Research Article

Norfloxacin Prodrug: Synthesis and Biological Evaluation

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ABSTRACT

An amide-based prodrug (2) was prepared following a single-step synthesis by condensing norfloxacin with 4-oxo-4-(4-phenoxyphenyl)butanoic acid (1). Its structure was established on the basis of modern analytical techniques; IR, NMR, Mass and elemental analysis. The prodrug (2) was evaluated for in-vitro antibacterial activity against some selected microbial strains. The prodrug exhibited significant antibacterial activity against the tested bacteria.

Keywords: Fluoroquinolone, butanoic acid, amide, synthesis, antibacterial.

INTRODUCTION

Prodrug designing is an exciting and fruitful area of medicinal chemistry research. It is a concept of retro-metabolic drug design that considers targeting, metabolism, duration of action, biological action, physico-chemical properties etc. into the drug design process[1-3]. Generally, in a prodrug, the carrier group or promoiety used is inert or non-toxic. The selection of promoiety depends on the objectives to be achieved in prodrug designing. In some cases the promoiety may be another drug and such derivatives have been termed as mutual prodrugs[4]. Prodrugs and mutual prodrugs may exhibit improved biological, pharmacokinetic, pharmacodynamic properties[1-7].

Now a days the problem of bacterial resistance to a large number of antibacterial agents available in the market is increasing[8-10]. Moreover, the incidence of bacterial infections has been increasing dramatically owing to variety of factors including an increase in the number of immuno-compromised hosts[10-14]. These points clearly indicate the need of more effective antimicrobial agents with a broad spectrum of activity[13,14].

In view of these observations and in continuation of our work on prodrugs[5-7], it was considered worthwhile to synthesize an amide-based prodrug comprising of norfloxacin and 4-oxo-4-(4-phenoxyphenyl)butanoic acid, with an aim of getting a useful drug, which may act with effectiveness against gram-positive and gram-negative bacteria (broad spectrum). An added advantage of using the prodrug could be its sustained release and even low doses might be effective.

MATERIALS AND METHODS

Synthesis

Melting points are uncorrected, and were taken in open capillary tubes. The IR spectra were recorded on Bruker, alpha E ATR FTIR spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker spectropsin DPX-300MHz with tetramethylsilane as internal standard in solvent $CDCl_3$. Mass spectrum was recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Elemental analysis 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 glass TLC plates, which was performed on silica gel G. Iodine chamber and UV-lamp were used for locating the spots. The reaction involved in synthesis is presented in **Scheme 1**. The required 4-oxo-4-(4-phenoxyphenyl)butanoic acid (**1**) was prepared by condensing diphenyl ether with succinic anhydride in presence of anhydrous aluminium chloride following Friedel-Craft's acylation reaction conditions[15].

Synthesis of Norfloxacin prodrug (**2**): Norfloxacin (638 mg; 2 mmol) was dissolved in dry pyridine (8 mL) and 4-oxo-4-(4-phenoxyphenyl)butanoic acid (**1**) (540 mg; 2 mmol) (**2**) was also dissolved separately in dry pyridine (7 mL). 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 reaction proceeded. The contents were further stirred for 3h. After completion of reaction, the reaction mixture was decomposed by adding into ice cold water (100 mL). A solid mass separated out, which was filtered, washed with water, dried and crystallized from methanol to furnish TLC pure reddish-brown crystals of (**2**).

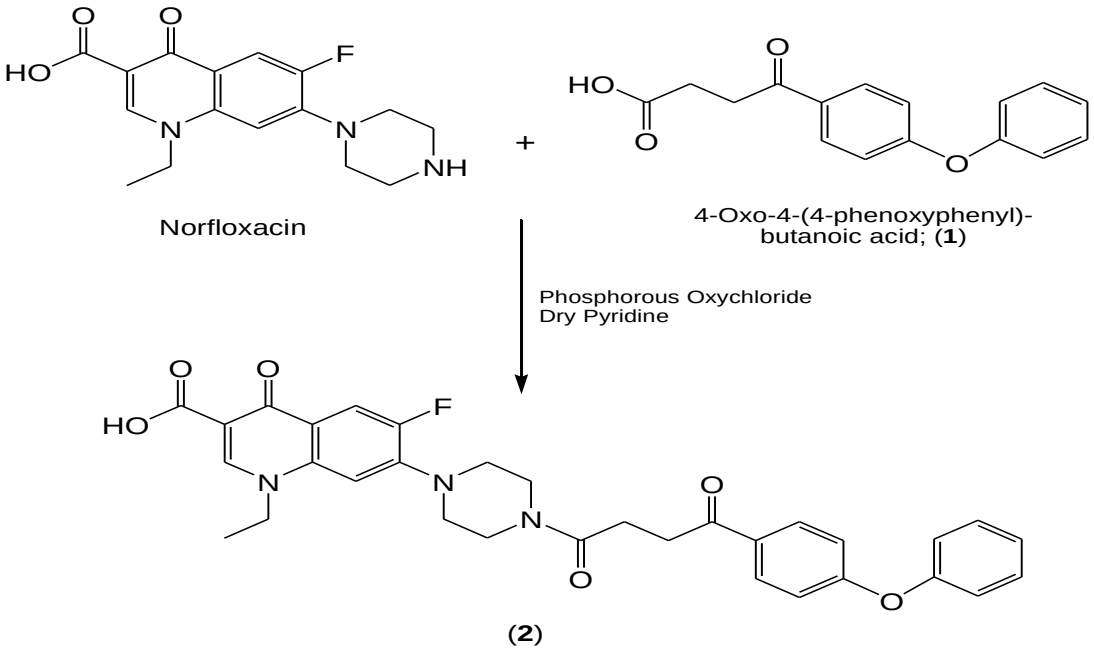
Antibacterial activity

The in-vitro antibacterial activity of the newly synthesized prodrug of norfloxacin (**2**) was determined against 4 bacterial strains; 2 gram positive bateria- *Staphylococcus aureus* (MTCC 96) & *Bacillus subtilis* (MTCC 121), 2 and gram negative bacteria- *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883). The test was carried out according to the turbidity method[16,17]. A solution of the compound/standard was prepared in dimethylformamide (DMF) and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile stoppered test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. Norfloxacin was used as standard drug for comparison. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37° for 24 h and examined for turbidity. The tubes with highest dilution showing no turbidity was the Minimum Inhibitory Concentration (*MIC*).

RESULTS AND DISCUSSION

Synthesis

The title prodrug (**2**) was synthesized in a single step as outlined in **Scheme 1**. Norfloxacin was condensed with 4-oxo-4-(4-phenoxyphenyl)butanoic acid (**1**) in dry pyridine in presence of $POCl_3$ at a temperature 0-5°C. Usual work up of the reaction mixture followed by crystallization from methanol gave the desired prodrug (**2**) as reddish brown crystals, Melting Point: 202-204° C. Rf value: 0.57 (Toluene: Ethyl acetate: Formic acid, 5:4:1), Yield: 52 %.



Scheme 1: Protocol for synthesis of norfloxacin prodrug (**2**).

Structure establishment of prodrug (2).

The structure of prodrug (**2**) was established on the basis of IR, ^{1}H-NMR, Mass and elemental analysis results.

***IR spectrum*:** The IR spectrum of the prodrug (**2**) showed following signals: 3293 (COOH), 3001 (aryl C-H), 2982 & 2847 (C-H), 1743 (C=O), 1639 (CONH), 1633 (C=O, pyridone), 1456 (C-N), 1218 (C-F).

***NMR spectrum*:** The ^{1}H-NMR spectrum of the prodrug (**2**) showed a triplet and a quartet located at δ 1.61 and δ 4.39 arising from the methyl and methylene group of ethyl moiety in norfloxacin. There were two triplets located at δ 2.86 and δ 3.44 integrating for the protons of two methylene groups. There appeared two multiplets located at δ 3.27 and δ 3.41 arising from the protons of four methylene groups of piperazines moiety. There was located a doublet at δ 6.85 arising from the lone proton ortho to fluorine atom. Another doublet located at δ 8.17 could be accounted for another lone proton meta to fluorine. There appeared three multiplets centered at δ 7.04 (H-2,6), 7.19 (H-4) and 7.71 (H-3,5) which integrated for five protons of the phenyl ring. Two doublets forming an A_2B_2 pattern located at 7.08 and 7.76 which could arise from the four protons of *p*-substituted phenyl ring. The lone proton of pyridine ring appeared as a singlet located at δ 8.73. These data are satisfactory for the structure assigned to the compound.

***Mass spectrum*:** The mass spectrum of (**2**) showed a molecular ion peak at m/z 571, and M^{+}+1 peak at m/z 572.

***Elemental analysis*:** The values were found within ±0.4% of the theoretical values, $C_{32}H_{30}FN_3O_6$, Calculated C, 67.24; H, 5.29; N, 7.35, Found C, 66.96; H, 5.18; N, 7.24.

Antibacterial activity

In-vitro antibacterial activity was carried out against the bacterial strains gram positive (*Staphylococcus aureus* & *Bacilluus subtilis*) and gram negative (*Escherichia coli* & *Klebsiella pneumonia*). Minimum inhibitory concentration was determined and results indicated that the prodrug (**2**) 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. Norfloxacin showed *MIC* value ranging from 3.12-6.25 μg/mL against the tested microbes. In-vivo antibacterial activities are required to further ascertain its usefulness; which are under progress in our laboratories.

CONCLUSION

Norfloxacin was successfully condensed with 4-oxo-4-(4-phenoxyphenyl)butanoic acid (**1**) in a single step to furnish an amide-based prodrug (**2**). The spectral data and microanalysis data were found to be in full agreement with the proposed structure. The prodrug (**2**) showed significant in vitro antibacterial activity against *S. aureus* and *E. coli* with *MIC* 6.25 μg/mL. It is expected that after in vivo hydrolysis (by amidases and/or other enzymes) the prodrug would release norfloxacin into the system which have established antibacterial activity. These results show the importance of exploring old drugs as safer templates to built new prodrug candidates.

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