SHORT NOTES

A FACILE SYNTHESIS OF MUTUAL PRODRUG OF DICLOFENAC SODIUM AND PARACETAMOL AND ITS PREFORMULATION STUDIES

ABSTRACT

Mutual prodrug was synthesized by esterifying diclofenac with paracetamol. *In vitro* hydrolysis of prodrug in HCl buffer (pH 1.2) and phosphate buffer (pH 7.4) showed that the drug was released more in pH 7.4. The purity of the prodrug was confirmed by TLC and characterized on the basis of IR spectroscopy and ^{1}H NMR spectroscopy. The physiochemical parameters were determined and the results showed that they are more lipophilic than the parent drug. The compound was also evaluated for anti-inflammatory and ulcerogenicity.

Keywords: Diclofenac, Paracetamol, Prodrug, Anti-inflammatory, Anti-ulcerogenic.

INTRODUCTION

A therapeutically significant drug may have limited utilization in clinical practice because of certain factors like poor organoleptic properties, poor bioavailability, short duration of action, non-specificity, incomplete absorption, poor aqueous solubility, high first-pass metabolism or other adverse effects. So a new method has to be used to improve the therapeutic efficacy by some modification. Mutual prodrug, is a type of carrier-linked prodrug, consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and *vice versa.* Mutual prodrug design is not really different from the general drug discovery process, is a unique substance which is observed to have desirable pharmacological effects, and studies of its properties lead to the design of better drugs. It is a better area of research, and its introduction in human therapy has given successful results in improving the clinical and therapeutic effectiveness of drugs suffering from some undesirable properties that otherwise hinder their clinical usefulness. Sometimes, an adequate pharmaceutical formulation can overcome these drawbacks, but often the galenic formulation is inoperant and a chemical modification of active molecule is necessary to correct its pharmacokinetic insufficiencies.

Diclofenac a widely used non steriodal anti-inflammatory drug (NSAID) is associated with major gastrointestinal side effects. The reaction range in both severity and frequency from relatively mild to the more serious conditions and in some cases it may develop life threatening states which lead to gastrointestinal tract ulceration and haemorrhage. The development of gastrointestinal tract ulceration and hemorrhage induced by NSAIDs is due to the inhibition of prostaglandin synthesis, as they have cyto-protective action on gastric mucosa. It also helps in the regulation of acid secretion and maintains mucosal integrity against stress, variety of chemicals and thermal injury[1, 2].

The gastric side effects of diclofenac are attributed due to the presence of free COOH group and the inhibition of endogenous prostaglandins. Therefore the possible way to solve this problem is to convert the carboxylic function to produce the prodrug with adequate stability at the acidic pH of stomach. Thus, this derivatisation may prevent local irritation of stomach mucosa and it is capable of releasing the parent drug spontaneously or enzymatically in the blood following its absorption[3-6].

The present article takes a review of various applications of mutual prodrugs and the developments in this field during the last few decades. Thus the present work is focused on the synthesis, physiochemical characterization, *in vitro* hydrolysis

and biological evaluation of mutual prodrug of diclofenac and paracetamol for the possible use with less or no gastric side effects.

MATERIALS AND METHODS

All melting points were determined in open capillary tube and are uncorrected. The IR spectra were recorded on Perkin-Elmer BXF FT-IR spectrophotometer. The ^{1}H NMR spectra (DMSO-d_6) were scanned on a Brucker AMX-400 spectrophotometer using TMS as internal standard and chemical shifts are expressed in δ ppm. Purity of the synthesized compounds was checked by TLC using silica gel-G as adsorbent and visualization was accomplished by iodine vapours.

Synthesis of Acid Chloride of Diclofenac (2)

0.05 mol of diclofenac (1) was taken in a beaker and 10 mL of thionyl chloride was added. This mixture was heated on waterbath with continuous stirring until evolution of sulphur dioxide and hydrochloric acid was completed. The resultant acid chloride was cooled and the excess of thionyl chloride was removed under reduced pressure. mp. 115-118^0. IR values (KBr, cm^{-1}): 3319 (N-H), 1694 (C=O), 1360 (C-N), 920 (C-Cl). ^{1}H NMR:δ 4.12(s, 2H, CH_2), 6.67 (s, 1H, NH), 6.98-7.28 (m, 7H, ArH) (Scheme 1).

Synthesis of Mutual Prodrug of Diclofenac and Paracetamol (3)

Equimolar quantities of diclofenac acid chloride and paracetamol was dissolved in 1, 4-dioxan and 10% sodium hydroxide solution respectively. The solution of acid chloride was added dropwise to the solution of paracetamol with continuous stirring for 1 h. The reaction mixture was stirred continuously for another 1 h and then 50% hydrochloric acid was added dropwise with continuous stirring so that the prodrug precipitates in the form of hydrochloride salt. mp. 174-176^0. IR values (KBr, cm^{-1}): 3323 (N-H), 1691 (C=O), 1351 (C-N), 1294 (C-O), 937 (C-Cl). ^{1}H NMR:δ 3-4 (s, 3H,CH_3), 6.54-6.58 (s, 2H, CH_2), 6.67-6.81 (d, 2H, NH), 6.94-7.36 (m, 11H, ArH).

Determination of Partition Coefficient

The partition coefficient of the prodrug was determined in two systems i.e., chloroform-hydrochloric acid buffer (pH 1.2) and chloroform-phosphate buffer (pH 7.4) at 25^0. The synthesized compound (100 mg) was added to 50 mL of aqueous phase (buffer 1.2, 7.4) and 50 mL of organic phase was added to it. The system was then shaken vigorously for 1 h and allowed to stand for 2 h for the separation of two phases. Prodrug concentration in hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) was determined by UV spectrophotometer at 276 nm for free diclofenac released after the hydrolysis of prodrug. Results of partition coefficient are shown in Table I. The partition coefficient was calculated as, partition coefficient=concentration of drug in organic phase/that in aqueous phase.

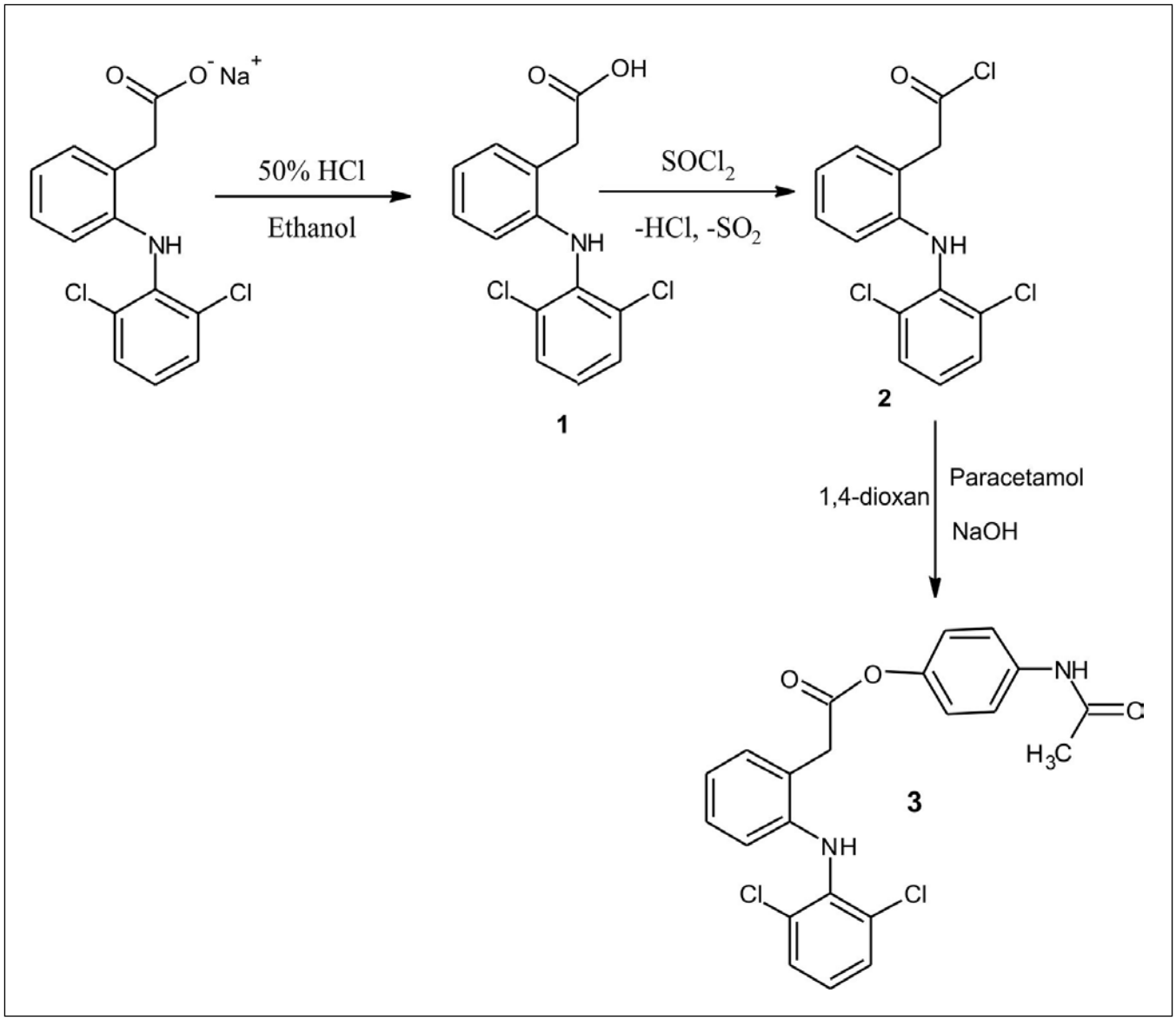
$$Cx = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

Dissolution Study

In vitro dissolution studies of the synthesized prodrug and diclofenac sodium was carried out in Labindia dissolution rate apparatus (IP Apparatus-1). Drug granules and prodrug granules were prepared using starch paste (10% w/v) as granulating fluid. The granules were suspended in the vessel containing 900 mL of dissolution medium at 37±0.5^0.

The dissolution media used 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 I.P[7]. The paddles were rotated at 100 rpm. Five milliliters of the sample were withdrawn at each time interval and replaced with equal volume of fresh dissolution medium.

The collected samples were suitably diluted with the dissolution medium and analyzed for the content of drug released by using UV-Visible spectrophotometer. Each granule was subjected to dissolution study two times and the mean values were taken. Results were shown in Table I. The dissolution rate profile of prodrug and diclofenac sodium is mentioned in Fig. 1.



Scheme 1

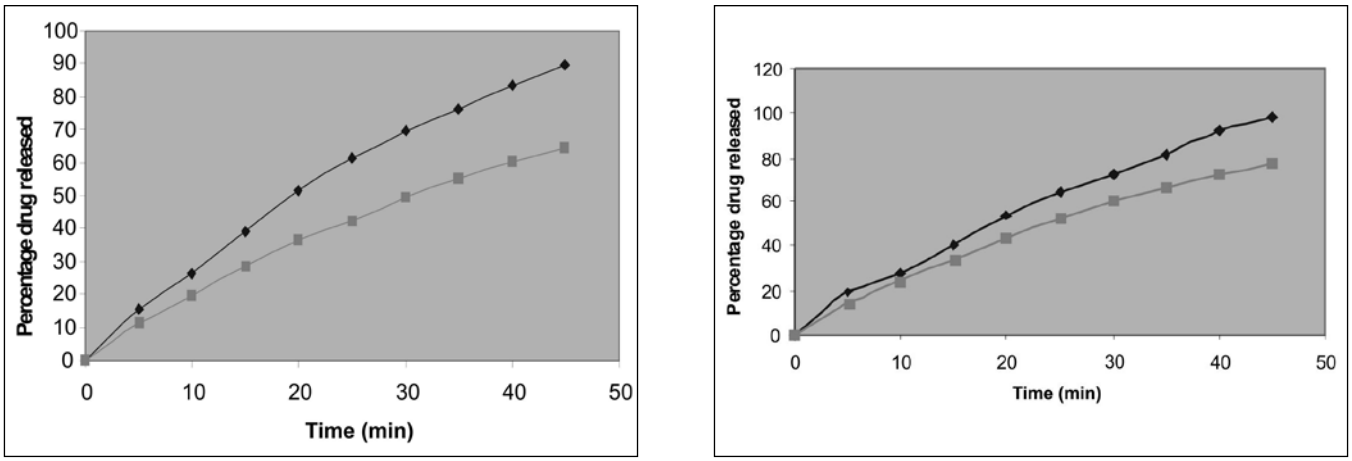
Table I: Dissolution rate studies of diclofenac sodium and mutual prodrug

Drug	Partition coefficient		pH	r	K	$t_{50\%}$ (min)
	$CHCl_3$-HCl buffer (pH 1.2)	$CHCl_3$-PO_4 buffer (pH 7.4)				
Diclofenac sodium	3.084	7.89	1.2	0.9997	0.022 min^{-1}	30.242
			7.4	0.9973	0.032 min^{-1}	21.084
Prodrug	0.859	5.072	1.2	0.9856	1.387 mg min^{-1}	25.233
			7.4	0.9124	1.503 mg min^{-1}	23.275

r = the correlation coefficient, k = the dissolution rate, $t_{50\%}$ = the time to dissolve 50% drug and prodrug is the conjugate of diclofenac and paracetamol

Table II: Anti-inflammatory activity and ulcer index of the mutual prodrug and diclofenac sodium

Treatment	Dose (mg/kg)	Mean % inhibition of paw volume				Mean ulcer index
		2 h	4 h	6 h	8 h	
Diclofenac sodium	4.5	27.00 ± 2.12	53.33 ± 2.43	76.08 ± 2.42	76.66 ± 2.06	6.3±0.49
Prodrug	6.3	24.16 ± 2.27	65.83 ± 1.72	81.33 ± 2.1	84.00 ± 2.48	1.4±0.23



(a) (b)

Fig. 1: Dissolution rate profile of prodrug and diclofenac sodium

In pH 1.2(a) and pH 7.4(b), the percentage drug released for prodrug (—♦—) and diclofenac sodium (—♦—)

Pharmacological Evaluation

Adult male wistar rats, weighing 150-260 g obtained from the animal house of Bapatla College of Pharmacy (1032/ac/07/CPCSEA); Bapatla, were maintained at a constant temperature of 26±2^0 and humidity 30-40% with 12 h light/dark cycle, throughout the experiments. The rats were fed with commercial rat feed and sterile water was given *ad libitum*. The experimental protocol was aproved (IAEC/II/BCOP/07-08) by Institutional Animal Ethics Committee (IAEC) of Bapatla College of Pharmacy; Bapatla and was in accordance with the guidelines of the CPCSEA.

Anti-inflammatory Activity[8]

Adult male wistar rats (150-260 g) were incorporated in the study. The animals were divided into two groups of six animals each. The groups were fasted overnight. The group 1 animals were treated with diclofenac sodium 4.5 mg/kg and group 2 animals were treated with its prodrug form 6.3 mg/kg orally. The animals were administered with the phlogistic agent carrageenan 1% (0.1 mL) in the subplantar region 1 h after oral administration of drugs. The paw volume was measured by mercury displacement method and the percent inhibition was calculated and were given in Table II.

Anti-ulcerogenic Potential[8]

The group subjected to anti-inflammatory evaluation were sacrified at the end of 8th h. The stomach was cut open along the greater curvature and was washed with distilled water. The ulcers were observed under magnification and the scores were given in Table II.

RESULTS AND DISCUSSION

Mutual prodrug in the form of diclofenac with paracetamol was synthesized. The structure of the prodrug was confirmed by infra-red spectroscopy and ^{1}H NMR spectroscopy. The results obtained were in accordance with the expected structure. The solubilities of the compound were determined in water, methanol, ethanol, chloroform, acetone, diethyl ether. Among the various solvents stated, prodrug has given a good solubility in organic solvents than to the polar solvents. Hydrolytic kinetics of the synthesized compounds showed that they did not undergo hydrolysis in hydrochloric acid buffer (pH 1.2) indicating that they are inert in acidic pH of the stomach. The prodrug is hydrolysed in the phosphate buffer (pH 7.4) with the zero order kinetics with the half life of 23.275 min.

The partition coefficient of the prodrug was determined in two systems, i.e.,chloroform/ hydrochloric acid buffer (pH 1.2) and chloroform/ phosphate buffer (pH 7.4) and it has shown high partition coefficient value in the chloroform/ hydrochloric acid buffer (pH 1.2). Therefore it confirms that the prodrug is more lipophilic than the parent drug.

From the Table II, it shows that the anti-inflammatory activity for the prodrug has given better activity than diclofenac. It also reveals that ulcerogenic activity produced by the prodrug is negligible when compared to diclofenac.

ACKNOWLEDGMENT

Principal and the management of Bapatla educational trust for providing all the necessary facilities in our work.

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Department of Pharmaceutical Chemistry
Bapatla College of Pharmacy
Bapatla, Guntur District-522101
Andhra Pradesh.
E-mail: vishak_dr@yahoo.co.in

Visagaperumal D.*, Mayuren C., Anbalagan N., Raghuveer Varma P., Srinivas B., Shanker Bontha V. B. and Ravikumar G.

*For correspondence

(Received 06 July 2010) (Accepted 05 July 2012)