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Synthesis and Antimicrobial Activity of N-Norfloxacin Mannich Bases of Isatin and Its Derivatives

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Schiff bases of isatin (and derivatives) with isoniazid and their N-norfloxacin Mannich bases were synthesized. Their chemical structures have been confirmed by IR, ^{1}H NMR and by elemental analyses. Antimicrobial evaluation was done against twenty six pathogenic bacteria and six pathogenic fungi. Most of the N-Mannich bases showed higher activity against most of the bacteria in comparison to norfloxacin. All the fungal strains were sensitive to the synthesized N-Mannich bases at the concentration of 50 µg/mL.

Key Words: N-Norfloxacin, Mannich bases, Antimicrobial activities.

INTRODUCTION

Isatin is an endogenous compound with a long history and a broad range of pharmaceutical actions. It has long range of action in CNS-MAO inhibition and as anticonvulsant and anxiogenic[1-4]. Isatin selectively damages *Echinococcus multilocularis* and may have role in chemotherapy of infections caused by parasites[5]. Some Mannich bases of isatin appear to act as antifungal, antibacterial and antiviral agents[6]. A series of 5-haloisatin was aminomethylated in position-1 and hydrazino group were introduced in positions-3. Synthesized N-Mannich bases and hydrazones were tested against various bacteria and fungi. Halogen in position-6 and amino moiety in position-1 showed better activity than unsubstituted isatin[7].

Isoniazid is a good antimicrobial compound. Many quinolones with C-7 substituent having a basic heterocyclic group have excellent antibacterial activity[8-12]. Considering the great antimicrobial properties of isatin (and its derivatives), isoniazid and norfloxacin (a quinolone), it was thought to synthesize N-Mannich bases of norfloxacin which contain isatin and isoniazid moieties. Isatin (and its derivatives) when treated with isoniazid gave Schiff bases (**I**). These Schiff bases when treated with norfloxacin in equimolar quantities gave N-norfloxacin Mannich bases of isatin and derivatives (**II**) (**Scheme-I**).

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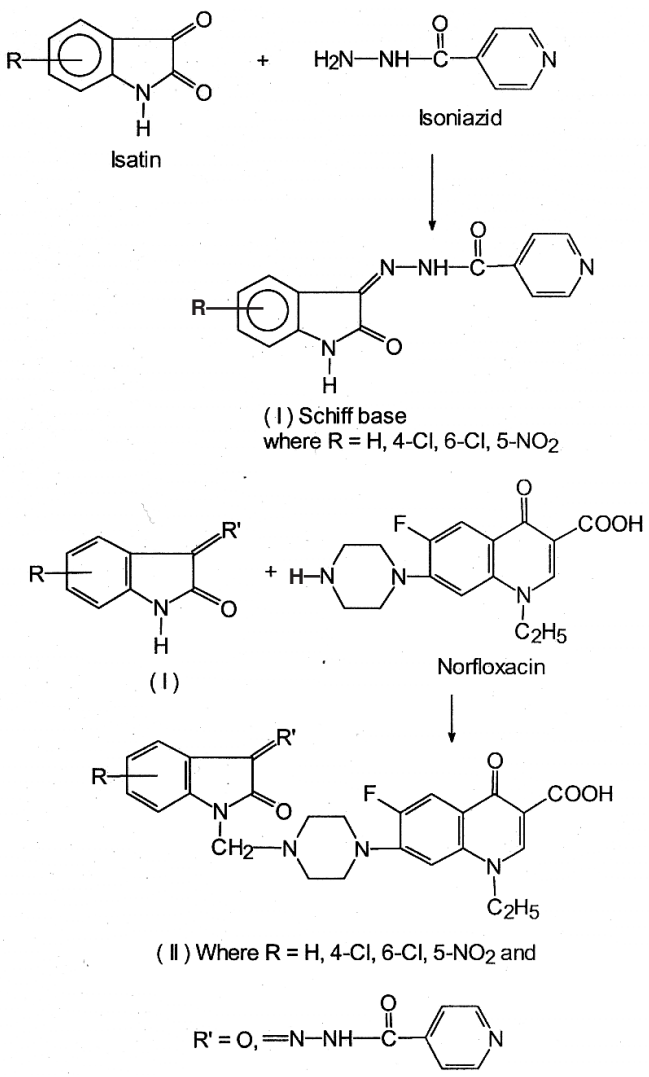


Scheme-I: Synthetic protocol of the title compounds

EXPERIMENTAL

Isatin, 4-chloroisatin, 6-chloroisatin were prepared by methods given in literature[13,14]. The melting points were determined by using Thomas Hoover melting point apparatus and are uncorrected.

Synthesis of Schiff bases of isatin with isoniazid (I): Isonicotinic acid hydrazide (13.7 g, 0.1 mol) was dissolved in ethanol in a 500 mL round bottom flask and to this, solution of respective isatin/substituted isatin (0.1 mol) was added. Few drops of glacial acetic acid were also added. Whole content was refluxed for 3 h with occasional shaking. The flask was

cooled to room temperature and kept in ice cold condition for 1 h. The solid mass was filtered off under suction, air dried and recrystallized from ethanol-water mixture (Table-1). The structures of the synthesized compounds **Ia-d** were confirmed by elemental analysis, IR spectra and ^{1}H NMR spectra.

TABLE-1
PHYSICAL DATA OF SCHIFF BASES OF ISATIN WITH ISONIAZID (**I**)

Compd. no.	R	m.f.*	Yield (%)	m.p. (ºC)	IR, ν_{max} (cm^{-1})
1a	H	$C_{14}H_{10}N_4O_2$	69	298	3200, 1660, 1600, 1540, 860
1b	4-Cl	$C_{14}H_9N_4O_2Cl$	65	296	3200, 1660, 1500, 1220-1240, 820
1c	6-Cl	$C_{14}H_9N_4O_2Cl$	75	298	3200, 1650, 1600, 1540, 850
1d	5-NO_2	$C_{14}H_9N_5O_4$	90	297	3200, 1600, 1500, 1520, 1340, 820

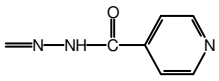
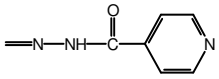
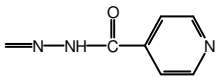
*All the compounds gave satisfactory elemental analyses and error was within ± 0.04 %.

Spectral analysis for 6-chloro-3-isonicotinic acid hydrazino isatin (Ic): IR (KBr, ν_{max}, cm^{-1}): 3200 (amide NH *str.*), 1650 (C=N *str.*), 1600 (aromatic C-H *str.*), 1540 (NH bend), 850 (aromatic C-H, out of the plane, bending). ^{1}H NMR δ: 6.8-7.1 (2H, d, indole ring, 4H, 5H), 7.6-7.7 (H, t, indole ring, 7H), 7.8 (2H, s, pyridine ring 3H, 5H), 8.7 (2H, pyridine ring 4H, 6H), 10.4 (1H, s, indole ring NH group) and 13.7 (1H, S or N-NH-C=O).

Synthesis of N-Mannich bases of isatin/isatin derivatives with norfloxacin (IIa-g): A solution of norfloxacin (2 mol) in glacial acetic acid was added to slurry of isatin derivatives (**Ia-d**) and 37 % formalin (1 mL) in little amount of tetrahydrofuran. The reaction mixture was refluxed for 1-3 h (1.5 h in case of isatin, 2-3 h in case of substituted isatin derivatives) over water bath. TLC control was performed to assess the completion of the reaction as indicated by complete disapearance of norfloxacin in the reaction mixture. The reaction mixture was concentrated to half of its volume and precipitated thus obtained was recrystallized from DMF-water mixture (Table-2).

Spectral analysis for 1-ethyl-6-fluoro-4-oxo-7-[N^4-{6′-chloro-3′-(isonicotinic acid hydrazide imino)isatinyl}-N^1-piperazinyl]-3-quinoline carboxylic acid (IIe): IR (KBr, ν_{max}, cm^{-1}): 3400 (NH *str.*), 2900 (olefinic CH_2 *str.*), 1730 (COOH group), 1700 (C=N *str.*), 1600 (C=N *str.*). ^{1}H NMR (δ ppm): 1.78 (3H, t, CH_3 group), 3.7-4.1 (8H, m, piperidine CH_2), 4.23 (2H, s, -N-CH_2-N), 4.88 (2H, q, CH_2 of N-methyl group), 6.8-7.2 (3H, m, indole ring), 7.5 (1H, d, 8H of quinoline nucleus), 7.7 (2H, s, pyridine ring 3H, 5H), 8.3 (1H, d, 2H of quinoline nucleus), 8.7 (2H, s, pyridine ring, 4H, 6H), 9.32 (1H, s, 2H of quinoline nucleus), 10.8 (1H due to COOH), 13.7 (1H, N-NH-C=O).

TABLE-2
PHYSICAL CHARACTERISTICS OF N-NORFLOXACIN MANNICH BASES (**II**)

Compd.	R	R^1	Yield (%)	m.p. (ºC)	m.f.*
IIa	H	=O	90	112	$C_{25}H_{23}N_4O_5F$
IIb	4-Cl	=O	87	152	$C_{25}H_{22}N_4O_5ClF$
IIc	4-Cl		85	182	$C_{31}H_{27}N_7O_5ClF$
IId	6-Cl	=O	70	160	$C_{25}H_{22}N_4O_5ClF$
IIe	6-Cl		95	180	$C_{31}H_{27}N_7O_5ClF$
IIf	5-NO_2	=O	70	200	$C_{25}H_{22}N_5O_7F$
IIg	5-NO_2		75	195	$C_{31}H_{27}N_8O_7F$

*All the compounds gave satisfactory C, H and N analyses and error was within ± 0.04 %.

Antimicrobial study

***in vitro* Antibacterial activity:** The *in vitro* antibacterial activity of the synthesized compounds was evaluated by agar dilution technique[15] against 26 pathogenic bacteria. The bacterial strains were procured from the bacterial repository of Department of Microbiology, IMS, BHU, Varanasi. The medium was prepared as per instructions of manufacturer of dry Mueller Hinton agar power (Hi Media). The conditions of the test samples used were from 5000 µg/mL to lower concentrations made by serial double dilutions with DMF. The minimum inhibitory concentration (MIC) was taken as lowest concentration (higher dilution) without visible growth. The study was simultaneously performed for the pure standard drug norfloxacin also. The MICs are reported in Table-3

***in vitro* Antifungal activity:** *in vitro* Antifungal study was done on five fungi strains. The synthesized compounds to be screened were dissolved in DMF. The concentration of drugs was 50 µg/mL. The antifungal study was done by tube slant culture method. The results of antifungal study are given in Table-4.

RESULTS AND DISCUSSION

Isatin norfloxacin Mannich base (**IIa**) showed moderate activity against *Shigella sonnei, V. cholerae* 01 Sal., *P. typhi, S. marscences, E. tarda*; less active against *B. subtitis, M. marganii* No significant increase in activity against other organisms in comparison to norfloxacin. 4-Chloroisatin sereis (**IIb** and **IIc**) showed higher activity against *S. sonnei, K. pneumoniae, M.*

TABLE-3
in vitro ANTIBACTERIAL ACTIVITIES OF N-NORFLOXACIN MANNICH BASES OF ISATIN AND ITS DERIVATIVES (**II**)

Microorganism/drugs	**IIa**	**IIb**	**IIc**	**IId**	**IIe**	**IIf**	**IIg**	NFX*
Bacillus subtilis	0.61	0.61	0.61	0.038	0.076	1.22	0.076	1.22
Staphylococcus aurreus	2500	312.5	312.5	312.5	312.5	312.5	312.5	312.5
Staphylococcus epidermis	2500	312.5	312.5	312.5	312.5	625	312.5	312.5
Escherichia coli	625	312.5	312.5	312.5	312.5	312.5	312.5	156.25
Pseudomonas aeruginosa	2.44	1.22	1.22	2.44	0.22	9.76	4.88	2.44
Vibrio cholerae 01	0.018	0.019	0.038	0.038	0.0182	0.152	0.07	0.038
Vibrio cholerae Inba	0.305	0.38	0.038	0.018	0.0182	0.152	0.07	0.038
Vibrio cholerae non 01	0.152	0.076	0.018	0.038	0.038	0.061	0.305	0.038
Vibrio cholerae 0139	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.152
Shigella sonnei	0.61	0.152	0.076	0.07	0.037	0.61	1.22	2.00
Shigella boydii	0.036	0.076	0.076	0.038	0.018	0.152	0.152	0.038
Shigella flexeneri	0.152	0.152	0.152	0.061	0.076	0.61	0.61	1.22
Shigella dysenteriae	9.76	0.038	0.076	0.076	0.018	1.22	1.22	1.22
Salmonella P. typhi A	0.152	0.152	0.152	0.61	0.076	0.61	0.61	1.22
Salmonella P. typhi B	0.122	0.152	0.037	0.037	0.018	1.22	1.22	0.61
Salmonella typhimurium	0.152	0.018	0.3	0.018	0.018	1.22	1.22	0.305
Salmonella enteridis	0.038	0.076	0.018	0.152	0.038	0.61	0.61	2.44
Klebseilla penumoniae	19.22	0.61	0.152	0.07	0.07	1.22	1.22	2.44
Placiomonas shigelloids	0.038	0.03	0.018	0.018	0.018	0.61	0.018	0.018
Morganella morganii	1.24	0.07	0.37	0.018	0.018	0.61	1.22	4.88
Serretia marscences	0.15	0.038	0.038	0.018	0.018	1.22	0.61	1.21
Aeromonas hydrophila	0.037	0.038	0.018	0.018	0.018	0.152	0.038	0.038
Edwardsiella tarda	0.61	0.076	0.038	0.018	0.018	0.61	0.61	1.22
St. aureus 25925	2500	2500	2500	1250	1250	2500	2500	2500
Proteus vulgaris	0.61	0.037	0.037	0.038	0.038	0.61	0.61	0.038
Citrobacter ferundii	1.22	0.15	0.018	0.076	1.22	0.152	0.61	0.152

*Norfloxacin, MIC's of the compound in μg/mL

TABLE-4
in vitro ANTIFUNGAL ACTIVITY OF N-NORFLOXACIN MANNICH BASES (**II**) 50 μg/mL)

Compd.	A	B	C	D	E
IIa	+	+	+	+	–
IIb	+	–	+	+	±
IIc	+	+	+	+	±
IId	+	+	+	±	–
IIe	+	+	+	+	±
IIf	+	+	+	±	±
IIg	+	+	+	±	±

A = *Echinococcus floculens*; B = *Microsporum audounii*;
C = *Microsporum ammi*; D = *Microsporum gypseum*; E = *Candida albicans*.
+Inhibition of growth; –No inhibition of growthl; ±Weak inhibition

marganii, S. marcences, E. tarda, moderate activity against *B. subtilis, Salmonella, P. typhi, Acromonas hydrophilla, C. ferundii* in comparison to norfloxacin 6-Cl isatin series (**IId** and **IIe**) showed higher activity against *Sh. sonnei, Sh. dysentriae, Sal. interidis, K. pneumoniae, M. marganii, S. marscences, E. tarda*, moderately active against *Sal. P. typhi, B. subtilis, Sal. P. tophi* in comparison to norfloxacin. Whereas, in case of 5-NO_2 series (**IIf** and **IIg**), higher activity was observed against *S. enteridis, V. cholerae* 0139, *Salmonella P. typhi A, M. marganii, S. marsceneces, E. tarda* less active against *V. cholerae* non 01, *P. vulgaris, S. boydii, B. subtilis, Sal p. typhi* B in comparison to norfloxacin.

In general, N-norfloxacin Mannich bases showed higher activity against most of the bacteria studied in comparison to norfloxacin. Among the compounds synthesized, **IIe**, 1-ethyl-6-fluoro-4-oxo-7-[N^4\{6′-chloro-3′-(isonicotinic acid hydrazideimino)isatinyl methyl\}N^1-piperazinyl]-3-quinoline carboxylic acid showed the most promising activity.

The results indicate the all the fungal strains were sensitive to the synthesized compounds. All the compounds were active against *E. fluoculens, M. ammi, M. audonii* but are moderately active against *M. gypseum* and *C. albicans* at 50 µg/mL.

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REFERENCES

1. V. Glover, S.K. Bhattacharya, M. Sandler and S.E. File, *Nature*, 292 (1981).
2. A.E. Medvedev, B. Goodwin, A.H.J. Clow, V. Glover and M. Sandler, *Biochem. Pharmacol.*, **44**, 590 (1992).
3. R.S. Verma and W.L. Nobles, *J. Med. Chem.*, **10**, 972 (1967).
4. R.S. Verma and W.L. Nobles, *J. Med. Chem.*, **65**, 881 (1975).
5. I. Delebra-Defayolle and M. Sarciron, *J. Antimicrob. Chemother.*, **23**, 237 (1987).
6. K.C. Joshi and P. Chand, *Pharmazie*, **1**, 37 (1982).
7. D. Maysinger, J. Ban and M. Marvin, *Arzneimittel Forsch.*, **30**, 32 (1980).
8. S.C. Cooper, D.T.W. Chu, L.F. Shen and A.G. Pernet, 26th Int. Cong., Antimicrob, Agents, Chemother, New Orleans, 28th Sept. to 1st Oct. (1986).
9. J.C. Wang, First Conference on DNA Topisomerase in Cancer Chemotherapy, NIH Publication No. 87-2943-44, pp. 3-6 (1987).
10. P.S. Sharma, *Ann. Int. Med.*, **3**, 336 (1989).
11. S. Krishna, J.M.E. Davis, P.C.Y. Chan, R.S. Wells and K.J.H. Robson, *Lancet*, **2**, 1231 (1988).
12. S.N. Pandeya and D. Sriram, *Acta Pharm. Turc.*, **33**, 340 (1988a).
13. A.E. Senear, J.F. Sarjent and J.B.G. Koept, *J. Am. Chem. Soc.*, **68**, 2695 (1946).
14. W.C. Sumpter and W.F. Jones, *J. Am. Chem. Soc.*, **65**, 1809 (1943).
15. A. Bany, In: Antibiotics in Laboratory Medicines, William and Wikins, Baltimore, edn. 5, p. 1 (1991).

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