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Synthesis and antimicrobial activity of Schiff and Mannich bases of isatin and its derivatives with pyrimidine

S.N. Pandeya [a,*], D. Sriram [a], G. Nath [b], E. De Clercq [c]

[a] *Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221 005, India*
[b] *Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India*
[c] *Rega Institute for Medical Research, Katholieke University-Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

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Abstract

Isatin and its derivatives have been reacted with 4-(4′-chlorophenyl)-6-(4″-methyl phenyl)-2-aminopyrimidine to form Schiff bases and the *N*-Mannich bases of these compounds were synthesized by reacting them with formaldehyde and several secondary amines. Investigation of antimicrobial activity of the compounds was made by the agar dilution method against 28 pathogenic bacteria, eight pathogenic fungi and anti-HIV activity against replication of HIV-1 (III B) in MT-4 cells. The compounds are significantly active against bacteria and fungi.

Keywords: Schiff bases; *N*-Mannich bases; Isatin; Antimicrobial activity

1. Introduction

Isatin (indole-2,3-dione), its Schiff and Mannich bases are reported to show a variety of biological activities, such as antibacterial [1], antifungal [2] and anti-HIV [3] activities. Pyrimidines are reported to have antibacterial [4], antifungal [5] and anti-HIV [6] activities. In view of these facts and as a continuation of previous efforts carried out in our laboratory [3,7], 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-aminopyrimidine has been synthesized from 3-(4′-chlorophenyl)-1-(4″-methyl phenyl) 2-propen-1-one. It was condensed with isatin and its 5-chloro and 5-bromo derivatives to form Schiff bases. The *N*-Mannich bases of the above Schiff bases were synthesized by condensing the acidic imino group of isatin with formaldehyde and secondary amines (Scheme 1). All compounds (Table 1) gave satisfactory elemental analysis. IR and ^{1}H NMR spectra were consistent with the assigned structures. All the synthesized compounds were screened for antibacterial, antifungal activity by the agar dilution method and anti-HIV activity against HIV-1 (III B) in MT-4 cells.

2. Experimental

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco infrared spectrometer in KBr. ^{1}H NMR spectra were recorded on a Jeol FX 90Q FT-NMR spectrometer (90 MHz) employing tetramethyl silane as the internal reference.

2.1. Synthesis of 3-(4′-chlorophenyl)-1-(4″-methyl phenyl)-2-propen-1-one

An aqueous solution of sodium hydroxide (10% w/v, 10 ml) was added to a solution of 4-chloro benzaldehyde (0.02 mol) and 4-methyl acetophenone (0.02 mol) in ethanol (6 ml). The reaction mixture was stirred at room temperature overnight and poured into water (100 ml). After neutralization with hydrochloric acid (10% w/v), a yellow solid was obtained which was recrystallized from water–ethanol; yield 85%, m.p. 125°C.

2.2. Synthesis of 4-(4′-chlorophenyl)-6-(4″methyl phenyl)-2-aminopyrimidine

A mixture of 3-(4′-chlorophenyl)-1-(4″-methylphenyl)-2-propen-l-one (0.01 mol) and guanidine hydrochloride

* Corresponding author. Fax: +91-542-316 427.
E-mail address: snpande@banaras.ernet.in (S.N. Pandeya)

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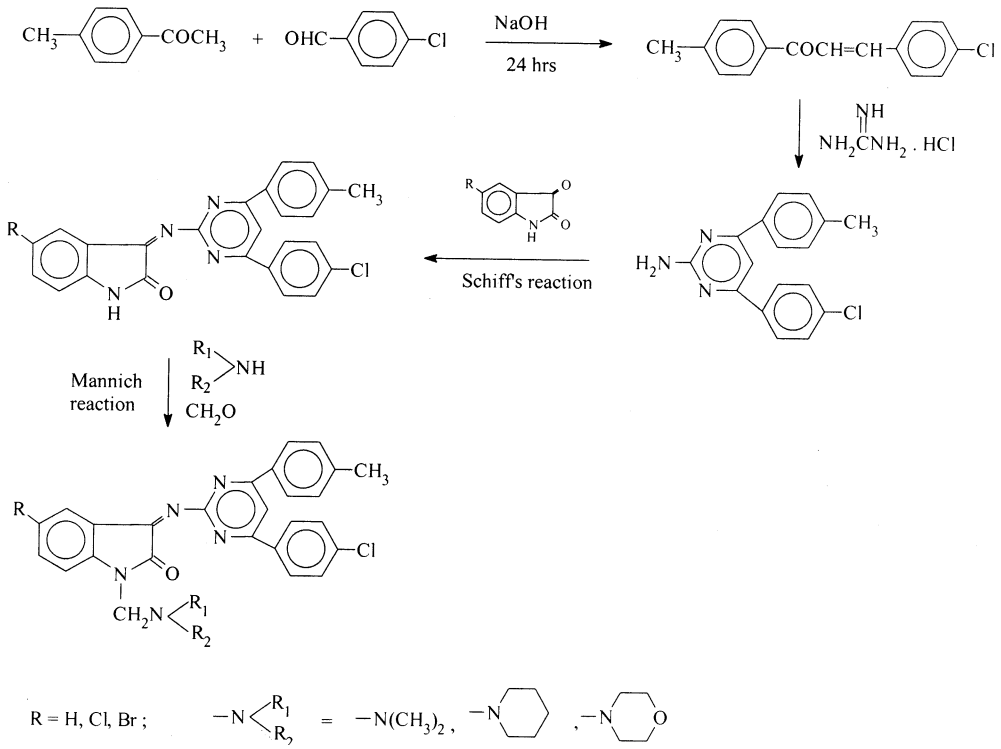
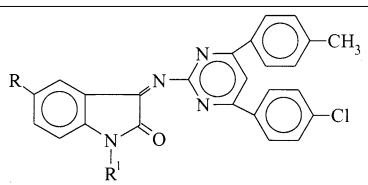


Scheme 1. Synthetic protocol of the compounds.

Table 1
Physical constants



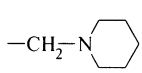
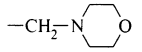
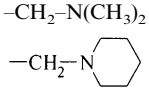
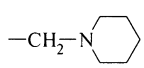
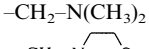
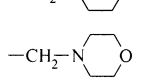
Code	R	R^1	Yield (%)	M.p. (°C)	Mol. Formula	R_f C_6H_6:C_2H_5OH (9.5:0.5)
S1	H	H	72.34	135	$C_{25}H_{17}ON_4Cl$	0.73
S2	H	–CH_2–$N(CH_3)_2$	79.00	122	$C_{28}H_{24}ON_5Cl$	0.63
S3	H		90.97	98	$C_{31}H_{28}ON_5Cl$	0.67
S4	H		47.41	138	$C_{30}H_{26}O_2N_5Cl$	0.75
S5	Cl	H	78.76	160	$C_{25}H_{16}ON_4Cl_2$	0.84
S6	Cl	–CH_2–$N(CH_3)_2$	75.08	134	$C_{28}H_{23}ON_5Cl_2$	0.72
S7	Cl		78.26	101	$C_{31}H_{27}ON_5Cl_2$	0.69
S8	Cl		76.26	118	$C_{30}H_{25}O_2N_5Cl_2$	0.75
S9	Br	H	57.31	209	$C_{25}H_{16}ON_4ClBr$	0.59
S10	Br	–CH_2–$N(CH_3)_2$	84.66	235	$C_{28}H_{23}ON_5ClBr$	0.75
S11	Br		81.11	109	$C_{31}H_{27}ON_5ClBr$	0.68
S12	Br		85.52	115	$C_{30}H_{23}O_2N_5ClBr$	0.75

Table 2
Antibacterial activity MIC values (μg ml^{-1}) [a]

Microorganisms/drugs		S1	S2	S3	S4	S5	S6	S7
1	*S. typhimurium*	156.25	156.25	156.25	78.12	156.25	156.25	156.25
2	*Vibrio parahaemolyticus*	78.12	156.25	78.12	39.06	78.12	39.06	39.06
3	*Salmonella paratyphi* B	56.25	156.25	156.25	156.25	156.25	156.25	78.12
4	*E. tarda*	1250	312.5	312.5	156.25	156.25	156.25	156.25
5	*V. cholerae* 0139	156.25	78.12	78.12	39.06	78.12	78.12	156.25
6	*S. aureus*	625	312.5	625	625	312.5	625	312.5
7	*E. coli* NCTC 10418	1953	19.53	9.76	9.76	19.53	9.76	9.76
8	*V. cholerae* non-01	2.44	2.44	9.76	9.76	2.44	4.88	9.76
9	*E. faecalis*	9.76	9.76	9.76	4.88	9.76	19.53	19.53
10	*Salmonella typhi*	156.25	156.25	78.12	156.25	156.25	39.06	78.12
11	*P. aeruginosa*	2500	5000	2500	1250	5000	1250	1250
12	*K. pneumoniae*	1250	1250	1250	1250	1250	625	625
13	*S. albus*	1250	625	625	625	1250	625	1250
14	*Salmonella enteritidis*	78.12	78.12	78.12	39.06	39.06	78.12	39.06
15	*A. hydrophila*	312.5	625	625	312.5	156.25	312.5	312.5
16	*V. cholerae*-01	19.53	19.53	9.76	4.88	9.76	9.76	4.88
17	*B. subtilis*	39.06	19.53	39.06	9.76	78.12	78.12	39.06
18	*Shigella sonnei*	312.5	312.5	625	312.5	312.5	625	312.5
19	*Shigella boydii*	156.25	156.25	156.25	78.12	78.12	39.06	78.12
20	*Plesiomonas shigelloides*	9.76	9.76	9.76	9.76	19.53	9.76	9.76
21	*P. rettgeri*	156.25	312.5	312.5	78.12	156.25	78.12	156.25
22	*Shigella flexneri*	156.25	156.25	312.5	156.25	156.25	156.25	156.25
23	*Proteus vulgaris*	312.5	312.5	312.5	156.25	312.5	312.5	156.25
24	*Enterobacter* spp.	625	625	625	312.5	625	625	625
25	*Morgonella morganii*	156.25	156.25	312.5	78.12	156.25	156.25	312.5
26	*Citrobacter freundii*	78.12	156.25	78.12	78.12	156.25	78.12	78.12
27	*Proteus morganii*	625	625	625	625	625	625	625
28	*S. paratyphi* A	156.25	78.12	156.25	312.5	78.12	78.12	156.25

[a] MIC, minimum inhibitory concentration.

(0.015 mol) was added to sodium hydroxide (0.045 mol in 2 ml of water) and ethanol (50 ml). The reaction mixture was refluxed for 6 h. The mixture was concentrated under reduced pressure and cooled, a yellow solid was obtained which was recrystallized from ethanol. Yield: 82%, m.p. 190°C. IR (KBr) (cm^{-1}): 3280 (NH_2), 1610 (ring C=C, C=N). ^1H NMR ($CDCl_3$) δ (ppm): 2.30 (3H, s, CH_3), 5.5 (2H, s, NH_2), 7.3–7.5 (4H, m, Ar-H of tolyl), 7.75 (1H, s, H-5), 7.9–8.0 (4H, Sym 2d, Ar-H of *p*-Cl-phenyl). *Anal.* ($C_{17}H_{14}N_3Cl$) C, H, N.

2.3. Synthesis of 3-[4′(4″-chlorophenyl)-6′-(4‴-methylphenyl) pyrimidin-2′-yl] iminoisatin (*S1*)

Equimolar quantities (0.03 mol) of isatin and 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-aminopyrimidine were dissolved in 75 ml of warm ethyl alcohol containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 18 h and set aside. The resultant solid was washed with dilute ethanol, dried and recrystallized from an ethanol–chloroform mixture. Yield: 72.34%; m.p. 135°C. IR (KBr) (cm^{-1}): 1640 (C=N), 1610 (ring C=C, C=N). ^1H NMR ($CDCl_3$) δ (ppm): 2.35 (3H, s, CH_3), 6.8–7.1 (4H, m, Ar-H isatin), 7.2–7.5 (4H, m, Ar-H, tolyl), 7.75 (1H, s, H-5′), 7.9–8.1 (4H, Sym 2d, Ar-H, *p*-chlorophenyl), 10.4 (1H, s, NH, D_2O exchangeable). *Anal.* ($C_{25}H_{17}ON_4Cl$) C, H, N.

Similarly, compounds **S5** and **S9** were synthesized using 5-chloro and 5-bromo isatin.

2.4. Synthesis of 1-piperidinomethyl-3-[4′(4″-chlorophenyl)-6′-(4‴-methylphenyl) pyrimidin-2′-yl] iminoisatin (*S3*)

A slurry consisting of **S1** (0.005 mol), tetrahydrofuran (5 ml) and 37% formalin (2 ml) was made. To this, piperidine (0.005 mol) was added dropwise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 h with occasional shaking after which it was warmed on a steam bath for 15 min. At the end of the period the contents were cooled and the product obtained was recrystallized from chloroform–petroleum ether. Yield: 90.97%; m.p. 98°C. IR (KBr) (cm^{-1}): 2870 (CH_2), 1645 (C=N); ^1H NMR ($CDCl_3$) δ (ppm): 1.8 (6H, m, $(CH_2)_3$), 2.3 (3H, s, CH_3), 2.6 (4H, t, CH_2NCH_2), 4.45 (2H, s, NCH_2N), 6.8–7.1 (4H, m, Ar-H, isatin), 7.1–7.5 (4H, m, Ar-H, tolyl), 7.7 (1H, s, H-5), 7.9–8.1 (4H, Sym 2d, Ar-H, *p*-Cl-phenyl). *Anal.* ($C_{31}H_{28}ON_5Cl$) C, H, N.

Table 3
Antibacterial activity MIC values in (μg ml^{-1}) [a]

	Microorganisms/drugs	S8	S9	S10	S11	S12	Sulphamethoxazole	Trimethoprim
1	*S. typhimurium*	39.06	156.25	156.25	156.25	156.25	5000	>5000
2	*V. parahaemolyticus*	39.06	156.25	78.12	78.12	78.12	1250	2.44
3	*S. paratyphi* B	78.12	156.25	156.25	156.25	39.06	5000	9.76
4	*E. tarda*	156.25	156.25	156.25	78.12	39.06	5000	312.5
5	*V. cholerae* 0139	19.53	156.25	156.25	78.12	39.06	>5000	39.06
6	*S. aureus*	78.12	312.5	625	625	78.12	5000	>5000
7	*E. coli* NCTC 10418	4.88	9.76	9.76	9.76	2.44	2500	19.53
8	*V. cholerae* non-01	1.22	0.61	0.61	1.22	0.3	312.5	1.22
9	*E. faecalis*	4.88	4.88	9.76	4.88	1.22	5000	78.12
10	*S. typhi*	39.06	156.25	156.25	39.06	19.53	2500	4.88
11	*P. aeruginosa*	1250	2500	2500	1250	312.5	78.12	5000
12	*K. pneumoniae*	312.5	625	1250	625	312.5	2500	5000
13	*S. albus*	1250	625	1250	625	625	2500	>5000
14	*S. enteritidis*	19.53	78.12	39.06	39.06	9.76	2500	4.88
15	*A. hydrophila*	156.25	156.25	312.5	312.5	312.5	2500	1250
16	*V. cholerae*-01	4.88	19.53	19.53	9.76	9.76	5000	5000
17	*B. subtilis*	39.06	39.06	9.76	19.53	9.76	5000	5000
18	*S. sonnei*	312.5	312.5	312.5	312.5	312.5	2500	9.76
19	*S. boydii*	19.53	39.06	78.12	39.06	9.76	2500	9.76
20	*P. shigelloides*	9.76	9.76	4.88	4.88	4.88	5000	4.88
21	*P. rettgeri*	78.12	78.12	78.12	156.25	39.06	2500	2500
22	*S. flexneri*	312.5	156.25	156.25	156.25	312.5	2500	156.25
23	*P. vulgaris*	156.25	312.5	312.5	312.5	312.5	2500	156.25
24	*Enterobacter* spp.	312.5	625	625	625	156.25	1250	156.25
25	*M. morganii*	312.5	156.25	156.25	78.12	156.25	2500	156.25
26	*C. freundii*	78.12	156.25	156.25	156.25	39.06	5000	19.53
27	*P. morganii*	312.5	625	312.5	312.5	156.25	5000	156.25
28	*S. paratyphi* A	156.25	312.5	156.25	156.25	39.06	2500	156.25

[a] MIC, minimum inhibitory concentration.

Similarly other Mannich bases **S2**, **S4**, **S6**–**S8** and **S10**–**S12** were prepared by using appropriate Schiff bases with formaldehyde and corresponding secondary amines in tetrahydrofuran as a solvent.

2.5. In vitro antimicrobial activity

Evaluation of antibacterial (28-bacteria) and antifungal (8-fungi) activity by the agar dilution method [8]. The microorganisms used were procured from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University. 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 minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculum.

2.6. Anti-HIV activity

The procedure to measure anti-HIV activity in MT-4 cells has been described previously [3]. Either mock-infected or HIV-1 infected MT-4 cells were incubated in the presence of various concentrations of test compounds and the number of viable cells was determined by the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method on day 5 after virus infection.

3. Results and discussion

All the synthesized compounds were tested for in vitro antibacterial activity by the agar dilution method. The MIC values of the synthesized compounds against 28 pathogenic bacteria are presented in Tables 2 and 3. Also included is the activity of reference compounds sulphamethoxazole and trimethoprim. It has been observed that all the compounds tested showed mild to moderate activity against the tested bacteria. All the tested compounds showed more activity (less MIC) than sulphamethoxazole except *Pseudomonas aeruginosa*. When compared to trimethoprim all the compounds were more active against *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus albus*, *Aeromonas hydrophila*, *Vibrio cholerae*-01, *Bacillus subtilis* and *Proteus rettgeri*; nine compounds

Table 4
Antifungal activity MIC values in (μg ml^{-1})

Drugs/ microorganism	*Candida albicans*	*Aspergillus niger*	*Cryptococcus neoformans*	*M. audouinii*	*T. mentagrophytes*	*Epidermophyton floccosum*	*M. gypseum*	*Histoplasma capsulatum*
S1	39.06	39.06	39.06	9.76	4.88	19.53	4.88	39.06
S2	78.12	39.06	19.53	4.88	9.76	9.76	4.88	19.53
S3	78.12	78.12	19.53	9.76	9.76	9.76	2.44	19.53
S4	39.06	39.06	39.06	9.76	9.76	4.88	2.44	39.06
S5	78.12	39.06	39.06	4.88	4.88	19.53	4.88	78.12
S6	78.12	39.06	39.06	2.44	2.44	19.53	4.88	78.12
S7	78.12	78.12	39.06	4.88	2.44	39.06	1.22	78.12
S8	78.12	78.12	78.12	4.88	4.88	9.76	4.88	78.12
S9	39.06	39.06	19.53	4.88	4.88	19.53	2.44	39.06
S10	39.06	39.06	9.76	9.76	2.44	4.88	4.88	39.06
S11	19.53	39.06	19.53	4.88	2.44	19.53	9.76	78.12
S12	39.06	39.06	39.06	9.76	9.76	19.53	9.76	39.06
Clotrimazole	0.3	2.44	2.44	4.88	2.44	2.44	2.44	19.53

Table 5
Anti-HIV activity

Drug	ED_{50} [a] (μM)	CD_{50} [b] (μM)	SI [c]
S1	>9	9.4	<1
S2	>10	10.1	<1
S3	>1	1.1	<1
S4	>3	2.7	<1
S5	>8	8.2	<1
S6	>7	6.8	<1
S7	>3	2.6	<1
S8	>3	3.0	<1
S9	>10	10.4	<1
S10	>13	12.9	<1
S11	>1	<1	<1
S12	>2	1.9	<1

[a] Effective dose of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.

[b] Cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%.

[c] Selectivity index or ratio of CD_{50} to ED_{50}.

(**S4**–**S12**) were more active against *Edwardsiella tarda*, *Escherichia coli* NCTC 10418; and three compounds (**S9**, **S10**, **S12**) were more active against *V. cholerae* non-01. Compound **S12** was found to be the most active antibacterial agent. In general the order of antibacterial activity of the substituents at the 5th position is Br > Cl > H. In case of substituents at the 1st position, the morpholinomethyl group showed good activity.

The antifungal activity of the compounds was studied for the eight pathogenic fungi. The results are summarized in Table 4. Clotrimazole was used as the reference for inhibitory activity against fungi. All the compounds showed significant antifungal activity. When compared to clotrimazole, six compounds were equipotent (4.88 μg ml^{-1}) and one compound (**S6**) was more active against *Microsporum audouinii*. The compounds showed good activity against dermatophytes like *Microsporum gypseum*, *M. audouinii* and *Trichophyton mentagrophytes* with an MIC of less than 10 μg ml^{-1}.

The synthesized compounds were evaluated for their inhibitory effect of the replication of HIV-1 in human MT-4 cells. None of the compounds showed marked anti-HIV activity at a concentration significantly below their toxicity threshold (Table 5).

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