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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of 9-O-glycosyl-berberine derivatives and bioavailability evaluation

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Abstract To increase the bioavailability of berberine, three new 9-O-glycosyl-berberine derivatives, 9-O-glucosyl-(**4a**), 9-O-arabinosyl-(**4b**), and 9-O-erythrol-(**4c**) were obtained and confirmed by UV, ^{1}HNMR, ^{13}CNMR, and MS. The pharmacokinetic profiles of these synthetic compounds have been evaluated compared with berberine (**1**) and 9-O-alkyl-berberine (**5**) derivatives, which showed that maximum concentration (C_{max}) and area under concentration–time curve (AUC) of 9-O-glycosyl-berberine increased dramatically. The results indicated that hydrophilic modification could significantly improve the bioavailability of berberine; 9-O-glycosyl-berberine might be a promising prodrug.

Keywords Berberine · Structural modification · Bioavailability · 9-O-glycosyl-berberine

Introduction

Berberine (**1**) is one of the most important alkaloids existed in *Coptis chinensis* French. Reportedly, it has many important bioactivities, such as hypoglycemic, antihyperlipidemics, anti-bacterial, anti-inflammatory, and antineoplastic (Cernakova and Kostalova, 2002; Zhang *et al*., 2010; Kong *et al*., 2004) effects, etc. The structure of **1** is shown in Fig. 1. Previous studies indicated that the low bioavailability is one of the most important factors influencing the therapeutic effects of **1** in vivo (Xiong *et al*., 1989; Wang *et al*., 2005; Sheng and Sun, 1993; Bao *et al*., 1997). It also leads to high dosage for hypoglycemic and antihyperlipidemics oral treatment (Qian, 2010; Shen *et al*., 1996). Consequently, many researchers attempted to increase the bioavailability of **1** to improve the potency and decrease the oral dosage. It was found that coadministration with oryzanol could improve the absorption of **1** (Li *et al*., 2000).Our results indicated that some additives, such as glutin, could increase the blood concentration and bioavailability of **1** (Li *et al*., 2009). Studies on metabolites of **1** from human and rat urine indicated that they are existed as glucoside and sulfate, moreover 9-O-glucosyl-berberine (**4a**) shows the highest concentration (Pan *et al*., 2002; Qiu *et al*., 2005). Therefore, the transformation from **1** to its water-soluble derivatives supplied a new idea to increase the bioavailability of berberine (**1**) by means of changing in hydrophilicity.

Studies upon structural modifications of **1** have been reported frequently, which focus on the field of alkyl and aromatic substitution. A compound possessing palmitate at the C_9-position showed an effective inhibition on low-density lipoprotein receptor (LDLR) expression (Li *et al*., 2010). The 9-substituted derivatives of **1**, especially with terminal amino group at C_9-position, would significantly improve the binding affinity with G-quadruplex, resulting in increasing inhibitory effects on the amplification of telo21 DNA and on the telomerase activity (Zhang *et al*., 2007). Introducing benzyl group into C_7-position showed an effective hypoglycemic activity in vitro (Bian *et al*., 2006). Substitution on C_8 and C_{13}-position with alkyl or phenyl

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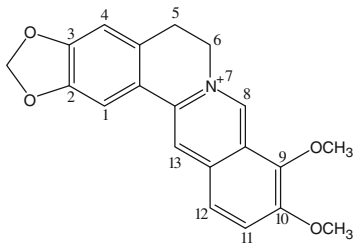


Fig. 1 Structure of berberine

group and on C_{12}-position with bromine group showed antibacterial activity (Iwasa *et al.*, 1998a, b). A series of compounds bearing 9-O-acyl and 9-O-alkyl-substituents were synthesized and tested by Kim. It suggested that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal antimicrobial activity (Kim *et al.*, 2002). Our experiments on 8-alkyl-substituents suggested that 8-octyl-berberine significantly increases the activity of anti-bacterial and antiatherosclerosis (Yang *et al.*, 2007).

In current study, hydrophobic alkyl and hydrophilic glycosyl as substituent groups were introduced into C_9-position of **1** to synthesize 9-O-glycosyl-(**4a–c**) and 9-O-alkyl-berberine (**5a–b**) derivatives. Then, the effects of hydrophilicity on the bioavailability of berberine were investigated to develop new prodrugs with an improved bioavailability.

Results and discussion

Synthesis

In this study, five novel compounds have been synthesized, and compounds **4a–c** were synthesized for the first time. Schemes 1 and 2 illustrated the synthetic route for the preparation of target compounds. The spectra data and other characteristic parameters of compounds **4a–c** and **5a–b** were shown in the Experimental section. Compared with ^{1}H and ^{13}C NMR data of **1**, the peak data of 9-OCH_3 disappeared and that of hydrogen or carbon in glucose, arabinose or erythritol which has not been covered by solvent peak emerged in the ^{1}H and ^{13}C NMR data of compounds **4a–c**. All spectral data can be analyzed reasonably by their molecular structures. As a consequence, we concluded that compounds **4a–c** were synthesized successfully.

Optimization of intermediate products synthesis

These studies were carried out on 9-O-(2′,3′,4′,6′-O-Tetraacetyl-glucosyl)-berberine (**3a**), 9-O-(2′,3′,4′-O-triacetyl-arabinosyl)-berberine (**3b**), and 9-O-(2′,3′,4′-triacetyl-erythritol)-berberine (**3c**) to investigate the optimized reaction condition. The synthetic procedure of intermediate products **3** were the critical process for synthesis of end product **4**. The yields of compound **3** were related to temperature and solvent. Tables 1 and 2 illustrated the yields of compound **3a** affected by different temperature and solvents, respectively. It was concluded from experimental results that with DMF as solvent at 45°C, we could obtain the highest yield of compound **3**.

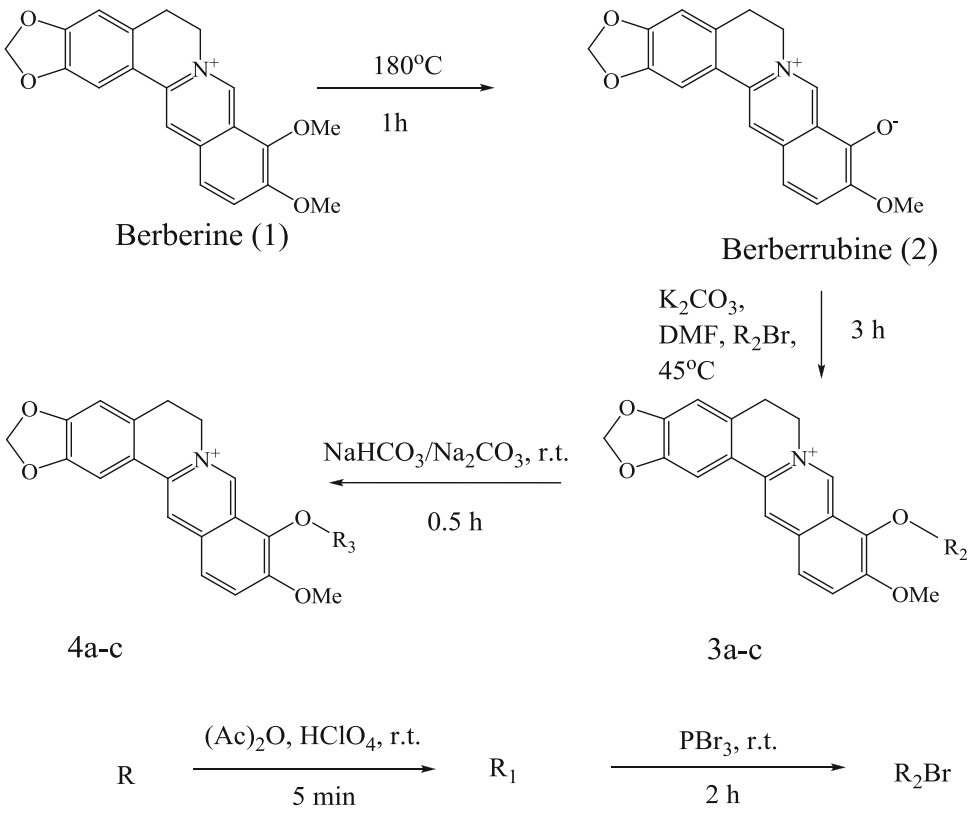
In vivo pharmacokinetic evaluation

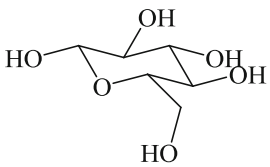
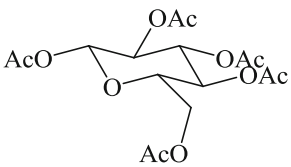
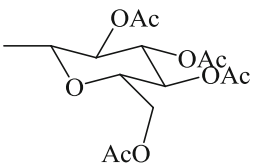
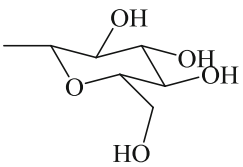
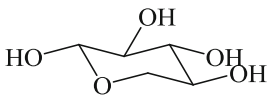
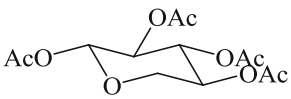
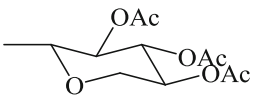
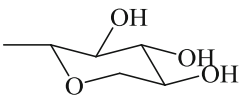
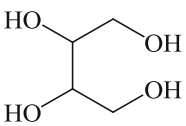
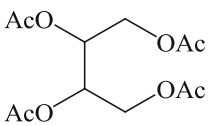
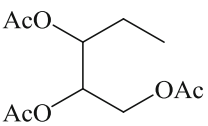
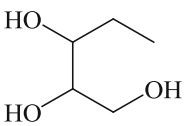
These studies were carried out on six compounds **1**, **4a–c**, **5a**, and **5b** by way of oral administration for SPF rats to investigate the in vivo pharmacokinetics. The pharmacokinetic parameters were listed in Table 3. Large amount of **1** were detected in plasma after **4a–c** treatment, while **5a** and **5b** had not degraded in plasma.

The tested results of pharmacokinetic experiments in vivo demonstrated that the maximization plasma concentration (C_{max}) and area under concentration–time curve (AUC) of all glycosylated synthesized derivatives **4a–c** were remarkably improved after oral administration. Compound **4a** showed the highest C_{max} and AUC, which were 9.3 and 11.1 times higher than that of compound **1**, respectively. Compared with compound **1** and **4**, tested results above indicated that compound **5** modified with hydrophobic alkyl groups had lower plasma concentration and bioavailability. Above results demonstrated that glycosylated modification would enhance the hydrophilic activity of a compound, thus improve the dissolution rate of the compound in plasma, which is one of the factors that lead to the increasing of bioavailability. On the contrary, the hydrophobic structural modification on 9-O-position of berberine decreased the bioavailability.

Conclusion

In conclusion, the current research undertakes the synthesis of five novel derivatives of berberine. Three of them, compounds **4a–c**, were synthesized for the first time. All the newly synthesized compounds have been tested for their in vivo pharmacokinetic pattern. From the perspective of present investigation, it can be inferred that the introduction of hydrophobic alkyl groups will reduce the plasma concentration and bioavailability of berberine, but the glycosylated modification will improve the dissolution rate of the compound in plasma and furthermore, improve its bioavailability.



Comp	R	R_1	R_2	R_3
3a/4a				
3b/4b				
3c/4c				

Scheme 1 Synthetic route of 9-O-glycosyl-berberine

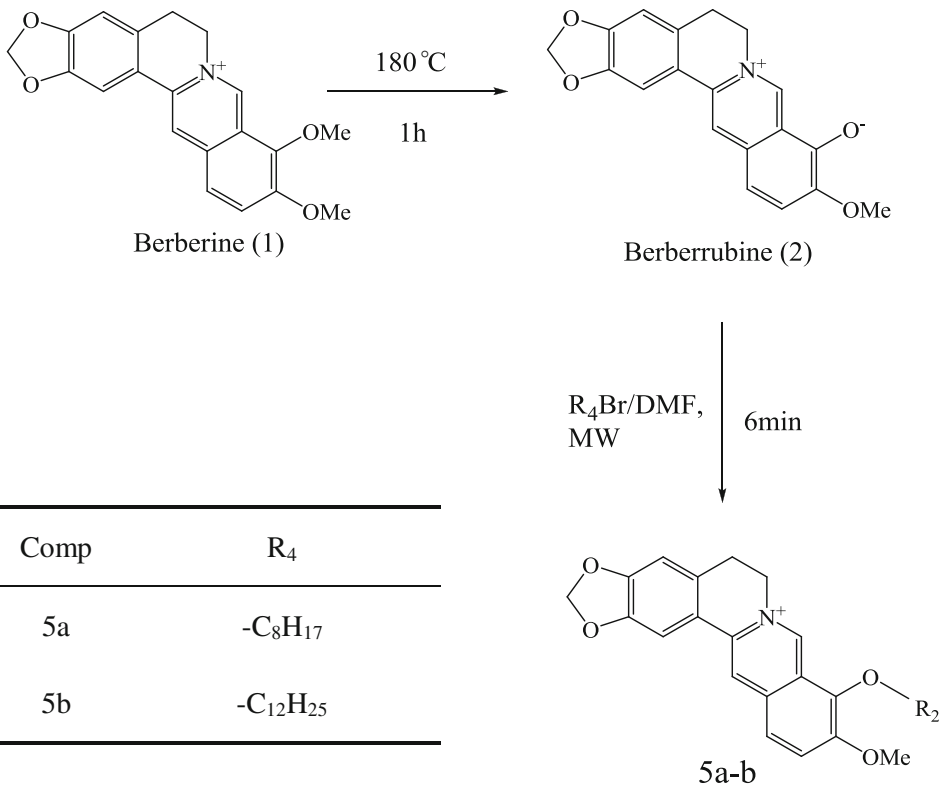
Materials and methods

Experimental

Berberine standard and monosaccharide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Berberine used for synthesis was extracted from *Coptis chinensis* French, whose purity was over 98% analyzed by HPLC. Other reagents were from Pharmaceutical and Chemical Reagent Inc. (Chongqing, China), which were of analytical grade.

UV, ^{1}H NMR, ^{13}C NMR, Mass spectrometry (MS), and TLC were used to identify the structures of new compounds. UV spectra were recorded on a Hitachi U-1800

Scheme 2 Synthetic route of 9-O-alkyl-berberine



Comp	R_4
5a	$-C_8H_{17}$
5b	$-C_{12}H_{25}$

Table 1 Yields of compound **3a** at different temperatures with DMF as solvent

	Yields at different temperature (%)				
Temperature (°C)	25	35	40	45	50
Yields	63[a]	58	71	85	21

[a] The reaction conducted at 25°C was lasted for 8 h while others were lasted for 3 h

Table 2 Yields of compound **3a** with different solvents at 45°C

	Yields with different solvents (%)			
Solvent	CH_3Cl	DMF	DMSO	CH_3OH
Yields	42	85	84	11

Table 3 Pharmacokinetic parameters of compounds **1**, **4a–c**, and **5a–b**

Compound	T_{max} (h)	C_{max} (μg/l)	AUC (μg h/l)	$t_{1/2}$ (h)
1	2	6.79	24.184	7.88
4a	3	63.42	268.94	7.58
4b	3	48.74	205.146	7.43
4c	3	37.49	180.316	7.31
5a	3	4.39	17.329	7.85
5b	3.5	3.71	14.592	7.86

UV apparatus. The ^1H and ^{13}C NMR spectra were recorded on Bruker Model Advance DMX300 Spectrometer (300M) using TMS as an internal standard and DMSO-d_6 or methonal-d_4 as solvent. MS spectra were collected on Shimadzu LCMS-2010A instrument. TLC analysis was used to confirm the purity of the compounds, performed on silica gel-GF_{254} thin layer chromatogram and spread with corresponding mobile phase. The mobile phase for 9-O-alkyl-berberine was C_6H_6/EtOAc/i-PrOH/MeOH/NH_4OH (6:3:1.5:1.5:0.5), and 9-O-glycosyl-berberine derivatives were EtOAc/MeOH/light petroleum/NH_4OH (6:3:3:0.5).

Synthesis of acetyl-glycosyl bromide (glycosylated reagent)

Perchloric acid (0.15 ml) was dropped slowly into monosaccharide (glucose, arabinose, or erythrol, 0.27 mol) in 20 ml acetic anhydride at 0°C, then kept stirring at room temperature for 5 min. Phosphorus tribromide (8.5 ml) was then dropped into reaction solution at 0°C, and kept stirring at room temperature for 2 h. The reaction solution was then diluted by 50 ml $CHCl_3$, washed by sodium bicarbonate solution at 0°C, dehydrated the solution, and kept in freezer.

Synthesis of compound **3**

Berberrubine (**2**) was obtained from compound **1** according to previous literature (Iwasa *et al.*, 1996). Glycosylated

reagent (0.27 mol in 50 ml $CHCl_3$) was dropped slowly into compound **2** (0.743 g, 2.7 mmol) dissolved in 20 ml DMF with 3.2 g K_2CO_3 as catalyst at 0°C under magnetic stirring and nitrogen protection. After 3 h stirring at 45°C, reaction solution was washed by distilled water and then dehydrated. Organic phase was concentrated and purified by a silica gel column (3 × 75 cm, 100 ml of 200–300 mesh silica gel), and the eluent was 400 ml EtOAc/MeOH/light petroleum(2:1:1). The resulting eluent was concentrated and crystallized twice with ethanol at 6–8°C to collect compound **3**.

Synthesis of compound **4**

Compound **3** (2 mmol) was dissolved in 50% methanol with 0.25 g $NaHCO_3$ and 0.11 g Na_2CO_3. After 0.5 h stirring below 40°C, the reaction solution was concentrated and crystallized twice with ethanol at 8°C to get Compound **4a**–**c**.

*9-O-glucosyl-berberine **4a***

Yield, 71%. Rf, 0.15; UV (MeOH) λmax 230, 265, 350 nm; ^{1}H NMR: δ (ppm) (MeOD), 9.836(1H, m, 8-Ar–H), 8.714(s, 1H, 13-Ar–H), 8.126(m, 1H, 1-Ar–H), 8.097(m, 1H, 4-Ar–H), 7.673(s, 1H, 12-Ar–H), 6.969(s, 1H, 11-Ar–H), 6.113(s, 2H, 2,3-OCH_2O–), 5.176(s, $J = 7.0$ Hz, 1H, Glu-1′–CH,), 4.121(t, 4H, 6-CH_2–, Glu-4′,5′-H), 3.595–3.427(t, 13H, 10-OCH_3, Glu-2′,3′,4′,5′-H, Glu-2′,3′,4′,5′-OH, Glu-5′-CH_2); ^{13}CNMR δ(ppm): 26.46, 55.45, 57.18, 62.86, 71.30, 72.59, 75.23, 80.22, 102.27, 104.34, 106.46, 109.10, 119.71, 120.33, 121.75, 123.42, 126.83, 131.62, 133.14, 137.55, 142.99, 145.36, 147.78, 149.91, 151.86; MS (*m/z*) $[M]^+$: Calcd: 484.4784; Found: 484.

*9-O-arabinosyl-berberine **4b***

Yield 69%. Rf, 0.13; UV (MeOH) λmax 235, 265, 350 nm; ^{1}H NMR: δ (ppm) (MeOD), 10.304(m, 1H, 8-Ar–H), 9.387(s, 1H, 13-Ar–H), 9.188(m, 1H, 1-Ar–H), 7.840(m, 1H, 4-Ar–H), 7.663(s, 1H, 12-Ar–H), 7.123(s, 1H, 11-Ar–H), 6.241(s, 2H, 2,3-OCH_2O–), 4.694(m, 2H, 6-CH_2–), 3.940–3.865(s, 4H, 10-OCH_3, Ara-2′-H), 3.191(s, 2H, 5-CH_2–); ^{13}CNMR δ(ppm): 26.46, 55.45, 57.18, 66.71, 70.30, 71.23, 72.41, 102.27, 104.34, 106.46, 109.10, 119.71, 120.33, 121.75, 123.42, 126.83, 131.62, 133.14, 137.55, 142.99, 145.36, 147.78, 149.91, 151.86; MS (*m/z*) $[M]^+$: Calcd: 454.4526; Found: 454.

*9-O-erythrol-berberine **4c***

Yield 59%. Rf, 0.18; UV (MeOH) λmax 232, 264, 350 nm; ^{1}H NMR: δ (ppm) (MeOD), 10.296(m, 1H, 8-Ar–H), 9.386(s, 1H, 13-Ar–H), 9.180(m, 1H, 1-Ar–H), 7.834(m, 1H, 4-Ar–H), 7.663(s, 1H, 12-Ar–H), 7.123(s, 1H, 11-Ar–H), 6.242(s, 2H, 2,3-OCH_2O–), 4.695(s, 2H, 6-CH_2–), 4.214(m, $J = 7.2$ Hz, 1H, Ery-1'-H), 3.865(s, 5H, 10-OCH_3, Ery-1′-H, Ery-4′-H), 3.192(s, 2H, 5-CH_2–); ^{13}CNMR δ(ppm): 26.46, 55.45, 57.18, 62.18, 64.75, 72.81, 73.64, 102.27, 106.46, 109.10, 119.71, 120.33, 121.75, 123.42, 126.83, 131.62, 133.14, 137.55, 142.99, 145.36, 147.78, 149.91, 151.86; MS (*m/z*) $[M]^+$: Calcd: 426.4426; Found: 426.

Synthesis of 9-O-alkyl-berberine (compound **5**)

Bromoalkane (bromooctane or bromododecane, 1.0 ml) were added into compound **2** (1.0 g) and dissolved in 10 ml DMF. After 6 min microwave heating at 400 W, the reaction solution were cooled and filtrated. The filtrate was concentrated and filtrated again with diethyl ether. Collected the sediments and crystals received from filtration and then crystallized twice with ethanol at 6–8°C to collect compound **5**.

*9-O-octyl-berberine **5a***

Yield 48%, Rf, 0.43; ^{1}HNMR: δ (ppm) (DMSO-d_6), 9.73(s, 1H, 8-Ar–H), 8.92(s, 1H, 13-Ar–H), 8.18(s, 1H, 1-Ar–H), 7.96(s, 1H, 4-Ar–H), 7.78(d, 1H, 12-Ar–H), 7.08(d, 1H, 11-Ar–H), 6.15(s, 2H, –OCH_2O–), 4.93(t, 2H, 6-CH_2–), 4.25 (t, 2H, –OCH_2–), 4.03 (s, 3H, –OCH_3), 3.16(t, 2H, 5-CH_2–), 1.85(m, 2H, –O–C–CH_2–), 1.46(m, 2H, –CH_2–), 1.26(m, 8H, -$(CH_2)_4$–), 0.85(t, 3H, –CH_3); ^{13}CNMR δ(ppm): 14.02, 22.15, 25.35, 26.43, 28.75, 28.87, 29.56, 31.32, 55.42, 57.16, 74.35, 102.17, 105.52, 108.50, 120.31, 120.54, 121.75, 123.36, 126.83, 130.79, 133.14, 137.55, 142.99, 145.36, 147.78, 149.91, 150.48; MS (*m/z*) [M-Br]$^+$: Calcd: 434.2332; Found: 434.

*9-O-dodecyl-berberine **5b***

Yield 31.7%,Rf, 0.53; ^{1}HNMR: δ (ppm) (DMSO-d_6), 9.73(s, 1H, 8-Ar–H), 8.92(s, 1H, 13-Ar–H), 8.18(s, 1H, 1-Ar–H), 7.96(s, 1H, 4-Ar–H), 7.78(d, 1H, 12-Ar–H), 7.08(d, 1H, 11-Ar–H), 6.15(s, 2H, –OCH_2O–), 4.93(t, 2H, 6-CH_2–), 4.25 (t, 2H, –OCH_2–), 4.03 (s, 3H, –OCH_3), 3.16(t, 2H, 5-CH_2–), 1.85(m, 2H, –O–C–CH_2–), 1.45(m, 2H, –CH_2–), 1.22(m, 16H, –$(CH_2)_8$–),0.81(t, 3H, –CH_3); ^{13}CNMR δ(ppm): 14.02, 22.15, 25.35, 26.43, 28.75, 28.80, 28.81, 28.81, 28.82, 28.87, 29.56, 31.32, 55.42, 57.16, 74.35, 102.17, 105.52, 108.50, 120.31, 120.54, 121.75, 123.36, 126.83, 130.79, 133.14, 137.55, 142.99, 145.36, 147.78, 149.91, 150.48; MS (*m/z*) [M-Br]$^+$: Calcd: 490.2958; Found: 490.

Pharmacokinetic evaluation

Healthy SPF rats (200 ± 20 g) were purchased from Laboratory Animal Centre of Chongqing Medical University (Chongqing, China). They were housed in stainless cages in a room with controlled temperature (23 ± 2°C) and humidity (40–60%) and a 12 h light/dark cycle. The protocol complied with the guidelines of Chongqing City Laboratory Animal Administration Committee of China for the care and use of laboratory animals. The rats were randomly divided into six groups with six each for plasma drug concentration test. Compounds **1** and **4** were dissolved in 2% Tween-80 aqueous solution, while compound **5** was dispersed in 0.7% CMC-Na solution with hyperacoustic treatment for 1 h at 60°C, all compounds were given to rats by oral administration (100 mg/kg body wt), respectively. After 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 h, respectively, 0.4 ml plasma samples were taken from the orbital veins of rats for HPLC analysis (Wang *et al*., 2010) to investigate the pharmacokinetics of compounds **1**, **4**, and **5**.

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