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# Synthesis and Antibacterial Activity of Isatin-3-(isonicotinoyl) Hydrazone and substituted Isatin-3(Isonicotinoyl) Hydrazone

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**Isatin-3-(isonicotinoyl) hydrazone and substituted isatin-3(isonicotinoyl) hydrazone were synthesized and their antibacterial activity was screened. It was observed that the Mannich base with methtyl, isopropyl and isobutyl group substituted Istain –3 -(isonicotinoyl) hydrazone exhibited higher activity and inhibit the growth of almost all the tested bacteria. Among the series, the least active was the compound residues and the methyl derivative (II-20) is the most active.**

**Keywords:** Istain-3-(isonicotinoyl) hydrazone, antibacterial activity.

Antibacterial agents are among the most dramatic examples of the advances of modern medicines. Many infectious diseases are considered incurable and lethal are now amenable to treat with few pills. The remarkably powerful and specific activity of antimicrobial drugs is due to microorganisms or much important in them that in human[4,7,9-10].

The development of new drugs has been responsible for increasing human mortality and morbidity more than any other scientific endeavor in our lifetime. These products have dramatically improved the quality of life across all age ranges and socioecomics groups.

Isatin, also named indoline-2, 3-dione, is a bright coloured with a long history and broad range of pharmacological actions. Its structure was characterized in 1841 by Erdamann and Laurent through oxidation studies on indigo. Chemically, Isatin may be characterized as lactam of o-amino-benzoylformic acid. It possesses both amide group and a keto group.

Isatin and some of its derivatives have shown moderate activity against gram + ive bacteria. Isoniazid has bacteriostatic and bactericidal activity in vitro against M. tuberculosis. Isoniazid is one of the most effective antituberculars but, when it is administered alone, a quick emergence of resistant strain follows. Several hydrazones of isoniazid with Isatin have been prepared and the corresponding Mannich bases were also synthesized in order to evaluate the antibacterial properties of this drug with respect to isoniazid.

The invitro antibacterial activities of the synthesized compounds were evaluated against representative gram positive and gram-negative organisms. The study was performed to find and develop novel antibacterial agents of synthetic origin giving a broad spectrum of activity and high potency. In the preliminary studies, paper disc method was used for testing the sensitivity of the synthesized compounds against various microorganisms. The schiff base of Isatin with isoniazid was synthesized and then converted into its Mannich bases according to Scheme 1.

## MATERIAL AND METHODS

Melting points were taken by Open Capillary method and are uncorrected. UV Spectra were recorded on a Jasco 7800UV/Visible Spectrophotometer and IR Spectra on Jasco 5800 FT-IR using KBr discs. The 1HNMR Spectra

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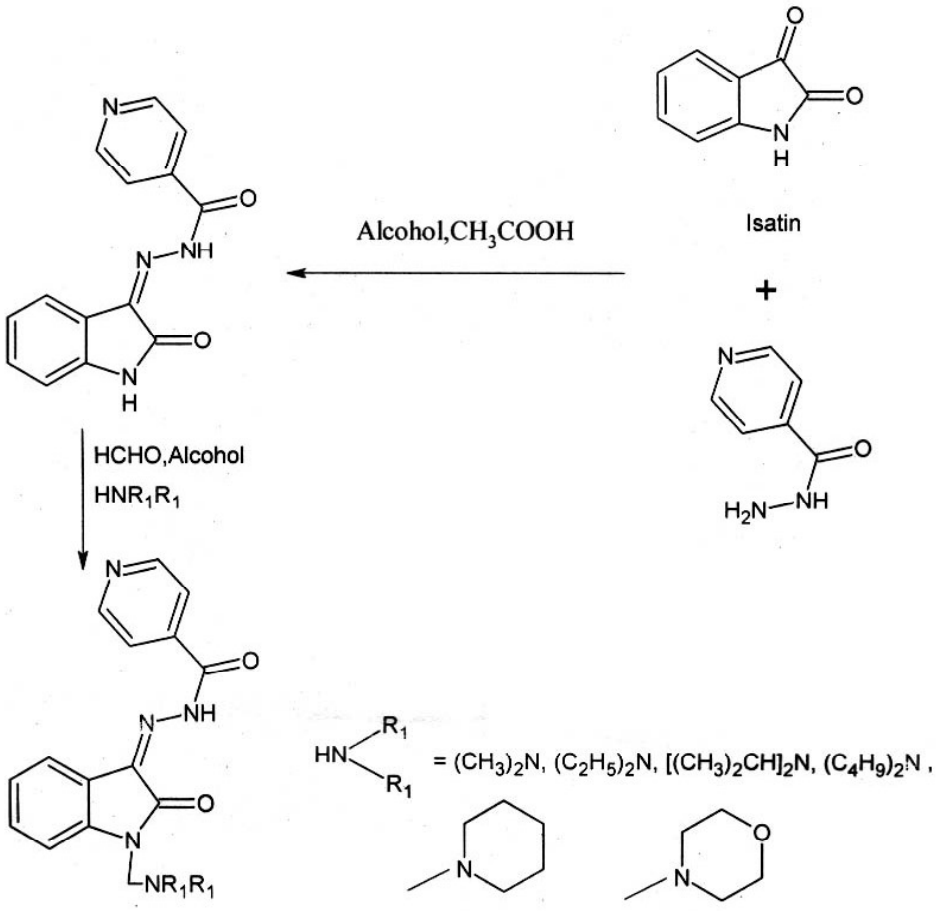


**Scheme 1.**

standard. Elemental analysis were determined with Perkin-Elmer model 240 analyser. The purity of the compounds was confirmed by Thin Layer Chromatography using silica gel plates and different solvent systems. Isatin was purchased from Ward, Blenkinsop and Co. Ltd.

**Chemical synthesis of Isatin-3-(isonicotinoyl) hydrazone(11-19)**

Equimolar quantities of isoniazid (13.7g, 0.1 mol) were and isatin (14.7g, 0.1 mol) dissolved in 75ml alcohol and few drops of gklacial acetic acid and refluxed for two hours on a water bath. It was allowed to cool and kept in ice-cold condition for one hour, filtered, air-dried and recrystallised from ethanol. M.P. 300°C, yield 80%.

Anal : $C_{14}H_{10}N_4O_2$
Calcd:C:63. 14,H:3.78.N:21.04%
Found: C: 63.49, H:3.94, N:20.83%

**Spectral Data**

UV (λmax MeOH): 237, 275,325
IR (KBr,cm-1): 3110s(N-H), 1720s ($CONH_2$), 1720m (C=O), 1670s (C=N), 15490s, 1460s., 750s, 660w. HNMR (DMSO-$d_6$,δppm): 7.0-7.5 (m, 4H, aromatic), 7.9-8.1 (m, 4H, Pyridinyl)9.0 (br, 1H,NH,indole). 11.1 (s, 1H=NNH $D_2O$ exchangeable). Similarly, Mannich bases of substituted Isatin-3-(isonicotinoyl) hydrazone were prepared corresponding isatin.

**Antibacterial Activity**

**Paper disc diffusion method**

This method involves small uniform Whatman AA disc containing a fixed concentration of the drug and observing the sensitivity and zone of inhibition after incubating the seeded media plates[6,9].

**Preparation of discs**

**Uniforms disc** (5mm) of Whatman AA

paper were cut and filled into vials plugged with cotton. These vials were kept in hot air oven at 160°C for 30 minutes for sterilization of the discs. Counted no. of disc were filled in different sterilized vials. Test samples were then prepared in N, N-dimethyl formamide (DMF) with a concentration of 10mg/ml. Known volume of these samples was added into each vial containing disc such that each saturated disc containing 100mg of the test samples. As a control, paper disc soaked in solvent alone was used.

**Culture media**

**Nutrient agar media (dehydrated)**

Nutrient agar media were used for subculturing the various strains of microorganisms from the laboratory stock culture.

**Direction's to rehydrate this medium**

The media (35g) was suspended in 100ml of distilled water and dissolved completely. It was sterilized in an autoclave at 15lbs per sq inch pressure at 121°C for 15 minutes.

**Mueller Hinton agar media (MHA)**

MHA media were used for testing the samples against gram-positive and gram-negative organisms. MHA media (37.5 g) were suspended in 1000ml of distilled water in a conical flask and plugged tightly with cotton. It was sterilized at 15lbs per sq-inch for 15 min. in an autoclave.

**Subculture process**

The strains cultured on nutrient agar media were further subcultured in peptone water (1%) for drug susceptibility testing. Using a sterile

**Table 1.** Characterisation data of Mannich bases of subsitituted isatin-2-(isonicotinoyl) hydrazone

| Comp. No. | R | M.P. (°C) | Yield (%) | Molecular formula | Analysis % Calcd. | Found | Rf Value | IR (KBr, $cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| II-20 | $N(CH_3)_2$ | 240 | 79 | $C_{17}H_{17}N_5O_2$ | C: 64.93<br>H: 5.29<br>N: 21.66 | C: 63.64<br>H: 5.42<br>N: 21.31 | 0.59 | 3450m (NH stretch), 2850m(CH3 stretch) 1720s(C=O),1680s (C=N),760, 650s (aromatic) |
| II-21 | $N(C_2H_5)_2$ | 150 | 73 | $C_{19}H_{21}N_5O_2$ | C: 64.93<br>H: 6.20<br>N: 19.97 | C: 64.65<br>H: 6.23<br>N. 19.72 | 0.81 | 3400s(NH Stretch), 1700s(C=O),1660m (C=N),1460m |
| II-22 | N[CH $CH_3)_2$] | 225 | 70 | $C_{21}H_{25}N_5O_2$ | C: 66.38<br>H: 6.63<br>N: 18.43 | C: 66.56<br>H: 6.36<br>N: 18.69 | 0.32 | 3210w(NH stretch) 1700s(C=O),1660s (C=N),1550m, 680m (aromatic) |
| II-23 | $N(C_4H_9)_2$ | 190 | 64 | $C_{23}H_{29}N_5O_2$ | C: 67.79<br>H: 7.17<br>N: 17.18 | C: 67.57<br>H: 7.48<br>N: 17.32 | 0.70 | 3410m(N-H stretch) 2950m(CH2 stretch) 1750m (C=O),1680s (C=N),760,680m (aromatic) |
| II-24 | $NC_5H_{10}$ | 120 | 63 | $C_{20}H_{21}N_5O_2$ | C: 66.09<br>H: 5.82<br>N: 19.27 | C: 66.15<br>H: 5.76<br>N: 19.05 | 0.80 | 3200s(NH stretch) 2950m(CH2 stretch) 1725s(C=O),1690m (C=N),156Os (aromatic |
| II-25 | $NC_4H_8O$ | 205 | 68 | $C_{19}H_{19}N_5O_2$ | C: 62.37<br>H: 5.23<br>N: 19.14 | C:62.11<br>H: 5.39<br>N: 19.34 | 0.34 | 3450s(NH stretch), 2850s, (CH2 stretch) 1700s(C=O),1680 m(C=N),1610s,161 0s,750m,680s (aromatic) |

platinum loop, a loopful of the colony of microorganism was taken and inoculated into the sterile plugged test tube containing peptone-water under aseptic condition's. These inoculated test tubes for each microorganism were then incubated at 37°C for 3hr.

**Preparation of petri dishes**

The sterile MHA medium was cooled to 60°C and poured 20ml each into previously sterilized and cooled petridishes and allowed the contents to solidify.

**Paper disc method**

The prepared petridishes were smeared with dilute suspension of different microorganisms in 1% peptone water using sterile cotton swabs. Drug saturated paper discs were then aseptically placed at specific marked spots on each plate and suitably spaced apart. The petridishes were incubated at 37°C for 34hr. The zone of inhibition around each disc observed after incubation was indicative of the drug sensitivity at that concentration.

**Broth dilution method**

Accurately weighed quantity of the test sample was taken and dissolved in 2ml of DMF so as to give a stock solution 500 μg/ml in sterile cotton plugged test tube. From the stock solution 1ml was diluted with DMF and 1:1 dilution was carried out upto 0-2 μg/ml.

The petridishes were sterilized in hot air oven at 160°C for 30min., cooled and marked 1to 25 on the bottom for the respective microorganisms. The test solution (1ml) serially diluted in test tube was added to the petridishes, diluted by addition of 19ml of MHA media, previously cooled to 60°C. The contents were mixed and uniformly allowed to solidify under aseptic conditions. The diluted bacterial suspension was inoculated as spots using sterilizes swabs. After inoculation of all microorganisms the petridishes

**Table 2.** Antibacterial activity of Mannich bases of isatin-3-(isonicotinoyl) hydrazone (II-19)

| Bacteria | II-19 | II-20 | II-21 | II-22 | II-23 | II-24 | II-25 |
|---|---|---|---|---|---|---|---|
| *Bacillus subtilis* | + | + | - | + | - | + | - |
| *Staphylococcus aureus* | + | + | + | + | + | + | - |
| *Staphylococcus albus* | + | + | + | + | + | + | + |
| *E.coli* | + | + | + | + | + | + | + |
| *E.coli* MCTC | + | + | + | + | + | + | + |
| *Proteus vulgaris* | + | + | + | + | + | + | - |
| *Pseudomonas aeruginosa* | - | + | + | + | + | + | - |
| *P. aeruginosa* MCTC | - | + | + | + | - | - | + |
| *Vibrio cholerae* | - | + | + | + | + | + | + |
| *Vibrio parahaemolyticus* | + | + | + | + | + | + | + |
| *Vibrio cholerae non* 01 | + | + | + | + | + | + | - |
| *Enterobacter* | + | + | - | + | + | + | - |
| *Shigella boydii* | + | + | + | + | + | - | + |
| *Shigella dysenteriae* | + | + | - | + | + | + | + |
| *Shigella flexuneri* | + | + | + | + | + | + | + |
| *Shigella sonnei* | + | + | - | + | + | + | + |
| *Salmonella paratyphi* A | + | + | - | + | + | + | + |
| *Salmonella paratyphi* B | + | + | + | - | + | - | - |
| *Salmonella enteridis* | + | + | - | + | + | + | - |
| *Salmonella typhimuricum* | + | + | - | + | + | + | - |
| *Klebsiella pneumoniae* | + | + | + | + | + | + | - |
| *Serratia marcescens* | + | + | + | + | + | + | + |
| *Citrobacter freundii* | + | + | + | + | + | + | - |
| *Morgenella morganii* | + | + | + | + | + | + | + |
| *Aeromonas hydrophile* | - | + | + | + | + | + | + |

Concentration 1000mg/ml; +=active - = inactive

were incubated at 37°C for 20hr. The minimum inhibitory concentration (MIC)[2,3,5] was considered to be the lowest concentration at which no growth of bacteria was noticed, disregarding a single or faint caused by inoculation.

## RESULTS AND DISCUSSION

Antibacterial activity of 3-(isonicotinoyl) hydrazone and its Mannich bases is shown in Table 2. At a glance, the Mannich base with methyl, isopropyl and isobutyl group exhibited higher activity and inhibit the growth of almost of all the bacteria tested. On the other hard, Mannich bases with cycloamino residues are less active and inhibit the growth of nearly 75% bacteria tested. In this series, the methyl derivative (II-20) is the most active.

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