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Synthesis, Characterization, Antibacterial and Antifungal Activities of Isatin Derivatives

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Abstract: Isatin is a dioxo- indole derivative; it is evident from literature that the presence of the indole nucleus found to have various pharmacological and biological activities like antimicrobial, anticonvulsant, MAO inhibitory, anticancer, antiviral activities. In view of these facts continuation of our work on some biologically active new Isatin derivatives (3)-3-hydrazinylidine-1, 3-dihyro-2h-benzo (g) indol-2-one has been condensed with different aryl aldehydes to form Schiff bases. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. All the synthesized compounds were screened for antibacterial, antifungal activity. It has been observed that all the compounds showed mild to moderate activity against the both bacteria and fungi. The compound Vi (R=4-N $(CH_3)_2$) showed more potent among all the test compounds.
Keywords: Schiff bases / Isatin / Antibacterial, Antifungal.

1.Introduction

Isatin (1H-Indole 2, 3-di-one) was first discovered by Erdmann[1] and Laurent[2] in 1841 by oxidation product of Indigo. In nature it is found in plants of genus Isatis. Isatin is an endogenous compound isolated in 1988[3]. Isatins and its Schiff bases are reported to show a variety of biological activities like antibacterial[4-6], antifungal[7-9], antiprotozoal[10-11], antiviral[12-14], anthelmintic[15-16] and CNS activities[17-18]. In view of these facts continuation of our work on some biologically active Isatin derivatives (3)-3-hydrazinylidine-1, 3-dihyro-2h-benzo (g) indol-2-one has been condensed with different aryl aldehydes to form Schiff bases was shown in the **scheme-I.** The chemical structures of synthesized compounds were confirmed by means of ^{1}HNMR, IR, mass spectral data and elemental analysis. All the synthesized compounds were screened for antibacterial and antifungal activity by an agar diffusion method[19].

2. Experimental protocols

2.1 Chemistry :

Melting points were determined in open capillary tubes by using Toshniwal or Cintex melting point apparatus and were uncorrected. Purity of compounds was checked by TLC on silica Gel- coated plates. IR spectra were recorded on IR thermo Nicolet nexus 6705 series FT-IR spectrometer using KBr discs. PMR spectra were recorded on Brucker Avance -300 MHz instrument using TMS as an internal standard (chemical shifts in delta, ppm) and the mass spectra was recorded on Liquid Chromatography Mass Spectrometer.

2. 1.1 Synthesis of 1H-benzo[g]indole-2, 3-dione (III):

a. Synthesis of (2)-2-(hydroxyimino)-N-(naphthalene-1-yl) ethanamide (II) General Procedure:[20]

In a 5 lit. R.B. flask were placed chloralhydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate followed by a solution of an alpha-Naphthyl amine (0.5 mol) (I) in water and concentrated hydrochloric acid (0.52 mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in water was added. The contents of flask were heated over a wire-gauge by a mecker burner. So that vigorous boiling begins in about 45 minutes. After 1-2 minutes of vigorous boiling the reaction was complete. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent (s).

IR (KBr) Cm^{-1}: 3166.10(N-H), 1682.58(C=O), 1571.91(C=N), Mass spectra: Molecular ion (M^+) at m/z = 214

Elemental Analysis:

%	C	H	O	N
Calculated	67.28	4.70	14.93	13.07
Found	67.28	4.67	14.95	13.08

b. Synthesis of 1H-benzo[g]indole-2, 3-dione (III) – General Procedure: [21]

Sulphuric acid was warmed to 50^0 C in flask fitted and to this, finely powdered and (2)-2 (hydroxyimino)-N-(naphthalen-1-yl) ethanamide (0.46 mol) (II) was added at such a rate so as to maintain the temperature between 60 and 70^0 C. Then, the reaction mixture was cooled to room temperature and poured on crushed ice. After standing for about half-an–hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by recrystallisation from methanol.

IR (KBr) Cm^{-1}: 3195.58(N-H Streching), 1717.33(C=O Streching), 1720.08(C=O Streching), 1623.98(C=C Streching); ^{1}HNMR: δ 6.6-6.9 (m , 6H, Ar-H), δ 7.2 (s,1H, NH); Mass spectra: Molecular ion (M^+) at m/z = 197

Elemental Analysis:

%	C	H	O	N
Calculated	73.09	3.57	16.22	7.10
Found	73.09	3.55	16.24	7.10

2.1.2 Synthesis of (3)-3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one [21] (IV):

Equimolar quantity (0.004 mol) of 1H-benzo[g]indole-2, 3-dione (III) and hydrazine were dissolved in 10 ml of warm methanol and refluxed for 30 min. After standing for approximately 24hr at room temperature, the product was separated by filtration. The compound was vacuum dried and recrystallized from warm methanol.

IR (KBr) Cm^{-1}: 3415.55(NH_2), 3195.58(NH), 1720.08(C=O), 1587.11 (C=N), 1623.98(C=C).

Elemental Analysis:

%	C	H	O	N
Calculated	68.24	4.29	7.57	19.89
Found	68.24	4.29	7.58	19.90

2.1.3Synthesis of (3)-3-[(2)-(phenylmethylidene) hydrazono]-1,3-dihydro-2*H*-benzo[*g*]indol-2-ones (v):

Equimolar quantity (0.01 mol) of (3)-3-hydrazinylidene-1,3-dihydro-2H-benzo[g]indol-2-one (IV), an appropriate aromatic aldehydes (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4hrs. After standing for approximately 24hr at room temperature, the products were separated by filtration. The products obtained were vacuum dried and recrystallized from warm methanol. The synthesized compounds were characterized by the physical and spectral data.

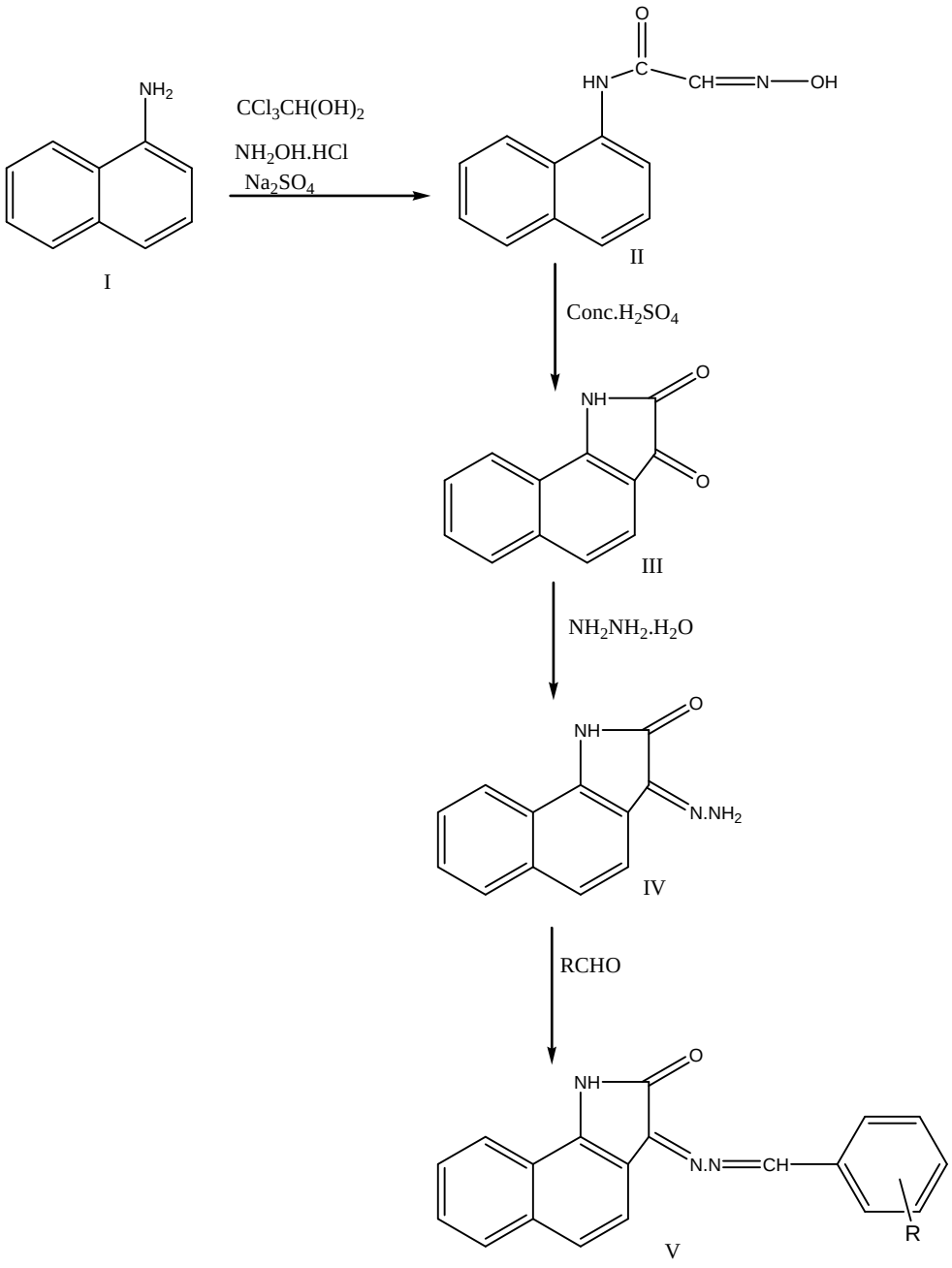
V(i) IR (KBr, cm^{-1}): 3150 (N-H str), 3055 (Ar-CH str), 1698 (C=O str), 1613(C=C str) 1568(C=N); ^{1}HNMR ($CDCl_3$, δ, ppm): 8.21 [s,1H, N=CH], 8.02 [s, 1H, -NH-], 4.2 [2H, H-2"& H-6"; Ar-H], 7.03-7.68[m,6H,4,5,6,7,8,9-H], 6.61[2H, H-3", H-5", Ar-H]

V (h) IR (KBr, cm^{-1}) 3150 (N-H str), 1698 (C=O str), 1568 (C=N str), 3600 (C-OH str), ^{1}HNMR ($CDCl_3$, δ, ppm): 8.21 [s,1H, N=CH], 8.02 [s, 1H, -NH-], 7.42[2H, H-2"& H-6"; Ar-H], 7.03-7.68 [m, 6H,4,5,6,7,8,9-H], 6.61 [2H, H-3", H-5", Ar-H], 3.42 [1H-OH]

Elemental Analysis:

%	C	H	O	N
Calculated	73.67	5.29	4.67	16.36
Found	73.68	5.26	4.67	16.37

Scheme-I



R
H, 4 -OCH_3, 4 -Cl,
4-NO_2, 4 -F, 2 -OH, 4 -$N(CH_3)_2$
4-OH 3-OCH_3, 3-OCH_3 4-OCH_3, CH=CH -C_6H_5

3. Biological Evaluation

3.1. Invitro antibacterial activity

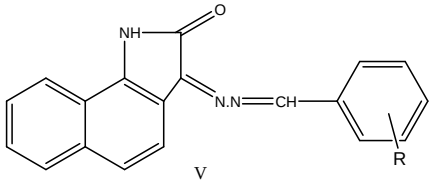
Compounds were evaluated for their *in vitro* antibacterial activity against 4 pathogenic bacteria by nutrient agar diffusion method. All bacteria were grown on nutrient agar (Himedia) plates. The test solutions at different concentrations was added to plates and incubated at 37 ± 1^0 C for 24 hrs. The diameter of zone of inhibition surrounding each of discs was measured with the help of an antibiotic zone recorder.

3.2. In vitro antifungal activity

Compounds were evaluated for their *in vitro* antifungal activity against 4 pathogenic fungi by agar diffusion method. All fungi were grown on Sabourad dextrose agar (Hi-media) plates (25^0 C for 48 hrs) the test solution at different concentrations was added to plates and incubated at 25^0C for 48 hrs the diameter of zone of inhibition surrounding each of discs was measured with the help of an antibiotic zone recorder.

Physical data

Table-I: Physical data of (3)-3-[(2)-(phenylmethylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-ones (v):



V

S.No.	Compounds	Substituents R	Mol. Formula	Mol. Weight	M.P.(^{0}C)	Yield (%)
1.	Va	H	$C_{19}H_{13}N_3O$	299.32	140-144	67
2.	Vb	4-OCH_3	$C_{20}H_{15}N_3O_2$	329.35	146-148	54
3.	Vc	4-OH, 3-OCH_3	$C_{20}H_{15}N_3O_3$	345.35	128-132	91
4.	Vd	4-Cl	$C_{19}H_{12}ClN_3O$	333.77	120-124	48
5.	Ve	4-NO_2	$C_{19}H_{12}N_4O$	344.32	152-154	76
6.	Vf	4-F	$C_{19}H_{12}FN_3O$	317.31	170-172	92
7.	Vg	3-OCH_3, 4-OCH_3	$C_{21}H_{17}N_3O_3$	359.37	124-126	46
8.	Vh	2-OH	$C_{19}H_{13}N_3O_2$	315.32	144-146	72
9.	Vi	4-N(CH_3)$_2$	$C_{21}H_{18}N_4O$	342. 19	158-160	94
10.	Vj	1-CH═CH-	$C_{21}H_{15}N_3O$	325.13	148-150	64

4. Results and Discussion

As many as new ten 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 I.

All the synthesized compounds were tested for *in vitro* antibacterial activity by the agar diffusion method. The results are summarized in table II that includes the activity of reference compound Ampicillin.

The tested compounds exhibited mild to moderate antibacterial activity against the all four strains of bacteria. The compound Vi (R=4-N (CH_3)$_2$) tested against both gram positive and gram negative organisms, showed more potent antibacterial activity. It has also been observed that compounds Vh and Ve showed activity against both bacteria.

All the synthesized compounds were tested for antimicrobial activity by the agar diffusion method. The results are summarized in table III that includes the activity of reference compound Clotrimazole. The antifungal activity of synthesized compounds was studied against Candida albicans and yeast, Clotrimazole was used for reference for inhibitory activity against fungi. It was observed that all the compounds showed mild to moderate antifungal activity. The compound compound Vi (R=4-N $(CH_3)_2$) showed more potent activity against both *C. albicans* and Yeast. It has also been observed that compounds Va, Vb and Vh showed activity against both organisms.

The antimicrobial study revealed that 3rd position of bezo-indole 2-one with para-dimethyl amino, hydroxy benzaldehyde derivatives produced more active compounds in a series.

Table-II: Antibacterial activity of (3)-3-[(2)-(phenylmethylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-ones (V):

S.No	Substituents		Concentration	Zone of inhibition (in mm)			
	Compound No	R	Conc. in µg/ml	B. subtilis	S. aureus	E.coli	P.vulgaris
1	Va	H	100	8	10	8	9
			150	10	12	10	10
			200	13	13	11	12
2	Vb	4-OCH_3	100	9	8	7	8
			150	11	10	9	9
			200	12	11	10	11
3	Vc	4-OH, 3-OCH_3	100	11	10	9	8
			150	13	12	12	11
			200	16	14	14	13
4	Vd	4-Cl	100	7	8	8	9
			150	9	10	10	11
			200	11	12	11	14
5	Ve	4-NO_2	100	8	10	9	10
			150	10	12	11	13
			200	12	14	12	15
6	Vf	4-F	100	10	11	10	9
			150	13	14	13	11
			200	15	16	15	14
7	Vg	3-OCH_3, 4-OCH_3	100	9	7	8	9
			150	12	10	11	11
			200	14	13	12	13
8	Vh	2-OH	100	11	10	10	9
			150	14	13	14	12
			200	16	15	15	14
9	Vi	4-$N(CH_3)_2$	100	12	11	10	10
			150	14	13	12	12
			200	18	18	16	15
10	Vj	1-CH=CH-	100	8	9	10	8
			150	9	11	11	10
			200	11	13	12	11
Standard drug: Ampicillin (10 µg/ml) shows 20 mm Zone of inhibition							

Table-III: Antifungal activity of (3)-3-[(2)-(phenylmethylidene) hydrazono]-1, 3-dihydro-2*H*-benzo[*g*]indol-2-ones (V):

S. No	Substituents		Concentration	Zone of inhibition (in mm)	
	Compound No	R	Conc. in µg/ml	Candida albicans	Yeast
1	Va	H	100	9	9
			150	12	10
			200	14	12
2	Vb	4-OCH_3	100	8	8
			150	11	10
			200	13	12
3	Vc	4-OH, 3-OCH_3	100	9	9
			150	10	11
			200	12	12
4	Vd	4-Cl	100	9	8
			150	10	10
			200	12	11
5	Ve	4-NO_2	100	10	8
			150	11	10
			200	12	11
6	Vf	4-F	100	8	7
			150	10	9
			200	11	10
7	Vg	3-OCH_3, 4-OCH_3	100	9	8
			150	10	10
			200	12	11
8	Vh	2-OH	100	8	8
			150	10	10
			200	13	12
9	Vi	4-$N(CH_3)_2$	100	12	9
			150	14	11
			200	16	13
10	Vj	1-CH=CH-	100	10	9
			150	11	10
			200	13	12
Standard drug: Clotrimazole (10 µg/ml) shows 22 mm Zone of inhibition					

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