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Two-step enzymatic selective synthesis of water-soluble ketoprofen–saccharide conjugates in organic media

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ABSTRACT

Ketoprofen–saccharide conjugates were synthesized by selectively enzymatic hydrolysis and acylation. Firstly, the (*S*)-ketoprofen vinyl ester was prepared by enzymatic hydrolysis of (*R*,*S*)-ketoprofen vinyl ester. Then enzymatic transesterification of (*S*)-ketoprofen vinyl ester with a series of saccharides were performed by the catalysis of a commercial protease from *Bacillus licheniformis* (BLP) in organic medium mixture of pyridine and *tert*-butanol. The ketoprofen was selectively conjugated onto the primary hydroxyl group of saccharides and with high yield after 72 h. Partition coefficient determination showed that all the products have better water solubility than parent ketoprofen. Chemical hydrolysis experiment indicated that 50% ketoprofen could be release from ketoprofen glucoside and maltoside in aqueous buffer (pH 7.4) within 48 h.

1. Introduction

Ketoprofen is one of the commercially available 2-arylpropionic acids non-steroidal anti-inflammatory drugs (NSAIDs), which is widely used for alleviation of pain and inflammation associated with tissue injury.[1] However, previous pharmacological study of ketoprofen has indicated that the gastrointestinal (GI) ulceration and hemorrhage due to the locally direct contact effect induced by the acidic moiety.[2] An alternative method of avoiding GI side effects is to administer the drug via the transdermal route. However, the therapeutic efficacy of ketoprofen, especially its application onto the skin, is mainly limited by its poor aqueous solubility and low permeability.[3]

Therefore, various methods such as salt formation, solid dispersion with a polymer and the prodrug approach have been used to increase its solubility and bioavailability.[4–6] Among these methods, preparation of ester prodrug, especially for its functionalization with appropriate hydrophilic groups is one of the most effective and promising way to enhance the solubility of drug and then to exploit their properties as well as possible. Furthermore, preparation of ester prodrugs can temporarily mask the acidic moiety and decrease the side effects.[7] Appropriately designed prodrugs, such as oligoethylene ester of ketoprofen,[8] sugar-surfactant/ketoprofen catanionic assembly,[9] ketoprofen acyloxyalkyl ester[10] and amide derivative of ketoprofen with L-cysteine ethyl ester[11], have been reported to show good water stability, rapid enzymatic hydrolysis and increased flux through excised human skin.

Carbohydrate plays an important role in biological molecular recognition and exhibits several desirable biological properties such as non-toxicity, good biocompatibility and biodegradability.[12–14] Since a good hydrophilic–lipophilic balance of the prodrug is essential to give an acceptable bioavailability,[15,16] the selection of hydroxyl compound (alcohol) is important in the study of prodrug. Sugars are hydrophilic compounds and it is particularly effective for the improvement of drugs dissolution behavior to form ester prodrugs. Multivalent sugar-based therapeutic agents have been reported in recent years.[17–19] Thus we considered it worthy of interest to evaluate sugar as a potential carrier for improving drug dissolution behavior.

However, preparation of optically active ketoprofen prodrug containing sugar has still received limited attention. The preparation of optically active ketoprofen has become an important subject due to the bioactivity of ketoprofen is mainly *S*-enantiomer.[20] The application of enzymes in the resolution of ketoprofen has great advantages such as the simplicity of process and the high enantioselectivity of biocatalysis. An irreversible enzymatic resolution method has been developed to prepare optically active ketoprofen vinyl ester in our previous study.[21,22]

Herein, we wish to report the two-step selectively enzymatic synthesis optically active ketoprofen–saccharide conjugates with high water solubility. (*S*)-Ketoprofen vinyl ester (**2**) was prepared from resolution of (*R*,*S*)-ketoprofen vinyl ester (**1**) by using Lipozyme® immobilized from *Mucor miehei* (MML). The protease from *Bacillus licheniformis* (BLP) was used to catalyze the transesterification of (*S*)-ketoprofen vinyl ester with a series of saccharides in organic media. (*S*)-ketoprofen was regioselectively conjugated onto the primary hydroxyl group of saccharides through the

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ester-linkage and afforded ester type ketoprofen–saccharide conjugates. The obtained ketoprofen–saccharide conjugates have better water solubility than parent ketoprofen and thus suitable for potentially pharmaceutical application.

2. Results and discussion

2.1. Selective enzymatic synthesis of ketoprofen–saccharide conjugates

Two-step enzymatic selective synthesis of ketoprofen–saccharide conjugates was shown in Scheme 1. At the first step, optically active (*S*)-ketoprofen vinyl ester was prepared by enzymatic irreversible resolution. Lipozyme® immobilized from *M. miehei* was used to catalyze the hydrolysis of (*R*,*S*)-ketoprofen vinyl ester in the solvent composed of 2.5% water in dioxane. We obtained bioactive *S*-enantiomer with good optical purity under the optimized conditions based on the previous studies.[21,22] The (*S*)-ketoprofen vinyl ester obtained was used in the subsequent transesterification with a series of saccharides catalyzed by BLP under mild conditions. The products were purified by silica gel chromatography and characterized by MS and NMR spectrometries. Vinyl ester had been chosen as active acyl substrate in the enzymatic hydrolysis and transesterification reaction because the vinyl alcohols could instantly tautomerize to volatile acetaldehyde, which is thermodynamically favourable to product synthesis and make the process irreversible.

After the reaction conditions such as enzyme sources, solvent and reaction time were optimized, we obtained a series of ketoprofen–saccharide conjugates with good yields. The acylation position and yields of products are shown in Table 1. The acylation position in compounds **3a**–**g** was characterized by NMR. According to the general strategy described by Yoshimoto et al.[23], acylation of a hydroxyl group of sugar would lead to the *O*-acylated carbon downfield while the adjacent carbon upfield in the ^{13}C NMR. Analysis of ^{13}C NMR spectra of all the products revealed that the reactions occurred on the primary hydroxyl position of the saccharides. For example, in the ^{13}C NMR spectrum of **3b**, the peak of C6α (C6β) results in a downfield shift from 60.72 (60.94) ppm to 64.9 (65.0) ppm, while the peak of C5α (C5β) results in an upfield shift from 73.2 (76.9) ppm to 69.7 (74.0) ppm, which shows that the acylation position of **3b** is the C-6 hydroxyl of glucose. In the ^{13}C NMR spectrum of **3e**, the peak of C6′ results in a downfield shift from 62.4 ppm to 64.5 ppm, while the peak of C5′ results in a upfield shift from 77.2 ppm to 72.9 ppm, which shows that the acylation position of **3e** is the C6′ hydroxyl of lactose.

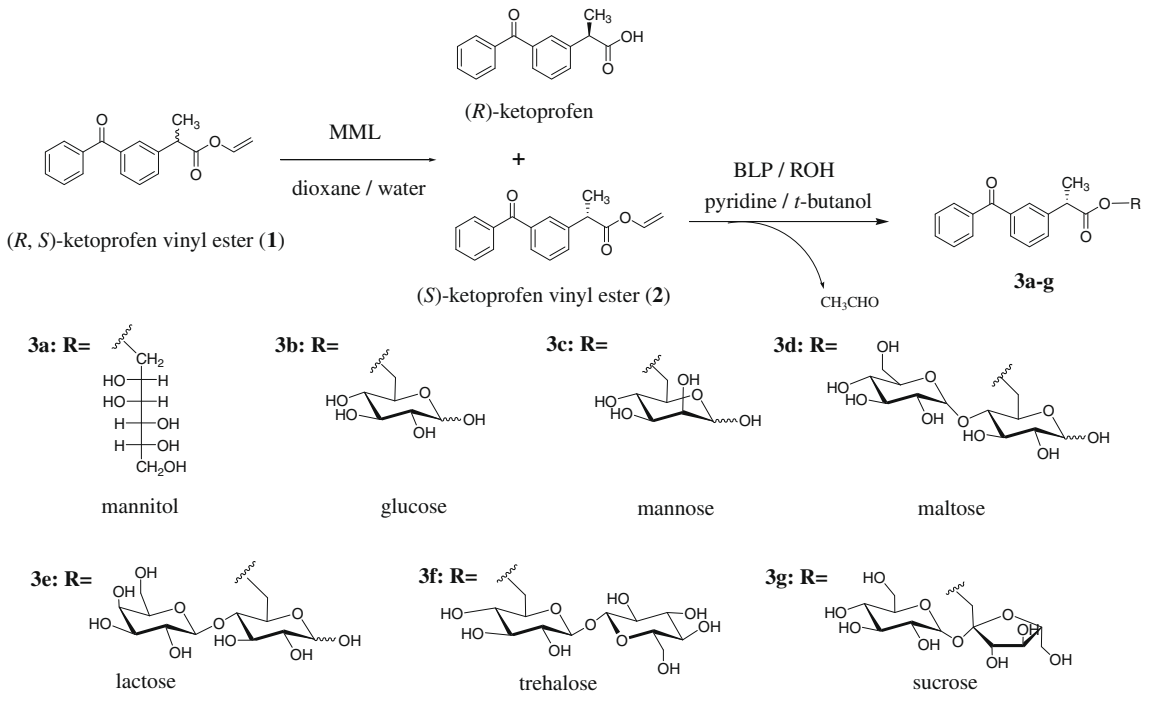
Table 1
The acylation position and yields of ketoprofen–saccharide conjugates

Entry	Compound	Acylation position	Yield[a] (%)
1	**3a**	1	73.5
2	**3b**	6	73.8
3	**3c**	6	87.4
4	**3d**	6′	37.1
5	**3e**	6′	22.8
6	**3f**	6	33.2
7	**3g**	1′	50.0

Reaction condition: 3.2 mmol (*S*)-ketoprofen vinyl ester, 0.8 mmol saccharide, 400 μL BLP, 40 mL pyridine/*t*-butanol (1:1, v/v), 50 °C, 96 h.
[a] Determined by HPLC.

2.2. Selection of biocatalysts

One of the most important parameters for enzyme-catalyzed reaction is the selection of biocatalyst. Eight commercial enzymes had been tested for their ability to catalyze the transesterification of (*S*)-ketoprofen vinyl ester with glucose in pyridine, which is widely used in the synthesis of sugar esters due to its good solubility for sugar. The results were listed in Table 2. It clearly shows that no product can be detected without enzyme participation. The best result was obtained with protease from *Bacillus licheniformis* (BLP). Lipase from *Candida antarctica* (CAL-B), protease from *Bacillus sp.*, and lipase from *Candida rugosa* also exhibited moderate activities (Table 2, entries 3–5), while other proteases and lipases showed



Scheme 1. Enzymatic synthesis of ketoprofen–saccharide conjugates.

Table 2
Effect of enzyme sources on the transesterification reaction

Entry	Enzyme	Yield[a] (%)
1	Protease from *Bacillus licheniformis*	17.2
2	Protease from *Bacillus subtilis*	Trace
3	Protease from *Bacillus* sp.	12.1
4	Lipase from *Candida rugosa*	12.0
5	Lipase from acrylic resin from *Candida antarctica*	13.0
6	Lipase from porcine pancreas	10.0
7	Lipozyme® immobilized from *Mucor miehei*	8.0
8	Amano lipase M from *Mucor javanicus*	9.0
9	No enzyme	0

Reaction conditions: 0.16 mmol (*S*)-ketoprofen vinyl ester, 0.04 mmol glucose, 20 mg (μL) enzyme, 2 mL pyridine, 50 °C, 72 h.
[a] Determined by HPLC.

quite low catalytic activities for the reaction. Thus, we chose BLP for the further investigation of the transesterification of ketoprofen vinyl ester with other mono- and di-saccharides.

2.3. Effect of reaction media

Enzymatic synthesis of sugar ester is limited by the fact that the activities of biocatalysts may obviously decrease in polar solvents while the sugar is soluble. Owing to the solubility of saccharides, polar solvents such as DMF, DMSO or pyridine usually are used in the enzyme-catalyzed acylation of sugars.[24,25] Tertiary alcohol or mixtures solvents as reaction media have also been reported.[26–28] Since BLP showed the best activity for the transesterification of ketoprofen vinyl ester with glucose, it was selected as the biocatalyst for further optimization study to improve the yield.

Firstly, four pure solvents (DMF, DMSO, pyridine and *t*-butanol), which were commonly used in the sugar esterification, were investigated for the transesterifications of glucose with (*S*)-ketoprofen vinyl ester (Table 3, entries 1–4). The reaction carried out in pyridine or *t*-butanol attained considerable yields, while almost no product was detected in DMF or DMSO. Therefore we considered using binary mixtures of solvent as reaction media in order to improve the yield of product. The best result was obtained in the mixture of equal volume pyridine and *t*-butanol (Table 3, entry 7) and the yield of 6-*O*-ketoprofen glucoside reached 70% after 72 h. In addition, different ratios of pyridine to *t*-butanol (from 1/3 to 3/1) were examined by the catalysis of BLP at 50 °C. Obviously, different ratios of pyridine to *t*-butanol (Table 3, entries 7–11) obtained the better yields than single solvent, and the highest yield was reached at a ratio of 1/1 up to 70%.

Table 3
Effect of reaction media on the yield of 6-*O*-ketoprofen glucoside

Entry	Solvents	Yield[a] (%)
1	Pyridine	17.2
2	*t*-Butanol	10.1
3	DMF	4.4
4	DMSO	0
5	DMF/*t*-butanol (1:1, v/v)	16.9
6	Pyridine/DMF (1:1, v/v)	Trace
7	Pyridine/*t*-butanol (1:1, v/v)	70.0
8	Pyridine/*t*-butanol (3:1, v/v)	46.2
9	Pyridine/*t*-butanol (2:1, v/v)	47.4
10	Pyridine/*t*-butanol (1:2, v/v)	50.0
11	Pyridine/*t*-butanol (1:3, v/v)	38.3

Reaction conditions: 0.16 mmol (*S*)-ketoprofen vinyl ester, 0.04 mmol glucose, 20 μL BLP, 2 mL solvent, 50 °C, 72 h.
[a] Determined by HPLC.

2.4. Time course of acylation of saccharides

The time course of the transesterification of ketoprofen vinyl ester with different saccharides under the optimal condition was shown in Figure 1. After 72 h the yield reached the plateau and ketoprofen mannoside could be obtained with the highest yield (87.4%). Obviously, mono-saccharides esters (**3a**, **3b** and **3c**) were obtained with higher yields than di-saccharides esters (**3d**, **3e**, **3f** and **3g**). Thus, the optimal reaction time is 72 h.

2.5. Partition coefficient

Octanol–water partition coefficient (log*P*) is one of the essential physicochemical parameters for the estimation bioavailability of a prodrug molecule. Thus, log*P* of compound **3a**–**g** were determined and compared to that of the parent drug (Table 4). The results indicated that these ketoprofen–saccharide conjugates exhibited a lower partition coefficient than ketoprofen, which implied their water solubility was increased. And ketoprofen–disaccharide conjugates have better water solubility than ketoprofen–monosaccharide conjugates. The improvement of aqueous solubility may facilitate biomembrane transport and make ketoprofen prodrugs process higher bioavailability.

Both the CLOGP and molinspiration programs are unable to distinguish between stereoisomers, such as ketoprofen glucoside and ketoprofen maltoside. The difference of experimental partition coefficients between stereoisomers of ketoprofen–saccharide conjugates clearly indicated that spatial structure of compound plays an important role in the physicochemical properties of these compounds.

2.6. Chemical hydrolysis of prodrug

An essential prerequisite for successful prodrug is that prodrug reconversion into the parent drug occurs in vivo. The designed esters should undergo enzymatic hydrolysis to release the parent drug after entering circulation. Different models have been developed to assess the susceptibility of ester prodrugs in undergoing bioconversion in vivo. In this experiment, the ketoprofen maltoside (**3d**) and glucoside (**3b**) were hydrolysis in the phosphate buffer at pH 7.4. As shown in Figure 2, the release amount of (*S*)-ketoprofen is 47.5% from maltose ester and 37.5% from glucose ester within 24 h, almost 90% of (*S*)-ketoprofen can be released within 7 days.

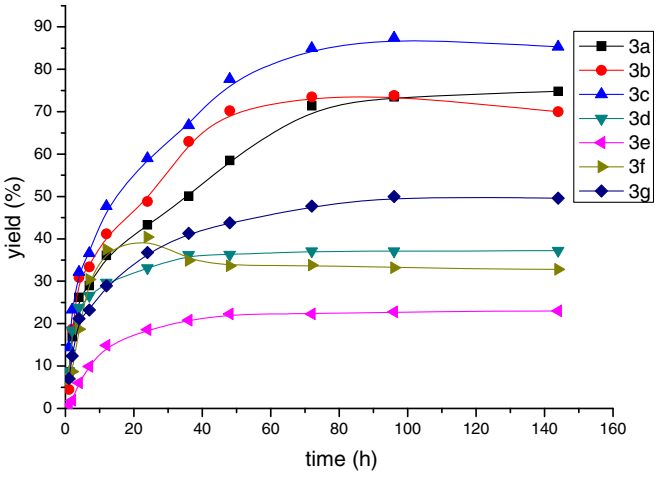


Figure 1. Time course of the saccharides with (*S*)-ketoprofen vinyl ester catalyzed by BLP. Reaction conditions: 3.2 mmol (*S*)-ketoprofen vinyl ester, 0.8 mmol saccharide, 400 μL BLP, 40 mL pyridine/*t*-butanol (1:1, v/v), 50 °C.

Table 4
Partition coefficients (log*P*) of ketoprofen and ketoprofen–saccharide conjugates

Compound	Predicted		Experimental[c]
	CLOGP[a]	Molinspiration[b]	
Ketoprofen	2.76	3.59	3.12[d]
3a	1.16	0.79	0.78
3b	1.00	1.25	1.26
3c	1.00	1.25	1.00
3d	−1.18	−0.68	−0.13
3e	−1.18	−0.68	−0.43
3f	−0.05	−0.46	−0.25
3g	−0.64	−0.83	−0.24

[a] Calculated using CLOGP software.
[b] Calculated using molinspiration (http://www.molinspiration.com/cgi-bin/properties).
[c] At pH 7.0 phosphate buffer.
[d] From in-built database of CLOGP.

Based on the data of the chemical hydrolyses, it was concluded that the derivatives could effectively release the ketoprofen.

3. Conclusions

In conclusion, a selectively enzymatic method for ketoprofen modification with different saccharides was described. Seven saccharide conjugates of ketoprofen were synthesized using the protease from *B. licheniformis* in a mixture of pyridine/*t*-butanol (1:1, v/v) at 50 °C. Ketoprofen was selectively conjugated onto the primary hydroxyl group of saccharides and afforded ester type ketoprofen–saccharide conjugates. Their lower partition coefficients show an increased hydrophilicity, and the ability to release ketoprofen in the buffer suggests that the derivatives are anticipated to be new candidates for potential high bioavailability prodrugs.

4. Experimental

4.1. Materials

Protease from *B. licheniformis*, protease from *Bacillus* sp., protease from *B. subtilis*, lipase from acrylic resin from *C. antarctica*, lipase from *C. rugosa* and lipase from porcine pancreas were purchased from Sigma. Lipozyme® immobilized from *M. miehei* was purchased from Fluka. Amano lipase M from *M. javanicus* was purchased from Amano. Racemic ketoprofen was obtained from Wuhan Gang Zheng Biology Technology Co. Ltd. (China). All organic solvents were analytical grade and were dried over 3 Å molecular sieves for 24 h prior to use.

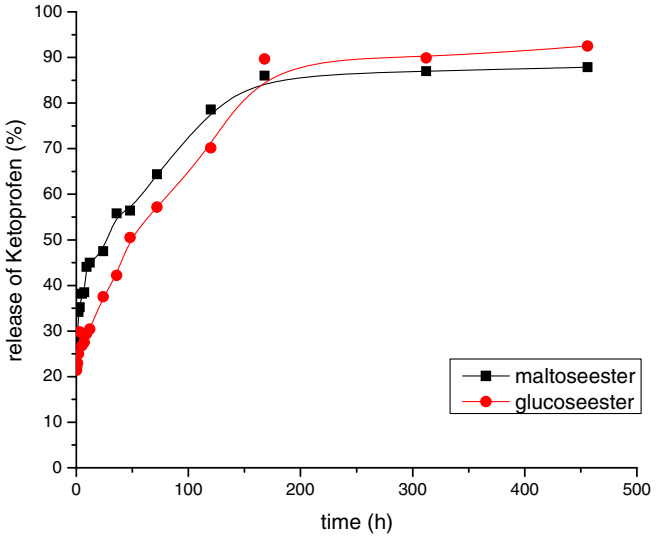


Figure 2. Hydrolysis of ketoprofen glucoside and maltoside at pH 7.4 phosphate buffer.

4.2. Analytical methods

All reactions were monitored by TLC on silica gel plates eluted with ethyl acetate/methanol/water (17/2/1, v/v). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DMX 400. Spectra were run in DMSO-d_6 and referenced to an internal TMS standard. High resolution mass spectrometry (HRMS) data were recorded on a Bruker Daltonics Bio TOF mass spectrometer. The quantitative analysis of samples was made by HPLC on a reverse phase column (Welchrom-C18, 5 μm, 4.6 × 150 mm, Welchrom) using shimadzu LC-2010A$_{HT}$ equipped with a UV detector at 254 nm. For the analysis of sugar esters of ketoprofen, methanol/water 50:50 (v/v) was used as eluent (flow rate, 0.8 mL/min).

4.3. Synthesis of (*R,S*)-ketoprofen vinyl ester (1)

Ketoprofen vinyl ester was synthesized and purified as described by Yang et al.[29] (*R,S*)-ketoprofen (0.02 mol) and $Hg(OAc)_2$ (0.22 g) were dissolved in 80 mL vinyl acetate. After stirring the mixture for 30 min at room temperature, 0.5 mL of concentrated H_2SO_4 was added dropwise and the solution was refluxed for 6 h. Then the mixture was cooled to room temperature and NaOAc (5 g) was added to quench the catalyst. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl ether 20/1, v/v).

4.4. Enzymatic preparation of (*S*)-ketoprofen vinyl ester (2)

1 g of (*R,S*)-ketoprofen vinyl ester was added to 20 mL mixture dioxane/water (97.5/2.5, v/v). The reaction was initiated by adding 200 mg Lipozyme® immobilized from *M. miehei* and the suspension was shaken at 25 °C for 4 d. The solution was filtered and concentrated. The product was purified by silica gel column chromatography (petroleum ether/ethyl ether 20/1, v/v). The enantiomer of ketoprofen and ketoprofen vinyl ester were analyzed using a chiral column (chiralcel OD-H, 0.46 cm × 25 cm, DAICEL) and were detected at 254 nm. The mobile phase was *n*-hexane/isopropanol (90/10, v/v) with a flow rate of 0.8 mL/min. (*S*)-ketoprofen vinyl ester: light yellow liquid; (yield: 90%; ee: 90%). ^{1}H NMR ($CDCl_3$): 7.81–7.46 (m, 9H, ArH), 7.26 (dd, J = 6.3 Hz, J = 14.0 Hz, 1H, –CH=CH_2), 4.58 (dd, J = 1.6 Hz, J = 6.2 Hz, 1H, –CH=CH_2), 3.87 (m, 1H, –C_6H_4CH), 1.58 (d, J = 7.2 Hz, 3H, –CH_3).

4.5. General method for the enzymatic synthesis of ketoprofen sugar conjugates

The typical reaction mixture consisted of 4 mmol (*S*)-ketoprofen vinyl ester, 1 mmol of saccharide, 200 μL of protease from *Bacillus licheniformis* and 25 mg/mL of 3 Å molecular sieves in 20 mL of reaction medium mixture of 1:1 pyridine and *t*-butanol. The mixtures were kept at 50 °C and shaken at 200 rpm. The reaction mixture was then filtered and removed the organic solvent in vacuum. All reactions were detected by TLC plates using ethyl acetate/methanol/water (17:2:1, v/v) as eluent.

4.5.1. 1-*O*-Ketoprofen mannitol ester (3a)

Yellow solid. ^{1}H NMR (DMSO-d_6): 7.76–7.52 (m, 9H, Ar–H), 4.77 (s, 1H, 2-OH), 4.41 (d, J = 4.0 Hz, 1H, 3-OH), 4.39 (m, 2H, 4-OH, 5-OH), 4.28 (d, J = 8.0 Hz, 1H, 6-OH), 4.14 (d, J = 4.0 Hz, 1H, 1-H),

3.95 (m, 2H, -CH, 6-H), 3.69 (m, 1H, 3-H), 3.66–3.53 (m, 3H, other H of mannitol), 3.48 (m, 1H, 5-H), 3.43 (m, 1H, 6-H), 1.45 (d, J = 6.8 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6): 195.6 (CO), 173.8 (CHCO), 141.2, 137.1, 137.0, 132.7, 131.9, 129.6, 128.7 (CH), 71.3 (C-5), 71.2 (C-3), 69.6 (C-4), 68.4 (C-2), 67.8 (C-1), 63.8 (C-6), 44.4 (CH), 18.7 (CH_3). HRMS (m/z): Calcd for $C_{22}H_{26}O_8$: 418.1628; Found: 418.1636.

4.5.2. 6-*O*-Ketoprofen glucoside (3b)

Yellow solid. ^{1}H NMR (DMSO-d_6): 7.75–7.52 (m, 9H, Ar–H), 6.66 (s, 0.4H, 1β-OH), 6.33 (s, 0.6H, 1α-OH), 5.04 (m, 0.6H, 1α-H), 4.95 (m, 1.2H, other β-OH), 4.78 (s, 0.5H, 3α-OH), 4.51 (d, J = 6.1 Hz, 0.6H, 2α-OH), 4.42–4.35 (m, 0.7H, 6β-H and 6′β-H), 4.28 (d, J = 8.0 Hz, 0.6H, 6α-H), 4.03 (m, 0.6H, 6′α-H), 3.94 (m, 1H, –CH), 3.78 (m, 0.6H, 5α-H), 3.55 (m, 0.7H, 3β-H and 4β-H), 3.30–2.90 (m, 4.4H, other H of glucose), 1.45 (d, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO-d_6): 196 (CO), 174 (CHCO), 141.6, 137.7, 133.3, 132.4, 130.2, 129.4, 128.6 (CH), 97.4 (C-1β), 92.8 (C-1α), 76.9 (C-3β), 75.2 (C-2β), 74.0 (C-5β), 73.3 (C-3α), 72.6 (C-2α), 71.0 (C-4α), 70.6 (C-4β), 69.7 (C-5α), 65.0 (C-6β), 64.9 (C-6α), 44.8 (CH), 19.4 (CH_3). HRMS: Calcd for $C_{22}H_{24}O_8$: 416.1471; Found: 416.1463.

4.5.3. 6-*O*-Ketoprofen mannoside (3c)

Yellow solid. ^{1}H NMR (DMSO-d_6): 7.75–7.52 (m, 9H, Ar–H), 6.35 (d, J = 4.4 Hz, 0.64H, 1α-OH), 6.22 (d, J = 8.4 Hz, 0.24H, 1β-OH), 4.89–4.85 (m, 1.7H, H-1 and 4-OH), 4.65 (d, J = 4.0 Hz, 0.3H, 3β-OH), 4.62 (d, J = 4.1 Hz, 0.7H, 3α-OH), 4.56 (d, J = 6.8 Hz, 1H, 2α-OH), 4.52 (d, J = 4.0 Hz, 0.3H, 2β-OH), 4.45 (d, J = 11.5 Hz, 0.7H, 6α-H), 4.30–4.11 (m, 0.6H, 6β-H and 6′β-H), 4.03 (m, 0.3H, 5β-H), 3.95 (m, 1.7H, 6′α-H, -CH), 3.55 (m, 0.7H, 5α-H), 3.45–3.3 (m, 2H, 2-H, 3-H), 3.29 (m, 1H, 4-H), 1.43 (d, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO-d_6): 196.1 (CO), 174.1 (CHCO), 141.6, 137.6, 137.4, 133.2, 132.4, 130.1, 129.3, 129.1, 128.9 (CH), 94.6 (C-1), 74.5 (C-5β), 74.0 (C-3β), 72.0 (C-2β), 71.8 (C-2α), 70.9 (C-5α), 70.8 (C-3α), 67.6 (C-4α), 67.3 (C-4β), 65.4 (C-6β), 65.3 (C-6α), 44.8 (CH), 19.4 (CH_3). HRMS: Calcd for $C_{22}H_{24}O_8$: 416.1471; Found: 416.1480.

4.5.4. 6′-*O*-Ketoprofen maltoside (3d)

White powder. ^{1}H NMR (DMSO-d_6): 7.76–7.55 (m, 9H, Ar–H), 6.78 (m, 0.3H, 1β-OH), 6.35 (m, 0.3H, 1α-OH), 5.62 (d, J = 4.0 Hz, 0.3H, 2α-OH), 5.52–5.47 (m, 1H, 2β-OH), 5.44–5.34 (m, 1H, 3-OH), 5.18 (m, 0.5H, 1α-H), 5.03 (m, 1H, 4′-OH), 4.97 (m, 1H, 1′-H), 4.92 (m, 1H, 3′-OH), 4.61 (m, 0.3H, 1β-H), 4.52–4.48 (m, 1H, 6β-OH), 4.45–4.20 (m, 1H, 6α-OH), 4.14–4.08 (m, 0.5H), 4.04–3.98 (m, 1.5H, -CH), 3.76–3.59 (m, 3H, 5′α-H, 5′β-H, 5α-H, 6α-H), 3.59–3.36 (m, 4H, 3′α-H, 3′β-H, 3β-H), 3.32–3.19 (m, 3H, 4β-H, 4α-H, 2′α-H, 2′β-H, 2β-H, 2α-H), 3.10–2.93 (m, 1.5H, 4′α-H, 4′β-H), 1.45 (d, J = 6.8 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6): 196.1 (CO), 174.0 (CHCO), 141.5, 137.6, 137.4, 133.2, 132.4, 130.1, 129.4, 129.1, 128.9 (CH), 101.2 (C-1′), 97.3 (C-1β), 92.6 (C-1α), 81.4 (C-4α), 81.1 (C-4β), 77.0(C-3β), 76.7 (C-5β), 73.9 (C-3′), 73.6 (C-3α), 72.8 (C-2′), 72.6 (C-2α), 71.1 (C-5′), 70.8 (C-5α), 70.4 (C-4′), 64.9 (C-6′), 60.2 (C-6α,C-6β), 44.7 (CH), 19.2 (CH_3). HRMS: Calcd for $C_{28}H_{34}O_{13}$: 578.1999; Found: 578.2011.

4.5.5. 6′-*O*-Ketoprofen lactoside (3e)

White powder. ^{1}H NMR (DMSO-d_6): 7.75–7.54 (m, 9H, Ar–H), 6.70 (d, J = 4.0 Hz, 0.5H, 1β-OH), 6.37 (s, 0.5H, 1α-OH), 5.18 (s, 1H, 2′-OH), 4.95–4.90 (m, 2H, 1α-H, 2β-OH), 4.80 (m, 1H, 3′α-OH, 3′β-OH), 4.60 (m, 1H, 2α-OH, 6β-OH), 4.47 (m, 1H, 4α-OH, 3β-OH), 4.35–3.97 (m, 5H, -CH, other H of lactose), 3.72 (m, 2H, 5α-H), 3.64–3.58 (m, 3H, 3α-H, 6α-H, 5′α-H), 3.34 (m, 2.5H, 5′β-H, 2′-H), 3.27 (m, 2H, 2α-H, 4α-H), 3.00 (m, 0.5H, 2β-H), 1.45 (d, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO-d_6): 196.1 (CO), 174.0 (CHCO), 141.5, 137.6, 137.4, 133.2, 132.7, 132.5, 130.1, 129.3, 128.9 (CH), 104.0 (C-1′), 97.2 (C-1β), 92.5 (C-1α), 81.3 (C-4α), 80.9 (C-4β), 75.3 (C-5β), 75.1 (C-3β), 75.0 (C-2β), 73.2 (C-3′), 72.9 (C-5′), 71.7 (C-5α), 70.7 (C-3α), 70.6 (C-2′), 70.2 (C-2α), 68.7 (C-4′), 64.5 (C-6′), 60.9 (C-6α & C-6β), 44.7 (CH), 18.9 (CH_3). HRMS: Calcd for $C_{28}H_{34}O_{13}$: 578.1999; Found: 578.1998.

4.5.6. 6-*O*-Ketoprofen trehaloside (3f)

White powder. ^{1}H NMR (DMSO-d_6): 7.76–7.51 (m, 9H, Ar–H), 5.05 (s, 1H, 1′-H), 4.90 (s, 1H, 1-H), 4.83 (m, 2H), 4.77 (m, 2H), 4.69 (m, 2H), 4.35 (m, 1H), 4.33–4.04 (m, 2H), 3.96 (m, 2H, –CH, 6-H), 3.63 (s, 1H, 2-H), 3.52–3.47 (m, 4H, 4-H), 3.23 (d, J = 4.0 Hz, 2H), 3.14 (m, 2H, 4′-H), 1.43 (d, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO-d_6): 195.6 (CO), 173.3 (CHCO), 141.1, 137.1, 137.0, 132.7, 131.9, 129.6, 128.7, 128.5, 128.4 (CH), 93.3 (C-1′), 93.2 (C-1), 72.8 (C-3′), 72.7 (C-3), 72.5 (C-5′), 71.5 (C-2′), 71.4 (C-2), 70.1 (C-4′), 70.1 (C-4), 69.7 (C-5), 63.5 (C-6), 60.7 (C-6′), 44.4 (CH), 18.5 (CH_3). HRMS: Calcd for $C_{28}H_{34}O_{13}$: 578.1999; Found: 578.1982.

4.5.7. 1′-*O*-Ketoprofen sucroside (3g)

White powder. ^{1}H NMR (DMSO-d_6): 7.76–7.51 (m, 9H, Ar–H), 5.27 (d, J = 4.0 Hz, 1H, 1-H), 5.15 (s, 1H, 3-OH), 4.82–4.76 (m, 4H), 4.39 (s, 2H, 3′-OH), 4.28–4.20 (m, 1H), 4.04–3.89 (m, 2H, –CH, 3′-H), 3.80–3.71 (m, 1.5H, 6-H), 3.67–3.63 (m, 2H, 1′-H), 3.55–3.51 (m, 3H, 4′β-H, 5-H), 3.44 (m, 2.5H, 4-H, 3-H), 3.16–3.10 (m, 2H), 1.46 (d, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO-d_6): 196.2 (CO), 173.4 (CHCO), 141.4, 137.6, 137.4, 133.2, 132.4, 130.1, 129.3, 129.0, 128.9 (CH), 102.5 (C-2′), 92.5 (C-1), 83.1 (C-5′), 77.0 (C-3′), 73.5 (C-4′), 73.4 (C-3), 73.2 (C-5), 71.8 (C-2), 70.2 (C-4), 63.1 (C-1′), 62.3 (C-6′), 60.9 (C-6), 44.8 (CH), 18.8 (CH_3). HRMS: Calcd for $C_{28}H_{34}O_{13}$: 578.1999; Found: 578.1983.

4.6. *n*-Octanol/water partition coefficient (log*P*)

Partition coefficient was determined in *n*-octanol–water system using the shake flask method. Equal volumes of *n*-octanol and phosphate buffer (pH 7.0) were saturated for a period of 24 h. Solutions of the prodrugs were prepared in the pre-saturated *n*-octanol phase, and it was shaken with the phosphate buffer at 30 °C for 1 h in a 10 mL tube. Two layers were separated by centrifugation. After the two phases were separated, the concentrations of prodrugs in octanol phase (C_0) and the concentrations of prodrugs in water phase (C_1) are detected by HPLC. The partition coefficients (log$P$) can be calculated with following equation:

$$P = C_0V_0/C_1V_1$$

V_0 is the volume of octanol phase and V_1 is the volume of water phase.

CLOGP and molinspiration software were used to predict the partition coefficients for these prodrugs.

4.7. Chemical hydrolysis study in aqueous buffers

The chemical hydrolysis rates of ester prodrugs **3b** and **3d** were determined by monitoring the production of ketoprofen with HPLC methods described above. The chemical hydrolysis of the ester prodrugs was studied at pH 7.4 using phosphate buffer. 15 mg of sample for hydrolysis was dissolved in 70 mL of buffer solution, which were shaken at 37 °C. At appropriate time intervals, samples were withdrawn and diluted with mobile phase for direct analysis by HPLC.

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