Iranian Journal of Pharmaceutical Research (2006) 4: 249-254
Received: August 2005
Accepted: July 2006

Original Article

Synthesis and Antimicrobial Activity of Some New Isatin Derivatives

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Abstract

Some new 3-[(5-benzylidene-2-phenyl)-3, 5-dihydro-4-*H*-imidazol-4-one-3-(4-bezoylhydrazono)]-indole-2-ones **(VIII)** have been synthesized from different isatinhydrazones **(II)** by condensing with 2-phenyl-5-benzylidene- 3-N (4-acetyl phenyl)-1, 5-dihydro-imidazol-4-one **(VII)**. Their chemical structures have been confirmed by IR, ^{1}HNMR, 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-Br substitution showed the most favourable antimicrobial activity.

Keywords: Isatin; Isatin hydrazone; Imidazolone; Antibacterial; 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), anti-fungal (5), anti-tubercular (6), anti-depressant (7). Isatin hydrazones have been reported to possess anticonvulsant (7) activity also. In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize some new 3–[(5-benzylidene -2-phenyl)-3, 5-dihydro-4*H*-imidazol-4-one-3-(4-benzoylhydrazono)]-indole -2-ones **(VIII)**. All the synthesized compounds were screened for their in vitro anti-bacterial and anti-fungal activity.

Experimental

All the melting points were determined by open capillary using Toshniwal 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 8400 Shimadzu spectrophotometer. 1H NMR spectra were recorded on 300 MHz Bruker DPX using $CDCl_3$ and MASS spectra were recorded on VG AUTOSPEC using EI-MS mode. Elemental analysis was performed on Perkin-Elmer Series 2400.

4-Benzylidine-2-phenyloxazol-5-one **(V)** & methyl p-aminobenzoate **(VI)** were synthesized by the methods available in the literature (8). Synthesis of the title compounds has been depicted as shown in Figure 1.

Synthesis of isatins (Indole- 2, 3-diones) (III)

The different isonitrosoacetanilides were prepared from the respective aromatic amines **(I)** viz. aniline, p-bromoaniline and p-toluidine etc. on reaction with chloralhydrate and hydroxylamine hydrochloride. Each of the isonitrosoacetanilide **(II)** was subjected to a

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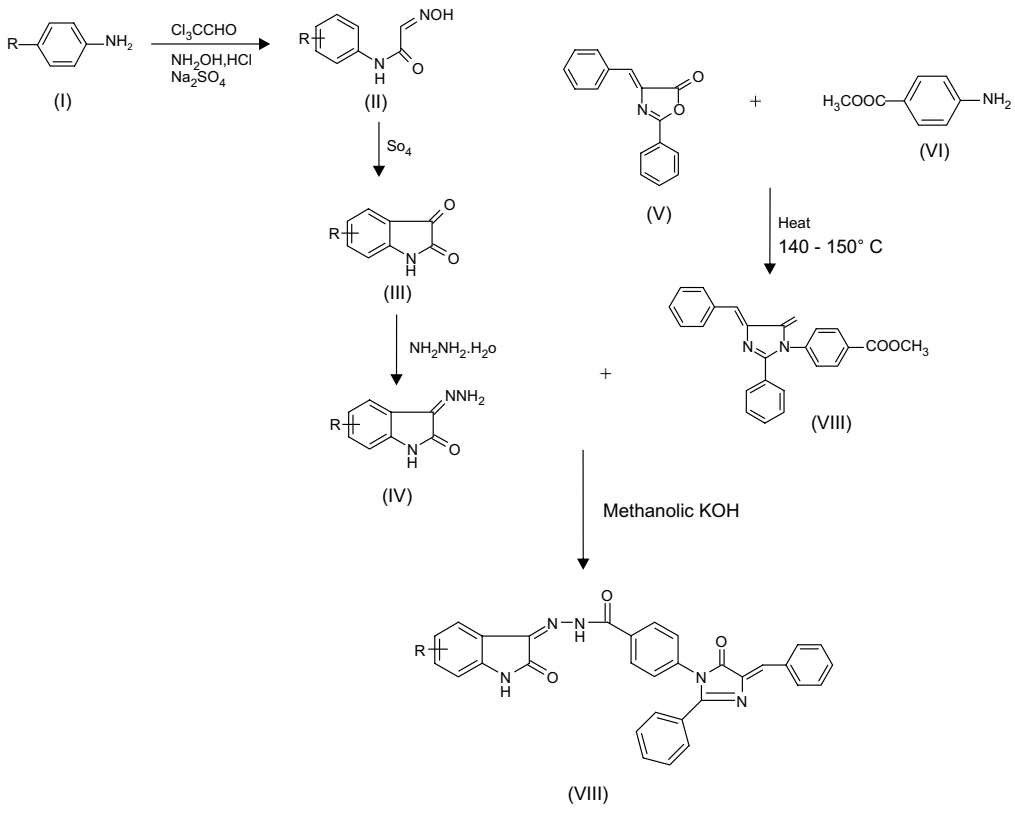

Figure 1. Schematic steps of isatin derivatives synthesis.

dehydrative cyclization using sulphuric acid (d 1.84) to yield the corresponding isatin **(III)**. All these isatin thus prepared were identified by their physical constants reported in the literature (9).

Synthesis of isatin hydrazones (IV): General Procedure

An appropriate isatin (indole-2,3-dione) (V, 0.01 mol) was dissolved in alcohol (20 ml) and added hydrazine hydrate (99%, 0.015 mol) while shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 h. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and purified by recrystallization from chloroform. (IV) M.W: 161, M.F: $C_8H_7N_3O$, M.P 220° C. Yield 74.5%.

The compounds thus obtained were characterized by comparison with their physical constants reported in the literature (10, 11).

Synthesis of 2-phenyl 5-benzylidene 3N-(4-acetyl phenyl)-1, 5-dihydro-imidazole-4-one (VII)

An equimolar mixture of 4-benzylidine-2-phenyloxazol-5-one **(V)** and Methyl p-aminobenzoate **(VI)** was heated on oil bath at 140° C for 40-50 min. The resulting jelly like mass was recrystallized from methanol. **(VII)** M.W: 382, M.F: $C_{24}H_{18}N_2O_3$, M.P.:181° C. IR(KBr in cm^{-1}): 1280 (O-C=O bending), 1712, (C=O streching), 2923 (Ar-CH streching): ^1H NMR ($CDCl_3$) (δppm):3.901 (s, 3H, CH_3) , 2.597 (s, 1H ,CH), 7-8 (m,14H ,Ar –CH); MS: (m/z) 382 (M^+); Analysis ($C_{24}H_{18}N_2O_3$) Cal.(Found)%: C 75.39 (75.35), H 4.7 (4.2), N 7.3 (7.01).

Synthesis of 3–[(5-Benzylidene-2-phenyl)-3,5-dihydro-4H-imidazole-4-one-1-(4-benzoylhydrazono)]-indole-2-ones (VIII)

A mixture of equimolar quantity of isatin hydrazones (II, 0.01 mol) and 2-phenyl 5-bezylidene 3N (4-acetyl phenyl)-1, 5-dihydro-imidazole-4-one (V, 0.01 mol), was dissolved in methanol containing a catalytic amount of

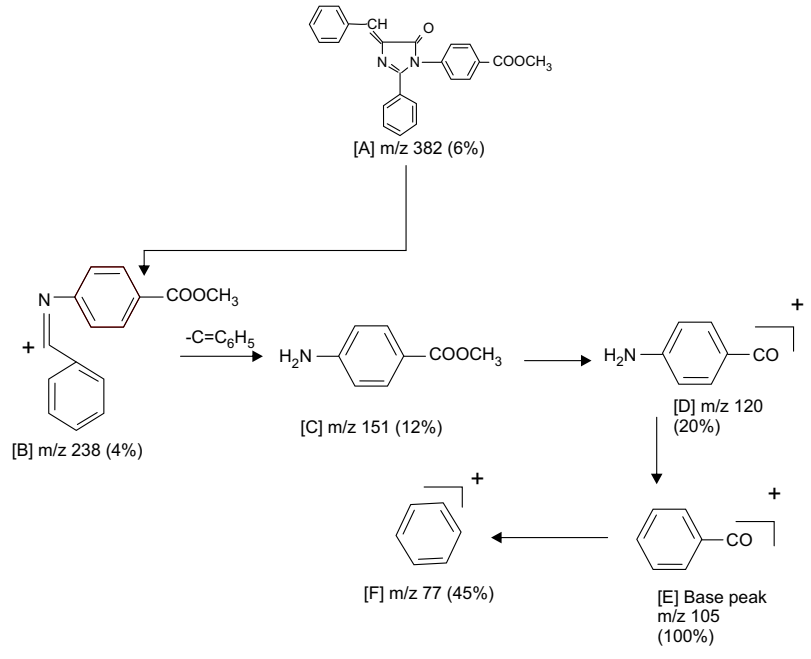

Figure 2. Mass spectral fragmentation pattern of 2-phenyl 5-benzylidene 3-N-(4-acetyl phenyl)-1,5-dihydro-imidazole-4-one (VII).

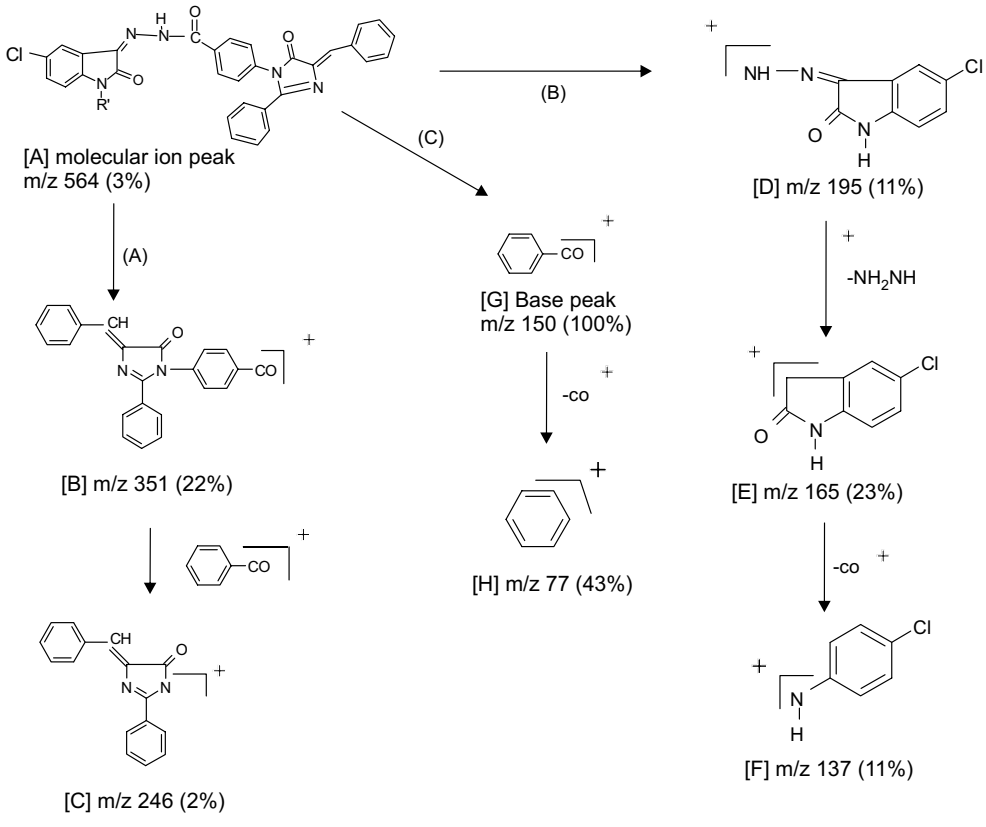

Figure 3. Mass spectral fragmentation pattern of 3–[(5-Benzylidene-2-phenyl) 3,5-dihydro-4H-imidazole-4-one-1-(4-benzoylhydrazono)]-indole-2-ones (VIIIb).

Table 1. Physical data and elemental analysis of synthesized compounds (VIIIa-n).

Sr. No	Compound code	R	R´	M.P. (ºC)	MOLECULAR FORMULA	M.W.	YIELD (%)	*Elemental Analysis[a] Found (Calculated) (%)*		
								C	H	N
1	VIIIa	H	H	280	$C_{31}H_{21}O_3N_5$	511	85	72.79 (72.75)	4.10 (4.05)	13.69 (13.71)
2	VIIIb	5-Cl	H	252	$C_{31}H_{20}O_3N_5Cl$	546	75	68.13 (68.20)	3.66 (3.60)	12.82 (12.80)
3	VIIIc	5-F	H	237	$C_{31}H_{20}O_3N_5F$	529	78	70.32 (70.25)	3.78 (3.85)	13.23 (12.21)
4	VIIId	5-Br	H	265	$C_{31}H_{20}O_3N_5Br$	591	80	62.94 (62.90)	3.38 (3.34)	11.84 (11.86)
5	VIIIe	4-Cl,5-F	H	232	$C_{31}H_{19}O_3N_5FCl$	565	74	65.84 (64.88)	3.36 (3.33)	12.38 (12.35)
6	VIIIf	5-CH_3	H	198	$C_{32}H_{23}O_3N_5$	526	69	73.00 (72.98)	4.37 (4.40)	13.30 (13.32)
7	VIIIg	5-NO_2	H	270	$C_{31}H_{20}O_5N_6$	557	65	66.78 (66.74)	3.59 (3.56)	15.08 (15.12)
8	VIIIh	5-COOH	H	> 300	$C_{32}H_{21}O_5N_5$	558	78	69.06 (69.09)	3.37 (3.34)	12.58 (12.62)
9	VIIIi	7-COOH	H	215	$C_{32}H_{21}O_5N_5$	558	72	69.06 (69.08)	3.37 (3.40)	12.58 (12.62)
10	VIIIj	7-$COOCH_3$	H	200	$C_{33}H_{23}O_5N_5$	570	52	66.47 (66.50)	4.03 (4.05)	12.28 (12.24)
11	VIIIk	H	Methyl	152	$C_{33}H_{23}O_4N_5$	554	72	71.48 (71.45)	4.15 (4.12)	12.63 (12.66)
12	VIIIl	H	Methyl	143	$C_{32}H_{23}O_3N_5$	526	77	73.00 (72.97)	4.37 (4.35)	13.30 (13.32)
13	VIIIm	5-Br	Methyl	183	$C_{33}H_{22}O_4N_5Br$	634	70	62.46 (62.44)	3.47 (3.50)	11.04 (11.06)
14	VIIIn	5-Br	Methyl	187	$C_{32}H_{22}O_3N_5Br$	606	71	63.36 (63.35)	3.63 (3.66)	11.55 (11.58)

[a] All compounds gave satisfactory elemental analysis (± 0.4%).

potassium hydroxide and heated under refluxed for 5-6 h. The reaction mixture was cooled and neutralized with concentrated hydrochloric acid. The resultant product was then filtered, dried and purified by recrystallization from methanol.

(VIIIb) M.W:- 546, M.F $C_{31}H_{20}ClN_5O_3$, M.P.: 252°C, IR (KBr in cm^{-1}): 1280.65 (O-C=O bending), 1712 (CO streching), 2923 (ArCH streching):, ^1H NMR ($CDCl_3$) (δppm):3.90 (s, 3H, CH_3), 2.1(s, 1H ,CH), 7-8 (m,14H , Ar–H) , MS (m/z): 546 (M^+)

In vitro antimicrobial activity

Evaluation of antibacterial (4-bacteria) and antifungal (2-fungi) activities was done by the disk diffusion technique (12). The microorganisms used were purchased from National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory (NCL), Pune (India).

The tested compounds solutions were prepared in dimethylformamide and evaluated them for their *in vitro* antibacterial and antifungal activities against *Bacillus subtillis* NCIM 2250, *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2109, *Salmonella typhi* NCIM 2501, *Aspergillus niger* NCIM 501 and *Candida albicans* NCIM 7431, respectively.

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

Table 2. In *Vitro* Anti microbial activity of 3-imino –[(4-Benzylidene-2-phenyl-imidazole- 5-one-1-(4-bezoylhydrazono)]-indole-2-ones (VIIIa-n)[a].

Compound code	R	R´	In *Vitro* Antibacterial Activity				IN *VITRO* ANTIFUNGAL ACTIVITY	
			S. aureus	*E. coli*	*S. typhi*	*B. subtillis*	*A.. niger*	*C. albicans*
VIIIa	H	H	10 ± 0.66[b]	10 ± 1.33	12 ± 0.66	NA	12 ± 1.33	10 ± 0.66
VIIIb	5-CL	H	14 ± 0.66	11 ± 1.33	11 ± 1.33	13 ±1.33	11 ± 0.66	13 ± 0.66
VIIIc	5-F	H	14 ± 1.33	11 ± 0.66	12 ± 0.66	11 ± 1.33	11 ± 0.66	13 ± 1.33
VIIId	5-Br	H	12 ± 1.33	12 ± 0.66	11± 1.33	13 ± 1.33	12 ± 1.33	12 ± 0.66
VIIIe	4-Cl,5-F	H	NA[c]	NA	NA	12 ± 0.66	NA	NA
VIIIf	5-CH_3	H	NA	NA	11±1.33	NA	NA	NA
VIIIg	5-NO_2	H	12 ± 0.66	NA	NA	12 ± 0.66	NA	12 ± 0.66
VIIIh	5-COOH	H	11 ± 1.33	NA	10 ± 1.33	12 ± 1.33	11 ± 0.66	11 ± 0.66
VIIIi	7-COOH	H	NA	NA	NA	NA	NA	NA
VIIIj	7-$COOCH_3$	H	NA	NA	NA	NA	NA	NA
VIIIk	H	Acetyl	NA	NA	NA	NA	NA	NA
VIIIl	H	Methyl	NA	NA	NA	NA	NA	NA
VIIIm	5-Br	Acetyl	NA	NA	NA	NA	NA	NA
VIIIn	5-Br	Methyl	NA	12 ± 1.33	11 ± 0.66	NA	12 ± 1.33	NA
Gentamicin (10 µg/disc)				20 ± 1.33	22 ± 0.66	21 ± 0.66	-------	-------
Amphotericin B (20 µg/disc)				-------	-------	-------	15 ± 1.33	16 ± 0.66

[a]Concentrations of test compound: 100 µg/disc
[b]Zone of inhibition in mm (Mean ± S.D, n=3).
[c]NA=Not Active

Results and Discussion

As many as new fourteen 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 Gentamicin.

The tested compounds exhibited mild to moderate antibacterial activity against all four strains of bacteria.The compounds, VIIIb, VIIIc, VIIId and VIIIg tested against *S.aureus*, showed highest activity. It has also been observed that compounds, VIIIb and VIIId showed activity against *B.subtilis*.

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 gentamicin.

The antifungal activity 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 VIIIb, VIIIc and VIIId had highest activity against *C. albicans* and VIIIa, VIIId and VIIIn showed good activity against *A. niger*.

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

Acknowledgement

The authors are very much thankful to the Principal, R.C.Patel College of Pharmacy, Shirpur for providing necessary facilities during the work.

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