

## SYNTHESIS AND PHARMACOLOGICAL SCREENING OF NEW ISATIN-3- [N<sup>2</sup>-(BENZIMIDAZOL-1-ACETYL)]HYDRAZONE

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**ABSTRACT.** Twenty new isatin-3-[N<sup>2</sup>-(benzimidazol-1-acetyl)]hydrazones (**IV**) were synthesized from ten different isatin-3-[N<sup>2</sup>-(chloroacetyl)] hydrazones (**III**) by reacting with benzimidazole and 2-methyl benzimidazole. The intermediates were obtained from isatin hydrazones (**II**) on condensation with chloroacetyl chloride. These compounds were characterized by IR, <sup>1</sup>H NMR and mass spectra. All the compounds were screened for antimicrobial, antioxidant and cytotoxic activity. Some of the new compounds showed promising antibacterial and antifungal activity.

**KEY WORDS:** Antibacterial activity, Antifungal activity, Antioxidant activity, Cytotoxic activity, Benzimidazole, Isatin

### INTRODUCTION

In recent years, several benzimidazole derivatives have been synthesized and reported to possess varied biological and pharmacological properties. Benzimidazole nucleus is one of the bioactive heterocyclic compounds that exhibit a range of biological activities. The pharmacological activities of the benzimidazole containing moiety have been well documented. It is reported that benzimidazole derivatives were active against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [1]. Anthelmintic activities of few synthesized benzimidazole derivatives were also reported [2]. Further, in-vitro antiprotozoal activity of synthesized N-azopridinium salts and several betaines were reported adding to the existing pool of biological effects. Few other observations can be made from the available literature, that the benzimidazole molecule posses different biological activities such as antihistaminic activity [4], antitumor activity [5], cytotoxic activity [6], antitubercular activity [7], antifungal [8], antiviral [9] and anti-inflammatory [10] activities.

An interesting observation one could make from a careful survey of the literature is the absence of any report on isatin derivatives containing benzimidazole system in the side chain at 3<sup>rd</sup> position. So, it has been felt worthwhile to undertake the present work of synthesizing such compounds for the first time by appropriate synthetic routes, with a view to evaluate for antibacterial, antioxidant and anticancer activities. The required isatins (**I**) and their hydrazones (**II**) were prepared by the standard methods available in literature. Isatin hydrazones on treatment with chloroacetylchloride afforded their respective isatin-3-[N<sup>2</sup>-(chloro acetyl)] hydrazones (**III**). Each of these compounds were reacted with benzimidazole and 2-methyl benzimidazole in presence of dry acetone with an anhydrous potassium carbonate to get the respective isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazones (**IV**). The intermediates and the title compounds were purified by recrystallization and characterized by analytical and spectral (IR, NMR and mass) data.

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## EXPERIMENTAL

### *Materials and instruments*

All the chemicals were of synthetic grade and commercially procured from Sigma Aldrich, Mumbai, India. The melting points were determined by open capillary method and were uncorrected. Infrared spectra were recorded on FTIR (Bruker Alpha-E) by KBr disc method. <sup>1</sup>H NMR spectra were recorded at 400MHz in CDCl<sub>3</sub> as solvent and TMS as an internal standard using Bruker Advance 400 instrument. In addition, mass spectra were recorded on PEP-SCIUX-APIQ pulsar mass spectrophotometer. Elemental analysis was performed on Perkin-Elmer EAL240 elemental analyzer.

### *Synthesis of compounds*

*Synthesis of isatin hydrazones (II).* An appropriate isatin (indole-2,3-dione) (**I**, 0.01 mol) was dissolved in alcohol (20 mL) and added hydrazine hydrate (99%, 0.015 mol) while shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 hours. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and purified by recrystallization from chloroform. The compounds thus obtained were characterized by comparison with their physical constants reported in the literature.

*Synthesis of isatin-3-[N<sup>2</sup>-(chloroacetyl)] hydrazones (III).* An appropriate isatin hydrazone (**II**, 0.01 mol) was heated under reflux with chloroacetyl chloride (0.01 mol) in dry benzene under anhydrous conditions using calcium chloride guard-tube for 2 hours. The product thus formed was filtered and washed with small portions of benzene to remove the un-reacted chloroacetyl chloride. It was purified by recrystallization from suitable solvent(s).

*Synthesis of benzimidazole.* O-phenylenediamine of weight 27 g (0.25 mol) is placed in a 250 mL round bottomed flask and added 17.5 g (16 mL, 0.34 mol) of formic acid (90%). The reaction mixture was refluxed on a water bath for 2 hours, cooled, then added 10 percent sodium hydroxide solution slowly, with constant rotation of the flask, until the mixture was just alkaline to litmus. Later, filter off the crude benzimidazole at the pump, washed with ice-cold water thoroughly. It was completely dried and purified by recrystallization from boiling water. The yield of pure benzimidazole, m.p. 171 °C (lit. 172 °C), is 25 g (85%). Adopting this procedure, 2-methylbenzimidazole was also prepared by the reaction of O-phenylenediamine and acetic acid. It was recrystallized from 10% aqueous ethanol, the yield is 2.2 g (56%), m.p. 176 °C (lit. 176 °C).

*Synthesis of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (IV).* A mixture of an appropriate isatin-3-[N<sup>2</sup>-(chloroacetyl)]hydrazone (**III**, 0.01 mol) and a benzimidazole (0.01 mol) or 2-methyl benzimidazole (0.01 mol) in dry acetone (20 mL) and anhydrous potassium carbonate, heated under reflux on a water bath for 5 h. The solvent was evaporated and poured in crushed ice. The product thus formed was filtered, washed with cold water and dried. The compound was purified by recrystallization from suitable solvent(s).

### *Biological evaluation*

All the compounds were screened for antibacterial, antifungal, antioxidant and cytotoxic activity by following the standard protocols as available in the literature.

**Antibacterial activity.** The antibacterial activity of synthesized compounds was conducted against two gram positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria viz., *Escherichia coli* and *Proteus vulgaris* by using cup plate method. Ampicillin sodium was employed as standard to compare the results. Both the test and standard compounds were studied at 10 µg/mL/cup.

**Antifungal activity.** All those compounds screened for antibacterial activity were also tested for their antifungal activity by using potato-dextrose-agar medium against clotrimazole as standard. Both the test and standard compounds were studied at the same dose of 10 µg/mL. The fungi employed for screening were: *Aspergillus niger* and *Cunninghamella verticillata*.

**Antioxidant activity.** The model of scavenging the stable DPPH (1,1-diphenyl-2-picryl-hydrazil) radical is a widely used method to evaluate antioxidant activities in a relatively shorter time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be owing to their hydrogen donating ability. The reduction in absorbance is calculated as percentage inhibition as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

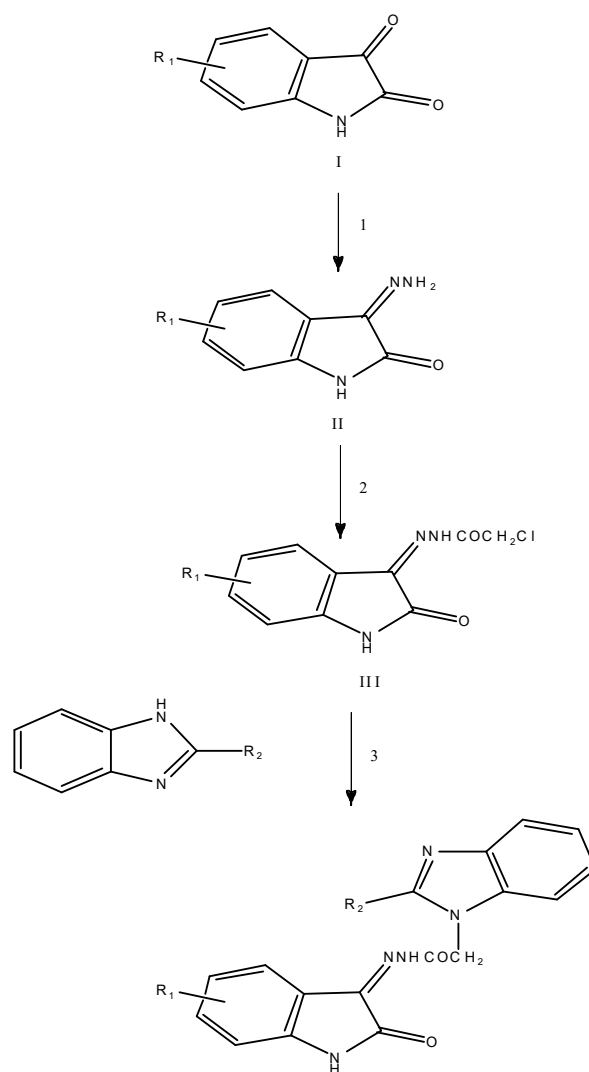
**Cytotoxic activity.** Cytotoxic activity was performed on against HBL-100 cell lines and HeLa cell lines using Microculture tetrazolium assay (MTT) method. It is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells.

The reaction sequence used in the synthesis of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methyl benzimidazol-1-acetyl)]hydrazone (**IV**) is depicted in the Scheme 1. The required isatin-3-[N<sup>2</sup>-(chloroacetyl)] hydrazones (**III**) have been prepared by a reaction of respective isatin hydrazones with chloroacetyl chloride. The isatin hydrazones (**II**) on the other hand, have been obtained by the reaction of respective isatins (**I**) with hydrazine hydrate (99%). These compounds (**XVI**) have been purified by recrystallization from suitable solvent(s) and characterized by their analytical and spectral data.

The IR spectrum of the isatin-3-[N<sup>2</sup>-(chloroacetyl)]hydrazone (**III**, R<sub>1</sub> = H) exhibited the absorption frequencies (in cm<sup>-1</sup>) at: 3238 (NH), 1700 (C=O, lactam), 1666 (C=O, acid hydrazide), 1624 (C=C, aromatic), 1533 (C=N), 951 (C=C, aromatic). Each of the isatin-3-[N<sup>2</sup>-(chloroacetyl)]hydrazones (**XVI**) has been subjected to a nucleophilic substitution reaction with benzimidazole and 2-methyl benzimidazole in dry acetone and anhydrous potassium carbonate to get their respective isatin-3[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (**IV**); similarly twenty compounds were prepared. The resultant products have been purified by recrystallization from suitable solvents and characterized by their physical data (Table 1) and spectral (IR, NMR and mass) data as follows:

**Iva.** Yield 85%, mp 262 °C; IR(KBr) cm<sup>-1</sup>: 3422(-NH str), 1696 (C=Ostr, lactam), 1625 (C=O, acid hydrazine), 1502 (C=Nstr); <sup>1</sup>HNMR (δppm): 12.71 (s, 1H, lactam), 11.35 (s, 1H, NHCO), 8.33 (s, 1H, -N-CH-N), 6.97-8.27 (m, 8H, Ar-H), 5.82 (s, 2H, COCH<sub>2</sub>); EI-MS: 319(M<sup>+</sup>).

**Ivb.** Yield 80%, mp 241 °C; IR (KBr) cm<sup>-1</sup>: 3422 (-NH str), 1696 (C=Ostr, lactam), 1625 (C=O, acid hydrazine), 1502 (C=Nstr); <sup>1</sup>HNMR (δppm): 12.69 (s, 1H, lactam), 11.21 (s, 1H, NHCO), 8.22 (s, 1H, -N-CH-N), 6.80-8.17 (m, 8H, Ar-H), 5.71 (s, 2H, COCH<sub>2</sub>); EI-MS: 319 (M<sup>+</sup>).



Scheme 1. Synthesis of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-cetyl)]hydrazone (IV), IVa-t R<sub>1</sub> = H, 5-CH<sub>3</sub>, 5-Cl, 5-Br, 5-NO<sub>2</sub>, 5-SO<sub>2</sub>NO<sub>2</sub>, 5-COOH, 5-COOCH<sub>3</sub>, 6-Br, 7-CH<sub>3</sub>; R<sub>2</sub> = H, CH<sub>3</sub>. Reagents and conditions: 1) isatin (I, 0.01 mol), ethanol, hydrazine hydrate (99%, 0.015 mol), Δ10 min, 2) chloroacetyl chloride (0.01 mol), dry benzene, reflux 2h and 3) benzimidazole or 2-methyl benzimidazole (0.01mol), dry acetone, anhydrous potassium carbonate reflux 5 h.

**IVc.** Yield 78%, mp 212 °C; IR (KBr) cm<sup>-1</sup>: 3410 (-NH str), 1690 (C=Ostr, lactam), 1610 (C=O, acid hydrazine), 1480 (C=Nstr); <sup>1</sup>HNMR (δppm): 12.69 (s, 1H, lactam), 11.21 (s, 1H, NHCO), 8.22 (s, 1H, -N-CH-N), 6.80-8.17 (m, 8H, Ar-H), 5.71 (s, 2H, COCH<sub>2</sub>); EI-MS: 353 (M<sup>+</sup>).

**IVd.** Yield 64%, mp 279 °C; IR (KBr) cm<sup>-1</sup>: 3435 (-NH str), 1710 (C=Ostr, lactam), 1640 (C=O, acid hydrazine), 1515 (C=Nstr); <sup>1</sup>HNMR (δppm): 12.85 (s, 1H, lactam), 11.40 (s, 1H, NHCO), 8.50 (s, 1H, -N-CH-N), 6.98-8.30 (m, 8H, Ar-H), 5.90 (s, 2H, COCH<sub>2</sub>); EI-MS: 398 (M<sup>+</sup>).

**IVe.** Yield 88%, mp 219 °C; IR (KBr) cm<sup>-1</sup>: 3405 (-NH str), 1680 (C=Ostr, lactam), 1605 (C=O, acid hydrazine), 1475 (C=Nstr); <sup>1</sup>HNMR (δppm): 12.65 (s, 1H, lactam), 11.15 (s, 1H, NHCO), 8.10 (s, 1H, -N-CH-N), 6.75-8.05 (m, 8H, Ar-H), 5.62 (s, 2H, COCH<sub>2</sub>); EI-MS: 364 (M<sup>+</sup>).

#### Biological activity

**Antibacterial activity.** The antibacterial activity data of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methyl benzimidazol-1-acetyl)] hydrazones (**IV**, Table 2) indicates that the compounds have a noticeable degree of inhibition, specifically against gram-positive strain, i.e. *B. subtilis*. Most significant of them has been found to be the compound **IVa** showed greater inhibitory effect against the organisms employed, particularly against *B. subtilis* and *S. aureus* with the zones of inhibition of 22 and 20 mm, respectively, which has been comparable to that of the standard employed at the concentration of 10 µg/mL. This has been closely followed by compound **IVd** with a 5-bromo substituent in indolinone which showed significant inhibitory effect specifically against *B. subtilis* and *E. coli* with zones of inhibition of 18 mm, each. Some of the compounds showed moderate antibacterial activity against both the gram-negative organisms *E. coli* and *P. vulgaris*. The compounds with 5-carboxylic acid and 5-carbomethoxy substituents on indolinone have not exhibited any activity against gram-negative organisms. Recently, Guo *et al.* [11] has excellently reviewed antibacterial potential of isatin derivatives. Intriguingly, azo linked substituted benzimidazole, benzoxazole, and benzothiazole synthesized by Mishra *et al.* [12] through diazo coupling also exhibited in vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia Coli* strains. Mannich bases of benzimidazole derivatives have also shown in vitro antibacterial activity against *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa* organisms [13].

**Antifungal activity.** The antifungal activity data of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazones (**IV**) as depicted in Table 2 shows that the compounds of this series have shown antifungal action against *A. niger* and *C. verticulata* except compounds **IVh**, **IVq** and **IVr**, of course, with a degree of variation in their action. Compounds **IVc** (R<sub>1</sub> = 5-Cl, R<sub>2</sub> = H) and **IVm** (R<sub>1</sub> = 5-Cl, R<sub>2</sub> = CH<sub>3</sub>) have more activity against *C. verticulata* with the zones of inhibition of 17 mm and 16 mm respectively among all the test compounds. The data shows that this series of compounds have been found to be comparatively more effective among all the series tested for antifungal activity. In an interesting work reported by Jarrahpour *et al.* [14], twelve new bis-Schiff bases of isatin, benzyisatin and 5-fluoroisatin 3a-3l were prepared by condensation of isatin, benzyisatin and 5-fluoroisatin with primary aromatic amines. Though these compounds exhibited antiviral and anticancer activity, the candidates failed to show antifungal activity when tested against *S. cerevisiae* (ATCC 28383) or *C. albicans* (CIP 1180-79). However, Kaplancıklı *et al.* [15] reported few benzimidazole-thiazole derivatives with proven anticandidal activity. Several bisbenzimidazole compounds exhibited moderate to excellent antifungal activities against fungal strains, with MIC values ranging from 15.6 to 0.975 µg/mL and the antifungal activity were found to depend on alkyl chain length [16].

**Antioxidant activity.** The  $IC_{50}$  values of antioxidant activity of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone derivatives (**IV**) are in the range of 10.45 to 17.56  $\mu$ M as shown in Table 3. The compound **IVa** ( $R_1 = R_2 = H$ ) has shown highest percentage of free radical scavenging activity at a concentration of 10.45  $\mu$ M among these compounds. This has been followed by the compounds **IVc**, **IVd** and **IVn** with an  $IC_{50}$  of 10.52, 10.61 and 10.98  $\mu$ M, respectively. In an attempt to synthesize gastro-sparing antiinflammatory agents, Sharma *et al.* [17] has reported antioxidant activity of 5-methanesulphonamido benzimidazole derivatives. Other benzimidazole derivatives has also shown remarkable in vitro antioxidant activity [18-20].

**Cytotoxic activity.**  $IC_{50}$  values of cytotoxic activity of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (**IV**) are given the Table 3. They are ranging from 345.59 to 473.9  $\mu$ M. The compound **IVm** ( $R_1 = 5-Br$ ,  $R_2 = CH_3$ ) has shown highest cytotoxic activity among the series at a concentration of 345.59  $\mu$ M against HeLa cell lines. This has been followed by the compound **IVd** ( $R_1 = 5-Br$ ,  $R_2 = H$ ) with  $IC_{50}$  of 347.69  $\mu$ M against HBL-100 cell lines. The compounds **IVe**, **IVg**, **IVh**, **IVp**, **IVq**, **IVr** and **IVt** have not shown any activity against HBL-100 cell lines and HeLa cell lines. Recent literature also revealed potential cytotoxic activity of several benzimidazole derivatives [21, 22]

Table 1. Physical data of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (**IV**).

S. No.	Compd.	R <sub>1</sub>	R <sub>2</sub>	Mol. Formula	m.p. (°C)	Yield (%)
1	<b>IV a</b>	H	H	C <sub>17</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	262	85
2	<b>IV b</b>	5-CH <sub>3</sub>	H	C <sub>18</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	241	80
3	<b>IV c</b>	5-Cl	H	C <sub>17</sub> H <sub>12</sub> N <sub>5</sub> O <sub>2</sub> Cl	212	78
4	<b>IV d</b>	5-Br	H	C <sub>17</sub> H <sub>12</sub> N <sub>5</sub> O <sub>2</sub> Br	279	64
5	<b>IV e</b>	5-NO <sub>2</sub>	H	C <sub>17</sub> H <sub>12</sub> N <sub>6</sub> O <sub>4</sub>	210	88
6	<b>IV f</b>	5-SO <sub>2</sub> NH <sub>2</sub>	H	C <sub>17</sub> H <sub>14</sub> N <sub>6</sub> O <sub>4</sub> S	272	70
7	<b>IV g</b>	5-COOH	H	C <sub>18</sub> H <sub>13</sub> N <sub>5</sub> O <sub>4</sub>	222	60
8	<b>IV h</b>	5-COOCH <sub>3</sub>	H	C <sub>19</sub> H <sub>15</sub> N <sub>5</sub> O <sub>4</sub>	229	65
9	<b>IV i</b>	6-Br	H	C <sub>17</sub> H <sub>12</sub> N <sub>5</sub> O <sub>2</sub> Br	248	84
10	<b>IV j</b>	7-CH <sub>3</sub>	H	C <sub>18</sub> H <sub>15</sub> N <sub>5</sub> O <sub>2</sub>	238	82
11	<b>IV k</b>	H	CH <sub>3</sub>	C <sub>18</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	259	80
12	<b>IV l</b>	5-CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>17</sub> N <sub>5</sub> O <sub>2</sub>	245	82
13	<b>IV m</b>	5-Cl	CH <sub>3</sub>	C <sub>18</sub> H <sub>14</sub> N <sub>5</sub> O <sub>2</sub> Cl	240	75
14	<b>IV n</b>	5-Br	CH <sub>3</sub>	C <sub>18</sub> H <sub>14</sub> N <sub>5</sub> O <sub>2</sub> Br	265	65
15	<b>IV o</b>	5-NO <sub>2</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>14</sub> N <sub>6</sub> O <sub>4</sub>	205	75
16	<b>IV p</b>	5-SO <sub>2</sub> NH <sub>2</sub>	CH <sub>3</sub>	C <sub>18</sub> H <sub>16</sub> N <sub>6</sub> O <sub>4</sub> S	276	72
17	<b>IV q</b>	5-COOH	CH <sub>3</sub>	C <sub>19</sub> H <sub>15</sub> N <sub>5</sub> O <sub>4</sub>	210	65
18	<b>IV r</b>	5-COOCH <sub>3</sub>	CH <sub>3</sub>	C <sub>20</sub> H <sub>17</sub> N <sub>5</sub> O <sub>4</sub>	232	68
19	<b>IV s</b>	6-Br	CH <sub>3</sub>	C <sub>18</sub> H <sub>14</sub> N <sub>5</sub> O <sub>2</sub> Br	228	75
20	<b>IV t</b>	7-CH <sub>3</sub>	CH <sub>3</sub>	C <sub>19</sub> H <sub>17</sub> N <sub>5</sub> O <sub>2</sub>	252	78

Table 2. Antibacterial activity and antifungal of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (IV).

S. No.	Compd.	R <sub>1</sub>	R <sub>2</sub>	Zone of inhibition (in mm)					
				Bacterial species				Fungal species	
				<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>A. niger</i>	<i>C. verticillata</i>
1	IV a	H	H	22	20	18	17	15	14
2	IV b	5-CH <sub>3</sub>	H	15	13	12	11	12	13
3	IV c	5-Cl	H	16	15	14	12	13	17
4	IV d	5-Br	H	18	17	18	16	10	14
5	IV e	5-NO <sub>2</sub>	H	13	11	--	--	8	10
6	IV f	5-SO <sub>2</sub> NH <sub>2</sub>	H	13	12	11	--	10	12
7	IV g	5-COOH	H	11	--	--	--	8	6
8	IV h	5-COOCH <sub>3</sub>	H	13	--	--	--	--	--
9	IV i	6-Br	H	17	16	15	13	9	12
10	IV j	7-CH <sub>3</sub>	H	16	15	--	--	10	8
11	IV k	H	CH <sub>3</sub>	18	17	16	15	14	12
12	IV l	5-CH <sub>3</sub>	CH <sub>3</sub>	16	15	--	--	11	12
13	IV m	5-Cl	CH <sub>3</sub>	16	15	14	13	14	16
14	IV n	5-Br	CH <sub>3</sub>	18	16	17	15	12	13
15	IV o	5-NO <sub>2</sub>	CH <sub>3</sub>	13	12	14	13	10	7
16	IV p	5-SO <sub>2</sub> NH <sub>2</sub>	CH <sub>3</sub>	15	16	--	--	11	7
17	IV q	5-COOH	CH <sub>3</sub>	12	--	--	--	--	--
18	IV r	5-COOCH <sub>3</sub>	CH <sub>3</sub>	14	12	--	--	--	--
19	IV s	6-Br	CH <sub>3</sub>	17	16	16	14	10	11
20	IV t	7-CH <sub>3</sub>	CH <sub>3</sub>	18	15	16	15	11	7
21	Ampicillin (10 µg/cup)			22	20	18	17	--	--
22	Clotrimazole (10 µg/cup)			--	--	--	--	19	20

Table 3. Antioxidant activity and cytotoxic activity of isatin-3-[N<sup>2</sup>-(benzimidazol/2-methylbenzimidazol-1-acetyl)]hydrazone (IV).

S. No.	Compd.	R <sub>1</sub>	R <sub>2</sub>	Antioxidant activity	Cytotoxic activity	
				IC <sub>50</sub> value (µM)	HBL-100 cell lines IC <sub>50</sub> values (µM)	HeLa cell lines IC <sub>50</sub> values (µM)
1	IV a	H	H	10.45	433.13	411.84
2	IV b	5-CH <sub>3</sub>	H	15.07	441.86	NA
3	IV c	5-Cl	H	10.52	365.28	349.84
4	IV d	5-Br	H	10.61	347.69	325.46
5	IV e	5-NO <sub>2</sub>	H	13.39	NA	NA
6	IV f	5-SO <sub>2</sub> NH <sub>2</sub>	H	15.6	NA	NA
7	IV g	5-COOH	H	16.61	NA	NA
8	IV h	5-COOCH <sub>3</sub>	H	17.56	NA	NA
9	IV i	6-Br	H	11.43	367.15	373.99
10	IV j	7-CH <sub>3</sub>	H	14.91	473.9	NA
11	IV k	H	CH <sub>3</sub>	11.63	454.48	463.36
12	IV l	5-CH <sub>3</sub>	CH <sub>3</sub>	14.12	NA	NA
13	IV m	5-Cl	CH <sub>3</sub>	11.39	375.59	345.59
14	IV n	5-Br	CH <sub>3</sub>	10.98	357.79	355.17
15	IV o	5-NO <sub>2</sub>	CH <sub>3</sub>	14.37	401.58	NA
16	IV p	5-SO <sub>2</sub> NH <sub>2</sub>	CH <sub>3</sub>	15.13	NA	NA
17	IV q	5-COOH	CH <sub>3</sub>	16.92	NA	NA
18	IV r	5-COOCH <sub>3</sub>	CH <sub>3</sub>	17.43	NA	NA
19	IV s	6-Br	CH <sub>3</sub>	13.67	391.56	365.49
20	IV t	7-CH <sub>3</sub>	CH <sub>3</sub>	15.05	NA	NA
21	Ascorbic acid			5.87	--	--
22	Cisplatin			--	25.00	25.00

### CONCLUSION

The synthesized novel isatin-3-[N<sup>2</sup>-(benzimidazol-1-acetyl)] hydrazones were found to possess remarkable biological activities. The compounds showed antibacterial activity against *B. subtilis*, *S. aureus*, *E. Coli* and *P. vulgaris* and antifungal activity against *A. Niger* and *C. verticillata*. The results were promising for antioxidant activity in free radical scavenging assay. The compounds possessed cytotoxic activity also against HeLa cell lines.

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