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Synthesis and Antimicrobial Activity of Some New Isatins Derivatives

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ABSTRACT

*Some new 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2-oxo-1, 2-dihydro-indole-3-ylidene) hydrazide (**III**) have been synthesised from different isatin derivatives (**I**) by condensing with 3-[2-(1, 3-diox-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid hydrazide (**II**). Their chemical structures have been confirmed by IR, ^{1}H NMR, Mass and by elemental analysis. Investigation of antimicrobial activity of compounds was done by the disk diffusion technique. Among the compounds tested, the compound with 5-Cl, 5-NO_2, 5-I substitution showed the most favourable antimicrobial activity.*

Keywords: Isatin, Ethylphthalimide hydrazone, Antibacterial and Antifungal.

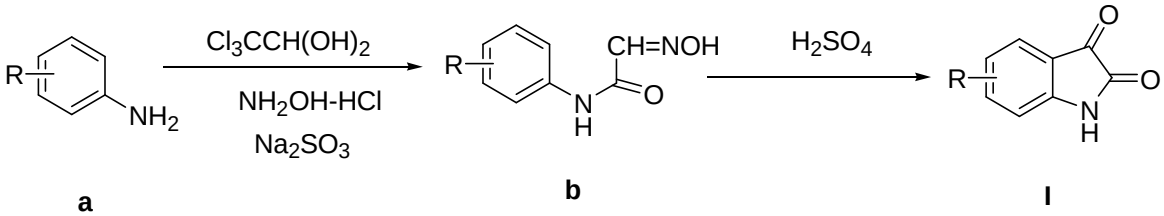
INTRODUCTION

It is evident from literature, that isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial [1], anti-inflammatory [2], analgesic[3], anti-viral[4], antifungal[5], antitubercular[6] and antidepressant[7]. Isatin hydrazone have been reported to possess anticonvulsant [7] activity also. In view of these fact prompted us to synthesize some new 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2-oxo-1, 2-dihydro-indole-3-ylidene) hydrazide (**III**). All the synthesized compounds were screened for their *in vitro* anti-bacterial and anti-fungal activity.

MATERIALS AND METHODS

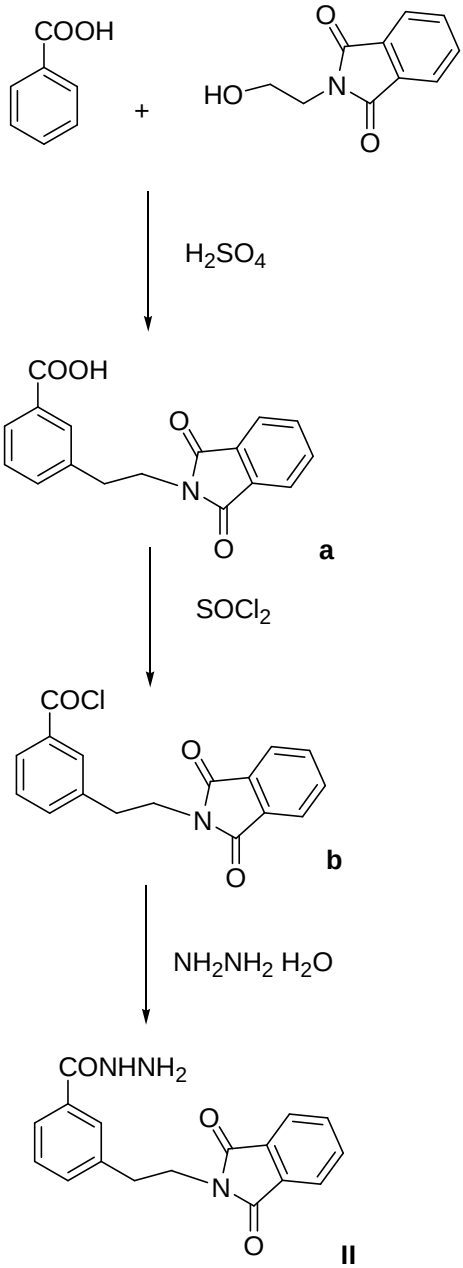
All the melting points were determined by open capillary using V-Scientific Melting Point apparatus and are uncorrected. Purity of compounds was checked by TLC on silica Gel-coated plates. IR spectra were recorded in KBr on FTIR Prestige-21 Simadzu spectrophotometer. ^{1}H NMR spectra were recorded on 500 MHz Bruker using $CDCl_3$/MeOD and Mass spectra were recorded on using EI-MS mode. Elemental analysis was performed on Perkin-Elmer Series 2400.

Scheme-I



a **b** **I**

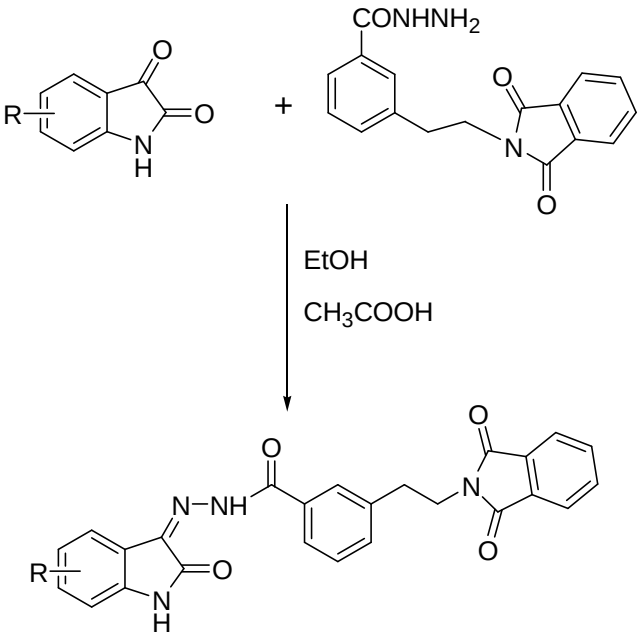
Scheme-II



Synthesis of Isatin (Indole-2, 3-diones): **(Ia-h)**

The different isonitrasoacetinilides **(b)** were synthesized from the respective aromatic amines (**a**) viz. on reaction with chloralhydrate and hydroxylamine hydrochloride. Each of isonitrasoacetinilides **(b)** was subjected to a dehydrative cyclization using sulphuric acid (d 1.84) to yield the corresponding substituted isatin **(I)**. All these isatin thus prepared were identified by IR and their physical constant reported in the literature [9]. As shown in **scheme-I**.

Scheme-III



Synthesis of 3-[2-(1, 3-diox-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid hydrazide: **(II)**
As the literature serve the synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindo-2-yl) ethyl] benzoic acid hydrazide by the process [8].

(a) Take N-Hydroxyethyl Phthalimide (0.1mol) and Benzoic acid (0.1mol) was dissolved in conc.sulfuric acid (50ml). Stir it overnight on mechanical stirrer after completion of reaction, poured into crushed ice and stirred it up to 30min and filter the reaction mass and wash with cold water. Recrystalised in methanol and get compound **(a)**.

(b) Take compound **(a) (**0.3mol**)** in carbon tetrachloride (50ml) and slowly add thionyl chloride (0.5mol), and was heat up to reflux and maintain for 3hrs, after distillation of solvent residue i.e., compound **(b)** was use for next step without purification.

Take above compound **(b)** and slowly add of hydrazine hydrate (0.3mol) at 0-5^{0}C temperature and was stirred for 60min. Solid materials filtered and wash with 10% $NaHCO_3$ solution. Purified in methanol to obtain pure crystalline compound **(II)**. As shown in **Scheme-II.**

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2-oxo-1, 2-dihydro-indole-3-ylidene) hydrazide: (**IIIa-h**) (General Procedure)
A mixture of equimolar quantity of isatin derivatives (0.01mol) **I** and 3-[2-(1, 3-dioxo1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid hydrazide (0.01mol) **II,** was dissolved in ethanol and add 2-3 drops of glacial acetic acid and refluxed for 3-4h.The reaction mixture was cooled and further cool it up to 0-5^0 C and filter it and recrystalised from hot ethanol [10]. As shown in **Scheme-III.**

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2-oxo-1, 2-dihydro-indole-3-ylidene) hydrazide: (**IIIa**)
IR (KBr) 3358.07 (-NH), 2806.43 (aromatic –CH starching), 1710.86 (-C=O, Hydrazide), 1685.79 (-C=O, Isatin), 1658.78 (-C=O, Amide), 1589.34 (-C=N-, Conjugation), 1483.26 &

1465.90(-C=C-, ring skeletal vibration); 1HNMR (CDCl3/MeOD) ppm: 3.357(2H,s,-CH_2), 4.203(2H,s,-CH_2), 6.759-7.780(9H,m,ArH), 7.796(1H,s,-NH),MS m/z:438.1(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-chloro-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIb**)

IR (KBr) 3404.36 (-NH), 2821.86 (aromatic –CH starching), 1734.01 (-C=O, Hydrazide), 1689.64 (-C=O, Isatin), 1668.43 (-C=O, Amide), 1598.99 (-C=N-, Conjugation), 1473.62 & 1454.33(-C=C-, ring skeletal vibration),565.14(-C-Cl); 1HNMR (CDCl3/MeOD) ppm: 3.355(2H,s,-CH_2), 4.202(2H,s,-CH_2), 6.755-7.784(8H,m,ArH), 7.796(1H,s,-NH), MS m/z:472.1(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-bromo-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIc**)

IR (KBr) 3400.50 (-NH), 2848.86 (aromatic –CH starching), 1703.00 (-C=O, Hydrazide), 1691.57 (-C=O, Isatin), 1668.43 (-C=O, Amide), 1598.99 (-C=N-, Conjugation), 1471.69 & 1438.90(-C=C-, ring skeletal vibration),650.01(-C-Br); 1HNMR (CDCl3/MeOD) ppm: 3.359(2H,s,-CH_2), 4.209(2H,s,-CH_2), 6.755-7.780(8H,m,ArH), 7.792(1H,s,-NH),MS m/z:517.9(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-methyl-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIId**)

IR (KBr) 3390.86 (-NH), 2860.43 (aromatic –CH starching), 1710.86 (-C=O, Hydrazide), 1631.78 (-C=O, Isatin), 1620.21 (-C=O, Amide), 1577.77 (-C=N-, Conjugation), 1481.33(-C=C-, ring skeletal vibration), 2924.09(-CH_3); 1HNMR (CDCl3/MeOD) ppm:2.363(3H,s,-CH_3) 3.355(2H,s,-CH_2), 4.202(2H,s,-CH_2), 6.751-7.784(8H,m,ArH), 7.796(1H,s,-NH),MS m/z:453.7(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-flouro-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIe**)

IR (KBr) 3375.43 (-NH), 2816.07 (aromatic –CH starching), 1714.72 (-C=O, Hydrazide), 1689.64 (-C=O, Isatin), 1622.13 (-C=O, Amide), 1560.41 (-C=N-, Conjugation), 1473.62(-C=C-, ring skeletal vibration),1280.73(-C-F); 1HNMR (CDCl3/MeOD) ppm: 3.350(2H,s,-CH_2), 4.203(2H,s,-CH_2), 6.759-7.787(8H,m,ArH), 7.796(1H,s,-NH),MS m/z:456.8(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-nitro-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIf**)

IR (KBr) 3400.50 (-NH), 2833.43 (aromatic –CH starching), 1707.00 (-C=O, Hydrazide), 1641.42 (-C=O, Isatin), 1642.00 (-C=O, Amide), 1593.20 (-C=N-, Conjugation), 1462.04(-C=C-, ring skeletal vibration),1354.03(-C-NO_2); 1HNMR (CDCl3/MeOD) ppm: 3.355(2H,s,-CH_2), 4.203(2H,s,-CH_2), 6.759-7.781(8H,m,ArH), 7.796(1H,s,-NH),MS m/z:484.0(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (5-iodo-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIg**)

IR (KBr) 3402.43 (-NH), 2779.42 (aromatic –CH starching), 1739.79 (-C=O, Hydrazide), 1689.64 (-C=O, Isatin), 1660.50 (-C=O, Amide), 1564.27 (-C=N-, Conjugation), 1467.83 & 1433.11(-C=C-, ring skeletal vibration),555.50(-C-I); 1HNMR (CDCl3/MeOD) ppm: 3.353(2H,s,-CH_2), 4.205(2H,s,-CH_2), 6.755-7.784(8H,m,ArH), 7.791(1H,s,-NH),MS m/z:565.3(M^+)

Synthesis of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (7-chloro-2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazide: (**IIIh**)

IR (KBr) 3421.72 (-NH), 2845.00 (aromatic –CH starching), 1724.36 (-C=O, Hydrazide), 1691.57 (-C=O, Isatin), 1649.14 (-C=O, Amide), 1597.06 (-C=N-, Conjugation), 1483.26 & 1442.75(-C=C-, ring skeletal vibration),570.93(-C-Cl); 1HNMR (CDCl3/MeOD) ppm: 3.350(2H,s,-CH_2), 4.207(2H,s,-CH_2), 6.759-7.787(8H,m,ArH), 7.794(1H,s,-NH),MS m/z:472.6(M^+)

Table 1. Physical data and elemental analysis of synthesized compound (IIIa-h)

Sr No	Compound code	R	MP (^{0}C)	Molecular Formula (MF)	Molecular Weight (MW)	Yield (%)	Elemental analysis Found (Calc.)		
							%C	%H	%N
1	IIIa	H	226-228^{0}C	$C_{25}H_{18}N_4O_4$	438.43	79%	67.82 (68.49)	4.08 (4.14)	12.36 (12.78)
2	IIIb	5-Cl	233-235^{0}C	$C_{25}H_{17}N_4O_4Cl$	472.88	85%	63.02 (63.50)	3.29 (3.62)	11.53 (11.85)
3	IIIc	5-Br	220-223^{0}C	$C_{25}H_{17}N_4O_4Br$	517.33	82%	57.65 (58.04)	3.10 (3.31)	10.39 (10.83)
4	IIId	5-CH_3	202-204^{0}C	$C_{26}H_{20}N_4O_4$	452.46	78%	68.79 (69.02)	4.39 (4.46)	12.27 (12.38)
5	IIIe	5-F	235-237^{0}C	$C_{25}H_{17}N_4O_4F$	456.43	78%	64.88 (65.79)	3.43 (3.75)	12.10 (12.28)
6	IIIf	5-NO_2	277-279^{0}C	$C_{25}H_{17}N_5O_6$	483.43	84%	63.92 (62.11)	3.11 (3.54)	14.28 (14.49)
7	IIIg	5-I	239-241^{0}C	$C_{25}H_{17}N_4O_4I$	564.33	82%	53.39 (53.21)	3.22 (3.04)	9.88 (9.93)
8	IIIh	7-Cl	198-200^{0}C	$C_{25}H_{17}N_4O_4Cl$	472.88	74%	63.28 (63.50)	3.39 (3.62)	11.19 (11.85)

In Vitro Antimicrobial Activity

Evaluation of antibacterial (3-bacteria) and antifungal (2-fungi) activities was done by the disk diffusion technique[12]. The microorganisms used were purchased from (1) N-broth i.e. Nutrient broth medium (Titan Biotech Ltd., Delhi). (2) Sabouraud's dextrose broth medium (Titan Biotech Ltd., Delhi). (3) Antibacteriological grade Agar-Agar (Qualigens-Glaxo, Mumbai).

Table 2. *In Vitro* antimicrobial activity of 3-[2-(1, 3-dioxo-1, 3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2-oxo-1, 2-dihydro-indole-3-ylidene) hydrazide (IIIa-h)

Compound Code	R	*E-coli*	*B-Subtillis*	*S-Aureus*	*S-Cerevisiae*	*A-niger*
IIIa	H	+	++	++	+	+
IIIb	5-Cl	++	++	+	++	+
IIIc	5-Br	++	+	++	+	++
IIId	5-CH_3	+	+	++	++	++
IIIe	5-F	++	+	+	++	+
IIIf	5-NO_2	++	+	++	++	+
IIIg	5-I	+	++	++	+	++
IIIh	7-Cl	++	+	++	+	++

**Effectively was classified in to three zones on the bases of the diameter of zone of inhibition*

+ + + : *Most effective*
+ + : *Moderate effective*
+ : *Slightly effective*
- : *Non effective*

The tested compounds solution were prepared in dimethylformamide(DMF) and evaluated them for their *in vitro* antibacterial and antifungal activity against *Baccillus subtillis* NCIM 2250, *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2109, *Aspergillus niger* NICM 501 and *Candida albicans* NICM 7431, respectively.

All bacteria were grown on Mueller-Hinton agar (Hi-Media) plates (37^0C, 24h) and fungi were grown on sabouraud dextrose agar (Hi-Media) plates (26^0, 48-72h). The results were established by the presence of clear zone of inhibition around the active compounds.

RESULTS AND DISCUSSION

As many as new eight compounds were synthesized by adopting similar above procedure and then characterized by their physical, analytical and spectral data. The details of some of the representative compounds are given in the experimental section. Their physical and elemental analysis data are presented in **Table 1**.

All the synthesized compounds were tested for in vitro antimicrobial activity by the disk diffusion technique. The results are summarized in **Table 2** that includes the activity of reference compound Gentamicine.

The tested compounds exhibited mild to moderate antibacterial activity against all three strains of bacteria. The compounds, IIIa, IIIc, IIId, IIIf, IIIg and IIIh tested against *S.aureus*, showed highest activity. It has also been observed that compounds, IIIa, IIIb and IIIg against *B.subtills*.

All synthesized compounds were tested for in vitro antimicrobial activity by the disk diffusion technique. The results are summarised in **Table 2** that includes the activity of reference compound Gentamicine.

The antifungal of the compounds was studied for the two pathogenic fungi. Amphotericin B was used as reference for inhibitory activity against fungi. It was observed that compounds IIIb, IIId, IIIe and IIIf had highest activity *S-Cerevisiae* and IIIc, IIId, IIIg and IIIh showed good activity against *A.niger*.

CONCLUSION

The antimicrobial study revealed that substitution in the 5^{th} position of isatin with chlorine, bromine or fluorine produced more active compounds in a series.

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