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Hypocrellin derivative with improvements of red absorption and active oxygen species generation

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Abstract—A novel diamino-substituted hypocrellin derived from hypocrellin B (HB) was synthesized by a mild method. The red absorption of the resulting product was significantly enhanced relative to the parent hypocrellins and any other hypocrellin derivatives, and the active oxygen species generating abilities were enhanced distinctly, which will remarkably improve its photodynamic therapy effectiveness.

In the treatment of cancer, one protocol that can better differentiate cancerous and normal tissue is photodynamic therapy (PDT). PDT combines light and endogenous oxygen with a photosensitizer localized in or around the tumor.

Photofrin® is the only photosensitizer to be approved by the US FDA so far.[1] Some properties of Photofrin, including undesirable side effects, could be improved upon and much work has been devoted to develop new photosensitizers.[2–4]

The naturally occurring polycyclic quinones, hypocrellins, have gained considerable attention because of their light-induced antitumor and antiviral activity, most notably against the human immunodeficiency virus, HIV.[5,6] Diwu et al. reported that the perylenequinone dyes showed certain tumor-selective cell-killing in the primary tests both in vitro and in vivo.[4,7–9] Potentially drug selectivity can be further enhanced via the strategy that is based the lower pH value characteristic of tumor cells than normal cells. It was found that hypocrellins and their derivatives display much higher resistance to photobleaching at lower pH value in the range characteristic of tumors. Upon irradiation the phenolic hydroxyl groups of hypocrellins become much more susceptible to dissociation into a nonphototoxic anionic species (vide supra). This ionization occurs more readily in normal tissues where pH value is higher on average than in tumors. These two factors have a great potential for enhancing the relative concentration and lifetime of the hypocrellins in tumors over those in normal tissues.

However, hypocrellins exhibit little absorption in the photodynamic window (600–900 nm), which limits their applications in PDT.

In recent 20 years, many hypocrellin derivatives have been synthesized,[10–14] but with few promising results. We have attempted to prepare the primary amino-substituted hypocrellins, because quantum chemistry calculations predicted that these compounds might have much improved absorption in the photodynamic window.

To overcome these limitations and to extend the photoresponse of the PDT agents, an electron-donating alkylamino group was introduced into the peri-hydroxylated perylenequinone ring of HB, which made the intramolcular charge transfer (ICT) readily between the alkylamino group and the carbonyl group in HB. The ICT distinctly red-shifted the absorption spectra of the sensitizers.[15,16] While, the addition of an alkylamino group decreases the generation yield of singlet oxygen in the dyes photosensitization process.

This paper describes a useful method by which the photoresponse of the dye is enlarged remarkably, and the product exhibits much higher singlet oxygen generating abilities than the other amino-substituted

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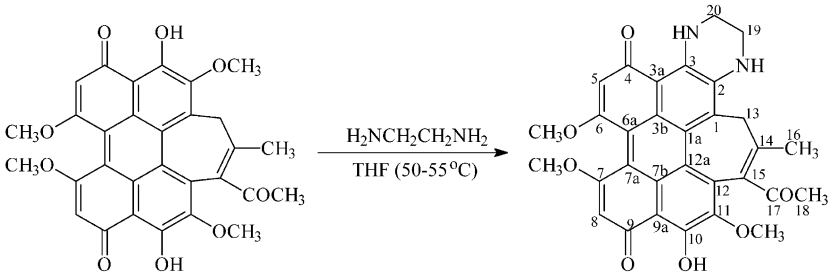

Figure 1. Pathway for the ethyldiamino-substituted hypocrellin B (EDAHB).

hypocrellins. The reaction of HB and ethylenediamine occurred at 50 °C with good selectivity. The chemical structure of hypocrellin suggests multiple possible reaction sites, such as the aromatic ring, quinonoid carbonyl groups[15] and the attached ring.[17] The yields in previous researches were rather low due to polymerization during the reaction at high temperature or in strongly basic media. The current one-step method for the amino substitution described in Figure 1 has a high yield, which is also noteworthy.

Comparison of ^{1}H NMR spectra from the parent HB, the product showed only three methoxy groups. The quinonoid carbonyl groups were retained in the amino-substituted HB as evidenced by its IR spectrum, the band 1708 cm^{-1} was retained, and its ^{13}C NMR, the 204.0 ppm peak was retained, which was the characteristics of carbonyl group in the acetyl group at 17-position in hypocrellin. Its molecular ion peak, UV–vis spectrum, ^{13}C NMR data and element analysis were used to assign its structure as 2-demethoxy-2,3-ethylenediamino HB (**EDAHB**).

EDAHB showed a very strong broad absorption band at 640 nm with $\varepsilon = 36{,}300\ M^{-1}cm^{-1}$, which is much stronger than any other hypocrellin derivatives (Fig. 2). Moreover, **EDAHB** was tested using spin trapping EPR technique[18,19] and DPA-bleaching method[20] to determine whether they possessed photodynamic activities. The results indicated that they displayed strong EPR signals due to the singlet oxygen and superoxide anion radicals, which are essential for a potent photodynamic agent. The $^{1}O_2$-generation quantum yield of **EDAHB** was determined to be 0.82, which was much higher than any other amino-substituted hypocrellins. The $O_2^{\cdot -}$ generation quantum yield of **EDAHB** was at least 2.5 times as effective as that of the parent hypocrellin B. All these results qualify **EDAHB** as more potent photodynamic therapeutic anticancer agent than its parent hypocrellins. The cellular and animal studies are ongoing and satisfactory results are expected.

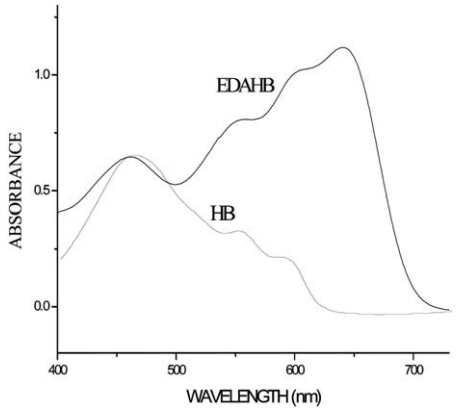

Figure 2. Absorption spectra of HB and ethylenediamino-substituted HB (EDAHB).

1. Experimental

1.1. Synthesis of EDAHB

HB (200 mg) was dissolved in fresh distilled tetrahydrofuran (250 mL) containing ethylenediamine (20 mL). The resulting solution was stirred for 12 h at 50 °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 black solid, which was separated by column chromatography on silica gel using ethyl acetate as eluent. The product **EDAHB** was obtained with the yield of 56% and identified by ^{1}H NMR, ^{13}C NMR, UV–vis, IR, mass spectra and elemental analysis. Data for **EDAHB**: UV–vis ([$CHCl_3$], λ_{max}, nm [logε]): 462 [4.34], 555 [4.43], 605 [4.53], 641 [4.56]; IR ([KBr], ν_{max}, cm^{-1}): 3384, 2928, 1708, 1606, 1513, 1452; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.58 (3H, s, 16-CH_3), 2.30 (3H, s, 18-CH_3), 3.60–3.80 (4H, m, N-CH_2), 3.95–4.15 (9H, 3s, 6,7,11-OCH_3), 5.10 (NH), 5.20–6.18 (2H, 13-CH_2), 6.32 (1H, s, 5(8)-H), 6.42 (1H, s, 8(5)-H), 11.90 (1H, 3-NH), 16.94 (1H, 10-OH); ^{13}C NMR (300 MHz, $CDCl_3$, δ, ppm): 27.4 (C-16), 29.2 (C-18), 38.2 (C-19), 39.2 (C-20), 55.4 (7-OCH_3), 55.8 (6-OCH_3), 61.0 (11-OCH_3), 101.2 (C-5), 102.8 (C-9a), 105.8 (C-8), 107.5 (C-3a), 115.4 (C-6a), 117.8 (C-7a), 119.4 (C-1a), 120.8 (C-13), 121.6 (C-12a), 134.5 (C-1), 135.1 (C-12), 142.7 (C-2), 145.2 (C-11), 164.5 (C-7b), 165.0 (C-3b), 168.2 (C-6), 168.2 (C-7), 184.3 (C-10), 185.4 (C-4), 185.4 (C-9), 204.0 (C-17); *m/z* (FAB-MS) 539.1802 (M + 1. $C_{31}H_{27}N_2O_7$ requires 539.1812). Anal. cacld for $C_{31}H_{26}N_2O_7 \cdot 2H_2O$: C, 64.80; H, 5.27; N, 4.87. Found: C, 64.48; H, 5.35; N, 4.68.

1.2. Determination of active oxygen species generation

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 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 **EDAHB**.[18] Typically, the reaction solution consisted of 0.1 mM **EDAHB** and 20 mM TEMP. The DPA bleaching method was used to confirm the $^1O_2$-generation quantum yields further; the details were described in a previous report.[20] The photo-oxidation of DPA sensitized by 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 $O_2\cdot^-$ by **EDAHB**.[19] Typically, the reaction solution consisted of 1 mM **EDAHB** and 40 mM DMPO.

Acknowledgements

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