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Original Article

Synthesis, characterization and evaluation of amide based prodrugs of Norfloxacin

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ABSTRACT

A series of new prodrugs (**3a-e**) of norfloxacin (**1**) was synthesized using 4-oxo-4-substituted-phenylbutanoic acids (**2a-e**) as promoiety. An amide-linkage was established between the secondary amino group of **1**with the free carboxylic group of **2** following a single-step synthesis. The structure of the synthesized prodrugs was established on the basis of IR, ^{1}HNMR and mass spectral data. The title compounds (**3a-e**) were evaluated for *in-vitro* antibacterial activity against some selected pathogenic bacterial strains. The minimum inhibitory concentration (*MIC*) results indicated that two compounds (**3c** & **3d**) were excellent in their antibacterial action.

Key words: Aroyl propionic acid, Norfloxacin, quinolone, antibacterial.

INTRODUCTION

Norfloxacin is a broad spectrum synthetic antibiotic that belongs to the fluoroquinolone class of antibacterial drugs. It is an important drug for the treatment of variety of infections but its blood levels and urinary recoveries after oral administration are not sufficient that limits its clinical usefulness [1,2]. In order to have better oral bioavailability, optimum blood concentration and sustained release for better clinical results of this antibiotic, several attempts have been made in the past to synthesize prodrugs and mutual prodrugs of norfloxacin [2-4].

Prodrug design comprises an important and fruitful area of drug research devoted to optimization of drug delivery where the pharmacologically inactive derivative of the active drug undergoes chemical and/or enzymatic biotransformation, resulting in the release of active drug in the body [5]. In a prodrug approach or bioreversible chemical derivatization, generally an inert or non-toxic carrier group or promoiety is linked to an active drug which undergoes enzymatic hydrolysis *in vivo* to release the active drug. The parent drug after detachment from the carrier or promoiety then shows the desired biological response. A mutual prodrug consists of two pharmacologically active agents coupled together in the form of a single molecule and each acts as promoiety for the other agent [6-8]. Amide-based prodrugs have desirable characteristics with reasonable *in vitro* chemical stability which allows them to be formulated with adequate shelf lives. They also function as amidase substrate and are very labile to hydrolysis *in vivo* [5]. The amide derivatives are converted to the parent compounds *in vivo* by hydrolytic mechanisms. Aroyl propionic acids have been found to be good promoities for prodrug synthesis and several prodrugs prepared with them have shown potential in the preclinical studies [9,10]. Further, substitution of piperazinyl ring at C-7 position of fluoroquinolones can have great impact on potency, solubility, spectrum and pharmacokinetic profile of this class of antibiotics [11].

In view of these observations and in continuation of our work on prodrugs [7, 8, 10, 12], it was considered worthwhile to design and synthesize various amide-based prodrugs of norfloxacin by using 4-oxo-4-substituted-phenyl propionic acids as promoiety. Therefore, the present study aimed to synthesize amide prodrugs of the commonly used antibiotic norfloxacin and to evaluate their *in-vitro* antibacterial activity against some selected pathogenic bacterial strains in order to improve the pharmacokinetic and biological profile of norfloxacin. It is also anticipated that these amide

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prodrugs would have sustained release and thus longer duration of action and even low doses might be required to produce the desired effects in antimicrobial therapy.

MATERIALS AND METHODS

General: Norfloxacin was a gift sample from Arbro Pharmaceuticals, Ltd, New Delhi, India. Melting points were recorded in open capillary tubes and are uncorrected. The IR spectra were recorded on Shimadzu FTIR in KBr pellets (anhydrous). ^{1}H NMR spectrum was recorded on Bruker spectrospin DPX-300MHz with tetramethylsilane (TMS) as internal standard in solvent DMSO-d_6 and chemical shifts were recorded in δ ppm. Mass spectrum was recorded on a Jeol JMS-D 300 instrument. 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 TLC, which was performed on silica gel. Iodine chamber and UV-lamp were used for visualization of TLC spots. Dry solvents were used throughout the study. 4-Oxo-4-substituted-phenylbutanoic acids (**2a-e**) were prepared as per the reported method in literature [9,10]. The reaction involved in synthesis of prodrugs is given in **scheme 1**.

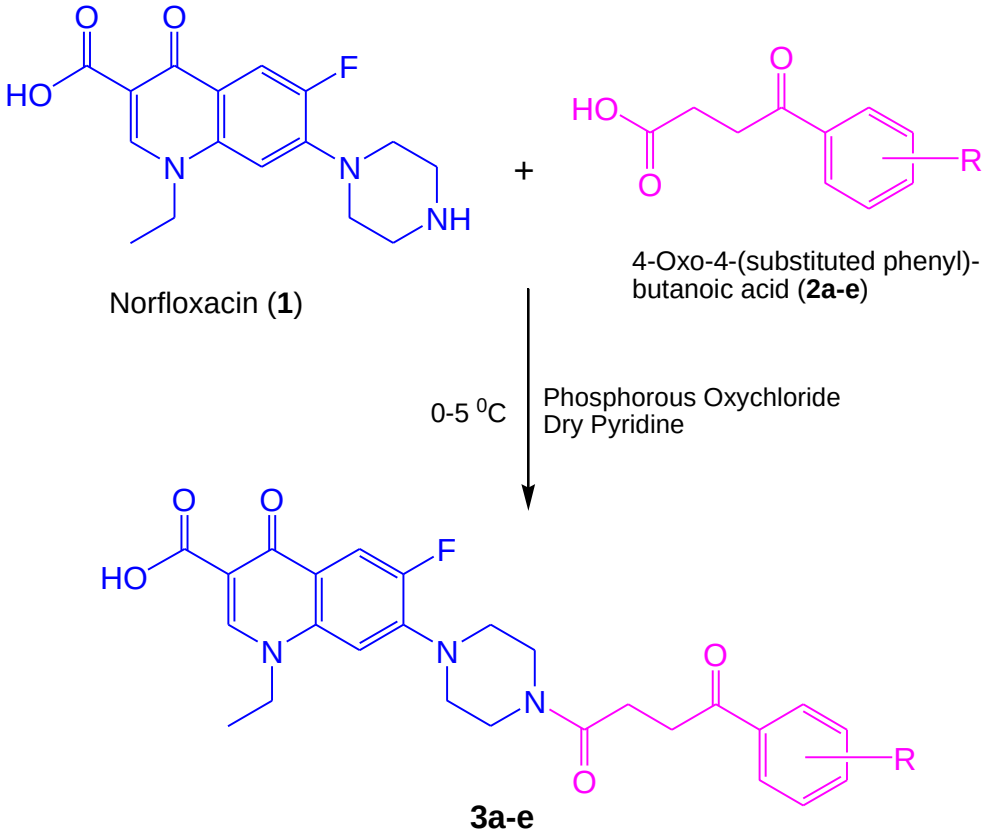
Chemistry:

*General procedure for the synthesis of prodrugs (**3a-e**)*: Norfloxacin (**1**) (638 mg; 2 mmol) was dissolved in dry pyridine (8 mL), and 4-oxo-4-substituted-phenylbutanoic acid (equimolar; 2 mmol) (**2**) was also dissolved separately in dry pyridine (4 mL). Both the solutions were mixed together and stirred magnetically. Phosphorous oxychloride (0.6 mL) was added dropwise maintaining the temperature 0-5° C while stirring. The contents were stirred for another hour and left overnight. It was poured into ice cold water and a solid mass separated out, which was filtered, washed, dried and crystallized from methanol:acetone mixture (1:1) to furnish TLC pure **3a-e**.

***In-vitro* antibacterial activity:** The bacterial strains gram positive; *Staphylococcus aureus* (MTCC 96) & *Bacilluus subtilis* (MTCC 121) and gram negative: *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883) were used. The test was carried out according to the turbidity method [13]. A solution of the compound 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. 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

Chemistry: The title prodrugs (**3a-e**) were synthesized in a single step as outlined in **Scheme 1**. The promoities, aroylpropionic acids (4-oxo-4-substituted-phenylbutanoic acid; **2a-e**), were synthesized following literature method [9,10]. Norfloxacin (**1**) was condensed with compound **2a-e** 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:acetone mixture yielded the desired prodrug (**3a-e**) as colored crystalline compounds (**Scheme 1**). Characterization of the synthesized prodrugs was carried out; their physical and spectral data are presented in **Table 1**. The formation of the prodrugs was confirmed by the IR, ^{1}H NMR, Mass spectroscopic data and elemental analysis results.



Scheme 1: Protocol for synthesis of norfloxacin prodrugs (**3a-e**).

Structure elucidation of 3a-e. The following generalizations could be made after analyzing the spectral data of the synthesized compounds;

IR spectra: Infra-red spectra of **3a-e** showed the characteristic bands at around 3280 cm^{-1} for carboxylic O-H stretching, 3025 cm^{-1} for aromatic C-H stretching, 2985 & 2830 cm^{-1} for asymmetric & symmetric, respectively, for C-H stretching of methyl and methylene of ethyl and piperazines moieties, 1740 cm^{-1} for C=O stretching in ester group, 1655 cm^{-1} for C=O stretching in tertiary amide, 1625 cm^{-1} for pyridone C=O stretching , 1465 cm^{-1} for C-N stretching, 1240 cm^{-1} for C-F and carboxylic C-O stretching.

NMR spectra: The ^{1}H NMR spectra of **3a-e** showed a triplet and a quartet located at around δ 1.61 and δ 4.63 arising from the methyl and methylene group of ethyl moiety in norfloxacin. There were two triplets located each around at δ 2.85 and δ 3.41 integrating for the protons of two methylene groups. There appeared two multiplets located around at δ 3.31 and δ 3.88 arising from the protons of four methylene groups of piperazines moiety. There was located a doublet around at δ 6.88 arising from the lone proton *ortho* to fluorine atom. Another doublet located around at δ 8.16 could be accounted for another lone proton *meta* to fluorine. The lone proton of pyridine ring appeared as a singlet located around at δ 8.70. Protons of substituted phenyl ring appeared at appropriate places. These data are satisfactory for the structure assigned to the prodrugs.

Mass spectra: The mass spectrum of the compounds **3a-e** showed a molecular ion peak located at m/z M+ and M+1 in good intensity.

Elemental analysis: The CHN values of **3a-e** were found within ±0.4% of the theoretical values.

Table 1. Physical and spectral data of norfloxacin prodrugs **(3a-e).**

Prodrug	R	M.p. (°C)/ Yield (%)	Mol. Formula/ Elemental analysis	IR (cm^{-1})	^{1}H-NMR (δ ppm)[a]	Mass (m/z)
3a	CH_3–	204-206 / 46	$C_{27}H_{28}FN_3O_5$/ Calc: C, 65.71; H, 5.72; N, 8.51. Found: C, 65.54; H, 5.68; N, 8.46.	3281 (-OH, carboxylic), 3018 (aryl C-H), 2986 & 2834 (C-H), 1731 (C=O), 1658 (CONH), 1621 (C=O, pyridone), 1468 (C-N), 1237 (C-F).	1.61 (t, 3H, -$CH_2\underline{CH_3}$) 2.37 (s, -CH_3, tolyl), 2.87 & 3.39 (t, each, 2x -CH_2-), 3.31 & 3.86 (m, each, 4x-CH_2-**,** piperazines moiety), 4.62 (q, 2H, -$\underline{CH_2}CH_3$), 6.87 (d, 1H, proton *ortho* to fluorine), 7.62 & 8.01 (d, each, A_2B_2, 4H, *p*-tolyl), 8.14 (d, 1H, proton *meta* to fluorine), 8.68 (s, 1H, pyridine ring).	493 (M^+), 494 (M+1).
3b	C_2H_5–	178-180 / 50	$C_{28}H_{30}FN_3O_5$/ Calc: C, 66.26; H, 5.96; N, 8.28. Found: C, 65.98; H, 6.12; N, 8.15.	3275 (-OH, carboxylic), 3020 (aryl C-H), 2978 & 2825 (C-H), 1742 (C=O), 1663 (CONH), 1625 (C=O, pyridone), 1474 (C-N), 1242 (C-F).	1.26 & 1.59 (t, each, 2x-$CH_2\underline{CH_3}$), 2.72 & 4.59 (q, each, 2x-$\underline{CH_2}CH_3$), 2.85 & 3.33 (t, each, 2x -CH_2-), 3.29 & 3.84 (m, each, 4x-CH_2-**,** piperazines moiety), 6.86 (d, 1H, proton *ortho* to fluorine), 7.51 & 7.94 (d, each, A_2B_2, 4H, *p*-ethylphenyl ring), 8.12 (d, 1H, proton *meta* to fluorine), 8.66 (s, 1H, pyridine ring).	507 (M^+), 508 (M+1).
3c	CH_3O–	220-222 / 52	$C_{27}H_{28}FN_3O_6$/ Calc: C, 63.65; H, 5.54; N, 8.25. Found: C, 63.53; H, 5.58; N, 8.16.	3278 (-OH, carboxylic), 3027 (aryl C-H), 2981 & 2830 (C-H), 1738 (C=O), 1651 (CONH), 1631 (C=O, pyridone), 1463 (C-N), 1239 (C-F).	1.63 (t, 3H, -$CH_2\underline{CH_3}$) 3.89 (s, 3H, -OCH_3), 2.88 & 3.45 (t, each, 2x -CH_2-), 3.32 & 3.88 (m, each, 4x-CH_2-**,** piperazines moiety), 4.64 (q, 2H, -$\underline{CH_2}CH_3$), 6.89 (d, 1H, proton *ortho* to fluorine), 7.63 & 8.12 (d, each, A_2B_2, 4H, *p*-anisyl), 8.16 (d, 1H, proton *meta* to fluorine), 8.71 (s, 1H, pyridine ring)**.**	509 (M^+), 510 (M+1).
3d	Cl–	192-194 / 56	$C_{26}H_{25}ClFN_3O_5$/ Calc: C, 60.76; H, 4.90; N, 8.18. Found: C, 60.54; H, 4.75; N, 8.24.	3280 (-OH, carboxylic), 3032 (aryl C-H), 2994 & 2841 (C-H), 1743 (C=O), 1655 (CONH), 1627 (C=O, pyridone), 1471 (C-N), 1246 (C-F), 748 (C-Cl).	1.62 (t, 3H, -$CH_2\underline{CH_3}$) 2.89 & 3.47 (t, each, 2x -CH_2-), 3.35 & 3.92 (m, each, 4x-CH_2-**,** piperazines moiety), 4.68 (q, 2H, -$\underline{CH_2}CH_3$), 6.88 (d, 1H, proton *ortho* to fluorine), 7.66 & 8.23 (d, each, A_2B_2, 4H, *p*-chlorophenyl), 8.18 (d, 1H, proton *meta* to fluorine), 8.74 (s, 1H, pyridine ring)**.**	513 (M^+), 514 (M+1), 515 (M+2).
3e	2,4-di CH_3–	226-228 / 44	$C_{28}H_{30}FN_3O_5$/ Calc: C, 66.26; H, 5.96; N, 8.28. Found: C, 66.10; H, 5.82; N, 8.17.	3282 (-OH, carboxylic), 3020 (aryl C-H), 2985 & 2835 (C-H), 1733 (C=O), 1656 (CONH), 1623 (C=O, pyridone), 1465 (C-N), 1234 (C-F).	1.60 (t, 3H, -$CH_2\underline{CH_3}$) 2.38 & 2.52 (s, each, 2x-CH_3, *m*-xylene), 2.85 & 3.38 (t, each, 2x -CH_2-), 3.31 & 3.85 (m, each, 4x-CH_2-**,** piperazines moiety), 4.59 (q, 2H, -$\underline{CH_2}CH_3$), 6.85 (d, 1H, proton *ortho* to fluorine), 7.12 (m, 2H, H-3,5, *m*-xylene), 7.74 (d, 1H, H-6, *m*-xylene), 8.13 (d, 1H, proton *meta* to fluorine), 8.65 (s, 1H, pyridine ring).	507 (M^+), 508 (M+1).

[a]s = singlet; d = doublet; q = quartet; t = triplet; m = multiplet.

Antibacterial activity: Prevalence of infectious diseases accompanied with increasing incidences of resistance to a large number of antibacterial agents are becoming a major concern worldwide [14]. Thus, there is a need to develop safer and effective antimicrobial agents with a broad spectrum of activity. Chen et al., in 2001 prepared a number of 7- substituted fluoroquinolone derivatives and evaluated for antibacterial and cytotoxic activity. They observed that synthesized compounds exhibit better activity against methicillin-resistant *Staphylococcus aureus* than norfloxacin [11]. Literature survey revealed that 4'-methylnorfloxacin and other 4-substituted piperazin-1-yl prodrugs of norfloxacin were prepared to improve the bioavailability of the parent fluoroquinolone [15,16]. In the present study, amide prodrugs of norfloxacin are prepared by coupling it with 4-oxo-4-substituted-phenylbutanoic acids in a hope to improve the spectrum and pharmacokinetic profile of norfloxacin. *In-vitro* antibacterial activity of the compounds **3a-e** was carried out against the four bacterial strains; two Gram positive (*Staphylococcus aureus* & *Bacilluus subtilis*), and two Gram negative (*Escherichia coli* & *Klebsiella pneumonia*) as per the reported method [13]. Minimum inhibitory concentration (*MIC*) was determined, and results indicated that prepared amide prodrugs possess good to excellent antibacterial activity against all the tested bacterial strains. Compound **3d** showed excellent activity against *S. aureus*, *E. coli*, and *B. subtilis* with *MIC* 6.25 µg/mL, and good activity against *K. pneumonia* with *MIC* 12.5 µg/mL. Another compound **3c** showed very good activity against *E. coli* with *MIC* 6.25 µg/mL, and good activity against *S. aureus*, *B. subtilis* and *K. pneumonia* with *MIC* 12.5 µg/mL. Norfloxacin showed *MIC* value ranging from 3.12-6.25 µg/mL against the tested microbes. Though *in-vitro* antibacterial evaluation, the synthesized compounds are found to be less active than the parent drug norfloxacin but it is expected that after *in- vivo* hydrolysis (by amidases and/or other enzymes) the prodrug would break into its parent compounds which have established antibacterial activity. *In vivo* studies are in progress in our laboratory to establish the suggested hypothesis.

CONCLUSION

Five new amide-based prodrugs (**3a-e**) were successfully synthesized by condensing norfloxacin with 4-oxo-4-substituted-phenylbutanoic acid (**2a-e**) in a single step. Two prodrugs (**3c** & **3d**) showed excellent antibacterial activity against the tested bacteria. Hydrolysis studies are required to assess the fate of the prodrugs in the system.

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