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*Original Article*

# Amide Based Mutual Prodrug: Synthesis and Antimicrobial Evaluation

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**Abstract**

An amide-based mutual prodrug (NA-D) was synthesized by condensing nalidixic acid with dapsone with an aim of preparing a useful drug, which could show broad spectrum of antimicrobial activity. Its structure was established on the basis of elemental analysis, $^{1}$H NMR and Mass spectral data results. The mutual prodrug (NA-D) was also evaluated for in-vitro antibacterial and antifungal activity.

**Keywords**: Dapsone; Amide; Prodrug; Bacterial; Fungal.

## Introduction

Prodrug research is an important and fruitful area of research. A prodrug may be defined as a biologically inactive derivative of a drug that requires a chemical or enzymatic metabolism within the body to release the active drug [1]. Prodrugs may have improved pharmacokinetic, pharmacodynamic, physicochemical, and other properties over the parent molecule [1-3]. In a prodrug, generally the carrier group (promoiety/linker) used is inert or biologically inactive molecule [3]. However, in some cases the prodrug consists of two pharmacologically active agents joined together as a single molecule. In such prodrugs, each acts as promoiety for the other agent [4]. These prodrugs are known as mutual prodrugs [4,5]. Mutual prodrug concept has been successfully applied to a number of NSAIDs to get compounds with improved pharmacological profile including reduced GIT toxicity [1-6].

Microbial resistance to antibiotics has become a huge problem in treating infections in recent times [7]. Tuberculosis (TB) is a global emergency and is amongst the worldwide health threats today [8]. Searching new compounds, which could combine a non specific activity against a broad spectrum of microbes and low toxicity, seems to be a promising way to tackle the problem. Nalidixic acid (1,8-naphthyridine derivative) was the first synthetic quinolone derivative introduced for the treatment of UTI (urinary tract infections) in 1963 [9]. It is particularly effective against gram-negative bacteria particularly *Escherichia coli* and resistant to most of the pseudomonas species [10,11]. The derivatives of nalidixic acid also show significant antimicrobial activities [12].

On the other hand, dapsone is one of the important antimicrobial agents used to kill *M. leprae*. Dapsone derivatives also show potential antimicrobial activities [13,14].

In view of these observations and in continuation of our work on mutual prodrugs [5,15], it was considered worthwhile to synthesize a mutual

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prodrug chemically combining nalidixic acid with dapsone in a single structure with an aim of preparing a promising antimicrobial compound which might act with effectiveness on both the gram-positive and gram-negative bacteria, and also have antifungal activity.

## 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 dapsone (249 mg; 1 mmol) (**2**) was also dissolved separately in dry pyridine (4 mL). Both the solutions were mixed together and stirred magnetically. Phosphorous oxychloride (0.9 mL) was added dropwise maintaining the temperature 0-5° 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 separated out, which was filtered, washed with plenty of water, dried and crystallized from methanol to give TLC pure reddish-brown crystals of the mutual prodrug (NA-D).

*In vitro antibacterial activity*

The antibacterial activity of NA-D was evaluated against four bacterial strains, gram positive - *Staphylococcus aureus* (MTCC 96) & *Bacilluus subtilis* (MTCC 121), and gram negative - *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883). The assay was carried out following the turbidity method [16]. Nalidixic acid was used as a standard drug. A solution of NA-D / standard drug 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 nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and control were inoculated with approximately $5x10^5$ c.f.u. of actively dividing bacterial cells. The cultures were incubated at 37°C for 24 h and the growth was monitored. The highest dilution (lowest concentration) at which the growth of bacteria arrested was taken as minimum inhibitory concentration (*MIC*).

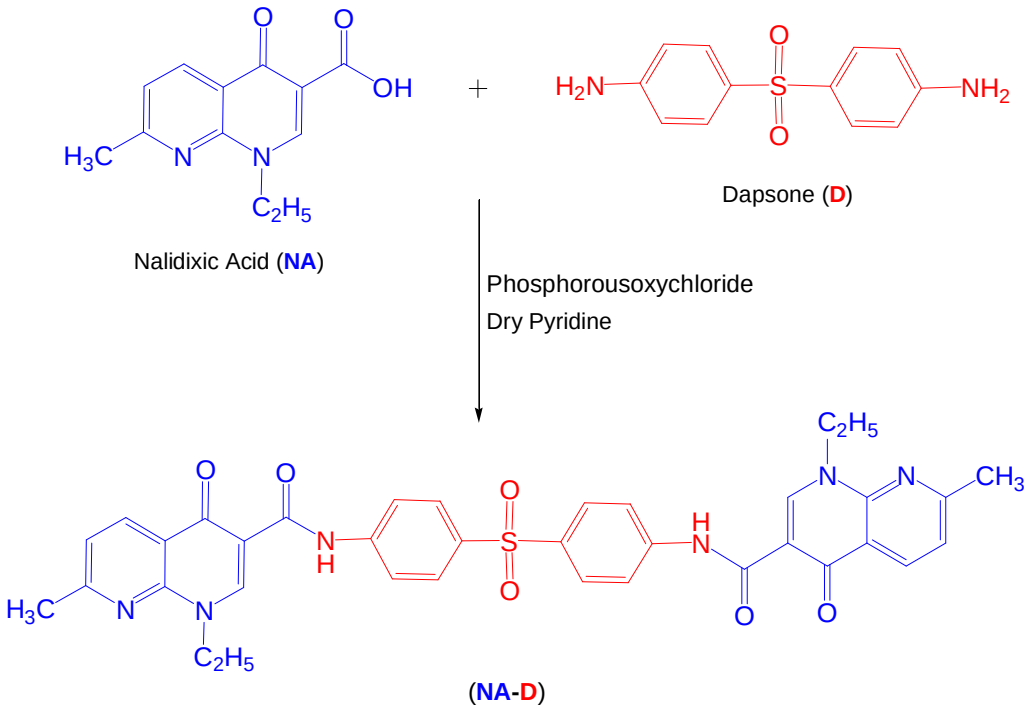
*In vitro antifungal activity*

Antifungal activity was evaluated against three fungal strains; *Candida albicans*, *Aspergillus niger* and *Rhizopus oryza [17,18]*. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was utilized to prepare a suspension of spores of fungal strain for lawning. A loopful of the fungal strain was transferred to 3 mL saline to obtain a suspension. The nutrient broth, which contained logarithmic serially two fold diluted amount of NA-D / standard drug and control was inoculated with approx. $1.6x10^4$-$6x10^4$ c.f.u. / mL. The cultures were incubated at 37°C for 48 h and the growth was monitored. Griseofulvin was used a s standard drug for comparison. The highest dilution (lowest concentration) at which the growth of fungi arrested was regarded as minimum inhibitory concentration (*MIC*).

## Results and Discussion

*Synthesis*

Nalidixic acid was condensed with dapsone in minimum quantity of dry pyridine in presence of phosphorous oxychloride ($POCl_3$) following single step synthesis method (*Scheme 1*). Usual work up of the reaction mixture followed by crystallization from methanol furnished the mutual prodrug (*NA-D*) as reddish-brown crystals, Melting Point: 214-216° C, Rf value: 0.67 (Toluene: Ethyl acetate: Formic acid, 5:4:1), Yield: 54 %.




**Scheme 1:** Protocol for synthesis of mutual Prodrug (NA-D)

*Structure establishment of the mutual prodrug (NA-D)*

*NMR spectrum:* The $^1$H NMR spectrum of the mutual prodrug (NA-D) showed a triplet and a quartet located at d 1.55 and d 4.81 arising from the methyl and methylene group of ethyl moieties ($2xC_2H_5$) in nalidixic acid. There was a broad singlet located at d 2.75 integrating for the methyl groups ($2xCH_3$) of nalidixic acid skeleton. Four protons of the dapsone ring appeared as doublets ($2xA_2B_2$ pattern) each at $\delta$ 7.67 and $\delta$ 8.02. There were two *ortho*-coupled doublets each at $\delta$ 7.62 and $\delta$ 8.63 arising from the two *ortho*-coupled protons of the nalidixic acid system. A singlet located at d 8.95 could be accounted for the lone proton of the nalidixic acid system. Two broad singlets located each at $\delta$ 9.81 and 9.98 could be accounted for 2x -NH- protons of the dapsone moiety.

*Mass spectrum:* The mass spectrum of the mutual prodrug (**NA-D**) showed a molecular ion peak located at m/z 676.

*Elemental analysis:* The values were found within ±0.4% of the theoretical values, $C_{36}H_{32}N_6O_6S$, Calculated C, 63.89; H, 4.77; N, 12.42, Found C, 63.54; H, 4.62; N, 12.58.

*Microbiology*

The synthesized mutual prodrug (**NA-D**) was evaluated for its in vitro antibacterial activity against the bacterial strains gram positive (*Staphylococcus aureus* & *Bacilluus subtilis*), gram negative (*Escherichia coli* & *Klebsiella pneumonia* ), and in vitro antifungal activity against *Candida albicans*, *Rhizopus oryza*, and *Aspergillus niger*. Minimum inhibitory concentration (*MIC*) was determined and results showed that the mutual prodrug (*NA-D*) was good against *B. subtilis* & *E. coli* with *MIC* 12.5 mg/mL, and significant activity against *S. aureus* with *MIC* 25 µg/mL. Nalidixic showed *MIC* 3.12 mg/mL against *E. coli*, *MIC* 6.25 mg/mL against *S. aureus* & *B. subtilis*, and *MIC* 12.5 µg/mL against *K. pneumonia*.

In antifungal assay, the mutual prodrug (*NA-D*) showed good activity against *C. albicans* with *MIC*12.5 mg/mL, and appreciable activity against *R. oryza* with *MIC* 25 µg/mL. Griseofulvin showed *MIC* 6.25 mg/mL against all the tested three fungal strains.

In vitro and in vivo hydrolysis studies are under progress in our laboratory to assess the fate of the *NA-D* in the system.

## Conclusion

Nalidixic acid and dapsone were condensed together through an amide-linkage (-CONH-) to get the mutual prodrug (*NA-D*). Spectral and analytical data were found in agreement with the proposed structure. In-vitro antibacterial activity of NA-D

against some selected bacteria showed good antibacterial and significant antifungal activities with *MIC* ranging from 12.5-25mg/mL. In vitro and in vivo hydrolysis studies are required to assess the fate of the *NA-D* in the system. The present work shows the pharmaceutical potential of mutual prodrugs.

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