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Butylamino-demethoxy-hypocrellins and photodynamic therapy decreases human cancer in vitro and in vivo

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Abstract

2-Butylamino-2-demethoxy-hypocrellin A (BAHA) and B (BAHB) are new photosensitizers synthesized by a mild reaction of hypocrellins and butylamine. In BAHA and BAHB, the peri-hydroxylated perylenequinone structure of the parent hypocrellins is preserved and the red absorption is enhanced distinctly. Electron paramagnetic resonance spin trapping measurements and 9,10-diphenylanthracene bleaching studies were used to investigate the photodynamic action of BAHA and BAHB in the presence of oxygen. Singlet oxygen ($^1O_2$) and superoxide anion radical ($O_2^{\bullet-}$) produced by illuminating BAHA and BAHB in aerobic solution have been observed. Compared with hypocrellin A and B, BAHA and BAHB primarily remained able to generate $^1O_2$ and enhanced distinctly the $O_2^{\bullet-}$-generating abilities. The photodynamic action of BAHA and BAHB in the therapy of cancer was investigated in vitro and in vivo. Both in vitro and in vivo results revealed a significant decrease in cancer cell growth. Laser or dye alone had no effect, indicating that intratumor BAHA and laser therapy may prove useful in unresectable cancer.

Keywords: Hypocrellin; Photodynamic therapy; Pancreatic adenocarcinoma cancer; Electron paramagnetic resonance

1. Introduction

Photofrin is the only photosensitizer to be approved by the US FDA so far [1]. The naturally occurring polycyclic quinones, hypocrellins (including hypocrellin A and B) (Fig. 1), have gained considerable attention because of their light-induced antitumor and antiviral activities, most notably against the human immunodeficiency virus, HIV [2,3]. The possible role of labile protons in the photodynamic activity of the polycyclic quinones was discussed in detail in the literature [4–7]. The hypocrellins exhibit several advantages over Photofrin, including ready preparation, easy purification, low aggregation tendency and rapid metabolism in vivo [8,9]. However, their low absorption in the photodynamic window (600–900 nm) limits their application in photodynamic therapy (PDT).

In the last 20 years, a variety of hypocrellin derivatives were demonstrated to be photodynamically active and efficient $^1O_2$ generators [10–14]. It was found that some amino-substituted hypocrellin derivatives reported by Z.J. Diwu possessed higher photo-

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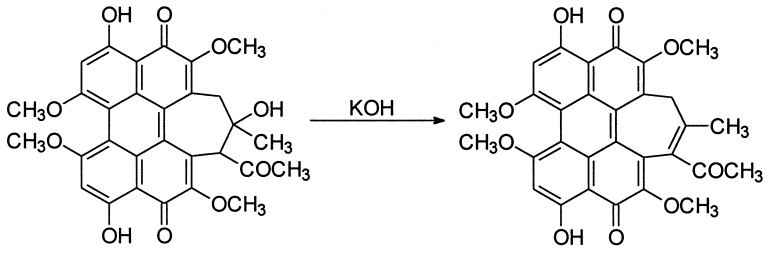


HA **HB**

Fig. 1. Structures of hypocrellin A and B.

dynamic activity than the other derivatives [15]. However, the original *peri*-hydroxylated perylenequinone structure of hypocrellin was modified by the amino substitution, which impeded the intramolecular proton transfer process.

In the present paper 2-butylamino-2-demethoxyhypocrellin A (BAHA) and B (BAHB) were synthesized by a mild method (Fig. 2), in which the perihydroxylated perylenequinone structure of parent hypocrellins was preserved and the photoresponse of the dye was enhanced markedly. Their photochemical and photobiological properties were studied. HeLa, MGC803 and human pancreatic tumor cell line Capan-1 cells were employed to investigate the phototoxicity of BAHA and BAHB in vitro, and the photodynamic therapy of transplanted human pancreatic cancer on nude mice in vivo was performed using BAHA as the sensitizer. Satisfactory results were obtained.

2. Materials and methods

2.1. Chemicals

HA was isolated from the fungus sacs of *Hypocrella bambusae* and recrystallized twice from acetone before use. HB was prepared by dehydration of HA in alkaline solution [16]. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone (TEMP), *p*-benzoquinone (PQ), 9,10-diphenylanthracene (DPA) and 1,4-diazabicyclo[2,2,2]-octane (DABCO) were purchased from Aldrich (Milwaukee, WI, USA). All the solvents were purchased from Beijing Chemical Plant (Beijing, PR China).

2.2. Measurements

Ultraviolet-visible (UV-Vis) absorption spectra were recorded with a Shimadzu UV-160A spectrophotometer. Infrared (IR) spectra were measured with a Perkin-Elmer 557 grating spectrophotometer. Mass spectra (MS) were conducted on FAB MS AEI-MS 50 Kratons and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a Varian XL-300 spectrophotometer.

2.3. Synthesis of BAHA and BAHB (Fig. 2)

Hypocrellin A or B (300 mg) was dissolved in freshly distilled pyridine (120 ml) containing *n*-butylamine (15 ml) and the resulting solution was stirred for 15 h at 50–55°C in the dark. The solvent was removed under reduced pressure. Then chloroform was added and the solution was washed with dilute hydrochloric acid several times until the pH value of the water layer was neutral. Chloroform was evaporated to afford a brown solid, which was separated by TLC on a 1–2% KH_2PO_4 silica gel plate using petroleum ether/ethyl acetate/95% ethanol (4:2:1 v/v/v) as eluent. The products BAHy (BAHA and BAHB) were obtained and identified by satisfactory data of ^{1}H-NMR, UV-Vis, IR and mass spectra.

2.4. Cyclic voltammogram measurement

Potentiostat/Galvanostat Model 283 from EG&G Instrument was used here. The counter electrode was a Pt spiral and the reference electrode a saturated calomel electrode (SCE). The supporting dielectric solution was 0.1 mol/l lithium perchlorate–acetonitrile solution.

2.5. Determination of active oxygen species generation

Electron paramagnetic resonance (EPR) spectra were recorded with a Bruker Model ESP 300E spectrometer at room temperature. Samples were introduced into the specially made quartz cup and illuminated directly inside the microwave cavity. All samples were purged with purified O_2 for 30 min in the dark and irradiated directly in the cavity of the EPR spectrometer with a Q-switched Nd:YAG nanosecond laser apparatus (full width at half-maximum, 35 mJ pulse^{-1}; $\lambda = 532$ nm).

The EPR measurement of spin trapping by TEMP was employed to determine the formation of $^1O_2$ by the sensitizers [17]. Typically, the reaction solution consisted of 0.1 mM sensitizer and 20 mM TEMP. The DPA bleaching method was used to confirm the $^1O_2$ generation quantum yields further; the details have been described in a previous report [18]. The photo-oxidation of DPA sensitized by a sensitizer was carried out on a 'merry-go-round', in which the samples were illuminated by 436 nm light. These two methods gave consistent results.

The EPR measurement of spin trapping of DMPO was used to detect the generation of $O_2^{\bullet -}$ by BAHy [19]. Typically, the reaction solution consisted of 1 mM BAHy and 40 mM DMPO.

2.6. HeLa and MGC803 cell survival studies

Cell survival was estimated by the MTT assay [20,21]. Cells were transferred to flat-bottomed 96-well plates, 100 μl in each well containing 1000 cells. Twenty hours later, 10 μl of MTT solution (10 mg/ml in fetal bovine serum (PBS)) were introduced into the wells for 4 h at 37–38°C. The wells were drained carefully and 150 μl DMSO were added per well to prevent MTT reduction and to dissolve the blue formazan crystals produced by mitochondrial hydrogenases in living cells only. The plate was shaken at room temperature for 10 min and read immediately at 595 nm on a Bio-Rad model 3550 microplate reader. Samples of BAHA were measured in 12 replicates and each experiment was repeated at least twice. Survival of PDT-treated cells was normalized against cells incubated with photosensitizer alone. The dark toxicity was assessed separately, and a similar procedure for cell exposure to graded doses of the photosensitizers for 4 h was followed.

2.7. Capan-1 cell survival study

Human pancreatic tumor Capan-1 cells were presented by the Department of Visceral and Transplantation Surgery, University of Bern, Switzerland. The cells were cultured in RPMI 1640 medium supplemented with 5% FBS, 100 μg/ml of penicillin, 100 μg/ml of streptomycin at 37°C in a Forma Scientific water-jacketed 5% carbon dioxide incubator.

Exponentially growing cells in 35 mm dishes (NUC) were incubated with increasing concentrations of BAHB from 2×10^{-6} M to 16×10^{-6} M in RPMI 1640 medium (FBS-free) for 4 h. The cells were irradiated with different doses of light from 2 J/cm^2 to 16 J/cm^2. The control group contained cells without laser treatment. A second group of controls underwent treatment with He-Ne632.8 laser therapy without addition of BAHB. The light source was a Red light Treatment Instrument (Institute of Electronics, Academia Sinaca China); its total power output was more than 90% of the ampul. After irradiation, the cells were placed in RPMI 1640 medium containing 5% FBS and incubated for 24 h before survival assessment. Cells survival was estimated by the MTT assay as mentioned above.

2.8. Animal model

Six-week-old athymic mice were purchased from the Institute of Animal Model, Chinese Academy of Medical Sciences and quarantined for 4 days in filter top cages. Twelve hour light/dark cycles were maintained in the mice facility. Mice had free access to water and rodent chow at all times during the experiment. The mice consisted of females and males and weighed between 16 and 18 g.

2.9. Intratumor photodynamic therapy of subcutaneous and transplanted human pancreatic tumor nodules

2.9.1. Subcutaneous tumor implantation

Capan-1 cells were trypsinized and washed twice with RPMI 1640 before resuspension in medium and

counted on a hemocytometer. Mice underwent subcutaneous injection of 1 million cells in 200 μl of RPMI 1640 in the underbelly. Forceps were used to pinch the skin closed after injection of Capan-1 cells to prevent leakage of cells. Mice were maintained for 4 weeks after implantation of human pancreatic cancer cells to allow growth of tumor nodules.

2.9.2. Animal grouping

When the nodule grew up to approx. 0.2 cm^3, the nude mice were grouped stochastically: group A: no treatment was applied to the nude mice; group B: only laser light was applied to the nude mice; group C: photodynamic therapy with HA and laser light was applied to the nude mice; group D: the same as group C but HA was replaced by BAHA.

2.9.3. Intratumor photodynamic therapy of subcutaneous human pancreatic tumor nodules

After a 1.5 month incubation period and growth of human pancreatic cancer nodules, a subset of mice were randomly injected with DMSO solution of dye 30 mg/kg and kept in the dark for 2 h. Light generated by a He-Ne pumped dye laser (632.8 nm) was supplied on the sole tumor area via a quartz fiber, which delivered 100 mW. Mice underwent intraperitoneal injections of 0.20 ml of anesthesia consisting of 1.0 ml 70% ethanol, 6.0 ml distilled water, and 1.6 ml sodium pentobarbital as previously reported [22]. Tumor nodules were measured in three dimensions by calipers. A minilaparotomy was performed in the upper abdomen to allow direct measurement of intrapancreatic tumors prior to injection of sensitizer. The measurements obtained at the 21 day interval served as the control size. Tumors received one dose of 120 J each of laser light for 20 min set at 100 mW. Mice were allowed to recover after photodynamic therapy treatments. Mice were sacrificed 21 days after laser treatment and tumors measured by calipers in three dimensions.

3. Results and discussion

3.1. Characteristics of BAHA and BAHB

Amination of hypocrellins (including HA and HB) in pyridine afforded the aminated hypocrellins BAHy (Fig. 2). The chemical structure of hypocrellins suggests three possible reaction sites, such as the aromatic ring, quinonoid carbonyl groups and the attached ring. Based on UV, IR, ^{1}H-NMR and MS data, the aminated hypocrellins were identified to be 2-butylamino-2-demethoxy HA (BAHA) and HB (BAHB). For BAHA: FAB MS (M^+, 587); IR cm^{-1} (KBr, ν_{max}): 3419, 2922, 1680, 1608; UV-Vis: ([$CDCl_3$] λ_{max}, nm [log ε]): 466 (4.05), 577 (4.12), 641 (4.06); ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 1.00 (3H, t, CH_3-22), 1.49 (2H, m, CH_2-21), 1.68 (2H, m, CH_2-20), 1.79 (3H, s, 16-CH_3), 1.91 (3H, s, CH_3-18), 2.55 (1H,d, H-13a), 3.08 (1H,d, H-13b), 3.60 (2H, m, NCH_2), 4.01 (3H, s, OCH_3-6), 4.08 (3H,

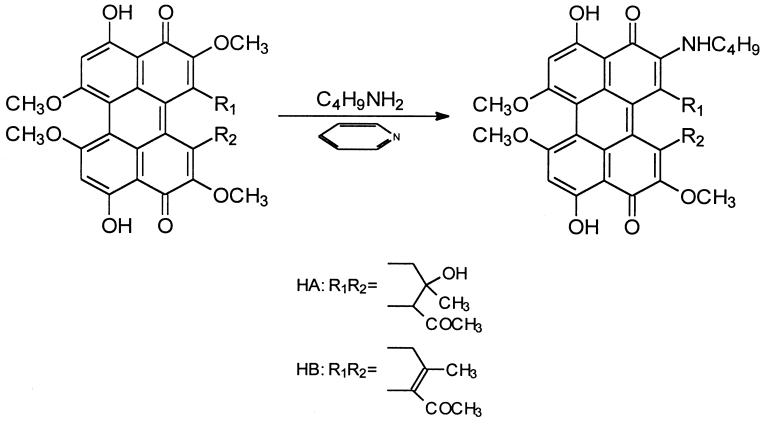


Fig. 2. Pathway for the amination of hypocrellin A and B.

s, OCH_3-7), 4.15 (3H, s, OCH_3-11), 6.32 (1H, s, H-5(8)), 6.80 (1H, s, H-8(5)), 15.78 (1H, OH-9(10)), 16.07 (1H, OH-3(4)); for BAHB: FAB MS (M^+, 569); IR cm^{-1} (KBr, ν_{max}): 3340, 2922, 1706, 1600; UV-Vis: ([$CDCl_3$] λ_{max}, nm [log ε]): 463 (4.06), 583 (4.09), 621 (4.10); ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 1.07 (3H, t, CH_3-22), 1.50 (2H, m, CH_2-21), 1.78 (3H,s, CH_3-16), 1.95 (2H, m, CH_2-20), 2.34 (3H, s, CH_3-18), 2.80 (1H, d, H-13a, J_{AB} = 11.5), 3.70 (2H, m, NCH_2), 3.90 (1H, d, H-13b, J_{AB} = 11.5), 4.05 (6H, s, OCH_3-6,7), 4.09 (3H, s, OCH_3-11), 6.38 (1H, s, H-5(8)), 6.60 (1H, s, H-8(5)), 15.8 (1H, OH-9(10)), 16.1 (1H, OH-3(4)).

3.2. Absorption spectra

Hypocrellins exhibited three absorption bands at 450 nm, 535 nm, and 584 nm in the region of the wavelength between 400 and 800 nm. When an electron-donating butylamino group was introduced into the hypocrellin molecules, the longer wavelength absorption band at 584 nm was enhanced markedly and a new band at 628 nm appeared, which expanded the photoresponse of hypocrellins that was essential for the photodynamic agents. The characteristics of the absorption spectra of BAHy are depicted in Table 1.

Table 1
Absorption spectra characteristics of hypocrellins and their aminated hypocrellins (in chloroform)

Compound	Absorption wavelength (nm) [log ε]
HA	465 [4.46], 539 [4.12], 580 [4.11]
HB	466 [4.36], 548 [3.70], 580 [3.52]
BAHA	466 [4.05], 577 [4.12], 641 [4.06]
BAHB	463 [4.06], 583 [4.09], 621 [4.10]

3.3. EPR measurements of active oxygen species generated during the photosensitization of BAHA and BAHB

3.3.1. Generation of singlet oxygen species

Illumination of BAHy (0.1 mM) containing 10 mM TEMP, an efficient $^1O_2$ tripper [17], in an oxygen-saturated DMSO solution with 532 nm laser at room temperature led to the generation of a strong EPR signal (Fig. 3). The intensity of the EPR signals increased rapidly during photoirradiation and decreased very slowly in the dark and it depended on the concentration of the sample and the illumination time. As shown in Fig. 3, an EPR spectrum of three equal intensity lines, characteristic of a nitroxide radical, was observed. The g factor and hyperfine splitting constant (a_N = 13.8 G) of the EPR signals were

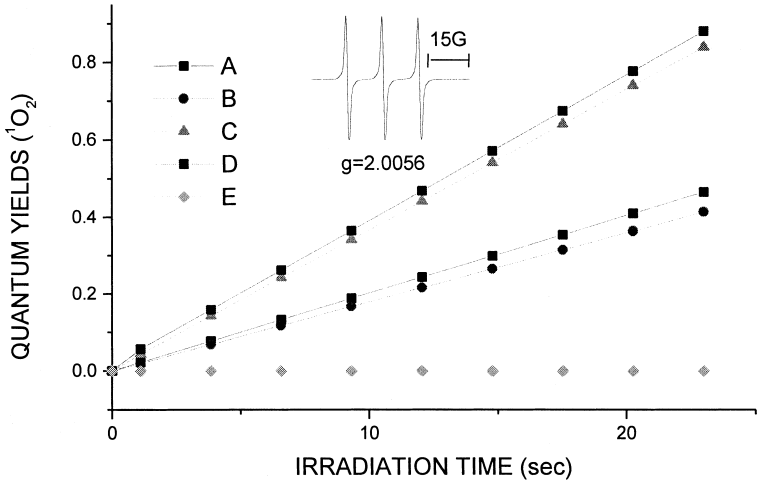
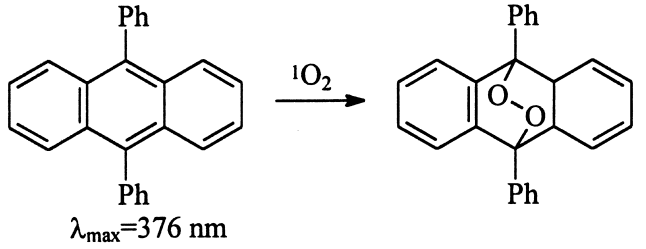


Fig. 3. Intensity of the spin adduct TEMPO against duration of laser (532 nm excitation) exposure for different sensitizers in aerobic chloroform. A, BAHA (0.1 mM); B, BAHB (0.1 mM); C, HA (0.1 mM); D, HB (0.1 mM); E, in the presence of DABCO (0.5 mM). Spectrometer settings: microwave power, 5 mW; modulation amplitude, 10 G; time constant, 0.128 s.

found to be same as those of authentic TEMPO [17]. Therefore, the EPR signals generated during the irradiation of the BAHy solution containing TEMP were attributed to TEMPO. Thus, $^1O_2$ should be implicated in the formation of TEMPO. The EPR signals were suppressed by $^1O_2$ scavengers, such as DABCO, further confirming that TEMPO was derived from the reaction of TEMP with $^1O_2$ generated by the irradiation of BAHy. Control experiments indicated that BAHy, oxygen and light were all essential for the formation of TEMPO, indicating that the formation of the nitroxide radical was a photodynamic process. The quantum yields of $^1O_2$ generation (Table 2) were further determined by the DPA bleaching method with HB as the reference ($\Phi_{^1O_2} = 0.76$) [18], as well as by EPR measurements.



(1)

The reaction was followed spectrometrically by observing the decrease of the absorbance of DPA at 376 nm (where HA, HB and BAHy all have the lowest absorption) as a function of irradiation time.

Table 2
Quantum yields of $^1O_2$ generation by hypocrellins and their aminated hypocrellins

Compound	Quantum yield ($^1O_2$)
HA	0.80
HB	0.76
BAHA	0.46
BAHB	0.44

3.3.2. Generation of superoxide anion radical

Illumination of an oxygen-saturated DMSO solution of BAHy (0.1 mM) and DMPO (40 mM) with laser ($\lambda = 532$ nm) in the EPR cavity gave rise to a typical $3\times2\times2$ EPR spectrum (Fig. 4), which was the characteristic of the DMPO/$^{\bullet}$OOH radical adduct [19]. In the presence of *p*-benzoquinone, which acted as an $O_2^{\bullet-}$ quencher [23], the EPR signals were inhibited, which confirmed the formation of superoxide anion radical detected by the DMPO spin trap. Fig. 4 shows that the generation ability of superoxide of BAHy was at least 3 times as effective as that of the parent hypocrellins. As we know, $O_2^{\bullet-}$ could be produced by hypocrellins via the reaction shown in Eqs. 2, 3 and 4. When the butylamino group was introduced into the hypocrellin molecule, the oxidation potential of the dyes decreased to 0.83 V (BAHA) and 0.81 V (BAHB) from 1.35 V (HA) and 1.32 V (HB) compared with their parent compounds (Table 3), which made the electron transfer

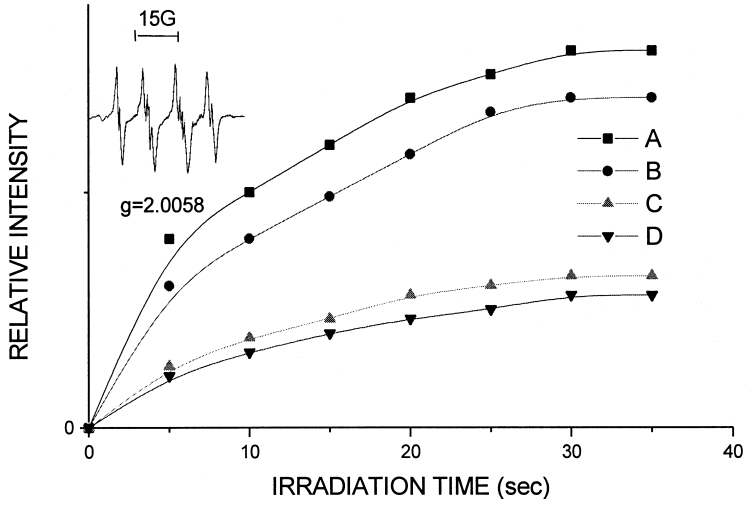


Fig. 4. Intensity of the spin adduct superoxide during irradiation of a DMSO solution of hypocrellins in the presence of DMPO (50 mM). A, BAHA (0.1 mM); B, BAHB (0.1 mM); C, HA (0.1 mM); D, HB (0.1 mM). Spectrometer settings are the same as in Fig. 3.

Table 3
Relevant redox potentials and singlet state energy of hypocrellins and their aminated hypocrellins

Compound	E_{OX} (V)	E_{Re} (V)	E_{0-0} (eV)
HA	1.35	−0.58	1.84
HB	1.32	−0.53	1.82
BAHA	0.83	−0.53	1.63
BAHB	0.81	−0.51	1.60
O_2		−0.50	

readily from the excited state of BAHy to the oxygen molecule as shown in Eq. 5 ($\Delta G = -0.30$ eV < 0), while for hypocrellins this is not allowed thermodynamically ($\Delta G > 0$). It may explain the enhancement of the $O_2^{\bullet -}$ generation ability of BAHy.

$$BAHy + h\nu \rightarrow BAHy^* \quad (2)$$

$$BAHy^* + BAHy \rightarrow BAHy^{\bullet +} + BAHy^{\bullet -} \quad (3)$$

$$BAHy^{\bullet -} + O_2 \rightarrow BAHy + O_2^{\bullet -} \quad (4)$$

$$BAHy^* + O_2 \rightarrow BAHy^{\bullet +} + O_2^{\bullet -} \quad (5)$$

3.4. HeLa and MGC803 cell survival studies

Some earlier studies have reported the photopotentiation factors (LD_{50} dark/LD_{50} light) of parent hypocrellins and their 16 new derivatives, in which HA was characterized by 4-fold photopotentiation [24]. The photopotentiation factors of HA and BAHA were studied by Zhang Zhiyi et al., our co-workers, who chose a dose of 4 J/cm^2 red light for further studies, since at this dose HA also demonstrated about 4-fold photopotentiation in our study as described above. The cell survival studies of HA and BAHA have been reported [25]. The MGC803 and HeLa cancer cells were employed to study the photobiological properties of HB and its aminated derivative. BAHB exhibited much higher phototoxicity than their parent hypocrellins, with 50% cell killing at a concentration of 0.25–0.5 μM at a light dose of 4 J/cm^2. Therefore BAHy were characterized by 100–200- and 200–400-fold photopotentiation factor, respectively. The detailed results, which are shown in Table 4, qualified BAHy as more ideal photodynamic therapeutic anti-cancer agents than their parent hypocrellins.

3.5. Human pancreatic cancer in vitro and in vivo

Pancreatic adenocarcinoma remains one of the devastating neoplasms of the gastrointestinal tract. The incidence of pancreatic cancer is nine per 100 000 and has remained steady since 1973 [26,27]. With an overall median survival of 6 months and a discouraging 5 year survival rate after radical surgery, the prognosis in pancreatic cancer remains pessimistic [28,29]. Adjunctive treatment to pancreatic resection has extended life approx. 9 months [30]. Intraoperative radiotherapy in conjunction with resective surgery has reported 5 year survival rates of 15.3–20.2% [31]. The search for other treatment modalities may alter the outcome of patients with pancreatic malignancies. Owing to the relatively selective targeting of neoplastic tissue reported in the literature [32], PDT was regarded as a promising method. We hypothesized that BAHy and laser phototherapy would decrease pancreatic cancer growth.

3.5.1. Capan-1 cell survival study

Without light illumination BAHB did not affect tumor cell growth distinctly. With red light stimulation, BAHB appeared effective in a dose-dependent manner from 0 to 16×10^{-6} mol/l (above 90% inhibition in three cell lines) (Fig. 5). The survival rate of

Table 4
Results of the cell survival studies of hypocrellins and their aminated hypocrellins

Compound	Dark toxicity LD_{50} (μM)	Phototoxicity LD_{50} (μM)	Photopotentiation factors
HA	17	4.25	4
HB	20	2	10
BAHA	100	0.25–1.0	100–200
BAHB	> 100	0.25–0.5	200–400

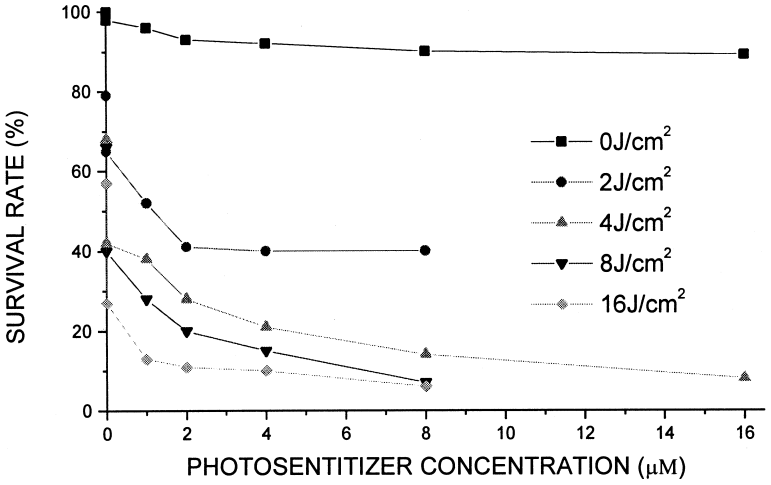


Fig. 5. Effects of increasing concentration of BAHB on Capan-1 cell survival of irradiated pancreatic tumor cells.

PDT for the Capan-1 cells decreased along with the increase of the concentration of BAHB and dose of light. Under low concentration of BAHB the curve declined rapidly, and slowed down to reach a plateau. An early and strong necrosis of Capan-1 cells in vitro was achieved after PDT at 16 J/cm^2, in spite of a relatively low dye concentration (2×10^{-6} mol/l).

3.5.2. *Intratumor photodynamic therapy of subcutaneous human pancreatic tumor nodules*

3.5.2.1. Growth of pancreatic tumor nodules. Incubated with pancreatic cells, the nodules appeared and accrued rapidly after 1 week incubation period. When the nodule grew up to approx. 0.2 cm^3, its volumes were listed and the nude mice were distributed for further treatment.

3.5.2.2. Intratumor photodynamic therapy of subcutaneous human pancreatic tumor nodules. Fig. 6 shows that there were no perceptible differences between the nude mice ($P>0.05$) before treatment. The nodule volumes were listed after 3, 7, 14 and 21 day photodynamic therapeutic treatment, respectively. Upon exposure to laser light, the nodules of the nude mice that had undergone BAHA injection decreased to some extent. Differences in nodule size became notable as a function of time. The data showed a significant decrease in the growth of pancreatic nodules. Fig. 7 displays the nude mice with orthotopically transplanted pancreatic cancer undergoing intratumor photodynamic therapy after direct injection of BAHA compared with those without treatment. A control experiment indicated that light, sensitizer and oxygen were all important for the photodynamic therapy.

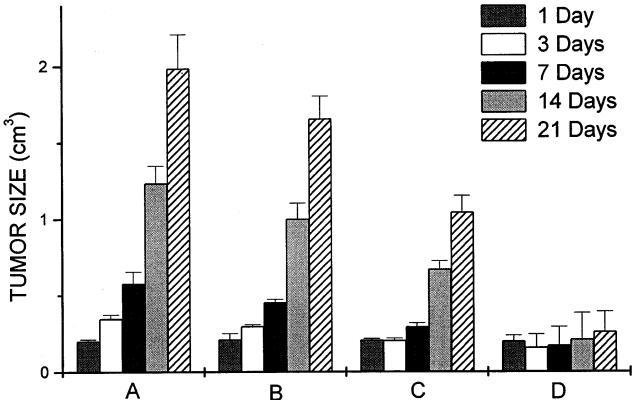


Fig. 6. Intratumor photodynamic therapy of subcutaneous and orthotopic human pancreatic tumor nodules in vivo. A, no treatment was applied to the nude mice; B, only laser light was applied to the nude mice; C, photodynamic therapy with HA (30 mg/kg) and laser light was applied to the nude mice; D, the same as C but HA was replaced by BAHA (30 mg/kg). Settings: $\lambda=632.8$ nm; optical energy density: 120 J/cm^2; illumination area: 2 cm^2; illumination mode: ambulating above the center of the tumor.

4. Conclusion

A simple and mild amination pathway was employed to synthesize 2-butylamino-2-demethoxy-hypocrellin A and B (BAHy). In BAHy molecules the peri-hydroxylated perylenequinone structure of the parent hypocrellin was preserved, which may play an important role in their photodynamic action [4–7]. Moreover, when an electron-donating butylamino group was introduced into hypocrellin molecules, it not only expanded the photoresponse of hypocrellins, but also enhanced their ability to generate

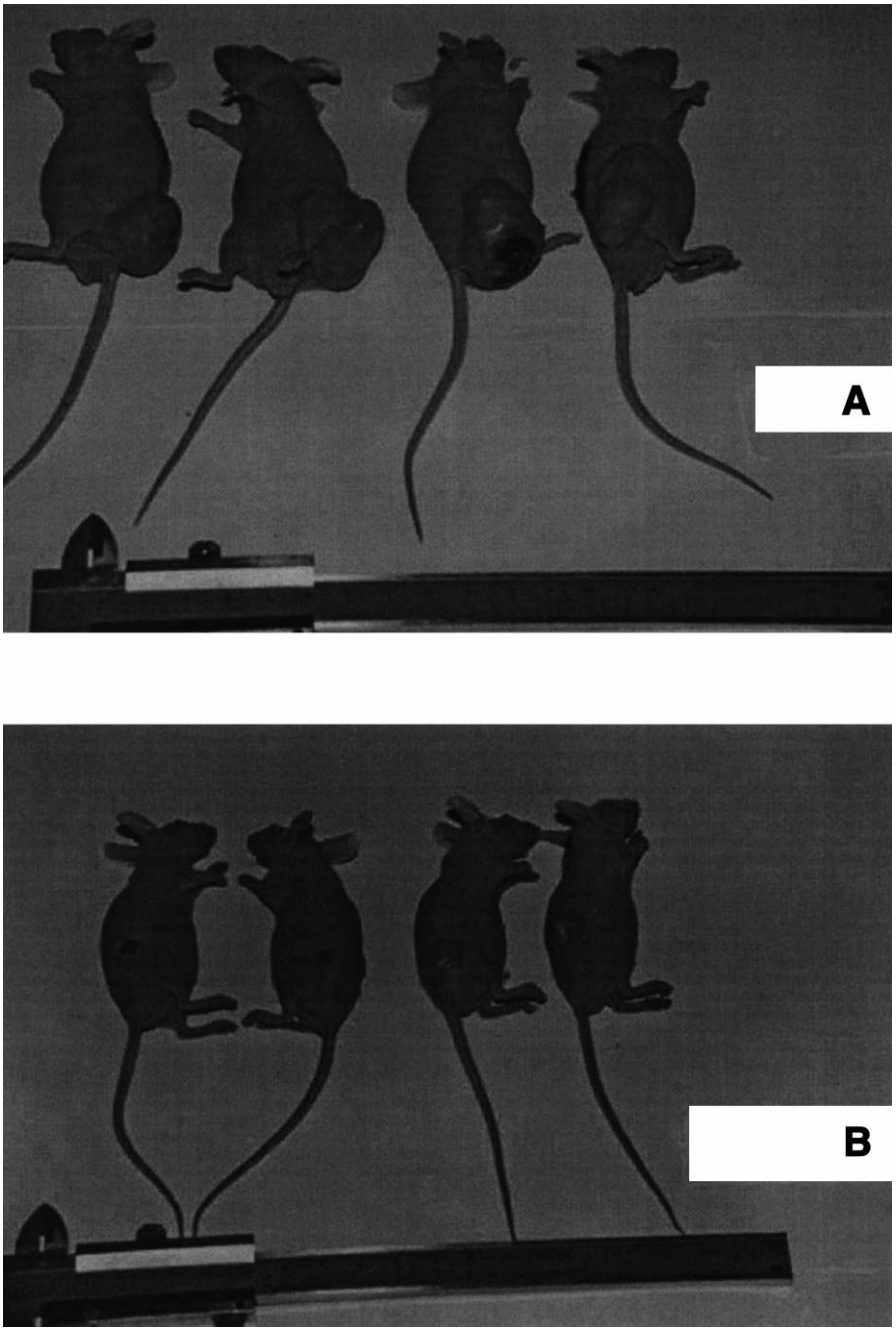


Fig. 7. Nude mice with orthotopically transplanted pancreatic cancer undergoing (A) no photodynamic therapy, (B) intratumor photodynamic therapy after direct injection of BAHA.

$O_2^{\bullet-}$. Both in vitro and in vivo results revealed that BAHy exhibited much higher photodynamic activity than their parent hypocrellins. It is suggested that the type I mechanism played a significant role in photodynamic therapy as well as the type II mechanism. All the results qualified the aminated hypocrellins as more potent photodynamic therapeutic agents than their parent hypocrellins.

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