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Original article

Design, synthesis and biological evaluation of brain targeting L-ascorbic acid prodrugs of ibuprofen with "lock-in" function

Yi Zhao [a, 1], Boyi Qu [a, 1], Xueying Wu [b], Xiaocen Li [a], Qingqing Liu [a], Xiuxiu Jin [a], Li Guo [a], Li Hai [a, *], Yong Wu [a, *]

[a] Key Laboratory of Drug Targeting of Education Ministry, West China School of Pharmacy, Sichuan University, Chengdu 610041, PR China
[b] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China

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ABSTRACT

A novel brain targeting L-ascorbic acid derivatives with "lock-in" function were designed and synthesized as prodrugs to achieve the effective delivery of ibuprofen to brain by glucose transporter 1 ($GLUT_1$) and the Na^+-dependent vitamin C transporter $SVCT_2$. Ibuprofen-loaded four prodrugs were tested in the animals. Results from the in vivo distribution study after i.v. administration of these four prodrugs and naked ibuprofen indicated that four prodrugs exhibited excellent transport ability across the BBB and significantly increased the level of ibuprofen in brain. Among them, prodrugs **4** showed higher brain concentration. Both biodistribution data and pharmacokinetic parameters suggested that L-ascorbic acid thiamine disulfide delivery system was a promising carrier to enhance CNS drug's delivery ability into brain.

1. Introduction

Over the past few decades, with the rapid development of medical science, many diseases are gradually being overcome. But the treatment of central nervous system (CNS) diseases, such as Alzheimer's disease (AD) and Parkinson's disease, is still a major challenge for the medical field. One of the crucial problems in drug delivery into the CNS is the low permeability of drugs due to the blood–brain barrier (BBB). The BBB plays an important role in its further protecting action towards the brain microenvironment, the function of BBB is not only a physical barrier but also a biochemical barrier [1]. Difficulties in crossing the BBB often hinder the bioavailability of drugs in the treatment of CNS diseases, such as those diseases mentioned above. Numerous studies [2,3] have suggested that chronic intake of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, could reduce the risk or even delay the onset of CNS diseases [4,5]. Therefore, ibuprofen may be seen as a very promising drug for the treatment of CNS diseases [6–8]. In order to achieve the satisfactory therapeutic effect, ibuprofen in the CNS needs to reach a higher concentration. However, because of the poor permeability of NSAIDs, the larger doses are required to achieve the desired therapeutic effect. This will cause a lot of gastrointestinal adverse effects and some toxicity, and may cause some damage to the body [9]. Therefore, the application of ibuprofen has been greatly restricted, and a new promising strategy should be developed to overcome the problems of low BBB permeability of ibuprofen and also to improve brain delivery of ibuprofen for the treatment of CNS diseases.

L-Ascorbic acid (AA), which is usually mentioned as vitamin C, is essential for many enzymatic reactions, and it serves to scavenge free radicals in order to protect tissues from oxidative damage [10]. In recent studies, the highest concentration of AA can be found in brain tissue, where it acts as an antioxidant and plays an important physiological function [11,12]. According to the recent researches on AA, the transport and storage mechanisms of AA in the brain have been clearly explained. It is generally believed that the transportation of AA in the brain is mainly based on two distinct ways: the facilitative sugar transporters of the GLUT type can transport the oxidized form of AA, dehydro-ascorbic acid (DHAA), which is then reduced into AA, and will retain in the brain and enhance to its CNS level [13,14]; while Na^+-dependent vitamin C transporter $SVCT_2$ transports AA directly into brain [15]. Based on

* Corresponding authors.
E-mail address: wyong@scu.edu.cn (Y. Wu).
[1] These authors contributed equally to this study and share first authorship.

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the above two transport ways, let us have a deeper understanding of the AA.

Recently, much studies reported that AA could be used as a carrier to improve its BBB permeation properties and then to promote brain drug delivery [16,17]. It was reported that the hydroxyl groups of enediol lactone in C2 and C3 are the important reaction sites and are required for the reducing properties of AA in the redox process while the C6-hydroxyl group of AA is not critical for the transport [18,19]. Because of these reports, most researchers focused their attention on the preparation of C6–*O*-modified AA, while we are also more curious about the preparation of C5–*O*-modified AA, and also C5&C6–*O*-di-derivatized AA (Fig. 1). Our previous work gave the answer for these ideas: it was showed that C5–*O*-ibuprofen-AA (Prodrug **2**), had the similar or even better targeting ability than C6–*O*-ibuprofen-AA (Prodrug **1**), which indicated that C5–OH of AA is a preferred position for modification. The RE, relative uptake efficiency $((AUC_{0-t})_{pro}/(AUC_{0-t})_{ibu})$ were enhanced to 2.03, 2.25 times than that of ibuprofen. Furthermore, C5–O-&C6–O-di-ibuprofen-AA (Prodrug **3**), whose C5–*O*&C6–*O* positions were both modified, also had good targeting ability for brain. And the RE was enhanced to 3.51 times than that of ibuprofen [20]. Therefore, the above results indicated that C5&C6-hydroxyl groups of AA may be both not critical for the transport.

Although our previous work has shown that our design is feasible, our group is still exploring how to further improve the carrier's BBB permeation. $GLUT_1$ and $SVCT_2$ are bidirectional transporters [21,22] which mediate the blood-to-brain and brain-to-blood transport of AA in either direction across the BBB. This indicated that the AA mediated ibuprofen would be pumped out to the outside of the brain after their entry, which prevented the concentration of prodrugs in the brain. In order to avoid the bidirectional delivery, a lipophilic thiamine disulfide system (TDS) [23] with the ability of "lock-in" attracted our attention. TDS is more stable and convenient for conservation in the air, which can be reduced by disulfide reductase and then ring-closed to be a thiazolium once entered the CNS, and then "locked" in the brain and can not be transported outside across the BBB. Then, followed by simple hydrolysis, the prodrug system can release the active drug to play a therapeutic role [24]. This TDS modified prodrug system seems to be a good way for the delivery, which not only can increase the lipophilic prodrug itself, making it easy to cross the BBB, but also prevent the bidirectional delivery of AA, improving the brain drug concentration. Hence, in the present study TDS was introduced into AA delivery system to form a novel brain targeting L-ascorbic acid prodrug (Prodrug **4**, Fig. 2) with ester linkage between ibuprofen and AA moieties with "lock-in" function. After changing of the behavior of prodrugs form bidirectional to unidirectional, this will enhance the concentration of the active drugs in brain (Fig. 3).

Based on the assumption above, we focused on the AA prodrug of ibuprofen with "lock-in" function, which linked TDS at C-6 position of AA and C5–O-&C6–O-di-ibuprofen-modified AA. In order to observe the effect of prodrug **4**, we also synthesized the reported

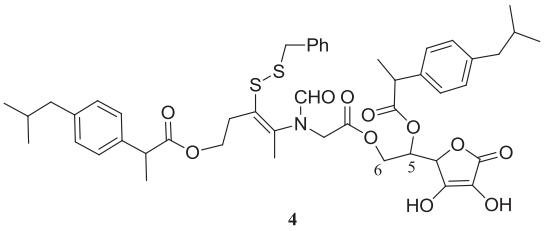

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Fig. 2. The structure of prodrug **4**.

prodrug **1**, **2**, **3** [20] as the reference. In this paper these four designed prodrugs were described in detail. Furthermore, the brain biodistribution and pharmacokinetics of these prodrugs in mice after i.v. administration were discussed.

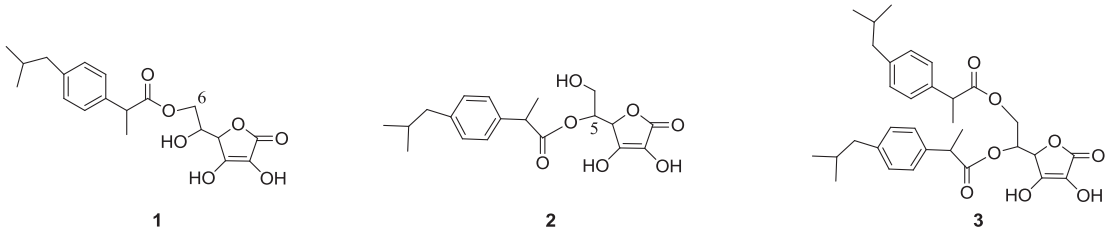
2. Chemistry

The C6–*O*-modified AA prodrug **1**, C5–*O*-modified AA prodrug **2** and C5&C6–*O*-di-derivatized AA prodrug **3** were synthesized according to the reported procedures in the reference 20. The synthetic routes of prodrug **1**, **2**, **3** were outlined in Schemes 1 and 2.

The synthetic route of prodrug **1** and **3** started from the available material AA. Briefly, in the presence of acetyl chloride, the hydroxyl groups of the 3, 4 positions of AA reacted with acetone to generate the 3,4-position isopropylidene protected AA ketol **5**. The ketol **5**, under the conditions of K_2CO_3 in acetone, was coupled with benzyl bromide to yield 2,3-*O*-di-benzyl protected derivative **6** in reflux, which deprotected the isopropylidene group under the action of HCl to give intermediate **7**. Then, coupled with the different number of equivalents of ibuprofen, intermediate **7** to get compound **8** or compound **9** by using DCC as a condensing agent, respectively. Finally, the debenzylation of the 2,3-*O*-di-benzyl groups of **8** and **9** with 10% Pd/C reached the prodrug **1** and **3**, respectively.

The synthesis of prodrug **2** was different from prodrug **1** and **3**, where it focused on the protection of C6-hydroxyl group of AA with suitable protecting group, thus exposing C6-hydroxyl group to couple with ibuprofen. Initially, we tried to introduce a phenyl group at the C3, C4 and C6 hydroxyl group at the same time, which can be removed together at the last step. Unfortunately, due to the difficult to control, we did not get a satisfactory yield of the product. Consequently, trityl chloride was chosen to selectively protect the C6-hydroxyl group of intermediate **7** to obtain compound **10**. Similarly, compound **10** was coupled with ibuprofen used DCC as dehydrating agent for ester condensation to get compound **11**. After the detritylation of compound **11**, 2,3-*O*-di-benzyl derivative **12** was obtained. Finally, the 2,3-*O*-di-benzyl groups of **12** was removed under hydrogen catalyzed by 10% Pd/C to provide prodrug **2.**

For the synthesis of the prodrug **4**, the key intermediate acid **18** is essential to achieve the "lock-in" functions. The synthetic route used to prepare key intermediate acid **18** has been illustrated in



1 2 3

Fig. 1. The structure of prodrugs **1**, **2**, **3**.

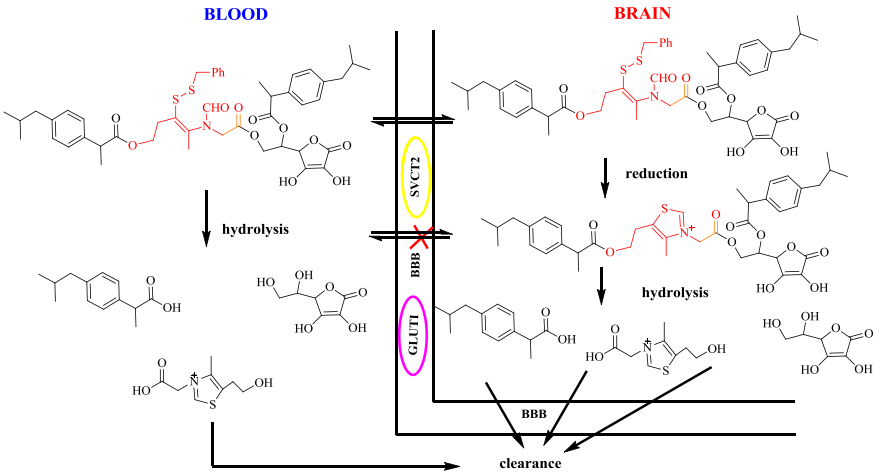
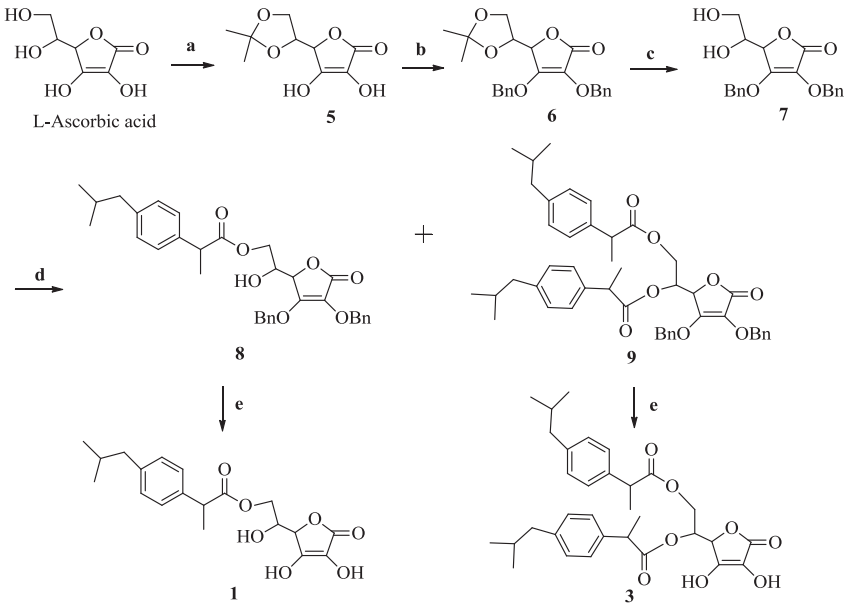
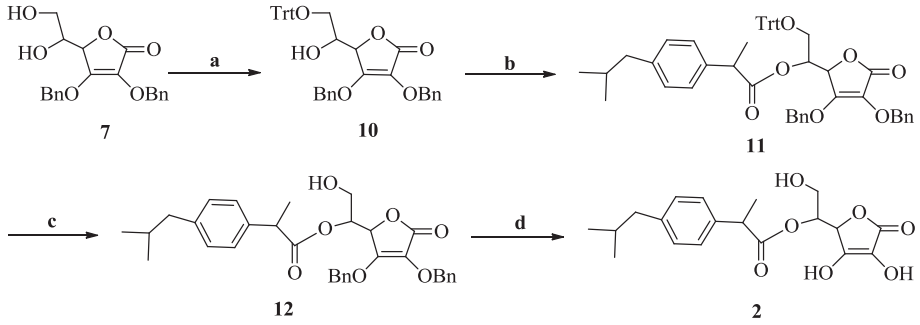


Fig. 3. Distribution, sequential metabolism and brain "lock-in" pathways of prodrug **4**.

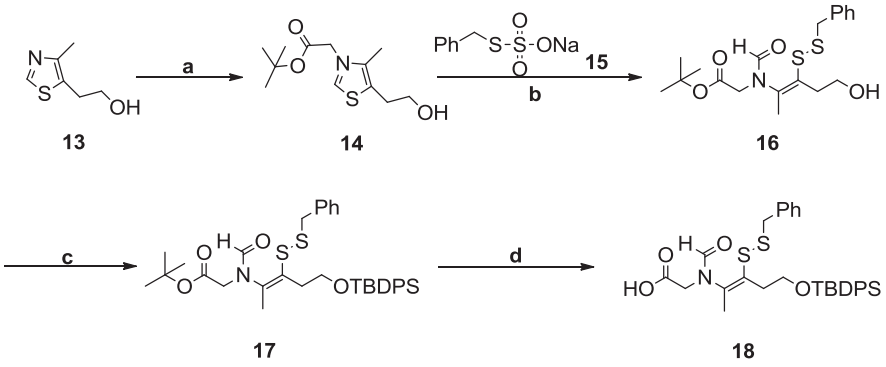


Scheme 1. The synthetic route of prodrug **1 and 3**. Reagents and conditions: (a) Acetone, acetyl chloride, r.t.; (b) BnBr, K_2CO_3, acetone, reflux; (c) HCl, CH_3CN, 30 °C; (d) DCC, DMAP, overnight; (e) 10% Pd/C, H_2, 0.4 MPa.

Scheme 3. It was started from the commercially available materials thiazole alcohol derivative **13**, which was reacted with tert-butyl bromoacetate in acetonitrile to yield tert-butyl ester **14**. Then, by the reaction of benzyl bromide with sodium thiosulfate to afford sodium S-benzyl sulfothioate **15**, which was coupled with tert-butyl ester **14** under alkaline conditions to obtain the ring-opened compound **16**. TBDPS-Cl was coupled with the hydroxy group of compound **16** to produce the TBDPS-protected compound **17**. Finally, after the hydrolysis of the compound **17**, the key intermediate acid **18** was successfully obtained.



Scheme 2. The synthetic route of prodrug **2**. Reagents and conditions: (a) TrtCl, Et_3N, DCM, r.t.; (b) DCC, DMAP, overnight; (c) HCl, CH_3CN, 50 °C; (d) 10% Pd/C, H_2, 0.4 MPa.



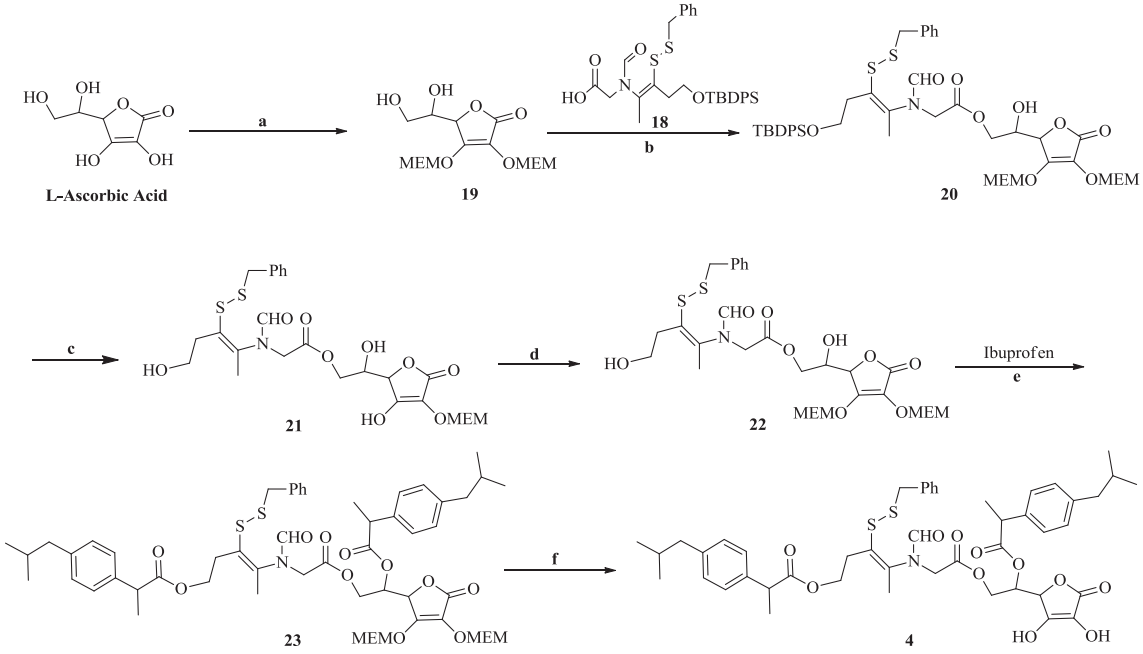
Scheme 3. The synthetic route of key intermediate acid **18**. Reagents and conditions: (a) tert-butyl bromoacetate, CH_3CN, 85 °C, 20 h, reflux; (b) S-benzyl sulfothioate **15**, NaOH, EA, 25 °C, 18 h; (c) imidazole, TBSCl, DCM, r.t., 3 h; (d) TFA, DCM, r.t., 5 h.

The synthetic pathway of prodrug **4** has been displayed in Scheme 4. AA as the starting material first reacted with 2-methoxyethoxymethyl chloride (MEMCl) to give the 2,3-hydroxy group protected AA **14**. The small steric hindrance 6-hydroxy group of AA coupled with the key intermediate **18** by using DCC as dehydrating agent for ester condensation to get ester **20**. To take off the TBDPS protecting group selectively, a number of conditions including different kinds and concentrations of the acids were tried. Finally the condition of 50% AcOH was chosen to take off TBDPS group and one MEME group at 2-hydroxy to produce the intermediate **21** with about 60% yield. Then, the exposed 2-hydroxyl group of intermediate **21** reacted with MEMCl again to yield the diol **22**. Subsequently, the diol **22** coupled with ibuprofen by condensation reaction also as the previous methods to obtain the C5–O-&C6–O-di-ibuprofen ester **23**. Finally, under the conditions of 1% HCl–MeOH, we removed the 2,3-hydroxy protecting group to reached anticipated prodrug **4**. All the title compounds and important intermedium were characterized by their respective IR, ^{1}H NMR, ^{13}C NMR and MS.

3. Results and discussion

3.1. Stability in different buffer solutions

In order to investigate the chemical stability of several prodrugs, prodrugs **1**, **2**, **3**, **4** were incubated in pH 2.21, 5.86, 7.33 and 7.88 phosphate buffers, respectively. These solutions were maintained in 37 °C, and the aliquots were withdrawn in predetermined time intervals, then the concentrations of the prodrugs were determined by HPLC method. The half-lives ($t_{1/2}$) and the pseudo first order rate constants (K_{disapp}) of these prodrugs in aqueous solutions which calculated by linear regression of Ln of peak area against time in hours are illustrated in Table 1. All the prodrugs appeared to be highly stable in PH 2.21 buffer solution, and moderately stable in pH 5.86 and 7.43, and instable in pH 7.88 buffer solution. According to this result, the slow hydrolysis of the prodrugs at pH 7.4 ensured that ibuprofen parent drug could be sustained and slowly released in the physiological environment.



Scheme 4. The synthetic route of prodrug **4**. Reagents and conditions: (a) MEMCl, DIEA, DCM, r.t., 1 h; (b) intermediate acid **18**, DCC, DMAP, r.t., overnight; (c) 50% AcOH, 50°C, 3.5h; (d) MEMCl, DIEA, DCM, r.t., 1 h; (e) ibuprofen, DCC, DMAP, DCM, r.t., overnight; (f) 1% HCl–EtOH, 70 °C, 1 h.

Table 1
Chemical stability of prodrugs at 37 °C.

Compound	PH value	K_{disapp} (h^{-1})	$t_{1/2}$ (h)
Prodrug 1	2.21	1.73×10^{-2}	40.1
	5.86	3.76×10^{-2}	18.4
	7.33	4.67×10^{-2}	14.8
	7.88	8.42×10^{-2}	8.2
Prodrug 2	2.21	1.61×10^{-2}	43.0
	5.86	3.36×10^{-2}	20.6
	7.33	4.30×10^{-2}	16.1
	7.88	7.15×10^{-2}	9.7
Prodrug 3	2.21	1.76×10^{-2}	39.5
	5.86	3.66×10^{-2}	18.9
	7.33	5.58×10^{-2}	12.4
	7.88	8.65×10^{-2}	8.1
Prodrug 4	2.21	1.29×10^{-2}	53.9
	5.86	2.89×10^{-2}	23.9
	7.33	4.27×10^{-2}	16.2
	7.88	6.87×10^{-2}	10.1

3.2. Metabolism studies of the prodrugs

3.2.1. Metabolic stability

To investigate the metabolic stability of the prodrugs **1**, **2**, **3**, **4** in mice plasma extract and brain homogenate at 37 °C, the pharmacokinetics of these prodrugs were detected. The half-lives ($t_{1/2}$) and the pseudo first order rate constants (K_{disapp}) of these prodrugs in aqueous solutions which calculated by linear regression of Ln of peak area against time in minutes, the metabolic stability data for these compounds were summarized in Table 2. As expected, these four kinds of prodrugs **1**, **2**, **3**, **4** showed considerable stability in plasma, where $t_{1/2}$ were found to be 51.4–44.2 min. Therefore, these prodrugs have sufficient time to reach the brain before decomposition and also gain more opportunities to get through BBB to release the carried parent drug while in the process of delivery and circulation.

In brain homogenate prodrugs **1**, **2**, **3** showed a moderate stability, illustrating that these three compounds were metabolized with a low rate and rarely hydrolyzed, probably due to the weakness or loss of esterase in brain. For this reason, it may take some time to release the active drug after the prodrugs were transported into the brain. Furthermore, it may cause that a portion of the prodrugs were excreted again to the outside of the brain, which prevented the concentrating of parent drug in brain effectively. In contrast, prodrug **4** converted swiftly after entering the brain, and almost completely converted in 30 min, resulting from the disulfide reductase which catalyzed the reduction and cyclization reaction of prodrug **4** to become the thiazolium. Upon completion of this transformation, the drug would be "locked" in the brain and would not be transported outside the brain again, thereby improved the concentration of active drug.

Table 2
Metabolic stability of prodrugs in mice plasma extracts and brain homogenate at 37 °C.

Compound	Biological matrix	K_{disapp} (min^{-1})	$t_{1/2}$ (min)
Prodrug 1	Blood	1.49×10^{-2}	46.6
	Brain	3.46×10^{-2}	20.1
Prodrug 2	Blood	1.35×10^{-2}	51.4
	Brain	4.16×10^{-2}	16.7
Prodrug 3	Blood	1.57×10^{-2}	44.2
	Brain	2.48×10^{-2}	27.9
Prodrug 4	Blood	1.84×10^{-2}	27.8
	Brain	5.46×10^{-2}	12.7

3.3. In vivo studies

3.3.1. Methodology evaluation

The chromatograms of blank mouse plasma and brain tissues showed no peaks at the retention time of ibuprofen and the internal standard naproxen. A good baseline separation of ibuprofen, naproxen, and other major components from mouse plasma and brain tissue samples was achieved under this chromatographic condition.

3.3.2. Pharmacokinetics in plasma and brain of each prodrug

For in vivo study, prodrugs **1**, **2**, **3**, **4** and ibuprofen original drug were injected through caudal vein of the mice with a single dose equivalent to 48 mmol/g body weight of ibuprofen respectively. Mice from each group were sequentially sacrificed at 5 min, 10 min, 30 min, 45 min, 60 min, 90 min, 120 min and 240 min after injection, blood sample was collected from the eye socket of mice, and placed in heparin tubes. Then blood and brain were collected to analyze the concentration of ibuprofen at different intervals by HPLC method.

For better expression of the in vivo behavior of each prodrug, the plasma pharmacokinetics of ibuprofen and various prodrugs in mice were assessed (Fig. 4). Pharmacokinetics parameters of ibuprofen in mice were reported in Table 3 after i.v. administration of ibuprofen and each prodrug (Prodrugs **1**, **2**, **3**, **4**).

The curves showed that the ibuprofen concentrations of prodrugs **1**, **2**, **3** and **4** were higher than that of ibuprofen and also showed slowly declined concentrations compared to the fast metabolism of ibuprofen drug. Because the prodrugs had certain stability in plasma, the degradation rate slowed down, increasing the chances of prodrugs to be rapidly transported across the BBB and the effective concentration of ibuprofen drug. On the other hand, the calculated result showed that the AUC_{0-t} of ibuprofen in four types of prodrugs were much higher than that of naked ibuprofen within 240 min after i.v. administration. Free ibuprofen of prodrugs **1**, **2**, **3** and **4** presented with an area ratio of 2.71, 5.05, 3.75, and 8.42, and the C_{max} for free ibuprofen of prodrugs were 2.72, 4.26, 7.40, and 8.67 times that of ibuprofen original drugs. These data further indicated that the prodrugs could be maintained stable in plasma. The mean residence times (MRT) of ibuprofen in plasma after i.v. administration of prodrugs **1**, **2**, **3**, **4** were 1.06, 1.17, 0.95 and 1.12 times that of ibuprofen, respectively. The half lives (t1/2) and MRT of ibuprofen in these prodrugs indicated that all the prodrugs could keep relatively high concentration in plasma during the text time, so they all could delay the plasma clearance of ibuprofen which is a well-known quick metabolized drug. Among them prodrug **4** showed the best stability, probably due to its

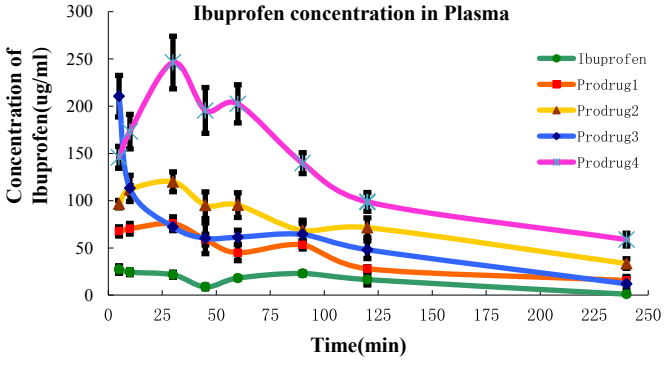


Fig. 4. The concentration curves of ibuprofen in plasma versus time after i.v. injection of prodrugs and ibuprofen in mice.

Table 3
The pharmacokinetic parameters of ibuprofen in blood after administration of ibuprofen and prodrugs. $^{*}P < 0.05$ versus ibuprofen; $^{**}P < 0.01$ versus ibuprofen; $^{***}P < 0.001$ versus ibuprofen.

Compounds	$AUC_{(0-t)}$ (μg/ml·min)	MRT (h)	C_{max} (ng/ml)	T_{max} (h)
Ibuprofen	3405.999 ± 517.582**	78.89 ± 4.113	28.429 ± 2.297	6.667
Prodrug 1	9228.515 ± 171.038	83.414 ± 4.432	77.376 ± 5.786**	23.333
Prodrug 2	17210.306 ± 452.971**	91.942 ± 4.01	121.147 ± 9.224**	23.333
Prodrug 3	12786.652 ± 1280.341***	75.088 ± 4.419	210.488 ± 21.952***	5
Prodrug 4	30116.755 ± 767.929**	88.606 ± 4.84	246.528 ± 26.776***	30

unique chemical structure extending the metabolic processes of ibuprofen.

3.3.3. *Distribution in brain of different prodrugs in mice*

For further evaluation of the possibility of the AA-TDS-ibuprofen prodrugs being transported across BBB, the distributions in brain of prodrugs **1**, **2**, **3**, **4** and ibuprofen original drug were determined. The distribution of ibuprofen was measured at 5 min, 10 min, 30 min, 45 min, 60 min, 90 min, 120 min and 240 min after i.v. injection. The concentrations of ibuprofen in brain versus time curves were displayed in Fig. 5. After i.v. administration of prodrugs **1**, **2**, **3**, **4** and ibuprofen, the pharmacokinetic parameters, relative uptake efficiencies (RE) and concentration efficiencies (CE) of ibuprofen in brain were reported in Table 4.

In brain, it is obvious that all the four prodrugs could be delivered to the brain after i.v. administration. The concentration of released ibuprofen from the four prodrugs at any interval was much higher than that from naked ibuprofen during 240 min. The AUC_{0-t} and C_{max} of ibuprofen after i.v. administration of prodrugs were fairly higher than that after the injection of naked ibuprofen, and it was worth noting that the curves showed an increasing trend from prodrug **1**–**4**. The relative uptake efficiencies (REs) were enhanced to 2.03, 2.25, 3.52, and 4.07 times that of naked ibuprofen for prodrugs **1**, **2**, **3**, **4**, respectively. The concentration efficiencies (CEs) were also enhanced to 2.62, 4.19, 4.96, and 7.46 times that of ibuprofen. Therefore, these data further indicated our design is feasible, that all four kinds of prodrugs could maintain sufficient levels and deliver ibuprofen into the brain. The pharmacokinetic parameters suggested that the brain targeted abilities of these designed prodrugs had a descending trend as prodrug **4** > prodrug **3** > prodrug **2** > prodrug **1**.

The result highlighted that prodrug **2**, C5—O-ibuprofen-AA, had a better targeting ability than prodrug **1**, which indicated C5—OH of AA a preferred position for modification than C6—OH. Additionally, the targeting ability for brain of prodrug **3**, whose C5—O&C6—O positions were both modified, only showed a marginally improved compared to the prodrug **2**, but also indicated that C5&C6-hydroxyl groups of AA are both not critical for the transport. Among all the prodrugs, prodrug **4** showed the best targeting ability for brain, which may be attributed to the characteristics of the compound. The higher REs and CEs of modified prodrug **4** demonstrated that TDS modified prodrug not only increases the lipophilic drug itself, but also prevent the bidirectional delivery of the drug, improving the brain ibuprofen concentration. Details above also indicated that more ibuprofen was distributed into brain.

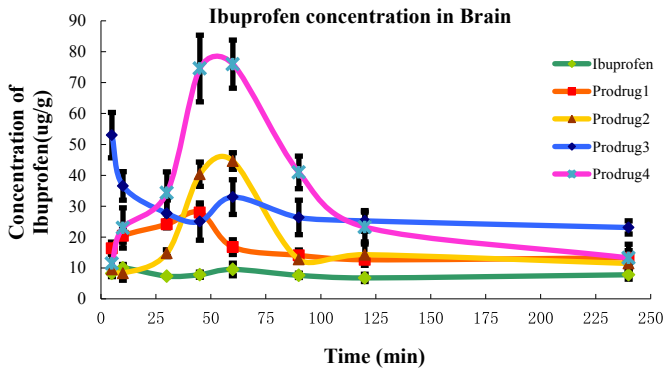


Fig. 5. The concentration curves of ibuprofen in brain versus time after i.v. injection of prodrugs and ibuprofen in mice.

4. Conclusion

In order to develop a more optimal brain targeting drug delivery system, we designed and successfully synthesized a serial of brain targeting L-ascorbic acid-prodrugs **1**, **2**, **3** and **4** of ibuprofen. After i.v. administration of the prodrugs **1**, **2**, **3** and **4**, the results showed that the concentrations of ibuprofen in brain were significantly higher than that of naked ibuprofen with the same dose. The prodrugs exhibited excellent transport ability across the BBB, among which the biodistribution data and pharmacokinetic parameters indicated that prodrugs **4** had higher brain concentration and AUC_{0-t} than those of other four ones. Prodrugs **4** seemed to be easier to be transported into CNS. Briefly, the L-ascorbic acid moiety of prodrugs **4** could be recognized by the GLUT and $SVCT_2$, which efficiently transported this prodrug into the brain, then TDS modified prodrug was locked in the brain through reduction and cyclization processes, contributing to the brain bioavailability of ibuprofen. These expected results strongly suggest that prodrug **4** was a potent brain targeting prodrug which could enhance drug's delivery ability into brain.

Generally, this work presented a way that the synthesis of L-ascorbic acid-TDS-prodrugs may be used as potential means to improve the therapeutic efficiency in brain diseases.

5. Experimental protocols

5.1. *Chemistry*

All liquid reagents were distilled before use. All unspecified reagents were from commercial resources. TLC was performed using precoated silica gel GF254 (0.2 mm), while column chromatography was performed using silica gel (100—200 mesh). The melting point was measured on an YRT-3 melting point apparatus (Shantou Keyi instrument & Equipment Co. Ltd, Shantou, China). IR spectra were obtained on a Perkin Elmer983 (Perkin Elmer, Norwalk, CT, USA). Elemental analyses were performed by Atlantic Microlab (Atlanta, GA, USA). ^{1}H NMR spectra were taken on a Varian INOVA400 (Varian, Palo Alto, CA, USA) using $CDCl_3$, d-DMSO and D_2O as solvent. Chemical shifts are expressed in δ (ppm), with tetramethylsilane (TMS) functioning as the internal reference, and coupling constants (J) were expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument (Agilent, Palo Alto, CA, USA). Ibuprofen and naproxen were obtained from National Institute for Food and Drug Control. Soybean phospholipids

Table 4
The pharmacokinetic parameters of ibuprofen in brain after administration of ibuprofen and prodrugs. $^{*}P < 0.05$ versus ibuprofen; $^{**}P < 0.01$ versus ibuprofen; $^{***}P < 0.001$ versus ibuprofen.

Compounds	$AUC_{(0-t)}$(μg/g·min)	MRT (min)	C_{max} (μg/g)	T_{max} (min)	Re	CE
Ibuprofen	1856.215 ± 160.2364	116.826 ± 5.111	10.6812 ± 1.4904	25	–	–
Prodrug 1	3767.9842 ± 167.8195**	106.204 ± 5.435	27.9772 ± 2.047	45	2.029928753	2.619293712
Prodrug 2	4183.3895 ± 319.0054**	101.489 ± 2.907	44.7925 ± 2.645	55	2.25372034	4.193583118
Prodrug 3	6526.7422 ± 468.2708***	109.097 ± 2.174	53.0288 ± 7.3462*	5	3.516156372	4.964685616
Prodrug 4	7557.692 ± 655.9545***	90.279 ± 6.508	79.6932 ± 9.0269**	55	4.071560676	7.46107179

(SPC) were purchased from Kelong Chemical. Cholesterol (CHOLE) was purchased from Bio Life Science& Technology Co., Ltd (Shanghai, China).

*5.1.1. Synthesis of compound **5** [25]*

To a stirred suspension of L-ascorbic acid (20 g, 114 mmol) in distilled acetone (150 ml) was added acetyl chloride (0.4 ml, 5.7 mmol). After stirred at room temperature for 20 h, the mixture was filtrated. The filter cake was washed with cooled acetone and dried to afford **5** as a white solid, which was used without purification. Yield (73.3%), m.p: 202–204 °C.

*5.1.2. Synthesis of compound **6** [25]*

Compound **5** (6 g, 27.75 mmol) and K_2CO_3 powder (11.3 g, 81.5 mmol) were suspended in acetone (distilled, 100 ml). After reflux, benzyl bromide (8.74 ml, 73.6 mmol) was added and the mixture was allowed to reflux for another 4 h. The solvent was removed under reduced pressure. The residue was treated with proper H_2O and Et_2O. After filtrated, the title compound **6** was afford as a white solid, which was used without purification. Yield (44.1%).

*5.1.3. Synthesis of compound **7** [25]*

The tetra protected L-ascorbic acid compound **6** was dissolved in CH_3CN (200 ml), then the aqueous solution of HCl (20 ml, 2 mol/L) was added and the reaction mixture was stirred for 3 h at 30 °C. The solvent was removed under reduced pressure and the residue was diluted in EtOAc. The organic layer was washed successively with H_2O and brine, then was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **7** as a yellow oil which slowly solidified. Yield (98%), m.p: 67–69 °C.

*5.1.4. Synthesis of compound **8** and **9***

To a stirred solution of ibuprofen (0.94 g, 4.57 mmol) in DCM (30 ml) was added DCC (1.14 g, 5.48 mmol) and DAMP (55.8 mg, 0.457 mmol) and the mixture was stirred for 30 min. Compound **7** (1.5 g, 4.21 mmol) was added dropwise in DCM (10 ml) and the reaction mixture was stirred overnight at room temperature and then filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography with petroleum–ethylacetate to give compound **8** as a pale-yellow oil (43.6%), as well as compound **9** as a colorless oil (25%). Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.82–0.85 (m, $J = 6$ Hz $(CH_3)_2$), 1.39–1.41 (d, 3H, $J = 6.8$ Hz, CH_3), 1.76–1.82 (m, 1H, C**H**$(CH_3)_2$), 2.40 (d, 2H, $J = 7.2$ Hz, C**H**$_2$Ar), 3.77–3.81 (m, 1H, C**H**CH_3), 3.97–3.81 (m, 2H, 6-CH_2), 4.46–4.48 (m, 1H, Hz, 5C–H), 4.86–4.92 (d, 2 × 2H, $J = 11.2$ Hz, OC**H**$_2$Ar), 5.23 (s, 1H, 4C–H), 7.07 (m, 4H, Ar–H),7.10 (d, 2H, $J = 8$ Hz, Ar–H), 7.19 (d, 2H, $J = 7.6$ Hz, Ar–H),7.37 (m, 6H, Ar–H); MS (*m/z*): 567.3 ([M+Na]$^+$); compound **9**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.86–0.89 (d, 12H, $J = 6.4$ Hz, 2 × $(CH_3)_2$), 1.35–1.43 (m, 6H, 2 × CH_3), 1.74–1.82 (m, 2H, 2 × C**H**$(CH_3)_2$), 2.36–2.43 (d, 4H, $J = 6.4$ Hz, 2 × C**H**$_2$Ph), 3.58–3.64 (m, 2H, 2 × C**H**CH_3), 4.27–4.35 (m, 2H, 6-CH_2), 4.56–4.61 (m, 1H, 5C–H), 4.84–5.07 (d, 2 × 2H, $J = 11.2$ Hz, OC**H**$_2$Ar), 5.30–5.35 (m, 1H, 4C–H), 6.98–7.16 (m, 10H, Ar–H); 7.32–7.37 (m, 8H, Ar–H). MS (*m/z*): 755.3 ([M+Na]$^+$).

*5.1.5. Synthesis of prodrug **1***

To a solution of compound **8** (200 mg) in MeOH (15 ml) in an autoclave vessel was added 10% Pd/C (30 mg), and hydrogen pressure of 4 bar was maintained for 4 h at room temperature. Pd/C was filtered and the filtrate was concentrated to afford prodrug **1** as a white solid (74.7%) m.p: 126–128 °C. IR (KBr, ν cm^{-1}): 3393, 3217, 2955, 2868, 1762, 1710, 1665, 1511, 1364, 1171, 1035, 819, 667; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83–0.86 (t, 6H, $J = 6$ Hz, $(CH_3)_2$), 1.38–1.40 (d, 3H, $J = 6.8$ Hz, CH_3), 1.79 (m, 1H, C**H**$(CH_3)_2$), 2.40 (d, 2H, $J = 7.2$ Hz, C**H**$_2$Ph), 3.76–3.81 (q, 1H, $J = 6.8$, 13.6 Hz, C**H**CH_3), 3.91 (s, 1H, 5C–OH), 3.97–3.81 (m, 2H, 6-CH_2), 4.47 (q, 1H, $J = 1.2$, 8 Hz, 5C–H), 5.33 (s, 1H, 4C–H), 7.10 (d, 2H, $J = 8$ Hz, Ar–H), 7.19 (d, 2H, $J = 7.6$ Hz, Ar–H), 8.41 (bs, 1H, 2C–OH), 11.09 (bs, 1H, 3C–OH); ^{13}C NMR ($CDCl_3$, ppm): 18.11 (1C), 22.32 (1C), 22.36 (1C), 30.11 (1C), 44.83 (1C), 44.99 (1C), 66.84 (1C), 68.75 (1C), 74.34 (1C), 118.36 (1C), 127.15 (1C), 127.24 (1C), 128.64 (1C), 129.37 (1C), 137.20 (1C), 140.66 (1C), 153.66(1C), 174.98 (1C), 179.93 (1C) .MS (*m/z*): 387.4 ([M+Na]$^+$).

*5.1.6. Synthesis of prodrug **3***

To a solution of compound **9** (280 mg) in MeOH (15 ml) in an autoclave vessel was added 10% Pd/C (40 mg), and hydrogen pressure of 4 bar was maintained for 5 h at room temperature. Pd/C was filtered and the filtrate was concentrated to afford prodrug **2** as a colorless semi-solid (75.8%) IR (KBr, ν cm^{-1}): 3439, 3294, 3089, 3049, 2955, 2930, 2865, 2725, 1905, 1743, 1654, 1537, 1513, 1381, 1202, 1072, 1022, 849, 630, 596; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.87–0.88 (d, 12H, $J = 6.4$ Hz, 2 × $(CH_3)_2$), 1.37–1.45 (m, 6H, 2 × CH_3), 1.77–1.85 (m, 2H, 2 × $CH(CH_3)_2$), 2.43 (d, 4H, $J = 6.4$ Hz, 2 × C**H**$_2$Ph), 3.59–3.66 (m, 2H, 2 × C**H**CH_3), 4.22–4.37 (m, 2H, 6CH_2), 4.61–4.67 (m, 1H, 5C–H), 5.35–5.39 (m, 1H, 4C–H), 7.04–7.16 (m, 8H, Ar–H); ^{13}C NMR ($CDCl_3$, ppm): 18.11 (1C), 20.81 (1C), 22.22 (1C), 22.26 (1C), 22.33 (1C), 22.36 (1C), 30.12 (1C), 30.16 (1C), 44.78 (1C), 44.94 (1C), 45.49 (1C), 49.78 (1C), 61.97 (1C), 69.26 (1C), 71.08 (1C), 118.76 (1C), 126.84 (1C), 127.12 (1C), 127.19 (1C), 127.29 (1C), 129.25 (1C), 129.29 (1C), 129.35 (1C), 129.59 (1C), 136.98 (1C), 138.64 (1C), 140.48 (1C), 140.78(1C), 154.05(1C), 173.94 (1C), 175.73 (1C), 179.97 (1C). MS (*m/z*): 575.3 ([M+Na]$^+$), 591.5 ([M+K]$^+$).

*5.1.7. Synthesis of compound **10** [25]*

To a stirred solution of (**6**) (670 mg, 1.88 mmol) and Et_3N (0.57 ml, 4.08 mmol) in DCM (15 ml) was added TrtCl (704 mg, 2.45 mmol) in DCM (10 ml) at 0 °C. Then the reaction mixture was stirred overnight at room temperature. The reaction mixture was washed successively with H_2O and brine, then was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography with petroleum–ethylacetate to afford compound **10** as a white solid (68.4%) m.p: 68–70 °C.

*5.1.8. Synthesis of compound **11***

To a stirred solution of ibuprofen (0.65 g, 3.15 mmol) in DCM (22 ml) was added DCC (0.65 g, 3.15 mmol) and DAMP (32 mg, 0.26 mmol). After the mixture was stirred for 30 min, compound **10** (1.57 g, 2.62 mmol) in DCM (10 ml) was added dropwise. The reaction mixture was refluxed overnight and then filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography with petroleum–ethylacetate to give compound **11** as a pale-yellow oil. (77.5%) MS (*m/z*): 809.4 ($[M+Na]^+$).

*5.1.9. Synthesis of compound **12***

To a stirred solution of compound **11** (1.6 g, 2.03 mmol) in $CNCH_3$ (30 ml) was added HCl (7 ml, 1.5 mol/L). After the reaction mixture was stirred for 4 h at 50 °C, the solvent was removed under reduced pressure and the residue was diluted in EtOAc. The organic layer was washed successively with H_2O and brine, then was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography with petroleum–ethylacetate to give compound **12** as a yellow semi-solid. (78.6%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.79–0.81 (m, 6H, $(CH_3)_2$), 1.46–1.48 (d, 3H, J = 7.2 Hz, CH_3), 1.66–1.71 (m, 1H, C**H**$(CH_3)_2$), 2.27 (d, 2H, J = 7.2 Hz, C**H**$_2$Ph), 3.68–3.73 (m, 1H, C**H**CH_3), 3.86 (s, 2H, 6-CH_2), 4.58–4.61 (s, 1H, 5C–H), 4.85–4.94 (d, 2 × 2H, J = 10.8 Hz, OC**H**$_2$Ar), 5.16 (s, 1H, 4C–H), 6.99 (d, 2H, J = 8 Hz, Ar–H), 7.06–7.08 (m, 2H, Ar–H), 7.17 (d, 2H, J = 8 Hz, Ar–H), 7.35–7.41 (m, 8H, Ar–H); MS (*m/z*): 567.4 ($[M+Na]^+$).

*5.1.10. Synthesis of prodrug **2***

To a solution of compound **12** (100 mg) in MeOH (10 ml) in an autoclave vessel was added Pd/C (10%, 15 mg), and hydrogen pressure of 4 bar was maintained for 3.5 h at room temperature. Pd/C was filtered and the filtrate was concentrated to afford prodrug **2** as a white solid. (80.7%) IR (KBr, ν cm^{-1}): 3419, 3056, 3025, 2956, 2929, 2870, 1902, 1799, 1738, 1513, 1381, 1167, 1071, 848, 631; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.83–0.88 (q, 6H, J = 6.8, 13.2 Hz, $(CH_3)_2$), 1.39 (d, 3H, J = 6.8 Hz, CH_3), 1.77–1.80 (m, 1H, C**H**$(CH_3)_2$), 2.38 (d, 2H, J = 7.2 Hz, C**H**$_2$Ph), 3.67–3.71 (m, 1H, C**H**CH_3), 3.72 (s, 2H, 6-CH_2), 4.98 (s, 1H, 5C–H), 5.18 (s, 1H, 4C–H), 7.03 (d, 2H, J = 8 Hz, Ar–H), 7.11 (d, 2H, J = 7.6 Hz, Ar–H); ^{13}C NMR ($CDCl_3$, ppm): 18.32 (1C), 22.72 (1C), 22.76 (1C), 30.81 (1C), 44.83 (1C), 46.78 (1C), 62.64 (1C), 69.65 (1C), 71.34 (1C), 118.56 (1C), 127.19 (1C), 127.48 (1C), 129.34 (1C), 129.67 (1C), 138.64 (1C), 140.78 (1C), 154.36(1C), 174.92 (1C), 178.64 (1C) .MS (*m/z*): 387.4 ($[M+Na]^+$); MS (*m/z*): 387.4 ($[M+Na]^+$).

*5.1.11. Synthesis of compound **14–16** [17]*

The synthesis of TDS compound **14–16** were reported in our previous work [17].

*5.1.12. Synthesis of compound **17***

To a solution of compound **16** (6 g, 0.015 mol) and imidazole (4.2 g, 0.062 mol) in DCM (70 ml) was added TBDPS-Cl (6 ml), then the reaction mixture was stirred for 3 h at 20 °C. After the reaction was completed, the reaction solution was washed with H_2O and brine, then was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the pure semi-solid product. (82.6%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.97 (s, 9H, $SiC(CH_3)_3$), 1.38 (s, 9H,$OC(CH_3)_3$), 1.86 (s, 3H, $NCCH_3$), 2.65 (t, 2H, $SCCH_2$), 3.66 (t, 2H, CH_2C**H**$_2O$), 3.73 (s, 2H, SC**H**$_2$Ar), 3.85 (s, 2H, $COCH_2N$), 7.12–7.14 (m, 2H, Ar–H), 7.17–7.21 (m, 3H, Ar–H), 7.29–7.35 (m, 6H, Ar–H), 7.57–7.59 (m, 4H, Ar–H), 7.92 (s, 1H, CHO). MS (*m/z*): 658.2 ($[M+Na]^+$).

*5.1.13. Synthesis of compound **18***

To a solution of compound **17** (6.4 g) in DCM (300 ml) was added TFA (12 ml), the reaction mixture was stirred for 5 h at room temperature. The reaction was monitored by TLC, controlling the amount of the impurities. The reaction solution was washed with H_2O and brine, then was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to yield compound **18** as a yellow syrup. (38.6%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.03 (s, 9H, $SiC(CH_3)_3$), 1.88 (s, 3H, $NCCH_3$), 2.70 (t, 2H, $SCCH_2$), 3.72 (t, 2H, CH_2C**H**$_2O$), 3.79 (s, 2H, SC**H**$_2$Ar), 4.02 (s, 2H, $COCH_2N$), 7.18–7.20 (m, 2H, Ar–H), 7.23–7.27 (m, 3H, Ar–H), 7.35–7.42 (m, 6H, Ar–H), 7.63–7.65 (m, 4H, Ar–H), 7.93 (s, 1H, CHO). MS (*m/z*): 602.1 ($[M+Na]^+$).

*5.1.14. Synthesis of compound **19** [26]*

In 30 ml of DCM was added ascorbic acid (1.5 g, 8.52 mmol) and DIPEA (3.693 ml, 20.5 mmol), the reaction mixture was stirred for 15 min at room temperature. Then, MEMCl (2.1 ml, 18.74 mmol) was added to the reaction solution, the reaction mixture was stirred for 30 min again at room temperature. The reaction solution was washed with H_2O, the organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to obtain a pale-yellow oil (70.3%).

*5.1.15. Synthesis of compound **20***

To a stirred solution of compound **18** (2 g, 2.113 mmol) in DCM (distilled, 20 ml) was added DCC (524.8 mg, 2.536 mmol) and DAMP (25.8 mg, 0.2113 mmol), then the mixture was stirred for 30 min. Compound **19** (745 mg, 2.113 mmol) was added dropwise in DCM (distilled, 20 ml) and the reaction mixture was refluxed overnight and then filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography to reach the compound **20** as colorless oil. (52.1%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.04 (s, 9H, $SiC(CH_3)_3$), 1.91 (s, 3H, $NCCH_3$), 2.72 (t, 2H, $SCCH_2$), 3.35 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3), 3.55–3.58 (m, 4H, OCH_2CH_2O), 3.73 (t, 2H, CH_2C**H**$_2O$), 3.81 (s, 2H, SC**H**$_2$Ar), 3.84–3.90 (m, 4H, OCH_2CH_2O), 4.02 (s, 2H, $COCH_2N$), 4.10–4.17 (m, 2H, 6-CH_2), 4.38–4.42 (m, 1H, 5C–H), 4.73 (s, 1H, 4C–H), 5.26 (s, 2H, OCH_2O), 5.36 (d, 1H, J = 5.6 Hz, OCH_2O), 5.76 (d, 1H, J = 6 Hz, OCH_2O), 7.19–7.21 (m, 2H, Ar–H), 7.24–7.27 (m, 3H, Ar–H), 7.36–7.45 (m, 6H, Ar–H), 7.64–7.65 (m, 4H, Ar–H), 7.92 (s, 1H, CHO). MS (*m/z*): 936.3 ($[M+Na]^+$).

*5.1.16. Synthesis of compound **21***

In 100 ml of 50% AcOH was added compound **20** (900 mg, 0.984 mmol), the reaction mixture was stirred for 3.5 h at 50 °C. The reaction was monitored by TLC, and was adjusted to neutral with saturated sodium carbonate solution. The reaction solution was extracted with ethylacetate, and then the organic layer was washed successively with H_2O and brine, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to get the compound **21** as a pale-yellow oil. (58.4%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.02 (s, 3H, $NCCH_3$), 2.72 (t, 2H, $SCCH_2$), 3.45 (s, 3H, OCH_3), 3.69–3.73 (m, 4H, OCH_2CH_2O), 3.89 (s, 2H, SC**H**$_2$Ar), 3.97 (m, 2H, CH_2C**H**$_2O$), 4.05 (s, 2H, $COCH_2N$), 4.16–4.23 (m, 2H, 6-CH_2), 4.38–4.42 (m, 1H, 5C–H), 4.74 (s, 1H, 4C–H), 4.95 (s, 2H, OCH_2O), 7.27–7.35 (m, 5H, Ar–H), 8.02 (s, 1H, CHO). MS (*m/z*): 610.1 ($[M+Na]^+$).

*5.1.17. Synthesis of compound **22***

In 5.6 ml of DCM was added compound **21** (70 mg, 0.1192 mmol) and DIPEA (25 ul, 0.12 mmol), the reaction mixture was stirred for 15 min at room temperature. Then, MEMCl (15 ul, 0.13 mmol) was added to the reaction solution, the reaction mixture was stirred for

30 min again at room temperature. The reaction solution was washed with H_2O, and the organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to obtain a pale-yellow oil. (85.3%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.96 (s, 3H, $NCCH_3$), 2.72 (t, 2H, $SCCH_2$), 3.37 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 3.55–3.57 (m, 4H, OCH_2CH_2O), 3.72 (t, 2H, $CH_2C\mathbf{H}_2O$), 3.82–3.86 (m, 4H, OCH_2CH_2O), 3.89 (s, 2H, $SC\mathbf{H}_2Ar$), 4.12 (s, 2H, $COCH_2N$), 4.16–4.18 (m, 2H, 6-CH_2), 4.41–4.45 (m, 1H, 5C–H), 4.73 (s, 1H, 4C–H), 5.24 (s, 2H, OCH_2O), 5.38 (d, 1H, J = 6 Hz, OCH_2O), 5.74 (d, 1H, J = 6 Hz, OCH_2O), 7.26–7.34 (m, 5H, Ar–H), 8.03 (s, 1H, CHO). MS (m/z): 698.2 ([M+Na]$^+$).

5.1.18. Synthesis of compound **23**

To a stirred solution of ibuprofen (55.7 mg, 0.27 mmol) in DCM (distilled, 5 ml) was added DCC (55.9 mg, 0.27 mmol) and DAMP (1.98 mg, 0.0162 mmol). After the mixture was stirred for 30 min, compound **22** (73 mg, 0.108 mmol) was added dropwise in DCM (distilled, 10 ml) and the reaction mixture was refluxed overnight, and then filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography with dichloromethane-methanol to yield compound **23** as a white semi-solid. (87.5%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.88–0.89 (d, 12H, J = 6.4 Hz, 4 × $(CH_3)_2$), 1.45–1.46 (d, 6H, J = 6.8 Hz 2 × CH_3), 1.58–1.62 (m, 2H, 2 × C**H**$(CH_3)_2$), 1.85 (s, 3H, $NCCH_3$), 2.43 (d, 4H, J = 6.4 Hz, 2 × C$\mathbf{H}_2$Ph), 2.70 (t, 2H, $SCCH_2$), 3.35 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3), 3.45–3.47 (m, 4H, OCH_2CH_2O), 3.55 (t, 2H, $CH_2C\mathbf{H}_2O$), 3.64–3.68 (m, 4H, OCH_2CH_2O), 3.69–3.72 (m, 2H, 2 × C**H**CH_3), 3.83 (s, 2H, $SC\mathbf{H}_2Ar$), 4.13 (s, 2H, $COCH_2N$), 4.34–4.39 (m, 2H, 6-CH_2), 4.95–4.94 (m, 1H, 5C–H), 5.03 (s, 1H, 4C–H), 5.28 (s, 2H, OCH_2O), 5.23 (d, 1H, J = 6.4 Hz, OCH_2O), 5.43 (d, 1H, J = 6 Hz, OCH_2O), 7.06–7.17 (m, 8H, Ar–H), 7.22–7.31 (m, 5H, Ar–H), 7.78 (s, 1H, CHO); MS (m/z): 1074.44 ([M+Na]$^+$).

5.1.19. Synthesis of prodrug **4**

In 1.44 ml of 1% HCl–EtOH was added compound **23** (60 mg, 58.6 mmol), and the reaction mixture was stirred for 1 h at room temperature at 70 °C. The reaction was monitored by TLC and was concentrated under reduced pressure. The residue was purified by column chromatography with dichloromethane-methanol to get prodrug **4** as a white semi-solid. (61.3%) ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.87–0.88 (d, 12H, J = 6.4 Hz, 4 × $(CH_3)_2$), 1.44–1.46 (d, 6H, J = 6.8 Hz 2 × CH_3), 1.58–1.61 (m, 2H, 2 × C**H**$(CH_3)_2$), 1.81 (s, 3H, $NCCH_3$), 2.42 (d, 4H, J = 6.4 Hz, 2 × C$\mathbf{H}_2$Ph), 2.68 (t, 2H, $SCCH_2$), 3.39 (t, 2H, $CH_2C\mathbf{H}_2O$), 3.63–3.69 (m, 2H, 2 × C**H**CH_3), 3.84 (s, 2H, $SC\mathbf{H}_2Ar$), 4.12 (s, 2H, $COCH_2N$), 4.37–4.39 (m, 2H, 6-CH_2), 4.51–4.52 (m, 1H, 5C–H), 5.45 (s, 1H, 4C–H), 7.04–7.09 (m, 4H, Ar–H), 7.13–7.16 (m, 4H, Ar–H), 7.21–7.29 (m, 5H, Ar–H), 7.78 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, ppm): 18.12 (1C), 18.59 (1C), 20.81 (1C), 22.23 (1C), 22.28(1C), 22.34 (1C), 22.46 (1C), 29.65 (1C), 30.11 (1C), 30.16 (1C), 44.24 (1C), 44.91 (1C), 45.03 (1C), 45.49 (1C), 45.76 (1C), 46.24 (1C), 62.45 (1C), 63.32 (1C), 70.23 (1C), 71.87 (1C), 102.25 (1C), 118.72 (1C), 126.84 (1C), 127.09 (1C), 127.12 (1C), 127.19 (1C), 127.29 (1C), 127.75 (1C), 128.42 (1C), 128.60 (1C), 128.81 (1C), 129.25 (1C), 129.39 (1C), 129.45 (1C), 129.69 (1C), 136.07 (1C), 137.47 (1C), 138.84 (1C), 139.86 (1C), 140.48 (1C), 140.66 (1C), 153.98 (1C), 162.95 (1C), 167.62 (1C), 173.64 (1C), 174.71 (1C), 179.27 (1C). MS (m/z): 898.4 ([M+Na]$^+$).

5.2. In vitro studies

5.2.1. Instrumentation and method

The waters liquid chromatographic system employed was an LC-10A liquid chromatographic system (Shimadzu Japan). The analysis was carried out on a SinoChrom ODS-C18 column (200 mm × 4.6 mm, 5 mm), thermostated at 25 °C. The mobile phase was composed of methanol/water (70:30), and the pH was adjusted to 2.86 with 10% phosphoric acid at a flow rate of 1.0 ml/min and the UV detector was set to monitor the signal at 219 nm corresponding to the maximum absorbance for the ibuprofen derivatives. The apparent pseudo first order rate constants of disappearance of prodrugs (K_{disapp}, h^{-1} or min^{-1}) was determined by calculation from linear regression of the natural logarithm of absorbance against time in hours or minutes.

5.2.2. Stability in different buffer solutions

The stability in phosphate buffer solution was investigated at four pH values: 2.21, 5.86, 7.33 and 7.88. Precisely, 1 ml methanol solution (250 μm/ml) of the synthesized compound was added into 4 ml different buffer at 37 °C. After mixing, it was kept in a 37 ± 1 °C constant water bath, and the 200 μl sample was withdrawn at the following time points: 0, 1, 3, 6, 12, and 24 h. The disappearance of ibuprofen esters was monitored by HPLC method as described.

5.2.3. Stability in plasma extract and brain homogenate

Blood was drawn from mice though orbital sinus and was collected in a heparinized tube paved with heparin sodium. Samples were centrifuged at 15,000 rpm for 15 min to separate plasma which was diluted with double volumes of water. The brain was removed and homogenized in cold phosphate buffer of pH 7.4 with proportion of 1:5 (w/v). Samples were then placed on ice and used immediately. 1 ml methanol solution (250 μm/ml) of the compound was added to 4 ml of plasma, or brain homogenate and gently vortexed. Samples were incubated at 37 °C and 200 μl aliquots were removed after 0, 5, 15, 30, 60, and 90 min, respectively. Then 200 μl of acetonitrile was added to each aliquot and vortexed. Samples were centrifuged for 15 min to remove proteins and the supernatants were analyzed by HPLC method as described.

5.3. Biodistribution studies in vivo

5.3.1. Test animals

Adult Kunming mice weighing 20–22 g were obtained from the animal center of Sichuan University. The animals were left for two days to acclimatize to animal room conditions and were maintained on standard pellet diet and water ad libitum. Food was withdrawn on the day before the experiment, but free access to water was allowed. Since the experiment could be completed within 24 h, there was no significant change in the mices' body weight during the experiment. All animals received human care, and the study protocols complied with the guidelines of Sichuan University animal ethical experimentation committee. Throughout the experiments, the animals were handled according to the requirements of the National Act on the use of experimental animals (People's Republic of China).

5.3.2. HPLC analysis of prodrugs and ibuprofen

The waters liquid chromatographic system was carried out according to the Section 5.2.1. The mobile phase was composed of methanol/water (70:30), and the pH was adjusted to 2.86 with 10% phosphoric acid at a flow rate of 1.0 ml/min and the UV detector was set to monitor the signal at 219 nm corresponding to the maximum absorbance for the ibuprofen derivatives.

5.3.3. Sample preparation

Blood was collected from the eye socket of mouse into a tube containing heparin, and centrifuged at 5000 rpm for 5 min. The supernatant was collected as plasma sample. The animals were killed by cervical dislocation, and the organs were removed and flushed with water for three times to remove the blood remained

[27] and then the brains were roll over on the filter paper carefully to remove the main vessel. All the tissues were homogenized with triple amount of water. An aliquot of 50 μl of internal standard (naproxen) was added into 500 μl plasma or 500 μl organ homogenate, the mixture containing ibuprofen was vortexed with 1.5 ml methanol for 5 min. Hydrolysis was performed, before analyzing prodrugs. Then an aliquot of 35 ml of 6 M NaOH aqueous solution was added to the spiking mixture. After 10 min of hydrolysis at room temperature, ibuprofen was released from the prodrugs, and 35 ml of 6 M HCl was added to neutralize NaOH. Subsequently, the mixture was vortexed with 1.5 ml methanol for another 5 min. After that, the mixture was centrifuged at 15,000 rpm for 10 min. The separated supernatant was evaporated to dryness at 40 °C under air flow. The residues were redissolved in 200 μl methanol and centrifuged at 15,000 rpm for 10 min, and then 50 μl of the supernatant was injected into the HPLC system for analysis.

5.3.4. Body distribution study

Twenty-four mice were randomly assigned to 8 groups for each prodrug or ibuprofen. **Prodrug 1**, **2**, **3**, **4** and ibuprofen were given to the mice via the tail vein, each was equivalent to the administration dose of ibuprofen of 48 mmol/g body weight at 5 min, 10 min, 30 min, 45 min, 60 min, 90 min, 120 min and 240 min after injection, blood sample was collected from the eye socket of mice, and placed in heparin tubes. After exsanguinations, the mice were killed by cervical dislocation and the organ were removed and washed twice with water. The organs were weighed and prepared as described earlier. The concentration the ibuprofen was analyzed by HPLC.

5.4. Statistical analysis

The area under the concentration–time profile (AUC_{0-t}), maximal concentration (C_{max}), and mean residence times (MRT) were calculated by Data and max Statistics (DAS3.25, Shanghai, China). Statistical evaluation was performed using analysis of variance followed by *t*-test. A value of $p < 0.05$ was considered significant. The relative uptake efficiency (RE) and concentration efficiency (CE) were calculated to evaluate the brain targeting property of prodrugs. The value of RE and CE were defined as follows:

$$RE = (AUC_{0-t})s/(AUC_{0-t})c$$

$$CE = (C_{max})s/(C_{max})c,$$

where s and c represented sample (the prodrugs) and control (ibuprofen), respectively.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.072.

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