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Ciprofloxacin Prodrug Via Mannich Reaction

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A prodrug of ciprofloxacin was prepared using Mannich reaction, refluxing ciprofloxacin as amine component with p-hydroxyacetophenone and aqueous formaldehyde in 1,2-dimethoxyethane for 30 h. The prodrug (mp. 230-232°) had increased solubility and dissolution rate over the parent drug. The pH-rate profile and *in vitro* antimicrobial activity of the prodrug were also studied.

The prodrug approach through formation of Mannich base has long been used to improve the solubility and dissolution rate of drugs[1]. However this approach has been mainly used for drugs having an active hydrogen viz., amides, amides and CH-acidic compounds. Such drugs are reacted with formaldehyde and an amine to give a Mannich base. In the present work, a prodrug of ciprofloxacin (CFLX) has been synthesized using it as an amine and condensing it with p-hydroxyacetophenone in presence of aqueous formaldehyde. CFLX is a widely used broad spectrum fluoroquinolone antibacterial drug and its hydrochloride or lactate salts are used in therapy[2].

EXPERIMENTAL

CFLX and CFLX.HCl.H_2O were gifts from Ranbaxy Research Laboratories (New Delhi). Other chemicals and solvents used were of reagent grade and obtained from commercial sources. Ultraviolet spectral measurements were performed with a Bausch and Lomb Spectronic 21 and a Beckman DU-64 double beam spectrophotometer. IR spectra were done using the KBr disc technique on a 5-DX Nicolet machine. ^{1}HNMR spectra were obtained using Brucker 300 MHz spectrometer. The mass spectra were recorded on Jeol JMS D-300. A Perkin Elmer 240 C elemental analyser was used For C,H,N analysis. An Electrolab TDT-6 tablet dissolution apparatus was used for dissolution studies.

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Synthesis of Mannich Base

CFLX.HCl.H_2O (0.036 mole) was added to a mixture of p-hydroxyacetophenone (0.01 mole), aqueous formaldehyde 37% w/v (0.02 mole and 70 ml of 1,2-dimethoxyethane (as solvent and refluxed for 30 h. The solvent was evaporated under reduced pressure and the residue was poured into dilute hydrochloric acid. It was extracted with ether to remove any unreacted ketone. The mixture was made alkaline with sodium bicarbonate and the mannich base was extracted with ether. The product obtained upon evaporation of ether extract was recrystalised from dimethyl formamide as needle shaped crystals. Yield 72%, m.p. 230-232°.

$C_{26}H_{26}FN_3O_5$ requires, C 65, 14; H, 5.43; N, 8.77; Found C, 65.6; H, 5.40; N, 8.64. IR(KBr) broad band at 3515 (OH), 3100 (COOH) and strong band at 1750 (C=O), 1490 and 1220 cm^{-1}. ^{1}HNMR (in TFA) 9.23 (1H, br. s. H-2), 8.30 (1H, m, H-2"), 7.93 (2H, m. H-3", H-5"); 7.90, (1H, br, s, H-8), 7.86 (1H, br. s. H-5), 7.03 (1H, m. H-6") 4.23 (1H, br. s. H-1"), 4.08 (2H, br. s. H_2-2"), 3.80 (6H, br. s. H_2-3', H_2-5, H_2-6'), 3.53 (2H, br. s. N-CH_2), 3.20 (2H, br. s. $COCH_2$), 1.63 (2H, br. s. H_2-2"), 1.40 (2H, br. s. H_2-3"), Mass spectrum m/z 479 (M^+) n.o., 462 (M^+-OH); 446 (462-OH)$^+$ and further fragmentation pattern was consistent with the assigned structure. The base peak at 122 (rel. int. 99.6) and peak at 149 (rel. int. 35.6) confirms the attachment of methylene group and p-hydroxyacetophenone.

Preformulation Studies

Solubility determination

The aqueous solubility of the CFLX mannich base was compared with CFLX free base by shaking 100 mg of each with 150 ml water at 25 ±1° over a period of 48 hours. Aliquots were withdrawn at various time intervals and absorbance was read at 272 nm (Fig. 1).

Dissolution rate

Disc (13 mm diameter) of CFLX mannich base and CFLX was prepared by compressing 100 mg of each in an IR hydraulic press at 400 Kg/cm² for 15 seconds. The dissolution medium was 500 ml distilled water at 37± 0.5° and the paddle speed was 50 rpm. At appropriate time intervals, 5 ml sample was withdrawn and replaced by fresh dissolution medium. The absorbance of the sample was read at 272 nm (Fig. 2).

Apparent Dissociation Constant

The pKa' value of CFLX mannich base was determined spectrophotometrically at 25 ±1°. A wavelength (240 nm) was chosen where the absorbance of the three species (cation, zwitterion and anion) varied the greatest. The change in absorbance with pH was monitored. The ionic strength was kept constant at 0.15 with NaCl.

Kinetic Studies and pH-rate profile

A UV spectrophotometric method was used to study the degradation kinetics of CFLX mannich base using a kinetic module attached to Beckman DU 64 spectrophotometer. Absorbance was noted at 210 nm (this being the wavelength at which the absorption of CFLX and CFLX mannich base differed maximally) at various time intervals for 1 h at 25 ±0.5°. For pH-rate profile, buffer solutions of pH 1.2 6.0, 6.6, 7.4 and 8.8 were prepared (phosphate buffer upto 6.6 and tris buffer for other pHs) and the rate of degradation was followed spectrophotometrically (Fig. 3).

Minimum Inhibitory Concentration (MIC) and *in vitro* antibacterial activity

Antibacterial activity of CFLX mannich base was compared with that of CFLX against two Gram-ve organisms, *E. coli* (NCTC 10418) and *P. aeruginosa*, (a clinical isolate) and one Gram+ve organism *S. aureus* (NCTC 6571) using agar plate diffusion method. Mueller Hinton Agar was used as bacteriological medium. The drug was

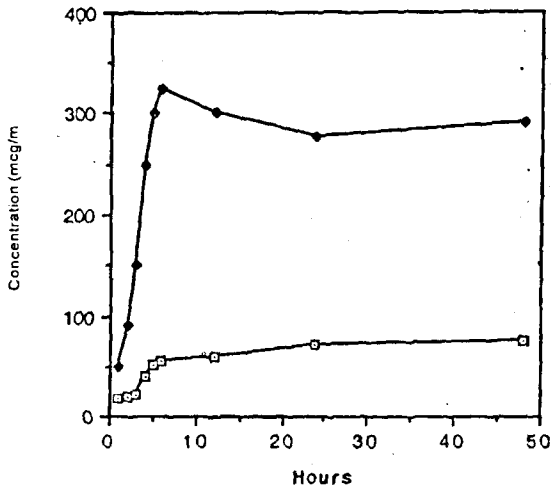


Fig. 1 : Solubility studies of CFLX and its prodrug
-□- CFLX -◆- CFLX prodrug

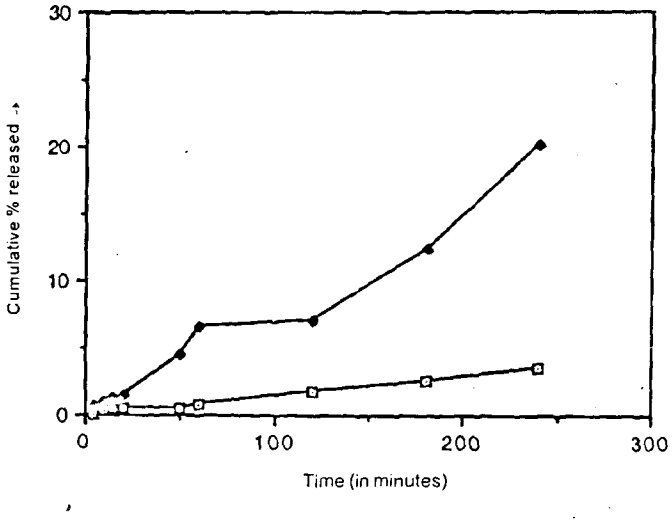


Fig. 2 : Intrinsic dissolution rate of CFLX and its prodrug
-□-CFLX -◆- prodrug of CFLX

dissolved in isotonic sodium phosphate buffer of pH 7.4 MIC of CFLX mannich base was determined according to the method of Gotto *et al*[3].

Toxicological Studies

Acute toxicity studies were carried out to determine the LD_{50} value of CFLX mannich base and compare it with the reported value of CFLX according to the method of Behrens-Kaerber[4]. Suspension of the drug in 0.2% carboxymethylcellulose was prepared in five different concentrations (1000, 2000, 3000, 4000, 5000 mg/kg)

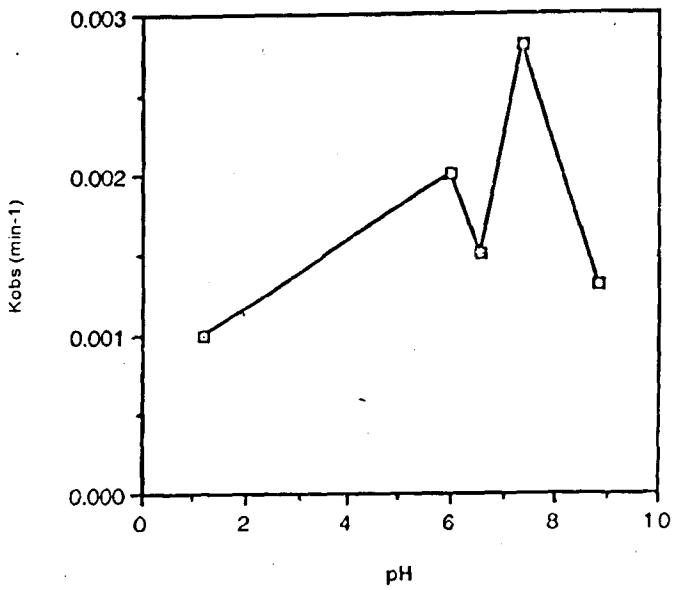
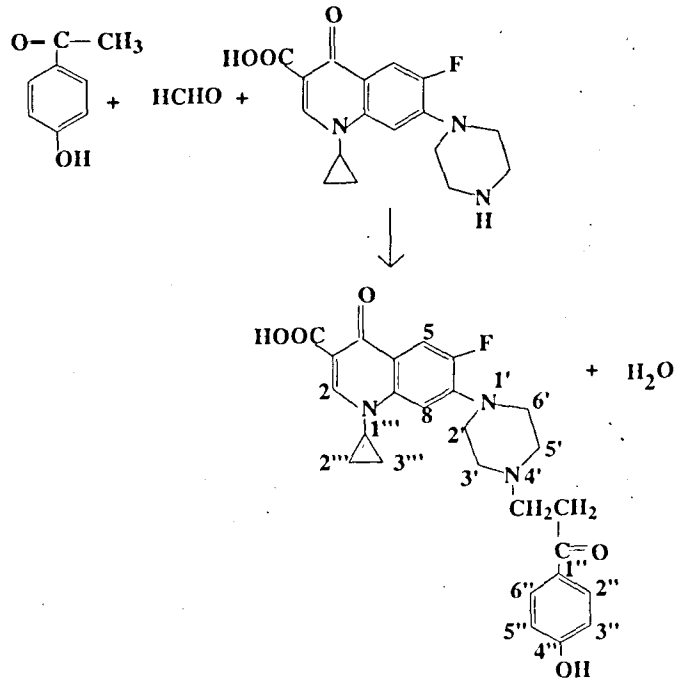


Fig. 3 : pH - rate profile of CFLX prodrug



SCHEME

and was given to male mice (five in each group) at a dose of 0.1 ml/10 g of the body weight. Mortality upto 7 days after administration was monitored.

RESULTS AND DISCUSSION

Mannich reaction between p-hydroxyacetophenonone, aqueous formaldehyde and ciprofloxacin yielded a mannich base according to the following scheme.

The thin layer chromatography examination of the drug and the prodrug (dissolved in dichloromethane:methanol, 1:1) on silica gel G plates with developing solvent consisting of a mixture of ethanol 95% v/v, chloroform and dilute ammonia (70:20:10) gave single spots, with Rf values 0.25 and 0.60 respectively. The melting point of ciprofloxacin showed a decrease upon substitution at the secondary amine group of piperazine moiety.

The solubility measurements (Fig. 1) indicated almost four fold increase in the solubility of prodrug over CFLX. The dissolution rate of the prodrug also showed an increase as compared to the parent drug (Fig. 2). Ciprofloxacin being an amphoteric drug exhibits two pKa' values reported pKa'_1, 6.0 and pKa'_2 8.8)[2-5]. The prodrug showed three pKa' values 5.30, 6.4 and 8.1 as determined by the spectrophotometric method. The pH-rate profile of the prodrug is given in Fig. 3. The rate of decomposition was found to follow first order kinetics. The product is quite stable at gastric and intestinal pH. The $t_{1/2}$ at physiological pH (7.4) was 3.85 h.

The *in vitro* antibacterial activity of the prodrug was found to be similar to CFLX as nearly equal zones of inhibition were obtained (Fig. 4) against the three test organisms studied indicating that the condensation at the piperazine ring does not affect the antibacterial activity. It is possible that the prodrug converted to the parent drug during the 48 h incubation period resulting in equal zones of inhibition. The minimum inhibitory concentration MIC 90) of the prodrug was 0.2, 0.05 and 0.5 mcg/ml for *S. aureus, E. coli* and *P.aeruginosa* respectively. In the toxicological study, the LD_{50} value for the mannich base was>5000 mg/Kg (reported value for CFLX>5000 mg/Kg)[6].

The above study has utilized ciprofloxacin as a novel amine in the preparation of prodrug via Mannich reaction. The prodrug has improved solubility and dissolution rate while the antibacterial activity remained unchanged.

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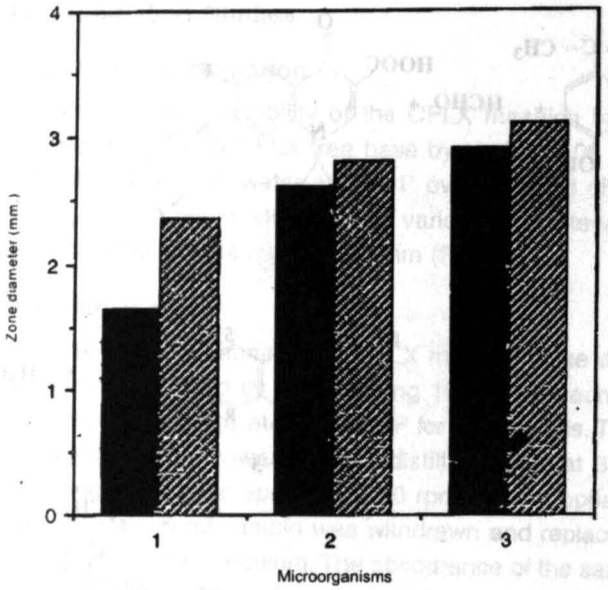

Fig. 4: Antibacterial activity of CFLX and its prodrug

■ **CFLX** ▨ **CFLX prodrug**

1 ***S. aureus*** **2** ***E. coli*** **3** ***P. aeruginosa***

ing facilities and thank Professor M.S.Y. Khan and Dr. Mohammed Ali (Faculty of Pharmacy) for spectral analysis and to Dr. S.C. Sharma (Faculty of Science) for help in kinetic studies. The help of Ranbaxy Research Laboratories, Gurgaon in providing drug samples is gratefully acknowledged.

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