Development of PEG Conjugates to Stabilize and Control Release of Pterostilbene

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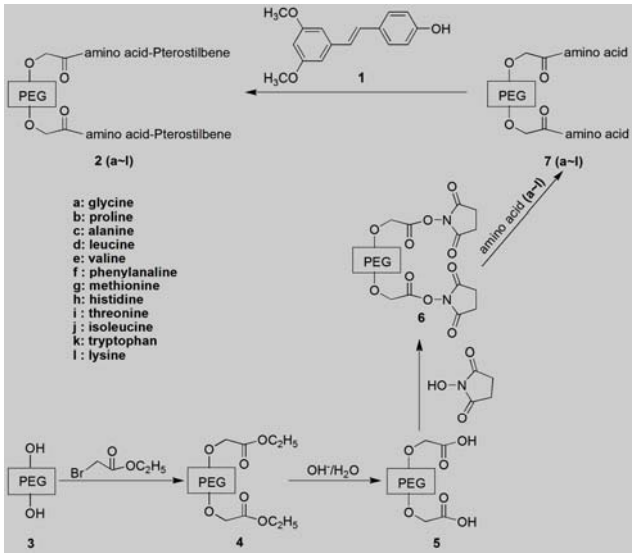
***Abstract* — In view of significant biological activities in preventing cancer, diabetes, cardiovascular diseases, pterostilbene has great potential applications in pharmaceutical fields. But its application is strictly restrained by poor solubility, stability and short half-life. In this paper we design a new PEG conjugate delivery system to overcome these shortcomings and realize a sustained release of pterostilbene. The spacers of PEG-pterostilbene conjugate are amino acids which are beneficial to the body and bromoacetic acid is used as linking arm between PEG and amino acids. The prepared conjugates were characterized by FT-IR, ^{1}H NMR and DSC. Free pterostilbene, loading capability, solubility, and in-vitro release performance of PEG conjugates were evaluated by the dialysis method. The results illustrated the PEG conjugates dramatically improved the stability and solubility of pterostilbene and the in-vitro release test showed the highest release rate of pterostilbene was 75.7% with lipase or 68.4% without lipase in pH 7.4 buffer within 72 h.**

Keywords - Pterostilbene; PEG conjugates; Characterization; Solubility; In vitro release

I. INTRODUCTION

Pterostilbene (trans-3,5-dimethoxyl-4′-hydroxylstilbene, 1 shown in Scheme 1), found existing in many plants and fruits, is a kind of phytoalexin and belongs to stilbenoid compound [1]. Substantial evidence suggests that pterostilbene may have numerous preventive and therapeutic properties in a vast range of human diseases including HeLa cell apoptosis in human neutrophils [2], preventing azoxymethane (AOM)-induced colon tumorigenesis [3-4], possessing anti-cancer abilities of gastric cancer [5-7], B16 melanoma [8], multi-drug resistant leukemia [9], lung cancer and breast cancer cells [10,11]. Pterostilbene still has anti-atherosclerosis and is more active than ciprofibrate, a common hypolipidemic drug [12]. In addition, pterostilbene has been reported for treatment of disorders including arthritis and pain [13,14], and more efficient in preventing skin diseases acting as a skin photoprotector [15]. Pterostilbene has great application prospect of pharmaceutical and nutrition food but the poor water solubility and stability of pterostilbene seriously limit its applications and bioavailabilities. Therefore, looking for a suitable delivery system to avoid the shortcomings of Drug modified by macromolecules to form conjugates is a prospective way to elevate the solubility, stability and further to enhance its bioavailability. After polymer conjugate was proposed [16], many macromolecules have also been used as carriers to bind drugs to improve the solubility and stability of them [17-19]. Polyethylene glycol (PEG), one of the accepted oral vehicle material by the FDA, is a highly water-soluble, biocompatible, non-teratogenic, non-immunogenic, antigenic and non-toxic macromolecule with easily eliminating from human body [20,21]. PEG conjugates could enhance water solubility of drugs due to the highly hydrophilic character of PEG, the altered distribution and biological behavior of drugs in aqueous solutions [22]. Additionally, PEG conjugates decrease the elimination or breakdown of drugs and facilitate the sustained or controlled release of drugs [23]. Several studies have been reported on PEG-modified drugs and the PEG conjugates have greatly improved the bioavailablity of paclitaxel, camptothecin and doxorubicin in clinics [24]. On this view, PEG modified pterostilbene has great potential in solubilizing, stabilizing and control release ability. The present study aimed at developing a set of PEG modified pterostilbene conjugates (as shown in Scheme 1) with carboxymethyl as linking arms and 12 amino acids as spacers to increase the solubility, stability and accomplishment of the controlled release of pterostilbene for applications in the pharmaceutical, food and nutrition fields.

The prepared PEG-pterostilbene conjugates are expected to release the corresponding beneficial amino acids accompanying with pterostilbene in the body. The reason why amino acid is used as spacer is it is biocompatible and essential to health; in particular, in favor of drug absorption with no side effects [25]. It has not been reported by literature so far on the PEG-matrix as a delivery system for pterostilbene, including the preparation and the in vitro release evaluation. The prepared PEG-pterostilbene conjugates were characterized by FT-IR, 1H NMR, DSC, and detected by UV and HPLC.



Scheme1. Synthetic route of PEG-amino acidyl-pterostilbene conjugates.

II. EXPERIMENTS

A. Materials

Pterostilbene (98.0% purity, HPLC) was synthesized in our laboratory according to a previously published method [26]. Analytic grade PEG2000 was purchased from Yongda Chemical Ltd. Chemical grade ethyl bromoacetate was purchased from OL-Chem Co. Ltd., Shanghai, China. Amino acids and some reagents including N-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC) and N,N-4-dimethylaminopyridine (DMAP) were all of industrial grade and purchased from Gongjia Chemical Co., Shanghai, China. Iso-propanol, pyridine, chloroform, anhydrous ether, dichloromethane, ethyl acetate, dimethyl formamide (DMF) and other chemicals were of analytical grade and purchased from Shijiazhuang Modern Reagent Co. Ltd., Shijiazhuang, China. Lipase with an activity of 100,000 U/g was purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. The dialytic bag (cut-off molecular weight of 3500) was purchased from Solarbio Science & Technology Co., Ltd., Beijing, China. Acetonitrile and methanol used in HPLC analysis were chromatographically pure and purchased from Oceanpak Alexative Chemical Co., Ltd., Gothenburg, Sweden.

B. Instrumentation

FT-IR spectra were recorded with a FT-IR spectrophotometer (FTS135, Bio-Rad, America) from 400 to 4000 cm^{-1} by using KBr as the sample holder. ^1H NMR spectrum were identified by Nuclear Magnetic Resonance Spectrometer (AVANCEDMX-500WB, Bruker, America) at room temperature by using $CDCl_3$ as the solvent and TMS as an internal standard. Multiplicities of proton resonance were designated as singlet (s), doublet (d), triplet (t), and multiplet (m). By using a DSC (SDT-2960, TA Co., America) the performance of conjugates of PEG-pterostilbene were tested as well. Samples were heated from 30 to 200 °C at a heating rate of 10 °C min^{-1} with a protective gas atmosphere of nitrogen. Ultraviolet spectrophotometer (UV 2501PC, Shimadzu, Japan) was used to determine the contents of pterostilbene in PEG composites and the water solubility of conjugates, and the maximum absorption of that was detected at a wavelength of 319 nm. Detected the amount of pterostilbene in the *in vitro* release test through high-performance liquid chromatography (HPLC, 1260 LC, Agilent, America) at 319 nm using a mixture of acetonitrile and distilled water (V/V=40/60) as the mobile phase with the flow rate of 1.0 $mL \cdot min^{-1}$. The HPLC column was a Phenomenexluna-C18 (250×4.6 mm, 5 μm) and the sample injection volume was 20 μL.

C. Preparation of PEG-amino acidyl-pterostilbene conjugates

PEG-amino acidyl-pterostilbene conjugates were synthesized according to the route shown in Scheme 1. Selected amino acids were glycine, proline, alanine, leucine, valine, phenylalanine, methionine, histidine, threonine, isoleucine, tryptophan and lysine.

*1) Synthesis of PEG-ethyl acetate (compound **4**)*

The dry PEG2000 (45 g, 0.0225 mol) was dissolved in 450 mL toluene. A 90 mL aliquot of toluene was removed by vacuum distillation at the same time take away the water. Then the residue was naturally cooled to room temperature, and potassium *tert*-butoxide (7.81 g, 0.069 mol) *tert*-butanol solution (45 mL) was added into it under stirring for 1 h. Subsequently, ethyl bromoacetate (40.58 g, 0.24 mol) was added by dropwise into the mixture and refluxed for 24 h. The obtained suspension was filtered, then *tert*-butanol, toluene and excess ethyl bromoacetate was separated from the filtrate by distilled under vacuum. The residue was dissolved in dichloromethane and recrystallized by adding anhydrous ether to obtain PEG2000-ethyl acetate (**4**, 45.69 g, 93.5%).

*2) Synthesis of PEG-acetic acid (compound **5**)*

PEG2000-ethyl acetate (45.69 g) was dissolved in 75 mL distilled water, and the pH of the solution was adjusted to 10 with a 0.1 $mol \cdot L^{-1}$ NaOH aqueous solution. Then the pH of the mixture was adjusted to 2 with a 1% oxalic acid aqueous solution in an ice water bath and continue to stir the mixture at room temperature for another 20 min. The mixture was extracted by dichloromethane (20 mL×3), and the organic phase was brine-washed until neutral, dried by anhydrous sodium sulfate, filtered and condensed. The crude product was recrystallized by anhydrous ether to give PEG2000-acetic acid (**5**, 40.38 g, 90.7%).

*3) Synthesis of PEG-carboxylmethyl-threonine (compound **6**)*

Compound **5** (31.14 g, 14.72 mmol) was dissolved in 75 mL dichloromethane. DCC (6.15 g, 29.85 mmol) and a DMF solution (30 mL) containing NHS (3.62 g, 31.41 mmol) were added into the solution below 0 °C, and then

the mixed solution was naturally warmed to room temperature and lasted for 24 h. The mixture was filtered, then the excessive DCC in the filtrate is neutralized with acetic acid aqueous solution (150 mL, 10%) and continuously stirred for 30 minutes. The organic phase was separated and brine-washed until neutral, dried by anhydrous sodium sulfate, filtered and condensed. The residue was recrystallized by ether to obtain PEG2000-carboxylmethyl active ester (**6**, 25.06 g, 73.7%).

*4) Synthesis of PEG-carboxylmethyl-threonine (compound **7i**)*

Compound **6** (9 g, 3.9 mmol) in 90 mL of DMF was stirred and cooled to 0~5 °C. 1 mol·L^{-1} $NaHCO_3$ (30 mL) aqueous solution containing threonine (1.86 g, 15.62 mmol) was dropped into the mixture and naturally warmed to room temperature for 24 h, and then the pH of the mixture was adjusted to 2 with 1 mol·L^{-1} hydrochloric acid. The mixture was extracted by dichloromethane (20 mL×3) and the organic phase was brine-washed until neutral, dried by anhydrous sodium sulfate, filtered and condensed. The residue was recrystallized by ether to obtain PEG2000-carboxylethyl-threonine (**7i**, 5.43 g, 60%).

Threonine was replaced by other amino acids such as glycine, proline, alanine, leucine, valine, phenylalanine, methionine, histidine, isoleucine, tryptophan or lysine to prepare the relevant PEG-carboxymethyl-amino acid (**7a-h** and **7j-l**) with similar preparation methods and different yields (**7a**, 48%; **7b**, 67%; **7c**, 55%; **7d**, 59%; **7e**, 63%; **7f**, 49%; **7g**, 68%; **7h**, 57%; **7i**, 53%; **7j**, 64%; **7k**, 64%; 7**l**, 61%).

*5) Synthesis of PEG-carboxymethyl-threonine-pterostilbene (**compound 2i**)*

Compound **7i** (0.75 g, 0.33 mmol) was dissolved in 22 mL of dichloromethane and cooled to 0~5 °C. DMF (7.5 mL) containing pterostilbene (0.165 g, 0.645 mmol), DCC (0.135 g, 0.66 mmol) and DMAP (0.075 g, 0.66 mmol) were added to the solution and reacted at room temperature for 24 h. After the reaction was done, the excess DCC was decomposed by 10% acetic acid aqueous solution (30 mL) and the pH of the solution was adjusted **3** with a 2% hydrochloric acid aqueous solution.Then the mixture was extracted by dichloromethane (10 mL×3) and the organic phase was collected and brine-washed until neutral, dried by anhydrous sodium sulfate, filtered and condensed under vacuum. The residue was recrystallized by *i*-propanol to obtain PEG2000-carboxymethyl-threoninely-pterostilbene (**2i**, 0.56 g, 60%).

Threonine was replaced by other amino acids such as glycine, proline, alanine, leucine, valine, phenylalanine, methionine, histidine, isoleucine, tryptophan or lysine to prepare the relevant PEG-carboxymethyl-amino acidyl-pterostilbene conjugates (**2a-h and 2j-l**) with similar preparation methods and different yields (**2a**, 46%; **2b**, 45%; **2c**, 56%; **2d**, 60%; **2e**, 38%; **2f**, 32%; **2g**, 40%; **2h**, 39%; **2i**, 46%; **2j**, 41%; **2k**, 29%; 2**l**, 50%).

D. Water solubility

Appropriate amounts of PEG-amino acidyl-pterostilbene conjugate was immersed in distilled water (1.0 mL) at 37±1 °C for 24 h with continuous agitating. And the water solubilities of the conjugates were determined by ultraviolet spectrophotometer [27].

E. Free dose rate of pterostilbene in PEG-amino acidyl-pterostilbene conjugates

A known amount of PEG-carboxymethyl-amino acidyl-pterostilbene conjugate was weighed, dissolved in methanol and diluted to a constant volume. The content of free pterostilbene was detected by HPLC and the concentration of free pterostilbene was determined using the calibration curve method. The free dose rate (FR) of pterostilbene in PEG-carboxymethyl-amino acidyl-pterostilbene conjugate was calculated according to the formula 1, where FR is defined as the free dose rate of pterostilbene in PEG-carboxymethyll-amino acidyl-pterostilbene conjugate; C stands for the concentration of pterostilbene; V and M respectively represented the solution volume and the mass of the PEG-carboxymethyl-amino acidyl-pterostilbene conjugate.

$$FR = \frac{C \times V}{M} \times 100\,\% \tag{1}$$

F. Loading rate of pterostilbene in PEG-amino acidyl-pterostilbene conjugates

The determination of pterostilbene in PEG-amino acidyl-pterostilbene conjugates by ultraviolet spectrophotometry was to measure the concentration of organic substance based on the feature that organic substance could absorb. A known amount of PEG-carboxymethyl-amino acidyl-pterostilbene conjugate was dissolved in a certain volume of ethanol and determined using an ultraviolet spectrophotometer. The concentration corresponding to each absorbance were calculated using the calibration curve method. The loading rate (LD) was calculated according to the formula 2, where C_1 is defined as the concentration of pterostilbene and V_1 is the metered volume of the ethanol solution, M_1 is the mass of PEG-carboxymethyl-amino acidyl-pterostilbene conjugate.

$$LD = \frac{C_1 \times V_1}{M_1} \times 100\,\% \tag{2}$$

G. Controlled release in vitro of PEG-amino acidyl-pterostilbene conjugates

Dynamic dialysis method simulating intestinal conditions (pH 7.4, p-osphate buffered saline, PBS) was used to determine the releasing characteristic of the prepared PEG-amino acidyl-pterostilbene conjugates in vitro. PBS buffer solution (15 mL) containing PEG-amino acidyl-pterostilbene conjugate (0.25 g) and lipase (0.25 g) was put into a dialytic bag and the bag was sealed. Then the bag was placed in a shaking basket with 60 mL PBS buffer solution and was shaken continuously at 37±0.5 ºC. At predetermined time intervals (0.5 h, 2.0 h, 4.0 h, 12.0 h, 36.0 h, 60.0 h, 72.0 h), release medium (7.5 mL) was taken

out and the same amount of warmed fresh buffer solution (37±0.5 ^{o}C) was added to keep the system at a constant volume. The withdrawn release medium (7.5 mL) was diluted with HPLC mobile phase to 50 mL, sonicated in an ultra-sonic bath, after micropore filter, and then was analyzed by HPLC. The concentrations corresponding to each time interval Data were evaluated using the calibration curve method to calculate. The accumulated amount of pterostilbene released from the dialytic bag was calculated from the formula 3, where Q_t stands for the accumulated amount of pterostilbene released from the dialytic bag; Ct is defined as the concentration of pterostilbene in buffers at t (time); C_{t-1} is defined as the content of that in taken out buffer solution at time t-1; V_0 is total volume of buffer solution; V is the volume of sample having been taken out. Each experiment was repeated for three times.

$$Q_t = C_t V_0 + \sum C_{t-1} V \qquad (3)$$

The *in vitro* release of PEG-amino acidyl-pterostilbene conjugate without lipase catalytic hydrolysis was observed following the same method in the absence of lipase.

III. RESULTS AND DISCUSSION

A. FT-IR Spectroscopy Analysis

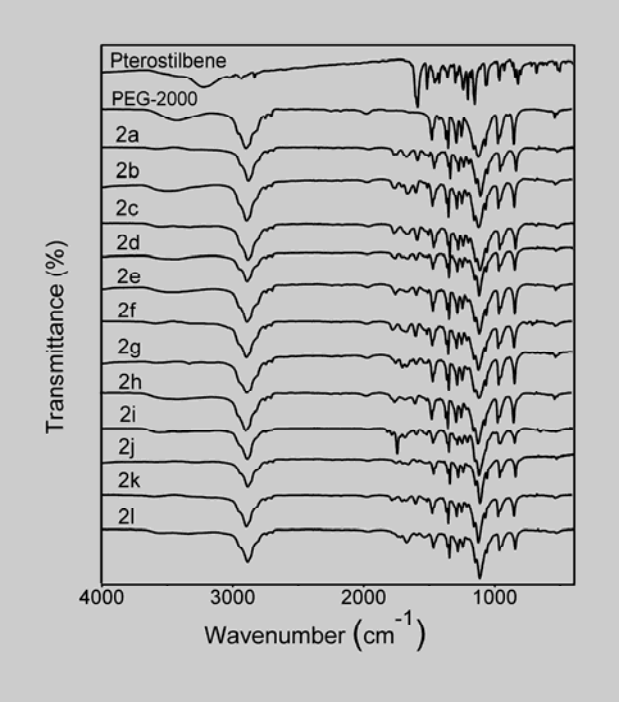


Figure1. FT-IR spectra of pterostilbene, PEG and PEG conjugates 2(a~l)

The FT-IR spectra of PEG-amino acidyl-pterostilbene conjugates are shown in Figure 1. As seen from the pterostilbene spectrum in Figure 1, the peak at 3319 cm^{-1} is attributed to the stretching vibration of hydroxyl group on pterostilbene. The peaks at 1600~1456 cm^{-1} c are assigned to skeleton vibration of the benzene ring and conjugated olefin, and the peak at 962 cm^{-1} is a typical absorption peak of trans olefin. In the PEG spectrum, an absorption band of hydroxyl group at 3465 cm^{-1} is relatively weak, the peak at 2881 cm^{-1} can be attributed to methylene, and there was a moderate strong peak at 1114 cm^{-1} correspond to stretching vibrations of a C-O ether bond. In the spectra of prepared PEG conjugates (**2a**~**l**), the peaks at 3260~3400 cm^{-1} (-OH) and at 2886 cm^{-1} (-CH_2) were enhanced because of linking with pterostilbene and methylenes from PEG, respectively. The characteristic peaks at 1735~1775 cm^{-1} were ascribed to carbonyl groups and ester groups. From 1580 cm^{-1} to 1680 cm^{-1} there were strong amide peaks, and relatively strong absorptions of approximately 1115 cm^{-1} illustrated the presence of C-N bonds. The peaks at 1450~1600 cm^{-1} were the characteristic peaks of the benzene ring and also indicated that pterostilbene was present in the conjugates.

B. ^{1}H NMR data of PEG conjugates

^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ:

2a ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.21~1.32 (H of glycine), 3.64 (-CH_2 of PEG), 4.02~4.29 (-CH of glycine and -CH_2 of carboxymethyl), 4.57~4.82 (H from -OH of pterostilbene), 6.38~7.49 (H from pterostilbene), 7.79~8.01 (H from amide).

2b ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.19~2.06 (H from tetrahydropyrrole), 3.62 (-CH_2 of PEG), 3.96~4.30 (-CH_2 of carboxymethyl), 4.69 (H from tetrahydropyrrole), 6.50~7.58 (H from pterostilbene), 7.75 (H from amide).

2c ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.11~1.92 (-CH_3 from analine), 3.61~3.62 (-CH_2 of PEG), 3.90~4.27 (-CH_2 of carboxymethyl), 4.52 (H from -OH of pterostilbene), 4.69 (chiral H from analine), 7.00~7.28 (H from pterostilbene), 7.72 (H from amide).

2d ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.07~1.93 (-CH_3 and -CH_2 of leucine), 3.63 (-CH_2 of PEG), 3.93~4.21 (-CH_2 of carboxymethyl), 4.40 (chiral H from leucine), 4.80~5.10 (–OH of pterostilbene), 6.38~7.59 (H from pterostilbene).

2e ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.11~1.38 (-CH_3 of valine), 3.64 (-CH_2 of PEG), 4.14~4.34 (-CH_2 of carboxymethyl), 4.40~4.42 (chiral H from valine), 4.80~5.17 (–OH of pterostilbene), 6.39~7.63 (H from pterostilbene).

2f ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 3.63 (-CH_2 of PEG), 3.91~4.29 (-CH_2 of carboxymethyl), 4.41 (chiral H from phenylanaline), 4.62~4.83 (-OH of pterostilbene), 6.38~7.63 (H from pterostilbene and benzene ring of phenylanaline).

2g ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 2.02 (-CH_3 of methionine), 2.11~2.14 (-CH_2 of methionine), 3.63 (-CH_2 of PEG), 3.91~4.36 (-CH_2 of methionine, -CH_2 of carboxymethyl, chiral H from methionine), 4.84~4.96 (-OH of pterostilbene), 6.36~7.46 (H from pterostilbene), 7.96~8.08 (H from amide).

2h ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 3.64 (-CH_2 of PEG), 3.92~4.17 (-CH_2 of carboxymethyl), 4.24~4.42 (chiral H from histidine), 4.65~4.90 (-OH of pterostilbene), 6.42~7.48 (H from imidazole ring and pterostilbene), 7.75 (H from amide).

2i ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.01~1.27 (-

CH_3 from threonine), 3.63 (-CH_2 of PEG), 3.97~4.01 (-CH_2

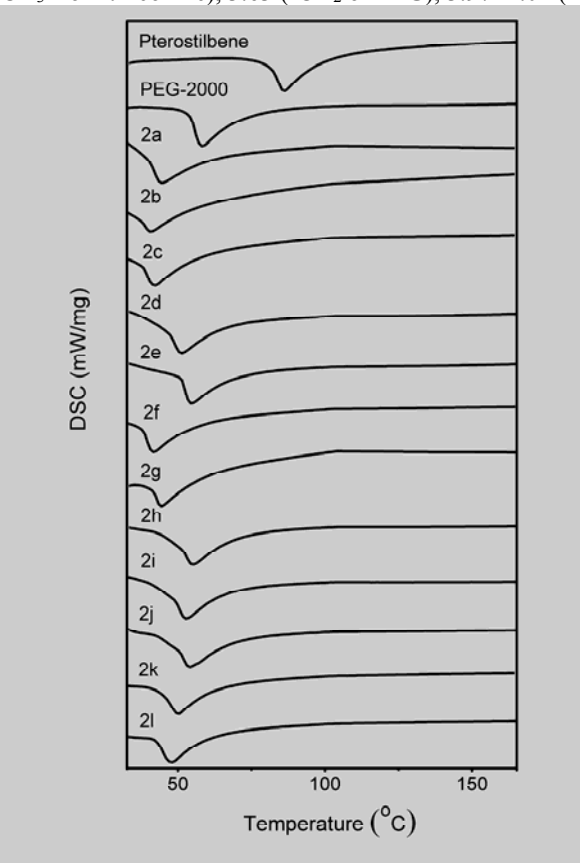


Figure2. DSC of pterostilbene, PEG and PEG conjugates 2(a~l)

of carboxymethyl), 4.09~4.40 (methenyl of threonine), 4.84~4.94 (-OH of pterostilbene), 6.37~7.47 (pterostilbene-H), 7.79~7.80 (H from amide).

2j ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 0.97~1.00 (5-CH_3 of isoleucine), 1.01~1.09 (3-CH_3 of isoleucine), 1.30~1.36 (4-CH_2 of isoleucine), 1.61~2.04 (3-CH of isoleucine), 3.64 (-CH_2 of PEG), 3.93~4.18 (-CH_2 of carboxymethyl), 4.20~4.42 (chiral H from isoleucine), 4.79~4.82 (-OH of pterostilbene), 6.39~7.49 (H from pterostilbene), 7.89~7.91 (H from amide).

2k ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 3.64 (-CH_2 of PEG), 3.97~4.41 (-CH_2 of carboxymethyl and C-3H of trypophane), 4.71 (C-2H), 6.38~7.47 (H from indole and pterostilbene), 7.73~7.80 (H from amide).

2l ^{1}H NMR ($CDCl_3$, 500 MHZ, ppm) δ: 1.17~2.00 (-CH_2 of lysine), 2.52~2.67 (C-6-H of lysine), 3.64 (-CH_2 of PEG), 3.83~4.42 (-CH_2 of carboxymethyl and chiral H of lysine), 4.80~4.99 (-OH of pterostilbene), 6.39~7.65 (pterostilbene-H).

C. Thermal Analysis

Differential scanning calorimetry can be conducted to obtain weight change and the melting point of samples according to the endothermic performances, and they can also be used to determine if pterostilbene has been reacted with PEG-acetic acid successfully to form the conjugates. As shown in Figure 2, the melting point endothermic peaks of pterostilbene and PEG appeared at 86 °C and 57 °C, respectively. In relative DSC curves of PEG-amino acidyl-pterostilbene conjugate, there was one endothermic peak appeared approximately 41 °C~55 °C attributed to the solvate which can not be volatilized completely by vacuum drying, however, the endothermic peaks at the temperatures of 86 °C and 57 °C were disappeared. These results clearly indicate that pterostilbene is linked with PEG through a connecting arm to form new compounds.

D. Water solubility, free dose rate and loading rate of pterostilbene in PEG conjugates

As shown in Table 1, the water solubilities of the prepared PEG conjugates are greater than 900 mg·mL-1, which is much higher than that of pterostilbene (0.069 mg·mL-1, CAS: 537-42-8). In addition, the free dose rate and loading rate of pterostilbene in PEG-carboxymethyl-amino acidyl conjugates are also shown in Table 1.

TABLEI. WATER SOLUBILITY, FREE DOSE RATE AND LOADING RATE OF PEG CONJUGATES, n=3

Conjugate	Water solubility/mg·mL^{-1}	Loading rate/%	Free dose rate/%
2a	> 900	8.44 ± 0.04	1.3327 ± 0.0048
2b	> 900	7.50 ± 0.06	0.4678 ± 0.0032
2c	> 900	8.00 ± 0.04	0.7706 ± 0.0010
2d	> 900	14.28 ± 0.03	0.3164 ± 0.0021
2e	> 900	4.81 ± 0.02	0.3846 ± 0.0031
2f	> 900	7.98 ± 0.04	0.2748 ± 0.0042
2g	> 900	6.53 ± 0.06	0.8027 ± 0.0010
2h	> 900	4.29 ± 0.04	1.1972 ± 0.0045
2i	> 900	7.91 ± 0.03	0.2287 ± 0.0011
2j	> 900	6.33 ± 0.05	0.2051 ± 0.0010
2k	> 900	7.99 ± 0.03	0.2229 ± 0.0023
2l	> 900	5.08 ± 0.02	0.5815 ± 0.0033

E. Controlled release in vitro of conjugates

The *in vitro* release of PEG-amino acidyl-pterostilbene conjugate was investigated to determine if the conjugate can prolong the release of pterostilbene. And the study was conducted in a buffer solution of pH 7.4 within 72h with or without lipase as catalyst. As shown in Figure 3 and 4, the release of PEG-carboxymethyl-histidine-pterostilbene (**2h**) was the highest with lipase (75.7%) or without lipase (68.4%), whereas the release of **2k** and **2d** was the lowest and the release rates of them were both about 26% in the presence of lipase but the release rates were only 18% in the absence of lipase. There may be two reasons for the difference in release performance, one may be the different hydrolyzing ability of the ester bond between the amino acid and pterostilbene, and the other may be the function,

wrapping pattern and spatial structure of the macromolecular carrier, which can affect the hydrolysis of some conjugates and pterostilbene release [28]. In addition, most of the release rate of conjugates in the presence of lipase was higher than that in the absence of lipase, and the difference in release performance ranged from 2.2% to 25.4%. However, the release of pterostilbene with lipase form some conjugates such as **2b** and **2i** were slightly lower than that without lipase. Therefore, the release rate may mainly depend on the structure of the amino acid rather than the linking arm.

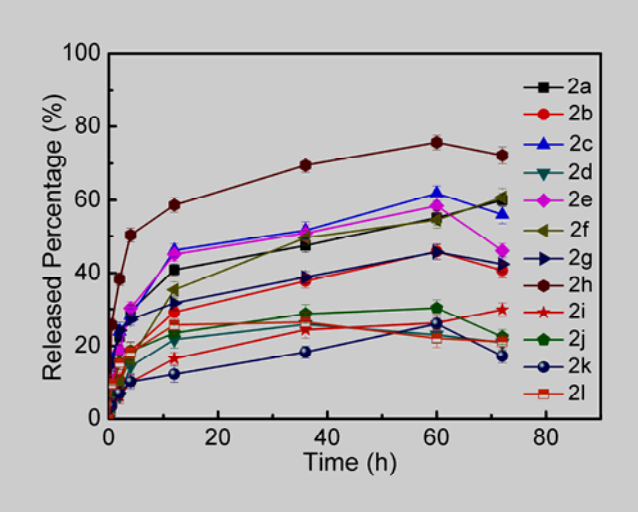


Figure3. Release of pterostilbene from PEG-carboxymethyl-amino acidyl-pterostilbene with lipase

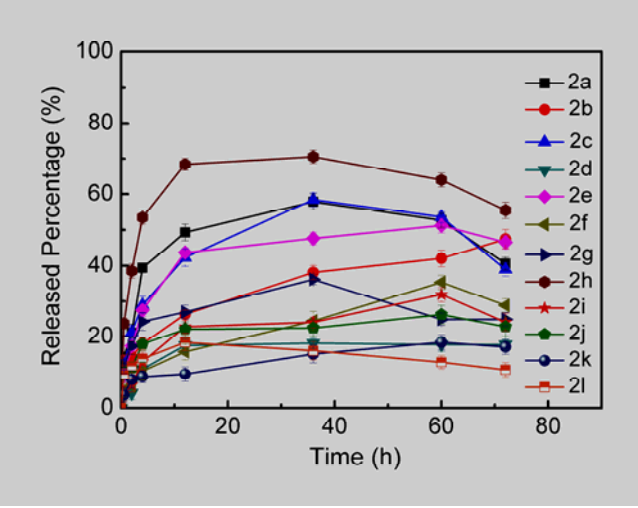


Figure4. Release of pterostilbene from PEG-carboxymethyl-amino acidyl-pterostilbene without lipase.

IV. CONCLUSION

A novel PEG-amino acidyl-pterostilbene conjugates was prepared using bromoacetic acid as linking arm to improve the water solubility, stability of pterostilbene and achieve the controlled release of pterostilbene. The prepared conjugates were characterized by FT-IR, ^{1}H NMR and DSC, and the results indicate that the solubility and stability of PEG conjugates was greatly increased. The *in vitro* release performance of PEG-pterostilbene conjugates was evaluated by HPLC and the release rate of pterostilbene from PEG conjugates in the presence of lipase was 75.7%, which was higher than that in the absence of lipase under the same condition. Hence, the PEG conjugates can successfully achieve the controlled release of pterostilbene and effectively prolong its half-life.

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