ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
Volume 4, Issue 2, 2015, 43-46

Research Article

STUDIES ON AN AMIDE BASED MUTUAL PRODRUG: SYNTHESIS AND EVALUATION

Asif Husain[1]*, Aftab Ahmad[2], Shah Alam Khan[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard University, New Delhi-110 062, India

[2]Jeddah Community College, King Abdul Aziz University, Jeddah 21589, Kingdom of Saudi Arabia

[3]Department of Pharmacy, Oman Medical College, Muscat, Sultanate of Oman

Received 02 April 2015; Accepted 15 April 2015

ABSTRACT

The aim of this study has been to prepare a useful drug, which could have broad spectrum of antimicrobial activity including antitubercular action. Thus, an amide-based mutual prodrug (NA-INH) was synthesized by condensing isoniazid with nalidixic acid following single-step synthesis. Its structure was established on the basis of elemental analysis, ^{1}H NMR and Mass spectral data results. The mutual prodrug (NA-INH) was also evaluated for in-vitro antibacterial activity including anti-mycobacterial and antifungal activity.

Key words: Nalidixic acid, amide, prodrug, antibacterial, antiTB.

INTRODUCTION:

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 it may have improved pharmacokinetic, pharmacodynamic and other properties over the parent molecule[1-3]. Generally, in a prodrug, the carrier group or promoiety used is inert or non-toxic. 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[4]. Mutual prodrug concept has been successfully applied to various NSAIDs to get agents with improved pharmacological profile including reduced GIT side effects[4-6].

In recent years the bacterial resistance to antibiotics has become one of the most important problems of infections treatment[7]. Tuberculosis (TB) is a global emergency and is amongst the worldwide health threats today. TB remains the number one killer infectious disease affecting adults in developing countries[8]. Over the last few decades current control efforts are severely hampered due to *Mycobacterium tuberculosis* is a leading opportunistic infection in patients with the acquired immune deficiency syndrome and also due to the spread of multidrug-resistant strains (MDR-MTB)[8-10]. Designing new compounds, which would combine a non specific activity against a broad spectrum of bacteria and low toxicity, seems to be a promising way to combat that problem.

Nalidixic acid (1,8-naphthyridine derivative) was the first synthetic quinolone derivative introduced for the treatment of UTI (urinary tract infections) in 1963[11]. It is particularly effective against gram-negative bacteria particularly *Escherichia coli* and resistant to most of the pseudomonas species[12,13]. Nalidixic acid and its derivatives also show significant antibacterial including antitubercular activities[14].

On the other hand, Isonicotinoylhydrazide (Isoniazid: INH) is one of the most potent anti-TB drugs, used to kill the *M. tuberculosis*[14,15]. It is the first line antitubercular medication used in the treatment and prevention of Tuberculosis. Despite the various drugs currently under evaluation, isoniazid is still the key and most effective component in all multi-therapeutic regimens recommended by the WHO. Isoniazid derivatives show potential antitubercular activities[15,16].

In view of these observations and in continuation of our work on mutual prodrugs [5,17], it was considered worthwhile to synthesize a mutual prodrug clubbing isoniazid with nalidixic acid in a single structure with an objective of getting a compound which may act with effectiveness on both the gram-positive and gram-negative bacteria including *M. tuberculosis*.

*Corresponding author: Dr. Asif Husain | E-mail: drasifhusain@yahoo.com, ahusain@jamiahamdard.ac.in

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 isoniazid (274 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 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 water, dried and crystallized from methanol to give TLC pure light brown colored crystals of the mutual prodrug (**NA-INH**).

Antibacterial activity:

In vitro antibacterial activity was determined against the bacterial strains gram positive; *Staphylococcus aureus* (MTCC 96) & *Bacilluus subtilis* (MTCC 121) and gram negative: *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883). The test was carried out according to the turbidity method[18]. Nalidixic acid was used a s standard drug for comparison. 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 nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5x10^5$ c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (*MIC*).

Antifungal activity:

In vitro antifungal activity was determined against *Candida albicans*, *Aspergillus niger* and *Rhizopus oryza* [19,20]. 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 used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound/drug and controls was inoculated with approximately $1.6x10^4$-$6x10^4$ c.f.u./mL. The cultures were incubated for 48 h at 37°C and the growth was monitored. Griseofulvin was used a s standard drug for comparison. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (*MIC*).

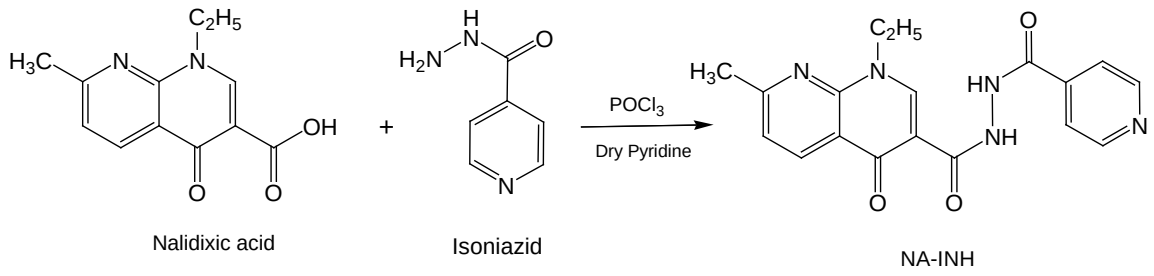
Antitubercular activity:

In vitro antitubercular activity[21,22] was determined against *M. tuberculosis* H37Rv (ATCC 27294) in Middle brook 7H11 agar medium with OADC (oleic acid albumin dextrose catalase) growth supplement. 10 fold serial dilutions of the mutual prodrug/isoniazid (in DMSO/water mixture) were incorporated into the agar medium. Inoculum of *M. tuberculosis* H37Rv were prepared from fresh Middlebrook 7H11 agar slants with OADC growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10-2 to give a concentration of approximately 10^7 cfu/mL. A 5 µL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 30 days. Isoniazid was used as standard drug for comparison. The minimum inhibitory concentration (*MIC*) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth.

RESULTS AND DISCUSSION:

Synthesis:

Nalidixic acid was condensed with isoniazid 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 (NA-INH) as dark red-colored fine needles, Melting Point: 172-74° C, Rf value: 0.79 (Toluene: Ethyl acetate: Formic acid, 5:4:1), Yield: 58.42 %.



Scheme 1: Protocol for synthesis of the mutual prodrug (NA-INH).

Structure establishment of the mutual prodrug (NA-INH):

NMR spectrum: The ^{1}H NMR spectrum of the mutual prodrug (NA-INH) showed a triplet and a quartet located at δ 1.57 and δ 4.83 arising from the methyl and methylene group of ethyl moiety in nalidixic acid. There was a singlet located at δ 2.77 integrating for 3 protons of the methyl group of nalidixic acid skeleton. Four protons of the 4-pyridyl ring appeared as doublets at δ 7.98 and δ 8.55. There could be located two *ortho*-coupled doublets at δ 7.61 and δ 8.65 arising from the two *ortho*-coupled protons of the nalidixic acid system. A singlet located at δ 8.98 could be accounted for the lone proton of the nalidixic acid system. Two singlets located at δ 9.74 and 9.93 could be accounted for 2x -NH- protons of the isoniazid moiety.

Mass spectrum: The mass spectrum of the mutual prodrug (NA-INH) showed a molecular ion peak located at m/z 351. The other two diagnostic peaks were located at m/z 306 and 215.

Elemental analysis: The values were found within ±0.4% of the theoretical values, $C_{18}H_{17}N_5O_3$, Calculated C, 61.53; H, 4.88; N, 19.93, Found C, 61.34; H, 4.61; N, 20.15.

Microbiology:

The newly prepared mutual prodrug (**NA-INH**) was screened for its in vitro antibacterial activity against the bacterial strains gram positive (*Staphylococcus aureus* & *Bacilluus subtilis*), gram negative (*Escherichia coli* & *Klebsiella pneumonia*), in vitro antifungal activity against *Candida albicans*, *Rhizopus oryza*, and *Aspergillus niger*, and in vitro antimycobacterial activity against *M. tuberculosis* H37Rv. Minimum inhibitory concentration was determined and results indicated that the mutual prodrug (**NA-INH**) showed very good activity against *S. aureus, B. subtilis* & *E. coli* with *MIC* 6.25 μg/mL, and good activity against *K. pneumonia* with *MIC* 12.5 μg/mL. Nalidixic showed *MIC* 3.12 μg/mL against *E. coli*, *MIC* 6.25 μg/mL against *S. aureus* & *B. subtilis*, and *MIC* 12.5 μg/mL against *K. pneumonia*.

In antifungal assay, the mutual prodrug (**NA-INH**) showed good activity against *C. albicans* with *MIC*12.5 μg/mL, and appreciable activity against *R. oryza* & *A. niger* with *MIC* 25 μg/mL. Griseofulvin showed *MIC* 6.25 μg/mL against all the three fungal strains. The antimycobacterial activity of **NA-INH** revealed *MIC* 10 μg/mL against *M. tuberculosis* H37Rv, while the standard drug isoniazid showed *MIC* 0.10 μg/mL.

In vitro and in vivo hydrolysis studies are under progress in our laboratory to assess the fate of the NA-INH in the system. Preliminary in vitro hydrolysis studies in acidic and basic buffer systems shows that the NA-INH is stable in these buffer systems.

CONCLUSION:

Nalidixic acid and isoniazid were successfully condensed together through an amide-linkage (-CONH-) to get a new mutual prodrug (**NA-INH**). In-vitro antibacterial activity of the compound against some selected bacteria showed very good antibacterial and significant antifungal activities with *MIC* ranging from 6.25-12.5μg/mL. The prodrug also showed remarkable antitubercular activity against *M. tuberculosis* H37Rv with *MIC* 10 μg/mL. In vitro and in vivo hydrolysis studies are required to assess the fate of the **NA-INH** in the system. The present work sheds the light on the pharmaceutical potential of mutual prodrugs comprising of classical agents.

Acknowledgements

One of the authors (AH) is thankful to Hamdard National Foundation (HNF), New Delhi for financial support.

REFERENCES:

1. Kristiina M. Huttunen, Hannu Raunio and Jarkko Rautio, Prodrugs—from Serendipity to Rational Design, Pharmacological Reviews, 2011, 63(3), 750-71.
2. Jarkko Rautio, Hanna Kumpulainen, Tycho Heimbach, Reza Oliyai, Dooman Oh, Tomi Järvinen & Jouko Savolainen Prodrugs: design and clinical applications. Nature Reviews Drug Discovery 2008, 7, 255-70.

3. Jana S, Mandlekar S, Marathe P. Prodrug design to improve pharmacokinetic and drug delivery properties: challenges to the discovery scientists. Curr Med Chem. 2010;17(32):3874-908.
4. Bhosle, D.; Bharambe, S.; Gairola, Neha; Dhaneshwar, Suneela S. Mutual Prodrug concept: fundamentals and applications, Ind. J. Pharm. Sci., 2006, 68(3), 286.
5. Asif Husain, Priyanka Ahuja, M. Shaharyar, Aftab Ahmad, Ibraheem Ahmed I. Mkhalid, M.M. Alam, M. Akhter, and M.S. Zaman (2014), Synthesis, biological activities and pharmacokinetics studies of a mutual prodrug of aceclofenac and paracetamol. *Med. Chem. Res.*, 23(3) 1077-1083.
6. Halen PK, Murumkar PR, Yadav MR, Giridhar R., Prodrug designing of NSAIDs. Mini Rev. Med. Chem, 2009; 9(1): 124-39.
7. Davies J. Nature, 1996; 383: 219-20.
8. Zaman K., Tuberculosis: A global Health Problem, J Health Popul Nutr., 2010, 28(2), 111-13.
9. Janin YL. Antituberculosis drugs: Ten years of research. Bioorg Med Chem. 2007; 15(7): 2479-513.
10. Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martin-Casabona N, et al. Worldwide emergence of extensively drug-resistant tuberculosis. Emerg Infect Dis.2007;13:380–7
11. G.Y. Leisher, E.J. Froelich, M.D. Gruett, J.H. Bailey, P.R. Brundage, J. Med. Pharm. Chem. 5 (1962) 1063e1065.
12. G.C. Crumplin, J.T. Smith, Antimicrob. Agents Chemother. 8 (3) (1975)
13. N. Aggarwal, R. Kumar, C. Srivastava, P. Dureja, J.M. Khurana, J. Agric. Food Chem. 58 (2010).
14. Zhang Y, The magic bullets and tuberculosis drug targets, Annu. Rev. Pharmacol. Toxicol. 2005, 45, 529-64.
15. Asif M., A Review on Antitubercular Agent Isoniazid and Its Derivatives, Mini-Reviews Med. Chem., 2015, 15(14). **DOI:** 10.2174/1389557514666140613114447
16. Hearn MJ, Cynamon MH, Chen MF, et al. Preparation and antitubercular activities in vitro and in vivo of novel Schiff bases of isoniazid. Eur J Med Chem. 2009; 44(10): 4169-4178.
17. Asif Husain, Aftab Ahmad, Shah Alam Khan, Mohammad Sarafroz. Synthesis and evaluation of a mutual prodrug. *Int. J. Pharmacy,* 2015, 5(2), 389-392.
18. Colle JG, Duguid JP, Fraser AG, Marmion BP. Laboratory strategies in diagnosis. In: Mackie TJ, MacCartney JE, eds. Practical Medical Microbiology, 13th ed. London: Churchill Livingstone, 1989;601–649.
19. Khan ZK. In vitro and vivo screening techniques for bioactivity screening and evaluation. In: *Proc. Int*. Workshop UNIDO-CDRI, 1997, 210–11.
20. Varma RS, ed. Antifungal Agents: Past, Present and Future Prospects Lucknow, India: National Academy of Chemistry & Biology, 1998.
21. W. K. Elmer, D. A. Stephen, M. J. William, C. S. Paul, C. W. Washing, Text Book of Diagnostic Microbiology, 5th ed, Lippincot Publishers, 2002.
22. J. B. Ellen, R. P. Lancer, M. F. Sydney, Bailey and Scott`s Diagnostic Microbiology, 9th ed, 2000.

