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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF MUTUAL AMIDE PRODRUG OF SALICYLIC ACID AND ISONIAZID

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ABSTRACT

The aim of the present work was to synthesize and evaluate the anti microbial activity of mutual amide prodrug of Salicylic acid (I) with Isoniazid (II). The mutual prodrug (III) was synthesized by single step process by the condensation of salicylic acid (I) with Isoniazid (II) in the presence of POCl3 with continuous stirring for 3 hours.The structure of synthesized prodrug was confirmed by IR, H^1NMR and Mass spectral data and characterized by some physicochemical properties including the Melting point and Rf value. The prodrug (III) was evaluated for anti microbial activity against gram positive (*Staphyloccoccusaureus)* and gram negative bacteria strain (*Escherichiacoli)* and fungal activity against *Candidaalbicans* by cup plate and serial dilution method. The prodrug (III) showed less anti bacterial activity with Zone of inhibition 20mm when compared with standard Ampicillin with zone of inhibition 22mm against (*Escherichia coli)*and potent antifungal activity against (*Candida albicans)* with zone of inhibition 30mm. The prodrug (III) showed significant antibacterial and antifungal activity with MIC 6.25µg/mlagainst (*Staphyloccoccusaureus)* and (*candidaalbicans*).

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. Salicylic acid is a compound mainly used in organic synthesis. It is an active metabolite of aspirin which actin partasa prodrug in salicylic acid[13] Salicylic acid is a medication used to treat the warts, psoriasis dandruff. Chemically it is2-Hydroxy benzoic acid. The Salicylic acid was active against whole bacterial spectrum responsible for complicated against antibiotics and mainly acts on NSAIDs and production of other pharmaceuticals ,including 4-amino salicylic acid sandulpiridelandetimide(via Salethamide). On the other hand, Isonicotinoyl hydrazide (Isoniazid:INH) is one oft he 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 componentinally multi-therapeutic regimens recommended by the WHO. Isoniazid derivatives show potential antitubercular activities[15,16] Inview of these observations and In continuation of our work on mutual prodrugs it was considered worth

while to synthesize a mutual prodrug clubbing salicylic acid with Isoniazid in a single structure with an objective of getting a compound which may act as effective of anti microbial activity against both gram-positive and gram-negative bacteria etc.

2.0 EXPERIMENTAL WORK

2.1 Materials and Methods:

Drug was gifted from micro-labs Sikkim as gift Sample Salicyclic acid and(INH) was procured from Cipla pharmaceuticals as a gift sample for project purpose. Pocl3 was purchased from Hi- media chemicals. Dry solvents were used through out the study. Purity of the prodrug was monitored by TLC analysis using precoated aluminium plates (Cipla) coated with silicagel (kiesel gel 60). Melting points were determined in open capillaries using SRS Digimelt 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 (Bruker400) using TMS as internal reference. Chemicalshifts ($^{\delta}$)values are given in parts per million (ppm) using DMSOD6 as solvent and coupling constants in Hz. Splitting patterns are designed as follows :S, singlet; d, double t; t,triplet; etc. Mass spectral data was obtained on LC-MS (Agilent).

Synthesis of prodrug: ***N^{1}***–(2-hydroxy benzoyl) Isonicotino hydrazide. (III). Salicylic acid (1000mg;5mmol) and Isoniazid (822 mg;5mmol) were dissolved separately15 ml each in dry pyridine. Both the solution were mixed together and stirred magnetically. Phosphorousoxychloride (2ml) was added drop wise to the above content and stirred at temperature10^{O}c. The contents were stirred for another three hours andl eft ove rnight .It was poured into ice cold water and a solid mass separated out. The mass was filtered and w a s h e d with water and crystallized form was collected while washing with distilled water.

Melting point Prodrug (III) 106^{0}c. **RF**: (toluene4:ethylacetate4:formicacid2) Rf: (III) 0.622. **(FT-IR4000)** Vmax, cm^{-1}3321.634 (2^{O}amines or amide N-H Str), 2849.970 (C-H Str alkanes), 1606,1641 (C=C alkenes, C=O Str amide).**^{1}H NMR (400MHz,DMSOD6)** $^{\delta}$(ppm): 13.5 (Singlet O-H),11.68 (doublet –CONH-), 7.2 (Ar-H Singlet),12-12.5 (Pyridyl protons doublet).

LC-MS Agilent:M/z = 257 (M^{+}).

2.3 ANTI-MICROBIAL STUDIES

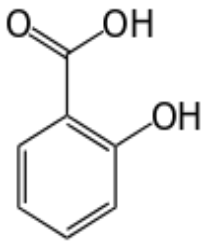
Cup plate method: *In Vitro* antibacterial activity was determined against gram positive strains of bacteria; *Staphylococcus aureus* (MTCC95) and gram negative: *E-coli* (MTCC3160). The test was carried out according to the zone of inhibition method. Ampicillin was used as a standard drug. Drugs and 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 30minutes. 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. The zone of inhibition was performed.

Serial dilution Method: *In Vitro* anti bacteria lactivity was performed against gram –ve bacteria *E-coli* and gram +ve bacteria *Staphylococcu saureus* and nutrient broth was used as liquid culture. Prodrug (SA-INH) was determined by using serial dilution method. Serial dilution method is mainly based on MIC minimum inhibitory concentration. The MIC was performed against *Escherichiacoli gram-ve, Staphylococcus aureus gram+ve* as well as antifungal activity against *candidaalbicans* In vitro anti fungal activity was performed against *candidaalbicans* ,and. Sabourands agar media was prepared by dissolving peptone(0.5g),D-glucose(2g), Agar (1g) ,in distilled water 50ml and adjustied pH glucose (2g), Agar (1g), in distilled water 50ml and adjustied pH 6.

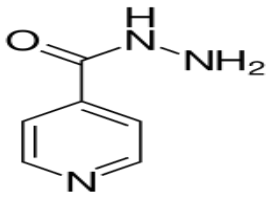
3. RESULTSAND DISCUSSION:

In the present study the synthesis of the mutual amide prodrug (III) were carried out via stirring process (AsifHusainetal;2015).

(–CONH-) amide linkage was formed Fig:1, The prodrug(III) showed the yields(42-70%).

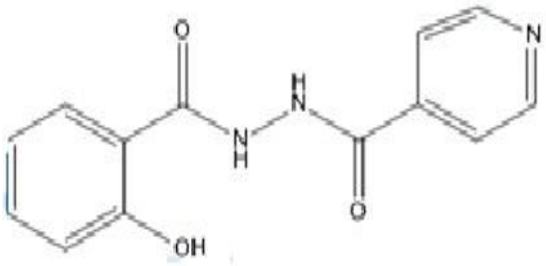


Salicylicacid(I)



Isoniazid(II)

$POCl_3$



Mutual amide prodrug (III)

Fig:1 Schematic reaction of Mutual amide prodrug

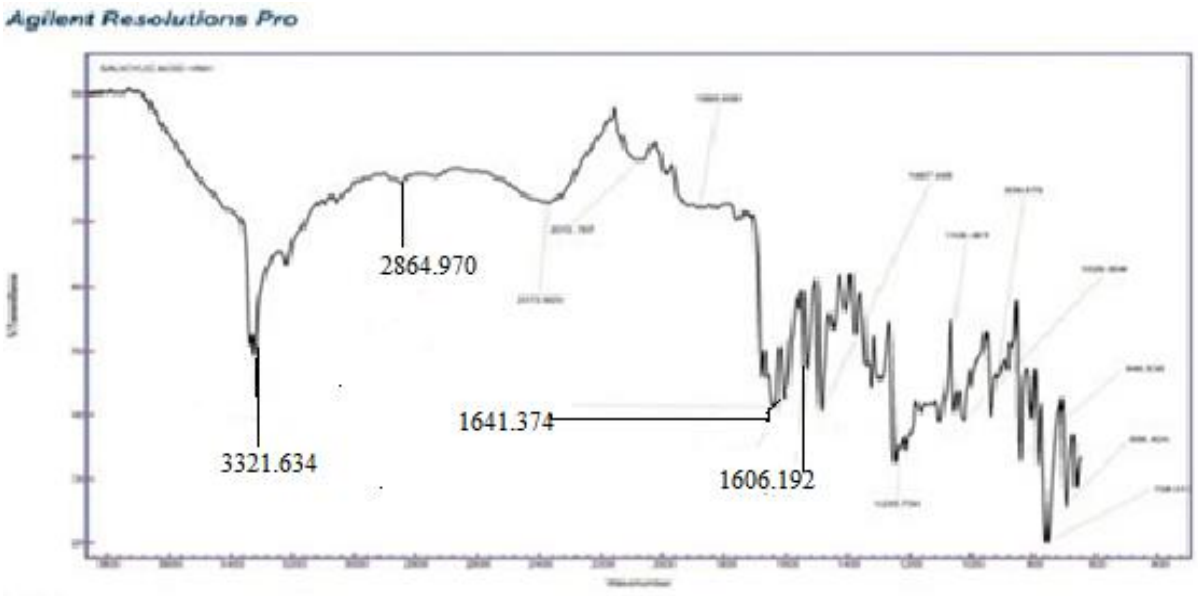


Fig2: IR Spectrum of Mutual amide prodrug (III).

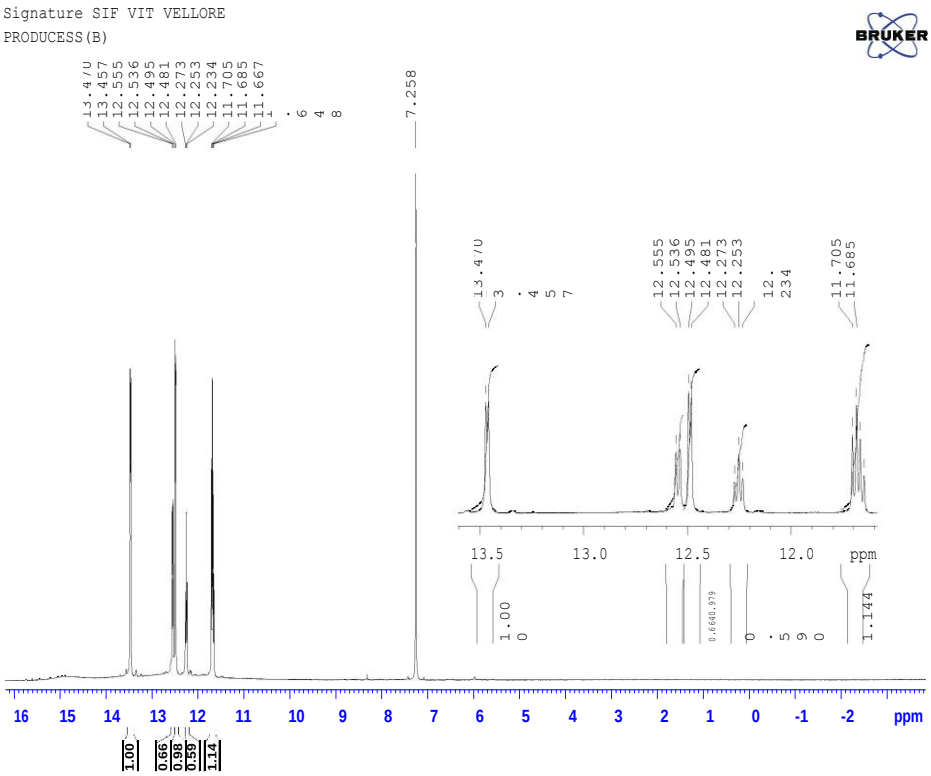


Fig: 3 NMR Spectrum of prodrug (III).

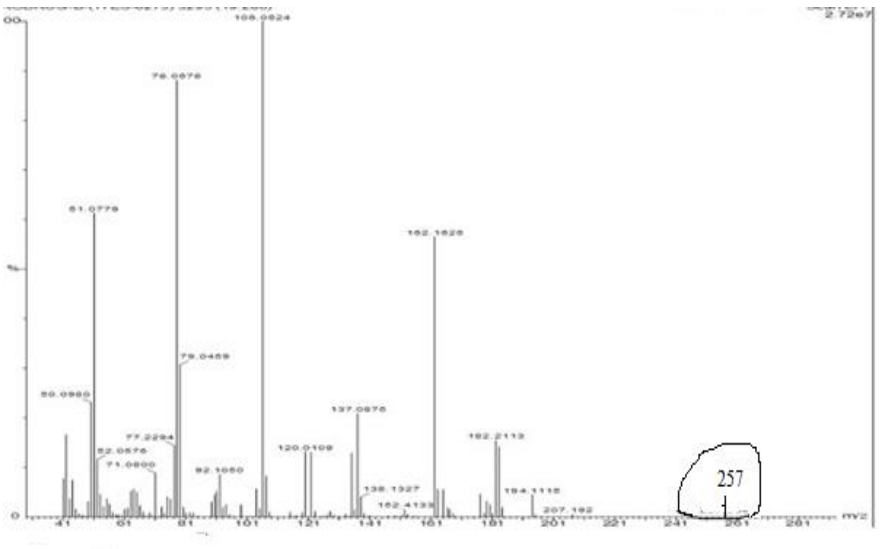


Fig4: Mass spectrum of prodrug (III)

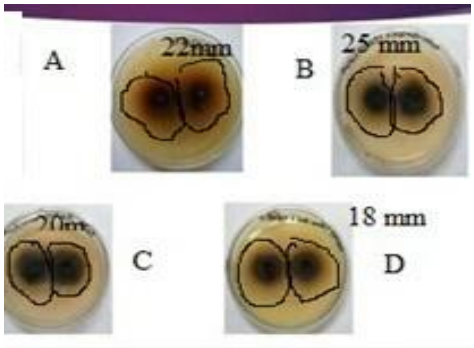


Fig:5 Anti bacterial activity of (III)

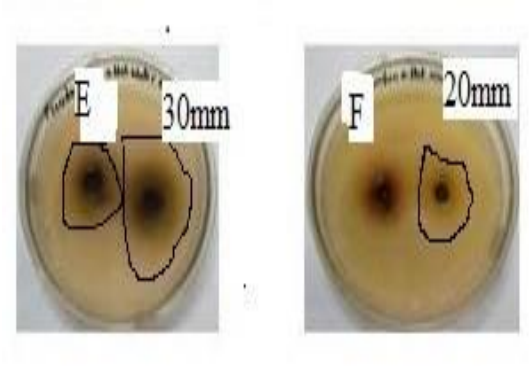


Fig: 6 Anti fungal activity(III)

Fig: 7 Against *S-aureus* with 6.25 µg/mL (III).

Fig: 8 Against C-*albicans* with MIC 6.25

The IR Spectra of the title compound showed intensive band at 3500-3400Cm^{-1} signable to the secondary amide group.1606, 1641 represents formation of (C=O amide Str), **Fig: 2** ^{1}H NMR Spectrum of the compounds supported the structure of prodrug III) Showed (singlet O-H13.5ppm), (-CONH-) doublet at 11.68) **Fig: 3** The mass spectrum of prodrug (III) showed the molecular ion peak at their molecular weights as M^{+}**Fig: 4**

Cup plate method:

Newly synthesized prodrug (III) was examined for antibacterial l as well as antifungal activity and the results were summarized in **Fig: 5&6**. The antibacterial screening results revealed that the prodrug (III) showed less antibacterial activity with zone of inhibition 20mm (C) against *Escherichiacoli* and *Staphylococcus aureus* with ZOI 18mm(D) compared with standard ampicillin with ZOI 22mm (A) and ZOI 25mm (B)respectively and also prodrug (III)showed potent antifungal activity against *Candidaalbicans* with zone of inhibition 30mm compared with standard fluconazole against *Candida albicans* with zone of inhibition 20mm(F).

CONCLUSION:

Salicylic acid (I) was successfully condensed with Isoniazid (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 less antibacterial activity with zone of inhibition 20mm against *Escheriachia coli* and showed less zone of inhibition18mm against *Staphylococcus aureus*. Compared with standard Ampicillin. The prodrug showed Impressive antifungal activity with zoneof inhibition 30mm when compared with standard fluconazole against *Candida albicans* 20mm. The prodrug (III) showed very good antibacterial activity with MIC6.25µg/ml against *Staphylococcus aureus* and *candida albicans* when compared with standard fluconazole and Ampicillin.

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