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STUDIES ON ANTIMICROBIAL POTENTIAL OF NEW ISATIN DERIVATIVES

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ABSTRACT

In a present investigation, a series of new isatin derivatives (**3a-i**) were prepared by condensing substituted isatins with benzoyl hydrazides. The structures of the synthesized compounds were established on the basis of modern analytical techniques. The title compounds were evaluated for antimicrobial activity 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 by MABA against *Mycobacterium tuberculosis* strain H_{37}Rv. The findings are interesting and show that the compounds are biologically active.

KEYWORDS: Oxindole, hydrazide, antibacterial, antifungal, antitubercular activity.

INTRODUCTION

Tuberculosis (TB) is one of the most prevalent communicable infectious diseases on earth. TB exists the largest infectious disease which causes high mortality in human being and causes 3 million casualties every year, in other words we can say five casualties every minute. TB is in the top three causes of death from a single infectious agent, with malaria and AIDS. Approximately 8 to 10 million people get infected every year by this pathogen. In India about 5,00,000 deaths occurring annually due to TB. Seeing the serious situation World Health Organization (WHO) in 1993 announced TB a universal emergency. A report from

WHO shows that the total number of new cases of TB around the world has increased rapidly in recent years.[1]

In our world, heterocyclic compounds containing nitrogen come in the important categories of compound in the field of medicinal chemistry due to their potential applications as biologically as well industrially.[2] The biologically active chemical, isatin is generated by a strain *Alteromone,* dwelling the surface of unborn off-spring of the cardiean shrimp *palaemon macrodectylus* and reported to have antifungal activity.[3] Synthetic isatin derivatives have been reported to show numerous pharmacological actions[4-15] including antibacterial, anti viral, anti HIV and anti cancer activities. The hydrazide derivatives of substituted isatins show potential antibacterial, antifungal and anticancer activities.[14,15]

In view of these points, it was thought worthwhile to study some newer isatin derivatives as antimicrobial including antitubercular agents.

MATERIALS AND METHODS

Experimental

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 chemical analysis.

Synthesis of 5-substituted-1-(4'-substituted benzyl)-2,3-dihydro-2,3-dioxindole (2)

A mixture of 5-substituted-1*H*-indole-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 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 5-substituted-1-(4'-substituted benzyl)-2,3-dihydro-2,3-dioxindole-3-(4'-substituted benzoyl hydrazide (3a-i)

Substituted benzyl isatin (**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.

BIOLOGICAL STUDIES

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.[16] 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 3**.

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.[16] 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, 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 4**.

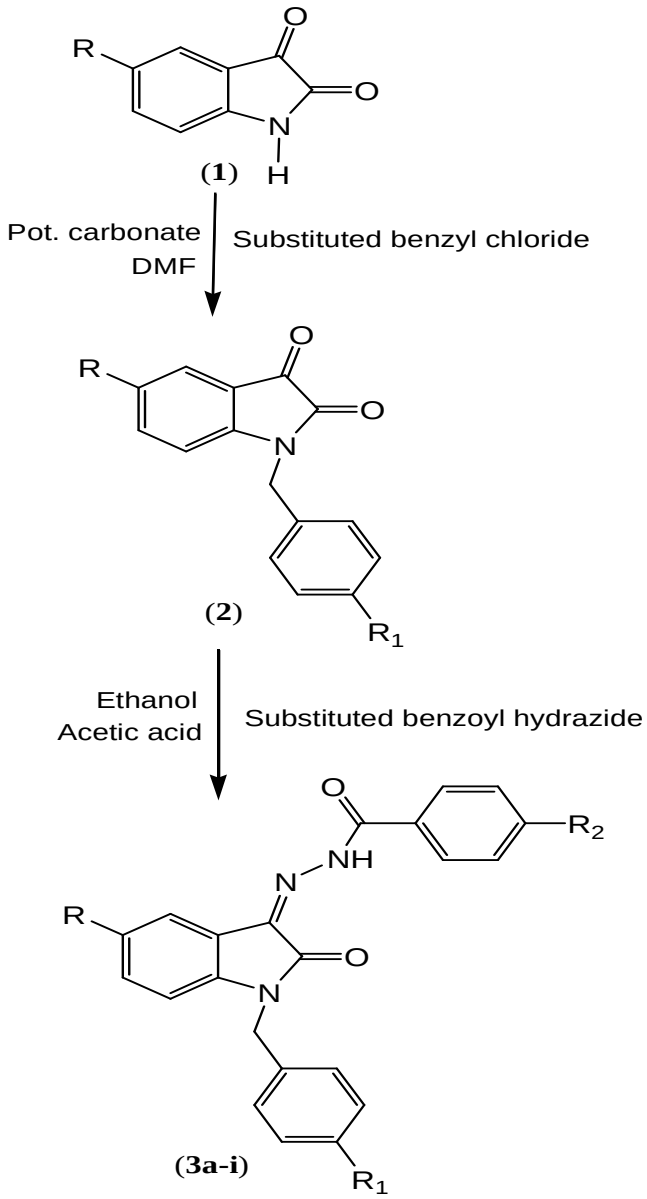
Antimycobacterial activity

Potent derivatives which have revealed moderate antifungal and antibacterial activity with comparison of standard drugs were further screened for 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.[17,18] 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 5**. Isoniazid and gentamicin were taken as standard drugs for comparison.

RESULTS AND DISCUSSION

Chemistry

The synthesis of title compounds is on account of the powerful biological activity of indole and carried out using a general, simple and straightforward pathway. 5-Substituted-1*H*-indole-2,3-dione (substituted isatins) was used basic material for the synthesis of resultant derivatives. The physiochemical evaluation of the resultant compound was shown in **Table 1**. The treatment of 5-substituted-2,3-dihydro-2,3-dioxindole (substituted isatins) and 4-substituted benzyl chloride in DMF with K_2CO_3 yield substituted benzyl isatins (**Scheme 1**). All final compounds were pure and stable. The structure elucidation of the final derivatives was certified by IR, ^{1}H-NMR and Mass spectroscopy. IR spectral peaks of the compound were recognized from 1705-1690 cm^{-1} for C=O stretching, 3375-3200 cm^{-1} for N-H stretching, 3075-2850 cm^{-1} for C-H aliphatic and aromatic correspondently, some stretching bands were also found for C=C and C=N at 1575-1490 cm^{-1} (**Table 2**). In ^{1}H-NMR spectra typical proton signals for C-H aliphatic, aromatic and N-H were observed near 2.36-3.68, 8.06-6.30, 10.25-11.30 δ ppm and N-H protons were exchangeable with D_2O. Mass spectra revealed exact molecular ion peaks (**Table 2**).



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

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

Compound	R	R_1	R_2	Yield (%)	M.p (°C)	Mol. Formula	R_f value
3a	H	H	Cl	84	220	$C_{22}H_{16}N_3O_2Cl$	0.68
3b	Cl	H	Cl	81	223-25	$C_{22}H_{15}N_3O_2Cl_2$	0.74
3c	CH_3	H	Cl	78	211-13	$C_{23}H_{18}N_3O_2Cl$	0.76
3d	H	Cl	Cl	74	208-10	$C_{22}H_{15}N_3O_2Cl_2$	0.70
3e	Cl	Cl	Cl	81	242	$C_{22}H_{14}N_3O_2Cl_3$	0.74
3f	CH_3	Cl	Cl	83	218-20	$C_{23}H_{17}N_3O_2Cl_2$	0.69
3g	H	CH_3	Cl	82	203-5	$C_{23}H_{18}N_3O_2Cl$	0.66
3h	Cl	CH_3	Cl	77	221-23	$C_{23}H_{17}N_3O_2Cl_2$	0.71
3i	CH_3	CH_3	Cl	69	199-200	$C_{24}H_{20}N_3O_2Cl$	0.72

Table 2: Spectral data of the title compounds (3a-i).

3a	IR (ν, cm^{-1}) : 3359, 3220, 3051, 2833, 1683, ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.92 (s, 2H, CH_2), 6.74-7.10 (m, 13H, Ar-H), 14.02 (s, 1H, NH).
3b	IR (ν, cm^{-1}) : 3237, 3044, 2921, 1703, ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.94 (s, 2H, CH_2), 6.78-7.91 (m, 12H, Ar-H), 13.94 (s, 1H, NH), MS: $[M/]^+$ at *m/z* 422.
3c	IR (ν, cm^{-1}) : 3217, 3073, 2884, 1671, ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.32 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 6.68-8.04 (m, 12H, Ar-H), 14.08 (s, 1H, NH).
3d	IR (ν, cm^{-1}) : 3228, 3055, 2836, 1682, ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.90 (s, 2H, CH_2), 6.73-8.02 (m, 12H, Ar-H), 14.00 (s, 1H, NH).
3e	IR (ν, cm^{-1}) : 3236, 3046, 2918, 1704, ^{1}H NMR (δ, ppm/ DMSO-d_6): 4.76 (s, 2H, CH_2), 6.55-7.89 (m, 11H, Ar-H), 13.84 (s, 1H, NH, exchangeable with D_2O).
3f	IR (ν, cm^{-1}) : 3213, 2894, 2828, 1728, ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.26 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.57-7.96 (m, 11H, Ar-H), 13.95 (s, 1H, NH), MS: $[M/]^+$ at *m/z* 437.
3g	IR (ν, cm^{-1}) : 3264, 3100, 2940, 1710, ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.24 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.74-7.97 (m, 11H, Ar-H), 14.04 (s, 1H, NH).
3h	IR (ν, cm^{-1}) : 3308, 3033, 2972, 1685, ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.29 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.75-7.10 (m, 11H, Ar-H), 14.04 (s, 1H, NH).
3i	IR (ν, cm^{-1}) : 3270, 3055, 2965, 1691, ^{1}H NMR (δ, ppm/ DMSO-d_6): 2.27 (s, 6H, CH_3), 4.85 (s, 2H, CH_2), 6.57-7.96 (m, 11H, Ar-H), 14.01 (s, 1H, NH).

Biological activity

All the final synthesized derivatives were taken for preliminary screening to evaluate antibacterial activity by cup plate method, in the nutrient agar medium against three Gm+ and three Gm- bacterial strains at 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 **3b, 3c, 3d, 3e** and **3g** 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 3**). The area of inhibition (mm) of each derivative was ascertained and compared with standard drug ketoconazole. The compounds **3b, 3c, 3d, 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 potent derivatives and standard drugs against verified strains are shown in **Table 4**.

Anti-tubercular 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 32 to 72%. Compound **3c** comes forth as most active analogue in this research with 72% inhibition against *M. tuberculosis* H37Rv in comparison with standard drugs isoniazid as well as gentamicin (**Table 5**). The findings show that isatin derivative with chloro and methyl group **(3c)** revealed comparable activity with the referred drugs.

Table 3: Zone of inhibition of newly synthesized isatin derivatives (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	14	18	14	18	16	16	14	14	12
3b	42	40	38	45	34	34	44	42	35
3c	40	37	39	45	30	35	45	40	39
3d	43	39	38	44	33	34	40	41	37
3e	42	40	36	46	34	34	42	44	37
3f	21	20	18	15	14	10	16	10	18
3g	41	39	37	44	34	34	42	44	35
3h	24	16	21	25	15	18	20	16	21
3i	43	38	36	42	31	35	41	42	38
Std.	45[a]	42[a]	40[a]	50[b]	35[b]	40[b]	45[b]	45[b]	40[b]

[a]Ketoconazole, [b]Ampicillin

Table 4: MIC values of potent isatin derivatives 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*
3b	20	20	30	15	25	30	10	15	25
3c	25	30	25	15	35	30	10	20	15
3d	20	25	30	15	30	30	20	20	20
3e	20	20	35	15	25	30	15	10	20
3g	25	25	30	15	25	30	15	10	25
3i	20	25	35	20	35	30	20	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 5: Anti-tubercular activity of potential isatin derivatives and standard drugs against $H_{37}Rv$ strain.

Compound	Concentration (µg/ml)	Percentage inhibition
3b	6.25	66
3c	6.25	72
3d	6.25	65

3e	6.25	58
3g	6.25	68
3i	6.25	32
Isoniazid	0.031	95
Gentamicin	6.0	99

CONCLUSION

A series of nine compounds (**3a-i**) was prepared by reacting substituted 1-benzyl-2,3-dihydro-2,3-dioxindole with different substituted benzoyl hydrazide by easy, useful method and characterized by TLC, M.P. and spectral analysis. All the synthesized compounds were evaluated for their in-vitro antimicrobial activity against different fungal and bacterial strains. Compounds **3b, 3c, 3d, 3e** and **3g** were highly active against gram positive and gram negative bacteria, compounds **3b, 3c, 3d, 3e** exhibited potent antifungal activity, whereas compound **3c** is active against *M. tuberculosis* H37Rv.

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