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Original Article

Antimicrobial and anti-tubercular activity of some new indole derivatives

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ABSTRACT

Isatin, an indole derivative, is a versatile compound and has a diversity of applications. In a present investigation, a series of new isatin derivatives **(3a-i)** were prepared by condensing substituted isatins with benzoyl hydrazides. We designed to investigate new isatin derivatives with the inhibitory effect on bacteria and fungi. The chemical structures of the synthesized compounds were affirmed on the basis of modern analytical technique. The title compounds were evaluated for antimicrobial activity by cup plate method against some selected gram (+) gram (-) bacterial strains, and fungal strains. Preliminary screening was carried out for all the compounds and potent compounds were further evaluated for MIC. Anti-tubercular activity was also determined against *Mycobacterium tuberculosis* strain $H_{37}Rv$. The results are promising and show that the compounds are biologically active.
Key words: Oxindole, hydrazide, antibacterial, antifungal, TB.

INTRODUCTION

Overtime antibacterial infection generates severe defects in cell mediated immunity and brings to profound depletion of CD4 T-lymphocytes (T-cells) causing infection like tuberculosis (TB), bacterial, fungal and finally leads to death. According to world health organization (WHO), TB is the leading infectious disease among humans and world's one third population is being infected with mycobacterium tuberculosis [1]. Less developed countries are mostly exist with TB while in other parts of the world does it very often.
Isatin is 1*H*-indole-2,3-dione or an indole derivative. It has been a much known compound for about hundred years and is found in a variety of heterocyclic compounds with potent biological effects in mammals [2-5]. Synthetic isatin derivatives have been reported to show numerous pharmacological actions including antibacterial, antifungal, antiviral, anticonvulsant and anti-HIV activities [3-16]. The hydrazide derivatives of substituted isatins show potential antibacterial, antifungal and anticancer activities [17-18]. All concerned reports encouraged us to study some newer isatin derivatives as antimicrobial including antitubercular agents.

MATERIALS AND METHODS

Chemistry: Melting points were determined in open capillary tubes on Thomas-Hoover melting point apparatus, and are uncorrected. The purity of derivatives was checked by Thin layer chromatography (TLC) by using silica gel G coated glass plates taking mobile phase $CHCl_3$:CH_3OH (9:1). The spots were visualized in UV chamber or exposure to iodine vapors. IR spectra (in KBr) were recorded on a Jasco 460 FTIR spectrophotometer. ^{1}H-NMR spectra (DMSO/$CDCl_3$) were taken on a 400 MHz Bruker spectrometer and LCMS were entrusted on LCMS-2010A Shimadzu. All the compounds have presented satisfactory spectral/chemical results.

Synthesis of 1-(4-substituted benzyl)-5-substituted-indoline-2,3-dione (2): A mixture of 5-substituted-indoline-2,3-dione (substituted isatin) (**1**) (0.005 mol), 4-substituted benzyl chloride (equimolar; 0.005 mol), potassium carbonate (2.0 gm) and dimethyl formamide (DMF) (20 ml) was refluxed for 2h in a round bottom flask. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into 100 ml of ice cold water. A precipitate separated

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out which was filtered, washed with water, dried and recrystallized from ethanol-water mixture to furnish TLC pure compound **2**.

General procedure for synthesis of *N*[1]-[1-(4-substituted benzyl)-5-substituted-2-oxoindolin-3-ylidene]-4-substituted-benzohydrazide (3a-i): Compound (**2**) (0.005 mol) and substituted benzoyl hydrazide (equimolar; 0.005 mol) were dissolved in 50 ml of ethanol. Glacial acetic acid (4-5 drops) was added and the reaction mixture refluxed on a water bath for 2-4 h. After completion of the reaction, it was kept at room temperature for approximately 30 minutes, the colored solution slowly changed in to some feathery solid crystals, the solid product was segregated by filtration and recrystallized with ethanol:chloroform.

N[1]-(1-benzyl-2-oxoindolin-3-ylidene)-4-bromobenzohydrazide **(3a)**: Yield 71%; yellow fluffy solid; m.p 208-210 ºC; IR (KBr): ν/cm^{-1}; 3247 (NH), 3098 (CH, Ar), 2855 (CH, Ali), 1708 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.91 (s, 2H, CH_2), 6.72-7.98 (m, 13H, Ar-H), 14.02 (s, 1H, NH).

N[1]-(1-benzyl-5-chloro-2-oxoindolin-3-ylidene)-4-bromobenzohydrazide **(3b)**: Yield 74%; dark yellow fluffy solid; m.p 214 ºC; IR (KBr): ν/cm^{-1}; 3253 (NH); 3086 (CH, Ar); 2917 (CH, Ali); 1704 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.83 (s, 2H, CH_2), 6.86-8.12 (m, 12H, Ar-H), 13.96 (s, 1H, NH); MS: 469 (M^+).

N[1]-(1-benzyl-5-methyl-2-oxoindolin-3-ylidene)-4-bromobenzohydrazide **(3c)**: Yield 68%, pale yellow fluffy solid, m.p 215-217 ºC, IR (KBr): ν/cm^{-1}; 3294 (NH); 3057 (CH, Ar); 2891 (CH, Ali); 1703 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.33 (s, 3H, CH_3), 4.86 (s, 2H, CH_2), 6.63-8.06 (m, 12H, Ar-H), 14.05 (s, 1H, NH); MS: 449 (M^+).

N[1]-[1-(4-chlorobenzyl)-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3d)**: Yield 78%, yellow fluffy solid, m.p 252-253 ºC, IR (KBr): ν/cm^{-1}; 3360 (NH); 3075 (CH, Ar); 2846 (CH, Ali); 1696 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.92 (s, 2H, CH_2), 6.74-7.98 (m, 12H, Ar-H), 14.02 (s, 1H, NH)

N[1]-[1-(4-chlorobenzyl)-5-chloro-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3e)**: Yield 82%, dark yellow crystalline solid, m.p 238-40 ºC, IR (KBr): ν/cm^{-1}; 3357 (NH); 3070 (CH, Ar); 2912 (CH, Ali); 1707 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.88 (s, 2H, CH_2), 6.81-7.99 (m, 11H, Ar-H), 13.89 (s, 1H, NH); MS: 502 (M^+).

N[1]-[1-(4-chlorobenzyl)-5-methyl-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3f)**: Yield 76%, pale yellow fluffy solid, m.p 220 ºC, IR (KBr): ν/cm^{-1}; 3348 (NH); 3046 (CH, Ar); 2991 (CH, Ali); 1668 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.28 (s, 3H, CH_3), 4.75 (s, 2H, CH_2), 6.58-8.16 (m, 11H, Ar-H), 13.97 (s, 1H, NH).

N[1]-[1-(4-methylbenzyl)-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3g)**: Yield 66%, creamy fluffy solid, m.p 228-30 ºC, IR (KBr): ν/cm^{-1}; 3351 (NH); 3085 (CH, Ar); 2928 (CH, Ali); 1695 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.25 (s, 3H, CH_3), 4.91 (S, 2H, CH_2), 6.75-7.98 (m, 11H, Ar-H), 14.01 (s, 1H, NH); MS: 449 (M^+).

N[1]-[1-(4-methylbenzyl)-5-chloro-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3h)**: Yield 75%, yellow fluffy solid, m.p 210 ºC, IR (KBr): ν/cm^{-1}; 3356 (NH); 3084 (CH, Ar); 2929 (CH, Ali); 1696 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.27 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.76-7.99 (m, 11H, Ar-H), 14.01 (s, 1H, NH); MS: 483 (M^+).

N[1]-[1-(4-methylbenzyl)-5-methyl-2-oxoindolin-3-ylidene]-4-bromobenzohydrazide **(3i)**: Yield 71%, creamy crystalline solid, m.p 194-196 ºC, IR (KBr): ν/cm^{-1}; 3352 (NH); 3073 (CH, Ar); 2976 (CH, Ali); 1697 (C=O); ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.21 & 2.36 (s, each, 6H, 2xCH_3), 4.85 (s, 2H, CH_2), 6.47-7.97 (m, 11H, Ar-H), 14.03 (s, 1H, NH).

MICROBIOLOGY

In vitro antimicrobial activity: All newly synthesized isatin derivatives were evaluated of antimicrobial activity for three gram positive bacterial strains, *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 9372), *Streptococcus pyrogens* (ATCC 19615) gram negative bacterial strains, *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 3882) and fungal strains, *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231), *Trichoderma viridae* (IAM 5061) in the nutrient agar medium for bacteria and in sabouraud agar medium for fungi by cup plate method [19]. For bacteria and fungi ampicillin and ketoconazole were used as standard drugs. Initial screening of isatin derivatives and standard drugs was carried out at fixed concentration of 1000 µg/ml. The zone of inhibition was taken in record by measuring the diameter in millimeter (mm) after 24 h for bacteria and 72 h for fungi. Measurements of results are shown in **Table 2**.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration of influential derivatives against all bacterial and fungal strains was ascertained by liquid dilution method [19]. Stock solutions of resultant compounds along with 2.5, 0.5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml concentrations were prepared with suitable solvent. The concentrations of standard drugs Ampicillin and ketoconazole were prepared in the same concentrations and solvent. Bacterial and fungal strains inoculums were also prepared. Test tubes, to a series, containing 1 ml each of isatin derivative solution with varied concentrations, 0.2 ml of the inoculums, and 3.8 ml of the sterile water were added to each of the test tube. To find out the presence of turbidity, these test tubes were incubated for 24 h. same treatment was adopted for remaining derivatives with standard drugs for comparison. The growth of the microorganism in tubes was determined visually and the minimum concentration, at which no growth was seen, called minimum inhibitory concentration (MIC) and was considered as the MIC values. MIC values (µg/ml) for powerful derivatives and standard drugs against all experimented strains are summarized in the **Table 3**.

Anti-mycobacterial activity: Potent derivatives which have revealed moderate antifungal and antibacterial activity with comparison of standard drugs were further screened for their in vitro antimycobacterial activity. Using BACTEC 12B Medium, Microplate Alamar Blue Assay (MABA), initial screening of all potent derivatives against *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) had been performed at fixed concentration of 6.25 µg/ml [20,21]. Stock solutions of final derivatives were prepared in DMSO. Minimum inhibitory concentration (*MIC*) was detected visually and explained as the minimum concentration that checked a color alteration. The least drug concentration produces effect in an inhibition more than 90% was taken in consideration the MIC. The inhibition percentage of bacterial growth at 6.25µg/ml of all the screened synthesized derivatives along with the MIC of standard drugs was presented in **Table 4**. Isoniazid and gentamicin were taken as standard drugs for comparison.

RESULTS AND DISCUSSION

Chemistry: The synthesis of title compounds is on account of the potent biological activity of indole and carried out using a simple, straight and general pathway. 5-Substituted-indoline-2,3-dione (substituted isatins) was used basic material for the synthesis of title compounds. The physiochemical parameters of the resultant compounds are shown in **Table 1**. The treatment of 5-substituted-indoline-2,3-dione (**1**) with 4-substitutedbenzyl chloride in DMF in presence of Na_2CO_3 yielded 1-(4-substituted benzyl)-5-substituted-indoline-2,3-dione (**2**). Different benzoyl hydrazides were reacted with compound (**2**) in ethanol in presence of glacial acetic acid furnished the title compounds; N^1-[1-(4-substituted benzyl)-5-substituted-2-oxoindolin-3-ylidene]-4-substituted-benzo hydrazide (**3a-i**). The steps involved in the synthesis are depicted in **Scheme 1**. The structure elucidation of final derivatives was done by IR, ^{1}H-NMR and Mass spectroscopy. IR spectral peaks of the compound were recognized from 1708-1695 cm^{-1} for C=O stretching, 3360-3247 cm^{-1} for N-H stretching, 3086-2846 cm^{-1} for C-H aliphatic and aromatic. In ^{1}H-NMR spectra typical proton signals for CH_3, CH_2, aromatic and N-H were observed around δ 2.2, 4.9, 6.6-8.0, 14, respectively. Mass spectra revealed molecular ion peaks in reasonable intensity.

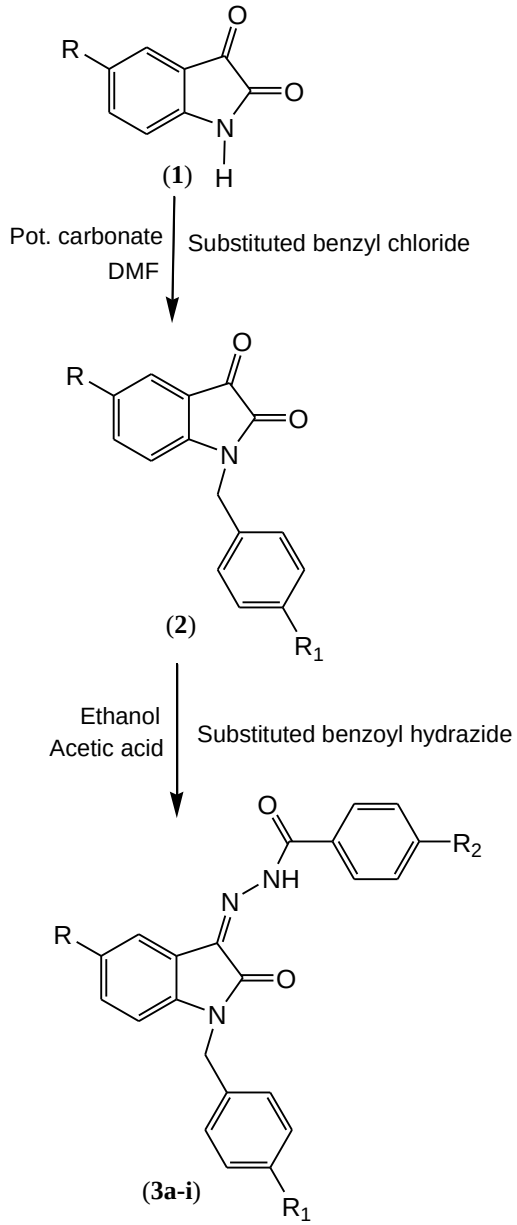
Antimicrobial activity: All the final synthesized derivatives were screened for their antibacterial activity by cup plate method, in the nutrient agar medium against 3 Gm+ and 3 Gm- bacterial strains at the concentration of 1000µg/ml. The zone of inhibition (mm) of each derivative was ascertained and compared with ampicillin taken as standard drug for bacteria. DMSO was used to prepare stock solutions of tested derivatives. The findings of antibacterial evaluation revealed that most of the derivatives have variable activity against bacterial strains. Compounds **3a, 3c, 3e** and **3f** are the best active derivatives which present excellent activity against the bacteria in comparison to standard drug ampicillin. All the final compounds were examined for antifungal activity using cup plate method, in the sabouraud agar medium against three pathogenic fungal strains **(Table 2).** The area of inhibition (mm) of each derivative was ascertained and compared with standard drug ketoconazole. The compounds **3a, 3c, 3e** were found active derivatives against the fungal strains used. These potent derivatives were taken forward to determine MIC value by liquid dilution method. The comparison of the MIC (µg/ml) of powerful derivatives and standard drugs against the test strains are shown in **Table 3**.

Antitubercular activity was performed only on potent derivatives, stock solution of test derivatives were prepared in DMSO. All the potent derivatives evaluated at 6.25µg/ml, present the percentage of inhibition varying from 38 to 70%. Compound **3c** comes forth as most active analogue with 70% inhibition against *M. tuberculosis* H37Rv, in comparison with standard drugs isoniazid and

gentamycin **(Table 4)**. The findings show that isatin derivative with bromo and methyl group **(3c)** revealed comparable activity with the standard drugs.

CONCLUSION

A series of nine compounds **(3a-i)** was prepared starting from 5-substituted-indoline-2,3-dione (substituted isatins) by simple and easy methods, and characterized by physico-chemical and spectral analysis. All the synthesized compounds were evaluated for their in vitro antimicrobial activity against different fungal and bacterial strains. The active compounds were further evaluated for their antitubercular activity. Antimicrobial activity results showed that compounds **3a, 3c, 3e, 3f** were good against gram positive and gram negative bacteria, compounds **3a, 3c, 3e** exhibited potent antifungal activity, whereas compound **3c** was highly active against *M. tuberculosis* H37Rv. It is conceivable that further derivatization of these compounds could result potential antimicrobial agents.



Scheme 1: Protocol for synthesis of title compounds (3a-i).

Table 1: Physico-chemical data of the title compounds (3a-i).

Compd.	R	R_1	R_2	Mol. Formula	R_f value
3a	H	H	Br	$C_{22}H_{16}N_3O_2Br$	0.62
3b	Cl	H	Br	$C_{22}H_{15}N_3O_2ClBr$	0.74
3c	CH_3	H	Br	$C_{23}H_{18}N_3O_2Br$	0.79
3d	H	Cl	Br	$C_{22}H_{15}N_3O_2ClBr$	0.76
3e	Cl	Cl	Br	$C_{22}H_{14}N_3O_2Cl_2Br$	0.71
3f	CH_3	Cl	Br	$C_{23}H_{14}N_3O_2Cl_3$	0.66
3g	H	CH_3	Br	$C_{23}H_{18}N_3O_2Br$	0.73
3h	Cl	CH_3	Br	$C_{23}H_{17}N_3O_2ClBr$	0.68
3i	CH_3	CH_3	Br	$C_{24}H_{20}N_3O_2Br$	0.71

Table 2: Zone of inhibition of title compounds (3a-i) against different bacterial and fungal strains.

Compd.	Zone of inhibition/mm @1000µg/ml								
	Fungi			Gram positive bacteria			Gram negative bacteria		
	A. niger	*T. viridae*	*C. albicans*	*S. a*	*S. p*	*B. s*	*S. t*	*K. pn*	*E. coli*
3a	43	39	37	45	31	36	44	40	36
3b	14	12	10	21	20	12	21	20	11
3c	42	40	36	46	34	34	43	43	37
3d	16	18	15	16	22	10	22	12	14
3e	45	37	38	48	33	36	42	42	37
3f	40	38	40	44	32	35	42	41	39
3g	41	39	35	45	31	35	41	41	37
3h	20	18	14	16	14	14	14	16	18
3i	42	40	35	43	30	34	43	43	36
Std.	45[a]	42[a]	40[a]	50[b]	35[b]	40[b]	45[b]	45[b]	40[b]

[a] ketoconazole, [b] Ampicillin

Table 3: MIC values of potent title compounds and standard drugs.

Compd.	MIC @µg/ml								
	Fungi			Gram positive bacteria			Gram negative bacteria		
	A. niger	*T. viridae*	*C. albicans*	*S. a*	*S. p*	*B. s*	*S. t*	*K. pn*	*E. coli*
3a	20	25	30	15	35	25	10	20	20
3c	20	20	35	15	25	30	15	15	20
3e	15	30	30	10	30	25	15	15	20
3f	25	25	25	15	35	30	15	20	15
3g	25	25	35	15	35	30	20	20	20
3i	20	20	35	20	35	30	15	15	20
Std	15[a]	20[a]	25[a]	10[b]	25[b]	20[b]	10[b]	10[b]	15[b]

[a] ketoconazole, [b] Ampicillin

Table 4: Anti-tubercular activity of potent compounds and standard drugs against $H_{37}Rv$ strain.

Compd.	Concentration (µg/ml)	Percentage inhibition
3a	6.25	50
3c	6.25	70
3e	6.25	54
3f	6.25	47
3g	6.25	65
3i	6.25	59
Isoniazid	0.031	95
Gentamycin	6.0	99

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