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Synthesis and Biological Properties of Ketoprofen Glucopyranoside Conjugates

JAGDISH K. SAHU and LOPAMUDRA BANERJEE*
Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutical Sciences
Dr. Hari Singh Gour University, Sagar-470 003, India
Tel: (91)(758)2264124; (91)9425171057
E-mail: lopamudrabanerjee@rediffmail.com

Glucopyranoside conjugates of ketoprofen have been synthesized and evaluated for antiinflammatory, analgesic and ulcerogenic activities. The results designate significant augmentation in analgesic and antiinflammatory activity and diminution in gastrointestinal toxicity.

Key Words: Ketoprofen, Glucopyranoside, Analgesic, Antiinflammatory, Ulcerogenic activity.

INTRODUCTION

Traditional non-steroidal antiinflammatory drugs (NSAIDs) are used in treatment of mild to moderate pain and as an adjunct to opioids in the management of moderate to severe pain[1]. The clinical effects of NSAIDs are based on the inhibition of the enzyme cyclooxygenase (COX), which catalyzes the rate limiting step in the formation of prostanoids, prostaglandins and thromboxane A2 (TxA2)[2,3]. Prostaglandins are ubiquitous compounds that mediate a variety of physiologic and pathologic processes. Under normal physiologic conditions, prostaglandins play an essential homeostatic role in cytoprotection of gastric mucosa, hemostasis, renal function, gestation and parturition[4-6].

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[7]. Ketoprofen (1), a 2-arylpropinoic acid, belongs to the family of non-steroidal antiinflammatory drugs (NSAIDS). It has similar pharmacological actions to other drugs in this class such as ibuprofen, fenoprofen and naproxen. Ketoprofen has been used extensively in treatment of chronic rheumatoid arthritis and various painful conditions in various species. The principal mechanism of action is considered to be *via* inhibition of cyclo-oxygenase mediated generation of prostanoids[8]. Temperature orarily masking the acidic group of NSAIDs with a view to decrease the gastrointestinal toxicity due to direct injury has been postulated[9-12]. The purpose of this study was to mask the free acidic group by synthesizing its glucopyranoside derivative and evaluate it's anti-inflammatory activity, analgesic activity and gastrointestinal toxicity.

EXPERIMENTAL

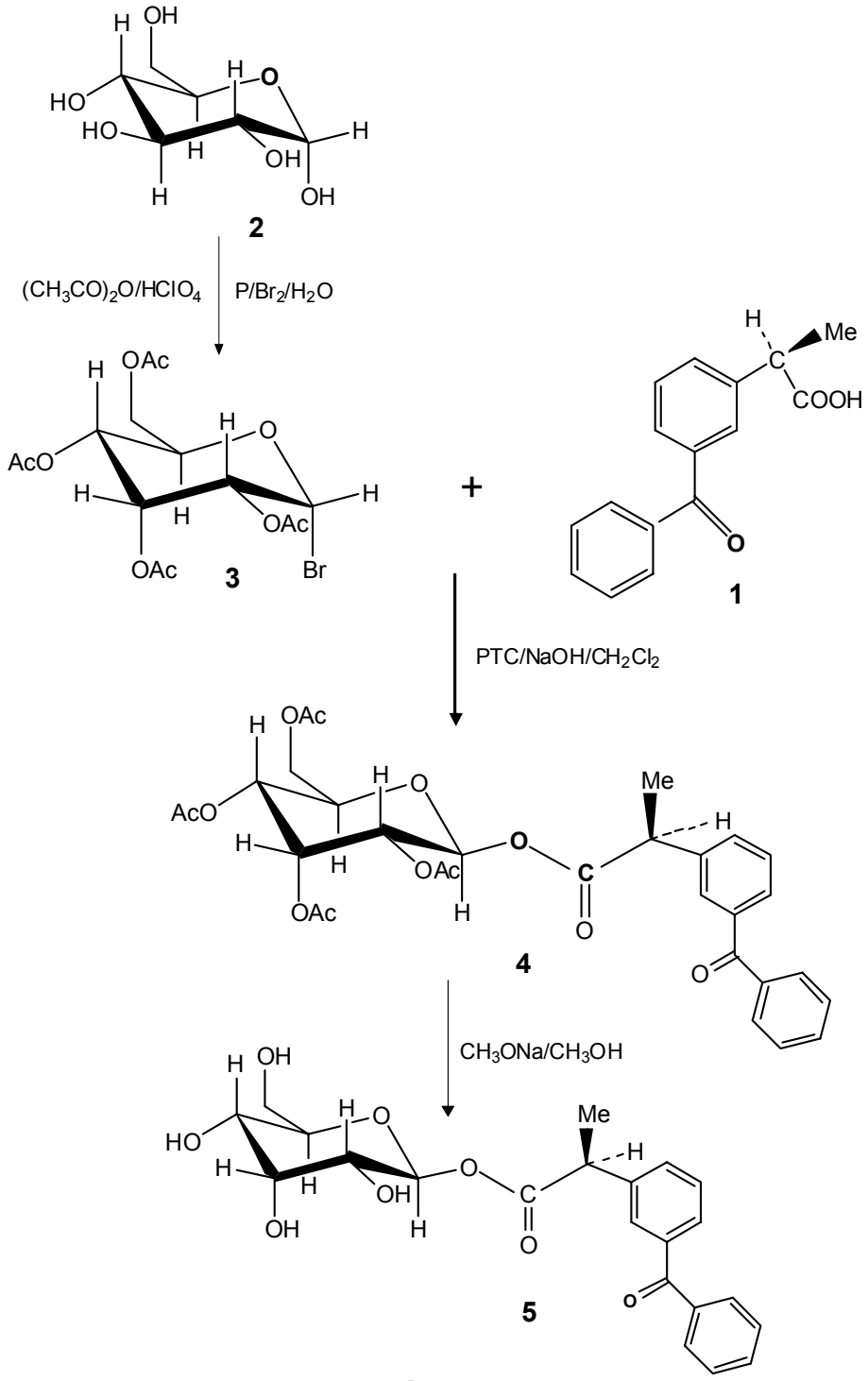
Melting points were determined by superfit melting point determination apparatus in open capillary tube and were uncorrected (Table-1). IR spectra were taken on Nujol mulls between salt plates. ^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance II 400 spectrometer. The chemical shift are reported in part per million (δ, ppm) downfield from tetramethylsilane, which was used as internal standard. Mass spectra were also recorded in $CDCl_3$ solution.

Synthetic procedure (Scheme-I)

Synthesis of 2,3,4,6-tetra-*o*-acetyl α-D-glucopyranosyl bromide (3): Acetic anhydride (400 mL) was placed in a two-necked flask kept in an ice-salt bath on a magnetic stirrer. The temperature was maintained at 4 ± 1 °C and 2.4 mL of 60 % perchloric acid is added drop-wise. The reaction mixture was allowed to reach room temperature gradually. α-D-Glucose (**2**) (100 g) as dry powder was added in portions, with continuous stirring, the temperature being maintained at 30-40 °C. Red phosphorus (31 g) was added immediately followed by bromine (58 mL) drop wise, keeping the temperature maintained at 20 °C. Water (36 mL) was added over a period of 0.5 h, stirring and cooling being continued and the temperature being maintained below 20 °C. The reaction mixture was allowed to stand for 2 h at room temperature, then dichloromethane (300 mL) was added and then filtered. The filtrate was washed twice with iced water. The dichloromethane layer was run in saturated solution of $NaHCO_3$ and some crushed ice. After the vigorous evolution of CO_2, the dichloromethane layer was run into a flask containing 10 g of powdered activated silica gel and filtered after 10 min. The solvent was removed by the use of rotary evaporator. The syrupy mass crystallized as a thick layer around the inside of the flask. Portions of the solid was transferred to a pestle mortar and ground with a 2:1 mixture of light petroleum (B.P. 60-80 °C) and dry ether. The combined slurry was filtered and washed with a light-petroleum ether solvent mixture and then with 50 mL of previously chilled (0 °C) dry ether.

Synthesis of ketoprofen tetraacetyl β-D-glucopyranosyl derivative (KTAG/4): To a solution of ketoprofen (0.61 g, 3 mmol) and 2,3,4,6-tetra-O-acetyl α-D-glucopyranosyl bromide (1.64 g, 4 mmol) in dichloromethane was added tetrabutyl ammonium bromide (0.644 g, 2 mmol) with stirring at 5 °C. Aqueous sodium hydroxide (10 %, 10 mL) was added to it drop wise over a period of 0.5 h and the reaction mixture further stirred for 24 h. The organic layer separated out was washed with water followed by 5 % aqueous $NaHCO_3$ solution again with water. The product was dried and concentrated *in vacuo*. A semi-solid mass so obtained was purified on a column of silica gel and crystallized from ethanol as colourless needles. Yield was 81.5 %.

IR (KBr, ν_{max}, cm^{-1}): 3060, 2961, 2874, 1651, 1594, 1461 and 1381. NMR ($CDCl_3$): 2.01 (s, 12H, $OCOCH_3$), 7.31-7.70 (m, 9H, aromatic), 3.78 (m, 1H, CH), 1.52 (m, 3H, CH_3), 4.09 (s, 2H, CH_2). Mass: 584.4 (M^+).



Scheme-I

Synthesis of ketoprofen β-D-glucopyranosyl derivative (KG/5): The tetra acetyl derivative was deacetylated by adding 1.45 mL of 0.5 % sodium methoxide solution and kept at room temperature for 45 min. The reaction mixture was neutralized with Amberlite IR 120, SD Fine, filtered and concentrated *in vacuo*. A semi-solid mass so obtained was crystallized from absolute ethanol as colourless compound. Yield was 67 %.

IR (KBr, ν_{max}, cm^{-1}): 3405, 2961, 2874, 1742, 1461 and 1200. NMR ($CDCl_3$): 2.0 (s, 4H, OH), 7.31-7.70 (m, 9H, aromatic), 3.78 (m, 1H, CH), 1.52 (m, 3H, CH_3), 3.79 (m, 2H, CH_2). Mass: 416.1 (M^+).

Biological evaluation

Antiinflammatory activity: Antiinflammatory activity was determined by Carrageenan induced rat paw edema method. Six male Sprague-Dawley rats with a body weight between 100 and 150 g were used for each test and control groups. Just before injection of the test compounds the volume of the paw was measured plethysmometrically[13]. Animals were pretreated intraperitoneally with 1 mL of 20 mL/kg of either test compounds or ketoprofen drug. The control group received the same volume of the vehicle. Edema was induced after 1 h by sub planter injection of 0.05 mL of a 1 % solution of carrageenan into the left hind paw. The increase in paw volume was determined after 2, 4, 6, 8 and 24 h. The percentage anti-inflammatory activity was calculated by the formula: antiinflammatory activity = (1-dt/dc)/100 where dt is the difference of paw volume in drug/test compounds treated groups and dc is the difference in paw volume of the control group.

Analgesic activity: Analgesic activity was determined in mice by Tail flick method. The pre-screened animals (reaction time: 3-4 s) were divided into control, standard and test groups. Ketoprofen 20 mg/kg acted as the standard drug. The drugs were administered intraperitoneally. The tail flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 s to avoid tissue damage[14].

Ulcerogenic activity: The Albino rats of either sex (100-150 g) were fasted for 12 h. They're administered orally an aqueous suspension of the drug twice a day over a two-day period. The rats were then sacrificed the day after giving the final dose. To determine the gastric mucosal damage, rat stomachs were removed, opened along the length of greater curvature and cleaned of the debris, washed and examined under a microscope (10x) and the ulcers were scored as

0.0	Normal colour stomach	1.5	Hemorrhagic streaks
0.5	Red colouration	2.0	Ulcers > 3mm but < 5mm
1.0	Spot ulcers	3.0	Ulcers > 5mm

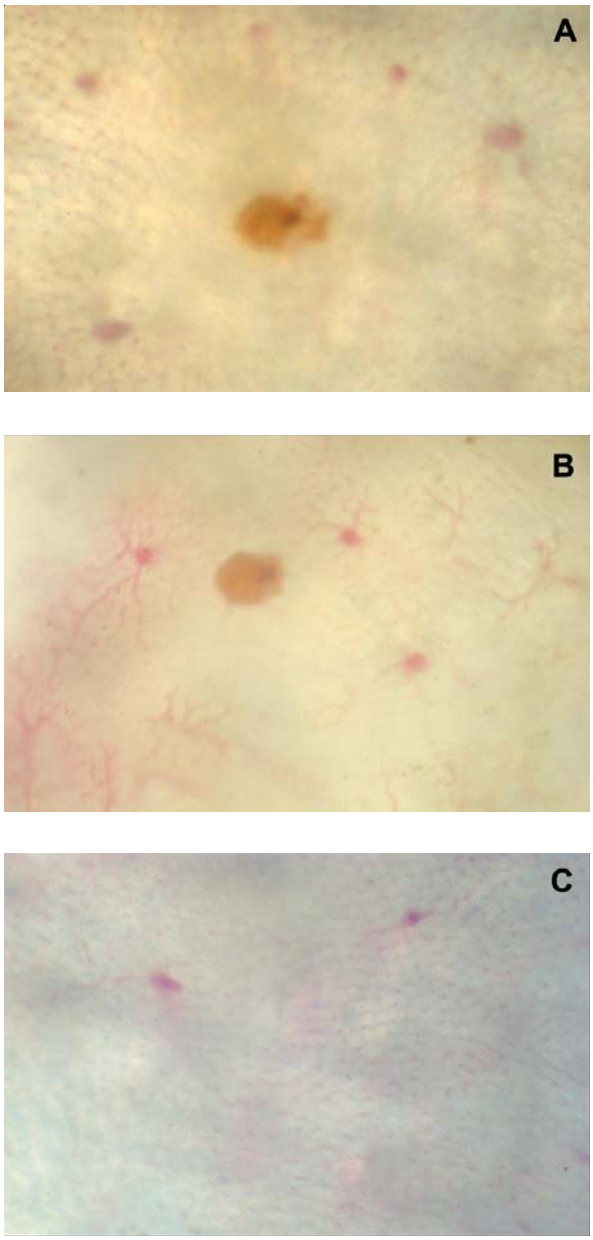


Fig. 1. Photograph showing (A) ulcers induced by ketoprofen (pure drug) (B) ulcers induced by KTAG (intermediate conjugate) (C) ulcers induced by KG (fingal conjugate)

The mean ulcer score for each animal was expressed as Ulcer/Lesion Index (LI). For assessing the incidence of ulceration, the rats showing ulcers greater than 0.5 mm in gastric mucosa were considered to have a positive ulcerogenic response[15].

RESULTS AND DISCUSSION

The glucopyranoside derivatives were synthesized successfully and evaluated for their analgesic, antiinflammatory and gastrointestinal toxicity. They showed improved antiinflammatory and analgesic activity over the parent drug. The test compound **5** showed 64.28 % inhibition in edema as compared to 59.52 and 52.38 % inhibition by **4** and **1**, respectively in the case of antiinflammatory activity. In the tail flick method, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. The test drug produced a dose dependent increase in the reaction time at various time intervals of observation. In comparison to ketoprofen, the test compound **5** was found to be considerably less ulcerogenic indicating that GI toxicity due to direct contact of the carboxylic group has been reduced. The results are mentioned in Fig. 1 and Table-1.

TABLE-1
COMPARATIVE CHART OF PREPARED COMPOUNDS **5**, **4** and KETOPROFEN **1** WITH RESPECT TO THEIR MELTING POINT AND BIOLOGICAL PROPERTIES

Compound	Ketoprofen	**4**	**5**
m.p. (ºC)	96	112-114	163-164
Antiinflammatory activity (%)	52.38	59.52	64.28
Analgesic activity (%)	45.67	71.87	81.73
% of Animals with ulcer	100	66.66	33.33
Ulcer Index	6.0	4.0	1.5

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