Borinated Porphyrins and Chlorins as Potential Anticancer Drugs†

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1 This paper is dedicated to Professor Sang Chul Shim on the occasion of his honorable retirement.

Abstract: Boronated derivatives of porphyrins and structurally close chlorins have been the subject of an extensive investigation as tentative agents for binary anticancer treatment, i.e., photodynamic therapy (PDT) and boron neutron capture therapy (BNCT). Use of these compounds is based on the accumulation of porphyrins in malignant tumors and activation by thermal neutrons (BNCT) or laser light (PDT). The path length of particles 23He and 7Li (BNCT) or singlet oxygen 1O (PDT) generated in the tumor is comparable with the cell diameter. This provides an opportunity to selectively destroy the tumor with minimal damage to healthy tissues. Some porphyrin-containing pharmaceuticals such as Fotogem® or its analogue Photofrin® are used in the clinic as PDT agents. Moreover, the second generation of the chlorin-based photosensitizers (PSs) for PDT is under development. Chlorins are more promising PSs for PDT because they absorb light in the red spectral region (λ = 650-660 nm). This property of chlorins, first, provides deeper penetration of tissue photodamage compared with that achievable with porphyrins. Second, radiation used for excitation of chlorins is less absorbable by surrounding tissues. Therefore, the synthesis of boronated porphyrins and chlorins makes it possible to obtain compounds with the properties advantageous for both BNCT and PDT: the tropicity to neoplasms, phototoxicity, and the ability to generate local radioactive reaction and cytotoxic particles generated by thermal neutrons. To develop an efficient dual sensitizer for BNCT/PDT, general (dark) toxicity of the porphyrin-type structures should be low, as higher doses of the drug are usually required for BNCT compared to PDT.

Keywords: Carboranes, Porphyrins, Drug resistance, Photodynamic therapy, Boron neutron capture therapy

Introduction

Boronated derivatives of porphyrins and structurally close chlorins have been the subject of an extensive investigation as tentative agents for binary anticancer treatment, i.e., photodynamic therapy (PDT) and boron neutron capture therapy (BNCT). Use of these compounds is based on the accumulation of porphyrins in malignant tumors and activation by thermal neutrons (BNCT) or laser light (PDT). The path length of particles 23He and 7Li (BNCT) or singlet oxygen 1O (PDT) generated in the tumor is comparable with the cell diameter. This provides an opportunity to selectively destroy the tumor with minimal damage to healthy tissues. Some porphyrin-containing pharmaceuticals such as Fotogem® or its analogue Photofrin® are used in the clinic as PDT agents. Moreover, the second generation of the chlorin-based photosensitizers (PSs) for PDT is under development. Chlorins are more promising PSs for PDT because they absorb light in the red spectral region (λ = 650-660 nm). This property of chlorins, first, provides deeper penetration of tissue photodamage compared with that achievable with porphyrins. Second, radiation used for excitation of chlorins is less absorbable by surrounding tissues. Therefore, the synthesis of boronated porphyrins and chlorins makes it possible to obtain compounds with the properties advantageous for both BNCT and PDT: the tropicity to neoplasms, phototoxicity, and the ability to generate local radioactive reaction and cytotoxic particles generated by thermal neutrons. To develop an efficient dual sensitizer for BNCT/PDT, general (dark) toxicity of the porphyrin-type structures should be low, as higher doses of the drug are usually required for BNCT compared to PDT.
A number of synthetic routes toward the boronated porphyrins and chlorins have been developed but the suitability of these compounds for medicinal application can be estimated only after the in vitro and in vivo biological testing. We have developed a strategy of synthesis of carboranylporphyrins via classical route using condensation of carborane aldehydes with pyrrole and by introducing the carborane polyhedra into the synthetic or natural porphyrins. In this review our recent results of synthesis and anticancer properties of boronated porphyrins and chlorins are analyzed.

**Boronated Derivatives of 5,10,15,20-Tetraphenylporphyrin**

We developed a series of carboranylporphyrins based on functional derivatives of 5,10,15,20-tetraphenylporphyrin and various neutral and anionic polyhedral carboranes. Our choice of 5,10,15,20-tetraphenylporphyrin as a basic compound for chemical modifications was dictated by (i) availability of this compound, (ii) suitability for introducing different functional groups, and (iii) easiness of obtaining final products with reasonable yields and high purity.

To obtain functionally substituted hydrophilic boronated porphyrins, we employed general approach based on the interactions of carborane carbanions with aldehydes. The reaction of 2-formyl-5,10,15,20-tetraphenylporphyrin (1), its copper (2) and cobalt (3) complexes with 1-lithium-2-methyl-o-carborane or 1-isopropyl-7-lithium-m-carborane yielded neutral carboranylporphyrin alcohols (Scheme 1).

The anionic nido-carboranylporphyrin alcohols 9-11 were synthesized by deboronation of closo-analogues 4, 6 and 7 with Bu4NF·2H2O in THF and isolated as tetrabutylammonium salts (Scheme 1).

Also, we synthesized a new type of anionic carboranylporphyrin alcohols as synthones for water soluble analogues. We used hydrophilic closo-monocarbon carborane anion, closo-CB11H11−, which is isoelectronic to neutral closo-C2B10H12 carboranes. This compound was chosen because (i) monocarbon carborane is stable in air and aqueous media, suggesting its stability in the body; (ii) some salts of monocarbon carborane and its hydrophilic derivatives are water soluble due to anionic charge; this is advantageous over neutral carboranes and allows for obtaining hydrophilic boronated porphyrins. We found that the reaction of 1-lithium- closo-monocarbon carboranyl cesium with formylporphyrins 1-3 in THF resulted in the formation of anionic monocarbon carboranylporphyrin alcohols 12-14 in high yields (70-85%) (Scheme 2).

Among the novel monocarbon carborane substituted porphyrins, only cesium salt of monocarbon carborane alcohol 12 was, to some extent, soluble in water. Our preliminary data suggest that substitution of cesium cation by sodium or lithium cations results in higher amphiphilicity (not shown). All carboranylporphyrins were tested for their ability to kill cultured human tumor cells. The compounds showed differential activity. We found that closo-alcohols 4, 6, 8 as well as nido-alcohols 9, 10 demonstrated little or no cytotoxicity for leukemia and breast carcinoma cell lines (not shown). However, 5,10,15,20-tetraphenylporphyrin caused death of K562 leukemia cells with IC50 = 52.6 ± 4.3 μM as determined in the colorimetric test based on conversion of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide into formazan (MTT-test) after 72 h of continuous exposure (Figure 1A). Comparison of activities of closo- and nido-carboranyl-substituted derivatives of Cu-5,10,15,20-tetraphenylporphyrin complex (compounds 5, 7 and 11) and monocarbon carborane substituted copper (II) complex of 5,10,15,20-tetraphenylporphyrin (13) identified compound 13 as the most active (IC50 = 4.6 ± 2.2 μM) (Figure 1A). Next, 13 demonstrated higher activity for MCF-7 breast...
carcinoma cell line than structurally related cobalt (II) salt 12 and metal free 14 carborane derivatives of 5,10,15,20-tetraphenylporphyrin (Figure 1B). Importantly, 13 was virtually inert for non-malignant human skin fibroblasts at concentrations up to 100 μM whereas this compound potently killed ovarian carcinoma CaOv cells (Figure 1C). Only at higher concentrations at which 13 formed precipitates in aqueous solutions, this agent was toxic for fibroblasts. These experiments provide evidence that monocarbon carboranes conjugated with Cu (II) salt of 5,10,15,20-tetraphenylporphyrin could be perspective for further investigation as anticancer agents.

Pleiotropic refractoriness of tumor cells to exogenous stimuli remains a major reason for therapeutic failure. The transmembrane transporter P-glycoprotein (Pgp; ABCB1) frequently mediates the intrinsic (prior to chemotherapy) resistance to apoptosis as well as multidrug resistance (MDR) acquired in the course of treatment. To study the potency of carboranylporphyrins for sublines with Pgp-mediated MDR, we chose 13 because this compound was the most active against parental leukemia and breast cancer cells. We first addressed the role of Pgp in the cytotoxicity of 13 by comparing the survival of K562 cells and the K562i/S9 subline that expresses Pgp without selection.13 The K562i/S9 cells were significantly more resistant than K562 cells to vincristine, the conventional chemotherapeutic drug transported by Pgp (IC₅₀ = 56.1 ± 4.5 nM versus 6.2 ± 2.1 nM, respectively; resistance index 9.0). In striking contrast, survival of both cell lines in the presence of 13 differed only moderately; the respective IC₅₀s were 10.5 ± 2.0 μM versus 5.2 ± 1.7 μM; resistance index 2.0). These data suggest that 13 is a weaker substrate of Pgp-mediated transport than vincristine. Nevertheless, inhibition of Pgp function with verapamil (VER) dramatically potentiated the cytotoxicity of 13. Death of K562i/S9 cells after 24 h of exposure to 4 μM 13 + 20 μM VER (Figure 2) was almost as pronounced as death of the same cells treated with 16 μM 13 alone. Only marginal MTT conversion (Figure 2) and clearly detectable morphological signs of apoptosis such as cell shrinkage and nuclear fragmentation were found in K562i/S9 cells treated with 2 μM 13 + 20 μM VER for 48 h. No significant changes in MTT reduction were detected in these cells after exposure to 4 μM 13 or 20 μM VER alone (Figure 2).

To study the potency of 13 for cells that acquired Pgp-mediated MDR during multistep selection with conventional drugs, we compared the cytotoxicity of 13 for MCF-7 cells and the MCF-7Dox variant selected for long-term survival in the presence of doxorubicin (DOX). The MCF-7Dox subline displayed Pgp-mediated MDR as determined by higher resistance to Pgp-transported chemotherapeutics DOX, vincristine, mitoxantrone and taxol (Table 2), elevated amount of Pgp and increased efflux of Pgp-transported fluorescent dye rhodamine 123.13 The MCF-7Dox subline displayed Pgp-mediated MDR as determined by higher resistance to Pgp-transported chemotherapeutics DOX, vincristine, mitoxantrone and taxol (Table 2), elevated amount of Pgp and increased efflux of Pgp-transported fluorescent dye rhodamine 123.13 The MCF-7Dox subline displayed Pgp-mediated MDR as determined by higher resistance to Pgp-transported chemotherapeutics DOX, vincristine, mitoxantrone and taxol (Table 2), elevated amount of Pgp and increased efflux of Pgp-transported fluorescent dye rhodamine 123.13 The MCF-7Dox subline displayed Pgp-mediated MDR as determined by higher resistance to Pgp-transported chemotherapeutics DOX, vincristine, mitoxantrone and taxol (Table 2), elevated amount of Pgp and increased efflux of Pgp-transported fluorescent dye rhodamine 123.13

Thus, although carboranylporphyrins are currently studied as tentative anticancer agents, anionic monocarbon carboranes such as CB₁₁H₁₂⁻ are poorly investigated. We demonstrate that this class of boron containing porphyrins could be...
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perspective since 13, the water soluble Cu (II) salt of monocarboranyl substituted 5,10,15,20-tetraphenylporphyrin, potently killed human tumor cells otherwise resistant to many apoptotic stimuli.

A key prerequisite for overcoming pleiotropic irresponsiveness should be delivery of the amount of the drug sufficient to activate as many death pathways as possible.

Our results provide evidence that 13 is relatively poor substrate for Pgp-mediated efflux. Indeed, the cytotoxicity of 13 for Pgp-negative and -positive cells did not differ as substantially as it did for Pgp transported drugs, making 13 (and potentially other agents of this class) perspective for circumventing the resistance.

Our data demonstrate that, for tumor cells, boronated porphyrins are toxic even as single agents; one may expect that this activity would potentiate the efficacy of these compounds as photo/radiosensitizers in binary treatments. The potency of Cu(II) salt of monocarboranyl substituted 5,10,15,20-tetraphenylporphyrin for cells with altered stress response proves the applicability of this chemical class for circumventing anticancer drug resistance.

It should be noted that the efficacy of binary treatments depends on the accumulation of the sensitizing agent in the tumor cells. Direct calculations revealed that the therapeutic efficacy of boronated porphyrins is higher if the agent is accumulated in the nucleus compared with the cytoplasmatic localization. Furthermore, generation of free oxygen burst in the vicinity of the nucleus would enhance the cytotoxic effect. Despite an extensive search for new compounds for BNCT and PDT, the problem of optimization of boron delivery systems has not been addressed in detail. Therefore, optimization of boronated porphyrins as photo/ radiosensitizers presumes the design of DNA-interacting compounds. The desired selectivity can be attained by attaching carborane cages to various tumor-seeking biomolecules or by introducing functional groups into the carborane cage. The key point is to choose appropriate functionalities whose transformations eventually yield active compounds. We chose the isocyanate group, an attractive moiety from the standpoint of design of non-toxic compounds for BNCT and PDT. We found that 1-lithium-9-isocyanato-<em>c</em>- and <em>m</em>-carboranes readily reacted with the aldehyde group of porphyrin (1) and its metal complexes (2, 15-17) to form corresponding porphyrin alcohols 18-27, containing N–C–O group at the boron atom of carborane polyhedron (Scheme 3).

Isocyanates (19, 24) can be readily transformed to urethanes (28-29) and water soluble ammonium derivatives (30-31) in quantitative yields (Scheme 4).

Cytotoxicity of compounds 18-27 was studied using human K562 leukemia cells and non-malignant skin fibroblasts. Importantly, 18, 20-23, 25-27 were not toxic for fibroblasts whereas 19 and 24 killed leukemia cells. These data imply that the compounds of this series can potentially be useful given their differential cytotoxicity for non-malignant and tumor cells.

Binding of compounds 19, 22, 24 and 27 with double stranded DNA was investigated using spectrophotometry. Compounds 19, 22, 24 caused a decrease of DNA absorption without shifting its maximum, suggesting that these agents do not interact with DNA. In contrast, 27 shifted the maximum from 260 to 272 nm, indicating the formation of a complex between this compound and the duplex DNA.

So, the introduction of isocyanatocarboranes into the porphyrin macrocycle allow to obtain carboranylporphyrins with two functional groups, one of which bound to the boron atom of polyhedron. This allows for modifying hydrophobic/hydrophilic properties of boronated conjugates as well as to improve their selective uptake by tumor cells.

**Scheme 3.** Conjugation of 2-formyl 5,10,15,20-tetraphenylporphyrin with 9-isocyanato-carboranes.

**Figure 4.** VER sensitizes MCF-7Dox cells to compound 13.
A major problem that limits clinical use of porphyrin-based compounds is general (dark) toxicity. Although the boronated porphyrins demonstrate therapeutic efficacy due to high ratio of tumour-to-tissue content and the ability to generate intratumoural ionization processes, these compounds (13 for example) may cause toxicity prior to irradiation. Photofrin®, a mixture of porphyrin oligomers derived from natural products, recently entered clinical trials as a photosensitizer for PDT of bladder, stomach, lung, esophageal and cervical tumours. However, skin photosensitivity emerged as an unfavourable effect, and a series of novel porphyrins and chlorins have been synthesized to obtain active antitumour compounds with attenuated general toxicity.

Studies of BOPP [tetrakis(carboxylate ester of 2,4-bis(α,β-dihydroxyethyl) deuteroporphyrin IX disodium salt], a water-soluble boronated porphyrin, demonstrated its excellent characteristics such as the selective tumour uptake, mitochondrial localization and anticancer effect in PDT of experimental intracranial tumours and in phase I clinical trials. Still, thrombocytopenia was a dose-limiting factor, and skin photosensitivity should be taken into consideration.

For this reason we have developed the synthesis of carboranyl and monocarbon carboranyl derivatives of protohemin IX (32), a component of heme containing proteins. Using the activation of porphyrin (32) carboxylic groups with di-tert-buty1 pyrocarbonate (Boc₂O) or pivaloyl chloride the neutral and anionic congeners in which the boron polyhedra are linked to the porphyrin ring by ester (35) or amide bonds (36) were prepared (Scheme 5).

Hydrophilic boronated derivatives (40, 41) of protohemin IX were prepared by the direct introduction of anionic closo-monocarbon carborane polyhedron into the porphyrin system (Scheme 6). In this case we prepared only mono-substituted zwitter-ionic monocarbon carboranylporphyrins 40 and 41.

The water soluble 1,3,5,8-tetramethyl-2,4-divinyl-6(7)-[2'-closo-monocarbon-carborane-1''-yl]methoxycarbonyl-ethyl]-7(6)-(2'-carboxyethyl)porphyrin Fe (III) (40) exerted no discernible cytotoxicity for cultured mammalian cells, nor did it cause general toxicity in rats. Importantly, 40 demonstrated the dose dependent activity as a phototoxin in PDT of M-1 sarcoma bearing rats. In animals injected with 20 mg/kg of 9 the tumours shrunk by day 3 after one single irradiation of the tumour with red laser light. By days 7-14 post irradiation 77.8% of rats were tumour free; no
recurrence of the disease was detectable within at least 90 days. Protohem IX alone was without effect, indicating that boronation is important for the phototoxic activity of 9. The applicability in PDT broadens the therapeutic potential of boronated porphyrins beyond their conventional role as radiosensitizers in boron neutron capture therapy.

Aiming at optimization of antitumour characteristics of compound 40 we developed the methods of conjugation of L-amino acids with carboxy group of porphyrin 40. We hypothesized that amino acid residues should ensure high amphiphilicity and therefore good tissue accumulation of the conjugates. Moreover, it has been suggested that amino acids facilitate transmembrane transport and stabilize carboranyl/porphyrin-DNA complexes.

We obtained the amide L-amino acid derivatives of 40 in the reaction of methyl esters of serine, valine and phenylalanine with Boc-activated carboxy group of 40 (ref. 25; Scheme 7).

The reaction of compound 40 with oxazaborolidine complexes of L-serine (46) or L-threonine (47) and subsequent hydrolysis of intermediates 48 and 49 yielded the amino acid conjugates 50 and 51 in which the amino acid residue was linked to the porphyrin macrocycle via ester bond (Scheme 7). Importantly, 50 and 51 contain free amino- and hydroxy groups that increases their water solubility.

Screening of novel compounds for cytotoxicity revealed that their potency was 43 > 44 >> 45, 50, 51. Compound 43 triggered complex pattern of cell death. This multiplicity of death pathways induced by 43 implies that our novel amino acid derivatives of boronated porphyrins can be potent for tumor cells in which death signaling was impaired during their natural history and/or preceding therapy.
Finally, we obtained previously unknown carbamine derivatives of chlorin ε, by alkylation of amino and hydroxy groups of chlorins 53 and 54 with carbonyl/methyl triflate (Scheme 8). As a result carbamoylchlorins 55 and 56 were formed.

Both 55 and 56 did not cause cell death at concentrations of 1-50 μM during 72 h. It should be noted that compounds 55 and 56 are soluble in water at concentrations up to 25 mM. Thus, these carboranylchlorins are non-toxic for cultured cells at concentrations that do not affect the solubility. The toxicity of the new compounds and their efficacy in PDT and BNCT will be determined in animal studies.

Conclusion

In summary, progress in the synthesis of boronated conjugates of porphyrins and chlorins yielded the reliable methods for obtaining the compounds potent as cytotoxic agents for tumor cells in culture and as phototoxins in photodynamic therapy of tumor xenografts. Boronated porphyrins and chlorins deserve further development as promising class of antitumor agents due to their applicability as the chemotherapeutic drugs alone and photo- and radiosensitizers in binary treatment strategies.

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