Indian Journal of Chemistry
Vol. 46B, July 2007, pp. 1164-1168

Ester prodrugs of flurbiprofen: Synthesis, plasma hydrolysis and gastrointestinal toxicity

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Received 11 August 2006; accepted (revised) 20 February 2007

Nine alkyl ester prodrugs of flurbiprofen have been synthesized with an aim to reduce it's gastrointestinal side-effects. The synthesized prodrugs have been subjected to plasma hydrolysis and gastrointestinal toxicity studies. The chemical structures of the prodrugs have been varied in terms of lipophilicity and reactivity towards hydrolysis. The plasma hydrolysis studies indicate that methyl and propyl prodrugs of flurbiprofen undergo faster hydrolysis as compared to the remaining ester prodrugs. Reduction of ulcer index in rats indicate that *n*-propyl, *iso*-propyl, benzyl and cyclopentyl prodrugs of flurbiprofen are significantly ($p < 0.05$) less irritating to the gastric mucosa as compared to the parent drug, *i.e.*, flurbiprofen.

Keywords: Flurbiprofen, ester prodrugs, DCC coupling, plasma hydrolysis, ulcerogenicity

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in the world[1-3]. Prevalence studies have demonstrated that gastric or duodenal ulcers are present in 15-20% of patients taking NSAIDs chronically [4-9].

The major factor in the development of gastro-intestinal ulcers and haemorrhage induced by NSAIDs is inhibition of prostaglandin synthesis. NSAIDs produce gastrotoxicity by two different mechanisms: a direct contact mechanism and a generalized systemic action, which occurs after absorption[10,11]. The local contact effect, which is unrelated to the pharmacological activity, varies from drug to drug. This effect has been reported to be predominant in case of acidic NSAIDs and hence it's gastrointestinal tolerance can be improved by reducing the factors responsible for the local erosive effects.

Temporary masking of the free carboxylic acid group of NSAIDs can improve their gastrointestinal tolerability[12-14]. The ester prodrugs of flurbiprofen have been designed to achieve this very objective.

Results and Discussions

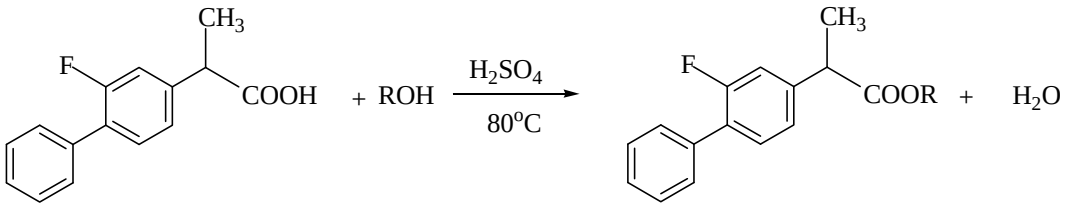
In the present study, the free acidic group of flurbiprofen was temporarily masked by a promoiety so as not to expose stomach's mucosa to this free carboxylic acid group. A series of nine ester prodrugs of flurbiprofen were synthesized. Nine different alcohols were selected for this purpose. The selection was done in such a manner that prodrugs with varying degrees of lipophilicity could be obtained. The nine alcohols that were selected were methanol, ethanol, *n*-propanol, *iso*-propanol, *iso*-butanol, *tert*-butanol, benzyl alcohol, cyclopentanol and cyclohexanol. Two different synthetic procedures were followed in order to carry out esterification of flurbiprofen:

(i) Direct esterification: Three esters (methyl, ethyl and *n*-propyl) of flurbiprofen were synthesized using direct esterification method (**Scheme I**).

(ii) Coupling *via* carbodiimide: Six esters (*iso*-propyl, *iso*-butyl, *tert*-butyl, benzyl, cyclopentyl and cyclohexyl) of flurbiprofen were synthesized using N,N-dicyclohexylcarbodiimide (DCC) as coupling agent (**Scheme II**).

In vitro plasma hydrolysis on synthesized prodrugs

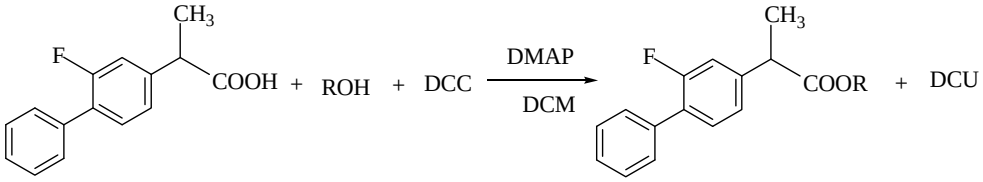
Synthesized prodrugs were subjected to *in vitro* hydrolysis enzymatically as well as non-enzymatically (control) in 0.01 M sodium phosphate buffer (*p*H 7.4) in order to verify their *in vivo* efficacy[15].



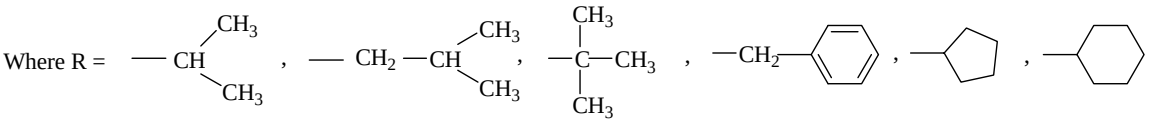
Flurbiprofen Ester Prodrug of Flurbiprofen

Where R = -CH_3, -C_2H_5, -C_3H_7

Scheme I



Flurbiprofen Alcohol Ester Prodrug of Flurbiprofen



Scheme II

Hydrolysis of ester prodrugs were carried out in 50% human plasma in a water bath shaker at 37° ± 2°C. Control incubation for hydrolysis of flurbiprofen in human plasma was carried out in 0.01 M sodium phosphate buffer alone.

The reaction was initiated by adding 50 µL stock solution each of esters and ketoprofen (internal standard) in methanol to 5 mL of 50% human plasma preheated to 37°C. Samples were kept at 37°C and after appropriate time intervals (0 hr, 1 hr, 2 hr, 4 hr and 6 hr) aliquots of 250 µL were withdrawn and mixed with 0.5 mL of 2% zinc sulfate solution. Samples were centrifuged at 3000 rpm for 10 min. The supernatant was analyzed using HPLC. Quantitation of compounds was carried out by measurement of peak area in relation to those of ketoprofen chromatographed under similar conditions. **Table I** shows the results that were obtained at the end of 6 hr.

The results indicate that the ester prodrugs of flurbiprofen were not significantly hydrolyzed into parent drug, *i.e.*, flurbiprofen in the phosphate buffer, *p*H 7.4. This proves that ester prodrugs of flurbiprofen are chemically stable and do not undergo hydrolysis non-enzymatically at *p*H 7.4.

Hydrolysis of prodrugs to flurbiprofen that has occurred in 50% human plasma is solely due to the action of esterases present in plasma.

The hydrolysis rate of flurbiprofen ester prodrugs in 50% plasma prepared in 0.01 M phosphate buffer (*p*H 7.4) was found to be in the increasing order as follows:

iso-Propyl ester < Benzyl ester < *iso*-Butyl ester < Ethyl ester < Propyl ester < Methyl ester.

The data from the hydrolysis study suggests that a decrease in the alkyl chain group of esters results in faster hydrolysis (except ethyl ester). The results that were obtained also indicate that flurbiprofen esters

Table I

Ester Prodrug	Time (hr)	(Flurbiprofen:Ketoprofen)/ (Ester: Ketoprofen)	
		Buffer	50% Human plasma
Methyl	0	0.13	0.12
	6	0.14	0.81
Ethyl	0	0.09	0.05
	6	0.1	0.31
Propyl	0	0.14	0.10
	6	0.14	0.47
iso-Propyl	0	0.00	0.015
	6	0.033	0.035
iso-Butyl	0	0.00	0.00
	6	0.01	0.17
tert-Butyl	0	0.00	0.00
	6	0.00	0.00
Benzyl	0	0.01	0.00
	6	0.01	0.16
Cyclopentyl	0	0.00	0.00
	6	0.00	0.00
Cyclohexyl	0	0.00	0.00
	6	0.00	0.00

Table II

Group	Treatment	Ulcer Index (Mean + SEM)
1	Flurbiprofen	3.07 + 0.63
2	Negative Control	0.37 + 0.37
3	Methyl Ester	1.90 + 0.09
4	Ethyl Ester	3.20 + 0.18
5	Propyl Ester	1.03 + 0.06 *
6	iso-Propyl Ester	0.98 + 0.17 *
7	iso-Butyl Ester	3.57 + 1.10
8	tert-Butyl Ester	1.89 + 0.97
9	Benzyl Ester	0.22 + 0.15 *
10	Cyclopentyl Ester	0.40 + 0.22 *
11	Cyclohexyl Ester	2.49+ 0.49

Values are expressed as Mean + SEM, (n = 6), by unpaired t-test, *($p < 0.05$) vs Flurbiprofen.

prepared with secondary and tertiary alcohols do not undergo hydrolysis in plasma. Since the esters prepared from secondary and tertiary alcohols are not bio-reversible, their role as potential prodrugs is questionable.

It should be noted that the hydrolysis data obtained in this study are of preliminary nature due to experimental limitations. Further work needs to be carried out in order to ascertain the statistical significance of the plasma hydrolysis study for alkyl esters of flurbiprofen. It appears from this preliminary study that methyl and propyl esters of flurbiprofen undergo faster hydrolysis as compared to the other seven esters.

Ulcerogenic Studies

The fasted rat model has been used for comparing ulcerogenic potential of flurbiprofen and it's ester prodrugs in laboratory animals[16]. The animals were divided into groups with 6 animals in each group. Control group was given only 0.5% carboxymethyl cellulose (CMC) suspension. Compounds were administered orally as a 1 mg/kg suspension/ emulsion in 0.5% CMC. The animals were fasted for 24 hr prior to dosing. However, water was given *ad libitum*. The animals were sacrificed 4 hr after dosing. Stomachs were removed and preserved on saline soaked filter paper until inspection. The stomach was cut open along larger curvature and washed with distilled water. The mucus was wiped off and the area of ulcers and stomachs were noted[16-18]. Ulcer Index (UI) was calculated using the following formula[19]:

$$UI = \frac{\text{Area of the ulcer}}{\text{Area of the stomach}} \times 100$$

UI of animals administered with ester prodrugs were compared with that of Flurbiprofen. Results were evaluated using unpaired t-test. The results are given in **Table II**.

Flurbiprofen treated group showed an ulcer index of 3.07. Direct contact mechanism as well as prostaglandin inhibition resulted in this gastrotoxicity.

Propyl, *iso*-propyl, benzyl and cyclopentyl esters of flurbiprofen were capable of significantly reducing gastrotoxicity of flurbiprofen. Reduction in ulcerogenic potential of these prodrugs is due to the temporary masking of free carboxyl group, which prevents direct contact mechanism.

This proves the hypothesis that esterification of acidic group reduces gastrotoxicity. Preparation of esters, a one-step simple reaction, has helped in significantly reducing the ulcerogenic potential of flurbiprofen. Hence, use of alkyl ester prodrugs of flurbiprofen may be considered as a potential alternative to the use of flurbiprofen in order to reduce gastrointestinal adverse effects.

Experimental Section

^{1}H NMR spectra were recorded in $CDCl_3$ on an EL Varian 300 MHz instrument using TMS as internal

standard. Chemical shift values are reported in δ, ppm. All reactions as well as column chromatography were followed by TLC using Merck pre-coated silica gel 60 F_{254} plates and spots were visualized by observing in UV cabinet under short UV. UV-Vis spectroscopic analysis was carried out using UV-Vis spectrophotometer Shimadzu UV 1601. IR spectra were recorded on an FTIR- 8400S instrument in NaCl windows and only the principal absorption level (cm^{-1}) has been listed. HPLC analysis was carried out to determine purity of synthesized compounds. It was also used for analysis of plasma hydrolysis samples. HPLC was carried out using Jasco HPLC with PU-2080 intelligent pump and UV-975 detector. The HPLC software used was Jasco Borwin Chromatograph (1.5 Version). The HPLC column used was RP C-18 (Thermo Electron Corporation, 250×4.6 mm, 5 μm).

General Methods for Synthesis of Ester Prodrugs

(i) Direct esterification

Methyl, ethyl and *n*-propyl esters of flurbiprofen were synthesized using direct esterification method. Flurbiprofen (0.004 mols) and the respective alcohol (0.25 mols) were taken in a dry round bottom flask fitted with a condenser. 1 mL of concentrated H_2SO_4 was gradually added to this reaction mixture. The reaction mixture was refluxed at 80°C, until esterification was complete. The progress of reaction was monitored by thin layer chromatography.

The reaction mixture was extracted with dichloromethane (30 mL). The organic layer was washed with 1% NaOH (30 mL). This was followed by water washings (30 mL×3). The organic layer was dried over anhydrous sodium sulfate. DCM was removed by distillation and the crude product was purified by column chromatography using silica gel as stationary phase and chloroform as eluent.

(ii) Coupling *via* carbodiimide

iso-Propyl, *iso*-butyl, *tert*-butyl, benzyl, cyclopentyl and cyclohexyl esters of flurbiprofen were synthesized by coupling reaction using dicyclohexylcarbodiimide (DCC). A solution of flurbiprofen, (6.7 mmols), N,N-dicyclohexylcarbodiimide (7.4 mmols), respective alcohol (7.4 mmols) and dimethylaminopyridine (DMAP, 0.7 μmol) in dichloromethane (30 mL) was taken in a dry round bottom flask. The reaction mixture was stirred at RT using a magnetic stirrer until esterification was complete. The progress of reaction was monitored by thin layer chromatography.

After completion of the reaction, N,N-dicyclohexylurea (DCU) was filtered off and filtrate was washed with water (50 mL×2), 5% acetic acid solution (50 mL×2), 1% NaOH solution (50 mL×2) and again with water (50 mL×3). The organic layer was then separated and dried over anhydrous sodium sulphate. DCM was removed by distillation and the crude product was purified by column chromatography using silica gel as stationary phase and chloroform as eluent[20,21].

Methyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 47.16%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.5 (d, 3H, >CH-CH_3), 3.6 (m, 3H, -OCH_3), 3.7 (q, 1H, >CH-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.68 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1737.74 cm^{-1} (-C=O).

Ethyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 57.7%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.2-1.3 (t, 3H, -CH_2-CH_3), 1.5 (d, 3H, >CH-CH_3), 3.7 (q, 1H, >CH-CH_3), 4.0-4.5 (m, 2H, -O-CH_2-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.70 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1731.96 cm^{-1} (-C=O).

Propyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 55.61%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 0.8-1.0 (t, 3H, -CH_2-CH_2-CH_3), 1.5 (d, 3H, >CH-CH_3), 1.6-1.8 [m, 2H, -CH_2-CH_2-CH_3], 3.7 (q, 1H, >CH-CH_3), 4.0-4.2 (t, 2H, -O-CH_2-CH_2-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.71 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1731.96 cm^{-1} (-C=O).

***iso*-Propyl 2-(2-fluoro-4-biphenylyl)propionate**

Yield 32.3%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.1-1.3 [m, 6H, O-CH-$(CH_3)_2$], 1.5 (d, 3H, >CH-CH_3), 3.7 (q, 1H, >CH-CH_3), 4.9-5.1 (s, 1H, O-CH-$(CH_3)_2$), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.70 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1730.03 cm^{-1} (-C=O).

***iso*-Butyl 2-(2-fluoro-4-biphenylyl)propionate**

Yield 35.7%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 0.8-0.9 [d, 6H, -CH_2-CH-$(CH_3)_2$], 1.5 (d,

3H, >CH-CH_3), 1.8-2.0 [m, 1H, -CH_2-CH-$(CH_3)_2$], 3.7 (q, 1H, >CH-CH_3), 3.8-4.0 [d, 2H, -CH_2-CH-$(CH_3)_2$], 7.1-7.6 (m, 8H, aromatic protons); R_f 0.73 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1733.89 cm^{-1} (-C=O).

tert-Butyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 28.9%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.5 (d, 3H, >CH-CH_3), 1.5-1.7 [m, 9H, -O-C-$(CH_3)_3$], 3.7 (q, 1H, >CH-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.76 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1731.96 cm^{-1} (-C=O).

Benzyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 48.4%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.5 (d, 3H, >CH-CH_3), 3.7 (q, 1H, >CH-CH_3), 5.1-5.2 (m, 2H, -CH_2-Ar), 7.1-7.6 (m, 13H, aromatic protons); R_f 0.71 (chloroform); UV-Vis (methanol): λ_{max} 247 nm with slight shouldering at 273-279 nm; IR (NaCl): 1734.85 cm^{-1} (-C=O).

Cyclopentyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 51.2%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.3-2.0 (m, 9H, -O-Cyclopentyl), 1.5 (d, 3H, >CH-CH_3), 3.7 (q, 1H, >CH-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.8 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1729.06 cm^{-1} (-C=O).

Cyclohexyl 2-(2-fluoro-4-biphenylyl)propionate

Yield 50.3%. Pale yellow aromatic oil. ^{1}H NMR ($CDCl_3$): δ 1.0-2.0 (complex spectra, 11H, -O-Cyclohexyl), 1.5 (d, 3H, >CH-CH_3), 3.7 (q, 1H, >CH-CH_3), 7.1-7.6 (m, 8H, aromatic protons); R_f 0.9 (chloroform); UV-Vis (methanol): λ_{max} 247 nm; IR (NaCl): 1728.10 cm^{-1} (-C=O).

Conclusions

The fact that prostaglandin synthesis inhibition is implicated both in pharmacological and ulcerogenic activity of NSAIDs makes prodrug designing a very tricky affair. The prodrug should hydrolyze in a manner, which prevents accumulation of active drug in gastric mucosa, but maintains pharmacological activity. The difference in susceptibility of the prodrug hydrolysis to enzymes present in gut, plasma and other biological tissues further complicates the problem. A very rapid hydrolysis kinetics within the biological system is certainly not the answer as build-up of flurbiprofen in gastric mucosa might still occur. A controlled hydrolysis kinetics, which helps the prodrug cross the mucosa in intact form, followed by elicitation of pharmacological activity with reduced gastrotoxicity, provides the answer. The role of propyl 2-(2-fluoro-4-biphenylyl)propionate in this context calls for further extensive studies.

Acknowledgements

The authors are grateful to FDC (Pvt.) Ltd., Mumbai and Taurus Laboratories Pvt. Ltd., Ahmedabad for providing gift samples of flurbiprofen. The authors would also like to thank Dr. Krishna R Iyer, Asst. Professor in Pharmaceutical Chemistry, Bombay College of Pharmacy, and Dr. D. P. Chaudhary of Haffkines Institute, for their useful suggestions and help.

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