

COMBINATION THERAPY OF TUMOR MODEL WITH PDT IN RADIOLOGICAL REGIONS

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Abstract

Physical methods in antitumor treatment are frequently used in combination (simultaneous or sequential) to achieve maximal effect. Therefore, the compound capable of sensitizing tumor cells to different types of energy (light, ionizing X-rays, thermal neutrons) would be applicable in more than one therapeutic modality. We synthesized a novel fluorinated boron-containing chlorin derivative [5,10,15,20-tetrakis[4-(1-carba-closo-dodecaboran-1-yl)tetrafluorophenyl]-17,18-dihydroporphyrin] tetrasodium (Compound-B). In this compound the boron atoms are represented by boron-10. Compound-B showed a good water solubility and cytoplasmic accumulation, as well as low-to null dark cytotoxicity. Compound-B potently (at low μM concentrations) sensitized the transplanted C6 glial tumor cells (a BALB-c-nu/nu murine model) to PDT and BNCT. Importantly, Compound-B also increased the efficacy of proton or carbon therapy. These results provide evidence that structural optimizations of known chemical classes may confer valuable characteristics to compounds initially designated for one particular therapeutic modality.

Key words: combination therapy, tumor model, radiological regions

1. INTRODUCTION

In an attempt to make a multifunctional photosensitizer for light, neutron and ionizing X-ray beams we synthesized {5,10,15,20-tetrakis[4-(1-carba-closo-dodecaboran-1-yl)tetrafluoro-phenyl]-17,18-dihydroporphyrin} tetrasodium (Compound-B) in which B atoms in the carborane cages were boron-10. This compound showed good water solubility, a feature that is not easy to achieve with chlorin derivatives with boron cages and fluorine atoms without our specific synthetic technologies [1, 2] and the patent [3].

We applied for different irradiation sources to bring experimental tumor bearing mice to four institutes: KU-RRI in Osaka Prefecture, Wakasa-wan Energy Research Center (WERC) in Fukui Prefecture, National Institute of Radiological Sciences, National Institute for Quantum and Radiological Science and Technology (NIRS-NIQRST) in Chiba Prefecture, and Takasaki Advanced Radiation Research Institute, National Institute for Quantum and Radiological Science and Technology (TARRI-NIQRST) in Gunma Prefecture with an originally developed diode laser (LD) in Japan. From these results in WERC, NIRS-NIQRST, and TARRI-NIQRST we received 3 patents [4-6] in Japan about the sensitizing effects of Compound-B against the heavy ionizing X-rays and the combination therapy with PDT.

2. EXPERIMENTAL DETAIL

2.1. Preparation of Tumor Samples and Cell plates

Rat glial tumor cells (C6 cell line) were cultured in a CO₂ incubator (5% CO₂, 37°C) in RPMI-1640 (Nacalai Tesque Inc., Kyoto, Japan) for 2-3 days to reach the concentration 2×10^6 cells/ml. A single cell suspension was prepared using trypsin-EDTA. The suspension (2×10^5 cells/0.1 ml) was injected s.c. in

both thighs of the nude BALB-c-nu/nu strain mouse (CLEA Japan Inc., Tokyo, Japan). When the tumors grew up to 7x7x7 mm (179.50) mm³ size within 3-4 weeks, animals were used for experiments with radiation.

Compound-¹⁰B (Figure 1) was developed in a collaboration with Nesmeyanov Institute of Organoelement Compounds and Blokhin Cancer Center (Russian Federation) and Department of Tumor Pathology, Faculty of Medical Sciences, National University of Fukui (Japan) for 3 years. Initially the compound was patented in Russian Federation (Patent No. 2402554 Oct. 27, 2010). Boron-10 cages were imported from Katchem Ltd. Co., Czech Republic (order funded by the Japanese partner). Compound-B with boron-10 was then synthesized in Nesmeyanov Institute of Organoelement Compounds, Russian Federation. The compound is readily soluble in aqueous solution and shows no general toxicity at up to 151 mg/kg body weight in the murine tumor model.

The combination treatments of photodynamic therapy (PDT) were added after these radiation treatments within 6 hrs in the case of animal and cell culture using a pulsed (0~1 kHz) semiconductor laser of 377.6 nm (~150 mW) and 657 nm (~578 mW) bring into the each neutron, proton, carbon beam radiation room in these buildings to do combination irradiations with laser and the other ionizing X-rays in the same time in the same position.

2.2. Radiation by neutron beam in Research Reactor Institute of Kyoto University (RIIKU)

Three C6 tumor mice bearing 6 tumors on both thighs were irradiated in the neutron beam for 20 min with two different detector plates attached to the skin surface. The plates contained a gold leaf for the thermal neutron beam and a thermoluminescence dosimeter (TLD) against γ -rays against each tumor. The radiation was started 36 hrs after the i.p. administration of 3.5 μ M Compound-B. The accumulated concentration of boron-10 into the tumor tissue were 2.04 ppm, and the radiated total dose was calculated as 0.245 Gy for 20 min irradiation. Irradiated mice were brought back to the animal room in the RI Research Center at University of Fukui to monitor the tumor size to estimate the antitumor effects at the second day of the neutron irradiation in RRIKU.

2.3. Radiation by proton beam in Wakasa-wan Energy Research Center (WERC)

The proton radiation in WERC was started 36 hrs after the i.p. administration of 3.5 μ M Compound-B. The proton of 200 MeV in WERC was irradiated 4 times a week of 5 Gy, total dose for each tumor was 20 Gy. The irradiated mice were brought back to the RI Research Center of University of Fukui to monitor tumor size (42 tumors) over 3 weeks to estimate the antitumor effects and the survival fraction from the second day after the irradiation of proton beam in WERC.

2.4. Radiation by Carbon Beam (2 and 5 Greys) in National Institute of Radiological Sciences, National Institute for Quantum and Radiological Science and Technology (NIRS-NIQRST)

The Carbon beam in NIRS-NIQRST was started 36 hrs after i.p. administration of 3.5 μ M Compound-B into mice bearing C6 tumor. The carbon beam irradiation was 2-5 Gy against the each tumor. The irradiated mice were kept in the animal room of NIRS-NIQRST to measure 60 tumors over 10 days to estimate the antitumor effects.

2.5. Irradiation by Carbon Beam (0.2, 0.5, 1.0, and 2.0 Grey) in Takasaki Advanced Radiation Research Institute, National Institute for Quantum and Radiological Science and Technology (TARRI-NIQRST) 6 hrs after the incorporation of 10 μ M Compounds (-I, -II, -III, -IV=-B) in C6 Glial Tumor Cells

Cell dishes (ϕ = 6 cm) with C6 cells were irradiated at TARRI-NIQRST. Living cells were stained with 4.2% crystal violet solution in methanol one week post irradiation in TARRI-NIQRST. Colonies of living cells were counted by the colony formation method for estimation of the survival.

3. RESULTS AND DISCUSSION

3.1. Radiation by neutron beam in Research Reactor Institute of Kyoto University (RRIKU)

The tumor growth curves of the C6 glial tumor model were drawn for ^{10}B capture neutron capture therapy (BNCT) using Compound- ^{10}B (Figure 2). This compound carries four cages, each of 11 ^{10}B atoms, totally 44 boron atoms. The C6 tumor incorporated 2.04 ppm of ^{10}B elements during 36 hr after i.p. administration of 3.3 μM Compound-B into BALB-c-nu/nu mice transplanted with C6 cells. Tumors were irradiated for 20 min with a thermal neutron beam, final dose 0.105 Gy. The effect of BNCT is shown with blue curve (Figure 2) whereas irradiation only (no Compound-B) is shown red.

However, the best antitumor effect was achieved when combined with PDT with LD laser (657 nm, 150 J/cm^2) after BNCT (Figure 2, green dots). If tumor cells invaded the muscle no antitumor effect was detectable (black point bars close to the red curve). Then, we tried the local injection of 0.5 μM Compound- ^{10}B combined with or without PDT (bar 5; Figure 3). The exponential slopes extrapolated from the growth curves in Figure 2 were plotted in Figure 3. We observed good therapeutic effect in the case of local injection against the metastatic (bars 2 and 5) and/or non-metastatic tumors (bars 4 and 6). The advantage of the local injection is a higher concentration of the sensitizer in the tumor.

3.2. Radiation by proton beam in Wakasawan Energy Research Center (WERC)

Next, we studied the efficacy of proton particles at WERC. The sensitizing effect of porphyrin derivatives against the ionizing particle (proton or carbon) radiation has not been reported. Compound- ^{10}B was administered i.p. 36 hr prior tumor irradiation (Figure 4).

We found an enhancing effect of Compound-B (compare the dashed green line and the solid green line in Figure 4). Again, a combination of PDT after proton irradiation increased the antitumor effect (solid red curve, Figure 4) as reported in our patent [4].

3.3. Radiation by Carbon Beam (2 and 5 Gy) in National Institute of Radiological Sciences, National Institute for Quantum and Radiological Science and Technology (NIRS-NIQRST)

Six C6 tumors in BALB-c-nu/nu mice were irradiated after i.p. administration of Compound-B. At 5 Gy, Compound-B enhanced the antitumor effect 2.76 times more than in the absence of Compound-B (Figure 5).

Furthermore, the combination effect of PDT after the carbon radiation showed synergy of carbon beam irradiation although the number of tumors ($N=2$) was small [4].

3.4. Irradiation by Carbon Beam (0.2, 0.5, 1.0, and 2.0 Grey) in Takasaki Advanced Radiation Research Institute, National Institute for Quantum and Radiological Science and Technology (TARRI-NIQRST) 6 hrs after the incorporation of 10 μM Compounds (-I, -II, -III, -IV=-B) in C6 Glial Tumor Cells

We have requested for the synthesis of Compound-B derivatives (-I, -II, -III, and -IV=-B) to find out the most active element against the carbon beam at Nesmeyanov Institute of Organoelement Compounds in Moscow as shown in Figure 6.

Compound-I is 5,10,15,20-tetraphenylchlorin, Compound-II is 5,10,15,20-tetrakis(pentafluorophenyl)chlorin with $4 \times 4 = 16$ fluorine elements, and Compound-III is boronated derivative of 5,10,15,20-tetraphenylchlorin with 4 boron cages ($11 \times 4 = 44$ boron atoms). Compound-B has 44 boron-10 atoms and 16 fluorine atoms as shown in Figure 1.

C6 glial tumor cells were incubated with these derivatives medium solution to check the cytotoxicity in colony formation after the irradiation in TARRI-NIQRST. From the survival fraction curves of the cytotoxicity from the colony formation data, the exponential slopes of enhancing cytotoxicity irradiated by carbon beam with different compounds were plotted as shown in Figure 7.

The slope of Compound-II was 53 and that of Compound-III was 46 (Figure 7) which means that fluorination adds some therapeutic. Compound-B (IV in Figure 7) (16 F and 44 ^{10}B atoms) provided the most pronounced effect.

The enhancement of the efficacy of ionizing radiation by fluorine and boron atoms is very interesting. To our knowledge this is the first observation of sensitization to ion beam radiation, which might be applicable for the clinic.

4. CONCLUSION

In collaboration between Japanese and Russian scientists Compound-¹⁰B and its derivatives were generated. This compound demonstrated a striking potential in sensitizing the experimental tumors not only to PDT and BNCT but also to ionizing irradiation with carbon or proton particles. “Three enhancers in one molecule” allow for combining several therapeutic modalities, e.g., PDT and gamma rays in a compact laser instrument with a thermal neutron source or synchrotron. The newly discovered effect of tumor sensitization by fluorinated boron-containing chlorin derivative deserves further investigation as a promising background for clinical use of heavy ionizing particle radiation therapy.

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FIGURES

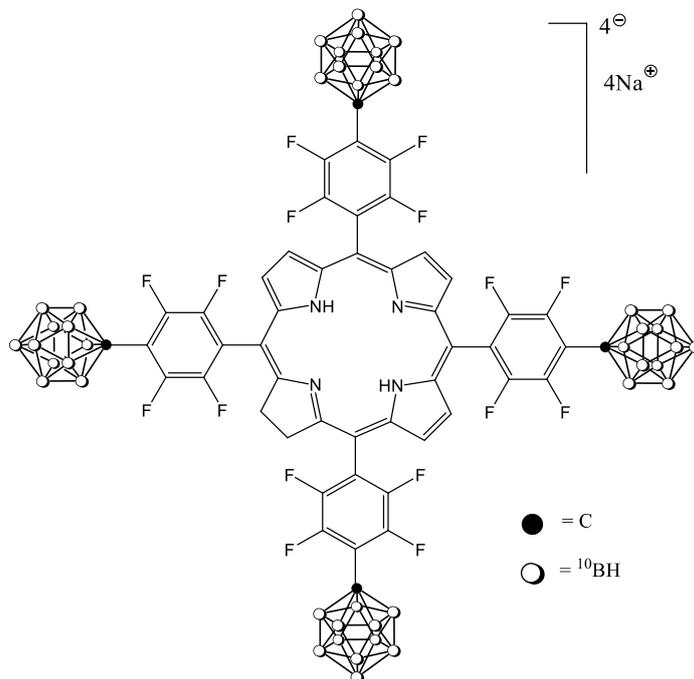


Figure 1. Structure of Compound-¹⁰B.

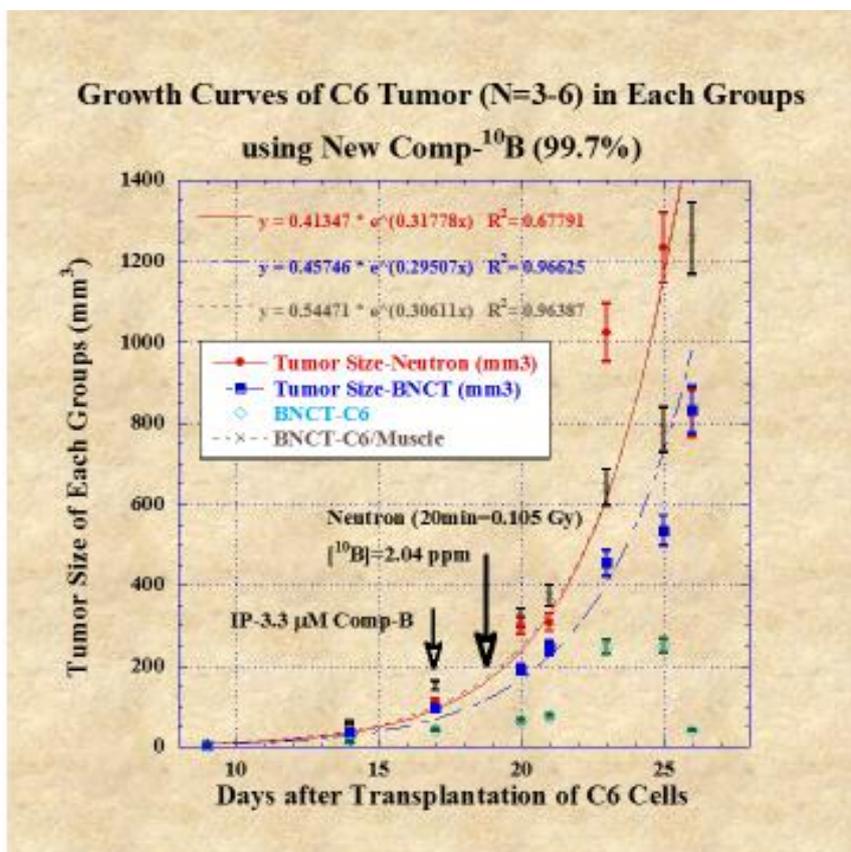


Figure 2. Growth Curves of C6 tumours after neutron radiation with or without PDT.

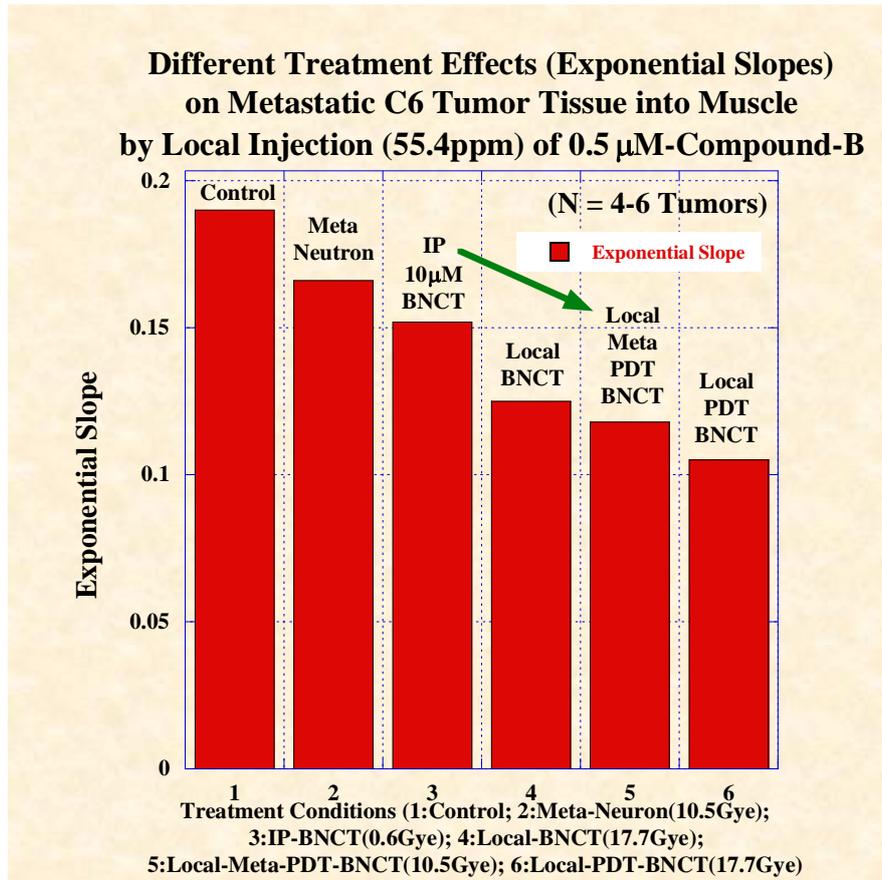


Figure 3. Different Treatments Effects (Exponential Slopes) on Metastatic C6 Tumor Tissue into Muscle by Local Injection (55.4ppm) of 0.5 μM-Compound-B

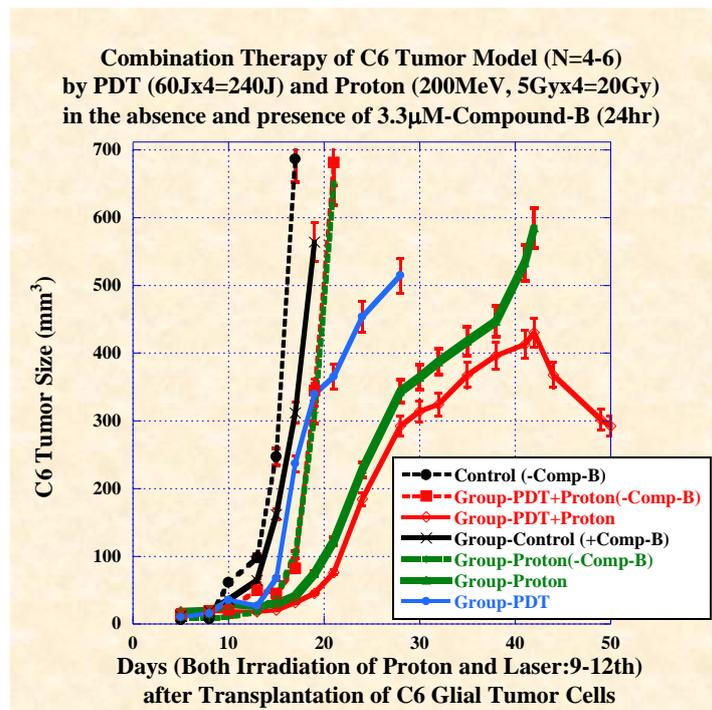


Figure 4. Growth Curves using Compound-¹⁰B Sensitizes for the C6 Tumors to irradiations of Protons Beam and Laser Light (PDT)

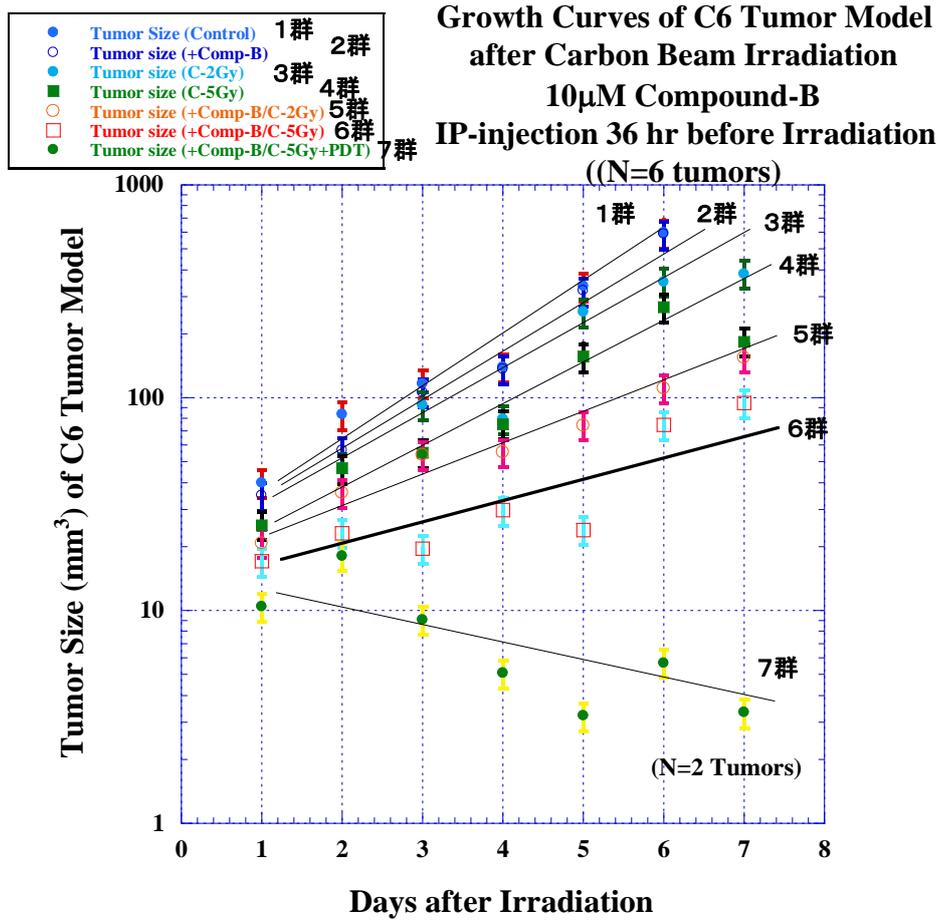


Figure 5. Growth Curves of C6 Tumors after Carbon Beam Irradiation (10 μ M Compound-¹⁰B i.p. 36 hr before Treatment)

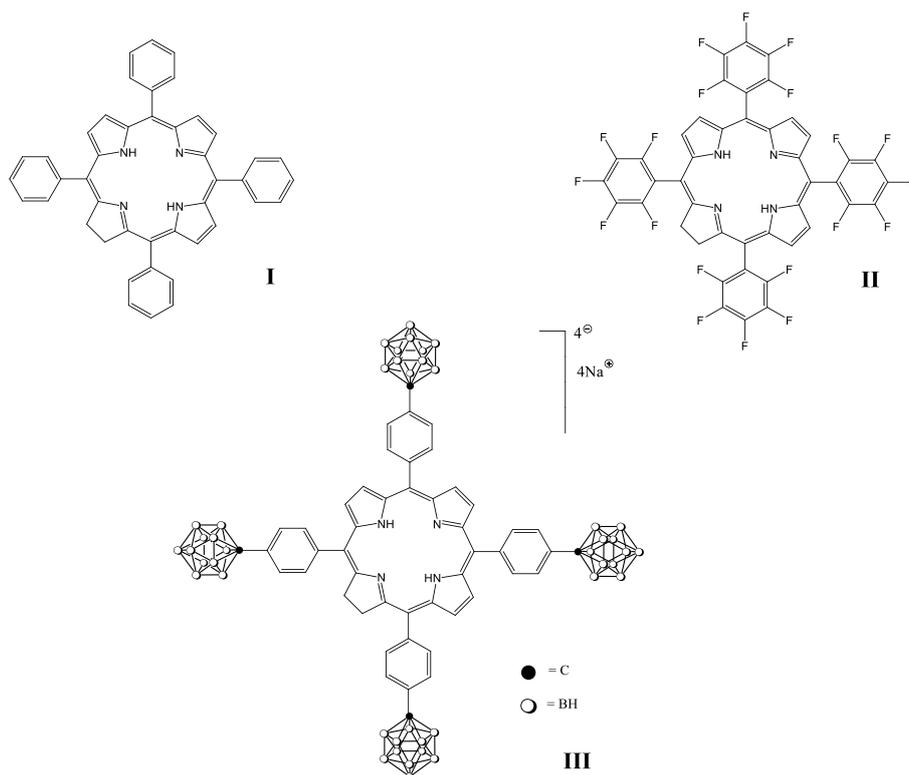


Figure 6. Chemical Structures of the other Compounds I-III to use in the Carbon Micro-Beams.

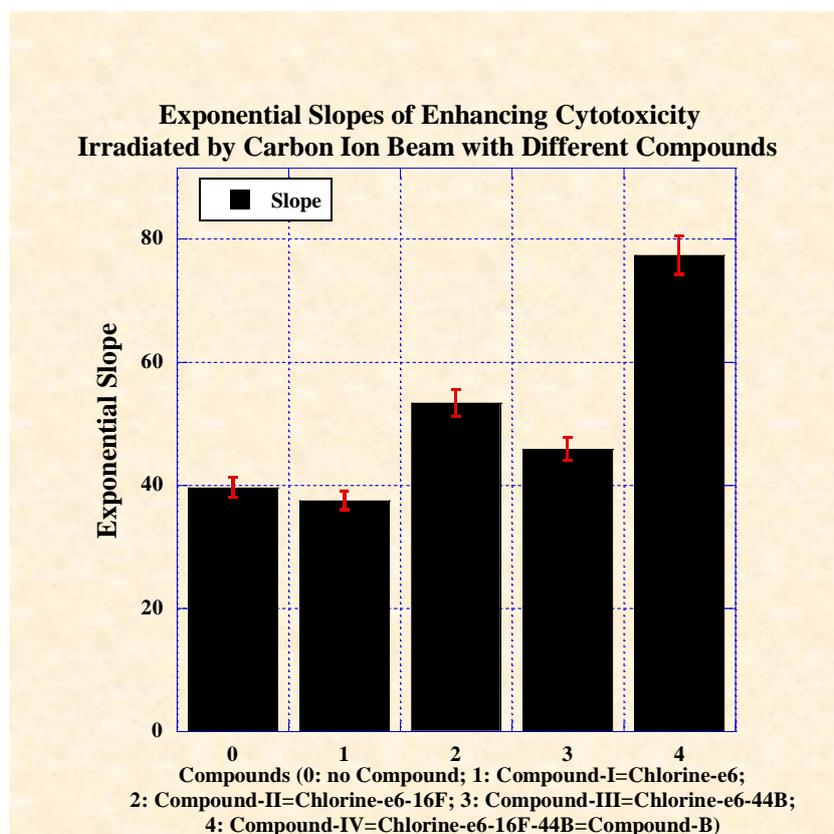


Figure 7. Exponential Slopes from the Each Survival Fractions of the Cytotoxicity. Irradiation with carbon beam in the presence of compounds I-IV.