A lipophilic prodrug of Danshensu: preparation, characterization, and in vitro and in vivo evaluation

GUO Xue-Jiao, FAN Xue-Jiao, QIAO Bin, GE Zhi-Qiang*

Department of Pharmaceutical Engineering, School of Chemical Engineering and Technology, Tianjin University, Education Ministry Key Laboratory of Systems Bioengineering, Tianjin 300072, China

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[ABSTRACT] Danshensu [3-(3, 4-dihydroxyphenyl) lactic acid, DSS], one of the significant cardioprotective components, is extracted from the root of Salvia miltiorrhiza. In the present study, an ester prodrug of Danshensu (DSS), palmitoyl Danshensu (PDSS), was synthesized with the aim to improve its oral bioavailability and prolong its half-life. The in vitro experiments were carried out to evaluate the physicochemical properties and stability of PDSS. Although the solubility of PDSS in water was only 0.055 mg·mL^{-1}, its solubility in FaSSIF and FeSSIF reached 4.68 and 9.08 mg·mL^{-1}, respectively. Octanol-water partition coefficient (log P) was increased from −2.48 of DSS to 1.90 of PDSS. PDSS was relatively stable in the aqueous solution in pH range from 5.6 to 7.4. Furthermore, the pharmacokinetics in rats was evaluated after oral administration of PDSS and DSS. AUC and t_{1/2} of PDSS were enhanced up to 9.8-fold and 2.2-fold, respectively, compared to that of DSS. C_{max} was 1.67 ± 0.11 μg·mL^{-1} for PDSS and 0.81 ± 0.06 μg·mL^{-1} for DSS. Thus, these results demonstrated that PDSS had much higher oral bioavailability and longer circulation time than its parent drug.

[KEY WORDS] Danshensu; Prodrug; Oral bioavailability; Circulation time

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[*Corresponding author] Tel: 86-22-87401546, Fax: 86-22-27403389, E-mail: gezhiq@tju.edu.cn
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Introduction

Danshensu (3-(3, 4-dihydroxyphenyl) lactic acid, DSS) is one of the main hydrophilic components of Radix Salvia miltiorrhiza (Danshen in China) which has been widely applied in clinical treatments in China and Japan for centuries. Its pharmacological activities have been comprehensively studied, including inhibiting platelet aggregation, decreasing the blood viscosity, scavenging oxygen free radicals, protecting the endothelial cell from homocysteine-induced dysfunction, improving heart function, and anti-inflammatory and anti-tumor activities [1-4]. However, the development of therapeutic DSS for clinical application still faces challenges because of its low oral bioavailability. DSS is administered parenterally (intravenous drip) in clinical practice due to its suboptimal biopharmaceutical properties, including the low oral bioavailability and short half-life [5]. It has been reported that the oral bioavailability of DSS in rats was only 11.09% and eliminated rapidly from the systemic circulation with t_{1/2} of 45.37 min [6]. In order to increase the circulation time and improve oral bioavailability of DSS in vivo, several studies have been conducted. It is found that DSS phospholipid complex (DPLC) could increase the oral bioavailability of DSS since the lipophilicity of the DSS mixture was enhanced with the presence of phospholipid [7]. Also, the oral bioavailability of DSS is increased from 11.09% to 18.62% with the addition of sodium caprate, a kind of absorption enhancer [6]. These studies have revealed that the excessive aqueous solubility could lead to the inefficient absorption through the small intestinal cell membranes. Although the oral bioavailability of DSS has been improved to some extent by the aforementioned formulations, the circulation time of DSS in vivo are not getting better and some absorption enhancers may cause damage and irritate the intestinal mucosal membrane [8]. Hence, for the drugs with such characteristics, it is meaningful to design and synthesize the lipophilic prodrugs for altering the physicochemical properties, promoting the intestinal membrane permeation, as well as achieving the desired circulation time [9].
The prodrug approach using reversible derivatives is useful in optimizing the clinical application of a drug [10]. Lipophilic prodrugs, also called drug-lipid conjugates, have the drug covalently bound to the lipid moiety, such as fatty acids, diglycerides, and phosphoglycerides [11]. The application of fatty acids in the formation of lipidic prodrugs has been extensively studied, such as the lipidic prodrug doxorubicin [12-13] and pomorphine [14]. As a result, it is useful to promote drug absorption and prolong the circulation time because the lipophilicity of the prodrug is enhanced dramatically, compared to the parent drug [11]. Although the lipophilic prodrug strategy by chemical modification has been widely used in the delivery of drugs, there are barely any reports on DSS prodrugs which can improve its oral bioavailability and extend its circulation half-life.

Hence, herein a palmitoyl prodrug of DSS (2-palmitoyl-3-(3, 4-dihydroxyphenyl) propanoic acid, PDSS) was designed and synthesized by introducing an alkyl-palmitoyl chain to the alcoholic hydroxyl of DSS. The palmitoylation of the drug could enhance its lipophilicity, thus improving the oral bioavailability and extending the circulation time of parent drug [15-16]. The reason that palmitic acid was used as the lipophilic moiety was its safety has been approved by the U. S. Food and Drug Administration. Moreover, the physicochemical properties, rate of hydrolysis of PDSS in vitro, as well as pharmacokinetics after oral administration in rats have been investigated. It was hoped that the lipophilic prodrug of Danshensu would have significant impact on its clinical application.

Materials and Methods

Chemicals and animals

DSS was purchased from Xi’an Honson Biotechnology (Xi’an, China). Palmitic anhydride (purity > 97%) and Carboxylesterase were purchased from Sigma-Aldrich (Shanghai, China). 4-Dimethylaminopyridine (DMAP, 99%), sodium borohydride (NaBH₄, 98%), pyridine (> 99%), and n-octanol (99%) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., China. Pyrene (97%) was purchased from Beijing Ou’he Technology Co., Ltd., China. HPLC grade acetonitrile (ACN) was bought from Merck (Darmstadt, Germany). Ultrapure water (Millipore, Schwabach, Germany) was used as the dispersion medium. All materials used in the present study were of the highest purity available unless otherwise noted.

Synthesis of PDSS

The synthesis process of PDSS is illustrated briefly in Fig. 1. DSS (5 mmol), palmitic anhydride (16 mmol), and DMAP (0.5 mmol) were dissolved in 10 mL of anhydrous pyridine. The reaction mixture was stirred magnetically at 80 °C for 12 h. After that, the resultant reaction mixture was cooled to room temperature, to which ethyl acetate (20 mL) was added. The organic phase was washed with 5% HCL (15 mL × 2) and distilled water (15 mL × 2) successively, dried over anhydrous sodium sulfate and then evaporated at 65 °C under reduced pressure to obtain the crude product. After purification by column chromatography, the intermediate product was successfully separated.

The intermediate product (3.5 mmol) was dissolved with 10 mL of methanol, to which NaBH₄ (3.5 mmol) was added in batches under the magnetic stirring in ice bath. After 10 min in ice bath, the reaction temperature was raised to the reflux temperature of methanol and maintained until the end of the reaction. The target product, PDSS (2-palmitoyl-3-(3, 4-dihydroxyphenyl) propanoic acid), was obtained using the same separation and purification method as described above.

Characterization and HPLC determination of PDSS

PDSS was characterized by nuclear magnetic resonance (NMR) spectroscopy (400 MHz), high resolution mass (HR-MS) spectroscopy, and fouriers transform infrared (FT-IR) spectroscopy. The purity of PDSS was determined by high performance liquid chromatography (HPLC; Waters e2695-2489uv; Waters Symmetry C18 5 μm, 46 mm × 250 mm column; eluent: 10% acetonitrile/90% H₂O/0.1% formic acid).

Melting point

The sample of PDSS was prepared through placing about 1 mg of PDSS on a glass slide, and the melting point was then determined by OptiMelt MPA100.

Equilibrium solubility in aqueous

Excess amounts of PDSS were added to 10 mL of distilled water in a closed Erlenmeyer flask. The oversaturated solution was placed in a shaking air bath at 25 °C and 60 r·min⁻¹ for 24 h. After solution reaching solubility equilibrium, the precipitates were removed from the suspension through centrifugation (13 000 r·min⁻¹ × 10 min) and the supernatant fluid was analyzed by HPLC [17].

Equilibrium solubility in FaSSIF and FeSSIF

Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) were prepared according to the manufacturer’s instructions. Ten milligram of...
PDSS was placed in a tube and 1 mL of FaSSIF or FeSSIF was added. Then the sample was sonicated for 1 h and shaken at room temperature for 4 h, placed for 12 h to reach equilibrium. The sample was filtered and the filtrate was analyzed by HPLC.[18]

**Partition coefficient (log P)**

The shake-flask method was used to determine the partition coefficient of PDSS between water and n-octanol. Distilled water (25 mL) and n-octanol (25 mL) were placed in a shaking bath at room temperature for 72 h, and the two saturated phases were separated completely. PDSS (10 mg) was dissolved in 5 mL of n-octanol saturated with water and diluted with 5 mL distilled water saturated with n-octanol.[19] After 72 h shaking in air, the two phases were separated and the concentration of PDSS in each phase was measured by HPLC. The partition coefficient was calculated according to the following equation:

\[ P = C_o/C_w \]

where \(C_o\) and \(C_w\) represent the concentration of PDSS in n-octanol and water, respectively. All experiments were conducted in triplicate.

**Critical micelle concentration**

The critical micelle concentration of PDSS in aqueous was determined by fluorescence spectroscopy using pyrene as a probe. Pyrene solution in acetone was added to tubes and the acetone was evaporated. The PDSS solutions at different concentrations were then added to the tubes. After that, the mixed solutions were sonicated at 60 °C for 1 h and let stand to equilibrium at room temperature for 12 h. The samples were then measured by fluorescence spectroscopy (Hitachi F-2500) at \(\lambda_{excitation} = 343\) nm, \(\lambda_{emission} = 350–450\) nm. The slit width for excitation and emission was 2.5 nm, and the speed of spectra scan was 300 nm min^{-1}.[20]

**Hydrolysis in different pH solutions**

Seven pH values (2.0, 4.2, 5.6, 6.8, 7.4, 8.0, and 9.0) were selected to evaluate the stability of PDSS in aqueous. PDSS methanolic stock solutions (5 mg·mL^{-1}; 0.2 mL each) were respectively placed into seven flasks containing 9.8 mL of buffer with various pH values, and shaken in air bath at 37 °C and 60 r·min^{-1} for 24 h. A mixture of 0.2 mL was collected every 2 h followed by HPLC analysis. All the experiments were conducted in triplicate.[19]

**Enzymatic hydrolysis in vitro**

Porcine liver esterase (20 mg, 360 units) was dissolved in 20 mL of 0.01 mol·L^{-1} sodium phosphate (pH 7.4) and pre-incubated in a shaking air bath (60 r·min^{-1}) for 10 min at 37 °C. The enzymatic hydrolysis was initiated by adding 100 μL of PDSS stock solution (10 mg·mL^{-1}). Two hundred microliters of reaction solutions were taken at predetermined time points and mixed with 800 μL of ice acetonitrile to stop the enzymolysis reaction. The mixture was centrifuged at 13 000 r·min^{-1} for 15 min.[21-22] The supernatants were analyzed by HPLC to determine the concentrations of PDSS and DSS.

**Animal experiments**

The in vivo studies were carried out using adult male Sprague-Dawley rats (body weight 250 ± 20 g; Beijing HFK Bioscience Co. Ltd., Beijing, China). The animal experiment protocol was according to the guidelines of the Animal Ethics Committee of the Tianjin Institute of Pharmaceutical Research. Twelve Sprague-Dawley rats were fasted for 24 h prior to use and randomly divided into two groups: group A received 30 mg·kg^{-1} of DSS orally and group B received 66 mg·kg^{-1} (equivalent to 30 mg·kg^{-1} of DSS) of PDSS orally. The blood samples were obtained via posterior orbital venous plexus at predetermined time points and placed in heparinized microcentrifuge tubes. The blood samples were centrifuged at 3 500 r·min^{-1} for 10 min to obtain the plasma. The plasma samples were stored at −20 °C until analysis.[6]

**Sample preparation and analysis**

The plasma samples were prepared through liquid-liquid extraction according to the method of Zhou et al.[23] with some modifications. One hundred microliters of 1% acetic acid was first added to 100 μL of plasma sample, followed by adding 100 μL of 3 mol·L^{-1} chlorhydric acid; the mixture was vortexed for 3 min to blend completely, extracted with 1 000 μL of ice ethyl acetate for 5 min, and centrifuged at 13 000 r·min^{-1} for 15 min. After that, 800 μL of the supernatant was collected and evaporated to dryness at 37 °C; 100 μL of mobile phase was used to re-suspend the residue for HPLC-MS analysis.

The DSS was separated on a Zorbax SB C18 column (2.1 mm × 100 mm, 1.8 μm) using an Agilent 1260 HPLC (Agilent, Santa Clara, CA, USA). The column temperature was kept at 30 °C. The mobile phase was MeOH/water (0.1% formic acid) (10/90, V/V). The flow rate was set at 0.2 mL·min^{-1} and the injection volume was 3 μL. The total elution time was 15 min.

The mass spectrometry detection was performed on a 6420A triple quadrupole (Agilent). An electrospray ionization source was used in negative ion mode. The capillary voltage was 4 000 V. Nitrogen was used as the drying and nebulizer gas with a flow rate of 9 L·min^{-1} and a nebulizer pressure of 30 psi. The gas temperature was set at 320 °C. The multiple reaction monitoring (MRM) mode was used for DSS quantification and the monitor ion was m/z 197.1 → 135.1.

**Statistical analysis**

PKSolver software (China Pharmaceutical University, Nanjing, China, version 2.0) was applied to analyze the DSS plasma concentration versus time profiles. The non-compartmental data analysis was employed to estimate the following pharmacokinetic parameters: the peak plasma concentration (C_{max}) and the time reaching C_{max} (T_{max}), the terminal elimination half-life (t_{1/2}), and area under the plasma concentration versus time curve from zero to infinity (AUC_{0-\infty}). These parameters were presented as the mean ± SD and analyzed by Student’s t-test to determine statistical significance, \(P < 0.05\) or \(P < 0.01\) was considered to be statistically significant.
Results and Discussion

Synthesis and characterization of PDSS

As shown in Fig. 1, the whole synthesis process was consisted of two steps: (i) introducing three palmitoyl chains to the hydroxyl of DSS and (ii) deacylating the palmitoyl chains from the benzene ring. In the second step, the selected reaction condition could protect the alcohol ester from reaction. After purification by column chromatography, a yield of 73% was obtained for PDSS.

The structure of PDSS was confirmed by 1H NMR, 13C NMR, HR-MS and FT-IR. 1H NMR (400 MHz, CDCl3): δ 6.79 (d, J = 8.0 Hz, 1H), 6.75–6.62 (m, 2H), 5.18 (t, J = 6.2 Hz, 1H), 3.16–3.00 (m, 2H), 2.38 (dd, J = 7.8, 6.3 Hz, 2H), 1.68–1.55 (m, 2H), 1.37–1.22 (m, 24H), 0.90 (t, J = 6.7 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 174.69, 174.39, 143.50, 143.05, 127.81, 122.15, 116.30, 115.45, 72.94, 36.50, 33.96, 31.93, 29.77–29.60, 29.48, 29.37, 29.25, 29.01, 24.68, 22.69, 14.11; HR-MS (ESI) m/z 459.272 7 [M + Na]+. According to Fig. 2, the main peaks from the IR (KBr) analysis (1 801, 1 742 cm−1) were consistent with the NMR spectra data. These spectrum data showed that PDSS was synthesized successfully through an ester bond. Its purity was determined to be 97.5% by HPLC analysis.

Aqueous solubility and partition coefficient

The determined values for aqueous solubility and partition coefficient are shown in Table 1. Aqueous solubility is often used as a reference parameter to predict the absorption and oral bioavailability of drugs. However, this parameter is insufficient to explain the membrane penetration capacities of drugs. For instance, for oral bioavailability of Class III drugs defined in the biopharmaceutical classification system (BCS), the determining factor is the permeation capacity through the intestinal cell membranes but not solubility in aqueous. Hence, the partition coefficient (log P) is a key parameter in the development of drugs due to the fact that the absorption, distribution, and metabolism of a drug involve its passage across biological membranes of the cell. It has been reported that drugs with log P values ranging from 0 to 3 have higher membrane permeability.[24] DSS, as the parent drug of PDSS, should be classified into Class III drug as its high aqueous solubility (> 50 mg·mL−1) and low lipophilicity (log P, −2.48). The introduction of the alkyl chain changed aqueous solubility and lipophilicity of DSS. The solubility of PDSS in water was much lower than that of DSS, but its log P (1.90) was transformed from negative value to positive value, which meant that the lipophilicity of PDSS was enhanced significantly. According to Overton’s rule, the easier it is for a drug to dissolve in a lipid, the easier and faster it will permeate the cell membrane. Therefore, log P transformation of PDSS will be beneficial to enhance penetration of the intestinal epithelial cells, consequently increasing its oral bioavailability.

Critical micelle concentration

The critical micelle concentration (CMC) of PDSS was measured by steady-state fluorescence spectroscopy of pyrene. The intensity ratio I3/I1 (I393/I373) could be an index of polarity of its solution environment [20]; the ratio I3/I1 increases with the increasing polarity of medium in which pyrene is dissolved. As demonstrated in Fig. 3, I3/I1 remained constant (about 1.0–1.2) at low concentration, indicating that pyrene was in aqueous environment. With the increase of concentration of PDSS, I3/I1 increased rapidly since the micelles had been formed and thus pyrene moved into the hydrophobic core of micelles. It was determined that the CMC of PDSS was 518 μmol·L−1 by the intersection of the two fitted straight lines in Fig. 3, suggesting that PDSS possess amphiphilic properties. CMC of DSS was not observed with this method because DSS did not possess lipophilic moiety in its molecule. Amphiphilicity contributes to the affinity of drugs to biological membranes and drugs lymphatic absorption[25].

Table 1 Physicochemical properties for DSS and PDSS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Aqueous solubilitya</th>
<th>FaSSIF solubilitya</th>
<th>FeSSIF solubilitya</th>
<th>Melting point</th>
<th>Log Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS</td>
<td>&gt; 50</td>
<td>−</td>
<td>−</td>
<td>84.2 ± 0.6</td>
<td>−2.48 ± 0.04</td>
</tr>
<tr>
<td>PDSS</td>
<td>0.055 ± 0.001</td>
<td>4.68 ± 0.07</td>
<td>9.08 ± 0.04</td>
<td>78.3 ± 0.9</td>
<td>1.90 ± 0.02</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 3).

1 Solubility determination was carried out in water at 37 °C, and the unit of solubility is mg·mL−1.

2 Partition coefficient values (P) were carried out in n-octanol/water at 37 °C.

3 Not determined.

![Fig. 2](image-url)
Fig. 3  Determination of the CMC of PDSS using fluorescence spectroscopy: I393/I373 ratios of pyrene solutions (2.0 \( \mu \text{mol} \cdot \text{L}^{-1} \)) versus the logarithm of the PDSS concentration (log C) at 25 °C

Solubility in FaSSIF and FeSSIF

Although aqueous solubility has traditionally been a standard medium in the BCS, biorelevant gastrointestinal media provide a more physiologically relevant estimate of the solubility in the intestinal environment \[26\]. Among the most widely used media, simulated intestinal fluids (FaSSIF and FeSSIF) are bio-relevant gastrointestinal media used to mimic the intestinal milieu in fasted and fed conditions, respectively \[27\]. In generally, solubility in bio-relevant media is normally increased compared to the solubility in aqueous buffers as a result of enhanced micellar solubilization \[28\]. As shown in Table 1, the solubility of PDSS in FaSSIF and FeSSIF were significantly higher than the solubility in water. Several papers \[19\] have addressed the influence of CMC, as the most important parameter, on the solubility enhancement in FaSSIF and FeSSIF. A compound with a high propensity to form micellar systems on their own exhibits good ability to be solubilized by associating with the FaSSIF (or FeSSIF) colloidal arrangements because the integration of the compound into the FaSSIF (or FeSSIF) micellar structure is a prerequisite for solubilization. Conversely, a compound with high or no observable CMC (i.e., do not form micelles) shows negligible solubility enhancement. Thus, PDSS with an acceptable solubility in FaSSIF or FeSSIF would be beneficial in improving oral bioavailability, even though its solubility in aqueous was only 0.055 mg·mL\(^{-1}\).

Hydrolysis in different pH solutions

The examination of prodrug degradation in aqueous is essential to appraise its stability, providing a practical basis for the safety of clinical use and storage. The pH value is one of the most significant factors that influence the hydrolysis of prodrug in water. The cleavage of the ester bond between DSS and palmitoyl chain of PDSS was determined at the pH values from 2.0 to 9.0 at 37 °C. By plotting the natural logarithm of the remaining concentration of PDSS (ln C) versus time at different pH, it could be found that the hydrolysis of PDSS followed apparent first-order degradation kinetics at pH values ranging from 2.0 to 9.0, and the apparent hydrolysis rate constant (\(K_{\text{obs}}\)) and the half-life of degradation could be calculate via the pseudo-first-order kinetics equation as shown in Table 2.

<table>
<thead>
<tr>
<th>pH</th>
<th>pseudo-first-order kinetics</th>
<th>(r^2)</th>
<th>(K_{\text{obs}}) (h(^{-1}))</th>
<th>(t_{1/2}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>(C_t = C_0 \cdot e^{-0.049t})</td>
<td>0.994</td>
<td>0.049</td>
<td>14.15</td>
</tr>
<tr>
<td>4.2</td>
<td>(C_t = C_0 \cdot e^{-0.026t})</td>
<td>0.989</td>
<td>0.026</td>
<td>26.66</td>
</tr>
<tr>
<td>5.6</td>
<td>(C_t = C_0 \cdot e^{-0.012t})</td>
<td>0.992</td>
<td>0.012</td>
<td>57.76</td>
</tr>
<tr>
<td>6.8</td>
<td>(C_t = C_0 \cdot e^{-0.014t})</td>
<td>0.992</td>
<td>0.014</td>
<td>49.51</td>
</tr>
<tr>
<td>7.4</td>
<td>(C_t = C_0 \cdot e^{-0.017t})</td>
<td>0.986</td>
<td>0.017</td>
<td>40.77</td>
</tr>
<tr>
<td>8.0</td>
<td>(C_t = C_0 \cdot e^{-0.040t})</td>
<td>0.952</td>
<td>0.049</td>
<td>14.15</td>
</tr>
<tr>
<td>9.0</td>
<td>(C_t = C_0 \cdot e^{-0.132t})</td>
<td>0.997</td>
<td>0.152</td>
<td>4.56</td>
</tr>
</tbody>
</table>

\(K_{\text{obs}}\): the observed hydrolysis rate constant; \(t_{1/2}\): half-life.

The stability profile of PDSS in different pH solutions was obtained by plotting the natural logarithm of the apparent hydrolysis rate constant (ln \(K_{\text{obs}}\)) versus the corresponding pH values. As shown in Fig. 4, the pH had a significant impact on the stability of PDSS, and neutral and acidic conditions were more favorable than alkaline condition. It can be seen that when the pH value gone through 5.6, the ln \(K_{\text{obs}}\) reached the lowest point, which meant that the hydrolysis reaction rate of PDSS was slowest, and the structure of PDSS was the most stable at pH 5.6 with a \(t_{1/2}\) value of 57.76 h. When pH was above 7.4, the PDSS hydrolysis rate rose quickly. The highest hydrolysis rate was obtained at pH 9 with a \(t_{1/2}\) value of 4.56 h. These results indicated that PDSS was more susceptible to cleavage in the alkaline environment and the relatively stable pH range was from 5.6 to 7.4.

Enzymatic hydrolysis in vitro

Carboxylesterases that show ubiquitous expression profiles are crucial in the biotransformation of ester-containing prodrugs into their therapeutically active forms in the body \[29\]. High levels of carboxylesterases are found in the liver, small intestine and lungs where prodrugs are hydrolyzed firstly before entering the systemic circulation, and they are also
Fig. 4 The pH-rate profile for the degradation of PDSS in PBS with different pH values. The pH values were 2.0, 4.2, 5.6, 6.8, 7.4, 8.0, and 9.0; the initial concentration of PDSS was 0.1 mg·mL⁻¹; the conditions were 60 r·min⁻¹, 24 h and 37 °C; samples (0.2 mL) were collected every 2 h followed by HPLC analysis. Data are expressed as mean ± SD (*n* = 3).

abundant in the human plasma. Thus, porcine liver carboxylesterase was chosen to inspect the prodrug-to-drug conversion at 37 °C for 48 h in the present experiment. The rate of enzymatic hydrolysis is shown in Fig. 5. The pseudo first-order rate constant was 0.045 mmol·L⁻¹·min⁻¹ (*R*² = 0.996) and the half-life was 15.40 h for PDSS. It was clear that the hydrolysis of PDSS was much faster in esterase solution than that in the buffer solution of pH 7.4. In addition, this enzymatic hydrolysis led to the expectation that there will be delayed release of DSS in vivo.

Fig. 5 Enzymatic degradation of PDSS (black line) in carboxylesterase solution to DSS (red line). The concentrations of PDSS and carboxylesterase were 50 μg·mL⁻¹, 18 U·mL⁻¹; the conditions were PBS 7.4, 60 r·min⁻¹, 48 h and 37 °C; samples (0.2 mL) were collected and followed by HPLC analysis. Data are expressed as means ± SD (*n* = 6).

Fig. 6 Mean plasma concentration of DSS versus time profiles after oral administration. Group A (black line), 30 mg·kg⁻¹ DSS; Group B (red line), 66 mg·kg⁻¹ PDSS, equivalent to 30 mg·kg⁻¹ DSS. Insert figure was magnification of the time from 0 h to 2 h. Data are expressed as means ± SD (*n* = 6).

**Pharmacokinetic properties**

The concentration of DSS in blood plasma was quantified using HPLC-MS. The plasma DSS concentration-time profiles upon oral administration to rats are shown in Fig. 6, and the pharmacokinetic parameters are shown in Table 3. There were significant differences in the *C*ₘₐₓ of DSS between Group A and Group B (0.81 and 1.67 μg·mL⁻¹, respectively, *P* < 0.01). *AUC*₀→∞ values were 1.20 ± 0.08 and 11.71 ± 0.24 μg·mL⁻¹·h⁻¹ for Group A and Group B, respectively. *AUC* is an important pharmacokinetic parameter to assess the exposure and circulating time of a drug. The approximate 10-fold increase in *AUC*₀→∞ (*P* < 0.01) was indicative of a significant enhancement in the oral bioavailability of DSS when it was in the form of a lipophilic prodrug. It was also worth noting that the DSS plasma concentration in Group A showed a *t*ₘₐₓ (the time at which *C*ₘₐₓ is reached) of 0.67 h and declined quickly with a *t*₁/₂ of 1.02 h, suggesting a short circulation time. However, the results obtained from Group B showed a slower rise with a *t*ₘₐₓ of 2.23 h with a *t*₁/₂ of 4.67 h, which indicated that circulation time of Group B was longer than that of Group A (*P* < 0.05).

**Table 3 Pharmacokinetic parameters of Groups A and B after oral administration.** Data are expressed as means ± SD (*n* = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>t</em>₁/₂ (h)</td>
<td>1.02 ± 0.06</td>
<td>2.23 ± 0.12</td>
</tr>
<tr>
<td><em>t</em>ₘₐₓ (h)</td>
<td>0.67 ± 0.01</td>
<td>4.67 ± 0.37</td>
</tr>
<tr>
<td><em>C</em>ₘₐₓ (μg·mL⁻¹)</td>
<td>0.81 ± 0.06</td>
<td>1.67 ± 0.11</td>
</tr>
<tr>
<td><em>AUC</em>₀→∞ (μg·h·mL⁻¹)</td>
<td>1.20 ± 0.08</td>
<td>11.71 ± 0.74</td>
</tr>
</tbody>
</table>

*a* 0.01 < *P* < 0.05 vs Group A; 
*b* *P* < 0.01 vs Group A;
*t*₁/₂: The elimination half-life time; 
*t*ₘₐₓ: Time to maximum plasma concentration; 
*C*ₘₐₓ: Maximum plasma concentration; 
*AUC*₀→∞: Area under the curve from zero to infinity.
Above results demonstrated that the lipophilic prodrug of DSS significantly promoted the absorption of DSS and prolonged the DSS residue time in plasma. The improvement in oral bioavailability of PDSS was probably due to the following reasons: (i) DSS is a drug with high aqueous solubility and poor membrane permeability. This kind of drugs is too hydrophilic to permeate transcellularly and can only cross the biological membrane paracellularly into the systemic circulation \[30\]. However, the total area of paracellular pathway is ranging from 0.1% to 1%, relative to the transcellular route in intestinal epithelium \[31\], thus limiting the absorption of drug. (ii) It is well-known that compounds with optimum lipophilicity (log P) and appropriate molecular weight have reasonable permeability \[32\]. The log P and molecular weight of PDSS were, 1.90 and 436.28, respectively; hence it is consistent with the Lipinski’s rule-of-5, which may subsequently improve the membrane permeability of PDSS. (iii) The lipophilic drugs can be also transported to the systemic circulation by intestinal lymphatic system. The main mechanism of the lymphatic transfer is the combination of lipophilic drugs and chylomicrons in enterocyte and subsequent absorbed by the lymphatic transfer is the combination of lipophilic drugs can be also transported to the systemic circulation. (iv) The lipophilic prodrug can help improve pharmacokinetic properties as well as oral bioavailability of DSS.

**Conclusion**

In the present study, the palmitoyl prodrug of DSS, PDSS, was successfully synthesized by introducing a palmitoyl chain to the alcoholic hydroxyl of DSS. The chemical structure of the prodrug was confirmed by NMR, FT-IR, and HR-MS analyses. The in vitro and in vivo studies demonstrated better physicochemical stability and improved pharmacokinetics of PDSS. Compared with the parent DSS, PDSS showed increased absorption efficiency, oral bioavailability and prolonged circulation time in plasma. Therefore, the development of the lipophilic prodrug can help improve pharmacokinetic properties as well as oral bioavailability of DSS.

**References**


