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SYNTHESIS AND EVALUATION OF A MUTUAL PRODRUG

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ABSTRACT

The aim of this study has been to synthesize a useful drug, which may act with effectiveness both on the gram-positive and gram-negative bacteria (broad-spectrum). An amide-based mutual prodrug (**3**) was synthesized by condensing sulfadiazine with nalidixic acid, and evaluated for *in-vitro* antibacterial activity with significant results. Hydrolysis kinetics of the mutual prodrug were also studied in acidic and basic buffers.

Key words: Quinolone, Sulfadiazine, Prodrug, Antibacterial

INTRODUCTION

The incidences of bacterial and fungal infections are increasing dramatically due to different factors including an increase in the number of immuno-compromised hosts[1,2]. Immunosuppression due to HIV-infection, malignancy, immunosuppressive therapies, broad-spectrum antimicrobial treatment and age, as well as invasive procedures and mucosal barriers places patients at high risk for microbial infections[3,4]. The increasing incidence of resistance to a large number of antibacterial agents is becoming another major concern[5,6]. These observations clearly indicate the need of as well as search for new and more effective antimicrobial agents with a broad spectrum of activity[7]. Nalidixic acid is effective against infections with gram-negative bacteria, but it is less effective against most of the gram-positive bacteria whereas sulfadiazine is a broad-spectrum antibacterial agent and orally effective against *Escherichia coli, Klebsiella species, Enterobacter species, Staphylococcus aureus, Proteus mirabilis* and *P. vulgaris*[8,9]. A prodrug is defined as a biologically inactive derivative of a drug candidate that requires a chemical or enzymatic transformation within the body to release the active drug, and has improved delivery properties over the parent molecule. Generally, in a prodrug, the carrier group or promoiety used is inert or non-toxic[10,11]. However, in certain cases the prodrug consists of two pharmacologically active agents coupled together in the form of a single molecule so that each acts as promoiety for the other agent. Such derivatives have been termed as mutual prodrugs[12,13].
In view of these observations and in continuation of our work on prodrugs[13], it was considered worthwhile to synthesize a mutual prodrug of nalidixic acid with sulfadiazine, with an objective of getting a compound which may act with effectiveness both on the gram-positive and gram-negative bacteria.

EXPERIMENTAL

Materials and Methods: Melting points were taken in open capillary tubes and are uncorrected. Dry solvents were used throughout the study. Microanalysis of the compounds was done on Perkin-Elmer model 240

analyzer and the values were found within ±0.4% of the theoretical values. ^{1}H NMR spectrum was 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. Spectral data are consistent with the assigned structure. 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. The reaction involved in synthesis is given in **scheme 1**.

Synthesis: Nalidixic acid (464 mg; 2 mmol) (**1**) was dissolved in dry pyridine (5 mL) and sulfadiazine (500 mg; 2 mmol) (**2**) was also dissolved separately in dry pyridine (5 mL). Both the solutions were mixed together and stirred magnetically. Phosphorous oxychloride (0.9 mL) was added dropwise maintaining the temperature below 5° C while stirring. The contents were stirred for another half-hour and left overnight. It was poured into ice cold water and a solid mass, which separated out, was filtered, washed, dried and crystallized from acetone followed by recrystalisation from methanol to give brown small needles of the mutual prodrug.

Hydrolysis studies in aqueous buffers: Hydrolysis kinetics of the synthesized mutual prodrug (**3**) were studied in acidic and basic buffer. Acidic buffer (pH 1.5) was prepared from conc. hydrochloric acid and basic buffer (pH 7.4) was prepared from Tris base (Tris hydroxymethyl amino methane) of 0.2 M strength. Microcentrifuge tubes (1.5 mL capacity) were used for sampling purpose. In each tube 1 mg of the drug was transferred and to it 1 ml of the buffer was added. Samples were kept on a mechanical shaker at a temperature of 37±0.5°C. The analysis was done at time intervals of 5 min, 30 min, 1 h, 3 h, 5 h, 7 h, 20 h and 50 h and subjected to HPLC analysis. Standard solutions were made in the solvent system, methanol: sodium hydroxide (0.05 M) [3:2 v/v]. The HPLC system consisted of a U.V. absorbance detector (programmable multiwavelength detector; Waters 490 E), data module (Waters 745 B), pump and column (Bondapak C_{18} column, particle size 10 µm, 30 cm x 3.9 mm I.D; Waters). Mobile phase was consisted of methanol : acetonitrile : potassium dihydrogen phosphate (0.015 M) [3:2:5 v/v/v] of pH 2.5 adjusted with o-phosphoric acid. Detection was done at U.V. 255 nm. The prodrug was eluted at the retention time of 11.3±0.2 min. Nalidixic acid and sulfadiazine were eluted at 9.4±0.3 min. and 5.12.7±0.2 min., respectively.

***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 [14,15]. 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

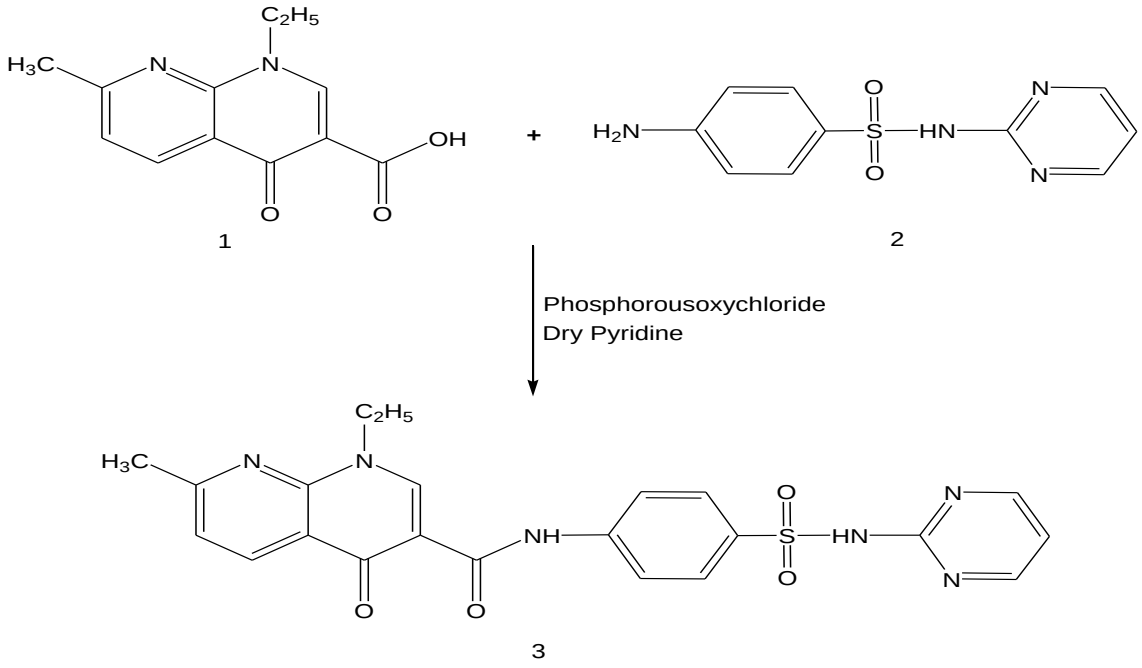
Synthesis: Nalidixic acid was condensed with sulfadiazine in dry pyridine in presence of phosphorous oxychloride ($POCl_3$) in a single step synthesis method (**Scheme 1**). Usual work up of the reaction mixture followed by crystallization with methanol furnished the desired compound (**3**) as dark red-colored fine needles, Melting Point: 212° C, Rf value: 0.79 (Toluene: Ethyl acetate: Formic acid, 5:4:1), Yield: 64.26 %.

Structure establishment of the mutual prodrug (3)

NMR spectrum: The ^{1}H NMR spectrum of the mutual prodrug (**3**) showed a triplet and a quartet located at δ 1.61 and δ 4.91 arising from the methyl and methylene group of ethyl moiety in nalidixic acid. There was a singlet located at δ 2.88 integrating for 3 protons of the methyl group of nalidixic acid skeleton. There could be located a triplet at δ 6.92 arising from the proton (H-4) of the diazine moiety. There appeared a multiplet at δ 7.29 arising from the protons (H-3,5) of diazine moiety. Four protons of the *p*-disubstituted benzene ring of sulfadiazine moiety appeared as doublets at δ 7.71 and δ 8.18. There could be located two *ortho*-coupled doublets at δ 7.66 and δ 8.75 arising from the two *ortho*-coupled protons of the nalidixic acid system. A singlet located at δ 9.47 could be accounted for the lone proton of the nalidixic acid system. NH-proton of the sulfonamide moiety appeared as a singlet at δ 9.91.

Mass spectrum: The mass spectrum of the mutual prodrug (**3**) showed a molecular ion peak located at m/z 464. The other two diagnostic peaks were located

at m/z 306 and 215. The fragmentation pattern has been shown in **Chart 1**.



1 2 3

Scheme 1: Protocol for synthesis of the mutual prodrug 3.

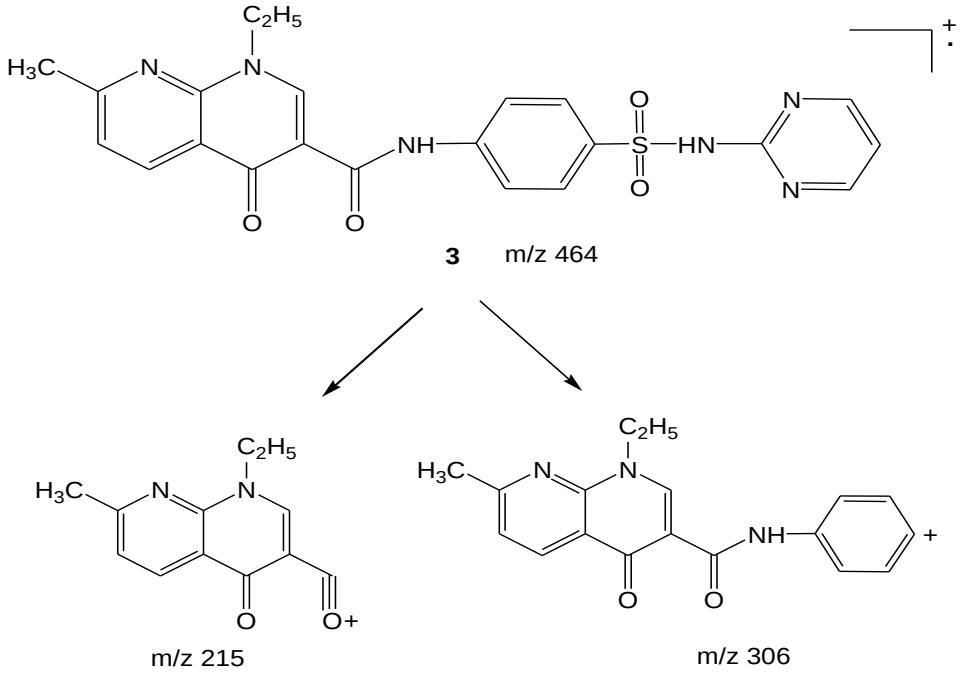


Chart 1: Mass fragmentation pattern of the mutual prodrug 3.

Elemental analysis: The values were found within ±0.4% of the theoretical values, $C_{22}H_{20}N_6O_4S$, Calculated C, 56.89; H, 4.34; N, 18.09, Found C, 56.64; H, 4.52; N, 17.88.

Hydrolysis study: *In-vitro* hydrolysis studies were carried out in aqueous buffer so as to study whether the mutual prodrug (**3**) hydrolyze in aqueous medium and to what extent or not, suggesting fate of the prodrug in the system. Hydrolysis kinetics of the synthesized prodrug (**3**) were studied in acidic buffer (pH 1.5) and basic buffer (pH 7.4). The hydrolysis of mutual prodrug (**3**) to its parent components (nalidixic acid & sulfadiazine) was not observed either in acidic or basic buffer suggesting that the drug was highly stable.

***In-vitro* 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 mutual prodrug (**3**) showed very good activity against *S. aureus, B. subtilis* & *E. coli* with *MIC*-12.5 µg/mL, and good activity against *K. pneumonia* (MIC-25 µg/mL). *In-vivo* antibacterial activities are required to further ascertain its usefulness; which are under progress in our laboratories.

CONCLUSION

Nalidixic acid and sulfadiazine were successfully condensed together through an amide-linkage to get a new mutual prodrug (**3**). *In-vitro* hydrolysis kinetics showed that the prodrug was resistant to hydrolysis in acidic and basic buffer system at pH 1.5 and 7.4, respectively, indicating its stability. *In-vitro* antibacterial activity of the compound against some selected bacteria showed significant antibacterial activities. The present work sheds the light on the pharmaceutical potential of mutual prodrugs comprising of classical agents.

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