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Synthesis, Characterization, and Antimicrobial Activity of Novel 2, 3-Indolindione Derivatives against Gram-Positive Bacteria and Fungi

Satish kumar Sharma[1*], Kashif Hussain[1]

[1]Department of Pharmacology, Glocal school of Pharmacy, Glocal University Saharanpur-247121 Uttar Pradesh

*Corresponding author: - Dr. Satish Kumar Sharma

Emil ID- satishdipsar55@gmail.com

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Abstract

The importance of the indole nucleus is well established in the field of pharmaceutical chemistry, in plants, and in animal biochemistry. The 2-Indolinone and 2, 3-indolindione are important derivatives, which possess antiviral, antitubercle, antifungal, and antibacterial activities. In the present work, fifty-seven derivatives of 2, 3-indolindione was prepared. The substitutions were made at positions such as 1 and 3. The azines (I-XVI) were prepared by substituting different aldehydes and ketones for Isatin-3-hydrazone. Azines (XVIIXXIV) were prepared by reacting Isatin and N-substituted Isatin with isonicotinyl hydrazide. Azines (XXV-XXXII) were prepared by the condensation of Isatin and N-substituted Isatin with each other. Isatin derivatives with chloroacetyl chloride (XXXIII-XXXIV) and thiadiazoline derivatives of Isatin were also prepared (XXXV-XXXIX). Besides all these, more Isatin derivatives and their Mannich bases were prepared (XXXX-LVII). All these new compounds have been screened for their antimicrobial activity against test organisms. Isatin-isocotinyl hydrazone (XVII), among new Isatin derivatives, was screened against *Mycobacterium* tuberculosis organisms. This strain was collected and isolated from the different body parts of Tuberculosis (TB) suffering twenty-five patients at Gulab Devi Hospital. This new compound was found more sensitive than isoniazid and Isatin drugs (Reference standard) and the Minimum Inhibitory Concentration (MIC) results of this new compound proved it more potent. Further research on this compound may be found the best antitubercular agent. Similarly, other new derivatives also showed different degrees of effectiveness according to different group substitutions, against Gram +ve and Grame-bacterial and fungal species. Further pharmacological and clinical studies will help us to find out the potency of these compounds.

Keywords: Isatin derivatives, Azines, Mannich bases, Isatin-isocotinylhydrazone and MIC.

1. Introduction

Herbal medicine is the oldest form of health care known to mankind. Herbs have been used by all cultures throughout the history and they constitute an integral part of the development

of modern civilization [Saklani *et al.*, 2011]. Medicinal plants are a source of great economic value all over the world. The sources of drugs is mainly depends on these natural products from plant, animal, microorganism and minerals, which is treatment of human and animal disease [Saklani *et al.*, 2014]. Indole is widely distributed in nature and provides a basic framework for a number of structural and functional units in plants as well as animals. Indole derivatives are found in many natural products. Indole itself has been obtained from many naturally occurring materials by methods, which suggest that the indole in many cases is the product of decomposition of its derivatives. It is found in *Robinia pseudacacia* [Eize *et al.*, 1910], the jasmines [Hess *et al.*, 1904] and certain citrus plant in the perfume of the *Hevea braziliensis* [Sack 1911: Sack 1911] and in orange blossom oil. Indole is also found in the wood of *Celtis reticulosa* [Herter *et al.*, 1909]. Indole is a non-basic nitrogenous compound in which a benzene ring and a pyrrole nucleus are fused in 2,3 positions of the pyrrole ring. It is a colourless crystalline solid, melts at 52°C, soluble in alcohol, benzene, ether and ligroin. It may be recrystallized from water. The formula that is generally accepted for indole was proposed by Baeyer and Emmerling [Bacyer 1869: Bacyer 1884]. A system of nomenclature devised by Baeyer and subsequently employed Fischer [Sumpler *et al.*, 1954] was cumbersome in that it employed independent numerical designations of each ring. Current practice concerning indole nomenclature is to number the position as shown in the formula above. The 2- and 3- position are also referring to as the α- and β- position, respectively.

Indole is the trivial name of a benzopyrrole in which the 2- and 3- carbon atoms of the nitrogen ring (Pyrrole) are members of a benzenoid nucleus. The preferred systematic name for this compound is l-benzo [b] pyrrole. Two systems have been generally employed in the nomenclature of indole compounds. In the more prominent and more recent, of the two systems, the atoms are numbered consecutively beginning with nitrogen counterclockwise around the two fused rings with bridgehead carbon. According to the older and more limited system, the two open carbons of pyrrole ring as designated α- and β- the substituted indoles are prefixed with N-, α- or β- depending on the point of attachment of substituents. In this treatment we have employed the former and more recent system. In both systems the radical name is formed in the customary fashion, e.g., 3-indolyl, 3-indolyl.Indole is a non-basic nitrogenous compound in which a benzene ring and a pyrrol nucleus are fused in 2,3 positions of the pyrrol ring. It is a colourless crystalline solid, melts at 52°C, soluble in alcohol, benzene, ether and ligroin. It may be recrystallized from water. The formula that is generally accepted for indole was proposed by Baeyer and Emmerling. A system of nomenclature devised by Baeyer [Bacyer 1869: Bacyer 1884] and subsequently employed Fischer [Sumpler *et al.*, 1954] was cumbersome in that it employed independent numerical designations of each ring. Current practice concerning indole nomenclature is to number the position as shown in the formula above. The 2- and 3- position are also referring to as the α- and β- position, respectively.

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The presence of reactive methylene and carbonyl group al position 3 in 2-indolinone and 2,3 -indolindione furnishes reaction site at position 3 for condensation reaction. Various substituted compounds of indole, 2-indolinone and 2,3 indolindione or Isatin have been shown to possess important pharmacological and microbiological activities by Sadler co-worker [Sadler 1968: Zam 1998] reported synthesis and pharmacological activity of Mannich bases by using indole 2,3 dione and 3-phenylimino-2-indolinone as amine moiety [Panda *et al.,* 1987] synthesized some new indoles derivatives, which were found antiviral. They also synthesized Mannich bases or morpholino, piperidino, pyrrolidino or 4 methyl-1-piperazino with appropriate 2-oxo-3 (3,5)-dinitrobenzoyl hydrazone-Substituted indole. These compounds showed antiviral activity against Sunnhemprosette (SRV) and Ranikhet disease (RDV) viruses. They observed that methyl group in position 5 increased the activity of compounds. Thus, the aim of the present work was to synthesize some indole 2,3-diones and analyze their antimicrobial potential.

2. Material and Methods

2.1 Material

All chemicals and solvents were of commercial reagent grade. Melting points were determined on Gallen Kamp apparatus. Infrared spectra were recorded in KBr disc on Shimadzu IR-460 Spectrophotometer. VIS/UV spectra were taken Oil Hitachi U-2000 spectrophotometer. Nitrogen or all these compounds was determined by using Kjedahl-2000 and Vapodest-2000 automatic instrument. Tile chloride ion in compounds was determined by gravimetric method. The determination of Carbon Hydrogen and Sulphur were arranged from the Atomic Energy Commission. The purity of the compounds was checked by Thin Layer Chromatography on pre-coated 20 x 20 cm plates of silica gel G-60 by E. Merck. Infrared spectra of the synthesized compounds were taken by KBr disc method.

2.2. General methods for the synthesis of derivatives

2.2.1. AZINES (I-XVI)

2.2.1.1. Reaction of Isatin-3-hydrazone with aldehyde / ketone

A. Isatin-3-hydrazone (0.1 mol) and appropriate aldehyde or ketone (0.1 mol) dimethylformamide (30 mL) and glacial acetic acid (10 mL) were kept at 60°C on steam bath for half an hour with vigorous stirring. The reaction mixture was poured into water (300 mL) and precipitated solid product was collected and recrystallized from suitable solvent to furnish the required azine.

B. Isatin (0.1 mol), hydrazone of appropriate aldehyde or ketone (0. l mol) anhydrous ethanol (50 mL) and acetic acid (2 mL) were refluxed on steam bath for 1 h. On cooling, the solid product separated out, collected and first washed with excess or water and then with cold ethanol (2-x 10 mL). The crude product was recrystallized from suitable

solvent. Similarly, all analogues 01 azines (I - XVI) were prepared by using different aldehydes and ketones.

2.2.1.2 AZINES (XVII-XXIV)

2.2.1.2.1 N-Substituted Isatin-3-isonicotinylhydrazones

Thin-layer chromatography (TLC) is a chromatographic technique used to separate and identify the components of a mixture. It is a simple, inexpensive, and versatile technique that is widely used in analytical chemistry. Isatin(0.1 mol), Isoniazid hydrochloride (0.1 mol) and dimethly formide (100 mL) were refluxed for 4 h completion of the reaction was monitored by TLC, using mobile phase Toluene: Ethyl acetate (4:3 v/v). On cooling orange-colored crystals were separated out, which were collected on Buchner funnel and washed with cold ethanol (3x10 mL). The product was recrystallized from dimethylformide to furnish 89.10% of compound XVII, m. p. 289-91°C. Similarly, analogues XVII - XXIV of this series were prepared by using different N-substituted Isatin [Ali *et al.*, 1994].

2.2.1.3 AZINES (XXV-XXXII)

2.2.1.3.1 N-Substituted Isatinazines

A. Isatinazine XXV

Isatin (0.1 mol) and Isatin-3-hydrazone (0.1 mol) were dissolved in dimethyl formamide and refluxed for 4 h. On cooling, the red crystalline product separated out, was collected and washed with water and recrystallized from dimethylformamide to give yield of 20 g (90%) of compound XXV, decomposed at 220-21°C. Similarly other analogues XXVI-XXXII were prepared by using Isatin or N-substituted Isatin with Isatin-3-hydrazones or N-substituted Isatin-3-hydrazones [Ali *et al.*, 1994].

B. Improved method of Isatinazine XXV

Isatin (0.2 mol) was dissolved in suitable amount of dimethylformamide followed by the addition of 2 mL of glacial acetic acid as catalyst. The contents were refluxed with gentle stirring and hydrazine hydrate (0.1 mol) was added drop-wise with in the period of half an hour. The contents were refluxed for 1.5h. A red crystalline product was obtained. The product was collected on Buchner funnel and washed with water. The pure product XXV was obtained after recrystallization from dimethyl formide, yield 91.0% yield, decomposed at 220-221°C.

2.2.2 MONOCHLOROACETYL CHLORIDE

Monochloroacetic acid (1 mol) in anhydrous benzene was refluxed and Thionyl chloride (1.5 mol) was added drop-wise during refluxing the reactants. The contents were constantly heated until the evaluation of SO_2 and HCl is ceased. The reaction was monitored by TLC to check the consumption of monochloroacetic acid. The product was distilled off, on isomental at 106°C. under strictly anhydrous conditions by using Guard tube filled with anhydrous $CaCl_2$.

N-Chloroacetylisatin XXXIII

Monochloroacetylchloride(0.15 mol) was added slowly with stirring to solution of Isatin (0.1 mol) in anhydrous toluene (100 mL) and heated under reflux for 4 h. On cooling to room

temperature, solid product separated out was collected and recrystallized from toluene to furnish the product XXXIII. 18.5 g (82%). m.p. 205 -206ºC [Ali *et al.*, 1994].

N-Chloroacetylisatin-3-Chloroacetylhydrazone XXXIV

Monochloroacetyl chloride(0.25 mol) was added slowly with constant stirring to a solution of Isatin-3-hydrazone (0.1 mol) in anhydrous toluene contents were heated under reflux for 3 h. Reaction mixture was allowed to cool to room temperature. Solid product separated out was collected on Buchner funnel washed with water and then with toluene (2x5 mL). The crude product subsequently recrystallized from toluene to give the product XXXIV 14.3 g. (85%), m.p. 164°C.

2.2.3 ISATIN DERIVATIVES OF THIADIAZOLINE

2.2.3.1 2-ACETYLAMINO-5-METHYL-1,3,4-THIADIAZOLINE XXXV

Thiosemicarbazide (15 g) and acetic anhyoride(30 mL) were heated under reflux for 2 h. On cooling, the resulting crystalline product was collected on buchner funnel and recrystallized from ethanol to give 15.6 g of compound XXXV m.p. 290ºC [Ali *et al.*, 1994].

2.2.3.2 2-AMINO-5-METHYL-1,3,4-THIADIAZOLINE XXXVI

The compound XXXV (15.6 g) and ammonium hydroxide (75 mL) were refluxed for an h. On cooling, white solid product was collected by filtration. Washed it with water and recrystallized from ethanol to give of 86.9 % of compound XXXVI m.p. 222ºC [Ali *et al.*, 1994].

2.2.3.2.3 3-[2-5- METHYLTHIADIAZOLYL) –IMINO] -2-INDOLINONE XXXVII

Isatin (0.1 mol), compound XXXVI (0.1 mol) and acetic acid (50 mL) were heated under reflux for 3 h. On cooling to room temperature light orange crystals were separated, collected on buchner funnel washed with water and recrystallized from acetic acid to furnish 21.32 g. (87.0%) or compound XXXVII, m.p. 287ºC.

2.2.3.2.43′-ACYL-5'-ACETYLAMINO)-I-ACETYL-SPIROINDOLINE-3-2' THIADIAZOLINE)-2-ONE. XXXVIII

Isatin-3-thiosemicarbazone (11.0 g) and acetic anhydride (65 mL) were heated under reflux for 2 h. On cooling, the resulting crystalline product was collected on buchner funnel and recrystallized from ethanol to furnish 25.0 g (80%) of compound XXXVII, m.p. 243ºC [Ali *et al.*, 1994].

2.2.3.2.5 5-AMINO-SPIRO INDOLINE-3, 2'-THIADIAZOLINE)-2 ONE XXXIX

The compound XXXVIII (16.5 g) and ammonium hydroxide (100 mL) were refluxed for 1.5 h, cooled to room temperature. The resulting crystalline product was collected by filtration, washed with water and recrystallized from ethanol to give 90% of compound XXXIX m.p. 206°C [Ali *et al.*, 1994].

2.2.4 ISATIN DERIVATIVES AND THEIR MANNICH BASES

2.2.4.1 MANNICH BASES

Derivatives of Isatin (0.05 mol) were suspended in ethanol (20 mL), 37.0% aqueous formaldehyde solution (7.5 mL) was added, followed by the addition or appropriate secondary amine (0.05 mol) with stirring. The contents were kept at 50ºC for 15 minutes. On

cooling the products were separated out, collected and washed with water The crude products (XXXXI, XXXIII- LVII) were recrystallized from suitable solvent.

2.2.4.2 Isatin-3-Oxime XXXX

Isatin (4.41 g) was dissolved in ethanol (50 mL). A solution of hydroxylammonium hydrochloride (8.49 g) dissolved in ethanol and neutralized with $NaHCO_2$ solution and was added to the Isatin solution drop-wise with stirring. The reaction mixture was stirred continuously for 3 h. The colour of tile solution turned yellow and solution was poured into cold water, a crystalline yellow product was separated, collected and washed with water. The crude product recrystallized from ethanol to furnish compound XXXX.

2.2.4.3 Isatin-3- Aniline XXXXII

Isatin (4.41 g), aniline (7.8 mL) in absolute ethanol (20 mL) in the presence or acetic acid (1 mL) were refluxed for 2 h. The mixture was allowed to cool to room temperature. The orange-coloured crystalline product separated, collected and washed with water. The crude product was recrystallized from ethanol to furnish compound XXXXII.

2.2.4.4 Isatin-3-aminophenol XXXXIV -XXXXVI

The Isatin derivatives of ortho-aminophenol, m-aminophenol and p-aminophenol were prepared under similar conditions as described for compound XXXXII.

2.3 Characterization of the synthesized compounds

Melting points were determined on Gallen Kamp apparatus.Infrared spectra of the synthesized compounds were taken by KBr disc method. 1 mg of compound mixed with 100 mg of spectroscopically pure KBr and ground in Agate pestle mortor to a very fine powder, a transparent disc was thus prepared under 60 tons/sq inch pressure, spectra were taken on Shimadzu IR-460 spectrophotometer. The synthesised compounds were purified by crystallization by conventional methods. λ_{max} of these compounds was determined using extra pure Dimethyl sulphoxide (DMSO) and ε_{max} was calculated. Further characterizations of the synthesized compounds were carried out by TLC.The λ_{max} and ε_{max} of TLC separated compounds was comparable to the λ_{max} and ε_{max} of crystallized derivatives or Isatin (authentic compounds) [Chandra *et al*., 2017].

2.4 Antimicrobial activity

All of the synthesized compounds were dried under vacuum. Their antimicrobial activity was studied in comparison to certain antibiotics i.e. Gentamicin, Cliadamicin, Polymyxin and Erythromycin and anti-fungal, agent that was Griseofulvin, using their reference standard secured from Drug Testing Laboratory. Nutrient agar medium was used for the growth of bacteria, while Sabouraud's Dextrose Agar (SDA) was used for the growth of Fungi. The slants of nutrient agar and Sabouraud's Dextrose agar were prepared taking about 10 mL of the respective medium in each test tube which were plugged with cotton, covered with butter paper, autoclaved and were allowed to cool at room temperature in inclined position [Clark 1982: Delaat 1976: Saklani 2012]. The pure cultures of the microorganisms were obtained from various sources as mentioned in the table 1.

Table 1: Source of Collection of various bacteria and Fungi used for testing the antimicrobial activity of newly synthetic indole-2,3-diones derivatives.

No	Name of Microorganisms	Characteristics	Source of Collection
	Bacteria		
1.	*Bacillus subtilis*(ATTC 6633)	Gram +ve bacilli	Glaxo Laboratories Lahore.
2.	*Bacillus pumilus*(ATCC 6538)	Gram +ve bacilli	Glaxo Laboratories Lahore.
3.	*Bacillus stearothermophilus*	Gram +ve bacilli	Drug Testing Lab. Lahore.
4.	*Staphylococcus aureus* (ATCC 6538)	Gram +ve cocci	Drug Testing Lab. Lahore.
5.	*Staphylococcuspyogenes*	Gram +ve cocci	Drug Testing Lab. Lahore.
6.	*Micrococcus luteus*(ATTC 9341)	Gram +ve cocci	Drug Testing Lab. Lahore.
7.	*Micrococcus flavus*(ATCC 10240)	Gram +ve cocci	Drug Testing Lab. Lahore.
8.	*Pseudomonas aeruginosa*(ATTCC 8739)	Gram -ve bacilli	Glaxo Laboratories Lahore.
9.	*Escherichia coli*(ATCC 9027)	Gram -ve bacilli	Glaxo Laboratories Lahore.
10.	*Proteus vulgaris*(OX 19)	Gram -ve bacilli	Drug Testing Lab. Lahore
11.	*Bordetellabornchiseptica*(ACT 4617)	Gram -ve cocci	Drug Testing Lab. Lahore
	Fungi		
12.	*Aspergillus niger*(ATCC 16404)	Vesicle andconidia are present	Drug Testing Lab. Lahore
13.	*Saccharomyces cerevisiae*(ATCC 2601)	Buddina like structure Yeast	Glaxo Laboratories Lahore
14.	*Candida albicans*(ATCC 10231)	Yeast	Glaxo Laboratories Lahore

2.4.1 Preparation of Inoculum

Procedure for preparation of inoculum was adopted as described by [Syed *et al.,* 1986]. Identified pure cultures were kept as stock slants. Fresh culture slants were prepared from stock slants, which were used as inoculum. The saline dilution of each culture used was made to appear in turbidity equivalent to Macferland reference standard [Melarland 1907: Brown 1919]. Fresh subculture of each microorganism was picked up with platinum sterilised wire loop and introduced to 5 mL saline water. it was shaken thoroughly and homogenised to prepare a suspension that was then incubated until its turbidity was visually equivalent to that of the Macferland reference standard. from this bacterial suspension, 1 mL was taken and mixed with 9 mL of saline water. In this way further 10-fold serial dilutions were prepared from each strain and the 10-fold dilution was used as an inoculum. Same procedure was adopted for fungi to prepare their inoculum.

2.4.2 Preparation of drug dilutions

The compounds were insoluble in water. their stock dilutions l mg/mL were prepared in sterile DMSO. The stock solution was used to prepare parallel dilutions with strength of 1000, 500, 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/ mL in a 1:1 mixture of phosphate buffer (pH 7.4) and DMSO. Same solvent was used to prepare standard solutions of Gentamicin, Clindamicin, Erythromycin, Polymyxin and Gircofulvin in concentration of l mg/mL.

2.4.3 Assay method

Microbiological activities of synthetic compounds were determined using diffusion method, which was improved by Flamming [Fleming *et al*., 1950]. Later Naqvi et al [Naqvi *et al.*, 1985] have also used well diffusion method for microbiological testing. The apparatus used was sterilized petridishes (100 mm), adjustable micro pipette and corkborer of uniform diameter (8 mm). The procedure was carried out under laminar flowhood to observe strict sterility conditions [Chandra *et al*., 2017].

2.4.4 Drug sensitivity

Culture plates were prepared by pouring 1 mL of inoculums (10′) of the test microorganisms in each petridish separately. The respective prepared medium maintained at about 45ºC was poured into each petridish. Then petridishes were rotated clockwise and anti-clockwise to mix the inoculums and medium thoroughly and allowed to solidify at room temperature. The petridishes containing solidified medium were divided into four equal parts. The wells were made at equal distances from the centre of the petridish aseptically with sterile cork borer. Each well of the petridish was filled with 1 mL of different concentrations of test compound separately. Petridishes were labelled accordingly and inoculated at 34ºC for 24 h in case of bacteria and at 25ºC for 72 h in case of Fungi. To check the sterility or the medium one plate was poured with medium only and taken as control, and another control plate containing microbial suspension was poured with DMSO along with sterile melted medium.

2.4.5 Minimum Inhibitory Concentration (MIC) of indole-2, 3-diones derivatives

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent, such as an antibiotic or antifungal drug, that prevents the visible growth of a microorganism *in-vitro*. The MIC range or newly synthesised compound was in between the two consecutive concentration of the compound with zone of inhibition and with no zone of inhibition. In case the least dilution or any compound showed zone or inhibition, further serial dilutions were prepared to find an MIC of the compound.

2.4.6 *In-vitro* model study on the mycobacterium sensitivity of ISAITN-3-ISONICOTINYLHYDRAZONE

2.4.6.1 Collection of samples

The samples composed of sputum, urine, pus or pleural fluids were obtained from the TB patients. A total number of 50 samples were collected. Out of these, twenty samples were of sputum, twelve of urine, eight of pus and eight samples were of pleural fluid. Properly sterile wide mouth plastic cups (25 mL), with black bottom and tight-fitting transparent lids, were supplied for the collection of sputum samples only. Patients were instructed to collect their 5 to 10 mL sputum early in the morning and to close their containers immediately after creating out the sputum by deep and forceful coughing.In the case of urine samples, the patients were provided with 50 mL sterilized test tubes with cotton plugs that were duly wrapped with butter paper. After careful collection of the samples, they were immediately brought to the Laboratory for examination.The pus and pleural fluids samples were provided in the sterilized container microsyringes/vials or test tubes) directly by the concerned physician, for the present experimentation.

2.4.6.2 Rapid identification of *mycobacterium* tuberculosis isolated from the collection of samples

Radiometric technique has introduced automation in mycobacteriology and has significantly shortened the time required for bacteriological investigation of mycobacterial diseases. This technique was developed by Deland and Wager [Deland *et al*., 1969] for automated detection of metabolism of bacteria by measuring the $^{14}CO_2$ liberated during the decarboxylation of labelled substance present in the medium. [Siddiqui 1981: Siddiqi 1985] also reported the evaluation of rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*.

2.4.6.3 Isolation of *mycobacterium* tuberculosis

Middlebrook reported Bacteriology of tuberculosis by different methods [Middlebrook *et al*., 1958]. Concentration method was used for the isolation of *Mycobacterium tuberculosis*. This method has also been used by Annie and Vested [Annie 1975: Siddqui 1977]. The spot specimen for smear and culture were processed by concentration method and exposed to Lowenstein-Jensen medium as recommended by [Siddiqui 1978: Siddiqui 1980].

2.4.6.4 Smear preparation

From each isolated specimen, duplicate smears were prepared, for Ziehl-Neelsen staining. Clean unscratched glass slide was selected and the reference number that was marked on the sample container was carefully written in pencil. Sputum specimen was inspected and solid purulent or blood-stained particles were picked up with the platinum loop and placed on the respective slide.

2.4.6.5 Identification of *mycobacterium* tuberculosis

"The identification of the tubercle organisms was carried out in three steps:

a) Direct Microscopic studies;
b) Culturing of *Mycobacterium* tuberculosis;
c) Using BACTEC Machine"

Stock solutions of this new compound, Isatin and INH as µreference drug) were prepared by dissolving 10 mg of each drug in 10 mL of sterile dimethyl sulphoxide [Piscopo 1987: Dobck 1983]. Each stock solution was diluted up to the concentration of 10 µg/mL. Serial tenfold of bacteria suspension dilutions (10 fold) were prepared up to 10^5 and two dilutions or inoculum i.e. l 0^5 and 10^1 were selected in the present studies for testing sensitivity of these drugs [Handbook of Tuber. 1962: David 1973].

2.4.6.6 Drug Sensitivity

Lowenstein-Jensen (LJ) medium is a selective and differential medium used to isolate and cultivate *Mycobacterium* species. The sterility of the prepared slants of LJ medium present in Biju bottles with and without drug was checked for purity by incubating them at 37°C for 24 h. For the 25 identified cultures of tubercle bacilli, 50 sets of slants were prepared for drug sensitivity, because each culture was used at a dilution of 10^5 and 10^3. One set comprised 10 slants of LJ medium, one control slant and nine test slants.New compound (XVII), "Isatin" and "INH" at the concentration i.e., l µg, 2 µg and 3 µg/100 mL were used in the studies. The

bacterial suspension prepared from each isolated sample was exposed to slant medium and incubated at 37°C for 4-5 weeks [Canetti *et al.,* 1969].

2.4.6.7 MIC of new compound ISATIN-3- ISONICOTINYLHYDRAZONE against isolated tubercle organism

When it was established that the tubercle organism isolated from selected specimen were sensitive to new compound (Isatin-3-isonicotinylhydrazone) [Ali *et al.,* 1994] even at the concentration of l µg/100 mL, tower dilutions of the compound were prepared to determine the minimum inhibitory concentration. For this purpose, 5 specimens were selected (S-2, S-8, U-2, P-2 and P-3) which were found to be highly sensitive to the test drug. MIC was determined in triplicates using-two concentrations, 10^5 and 10^1 of each cultured isolated tubercle, which were mixed separately in Biju bottle with 100 mL of LJ medium containing 1.0 µg, 0.75 µg, 0.05 µg, 0.25 µg and 0.1 µg of test drug. For each concentration of drug a control was also used. The tubes were incubated for 4-5 weeks to obtain net results [Canetti *et al.,* 1969].

3.0 Results and Discussion

3.1 AZINES (I-XVI) obtained from isatin-3-hydrazones with aldehyde/ketones

Azines (I-XVI) were prepared by the reactions of Isatin-3-hydrazones with different aldehydes and Ketones. Isatin-3-hydrazone is the basic unit of azines series. The solvent ofcrystallization, formula, molecular weight, m.p. and% yield of each compound is given in table-2. All these derivatives were identified by spectroscopic methods. antimicrobial action demonstrated against a variety of harmful bacteria and fungi to humans. The minimum inhibitory concentration (MIC) of each analogue was also determined and presented in table 3.

Table 2: Characteristic of Azines (I-XVI)

Compounds No.	Formula	Solvent of Crystallization	Molecular Weight	Melting Po. Int °C	% Yield
I	$C_8H_9N_3O$	Ethanol	161.08	222-233	93.00
II	$C_9H_7N_3O$	Acetone	173.08	194-195	90.10
III	$C_{10}H_{14}N_4$	Ethanol	187.10	202	92.00
IV	$C_{10}cl_3C_6N_3$	Ethanol	290.40	140 Dec.	89.68
V	$C_{14}H_{14}N_3$	Ethanol	201.12	199-200	90.52
VI	$C_{12}H_{13}N_2O$	Ethanol	215.14	207-209	89.52
VII	$C_{12}H_{12}N_3$	Aq. DMF	249.12	178	90.30
VIII	$C_{15}H_{10}N_4O_3$	Aq. DMF	294.12	242-243	86.70
IX	$C_{15}clH_{10}N_4$	Aq. DMF	283.57	232-234	90.00
X	$C_{16}H_{13}N_3$	Ethanol	263.14	175-180	93.03
XI	$C_{16}H_{13}N_3O_2$	Ethanol	279.14	185-187	90.80
XII	$C_{16}H_{12}N_3O_3$	Aq. DMF	294.14	270 Dec.	90.30
XIII	$C_{17}H_{13}N_3$	Ethanol	275.13	182-185	90.05
XIV	$C_{17}H_{15}N_4O$	Ethanol	292.17	230	92.60

XV	$C_{21}H_{15}N_3$	Ethanol	325.16	205	92.49
XVI	$C_{22}H_{14}N_3$	Ethanol	336.15	178-180	87.80

Table Error! No text of specified style in document.**: MIC (µg/mL) of different azines (I-XVI) against Test Microorganisms**

Concentrations µg/mL	**Compounds**															
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**	**XVI**
Bacillus subtilis	2.50	4.5-5.0	2.00	2.00-2.50	1.60	45.00	9.00	1.60	5.00	12.50	-	1.00	4.00	8.50	-	50.00
Bacillus pumilus	25.00	1.50	2.50	-	100.00	2.00	1.60	25.00	50.00	25.00	3.10	1.60	20.00	1.00	8.50	1.60
Staphylococcus aureus	-	10.00	3.10	1.00	6.30	50.00	8.00	6.30	20.00	-	20.00	2.00	3.10	8.50	1.00	-
Escherichia coli	-	12.50	8.50	12.50	20.00	-	12.50	9.00	35.00	-	9.50	1.60	7.50	12.50	-	20.00
Proteus vulgaris	20.00	50.00	300.00	170.00	159.00	130.00	80.00	-	6.30	200.00	56.00	1.00	100.00	12.50	200.00	180.00
Bardetellabronchiseptica	80.00	25.00	98.50	-	3.10	9.00	1.60	75.00	200.00	-	12.50	2.00	1.60	2.50	12.50	50.00
Aspergillus niger	100.00	85.00	2.50	1.30	12.50	6.30	25.00	40.00	20.00	25.00	5.50	4.00	6.30	2.50	3.10	5.00
Saccharomyces cerevisiae	70.00	25.00	12.50	30.00	100.00	25.00	35.00	100.00	85.00	120.00	25.00	1.60	4.50	3.10	6.30	85.00
Candida albicans	-	6.30	-	9.50	-	-	-	85.00	-	-	-	1.00	12.50	9.00	-	80.00

Mean reading of three replicates

An MlC result showed that compounds 1-V, VII-IX and XII-XIV were found most promising against *Bacillus subtilis*, while compound VI, X and XVI were found moderate active. The compounds II, Ill, VI, VII, XI, XII, XIV and XVI were found most promising against *Bacillus pumilus*, while the compound I, VIII, IX, X and XIII were found moderately active. However, the MIC result of compound V was found least active. An MIC result presented by compounds II-V, VII, VIII, XII-XVI were most promising against *Staphylococcus aureus*, whereas MIC of compounds VI, IX and XI were found moderate promising. An MIC result of compounds III, VIII and XI - XIII showed most promising against *Escherichia coli*, while

the compounds II, IV, V, VII, IX, XIV and XVI were found moderate promising active. The compounds IX and XII were found most promising against *Proteus vulgaris*, whereas an MIC result of compounds I, II, VII, XI and XIV were found moderate promising and compounds III–VI, X, XIII, XV and XVI were found least active. The MIC result of the present series of compounds can be compared with the compound 5-Nitrofurfurylidene-isatin-3-hydrazone tested against *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* reported by [Padhya *et a.,* 1973]. The new compounds IV, XII and XV also showed exactly the same MIC ranging from 1.0 to 2.0 µg/mL against *Staphylococcus aureus*(as reported for 5-Nitrofurfurylidene-3-hydrazone). The compounds of the present series VIII, XI-XIII showed more effective MIC results as 1.56 to 9.5 µg/mL against *Escherichia coli*, while reported [Padjya *et al.,* 1973] as 10 µg/mL. The compounds IV, XII and XIV were also more active (an MIC result from 1.0 to 12.5 µg/mL) than the already reported compounds MIC range from 10 to 20 µg/mL) against *Proteus vulgaris*.The MIC results of the compounds V-VII and XII - XIV were found most promising active, against *Bordetellabron chiseptica*, whereas compounds I, II, XI, XV and XVI were found moderate promising activity. However, an MIC result of compound IX was found least active. An MIC result of compounds III, IV, VI and XI - XVI were most promising against *Aspergillus niger*, while MIC result of compound II, V and VII - X were found moderate promising. However, compound I was found least active. The MIC result of the compound XII-XV was found most promising against *Saccharomyces cerevisiae*, while compounds I – IV, VI, VII, IX and XVI were found moderate promising active. However, MIC results of compounds V, VIII and X were found least active. The compounds II, IV, XII and XIV were found most promising against *Candida albicans*, while an MIC result of compounds VIII, XIII and XVI was found moderate promising. The *in-vitro* antibacterial activities of azines were studied by serial dilutions technique [Cruickshank 1960: Johnston 1064] examined antibacterial activity of a series or Isatinazine compounds prepared from 5-nitrofurfuraldhyde and other aldehydes. They reported that N-ethylisatin-5-nitrofurfurylidene hydrazone gave most promising *in-vitro* results regarding both activity and wide spectrum of effectiveness. While in our antimicrobial studies, Isatinazine series or analogues, were prepared by using various aldehydes as well as ketones such as formaldehyde, acetaldehyde, chloralaldehyde, benzaldehyde, anisaldehyde, 3-hydroxy-5methoxy-benzaldehyde, cinnamaldehyde and 4-dimethylamino) benzaldehyde. In present studies, azine derivatives gave different degree of efficiency against test organisms. The compound XII [Isatin-3-3hydroxy-5methoxybenzlidene), hydrazone] gave the most promising and broad-spectrum activities studies *in-vitro* studies. While compound VIII [Isatin-3-(4-nitro benzylidene) hydrazone] showed very poor response against the test organisms. The bacterial activity of azine data reported by [Phadhya *et al*, 1973] reveals that no significant change in antibacterial properties of the parent azine is produced by introducing CI or CH, in the Isatin moiety. Therefore, no such substitution was attempted in new derivatives. However, new azine compounds were synthesised by using different carbonyl compounds. Which showed promising antibacterial activities; moreover these new prepared compounds had proven antifungal activity that was even not reported by [Phadhya *et al.*, 1973].

3.2 N-substituted ISATIN-3-ISONICOTINYL HYDRAZONE

Azines (XVII - XXIV) are prepared by the condensation of Isatin or N-substituted Isatin with isonicotinyl hydrazide. The simplest analogue of the series Isatin-3-isonicotinyl hydrazone (XVII) which is synthesized with unsubstituted Isatin. Other analogues of these azines are prepared with N-substituted Isatin molecules such as N-Acetylisatin, N-benzoylisatin, N-Diethanolaminomethylisatin, N-morpholinomethylisatin. N-piperidinomethylisatin, N-Ephedrino methylisatin and N-diephenyl amino methylisatin. The solvent of recrystallization, m.p. formula, molecular weight and % yield of each analogue is given in table 4. Compounds are identified by spectroscopy method, the result are given in table 5.To estimate the efficacy of each analogue, the minimum inhibitory concentration (MIC) was determined and results are given in table 6.

Table 4: Characteristic of Azines (XVII-XXIV)

Compounds No.	Formula	Solvent of Crystallization	Molecular Weight	Melting Po. Int °C	% Yield
XVII	$C_{14}H_{10}N_4O_2$	Ethanol DMF	266.12	298-300	84.10
XVIII	$C_{16}H_{12}N_4O_1$	Ethanol DMF	308.13	155-158	88.42
XIX	$C_{21}H_{12}N_4O_1$	Acetone Ethanol	370.15	190-195	93.00
XX	$C_{19}H_{21}N_5O_4$	Ethanol	383.22	277	92.82
XXI	$C_{19}H_{19}N_5O_3$	Ethanol	365.20	298	87.20
XXII	$C_{20}H_{19}N_5O_2$	Ethanol	363.20	288-290	91.20
XXIII	$C_{24}H_{25}N_5O_1$	Ethanol Acetone	443.36	304	93.20
XXIV	$C_{27}H_{21}N_5O_2$	Ethanol	447.23	284	86.60

Table 5: VIS/UV Spectra and hRf values of Azines (XVII-XXIV)

Mobile Phase Toluene: Ethyl acetate (4:3)						VIS-UV Spectra	
Compound No.	hRf Values	Standard Deviation	UV Lamp		Iodine Vapours	λ_{max}	ε_{max}
			254 nm	366 nm			
XVII	57	± 2.1	+	+	+	446	2222
XVIII	29	± 1.5	+	F	-	371	1628
XX	40	± 2.3	+	+	+	452	2356
XXI	24	± 1.3	F	F	+	441	2263
XXII	16	± 2.0	+	+	+	422	2082
XXIII	50	± 1.0	-	+	+	448	2249
XXIV	66	± 2.8	+	+	+	450	2362

Solvent DMSO used for λ_{max}

F Fluorescent
\+ Visible
\- Invisible

Table 6: MIC (µg/mL) of different azines (XVII-XXIV) against various Test Microorganisms

Strains	Compounds							
	XVII	**XVIII**	**XIX**	**XX**	**XXI**	**XXII**	**XXIII**	**XXIV**
Bacillus subtilis	12.5	12.5	6.25	-	25	50	100	-
Staphylococcus aureus	1.56	6.25	12.50	51.0	1.56	-	40	-
Escherichia coli	6.25	1.56	6.25	3.12	6.25	25	3.12	45
Pseudomonas aeruginosa	3.12	6.25	1.56	-	85	160	20	200
Aspergillus niger	-	80	1.56	12.5	1.56	-	50	-
Candida albicans	-	140	-	20	1.56	3.12	1.56	45

In the present studies above mentioned hydrazone derivatives were condensed with isoniazid (1NH), which showed an increase in the antimicrobial activity. The action of 1NH is universally accepted against tubercle organism, but the present studies showed that its derivatives with Isatin are also effective against other test organisms and can be considered more effective as compared to the hydrazone derivatives reported.

It may be concluded from the antimicrobial result of azines (XVII - XXIV) that compound XIX is found to be most effective against all the used test organisms, except *Candida albicans* which is entirely ineffective. Therefore, the compound XIX can be considered as prime promising active compound which can be further used as potential and broad-spectrum antimicrobial agents.

The compound XXI showed most promising activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candido albicans*. This compound XXI may be the most suitable anti-fungal agent. However, this compound is round moderately effective against Bacilli. The compounds XVII and XVIII are most effective against *Staphylococcus aureus* and *Escherichia coli*. Moreover, these compounds (XVII, XVIII) also showed most promising against *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively.

The compounds XX, XXII and XXIII showed most promising activity only against *Candida albicans*, while compounds XXII were also found most effective against *Escherichia coli*. However, above mentioned compounds (XX, XXII and XXIII) were found moderately effective against most of the remaining test organisms.

3.3 N-substituted ISATIN AZINES

Azines are composed of hydrazones with carbonyl group (aldehyde and ketones). When N-substituted Isatin such as N-Piperdinomethylisatin, N-Morpholinomethylisatin N-Acetylisatin and N-Benzoylisatin reacted with Isatin-3-hydrazone, the compounds XXVI, XXVII, XXIX and XXXI were obtained. When N-substituted Isatin molecules were reacted with N-Substituted Isatin-3-hydrazones, compounds XXVIII, XXX and XXXII were produced. The suitable solvent or recrystallization, molecular weight, % yield and m.p of each analogue is given in table 7. These compounds were characterized by spectroscopic methods and results are given in table 8. Model study was conducted against Gram +ve and Gram -ve bacteria by selecting a few analogues (XXV, XXVII, XXVIII) from these N-substituted Isatinazines. Antimicrobial results are given in table 9.

Table 7: Characteristic of Isatinazines (XXV-XXXII)

Compounds No.	Formula	Solvent of Crystallization	Molecular Weight	Melting Po. Int °C	% Yield
XXV	$C_{16}H_{10}N_4O_2$	Ethanol DMF	290.12	220-221*	90.00
XXVI	$C_{22}H_{21}N_5O_2$	Ethanol	387.22	276*	87.00
XXVII	$C_{21}H_{19}N_5O_2$	Ethanol	389.21	151	91.00
XXVIII	$C_{26}H_{28}N_6O_4$	Aq DMF	488.29	170	90.00
XXIX	$C_{18}H_{12}N_4O_3$	DMF	332.14	210	78.63
XXX	$C_{20}H_{14}N_4O_4$	Acetic Acid	374.16	270	91.63
XXXI	$C_{23}H_{14}N_4O_2$	Ethanol	394.16	215	82.62
XXXII	$C_{30}H_{18}N_4O_4$	Pet. Ether	498.20	110	77.55

Table 8: VIS/UV Spectra and hR_f values of Isatinazines (XXV-XXXIII)

Mobile Phase Toluene:Ethylacetate (4 : 3 v/v)						VIS-UV Spectra	
Compound No.	hR_f Values	Standard Deviation	UV Lamp		Iodine Vapours	λ_{max}	ε_{max}
			254 nm	366 nm			
XXV	59	± 2.0	+	+	+	487	2624
XXVI	37	± 1.5	+	+	-	382	1604
XXVII	62	± 1.0	+	+	+	536	2681
XXVIII	44	± 3.0	+	F	+	464	2432
XXIX	51	± 0.7	+	+	+	425	2182
XXX	88	± 2.2	+	F	+	435	2095
XXXI	53	± 1.9	+	+	+	418	1969
XXXII	91	± 3.2	+	+	+	282	0826

Solvent DMSO used for λ_{max}

F Fluorescent

\+ Visible

\- Invisible

Table 9: Zones of inhibition (mm) of different synthesized Isatin derivatives (XXV, XXVII, XXVIII, XXXII and XXXIV) against various Microorganisms

Strains	XXV		XXVII		XXVIII		XXXIII		XXXIV		A		B	
	500 µg	100 µg	500 µg	100 µg	500 µg	100 µg	500 µg	100 µg	500 µg	100 µg	500 µg	100 µg	500 µg	100 µg
Bacillus subtilis	21.53 ±* 0.17	16.02 ± 0.12	24.30 ± 0.10	20.03 ± 0.29	10.62 ± 0.21	-	9.84 ± 0.05	-	16.31 ± 0.17	14.0 ± 0.04	30.92 ± 0.21	26.31 ± 0.15	26.05 ± 0.23	21.51 ± 0.15
Bacillus pumilus	17.5 ± 0.10	14.30 ± 0.20	20.0 ± 0.21	17.10 ± 0.30	17.90 ± 0.15	14.00 ± 0.20	16.51 ± 0.10	14.31 ± 0.16	18.00 ± 0.12	15.30 ± 0.13	27.30 ± 0.32	23.11 ± 0.32	22.20 ± 0.21	18.32 ± 0.11
Staphylococcus aureus	16.50 ± 0.12	13.20 ± 0.16	21.21 ± 0.23	16.00 ± 0.15	23.20 ± 0.12	20.20 ± 0.17	10.70 ± 0.13	-	11.52 ± 0.21	9.91 ± 0.26	31.26 ± 0.07	28.73 ± 0.07	25.62 ± 0.36	23.40 ± 0.30
Micrococcus flavus	11.53 ± 0.15	-	10.50 ± 0.17	-	19.40 ± 0.20	16.00 ± 0.16	17.40 ± 0.20	15.30 ± 0.27	19.50 ± 0.25	16.82 ± 0.20	25.72 ± 0.13	22.45 ± 0.12	22.34 ± 0.14	18.00 ± 0.12

Escherichia coli	19.70 ± 0.20	16.30 ± 0.10	9.82 ± 0.12	-	8.92 ± 0.23	-	8.73 ± 0.15	-	13.23 ± 0.30	10.80 ± 0.11	28.90 ± 0.17	20.72 ± 0.09	22.00 ± 0.02	18.71 ± 0.14
Proteus vulgaris	15.42 ± 0.17	12.00 ± 0.15	18.51 ± 0.11	15.52 ± 0.30	21.04 ± 0.15	18.30 ± 0.14	17.24 ± 0.17	15.02 ± 0.15	19.52 ± 0.07	16.83 ± 0.03	26.03 ± 0.10	21.90 ± 0.10	23.32 ± 0.15	18.73 ± 0.31
Bordetellabronchiseptica	10.93 ± 0.26	-	21.17 ± 0.15	19.01 ± 0.21	26.52 ± 0.11	24.00 ± 0.25	11.52 ± 0.08	-	10.01 ± 0.03	-	13.20 ± 0.32	28.73 ± 0.15	29.11 ± 0.23	26.40 ± 0.27

* Mean zone of inhibition (± S.D); - No zone of inhibition; A-Erythromycin (100µg, 500µg/mL); B-Polymixin (100µg, 500µg/mL)

From the above findings, it may be concluded that the compound XXVII is found to be the most effective against *Bacillus subtilis*, *Staphylococcus aureus* and *Bordetellabron chiseptica*, while moderately effective against *Bacillus pumilus* and *Proteus vulgaris* and least effective against *Micrococcus flavus* and *Escherichia coli* at the concentration of 500 µg/mL. but at the concentration of 100 µg/mL it was found moderately effective against all the test organisms except *Micrococcus flavus* and *Escherichia coli*. The compound XXVIII was found most effective against *Bacillus aureus*, *Proteus vulgaris* and *Bordetellabron chiseptica* while moderately effective against *Bacillus pumilus* and *Micrococcus flavus* and least effective against *Bacillus subtilis* and *Escherichia coli* at the concentration of 500 µg/mL, However, at the concentration of 100 µg/mL, it was found most effective against *Bordetellabronchiseptica* and moderately effective against *Staphylococcus aureus*, *Micrococcus flavus* and *Proteus vulgaris*. The compound XXV was found most effective only against *Bacillus subtilis* and moderately effective against all the remaining test organisms except *Micrococcus flavus* and *Bordetellabron chiseptica* at the concentration of 500 µg/mL. But at 100 µg/mL it was moderately effective against *Bacillus subtilis* and *Escherichia coli*.

3.4 MONOCHLOROACETYLATION

Monochloroacetylchloride was prepared from monochloroacetic acid with thionyl chloride. This product was further reacted with Isatin and Isatin-3-hydrazone to produce N-chloroacetylIsatin and N-chloroacetyl Isatin-3-chloroacetyl hydrazone.Solvent of crystallization molecular weight, % yield and m.p. of each of these compounds (XXXIII, XXXIV) is given in the table 10. The compound characterized by spectroscopic methods and results are given in table 11. The model study of these two chloroacetyl compounds was conducted against Gram +ve and Gram-ve bacteria at the concentration of 100 and 500 µg/mL. The reference standard Erthroymyein and polymyxin were used to check the effectiveness of these compounds.

Table 10: Characteristic of Chloroacetylation of Isatin and Isatin-3-hydrazone

Compounds No.	Formula	Solvent of Crystallization	Molecular Weight	Melting Po. Int °C	% Yield

XXXIII	$C_{10}H_9NO_2Cl$	DMF	223.52	205.6	85.32
XXXIV	$C_{12}H_9N_3O_2Cl_2$	Benzene, DMF	314.01	164	73.45

Table 11: VIS/UV Spectra and hR_f values of Isatin Derivatives (XXXIII, XXXIV)

Mobile Phase Toluene:Ethyl acetate (4 : 3 v/v)						VIS-UV Spectra	
Compound No.	hR_f Values	Standard Deviation	UV Lamp		Iodine Vapours	λ_{max}	ε_{max}
			254 nm	366 nm			
XXXIII	82	± 1.8	+	F	+	370	1599
XXXIV	43	± 3.0	+	+	+	407	1919

Solvent DMSO used for λ_{max}

F Fluorescent

\+ Visible

The Isatin derivatives were produced by the reaction or Isatin and Isatin-3-hydrazone with chloroacetylchloride, were screened against selected test microorganisms as a model study. These compounds showed antimicrobial activity against *Bacillus subtilis*. *Bacillus pumillus*. *Staphylococcus aureus*, *Micrococcus flavus*, *Escherichia coli*, *Proteus vulgaris* and *Bordetellabronchiseptica*. The efficacy of these compounds was estimated by using two reference standards i.e., Erythromycin and polymyxin. The concentrations of these two new derivatives and reference standards were used as described in results. The numerical values of the zones of inhibition of these compounds were graded into three categories as mentioned in the previous studies. The zones of inhibition more than or equal to 21.0 mm. 15.0 mm, and 8.5 mm were considered as "most effective", "moderately effective" and "least effective" respectively.*Bacillus subtilis* are spore forming species and were reported resistant to many antibiotics. This strain was moderately inhibited by the compound XXXIV at the concentration of 500 µg/mL, while this compound was found least effective at the concentration of 100 µg/mL. The compound XXXIII was found least effective at the concentration of 500 µg/mL, whereas it was ineffective at concentration of 100 µg/mL.The compound XXXIII and XXXIV showed moderate inhibitory effects against *Bacillus pumilus* at the concentration of 500 µg/mL. The compound XXXIV also showed moderate inhibitory effects at 100 µg/mL, but the compound XXXIII was found least effective at this concentration. The compound XXXIV was found least effective against *Staphylococcus aureus* at both concentrations of 100 µg and 500 µg/mL. The compound XXXIII was found least effective at the concentration 500 µg, while it was ineffective at 100 µg/mL. The zones of these compounds did not exceed the zones of inhibition of any reference standard against this strain. The compounds XXXIII and XXXIV were found moderately effective against *Micrococcus flavus* at both concentrations 100 µg and 500 µg/mL. The zones of inhibition obtained from the compound XXXIV at both concentrations were found more or less equal to the zone inhibition produced by the Polymyxin at the same concentrations, but they were less than the reference standard Erythromycin.The compound XXXIV was found least effective against *Escherichia coli* at both mentioned concentrations (100 µg, 500 µg/mL). The compound XXXIII was found least effective at the concentration of 500 µg/mL, while ineffective at the concentration of 100 µg/mL. Both these compounds showed poor results

against this test microorganism because these compounds did not exceed their zones of inhibition as compared to that of reference standards Polymyxin and Erythromycin.The compounds XXXIII and XXXIV were found moderately effective against *Proteus vulgaris* at the concentration of µg/mL. whereas these two compounds were found least effective against *Bordetellabron chiseptica* at the concentration of 500 µg/mL and ineffective at the concentration of 100 µg/mL.

3.5 THIADIAZOLINE derivativesof ISATIN

These are heterocyclic compounds, prepared by reacting cyclic thiosemicarbazide with Isatin or cyclization of Isatins-3-thiosemicarbazone.Their antimicrobial activity was also studied against Gram +ve and Gram -ve bacteria and fungi.The compound characterized by spectroscopic methods and results are given in table 12. Their minimum inhibitionconcentration was also studied, which is given in table 13.

Table 12: VIS/UV hR_1 Values of Thiadiazoline derivatives of Isatin (XXXV-XXXIX)

Mobile Phase Toluene:Ethyl acetate : Acetic acid (24 : 18 : 1 v/v)						VIS-UV Spectra	
Compound No.	HR_f Values	Standard Deviation	UV Lamp		Iodine Vaporus	λ_{max}	ε_{max}
			254 nm	366 nm			
XXXV	25	± 1.1	+	F	+	283	0916
XXXVI	19	± 3.0	+	+	+	290	0926
XXXVII	32	± 1.7	+	+	-	363	1105
XXXVIII	22	± 2.5	F	F	+	310	0980
XXXIX	47	± 1.4	F	F	+	310	1008

Solvent DMSO used for λ_{max}
F Fluorescent
\+ Visible
\- Invisible

Table 13: MIC (µg/mL) of Thiadiazoline derivatives of Isatin against various Microorganisms

Strains	Compounds			
	XXXVI	XXXVII	XXXVIII	XXXIX
Bacillus subtilis	140.00*	10.10	10.00	50
Bacillus pumilus	70.00	3.12	24.50	50.00
Staphylococcus aureus	170.00	1.50	10.00	25.00
Escherichia coli	200.00	10.00	12.50	20.00
Proteus vulgaris	500.00	10.00	8.00	25.00
Bordetellabronchiseptica	500.00	12.50	6.25	20.00
Aspergillus niger	100.00	50.00	12.50	100.00
Saccharomyces cerevisiae	100.00	50.00	12.50	-
Candida albicans	25.00	6.25	6.25	-

*** Mean reading of three replicates; - Experiment was not carried out**

It may be concluded that compound XXXVII is found to be the most effective against *Bacillus subtilis, Bacillus pumillus, Staphylococcus aureus, Escherichia coli. Bordetellabronchiseptica* and *Candida albicans,* while moderately effective against *Proteus*

vulgaris, *Aspergillus niger* and *Saccharomyces cerevisiae*. The compound XXXVIII was found most effective against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Bordetellabronchiseptica* and *Aspergillus niger*, while moderately effective against *Bacillus subtilis*, *Bacillus pumilus* and both the yeast. The compound XXXIX was found moderately effective against all the test bacteria, and *Aspergillus niger*, but ineffective against yeast However, compound XXXVI was found moderately effective against *Bacillus pumillus*. *Staphylococcus aureus*, and the fungi but it was least active against rest of the test microorganisms. The activity of the compounds XXXVI and XXXVII as well as compounds XXXVIII and compound XXXIX was compared. The compound XXXVI showed poor response as compared to compound XXXVII, it seems that the condensation of the compound XXXVI with Isatin enhance the antimicrobial activities. The comparative study of the compound XXXVIII and XXXIX showed the compound XXXVIII is more effective than compound XXXIX. It may be due to the absence of acetyl group in the later compound.

3.6 *In-vitro* model study onthe *mycobacterium*sensitivity of ISAITN-3-ISONICOTINYLHYDRAZONE

During research work, one of our Isatin derivatives (XVII) was prepared by reacting Isatin with Isoniazide as isoniazid is one of the best anti-tubercle drugs [Martindate *et al.*, 1972]. This new derivative was screened against *Mycobacterium tuberculosis* in Gulab Devi, Chest Hospital. This study was carried out on the susceptibility of tubercle organisms isolated from various sources of patients against this newly synthesized derivative (VXII) i.e., Isatin-3-isonicotinyl hydrazone [Ali *et al.*, 1994], to see whether Isatin enhances the antituberculosis activity of Isoniazid (INH) or not. These samples were composed of sputum, urine, pus and pleural fluid. Collected from patients suffering from tuberculosis. Their bacterial count and amount of growth in 4 mL of the medium was determined. In the case of sputum samples, out of 10 samples, 8 samples i.e., S-1, S-2, S-4-S-8 and S-10 showed a bacterial count from 11 to 100 per microscopic field, while remaining two S-3 and S-9 showed from l to 10. The 60% of these samples gave bacterial growth more than 900 in 4 mL of liquid medium (Contained in BACTEC 12 B Vial). The maximum population of tubercle organisms (995) was found in sputum sample S-7. Three urine samples (U-1, U-2, and U-4) out of six samples (or 50% of the urine samples) had growth above 900. The bacterial counts in the urine samples U-1, U-2, U-4, and U-6 ranged from 11 to 100 per microscopic field, while the counts in U-3 and U-5 were 1 to 1 0. Among these samples U-4 showed the maximum growth µ982). Similarly, out of 5 pus samples, only one µP-2) showed maximum amount of growth µ930), while other have shown less than 900. In 50% of these pus samples, the number of bacteria was between 11 and 100 per microscopic field. Only Pf-2, out of 4 pleural fluid samples, had a bacterial count of 11–100 per microscopic field. Less than 900 growth units had been seen in all pleural samples. It has been observed that the population of tubercle organisms in sputum samples is the highest, while the population in urine samples is second only to that in sputum samples, and the population in pus and pleural fluid samples is the lowest.

All the 25 selected specimens were tested for the sensitivity against a newly synthesized compound. INH µ as standard drug and Isatin. These medications' three distinct concentrations were used to test their efficacy, and the outcomes are shown in Table 14. The

reference standard medication, INH, as described in the literature, is used to determine the concentration. The recommended MIC of INH was 2 to 5 µg/100 ml [Martindale *et al*., 1972], so, a series of concentrations as l µg, 2 µg and 3 µg per 100 ml of the medium was used. When it came to the efficiency of medications against tubercle germs, the results indicated that some were "moderately effective" if there was growth, and "most effective" if there was no growth at all. However, medications were deemed "ineffective" when the organism grew un a manner akin to the growth of the control (i.e., when the drug was not used).

Table 14: Sensitivity of Different isolated Tubercle Organisms in dilutions of 10^5 and 10^3 against Isoniazid hydrochloride, Isatin and Isatin-3-isonicotinylhydrazone µ New Compound)

Specimen			Control Without Drug		Isatin-3-isonicotinythydrazone µg/100 ml						Isoniazid Hydrochloride µg/100 ml						Isatinµg/100 ml					
					1.0 µg		2.0 µg		3.0 µg		1.0 µg		2.0 µg		3.0 µg		1.0 µg		2.0 µg		3.0 µg	
Sr. No.	**Source**	**Code No.**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**	**10^5**	**10^3**
1	Sputum	S-1	+ +	+ + +	R	R	S	S	S	S	R	R	S	R	S	S	R	R	R	R	S	R
2		S-2	+ +	+ + +	S	S	HS	HS	HS	HS	S	R	S	S	HS	HS	R	R	S	R	S	S
3		S-3	+ +	+ + + +	R	R	S	S	HS	S	R	R	R	R	R	R	R	R	R	R	R	R
4		S-4	+ +	+ + + +	S	S	HS	S	HS	HS	R	R	HS	S	HS	HS	R	R	S	R	S	S
5		S-5	+ +	+ + + +	S	S	S	S	HS	HS	R	R	S	S	S	S	R	R	R	R	R	R
6		S-6	+ +	+ + + +	R	R	S	S	HS	S	R	R	R	R	S	S	R	R	R	R	R	R
7		S-7	+ +	+ + +	S	S	HS	HS	HS	HS	S	R	HS	S	HS	HS	R	R	S	R	S	S
8		S-8	+ +	+ + + +	S	S	HS	S	HS	HS	R	R	S	S	HS	S	R	R	S	R	S	S
9		S-9	+ +	+ + +	R	R	S	S	HS	HS	R	R	S	S	S	S	R	R	R	R	S	R
10		S-10	+ +	+ + +	R	R	S	S	HS	S	R	R	S	S	S	S	R	R	R	R	S	S
11	Urine	U-1	+ +	+ + + +	R	R	S	S	HS	S	R	R	S	R	S	S	R	R	R	R	S	R
12		U-2	+ +	+ + + +	S	S	HS	S	HS	HS	R	R	S	S	S	S	R	R	R	R	R	R

13		U-3	+ +	+ + + +	S	R	S	S	S	S	R	R	R	R	S	S	R	R	R	R	R	R
14		U-4	+ +	+ + + +	S	S	HS	S	HS	HS	R	R	S	S	HS	S	R	R	S	R	S	S
15		U-5	+ +	+ + + +	S	S	HS	HS	HS	HS	R	R	S	S	S	S	R	R	S	R	S	S
16		U-6	+ +	+ + + +	R	R	S	S	S	S	R	R	S	S	HS	S	R	R	R	R	S	R
17	Pus	P-1	+ +	+ + + +	S	R	S	S	S	HS	R	R	S	S	S	S	R	R	R	R	S	R
18		P-2	+ +	+ + +	S	S	HS	HS	HS	HS	R	R	S	S	HS	HS	R	R	S	R	S	S
19		P-3	+ +	+ + +	S	S	HS	S	HS	HS	R	R	S	S	HS	S	R	R	R	R	S	S
20		P-4	+ +	+ + + +	R	R	S	S	S	S	R	R	R	R	S	R	R	R	R	R	R	R
21		P-5	+ +	+ + + +	S	S	HS	S	HS	HS	S	R	S	S	HS	S	R	R	S	R	S	S
22	Pleural	Pf-1	+ +	+ + + +	S	R	HS	S	HS	HS	R	R	S	S	S	S	R	R	R	R	S	R
23	Fluid	Pf-2	+ +	+ + + +	S	S	S	S	S	HS	R	R	S	S	HS	S	R	R	R	R	S	R
24		Pf-3	+ +	+ + + +	S	R	HS	S	HS	HS	R	R	S	R	S	S	R	R	R	R	R	R
25		Pf-4	+ +	+ + +	S	R	HS	HS	HS	HS	R	R	S	S	HS	HS	R	R	R	R	S	S

HS Highly Sensitive µAbsence of growth) + + + + Maximum growth
S Sensitive Slight growth µbut less than control) + + +Moderate growth
R Resistant µSimilar growth as seen in control) + +Minimum growth

These drugs effects at the concentration of 3 µg/100 ml against sputum isolates showed that New compound and INH had similar response against both dilutions µ10^5, 10^3) of S-1, S-2, S-4 and S- 7, whereas their results against other sputum isolates were different. INH was most effective against isolates of samples S-2, S-4, S-5, and S-7-S-9, while the novel drug was most effective against isolates of samples S-2, S-4, and S-7 at each dilution. The New compound was also found most effective and moderately effective against S-3, S-6 and S-10 at a dilution of 10^5 and 10^3 respectively, but INH was found moderately effective against both

dilutions of isolates of S-l, S-5, S-6, S-9 and S-10, and had no response against S-3. These findings showed that the tubercle organisms from the S-3 sample were susceptible to the new chemical but resistant to INH. Isatin at this concentration was shown to be ineffective against the samples S-3, S-5, and S-6, but moderately effective against the samples S-2, S-4, S-7, S-8, and S-10 at both dilutions as well as against S-1 and S-9 at a dilution of 10^5.

The effect of the drug at the concentration of 2 µg/100 ml showed that the New compound was most effective against both dilutions of tubercle organism isolated from samples S-2 and S-7, whereas INH was found most effective against S-7 at a dilution of 10^5 and moderately effective against each dilutions of S-2. The New compound is found most effective against a dilution of 10^5 of S-8, whereas, INH is moderately effective against both dilutions. The New compound was found moderately effective against each dilution of S-3 and S-6, whereas, INH was found totally ineffective. This also confirmed that the tubercle organisms which were resistant to INH are found sensitive to New compound at the concentration recommended in literature µ2 to 5 µg/100 ml) [Verma *et al.,* 1967]. Isatin at this concentration 2 µg/ml is found moderately effective against a dilution of 10^5 isolates of samples S-2, S-4, S- 7 and S-8. It is also found totally ineffective against the rest of the samples.

The results of the drugs at the concentration of 1 µg/100 ml against tubercle organisms isolated from sputum samples, showed that New compound was found moderately effective against both the dilutions of isolates of samples S-2, S-4, S-5, S- 7 and S-8, whereas INH was found ineffective against all the isolates of sputum except against dilution of 10^5 of samples S-2 and S-7. However, Isatin has no response against all these sputum isolates at 1 µg/100 ml of the medium. These results showed that tubercle organism, which were found resistant to INH, were found sensitive to the new compound even at the lower concentration than recommended in literature [Martindale *et al*., 1972].

The effects of these drugs at the concentration of 3 µg/ 100 ml against urine isolates, showed that the New compound was most effective against each dilution or isolates of U-2, U-4 and U-5, Whereas INH is moderately effective against isolates U-2 and U-5, but it was also most effective against a dilution of 10^5 of U-4. Similarly New compound was found most effective and moderately effective against isolate of sample U-l at a dilution of 10^5 and 10^3 respectively, but INH was found moderately effective against U-1. New compound and INH gave similar results against the isolates of samples U-3 and U-6. Isatin at this concentration µ3 µg/100 ml) was found moderately effective against each dilution of U-4 and U-5, and it was also found moderately effective at a dilution of 10^5 of U-1 and U-6, However, it was ineffective against the isolates of the samples U-2 and U-3. The effects on the drugs at a concentration of 2 µg/100 ml showed that New compound was found most effective against each dilution of U-5, whereas INH was moderately effective. New compound was found most effective and moderately effective against the isolate of sample U-2 as well as U-3 at a dilution of 10^5 and 10^3 respectively; as compared to INH which was found moderately effective. The New compound was found moderately effective against each dilution of isolate of U-3, while INH was ineffective. It was also ineffective against a dilution of 10^3 of U-1. This also showed that new compound was more effective than INH, because the tubercle organisms, which were found resistant to INH, were found sensitive to new compound at the concentration as recommended in literature [Martindale *et al.,* 1972]. Isatin at this

concentration was found totally ineffective against all the urine isolates, except the dilution of 10^5 of U-4 and U-5.

The effects of these drugs at the concentration of 1 µg/100 ml showed that the New compound was found moderately effective against both dilutions of isolates of U-2, U-4 as well as U-5 and at dilution of 10^5 of U-3, whereas it was ineffective against U-6, while INH and Isatin at this concentration were found totally ineffective against the isolates of urine. So these results also indicate, that new compound was found active even at a concentration lower than that of recommended [Martindale *et al*., 1972].

The effects of these drugs at 3 µg/100 ml were examined against pus isolates, which showed that the New compound was most effective against each dilution of all the pus isolates, except P-4. The INH also showed similar results against only one isolate of sample P-2. This drug was also found most effective and moderately effective against the isolate of P-3 as well as P-5 at a dilution of 10^5 and 10^3 respectively. It was found moderately effective against each dilution of P-l and ineffective at a dilution of 10^3 of P-4. These results revealed that tubercle organisms, which were resistant to INH, were found sensitive to the New compound. Isatin was found moderately effective against each dilution of isolates of P-2, P-3 and P-5 and at a dilution of 10^5 of P-1, while it is found totally ineffective against P-4.

The effects of the drugs at the concentration of 2 µg/100 ml against isolates of pus samples, showed that New compound was most effective against each dilution of P-2 and one dilution 10^5 of isolate P-3 and P-5. Whereas INH was found moderately effective against each dilution of isolates of P-2, P-3 and P-.5 .The New compound and INH have similar results against P-1. Moreover New compound was found moderately effective against P-4, whereas INH was found totally ineffective. This indicates that the tubercle organisms, which were isolated from P-4 were resistant to INH , were found sensitive to the New compound.

The effects of these drugs at the concentration of l µg/100 ml showed that the New compound was moderately effective against about all the pus isolates, except one P-4 which was resistant. The INH was found totally ineffective against all the pus isolates, except a dilution of 10^5 of P-5. However Isatin at this concentration was found totally ineffective against all the pus samples. This result revealed that the tubercle organisms which were resistant to INH and Isatin, were found sensitive to new compound The effects of the drug at the concentration of 3 µg/100 ml the medium against pleural fluid isolates showed that the New compound was found most effective against all the isolates of pleural fluid at each dilution, whereas INH was found most effective against samples of Pf -4 only. It was found most effective and moderately effective against the isolates of Pf-2 at a dilution of 10^5 and 10^3 respectively, whereas INH was found moderately effective against the isolates of samples Pf-1 as well as Pf-3 at both dilutions. Isatin at this concentration was found moderately effective against both the dilutions of Pf-4 and a dilution of 10^5 of isolates of Pf-1 and Pf-2, but it was totally ineffective against Pf-3.

When these results are observed at the concentrations of 2 µg/100 ml, the New compound was found most effective against the isolates of Pf-4 at both dilutions. It was also found most effective and moderately effective against Pf-l as well as Pf-3 in the dilutions of 10^5 and 10^3 respectively. It was found moderately effective against isolate of Pf-2 at each dilution. Whereas INH was found moderately effective against all the isolates of pleural fluids at each dilution except Pf-3 at a dilution of 10^3. The New compound was found more effective than

INH, because tubercle organism isolated from Pf-3 were found resistant to INH, but sensitive to new compound at a concentration recommended [Martindale *et al.*, 1972]. However, Isatin was found ineffective against all these pleural fluid samples.

The effects of the drugs at the concentration of 1 µg/100 ml showed that the New compound was found moderately effective against isolate of Pf-2 at each dilution. It was found moderately effective and ineffective against Pf-1, Pf-3 as well as Pf-4 at a dilution of 10^5 and 10^3 respectively, whereas, INH and Isatin both were ineffective against all the isolates of pleural fluids. This outcome showed that pleural fluid isolates resistant to isatin and INH were sensitive to new substance at a concentration higher in the tower than suggested by previous research [Martindale *et al*., 1972].

Recent outbreaks of multidrug resistant (MDR) tuberculosis (TB) have major cases of concern. TB is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. It most commonly affects the lungs, but it can also affect other parts of the body, such as the brain, kidneys, and spine. The "World Health Organization" (WHO) declared tuberculosis is a "global emergency". Tuberculosis is leading cause of morbidity in developing countries, where 95% of tuberculosis occur. Development of effective and safer antituberculosis drugs that might have good patient compliance is a must to stop multidrug resistant lethal strains and also stop TB epidemic [Ravighone 1995: WHO 1996]. One survey report of Government of Pakistan [Report; 1962] indicates a high prevalence rate antituberculosis drug resistance. The problem of emergence of drug resistant strain of the tubercle bacilli may be responsible for the most serious deterrent, to the free use of antibacterial agent in the treatment or tuberculosis. It is essential to employ drugs according to a regimen best designed to delay the emergence of drug resistant organisms. To decide the most effective treatment regimen. The knowledge about the sensitivity or tubercle bacilli to the commonly available drugs is important drug treatment is so effective that if the organisms are sensitive to the drugs at the beginning of treatment nearly 100%, cure rate is expected [Malik *et al.*, 1986]. Wide spread development of drug resistant organism and other infections may aggravate this already difficult public health problem. A recent study reported that isoniazid resistance was 15 to 22% in previously unsuccessfully treated patients [Aziz *et al.*, 1986].

In Thailand, three studies were conducted on diagnosed and untreated patient and resistance of INH drug was determined. The first study on drug resistance was conducted in 1966-67 at the antituberculosis association which showed tubercle organisms were 14.6% resistant [Nuchprayoon *et al.*, 1969]. In 1977-80 another study was conducted which showed 18.03 % resistance of tubercle organisms to INH [Supcharoen *et al*., 1984]. Further studies performed in 1982-84 depicted 12.6% resistance or tubercle organisms to INH.Another study was conducted in central chest hospital 1979-81 on untreated and newly diagnosed patients. The tubercle organisms, average resistance turned out to be 14.7% to INH in three years duration [Punnotok *et al*., 1985]. In contrast study at the T.B control division in 1982 of resistance among previously treated tuberculosis patients showed a much higher prevalence rate, which was 84%, of INH [Jittinand *et al*., 1982]. The key factor in the prevention of resistance is compliance of all the part involved in TB control. This compliance should include program policy. Patient management and funds to support both TB programs and development of effective new safer drug. If the TB epidemic continue to be neglected in the developing countries, further generation will remember this decade as the time when humanity allow

deadly super bug that travel through the air to become drug resistant and incurable throughout the world [WHO 1996: WHO 1995: Neville 1994].

The drug resistance, which is rapidly increasing and contaminating the atmosphere with tubercle organism, invites the chemists and pharmacologists, to pay their due attention to develop pharmacologically specific and safer agents to knock out this highly resistant strain. These finding force us to produce INH derivatives by using suitable substituents. which increase the efficacy of the INH. Therefore, eight Isatin-3-isonicotinyl hydrazones (XVII-XXIV) were prepared. These analogues were synthesized by the condensation of Isatin and N-substituted Isatin with isonicotinyl hydrazide (INH) and the simplest analogues of series (XVII) was screened against *Mycobacterium* tuberculosis. As already mentioned, this strain was isolated from sputum, urine, pus and pleural fluid of the treated and untreated TB patient. The results obtained were discussed in the previous section which revealed that all the isolates of collected samples were found almost resistant to Isatin even at the highest concentration used (3 µg/100 mL).

The results of INH and new compound showed that INH was less effective than new compound. In many cases, INH was found resistant against *Mycobacterium tuberculosis*, while isolates from same samples were found sensitive to new compound even at the concentration of 1 µg/100 mL. New compound at the concentration of the l µg/100 mL was found moderately effective against both the dilutions of (10^3 and 10^5) isolate of the samples S-2, S-4, S-5, S-7, S-8, U-2, U-4, U-5, P-2, P-1, P-5 and Pf-2 and also effective to a dilution (10^5) of isolates of samples U-3, P-l, Pf- l, P1-2, Pf-5.

Conclusion

In order to determine the minimum inhibitory concentration of new compound, a few specimens S-2, S-8, U-2, P-2 and P-3 were selected, which were highly sensitive to the new compound at the concentration of 1 µg/mL. This concentration is less than the recommended concentration. Therefore, different concentrations of the new compound used, were 1.0, 0.75, 0.50, 0.25 and 0.10 µg/100 mL of the medium. The effects of these concentrations showed that the new compound was found effective up to the concentration of 0.50 µg/100 mL against each dilution of isolates S-8 and P-3 and a dilution (10^5) of isolate S-2, U-2, as well as P-2. However, the other concentration (0.25 µg and 0.1 µg) of the new compound were ineffective.

During our studies the new compound (Isatin-3-isonicotinylhydrazone) was found more effective than INH and Isatin against isolated tubercle organisms extracted from sputum, urine, pus and pleural fluid of TB patients It is already mentioned that many derivatives of Isatin were effective against *Mycobacterium* tuberculosis, but in our studies Isatin alone (without INH substituted) gave poor response at a concentration of 1µg-3µg /100 mL of the medium as compared to the new compounds. Isatin was found totally ineffective at a concentration of 1 µg/100 mL, whereas most of the tubercle isolates of sputum, urine, pus and pleural fluid were found sensitive to new compound. Its MIC was also determined which was as 0.50 µg to 0. 75 µg/100 mL as compared to standard MIC of INH i.e., 2 -5 µg/100 mL. The last new compound approved specifically for tuberculosis was rifampicin in 1966 which is also continuously developing resistance. Considering the results or present studies, Isatin-3-isonicotinylhydrazone already demonstrated as antibacterial and antifungal agents as

described in previous section, can also be regarded very active against tubercle organisms and should be given due consideration, while trying to develop any new ant tuberculosis therapy.

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References

1. Ali S., Alam M. (1994). Potential antibacterial agents-1: Structural modification and antibacterial activity of some isatin derivatives. *Arch. Pharm. Res*. 17, 131.
2. Annie L., Vested B.S. (1975). Procedures for the isolation and identification of Mycobacteria. *Dhew Publication No. (ISM),* 75.
3. Aziz A., Ishaq M., Jaffer N.A., Akhwand R. (1986). Clinical trial of two short course µ6 month) regimens and a standard regimen µ12 months) Chemotherapy in retreatment of pulmonary tuberculosis in Pakistan. Result 18 months after completion of treatment µLahore tuberculosis study). *Am. Rev. Resp Dis*. 135.
4. Bacyer A. (1869). Emmerting Synthesis of indole. *Ber;* 2, 679.
5. Baeyer A. (1884). The chemical nomenclature. *Ber;* 17, 960.
6. Bourrows W., Moulder W., Lewen M.R. (1968). Text book of Microbiology. 19th Ed. W.13 *sounder's company Philadelphia*. P.15.
7. Brown H.C. (1919). Opacity Standard. *Indian J. Medical Research*. 7, 238.
8. Canetti G. (1969). Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity test in tuberculosis programmes. *Bull. WIIO,* 41, 21.
9. Chandra S., Saklani S. (2017). Isolation and Identification of *Ficus palmata* Leaves and Their Antimicrobial Activities, *Journal of Scientific Research,* Vol- 9 (2): 193-200. http://doi:10.3329/jsr.v9i2.27806.
10. Clark F.J.C. Lucia, B. (1982). Primary culture media for routine urine processing. *J. Chin. Microbiol.* 16, 632.
11. Crowle A. J., Sbarbaro J.A., May M.H. (1988). Effects of isoliazid and of ceforanide 248. against virulent tubervle bacilli in cultured human macrophages. *Tubercle*. 69. 15.
12. Cruickshank. (1960). Mackie and McCartney's Handbook of Bacteriology. *E & S. Livingstone Edinburgh*. P. 407.
13. David H.L. (1973). Fundamentals of drug susceptibility testing in Tuberculosis Centre for Disease Control. µCDC). *Altanta, U.S.A.*
14. Delaat A.N.C. (1976). Microbiology for allied health profession. 2nd Ed. Ica and 229. *Febiger, philadephia*. P.389.
15. Deland F.H., Wagner Jr. (1969). Early detection of bacterial growth with carbon-14 labeled glucose, *Radiology*. 92. 154.
16. Dobck A.S., Klayman D.L., Dickson E.T. (1983). Thiosemicarbazone of 2-acetylpyridine, 2-acetylquinoline, 1-and 3 Acetylisoquinoline and related compounds

an inhibitors of clinically significant bacteria in-vitro. *Arveim-Forsch/Drug Res*. 33,1583.

17. Dobck S., Danicl I., Edward T. (1960). Inhibition of clinically significant bacterial organisms. In-vitro by 2-acetylpyridinethiosemicarbazones. Antimicrobial agents and chemotherapy. I, 27.

18. Eize, F. (1910). Oil of Robina Pseudacacia, *Chem-zig*, 34, 814.

19. Fleming. (1950). Pencilling, 2nd Ed., *Butterworths Co Ltd. London*. 91.

20. Handbook of Tuberculosis Lab. (1962). Methods. Va-Armed force cooperative study on Chemotherapy of tuberculosis. *U.S Printing Office, Washington* 25, D.C, USA.

21. Herter C.A. (1909). Note on the occurrence of skatol and indole in the wood of cellis Reticulosa. *J. Biel. Chem*. 5. 489.

22. Hess A. (1904). Jasmines flower Oil. *Ber:* 37, 1457.

23. Jittinand A., Kechararanta P. (1982). Primary drug resistance diagnosed in treated tuberculosis patients in TB Control Division. *Thia. J. Tuberc. Chest Dis*, 3, 143.

24. Johnston R.G., Kidd D. (1964). Antimicrobials Part II: Nitrofuran Analogues containing 2-hydrozonomethyl sidechain. *J. Chem. Soc.* 4734.

25. Malik M. A. (1967). Treatment of Tuberculosis. PMJR. P.254, Oct.

26. Martindale. (1972). The extra pharmacopoeia. 26th Ed. Department of pharmaceutical science, *London. WCI. P*. 1874.

27. Maysinger M., Movrin M., Saric M. (1980). Structural analogues of isatin and their antimicrobial activity. *J.Pharmazic*. 35, 14.

28. Melarland. (1907). Nephelometer an instrument for estimating the number for estimating the number of bacterial in suspension used for calculating the *opsonic index* and for vaccines. *J. America Medical association*. 14, 1176.

29. Middlebrook G. (1958). Bacteriology of tuberculosis laboratory method. *Am. J. Publ. Health*. 48, 844.

30. Naqvi B.S., Shaikh D., Shaikh R. (1985). Screening of Pakistani plants for antibacterial activity. Pakistan. J. *Sci. Ind. Res*. 28, 269.

31. Neville K., Bromberg A., Bromberg R. (1994). The third epidemic multidrug resistant tuberculosis. *Chest.* 105, 45.

32. Nuchprayoon C., Chinachoti N., Thanabodhi P. (1969). Primary drug resistance in tuberculosis: survey at a chest clinic in Bangkok." J. Med. Assoc. Thailand. 52, 646.

33. Padhya A.C., Mehta II.J., Dighe V.S. (1973). Antibacterial activity of Nitofurfurilidincisatin-3-hydrazone. *India, Sci. Cult*. 39, 55.

34. Panda A., Agarwal S., Saxena V.K. (1987). Synthesis and antiviral activity of some new indole derivatives. *India J. Pharm. Soci*. 93, 85.

35. Piscopo E., Diurno M.V., Mazzoni O. (1987). Studies on hetrocyclic compounds indole 2, 3 diones derivatives VIII: Structural modification and antimicrobial activity of indole 2, 3 diones derivative. *Boll. Soc. Ital. Biol. Sper*. 63, 883.

36. Punnotok J., Fuangtong P., Wongsangiem M. (1985). Primary drug resistance in newly diagnosed untreated tuberculosis in Central Chest Hospital. *Thia. J. Tuberc. Chest Dis*. 2, 69.

37. Ravighone M.C., Saider D.E., Kochi A. (1995). Global epidemiology of tuberculosis morbidity and mortality of world-wide epidemic. *JAMA*. 273, 220.

38. Report on Tuberculosis Survey. (1962). Directorate of Tuberculosis control. P.22.
39. Sack J. (1911). Indole in flower perfume. *Pharm. Weekblad*, 48, 775.
40. Sack J. (1911). Some communication on plant chemistry. *Pharm, Weekblad*, 48, 307.
41. Sadler R.W., Dixon G.J., Frank M. (1968). In- vivo antiviral properties of biologically active compounds II: Studies with influenza and vaccinia viruses. *J. Appl. Microbiology*, 16, 370.
42. Saklani S., Chandra S. (2011). Antimicrobial activity nutritional profile and quantitative study of different fractions of *Ficus palmata*, *International Research Journal of Plant Science*, 2(11), 332-337.
43. Saklani S., Chandra S. (2014). In vitro Antimicrobial activity, Nutritional value. Antinutritional value and phytochemical screening of *Pyracantha crenulata* fruit. *International Journal of Pharmaceutical Sciences Review and Research*, Vol-26 (1), 1-5.
44. Saklani S., Chandra S., Mishra A.P., Badoni P.P. (2012). Nutritional Evaluation, Antimicrobial activity and Phytochemical Screening of Wild edible fruit of *Myrica nagi* pulp, *International Journal of Pharmacy & Pharmaceutical Sciences*, 4 (3), 407-411.
45. Siddiqi S.H. (1981). Evaluation of Repid Radiometric Method for drug susceptibility testing of Mycobacterium tuberculosis. *J. Clin. Microbiol.* 13, 908.
46. Siddiqi S.H. (1985). Studies to further enhance mycobacterial growth in radiometric 71112 Medium. *Abstract U-46, A.S.M Meeting, Las Vegas*, NV.
47. Siddiqi S.H., Ahmad B. (1977). Detection of AFB position by direct and concentration Method. *Pakistan J. Med. Res*. 16. 58.
48. Siddiqui S.H. (1978). Comparison of Lowenstein Jensen and 711-10 Middlebrookmeida for primary isolation of tubercle bacilli from clinical specimens. *Pakistani J. Med. Res*. 17, 19.
49. Siddiqui S.H., Khan F.A (1980). Bacteriological Aspects of Renal Tuberculosis in the Punjab Arca. *J.P.M.A.* June.
50. Sumpler W.C., Miller F.M. (1954). Heterocyleic compounds with indole and carbazole systems. Interscience *Publisher INC; New Yark*. P-2.
51. Supcharoen S., Nuehprayoon C., Prijyanonda B. (1984). A study of correlation between primary drug resistance and result of treatment in patients with pulmonary tuberculosis. *Thia. J. Tuberc. Chest Dis*. 4, 167.
52. Syed M., Ilanif M., Chaudhary F.M. (1986). Antimicrobial activity of the essential oils of the umbelliferae family part 1: *Cuminum Cyminum, Coriandum sativum, foeniculum vulgare* and *Bunium persicum* oil. *Pakistan. J. Sci Ind. Res*. 29, 183.
53. Verma R. S., Nables W. L. (1967). Substituted N-Aminoethylisatins. *J. Med. Chem*. 10, 510.
54. WHO. (1995). Reported on the tuberculosis epidemic. Stop T.B at the source. *Genera. WHIO/TB/95*. 183 and 94. 184.
55. WHO. (1996). Reported on the tuberculosis epidermis, T.B groups risk. WHO March.
56. Zam A., Saleshicb M.F., Rahamathullah A. (1998). Synthesis and pharmacological activity of Mannich bases using indole 2,3 dione/3-phenylimino-2-indolione as amine moiety. *J. Inst. Chem. India*. 68, 114.