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HYDROLYSIS KINETIC STUDIES OF MUTUAL PRODRUGS OF DICLOFENAC SODIUM

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ABSTRACT

Mutual prodrugs of diclofenac with paracetamol, salicylamide and N-Mannich bases of salicylamide were synthesized with a view to reduce its ulcerogenic potential, which is attributed to the presence of free –COOH group and inhibition of endogenous prostaglandins. Therefore, blocking this group by synthesizing functional derivatives carboxylic acid, may reduce these side effects. *In vitro* hydrolysis of the prodrug in hydrochloric acid buffer pH 1.2 indicated that it was resistant to acidic hydrolysis and hence, may not produce irritation of the gastrointestinal tract, where as in phosphate buffer (pH 7.4), it is hydrolyzed following first order kinetics, releasing free drug and linker moiety.

Key words : Diclofenac sodium, Prodrug, Hydrolysis kinetics.

INTRODUCTION

Gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs[1]. The reactions range in both; severity and frequency from relatively mild to the more serious and in some cases, it may develop life threatening states, which lead to GIT ulceration and hemorrhages[2, 3]. The development of GIT ulceration and hemorrhages induced by NSAIDs is due to the inhibition of prostaglandin synthesis. Endogenous prostaglandins are known to have a cytoprotective action on gastric mucosa[4]. Prostaglandins regulate acid secretion and maintain mucosal integrity against stress, variety of chemicals and thermal injury.

NSAIDs are used for long term therapy of arthritis and other rheumatic diseases. There is always a need for safer NSAIDs and efforts are going on for developing safer NSAIDs. The prodrug approach is one of the most promising amongst these[5, 6]. In recent

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years, more emphasize is on design and development of mutual prodrugs, which involves combining of two different pharmacophore to give synergistic action. The gastric irritancy of diclofenac is attributed to the presence of free –COOH group and inhibition of endogenous prostaglandins. Therefore, blocking this group by synthesizing functional derivatives carboxylic acid may reduce these side effects[7].

The present work is aimed at concept of drug design through conjugation of two different pharmacophore having synergistic action. The physicochemical properties of a drug play a major role in the design, development of formulations and bioavailability. Therefore in addition to characterization of prepared structures, the physicochemical parameter like dissolution rates of prodrugs was studied.

EXPERIMENTAL

Materials and methods

All chemicals and solvents used were of General grade, obtained from Loba Chem (India) Ltd. All the solvents used in these studies were dried and purified, if necessary before use. Diclofenac sodium and paracetamol was procured as a gift sample from Wochardt Ltd (Aurangabad, Maharashtra, India), Melting points were determined using DBK programmed melting point apparatus and are uncorrected, Infrared spectra were recorded on FTIR 8400, Shimadzu corporation (Kyoto, Japan). Mass spectra was recorded on Jeol SX-120 mass spectrophotometer. Proton nuclear Resonance spectra were recorded with Burker DRX 300 (300 MHz FT NMR) using $CDCl_3$.Dissolution studies was carried out by dissolution test apparatus model No. DA-3 Veego Scientific Devices, (Mumbai, India).

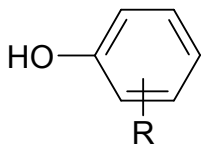
Synthesis of 2-[(2, 6-dichlorophenyl) amino] benzene acetyl chloride (II)

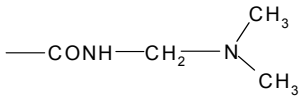
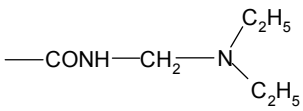
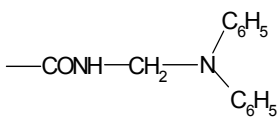
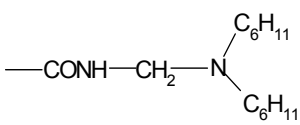
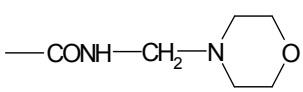
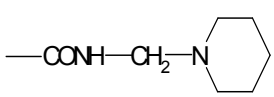
2-[(2, 6-dichlorophenyl) amino] benzene acetic acid, (2.96 g; 0.01 mole) was taken in a round bottom flask and dichloromethane (6.5 mL) and dimethylformamide (830 µL; 0.01 mol) were added to it to form a suspension. To this suspension, oxalyl chloride (2.53 g; 0.01 mole) in 10 mL dichloromethane was added to form a clear solution. It was then stirred for 30 min at room temperature using a magnetic stirrer. The solution is concentrated to give yellow solid of acid chloride of diclofenac, which was used for further reaction without purification. M. p. 109-111^{0}C, yield 76% and R_f value 0.83 (ethyl acetate : methanol : ammonia, (9 : 1 : 0.1) as mobile phase).

General method for synthesis of substituted hydroxyl benzamide derivatives (IIIb-g)

Appropriate secondary amines (0.01 mole) were gradually added to the solution of 2-hydroxy benzamide, (1.374 g, 0.01 mole) in methanol (12 mL), followed by addition of 38 % formaldehyde solution (0.8 mL, 0.01 mole). The reaction mixture was stirred for 1 hr at room temperature and allowed to stand overnight at 0^0C. Then precipitates were filtered, dried and recrystallized using ethanol. The titled compounds **(III b-g)** were prepared as per the procedure by Bahekar and Gaikwad[8].

Table 1 : Physico-chemical data for IIIb – g

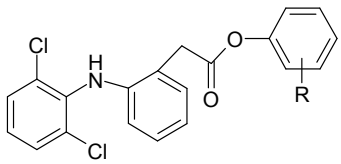


Compound	R	M. P. (^{0}C)	Yield (%)	R_f value
IIIb		135 - 137	52.71	0.931
IIIc		132 - 135	60.50	0.850
IIId		110 - 112	68.76	0.895
IIIe		80 - 83	55.40	0.910
IIIf		110 - 115	72.60	0.583
IIIg		90- 95	55.40	0.750

General procedure for synthesis of diclofenac esters (IV) (Scheme 1)

To the solution of 2-[(2, 6-dichlorophenyl) amino] benzene acetyl chloride **(II)** (3.14 g; 0.01 mole) in dry pyridine (30 mL), appropriate substituted hydroxybenzamide derivatives **(IVa-h)** (0.01) mole were added dropwise with constant stirring. Reaction mixture was allowed to stand overnight. Pouring it to crushed ice precipitated product and the solid obtained was filtered, washed with water and recrystallized from the ethanol. Physicochemical and spectral data for **(IVa-h)** are depicted in Table 2.

Table 3 : Showing K values and calculated half life of prodrugs



Time (hr)	pH 1.2		pH 7.4	
	K value	$t_{1/2}$ (hr)	K value	$t_{1/2}$ (hr)
Ia	0.1069	6.48	0.1785	3.88
Ib	0.144	4.81	0.1928	3.59
Ic	0.1186	5.84	0.1771	3.91
Id	0.1534	4.51	0.1873	3.69
Ie	0.1156	5.99	0.2078	3.33
If	0.0914	7.57	0.1283	5.39
Ig	0.1087	6.37	0.147	4.71
Ih	0.1025	6.75	0.1147	6.03

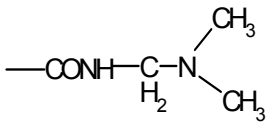
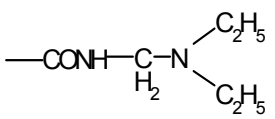
Procedure for synthesis of diclofenac amide (V) (Scheme 2)

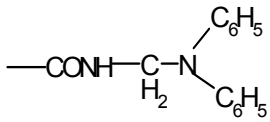
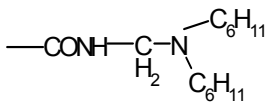
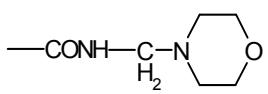
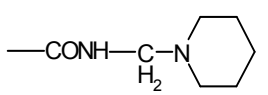
2-[(2, 6-dichlorophenyl) amino] benzene acetyl chloride (II) (3.14 g; 0.01 mole) was dissolved in the acetone (5 mL) and the solution was added dropwise to well stirred ice cooled liquid ammonia solution (20% W/V) for 30 min. The amide of diclofenac **(V)** was filtered and recrystallized from water-ethanol mixture (40 : 60 v/v), melting point 120-121 ^{0}C, yield 80% and R_f 0.63.

Method for synthesis of diclofenac amide Mannich base prodrugs (VI, Scheme 2)[9]

The amide **(V)** (2.95 g; 0.01 mole) para formaldehyde (2 g) and morpholine / piperidine (0.01 mole) were taken in 250 mL round bottom flask. Absolute alcohol (20 mL) and hydrochloric acid (1 mL) were then added to the flask. The content was refluxed for 2-3 hrs and allowed to cool. Acetone was slowly added to precipitate the product. The product was recrystallized from acetone/methanol mixture (20 : 80 v/v). Physicochemical data for **(VI a-b)** are given in Table 3.

Table 3: Physicochemical and spectra data

Comp.	R	M. P. (ºC)	Yield (%)	R_f value	^{1}H NMR (δ ppm), Mass, IR (cm^{-1}, KBr)
IVa		150-152	65	0.47	1.258 (s, 3H, CH_3), 3.771 (s, 1H, ArNH), 6.383 – 6.409 (d, 1H, CONH), 7.065 -7.114 (t, ArH), 7.176 – 7.252 (q, ArH), and 7.324-7.398 (m, ArH). 3420 (N-H Str), 2910.81 (C-H Str), 1731.96 (C=O Str). m/z 414
IVb		140-142	60	0.78	1.256 (s, 3H, CH_3), 6.502 – 6.531 (q, 1H, CONH), 7.347 – 7.548 (m, ArH). 3452.48 (N-H Str), 2918.50 (C-H Str), 1669.38 96 (C=O Str), m/z-472
IVc		145-146	70	0.82	1.248 – 1.279 (d, 2H CH_2), 3.779 (s, 1H, Ar_2NH), 6.386 – 6.412 (d, 1H CONH), 7.072 – 7.653 (m, ArH). 3410.40 (N-H Str), 1679.30 (C=O Str), m/z – 500

Comp.	R	M. P. (ºC)	Yield (%)	R_f value	^{1}H NMR (δ ppm), Mass, IR (cm^{-1}, KBr)
IVd		172-173	82	0.85	0.812-0.941 (d, 6H), 1.245 (s, 2H CH_2), 3.779 (s, 1H, Ar_2NH), 6.386-6.412 (d, 1H, CONH), 7.172-7.579 (m, ArH). 3325.12 (N-H Str), 2920.05 (C-H Str), 1696(C=O Str). m/z-596.
IVe		150-152	55	0.90	1.254-1.285 (d, 2H CH_2), 3.784 (s, 1H, Ar_2NH), 6.476-6.528 (m, 1H, CONH), 7.238-7.264 (m, ArH). 3395.65(N-H Str), 2508.30 (C=O Str), 1735.42 (C=O Str). m/z-607
IVf		162-168	55	0.66	2906.44(C-H Str), 1738.54 (C=O Str), 1618, 1572.00, 1462.23, 1410.28, 1308.62, 1240.51, 1165.21, 749.60, 669.25
IVg		138-140	72	0.85	3436.10 (N-H Str), 3060.24 (C-H Str), 1738.62 (C=O Str) 1623.82, 1554.55, 1454.23, 1309.80, 1153.07, 1183.51, 750.13, 669.25.

Cont...

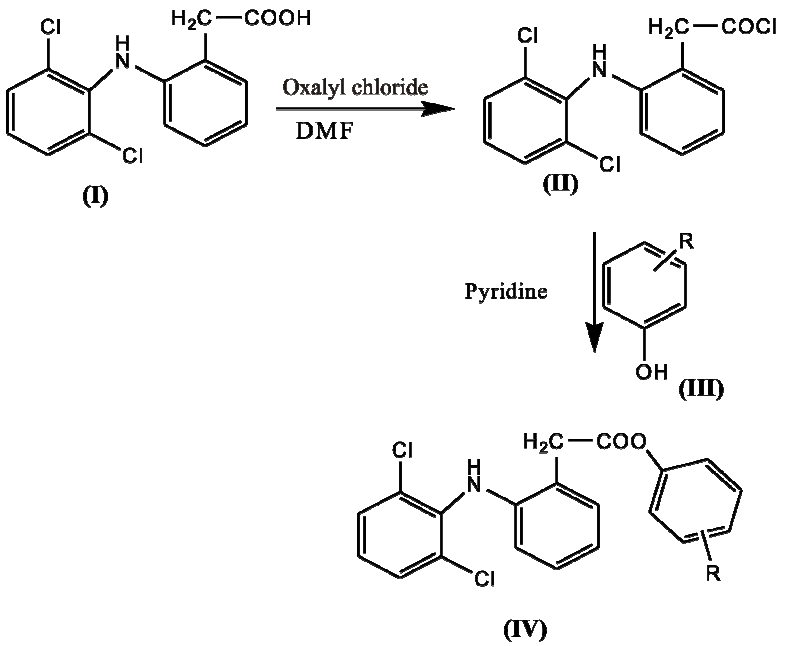
Comp.	R	M. P. (ºC)	Yield (%)	R_f value	^{1}H NMR (δ ppm), Mass, IR (cm^{-1}, KBr)
IVh	—NHCO—CH_3	167-169	69	0.54	1.217-1.257 (t 2H CH_2), 3.395 (s, 1H, Ar_2NH), 6.382-6.424 (d, 1H, CONH), 7.252 (s, ArH). 3400.36 (N-H Str), 2925.80 (C-H Str), 1699.17 (C=O Str). m/z-429

Dissolution rate studies[10, 11]

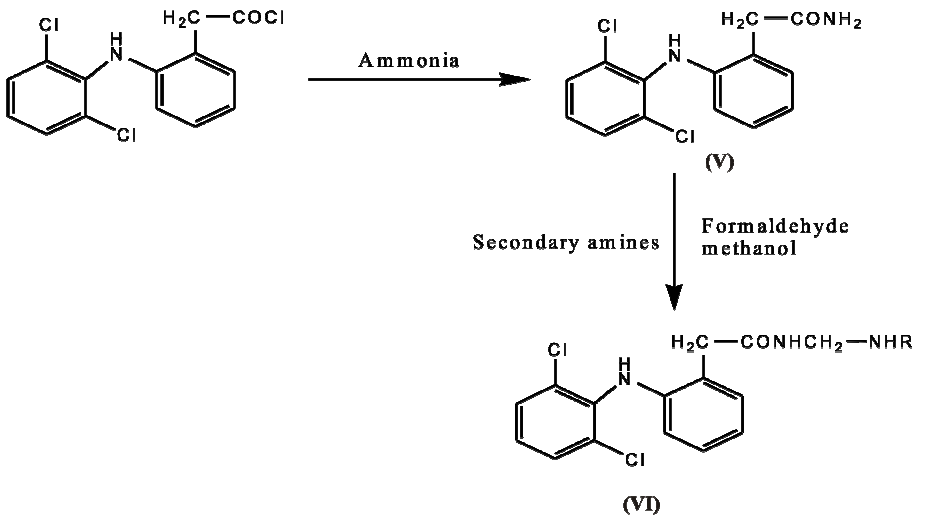
In vitro dissolution studies of synthesized prodrugs were carried out in DA-3, Veego Scientific Devices (Mumbai, India) dissolution rate apparatus (six stations). Drug pellets (10 mg) were prepared using hydraulic pressure (Shimadzu, Japan) by compressing at 8 ton/in^2 pressure for two minutes. The pellets were placed in the wire basket and suspended in the vessel containing 900 mL of dissolution medium at 37 ± 1^{0}C. The dissolution media were hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4). The hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) were prepared as per Indian Pharmacopoeia 1996.The baskets were rotated at 100 rpm; 5 mL of the sample was withdrawn at each time interval and replaced with equal volume of fresh dissolution medium. Prodrug concentration in the sample was determined by the UV spectrophotometer by transferring the withdrawn sample to separating funnel containing ethyl acetate (5 mL). Free diclofenac was released after hydrolysis and it was estimated at 276 nm. The release profile was plotted as percentage drug dissolved Vs time. The values of dissolution rate (K) and the time to dissolve 50 % drug ($t_{50\%}$) are given in Table 3.

RESULTS AND DISCUSSION

Mutual prodrugs of diclofenac with paracetamol, salicylamide and N-Mannich bases of slicylamide were synthesized. Thin layer chromatography was performed on pre-coated silica gel G glass plate to ascertain the purity of these compounds. The structures of the synthesized compounds were characterized by spectral (IR, NMR and MS) data and elemental analysis. Elemental analysis of the compounds was fond to be within permissible limits.



Scheme 1.General scheme for synthesis of diclofenac ester prodrug :



Scheme 2.General scheme for synthesis of diclofinac amides Mannich base prodrug

The hydrolysis studies conducted at selected pH values (1.2 and 7.4) at 37 ± 1^0C provided useful information relating to the likely stability of the compounds in the

gastrointestinal tract. Graphs were plotted with time in hours on X-axis and log of amount remaining (a - x) on Y-axis. From the pH rate profile, it was found that all the synthesized compounds are stable at low pH value (pH 1.2), while they undergo hydrolysis as the pH was increased (pH 7.4). This indicates that the synthesized compounds will be hydrolyzed and subsequently absorbed through intestine. At pH 1.2, the $t_{1/2}$ of the compounds was in between 4.51–12.0 hr, while at pH 7.4, the $t_{1/2}$ were in between 3.3–6.0 hr.

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