifted to a bigger extent (their positions were at 487 nm, 519 nm and 613 nm) than the Q bands in **7** and **8**, where their positions were at 520 nm, 590 nm and 638 nm, respectively. The ratio $\varepsilon_{Soret}/\varepsilon_{Q0}$ was the highest in the case of **7** (~2.75), while for **6** and **8** this parameter was ~1.8.

2.2. Biological testing

All novel compounds were tested for the ability to cause light-independent cytotoxicity (dark cytotoxicity) against human tumor cell lines. As shown in Table 1, Zn(II) complexes **8** and **10** caused death of HCT116 colon carcinoma cells at micromolar concentrations whereas Pd(II) or Sn(IV) containing carboranylchlorins **6** and **7** were virtually inert after 72 h of continuous cell exposure

Table 1
Dark cytotoxicity of metal carboranylchlorins for HCT116 cell line.

Compound	IC_{50} (μM)[a]
1	>12
2	>12
6	>12
7	>12
8	6.5 ± 2.1
9	5.8 ± 2.0
10	3.7 ± 1.1

[a] Mean ± standard deviation of three independent experiments (MTT-test), each concentration tested in duplicate.

(MTT-test; [28]. Similar results were obtained for MCF-7 breast carcinoma cell line (not shown). Therefore, **6** and **7**, the compounds that evoked low dark cytotoxicity, can be further investigated as tentative photosensitizers. Both compounds were readily soluble in aqueous solutions at >50 μM. In particular, we studied intracellular distribution of **6** in living tumor cells by confocal laser microscopy, taking advantage of the possibility to excite the photosensitizer with the microscope's monochromatic light source (light illumination, LI; λ = 633 nm). Weak diffuse staining of HCT116 cells loaded with **6** was visible at the time of the initial cell scanning (Fig. 1A). In striking contrast, rapidly after LI of cells the drug was detectable as bright cytoplasmic clusters, predominantly localized in the vicinity of the nuclei (Fig. 1B). This pattern of re-distribution of **6** remained unchanged during next 3 h at 37 °C, 5%CO_2 (not shown). Essentially the same patterns of intracellular distribution of **6** were detectable in MCF-7 cells (not shown).

To identify the cytoplasmic compartments associated with **6** in response to LI, MCF-7 or HCT116 cells were loaded with the lysosomal dye LysoTracker Green DND-26 and compound **6**. Thirty minutes after LI the lysosomes appeared as multiple, distinct cytoplasmic bodies (Fig. 2A); likewise, a punctate staining of the cytoplasm was observed when **6** was visualized (Fig. 2B). Merge of the two images demonstrated that **6** co-localized with the lysosomal dye in illuminated cells (Fig. 2C). Importantly, addition of propidium iodide (PI) revealed that virtually every cell became PI-positive already 10 min post LI (Fig. 2C) whereas in mock-treated cells (compound **6** alone or LI in the absence of **6**) only 2–3% cells were stained with PI (not shown). These events were paralleled by changes of mitochondrial shape and transmembrane electric potential $\Delta\Psi_m$ (Fig. 3). As soon as 5 min post LI the mitochondrial network was fragmented (Fig. 3B). Ten minutes post LI the brightness of mitochondria decreased dramatically (Fig. 3C). To quantitatively analyze the data using ImageJ 1.40g software, the threshold for 8 bit images was set to 50, and the range of the analyzed signal was 50–255. Total number and intensity of pixels within this range were compared in samples illuminated in the absence or presence of the photosensitizer. Ten minutes after cell illumination in the presence of **6** the number of pixels within the signal detection range increased 1.6-fold whereas mean intensity of the pixels dropped down to 74% of the initial pixel intensity. These data indicated that photoactivation of **6** altered the permeability and the shape of mitochondria, i.e., $\Delta\Psi_m$ dissipated. Thus, confocal microscopy demonstrated that photoactivation of **6** trig-

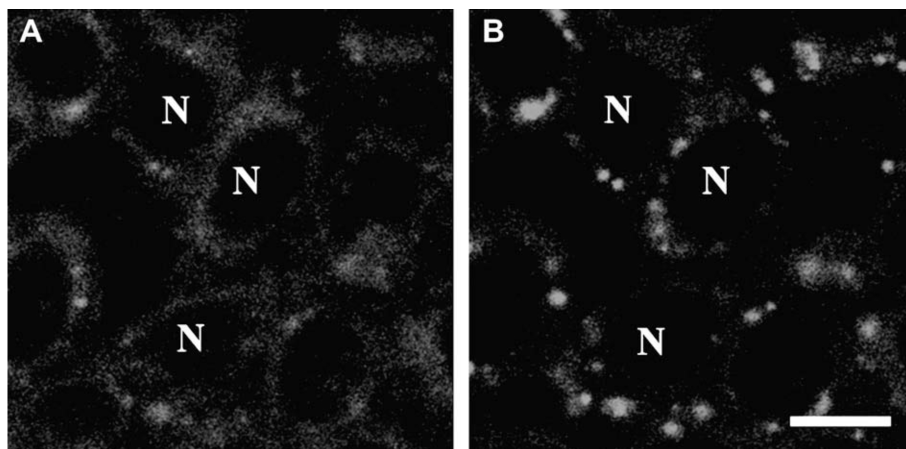


Fig. 1. Intracellular re-distribution of Pd (II) carboranylchlorin **6** after photoactivation. HCT116 cells were loaded with 1 μM of **6** for 1 h at 37 °C, 5% CO_2 followed by confocal microscopy. (A) Cells were illuminated during scanning. (B) Cells were imaged 20 min after the initial illumination. Compare a diffuse weak staining of the cytoplasm in A with distinct bright clusters around the nuclei in B. Shown is the optical section with the pinhole extended up to three Airy units. N, the nuclei in individual cells. Here and in Figs. 2 and 3: bar 10 μm.

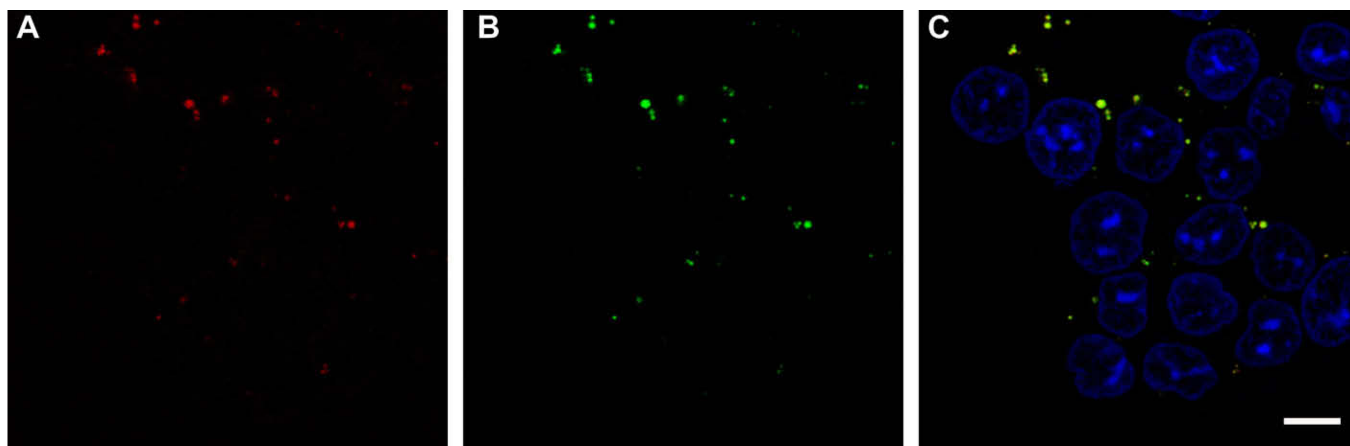


Fig. 2. Photoactivation causes an association of **6** with lysosomes and cell necrosis. MCF-7 cells were loaded with compound **6** (1 μM, 30 min), then LysoTracker Green DND-26 (1 μM, 10 min) and PI (10 μg/ml) were added, and cells were subjected to confocal microscopy. (A) LysoTracker (*green*); (B) compound **6** (*red*); (C) merge of A and B plus PI staining. Note almost completely disappeared red staining in C, the *yellow* compartments (co-localization of **6** with LysoTracker), and the nuclei stained with PI. Shown is the optical section. Pseudo colors were used to generate the confocal images.

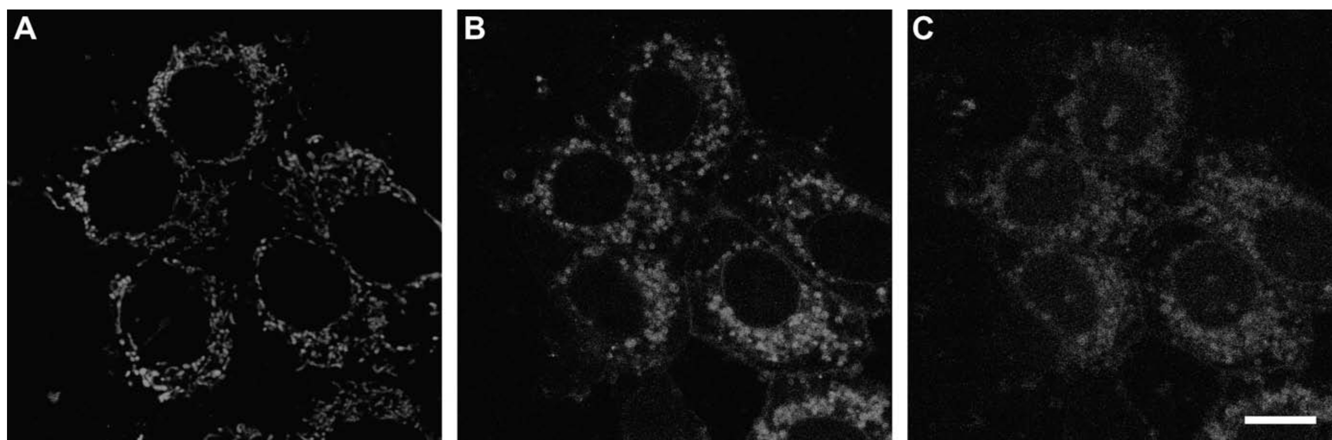


Fig. 3. Altered morphology of mitochondria and $\Delta\Psi_m$ dissipation after photoactivation of compound **6**. MCF-7 cells were loaded with compound **6** (1 μM, 30 min) and $\Delta\Psi_m$ sensitive Mitotracker Red CMX-Ros (100 nM, 10 min) followed by confocal microscopy. (A) cells were illuminated during scanning. (B) and (C) cells were imaged after an additional 5 min (B) or 10 min (C) at 37 °C, 5% CO_2. Note the fragmented mitochondria in B and C, and substantially decreased brightness of the mitochondrial dye in C. Shown are optical sections.

gered rapid association of the drug with lysosomes concomitant with $\Delta\Psi_m$ loss and cell necrosis. Exactly the same data were obtained after photoactivation of structurally close Pd(II) derivative of chlorin e_6 with neutral carborane (not shown), indicating that the electric charge of the carborane substituent is not critical for intracellular distribution or the mode of cell death induced by these metal carboranylchlorins. Finally, rapid photoinducible necrosis was reproduced in an independent experimental setting, namely, after LI of logarithmically growing tumor cells followed by flow cytometrical analysis of the plasma membrane permeability for PI (see Section 3). The Pd(II) or Sn(IV) carboranylchlorins (compounds **6** and **7**, respectively), each at 1 μM, sensitized HCT116 cells to illumination with monochromatic red light: as soon as 10–20 min post LI >98% cells were PI-positive, whereas in untreated cells or in cells illuminated in the absence of the photosensitizer the percentage of PI-stained cells was <5%. Nor did we observe any increase of PI staining in cells exposed to **6** or **7** alone without LI, which is in line with low dark cytotoxicity of these complexes after prolonged cell exposure (Table 1). Data obtained with MCF-7 and HCT116 cells were similar, indicating that the observed effects of drug photoactivation were independent of tissue origin.

Introduction of metals into the coordination sphere of the tetrapyrrole macrocycle can elevate the intratumoral accumulation of the complex [29] and increase the quantum yield of singlet oxygen [30], strongly suggesting that metallation is perspective for optimization of drugs for PDT. Based on the observations that boronation improved the photosensitizing potency of chlorins [12,23], we further modified the conjugates of chlorin e_6 and boron polyhedra by synthesizing their complexes with Pd(II), Sn(IV) or Zn(II) in the coordination sphere of the chlorin macrocycle. This study shows that our series of metal carboranylchlorins includes the compounds that cause dark cytotoxicity (such as Zn carboranylchlorin **8**) as well as the agents with negligible dark cytotoxicity (such as compounds **6** and **7**). Both categories of metal carboranylchlorins might be useful in anticancer treatment. The cytotoxic compounds are perspective as chemotherapeutic drugs alone and/or in combination with conventional drugs, as reported for structurally close metal complexes of boronated 5,10,15,20-tetraphenylporphyrin [13]. Among low toxic metal carboranylchlorins the potent photosensitizers can be selected. Rapid necrosis triggered by compounds **6** and **7** might be a desirable mechanism of tumor cell destruction in situations when apoptosis is hampered, e.g., in patients with residual or recurrent disease after the subsequent rounds of che-

motherapy or in pleiotropically resistant malignancies. Indeed, P-glycoprotein, an efflux pump frequently overexpressed in multidrug resistant cancers, can attenuate chemotherapy-induced apoptosis [31] but necrotic pathways remain functional [32,33]. Apparently, severe photodamage of critical organelles and the plasma membrane followed by necrosis, the phenomena observed instantly after photoactivation of novel metal carboranylchlorins, are important for the efficient eradication of tumors otherwise refractory to apoptotic stimuli. Thus, together with the applicability of metal carboranylchlorins as fluorescent/phosphorescent diagnostic probes, these compounds emerge as a promising class of agents in which each moiety, i.e., the metal ion, the chlorin macrocycle and the boron substituent, as well as the entire complex, is useful in cancer detection and treatment.

3. Experimental

^{1}H and ^{11}B NMR spectra were recorded on a Bruker Avance-400 spectrometer in $(CD_3)_2CO$. Chemical shifts (δ) are given in ppm relative to internal chloroform.IR spectra were recorded on a Specord M-82 of Carl Zeiss spectrometer in KBr tablets. The UV–Vis spectra were measured on a spectrophotometer Jasco UV 7800 in $CHCl_3$. Mass spectra were obtained using Vision 2000 (MALDI) mass spectrometer, the most intense peaks are given below for each compound. Merck silica gel L 0.040–0.08 mesh was used for column chromatography. The identities of new compounds were verified on TLC 60 F_{254} plates (Merck) in $CHCl_3$–CH_3OH (9:1) solvent system. The solvents were purified according to standard procedures. Synthesis of compounds **1–4, 9** and **10** has been reported earlier [23,26,34].

*3.1. General procedures of synthesis of boronated chlorin derivatives **6–8***

A solution of **3, 4** or **10** (0.15 mmol) and triflate **5** (0.2 mmol) in THF (8 ml) was stirred with BSA (0.2 mmol) at room temperature for 1 h. The reaction mixture was diluted with $CHCl_3$ (50 ml), washed with 3% aqueous CsCl solution (3 × 100 ml), dried over Na_2SO_4 and evaporated to dryness in vacuo. The crude product was purified by column chromatography on SiO_2 using $CHCl_3$–CH_3OH (9:1) as an eluent to give pure **6–8**, respectively.

*3.2. Palladium(II) complex of chlorin e_6 13(1)-N-{2-[N-(1-carba-closo-dodecaboran-1-yl)methyl]aminoethyl}amide-15(2), 17(3)-dimethyl ester (**6**)*

From 116 mg (0.15 mmol) chlorin **3**, 85 mg (0.2 mmol) triflate **5** and 0.5 ml (0.2 mmol) BSA, 122 mg (77%) of carboranylchlorin **6** was obtained. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): δ 9.75 (s, 1H, 10H), 9.73 (s, 1H, 5H), 9.67 (s, 1H, 20H), 8.16 (dd, 1H, J = 17.4, 11.8 Hz, 3^1H), 6.54 (br s, 1H, 13^1NH), 6.45 (d, 1H, J = 8.0 Hz, 3^2H(trans)), 6.22 (d, 1H, J = 12.2 Hz, 3^2H (cis)), 15^1CH_2: 5.51 (d, 1H, J = 19.1 Hz) and 5.38 (d, 1H, J = 19.2 Hz), 4.70 (q, 1H, J = 7.2 Hz, 18H), 4.53 (br d, 1H, J = 8.8 Hz, 17H), 4.40 (br s, 1H, 13^1NH), 4.30 (q, 2H, J = 7.2 Hz, 8^1CH_2), 4.08 (m, 4H, 13^2CH_2, 13^3CH_2), 3.80 (s, 3H, 15^3CH_3), 3.77 (s, 3H, 17^4CH_3), 3.61 (s, 3H, 12^1CH_3), 3.59 (s, 3H, 2^1CH_3), 3.38 (s, 3H, 7^1CH_3), 2.94 (s, 1H, 13^3NH), 2.60 (s, 2H, NH-*CH₂*-C(carborane)), 2.33 (m, 2H, 17^2CH_2), 2.29 (m, 2H, 17^1CH_2), 3.0–1.4 (br m, 11H, BH), 1.69 (d, 3H, J = 7.0 Hz, 18^1CH_3), 1.70 (t, 3H, J = 7.5 Hz, 8^2CH_3). ^{11}B NMR ($(CD_3)_2CO$, 128.38 MHz): δ −8.99 (d, 1B, J = 138.4 Hz), −13.16 (d, 5B, J = 146 Hz), −14.97 (d, 5B, J = 154 Hz). IR (KBr, cm^{-1}): ν 3313 (NH), 2517 (BH), 1721 (CO), 1610 (chlorin band), 1631 (amide I), 1520 (amide II). UV–Vis ($CHCl_3$, nm, $\varepsilon^* 10^{-3}$): λ_{max} 410 (77.8), 487 (6.8), 569 (9.74), 613 (44.0). MS (MALDI) m/z 926 (M−Cs$^+$).

*3.3. Tin(IV) dihydroxide complex of chlorin e_6 13(1)-N-{2-[N-(1-carba-closo-dodecaboran-1-yl)methyl]aminoethyl}amide-15(2), 17(3)-dimethyl ester (**7**)*

From 123 mg (0.15 mmol) chlorin **4**, 85 mg (0.2 mmol) triflate **5** and 0.5 ml (0.2 mmol) BSA, 99 mg (58%) of carboranylchlorin **7** was obtained. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): δ 10.09 (s, 1H, 10H), 10.03 (s, 1H, 5H), 9.21 (s, 1H, 20H), 8.21 (dd, 1H, J = 17.4, 11.6 Hz, 3^1H), 6.86 (br s, 1H, 13^1NH), 6.42 (d, 1H, J = 17.8 Hz, 3^2H(trans)), 6.27 (d, 1H, J = 11.8 Hz, 3^2H(cis)), 15^1CH_2: 5.61 (d, 1H, J = 18.5 Hz) and 5.47 (d, 1H, J = 18.7 Hz), 4.78 (q, 1H, J = 7.1 Hz, 18H), 4.51 (br d, 1H, J = 9.0 Hz, 17H), 4.30 (q, 2H, J = 7.1 Hz, 8^1CH_2), 4.02 (m, 4H, 13^2CH_2, 13^3CH_2), 3.76 (s, 3H, 15^3CH_3), 3.75 (s, 3H, 17^4CH_3), 3.62 (s, 3H, 12^1CH_3), 3.60 (s, 3H, 2^1CH_3), 3.37 (s, 3H, 7^1CH_3), 2.90 (s, 1H, 13^3NH), 2.66 (s, 2H, NH-*CH₂*-C(carborane)), 2.33 (m, 4H, 17^2CH_2, 17^1CH_2), 3.2–1.3 (br m, 11H, BH), 1.83 (d, 3H, J = 7.8 Hz, 18^1CH_3), 1.81 (t, 3H, J = 7.6 Hz, 8^2CH_3). ^{11}B NMR ($(CD_3)_2CO$, 128.38 MHz): δ −9.36 (d, 1B, J = 137 Hz), −13.35 (d, 5B, J = 130 Hz), −14.35 (d, 5B, J = 143 Hz). IR (KBr, cm^{-1}): ν 3378 (NH, OH), 2534 (BH), 1723 (CO), 1608 (chlorin band), 1628 (amide I), 1518 (amide II). UV–Vis ($CHCl_3$, nm, $\varepsilon^* 10^{-3}$): λ_{max} 410 (88.3), 509 (5.2), 591 (5.2), 637 (31.4). MS (MALDI) m/z 1009 (M−Cs$^+$).

*3.4. Zinc(II) complex of chlorin e_6 13(1)-N-{2-[N-(1-carba-closo-dodecaboran-1-yl)methyl]aminoethyl}amide-15(2), 17(3)-dimethyl ester (**8**)*

From 109 mg (0.15 mmol) chlorin **10**, 85 mg (0.2 mmol) triflate **5** and 0.5 ml (0.2 mmol) BSA, 133 mg (88%) of carboranylchlorin **8** was obtained. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): δ 9.61 (s, 1H, 10H), 9.60 (s, 1H, 5H), 8.73 (s, 1H, 20H), 8.18 (dd, 1H, J = 18.0, 12.1 Hz, 3^1H), 6.22 (d, 1H, J = 18.0 Hz, 3^2H(trans)), 6.01 (d, 1H, J = 12.1 Hz, 3^2H (cis)), 15^1CH_2: 5.45 (d, 1H, J = 18.9 Hz) and 5.23 (d, 1H, J = 18.7 Hz), 4.40 (br d, 1H, J = 9.1 Hz, 17H), 4.33 (q, 1H, J = 7.1 Hz, 18H), 3.92 (br s, 1H, 13^1NH), 3.83 (q, 2H, J = 7.5 Hz, 8^1CH_2), 3.77 (s, 3H, 15^3CH_3), 3.68 (s, 3H, 17^4CH_3), 3.62 (m, 4H, 13^2CH_2, 13^3CH_2), 3.59 (s, 3H, 12^1CH_3), 3.57 (s, 3H, 2^1CH_3), 3.39 (s, 3H, 7^1CH_3), 2.84 (s, 1H, 13^3NH), 2.83 (s, 2H, NH-C*H₂*-C(carborane)), 2.11 (m, 4H, 17^2CH_2, 17^1CH_2), 3.0–1.4 (br m, 11H, BH), 1.70 (d, 3H, J = 7.6 Hz, 18^1CH_3), 1.67 (t, 3H, J = 7,1 Hz). ^{11}B NMR ($(CD_3)_2CO$, 128.38 MHz): δ −9.02 (d, 1B, J = 136 Hz), −13.17 (d, 5B, J = 130 Hz), −14.39 (d, 5B, J = 154 Hz). IR (KBr, cm^{-1}): ν 3309 (NH), 2534 (BH), 1721 (CO), 1607 (chlorin band), 1658 (amide I), 1542 (amide II). UV–Vis ($CHCl_3$, nm, $\varepsilon^* 10^{-3}$): λ_{max} 418 (69.4), 522 (3.44), 597 (5.16), 638 (33.7). MS (MALDI) m/z 875 (M−Cs$^+$).

*3.5. 1-Trifluoromethanesulfonylmethyl-1-carba-closo-dodecaborate cesium (**5**)*

A solution of Tf_2O 3.9 g (14 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a mixture of 1-hydroxymethyl-1-carba-*closo*-dodecaborate cesium [35] 4 g (13 mmol), and Na_2CO_3 1.4 g (13 mmol) in CH_2Cl_2 (10 ml) at 0–3 °C. The mixture was refluxed for 7 h, the precipitate was filtered, dissolved in 3% aqueous CsCl solution (100 ml) and extracted with ethylacetate. The organic solvent was evaporated in vacuo, and recrystallization from ethylacetate/hexane gave 5 g (85.1%) of **5**. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): 3,59 (s, 2H, CH_2), 2.5–1.1 (m, 11H, BH). ^{11}B NMR ($(CD_3)_2CO$, 128.38 MHz): δ −8.92 (d, 1B, J = 138 Hz), −13.10 (d, 5B, J = 145 Hz), −14.90 (d, 5B, J = 153 Hz). IR (KBr, cm^{-1}): ν 2540 (BH), 1400, 1260, 1187 (OSO_2O), 1034 (CF). Anal. Calc. for $C_3H_{13}B_{11}CsF_3SO_3$: C, 8.23; H, 3.00; B, 27.15. Found: C, 7.92; H, 3.12; B, 26.98%.

3.5.1. Biological methods

The HCT116 human colon carcinoma and MCF-7 human breast carcinoma cell lines (American Type Culture Collection) were

propagated in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum (Invitrogen, USA), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO_2 in humidified atmosphere. Newly synthesized compounds were dissolved as 10 mM stock solutions in dimethyl sulfoxide, and serial aqueous dilutions were made immediately before experiments. The compounds were kept away from light. The experiments were performed in the dark. All chemicals were purchased from Sigma–Aldrich, USA unless specified otherwise. Dark cytotoxicity was tested in a 96-well format by reduction of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan (MTT-test) [28]. For confocal microscopy, cells were grown on round 24 mm glass coverslips for 48 h to reach ~50% confluency. To visualize mitochondria or lysosomes, cells in growth medium were stained with $\Delta\Psi_m$ sensitive MitoTracker Red CMX-Ros (100 nM, 10 min) or LysoTracker Green DND-26 (1 μM, 10 min), respectively. Both dyes were from Molecular Probes, USA. Digital images were acquired using Axiovert 200M LSM 510 Meta (Carl Zeiss AG, Germany). The 1024 × 1024 pixel confocal images were recorded with a 63× Plan-Apochromat oil immersion objective, NA 1.4. The fluorescence of **6** was excited with a 633 nm He–Ne laser, and emission was registered with the 650–710 nm band pass filter. For 633 nm laser line the density of light emission energy was 35 J/cm^2. Fluorescence of PI and Mitotracker Red CMX-Ros was excited with a 543 nm He–Ne laser, and emission was registered with the 565–615 nm band pass filter. The 488 nm line of argon laser and 500–530 nm band pass filter were used for LysoTracker Green DND-26 visualization. For simultaneous detection of the metal carboranylchlorin, PI and LysoTracker Green DND-26, three-channel acquisition of a series of optical sections was done using a line-by-line multi-track mode. The 488 nm, 543 nm and 633 nm laser lines for excitation with the appropriate set of dichroic mirror and band pass filters on the emission side were used. The pinhole setting for high-resolution images was according to the manufacturer's instructions, and for the time-lapse series the pinhole was three Airy units to extend the focal depth. Zeiss LSM 510 Meta Software release 3.2 was used for image processing and quantification.

For PDT in cell culture, HCT116 or MCF-7 cells were seeded overnight into a 35 mm Petri dish (Costar; 5×10^4 cells per dish) followed by the addition of **6** or **7** (final concentration 1 μM) for 30 min at 37 °C, 5% CO_2. After the completion of exposure the culture medium was replaced with 0.5 ml PBS, and cell monolayers were illuminated for 10 min using a monochromatic light source manufactured in Lebedev Institute of Physics, Russian Academy of Sciences (λ = 633 nm, density of light emission energy 200 J/cm^2). An aqueous solution of $NaNO_2$ was placed between the light source and the cells to protect the monolayer from warming. After illumination cells were replenished with fresh culture medium and further incubated at 37 °C, 5% CO_2. Control cells were left untreated (no drug, no light) or treated with 1 μM of **6** or **7** alone or illuminated in the absence of each of these compounds. For quantitation of cells with damaged plasma membrane, PI (10 μg/ml) was added, and cells were analyzed by flow cytometry on FL2 (FACSCalibur System, Becton Dickinson, USA). The experiments were performed five times with essentially the same results.

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