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## Synthesis and Antimicrobial Evaluation of Novel Isatin Derivatives.

Manju Pal\*, Sangh Partap, Neeraj Kant Sharma and KK Jha.

Department of Pharmaceutical Chemistry, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, 244001

### ABSTRACT

The proposed research program is continuation of the research work, which has been carried out on the synthesis and biological studies of some isatin (indole 2,3-diones). In this connection fifty-seven derivatives were prepared. The substitution was made at position such as 1 and 3. The isatin derivatives (MP V<sub>A-F</sub>) were prepared by the substituted of different aldehydes by reacting the mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one with the presence of with glacial acetic acid. Isatin derivatives with acid anhydride were prepared and isatin derivatives were also reacted with isatin having 85% yield. All these new compounds have been screened for their antimicrobial activity against test organism.

**Keyword:** isatin, Antimicrobial Activity, p-chloro aniline and hydrazine hydrate.

*\*Correspondence author*

## INTRODUCTION

Isatin chemically 1H-indole-2,3-dione, was first obtained by Erdman and Laurent in 1841 [1] as a product from the oxidation of indigo by nitric and chromic acids. In nature, isatin is found in plants of the genus *Isatis*, in *Calanthe discolor* LINDL and in *Couroupita guianensis*. [2]. Isatin and its derivatives possess a broad range of biological and pharmacological properties and are widely used as starting materials for the synthesis of a broad range of heterocyclic compounds and as substrates for drug synthesis [3]. Many methodologies have been adopted for the synthesis of isatin derivatives and to explore its possible role in treatment of various diseases. Among these the method developed by Sandmeyer [4] is the oldest and the most frequently used for the synthesis of isatin. It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid produce isatin.

Isatin nucleus having both the keto and lactam moiety has aroused tremendous curiosity due to its diverse biological and pharmacological studies. It is widely distributed in mammalian tissues and body fluids. [5]. Isatin has also drawn great attention from being discovered as a component of endogenous monoamine oxidase (MAO) inhibitory activity (tribulin) and subsequently identified as a selective inhibitor of MAO B at low concentrations [6]. A large number of human and animal diseases exist in both developed and developing countries [7] and a number of synthetic isatin derivatives were found to have variety of pharmacological properties including anticonvulsant [8], antibacterial, antifungal [9], antitubercular [10], anti-inflammatory [11], analgesic [12], antiviral and anti-HIV activities [13]. Some of the isatin derivatives possess promising antimicrobial activity like a series of chloroisatin-3-semicarbazone and hydrazones which has been designed for antitubercular activity against *Mycobacterium tuberculosis* [14] In view of the immense pharmacological activities exhibited by the molecules it was considered worthwhile to synthesize novel isatin derivatives and to explore their pharmaceutical importance in terms of antimicrobial activity for its use in various microbes generated ailments.

## MATERIALS AND METHODS

Melting points were measured using Kshitij Innovations apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: n-hexane (1:2) solvent system and iodine vapours as a visualizing agent. IR spectra were recorded using KBr pellets on a Shimadzu Affinity-1 FTIR spectrophotometer. <sup>1</sup>H-NMR spectra were recorded on a Bruker Advance 400 spectrometer at 400.13 MHz using CDCl<sub>3</sub> solvent and TMS as internal standard. Chemical shifts were expressed in δppm unit.

### Synthesis of (3Z)-3-[(4-chlorophenyl)imino]-1,3-dihydro-2H-indol-2-one (I)

Equimolar mixture of isatin and p-chloro aniline (0.01 mol) was dissolved in warm ethanol and water (3:1) and was then refluxed on a steam bath for 6 hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as chloroform and ethylacetate (3:1) as solvent system. The mixture was then kept for 24hrs

at room temperature. The product were separated by filtration and recrystallized from warm ethanol [15].

#### **Synthesis of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II):**

A mixture of (3Z)-3-[(4-chlorophenyl)imino]-1,3-dihydro-2H-indol-2-one (I) (0.05 mol) and hydrazine hydrate (3ml) in dioxane (30ml) was refluxed for 2hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (2:1) as solvent system. The separated solid was filtered off and recrystallized from dioxane to give as yellow crystals [16].

#### **Synthesis of (3Z)-3-[2-(4-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino) phenyl)hydrazinylidene]-1,3-dihydro-2H-indol-2-one (III):**

Equimolar quantities (0.01 mol) of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) and isatin were dissolved in warm ethanol and water (3:1) and was then refluxed on a steam bath for 6 hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as chloroform and ethylacetate (3:1) as solvent system. After standing for 24hrs at room temperature, the product was separated by filtration and recrystallized from warm ethanol [16].

#### **Synthesis of (3Z)-3-[(3-methyl-1H-imidazol-5-yl)imino]-1,3-dihydro-2H-indol-2-one (IV):**

A mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) (0.01mol) and acetic anhydride (2ml) was refluxed for 6 hr. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (1:1) as solvent system. After cooling the mixture was poured into ice-water and extracted with ether and the solvent was evaporated. The obtained solid was recrystallized from ethanol [17].

#### **Synthesis of (3Z) substituted phenyl-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (V<sub>A-F</sub>):**

A mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) (0.01mol) and substituted aromatic benzaldehyde (0.01mol) in 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr. The reaction mixture was cooled. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (1:1) as solvent system. The solid obtained was filtered off and purified by recrystallization from ethanol [39].

#### **Chemistry**

IR, <sup>1</sup>H-NMR and elemental analysis were consistent with the assigned structure.  
**(3Z)-3-[2-(4-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene] amino) phenyl)hydrazinylidene]-1,3-dihydro-2H-indol-2-one (III):** Yield: 85% ; Melting Point: 185 °C; IR(KBr, cm<sup>-1</sup>) – 3362, 3358, 3324 (N-H str); 3093, 3065, 3022, 2969, 2921, 2897, 2757, 2687(C-H str); 1736 (C=O str); 1628 (C=N str); 1612 (C=C str); 1383 (C-N str); 747, 749 (C-H bend); <sup>1</sup>H-NMR (DMSO,

$\delta$ ppm) – 7.543 (1H, S, Ar NH); 7.515- 6.442 (12H, m, Ar H); 6.418 (1H, S, Ali NH ); Anal. Calcd. for  $C_{22}H_{15}O_2N_5$ : C, 69.28; H, 3.96; N, 18.36; O, 8.39% **found**; C, 68.75; H, 3.45; N, 18.8; O, 9% Mol. Wt.: 381.

**Physical characteristics of (3Z)-3-[(3-methyl-1*H*-imidazol-5-yl)imino]-1,3-dihydro-2*H*-indol-2-one (IV):**

Yield: 54% ; Melting Point: 205 °C; IR(KBr,  $cm^{-1}$ ) – 3386, 3246 (N-H str); 1617 (C-N str); 1339 (C-N str), 1599 (C=C str); 1617 (C=N str); 2822, 3096, 3032, 2822 (C-H str); 1736 (C=O str), 1145 749 (C-H Bend);  $^1H$ -NMR (DMSO,  $\delta$ ppm) – 7.524 (1H, S, Ar NH); 11.021 (1H, S, Ar NH); 7.497- 6.867 (7H, m, Ar H); Anal. Calculated for  $C_{16}H_{13}ON_4$ - C, 69.55; H, 4.38; N, 20.28; O, 5.79% **found** C, 69; H, 4.05; N, 21.03; O, 5.92% ; Mol.Wt.: 254.

**Physical characteristics of (3Z)-3-({4-[(2*E*)-2-benzylidenehydrazinyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one (V<sub>A</sub>):**

Yield: 62% ; Melting Point: 165 °C; IR(KBr,  $cm^{-1}$ ) – 3386, 3277 (N-H str); 3092, 3062, 3023, 2890, 2961, 2819 (C-H str); 1728 (C=O str); 1585 (C=C str); 1613 (C=N str); 1342 (C-N str);  $^1H$ -NMR (DMSO,  $\delta$ ppm)– 8.107 (1H, S, Ar NH); 7.985 (1H, S, CH); 4.255 (1H, S, NH); 7.390 – 6.418 (13H, m, Ar H); Anal. Calculated for  $C_{21}H_{16}ON_4$  – C, 74.10; H, 4.974; N, 16.46; O, 4.70% **found** C, 74; H, 4.96; N, 16.12; O, 4.92%; Mol.Wt.: 340.

**Physical characteristics of (3Z)-3-({4-[(2*E*)-2-(2 chlorobenzylidene)hydrazinyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one (V<sub>B</sub>):**

Yield: 75% ; Melting Point: 170 °C; IR(KBr,  $cm^{-1}$ ) – 3389, 3357 (N-H str); 1731 (C=O str); 789 (C-Cl str); 1614 (C=N str); 2969, 2890, 2818, 2745, 3027, 3062, 3062 (C-H str); 1591 (C=C str), 1086 (C-Cl), 1340 (C-N str);  $^1H$ -NMR (DMSO,  $\delta$ ppm) – 8.625 (1H, S, Ar NH); 3.351 (1H, S, Ali NH); 2.670 (2H S, NH<sub>2</sub>), 3.327 (1H, S, Ar CH-Cl); 7.998 – 6.900 (12H, m, Ar H); Anal. Calculated for  $C_{21}H_{15}ON_4Cl$  – C, 66.79; H, 5.43; Cl, 9.96; N, 14.86; O, 2.96% **found** C, 66.79; H, 5.43; Cl, 9.96; N, 14.86; O, 2.96%; Mol.Wt.: 374.

**Physical characteristics of (3Z)-3-({4-[(2*E*)-2-(2-methoxybenzylidene)hydrazinyl]phenyl}imino)-1,3-dihydro-2*H*-indol-2-one (V<sub>C</sub>):**

Yield: 67% ; Melting Point: 160 °C; IR(KBr,  $cm^{-1}$ ) – 3421, 3393 (N-H str); 1724 (C=O str); 3098, 2948, 2886, 2805, 2673 (C-H str); 1269 (C-O-C str); 1591 (C=C str); 1616 (C=N str); 747 (C-H bend), 1329 (C-N str), 747 (C-H bend);  $^1H$ -NMR (DMSO,  $\delta$ ppm)– 7.210 (1H, S, Ar NH); 4.079 (1H, S, Ali NH), 2.275(1H, S, NH<sub>2</sub>); 3.462 (3H, S, OCH<sub>3</sub>), 7.18 – 6.789 (12H, m, Ar H); Anal. Calculated for  $C_{23}H_{18}O_2N_4$  – C, 71.34; H, 4.90; N, 15.13; O, 8.64% **found** C, 71.02; H, 4.96; N, 15.45; O, 8.57% ; Mol.Wt.: 382.

**Physical characteristics of (3Z)-3-({4-[(2*E*)-2-(2,4-dihydroxybenzylidene)hydrazinyl] phenyl}imino)-1,3-dihydro-2*H*-indol-2-one (V<sub>D</sub>):**

Yield: 33.8% ; Melting Point: 190 °C; IR(KBr,  $cm^{-1}$ ) – 3357 (O-H str); 3267 (N-H str); 3028, 3089, 2984, 2814, 2867 (C-H str); 1619 (C=C str); 1222, 1293 (C-O str); 730 (C-H bend),

1348 (C-O-H str), 1736 (1736), 1597 (C=N str); <sup>1</sup>H-NMR (DMSO, δppm)– 5.971, 5.499 (1H, S, Ar OH); 4.356 (1H, S, Ali NH); 7.077 (1H, S, Ar NH); 7.058 (1H, S, Ali CH); 7.039 – 6.450 (11H, m, Ar CH); Anal. Calculated for C<sub>21</sub>H<sub>16</sub>O<sub>3</sub>N<sub>4</sub> – C, 67.73; H, 4.33; N, 15.05; O, 12.89% **found** C, 67.76; H, 4.69; N, 15.05; O, 12.5%; Mol.Wt: 372.

**Physical characteristics of (3Z)-3-{{4-[(2E)-2-(3-methylbenzylidene)hydrazinyl] phenyl} imino}-1,3-dihydro-2H-indol-2-one (V<sub>E</sub>):**

Yield: 61% ; Melting Point: 145 °C; IR(KBr, cm<sup>-1</sup>)– 3362, 3358 (N-H str); 1738 (C=O str); 1628 (C=N str); 1592 (C=C str); 3065, 3022, 2969, 2921, 2837, 2897, 2757, 2687 (C-H str); 1334 (C-N str); 791 (C-H bend); <sup>1</sup>H-NMR (DMSO, δppm)– 7.987 (1H, S Ar NH); 3.568 (1H, Ali NH); 2.735 (2H, S, NH<sub>2</sub>); 2.275 (3H, S, Ali CH<sub>3</sub>); 9.474 (1H, S, CH); 7.654-6.870 (12H, m, Ar H); Anal. Calculated for C<sub>22</sub>H<sub>18</sub>ON<sub>4</sub> – C, 74.03; H, 5.52; N, 18.29; O, 4.17% **found** C, 74.03; H, 5.92; N, 15.79; O, 4.26%; Mol.Wt.: 354.

**Physical characteristics of (3Z)-3-[(4-{{(2E)-2-[4-(dimethylamino)benzylidene] hydrazinyl} phenyl)imino]-1,3-dihydro-2H-indol-2-one (V<sub>F</sub>):**

Yield: 72% ; Melting Point: 168 °C; IR(KBr, cm<sup>-1</sup>)– 3357 (N-H str)1684 (C=O str), 1654 (C=N str); 1588 (C=C str); 1250 (C-N-H str); 1364 (C-N str), 3357 (N-H str), 1684 (C=O str), 3197, 3179, 3156, 3150, 2674, 2974 (C-H str), 1269 (C-N str); <sup>1</sup>H-NMR (DMSO, δppm)– 4.079 (1H, S, Ali NH); 7.210 (1H, S, Ar NH); 3.462, 3.357 (6H, D, CH<sub>3</sub>); 7.186-6.789 (12H, m, Ar H); Anal. Calculated for C<sub>23</sub>H<sub>21</sub>ON<sub>4</sub> – C, 72.04; H, 5.52; N, 18.26; O, 4.17% **found** C, 72.73; H, 5.36; N, 18.42; O, 3.49%; Mol.Wt.: 383.

**Biological Screening**

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100µg/ml involving four bacterial strains *Bacillus subtilis* & *Staphylococcus aureus* (gram +ve) and *Escherichia Coli* & *Klebsiella Pneumoniae* (Gram -ve) using Ampicillin as standard at the same concentration. The work, in reference, was carried out by Agar disc diffusion method. The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

**Preparation of Nutrient Broth Medium:**

To prepare Nutrient Broth Medium, 10gm of beef extract, 10gm of peptone and 5 mg of sodium chloride were suspended in 1000 ml of purified water. It was mixed properly and heated up to boiling to dissolve the medium completely. The pH was adjusted (8.0 - 8.4) with 5M sodium hydroxide solution and boiled for 10 minutes. The mixture was filtered & sterilized by maintaining at 115°C for 30 minutes and the pH was adjusted to 7.3 ± 0.1.

**Preparation of Nutrient Agar Medium**

To prepare Nutrient Agar Medium, 5gm of beef extract, 5gm of Peptone, 10 gm of agar and 2.5 mg of sodium Chloride were suspended in 500 ml of purified water. It was mixed properly and heated upto boiling to dissolve the ingredients completely. The mixture

was filtered, sterilised by maintaining at 115° for 30 minutes and the pH was adjust to  $7.3 \pm 0.1$ . It was then cooled and poured into sterilized petridish. All the plates were kept for 4-5 hours in laminar airflow until the media got solidified. The plates were then kept in an incubator at 37°C.

#### **Preparation of solutions:**

A solution (100µg/ml) of standard drug (Ampicillin) was prepared by dissolving 10 mg of Ampicillin into 100 ml of sterile water.

#### **Preparation of test solution:**

10 mg of the synthesized compound(s) was dissolved in 100 ml of DMSO to prepare the test sample of 100 µg/ml concentration. A 24 hour old culture was used for the preparation of bacterial suspension. Likewise suspensions of all the organisms were prepared as per standard procedure.

#### **Preparation of discs:**

Discs of 6-7 mm in diameter were punched from No. 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Standard and test solutions were added separately to these discs which were air dried later on.

#### **Diffusion Test**

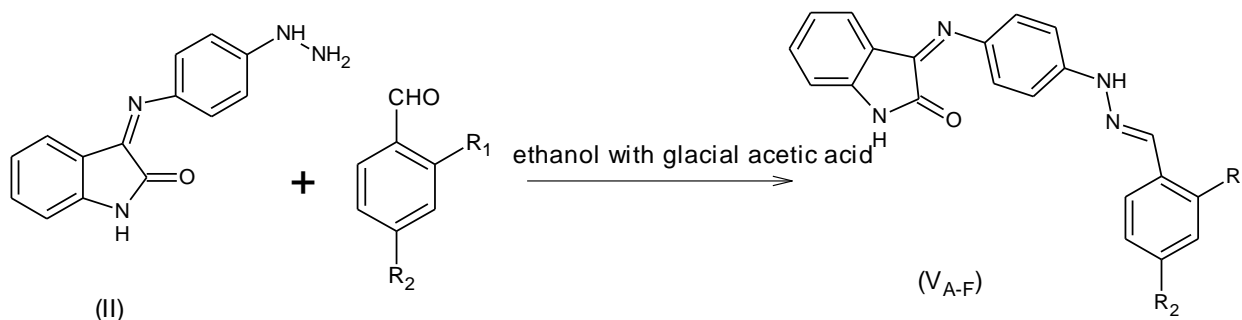
A filter-paper disk impregnated with the compound to be tested was placed on the surface of the agar carefully by using sterilized forceps. The compound diffused from the filter paper into the agar. The larger the clear area around the filter disk, the more effective was regarded the compound.

These petridishes were kept upto one hour for diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters. The size of the zone of inhibition was measured as a determination of compound's effectiveness.

### **RESULTS AND DISCUSION**

A mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) (0.01mol) and substituted aromatic benzaldehyde (0.01mol) in 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr. Substantial proof for the formation of  $V_A$ - $V_F$  has been provided by differences in melting points and yield value from that of parent compound. Compound  $V_A$  on reaction with various substituted aromatic benzaldehyde yielded which on 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr yielded novel isatin derivatives ( $V_A$ - $V_F$ ). The primary structural difference within this series involves the nature of various substituted aldehydes. Synthesized compounds were found to be crystalline in nature and easily soluble in methanol, DMF,

DMSO and DMF but insoluble in chloroform, ethyl acetate, benzene hexane and toluene. With the help of analytical techniques such as melting point, IR and <sup>1</sup>H-NMR, synthesized derivatives were characterized. All the compounds showed IR and NMR signals for different kinds of protons at their respective positions.



**Table 1: Antibacterial Activity of synthesized isatin derivatives (V<sub>A</sub>-V<sub>F</sub>)**

Sample	Zone of inhibition in mm			
	<i>B. Subtilis</i>	<i>S. Aureus</i>	<i>E. Coli</i>	<i>K. Pneumoniae</i>
Ampicilin	24	18	20	22
MP V <sub>a</sub>	13	11	12	10
MP V <sub>b</sub>	15	12	14	15
MP V <sub>c</sub>	14	10	10	11
MP V <sub>d</sub>	10	8	9	10
MP V <sub>e</sub>	16	12	12	14
MP V <sub>f</sub>	13	9	12	13

All compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group and ester linkage made the compounds to exhibit moderate to significant activity in comparison to standard drug Ampicillin. Compound MPV<sub>b</sub> and MPV<sub>e</sub> exhibited promising antibacterial activity however other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compound MPV<sub>b</sub>, MPV<sub>c</sub>, MPV<sub>d</sub>, MPV<sub>e</sub> and MPV<sub>f</sub> can be recommended for further studies. The above results established the fact that aromatic benzaldehyde substituted with various isatin (substituted) can be studied further to explore out newer antimicrobial compounds.

## CONCLUSION

The analytical and other informational data, available in literature so far, have rendered thiophene significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections etc. since last two decades immensely hiked interests of medicinal chemist and biochemist.

This particular research study, in reference, would extend great deal of help to researchers in reckoning and determining the best and most productive, economical, suggestive and conclusive access to various thiophenes of clinical importance superseding other compounds of their class.

Further combinatorial libraries of these compounds can be generated which can be

screened for optimal pharmacological activities by optimization techniques using 2D and 3D QSAR investigation.

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