Acta Pharmaceutica Turcica
47: 21- 29 (2005)

Synthesis and Evaluation of Schiff and N-Mannich Bases of Isatin as Potential Antimicrobial Agents

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Abstract

A series of schiff and N-Mannich bases of isatin (indol-2,3-dione) were designed with the aim to develop novel antimicrobial agents of synthetic origin having broad spectrum of activity and high potency. The chemical structure of all the compounds was established by means of UV, IR, ^{1}H NMR spectra and elemental analysis(C,H,N).The compounds were then screened for *in vitro* antibacterial & antifungal activity against 25 pathogenic bacteria and 5 pathogenic fungi respectively. Most of the compounds showed high to moderate activity against all tested microbes.

Key words: Isatin; Schiff bases, N -Mannich bases, Antimicrobial

Introduction

Isatin (indol-2,3-dione) is a versatile endogenous heterocyclic molecule identified in human and rat tissues. Several of its 1,3 and 5 derivatives were reported to elicit promising pharmacodynamic activities viz. Anticonvulsant (Gursoy *et al.*, 1996; Pandeya *et al.*, 2002) anti-HIV (Teitz *et al.*, 1994), antineoplastic (Popp and Pajouhesh, 1983), tuberculostatic (Varma and Nobles, 1962), anti-inflammatory (Kar *et al.*, 2003). At milimolar concentrations, isatin has been found to inhibit different enzymes, an effect that may contribute to its anti infective actions (Glover *et al.*, 1991). Therefore we aimed to prepare a novel series of N-substituted isatin-3-hydrazones by following Schiff & Mannich reaction and to establish their antimicrobial potency against both bacteria and fungi.

Materials and Methods

Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using silica gel G glass plates as stationary phase, chloroform and methanol (2:1) as mobile phase. Elemental analysis (CHN) were undertaken for all compounds and were within ± 0.4% of the calculated values. The IR spectra were recorded on a JASCO FTIR 5300

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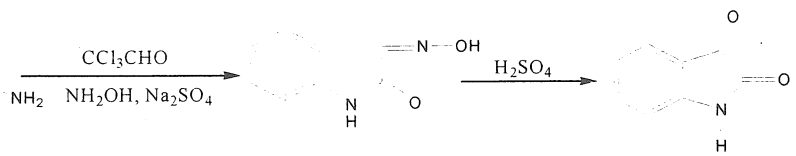
instrument using KBr and ^{1}H NMR spectra were recorded at 90 MHz on a Jeol FX 90Q FT-NMR spectrophotometer using $DMSOd_6$ as solvent and TMS as an internal standard.

Chemistry

The isatin was prepared by following the procedure reported earlier (Blatt, 1964) (Scheme 1).

Scheme 1.



Synthesis of hydrazides (I & III) and hydrazone (II)

Equimolar quantities of hydrazine hydrate (0.1 mol) and the acid chloride or aldehyde were kept in separate ice cold bath. Acid chloride or aldehyde was slowly transferred with stirring at 0°C for 15 min. It was then filtered and recrystallized with ethanol.

Synthesis of Schiff bases with hydrazides and hydrazones (1,3,4)

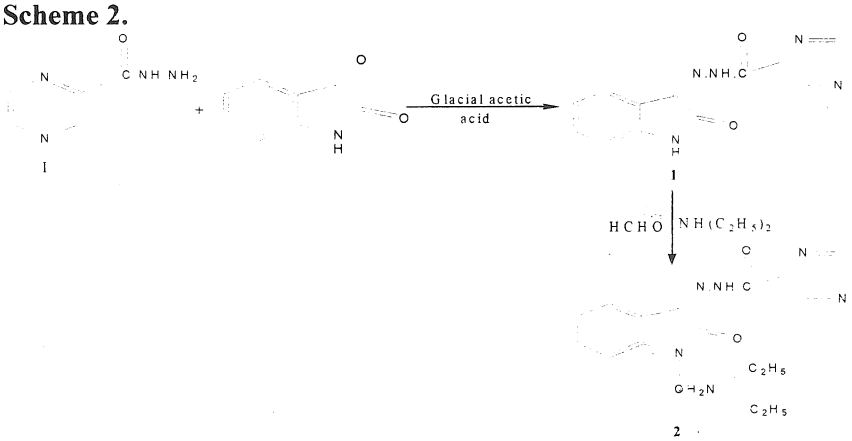
Hydrazides I & III (dissolved in ethanol) / hydrazones II were refluxed with equimolar quantities of isatin on a water bath for 2 h with occasional stirring. Then it is kept in ice cold conditions for 1 h Filtered, washed with ice cold water, dried and recrystallised from ethanol water mixture.

Synthesis of N-Mannich bases of Isatin-3-(substituted hydrazones/hydrazide) (2,5,6,7,8,9)

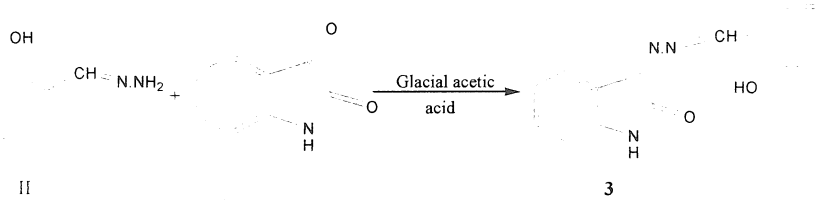
0.5 mol of dimethyl amine (5) / diethyl amine (2,6) / piperidine (9) / morpholine (7) / pyrazinamide (8) in little quantity of THF was added slowly to the slurry of isatin-3-(substituted hydrazides / hydrazone)derivatives, 37% formaldehyde in little quantity of THF. The reaction mixture was warmed on steam bath for 15 min. and then stirred at 0°C for 45 min and left overnight. The solid product was filtered and recrystallised from acetone-water mixture.

Percentage yield and melting points of the synthesized compounds are presented in table 1.

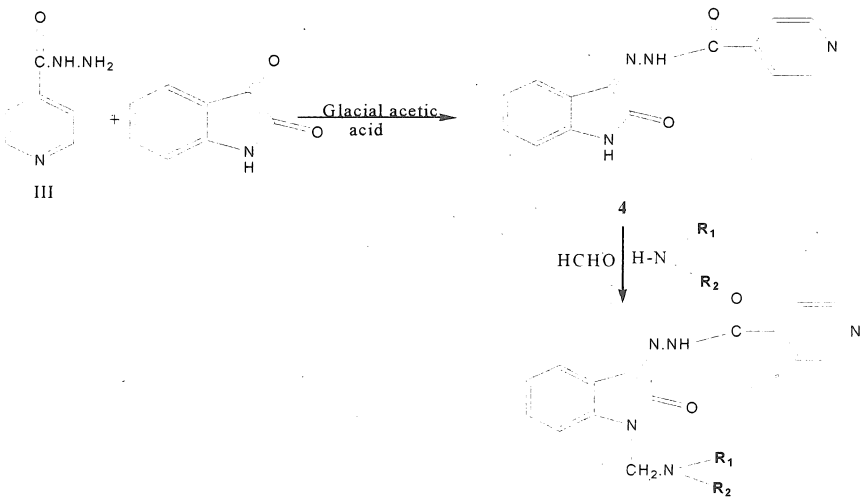
Scheme 2.



Scheme 3.



Scheme 4.



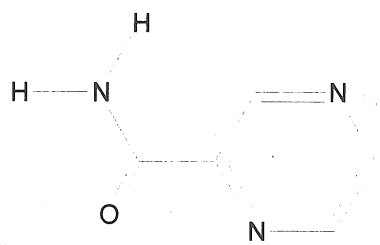
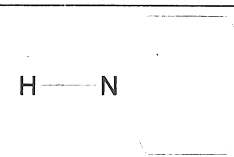
Compound	R_1 H-N R_2
5	CH_3 H—N—CH_3
6	C_2H_5 H—N—C_2H_5
7	
8	
9	

Table 1. Charactaristics of the synthesized compounds.

Compd.	Melting point (°C)	Yield (%)	Molecular weight (g)	Molecular formula	Elemental analysis Calculated% Found% C	N	H	λ_{max} in MeOH (nm)	R_f	R_m	$LogP_0$ $P_{0=}C_{org}/C_{aq}$ *
1	256	71	267.24	$C_{13}H_9N_5O_2$	58.43 58.50	3.39 3.30	26.21 26.30	-	0.51	- 0.017	-0.1957
2	71	57	352.39	$C_{18}H_{20}N_6O_2$	61.35 61.33	5.72 5.75	23.85 23.84	207.5 247.5	0.52	- 0.034	-0.2749
3	264	74	265.27	$C_{15}H_{11}N_3O_2$	67.92 67.91	4.18 4.18	15.84 15.94	-	0.47	0.052	-0.1123
4	296	68	266.25	$C_{14}H_{10}N_4O_2$	63.15 63.10	3.79 3.78	21.04 21.04	205, 271.5, 328.5	0.58	- 0.140	-0.0784
5	74	68	323.35	$C_{17}H_{17}N_5O_2$	63.15 63.15	5.30 5.28	21.66 21.69	210, 247.5, 327.5	0.57	- 0.122	-0.2174
6	77	62	351.40	$C_{19}H_{21}N_5O_2$	64.94 64.96	6.02 6.00	19.93 19.98	207.5, 245, 315	0.63	-0.23	-0.4828
7	98	57	365.39	$C_{19}H_{19}N_5O_3$	62.46 62.44	5.24 5.23	19.17 19.14	207.5, 252.5, 330	0.53	- 0.052	0.4678
8	81	69	401.38	$C_{20}H_{15}N_7O_3$	59.85 59.88	3.77 3.79	24.43 24.42	210, 267.5, 322.5	0.60	- 0.176	-0.2188

$CHCl_3$- phosphate buffer (pH7.4) partition coefficient

IR(KBr ν cm^{-1}) and ^1H NMR (DMSO-d_6, δppm)

1: 3450 & 3200 (amide NH asym. & sym.), 1728 (keto C=O), 1680 (imine C=N), 1610 (amide C=O), 1520 (pyrazine C=C), 860-660 (aromatic CH bending). ^1H NMR(DMSOd_6δ) 8.8-9.2 (m,3H,pyrazine ring), 7.2-7.7 (m,4H,indole ring), 7.9(s,1H,NH).

2: 3400 (NH), 2970 (aliphatic CH), 1710 (imine C=N), 1620 (amide C=O), 1480&1360 (CH_3 bend), 1460 (CH_2 bend), 1100 (amine CN), 760 (aromatic CH bending). ^1H NMR(DMSOd_6δ) 8.8-9.2 (m,3H,pyrazine ring), 7.2-7.7 (m,4H,indole ring), 4.1 (s,2H,CH_2 group), 2.6 (d,2H,ethyl CH_2 group), 1.2 (t,6H,ethyl CH_3).

3: 3650 (OH), 3400 (amide NH), 1620 (amide C=O), 1570 (imine C=N), 1480 (aromatic C=C), 3000 (aromatic CH), 780-720 (aromatic CH bending).

4: 3240 (amide NH), 1720 (keto C=O), 1650 (imine C=N), 1620 (amide C=O), 1540 (NH bend), 1460 (C-N), 890-740 (aromatic CH bending). ^1H NMR (DMSOd_6δ) 8.25 (s,1H,NH group), 7.9 (m,4H,pyridine ring), 7.3 (m,4H,indole ring).

5: 3200 (amide NH), 2850 (aliphatic CH_3 stretch), 1720 (amide C=O), 1670 (imine C=N), 1610 (aromatic C=C), 1550 (amide NH), 1460 &1340 (aliphatic CH_3), 1140 (amine CN), 760-650 (aromatic CH bending). ^1H NMR (DMSOd_6δ) 7.9 and 9.0 (d,4H,pyridine ring), 7.2-7.4 (m,4H,CH indole ring), 4.5 (s,2H,CH_2 group), 2.3 (s,6H,CH_3 group).

6: 3400 (NH), 3000 (CH_2), 1700 (amide C=O), 1600 (C=N), 1550 (amide NH bend), 1475&1380 (CH_3bend), 1460 (CH_2 bend), 1160 (amine CH). ^1H NMR(DMSOd_6δ) 7.85 and 9.0 (d,4H,pyridine ring), 7.2-7.4 (m,4H,CH indole ring), 4.0 (s,2H,CH_2 group), 2.8 (d,2H,ethyl CH_2 group), 1-1.3 (t,6H,ethyl CH_3).

7: 3450 (NH), 2850 (CH_2), 1700 (C=N), 1640 (amide C=O), 1610 (amide NH bend), 1120 (amine CN), 760 (aromatic CH bending). ^1H NMR(DMSOd_6δ) 7.85 and 9.0 (d,4H,pyridine ring), 7.2-7.4 (m,4H,CH indole ring), 4.0 (s,2H,CH_2 group), 2.4 and 3.8 (d,4H,morpholine ring).

8: 3420 (NH), 2950 (CH_2), 1700 (C=N), 1680& 1600 (amide C=O), 1610 (amide NH bend), 1120 (amine CN), 760 (aromatic CH bending). ^1H NMR(DMSOd_6δ) 7.85-9.0 (m,7H,hetero rings), 7.2-7.4 (m,4H,CH indole ring), 4.5(s,2H,CH_2 group)

9: 3400 (NH), 2950 (CH_2), 1700 (C=N), 1605 (amide C=O), 1560 (amide NH bend), 1460 (aromatic C=C bend), 1080 (amine CN), 760-680 (aromatic CH bending). ^1H NMR(DMSOd_6δ) 7.85 and 9.0 (d,4H,pyridine ring), 7.2-7.4 (m,4H,CH indole ring), 4.6 (s,2H,CH_2 group), 1.7 and 2.1 (d,4H,tetrahydropyridine ring)

Antimicrobial Screening

All the compounds were evaluated for their *in vitro* antibacterial activity against 25 pathogenic bacteria and antifungal activity against 5 pathogenic fungi by agar dilution method using Mueller-Hinton agar and Sabouraud's dextrose agar respectively (Barry, 1991). The strains for antimicrobial screening were procured from Institute of Medical Sciences, Banaras Hindu University. The activities are presented in table 2 & 3 .

Results and Discusion

Schiff bases of isatin were obtained successfully in pure form as indicated by TLC & IR data. In the preparation of mannich bases, the reaction was carried out in ice cold condition with little warming to obtain pure compounds avoiding the formation of by products. The spectral data of all the compounds were consistent with their structures. In UV spectra, the π→π* transition of benzene appeared in the region 210-230 nm and the absorption in the region 256-310 nm confirmed n→π* transition of heterocyclic ring. IR spectra showed characteristic peak for N-H stretching and C=O stretching in the region of 3500-3150 cm^{-1} and 1728-1710cm^{-1} respectively. The peaks at 1570 cm^{-1} and 1470 cm^{-1} confirmed the presence of C=N in schiff bases & -CH_2-

of mannich bases. These facts were also supported by characteristic NMR data, δ= 8.0 for NH (D_2O exchangeable), δ=4.4 for methylene protons and 7.2-7.5 for aromatic ring protons. In the antibacterial screening, the MIC of the compounds against susceptible strains was determined and compared with standard drugs such as norfloxacin & isoniazid. Most of the compounds showed activity against both G+ve & G-ve strains. Compound **1** was highly active against *E.Coli, Morgenella mergonii, A.hydrophila, Ser.marcescens & Citrobacter freundii* and compound **6** and **7** showed excellent activity against *S.aureus, S.albus, Morgenella mergonii* and various species of Shigella indicating broad-spectrum activity. Among the compounds tested for antifungal activity, compounds **5** & **6** showed activity against all tested fungi. These results revealed that the N-mannich bases of isatin-3-hydrazones (1,3 disubstituted) showed superior antimicrobial potency compared to schiff bases (3 substituted). Especially the N substituted isatin with isoniazid schiff bases (**5-8**) were very active against most of the tested bacteria even at MIC of less than 3μg/ml and were comparable to norfloxacin. This may be due to the fact that higher lipophilicity elicited by bulky substituents at N1 position as in compounds N-morpholino methyl Isatin-3-isonicotinyl hydrazone and N-piperidinomethyl isatin-3-isonicotinyl hydrazone favoured increased penetration into biological membranes thereby resulted in greater antimicrobial potency. Further *in vivo* antimicrobial studies on the above compounds are in progress.

Table 2. *In vitro* antibacterial activities (MIC) of synthesized compounds in μg/ml.
(-) : ineffective INH: Isoniazid NFXL: Norfloxacin

Organisms	**Compound**									**Reference**	
	1	2	3	4	5	6	7	8	9	INH	NFXL
B.Subtilis	157.8	111.7	320.2	5.9	5.2	<0.6	<0.6	1.2	2.5	2478.7	-
S.Aureus	2.4	< 7	2.5	5.9	2.6	2.4	<0.6	1.2	2.5	<9.68	<1.8
S.Albus	157.8	< 7	320.2	5.9	5.2	1.2	0.6	1.2	0.6	77.5	<1.8
E.Coli	<1.2	446.9	320.2	5.9	20.9	39	19.8	26.5	2.5	154.9	469.6
E.Coli MCTC10418	2.4	-	5.0	758.8	671.2	78	79	53	19.6	-	58.7
Ps. vulgaris	2.4	446.9	5.0	89.7	83.9	1.2	<4.9	6.6	2.5	2478.7	-
Ps.aeruginosa	57.8	446.9	320.2	<1.4	10.5	78	<1.2	26.5	2.5	309.8	58.7
Ps.aeruginosa MC10662	2.4	111.7	5.0	<1.4	10.5	312.2	79	26.5	<0.3	77.5	<1.8
V.cholerae	157.8	893.9	320.2	189.7	167.8	1.2	19.8	26.5	2.5	154.9	58.7
V.parahaem.	<1.2	< 7	320.2	<1.4	5.2	78	7.9	26.5	2.5	-	<1.8
V.cholerae nonO$_1$	2.4	893.9	320.2	758.9	335.6	4.8	39.5	26.5	2.5	309.8	58.7
Enterobacter	2.4	446.9	5.0	189.7	83.9	4.8	<1.2	26.5	1.2	2478.7	<1.8
Sh. boydii	157.8	111.7	160.1	5.9	167.8	<0.15	<1.2	6.6	1.2	154.9	<1.8
Sh. dysenteriae	157.8	446.9	5.0	5.9	5.2	78	9.9	53	<0.3	-	<1.8
Sh. flexneri	2.4	893.9	320.2	758.8	83.9	<0.6	19.8	1.2	4.9	<9.68	<1.8
Sh. sonnei	2.4	446.9	320.2	189.7	671.2	78	2.4	13.2	39.7	-	58.7
Salmonella para A	<1.2	446.9	5.0	5.9	2.6	<0.6	9.9	53	0.3	77.5	-
Salmonella para B	<1.2	111.7	2.5	5.9	5.2	78	<1.2	26.5	2.5	1239.4	-
Sal-. Enteridis	2.4	446.9	320.2	189.7	10.5	4.8	9.9	26.5	<0.3	154.9	58.7
Sal. Typhimurium	2.4	893.9	320.2	189.7	167.8	312.2	<1.2	26.5	9.8	-	58.7
Klb. Pneumoniae	2.4	55.9	320.2	5.9	167.8	<0.15	<1.2	1695.8	2.5	<9.68	<1.8
Ser. marcescens	<1.2	446.9	320.2	189.7	167.8	<0.15	<1.2	26.5	19.6	2478.7	<1.8
Citrobacter freundii	<1.2	893.9	2.5	189.7	671.2	78	9.9	26.5	19.6	2478.7	58.7
Morgenella mergonii	0.6	< 7	<1.2	<1.4	167.8	78	79	26.5	39.7	<9.68	58.7
Aeromona hydrophila	0.6	446.9	40.0	-	<2.6	<0.15	19.8	26.5	2.5	154.9	<1.8

Table 3. Antifungal activity after 7, 10 & 14 days

FUNGI	COMPOUND									
	Control	1	2	3	4	5	6	7	8	9
Candida albicans	+ + +	+ ± -	+ + +	+ + -	+ + -	+ + +	+ - -	+ ± -	+ + +	+ + +
Aspergillus flavus	+ + +	+ + -	+ + +	+ + +	- - -	+ + +	+ + +	+ ± -	± ± -	+ + +
Cryptococcus neoformans	+ + +	± - -	+ + +	- - -	- - -	+ + +	+ + +	+ ± -	+ + +	± - -
Histoplasma capsulatum	+ + +	± - -	+ + +	± - -	± - -	+ + +	+ + +	+ + +	+ + +	+ + +
Microsporum audouinii	+ + +	± - -	+ + +	- - -	- - -	+ + +	+ + +	+ + +	+ + +	+ + +

+) : Inhibition; -): no inhibition; ±) :weak inhibition ; Concentration : 100µg/ml.; Incubation : 37°C; NT: not tested ; Control : Nystatin

Acknowledgement

One of the authors (P.S Bhasin) is grateful to University Grant Commission, New Delhi for the award of Junior Research Fellowship.

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Received: 20.10.2004
Accepted: 01.03.2004