

Synthesis and Pharmacological Evaluation of Cyclodextrin Conjugate Prodrugs of Ibuprofen

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In the present investigation ibuprofen prodrugs of α -, β - and γ -cyclodextrins were synthesized. The primary hydroxy group of α -, β - and γ -cyclodextrins was used to block the acid group. The synthesis involved a series of protection and deprotection reaction. The esters were evaluated for stability in simulated gastric and intestinal fluid. The hydrolysis of cyclodextrin conjugates in colon is confirmed by the hydrolysis kinetics studies in rat faecal material. The esters were also evaluated for ulcerogenicity. Results of these studies established the primary aim of masking the ulcerogenic potential of free drug, by using 12-fold dose of the normal dose of ibuprofen and equivalent doses of the esters.

Cyclodextrins (CDs) belongs to family of cyclic-oligosaccharides; the most common being α -, β - and γ -CDs consisting of 6, 7 and 8 glucopyranosyl units, respectively, linked by a (1 \rightarrow 4) glucosidic bonds¹. CDs are obtained by enzymatic degradation of starch, glucosyl transferase a type of amylase of bacterial origin obtained usually from *Bacillus macerans*, *Bacillus megaterium*, *Klebsiella pneumoniae* M5, and *Bacillus sterothermophilus*. CDs are moderately soluble in water, methanol and readily soluble in strongly polar aprotic solvents². After oral administration, CDs are not hydrolyzed during their transit through the stomach, hydrolysis occurs only in colon by colonic microflora. The oral administration of CDs does not result in toxicity^{3,4}. Thus, CDs were thought to be one of the most suitable promoiety to reduce the ulcerogenic tendencies of ibuprofen since they eliminate the exposure of free drug in stomach and small intestine but release the drug in colon.

Ibuprofen is a nonsteroidal antiinflammatory and analgesic compound belonging to the family of phenyl and methyl acetic acid. It is also described as (RS)-2-(4-isobutylphenyl) propionic acid. The main side effects include GIT disturbance, peptic ulceration and gastric bleed-

ing. Ibuprofen is widely used in rheumatic disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis.

The major factor, which limits the use of ibuprofen, is its gastric effect due to local irritation of gastric mucosa by free-COOH group of the drug⁵⁻⁷. Hence efforts have been made to mask the free-COOH group by the primary hydroxyl group of CD and releasing the drug in colon and preventing the exposure of free drug to the stomach.

MATERIALS AND METHODS

All melting points were determined by open capillary method and are uncorrected. TLC ascertained the purity of the compounds on precoated silica gel-60 F₂₅₄ plates. Solvent used was butanol:ethanol:water:acetic acid (3:2:3:0.1). Thus different spots for reference and test substance were detected using iodine vapours or by charring the plates using 5% methanolic sulfuric acid. All the final compounds were re-crystallized from water, after extracting the impurities with ethyl acetate. The IR spectra of the synthesized compounds were recorded on a FT-IR spectrophotometer, in potassium bromide (anhydrous IR Grade) pellets. The NMR spectra of the synthesized compounds were determined on a FT-NMR spectrophotometer. The λ_{max} of the

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synthesized compounds was determined on UV/Vis double beam spectrophotometer by scanning the compounds between 400-200 nm in various solvents. Ibuprofen was obtained as a gift sample from Zyg Pharma Ltd., Indore. CDs were obtained as gift sample from S. A. Chemicals, Mumbai. All the other chemicals used were of synthetic grade.

Ibuprofen ester of α - and β -CD (SRD 1 and SRD 2):

Synthesis of ibuprofen ester of α - and β -CD involved 5 steps. First step involved tritylation of one of the primary hydroxyl groups of α - and β -CD (0.00514 mol, 5 g). This reaction involved reacting the α - and β -CD with trityl chloride (a) (0.00565 mol, 1.57 g) in pyridine (30 ml) by stirring it for 24 h⁸. The residue obtained was refluxed with n-hexane to remove trityl alcohol, the by-product. The solid was dried under vacuum to give tritylated α - and β -CD (5.03 g). Next step involved acetylation of tritylated α - and β -CD (0.00411 mol, 5 g) using acetyl chloride (b) (0.0698 mol, 4.9 ml) and triethyl amine (c) (0.698 mol, 9.7 ml) in ethylene dichloride (75-80 ml). The temperature was maintained at 0° throughout the reaction. The reaction mixture was filtered and dried under reduced pressure to give acetylated monotritylated α - and β -CD (6.2 g)⁹.

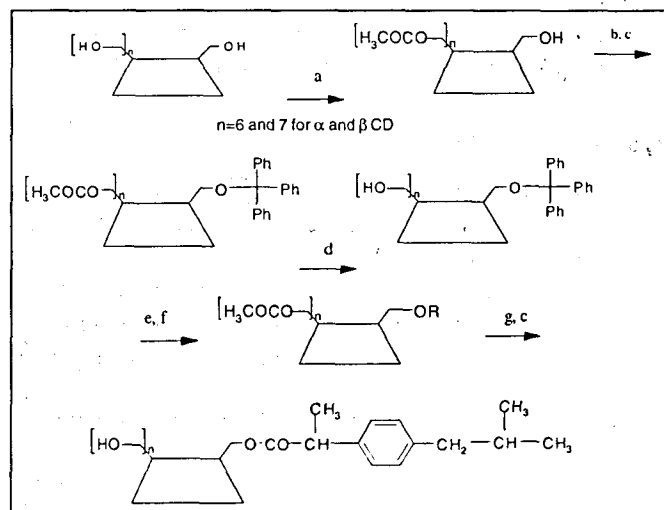
Acetylated monotritylated α - and β -CD (0.0031 mol, 6 g) were dissolved in N,N-dimethyl formamide (DMF, 200 ml) in round bottom flask. The cation exchange resin *T-63 (MP) (d) (3 g) was added slowly into the reaction flask. The reaction mixture was stirred at room temperature and TLC ascertained reaction completion. The reaction mixture was concentrated under vacuum after evaporation of the resin. The residue was refluxed with n-hexane in order to remove trityl alcohol liberated during reaction. The residue obtained after extraction was subjected to esterification reaction. [*T-63 (MP) Tulsion Thermax is a catalyst grade strong acid cation exchange resin]

Selectively detritylated α - and β -CD (0.0024 mol, 4 g) were dissolved in DMF (50 ml) in a round flask. 1,3-dicyclohexyl carbodiimide (DCC) (e) (0.0024 mol, 0.49 g) was dissolved in DMF. Both the solutions were ice cooled to 0°. The DCC solution and ibuprofen free acid (f) (0.0023 mol, 0.57 g) were added to CD solution¹⁰ at 0°. The reaction mixture was stirred at 0° for 2 h and then at room temperature for 12 h. The reaction mixture was then filtered to separate the precipitate of N,N-dicyclohexyl urea, the by-product. The filtrate was concentrated under reduced pressure, to give acetylated ibuprofen ester of α - and β -CD (3.7 g). These esters were deacetylated by dissolving the esters (0.0015 mol, 3 g) in methanol at room temperature and

reacting with ethylenediamine (g) (0.15 mol, 10.12 ml) and triethyl amine (c), (0.15 mol, 20.2 ml)¹¹. The reaction was stirred for 5 h and large amount of acetone (400 ml) was added to precipitate the deacetylated ester. Molecular formula of α - and β -CD ester of ibuprofen (SRD 1, SRD 2) is C₅₇H₈₇O₃₆ and C₄₉H₇₇O₃₈, respectively. Melting point of SRD 1 and SRD 2 is 257-258°, 260-262°, respectively. TLC; R_f: 0.59 and 0.70, respectively; butanol:ethanol:water:acetic acid (3:2:3:0.1) and λ_{max} in distilled water: 239.0 nm and 239.0 nm, respectively, in 0.05 M HCl buffer (pH 1.2): 242.0, 242.0 nm, respectively, 0.05 M phosphate buffer (pH 7.4): 242.0, 243.0, nm respectively. The chemical shift was in the ppm relative to TMS designated as 0.00. NMR revealed the presence of signals in ppm at δ =2.227 (2H, d, CH₂), 1.48 (3h, d, CH₃) and 7 (aromatic protons) respectively for SRD 1 and SRD 2. Schematic representation for synthesis of SRD 1 and SRD 2 is shown in Scheme 1.

Ibuprofen ester of γ -CD (SRD 3):

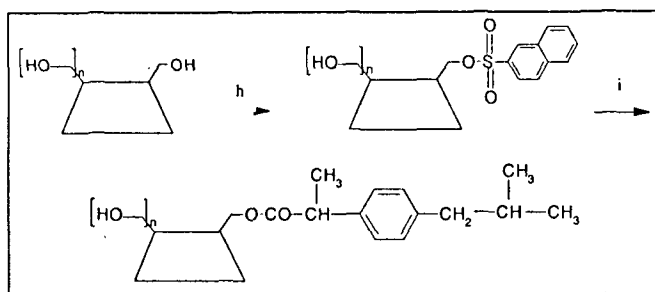
γ -CD (0.0008 mol, 4 g) was dissolved in dry pyridine (250 ml). To it 2-naphthalene sulfonyl chloride (h) (0.00924 mol, 2.09 g) was added. The reaction mixture was allowed to stir at room temperature for 8 h. the reaction mixture was concentrated under reduced pressure to give 2-naphthalene sulfonyl γ -CD (4 g). 2-naphthalene sulfonyl γ -CD (0.002



Scheme 1: Synthesis of α - and β -cyclodextrin esters of ibuprofen SRD 1 and SRD 2

The reagents for each steps are trityl chloride (a), acetyl chloride (b) and triethylamine (c), T-63 MP resin, (d), Ibuprofen free acid and, (e), 1,3-dicyclohexyl carbodiimide, (g) ethylenediamine and triethylamine. N is 6 for SRD1 and n is 7 for SRD 2.

mol, 3 g) was dissolved in DMF (100 ml) and ibuprofen sodium (i) (0.002 mol, 0.537 g) was added to the reaction solution and the mixture was stirred at 0° for 30 h¹². Reaction mixture was concentrated under reduced pressure and the final ester was precipitated using 500 ml of acetone. Molecular formula of ibuprofen ester of γ -CD (SRD 3) is C₆₁H₉₇O₄₁. Melting point: 205-206°, TLC; R_f: 0.50; butanol:ethanol:water:acetic acid (3:2:3:0.1) and λ_{max} in distilled water: 240.0 nm, in 0.05 M HCl buffer (pH 1.2): 250.0 nm and in 0.05 M phosphate buffer (pH 7.4): 254.5 nm. The chemical shift was in the ppm relative to TMS designated as 0.001. ¹H-NMR revealed the presence of signals in ppm at δ =2.55 (2H,CH₂), 3.48 (1H,OH) and 7 (aromatic protons). Schematic representation for synthesis of SRD 3 is shown in Scheme II.



Scheme 2: Synthesis of γ -cyclodextrin ester of Ibuprofen SRD 3

The reagents for each step are 2-naphthalenesulfonyl chloride (h), ibuprofen sodium (i). n is 8 for γ -CD

Hydrolysis kinetics:

Hydrolysis of α -, β - and γ -CD esters were studied in simulated gastric fluid and simulated intestinal fluid (0.05 M HCl buffer, pH 1.2 and 0.05 M phosphate buffer, pH 7.4). The esters showed negligible release in both the hydrolysis media. The hydrolysis of the esters was studied in rat faecal material (pH 7.4) to confirm the colonic hydrolysis of

the esters¹³. The release of drug in rat faecal material was almost complete, maximally being for γ -CD ester. The results of hydrolysis in rat faecal content are reported in Table 1.

Release studies in 0.05 M HCl buffer, pH 1.2 and 0.05 M phosphate buffer, pH 7.4:

Same procedure was followed for all the esters α -, β - and γ -CD esters were dissolved in HCl buffer and in phosphate buffer so that the final concentration of all the esters is equivalent to 1 μ g/ μ l of ibuprofen. To each of the flasks of dissolution apparatus (Veego Scientific DA 6D model) 800 ml of buffer was added when the temperature reached 37° the ester solution was added to the flask and stirred at 75 rpm. The esters showed negligible release in both the hydrolysis media.

Release study in rat faecal matter, pH 7.4:

All the esters were dissolved in phosphate buffer (pH 7.4) so that final concentration of solution was 250 μ g/ml. Fresh fecal material of rats was weighed (about 1 g) and placed in different sets of test tubes. To each test tube, 1 ml of the ester solution was added and diluted to 5 ml with phosphate buffer (50 μ g/ml). The sets of test tubes were incubated at 37° for different intervals of time. For analysis the free drug was extracted in 5 ml CHCl₃ and directly estimated on double beam UV-spectrophotometer (Jasco, V-530 model, Japan). The concentration of free drugs was determined using K and b values obtained from calibration curve.

Antiinflammatory activity:

The animal study protocols have been approved by the Institutional Animal Ethics Committee's (IAEC). Carrageenan-induced rat hind paw oedema method was used for determining antiinflammatory activity¹⁴. Sprague-Dawley rats of either sex (150-200 g) were taken in groups of six

TABLE 1: HYDROLYSIS KINETICS OF SRD 1, SRD 2 AND SRD 3 IN RAT FAECAL MATTER.

Compound	%Drug released with various time intervals in minutes									
	15	30	45	60	75	90	105	120	240	24 h
SRD 1	0	0	5.40	7.65	25.05	76.00	81.86	83.04	85.20	85.30
SRD 2	0	0	15.05	26.91	45.15	64.80	69.00	76.00	80.50	82.30
SRD 3	0	0	3.20	6.90	15.20	37.30	51.00	64.80	82.00	85.10

The hydrolysis of SRD 1, SRD 2 and SRD 3 in rat faecal contents was studied with time.

animals each. The synthesized compounds were suspended in 1 % solution of carboxy methyl cellulose (CMC) in distilled water. For control 1% solution of CMC in distilled water was given orally. Ibuprofen 2.6 mg/kg was used as reference drug. Thirty minutes after the drug administration, 0.1 ml of 1% w/v carrageenan solution was injected in the plantar region of the left hind paw of the animals. The inflammation was determined using a plethysmograph 3 h after injecting the phlogistic agents and compared with that of the control. The data was analyzed using student's "t" test and the level of significance was defined at $p < 0.05$. The results are summarized in Table 2.

Analgesic activity:

The analgesic activity was evaluated by Randall Selitto test¹⁵. The apparatus used for testing analgesic activity was a UGO Basile analgesymeter. The instrument is basically a device, which exerts a force that increases at constant rate (a certain number of grams per seconds). This force is continuously monitored by a pointer moving along a linear scale. The force is applied to the rat paw, which is placed on a small plinth under a cone shaped pusher with a rounded tip. The plinth is made of Teflon, which is biologically inert and has a very low friction coefficient. Thus if the animal suddenly withdraws its paw, it steps out easily without being injured. The mechanism, which exerts the force, is begun by depressing a pedal switch.

The solution of test compounds was prepared in 1 % CMC in distilled water. In all cases the control group received 1 % CMC in distilled water orally. Analgesic activity was evaluated using Carrageenan-induced hyperalgesia method of Randall and Sellito¹⁵. Sprague-Dawley rats of

either sex weighing 150-200 g were distributed in control and experimental group of six animals each. Animals were injected subcutaneously in plantar surface of the hind paw using 0.1 ml of 1 % carrageenan suspension in distilled water. The animals received the vehicle/drugs 2 h after Carrageenan administration. The pain threshold was measured every 2 h up to 6 h after drug administration. The results are given in Table 2.

Ulcerogenicity:

Ulcerogenicity was determined by the method reported by Rainsford *et al.*¹⁶ According to this method sensitivity of gastric mucosa to ulcerogenic -COOH group is increased by exposing the animals to a short period of physical stress (i.e. cold or restraint)¹⁶. Sprague Dawley rats of either sex (150-200 g) were selected. They were kept for fasting for 24 h. Following oral administration of drug that is 12 times the normal dose in case of test and vehicle dose in case of control, the animals were stressed by exposure to cold (-15° for 1.5 h). The animals were sacrificed 3 h after drug administration and the number and severity of gastric mucosal lesions were determined. The results are summarized in Table 3 and 4.

RESULTS AND DISCUSSION

The studies have revealed that the CD esters of ibuprofen have retained their pharmacological activity in terms of % inhibition of oedema and % analgesic activity as compared with the standard drug. For the esters the maximum activity was obtained after 6 h showing that absorption of drug does not occur in stomach. Ulcerogenicity studies showed that esters are not ulcerogenic (Table 2, 3 and 4). The photographic presentation of ulcers is depicted in

TABLE 2: ANTIINFLAMMATORY, ANALGESIC AND ULCEROGENIC ACTIVITY OF SRD 1, SRD 2 AND SRD 3.

Compound	Antiinflammatory activity (% inhibition of oedema)		Analgesic activity (%)	Ulcerogenic activity
	6h	24h		
Control	NIL	NIL	NIL	NIL
Ibuprofen	36.3	15.3	52.1±14.05	59.0±1.35
SRD 1	43.8	85.9	43.0±9.25	12.5±0.78
SRD 2	67.6	83.8	47.5±19.04	NIL
SRD 3	77.8	55.3	51.9±10.00	10.5±0.55

SRD 1, SRD 2 and SRD 3, Ibuprofen (2.6 mg/kg or equivalent) and vehicle were administered orally to Sprague Dawley rats and corresponding parameters were measured after specific time intervals after inducing either inflammation or hyperalgesia with carrageenan.

TABLE 3: ULCEROGENIC RESPONSE SCORE

Response	Ulcer <1 mm	Ulcer <1-2 mm	Ulcer <2-3 mm	Ulcer <3-4 mm	Ulcer <4-5 mm	Ulcer >5mm	Ulcer Perforated lesions
Score	1	2	3	4	5	10	25

TABLE 4: ULCEROGENIC ACTIVITY OF SRD 1, SRD 2 AND SRD 3

Compound Dose (mg/kg)	Ulcer Index \pm SD
*Control	Nil
Ibuprofen	59.0 \pm 1.35
SRD 1	12.5 \pm 0.78
SRD 2	Nil
SRD 3	10.5 \pm 0.55

*Average of six readings, $p < 0.05$

(figs. 1 and 2). *In vitro* hydrolysis studies have shown that the esters were quite stable in simulated gastric and

intestinal fluid where as they hydrolyzed almost completely in rat faecal contents representing the colon. Although, this is the method of confirming the hydrolysis of esters in colon, it gives an idea about the behavior of ester in gastrointestinal tract. This can be further confirmed by improved analysis, one approach to it is to isolate the specific strain of microorganism responsible for hydrolysis of CD and study the hydrolysis of conjugates in presence of them. The present study clearly indicates that conjugation of CDs with ibuprofen is a good method of masking the -COOH group and thus reducing the ulcerogenicity, a major drawback. The synthesized compound need to be further studied before being considered as potentially useful prodrugs.

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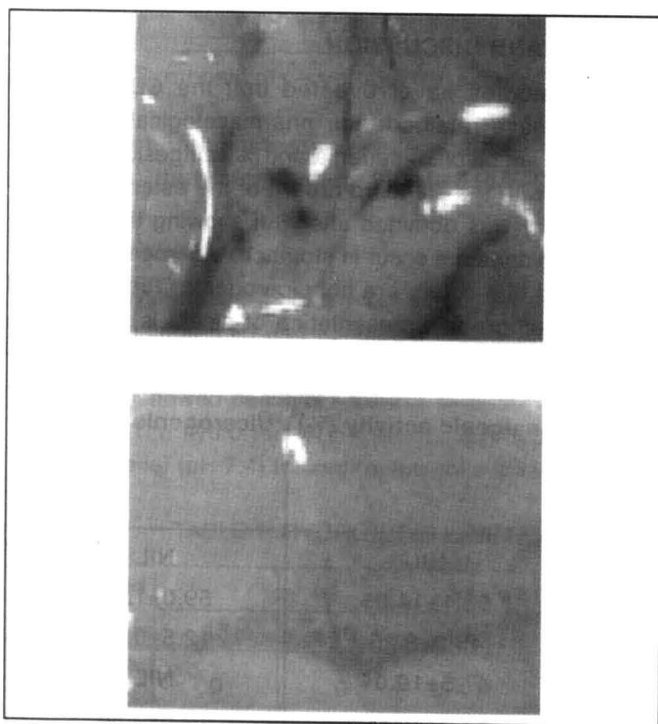


Fig. 1: Microphotographs of ulcers produced by ibuprofen and SRD 1

Top panel shows the ulcerogenic effect of ibuprofen treatment while the bottom panel depicts that after treatment with SRD 1

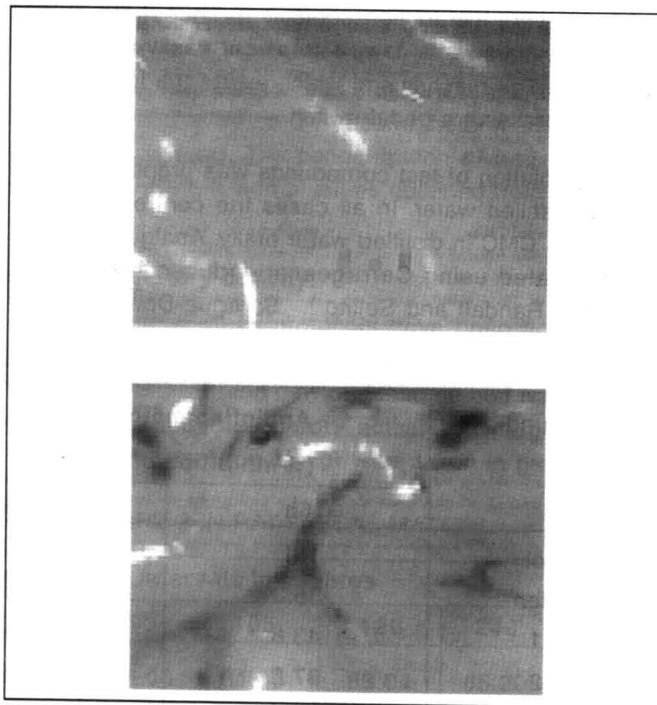


Fig. 2: Microphotographs of ulcers produced by SRD 2 and SRD 3

Top panel shows the ulcerogenic effect of SRD 2 treatment while the bottom panel depicts that after treatment with SRD 3

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