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SYNTHESIS AND CHARACTERISATION OF BIOLOGICALLY ACTIVE BENZOFURANYL INDOLINONES

K. M. Basavaraja*[1], Manjunatha Harihara Mathada[1], H. M. Naveena Kumari[1], Mahesh Kumar[1], K. T. Sudha[1]

[1]*Department of Chemistry/Industrial Chemistry, Vijayanagara Sri Krishnadevaraya University, Ballari, Karnataka, India.

ABSTRACT

In this article, we have synthesised 5-bromo-7-methoxy-*n*'-[(3*z*)-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene]-1-benzofuran-2-carbohydrazide (1) from 5-bromo-7-methoxy-1-benzofuran-2-carbohydrazide and indole-2, 3-dione in the presence of ethanol and glacial acetic acid. Then 5-Bromo-7-Methoxy-*N*'-[(3*z*)-2-Oxo-1, 2-Dihydro-3*h*-Indol-3-Ylidene]-1-Benzofuran-2-Carbohydrazide (1) was treated with secondary amines in the presence of formaldehyde and DMF to yield Benzofuranyl Indolinones. All the synthesised compounds were characterised by IR and NMR spectral data. Also all the compounds were screened for antibacterial (Cup-plate method) and antifungal activity (MIC method).

KEYWORDS

Benzofuran, Indolinone, Antibacterial activity and Antifungal activity.

Author for Correspondence:

Basavaraja K M,

Department of Chemistry/Industrial Chemistry,

Vijayanagara Sri Krishnadevaraya University,

Ballari, Karnataka, India.

Email: kmbasavaraja@gmail.com

INTRODUCTON

Nowadays the medicinal chemistry on newly synthesised benzofuran and their derivatives compounds becomes more progressive, and also researchers are finding different routes for synthesising biologically active benzofuran and their derivatives by the conventional and microwave methods. Manjunatha, *et al*[1]. Have synthesised biologically active benzofuran derivatives which contains methoxy and bromo group in the ring and these compounds exhibits promising antibacterial and antifungal activity for gram positive and gram negative microorganisms, which were compared with standard drugs.

Indolin-2, 3-dione is a major molecule for building potential bioactive agents, and its derivatives have shown the broad spectrum of bioactivity. Many of them were assessed anti-HIV, antiviral, antitumor, antifungal, anti-angiogenic, anticonvulsants, anti-Parkinson's disease therapeutic, and effective Sever Acute Respiratory Syndrome (SARS) corona virus 3CL protease inhibitor. These interesting properties prompting many researchers towards the synthesis and pharmacological screening of Indolin-2, 3-dione derivatives[2].

The analogues of 3-substituted-2-oxoindole show their evaluation as kinase inhibitors, anticancer and antiangiogenic agents[3]. Quantitative structure activity relationships (QSAR) of anti-cancer of isatin derivatives were discovered by multiple linear regressions (MLR) and genetic algorithm partial least squares (GA-PLS) methods[4]. The New 2-oxo-N'-(2-oxoindolin-5-substituted-3-ylidene)-2H-chromene-3-carbohydrazide Compounds exhibited good antiproliferative profile against colon HT-29, leukemia K562 and breast MDA-MB-468 cell lines. Additionally, these compounds displayed a moderate inhibitory activity for side population (SP) colon HT-29 cancer stem cells[5]. The newly synthesised two series of isatin-3'-oxime- and isatin-3'-methoxime-based hydroxamic acids were exhibits as histone deacetylase inhibitors and antitumor agents[6]. A series of Schiff bases Gyrase inhibitory activity antitubercular activity and pharmacophoric model building[7,8]. Of indoline-2, 3-dione were synthesized and investigated for their *Mycobacterium Tuberculosis* (Mtb) DNA.

Some of newly synthesised isatin derivatives delivered antioxidant activity and β-amyloid aggregation inhibitory activity in a decent manner[9,10]. Also, a series of novel 5-substituted-1-(arylmethyl/ alkylmethyl)-1H-indole-2, 3-dione-3-(N-hydroxy/methoxy thiosemicarbazone) analogues were evaluated for their anti-HIV activity and anti-tubercular activity in both log phase and starved cultures[11].

The Synthesis and biological evaluation of some new oxadiazole and pyrazole derivatives incorporating benzofuran moiety have shown moderate to good microbial inhibition for different concentrations[12]. Also DNA cleavage activity of the compounds 3-(7-methoxy-benzofuran-2-yl)-5-aryl-*4H*-[1, 2, 4] triazoles) have shown that, the untreated DNA does not show any cleavage (Lane-C), whereas all the compounds have exhibited cleavage activity on DNA[13].

MATERIAL AND METHODS

All chemicals and reagents used in the reactions were analytical grade and used without further purification. The purification of the synthesized compounds was performed by recrystallization with appropriate solvent system. Melting points of all the synthesized compounds were determined in open capillary tube and were found uncorrected. The synthesised products were characterized using ^{1}H NMR, FT-IR and The IR spectra were recorded on FTIR-8400 Perkin Elmer spectrophotometer using KBr disks. ^{1}HNMR were recorded on BRUKER 400 MHz using CDCl3 solvent with TMS as internal standard.

Synthesis of 5-bromo-7-methoxy-*N*'-[(3*z*)-2-oxo-1, 2-dihydro-3*h*-indol-3-ylidene]-1-benzofuran-2-carbohydrazide (1)

An equimolar mixture of 5-bromo-7-methoxy-1-benzofuran-2-carbohydrazide (0.01 mol) and (isatin) indole-2, 3-dione (0.01 mol) in ethanol (30mL) containing few drops of glacial acetic acid was refluxed on a water bath for 2 h. Crystalline solid separated on cooling was filtered and recrystallised from absolute ethanol. (Scheme No.1). Colour yellow, Yield 81%, melting point 199^{o}C. Molecular formula $C_{18}H_{12}BrN_3O_4$, IR (ν_{max}, cm-1) 3440(-NH), 1712 (C=O indolinone), 1675 (C=O Carbohydrazide), ^{1}H-NMR (400 MHz, CDCl3): δ 1.23 (s, 1H), 2.15 (s, 1H), 4.06 (s, 3H), 6.89-6.90 (m, 3H), 7.35-7.36 (m, 2H).

Synthesis of 5-bromo-7-methoxy-N-[1-(morpholinomethyl)-2-oxoindolin-3-ylidene] benzofuran-2-carbohydrazide (2a), 5-bromo-7-methoxy-N-[2-oxo-1-(piperidin-1-yl-methyl) indolin-Ylidene] benzofuran-2-carbohydrazide (2b) and 5-bromo-N-[1-(dicyclohexylaminomethyl)-2-oxoindolin-3-ylidene]-7-methoxybenzofuran-2-carbohydrazide (2c)

The compound 1 (0.01mol) was suspended in a minimum quantity of dimethylformamide (10mL). To this solution, slightly more than 0.01 mol of formaldehyde and corresponding secondary amines (Morpholine, Piperidine, Diphenylamine) (0.01 mol) was added with vigorous stirring. The reaction mixture was heated on a water bath for 35 min. and kept it overnight. The product thus obtained was purified by recrystallisation from absolute ethanol.

5-Bromo-7-methoxy-N-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene) benzofuran-2-carbohydrazide (2a)

Colour yellow, Yield 84%, mp 188^{o}C, Molecular Formula $C_{24}H_{23}BrN_4O_5$, IR (ν_{max}, cm-1) 3440(-NH), 1705 (C=O indolinone), 1580 (C=O Carbohydrazide), 2926 (CH_2), 1H-NMR (400 MHz, CDCl3): δ 1.23 (s, 2H), 1.53 (s, 4H), 2.00 (s, 1H), 2.15-0.00 (m, 1H), 3.83 (s, 3H), 4.10 (d, J = Hz, 2H), 6.35 (d, J = 10.80 Hz, 1H), 6.51 (d, J = 12.80 Hz, 1H), 6.74 (d, J = 7.20 Hz, 1H), 6.93 (d, J = 1.60 Hz, 1H), 7.09-7.10 (m, 1H), 7.24 (s, 2H).

5-Bromo-7methoxy-N-(2-Oxo-1-(Piperidin-1-Ylmethyl) Indolin-Ylidene) Benzofuran-2-Carbohydrazide (2b)

Colour brown, Yield 86%, mp 194^{o}C, Molecular Formula $C_{25}H_{25}BrN_4O_4$, IR (ν_{max}, cm-1) 3430(-NH), 1675 (C=O indoline), 1533 (C=O Carbohydrazide), 2984 (CH_2); 1H-NMR (400 MHz, CDCl3): δ 1.45 (m, 10H), 2.00 (s, 1H), 2.57 (s, 2H), 3.83 (s, 3H), 4.10 (d, J = Hz, 2H), 6.33 (d, J = 10.80 Hz, 1H), 6.55 (d, J = 12.80 Hz, 1H), 6.73 (d, J = 7.20 Hz, 1H), 6.92 (d, J = 1.60 Hz, 1H), 7.14-7.26 (m, 1H), 7.20 (s, 2H).

5-Bromo-N-(1-((Dicyclohexylamino) Methyl)-2-Oxoindolin-3-Ylidene)-7-Methoxybenzofuran-2-Carbohydrazide (2c)

Colour white, Yield 89%, mp 175^{o}C, Molecular Formula $C_{31}H_{35}BrN_4O_4$, IR (ν_{max}, cm-1) 3438(-NH), 1710 (C=O indolinone), 1590 (C=O Carbohydrazide), 2954 (CH_2); 1H-NMR (400 MHz, CDCl3): δ 1.21-1.49 (m, 12H), 2.57 (dd, 2H), 3.83 (s, 3H), 4.1 (d, J = Hz, 2H), 6.31 (d, J = 10.80 Hz, 1H), 6.53 (d, J = 12.80 Hz, 1H), 6.67 (d, J = 7.20 Hz, 1H), 7.00 (d, J = 1.60 Hz, 1H), 7.21-7.28 (m, 1H), 7.19 (s, 2H).

ANTIBACTERIAL ACTIVITY

Method of testing: Cup-Plate Method

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antibiotic.

A previously liquefied medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms between 40-50^{o}C and the inoculated medium was poured into Petri dishes to give a depth of 3 to 4 mm. ensuring that the layers of medium were uniform in thickness by placing the dishes on a levelled surface.

The Petri dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out the set agar in each Petri dish (three cups were numbered for the particular compounds, solvent, and a standard) using sterile pipettes, the standard (10µg/well) and the sample of known concentration (10mg/ml) were fed into the bored cups. The dishes were left standing for 2 h. At room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24 h at 37^{o}C. The zone of inhibition developed, if any, was then accurately

measured and recorded. Each zone of inhibition recorded was an average of three measurements. Zone of inhibition for DMF was done separately.

The antimicrobial activities of different extracts and fractions were compared with standard antibacterial agent *Penicillin* and *Streptomycin*. The zone of inhibition was calculated by measuring the minimum dimensions of the zone of no bacteria. The results are recorded in Table No.1.

ANTIFUNGAL ACTIVITY

The antifungal activities of the synthesized compounds were performed against standard fungal strains *S. aureus, E. coli,* in DMF by broth micro dilution method. The MIC determination of the tested compounds was investigated in comparison with *Griseofulvin* by broth micro dilution method. Double dilutions of the test compounds and reference drugs were prepared in Sabouraud's dextrose broth. 10 mg of each test compounds were dissolved in 1mL of DMF separately to prepare the stock solution. Further progressive dilutions with Sabouraud's dextrose broth were performed to obtain the required concentrations of 100, 50, 25, 10µg/mL. The Petri dishes were inoculated with 1-5 x 10^4 colonies forming units (cfu/mL) and incubated at 25^{o}C for 48-72 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound that yield no visible growth on the plates.

To ensure that the solvent had no effect on the fungal growth, a control was performed with the test medium supplemented with DMF at the same dilutions as used in the experiments. The results are incorporated in Table No.2.

RESULTS AND DISCUSSION

The synthesised compounds were confirmed by IR and NMR data. Further the synthesised compounds were screen for antibacterial and antifungal activity to the concentrations 50µg/mL and 100µg/mL. The compound 3 and 4 shows excellent antibacterial activity against the three organisms *S. aureus, E.coli, P. aureginosa* which are compared with the standard drugs *penicillin* and *streptomycin* to the concentrations 50µg/mL and 100µg/mL. And the antibacterial activity of the compounds 1 and 2 were prominent to the concentrations 50µg/mL and 100µg/mL against all the organisms. (Figure No.1 and No.2). The antifungal activity of the all the synthetic compounds exhibits predominant activity against the organisms *S. Aureus, and E. Coli,* to the concentrations 50µg/mL and 100µg/mL, these synthesised compounds were compared with standard drug *Griseofulvin* which is depicted in Figure No.3 and Figure No.4.

Table No.1: Antibacterial activity of synthesized Compounds

S.No	Compound	Zone of inhibition (in mm)					
		S. aureus		*E. coli*		*P. aureginosa*	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1	IS 01	14	16	12	14	13	15
2	IS 02	13	15	11	15	14	16
3	IS 03	14	17	13	16	12	14
4	IS 04	15	18	14	17	13	18
Standard							
5	*Penicillin*	15	21	16	20	17	23
6	*Streptomycin*	24	28	22	27	21	26
Control							
7	DMF	Nil	Nil	Nil	Nil	Nil	Nil

Table No.2: Antifungal activity of synthesised Compounds

S.No	Compound	Zone of inhibition (in mm)			
		S. aureus		*E. coli*	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1	IS 01	11	15	12	16
2	IS 02	13	16	11	15
3	IS 03	10	14	12	15
4	IS 04	13	17	12	18
Standard					
5	*Griseofulvin*	21	26	22	27
Control					
6	DMF	Nil	Nil	Nil	Nil

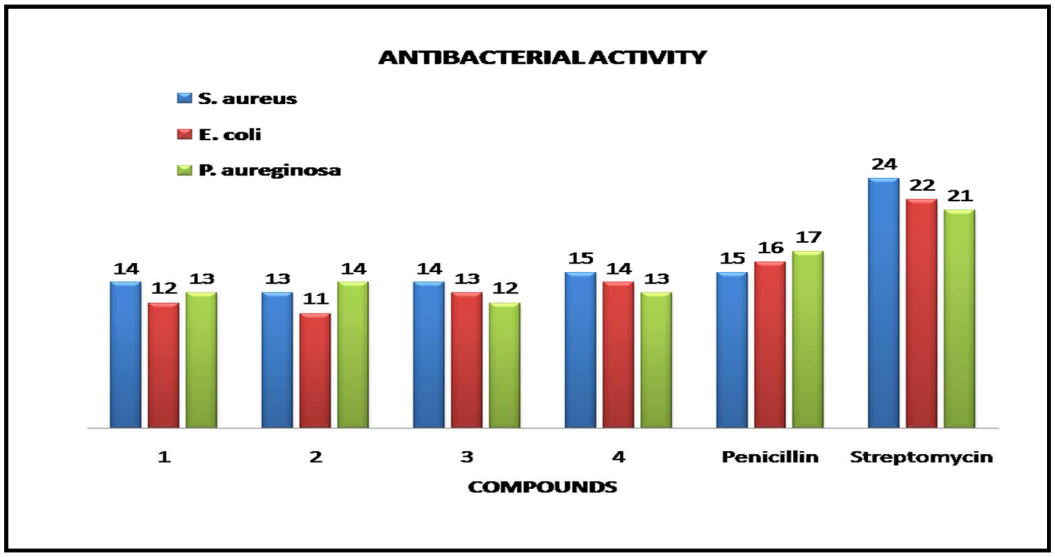


Figure No.1: Antibacterial activity of the Compounds 1, 2, 3, 4 using the organisms *S. aureus, E.coli,* and *P.aureginosa* (50 µg/ml), which are compared with standard drug *penicillin* and *streptomycin*

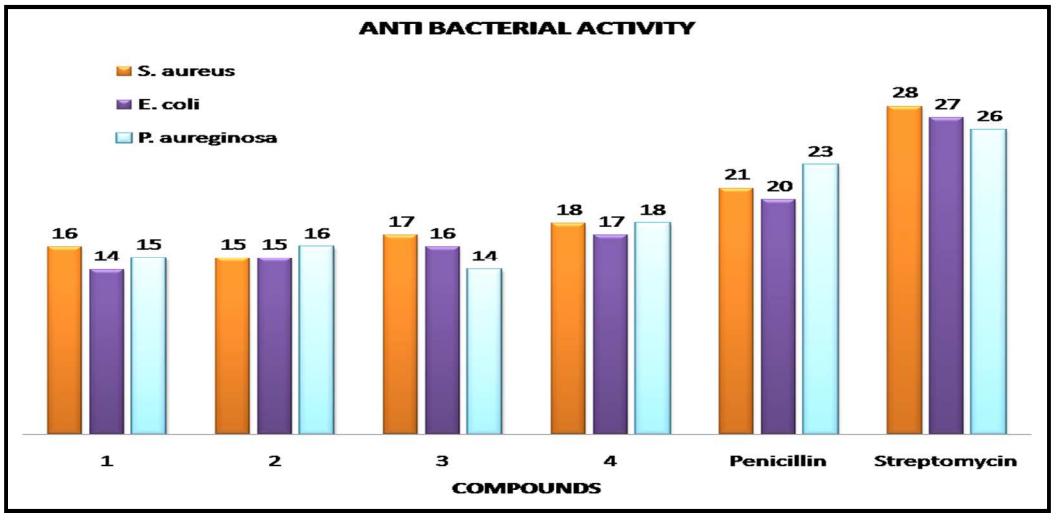


Figure No.2: Antibacterial activity of the Compounds 1, 2, 3, 4 using the organisms *S. aureus, E.coli,* and *P.aureginosa* (100 µg/ml), which are compared with standard drug *penicillin* and *streptomycin*

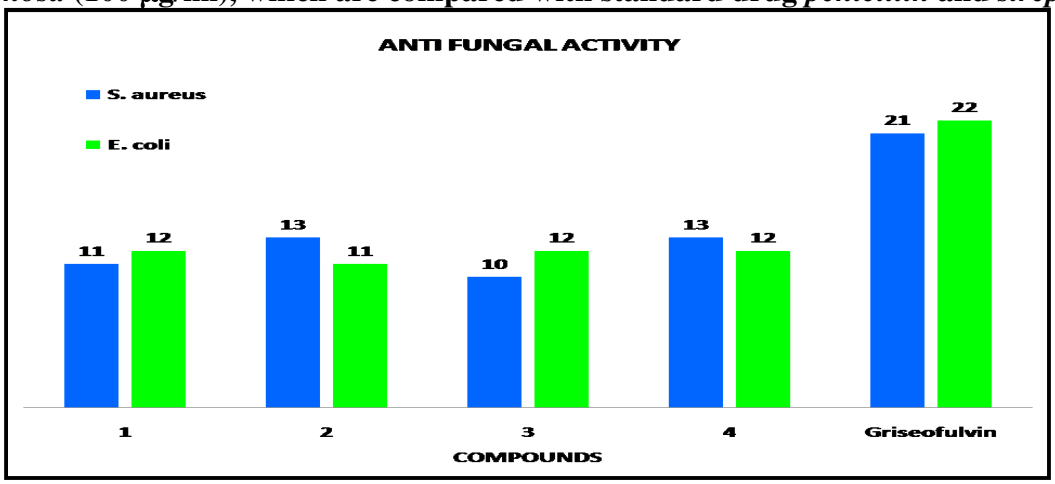


Figure No.3: Antifungal activity of the Compounds 1, 2, 3, 4 using the organisms *S.aureus and E.coli*, (50 µg/ml), which are compared with standard Drug *Griseofulvin*

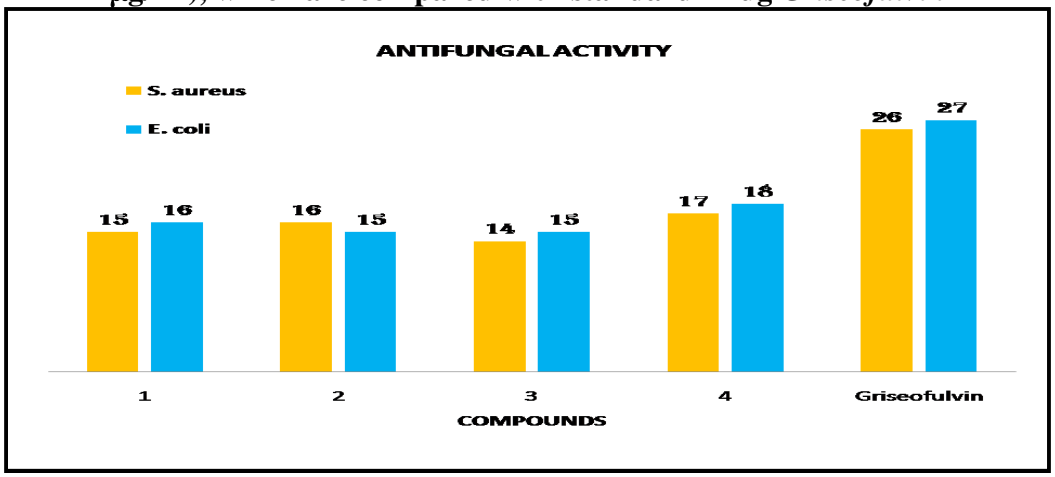
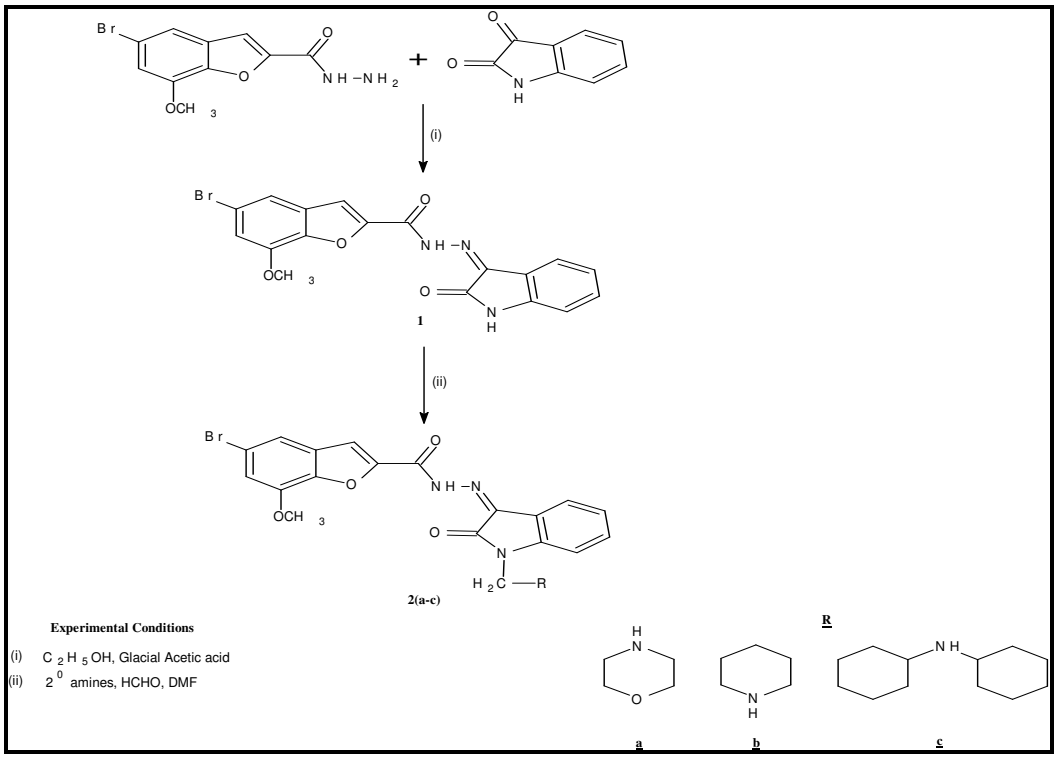


Figure No.4: Antifungal activity of the Compounds 1, 2, 3, 4 using the organisms *S.aureus and E.coli,* (100 µg/ml), which are compared with standard Drug *Griseofulvin*



Scheme No.1: Synthetic route of the titled compounds

CONCLUSION

In the conclusion it can stated that the, all the synthesised benzofurany indolinones exhibits moderate to high antibacterial and antifungal activity against all the organisms and which are compared with the standard drug. And these newly synthesised compounds are in concurrent with the assigned structures which are confirmed by IR and NMR spectral data.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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