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# Efficient synthesis of new 2,3-dihydrooxazole-spirooxindoles hybrids as antimicrobial agents.

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## Abstract

Two series of new 2,3-dihydrooxazole-spirooxindole derivatives were efficiently synthesized starting from N'-(2-oxoindolin-3-ylidene) benzohydrazide/N'-(2-oxoindolin-3-ylidene)-2-phenoxyacetohydrazide using designed synthetic route. Newly synthesized 2,3-dihydrooxazole-spirooxindole derivatives were screened for their antibacterial and antifungal activity against different pathogenic strain of bacteria and fungi. The minimum inhibitory concentration (MIC), minimum Bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined for the test compounds as well as for reference standards. Compounds **4e**, **4g**, **7g** have shown good antibacterial activity whereas compounds **4f**, **7b**, **7d** have displayed better antifungal activity.

## Keywords

2,3-dihydrooxazole-spirooxindole, Isatin, spirooxindole, hydrazide, schiff base, antimicrobial antibacterial activity and antifungal activity.

Efficient synthesis of drug-like small molecules has been the focus of the research for medicinal chemists and chemical biologists because, they play very important role in drug discovery processes.<sup>1</sup> These different drugs like bioactive compounds are broadly used to modulate enzyme or receptor function and can serve as important leads for drug development.<sup>2</sup> Isatin (1H-indole-2,3-dione) and its derivatives have been proven as privileged core structures and are found in many bioactive natural products and in various pharmaceutical agents.<sup>3</sup> The synthetic versatility of the isatin, due to its privileged scaffold, has led to the generation of a large number of structurally diverse derivatives which include analogues derived from either C-3 carbonyl modification with mono-, di-, and trisubstitution of the aryl ring and/or those obtained by derivatisation of the nitrogen of isatin.<sup>4</sup>

The most fascinating derivatization of isatin analogues is undoubtedly reaction on C-3 carbonyl center instead of C-2 carbonyl. This selective and smooth derivatization is possible because C-3 prochiral carbonyl center is more reactive towards nucleophilic addition than C-2 carbonyl.<sup>5</sup> The reactions on the C-3 carbonyl group of isatins, mostly by nucleophilic additions or spiro

annulations, transform it into various spiro-fused heterocyclic and carbocyclic frameworks including important 2-oxindole derivatives. The 2-oxindoles, which are spiro-fused to other cyclic frameworks have drawn remarkable interest of researchers around the globe working in the area of medicinal chemistry, chemical biology and synthetic organic chemistry because they are present in many bioactive natural products.<sup>6,7</sup> Some of such 2-oxindole derivatives of isatin are horsfiline, spirotryprostatins, gelseverine, gelsmine, rhynchophylline etc. These derivatives have been known for various types of bioactivity such as progesterone receptors modulators, anti- HIV, anticancer, antitubercular, antimalarial and MDM2 inhibitor.<sup>8,9</sup>

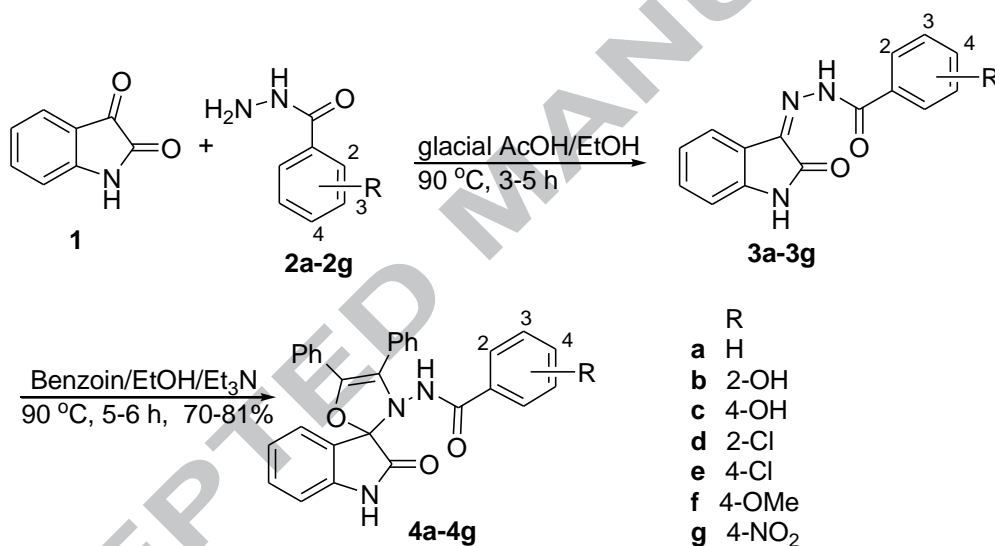
Indole nucleus itself is privileged structure and has been reported for various biological activities including fungicidal, bactericidal and herbicidal activities.<sup>10</sup> The spirooxindoles is among the most important class of naturally occurring substances, characterised by highly pronounced biological properties, and is also the core structure of many synthetic pharmaceuticals.<sup>11</sup> The various biological activities of spirooxindoles derivatives have attracted much attention from organic chemists and as a consequence a number of methods have been reported for the preparation of spirooxindoles fused heterocycles. Isatin perhaps is the single class of heterocyclic compound which has been most extensively employed as a starting material for the synthesis of a wide number of spiro-cyclic oxindoles frameworks, both carbocyclic and heterocyclic.<sup>12-14</sup> Oxazole-benzamide derivatives also constitute an important class of heterocyclic compounds and recently design, synthesis and structure-activity relationship of a series of oxazole-benzamide derivatives reported alongwith their bacterial and pesticidal activities.<sup>15</sup>

On the basis of the above literature abundance on bio-potentials of spiro-cyclic oxindoles and oxazole-benzamide framework, we were confident that the hybrids spiro-cyclic oxindoles fused with oxazole-benzamide framework would provide the important structural motifs for the discovery of novel bioprobes or therapeutic agents. In continuation of our research on efficient synthesis of drug like small molecules, we designed and synthesized spiro-cyclic oxindoles fused with oxazole-benzamide as novel 2,3-dihydrooxazole-spirooxindoles hybrids as antimicrobial agents.

## Chemistry

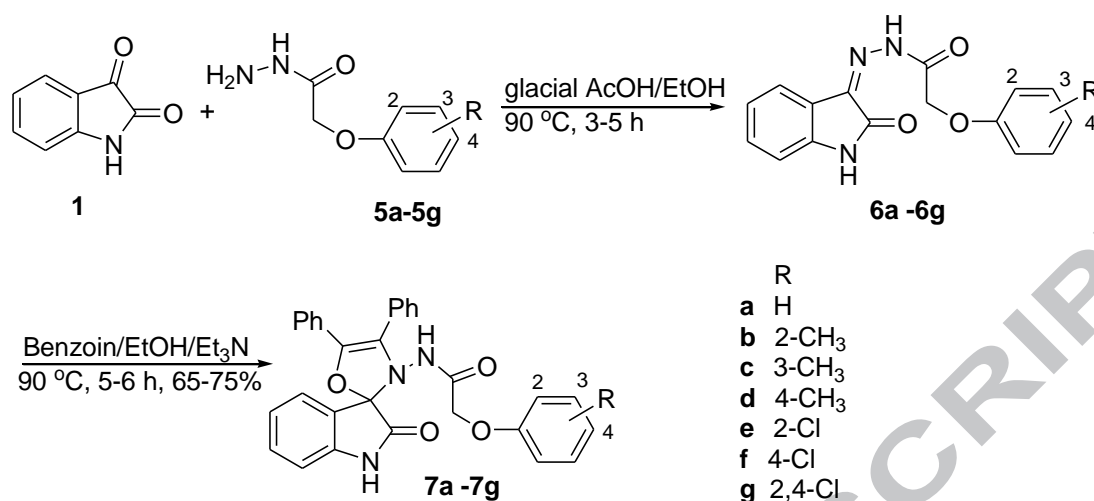
For the development of efficient and facile methods for the synthesis of designed novel 2,3-dihydrooxazole-spirooxindoles hybrids, we identified Isatin (indole-1*H*-2,3-dione) **1** as the key starting material that can be transferred to our designed antimicrobial molecules in two step via schiff base intermediate. The synthesis of intermediate schiff bases **3a-3g**<sup>16-18</sup> and **6a-6g**<sup>19-21</sup> was carried out by reacting Isatin **1** and corresponding commercially available analogues of benzohydrazides **2a-2g** and phenoxyacetohydrazides **5a-5g** respectively. These schiff bases then subsequently were coupled with benzoin at elevated temperature in presence of triethyl amine which furnish desired 2,3-dihydrooxazole-spirooxindoles derivatives **4a-4g** and **7a-7g** respectively in good yield (Scheme 1 and 2).

We initiated our synthetic protocol with commercially available Isatin **1** refluxing with different benzohydrazides **2a-2g** in ethanol in the presence of catalytic amount of acetic acid which furnished corresponding schiff bases **3a-3g** as reported in literature.<sup>16-18</sup> The schiff bases **3a-3g** were purified by crystallization and characterized using spectroscopic data. All the NMR and IR data of these schiff bases **3a-3g** were in consistent with the reported analytical data. After having characterized and pure schiff base **3a** in hand, it was refluxed with benzoin under various reaction conditions using different solvents (MeOH, Toluene, THF etc) but ethanol under reflux in basic condition for 6h was found better, which showed the formation of clean product (TLC). Reaction mixture was cooled to room temperature and after usual workup (experimental section) and purification it furnished 2,3-dihydrooxazole-spirooxindole derivative **4a** in very good isolated yield (81%). Similarly other schiff bases **3b-3g** were successfully transferred to their corresponding 2,3-dihydrooxazole-spirooxindoles **4b-4g** under reflux condition in ethanol using triethyl amine in very good isolated yields (71-81%) (Scheme 1).



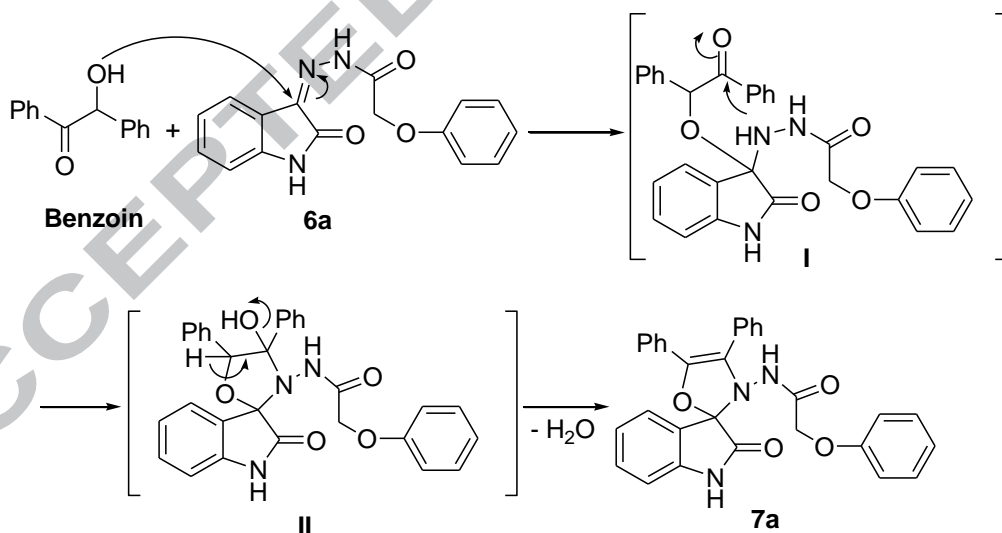
**Scheme 1.** Synthesis of **4a-4g**.

In parallel synthesis approach using similar reaction protocols Isatin **1** refluxed with different phenoxyacetohydrazides **5a-5g** in ethanol in the presence of catalytic amount of acetic acid which furnished corresponding schiff bases **6a-6g** as reported in literature.<sup>19-21</sup> The schiff bases **6a-6g** were purified by crystallization and characterized by comparing acquired analytical data with data reported in literature. All the prepared schiff bases **3a-3g** were refluxed with benzoin under previous optimized condition in ethanol in presence of Et<sub>3</sub>N for 6 h which result in the formation of nonpolar products on TLC. After usual workup and purification their corresponding 2,3-dihydrooxazole-spirooxindoles **7a-7g** were isolated in 65-75% isolated yields (Scheme 2).



**Scheme 2.** Synthesis of **7a-7g**.

The mechanism of this transformation can be postulated as follows: hydroxy group of benzoin in the presence of triethyl amine at elevated temperature attacking on imine carbon pushing imine double bond towards nitrogen resulting an unstable intermediate **I** (Figure 1). Intramolecular attack of hydrazine N-atom to carbonyl carbon of benzoin may resulting intermediate **II** which under high temperature reaction condition loses water molecule and furnishing stable 2,3-dihydrooxazole-spirooxindole derivative **7a** (Figure 1). All the compounds and intermediates were confirmed by spectral analysis.<sup>22</sup>



**Figure 1.** Proposed Mechanism

## Biology

The synthesized pure compounds were screened for antibacterial and antifungal activities adopting standard protocols.<sup>23</sup> The antibacterial activity, of prepared final pure compounds (**4a-**

**4g** and **7a-7g**), was performed against *Bacillus subtilis*, *Enterobacter* and *Klebsiella pneumoniae* using Ciprofloxacin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) were determined and the activity was reported in  $\mu\text{g/ml}$ . The nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and controls were inoculated with approximately  $5 \times 10^5$  c.f.u/ml of actively dividing bacteria cells. The cultures were incubated for 24 hour at  $37^\circ\text{C}$  and the growth was monitored visually and spectrophotometrically. The antibacterial results are summarized in table 1, only for those compounds which were found active against any strain of bacteria. It is evident from table 1 that compounds **4b**, **4e**, **4f**, **7a** and **7g** showed moderate activity against gram-positive bacteria *B. subtilis* whereas, **4e**, **4g**, **7c**, **7d** and **7g** were active against gram-negative *Enterobacter* ranging from 30-50  $\mu\text{g/ml}$  concentration and only one compound **7g** was found active against *K. pneumoniae* 25  $\mu\text{g/ml}$  concentration (Table 1).

**TABLE 1.** Antibacterial activity of novel 2,3-dihydrooxazole-spirooxindole derivatives

Compounds	Gram-Positive bacteria		Gram-Negative bacteria			
	<i>B. subtilis</i>		<i>Enterobacter</i>		<i>K. Pneumoniae</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
<b>4b</b>	70	100	40	50	n.a.	n.a.
<b>4e</b>	50	100	30	50	50	100
<b>4f</b>	100	200	50	100	n.a.	n.a.
<b>4g</b>	30	50	30	50	n.a.	n.a.
<b>7a</b>	50	100	40	100	n.a.	n.a.
<b>7b</b>	n.a.	n.a.	30	50	n.a.	n.a.
<b>7c</b>	n.a.	n.a.	40	100	n.a.	n.a.
<b>7d</b>	n.a.	n.a.	50	100	n.a.	n.a.
<b>7g</b>	25	50	40	100	25	50
Ciprofloxacin	6.25	12.50	10.25	25	6.25	25
DMSO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

MIC ( $\mu\text{g/ml}$ ), minimum inhibitory concentration i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC ( $\mu\text{g/ml}$ ) minimum bacteriostatic concentration i.e. the lowest concentration of the compound for killing the bacteria completely; n.a.-No activity detected.

The antifungal activity, of prepared final pure compounds, was performed against *Pyricularia oryzae*, *Pseudoperenospora cubensis*, *Sphaerotheca fuliginea* and *Phytophthora infestans* using Nystatin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) was determined and reported in  $\mu\text{g/ml}$ . Antifungal activity was carried out through disk diffusion method.<sup>24</sup> All fungal cultures were routinely maintained on sabouraud dextrose agar (SDA) and incubated at  $28^\circ\text{C}$ . The summarized data are presented in table 2. The antifungal activities are summarized in table 2, only for those compounds which were found active against any of these

strain of fungi. It is evident from table 2 that compounds **4b**, **4e-4g**, **7c-7d** and **7g** showed antifungal activity against used strains of fungi ranging from 8-18  $\mu\text{g/ml}$  in concentration and compound **7c** was the only active compound against *P. oryzae* and *P. infestans* in 20 and 12  $\mu\text{g/ml}$  respectively. Compound **7g** was found active against *S. fuliginea* 14  $\mu\text{g/ml}$  concentration (Table 2).

**TABLE 2.** Antifungal activity of novel 2,3-dihydrooxazole-spirooxindole derivatives

Compounds	Fungal Species and MIC ( $\mu\text{g/ml}$ )			
	<i>P. oryzae</i>	<i>P. cubensis</i>	<i>S. fuliginea</i>	<i>P. infestans</i>
<b>4b</b>	16	12	12	14
<b>4e</b>	14	12	12	14
<b>4f</b>	12	14	8	14
<b>4g</b>	16	18	12	12
<b>7a</b>	15	12	16	14
<b>7b</b>	17	8	12	18
<b>7c</b>	20	n.a.	n.a.	12
<b>7d</b>	8	12	12	14
<b>7g</b>	n.a.	n.a.	14	n.a.
Nystatin	20	18	18	18
DMSO	n.a.	n.a.	n.a.	n.a.

MIC ( $\mu\text{g/ml}$ ), minimum inhibitory concentration i.e. the lowest concentration of the compound to inhibit the growth of fungi. n.a.-No activity detected.

The structure-activity relationship (SAR) of the tested compounds for antibacterial activity can be summarized as follows: i) in the series of the 2,3-dihydrooxazole-spirooxindoles, the substitution at phenyl ring is important for better antibacterial activity; ii) 4-Cl and 4-NO<sub>2</sub> substitution in this series shown better activity than other groups against *B. subtilis* and *K. pneumoniae*; iii) Phenoxyacetohydrazides condensed 2,3-dihydrooxazole-spirooxindoles do not show any better antibacterial activity. Antifungal activity of these compounds was very much comparable to Nystatin a known antifungal agent. The mechanism responsible for the antibacterial activity of examined compounds is not known at the moment; work is in progress to investigate the mechanism of antibacterial action, and synthesis of more effective compounds.

In summary, an efficient, clean and convenient synthesis of novel 2,3-dihydrooxazole-spirooxindole derivatives has been developed. The procedure offers several advantages including simple reaction conditions as well as simple experimental and product isolation procedures, thus, making the synthesis of series of novel 2,3-dihydrooxazole-spirooxindoles in good to very good yields. These 2,3-dihydrooxazole-spirooxindoles were designed through the recombination of, cheap and readily available starting materials, benzoin and schiff bases of isatin, with corresponding analogues of benzohydrazides and phenoxyacetohydrazides. Subsequent preliminary anti-bacterial and anti-fungal screening done on these compounds which revealed



that newly generated compounds possess antimicrobial activity and these can be elaborated as potential antimicrobial agents. The complete library realization of these novel 2,3-dihydrooxazole-spirooxindole derivatives and associated detailed biological evaluations will be reported in due course of time.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://>.

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  22. Compound 4a. Amorphous solid (1.85 g, 81%). IR (KBr,  $\text{cm}^{-1}$ ): 1450, 1500, 1580 (C=C str) 1665, 1690 (C=O str), 2950 (=C-H str), 3200 (N-H str);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 5.99-6.06 (m, 2H, ArH), 7.19-7.27 (m, 3H, ArH), 7.31-7.45 (m, 6H, ArH), 7.52-7.63 (m, 3H, ArH), 7.78 (t, 1H,  $J = 7.5$  Hz, ArH), 7.91 (d, 2H,  $J = 7.5$  Hz, ArH), 7.92 (d, 2H,  $J = 7.5$  Hz, ArH);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 118.2, 127.7, 128.1, 128.9, 129.0, 129.3, 129.9, 130.0, 131.9, 132.7, 133.7, 135.1, 136.0, 140.2 195.3 (C=O), 195.6 (C=O); FAB HRMS  $m/z$ : calcd for  $\text{C}_{29}\text{H}_{21}\text{N}_3\text{O}_3$   $[\text{M} + \text{H}]^+$ , 460.1661; found, 460.1660.
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## Graphical Abstract

