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Brief Communication

Synthesis and biological evaluation of a mutual prodrug of norfloxacin and fenbufen

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Abstract

Objectives: The study aimed to synthesize a mutual prodrug of norfloxacin and fenbufen with an objective of obtaining an effective and safer anti-inflammatory drug with useful antimicrobial actions.

Methods: An amide-based mutual prodrug (**NF-FN**) was prepared following a single-step synthesis by condensing norfloxacin with fenbufen under appropriate laboratory conditions. Its structure was established on the basis of IR, NMR, Mass spectral data and elemental analysis. The prodrug (**NF-FN**) was evaluated for *in-vitro* antibacterial activity against two gram positive (*Staphylococcus aureus* & *Bacillus subtilis*) and two gram negative bacterial strains (*Escherichia coli* & *Klebsiella pneumonia*). The *in-vivo* anti-inflammatory activity and ulcerogenicity of the synthesized prodrug were investigated in Wistar albino rats at the doses of 10 and 30 mg/kg body weight, respectively.

Results: The synthesized prodrug (**NF-FN**) showed very good activity against *S. aureus* & *E. coli* with *MIC*-6.25 μg/mL, and good activity against *B. subtilis* & *K. pneumonia* with *MIC*-12.5 μg/mL. Its anti-inflammatory activity was found to be better than that of the parent drug fenbufen. It was also observed to less severe on gastric mucosa in comparison to reference drug, fenbufen.

Conclusion: The prodrug showed promising results as anti-inflammatory agent however, its antibacterial action was found to be slightly weaker than the other parent drug norfloxacin.

Keywords: Antibacterial; Anti-inflammatory; Cinopal; Fluoroquinolone

Introduction

Fenbufen (4-oxo-4-[biphenyl-4-yl] butanoic acid) is an aroylpropionic acid derivative having potent anti-inflammatory actions. It is used clinically to treat and manage various inflammatory conditions. It is available in the market under the name of Cinopal.[1] Fenbufen like other members of aroylpropionic acid class is good anti-inflammatory agent but causes gastrointestinal side effects.[1,2] One of the synthetic approaches commonly used to improve the NSAIDs safety profile involves chemical modification of the free carboxylic group.[3–5] Previously conducted research studies have clearly indicated that chemical derivatization of the carboxylate function of some NSAIDs could result in an increased anti-inflammatory activity with reduced ulcerogenic effect.[6,7]

Norfloxacin belongs to fluoroquinolone class of antimicrobial agents. A number of derivatives of norfloxacin have been synthesized with an attempt to improve its antimicrobial activities.[8,9] Several prodrugs derived from norfloxacin

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have also shown improved pharmacological and pharmacokinetic profile.[10,11]

Prodrug designing is a concept of retro-metabolic drug design that considers targeting, metabolism, duration of action, biological action, side effect, physico-chemical properties etc. into the drug design process.[12,13] Prodrug designing so far has been proved to be an exciting and fruitful area of medicinal chemistry research. Generally, in a prodrug, an inert or non toxic carrier group or promoiety is used, whose selection primarily depends on the objectives to be achieved in prodrug designing. In case of mutual prodrug, the promoiety may be another drug.[14] Prodrugs and mutual prodrugs may exhibit improved biological, pharmacokinetic, pharmacodynamic properties with or without minimum side effects.[15]

In view of these facts and in continuation of our work on design, synthesis and biologically evaluation of prodrugs,[10,11] it was considered worthwhile to synthesize a mutual prodrug comprising of norfloxacin and fenbufen i.e., two drugs in one with an aim of obtaining an effective and safer anti-inflammatory drug with useful broad spectrum antimicrobial activity against gram positive and gram-negative bacteria drug with useful antimicrobial actions against gram-positive and gram-negative bacteria (broad spectrum). Such drugs might be useful in the inflammatory conditions associated with infection. The other advantages of using the prodrug could be its sustained release, administration of one drug instead of two and low doses that might be required to produce the desired pharmacological effect.

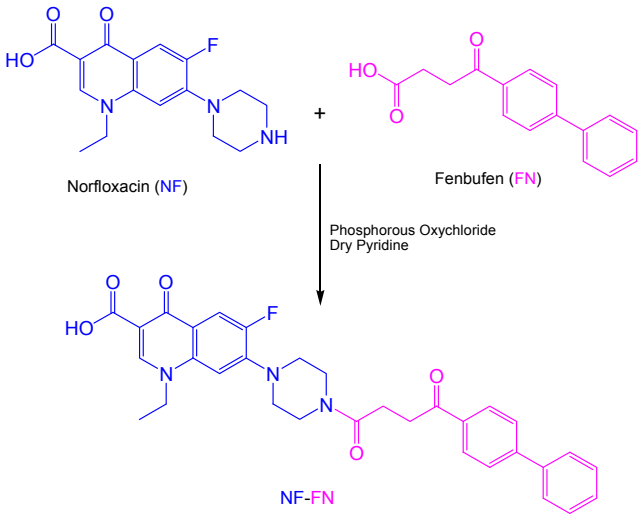
Materials and Methods

Synthesis

Melting points were recorded in open capillary tube and are uncorrected. The IR spectrum of the mutual prodrug was recorded in KBr pellet using a Win IR FTS135 spectrophotometer. ^{1}H NMR spectrum was recorded on Bruker spectrospin DPX-300 MHz (Rheinstetten, Germany) with tetramethylsilane (TMS) as an internal standard in solvent $CDCl_3$. Mass spectrum was recorded on a Jeol JMS-D 300 (Tokyo, Japan) instrument fitted with a JMS 2000 data system at 70 eV. Microanalysis of the compound was done on Perkin–Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. The progress of the reaction was monitored on silica gel G coated plates by TLC. Iodine chamber and UV-lamp were used for visualization of TLC spots. Dry solvents were used throughout the study. The reaction involved in the synthesis of the mutual prodrug is given in Scheme 1. The starting material **1**, fenbufen i.e., 4-oxo-4-(biphenyl-4-yl)butanoic acid required for the study was prepared as per the reported method.[3]

*Synthesis of norfloxacin prodrug (**NF-FN**)*

Fenbufen (508 mg; 2 mmol) (**1**) and norfloxacin **2** (638 mg; 2 mmol) were dissolved separately in dry pyridine (6 & 8 mL, respectively). Both the solutions were mixed together under ice cold conditions followed by dropwise addition of phosphorous oxychloride (0.5 mL) maintaining the temperature 0–5 °C while stirring on a magnetic stirrer. Initially the reaction mixture was colorless but slowly developed color as



Scheme 1: Protocol for synthesis of mutual prodrug (**NF-FN**).

reaction proceeded. The contents were then further stirred for 3 h. After completion of reaction, the reaction mixture was decomposed by adding on to the ice cold water (100 mL). A solid mass separated out, which was filtered, washed with water, dried and crystallized from methanol:chloroform mixture (1:1) to furnish TLC pure light reddish brown crystals of **NF-FN**.

Yield: 46%, m.p.: 228–229 °C. R_f: 0.38 (Toluene:Ethyl acetate:Formic acid, 5:4:1), IR (KBr/ν_{max}) cm^{-1}: 3281 (–COOH), 3018 (aryl C–H), 2986 & 2834 (C–H), 1731 (C=O), 1658 (CONH), 1621 (C=O, pyridone), 1468 (C–N) and 1237 (C–F), ^{1}H NMR ($CDCl_3$): (δ ppm) 1.60 (t, 3H, $-CH_2\underline{CH_3}$), 4.37 (q, 2H, $-\underline{CH_2}CH_3$), 2.87 & 3.42 (t, each, 2x-CH_2–), 3.29 & 3.87 (m, each, 4x-CH_2–, piperazine moiety), 6.87 (s, 1H, proton *ortho* to fluorine), 7.42–7.63 (m, 5H, Ar–H of *p*-phenyl ring), 7.69 & 8.08 (d, each, A_2B_2, 4H, *p*-benzoyl ring), 8.05 (s, 1H, proton *meta* to fluorine), 8.70 (s, 1H, pyridine ring), MS: m/z 555 (M^+), $C_{32}H_{30}FN_3O_5$; Calculated C, 69.18; H, 5.44; N, 7.56; Found C, 68.93; H, 5.32; N, 7.48.

Antibacterial activity

The *in-vitro* antibacterial activity of the newly synthesized mutual prodrug of (**NF-FN**) was determined against 4 bacterial strains; 2 gram positive bacteria – *Staphylococcus aureus* (MTCC 96) & *Bacillus subtilis* (MTCC 121), and two gram negative bacteria – *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883). The minimum inhibitory concentration (MIC) of the test compound and standard drug norfloxacin was determined according to the turbidity method.[16]

Anti-inflammatory activity

The synthesized mutual prodrug was also investigated for its anti-inflammatory activity by using carrageenan-induced paw edema method of Winter et al.[17] The animal studies were approved by the Institutional Animal Ethics Committee [IAEC] of Jamia Hamdard University, New Delhi and utmost care was taken to ensure that the animals were treated in the most humane and acceptable manner. The experiment was performed on Wistar Albino rats of