RESEARCH PAPER

Accepted 31 July 1999
Received 23 March 1999
Indian J. Pharm. Sci., 1999, 61 (6) 358-361

Synthesis, Antibacterial, Antifungal and Anti-HIV Activity of Schiff and Mannich Bases of Isatin with N-[6-chlorobenzothiazol-2-yl] Thiosemicarbazide

S.N. PANDEYA*, D. SRIRAM, G. NATH[a] AND E. DE CLERCQ[b]
Department of Pharmaceutics, Institute of Technology,
Banaras Hindu University, Varanasi-221 005, (India)
[a]Department of Microbiology, Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221 005, (India)
[b]Rega Institute for Medical Research, Katholieke University-Leuven, Belgium

Isatin (2,3-dioxoindole) has been reacted with N-(6-chloro benzothiazol-2-yl) thiosemicarbazide to form Schiff base and the N-Mannich bases of the above Schiff base were synthesized by reacting with formaldehyde and several secondary amines. The chemical structures of N-Mannich bases have been confirmed by means of IR, ^{1}H-NMR data and by elemental analysis. Investigation of antimicrobial activity of compounds was done by agar dilution method against 25 pathogenic bacteria, 8 pathogenic fungi and anti-HIV activity against replication of HIV-1 (III B) in MT-4 cells. Among the compounds tested 1-[N,N-dimethylaminomethyl]isatin-3-[1'(6"-chlorobenzothiazol-2"-yl) thiosemicarbazone] showed the highest antimicrobial activity.

Schiff and Mannich bases of isatin derivatives are reported to show variety of biological activities, like antibacterial[1], antifungal[2] and anti-HIV[3] activities. Benzothiazole derivatives are reported to have antibacterial[4], antifungal[5] and anti-HIV[6] activities. It is also reported that isatin-β-thiosemicarbazone has shown antimicrobial activity[7]. In view of the antimicrobial property of the above pharmacophores, it was envisaged that the combined effect of all the entities will result in enhanced antimicrobial activity. In the present study N-(6-chlorobenzothiazol-2-yl) thiosemicarbazide has been synthesized from p-chloroaniline. This has been condensed with isatin to form Schiff bases. The N-Mannich bases of the above Schiff base were synthesized by condensing with formaldehyde and secondary amines (scheme). All compounds gave satisfactory elemental analysis for C, H, N. IR and ^{1}H-NMR spectra were consistant with the assigned structures. All the synthesized compounds were screened for their antibacterial, antifungal activity by agar dilution method and anti-HIV activity against HIV-1 (III B) in MT-4 cells.

*For correspondence

EXPERIMENTAL

The melting points were determined by using Thomas Hoover melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments : IR, Jasco infrared spectrometer, Jeol FX 90 Q FT-NMR spectrometer (90 MHz).

2-amino-6-chloro-benzothiazole has been synthesized from p-chloro aniline according to the literature method[8].

Synthesis of N-(6-chloro benzothiazol-2-yl) thiosemicarbazide:

To a solution of 2-amino-6-chloro benzothiazole (0.01 mol) in DMF (10 ml) was added sodium hydroxide (0.01 mol) and carbon disulphide (0.75 ml). The mixture was stirred at 15-20° for 1 h. To the stirred mixture was added hydrazine hydrate (0.01 mol) and stirring continued at 60° for 1 h more. On adding water, a pale yellow solid separated out which when recrystallized from DMF-ethanol afforded pale yellow crystals, yield : 90%, m.p. 170°.

TABLE 1 - ANTIBACTERIAL ACTIVITY : MIC's in μg/mL

Microorganisms/Drugs	S-1	S-2	S-3	S-4	Sulpha-methoxazole	Trimethoprim
1. *Salmonella typhimurium*	312.5	312.5	312.5	156.25	5000	>5000
2. *Salmonella paratyphi* B	312.5	312.5	312.5	312.5	5000	9.76
3. *Edwardsiella tarda*	156.25	78.12	312.5	312.5	5000	312.5
4. *Staphylococcus aureus*	156.25	78.12	312.5	156.25	5000	>5000
5. *Escherchia coli* NCTC 10418	312.5	156.25	625	625	2500	19.53
6. *Vibrio cholerae non*-01	39.06	39.06	78.12	78.12	312.5	1.22
7. *Enterococcus faecalis*	39.06	19.53	39.06	39.06	5000	78.12
8. *Salmonalla typhi*	78.12	78.12	78.12	39.06	2500	4.88
9. *Pseudomonas aeruginosa*	312.5	78.12	156.25	156.25	78.12	5000
10. *Klebsiella pneumoniae*	312.5	156.25	156.25	156.25	2500	5000
11. *Salmonella enteritidis*	312.5	312.5	625	312.5	2500	4.88
12. *Aeromonas hydrophile*	78.12	78.12	156.25	156.25	5000	1250
13. *Vibrio cholerae*-01	78.12	19.53	78.12	156.25	5000	5000
14. *Bacillus subtilis*	39.06	78.12	78.12	156.25	2500	5000
15. *Shigella sonnei*	78.12	39.06	78.12	156.25	2500	9.76
16. *Shigella boyadii*	156.25	78.12	156.25	312.5	2500	9.76
17. *Plesiomonas shigelloides*	156.25	78.12	312.5	156.25	5000	4.88
18. *Proteus rettgeri*	156.25	156.25	312.5	156.25	2500	2500
19. *Shigella flexnari*	312.5	156.25	625	312.5	2500	156.25
20. *Proteus vulgaris*	156.25	156.25	312.5	312.5	2500	156.25
21. *Enterobacter*	156.25	78.12	156.25	156.25	1250	156.25
22. *Morgonella morgonii*	156.25	78.12	156.25	156.25	2500	156.25
23. *Citrobacter ferundii*	156.25	78.12	312.5	312.5	5000	19.53
24. *Proteus morgonii*	156.25	78.12	312.5	312.5	5000	156.25
25. *Salmonella paratyphi* A.	156.25	39.06	78.12	156.25	2500	256.25

MIC-Minimum inhibitory concentration

Synthesis of isatin 3[1'(6"-chloro benzothiazol-2"yl) thiosemicarbazone] (S-1) :

Equimolar quantities (0.02 mol) of isatin and N-(6-chloro benzothiazol-2-yl) thiosemicarbazide were dissolved in warm ethanol containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 26 h and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol chloroform mixture. Yield 69.12%; m.p. 135°. IR (KBr): 1640 cm^{-1} (C=N), 1015 cm^{-1} (C=S); ^{1}H-NMR ($CDCl_3$) δ ppm: 7.0-7.75 (m, 7H, Ar-H), 9.6 (s, 2H, 2x-NH D_2O exchangeable), 10.4 (s, 1H, NH of isatin, D_2O exchangeable); Mol formula ($C_{16}H_{10}ON_5S_2Cl$).

Synthesis of 1-[N,N-dimethylaminomethyl]isatin-3-[1' (6"-chloro benzothiazol-2"-yl] thiosemicarbazone (S-2) :

A slurry consisting of the S-1 (0.005 mol), tetrahydrofuran (5 ml) and 37% formalin (2 ml) was made. To this dimethylamine (0.005 mol) was added dropwise with cooling and shaking. The reaction mixture was

SYNTHETIC PROTOCOL OF THE COMPOUNDS

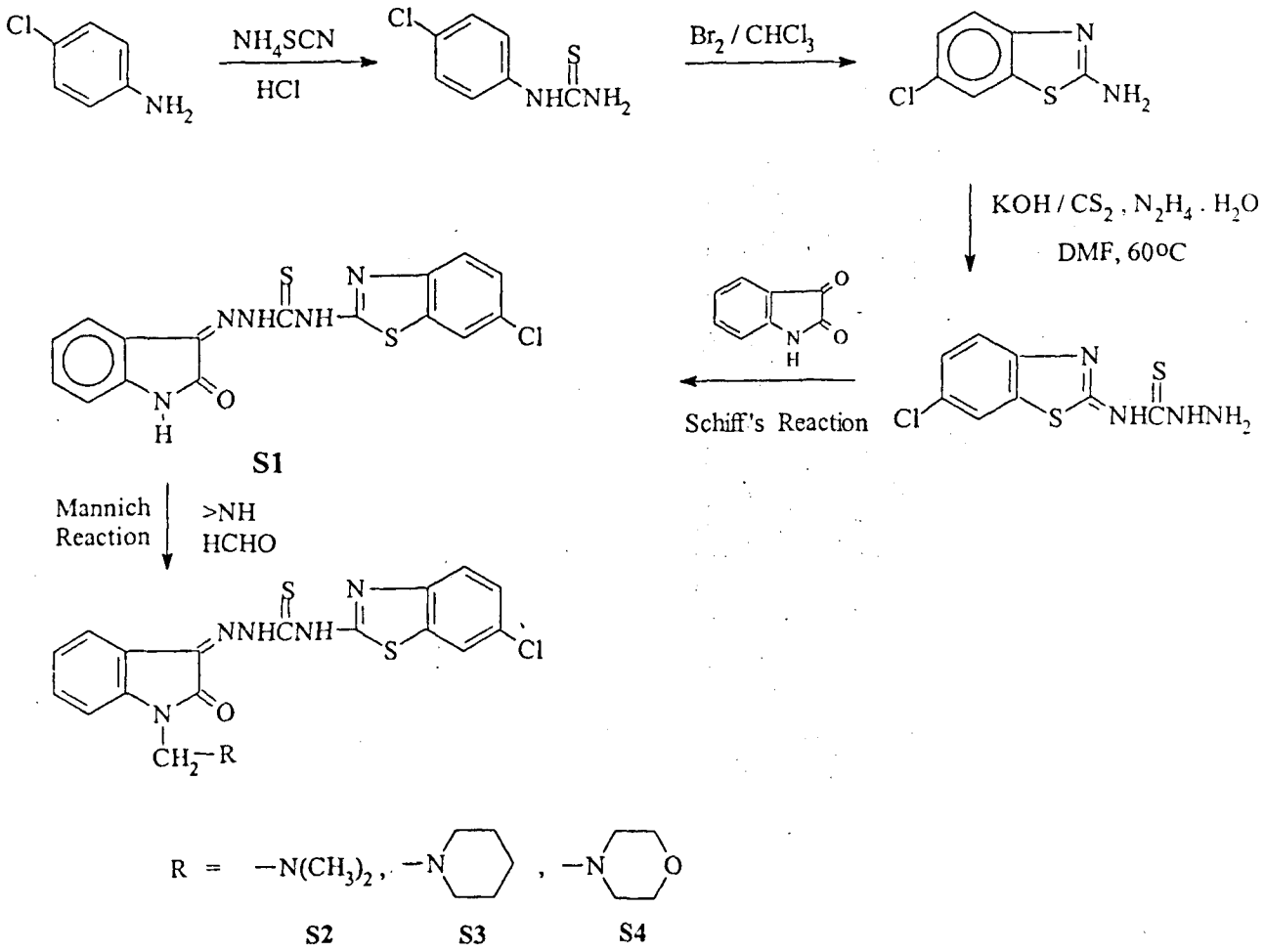

TABLE 2 - ANTIFUNGAL ACTIVITY OF THE COMPOUNDS : MIC's in µg/mL

Micro organism/Drugs	S-1	S-2	S-3	S-41	Clotrimazole
1. *Candida albicans*	9.76	9.76	9.76	19.53	0.3
2. *Aspergillus niger*	19.53	19.53	19.53	39.06	2.44
3. *Cryptococcus neoformans*	9.76	9.76	19.53	19.53	2.44
4. *Microsporum audouinii*	4.88	9.76	9.76	9.76	4.88
5. *Trichophyton mentagrophytes*	9.76	9.76	9.76	9.76	2.44
6. *Epidermophyton floccosum*	4.88	9.76	9.76	9.76	2.44
7. *Micosporum gypsum*	2.44	2.44	2.44	4.88	2.44
8. *Histoplasma capsulatum*	9.76	19.53	19.53	19.53	19.53

MIC-Minimum inhibitory concentration

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: 90.60%; m.p. 106°; IR (KBr) 1630 (C=N), 2850 cm^{-1} (CH_2), ^{1}H-NMR ($CDCl_3$) δ ppm: 2.5 (s, 6H, $N(CH_3)_2$), 5.2 (S, 2H, CH_2), 6.9-7.8 (m, 7H, Ar-H), 9.45 (s, 2H, 2x-NM D_2O exchangeable); Mol formula ($C_{19}H_{17}ON_6S_2Cl$).

Antimicrobial activity:

Evaluation of antibacterial (25-bacteria) and antifungal (8-fungi) activity was carried out by agar dilution method[9]. The microorganisms used were procured from Dept. of Microbology, Institute of Medical Sciences, Banaras Hindu University. All bacteria were grown on Mueller-Hinton Agar (Hi-Media) plates (37°, 24 h.) and fungi were grown on Sabouraud dextrose agar (Hi-media) plates (26°, 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.

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 concentration of test compounds and the number of viable cells was determined by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method on day five after virus infection.

RESULTS AND DISCUSSION

All the synthesized compounds were tested for *in vitro* antibacterial activity by agar dilution method. The MICs of the synthesized compounds against 25 pathogenic bacteria are presented in Table 1. Also included is the activity of reference compounds sulphamethoxazole and trimethoprim. It has been observed that all the compounds tested showed significant activity against 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, Pseudomonas aeruginosa, Aeromonas hydrophile, Vibrio cholerae-01, Bacillus subtilis and Proteus rettgeri,* compound S-2 was the most active antibacterial agent.

The antifungal activity of the compounds was studied with eight pathogenic fungi. The results are summerized in Table 2. Clotrimazole has been used as reference for inhibitory activity against fungi. All the compounds showed significant antifungal activity. When compared to clotrimazole, three compounds are equipotent (2.44 μg/ml) against *Microsporum gypsum* and three compounds are equipotent (19.53 μg/mL) and one compound (S-1, 9.76 μg/mL) are more active against *Histoplasma capsulatum.* The compounds showed good activity against dermatophytes like *Microsporum gypsum, Epidermophyton flocosum, Trichophyton mentagrophytes and Microsporum audouinii* with MIC of less than 10 μg/ml.

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

ACKNOWLEDGEMENTS

The authors would like to express their gratitude and thanks to the Head, Dept. of Pharmaceutics and Dept. of Microbiology, Banaras Hindu University for providing necessary facilities for this research work.

REFERENCES

1. Pandeya, S.N., Yogeesawari, P., Sriram, D. and Nath, G., **Boll. Chim. Farm.** 1998, 137, 685.
2. Piscopo, E., Diurno, M. V., Godliaridi, R., Caccinello, M. and Veneruso, G., **Bol. Soc. Ital Biol. Sper.** 1987, 63, 827.
3. Pandeya, S.N. Sriram, D., Clercq, E. De., Pannecouque, C. and Witvrouw, M., **Indian. J. Pharm. Sci.** 1998, 60, 207.
4. Moharram, H.H., **Arch. Pharmacol. Res.** 1990, 13, 14.
5. Yalcin, I., Oren, I., Senav, E., Akin, A. and Ucarturk, N., **Eur. J. Med. Chem.** 1992, 27, 395.
6. Getman, D.P., Decreescenzo, G.A., Freskos,J.N., Vazquez, M.L., Sikorski, J.A., Devadas, B. and Nagarajan, S., **US**, 5, 705, 500. 1998.
7. Teitz,Y., Ronen, P., Vasover, A., Stematsky, T. and Riggs, J.L., **Antiviral Res.** 1994, 24, 305.
8. Bhargava, P.N. and Baliga, B.T. **J. Indian. Chem. Soc.** 1958, 35, 807.
9. Barry, A., in **Antibiotics in laboratory medicine,** 5th Edn. William and Wilkins: Baltimore, MD, 1991, 1.