Synthesis of the prodrug ibuprofen \(\beta\)-D-glucopyranoside and its biological evaluation as a better moiety than the parent drug

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\(\beta\)-D-Glucopyranosyl derivative 5 of ibuprofen 1 has been synthesised by condensing 2,3,4,6-tetraacetyl glucopyranosyl bromide with sodium salt of ibuprofen by modified Koenig’s Knorr reaction in presence of phase transfer catalyst and subsequent deacetylation by 0.5% sodium methoxide. It has been tested for anti-inflammatory and analgesic activity and gastrointestinal toxicity. The results indicate a remarkable improvement in anti-inflammatory activity with almost negligible number of gastrointestinal ulcers as compared to the parent drug ibuprofen. The results of analgesic activity are also excellent and better than the parent drug. The in-vivo hydrolysis of 5 is also determined in rabbit by HPLC. Partition coefficient of 5 is determined in n-octanol-buffer system and \(LD_{50}\) in mice.

The present studies were undertaken in order to minimize the ulcerogenic potential of ibuprofen and render it water soluble properties through glucosidation for better absorption and sustained release. Non-steroidal anti-inflammatory drugs (NSAIDs) are associated with mild to life threatening states such as gastrointestinal ulceration and haemorrhage. This condition is manifested due to the inhibition of prostaglandin synthesis. Endogenous prostaglandins are known to have a cytoprotective action on the gastric mucosa. Prostaglandins help regulate acid secretion and maintain mucosal integrity against stress, a variety of chemicals and thermal injury.

It is a well accepted fact that gastrointestinal lesions produced by NSAIDs are due to two different mechanisms: (a) direct contact with gastric mucosa through oral dose and (b) systemic effect which may be manifested by after intravenous dosing.

Cioli et al. examined the importance of the direct contact effect in the gastrointestinal toxicity of ibuprofen 1 in rats. The study showed that 1 exerted greater toxicity on the stomach and the intestine by oral route than by intravenous route with similar anti-inflammatory action for both the routes.

Although considerable research has been directed at designing prodrugs of NSAIDs with reduced gastrointestinal toxicity, however, none of the approaches so far have resulted in an ideal prodrug. Several of these approaches show poor aqueous solubility as well as poor absorption.

Temporarily masking the acidic group of NSAIDs with a view to decrease the gastrointestinal toxicity due to direct injury has been postulated. The purpose of this study was to mask the free acidic group by synthesising its water soluble glucoside derivative 5 and evaluate it’s anti-inflammatory activity, analgesic activity and gastrointestinal toxicity and determine its hydrolysis in a biological system. Ibuprofen tetraacetyl-\(\beta\)-D-glucopyranoside 4 was synthesised by condensing 2,3,4,6-tetraacetyl-\(\alpha\)-D-glucopyranosyl bromide with 1 in presence of phase transfer catalyst (PTC) and 10% aq. sodium hydroxide in a two-phase, dichloromethane/water system as indicated in Scheme 1. A careful deacetylation of 4 as per the reported literature procedure gave ibuprofen-\(\beta\)-D-glucopyranoside 5.

### Biological Evaluations

#### Anti-inflammatory activity

Anti-inflammatory activity was determined by the method of Winter et al. against carrageenan induced rat paw edema. The dose of 1 was 20mg/kg administered orally and compounds 4 and 5 were administered orally in doses equivalent to 20mg/kg of 1. Rats in control group received an equal volume of the vehicle (0.5% CMC in normal saline; 10ml/kg). Percentage reduction in edema at 3 hr in comparison to control is presented in Table 1. Prodrug 4 showed 81.4% inhibition in edema as compared to 69.3% and 66.6% inhibition by 4 and 1, respectively.
KHAN et al.: SYNTHESIS OF THE PRODRUG IBUPROFEN β-D-GLUCOPYRANOSIDE

Scheme I

**Table I — Comparative chart of prodrug 5, ibuprofen 1 and 4 with respect to their m.p., dose and biological properties**

<table>
<thead>
<tr>
<th>Compd</th>
<th>Ibuprofen</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>77°</td>
<td>112-14°</td>
<td>163-64°</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>20</td>
<td>51*</td>
<td>36*</td>
</tr>
<tr>
<td>Anti-inflammatory activity (%)</td>
<td>66.6</td>
<td>69.3</td>
<td>81.4</td>
</tr>
<tr>
<td>Analgesic activity (%)</td>
<td>60.7</td>
<td>64.8</td>
<td>89.4</td>
</tr>
<tr>
<td>Gastric ulcers (average)</td>
<td>8.0</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>(score)</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* indicates molar equivalent of ibuprofen

**Analgesic activity**

Analgesic activity was determined in mice by acetic acid induced writhing method\(^\text{13}\). The dose of 1 was 20mg/kg administered orally and compounds 4 and 5 were administered orally in doses equivalent to 20mg/kg of 1. The decrease in number of writhings expressed as percentage protection by test compounds with reference to control is given in Table I. Prodrug 5 gave 89.4% protection while 4 and 1 gave 64.8% and 60.7% protection respectively.

**Gastrointestinal toxicity**

Subacute gastrointestinal toxicity studies were done by the method of Wilhemi et. al\(^\text{14}\). The animals were divided in groups with six animals in each group. Control group was given only 0.5% CMC suspension. The dose of 1 was 20mg/kg and compounds 4 and 5 were administered at doses equivalent to 20mg/kg of ibuprofen and were given orally once in a day for 10 days. The animals were fasted for 8 hr prior to dosing and for 4 hr post dosing. Food was available at all other times, free access to water was provided throughout the experiment. Four hours after the last dose, the animals were sacrificed using chloroform. The abdomen was opened at the midline and the stomach and the first 3cm of the duodenum were removed. The stomach was opened along the larger curvature and washed with distilled water. The mucus was wiped off and the numbers of ulcers were examined by means of a magnifying glass. All ulcers were counted and recorded as average number of ulcers per animal and assessed as score. The results are given in Table I.

**LD\(_{50}\) evaluation**

The study was carried out on Swiss albino mice (20-25g) by Miller and Tainter method\(^\text{15}\). Compound 5 was administered intraperitoneally (ip) to mice in ascending and widely spaced doses of 250, 500, 750, 1000 and 1250 mg/kg in 0.5% CMC suspension. The percentage mortality was calculated and converted to
prob val values from the probit tables. Graph was plotted between probit values against log doses and LD₉₀ value read as the dose, which corresponded to probit 5. The LD₉₀ value of 5 was found out to be 1047 mg/kg ip (Ibuprofen 320 mg/kg ip).

**In vivo pharmacokinetic studies of 5**

HPLC analysis of prodrug 5 in rabbit was performed by the method of Anwar et al. on a Shimadzu HPLC instrument. Two healthy rabbits weighing 2-3 kg were chosen for the study. The dose of 1 was 60 mg/kg and 5 was given in a dose equivalent to 60 mg/kg of 1. The animals were fasted overnight prior to experimentation. Drug suspensions in 0.5% CMC were given orally. Blood samples were withdrawn from marginal ear vein at predose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 hours. Plasma was separated by centrifugation at 4000 rpm and stored at −50 °C. Calibration curves for 5 and 1 were prepared in rabbit plasma in the concentration range of 10-40 μg/mL. In a 5mL centrifugal tube, 0.5 mL of plasma was taken and to it was added 1.5 mL of acetonitrile and vortexed for 2 min. The precipitated proteins were separated by centrifugation at 4000 rpm for 20 min. Separations were achieved on a µBondapak C₁₈ (reverse phase) analytical column with UV detector set at 220 nm. Mobile phase was acetonitrile: 0.1 M acetic acid (55:45). Flow rate was set at 1 mL/min, and the injection volume was 10 μL. Prodrug 5 gave a peak at retention time of 5.5 min and 1 at retention time of 8.5 min. The graphs of plasma concentration versus time were plotted for 1 and 5 and C₉₀, T₉₀ and t₁/₂ calculated (Figures 1 & 2). The prodrug 5 gave higher plasma levels of ibuprofen in comparison to ibuprofen indicating improved bioavailability. The C₉₀ for ibuprofen in case of the animal given prodrug 5 was more (34 μg/mL) when compared to C₉₀ for ibuprofen (27.8 μg/mL) in case of the animal given ibuprofen. T₉₀ was found to be the same in both the cases (1.5 hr). The prodrug was also detected in plasma from 0.5 hr to 3.0 hr.

**Partition co-efficient**

Partition co-efficient was determined in n-octanol/buffer system by conventional shake flask method. Standard curve for 5 in 0.05M phosphate buffer (pH 7.4) was prepared in the range of 10-100μg/mL. Absorbances were taken at 220 nm. A known amount of the prodrug 5 was dissolved in 100 mL phosphate buffer presaturated with octanol and above solution was added to a flask containing 100 mL of octanol presaturated with phosphate buffer. The flask was allowed to shake gently on a sideways mechanical shaker. Samples from aqueous phase were withdrawn over a period of 12 hr, centrifuged and absorbances taken at 220 nm. Concentrations of 5 in n-octanol were determined by subtracting its concentration in phosphate buffer. Partition co-efficient was determined by the formula given below:

\[ P_{octanol/buffer} = \frac{C_1}{C_2} \]

where C₁ is concentration in n-octanol and C₂ is concentration in buffer. The partition co-efficient of 5 was calculated as 0.176 (Log P = -0.75).

**Conclusion**

The glucoside derivative 5 was synthesised successfully and evaluated for its gastrointestinal toxicity. In comparison to ibuprofen, the prodrug 5 was found to be considerably less ulcerogenic indicating that GI toxicity due to direct contact of the carboxylic group has been reduced. It also showed improved anti-inflammatory and analgesic activity over ibuprofen. It gave higher plasma levels of ibuprofen in rabbit without altering the pharmacokinetic parameters of the parent compound. The higher LD₉₀ value for prodrug 5 (1047mg/kg
equivalent to 586mg/kg of ibuprofen) indicated improved therapeutic range over ibuprofen.

Experimental Section

Melting points were determined on a liquid paraffin bath in open capillaries and are uncorrected. NMR spectra were recorded on a Bruker Spectrospin 300 MHz instrument using TMS as internal standard. The chemical shift values are given in ppm (δ). For partition co-efficient experiment absorances were taken on a Perkin-Elmer Lambda Bio 20 spectrophotometer at 220 nm. Both the compounds 4 and 5 gave satisfactory elemental analysis.

Synthesis of β-D-glucoside pentacetate 2 and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 3. These compounds were synthesised by standard procedures.[1]

Synthesis of tetraacetyl β-D-glucopyranosyl derivative 4. To a solution of 1 (0.61g, 3mmole) and 3 (1.64g, 4mmole) in dichloromethane was added tetrabutylammonium bromide (0.644g, 2mmole) with stirring at 5°C. Aq. sodium hydroxide (10%, 10ml) was added to it dropwise over a period of 30 min and the reaction mixture further stirred for 24 hr. The organic layer was separated, washed with water, 5% aq. NaHCO₃, again with water, dried and concentrated in vacuo. A semi-solid mass so obtained was purified on a column of silica gel and crystallised from ethanol as colourless needles, yield 1.35g (85.5%), m.p. 112-14°C; NMR (CDCl₃): δ 0.8 (d, 6H, 2xCH₃), 1.49 (d, 3H, sec-methyl), 1.81 (m, 1H, -CH of isopropyl), 2.0 (s, 3H, OCCH₃), 2.01 (s, 3H, OCCH₃), 2.06 (s, 3H, OCCH₃), 2.03 (s, 3H, OCCH₃), 2.09 (s, 3H, OCCH₃), 2.42 (d, 2H, -CH₂ benzyl), 3.73 (q, 1H, -CH of propionic acid), 3.8 (m, 1H, H-5), 4.06 (d, 1H, H-6), 4.26 (d, 1H, H-6'), 5.1 (m, 3H, H-2, H-3, H-4), 5.68 (d, J=8Hz, 1H, H-1 of sugar), 7.08 and 7.17 (d, each 4H, aromatic protons).

Synthesis of β-D-glucopyranosyl derivative 5. The tetraacetyl derivative 4 was deacetylated with methanolic 0.5% sodium methoxide by following the reported procedure.[11] To a solution of 4 (3g) in absolute methanol (25ml) was added 1.45 ml of 0.5% of sodium methoxide solution and kept at room temperature for 45 min. The reaction mixture was neutralised with ion exchange resin (Amberlite IR 120, SD fine, H⁺ form), filtered and concentrated in vacuo. A semi-solid mass so obtained was crystallised from absolute ethanol as colourless compound, yield 1.35g (65%), m.p. 163-64°C; NMR (DMSO-d₆): δ 0.83 (d, 6H, 2xCH₃), 1.22 (s, 3H, sec-methyl), 1.77 (m, 1H, CH of isopropyl), 2.3 (d, 2H, CH₂ of benzyl), 3.5 (m, 1H, CH of propionic acid), 3.40 and 4.2 (m, 6H, H-2 to H-6 of sugar protons), 5.4 (d, J=7Hz, 1H, H-1 of sugar) 6.9 and 7.15 (d each, 4H, aromatic protons).

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References

1 Rainsford K D, Toxicol Pathol 16, 1988, 251.