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Preliminary communication

Pharmacological activity and hydrolysis behavior of novel ibuprofen glucopyranoside conjugates

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Abstract

Novel ester prodrugs (II, III and IV) of ibuprofen (I) were synthesized using α-methyl, ethyl and propyl glucopyranoside as promoieties and tested for their anti-inflammatory, analgesic and ulcerogenic activities. Study of their chemical hydrolysis in aqueous buffer (pH 3.0–10.0) showed that these compounds acted as true prodrugs of ibuprofen, giving the ibuprofen and alkyl glucopyranoside. Additionally, all the derivatives studied did cleave rapidly inside the biological system and on oral administration did elicit a pharmacological profile quite similar to that of ibuprofen, but, unlike this drug, they displayed reduced gastric ulceration. In conclusion, these alkyl glucopyranoside esters have promising properties as prodrugs for oral delivery of ibuprofen.

Keywords: Ester; Glucopyranoside; Ibuprofen; Non-steroidal anti-inflammatory drugs (NSAIDs); Prodrug; Ulceration

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (I) are widely used in the treatment of pain and inflammation in many conditions, including osteoarthritis (OA) and rheumatoid arthritis (RA) [1–3]. However, the problem of side effects after long-term administration of these drugs, such as irritation and ulceration of the gastrointestinal (GI) mucosa, has arisen in clinical trials [4,5]. These gastroenteropathies are generally believed to be resulted from the direct contact effect, which can be attributed to the combination of local irritation produced by the free carboxylic group in the molecular structure and by local blockage of prostaglandin biosynthesis in the GI tract [6–10]. Therefore, the development of new NSAIDs without these side effects has long been awaited.

The utilization of prodrugs to temporarily mask the acidic group of NSAIDs has been proposed as an approach to reduce or suppress the GI toxicity due to the direct contact effect and also increase their absorption values [11]. Ester prodrugs of ibuprofen have been synthesized with this very aim [12–14]. Therefore, these prodrugs will possess the potential to avoid ibuprofen-mediated GI damage, while maintaining their efficacy via the systemic action of ibuprofen. It has been demonstrated that some glycoamide esters represented potentially useful derivatives in overcoming GI injury associated with long-term administration of ibuprofen [14].

A pre-requisite for the prodrug effectiveness is the availability of chemical derivatives which satisfy the prodrug requirements, such as an appropriate aqueous solubility and lipophilicity [14]. However, most of the prodrugs of NSAIDs reported in literatures have poor or limited hydrosolubility [11,15]. The amphiphilic structure of glucopyranoside makes it particularly efficient for this very aim. Moreover, the hydroxyl group in the structure can serve as a handle for the prodrug formation of ibuprofen with glucopyranoside by means of lipase-catalyzed reactions [16]. It has shown that the glucopyranoside derivatives of ibuprofen exhibited lower toxicity than ibuprofen on GI mucosa [17,18]. However, most of the conventional chemical regioselective modifications of carbohydrates containing multiple hydroxyl groups of similar reactivity need the process of protection/deprotection and rigorous reaction conditions [19,20]. Thus, the use of enzyme in non-aqueous media has become an important approach to synthesize glucopyranoside derivatives due to simple feasibility and high selectivity [21,22]. These observations prompted us to enzymatically prepare and to evaluate glucopyranoside esters of ibuprofen as potential prodrugs.

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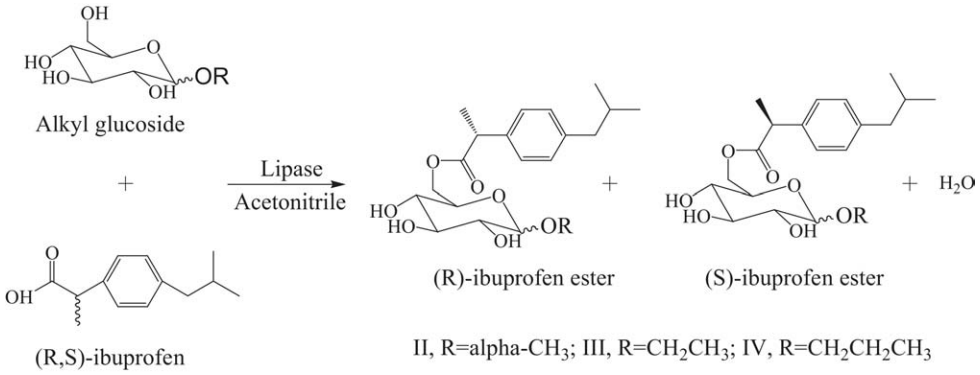

Scheme 1. Route of synthesis of ibuprofen glucopyranside derivative.

In this paper, in order to evaluate the potential of using alkyl glucopyranoside as promoiety in ibuprofen prodrugs design, three new ester prodrugs (II, III and IV) were synthesized in one step by directly coupling ibuprofen with appropriate alkyl glucopyranoside using immobilized lipase as a biocatalyst. These novel derivatives synthesized have been characterized to evaluate their hydrolysis stabilities in both in vitro and in vivo systems, as well as their anti-inflammatory and analgesic activity. Furthermore, we assessed the gastric ulcerogenic activity of the novel derivatives of ibuprofen.

2. Chemistry

The lipase-catalyzed synthesis of ibuprofen alkyl glucopyranoside esters was performed as described earlier with minor modification [23]. Ibuprofen α-methyl glucopyranoside ester (II), ibuprofen ethyl glucopyranoside ester (III) and ibuprofen propyl glucopyranoside ester (IV) were synthesized in one step by directly coupling racemic ibuprofen with appropriate alkyl glucopyranoside using immobilized lipase Novozym 435 in acetonitrile. The synthesis of ibuprofen alkyl glucopyranoside esters is illustrated in Scheme 1. The structures of the synthesized compounds were established by mass spectrometry (MS) and ^{1}H NMR. The enantiomeric purity of the product was analyzed by automatic polarimeter.

3. Biological investigations

The anti-inflammatory and anti-nociceptive activity of the compounds was performed on male ICR mice by Xu et al. [24] method, and gastric ulcerogenic activity was carried out on female Sprague–Dawley rats by Khan and Khan [17] method. In vitro hydrolysis studies of prodrugs were performed by HPLC method and in vivo hydrolysis were carried out on male Sprague–Dawley rats by HPLC.

4. Results and discussion

4.1. Hydrolysis studies

It has been reported that the essential pre-requisite for success in the use of prodrugs is that the masked compounds should be acid-stable to prevent the direct contact effects with the gastric mucosa as well as the local inhibition of the prostaglandins [25]. Therefore, pH values ranging from 3.0 to 10.0 were selected to mimic the appropriate clinical range. Furthermore, the chemical stability studies were carried out in aqueous buffer in order to indicate whether the prodrugs hydrolyze in aqueous medium and to what extent or not. The chemical degradation of ester prodrugs of ibuprofen followed first-order kinetics, and quantitatively converted to ibuprofen as revealed by HPLC analysis of the reaction solutions. Values of the rate parameter k_{obs} for hydrolysis of the various ester prodrugs at different pH and 37 °C are listed in Table 1 along with the half-lives of hydrolysis.

In the aqueous buffer solution of pH 7.4, all glucopyranoside ester prodrugs (II, III and IV) showed a high chemical stability, with half-lives ($t_{1/2}$) ranging from 13 to 41 days (Table 1). Furthermore, the degradation of all ester prodrugs (II, III and IV) indicted a substantially higher chemical stability in an aqueous buffer solution of down to pH 3.0 than pH 7.4. Percent release of ibuprofen following 6 days incubation in an aqueous buffer solution of pH ranged from 3.0 to 7.0 was negligible (< 5%) for all the three ester prodrugs. The acid stability implied that the compounds passed unhydrolyzed through the stomach on oral administration. In contrast, the ester prodrugs were found to be only an alkaline-catalyzed hydrolysis to give the ibuprofen and the alkyl glucopyranoside. Moreover, an increased tendency of enhanced rate of hydrolysis with increased the straight alkyl chain length of alkyl glucopyranoside is observed from methyl to propyl glucopyranoside ester at all pH values examined. For example, half-lives of three ibuprofen alkyl glucopyranoside esters (II, III and IV) ranged from 27.75 to 9.46 h at pH 10.0. From the above study, therefore, the prepared glucopyranoside esters of ibuprofen fulfilled

Table 1
Kinetic data for the hydrolysis of ibuprofen alkyl glucopyranoside esters at different pH and 37 °C

pH	II		III		IV	
	K_{obs} (h^{-1})	$t_{1/2}$ (h)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	K_{obs} (h^{-1})	$t_{1/2}$ (h)
7.4	0.69×10^{-3}	1010	1.43×10^{-3}	485	2.17×10^{-3}	320
8.0	1.78×10^{-3}	390	3.02×10^{-3}	229	4.00×10^{-3}	173
9.0	2.61×10^{-3}	266	3.61×10^{-3}	192	4.56×10^{-3}	152
10.0	2.50×10^{-2}	27.75	7.20×10^{-2}	9.62	7.33×10^{-2}	9.46

the requirement of prodrugs, since they showed good stability at acidic pH.

The hydrolysis studies were also studied in conscious rats. Although all ester prodrugs (II, III and IV) were quantitatively hydrolyzed to the parent drug ibuprofen by enzymatic conversion in vivo, their properties of in vivo ibuprofen releasing are dramatically different (Fig. 1). The C_{max} of ibuprofen was 65.7 mg l^{-1} with an $(AUC)_{0-t}$ of 148.4, whereas C_{max} of II was 59.6 mg l^{-1}, III was 35.8 mg l^{-1} and IV was 106.7 mg l^{-1} with an $(AUC)_{0-t}$ of 158.1, 153.0 and 238.5, respectively. The plasma levels of ibuprofen released from both ibuprofen (I) and ibuprofen α-methyl glucopyranoside ester (II) appeared to be very similar. However, higher plasma concentrations of ibuprofen were observed in rats treated with ibuprofen propyl glucopyranoside ester (IV) compared to animals treated with ibuprofen α-methyl glucopyranoside ester (II) indicating improved bioavailability. In contrast, the plasma levels of ibuprofen after oral administration of ibuprofen ethyl glucopyranoside ester (III) were lower than those after administration of ibuprofen α-methyl glucopyranoside ester (II). As shown in Fig. 1, ibuprofen ethyl glucopyranoside ester (III) releases ibuprofen slowly compared to ibuprofen propyl glucopyranoside ester (IV) or ibuprofen α-methyl glucopyranoside ester (II), due to the slow enzymatic conversion rate of the ester (III) to ibuprofen. Thus, ibuprofen ethyl glucopyranoside ester (III) exhibited sustained release characteristics following oral dosing to rats.

4.2. Acetic acid-induced writhing test in mice

The anti-nociceptive activity of ibuprofen and all its ester produgs (II, III and IV) was measured by their ability to inhibit acetic acid-induced writhing in mice. The vehicle control was considered to exhibit 100% writhing, and the protection afforded by the parent drug and prodrugs was calculated on a percentage basis. In nociception model, ibuprofen and all its prodrugs (II, III and IV) presented remarkable reduction of writhing number if compared to control group (Fig. 2). Ibuprofen

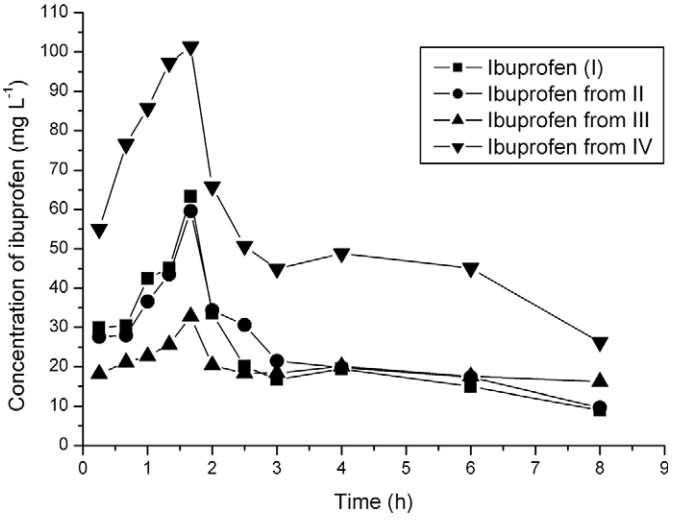

Fig. 1. Mean plasma levels of ibuprofen in rat following oral administration of the ibuprofen and its prodrugs at 200 mg kg^{-1} (molar equivalent to ibuprofen).

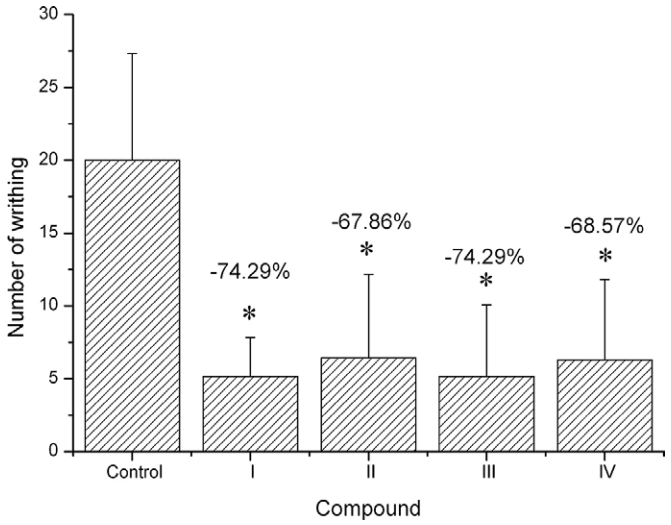

Fig. 2. Effect of ibuprofen and its prodrugs (II, III and IV) on acetic acid-induced writhing in mice. Data represent mean values (±S.D.) and percent inhibition (%) compared to the control animals. Statistical differences versus control group are indicated by asterisks ($N = 7$, one-way ANOVA, $P < 0.05$).

administrated 60 min prior to testing produced a significant decrease (74.29%) in writhing number versus control values (Fig. 2). Moreover, either ibuprofen (I) or ibuprofen ethyl glucopyranoside ester (III) were equally effective in reducing the nociceptive response at molecular equivalent quantity. In contrast, the protection of other two prodrugs II and IV was lower when compared to ibuprofen ethyl glucopyranoside ester (III); however, no appreciable difference could be observed among all prodrugs with respect to their analgesic activity (Fig. 2). The ready hydrolysis kinetics in vivo could be the right reason that all the prodrugs retained the analgesic activity profile of the parent drug.

4.3. Xylene-induced acute mice ear edema test

All the compounds were tested for their anti-inflammation in the xylene-induced mouse ear edema assay. In the control group, ear weight increased by 20.22 ± 2.61 mg ($N = 10$) at 2 h after application of xylene. In regard to ear edema, either ibuprofen or its glucopuranoside prodrugs (II, III and IV) were equally effective in reducing inflammation at molecular equivalent quantity. In mice administrated ibuprofen (I), the edema response was markedly inhibited with 45.9% reduction in edema formation compared with the control counterparts. In contrast, ibuprofen propyl glucopyranoside ester (IV) induced a higher reduction (52.67%) of the edema response than ibuprofen (I). The inhibition of edema response in ibuprofen ethyl glucopyranoside ester (III) was lower when compared to other two prodrugs II and IV; however, no significant difference was observed as the length of the straight alkyl chain length of alkyl glucopyranoside changed (Fig. 3). The anti-inflammation of all the compounds (II, III and IV) could partly rest with their behavior as prodrugs of ibuprofen (I). Indeed, as shown in Fig. 1, after 3 h of administration of the prodrugs (II, III and IV), they afforded a significant quantity of ibuprofen, although

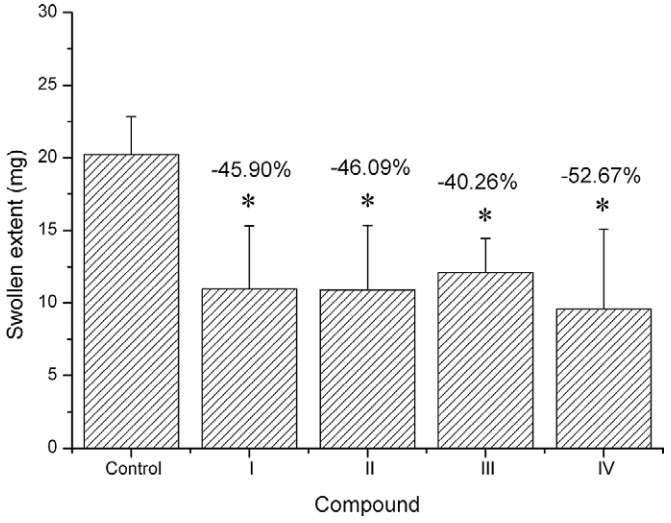

Fig. 3. Effect of ibuprofen and its prodrugs (II, III and IV) on xylene-induced ear edema in mice. Data represent mean values (±S.D.) and percent inhibition (%) compared to the control animals. Statistical differences versus control group are indicated by asterisks ($N = 10$, one-way ANOVA, $P < 0.05$).

to different degrees. These findings are particularly important for in clinical practice anti-inflammatory agents of ibuprofen.

4.4. Gastric ulcerogenic activity

The most frequently reported adverse events in ibuprofen recipients were GI disorders, although the incidence of these events was low [26]. In the present study, the ulcerogenic liability of the prepared prodrugs (II, III and IV) was tested in comparison to the parent drug ibuprofen (I) following oral administration in rats. The severities of gastric lesions were measured after the successive administration of the compounds for 4 days, and the results were expressed as the number of ulcers and ulcer index (UI).

Preliminary ulcerogenic experiments in rats revealed that administration of ibuprofen (125 mg kg^{-1} daily) over a period of 4 days resulted in a high incidence of gastric mucosa lesions (average number of ulcers was 15; mean UI was 27.12 mm). In contrast, the results of the ulcerogenic activities of compounds II, III and IV compared with ibuprofen indicated that replacement of a carboxylic residue by a glucopyranoside group could remarkably reduce the gastric ulceration of ibuprofen (Table 2). At molecular equivalent quantity, the number of ulcers in gastric mucosa was markedly smaller in animals treated with the products (II, III and IV), ranged from 4.83 to 7.00, when compared to the ibuprofen control group. In addition, the UI in animals treated with the ester prodrugs (II, III and IV) revealed values statistically smaller (average UI ranges between 7.97 and 13.34 mm), compared to that of animals treated with ibuprofen. Additionally, there was no appreciable difference in the reduction of ulceration in animals treated with the product IV in comparison to those with the other two products III and IV (Table 2). These data indicated that glucopyranoside ester prodrugs of ibuprofen protected the gastric mucosa from injury evoked by ibuprofen. In this respect, our results shed new light on the chemical requisite to design new agents of ibuprofen with reduced ulcerogenic effects in gastric mucosa.

Table 2
Ulcerogenic effect of ibuprofen and its prodrugs in rats ($N < 6$, mean ± S.D.)

Compound	Dose (mg kg^{-1})	Number of ulcer	UI (mm)
Ibuprofen	125.0	15.00 ± 6.78	27.12 ± 13.00
II	231.8	7.00 ± 3.22[a]	13.34 ± 6.31[a]
III	240.2	6.83 ± 3.66[a]	11.94 ± 6.79[a]
IV	248.8	4.83 ± 4.58[a]	7.97 ± 7.21[a]

[a] Significant difference compared to ibuprofen group. $P < 0.05$ (Student's *t*-test).

5. Conclusion

The preliminary biological and pharmacological evaluation of the alkyl glucopyranoside esters of ibuprofen reported here indicated that esterifcation of the free carboxylic group in ibuprofen suppressed its gastric ulceration without adversely affecting its anti-inflammatory and analgesic activity. In addition, all ester prodrugs displayed good resistance to acid-catalyzed hydrolysis. In conclusion, on the basis of the results obtained in this work, alklyl glucopyranoside appears to be suitable promoiety for ibuprofen prodrugs design.

6. Materials and methods

6.1. Materials

Novozym 435 (lipase B from *Candida antarctica*, a non-specific lipase immobilized on a macroporous acylic resin with a specific activity 10,000 propyl laurate unit (PLU) g^{-1} and water content 1–2% (W/W), PLU is based on a reaction between propyl alcohol and lauric acid) was supplied by Novo Nordisk Bioindustrials Inc.

Methyl α-D-glucopyranoside was obtained from Sigma, and racemic ibuprofen was obtained from Shandong Xinhua Pharmaceutical Co., Ltd. (China). (*S*)-Ibuprofen (purity, > 99.9%) was obtained from Hubei Biocause Pharmaceutial Co., Ltd. (China). Ethyl and propyl glucopyranoside were produced and purified in our lab. All organic solvents and reagents for the preparation of the buffers were obtained from commercial suppliers (Shanghai Chemical Company (China)) and were analytical grade or purer.

Sprague–Dawley rats and male ICR mice (18–25 g) were purchased from Shanghai Xipuer-Bikai Laboratory Animal Co., Ltd. (China). They were housed in groups of eight or 10 animals in plastic cages at 20–25 °C and maintained on a standard pellet diet with free access to water.

6.2. General procedures for synthesis of ibuprofen ester prodrugs

The lipase-catalyzed synthesis of ibuprofen alkyl glucopyranoside esters (II, III and IV) was performed as described earlier in [23]. Esterification was performed by mixing ibuprofen (1 mmol) with the corresponding alkyl glucopyranoside (0.5 mmol) in a stopped glass bottle as shown in Scheme 1.

To this reaction mixture was added 20.0 ml of acetonitrile. The mixtures were incubated in a thermoconstanter orbital shaker at 200 rpm and 50 °C. Reactions were initiated by addition of 0.2 g Novozym 435 lipase and terminated after 144 h. At the end of the reaction, the mixture was extracted with dichloromethane by stirring at ambient temperature for 20 min. Hereby, the immobilized lipase was separated by flotation from the reaction mixture allowing an easy recovering of the biocatalyst. After removal of organic solvent in vacuum, the crude products were purified by silica gel chromatography (ethyl acetate/methanol, 10:1, V/V).

After purification of the synthesized products by silica gel liquid chromatography, identity of products was confirmed by MS using Perkin–Elmer SCIEX API 100 for ESI measurements and by ^{1}H NMR in $CDCl_3$ using Brücker AM 500 spectrometer.

Optical rotations were measured at 589 nm (sodium line) and 25 °C in a WZZ-1S automatic polarimeter (Shanghai Precision Scientific Instrument Co. Ltd., China).

6.2.1. Methyl 6-O-(2′-(4′-isobutylphenyl) propionyl) D-α-glucopyranoside (II)

Synthesis was performed as described above starting from 1 mmol of racemic ibuprofen and 0.5 mmol of methyl α-D-glucopyranoside with yield of 65.1 mg (34.1%), white solid, the spectroscopic data were reported earlier in [23].

6.2.2. Ethyl 6-O-(2′-(4′-isobutylphenyl) propionyl) D-glucopyranoside (III)

Synthesis was performed as described above starting from 1 mmol of racemic ibuprofen and 0.5 mmol of ethyl glucopyranoside (mixtures of α- and β-ethyl glucopyranoside) with yield of 83.6 mg (42.2%), white solid, R_f: 0.69 (chloroform/methanol/water, 65:15:2); ^{1}H NMR data are as follows (500 MHz, $CDCl_3$): δ 7.19 (d, 2H, $J = 8.05$ Hz, H-2′, H-6′), 7.07 (d, 2H, $J = 7.94$ Hz, H-3′, H-5′), 4.74 (d, 0.67H, $J = 3.76$ Hz, H-1, β-H), 4.38 (d, 0.33H, H-1, α-H), 4.14–4.31 (3H, H-6a, H-6b, H-5), 3.73 (q, 1H, $J = 7.13$, ROOC-C$\underline{H}$(CH_3)-R′), 3.58 (3H, OH-2,3,4), 3.40 (2H, H-2, H-4), 3.22 (2H, RO-C$\underline{H}_2$$CH_3$), 3.07 (t, 1H, $J = 9.45$ Hz, H-3), 2.38 (d, 2H, $J = 7.15$ Hz, C$\underline{H}_2$$CH(CH_3)_2$), 1.78 (m, 1H, $J = 6.74$ Hz, CH_2C$\underline{H}$(CH_3)$_2$), 1.43 (d, 3H, $J = 7.10$ Hz, ROOC-CH(C$\underline{H}_3$)-R′), 1.22 (m, 3H, RO-CH_2C$\underline{H}_3$), 0.83 (d, 6H, $J = 6.60$ Hz, CH_2CH(C$\underline{H}_3$)$_2$). MS: m/z = 397.1 (M + H).

After acylation of ethyl glucopyranoside with ibuprofen, optical rotation of residual ibuprofen was analyzed. The specific rotations ($[\alpha]_D^{25}$ ($c = 1.0$ in ethanol)) of residual ibuprofen and (*S*)-ibuprofen were +20.6° and +56.0°, respectively. Then, the optical purity of product was 36.8%, and the ratio of *R*-enantiomer and *S*-enantiomer in the product was 2.16.

6.2.3. Propyl 6-O-(2′-(4′-isobutylphenyl) propionyl) D-glucopyranoside (IV)

Synthesis was performed as described above starting from 1 mmol of racemic ibuprofen and 0.5 mmol of propyl glucopyranoside (mixtures of α- and β-propyl glucopyranoside) with yield of 54.0 mg (26.3%), white solid, R_f: 0.71 (chloroform/methanol/water, 65:15:2); ^{1}H NMR data are as follows (500 MHz, $CDCl_3$): δ 7.19 (d, 2H, $J = 8.01$ Hz, H-2′, H-6′), 7.08 (d, 2H, $J = 7.94$ Hz, H-3′, H-5′), 4.77 (d, 1H, $J = 3.72$ Hz, H-1, β-H), 4.27 (3H, H-6a, H-6b, H-5), 3.70 (q, 1H, ROOC-C$\underline{H}$(CH_3)-R′), 3.70 (3H, OH-2,3,4), 3.39 (5H, H-2, H-3, H-4, RO-C$\underline{H}_2$$CH_2CH_3$), 2.40 (d, 2H, $J = 7.08$ Hz, C$\underline{H}$$_2$$CH(CH_3)_2$), 1.83 (m, 1H, $J = 6.75$ Hz, CH_2C$\underline{H}$(CH_3)$_2$), 1.48 (3H, $J = 6.96$ Hz, ROOC-CH(C$\underline{H}_3$)-R′), 0.88 (9H, CH_2CH(C$\underline{H}$$_3$)$_2$, RO-$CH_2CH_2C\underline{H}_3$). MS: m/z = 843.4 (2M + Na).

After acylation of propyl glucopyranoside with ibuprofen, optical rotation of residual ibuprofen was analyzed. The specific rotations ($[\alpha]_D^{25}$ ($c = 1.0$ in ethanol)) of residual ibuprofen and (*S*)-ibuprofen were +19.4° and +56.0°, respectively. Then, the optical purity of product was 34.6%, and the ratio of *R*-enantiomer and *S*-enantiomer in the product was 2.06.

6.3. HPLC procedure for the analysis of ester prodrugs of ibuprofen

Quantitative analysis of glucopyranoside ester prodrugs and ibuprofen was determined by HPLC on a reversed-phase column (Zorbax SB-C18, 5 um, 4.6 × 250 mm, Agilent, USA) using Agilent 1100 series (USA) equipped with a DAD detector at 254 nm. Elution was conducted with mixture of 20 mM sodium acetate solution (pH 2.5) and acetonitrile at a flow rate of 1.0 ml min^{-1} and ambient temperature. The compositions of the eluent were adjusted for each compound in order to provide an appropriate retention time and separation of ester prodrugs and ibuprofen. The volume of the injected sample was 10 μl. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. The concentration of ibuprofen was determined from the peak area based on calibration curve prepared using standard ibuprofen, as well as ibuprofen glucopyranoside esters solution in organic medium.

6.4. Hydrolysis study in aqueous buffer

The chemical hydrolysis of the ester prodrugs was studied at pH 3.0–10.0 using HCl, acetate, phosphate, and NaOH buffer. The total buffer concentration was 20 mM and constant ionic strength of 0.5 M for each sample was maintained by adding KCl. Samples for hydrolysis were prepared by adding the compound to 20 mM buffer solution. The solutions were sealed in screw-capped glass vials and then placed into a thermostatically controlled water bath at 37 °C. At appropriate intervals, samples were taken and assayed for the presence of ester and hydrolysis product by HPLC according to the previously described method. Pseudo first-order rate constants (K_{obs}) for the individual reactions were determined from the slope of linear plots of the logarithm of residual ester concentration against time. The corresponding half-life ($t_{1/2}$) was then obtained from the equation: $t_{1/2} = 0.693/k_{obs}$.

6.5. In vivo hydrolysis studies

The male Sprague–Dawley rats were used to compare the bioavailability of prodrugs with that of ibuprofen following oral administration. Rats weighing between 250 and 300 g were divided into four groups of three animals each. The animals were fasted overnight prior to experimentation. The dose of ibuprofen was 200 mg kg^{-1} and the ester prodrugs were given in a dose equivalent to 200 mg kg^{-1} of ibuprofen. Drug suspensions in 0.5% sodium carboxy methyl cellulose (CMC-Na) solution were given orally. At the specified intervals following dosing: 15, 40, 60, 80 and 100 min and 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 h, blood samples were withdrawn from cephalic vein into heparinized tubes and immediately centrifuged for 10 min at 5000 rpm to separate plasma. To 200 μl of plasma, 200 μl of acetonitrile was added and vortexed for 2 min. The precipitated proteins were separated by centrifugation at 1.2×10^4 rpm for 10 min. The clear supernatant obtained was analyzed for remaining ester derivative and formed ibuprofen using the HPLC procedures described above after filtering through 0.45 μm Millipore filter.

6.6. Pharmacological evaluation

6.6.1. Analgesic activity

Analgesic activity was determined in mice by acetic acid-induced writhing method. The method of Xu et al. [24] was used with minor modifications. The male ICR mice were divided in groups with seven animals in each group and intra-gastrically (i.g.) administrated 40 mg kg^{-1} of body weight of ibuprofen or molecular equivalent quantity of the ester as suspension/emulsion in 0.5% CMC-Na solution. Mice in control group received an equal volume of vehicle (0.5% CMC-Na solution). One hour later, 0.75% acetic acid (in physiologic saline solution) was intra-peritoneally (i.p.) administered. Each mouse was then placed in an individual clear plastic observational chamber, and the total number of writhing made by each mouse was counted for 15 min after acetic acid administration. The decrease in number of writhing was expressed as percentage protection by test compounds with reference to control group.

6.6.2. Anti-inflammatory activity

The anti-inflammation of ibuprofen and its prodrugs on topical acute edema was assessed using xylene-induced ear edema in mice. The method of Xu et al. [24] was used with minor modifications. The male ICR mice were divided in groups with 10 animals in each group and administrated orally 40 mg kg^{-1} of body weight of ibuprofen or equimolar dose of the ester as suspension/emulsion in 0.5% CMC-Na solution. Mice in control group received an equal volume of vehicle (0.5% CMC-Na solution). One hour later, 25 μl of xylene was applied to the anterior surface of the right ear. The left ear was left untreated. Two hours after xylene application, mice were sacrificed and both ears were removed. Circular discs were taken, using a cork borer with a diameter of 7 mm, and weighed. The difference in the weight of discs from the right treated and left untreated ears was calculated and used as a measure of edema. The level of inhibition of edema was calculated using the following formula, Inhibition (%) = 100[1 – (Et/Ec)], where Et and Ec represent average edema of the treated group and the control group, respectively.

6.7. Gastric ulcerogenic activity

Subacute GI toxicity studies were done using the method reported by Khan and Khan [17] with minor modifications. The female Sprague–Dawley rats (150–200 g) were divided in groups with six animals in each group and given orally 125 mg kg^{-1} of ibuprofen or molecular equivalent quantity of the ester as suspension/emulsion in 0.5% CMC-Na solution. Control group was administered only 0.5% CMC-Na solution. The volume given was 10 ml kg^{-1}. Rats were given orally once in a day for 4 successive days. The animals were fasted for 8 h prior to dosing and for 4 h post dosing. Food was available at all other times, free access to water was provided throughout the experiments. Four hours after the last dose, the animals were sacrificed by spinal decapitation and the stomach was extracted and dipped in 10% formalin for about 15 min and then cut out along its greater curvature. The number and the length of ulcers were detected by means of a 2 × 2 binocular magnifier. All ulcer > 0.5 mm were counted and recorded as average number of ulcers per compound. The severity of the gastric lesions was measured along its greatest length (< 1 mm = rating of 1, 1–/2 mm = rating of 2, > 2 mm = /rating according to their length in mm). The overall total of length was designated as the 'ulcer index' (UI).

6.8. Statistical analyses

Data are expressed as mean ± S.D. For all tests, differences with a probability of $P < 0.05$ were considered to be significant.

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