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Synthesis and Evaluation of Pharmacological Activities of Cyclodextrin Conjugates of Flurbiprofen.

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In the present investigation flurbiprofen prodrugs of α and γ cyclodextrins were synthesized. Here the primary hydroxy group of α and γ cyclodextrins were used to block the acid group. The synthesis involved a series of protection and deprotection reaction. The esters were evaluated for their 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 evaluated for their analgesic and antiinflammatory activities. The ulcerogenicity of the esters was also studied. The results of these studies revealed that the esters have comparable antiinflammatory and analgesic activity with the parent drug, flurbiprofen. The esters showed no ulcers even at a dose 12 times greater than normal dose.

Cyclodextrins (CDs) belong to a family of cyclic-oligosaccharides; the most common being α, β and γ consisting of 6, 7 and 8 glucopyranosyl units, respectively, linked by α (1→4) glucosidic bonds[1]. CDs are obtained by enzymatic degradation of starch. The enzyme used is cyclodextrin glucosyl transferase, a type of amylase of bacterial origin obtained usually from *Bacillus macerans, Bacillus negaterium, Klebsiella pneumoniae* M5, and *Bacillus streothermophyllus*. CDs are moderately soluble in water, methanol and readily soluble in strongly polar aprotic solvents[2]. After oral administration, CDs are not hydrolyzed during their transit through the stomach, hydrolysis occurring 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 promoieties to reduce the ulcerogenic tendencies of flurbiprofen since they eliminate the exposure of free drug in stomach and small intestine but release the drug in colon.

Flurbiprofen is a nonsteroidal antiinflammatory and analgesic compound belonging to the family of phenyl and methyl acetic acid. It is also described as 2-(2-fluoro biphenyl–4-yl) propionic acid. The main side effects include GIT disturbance, peptic ulceration and gastric bleeding. Flurbiprofen is widely used in rheumatic disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis. The major factor, which limits the use of flurbiprofen, is its gastric side effect due to local irritation of gastric mucosa by free -COOH group of the drug[5-7]. Hence efforts have been made to mask the free -COOH group by the primary hydroxy group of CD and releasing the drug in colon and preventing the exposure of free drug to the stomach.

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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 F_{254} 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 ethylacetate. 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 recorded on a FT-NMR

spectrophotometer. The λ_{max} of the synthesized compounds was determined on UV/Vis double beam spectrophotometer by scanning the compounds between 400-200 nm in various solvents. Flurbiprofen was obtained as a gift sample from FDC Limited. CDs were obtained as gift sample from S. A. Chemicals. All the other chemicals used were of synthetic grade.

Flurbiprofen ester of α CD (SD1):

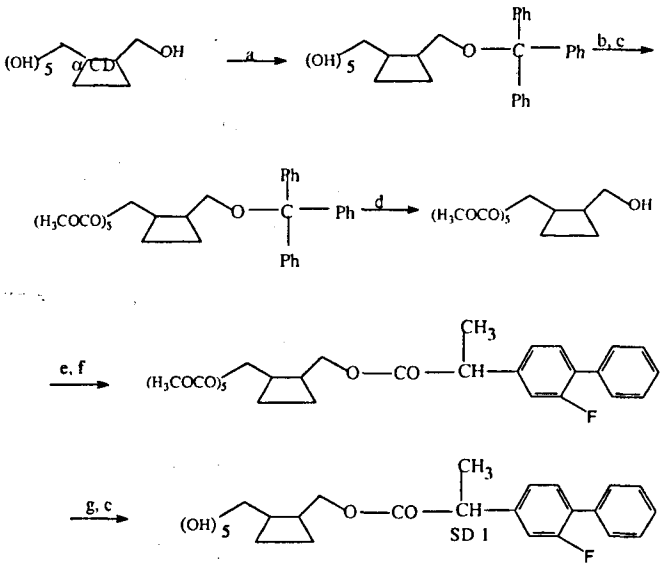
Synthesis of flurbiprofen ester of α CD involved 5 steps. First step involved tritylation of one of the primary hydroxyl groups of α CD (0.00514 mol, 5 g). This reaction involved reacting the α CD with trityl chloride (a) (0.00565 mol, 1.57 g) in pyridine (30 ml) by stirring it for 24 h[8]. The residue obtained was refluxed with n-hexane to remove trityl alcohol, the by-product. The solid was dried under vaccum to give tritylated α CD (5.03 g). Next step involved acetylation of tritylated α CD (0.00411mol, 5 g) using acetyl chloride (b) (0.0698 mol, 4.9 ml) and triethylamine (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 CD (6.2 g)[9].

Acetylated monotritylated α CD (0.0031 mol, 6 g) was 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 filteration 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.

Selectively detritylated α CD (0.0024 mol, 4 g) was 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 flurbiprofen free acid (f) (0.0023 mol, 0.57 g) were added to CD solution[10] 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 flurbiprofen ester of α CD (3.7 g). This ester was deacetylated by dissolving the ester (0.0015 mol, 3 g) in methanol at room temperature and reacting with ethylenediamine (g) (0.15 mol, 10.12 ml) and triethylamine (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 CD ester of flurbiprofen (SD1) is $C_{51}H_{72}FO_{31}$ and λmax in distilled water: 248.0 nm, in 0.05 M HCl buffer (pH 1.2): 240 nm and in 0.05 M phosphate buffer (pH 7.4): 244 nm. H-NMR revealed the presence of signals in ppm at δ 2.227 (2H, d, CH_2), 1.48 (3H, d, CH_3) and 7 (aromatic protons). Schematic representation for synthesis of SD1 is shown in Scheme 1.

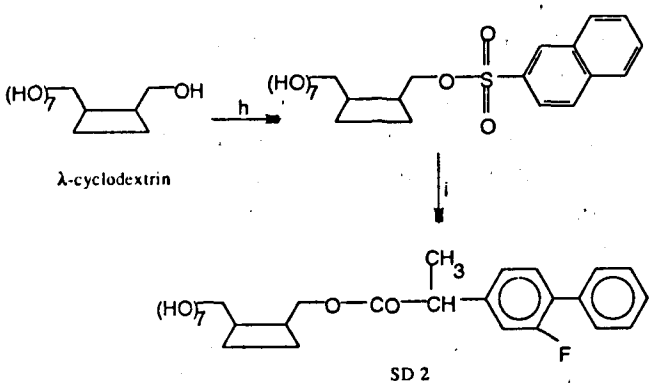
Flurbiprofen ester of γ CD (SD2):

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 mol, 3 g) was dissolved in DMF (100 ml) and flurbiprofen sodium (i) (0.002 mol, 0.537 g) was added to the reaction solution and the mixture was stirred at 0° for 30 h[12]. The reaction mixture was concentrated under reduced pressure and the final ester was precipitated using 500 ml of acetone. Molecular formula of flurbiprofen ester of γ-CD (SD2) is $C_{63}H_{92}FO_{41}$. Melting point: 198-200°, TLC; R_f: 0.49;



Scheme 1: Synthesis of SD 1.

Flurbiprofen esters of alpha-cyclodextrins were synthesized by the following five steps, 1.tritylation of alpha-cyclodextrin, 2. acetylation of 6-O-trityl-alpha-cyclodextrin, 3. detritylation of flurbiprofen acid, 4. esterification of flurbiprofen and 5. deacetylation of *flurbiprofen esters of alpha-cyclodextrins.*



Scheme 2: Synthesis of SD 2.

Flurbiprofen ester of gamma-cyclodextrins was synthesized using the following three steps, 1. synthesis of 2-napthalenesulphonyl gamma-cyclodextrin, 2. synthesis of sodium salt of flurbiprofen and 3. esterification of sodium salt of flurbiprofen.

eutanol:ethanol:water:acetic acid (3:2:3:0.1) and λmax in distilled water: 248 nm, in 0.05 M HCl buffer (pH 1.2): 252 nm and in 0.05 M phosphate buffer (pH 7.4): 258.5 nm. ^{1}H-NMR revealed the presence of signals in ppm at δ=2.55 (2H, CH_2), 3.48 (1H, OH) at and 7 (aromatic protons). Schematic representation for synthesis of SD1 is shown in Scheme 2.

Hydrolysis kinetics:

Hydrolysis of α-CD ester and γ-CD ester was studied in simulated gastric fluid (0.05 M HCl buffer, pH 1.2) and simulated intestinal fluid (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 esters[13]. 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 quoted in Table 1.

Antiinflammatory activity:

Carrageenan-induced rat hind paw oedema method was used for determining antiinflammatory activity[14]. Sprague-Dawley rats of either sex (150-200 g) were taken in groups of six 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. Flurbiprofen (4 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 agent 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.

TABLE 1: HYDROLYSIS KINETICS OF SD 1 AND SD 2 IN RAT FAECAL MATTER.

Time t (min)	% drug released	
	SD 1	SD 2
15	-	7.66
30	15.6	10.6
45	26.4	27.5
60	31.4	39.1
75	37.2	45.3
90	45.2	52.7
105	60.9	68.4
120	73.1	79.3
240	76.5	84.3
24 h	80.2	90.4

The hydrolysis of SD 1 and SD 2 in rata fecal contents were studied with time.

Analgesic Activity:

The analgesic activity was evaluated by Randall Selitto test[15]. 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 a constant rate (a certain number of grams per second). 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 solutions of test compounds were 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 Sellito[15]. 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 the planter surface of the hind paw using 0.1 ml of 1% carrageenan suspension in distilled water. The

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

Compound	Antiinflammatory activity (% inhibition of oedema)			Analgesic Activity (%)	Ulcerogenic activity
	3 h	6 h	24 h		
Control	Nil	Nil	Nil	Nil	Nil
Flurbiprofen	61.5±2.03	50.5±2.01	39.6±2.65	54.4±4.2	57±1.32
SD 1	43.9±1.34	53.0±3.1	43.4±3.44	45.5±2.4	1.4±0.87
SD 2	51.7±.9.71	68.8±1.43	58.5±2.78	58.3±3.11	1.2+0.66

SD 1, SD 2, flurbiprofen (4 mg/kg or equivalent) and vehicle were administered orally to Sprague Dawley rats and the corresponding parameters were measured after specific time intervals after inducing either inflammation or hyperalgesia with carrageenan

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 NSAIDs is increased by exposing the animals to a short period of physical stress (i.e. cold or restraint)[15]. 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.

RESULTS AND DISCUSSION

The studies have revealed that the CD esters of flurbiprofen 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 the drug did not occur in stomach. Ulcerogenicity studies showed that esters are not ulcerogenic (Table 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 (GIT). 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 NSAIDs 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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