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Synthesis of demethoxy-amino-substituted hypocrellins: novel photosensitizers for photodynamic therapy

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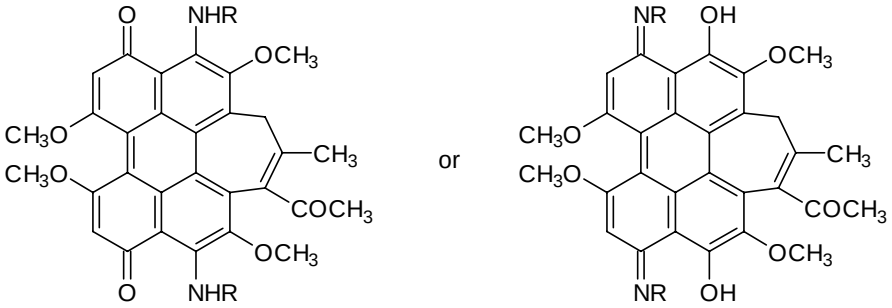
Abstract: A novel method was used to obtain demethoxy-amino-substituted hypocrellin derivatives. *Peri*-hydroxylated perylenequinone structure of hypocrellin was reserved when hypocrellin reacted with an amine in pyridine. The products were new compounds with significant cytotoxicity against tumor cells. Their structures have been established on the basis of their mass and ^{1}H NMR spectra.

Keywords: Hypocrellin, perylenequinone, photodynamic therapy, photosensitizer.

Gastric cancer was one of the most common malicious diseases around the world[1]. Photodynamic therapy (PDT) has been applied to the treatment of gastric tumors since 1980's and desirable results has been obtained. Hypocrellin B (HB), the natural perylenequinone pigment (PQP), was found to be new effective sensitizer in the photodynamic therapy by virtue of its photophysical, photochemical and photobiological properties[2].

Scheme 1. The structures of AHBDs



AHBDs

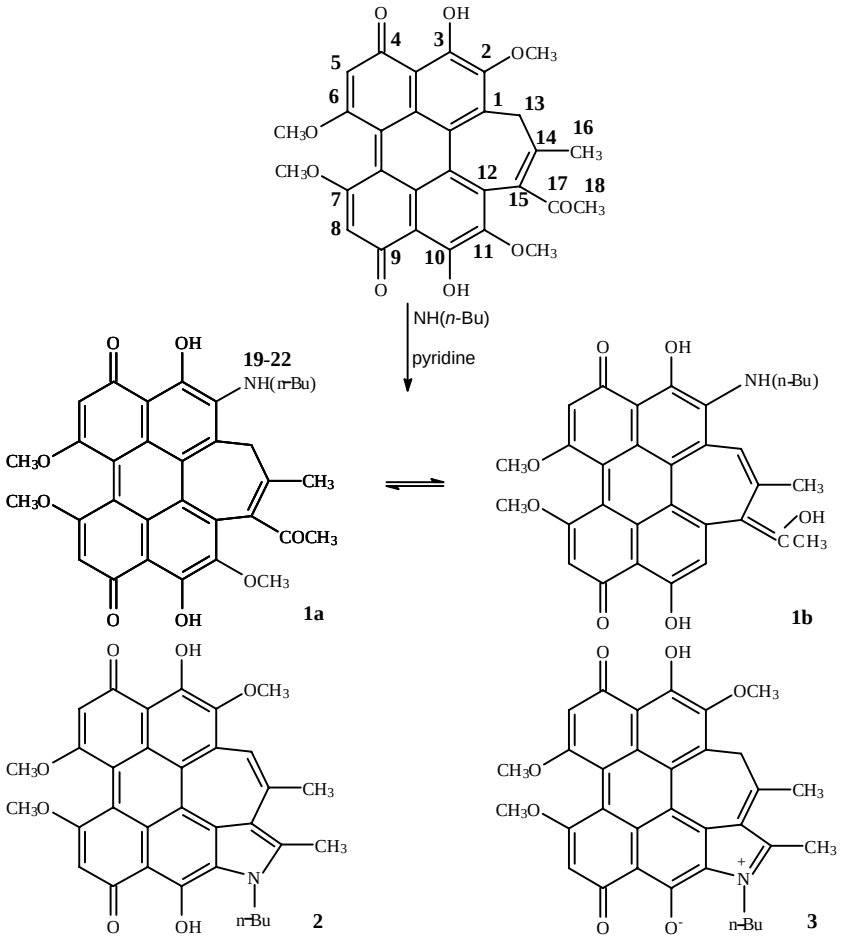
Nevertheless, natural PQP did not exhibit sufficiently strong absorptivity at

wavelength longer than 600 nm, which limited their applications in photodynamic therapy at present. Accordingly, structural modifications of hypocrellin B had been employed to resolve this problem. Some amino-substituted hypocrellin derivatives (AHBDs) (**Scheme 1**) had been synthesized in ethanol[3], which exhibited a considerable strong absorption at 632 nm.

However, the *peri*-hydroxylated perylenequinone structure of hypocrellin was altered since the quinonoid carbonyl groups participated in the amination. What is more, the low yield of the amination due to polymerization during the reaction was undesirable.

An improved method was employed to synthesize the amino-derivatives of hypocrellins with high absorptivity at wavelength longer than 600 nm, and the *peri*-hydroxylated perylenequinone structure was maintained at the same time. Pyridine was chosen as the reaction medium instead of ethanol or other solvents. As a result, the methoxy group at position 2 or 11 was substituted by amino group (DMAHs) in higher yield (**Scheme 1**), and no AHBDs were observed in this reaction.

Scheme 2. Pathway for the amination of HB



In our experiments, amination of HB with *n*-butylamine gave three products (DMAHs): **1**,**2**,**3,** (**Scheme 2**, arranged in the order of R_f values). By comparing with hypocrellins, we found that only three methoxy groups existed on each product from the data of ^{1}H-NMR spectra (**Table 1**). For **1**, its molecular ion peak (M^+, 587) and the absence of ^{1}HNMR of 2-OMe supported the structure we assigned to it. Furthermore, from the 17 sets of signals in ^{1}H-NMR spectrum we deduced that **1** consisted of two tautomers: ketone form (**1a**) and enol form (**1b**).

More detailed explanations were described as follows: in ^{1}H-NMR spectrum, H (13) had two series of signals, one showed the AB spin system (3.90 ppm and 2.80 ppm respectively) which was assigned to the ketone form, the other entered the chemical shift region of olefinic hydrocarbon (5.22 ppm) which was attributed to the enol form. Also the signal at 12.0 ppm was characteristic of hydroxyl group of enol form. No separated **1a** or **1b** was obtained due to the equilibrium between **1a** and **1b** in solution. As for **2** and **3**, molecular ion peaks were the same (M^+, 551), and the amino group was substituted at 11- position. Different from **1**, the resonance of C(17) of **3** in ^{13}C-NMR spectrum significantly displayed to upfield, *i.e.* 128.6 ppm , due to the elimination of the hydroxyl group on carbon (C(17)) and a new five-membered ring connected by nitrogen atom and carbon (C(17)) atom. COSY experiment was conducted to confirm the occurrence of a two-proton singlet at 4.68 ppm in ^{1}H-NMR spectrum of **3**, much downfield than HB, was characteristic of NCH_2. The absence of N-H (3340 cm^{-1}) in IR spectra was a convincing evidence for the above analysis. The differences between **2** and **3** suggested that there were still two hydrogens at position 13 (AB spin system) in **2**, while only one hydrogen in **3** and it appeared in the olefinic hydrogen region (4.2 ppm). In addition, **2** displayed two characteristic resonance in their ^{1}H-NMR spectra at 16.9 ppm and 17.2 ppm (3,9-OH) while only one at 17.12 ppm (3-OH) for **3** was observed, we inferred that **3** was an internal salt as the structure listed in **Scheme 2**. From the comparison of these NMR data, it had been indicated primarily that our previous proposal about the structure of **1**,**2**,**3** was rational.

Table 1. ^{1}H-NMR spectra data of hypocrellins and their derivatives

	HB	**1**	**2**	**3**
2-OMe	4.15(s)	~	4.15(s)	4.15(s)
4,9-OH	16.1,16.8	15.8,16.1	16. 9,17 .2	17.1
5,8-H	6.68(s)	6.38(s),6.60(s)	6.75(s),7.05(s)	6.65(s),7.05(s)
6,7-OMe	4.05(s)	4.05(s)	4.05(s)	4.05(s)
11-OMe	4.09(s)	4.09(s)	~	~
13-CH,	3.25(d),4.04(d)	2.80(d),3.90(d)	4.30(s)	2.65(d),3.75(d)
CH_2	J_{AB}=11.5	J_{AB}=11.5		J_{AB}=11.5
16-OH	~	12.0	~	~
16-Me	1.84(s)	1.95(s)	2.35(s)	2.63(s)
18-Me	2.38(s)	2.34(s)	2.58(s)	2.72(s)
19-CH_2	~	1.86(m)	1.92(m)	1.90(m)
20-CH_2	~	1.78(m)	1.85(m)	1.80(m)
21-CH_2	~	1.50(m)	1.45(m)	1.42(m)
22-CH_3	~	1.07(t)	1.05(t)	1.03(t)
NH	~	3.70	~	~

The products exhibited stronger absorption in the phototherapeutic window (600 - 900 nm) and cellular experiment had shown a much higher photopotentiation factor than HA (*i.e.*, more than 200 *versus* four at a dose of 4 J cm^{-2} of red light on human gastric adenocarcinoma MGC803 cells[4]). Previous study had shown that the light-mediated antiviral efficacy of HB was higher than that of HA[5] (HB are obtained by dehydration of HA in alkaline media[6]).

Experimental

HB (200mg, powder, stored in the dark) was dissolved in fresh distilled pyridine (250ml) containing *n*-butylamine (20 ml) and the resulting solution was stirred for 15h at 50℃ 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 water layer was neutral. Chloroform was evaporated to afford a black solid, which was separated by TLC on a 2% KH_2PO_4 silica gel plate using 4:2:1 petroleum ether/ethyl acetate/ 95% ethanol as eluent. Three products were obtained and identified by satisfactory NMR and mass spectra.

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Reference and notes

1. D. P. Kelsen, *Semin. Oncol.*, **1996,** *23*, 279.
2. L. J. Jiang, *Kexue Tongbao* (in Chinese), **1990**, *35* (22), 1681.
3. Z. J. DIWU, C. L Zhang, J. W. Lown, *Anti- Cancer Drug Design*, **1993**, *8*, 129.
4. W. G. Zhang, M. Weng, S. Z. Pang, M. H. Zhang, H. Y. Yang, H. X. Zhao, Z. Y. Zhang, *Journal of Photochemistry and Photobiology B: Biology*, **1998**, *44*, 21.
5. J. B. Hudson, V. Imperial, R. P. Haugland, Z. Diwu, *Photochemistry and Photobiology*, **1997**, *65*(2), 352.
6. K. Zhao, L. J. Jiang, *Youji Huaxue* (in Chinese), **1989**, *9*, 252.

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