Research Article

Synthesis, Characterization, Antimicrobial Evaluation and Forced Degradation Studies of Mutual Amide Prodrug of Moxifloxacin and Isoniazid

Elluru Himanesh Kumar[1*], S. Subramanyam[2] and K. Thamizhvanan[3]

[1]Department of Pharmaceutical Analysis, Sree Vidyanikethan college of pharmacy, A. Rangampeta, Tirupati – 517 102, Andhra Pradesh, India.

[2]Department of Pharmaceutical Chemistry, Sree Vidyanikethan College of Pharmacy, A. Rangampeta, Tirupati – 517 102, Andhra Pradesh, India.

[3]Department of Microbiology, Sree Vidyanikethan College of Pharmacy, A. Rangampeta, Tirupati – 517 102, Andhra Pradesh, India.

ABSTRACT

The aim of the present work was to synthesize and evaluate the antimicrobial activity and forced degradation studies of mutual amide prodrug of moxifloxacin Hcl with Isoniazid. The mutual prodrug (III) was synthesized by single step process by the condensation of moxifloxacinHcl (I) with Isoniazid (II) in the presence of $POCl_3$ with continuous stirring for 3 hours. The structure of synthesized prodrug was confirmed by IR, H^1 NMR and Mass spectral data and characterized by physicochemical properties including the Melting point and R_f value. The prodrug (III) was evaluated for antibacterial activity against gram positive *Bacillius substilis* and gram negative bacteria strain *Escherichia coli* and anti fungal activity against *Candida albicans* by cup plate and serial dilution method. The mutual prodrug (III) showed impressive antibacterial activity with Zone of inhibition 20mm when compared with standard ampicillin with zone of inhibition 12mm against *Escherichia coli* and exhibited less antifungal activity against *Candida albicans* with zone of inhibition 28mm and also showed significant antibacterial and antifungal activity with MIC 6.25µg/ml against *Bacillus substilis* and *candida albicans*. The forced degradation studies of prodrug (III) was performed by UV Spectrophotometer at wave length 362 nm under various stress conditions like acid, alkali, oxidation, photolytic, thermal. Among all the stress conditions the prodrug (III) was stable in alkali condition (0.1 N NaoH) with less percentage of degradation 69.41% .

Keywords: MoxifloxacinHCl , Isoniazid, $POCl_3$, Forced degradation studies.

INTRODUCTION

A mutual prodrug is a form of drug where two pharmacologically active agents are attached to each other in such a way that acts as a promoiety/carrier for each other. Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent developed by Bayer AG (initially called BAY 12-8039). It is marketed worldwide (as the hydrochloride) under the brand names **Avelox**, **Avalox**, and **Avelon** for oral treatment. In most countries, the drug is also available in parenteral form for intravenous infusion. Moxifloxacin is also sold in an ophthalmic solution (eye drops) under the brand names Vigamox, and Moxeza for the treatment of conjunctivitis (pink eye). Its antibacterial spectrum includes enteric Gram-(−) rods (*Escherichia coli*, *Proteus* species, *Klebsiaell* species), *Haemophilusinfluenzae*, atypical bacteria (*Mycoplasma Chlamydia*, *Legionella*, and *Streptococcus pneumoniae*, and anaerobic bacteria. It differs from earlier antibacterials of the

fluoroquinolone class such as levofloxacin and ciprofloxacin in having greater activity against Gram-positive bacteria and anaerobes Because of its potent activity against the common respiratory pathogen *Streptococcus pneumoniae*, it is considered a "respiratory quinolone. On the other hand, Isonicotinoylhydrazide (Isoniazid: INH) is one of the most potent anti-TB drugs, used to kill the M. tuberculosis14,15. 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 it was considered worthwhile to synthesize a mutual prodrug clubbing moxifloxacinHcl with Isoniazide in a single structure with an objective of getting a compound which may act as effective of antimicrobial activity against both gram-positive and gram negative bacteria etc.

EXPERIMENTAL

Drug was gifted from Micro-labs (Sikkim) as gift Sample MoxifloxacinHcl and (INH) was procured from Cipla pharmaceuticals as a gift sample for project purpose. $PocI_3$ was purchased from Hi-media chemicals. Dry solvents were used throughout the study. Purity of the prodrug was monitored by TLC analysis using precoated aluminium plates (Cipla) coated with silica gel (kiesel gel 60). Melting points were determined in open capillaries using SRS Digi melt apparatus and were uncorrected. IR Spectra were recorded as FT-IR Agilent carry 630 4100 Spectrophotometer. ^{1}H NMR spectra were carried out on Bruker-400 MHz NMR Spectrophotometer (Bruker 400) using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) using $DMSOD_6$ as solvent and coupling constants in Hz. Splitting patterns are designed as follows: S, singlet; d, doublet; t, triplet; etc. Mass spectral data was obtained on LC-MS (Agilent). UV Spectrophotometry (Agilent 360) FT-IR (Agilent carry 630).

Synthesis of mutual amide prodrug (5-chloro-1-cyclopropyl-6-fluoro-*N*^{1}-isonicotinoyl-8-methoxy-4-oxo-7-((4as,7as)-tetrahydro-1H-pyrrolo[3,4,-b]pyridine-6(2H,7H,7aH)-yl)-1,4-dihydroquinoline-3-carbohydrazide). (III)

Moxifloxacin Hcl (4g; 0.01 mol) and Isoniazid (2.7 g; 0.02mol) were dissolved separately 15 ml each in dry pyridine. Both the solution were mixed together and stirred magnetically. Phosphorous oxychloride (2ml) was added drop wise to the above contents and stirred at temperature 10^{o}c. The contents were stirred for another three hours and left overnight. It was poured into ice cold water and a solid mass separated out. The mass was filtered and recrystallized with $CHCl_3$. Scheme (1) and physical data of prodrug (III) was given in **Table 1.**

IR, ^{1}HNMR&Mass Spectra of prodrug (III)

The IR Spectrum of prodrug (III) peak at 3490cm^{-1} (Amide N-H Str), 1703.277 (C=0 keto), 1620 cm^{-1} (C=O amide).**^{1}H NMR (400 MHz, $DMSOD_6$)** δ (ppm): 6.5 (doublet H-C-F), 2.9 (doublet –CONH-).

Antimicrobial Activity

Cup plate Method

In-vitro antibacterial activity was evaluated by cup plate method against gram positives strains of bacteria. *Bacillius substilis* (MTCC 95) and gram negative: *E-coli* (MTCC 3160) and antifungal activity against *candida albicans*. Ampicillin and Fluconazole were used as a standard drugs. Mutual prodrug were dissolved in distilled water and prepared 100µg/ml concentration by diluting with DMSO. Agar agar and nutrient agar broth was prepared and sterilized by using autoclave at 121^o C for 30 minutes. Sterilized agar media were poured into sterile petri plates for solidification. The petri plates was inoculated under aseptic conditions and cavities were made for filling the sample. The sample solution was filled with micro pipettes in the cavities and incubated for 24hrs at 28^o C for antibacterial activity and 48 hrs at 32 ^{o} C for antifungal activity.

Serial dilution Method

In-vitro antibacterial was performed by serial dilution method against gram –ve bacteria *E-coli* and gram +ve bacteria *Staphylococcus aureus, and antifungal activity aganist candida albicans.* The inhibition of growth of micro-organisms was measured by MIC.

RESULTS AND DISCUSSION

In the present study the synthesis of the mutual amide prodrug (III) (–CONH-) amide linkage was formed **scheme 1**The prodrug (III) showed good yields (84%). The IR Spectra of the title compound showed intensive bands at 3490 Cm^{-1}, 1620cm^{-1} represents the presence of secondary amide

group and C=O carbonyl group respectively and the absence broad OH group at 3546 cm^{-1} . ^{1}H NMR Spectrum of the compounds supported the structure of prodrug (III) Showed (6.5 ppm H-C-F), (-CONH-) doublet at 2.9).

Newly synthesized prodrug (III) was examined by cup plate method for both antibacterial as well as antifungal activity and the results were summarized in **Fig: 1 & 2**. The antibacterial screening results revealed that the prodrug (III) showed impressive antibacterial activity against *Escherichia coli* and *Bacillius substilis* compared with standard ampicillin respectively. prodrug (III) showed less antifungal activity against *Candida albicans* when compared with standard fluconazole. **Table-2** prodrug (III) was evaluated by serial dilution method it showed potent antibacterial activity against *Bacillus substilis* when compared to standard ampicillin **Fig: 3 & 4** and also It showed potent antifungal activity against *Candida albicans* when compared to standard fluconazole. **Table 3**

FORCED DEGRADATION STUDIES BY UV SPECTROPHOTOMETER

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities.Longer term storage tests are usually used to measure similar properties when final formulations are involved because of the stringent FDA regulations. These tests are generally more expensive. (because of the time involved) than forced degradation which is therefore used for rapid selection and elimination tests

Procedure for Forced degradation studies

Acid Degradation

Weight accurately about 10mg of prodrug was transfered into a clean and dry standard flask. Add 2-5ml of distilled water sonicate for 10 minutes. And make up the volume with 0.1 N Hcl =100 µg/ml. (Standard). Pipette out 1ml from the standard solution make up the volume with 10 ml= 10 µg/ml (sample). Record both the absorbance standard as well as sample at 362nm at different time intervals.1,2,3,4,5 hrs.

Alkali Degradation

Weight accurately about 10mg of prodrug was transfered into a clean and dry standard flask. Add 2-5ml of distilled water sonicate for 10 minutes. And make up the volume with 0.1 N NaoH =100 µg/ml. (Standard). Pipette out 1ml from the standard solution make up the volume with 10 ml= 10 µg/ml (sample). Record both the absorbance standard as well as sample at 362nm at different time intervals.1,2,3,4,5 hrs.

Oxidative Degradation

Weight accurately about 10mg of prodrug was transfered into a clean and dry standard flask. Add 2-5ml of 20% H_2O_2sonicate for 10 minutes. And make up the volume with distilled water =100 µg/ml. (Standard). Pipette out 1ml from the standard solution make up the volume with 10 ml= 10 µg/ml (sample). Record both the absorbance standard as well as sample at 362nm at different time intervals.1,2,3,4,5 hrs.

Thermal Degradation

Weight accurately about 10mg of prodrug was placed into a hot air oven maintaining the temperature at 105 ^{0} C for 30 minutes. After 30 minutes transfer the prodrug into a clean and dry standard flask. Add 2-5ml of distilled water sonicate for 10 minutes. And make up the volume with distilled water =100 µg/ml. (Standard). Pipette out 1ml from the standard solution make up the volume with 10 ml with distilled water = 10 µg/ml (sample). Record both the absorbance standard as well as sample at 362nm at different time intervals.1,2,3,4,5 hrs.

Photolytic Degradation

Weight accurately about 10mg of prodrug was placed into a UV Chamber for 30 minutes. After 30 minutes transfer the prodrug into a clean and dry standard flask. Add 2-5ml of distilled water sonicate for 10 minutes. And make up the volume with distilled water =100 µg/ml. (Standard). Pipette out 1ml from the standard solution make up the volume with 10 ml with distilled water 10 µg/ml (sample). Record both the absorbance standard as well as sample at 362nm at different time intervals.1,2,3,4,5 hrs.

The newly synthesized prodrug (III) was examined for various stess conditions at wave length 362nm by using UV spectrophotometer following ICH Q1 guidelines. The commonly used stress conditions are Acid, Base, Oxidation, Thermal, Photolytic. Among this five stress conditions the prodrug (III) showed more stable in Alkali condition (0.1 N NaoH) with 69.41% when compare to other stress conditions. **Table 4**, **Fig: 5**

CONCLUSION

MoxifloxacinHcl (I) was successfully condensed with Isoniazide(II) in a single step

to furnish an amide-based prodrug (III) and structure was characterized by IR, NMR, and Mass spectral data. The mutual prodrug (III) showed impressive antibacterial activity with zone of inhibition 12mm against *Escheriachia coli* and showed impressive zone of inhibition 31mm against *Bacillius substilis* when compared with standard ampicillin **.** The prodrug showed less antifungal activity with zone of inhibition 28mm when compared with standard fluconazole against *Candida albicans* 40mm. The prodrug (III) showed very good antibacterial activity with MIC 6.25µg/ml against *Staphylococcus aureus* and impressive antifungal activity against *candida albicans* with MIC 6.25µg/ml when compared with standard fluconazole .Forced degradation of prodrug represents stable in alkali stress condition (0.1 N NaoH) 69.41% when compared with other stress conditions at wave length 362 nm by UV Spectrophotometer.

ACKNOWLEDGEMENT

I would like to thank to management of SreeVidyanikethancollege of pharmacy for providing me a good facilities to do my research work.

Table 1: Physical characterization data of prodrug (III)

Compound	Molecular formula	Mol. Wt	Solubility	Melting point	R_F
Prodrug (III)	$C_{27}H_{31}FN_6O_4Cl$	556	Completely soluble in distilled water	320^o C	0.545

Table 2: Antimicrobial activity of prodrug (III)

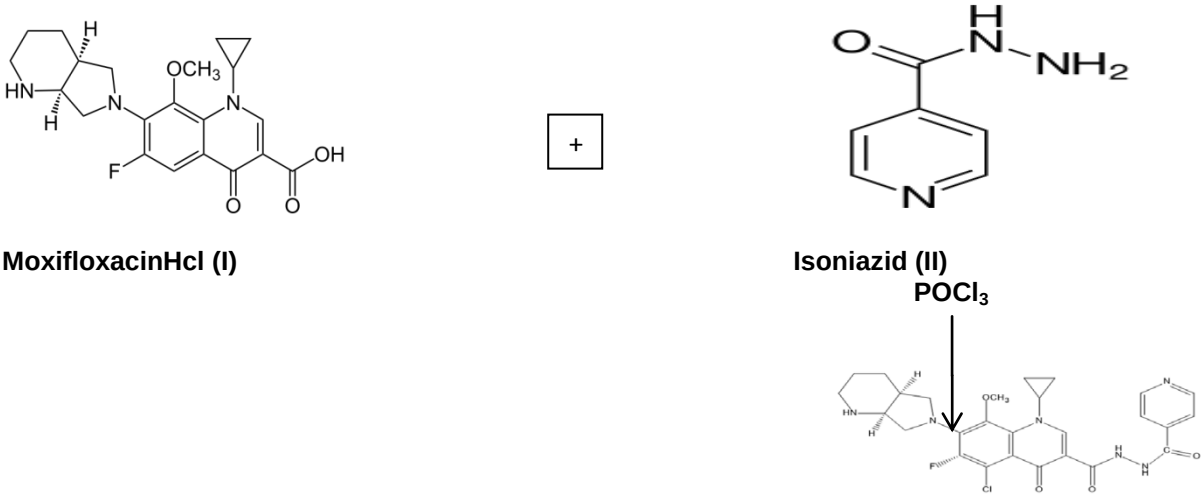
Compounds	Bacterial inhibition zone/mm		Compounds	Fungal inhibition zone/mm
	E-coli	*B-substilis*		*C- Albicans*
Ampicillin	12	20	Fluconazole	40
Prodrug	20	31	Prodrug	28

Table 3: Antimicrobial activity of prodrug (III)

Compounds	Bacterial inhibition MIC µg/mL		Compounds	Fungal inhibition MIC µg/mL
	E-coli	*B-substilis*		*C- Albicans*
Ampicillin	12.5	12.5	Fluconazole	12.5
Prodrug	6.25	6.25	Prodrug	6.25

Table 4: Forced degradation studies

S. No.	Degradation condition	Exposure	Time in Hrs	% Degraded
1	Acid	0.1 (N) Hcl	5	71.48
2	Alkali	0.1 (N) NaoH	5	69.41
3	Oxidation	40% H_2O_2	5	74.35
4	Thermal	At 60^0 c	5	71.09
5	Photolytic	UV Chamber	5	71.24



MoxifloxacinHcl (I) **Isoniazid (II)**

Scheme 1: Synthesis of prodrug (III)

Fig. 1: Antibacterial activity of (III)

Fig. 2: Antifungal activity (III)

Fig. 3: Against *B.substilis* with 6.25 µg/mL (III)

Fig. 4: Against *C-albicans*with MIC 6.25

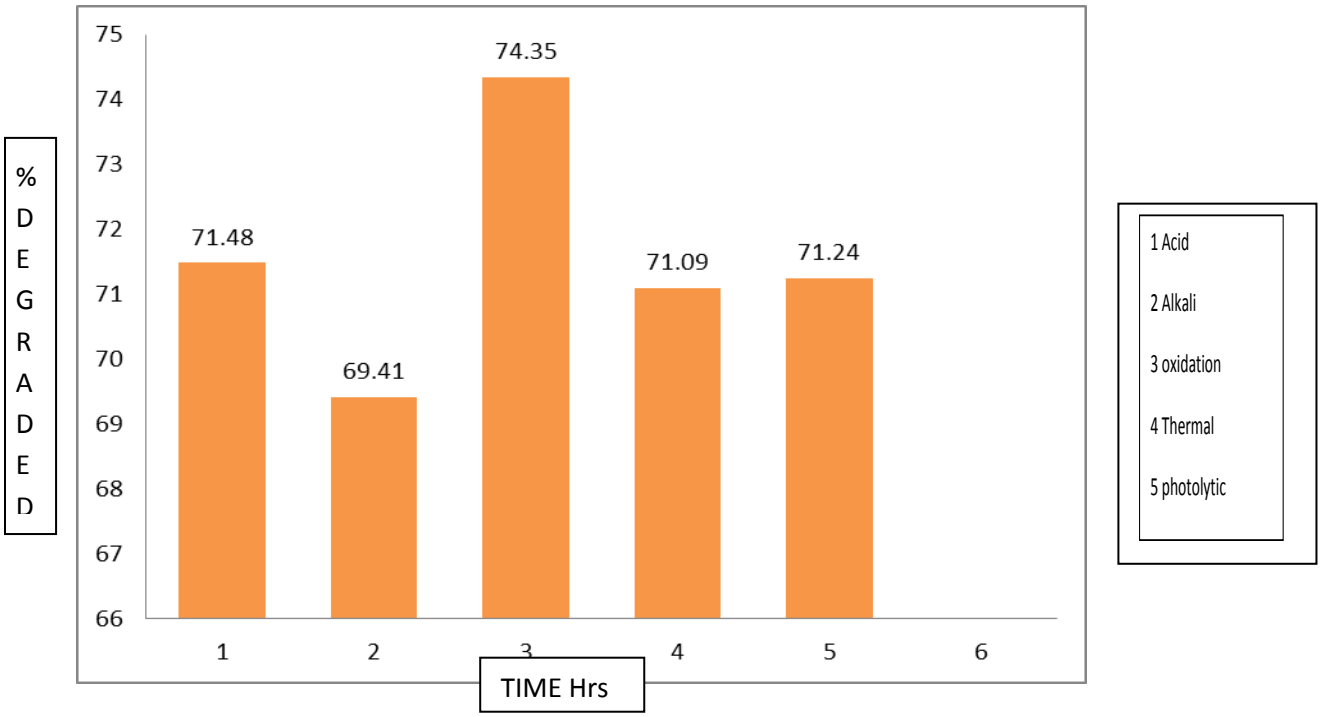


Fig. 5: Pictorial representation of various degradation conditions

REFERENCES

1. Pandeya SN, Sriram D, Nath G and De Clercq E. Farmaco. 1999;54:624.
2. Piscopo B, Diumo MV, Godliardi R, Cucciniello M and Veneruso G. *Bol Sper.* 1987;63:827.
3. 4-Ashok et al. Trisubstituted thiadiazole as bioactive agents oriental J Chem. 2007;23(1):183-190.
4. Auberson YP. Competative AMPA antagonism: a novel mechanism for antiepileptic drugs. Drugs Fut. 2001;26:463-471.
5. BerkMet al. Emerging options in the treatment of biopolardisorder Drugs. 2001;61:1407-1414.
6. AsifHussain et al. Studies on amide based mutual prodrugs: Synthesis and evaluation. Med Chem. 2015;4:43-46.
7. Zhang Y. The magic bullets and tuberculosis drug targets. Annu Rev Pharmacol Toxicol. 2005;45:529-564.
8. Ellen JB, Lancer RP, Sydney MF, Bailey and Scott S. Diagnostic Microbiology, 9th ed, 2000.
9. Halen PK, Murumkar PR, Yadav MR and Giridhar R. Prodrug designing of NSAIDs. Mini rev Med Chem. 2009:9(1):124-139.
10. Asif M. A Review on Antitubercular Agent Isoniazid and its derivatives. Mini–Reviews. Med Chem. 2015;15(14).
11. Aggarwal N, Kumar R, Srivastava C, Dureja P and Khurana JM. J Agric food Chem. 2010:58.
12. Jana S, Mandlekar S and Marathe P. Prodrug design to improve Pharmacokinetics and drug delivery properties: challenges to the discovery scientist. Curr Med Che. 2010;17(32):3874-3908.
13. Salicylic acid topical medical facts from Drugs.com. www.drugs.com. Retrieved 15 January 2017.
14. Isoniazid. The American Society of Health-System Pharmacists. Retrieved 8 December 2016.