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ORIGINAL ARTICLE

Benzofurano-isatins: Search for antimicrobial agents

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Abstract In an attempt to find a new class of antimicrobial agents, a series of novel *N*′-(5 or 7 substituted-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazides **3(a–p)** were synthesized by reacting benzofuran-2-carbohydrazide **1** with 5 and 7 substituted-isatins **2(a–p)**. The synthesized compounds were confirmed by melting point, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the synthesized compounds were screened for antimicrobial activity among the tested series, **3o** exhibited excellent antibacterial activity against *Escherichia coli* and *Pseudomonas vulgaris* while **3p** against *Bacillus subtilis*, *E. coli* and *P. vulgaris* (31.25 μg/mL) when compared with standards. Similarly **3o** and **3p** showed significant antifungal activity (31.25 μg/mL) when compared to fluconazole against *Aspergillus niger*.

1. Introduction

Human struggle against the affliction of disease, decay and death is eternal. The deterioration of human population due to an enhanced prevalence of infectious diseases is becoming a global problem. The contemporary treatment of infectious diseases involves administration of a multidrug regimen over a long period of time, which has led to the rapid emergence of multidrug-resistant strains plus a high level of patient non-compliance (Chambhare et al., 2003). The rising prevalence of multidrug resistant superbugs like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VREF) continues to provide impetus for the search and discovery of novel antimicrobial agents. A potential approach to overcome this resistance problem is to design new and innovative agents with a completely different mode of action so that no cross-resistance with the present therapeutics can occur (Khan et al., 2005).

Among an extensive diversity of heterocycles that have been explored for developing pharmaceutically important molecules, benzofuran derivatives have played an important role in medicinal chemistry. Benzofuran derivatives have drawn considerable attention due to their profound physiological and chemotherapeutic properties. Benzofuran has a variety of activities like antimicrobial (Khan et al., 2005; Alper-Hayta et al., 2008), anti-inflammatory (Jadhav et al., 2008), anticancer (Mahboobi et al., 2007; Asoh et al., 2009), antihistaminergic (Gfesser et al., 2005; Peschke et al., 2006; Cowart et al., 2005) and anticholinesterase (Luo et al., 2005; Belluti et al., 2005). Several attempts were made to study the effects of different functional groups on the homocycle and/or the heterocycle for bioactivity. On the other hand isatin, an indole

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derivative possesses good antimicrobial activity (Pandeya et al., 1999; Pandeya and Sriram, 1998; Mirjana et al., 2006). In the view of biological importance of these two moieties, the present work was undertaken to synthesize a new series of benzofurano-substituted isatins and to evaluate their possible anti-microbial activity.

2. Rationale and hypothesis

The chemistry of benzofurans available in a large number of natural products has attracted widespread interest due to their biological activities and their potential applications as pharmacological agents. Several benzofuran ring systems bearing various substituents at the C-2 position are widely distributed in nature. There are well known natural products having related benzofuran ring structures, the most recognized benzofurans are *ailanthoidol*, *amiodarone* and *bufuralol* compounds. *Ailanthoidol*, a neolignan with a 2-arylbenzofuran skeleton, was isolated from the Chinese herbal medicine *Zanthoxylum ailanthoides* (Fuganti and Serra, 1998). It has been reported that neolignans and lignans possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, antifungal and antifeedant activities (Kao and Chern, 2001). Cicerfuran, another antifungal benzofuran derivative, was first obtained from the roots of wild species of chickpea, *Cicer bijugum*, reported to be a major factor in the defence system against *Fusarium wilt* (Aslam et al., 2009) (Fig. 1).

Benzofuran derivatives are of special interest to natural product researchers for their biological activities and potential applications as pharmacological agents, e.g. corsifuran C (Xiao et al., 2008; Toshio et al., 2004; Kuete et al., 2009). Specifically, several benzofuran ring systems bearing various substituents at C-2 and C-3 positions are widely distributed in nature, the stilbenoids structural compounds, e.g. an oligostilbene derivative viniferin, are known to possess antimicrobial, antiviral, antioxidant, antifungal, and antitumor activities (Lin and Yao, 2006; Kim et al., 2008).

Isatin an endogenous compound identified in many organisms shows a wide range of biological activities (Pandeya et al., 2005). The isatin ring is a prominent structural moiety found in several pharmaceutically active compounds. This is mainly due to the easy synthesis and the importance of pharmacological activity. Therefore, the synthesis and selective functionalization of isatins have been the focus of active research area over the years (Lian-Shun et al., 2011). Isatin derivatives are reported to show antibacterial (Praveen et al., 2011) and antifungal (Amalraj et al., 2003) activities. Methisazone, for example plays an important role as prophylactic agent against several viral diseases (Foye and Sethi, 2002).

Prompted by the above-mentioned results, it was planned to combine two biologically active pharmacophore benzofuran and substituted isatins for the construction of some benzofuran–isatins. These combinations were suggested in an attempt to investigate the possible synergistic influence of such structure hybridizations on the anticipated activity, hoping to discover a new lead structure that would have a significant antimicrobial activity at very small concentration.

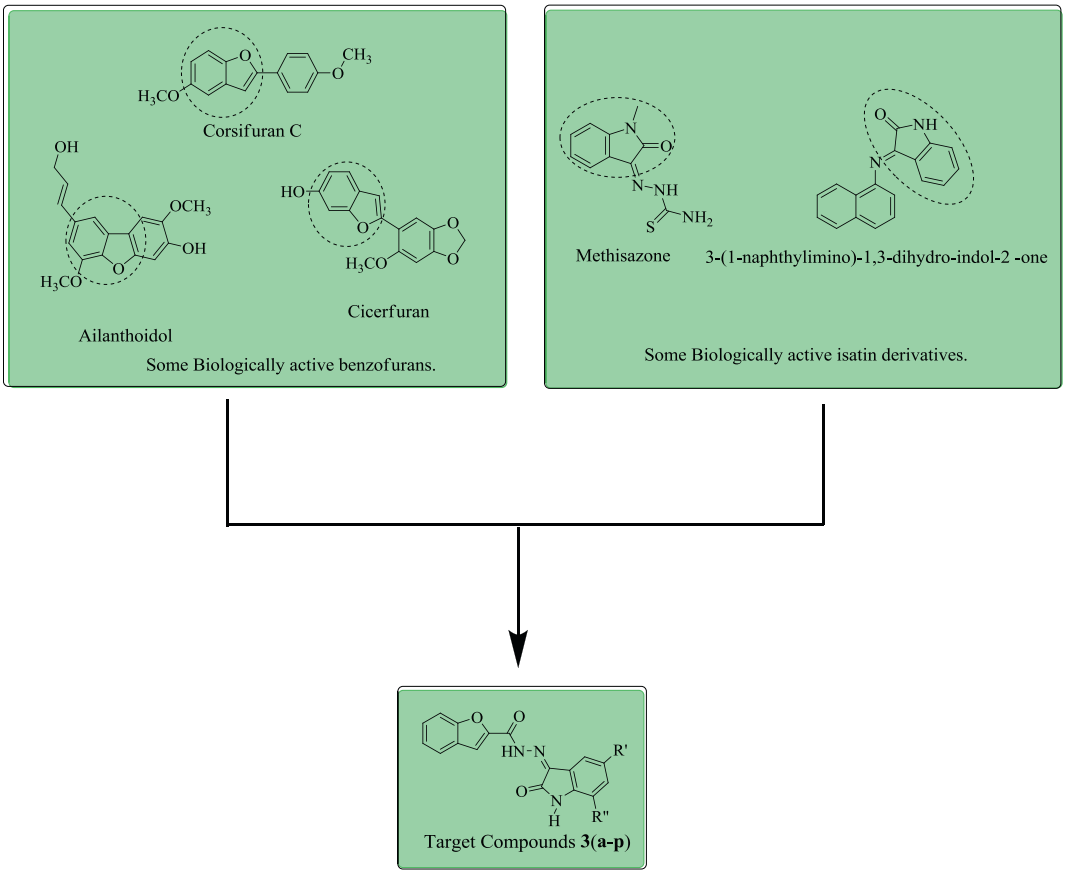


Figure 1 Reported and proposed structure **3(a–p)**.

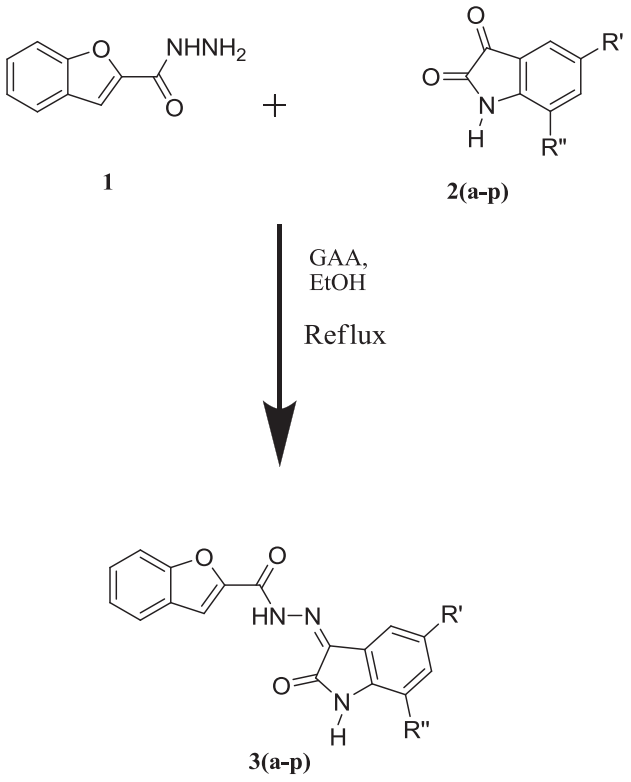
3. Chemistry

In the present work, 16 novel derivatives of benzofurano-substituted isatins were synthesized **3(a–p)**. The synthetic strategies adopted to obtain the target compounds are depicted in Scheme 1. The 5 and 7 substituted-isatins **2(a–p)** and benzofuran-2-carbohydrazide **1** were prepared as per the reported methods given by Henry and Blatt (1964) and Marvel and Heirs (1941), respectively. Finally 5 and 7 substituted-isatins **2(a–p)** and benzofuran-2-carbohydrazide **1** were refluxed in absolute ethanol with 2–3 drops of glacial acetic acid as a catalyst to afford corresponding *N*′-(5 and 7 substituted-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide **3(a–p)**. All the synthesized compounds were characterized by their physical (Table 1), analytical, and spectral data.

The mechanism of the reaction involves in the synthesis of *N*′-(5 and 7 substituted-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazides **3(a–p)** is nucleophilic addition of benzofuran-2-carbohydrazide to 5 and 7 substituted-isatins to give *N*′-(5 and 7 substituted-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazides as shown in Scheme 2.

4. Result and discussion

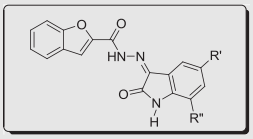
All the synthesized compounds were characterized by their physical and spectral data. The IR spectra of all the



Compounds	R'	R''	Compounds	R'	R''
a	H	H	**i**	H	Br
b	Br	H	**j**	H	Cl
c	Cl	H	**k**	H	OH
d	F	H	**l**	H	CH_3
e	CH_3	H	**m**	H	OCH_3
f	OCH_3	H	**n**	H	OC_2H_5
g	NO_2	H	**o**	H	NO_2
h	OH	H	**p**	H	F

Scheme 1

Table 1 Physical data of benzofurano-isatins **3(a–p)**.



Compounds	Molecular formula	Molecular weight	Yield (%)	Melting point (°C)
3a	$C_{17}H_{11}N_3O_3$	305.29	76.64	320–322
3b	$C_{17}H_{10}BrN_3O_3$	384.18	73.12	312–314
3c	$C_{17}H_{10}ClN_3O_3$	339.73	75.47	318–320
3d	$C_{17}H_{10}FN_3O_3$	323.28	64.45	339–341
3e	$C_{18}H_{13}N_3O_3$	319.31	74.16	315–317
3f	$C_{18}H_{13}N_3O_4$	335.31	70.15	320–322
3g	$C_{17}H_{10}N_4O_5$	350.29	72.60	306–308
3h	$C_{17}H_{11}N_3O_4$	321.29	71.14	311–314
3i	$C_{17}H_{10}BrN_3O_3$	384.18	71.40	316–318
3j	$C_{17}H_{10}ClN_3O_3$	339.73	75.58	321–323
3k	$C_{17}H_{11}N_3O_4$	321.29	70.57	315–317
3l	$C_{18}H_{13}N_3O_3$	319.31	71.23	313–315
3m	$C_{18}H_{13}N_3O_4$	335.31	69.76	319–321
3n	$C_{19}H_{15}N_3O_4$	349.34	63.12	329–331
3o	$C_{17}H_{10}N_4O_5$	350.29	71.34	309–311
3p	$C_{17}H_{10}FN_3O_3$	323.28	69.15	335–337

[a]Elemental analysis for C, H, N were within 0.4% of the theoretical values.

compounds **3(a–p)** showed appearance of band at 3290–3180 cm^{-1} due to amidal –NH– stretch. The title compounds were also confirmed by the appearance of bands at 1724–1697 cm^{-1} due to >C=O stretch of isatins. The ^1H NMR (DMSO-d_6) spectrum of compounds **3(a–p)** exhibited proton absorption singlet at 11.64–12.85 ppm and 10.97–10.01 ppm due to CONH- and indole –NH–, respectively. All other aromatic protons were observed as multiplet in the range 8.21–5.75 ppm. The ^{13}C NMR and mass spectra of the compounds **3(a–p)** were in agreement with the proposed structures.

In the present research we planned to combine two biologically active moieties such as benzofuran and isatin to observe influence over the biological activity since several benzofuran ring systems are known to possess significant antimicrobial activity as reported by Xiao et al. (2008), Toshio et al. (2004) and Kuete et al. (2009). Similarly isatin moiety also possesses good antimicrobial activity as described by Pandeya et al. (1999), Pandeya and Sriram (1998), Mirjana et al. (2006). Newly prepared compounds **3(a–p)** were screened for their antibacterial and antifungal activity. MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. The MIC values are generally within the range of 31.25–500 μg/mL against all evaluated strains. The results of the in vitro antibacterial activity screening of the novel series of *N′*-(5-bromo-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazides **3(a–p)** are summarized in Table 2, against the Gram-positive bacteria *S. aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633), the Gram-negative bacteria *Escherichia coli* (ATCC-25922) and *Pseudomonas vulgaris* (ATCC-27853). Anti-microorganism tests with 16 compounds have established some interesting structure–activity relationships. Compound **3o** with a nitro group at C-7 of isatins, displayed excellent antibacterial activities against *E. coli* and *P. vulgaris* with MIC values of 31.25 μg/mL as compared to the positive control drugs. The presence of fluoro group at the C-7 position, compound **3p**, also displayed good activities against *B. subtilis*, *E. coli* and *P. vulgaris* with an MIC value of 31.25 μg/mL as compared to standard drugs. Surprisingly both **3o** and **3p** are less active against *S. aureus*.

Among the tested series **3c**, **3d**, **3i**, **3j**, **3k**, **3m**, **3n** exhibited moderate antibacterial activity against Gram positive bacteria (*S. aureus* and *B. subtilis*) as well as Gram negative bacteria (*E. coli* and *P. vulgaris*) with MIC values of 62.50–125 μg/mL. However, rest of the compounds in the series were found to have less or poor activity against tested micro-organisms as compared to the standard drugs. It is interesting to note that the introduction of an electron withdrawing substituent to the aromatic ring at C5 and C7 positions resulted in compounds with an excellent antibacterial activity as it is confirmed by **3o** and **3p**. However, an electron releasing substituent gives compounds with poor activity, as it is observed in compounds **3e** and **3i**.

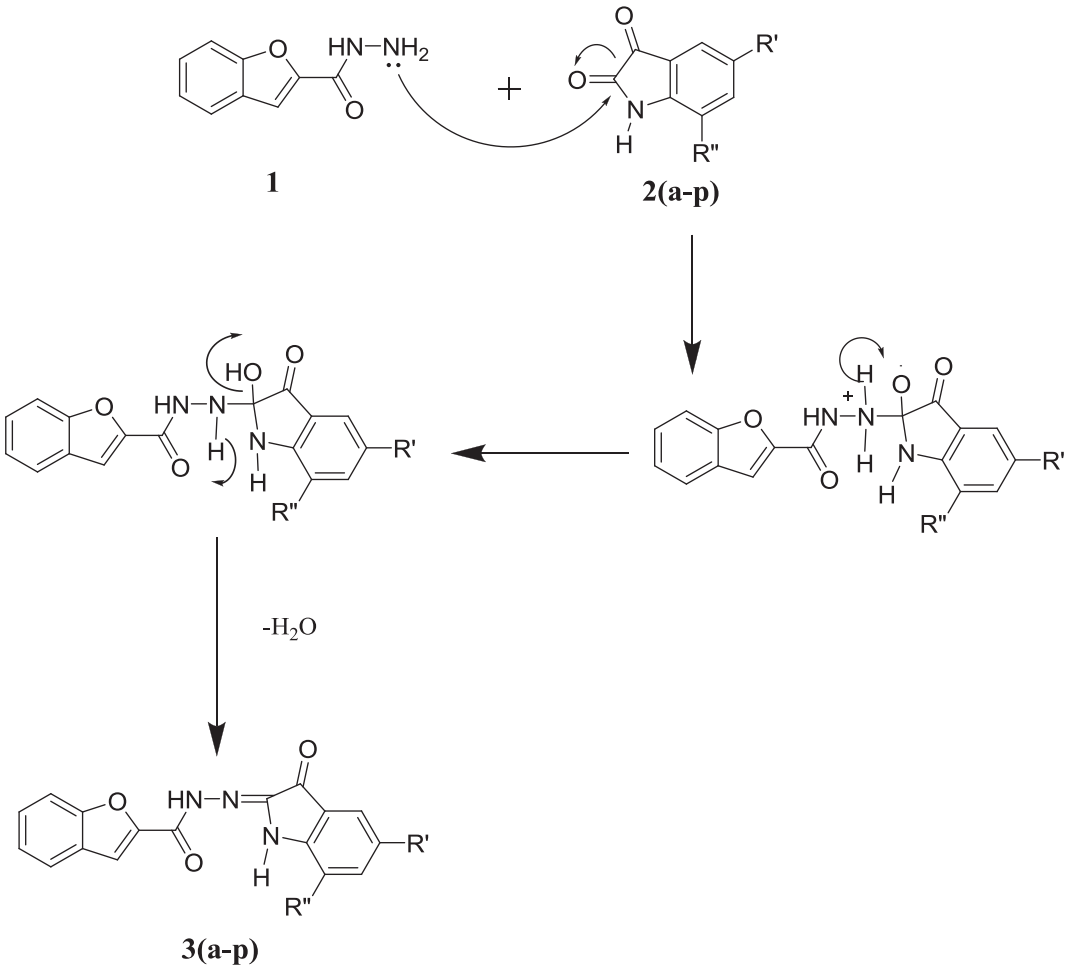
All the synthesized *N′*-(5-bromo-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazides **3(a–p)** were also screened for antifungal activity against two fungal species *Candida albicans* and *Aspergillus niger*. Compounds **3o** and **3p** displayed good activity (31.25 μg/mL) against *A. niger*. The results showed that compounds **3c**, **3d**, **3g**, **3i**, **3j**, **3m**, **3n** showed moderate activity (62.50–125 μg/mL) when compared to fluconazole against *A. niger*. In case of *C. albicans*, except **3o** and **3p**, all the tested compounds **3(a–p)** displayed weak anti-fungal activities as compared to standard (fluconazole).

5. Conclusion

In the present research, we report the synthesis and in vitro antimicrobial activity of a new series of novel benzofurano–isatins **3(a–p)**. In general, the results of the in vitro antibacterial activity are also encouraging, as out of 16 compounds tested, compound **3o** with a nitro group at C-7 of isatins exhibited significant antibacterial activity against *E. coli* and *P. vulgaris* with MIC values of 31.25 μg/mL. Similarly the presence of fluoro group at the C-7 position, compound **3p**, also displayed good activities against *B. subtilis*, *E. coli* and *P. vulgaris* with an MIC value of 31.25 μg/mL as compared to standard drugs. In case of antifungal activity compounds **3o** and **3p** displayed moderate activity (31.25 μg/mL) against *A. niger* when compared to fluconazole against *A. niger*. It is worth mentioning that the combination of two biologically active moieties benzofurano and isatin profoundly influences the biological activity. Possible improvements in the antimicrobial activity can be further achieved by slight modifications in the substituents and/or additional structural activity investigations to have good antifungal activity. Further developments on this subject to understand their mechanistic interactions are currently in progress.

6. Experimental

All chemicals and solvents were supplied by Merck, S.D. Fine Chemical Limited, Mumbai. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminium



Scheme 2

sheets with GF_{254} silica gel, 0.2 mm layer thickness (E. Merck). Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was acquired on a Shimadzu Infra Red Spectrometer, (model FTIR-8400S). Both ^{1}H NMR (DMSO) and ^{13}C NMR (DMSO) spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Punjab University (Chandigarh). Chemical shifts were measured relative to internal standard TMS (δ: 0). Chemical shifts are reported in δ scale (ppm). Mass (FAB) spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University.

*6.1. General procedure for the synthesis of N′-(5 or 7 substituted-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide **3**(**a**–**p**)*

A mixture of equimolar quantity of substituted indole-2,3-dione **2**(**a**–**p**) (1.47 g, 0.01 mol), benzofuran-2-carbohydrazide (1.82 g, 0.01 mol) was taken in a 100 mL round bottom flask in absolute ethanol (20 mL) with 2–3 drops of glacial acetic acid. The solution was refluxed for three hours and allowed to cool. The precipitated product was filtered, dried and purified by flash chromatography using mobile phase ethyl acetate:chloroform (1:8). The solid product was crystallized by using ethanol.

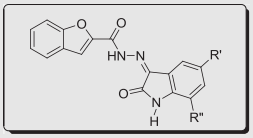
*6.1.1. N′-(2-Oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3a**)*

IR [KBr] ν_{max}: 3225 (NH stretch), 3116 (CH stretch), 1698 (C═O), 1670 (C═N); ^{1}H NMR (DMSO-d_6) δ: 12.85 (s, 1H, CONH), 10.18 (s, 1H, indole NH), 6.67–5.75 (m, 9H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 165.1, 158.5, 151.1, 127.4, 125.5, 124.5, 119.8, 110.9, 107.1 (benzofuran-2-carbohydrazide), 169.1, 140.5, 135.1, 130.0, 127.1, 122.1, 118.5, 116.1 (indoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{11}N_3O_3$: 305.0800; found: 305.0805.

*6.1.2. N′-(5-Bromo-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3b**)*

IR [KBr] ν_{max}: 3200 (NH stretch), 3027 (CH stretch), 1708 (C═O), 1668 (C═N), 742 (C–Br stretch); ^{1}H NMR (DMSO-d_6) δ: 11.97 (s, 1H, CONH), 10.67 (s, 1H, indole NH), 7.91–7.33 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 164.1, 155.1, 148.2, 127.2, 125.1, 124.6, 120.1, 110.5, 108.2 (benzofuran-2-carbohydrazide), 168.9, 141.6, 137.1, 134.5, 130.1, 117.1, 116.4, 115.9 (5-bromoindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}BrN_3O_3$: 382.9906; found: 382.9910.

Table 2 Minimum inhibitory concentration (MIC) of benzofuran–isatins **3(a–p)**.



Compounds	MIC corresponding effects on micro-organism (μg/mL)					
	Antibacterial activity				Antifungal activity	
	Gram positive		Gram negative			
	S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*C. albicans*	*A. niger*
3a	500	250	500	500	500	250
3b	250	250	250	250	250	250
3c	125	**62.50**	125	125	**250**	125
3d	125	**62.50**	**62.50**	**62.50**	250	**62.50**
3e	500	250	250	250	500	250
3f	250	250	250	500	500	250
3g	**62.50**	**62.50**	125	125	250	**62.50**
3h	125	**62.50**	125	125	250	125
3i	125	**62.50**	125	125	250	**62.50**
3j	**62.50**	125	**62.50**	125	250	**62.50**
3k	**62.50**	125	125	125	250	125
3l	500	250	250	250	500	250
3m	125	**62.50**	125	**62.50**	500	250
3n	125	**62.50**	125	**62.50**	500	250
3o	250	**62.50**	**31.25**	**31.25**	**62.50**	**31.25**
3p	250	**31.25**	**31.25**	**31.25**	**62.50**	**31.25**
Ampicillin	0.48	3.90	3.90	3.90	–	–
Norfloxacin	0.48	3.90	0.12	62.50	–	–
Fluconazole	–	–	–	–	0.98	1.95

*6.1.3. N′-(5-Chloro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3c**)*

IR [KBr] ν_{max}: 3197 (NH stretch), 3034 (CH strech), 1710 (C═O), 1682 (C═N stretch), 792 (C–Cl stretch); ^{1}H NMR (DMSO-d_6) δ: 11.76 (s, 1H, CONH), 10.49 (s, 1H, indole NH), 7.91–6.97 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 167.6, 156.1, 149.6, 125.5, 123.2, 122.1, 119.9, 111.1, 108.1 (benzofuran-2-carbohydrazide), 169.4, 138.9, 135.1, 133.1, 131.4, 128.5, 126.1, 119.6 (5-chloroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}ClN_3O_3$: 339.0411; found: 339.0416.

*6.1.4. N′-(5-Fluoro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3d**)*

IR [KBr] ν_{max}: 3219 (NH stretch), 3110 (CH strech), 1697 (C═O), 1667 (C═N stretch), 1010 (C–F stretch); ^{1}H NMR (DMSO-d_6) δ: 11.56 (s, 1H, CONH), 10.53 (s, 1H, indole NH), 7.94–7.12 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 165.4, 156.8, 148.2, 125.8, 123.5, 122.1, 120.5, 111.7, 108.2 (benzofuran-2-carbohydrazide), 169.3, 159.0, 137.1, 133.9, 118.9, 116.6, 111.9, 109.2 (5-fluroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}FN_3O_3$: 323.0706; found: 323.0701.

*6.1.5. N′-(5-Methyl-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3e**)*

IR [KBr] ν_{max}: 3227 (NH stretch), 3124 (CH stretch), 1700 (C═O), 1675 (C═N stretch); ^{1}H NMR (DMSO-d_6) δ: 11.64 (s, 1H, CONH), 10.28 (s, 1H, indole NH), 7.93–7.21 (m, 8H, Ar–H), 2.25 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ: 168.5, 155.7, 149.6, 127.2, 125.1, 124.1, 120.1, 111.7, 108.5 (benzofuran-2-carbohydrazide), 168.9, 138.5, 135.1, 133.4, 131.2, 127.1, 122.3, 115.9, 22.8 (5-methylindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{18}H_{13}N_3O_3$: 319.0957; found: 319.0963.

*6.1.6. N′-(5-Methoxy-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3f**)*

IR [KBr] ν_{max}: 3258 (NH stretch), 3068 (CH stretch), 1719 (C═O), 1651 (C═N stretch); ^{1}H NMR (DMSO-d_6) δ: 11.79 (s, 1H, CONH), 10.97 (s, 1H, indole NH), 8.15–7.81 (m, 8H, Ar–H), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ: 167.2, 156.9, 148.7, 127.4, 125.9, 124.0, 120.6, 111.3, 108.7 (benzofuran-2-carbohydrazide), 168.7, 155.9, 134.1, 132.8, 123.2, 117.4, 115.3, 110.7, 57.1 (5-methoxyindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{18}H_{13}N_3O_4$: 335.0906; found: 335.0911.

*6.1.7. N′-(5-Nitro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3g**)*

IR [KBr] ν_{max}: 3220 (NH stretch), 3056 (CH stretch) 1704 (C═O), 1677 (C═N stretch), 1520 (NO_2 stretch); ^{1}H NMR (DMSO-d_6) δ: 11.75 (s, 1H, CONH), 10.18 (s, 1H, indole NH), 7.91–7.02 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 165.7, 155.8, 148.7, 127.4, 124.9, 123.8, 120.5, 111.7, 108.1 (benzofuran-2-carbohydrazide), 169.7, 146.9, 142.3, 136.6, 124.9, 122.1, 120.0, 117.5 (5-nitroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}N_4O_5$: 350.0651; found: 350.0655.

*6.1.8. N′-(5-Hydroxy-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3h**)*

IR [KBr] ν_{max}: 3625 (OH stretch), 3194 (NH stretch), 3028 (CH stretch), 1710 (C═O), 1640 (C═N); ^{1}H NMR (DMSO-d_6) δ: 11.85 (s, 1H, CONH), 10.39 (s, 1H, indole NH), 7.91–7.02 (8H, m, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 167.5, 158.1, 151.0, 125.8, 123.9, 123.0, 120.6, 110.9, 108.2 (benzofuran-2-carbohydrazide), 169.7, 155.3, 135.9, 132.4, 121.9, 117.6, 116.9, 112.9 (5-hydroxyindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{11}N_3O_4$: 321.0750; found: 321.0756.

*6.1.9. N′-(7-Bromo-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3i**)*

IR [KBr] ν_{max}: 3185 (NH stretch), 3078 (CH stretch), 1710 (C═O), 1676 (C═N), 726 (C–Br stretch); ^{1}H NMR (DMSO-d_6) δ: 11.72 (s, 1H, CONH), 10.51 (s, 1H, indole NH), 7.72–7.35 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 164.9, 156.6, 149.3, 127.7, 124.4, 122.9, 119.8, 111.2, 108.1 (benzofuran-2-carbohydrazide), 169.5, 141.7, 135.9, 135.2, 129.1, 126.1, 123.9, 118.1 (7-bromoindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}BrN_3O_3$: 382.9906; found: 382.9911.

*6.1.10. N′-(7-Chloro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3j**)*

IR [KBr] ν_{max}: 3184 (NH stretch), 3067 (CH stretch), 1706 (C═O), 1683 (C═N); ^{1}H NMR (DMSO-d_6) δ: 11.95 (s, 1H, CONH), 10.62 (s, 1H, indole NH), 7.91–7.17 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 165.6, 156.3, 150.7, 127.7, 125.8, 123.9, 120.3, 110.7, 108.1 (benzofuran-2-carbohydrazide), 168.6, 138.7, 135.1, 133.9, 129.7, 126.1, 123.4, 118.0 (7-chloroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}ClN_3O_3$: 339.0411; found: 339.0416.

*6.1.11. N′-(7-Hydroxy-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3k**)*

IR [KBr] ν_{max}: 3249 (NH stretch), 3029 (CH stretch), 1713 (C═O), 1639 (C═N), ^{1}H NMR (DMSO-d_6) δ: 11.72 (s, 1H, CONH), 10.22 (s, 1H, indole NH), 7.84–7.11 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 165.7, 156.5, 149.8, 127.3, 125.1, 122.9, 120.5, 111.0, 108.1 (benzofuran-2-carbohydrazide); 169.0, 147.1, 137.0, 127.6, 124.3, 121.3, 118.0, 116.9 (7-hydroxyindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{11}N_3O_4$, 321.0750; found: 321.0756.

*6.1.12. N′-(7-Methyl-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3l**)*

IR [KBr] ν_{max}: 3195 (NH stretch), 3045 (CH stretch), 1724 (C═O), 1680 (C═N); ^{1}H NMR (DMSO-d_6) δ: 11.76 (s, 1H, CONH), 10.01 (s, 1H, indole NH), 7.71–7.03 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 167.8, 156.4, 149.4, 127.2, 124.1, 122.9, 119.7, 110.4, 107.8 (benzofuran-2-carbohydrazide), 169.4, 143.4, 134.1, 131.5, 128.1, 126.5, 124.1, 116.9, 19.4 (7-methylindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{18}H_{13}N_3O_3$: 319.0957 found: 319.0961.

*6.1.13. N′-(7-Methoxy-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3m**)*

IR [KBr] ν_{max}: 3278 (NH stretch), 3101 (CH stretch), 1712 (C═O), 1665 (C═N), ^{1}H NMR (DMSO-d_6) δ: 11.82 (s, 1H, CONH), 10.34 (s, 1H, indole NH), 8.21–7.72 (m, 8H, Ar–H), 3.89 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ: 167.1, 155.8, 149.5, 127.5, 125.0, 123.7, 120.5, 111.1, 108.3 (benzofuran-2-carbohydrazide), 169.6, 146.8, 134.9, 126.1, 125.2, 120.1, 117.9, 111.9, 56.7 (7-methoxyindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{18}H_{13}N_3O_4$: 335.0906; found: 335.0911.

*6.1.14. N′-(7-Ethoxy-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3n**)*

IR [KBr] ν_{max}: 3235 (NH stretch), 3081 (CH stretch), 1709 (C═O), 1671 (C═N), ^{1}H NMR (DMSO-d_6) δ: 11.82 (s, 1H, CONH), 10.34 (s, 1H, indole NH), 8.12–7.90 (m, 8H, Ar–H), 3.78 (q, 3H, OCH_2), 2.41 (t, 2H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 167.7, 156.2, 149.4, 127.1, 125.5, 124.2, 120.3, 111.0, 108.2 (benzofuran-2-carbohydrazide), 168.4, 148.2, 133.2, 127.1, 125.5, 121.8, 119.1, 117.0, 62.9, 16.9 (7-ethooxyindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{19}H_{15}N_3O_4$: 349.1063; found: 349.1069.

*6.1.15. N′-(7-Nitro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3o**)*

IR [KBr] ν_{max}: 3290 (NH stretch), 3064 (CH stretch), 1716 (C═O), 1658 (C═N), ^{1}H NMR (DMSO-d_6) δ: 11.65 (s, 1H, CONH), 10.45 (s, 1H, indole NH), 8.05–7.65 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 168.4, 158.1, 150.9, 127.1, 125.8, 124.1, 119.3, 110.5, 108.1 (benzofuran-2-carbohydrazide); 167.2, 143.3, 137.1, 133.9, 132.4, 126.9, 124.1, 117.1 (7-nitroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}N_4O_5$: 350.0651; found: 350.0656.

*6.1.16. N′-(7-Fluoro-2-oxoindolin-3-ylidene)benzofuran-2-carbohydrazide (**3p**)*

IR [KBr] ν_{max}: 3257 (NH stretch), 3058 (CH stretch), 1718 (C═O), 1666 (C═N); ^{1}H NMR (DMSO-d_6) δ: 11.98 (s, 1H, CONH), 10.72 (s, 1H, indole NH), 7.91–7.17 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 164.6, 154.2, 147.1, 126.8, 123.9, 122.5, 120.3, 110.8, 109.3 (benzofuran-2-carbohydrazide), 168.4, 161.4, 135.9, 125.1, 123.9, 122.0, 117.9, 115.8 (7-fluoroindoline-2,3-dione); HRMS (EI) m/z calcd for $C_{17}H_{10}FN_3O_3$: 323.0706 found: 323.0711.

7. Antimicrobial activity

All the test compounds were evaluated for the antibacterial activity against (Koneman et al., 1997; NCCLS, 2002) the Gram positive *S. aureus* (ATCC-25923) and *B. subtilis* (ATCC 6633); the Gram-negative bacteria *E. coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853); and for antifungal activity against two pathogenic fungi viz. *A. niger* and *C. albicans*. All the synthesized compounds were evaluated for antimicrobial activity against four bacterial and two fungal strains against norfloxacin and fluconazole as standard. Antimicrobial susceptibility testing was performed by the standardized disc diffusion and the agar dilution methods of the National Committee for Clinical Laboratory Standards. Inhibitory zone diameters were measured on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi, with conventional metrical filter paper discs (6 mm in diameter) containing specified doses of compounds.

7.1. Antibacterial testing

The Mueller Hinton Agar medium, the Petri-plates, filter paper discs and flask plugged with cotton were sterilized by autoclaving at 121 °C (151 lb/sq. inch). In each sterilized Petri plate (10 cm in diameter) about 30 mL of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, four discs of 6 mm diameter were placed on the medium which were previously dipped into the solution of test compounds which were prepared and labelled accordingly. The plates were kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 37 ± 1 °C for 24 h.

7.2. Antifungal testing

The Potato Dextrose Agar medium, the Petri-plates, filter paper discs and flask plugged with cotton were sterilized by autoclaving at 121 °C (151 lb/sq. inch). In each sterilized Petri plate (10 cm in diameter) about 30 mL of molten nutrient agar medium inoculated with respective strains of fungi was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, four discs of 6 mm diameter were placed on the medium which were previously dipped into the solution of test compounds which were prepared and labelled accordingly. The plates were kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 25 ± 1 °C for 24 h.

7.3. Minimum inhibitory concentration determination

The solution of the newly synthesized compounds and standard drugs was prepared at 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.98, 0.48, 0.24, 0.12 μg/mL concentrations in the wells of microplates by diluting in the liquid double

stranded nutrient broth. The bacterial suspensions used for inoculation were prepared of 105 cfu/mL by diluting fresh cultures at MacFarland 0.5 density (107 cfu/mL). Suspensions of the bacteria at 105 cfu/mL concentration were inoculated to the twofold diluted solution of the compounds. There were 104 cfu/mL bacteria in the wells after inoculations. Nutrient broth was used for diluting the bacterial suspension and for twofold dilution of the compound. DMSO, pure microorganisms and pure media were used as control wells. Ten microlitres bacteria inocula were added to each well of the micro dilution trays. The trays were incubated at 37 °C in a humid chamber and MIC endpoints were read after 24 h of incubation. For antifungal activity, same procedure was used. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported.

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