



# A FACILE GREEN SYNTHESIS PROCESS OF 3-SUBSTITUTED-3-HYDROXYINDOLIN-2-ONES AND THEIR *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

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Potassium carbonate catalyzed synthesis of 3-substituted-3-hydroxyindolin-2-ones (A-E) by treating substituted indole-2,3-diones with substituted aryl ketones in aqueous medium under sonication with an object to develop potent antioxidant, antibacterial and antifungal agents of synthetic origin. It is revealed from the antioxidant screening results that the **Compound A** (3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) and **Compound E** (5-Methyl-3-hydroxy-3-[2-oxo-2-(4-fluoro-phenyl)ethyl]indolin-2-one) manifested profound DPPH<sup>•</sup>, ABTS<sup>•+</sup> and NO radical scavenging activity. **Compound C** elicited the potent inhibitory action against all the bacterial pathogens. **Compound A** (3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) and **Compound C** (5-Methyl-3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) have showed equivalent activity comparable to standard drug Ampicillin against *Pseudomonas aurigonosa*. The advantages of this green method utilizing potassium carbonate as an inexpensive, safe, and efficient basic catalyst are high efficiency, mild reaction conditions, convenient operation and environmentally benign conditions.

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Owing to various disorders caused by free radicals and increased microbial resistance, new classes of antioxidant and antimicrobial agents with novel mechanisms are today's need to fight against various disorders caused by free radicals and multi drug-resistant infections.

The indole nucleus, a common and important structural functionality of a variety of both natural and unnatural products, is probably the most well-known heterocycles. Isatins are versatile substrates because they can be used for the synthesis of a large variety of heterocyclic compounds as raw materials for drug synthesis. Oxindole derivatives are known to possess a variety of biological activities.<sup>3</sup> In particular, the 3-substituted-3-hydroxyindolin-2-ones, a class of compounds bearing the indole skeletal structure, are found in several biologically active alkaloids and pharmacological agents (Figure 1; Table 1).<sup>4</sup>

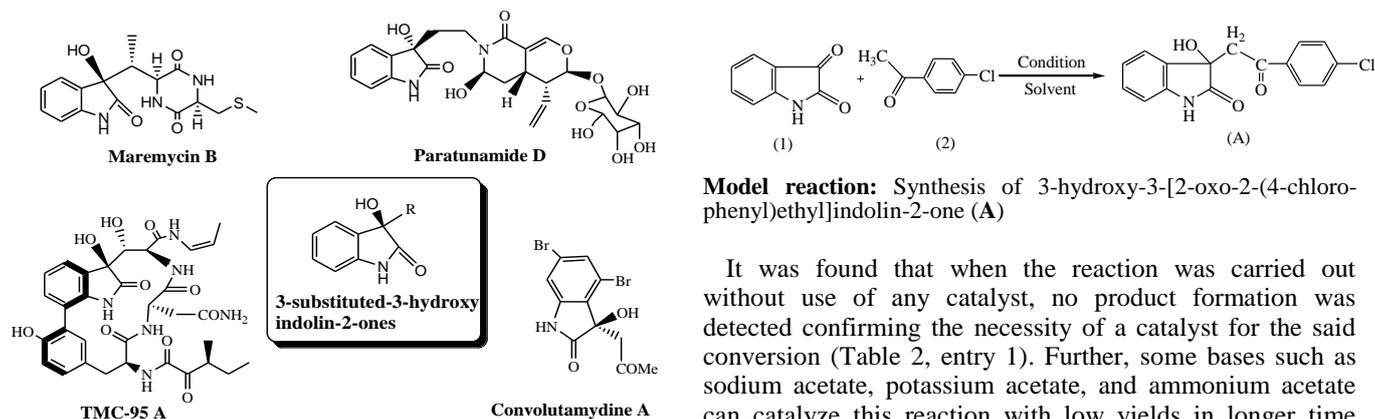
In context of this program, we have previously reported the synthesis of hydroxy derivatives and conversion of these into biodynamic heterocycles,<sup>5</sup> numerous methodologies have been developed and continue to be explored for the construction of this structure.<sup>6</sup> However, some of these methods suffer from certain drawbacks such as hazardous organic solvents, high cost, long reaction time, less selectivity and excess amount of base, unsatisfactory yields, cumbersome product isolation procedures, and environmental pollution. Therefore, there is still need for versatile, simple, and environmentally friendly processes for the synthesis of 3-substituted-3-hydroxyindolin-2-ones derivatives. Use of potassium carbonate as catalyst has inherent advantages including operational simplicity, low cost, and suitability in industrial applications.<sup>7</sup> An increasing number of examples are available in the literature where potassium carbonate alone has been used as a catalyst during organic transformations.<sup>8</sup>

## INTRODUCTION

After the discovery of the involvement of free radicals in oxidative tissue injury and diseases, the rapid development began in the area of free radical biology and medicine. Free radicals are produced as a result of normal biochemical reactions in the body. The human body uses an inherent antioxidant system to neutralize the excessive level of free radicals. In general, the cell is able to maintain an appropriate balance between oxidants and antioxidants under normal conditions. The imbalance between production of free radicals and the available antioxidant defence leads to a widely accepted phenomenon called oxidative stress, which is responsible for cellular and metabolic injury and accelerating aging, cancer, myocardial infarction, arthritis, cardiovascular diseases, neurodegenerative diseases and inflammation.<sup>1</sup> Around the world, life annoying contagious diseases caused by multi-drug-resistant pathogenic bacteria (Gram-positive/Gram-negative) increased at an alarming level. The presence and growth of pathogenic microorganisms (bacteria, mould, viruses, fungi) in food may cause its spoilage and results in a reduction in its quality and quantity.<sup>2</sup> This microbial contamination still poses important public health and economic concerns for human society.

**Table 1.** List of Compound Names, Bioactivity, Mode of Action

Entry	Natural Product	Target and mode of action	IC <sup>50</sup>
1	Maremycin A-D <sup>4a</sup>	Cytotoxicity Mouse fibroblasts L-929 Human Leukemia K562	50 µg mL <sup>-1</sup>
2	Paratunamide A-D <sup>4b</sup>	Cytotoxicity human epidermoid carcinoma KB cells	6 µg mL <sup>-1</sup>
3	Spiroepoxyindoles <sup>4c</sup>	Anti-fungal, anti-tubercular anti-cancer	-
4	TMC-95 A-D <sup>4d</sup>	Reversible (non-covalent) inhibitors of 20S proteasome	5.4 nM
5	Convolutamydine A-E <sup>4e</sup>	Inhibit differentiation of promyelocytic leukemia cells HL-60	12.5-25 µg mL <sup>-1</sup>
6	Spirotetrahydrofuryloxindole <sup>4f</sup>	Anti-cancer	16-32 µM
7	Spiroisoxazolidinyloxindole <sup>4g</sup>	anti-cancer, anti-tubercular	2-4 µM, 15-30 %

**Figure 1.** Examples of biologically active 3-substituted-3-hydroxyindolin-2-ones

An important technique that is widely used today in organic synthesis and has a profound impact on the way chemist approach organic and parallel synthesis, is "Ultrasonication", based on cavitation effects leading to mass transfer improvement. The benefits of this technology are reduction in reaction times, improved yields and suppression of side products, relative to traditional thermal heating.<sup>9</sup> Numerous examples under this condition for constructing the heterocycles with interesting properties have been reported in the literature.<sup>10</sup>

Considering the above points and in view of our quest for developing green protocols for heterocyclic frameworks,<sup>11</sup> herein, we report a greener, simple and efficient method for the synthesis of 3-substituted-3-hydroxyindolin-2-ones derivatives in water under ultrasonic irradiation by K<sub>2</sub>CO<sub>3</sub> and their evaluation for antioxidant and antimicrobial potential.

However, to the best of our knowledge there is no report available in the literature describing the antioxidant potential of 3-substituted-3-hydroxyindolin-2-ones compounds, but compound A have been reported for its synthetic origin<sup>6a</sup> and also for anticonvulsant activity<sup>6e</sup> while compound B,<sup>6a</sup> and compound E<sup>5c</sup> have been reported for their synthetic origin.

## RESULTS AND DISCUSSION

In order to study the present reaction in water with contemporary technique as ultrasound irradiation, we carried out the reaction of isatin **1** and 4-chloro acetophenone **2** as a model substrate in various catalysts and results are shown in Table 2.

**Model reaction:** Synthesis of 3-hydroxy-3-[2-oxo-2-(4-chlorophenyl)ethyl]indolin-2-one (**A**)

It was found that when the reaction was carried out without use of any catalyst, no product formation was detected confirming the necessity of a catalyst for the said conversion (Table 2, entry 1). Further, some bases such as sodium acetate, potassium acetate, and ammonium acetate can catalyze this reaction with low yields in longer time duration (Table 2, entry 2-4). Afterwards, evaluation of alkali metal carbonates such as sodium carbonate, potassium carbonate and cesium carbonate as catalyst was tried out as these catalysts find extensive use in synthetic organic chemistry.<sup>12</sup> Therefore, the synthesis of 3-hydroxy-3-[2-oxo-2-(4-Chloro-phenyl) ethyl]indolin-2-one (**A**) was carried out in aqueous medium under ultrasonic irradiation using these catalysts (Table 2, entry 5, 7 and 9). It was noticeable that a mixture of isatin **1** and 4-chloro acetophenone **2** in the presence of catalytic amount of potassium carbonate afforded (**A**) in satisfactory yields (69%) while with sodium carbonate and cesium carbonate, the product was formed in 60% and 55% yields respectively.

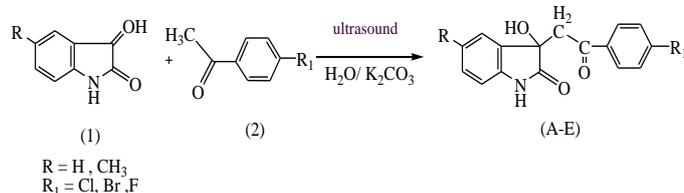
Further, optimization of the catalyst loading was done by using different concentration of potassium carbonate in the model reaction. It was found that increasing the amount of potassium carbonate from 10 to 20 and 25 mol%, the yields increased from 69% to 78% and 80%, respectively (Table 2, entries 9-11). Further increase in amount of catalyst does not seem to affect the overall yields of the product. Using 20 mol% of potassium carbonate in water under sonication is sufficient to push this reaction forward. More amount of the additive did not substantially improve the yield (entry 10 was considered to be better as almost same yield was obtained in case of entry 11 with longer reaction time).

In order to verify the effect of ultrasound irradiation on this reaction, the model reaction was also carried out in the absence of ultrasound under conventional manners both in stirring and refluxing conditions using water as a solvent (Table 2, entry 12, 13). As shown in Table 2, under refluxing conditions with high speed stirring, no reaction was observed at same time but a mixture of products was obtained after a prolonged reaction time. Thus, ultrasonic irradiation was found to have beneficial effect on the synthesis of 3-hydroxy-3-substituted indolin-2-one (**A-E**) derivatives which was superior to the traditional method with respect to yield, reaction time, particularly while considering the basic green chemistry concept.

Under the optimized reaction condition, we have synthesized 3-substituted-3-hydroxyindolin-2-ones by reaction of various aryl ketones and substituted isatins (Table 3) in good yields for screening them for antioxidant and antimicrobial potential. Compounds **A-E** are stable solids whose structures were established by IR,  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and mass spectroscopy and elemental analysis.

**Table 2** Optimization of reaction condition for model reaction generating **A**

No.	Catalyst	Condition	Time	Yield, %
1	no catalyst	ultrasound	8 h	Traces
2	AcONa	ultrasound	5 h	30
3	AcOK	ultrasound	4 h	45
4	AcONH <sub>4</sub>	ultrasound	6 h	40
5	Na <sub>2</sub> CO <sub>3</sub>	ultrasound	2 h	55
6	Na <sub>2</sub> CO <sub>3</sub>	rt, stirring	4 h	35
7	Cs <sub>2</sub> CO <sub>3</sub>	ultrasound	1 h	60
8	Cs <sub>2</sub> CO <sub>3</sub>	rt, stirring	3 h	42
9	K <sub>2</sub> CO <sub>3</sub>	ultrasound	38 min	69
	10 mol %			
10	K <sub>2</sub> CO <sub>3</sub>	ultrasound	35 min	78
	20 mol %			
11	K <sub>2</sub> CO <sub>3</sub>	ultrasound	40 min	80
	25 mol %			
12	K <sub>2</sub> CO <sub>3</sub>	rt, stirring	1 h	mixture of products
	20 mol %			
13	K <sub>2</sub> CO <sub>3</sub>	refluxing	1 h	mixture of products
	(20 mol %)			



**Scheme 1** Pathway for the synthesis of 3-substituted 3-hydroxyindolin-2-ones (**A-E**)

**Table 3** Synthesis of 3-substituted 3-hydroxyindolin-2-ones (**A-E**) catalyzed by K<sub>2</sub>CO<sub>3</sub>

Entry	R	R <sup>1</sup>	Time, min	M.p., °C	Yield, %
A	H	Cl	35	194	78 %
B	H	Br	37	192	79 %
C	Me	Cl	39	195	74 %
D	Me	Br	40	191	76 %
E	Me	F	36	177	70 %

### Antioxidant Activity

The antioxidant activities of oxindoles were determined as an index of pharmacological usefulness. Three model systems were used namely DPPH<sup>•</sup>, ABTS<sup>•+</sup> and NO scavenging activity. In the assessment of antioxidant activity, only synthetic relevant free radicals were used. The synthetic nitrogen-centered DPPH<sup>•</sup>, ABTS<sup>•+</sup> and NO radicals were used as indicator compounds in testing hydrogen transfer capacity that are related to the antioxidant activity. The antioxidant properties were expressed as EC<sub>50</sub> values. EC<sub>50</sub> is defined as the concentration of substrate that scavenges 50 percent free radicals. A lower value of EC<sub>50</sub>

indicates the greater antioxidant activity of a test substance. In results, we have found correlation between substitution in indole ring at 5<sup>th</sup> position and substitution of phenacyl ring at 4<sup>th</sup> position. Overall, all compounds have good DPPH<sup>•</sup> and NO scavenging activity whereas, all compounds have showed least ABTS<sup>•+</sup> scavenging activity.

### DPPH radical scavenging activity

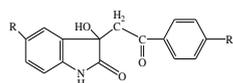
Although the DPPH radical scavenging abilities of all the spiroindoline derivatives were significantly lower than those of ascorbic acid (409.75±0.288) µg ml<sup>-1</sup>, but the Compound **A** (3-hydroxy-3-[2-oxo-2-(4-chlorophenyl)ethyl]indolin-2-one) and Compound **E** (5-methyl-3-hydroxy-3-[2-oxo-2-(4-fluorophenyl)ethyl]indolin-2-one) have highest DPPH radical scavenging activity as compared to other compounds. All compounds have -OH and -NH group in their structure, the difference in their structure is the -H, -CH<sub>3</sub> (electron donating group) in indole ring at 5<sup>th</sup> position and Cl, Br and F (electron withdrawing group) in phenacyl ring at 4<sup>th</sup> position. The exact reason of activity of synthesized compounds is yet not clear, but it was anticipated that compound **A** possessing H at 5<sup>th</sup> position of indole ring and Cl substitution at 4<sup>th</sup> position of phenacyl ring showed good activity compared to all compounds. Compound **E** and Compound **B** incorporating -CH<sub>3</sub> and H at 5<sup>th</sup> position of indole ring respectively with F and Br substitution at 4<sup>th</sup> position of phenacyl ring showed moderate activity, while compound **C** and compound **D** showed least activity, both compound have -CH<sub>3</sub> group, an electron donating group and moderate electron withdrawing group Cl, Br substitution at 4<sup>th</sup> position of phenacyl ring showed least activity compared to all compounds. (The results are shown in Table 4 and Figure 2).

### ABTS radical scavenging activity

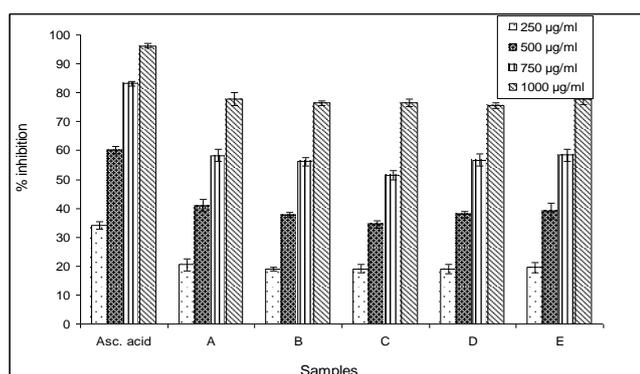
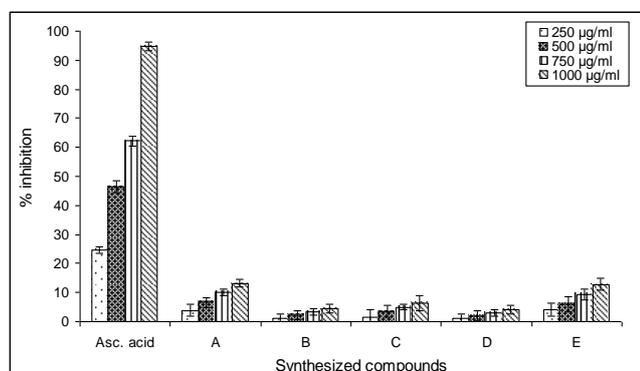
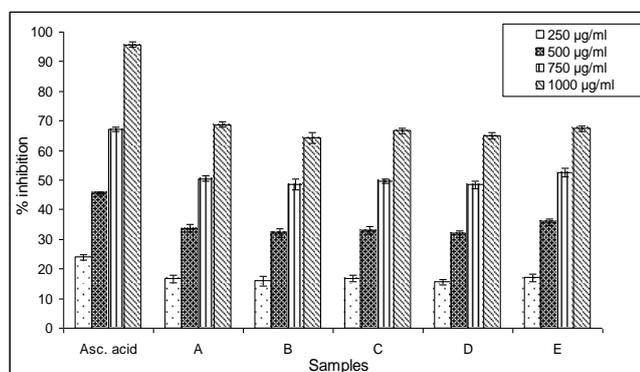
Among the tested compounds in ABTS assay all compounds showed least activity, but when compared the activity results of all compounds, Compound **A** possessing H at 5<sup>th</sup> position of indole ring and Cl substitution at 4<sup>th</sup> position of phenacyl ring showed good activity compared to all other compounds. Compound **E** has H at 5<sup>th</sup> position of indole ring and Cl substitution at 4<sup>th</sup> position of phenacyl ring showed good activity. Compounds **B**, **C**, **D** with -H, and -CH<sub>3</sub>, electron donating groups, in indole ring and p-Cl, p-Br, moderate electron withdrawing groups, in phenacyl ring showed least activity. (The results are shown in Table 4 and Figure 3).

### Nitric Oxide scavenging activity

Among the tested compounds, in NO assay, compound **E** (5-methyl-3-hydroxy-3-[2-oxo-2-(4-fluorophenyl)ethyl]indolin-2-one) showed good activity. Combination of without any substitution in indole ring with p-Cl and p-Br substitution in phenacyl ring i.e. compound **A** and compound **B** showed moderate activity. The incorporation of CH<sub>3</sub> group at 5<sup>th</sup> position in indole ring with p-Cl and p-Br substitution in phenacyl ring gives least activity. (The results are shown in Table 4 and Figure 4).

**Table 4** EC<sub>50</sub> values of synthesized compounds.

Compound	R	R <sub>1</sub>	EC <sub>50</sub> (μg ml <sup>-1</sup> )		
			DPPH	ABTS	NO
A	H	Cl	640.06±2.158	4117.08±3.856	732.30±1.237
B	H	Br	651.31±0.961	12463.75±1.462	734.30±1.641
C	CH <sub>3</sub>	Cl	675.45±1.593	8294.16±2.427	760.22±0.982
D	CH <sub>3</sub>	Br	665.86±1.688	12478.75±1.694	777.92±1.158
E	CH <sub>3</sub>	F	649.48±2.146	4492.72±1.245	726.61±1.238
Ascorbic acid	-	-	409.75±1.384	550.00±1.136	544.21±0.832

**Figure 2.** DPPH<sup>•</sup> scavenging activity (% inhibition) of synthesized compounds**Figure 3.** ABTS<sup>•+</sup> scavenging activity (% inhibition) of synthesized compounds**Figure 4** NO scavenging activity (% inhibition) of synthesized compounds

### Antimicrobial activity

The synthesized compounds A-E were evaluated for their *in vitro* antibacterial activity against four bacterial strains *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *in vitro* antifungal activity against four fungal strains *Aspergillus niger*, *Alternaria flavus*, *Rhizopus stonifer* and *Alternaria alternaria* by agar well diffusion method, using Ampicillin and Clotrimazole as standard for antibacterial and antifungal activities, respectively.

### Antibacterial activity for 3-substituted-3-hydroxyindolin-2-ones derivatives

Antibacterial activity of the synthesized five compounds against human bacterial pathogens as determined by agar well diffusion method with Ampicillin as reference control was investigated. Antimicrobial results revealed that the maximum antibacterial activity was observed for compound C against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compound A showed good antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Compound B and Compound D exhibited moderate activity against all tested antibacterial pathogens. Compound E did not show any activity against *Escherichia coli*. Compounds C, D, and E have not any antibacterial activity against *Klebsiella pneumoniae*. The structure activity relationship demonstrates that 3-substituted-3-hydroxyindolin-2-ones derivatives A (R=H, R<sub>1</sub>=Cl) and C (R=CH<sub>3</sub>, R<sub>1</sub>=Cl) showed good to moderate activity. From overall antibacterial activity results (Table 5), it was observed that compound C was the effective inhibitors against all the bacterial pathogens accept *Klebsiella pneumoniae* and has shown equivalent activity comparable to standard drug Ampicillin against *Pseudomonas aurigosa* but the activity of compound A was at par in case of *Pseudomonas aurigosa*.

### Antifungal activity for 3-substituted-3-hydroxyindolin-2-ones derivatives

The results of antifungal activity of synthesized compounds A-E revealed that only compound B showed moderate activity against *Alternaria flavus* and *Alternaria alternaria*. When the antifungal activity observed for all the compounds against *Aspergillus niger* Compound B does not show any resistance against this strain and compounds A, C,

**Table 5.** Effect of 3-hydroxy-3-phenacyl oxindoles derivatives (A-E) on the growth of some bacterial strains and fungicides

Compound	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)			
	<i>E. coli</i>	<i>P.aurigonosa</i>	<i>S. aureus</i>	<i>K.pneumonia</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>R. stonifer</i>	<i>A.alternaria</i>
A (AI)	19.33±1.69 (0.698)	19.00±1.00 (0.926)	19.83±1.11 (0.743)	10.23±0.31 (0.406)	10±0.99 (0.357)	NA	NA	10.44±0.07 (0.413)
B (AI)	19.91±0.87 (0.719)	13.21±1.69 (0.644)	16.28±0.73 (0.610)	18.45±0.33 (0.33)	NA	18.00±0.98 (0.666)	NA	18.23±0.22 (0.722)
C (AI)	12.00±0.77 (0.433)	20±0.33 (0.975)	21.08±0.67 (0.790)	NA	11.66±0.51 (0.416)	14.78±0.67 (0.547)	NA	11.22±31 (0.444)
D (AI)	18.66±0.87 (0.674)	17.55±0.91 (0.856)	14.22±0.35 (0.533)	NA	13.31±0.71 (0.698)	12.21±0.55 (0.452)	10.08±1.09 (0.555)	12.45±0.64 (0.493)
E (AI)	NA	12±0.45 (0.585)	15.00±1.00 (0.562)	NA	8.88±0.32 (0.317)	11.21±0.31 (0.415)	NA	7.77±0.29 (0.307)
Ampicillin	27.66±0.38	20.50±0.70	26.66±0.94	25.16±0.23	-	-	-	-
Clotrimazole	-	-	-	-	28.66±0.00	27.00±0.43	18.16±0.70	25.23±0.83

Mean± SE, NA= No activity, AI= activity index,

**D**, and **E** showed least activity against this strain when compared to reference strain i.e. Clotrimazole. When all the synthesized compounds were tested against *A. flavus*, compound **A** did not show any resistance against this fungal pathogen and compound **B** showed moderate activity while compounds **C**, **D**, and **E** showed least activity, while only compound **D** showed least resistance against *Rhizopus stonifer* while other compounds **A**, **B**, **C**, **E** did not show any resistance. With *Alternaria alternaria*, only compound **B** showed moderate activity while compounds **A**, **C**, **D**, **E** showed least resistance. The results of antifungal activity are shown in Table 5.

From the results of antimicrobial activities, it is revealed that the majority of the synthesized compounds having –CH<sub>3</sub>, an electron donating group in the indole ring and p-Cl, an withdrawing group substitution exhibited the maximum growth inhibitory activity. However, none of the compounds exhibited zone of inhibition more than that of the standards.

## EXPERIMENTAL SECTION

The melting points of synthesized compounds were determined in open capillary tubes using Toshniwal apparatus. The purity of compounds was checked on thin layers of silica gelG-coated glass plates with benzene ethylacetate (7:3) as eluent using iodine vapors as visualizing agents. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> + DMSO-d<sub>6</sub> using tetramethylsilane (TMS) as an internal standard on a Bruker spectrophotometer at 400 and 100 MHz respectively. Mass spectrum of representative compound was recorded on a Jeol SX-102 spectrometer at 70eV. Infrared (IR) spectra were recorded on a Shimadzu Fourier transform (FT)–IR 8400S spectrophotometer using KBr pellets.

Sonication was carried out with the help of a standard ultrasonic irradiation instrument SonaprosPR-1000 MP (Oscar Ultrasonics Pvt. Ltd.) operating at 750W and generating 23-KHz output frequency. It has the following characteristics: Standard Titanium horn with a diameter of 6

mm/12mm, replaceable flat stain less steel tip, and digital thermometer to determine temperature. The glass reactor was designed and made from borosil glass.

### General procedure for the synthesis of 3-hydroxy-3-substituted phenacyl indolin-2-one derivatives (A-E)

An equimolar mixture of isatin (2mmol, .294gm), p-Cl acetophenone (2mmol, .309gm) and K<sub>2</sub>CO<sub>3</sub> (20mol%, .055 gm) in 5ml water was introduced in a 20-mL, heavy-walled, pear shaped, two necked flask with non standard taper outer joint. The flask was attached to a 12mm tip diameter probe, and the reaction mixture was sonicated at ambient temperature for the specified period at 50% power of the processor and 230W output in a 4-s pulse mode. At the end of the reaction period, thin-layer chromatography (TLC) was checked, and the flask was detached from the probe. The contents were transferred to a beaker. The formed solid was filtered off, washed thoroughly with warm water (2×20ml), and then dried to obtain crude products which were purified by crystallization from ethanol to give 3-hydroxy-3-substituted phenacyl indolin-2-one derivatives, giving satisfactory spectral and elemental analysis.

**Synthesis of A<sup>6a</sup>.** Yellow crystalline solid; Yield: 78%; IR (KBr,  $\nu$  cm<sup>-1</sup>): 1640 (CONH), 1760 (COCH<sub>2</sub>), 3200 (NH), 3350 (OH). <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO-d<sub>6</sub>)  $\delta$ : 3.56 (d, *J* = 17.4 Hz, 1H), 4.06 (d, *J* = 17.4 Hz, 1H), 6.10 (brs, 1H), 6.79–7.91 (m, 8H, ArH), 10.30 (brs, 1H, NH); <sup>13</sup>C NMR(CDCl<sub>3</sub>+DMSO-d<sub>6</sub>)  $\delta$ : 44.2 (COCH<sub>2</sub>), 71.6 (Spiro C), 119.7, 121.9, 127.1, 128.0, 129.8, 133.2, 137.2, 141.2 (Aromatic C), 176.9 (CONH), 193.6 (COCH<sub>2</sub>). Anal. Calcd for C<sub>16</sub>H<sub>12</sub>ClNO<sub>3</sub>: C, 63.69; H, 4.01; N, 4.64. Found: C, 63.67; H, 4.07; N, 4.68; Mass (m/z): 301 (M<sup>+</sup>).

**Synthesis of B<sup>6a</sup>.** Yellow crystalline solid; Yield: 79%; IR (KBr,  $\nu$  cm<sup>-1</sup>): 1620 (CONH), 1720 (COCH<sub>2</sub>), 3200 (NH), 3380 (OH). <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO-d<sub>6</sub>)  $\delta$ : 3.55 (d, *J* = 17.4, 1H), 4.05 (d, *J* = 17.4 Hz, 1H), 6.09 (brs, 1H), 6.63–7.60 (m, 8H, ArH), 10.61 (brs, 1H, NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO-d<sub>6</sub>)  $\delta$ /ppm: 45.1 (COCH<sub>2</sub>), 73.0(Spiro C), 109.4, 121.2, 123.7, 127.6, 128.9, 129.9, 131.6, 131.8, 135.2, 142.9 (Aromatic C), 178.2 (CONH), 195.8 (COCH<sub>2</sub>).

Anal. Calcd for C<sub>16</sub>H<sub>12</sub>BrNO<sub>3</sub>: C, 55.51; H, 3.49; N, 4.05. Found: C, 55.57; H, 3.52; N, 4.08; MS (m/z): 345 (M<sup>+</sup>).

**Synthesis of C.** Yellow crystalline solid; Yield: 74%; IR (KBr,  $\nu$  cm<sup>-1</sup>): 1650 (CONH), 1720 (COCH<sub>2</sub>), 3200 (NH), 3340 (OH); <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 2.12 (s, 3H, -CH<sub>3</sub>), 3.54 (d, *J* = 17.4 Hz, 1H), 3.99 (d, *J* = 17.4 Hz, 1H), 6.05 (s, 1H, -OH), 6.67-7.82 (m, 7H, -Ar-H), 10.23 (s, 1H, -NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 21.9 (C-CH<sub>3</sub>), 46.2 (COCH<sub>2</sub>), 72.6 (Spiro C), 120.7, 121.9, 127.1, 128.3, 129.0, 129.8, 133.2, 137.2, 141.2 (Aromatic C), 176.7 (CONH), 193.8 (COCH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>14</sub>ClNO<sub>3</sub>: C, 64.70; H, 4.50; N, 4.48. Found: C, 64.67; H, 4.47; N, 4.44; Mass (m/z): 315 (M<sup>+</sup>).

**Synthesis of D.** Yellow crystalline solid; Yield: 76%; IR (KBr,  $\nu$  cm<sup>-1</sup>): 1640 (CONH), 1700 (COCH<sub>2</sub>), 3200 (NH), 3360 (OH); <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 2.14 (s, 3H, -CH<sub>3</sub>), 3.58 (d, *J* = 17.4 Hz, 1H), 4.09 (d, *J* = 17.4 Hz, 1H), 6.08 (s, 1H, -OH), 6.77-7.78 (m, 7H, -Ar-H), 10.23 (s, 1H, -NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 21.6 (C-CH<sub>3</sub>), 47.5 (COCH<sub>2</sub>), 72.7 (Spiro C), 120.8, 121.4, 127.8, 128.5, 129.5, 130.2, 133.8, 139.1 (Aromatic C), 176.4 (CONH), 192.9 (COCH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>14</sub>BrNO<sub>3</sub>: C, 56.70; H, 3.92; N, 3.89. Found: C, 56.67; H, 3.90; N, 3.84; Mass (m/z): 359 (M<sup>+</sup>).

**Synthesis of E<sup>5c</sup>.** Yellow crystalline solid; (Yield: 70%); IR (KBr,  $\nu$  cm<sup>-1</sup>): 1650 (CONH), 1780 (COCH<sub>2</sub>), 3210 (NH), 3360 (OH); <sup>1</sup>H NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 2.24 (s, 3H, -CH<sub>3</sub>), 3.51 (d, *J* = 17.4 Hz, 1H), 3.96 (d, *J* = 17.4 Hz, 1H), 5.80 (s, 1H, -OH), 6.67-7.82 (m, 7H, -Ar-H), 10.27 (s, 1H, -NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO- *d*<sub>6</sub>)  $\delta$ : 21.2 (C-CH<sub>3</sub>), 47.9 (COCH<sub>2</sub>), 72.9 (Spiro C), 115.8, 120.8, 127.7, 128.3, 130.0, 133.2, 133.8, 139.1, 166.5 (Aromatic C), 176.1 (CONH), 190.9 (COCH<sub>2</sub>). Anal. Calcd for C<sub>16</sub>H<sub>12</sub>FNO<sub>3</sub>: C, 68.22; H, 4.71; N, 4.68. Found: C, 68.26; H, 4.72; N, 4.71; Mass (m/z): 299 (M<sup>+</sup>).

#### Antioxidant activity

Antioxidant activities of test compounds were measured by estimating DPPH<sup>•</sup> and ABTS<sup>+•</sup> scavenging activity *in vitro* using ascorbic acid as reference compound. All experiments were made in triplicate and results of the present study were expressed as mean  $\pm$  SE.

#### DPPH<sup>•</sup> scavenging activity

Ability of test compound to scavenge the stable free radical DPPH<sup>•</sup> was measured by the method of Mensor *et al.*<sup>13</sup> Absorbance was recorded at 517 nm in a UV-Vis double beam spectrophotometer. The percent inhibition ( $\phi$ , in %) was calculated by using the following formula.

$$\phi = \frac{AC - AA}{AC} * 100$$

where

AC = absorption of control, and

AA = absorption of test.

#### ABTS radical scavenging activity

ABTS<sup>+•</sup> scavenging activity of synthesized compounds were measured by the method of Re *et al.*<sup>14</sup>

#### Nitric oxide scavenging activity

The interaction of test compounds with nitric oxide (NO) was assessed by the nitrite detection method. Sodium nitropruside (5 mM) in phosphate buffer spontaneously generates NO in an aqueous solution.<sup>15</sup> NO interacts with oxygen and produces nitrite ions, which can be estimated by use of Greiss reagent.

#### Antimicrobial activity

Antibacterial and Antifungal activity of synthesized compounds were studied.

#### Microorganisms Used

Clinical laboratory bacterial isolates of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungal isolates viz. *Aspergillus niger*, *A. flavus*, *Rhizopus stonifer* and *Alternaria alternaria* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College, Jaipur India.

#### Culture and Maintenance of Bacteria

Pure cultures of *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* obtained from S.M.S. Medical College, Jaipur, India were used as indicator organisms. These bacteria were grown in Nutrient agar medium (prepared by autoclaving 8% Nutrient agar of Difeco-Laboratories, Detroit, USA, in distilled water at 15 lbs psi for 25-30 min) and incubated at 37°C for 48 hrs. Each bacterial culture was further maintained on the same medium after every 48 h of transferring.

A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

#### Determination of Antibacterial Assay

*In vitro* antibacterial activity of the synthesized compounds were studied against gram positive and gram negative bacterial strains by the agar well diffusion method.<sup>16</sup> Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The compounds' were diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was melted and cooled to 48-50°C and a standardized inoculum (1.5 $\times$ 10<sup>8</sup> CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100  $\mu$ L) was introduced in the well (the wells were made through cork borer vertically to Petri plate up to 10 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of

the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the synthetic compound. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader in mm (the diameter is taken through the centre point of zone of inhibition and a zone of inhibition was calculated after adding the sample which can be visualized on the surface of the plate inoculated with bacteria and fungi). The experiment was performed three times to minimize the error and the mean values were presented.

#### Determination of Antifungal assay

Antifungal activity of the synthesized compounds were investigated by agar well diffusion method.<sup>17</sup> Fungus colonies were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 10<sup>6</sup> cells/mL. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone in mm. All experiments were made in triplicate and means were calculated.

#### CONCLUSION

We describe herein potassium carbonate catalysed highly efficient, green protocol for the synthesis of 3-substituted-3-hydroxyindolin-2-ones derivatives by the reaction of substituted indole-2, 3-diones and substituted aryl ketones in aqueous medium under ultrasound irradiation in excellent yields. The present methodology offered several advantages, such as simple procedure, lowcost, easywork-up, short reaction times, and milder conditions. We have also developed a novel and potent antioxidant and antimicrobial agents of synthetic origin. All desired products, showed good activities. **Compound A** (3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) and **Compound E** (5-methyl-3-hydroxy-3-[2-oxo-2-(4-fluorophenyl)ethyl]indolin-2-one) have highest DPPH<sup>•</sup>, ABTS<sup>•+</sup> and NO radical scavenging activity as compared to other compounds. **Compound C** (5-methyl-3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) was the effective inhibitors against all the bacterial pathogens. **Compound A** (3-hydroxy-3-[2-oxo-2-(4-Chloro-phenyl)ethyl]indolin-2-one) and **Compound C** (5-methyl-3-hydroxy-3-[2-oxo-2-(4-chloro-phenyl)ethyl]indolin-2-one) showed equivalent activity comparable to standard drug Ampicillin against *Pseudomonas aurigona*.

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