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Synthesis and Pharmacological Screening of New Isatin Derivatives

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Abstract
Isatin is a potential synthon for building synthetically a variety of chemical systems known for Their broader and pharmacological applications. Its derivatives demonstrate diverse biological and pharmacological activities, including anticonvulsant anti neoplastic, anti-inflammatory, analgesic, antimicrobial and antiviral properties. Schiff bases are aldehyde or ketone-like compounds in which the carbonyl group is replaced by imine or azomethine group. They are widely used for industrial purposes and exhibit a broad range of biological activities.

Keywords
Anti-inflammatory, anti-neoplastic, anticonvulsant, antimicrobial.

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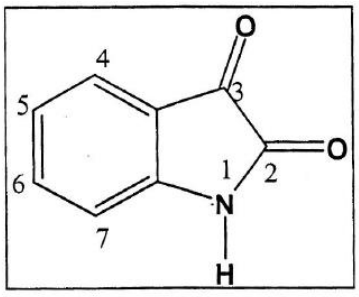
CHEMISTRY OF ISATIN

INTRODUCTION

Isatin (1H-indole-2, 3- dione) (I) was first discovered by Erdmann' and Laurent[2] in 1841, independently as a product from oxidation of indigo by nitric acid and chromic acids, Isatins are synthetically versatile substrates, where they can be used for the synthesis of a verity of heterocyclic compounds, such as indole is chemically benzenes fused with pyrrole, The IUPAC name of indole is 1H-benzo (b) pyrrole.

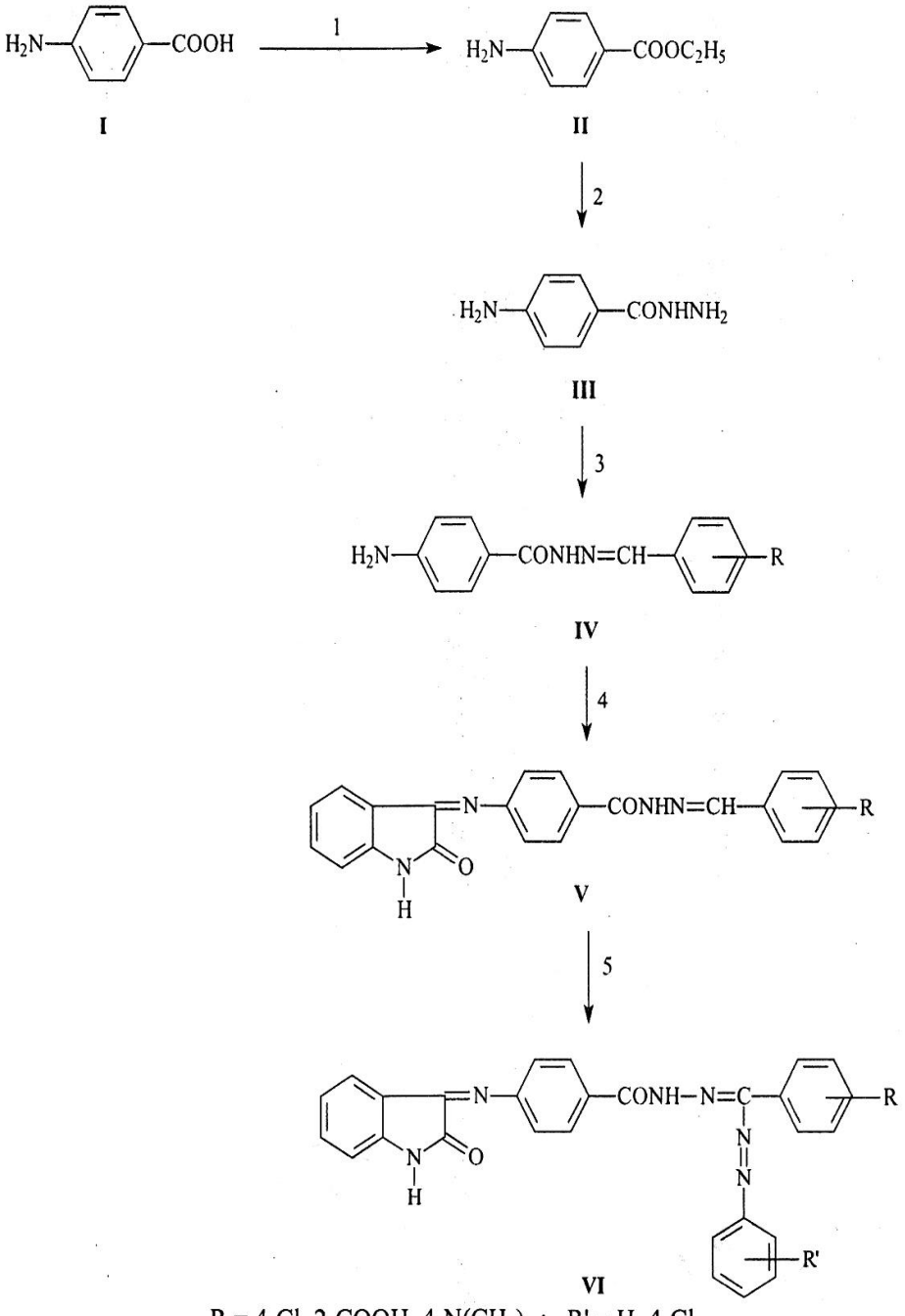


(I)

IH-indole-2, 3-Dione

Molecular formula	: $C_8H_5O_2N$
Molecular weight	: 147.13
Melting point	: 200^0C
Solubility	: Methanol

SCHEME



R = 4-Cl, 2-COOH, 4-N$(CH_3)_2$; R' = H, 4-Cl

1. Ethanol, conc. sulphuric acid;
2. Hydrazine hydrate, methanol;
3. various aromatic aldehydes, methanol and glacial acetic acid ;
4. Isatin, methanol & glacial acetic acid ;
5. Aromatic 1° amine, 10% sodium nitrate, 2N HCl and pyridine ;

EXPERIMENTAL PROCEDURE:

1) **Synthesis of ethyl p-amino benzoate (II):**
1.22 gm (0.01mol) of benzoic acid was taken in a 250 ml round bottomed flask. It is dissolved in 20 ml of ethanol. Then 2-3 ml of sulphuric acid was added. This reaction mixture was refluxed for 4 hrs. Then the reaction mixture was cooled, and the obtained liquid was extracted with carbon tetra chloride and sodium hydrogen carbonate. The liquid was collected. The ethyl ester of p-amino benzoic acid was crystallized out and filtered. It was separated and dried. The purity of the compound was checked by TLC and spectral data. m.p:91-93^{o}c, yield91%.

2) **Synthesis of 4-aminobenzoic acid hydrazide (III):**
A mixture of 4-aminoester (0.01mol) and hydrazine hydrate (99% 0.02 mol) in methanol was taken in a Clean 250ml RBFlask and reflex for about 1hr.The solvent was removed by evaporation. The resultant Compound thus obtained was washed with a little amount of methanol and dried. The purity of the compound was checked by TLC and spectral data.
The IR spectrum of the compound III (R=H) showed absorption bands (in cm^{-1}) at:3653(NH), 1170(C-N), 1512(C=C), 1723(C=O)

Synthesis of 4-amino-N'- [(Substituted phenyl) methylidene] benzo hydrazide (IV):
A mixture of 4-amino benzoic acid hydrazide (0.01mol) and an appropriate amount of aromatic aldehyde Was taken in a clean and dry RB Flask containing 25ml of methanol and 4-5 drops of glacial acetic acid. The reaction mixture was reflexed for 30minutes.The solid thus separated on cooling was filtered Wash with a little amount of cold methanol and dried. The purity of the recrystallized compound was Checked by TLC and spectral data. Various compounds have been prepared from the respective aromatic aldehydes viz., p-chloro benzaldehyde, Salicylaldehyde, p-dimethyl amino benzaldehyde. The IR spectrum of the compound IV (R=H) exhibited characteristic absorption bands (in cm^{-1}) At:3673(NH),2995(Ar-H),1513(C=C),1680(C=0)

3) **Synthesis of N'- [(Substitute phenyl) methylidene]-4-[(-2-oxo-1, 2dihydro-3H-indol 3-ylidene) amino] benzo hydrazide(V):**
A mixture of 4-amino-N- [(Substituted phenyl) methylidene] benzo hydrazide (0.01mol) and an appropriate isatin (0.01mol) in methanol (60ml) containing 3-4drops of glacial acetic acid was reflexed for 30minutes after completion of reaction, the product was filtered and washed a little amount of cold methanol and then recrystallized from methanol. The purity of the compound was checked by TLC. Three compounds were synthesized by adopting this procedure and characterized by physical data and spectral data.
The IR spectrum of the compound V(R=H) exhibited characteristic absorption bands (in cm^{-1}) At: 3674 (NH), 1711(C=O), 1535(C=N), 1167(C-N), 1482(C=C)

5) **Synthesis of N'- [(substituted phenyl) methylidene] -4- [-2-oxo-1, 2 dihydro-3H-indol-3-ylidene) amino] benzo hydrazide formazan (VI):**

a) Preparation of benzene diazonium chloride:
It is prepared by treating a solution of aniline in dil. Hydrochloric acid with sodium nitrite solution at 0-5^{o}C. Sodium nitrite reacts with hydrochloric acid to produce nitrous acid. The nitrous acid then reacts with aniline to give the diazonium salt.

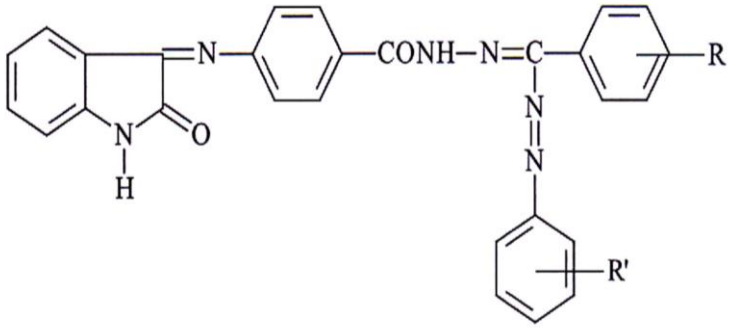
Procedure:
Dissolve 5ml of aniline in Di hydrochloric acid (20ml concentrated hydrochloric acid+20ml water) in a Beaker. Place the beaker in ice bath and set up the apparatus. Cool the contents of the beaker to about 5^{o}C. Slowly add with constant stirring sodium nitrite solution (10 gm in 50ml water) previously cooled to 5^{o}C.make sure that temperature does not rise above 10^{o}C.
The solution of benzene diazonium chloride so obtained is used immediately for further reaction. The dry diazonium salt is unstable and explosive and is seldom separated in solid state.

b)Synthesis of N'-[(Substituted phenyl) methylidene]-4- [(-2-oxo-1,2dihydro-3H-indol-3-ylidene) amino]benzo hydrazide formazan (VI):
The diazonium salt solution of an appropriate aryl amine (0.015mol) was added dropwise with continuous stirring to a solution of N'-[(Substituted phenyl) methylidene]-4- [(-2-oxo-1,2dihydro-3H-indol-3 ylidene) amino] benzohydrazide (0.01mol)in pyridine(20ml)keeping the mixture below 12^{o}c.
The reaction mixture was allowed to stand overnight and poured in ice cold water (250ml)with constant stirring. The dark colored solid thus separated was filtered, washed with cold water followed by hot water, dried well in air and recrystallized from methanol. As many as 6compounds have been prepared adopting the above method and the physical data is presented in table-1 and spectral data.

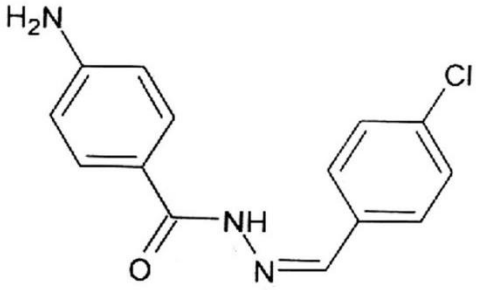
The IR spectrum of the N'-[(4-chlorophenyl) methylidene]-4- [-2-oxo-1,2dihydro-3H-indol-3 -ylidene) amino] benzo hydrazide formazan (VI a) :(R=H) exhibited characteristic absorption bands (in cm^{-1}) At: 3660(NH), 3029(=C-H), 1819 (C=O), 1584(N=N), 1166(C-N)

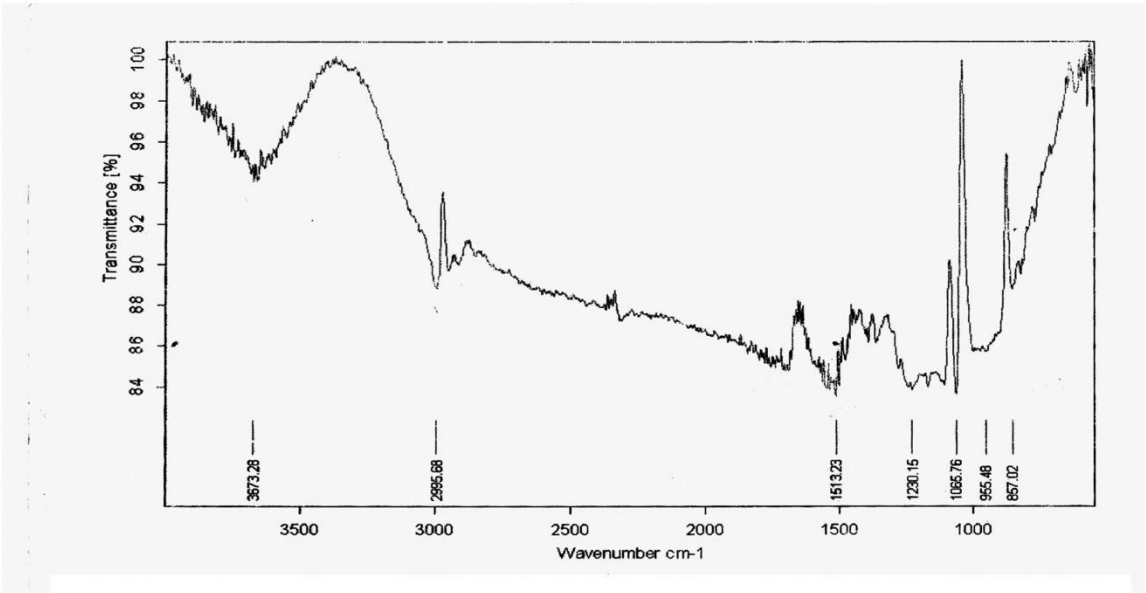
Table-1

Physical data of N'-[(Substitutedphenyl)methylidene]-4-[(-2-oxo-1,2 dihydro-3H-indol-3-ylidene)amino]benzohydrazide formazan (VI)$_{a-f}$

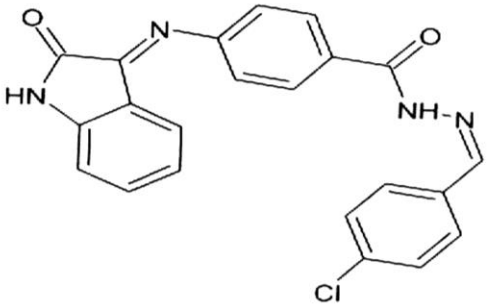


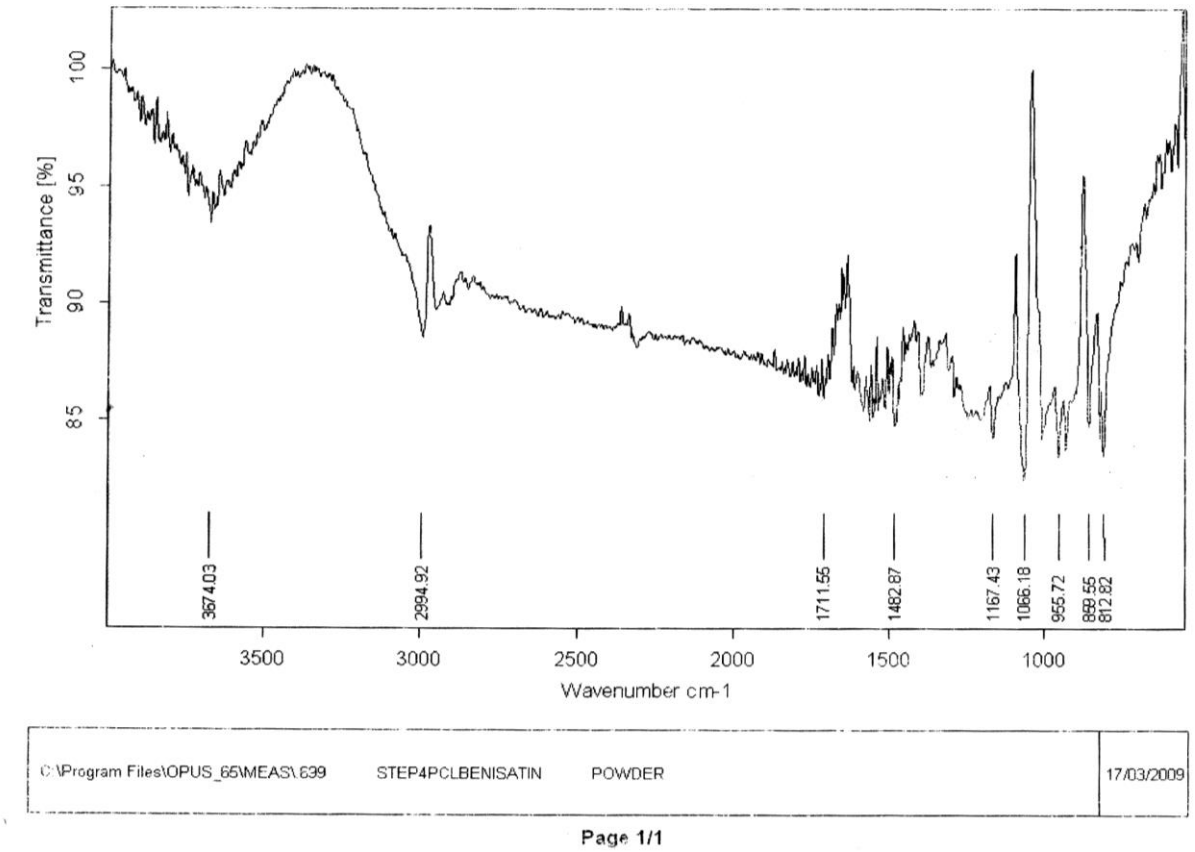
S.No.	Compound	Substituents		Molecular Formula	Molecular weight (grams/ mole)	Melting point (°C)	Rf Value	% Yield
		R	R'					
1.	**VIa**	4-Cl	H	$C_{28}H_{19}ClN_6O_2$	506	187-189	0.913	60
2.	**VIb**	2-COOH	H	$C_{29}H_{20}N_6O_4$	516	119-121	0.70	68
3.	**VIc**	4-N$(CH_3)_2$	H	$C_{30}H_{25}N_6O_2$	501	125-127	0.62	65
4.	**VId**	4-Cl	4-Cl	$C_{28}H_{18}Cl_2N_6O_2$	540	209-211	0.882	70
5.	**VIe**	2-COOH	4-Cl	$C_{29}H_{19}ClN_6O_4$	550	201-203	0.947	65
6.	**VIf**	4-N$(CH_3)_2$	4-Cl	$C_{30}H_{24}ClN_6O_2$	535	177-179	0.875	62



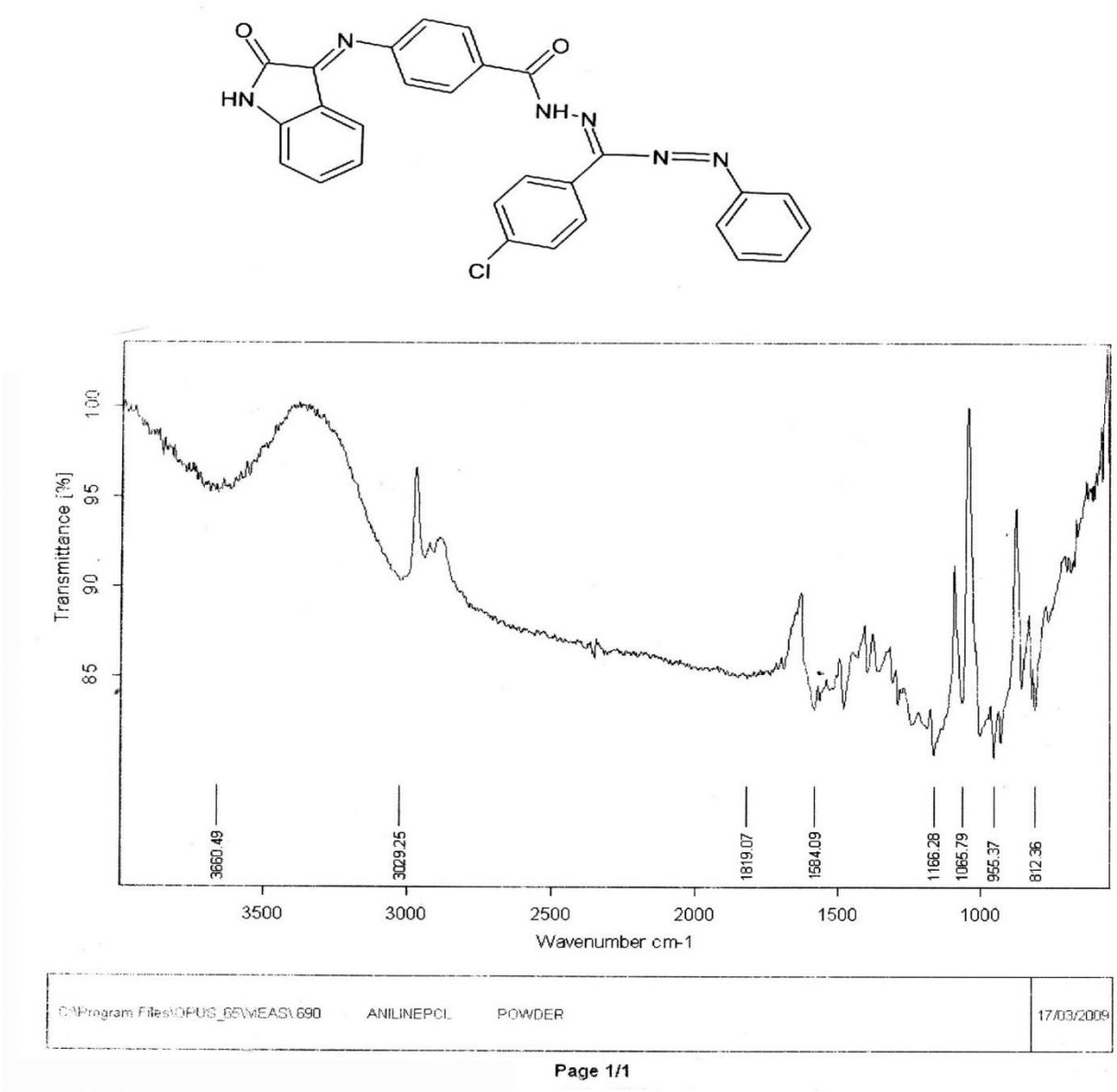


IR Spectrum of 4-amino-N'-[(4-chlorophenyl)methylidene]benzohydrazide(IV)





IR Spectrum of N'-[(4-chlorophenyl)methylidene]-4-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]benzohydrazide(Va)



IR spectrum of N'-[(4-chlorophenyl)methyledene]-4-[(-2-oxo-1,2dihydro-3H-indo-3-ylidene)amino]benzohydrazide formazan(VIa)

BIOLOGICAL ACTIVITY

ANTI-BACTERIAL ACTIVITY:

The antibacterial activity of N'-[(Substituted phenyl) methylidene]-4-[(-2-oxo-1,2 dihydro-3H-indol-3-ylidene)amino] benzohydrazide formazan (VIa-f) was screened against four different strains of bacteria by agar diffusion method(Table-2).

Two Gram-Positive Bacteria: ***Bacillus subitilis*** and ***Staphylococcus aureus.***

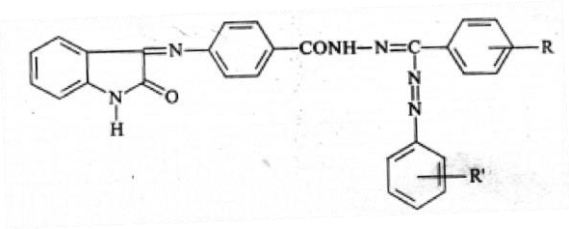
Two Gram-Negative Bacteria: ***Escherichia coli*** and ***Proteus vulgaris.***

ANTI-FUNGAL ACTIVITY:

The antifungal activity of N'-[(Substitutedphenyl)methylidene]-4-[(-2-oxo-1,2 dihydro-3H-indol-3-ylidene)amino]benzohydrazide formazan (VIa-f) was screened against 2 strains of fungi by agar diffusion method (Table-3).

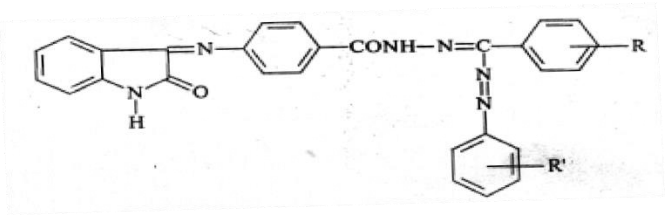
1. ***Candida aldicans***
2. ***Yeast (Saccharomyces cerevisiae).***

Table–II: ANTIBACTERIAL ACTIVITY OF N'-[(SUBSTITUTEDPHENYL) METHYLIDENE] -4-[(-2-OXO-1,2 DIHYDRO-3H-INDOL-3-YLIDENE) AMINO] BENZOHYDRAZIDE FORMAZAN (VI a-f)



S.No.	Compound	Substituents		Concentration (µg/ml)	Zone of inhibition B. subtilis (mm)	Zone of inhibition *E. coli* (mm)
		R	R'			
				100	3	3
1	VIa	4-Cl	H	150	9	9
				200	10	10
				100	10	8
2	VIb	2-COOH	H	150	11	10
				200	14	13
				100	11	11
3	VIc	4N(CH3)2	H	150	14	12
				200	16	14
				100	9	9
4	VId	4-Cl	4-Cl	150	9	11
				200	10	11
				100	10	12
5	VIe	2-COOH	4-Cl	150	11	12
				200	12	13
				100	10	13
6	VIf	4-N(CH3)2	4-Cl	150	13	14
				200	16	16
7	Ampicillin (std)			10	22	19

Table – III: ANTI-FUNGAL ACTIVITY OF N'-[(SUBSTITUTEDPHENYL) METHYLIDENE] -4-[(-2-OXO-1,2 DIHYDRO-3H-INDOL-3-YLIDENE) AMINO] BENZOHYDRAZIDE FORMAZAN (VI a-f)



S.No.	Compound	Substituents		Concentration (g/ml)	Zone of inhibition Candida albicans (mm)	Zone of inhibition Aspergillus Niger (mm)
		R	R'			
				100	5	5
1	VIa	4-Cl	H	150	7	6
				200	8	7
			H	100	6	7
2	VIb	2-COOH	μ	150	7	7
				200	9	8
				100	7	7
3	VIc	4-N(CH3)2	H	150	8	9
				200	10	10
				100	6	7
4	VId	4-Cl	4-Cl	150	8	6
				200	9	8
				100	5	6
5	VIe	2-COOH	4-Cl	150	7	7
				200	8	7
				100	6	6
6	VIf	4-N(CH3)2	4-Cl	150	7	7
				200	8	7
7	Ketacanazole (std)			10	14	13

RESULTS, DISCUSSIONS AND CONCLUSIONS:

The following conclusions have been drawn from the results of this investigation.

- Synthetic work of the study has positively undergone as per the planning and as such in the reactions carried out, the expected compounds alone could be obtained and characterized by spectral data.
- All the synthesized compounds were tested for invitro antimicrobial activity by the agar diffusion method.
- The zone of inhibition values of synthesized compounds against Bacillus aubtilis (Gram Positive) and *E. coli* (Gram Negative) were presented in table-II Amplicillin was used for the reference for inhibitory activity against bacteria.
- All the compounds exhibited mild to moderate activity against bacteria compound Vic (R=4-$N(CH_3)_2$), (R=H) was found to be active against gram positive and compound VI f (R=4-$N(CH_3)_2$ R'=4-cl was active against gram negative bacteria among all the test compounds. This was followed by compounds VI d (R=4-cl), (R'=4-cl) and (R=2-COOH), (R'=4-cl).
- The antifungal activity of the compounds studied against Candida albicans and aspergillums Niger. Clorimazole was used for the reference for inhibitory activity against fungi.
- All the compounds showed mild to moderate antifungal activity. Compound Vic (R=4-$N(CH_3)_2$), (R'=H) was found to be the most active antifungal activity. Compound Vic (R=4-$N(CH_3)_2$), (R'=H) was found to be the compounds VIb (R=2-COOH), (R'-H) VId(R=4-cl), (R'=4-cl)

- It was felt necessary from the results of the present antimicrobial investigation that there is a need for further advances studies at least on few of the test compounds which were found to be superior.

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