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Original article

Design, synthesis and biological evaluation of brain-specific glucosyl thiamine disulfide prodrugs of naproxen

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ABSTRACT

Glucosyl derivates exhibited favorable distribution to the brain. However, bidirectional transport of glucose transporter 1 might decrease concentrations of the prodrugs in brain before the release of parent drugs. To overcome this defect, glucosyl thiamine disulfide prodrugs **1a**–**1c** incorporating naproxen were designed and synthesized. Furthermore, prodrug **2** and **3** were also prepared as control. The favorable physicochemical properties of these prodrugs were verified by stability and metabolism studies. Results from the in vivo distribution study indicated that **1a**–**1c**, and **1b** in particular, significantly increased the level of naproxen in brain when compared to **2** and **3**. The study suggested glucosyl thiamine disulfide was a promising carrier to enhance the brain bioavailability of central nervous system active drugs.

1. Introduction

Over the past few years, studies have suggested that the chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as naproxen, could reduce the risk or even delay the onset of Alzheimer's disease [1]. Meanwhile, several mechanisms have been proposed for the neuroprotective activity of NSAIDs [2,3] and it was supposed that their neuroprotective effect might be independent of the ability to inhibit the cyclooxygenase (COX) enzyme isoforms [4]. It was also observed that these drugs possess antioxidant activity and regular NSAID use could decrease the risk of developing Parkinson's disease [5].

To achieve satisfactory therapeutic effect against these neurodegenerative diseases, NSAIDs should be delivered into the central nervous system (CNS) with a certain degree, particularly to the brain parenchyma. However, the blood–brain barrier (BBB), formed by brain vessel endothelial cells linked together by tight junctions [6], obstructs the penetration of these polar compounds. There is a promising way, among others [7–9], to convert drugs which do not enter the brain, into drugs with capacity to cross the BBB. The method involves the modification of drug structures, so that resulting molecules become substrates for the endogenous BBB transporters, such as carboxylic acid, amino acid and hexose derivatives or even small molecular weight peptides [10–12]. Among these strategies, the hexose derivatives, particularly, could be transported across the BBB with the most high efficient. It is well known that the large and uninterrupted energy demand of the brain is provided almost exclusively by glucose, which is transported through the BBB by glucose transporter 1 ($GLUT_1$), and it is estimated that there is a 15–3000 fold higher transport value for the $GLUT_1$ than for the other transporters [13]. Glucosylated derivatives of the drugs have, indeed, been proposed as a good strategy to overcome the BBB with high transport efficiency and easy preparation [14–16]. However, the $GLUT_1$ are found to be generally bidirectional transporters that mediate the blood-to-brain and brain-to-blood transport of the glucose in either direction across the BBB, owing to the expression of the $GLUT_1$on both the luminal and abluminal membranes of the endothelial cells forming the BBB [17]. This conclusion indicated that the glucosylated NSAIDs would face a problem similar to the glucose that the compounds would be pumped out to the bloodstream after their entry in CNS, thus prevented the prodrugs concentrating in the brain. Given these properties of the prodrugs, we have reason to believe that there must be an elevated concentration of the relative active drugs in brain after the change of behavior of the prodrugs form bidirectional to unidirectional (blood-to-brain) during their transport processes.

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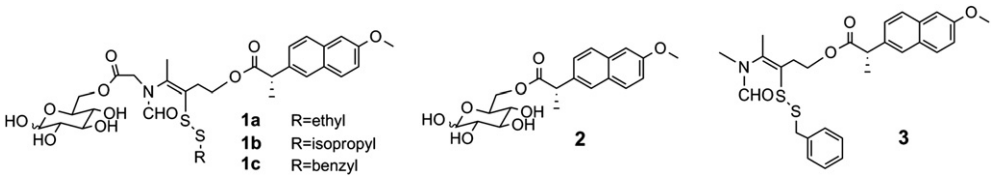


Fig. 1. Structure of the designed prodrugs of naproxen **1a**–**1c**, **2** and **3**.

Studies have proposed that analogs with hydrophobic alkyl substituents at the C-6 position possessed high affinity for the $GLUT_1$ [18], and vice versa [19]. For the purpose of unidirectional delivery, a lipophilic thiamine disulfide system with ability of "lock-in" was introduced in and attached to the glucose moiety at the C-6 position for the novel brain targeted carrier. The thiamine disulfide system (TDS), established by Toyoaki Ishikura and coworkers [20], was reduced and ring-closed to be a thiazolium once entered the BBB by disulfide reductase, and then the prodrug with this system was "locked" in the brain where it can serve a sustained release of the active drug via hydrolysis. The conversion reaction is similar to that of dihydropyridine-pyridinium salt type of redox system (CDS) [21], however, the reactions of TDS with thiamine and CDS are reduction and oxidation, respectively. Both the CDS and the TDS appeared to be a good prodrug approach, in which the increased lipophilic nature of a prodrug may facilitate passive diffusion across the blood–brain barrier with subsequent conversion to the active drug by simple chemical hydrolysis. Building upon the knowledge garnered from these studies, we have designed the glucosyl thiamine disulfide prodrugs **1a**–**1c** (Fig. 1) to mediate the penetration of naproxen, a well known NSAID, into the brain for several reasons. First, the TDS is more stable and convenient for conservation than the CDS in the air. Second, it is notable that the efficiency of the TDS delivering drug to brain is somehow lower than the $GLUT_1$ since brain endothelium transports about ten times its weight of glucose per minute [22], thus their conjunction might make an enhanced level when delivering naproxen to CNS. Third, it is reported that the concentration of reductive substances in the blood is rather low, 10 μM, which is not high enough to reduce the prodrugs to thiazolium form through disulfide [23], so the disulfide linkage in the TDS is relatively stable in plasma compared to other organs where the enzyme disulfide reductase is more abundant [24].

The carboxyl group of naproxen was attached to the linker by ester bond, which is expected to be swiftly cleaved in the brain with reasonable stability in the plasma. Fig. 2 illustrates the distribution, sequential metabolism and brain "lock-in" pathways of the prodrugs **1a**–**1c** to brain. Generally, the reduction of the disulfide to the quaternary thiazolium salt is crucial to this process and considered to be more swift than the hydrolysis while both reactions would proceed in the body simultaneously [20]. In order to check the evaluated extent of naproxen by **1a**–**1c** and determine their brain specific ability, the prodrug **2** just with a glucose moiety and the prodrug **3** with the original TDS were also designed as control compounds.

In this paper, these designed compounds were sequentially synthesized. The in vitro stabilities of these prodrugs were discussed. Furthermore, this account also included the biodistribution and pharmacokinetics of these prodrugs in mice after i.v. administration.

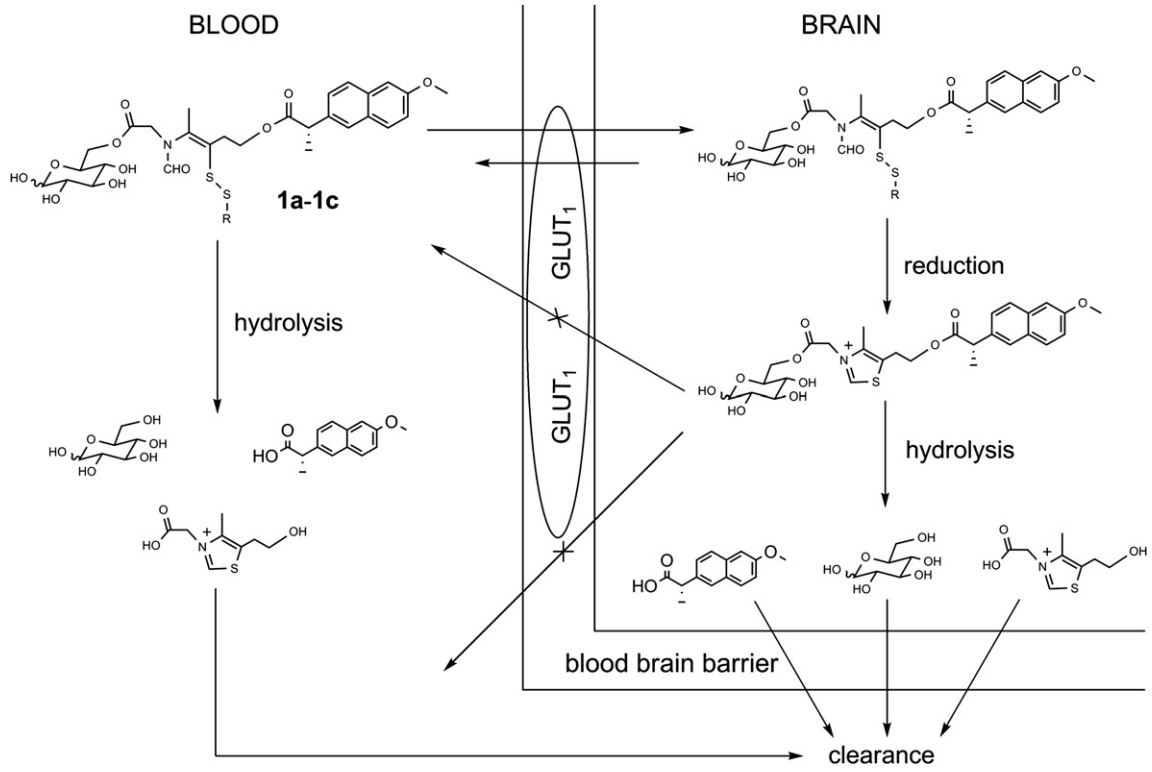
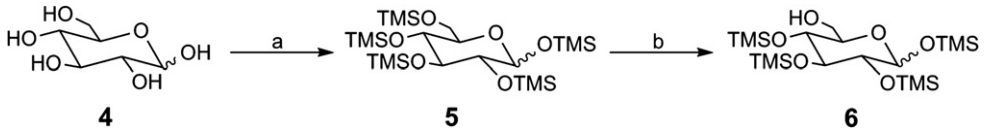


Fig. 2. Distribution, sequential metabolism and brain "lock-in" pathways of brain-specific glucosyl thiamine disulfide prodrugs of naproxen **1a**–**1c**.

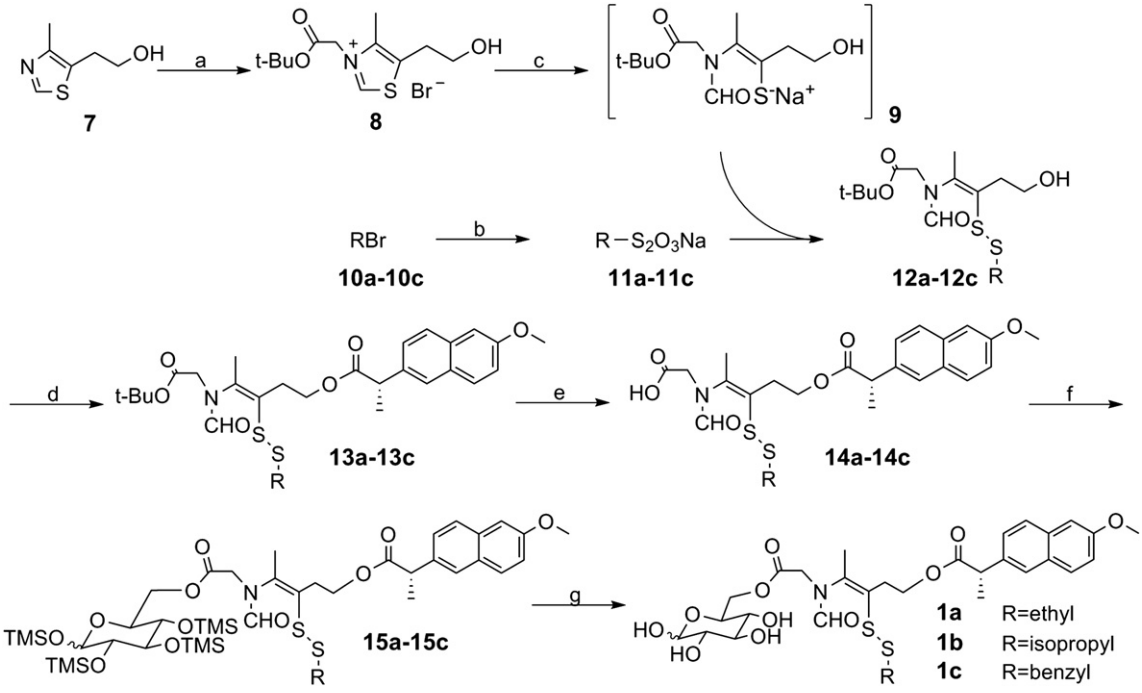


Scheme 1. Synthetic pathway to compound **6**. Reagents and conditions: (a) TMSCl, HMDS, pyridine, r.t, overnight, 93.1%; (b) acetone, methanol, acetic acid, 40 °C, 4 h, 75.2%.

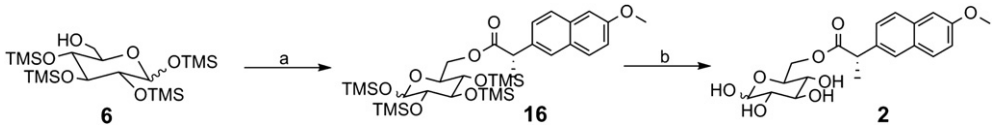
2. Chemistry

For the coupling reaction between the TDS and glucose derivative with a free hydroxyl group at C-6 position, the key intermediate 1,2,3,4-tetra-*O*-trimethylsilyl-D-glucopyranose **6** was involved in the synthetic works (Scheme 1). D-glucose **4** was etherified with chlorotrimethylsilane and hexamethyl disilazane in pyridine to give penta-*O*-trimethylsilyl-D-glucopyranose **5**. Our initial attempts for selective methanolysis of compound **5** at C-6 position under basic condition by potassium carbonate in methanol [25] was thwarted by the high reaction activity that caused a mixture of partially silylated products. A comparatively smooth and feasible pathway to the key intermediate **6**, however, was obtained when the persilylated glucose was treated by the mixture of acetone, acetic acid and methanol.

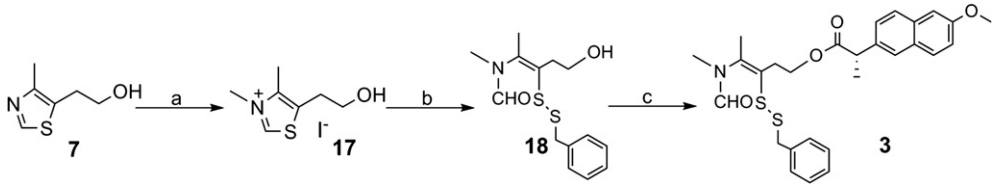
The synthetic route of prodrugs **1a**–**1c** was described in Scheme 2. The starting materials 5-(2-Hydroxyethyl)-4-methylthiazole **7** and alkyl bromides **10a**–**10c** were commercially available. Compound **7** was carried out with *t*-butyl bromoacetate in acetonitrile to generate the quaternary thiazolium salt **8** in satisfied yield. With the protection of argon gas, hydrolysis of compound **8** in aqueous solution of sodium hydroxide furnished the transient sodium salt **9**, which substituted with thiosulfate salts **11a**–**11c** prepared from bromoethane **10a**, isopropyl bromide **10b** and benzyl bromide **10c** by sodium thiosulfate respectively, resulting in intermediates **12a**–**12c**. It was notable that we failed to afford compounds **12a**–**12c** by this way without the stringent anaerobic conditions, because of the transient sodium salt **9** was a thiol nucleophile, which was not stable in the air or in the presence of oxidant, thus formed the corresponding disulfide. By the use of DCC as a condense agent, compounds **13a**–**13c** were afforded as coupling products of compounds **12a**–**12c** and naproxen. We were unable to get acids **14a**–**14c** through the action of trifluoroacetic acid and subsequent *p*-toluenesulfonic acid, that the products were too complicated. Hydrolysis of compounds **13a**–**13c** with sulfuric acid or hydrochloric acid, although successful, was unfruitful because these reactions could only be partially undertaken in room temperature, and there were numerous side products if temperature was raised.



Scheme 2. General synthetic route for synthesis of compounds **1a**–**1c**. Reagents and conditions: (a) *t*-butyl bromoacetate, CH_3CN, reflux, 1.5 h, 91.4%; (b) $Na_2S_2O_3 \cdot 5H_2O$, ethanol, PEG400, 6 h, 83.7–95.2%; (c) NaOH, H_2O, r.t, 30 min, two steps 79.6–86.2%; (d) DCC, DMAP, naproxen, THF, r.t, overnight, 80.5–87.6%; (e) 70%$HClO_4$, CH_2Cl_2, r.t, 1 h, 70.3–76.4%; (f) DCC, DMAP, compound **6**, THF, 60 °C, overnight, 35.8–55.4%; (g) TFA, CH_2Cl_2, 0 °C, 1.5 h, 75.8–90.2%.



Scheme 3. Synthetic pathway to compound **2**. Reagents and conditions: (a) DCC, DMAP, naproxen, CH_2Cl_2, r.t, overnight, 75%; (b) TFA, CH_3NO_2, CH_2Cl_2, 0 °C, 2 h, 91.4%.



Scheme 4. Synthetic pathway to compound **3**. Reagents and conditions: (a) iodomethane, 70 °C, 2 h, 96.3%; (b) NaOH, **11c**, H_2O, r.t, 2 h, 76%; (c) DCC, DMAP, naproxen, dichlormethane, r.t, overnight, 87.9%.

Exchanging the hydrolysis reagent with perchloric acid which possessed strong acidity and oxidation that was detrimental to disulfide linkages in these compounds, however, interestingly, provided the solution to the problem mentioned above affording the acids **14a**–**14c** in 70.3–76.4% yields. With the compounds **14a**–**14c** and saccharide **6** in hand, we were ready to make the following condensation in the presence of DCC and DMAP in tetrahydrofuran which furnished the compound **15a**–**15c**, and these, when subjected to the conditions of trifluoroacetic acid in dichloromethane, was readily underwent a deprotection reaction to get the target compounds **1a**–**1c**.

The synthesis of prodrugs **2** and **3** are illustrated in Schemes 3 and 4 respectively. Briefly, the prepared saccharide **6** condensed with naproxen by DCC and DMAP, and then, was deprotected by trifluoroacetic to afford ester **2**. Quaterisation of compound **7** with iodomethane furnished salt **17**, which was attacked by benzyl bromide **11c** under basic condition to get compound **18**. This compound was conjugated with naproxen in the presence of DCC and DMAP to afford the corresponding disulfide **3**. 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

To characterize chemical stabilities of the prodrugs, compounds **1a**–**1c**, **2** and **3** were incubated in pH 2.5, 5.0, 7.4, and 8.00 phosphate buffers, respectively. These solutions were maintained in 37 °C, and the aliquots were withdrawn in limited time intervals, thus to determine the concentrations of the prodrugs by HPLC method. The pseudo first order rate constants (K_{disapp}) and half lives ($t_{1/2}$) of these compounds in aqueous solutions, calculated by linear regression of Ln of peak area against time in minutes, are shown in Table 1. The compounds appeared to be stable in pH 7.4 buffer solutions, moderately stable in pH 5.0, and instable in pH 2.5 and 8.0 buffer solution. The slow hydrolysis rates to parent drug of the prodrugs at pH 7.4 indicated the sustained release of naproxen in the physiological environment. Generally, the stability of these compounds was depended on the pH deviation from neutral value, the larger the deviation was, the more unstable these compounds were, and hence, the prodrugs will not be administered orally.

Table 1
Chemical stability of compounds **1a**–**1c**, **2** and **3** at 37 °C.

Compound	pH value	Kinetic constant	
		K_{disapp} (h^{-1})	$t_{1/2}$ (h)
1a	2.5	4.34×10^{-2}	16.0
	5.0	2.95×10^{-2}	23.5
	7.4	2.55×10^{-2}	27.2
	8.0	2.77×10^{-2}	25.0
1b	2.5	3.19×10^{-2}	21.7
	5.0	2.04×10^{-2}	34.0
	7.4	1.36×10^{-2}	51.0
	8.0	2.42×10^{-2}	28.6
1c	2.5	3.39×10^{-2}	20.4
	5.0	2.87×10^{-2}	24.1
	7.4	2.30×10^{-2}	30.1
	8.0	3.56×10^{-2}	19.5
2	2.5	1.30×10^{-2}	53.3
	5.0	6.45×10^{-3}	107
	7.4	6.07×10^{-3}	114
	8.0	2.91×10^{-2}	23.8
3	2.5	5.05×10^{-2}	13.7
	5.0	2.81×10^{-2}	24.7
	7.4	1.11×10^{-2}	62.4
	8.0	2.05×10^{-2}	33.8

3.2. Metabolism studies of the prodrugs

3.2.1. Metabolic stability

To determine the extent of metabolism of these prodrugs after administration, we assayed pharmacokinetics of the prepared compounds **1a**–**1c**, **2** and **3** in mice plasma extract and brain homogenate at 37 °C. Such study could inform about the rate of their conversion into the corresponding quaternaries and hence the release of the carried parent drug while in the process of circulation or delivery. Pseudo first order rate constants of disappearances (K_{disapp}) of the prodrugs and their half lives ($t_{1/2}$) were calculated by linear regression of Ln of peak area against time in minutes. Data concerning the metabolic stability of these compounds were outlined in Table 2. Prodrugs **2** showed satisfactory stability ($K_{disapp} = 7.82 \times 10^{-3}$ min^{-1}, $t_{1/2} = 88.6$ min) in plasma while compounds **1a**–**1c** decomposed with a significantly higher rate ($t_{1/2} < 60$ min) during 90 min. The instability is due to the existence of ester linkages and disulfide in **1a**–**1c** which accelerated the decomposition of these compounds. However, in spite of that the disulfide of **1a**–**1c** and **3** could be profitless to their stability, it is relatively reasonable to give enough time ($t_{1/2} = 39.4$–59.2 min) for the compounds to be distributed and reach the brain before complete decomposition.

Table 2
Metabolic stability of compounds **1a**–**1c**, **2** and **3** in mice plasma extract and brain homogenate at 37 °C.

Compound	Biological matrix	Kinetic constants	
		K_{disapp} (min^{-1})	$t_{1/2}$ (min)
1a	Plasma	1.77×10^{-2}	39.4
	Brain	4.74×10^{-2}	14.6
1b	Plasma	1.46×10^{-2}	47.5
	Brain	3.37×10^{-2}	20.6
1c	Plasma	1.53×10^{-2}	45.3
	Brain	3.71×10^{-2}	18.7
2	Plasma	7.82×10^{-3}	88.6
	Brain	6.83×10^{-3}	101
3	Plasma	1.17×10^{-2}	59.2
	Brain	2.76×10^{-2}	25.1

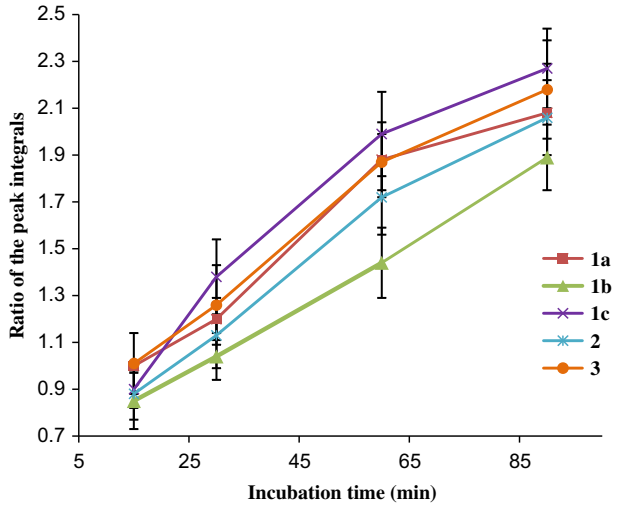


Fig. 3. Regeneration of naproxen by the prodrugs in brain homogenate. Error bars show the value of SD ($n = 3$).

In brain homogenate, **1a**–**1c** and **3** decomposed swiftly ($t_{1/2}$ = 14.6–25.1 min), and were almost completely consumed by 90 min, which probably due to the abundance of disulfide reductase which proceeded the reduction and cyclization of the thiamine disulfide component to corresponding thiazolium in brain homogenate. These four prodrugs could be reduced to strongly polar product with high rates, and thus, contributed to the "lock-in" effect in brain before they were effluxed to periphery. In contrast, compound **2** was metabolized with a low rate ($K_{disapp} = 6.83 \times 10^{-3}$ min^{-1}) that was even slower than the clearance in plasma, possibly the result of the lack of esterase in brain in comparison with that in plasma. The certain time of the prodrug exposed in brain with the original form might enhance its efflux by $GLUT_1$, and as a result, the parent drug could not be concentrated in brain effectively.

3.2.2. Release of the parent drug by prodrugs in brain homogenate

To study the release of naproxen by the prodrugs, the peak in the HPLC corresponding to the parent compound was also analyzed. The certain increase in peak area over time was observed, indicating that the prodrug was indeed converted to the parent drug by the esterase in mice brain homogenate. Although the regeneration of naproxen seemed not fast, the concentration increased steadily as time went by, which might indicate sustained release of naproxen by the prodrugs in the brain. Peak integrals were normalized by dividing by the area for the peak of **1a** at 15 min. The ratios are shown in Fig. 3. Compared with the quick clearance of **1a**–**1c** and **3** in brain homogenate, the regeneration of naproxen by these four prodrugs were shown to be approximate to, or even slower than that of **2**. On the other hand, the trend of naproxen by **2** increased moderately in the five prodrugs, however, was not in conformity with the relative stability of prodrug **2** in brain homogenate.

Several conclusions could be deduced from the above results: first, the instability of **2** in brain was mainly caused by hydrolysis of the esterase. Second, the disulfide reductase conducted reduction and ring closure of **1a**–**1c** and **3** predominated their clearance in brain with high efficiency, since the decomposition of the prodrugs was consisted by reduction and hydrolysis, and the esterolysis carried out with relatively low rate. Finally, the release process of parent drug was unlikely to be seriously affected by the formation of thiazolium.

As the above studies in vitro demonstrated that these prodrugs possessed preliminary and favorable physicochemical properties, we proceeded with in vivo distribution studies.

Table 3
Pharmacokinetic parameters of naproxen in plasma after administration of naproxen and prodrugs ($n = 3$).

Compound	AUC_{0-t} (μg/mL*min)	MRT (min)	$t_{1/2}$ (min)
1a	12914.16 ± 258.07$^{*, **, ***}$	55.57 ± 1.80*	44.43 ± 1.04$^{*, ***}$
1b	16185.44 ± 432.34$^{*, **, ***}$	64.53 ± 2.42$^{*, ***}$	63.32 ± 3.64$^{*, **, ***}$
1c	13348.87 ± 405.56$^{*, **, ***}$	54.56 ± 4.03*	59.33 ± 1.98$^{*, **, ***}$
2	10353.27 ± 141.95*	51.99 ± 1.32*	40.32 ± 1.81*
3	8834.70 ± 226.44*	45.24 ± 1.92	33.55 ± 1.13*
Naproxen	3812.35 ± 167.65	36.67 ± 4.11	23.55 ± 0.82

$^{*}p < 0.001$ with respect to naproxen.
** $p < 0.001$ with respect to prodrug **2**.
*** $p < 0.001$ with respect to prodrug **3**.

3.3. In vivo studies

3.3.1. Pharmacokinetics in plasma of the prodrugs

The compounds were injected through caudal vein of the mice with a single dose equivalent to 10 mg/kg body weight of naproxen, and then blood and organs were collected to analyze the concentration of naproxen at different intervals by HPLC method. Because these prodrugs could only particularly released parent drug in biological matrix within limited period, all the testing compounds

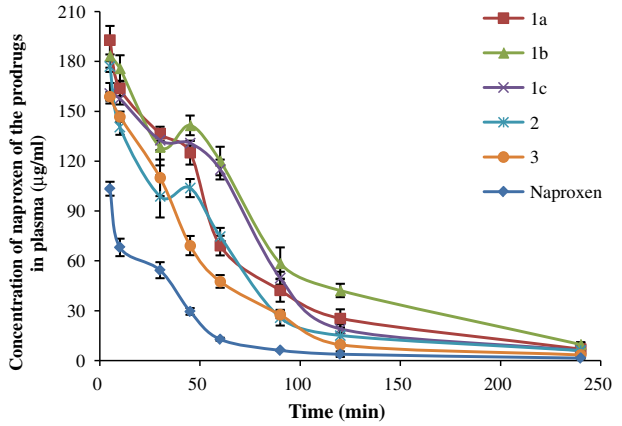


Fig. 4. Concentration curve of naproxen in plasma versus time after administration of naproxen and the prodrugs. Error bars show the value of SD ($n = 3$).

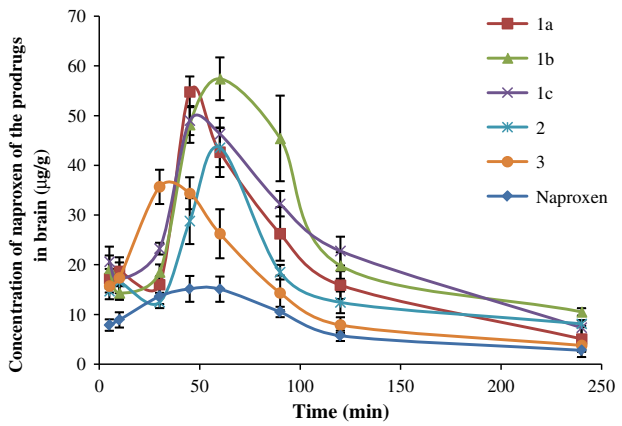


Fig. 5. Concentration curve of naproxen in brain versus time after administration of naproxen and the prodrugs. Error bars show the value of SD ($n = 3$).

Table 4
Pharmacokinetic parameters of naproxen in brain after administration of naproxen and prodrugs ($n = 3$).

Compound	AUC_{0-t} (μg/g*min)	MRT (min)	C_{max} (μg/g)	T_{max} (min)	RE	CE
1a	4716.88 ± 232.36*	83.56 ± 5.75	54.79 ± 3.09*	30	2.50	3.21
1b	6140.77 ± 236.92*, **,***	90.32 ± 7.37	57.40 ± 3.51*	60	3.25	3.36
1c	5669.45 ± 114.23*, **,***	89.80 ± 2.09	49.03 ± 2.91*	30	3.01	2.87
2	3930.87 ± 91.42*	93.30 ± 3.03	43.58 ± 3.93*	60	2.08	2.55
3	3307.75 ± 382.24	73.63 ± 4.45	36.05 ± 2.79*	30	1.75	2.11
Naproxen	1886.62 ± 15.78	84.34 ± 6.55	17.07 ± 0.76	45	–	–

*$p < 0.001$ with respect to naked naproxen.
** $p < 0.001$ with respect to prodrug **2**.
*** $p < 0.001$ with respect to prodrug **3**.

were hydrolyzed to naproxen by additional base in order to get convenience for detection and accuracy of HPLC analysis. To understand the in vivo behavior of different prodrugs, we assessed the plasma pharmacokinetics of naproxen and the designed compounds in mice (Fig. 4). The curves displayed that naproxen could be quickly metabolized while the prodrugs showed certain stability which would increases the chance to be transported across BBB. The slowly declined concentrations in plasma could be explained by that decomposition of these prodrugs extended the metabolism course of naproxen.

Pharmacokinetic parameters of naproxen in blood were reported in Table 3. Free naproxen of prodrugs **1a**–**1c**, **2**, **3** and naproxen presented with an area under the concentration–time profile (AUC_{0-t}) ratio of 3.38, 4.25, 3.50, 2.72 and 2.31, respectively, which indicated the prolonged plasma maintenance of the prodrugs. The mean residence times (MRT) of naproxen in plasma after i.v. administration of **1a**–**1c**, **2** and **3** were 1.52, 1.76, 1.49, 1.42 and 1.23 times that of naproxen, respectively. The half lives ($t_{1/2}$) and MRT of naproxen by these prodrugs indicated that **2** and **3** were cleaned more quickly than **1a**–**1c** which verified well with the fact that the complicated decomposition course of the **1a**–**1c** could more effectively delay the clearance of naproxen. The MRT of naproxen by the prodrugs corroborated that they could maintain certain level and get sufficient time to cross the BBB before they decomposed during the texting time.

3.3.2. *Biodistribution studies of prodrugs*

To further evaluate the possibility of prodrugs being transported across BBB, the tissue distribution of various prodrugs and naproxen after injection were detected in fixed time intervals. The concentrations of naproxen in brain versus time curves were displayed in Fig. 5. The results suggest that all of the prodrugs could be delivered to the brain following i.v. administration. Compared with the concentration curve of naproxen after administration of naked

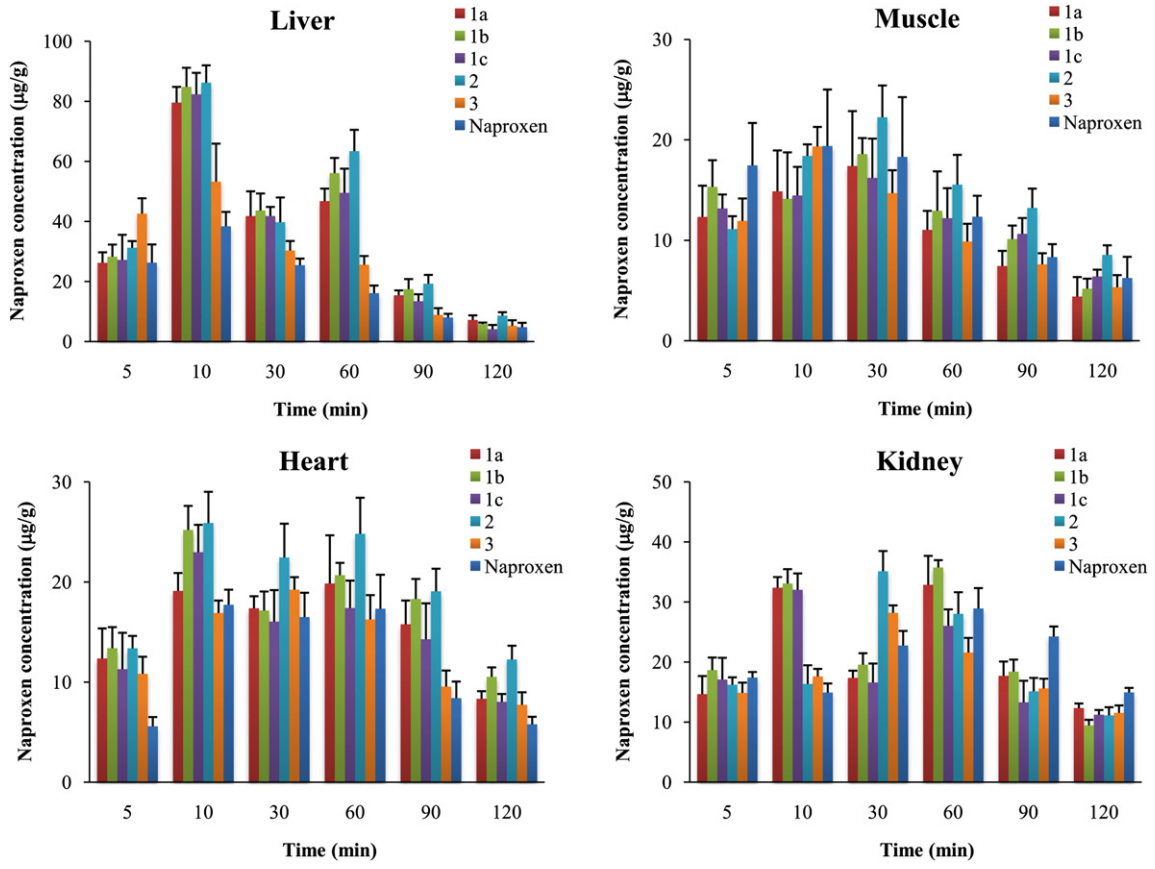


Fig. 6. The levels of naproxen by the prodrugs and naked naproxen in different tissues at different time. Error bars show the value of SD ($n = 3$).

naprxoen, prodrugs exhibited much higher concentrations at different time interval. The pharmacokinetic parameters, relative uptake efficiencies (REs), and concentration efficiencies (CEs) of naproxen in brain were reported in Table 4. The AUC_{0-t} and C_{max} of naproxen in brain after i.v. administration of prodrugs were significantly higher than that after the injection of naked naproxen. The REs were enhanced to 2.50, 3.25 and 3.01 times that of naproxen for prodrugs **1a**, **1b**, and **1c** respectively. The CEs were increased to 3.21, 3.36 and 2.87 times that of naproxen. The results indicated that prodrugs **2** and **3** also delivered naproxen across the BBB, but to a significantly lower extent than that observed for prodrugs **1a**–**1c**. This may be attributed to the peculiarity of these two compound, that the delivery of prodrug **2** by $GLUT_1$ was bidirectional even though with highly efficient, while prodrug **3** transferred naproxen to CNS simply by its lipophilicity. The naproxen level of prodrug **3** was lower than that of prodrug **2** may be explained by their different delivering efficiencies. The pharmacokinetic parameters suggest that the brain targeted abilities of these designed prodrugs had a descending trend of **1b** > **1c** > **1a** > **2** > **3**. The prodrug **1b** seems to be better transported into CNS than prodrug **1c** and **1a**, this may be explained by the difference between the alkyl groups substituted at S site of these prodrugs. The essential steric hinderance produced by the isopropyl group of prodrug **1b** make that disulfide reductase with relatively low level in blood could not reduced the compound effectively, which prolonged the circulation time of **1b** and increased the possibility to cross the BBB. It is notable that although the benzyl group in **1c** possessed comparable steric hinderance, the chemical activity of this group hindered its stability in blood. Additionally, transport of a compound through the BBB is associated with the lipophilicity and the molecular weight of the compound [26]. It is considered that prodrug **1b** which carried the lipophilic isopropyl group with a reduced molecular weight compared with prodrug **1c**, is more suitable for entering brain.

The concentrations of naproxen of prodrugs in other tissues at different times are summarized in Fig. 6. In liver, concentrations of naproxen dramatically increase immediately after administration of the prodrugs while they decreased rapidly, which is possibly attributed to the high metabolic capability of liver. It is also observed that naproxen of these prodrugs in muscle could be detected during the texting time. This phenomenon could be explained by the drug latentiation that regeneration of naproxen from the prodrugs in muscle occurred more slowly than that in internal bodily organs. On the other hand, the concentrations of prodrugs in kidney and heart were comparable to or slightly higher than that of naked naproxen, which indicated the proximate toxic effect of the parent drug in non-targeted tissues.

4. Conclusion

In the present work, a serials of brain-targeted prodrugs of naproxen was described on the hypothesis that the glucosyl thiamine disulfide could produce an enhancement in the brain targeted delivery mediated by the $GULT_1$, and hence, to elevate the concentration of naproxen in brain. Briefly, the glucose moiety of compounds **1a**–**1c** could be recognized by the $GULT_1$ presented on the BBB, which efficiently transported these prodrugs into the brain. In the brain, the thiamine disulfide component of the compounds could be immediately reduced by the abundant disulfide reductase, and simultaneously underwent a cyclization to form the corresponding thiazolium. Such a swift transformation might contribute to the brain bioavailability of naproxen.

The trials in vitro showed that these prodrugs were stable in neutral solution, and could instantly release naproxen in physiological environment. Moreover, the results showed that **1a**–**1c** were relatively stable in plasma extract during the texting time, which was propitious to increase the chance to be delivered across the BBB. In contrast, these prodrugs quickly decomposed in brain homogenate by the disulfide reductase. The instability indicated the "lock-in" effect of these compounds after they entered the CNS. These properties render the prodrugs servable by injection.

In vivo, the results displayed that designed compounds **1a**–**1c**, **2**, **3** could enhance the delivery of naproxen into brain. However, **1a**–**1c** and **2** presented with AUC_{0-t} ratios of 1.20, 1.56, 1.44 while the values between **1a**–**1c** and **3** was calculated as 1.42, 1.86, 1.71, respectively. The prodrugs **1a**–**1c** exhibited excellent transport ability across the BBB, among which prodrug **1b** seemed to be better transported into CNS than other derivatives, this was attributed to the specific substituted group at S site with suitable steric hinderance and molecular weight.

Generally, the increased distribution in brain after the injection compounds **1a**–**1c** suggested that the glucosyl thiamine disulfide moiety could act as a vector, transporting the prodrugs across the BBB, and beyond which, the designed prodrugs could constantly release naproxen.

5. Experimental protocols

5.1. Chemistry

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 a YRT-3 melting point apparatus (Shantou Keyi instrument & Equipment Co. Ltd, Shantou, China). IR spectra were obtained on a Perkin Elmer 983 (Perkin Elmer, Norwalk, CT, USA). ^{1}H NMR spectra were taken on a Varian INOVA400 (Varian, Palo Alto, CA, USA) using $CDCl_3$, d_6-DMSO and D_2O as solvent. Chemical shifts are expressed in δ (ppm), with tetramethylsilane (TMS) functioning as the internal reference, coupling constants (J) were expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument (Agilent, Palo Alto, CA, USA).

Naproxen was purchased from ChengDu GuoJia United Pharmaceutical Ltd. (Chengdu, P. R. China), while 5-(2-hydroxyethyl)-4-methylthiazole were purchased from AstaTech (Chengdu) Pharmaceutical Co. Ltd. (Chengdu, P. R. China).

5.1.1. *penta-O-Trimethylsilyl-α-D-glucopyranose (**5**)*

A mixture of chlorotrimethylsilane (14.1 mL, 111 mmol) and hexamethyl disilazane (7.8 mL, 37 mmol) was carefully added to a vigorously stirred solution of D-glucose (3.33 g, 18.5 mmol) in pyridine 8 mL at 0 °C. The temperature was slowly raised to room temperature and the solution was stirred overnight. Solvent and excess reagents were evaporated under reduced pressure. The resulting residue was dissolved in ether, washed with saturated $NaHCO_3$ solution and water, dried on anhydrous Na_2SO_4. Organic layer was distilled to get **5** (9.3 g, 93.1%) as a syrup.

5.1.2. *1,2,3,4-tetra-O-Trimethylsilyl-α-D-glucopyranose (**6**)*

To a solution of compound **5** (5.00 g, 9.30 mmol) in acetone (30 mL) were added methanol (35 mL) and acetate (2.0 mL), and the mixture was stirred for 2 h at 50 °C. After the addition of solid sodium carbonate (2.50 g), the mixture was concentrated and the residue was purified by column chromatography to yield **6** as a oil (3.26 g, 75.2%). IR (KBr pellets cm^{-1}): υ 3472, 2971, 2910, 1249, 1100; ^{1}H NMR ($CDCl_3$, ppm): 5.16 (d, 1 H, **H**-1, J = 3.2 Hz), 3.80 (t, 1 H, **H**-2, J = 8.8 Hz), 3.73–3.76 (m, 2 H, **H**-4, **H**-5), 3.68 (dd, 1 H, **H**-6, J_1 = 4.8 Hz, J_2 = 12 Hz), 3.66 (t, 1 H, **H**-3, J = 9.2 Hz), 3.34 (dd, 1 H, **H**-6′, J_1 = 2.8 Hz, J_2 = 9.2 Hz), 0.14–0.19 (m, 36 H, $Si(CH_3)_3$); MS (m/z): 466 ($[M + 1]^+$); $[\alpha]_D^{20}$ +35°(c = 1.0, $CHCl_3$).

*5.1.3. 3-(2-tert-Butoxy-2-oxoethyl)-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide (**8**)*

A solution of 5-(2-hydroxyethyl)-4-methylthiazole **7** (10 g, 51.5 mmol) and *t*-butyl bromoacetate (7.3 g, 51.5 mmol) in acetonitrile (75 mL) was heated to reflux and stirred for 1.5 h, then After completion of the reaction, the system was cooled to room temperature and EA (50 mL) was added to precipitate product. The precipitation was filtered, and then dried in a vacuum desiccator to yield a yellow solid (15.8 g, 91.4%). Mp: 148–150 °C; ^{1}H NMR (D_2O, ppm): 5.42 (s, 2 H, COC**H**$_2$), 3.92 (t, 2 H, CH_2C**H**$_2$OH, J = 6.0 Hz), 3.22 (t, 2 H, C**H**$_2$$CH_2$OH, J = 5.6 Hz), 2.50(s, 3 H, CC**H**$_3$), 1.54 (s, 9 H, OC(C**H**$_3$)$_3$).

*5.1.4. General procedure for synthesis of compounds **11a–11c***

A solution of sodium thiosulfate pentahydrate (80 mmol) in water (40 mL) was added to the mixture of alkyl bromide (72 mmol), PEG400 (0.5 mL) and ethanol (20 mL) under stirring and argon protection, which was heated to 110 °C and maintained for 5 h. The solvent was evaporated under vacuum to result a yellow solid, then was filtered. The filter cake was washed by the mixture of ethanol and water (v/v = 1:1, 50 mL) and dried in a vacuum desiccator to yield **11a–11c** as white solid. Compound **11a** (6.21 g, 95.2%), **11b** (5.95 g, 83.7%), **11c** (8.23 g, 91%).

*5.1.5. General procedure for synthesis of **12a–12c***

To a solution of quaternary ammonium salt **8** (8.90 mmol) and sodium hydroxide (17.8 mmol) in water (20 mL) was added sodium alkyl thiosulfate (26.7 mmol) under argon protection. The reaction mixture was stirred for 1.5 h at room temperature. The resultant oily substance was extracted with ethyl acetate (30 mL) and the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by column chromatography with petroleum–acetone (10:1) as eluent gave a yellow syrup.

*5.1.5.1. tert-Butyl 2-(N-(3-(ethyldisulfanyl)-5-hydroxypent-2-en-2-yl)formamido)acetate (**12a**).* Yield: 79.6%. ^{1}H NMR ($CDCl_3$, ppm): 8.04 (s, 1 H, C**H**O), 4.04 (s, 2 H, COC**H**$_2$), 3.81 (t, 2 H, CH_2C**H**$_2$OH, J = 6.4 Hz), 2.89 (t, 2H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 2.65 (q, 2 H, SC**H**$_2$$CH_3$, J = 7.2 Hz), 2.07 (s, 3 H, CC**H**$_3$), 1.47 (s, 9 H, OC(C**H**$_3$)$_3$), 1.29 (t, 3 H, SCH_2C**H**$_3$, J = 7.2 Hz); MS (m/z): 336 ($[M + 1]^+$).

*5.1.5.2. tert-Butyl 2-(N-(3-(isopropyldisulfanyl)-5-hydroxypent-2-en-2-yl)formamido)acetate (**12b**).* Yield: 83.9%. ^{1}H NMR ($CDCl_3$, ppm): 8.07 (s, 1H, C**H**O), 4.05 (s, 2H, COC**H**$_2$), 3.79 (t, 2H, CH_2C**H**$_2$OH, J = 6.4 Hz), 2.95–2.92 (m, 1 H, SC**H**$(CH_3)_2$), 2.89 (t, 2 H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 2.05 (s, 3 H, CC**H**$_3$), 1.47 (s, 9 H, OC(C**H**$_3$)$_3$), 1.27 (t, 6 H, $SCH(CH_3)_2$, J = 6.8 Hz); MS (m/z): 350 ($[M + 1]^+$).

*5.1.5.3. tert-Butyl 2-(N-(3-(benzyldisulfanyl)-5-hydroxypent-2-en-2-yl)formamido)acetate (**12c**).* Yield: 86.2%. ^{1}H NMR ($CDCl_3$, ppm): 7.96 (s, 1 H, C**H**O), 7.30–7.24 (m, 5 H, Ar–**H**), 3.97 (s, 2 H, C**H**$_2$Ar), 3.87 (s, 2 H, COC**H**$_2$), 3.69 (t, 2 H, CH_2C**H**$_2$OH, J = 6.4 Hz), 2.71 (t, 2 H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 1.98 (s, 3 H, CC**H**$_3$), 1.45 (s, 9 H, OC(C**H**$_3$)$_3$); MS (m/z): 398 ($[M + 1]^+$).

*5.1.6. General procedure for synthesis of compounds **13a–13c***

A stirred solution of naproxen (9.21 mmol) in dry tetrahydrofuran (40 mL) was treated with the coupling product (**12a–12c**) (7.67 mmol), DCC (2.37 g, 11.5 mmol) and DMAP (12.0 mg, 0.1 mmol). The mixture was kept at room temperature for overnight and then filtered. The filter cake was washed by dry dichloromethane (10 mL), then the filtrates were combined and concentrated under reduced pressure. The residue was purified by column chromatography with petroleum–ethyl acetate (12:1) to get **13a–13c** as white solid.

*5.1.6.1. (S)-4-(N-(2-tert-Butoxy-2-oxoethyl)formamido)-3-(ethyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**13a**).* Yield: 87.6%. Mp: 80–82 °C; ^{1}H NMR ($CDCl_3$, ppm): 7.90 (s, 1H, C**H**O), 7.77 (dd, 2H, Ar–**H**, J_1 = 3.2 Hz, J_2 = 8.8 Hz), 7.64 (s, 1H, Ar–**H**), 7.37 (dd, 1H, Ar–**H**, J_1 = 1.8 Hz, J_2 = 8.4 Hz), 7.14 (dd, 1H, Ar–**H**, J_1 = 2.8 Hz, J_2 = 11.2 Hz), 7.12 (s, 1H, Ar–**H**), 4.23 (t, 2H, CH_2C**H**$_2$O, J = 6.8 Hz), 3.91 (s, 3H, OC**H**$_3$), 3.89 (s, 2H, COC**H**$_2$), 3.82 (q, 1 H, C**H**CH_3, J = 7.2 Hz), 2.87 (t, 2 H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 2.58 (q, 2 H, SC**H**$_2$$CH_3$, J = 7.6 Hz), 1.86 (s, 3 H, CC**H**$_3$), 1.57 (d, 3 H, CHC**H**$_3$, J = 7.6 Hz), 1.46 (s, 9 H, OC(C**H**$_3$)$_3$), 1.22 (t, 3 H, SCH_2C**H**$_3$, J = 7.6 Hz); MS (m/z): 548 ($[M + 1]^+$).

*5.1.6.2. (S)-4-(N-(2-tert-Butoxy-2-oxoethyl)formamido)-3-(isopropyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**13b**).* Yield: 80.5%. Mp: 85–86 °C; ^{1}H NMR ($CDCl_3$, ppm): 8.01 (s, 1 H, C**H**O), 7.69 (dd, 2 H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 7.63 (s, 1H, Ar–**H**), 7.36 (d, 1H, Ar–**H**, J = 8.4 Hz), 7.13 (d, 1H, Ar–**H**, J = 8.8 Hz), 7.11 (s, 1H, Ar–**H**), 4.89 (s, 2H, COC**H**$_2$), 4.21 (t, 2H, CH_2C**H**$_2$O, J = 6.4 Hz), 3.91 (s, 3H, OC**H**$_3$), 3.82 (q, 1H, C**H**CH_3, J = 7.2 Hz), 2.87 (t, 2H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 2.84–2.81 (m, 1 H, SC**H**$(CH_3)_3$), 1.85 (s, 3 H, CC**H**$_3$), 1.56 (d, 3 H, CHC**H**$_3$, J = 7.2 Hz), 1.46 (s, 9 H, OC(C**H**$_3$)$_3$), 1.21 (d, 6 H, $SCH(CH_3)_2$, J = 6.8 Hz); MS (m/z): 562 ($[M + 1]^+$).

*5.1.6.3. (S)-4-(N-(2-tert-Butoxy-2-oxoethyl)formamido)-3-(benzyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**13c**).* Yield: 81.4%. Mp: 89–90 °C; ^{1}H NMR ($CDCl_3$, ppm): 7.84 (s, 1H, C**H**O), 7.69 (dd, 2H, Ar–**H**, J_1 = 3.6 Hz, J_2 = 8.4 Hz), 7.63 (s, 1H, Ar–**H**), 7.35 (dd, 1H, Ar–**H**, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 7.28–7.11 (m, 7H, Ar–**H**), 4.14 (t, 2H, CH_2C**H**$_2$O, J = 6.8 Hz), 3.91 (s, 3H, OC**H**$_3$), 3.86 (s, 2H, C**H**$_2$Ar), 3.82 (s, 2H, COC**H**$_2$), 3.80 (q, 1H, C**H**CH_3, J = 7.2 Hz), 2.68 (t, 2 H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 1.78 (s, 3 H, CC**H**$_3$), 1.57 (d, 3 H, CHC**H**$_3$, J = 6.8 Hz), 1.46 (s, 9 H, OC(C**H**$_3$)$_3$); MS (m/z): 610 ($[M + 1]^+$).

*5.1.7. General procedure for synthesis of compounds **14a–14c***

With the protection of argon gas, to a solution of the esterified product (**13a–13c**) (5.23 mmol) in dichloromethane (20 mL) was dropped with the dichlormethane solution (10 mL) of 70% perchloric acid (0.3 mL, 5.23 mmol). The mixture was stirred for 1 h at room temperature until completion of the reaction (monitored by TLC), then the sodium bicarbonate (0.44 g, 5.23 mmol) was added to adjust the pH to neutral and a further addition of silica gel (4 g) was performed. The system was concentrated under reduced pressure to afford a pale-yellow powder, which was purified by the column chromatography with petroleum–acetone (5:1) as eluent to get a colorless oil.

*5.1.7.1. (S)-2-(N-(3-(Ethyldisulfanyl)-5-(2-(6-methoxynaphthalen-2-yl)propanoyloxy)pent-2-en-2-yl)formamido) acetic acid (**14a**).* Yield: 76.4%. ^{1}H NMR ($CDCl_3$, ppm): 7.87 (s, 1 H, C**H**O), 7.67 (dd, 2H, Ar–**H**, J_1 = 5.2 Hz, J_2 = 8.4 Hz), 7.62 (s, 1H, Ar–**H**), 7.37 (dd, 1H, Ar–**H**, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 7.13 (dd, 1H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 7.11 (s, 1H, Ar–**H**), 4.24 (t, 2H, CH_2C**H**$_2$O, J = 6.4 Hz), 4.02–3.95 (m, 2H, COC**H**$_2$), 3.89 (s, 3H, OC**H**$_3$), 3.82 (q, 1H, C**H**CH_3, J = 7.2 Hz), 2.86 (t, 2H, C**H**$_2$$CH_2$OH, J = 6.4 Hz), 2.56 (q, 2 H, SC**H**$_2$$CH_3$, J = 7.2 Hz), 1.79 (s, 3 H, CC**H**$_3$), 1.56 (d, 3 H, CHC**H**$_3$, J = 7.6 Hz), 1.20 (t, 3 H, SCH_2C**H**$_3$, J = 7.6 Hz); MS (m/z): 492 ($[M + 1]^+$).

*5.1.7.2. (S)-2-(N-(3-(Isopropyldisulfanyl)-5-(2-(6-methoxynaphthalen-2-yl)propanoyloxy)pent-2-en-2-yl)formamido) acetic acid (**14b**).* Yield: 70.3%. ^{1}H NMR ($CDCl_3$, ppm): 7.86 (s, 1 H, C**H**O), 7.69 (dd, 2H, Ar–**H**, J_1 = 4.8 Hz, J_2 = 8.8 Hz), 7.63 (s, 1H, Ar–**H**), 7.35 (dd, 1H, Ar–**H**, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 7.13 (dd, 1H, Ar–**H**, J_1 = 2.8 Hz, J_2 = 9.2 Hz), 7.11 (s, 1H, Ar–**H**), 4.23 (t, 2 H, CH_2C**H**$_2$O, J = 6.4 Hz), 3.98 (s, 2H, COC**H**$_2$), 3.91 (s, 3H, OC**H**$_3$), 3.82 (q, 1H, C**H**CH_3, J = 7.2 Hz), 2.87 (t, 2H,

C**H**$_2$CH$_2$**H**OH, J = 6.4 Hz), 2.84–2.79 (m, 1H, SC**H**(CH$_3$)$_3$), 1.78 (s, 3H, CC**H**$_3$), 1.56 (d, 3H, CHC**H**$_3$, J = 7.2 Hz), 1.21 (d, 6 H, SCH(C**H**$_3$)$_2$, J = 6.8 Hz); MS (m/z): 506 ([M + 1]$^+$).

*5.1.7.3. (S)-2-(N-(3-(Benzyldisulfanyl)-5-(2-(6-methoxynaphthalen-2-yl)propanoyloxy)pent-2-en-2-yl)formamido) acetic acid (**14c**).* Yield: 75.9%. ^{1}H NMR (CDCl$_3$, ppm): 7.77 (s, 1 H, C**H**O), 7.68 (dd, 2H, Ar–**H**, J_1 = 4.8 Hz, J_2 = 8.4 Hz), 7.62 (s, 1H, Ar–**H**), 7.35 (dd, 1H, Ar–**H**, J_1 = 1.4 Hz, J_2 = 8.4 Hz), 7.26–7.10 (m, 7H, Ar–**H**), 4.15 (t, 2H, CH$_2$C**H**$_2$O, J = 6.0 Hz), 3.90 (s, 3H, OC**H**$_3$), 3.85 (s, 2H, C**H**$_2$Ar), 3.82 (s, 2H, COC**H**$_2$), 3.79–3.77 (m, 1H, C**H**CH$_3$), 2.66 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.4 Hz), 1.69 (s, 3H, CC**H**$_3$), 1.55 (d, 3H, CHC**H**$_3$, J = 6.8 Hz); MS (m/z): 554 ([M + 1]$^+$).

*5.1.8. General procedure for synthesis of compounds **15a–15c***

A mixture of the prepared acid (**14a–14c**) (3.60 mmol), DCC (0.97 g, 4.68 mmol) and DMAP (12 mg, 0.1 mmol) in dry tetrahydrofuran (40 mL) was stirred for 0.5 h. The solution of trimethylsilyl glucopyranose **6** (1.69 g, 3.60 mmol) in tetrahydrofuran (20 mL) was dropped into the reaction system, and then the temperature was slowly raised to 60 °C and the solution was stirred overnight. Solvent was evaporated under reduced pressure to result a yellow syrup. The desired derivative was purified by the column chromatography with petroleum–acetone (20:1) as eluent to give a colorless oil.

*5.1.8.1. (S)-4-(N-(2-(1,2,3,4-tetra-O-Trimethylsilyl-α-D-glucopyranosyl)-2-oxoethyl)formamido)-3-(ethyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**15a**).* Yield: 55.4%. ^{1}H NMR (CDCl$_3$, ppm): 7.91 (s, 1 H, C**H**O), 7.70 (dd, 2H, Ar–**H**, J_1 = 3.2 Hz, J_2 = 8.4 Hz), 7.64 (s, 1H, Ar–**H**), 7.37 (dd, 1H, Ar–**H**, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 7.15 (dd, 1H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 7.12 (s, 1H, Ar–**H**), 4.99 (d, 1H, **H**-1, J = 2.8 Hz), 4.41 (dd, 1H, **H**-6, J_1 = 2.0 Hz, J_2 = 11.6 Hz), 4.23 (t, 2H, CH$_2$C**H**$_2$O, J = 6.4 Hz), 4.12 (t, 1H, **H**-2, J = 5.6 Hz), 4.10–4.09 (m, 2H, COC**H**$_2$), 3.96–3.94 (m, 1H, **H**-6′), 3.92 (s, 3H, OC**H**$_3$), 3.82 (q, 1H, C**H**CH$_3$, J = 7.2 Hz), 3.79 (t, 1H, **H**-3, J = 9.2 Hz), 3.41 (t, 1H, **H**-4, J = 9.2 Hz), 3.36 (dd, 1H, **H**-5, J_1 = 3.2 Hz, J_2 = 9.2 Hz), 2.87 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.8 Hz), 2.58 (q, 2H, SC**H**$_2$CH$_3$, J = 7.2 Hz), 1.86 (s, 3H, CC**H**$_3$), 1.57 (d, 3H, CHC**H**$_3$, J = 8.0 Hz), 1.22 (t, 3H, SCH$_2$C**H**$_3$, J = 7.6 Hz), 0.19–0.09 (m, 36H, Si(C**H**$_3$)$_3$); MS (m/z): 942 ([M + 1]$^+$).

*5.1.8.2. (S)-4-(N-(2-(1,2,3,4-tetra-O-Trimethylsilyl-α-D-glucopyranosyl)-2-oxoethyl)formamido)-3-(isopropyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**15b**).* Yield: 53.4%. ^{1}H NMR (CDCl$_3$, ppm): 7.92 (s, 1 H, C**H**O), 7.70 (dd, 2H, Ar–**H**, J_1 = 2.8 Hz, J_2 = 8.4 Hz), 7.64 (s, 1H, Ar–**H**), 7.36 (d, 1H, Ar–**H**, J = 8.4 Hz), 7.14 (dd, 1H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 7.12 (s, 1H, Ar–**H**), 4.99 (d, 1H, **H**-1, J = 3.2 Hz), 4.40 (d, 1H, **H**-6, J = 11.2 Hz), 4.23 (t, 2H, CH$_2$C**H**$_2$O, J = 6.4 Hz), 4.11 (t, 1H, **H**-2, J = 5.6 Hz), 4.09–4.05 (m, 2H, COC**H**$_2$), 3.96–3.94 (m, 1H, **H**-6′), 3.91 (s, 3H, OC**H**$_3$), 3.82 (q, 1H, C**H**CH$_3$, J = 6.8 Hz), 3.78 (t, 1H, **H**-3, J = 8.8 Hz), 3.40 (t, 1H, **H**-4, J = 9.2 Hz), 3.36 (dd, 1H, **H**-5, J_1 = 3.2 Hz, J_2 = 9.2 Hz), 2.86 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.8 Hz), 2.84–2.81 (m, 1H, SC**H**(CH$_3$)$_3$), 1.90 (s, 3H, CC**H**$_3$), 1.56 (d, 3H, CHC**H**$_3$, J = 7.2 Hz), 1.21 (d, 6H, SCH(C**H**$_3$)$_2$, J = 6.8 Hz), 0.18–0.13 (m, 36H, Si(C**H**$_3$)$_3$); MS (m/z): 956 ([M + 1]$^+$).

*5.1.8.3. (S)-4-(N-(2-(1,2,3,4-tetra-O-Trimethylsilyl-α-D-glucopyranosyl)-2-oxoethyl)formamido)-3-(benzyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**15c**).* Yield: 35.8%. ^{1}H NMR (CDCl$_3$, ppm): 7.85 (s, 1 H, C**H**O), 7.69 (dd, 2H, Ar–**H**, J_1 = 3.6 Hz, J_2 = 8.4 Hz), 7.63 (s, 1H, Ar–**H**), 7.36 (d, 1H, Ar–**H**, J = 8.4 Hz), 7.28–7.11 (m, 7H, Ar–**H**), 4.99 (d, 1H, **H**-1, J = 2.8 Hz), 4.40 (d, 1H, **H**-6, J = 11.2 Hz), 4.14 (t, 2H, CH$_2$C**H**$_2$O, J = 6.4 Hz), 4.08 (t, 1H, **H**-2, J = 5.6 Hz), 4.07–4.04 (m, 2H, COC**H**$_2$), 3.95–3.93 (m, 1H, **H**-6′), 3.91 (s, 3H, OC**H**$_3$), 3.82 (s, 2H, C**H**$_2$Ar), 3.81–3.79 (m, 1H, C**H**CH$_3$), 3.78 (t, 1H, **H**-3, J = 4.8 Hz), 3.41 (t, 1H, **H**-4, J = 9.2 Hz), 3.36 (dd, 1H, **H**-5, J_1 = 3.2 Hz, J_2 = 9.2 Hz), 2.68 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.4 Hz), 1.78 (s, 3H, CC**H**$_3$), 1.56 (d, 3H, CHC**H**$_3$, J = 6.8 Hz), 0.17–0.13 (m, 36H, Si(C**H**$_3$)$_3$); MS (m/z): 1004 ([M + 1]$^+$).

*5.1.9. General procedure for synthesis of compounds **1a–1c***

The prepared ester (**15a–15c**) (2.0 mmol) was dissolved in dichlormethane (30 mL), which was cooled to 0 °C. To the vigorously stirred solution was dropped in trifluoroacetic acid (1.8 mL, 24 mmol) and kept for 1.5 h. Solvent and excess reagents were evaporated under reduced pressure. The resulting syrup was purified by the column chromatography with dichlormethane-methanol (40:1) to yield **1a–1c** as a pale-yellow solid.

*5.1.9.1. (S)-4-(N-(2-(α-D-Glucopyranose-6-yl)-2-oxoethyl)formamido)-3-(ethyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**1a**).* Yield: 90.2%. Mp: 64–66 °C; IR (KBr pellets cm^{-1}): υ 3421, 2960, 2932, 1733, 1666, 1453, 1266; ^{1}H NMR (DMSO-d$_6$, ppm): 7.85 (s, 1H, C**H**O), 7.78 (dd, 2H, Ar–**H**, J_1 = 7.6 Hz, J_2 = 8.4 Hz), 7.71 (s, 1H, Ar–**H**), 7.37 (dd, 1H, Ar–**H**, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 7.29 (d, 1H, Ar–**H**, J = 2.0), 7.16 (dd, 1H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 4.91 (d, 1H, **H**-1, J = 3.2 Hz), 4.40–4.31 (m, 1H, **H**-6), 4.21 (t, 2H, CH$_2$C**H**$_2$O, J = 6.4 Hz), 4.08 (t, 1H, **H**-2, J = 6.4 Hz), 4.05 (s, 2H, COC**H**$_2$), 3.91 (q, 1H, C**H**CH$_3$, J = 7.2 Hz), 3.86 (s, 3H, OC**H**$_3$), 3.83–3.79 (m, 1H, **H**-6′), 3.45 (t, 1H, **H**-3, J = 8.8 Hz), 3.15 (dd, 1H, **H**-5, J_1 = 3.6 Hz, J_2 = 9.6 Hz), 3.06 (t, 1H, **H**-4, J = 9.2 Hz), 2.83 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.4 Hz), 2.60 (q, 2H, SC**H**$_2$CH$_3$, J = 7.2 Hz), 1.88 (s, 3H, CC**H**$_3$), 1.46 (d, 3H, CHC**H**$_3$, J = 6.8 Hz), 1.13 (t, 3H, SCH$_2$C**H**$_3$, J = 6.8 Hz); ^{13}C NMR (DMSO-d$_6$, ppm): 174.3 (1C, **C**OCH), 168.5 (1C, **C**OCH$_2$), 162.6 (1C, **C**HO), 157.4 (1C, Ar–**C**O), 138.3 (1 C, N**C**=C), 135.7 (1C, Ar–**C**), 133.6 (1C, Ar–**C**), 129.4 (1C, Ar–**C**), 128.6 (1C, Ar–**C**), 127.3 (1C, Ar–**C**), 126.4 (1C, Ar–**C**), 125.9 (1C, Ar–**C**), 119.1 (1C, Ar–**C**), 105.9 (1C, Ar–**C**), 97.3 (1C, C=**C**S), 92.6 (1C, HO**C**HO), 76.6 (1C, **C**OH), 72.7 (1C, **C**OH), 70.7 (1C, **C**OH), 69.3 (1C, **C**OH), 65.3 (1C, CH**C**H$_2$O), 62.4 (1C, CH$_2$**C**H$_2$O), 55.4 (1C, O**C**H$_3$), 45.2 (1C, S**C**), 44.7 (1C, CO**C**H$_2$N), 40.3 (1C, CO**C**H), 29.5 (1C, C=C**C**CH$_2$), 18.6 (C=C**C**H$_3$), 18.5 (1 C, CH**C**H$_3$), 14.1 (1C, SC**C**H$_3$); MS (m/z): 654 ([M + 1]$^+$).

*5.1.9.2. (S)-4-(N-(2-(α-D-Glucopyranose-6-yl)-2-oxoethyl)formamido)-3-(isopropyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**1b**).* Yield: 83.6%. Mp: 56–58 °C; IR (KBr pellets cm^{-1}): υ 3418, 2962, 2930, 1733, 1669, 1454, 1266; ^{1}H NMR (CDCl$_3$, ppm): 7.86 (s, 1H, C**H**O), 7.78 (dd, 2H, Ar–**H**, J_1 = 7.2 Hz, J_2 = 8.4 Hz), 7.71 (s, 1H, Ar–**H**), 7.37 (d, 1H, Ar–**H**, J = 7.2 Hz), 7.28 (d, 1H, Ar–**H**, J = 2.0), 7.15 (dd, 1H, Ar–**H**, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 4.91 (d, 1H, **H**-1, J = 3.4 Hz), 4.32 (d, 1H, **H**-6, J = 11.2 Hz), 4.17 (t, 2H, CH$_2$C**H**$_2$O, J = 6.4 Hz), 4.09 (t, 1H, **H**-2, J = 5.2 Hz), 4.06 (m, 2H, COC**H**$_2$), 3.91 (q, 1H, C**H**CH$_3$, J = 6.8 Hz), 3.86 (s, 3H, OC**H**$_3$), 3.83–3.79 (m, 1H, **H**-6′), 3.45 (t, 1H, **H**-3, J = 9.2 Hz), 3.14 (dd, 1H, **H**-5, J_1 = 5.2 Hz, J_2 = 8.8 Hz), 3.06 (t, 1H, **H**-4, J = 9.6 Hz), 2.93–2.89 (m, 1H, SC**H**(CH$_3$)$_3$), 2.83 (t, 2H, C**H**$_2$CH$_2$OH, J = 6.8 Hz), 1.87 (s, 3H, CC**H**$_3$), 1.46 (d, 3H, CHC**H**$_3$, J = 7.2 Hz), 1.15 (d, 6H, SCH(C**H**$_3$)$_2$, J = 6.8 Hz); ^{13}C NMR (DMSO-d$_6$, ppm): 174.3 (1C, **C**OCH), 168.5 (1C, **C**OCH$_2$), 162.6 (1C, **C**HO), 157.4 (1C, Ar–**C**O), 137.4 (1C, N**C**=C), 135.8 (1C, Ar–**C**), 133.6 (1C, Ar–**C**), 129.4 (1C, Ar–**C**), 128.5 (1C, Ar–**C**), 127.3 (1C, Ar–**C**), 126.4 (1 C, Ar–**C**), 125.9 (1C, Ar–**C**), 119.1 (1C, Ar–**C**), 105.9 (1 C, Ar–**C**), 97.1 (1C, C=**C**S), 92.6 (1C, HO**C**HO), 76.6 (1C, **C**OH), 72.7 (1C, **C**OH), 70.7 (1C, **C**OH), 69.3 (1C, **C**OH), 65.3 (1C, CH**C**H$_2$O), 62.3 (1C, CH$_2$**C**H$_2$O), 55.4 (1C, O**C**H$_3$), 45.2 (1C, S**C**), 44.7 (1C, CO**C**H$_2$N), 40.9 (1C, CO**C**H), 29.2 (1C, C=C**C**CH$_2$), 22.1 (2 C, SC(**C**H$_3$)$_2$), 18.6 (2C, C=C**C**H$_3$, CH**C**H$_3$); MS (m/z): 668 ([M + 1]$^+$).

*5.1.9.3. (S)-4-(N-(2-(α-D-Glucopyranose-6-yl)-2-oxoethyl)formamido)-3-(benzyldisulfanyl)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**1c**).* Yield: 75.8%. Mp: 56–58 °C; IR (KBr pellets cm^{-1}): υ 3405, 2918, 2850, 1732, 1669, 1454, 1266; ^1H NMR ($CDCl_3$, ppm): 7.81 (s, 1 H, C**H**O), 7.77 (dd, 2H, Ar–**H**, $J_1 = 5.2$ Hz, $J_2 = 8.8$ Hz), 7.70 (s, 1H, Ar–**H**), 7.36 (d, 1H, Ar–**H**, $J = 8.0$ Hz), 7.27–7.14 (m, 7H, Ar–**H**), 4.91 (d, 1H, **H**-1, $J = 3.2$ Hz), 4.38 (d, 1H, **H**-6, $J = 12.0$ Hz), 4.32 (t, 2H, $CH_2C\mathbf{H_2}O$, $J = 8.0$ Hz), 4.16 (t, 1H, **H**-2, $J = 5.4$ Hz), 4.12–4.09 (m, 1H, **H**-6′), 4.04 (m, 2H, $COC\mathbf{H_2}$), 3.91 (s, 2H, $C\mathbf{H_2}Ar$), 3.86 (s, 3H, $OC\mathbf{H_3}$), 3.82–3.79 (m, 1H, $C\mathbf{H}CH_3$), 3.46 (t, 1H, **H**-3, $J = 9.2$ Hz), 3.14 (d, 1H, **H**-5, $J = 8.0$ Hz), 3.07 (t, 1H, **H**-4, $J = 9.2$ Hz), 2.72 (t, 2H, $C\mathbf{H_2}CH_2OH$, $J = 6.2$ Hz), 1.84 (s, 3H, $CC\mathbf{H_3}$), 1.45 (d, 3H, $CHC\mathbf{H_3}$, $J = 6.8$ Hz); ^{13}C NMR (DMSO-d_6, ppm): 174.1 (1C, **C**OCH), 168.4 (1C, **C**OCH_2), 162.7 (1C, **C**HO), 157.6 (1C, Ar–**C**O), 137.5 (1C, N**C**=C), 137.1 (1C, Ar–**C**), 135.8 (1C, Ar–**C**), 133.6 (1C, Ar–**C**), 129.4 (1C, Ar–**C**), 128.5 (1C, Ar–**C**), 128.2 (2C, Ar–**C**), 127.3 (1C, Ar–**C**), 127.1 (1C, Ar–**C**), 126.9 (2C, Ar–**C**), 126.4 (1C, Ar–**C**), 125.9 (1C, Ar–**C**), 119.1 (1C, Ar–**C**), 105.9 (1C, Ar–**C**), 97.1 (1C, C=**C**S), 92.8 (1C, HO**C**HO), 76.6 (1C, **C**OH), 72.4 (1C, **C**OH), 70.6 (1C, **C**OH), 69.3 (1C, **C**OH), 65.6 (1C, $CH\mathbf{C}H_2O$), 62.4 (1C, $CH_2\mathbf{C}H_2O$), 55.4 (1C, $O\mathbf{C}H_3$), 43.5 (1C, S**C**), 44.7 (1C, $CO\mathbf{C}H_2N$), 40.5 (1C, CO**C**H), 29.1 (1 C, C=**C**CH_2), 18.7 (1C, C=**C**CH_3), 18.5 (1C, $CH\mathbf{C}H_3$); MS (*m*/*z*): 716 ($[M + 1]^+$).

*5.1.10. 1-(1,2,3,4-tetra-O-Trimethylsilyl-α-D-glucopyranosyl)-2-(6-methoxynaphthalen-2-yl)propionate (**16**)*

Compound **16** was prepared according to the procedure described for the synthesis of **15a**–**15c**. After column chromatography (petroleum–ethyl acetate 60:1), compound **16** was obtained as a solid (760 mg, 75%). Mp: 104–106 °C; ^1H NMR ($CDCl_3$, ppm): 7.69 (s, 1H, Ar–**H**), 7.68–7.67 (m, 2H, Ar–**H**), 7.42 (dd, 1H, Ar–**H**, $J_1 = 1.6$ Hz, $J_2 = 8.8$ Hz), 7.12 (dd, 1H, Ar–**H**, $J_1 = 2.4$ Hz, $J_2 = 8.8$ Hz), 7.10 (s, 1H, Ar–**H**), 4.99 (d, 1H, **H**-1, $J = 2.8$ Hz), 4.45 (dd, 1H, **H**-6, $J_1 = 2.0$ Hz, $J_2 = 11.6$ Hz), 3.93 (t, 1H, **H**-2, $J = 4.8$ Hz), 3.90 (s, 3H, $OC\mathbf{H_3}$), 3.90–3.89 (m, 1H, $C\mathbf{H}CH_3$), 3.87 (dd, 1H, **H**-5, $J_1 = 2.8$ Hz, $J_2 = 6.8$ Hz), 3.74 (t, 1H, **H**-3, $J = 7.6$ Hz), 3.34 (t, 1H, **H**-4, $J = 9.2$ Hz), 3.32 (dd, 1H, **H**-6′, $J_1 = 3.2$ Hz, $J_2 = 9.2$ Hz), 1.58 (d, 3H, $CHC\mathbf{H_3}$, $J = 6.8$ Hz), 0.14–0.11 (m, 36H, $Si(C\mathbf{H_3})_3$); MS (*m*/*z*): 681 ($[M + 1]^+$).

*5.1.11. (S)-1-(α-D-Glucopyranose-6-yl)-2-(6-methoxynaphthalen-2-yl)propionate (**2**)*

Compound **2** was obtained through deprotection of **16** (750 mg, 1.1 mmol) by trifluoroacetic acid (1.6 mL, 21 mmol) in nitromethane (10 mL) and dichlormethane (30 mL), as described for **1a**–**1c**, Work-up and purification by column chromatography (dichlormethane–methanol 40:1), afforded **2** as a white solid (394 mg, 91.4%). Mp: 108–110 °C; ^1H NMR ($CDCl_3$, ppm): 7.72–7.70 (m, 3H, Ar–**H**), 7.43 (d, 1H, Ar–**H**, $J = 8.0$ Hz), 7.15 (d, 1H, Ar–**H**, $J = 8.8$ Hz), 7.12 (s, 1H, Ar–**H**), 5.07 (d, 1H, **H**-1, $J = 3.2$ Hz), 4.61 (dd, 1H, **H**-6, $J_1 = 4.0$ Hz, $J_2 = 12.0$ Hz), 4.47 (t, 1H, **H**-2, $J = 7.6$ Hz), 4.18 (d, 1H, **H**-5, $J = 11.2$ Hz), 3.92 (s, 3H, $OC\mathbf{H_3}$), 3.85–3.82 (m, 1H, $C\mathbf{H}CH_3$), 3.62 (t, 1H, **H**-3, $J = 9.2$ Hz), 3.19 (dd, 1H, **H**-6′, $J_1 = 3.6$ Hz, $J_2 = 9.2$ Hz), 3.01 (t, 1H, **H**-4, $J = 9.6$ Hz), 1.59 (d, 3H, $CHC\mathbf{H_3}$, $J = 6.2$ Hz); ^{13}C NMR (DMSO-d_6, ppm): 173.8 (1C, **C**OCH), 157.4 (1C, Ar–**C**O), 135.6 (1C, Ar–**C**), 133.5 (1C, Ar–**C**), 129.4 (1C, Ar–**C**), 128.7 (1C, Ar–**C**), 127.2 (1C, Ar–**C**), 126.4 (1C, Ar–**C**), 125.9 (1C, Ar–**C**), 119.2 (1C, Ar–**C**), 105.4 (1C, Ar–**C**), 92.6 (1C, HO**C**HO), 76.5 (1C, **C**OH), 72.7 (1C, **C**OH), 71.1 (1C, **C**OH), 69.5 (1C, **C**OH), 65.7 (1C, $CH\mathbf{C}H_2O$), 55.4 (1C, $O\mathbf{C}H_3$), 41.2 (1C, CO**C**H), 18.5 (1C, $CH\mathbf{C}H_3$); MS (*m*/*z*): 393 ($[M + 1]^+$).

*5.1.12. N,4-Dimethyl-5-(2-(hydroxy) ethyl) thiazolium iodide (**17**)*

5-(2-hydroxyethyl)-4-methylthiazole **7** (3.0 g, 21.0 mmol) and methyl iodide (2.64 mL, 42.0 mmol) were mixed and refluxed for 2 h. After evaporation of excess methyl iodide, to the residual brown syrup was added ether (50 mL) and stirred for 30 min, the precipitate was filtered to get **17** as a pale-yellow solid (5.76 g, 96.3%). Mp: 82–84 °C; ^1H NMR (D_2O, ppm): 4.14 (s, 3 H, $C\mathbf{H_3}N$), 3.90 (t, 2 H, $CH_2C\mathbf{H_2}O$, $J = 5.6$ Hz), 3.19 (t, 2 H, $C\mathbf{H_2}CH_2O$, $J = 6.0$ Hz), 2.53 (s, 3 H, $CC\mathbf{H_3}$).

*5.1.13. N-(3-(Benzyldisulfanyl)-5-hydroxypent-2-en-2-yl)-N-methylformamide (**18**)*

The synthesis of compound **18** was analogous to the procedure described for the synthesis of compound **12a**–**12c**. Purification of the reaction residue by column chromatography with petroleum–acetone (15:1) as eluent gave a yellow oil (540 mg, 76%). ^1H NMR ($CDCl_3$, ppm): 7.87 (s, 1H, C**H**O), 7.32–7.24 (m, 5H, Ar–**H**), 3.86 (s, 2H, Ar$C\mathbf{H_2}$), 3.70 (t, 2H, $CH_2C\mathbf{H_2}O$, $J = 6.8$ Hz), 2.92 (s, 3H, $NC\mathbf{H_3}$), 2.70 (t, 2H, $C\mathbf{H_2}CH_2O$, $J = 6.4$ Hz), 1.92 (s, 3H, $CC\mathbf{H_3}$); MS (*m*/*z*): 298 ($[M + 1]^+$).

*5.1.14. S-3-(Benzyldisulfanyl)-4-(N-methylformamido)pent-3-enyl 2-(6-methoxynaphthalen-2-yl)propanoate (**3**)*

Compound **3** was prepared from **18** as described for **13a**–**13c**, and purified by column chromatography (petroleum–acetone 12:1). Thus, **3** was obtained as a syrup (811 mg, 87.9%). ^1H NMR ($CDCl_3$, ppm): 7.93 (s, 1H, C**H**O), 7.70–7.62 (m, 4H, Ar–**H**), 7.35 (dd, 1H, Ar–**H**, $J_1 = 1.6$ Hz, $J_2 = 8.8$ Hz), 7.24–7.18 (m, 4H, Ar–**H**), 7.14 (dd, 1H, Ar–**H**, $J_1 = 2.4$ Hz, $J_2 = 8.8$ Hz), 7.10 (s, 1H, Ar–**H**), 4.16 (t, 2H, $CH_2C\mathbf{H_2}O$, $J = 6.4$ Hz), 3.92 (s, 3H, $OC\mathbf{H_3}$), 3.81 (q, 1H, $C\mathbf{H}CH_3$, $J = 5.6$ Hz), 3.79 (s, 2H, Ar$C\mathbf{H_2}$), 2.72 (s, 3H, $NC\mathbf{H_3}$), 2.66 (t, 2H, $J = 6.4$ Hz, $C\mathbf{H_2}CH_2O$), 1.58 (s, 3H, $CC\mathbf{H_3}$), 1.56 (d, 3H, $CHC\mathbf{H_3}$, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6, ppm): 173.6 (1C, **C**OCH), 162.6 (1C, **C**HO), 157.7 (1C, Ar–**C**O), 136.5 (1C, N**C**=C), 137.1 (1C, Ar–**C**), 136.1 (1C, Ar–**C**), 133.4 (1C, Ar–**C**), 129.4 (1C, Ar–**C**), 128.6 (1C, Ar–**C**), 128.2 (2C, Ar–**C**), 127.3 (1C, Ar–**C**), 127.1 (1C, Ar–**C**), 126.9 (2C, Ar–**C**), 126.4 (1C, Ar–**C**), 125.9 (1C, Ar–**C**), 119.2 (1C, Ar–**C**), 105.9 (1C, Ar–**C**), 97.6 (1C, C=**C**S), 62.4 (1C, $CH_2\mathbf{C}H_2O$), 55.4 (1C, $O\mathbf{C}H_3$), 43.5 (1C, S**C**), 40.5 (1C, CO**C**H), 31.6(1C, N$\mathbf{C}H_3$), 29.2 (1 C, C=**C**CH_2), 18.8 (1C, C=**C**CH_3), 18.6 (1C, $CH\mathbf{C}H_3$); MS (*m*/*z*): 510 ($[M + 1]^+$).

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 μm), thermostated at 25 °C. The solution of methanol-water (80:20) was used as the mobile phase at a flow rate of 1.0 mL/min and the UV detector was set to monitor the signal at 238 nm corresponding to the maximum absorbance for the naproxen 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: 2.5, 5.0, 7.4, and 8.0. 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 naproxen 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 15000 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.

5.2.4. Release of the parent drug by prodrugs in brain homogenate

The prodrugs were treated in brain homogenate according to the procedure in Section 5.2.3, and the peak of naproxen was analyzed by HPLC described in Section 5.3.2. Peak integrals were normalized by dividing by the area for the peak of **1a** at 15 min.

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 the animal center of Sichuan University. Throughout the experiments, the animals were handled according to the international ethical guidelines for the care of laboratory animals.

5.3.2. HPLC analysis of prodrugs and naproxen

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. The elution was monitored at 35 °C at a flow rate of 1.0 mL/min. The detector was set to monitor the signal at 230 nm corresponding to the maximum absorbance for naproxen.

5.3.3. Biodistribution studies of the prodrugs and naproxen

Mice were randomly divided into 6 groups, 24 in each group for different sampling time and was housed in one cage. Each animal was injected with the synthesized prodrugs or naproxen in a mixture of DMSO-water (1:2) through the tail vein at a single dose equivalent to 10 mg/kg body weight of naproxen. At appropriate time interval (5, 10, 30, 45, 60, 90, 120 and 240 min), the animal was sacrificed and 1 mL blood samples withdrawn from the orbital sinus were collected was collected in heparinized tube. Plasma was immediately separated by centrifugation and diluted with methanol to 1:3 which was stored at −20 °C until assay. Meanwhile, the tissue sample was removed, weighed. Each tissue sample was homogenized and diluted with methanol to 1:3 (g/mL). The homogenates were also stored at −20 °C until assay. Before analyzing prodrugs, hydrolysis was performed. The plasma samples and homogenates were added with an aliquot of 6 M NaOH aqueous solution to 1:4 (mL/mL or g/mL). After 5 min of hydrolysis at room temperature, naproxen was released from the prodrugs, then another aliquot of 6 M HCl aqueous solution was added to neutralize NaOH, and thus form the proportion of 1:5 (mL/mL or g/mL). After deproteinization, the mixture was centrifuged at 15000 rpm for 15 min. 200 μL aliquots were withdrawn, then 200 μL of acetonitrile was added to each aliquot and vortexed. Samples were centrifuged for further 15 min to remove residual proteins and the supernatants were analyzed by HPLC method as described.

5.4. Statistical analysis

The AUC_{0-t}, C_{max} and MRT were calculated by Data and max Statistics (DAS 2.0, Shanghai, China). Statistical evaluation was performed using analysis of variance followed by *t*-test. A value of $p < 0.05$ was considered significant. The RE and CE were calculated to evaluate the brain targeting property of liposomes. The value of RE and CE were defined as following:

$$RE = (AUC_{0-t})_S/(AUC_{0-t})_C$$

$$CE = (C_{max})_S/(C_{max})_C$$

Where s and n represented sample (the prodrugs) and control (naproxen), respectively.

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