Scientia Pharmaceutica (Sci. Pharm.) **70**, 39–48 (2002)

Synthesis and Antituberculous activity of N-Mannich bases of 3-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl] iminoisatin derivatives

D.Sriram[a]*, P.Yogeeswari[a], S.N.Pandeya[b] and S.Ananthan[c]

a. Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Sciences, Pilani, Rajasthan-333031, (INDIA). b. Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, (INDIA). c. National Institute of Allergy and Infectious Diseases, TAACF, Southern Research Institute, 2000, 9th Avenue South, Birmingham, AL 35255, (USA).

Summary

Isatin, its 5-chloro and 5-bromo derivatives have been reacted with 4-(4-chlorophenyl)-6-(4-methylphenyl)-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. The compounds were evaluated *in vitro* against *Mycobacterium tuberculosis* H37Rv at 6.25 μg/ml in BACTEC 12B medium using the BACTEC 460 radiometric system. Among the compounds tested 3-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl]iminoisatin (**S1**) showed promising activity with 97% inhibition at a concentration of 6.25 μg/ml.

Keywords: Isatin, Mannich bases, Schiff bases, Antituberculous.

1. Introduction

Tuberculosis is making a worldwide resurgence. Several factors may be responsible for the increase in cases rates, including infection with human immunodeficiency virus, changing economic and social circumstances and decline in tuberculosis control programs[1]. In addition, outbreaks of multi-drug resistant tuberculosis have been identified[2]. When the AIDS pandemic began, one third of the world population was infected with *Mycobacterium tuberculosis*. Each year, eight to ten million persons developed active disease and three million persons died from tuberculosis. Currently available first-line antituberculous agents, such as rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide are highly effective and generally well tolerated. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to any of these first-line drugs and because second-line drugs such as p-aminosalicylic acid, amikacin, cycloserine, capreomycin and ethionamide are less effective and more toxic[3]. The infuriating truth is that the pharmaceutical industry has produced nothing in the way of new tuberculosis drugs since rifampicin was introduced in the 1960s, even though rifapentine, a long-acting rifamycin drug was more recently investigated by Hoechst Marion Roussel. In the course of screening to discover new compounds employed in the chemotherapy of tuberculosis, we identified some Isatin derivatives, which inhibited *in-vitro Mycobacterium tuberculosis* H37 Rv[4]. Thus taking into account our experience in the field of the synthesis of new Isatin derivatives[5-13], we present results concerning the synthesis of isatin derivatives bearing pyrimidine nucleus and the *in-vitro* antituberculous activity of the first representative compounds of this family.

2. Investigation and Results

The N-Mannich bases of 3-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl]iminoisatin derivatives were prepared as depicted in the Scheme. All the compounds resulting from the reactional sequences were evaluated *in-vitro* against *Mycobacterium tuberculosis* H37 Rv at 6.25 μg/ml in BACTEC 12B medium using the BACTEC 460 radiometric system. Rifampicin, Isoniazid, Sparfloxacin, Ofloxacin, Tobramycin and Clarithromycin were used as the reference. The results are summarized in Table 1.

Table 1. *In-vitro* Antituberculous activity of the synthesized compounds

Compound code	Concentration, μg/ml	Inhibition
S1	6.25	97
S2	6.25	90
S3	6.25	89
S4	6.25	86
S5	6.25	90
S6	6.25	87
S7	6.25	87
S8	6.25	86
S9	6.25	92
S10	6.25	88
S11	6.25	88
S12	6.25	91
Rifampicin	0.25	98
Isoniazid	0.031	95
Tobramycin[1]	10.0	99
Clarithromycin[1]	26.0	99
Ethionamide[1]	1.17	99
PAS[1]	2.31	99
Ethambutol[1]	1.17	99
Gentamycin[1]	6.0	99
Doxycyline[1]	12.0	99

[1]The concentatrion represent their MICs

3. Discussion

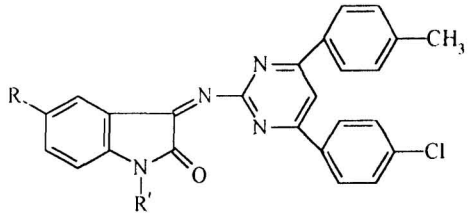
All the synthesized compounds tested in preliminary screening at 6.25 μg/ml showed percentage of inhibition ranging from 86 to 97%. The compound **S1** emerged as highly active analogue in this series with 97% inhibition and the MIC determintion are still under study at TAACF, USA. This compound (**S1**) was found to be equipotent with Gentamycin, as active as Doxycyline Tobramycin and Clarithromycin against *M. tuberculosis* H37 Rv. In general the Schiff bases (**S1, S5, S9**) were more active than Mannich bases and with regard to the substitutents at the 5th position, the order of activity was found to be H > Br > Cl. Among the Mannich bases the order of activity was as dimethylamino > piperidino > morpholino derivatives. From a preliminary examination of these results, it appears that compound **S1** showed potent activity against *M. tuberculosis* H37 Rv. These results need to be refined in terms of minimum inhibitory concentration and toxicity. Further studies to acquire more information about the structure-activity relationships are in progress in our laboratories.

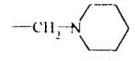
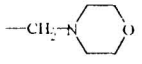
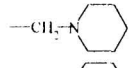
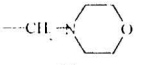
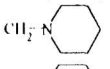
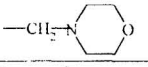
4. Experimental

4.1. Chemical Procedures

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 (90MHz) employing tetramethylsilane as the internal reference. Elemental analyses were undertaken for all the compounds and were within 0.4% of the calculated values. TLC was carried on silica gel chromatograms.

Table 2. Physical constants of the synthesized compounds



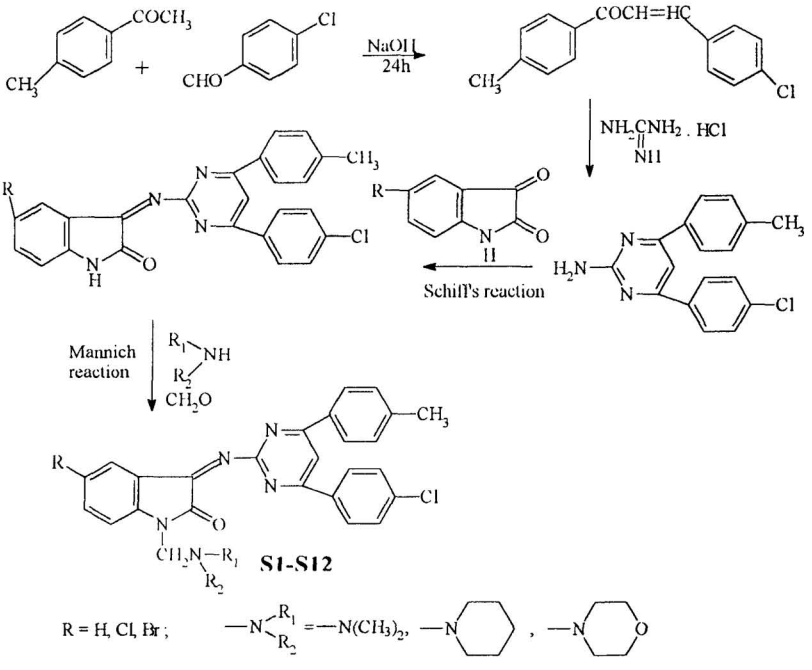
Code	R	R'	Yield (%)	M.P. (°C)	Mol. For.	R_f[a]
S1	H	H	72	135	$C_{25}H_{17}ON_4Cl$	0.73
S2	H	$-CH_2N(CH_3)_2$	79	122	$C_{28}H_{24}ON_5Cl$	0.63
S3	H		91	98	$C_{31}H_{28}ON_5Cl$	0.67
S4	H		47	138	$C_{30}H_{26}O_2N_5Cl$	0.75
S5	Cl	H	79	160	$C_{25}H_{16}ON_4Cl_2$	0.84
S6	Cl	$-CH_2N(CH_3)_2$	75	134	$C_{28}H_{23}ON_5Cl_2$	0.72
S7	Cl		78	101	$C_{31}H_{27}ON_5Cl_2$	0.69
S8	Cl		76	118	$C_{30}H_{25}O_2N_5Cl_2$	0.76
S9	Br	H	57	209	$C_{25}H_{16}ON_4ClBr$	0.59
S10	Br	$-CH_2N(CH_3)_2$	85	235	$C_{28}H_{23}ON_5ClBr$	0.74
S11	Br		81	109	$C_{31}H_{27}ON_5ClBr$	0.68
S12	Br		86	115	$C_{30}H_{25}O_2N_5ClBr$	0.78

[a]In TLC eluant used was benzene : ethanol (9.5:0.5).

4.1.1. Synthesis of 3-(4-chlorophenyl)-1-(4-methylphenyl)-2-propen-1-one

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

Scheme. Protocol of the synthetic compounds



4.1.2. Synthesis of 4-(4-chlorophenyl)-6-(4-methylphenyl)-2-aminopyrimidine

A mixture of 3-(4-chlorophenyl)-1-(4-methylphenyl)-2-propen-1-one (0.01mol) and guanidine hydrochloride (0.015mol) was added to sodium hydroxide (0.045mol in 2ml of water) and ethanol (50ml). The reaction mixture was refluxed for 6h. The mixture was concentrated under reduced pressure and cooled, and a yellow solid was obtained which was recrystallised in ethanol. Yield: 82%, m.p. 185-190°C, IR (KBr): 3280 cm^{-1} (NH_2), 1610 cm^{-1} (ring C=C, C=N); ^{1}H-NMR ($CDCl_3$) δppm: 2.30 (s, 3H, CH_3), 5.5 (s, 2H, NH_2), 7.3-7.5 (m, 4H, ArH of p-toluyl), 7.75 (sym 2d, 4H, ArH of p-Cl-phenyl); Anal. ($C_{17}H_{14}N_3Cl$) C, H, N.

4.1.3. Synthesis of 5-chloro-3-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl]iminoisatin

Equimolar quantities (0.03mol) of 5-chloroisatin and 4-(4-chlorophenyl)-6-(4-methylphenyl)-2-aminopyrimidine were dissolved in 75ml of warm ethanol containing 1ml of glacial acetic acid. The reaction mixture was refluxed for 18h and set aside. The resultant solid was washed with dilute ethanol, dried and recrystallised from ethanol-chloroform mixture. Yield: 78.16%; m.p.: 160°C; IR (KBr): 1640 cm^{-1} (C=N), 1610 cm^{-1} (ring C=C, C=N); ^{1}H-NMR ($CDCl_3$) δppm: 2.35 (s, 3H, CH_3), 6.8-7.1 (m, 3H, ArH of Isatin), 7.2-7.5 (m, 4H, ArH of p-toluyl) 7.75 (s, 1H, 5'H), 7.9-8.1 (sym 2d, 4H, ArH of p-chlorophenyl), 10.4 (s, 1H, NH, D_2O exchangeable); Anal. ($C_{25}H_{16}N_4OCl_2$) C, H, N.

Similarly compounds **S1** and **S9** were synthesized by using Isatin and 5-bromoisatin respectively.

4.1.4. Synthesis of 5-chloro-1-piperidinomethyl-3-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl] iminoisatin (S7)

A slurry consisting of the **S5** (0.005mol), tetrahydrofuran (5ml) and 37% formalin (2ml) was made. To this piperidine (0.005mol) was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1h with occasional shaking after which it was warmed on a steam bath for 15min. At the end of the period the contents were cooled and the product obtained was recrystallised from chloroform-petroleum ether. Yield: 78.26%; m.p.: 101°C; IR (KBr): 2870 cm^{-1} (CH_2), 1645 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δppm: 1.8 (m, 6H, $(CH_2)_3$), 2.3 (s, 3H, CH_3), 2.6 (t, 4H, CH_2NCH_2), 4.45 (s, 2H, NCH_2N), 6.8-7.1 (m, 4H, ArH of isatin), 7.1-7.5 (m,

3H, ArH of p-toluyl), 7.9-8.1 (sym 2d, 4H, ArH of p-Cl-phenyl); Anal. ($C_{31}H_{27}ON_5Cl_2$) C, H, N.

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.

4.2. Biological Evaluation

4.2.1. Antituberculous Screening

Antituberculous activity was determined using the BACTEC 460 system[14-15] as modified below. Stock solutions of test compounds were prepared in dimethylsulphoxide (DMSO) at 1mg/ml and sterilized by passage through 0.22mm PFTE filters (Millex-FG, Millipore, Bedford, MA). Fifty microlitres were added to 4ml radiometric 7H12 broth (BACTEC 12B; Becton Dickinson Diagnostic Instrument Systems, Sparkes, MD) to achieve a final concentration of 12.5μg/ml. Controls received 50ml DMSO. Rifampin (Sigma Chemical Co. St. Louis, MO) was included as a positive drug control. Rifampin was solubilized and diluted in DMSO and added to BACTEC 12B broth to achieve a range of concentrations for determination of minimum inhibitory concentration (MIC, lowest concentration inhibiting 99% of the inoculum). *Mycobacterium tuberculosis* H37 Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) was cultured at 37°C on a rotary shaker in Middlebrook 7H9 broth (Difco Laboratories, Detroit MI) supplemented with 0.2% v/v glycerol and 0.05% v/v Tween80 until the culture turbidity achieved an optical density 0.45-0.55 at 550nm. Bacteria were then pelleted by centrifugation, washed twice and resuspended in one-fifth of the original volume in Dulbecco's phosphate-buffered

saline (PBS, Irvine Scientific, Santa Ana, CA). Large bacterial clumps were removed by passage through an 8mm filter (Nalgene, Rochester, NY) and samples were frozen at –80°C. Cultures were thawed and an appropriate dilution performed such that a BACTEC 12B vial inoculated with a 0.1ml would reach a growth index (GI) of 999 in five days. One-tenth milliliter of the diluted inoculum was used to inoculate 4ml fresh BACTEC 12B broth containing test compounds. An additional control vial was included which received a further 1:100-diluted inoculum (as well as 50ml DMSO) for use in calculating the MIC of rifampin by established procedures[8]. Cultures were incubated at 37°C and the GI determined daily until control cultures achieved a GI of 999. Assays were usually completed in 5-8days. Percent inhibition was defined as (1-GI of test sample/ GI of control) × 100. MIC of rifampin was defined as the lowest concentration of compound effecting a reduction in the daily change in GI which was less than that observed with a 1:100-diluted control culture on the day the latter reached a GI of atleast 30.

Acknowledgement

We are grateful to TAACF for the Antituberculous evaluations.

Reference

1. Brudney K., Dobkin J., (1991) Am. Rev. Respir. Dis. 144, 745.
2. Gangadharam P. R. J., (1988) Peterson P. K., Verhoef J., (Eds.) The Antimicrobial Agent Annual, 3rd edition, Elsevier, Amsterdam/Main, 15.
3. Mandell G. L., Sande M. A., (1990) Gilman A. G., Goodmann L.S., Rall T. W., Nies A. S., Taylor P., (Eds.) The Pharmacological basis of Therapeutics, 8th edition Pergamon Press, New York/ Main, 1146.

4. Pandeya S. N., Sriram D., Yogeeswari P., Ananthan S., (2001) Chemotherapy 47, 266.

5. Pandeya S. N., Sriram D., (1998) Acta Pharm. Turc. 40, 33.

6. Pandeya S. N., Sriram D., De Clercq E., Pannecouque C., Witvrouw M., (1998) Indian J. Pharm. Sci. 60, 207.

7. Pandeya S. N., Yogeeswari P., Sriram D., Nath G., (1998) Boll. Chim. Farm. 137, 321.

8. Pandeya S. N., Yogeeswari P., Sriram D., De Clercq E., Pannecouque C., Witvrouw M., (1999) Chemotherapy 45, 192.

9. Pandeya S. N., Sriram D., Nath G., De Clercq E., (2000) Eur. J. Med. Chem. 35, 265.

10. Pandeya S. N., Sriram D., Nath G., De Clercq E., (2000) Arzneim.-Forsch./Drug Res. 50(1), 55.

11. Pandeya S. N., Sriram D., Nath G., De Clercq E., (1999) Eur. J. Pharm. Sci. 9, 25.

12. Pandeya S. N., Sriram D., Nath G., De Clercq E., (1999) Sci. Pharm. 67, 103.

13. Pandeya S. N., Sriram D., Nath G., De Clercq E., (1999) Pharm. Acta Helv. 74, 11.

14. Inderleid C, B., Nash K. A., (1996) Lorian V., (Eds.) Antibiotics in Laboratory Medicine, 4th edition, Williams & Wilkins, Baltimore/Main, 127.

15. Inderleid C. B., Salfinger M., (1995) Murray P. R., Baron E. J., Pfaller M. A., Tenover F. C., Yoken R. H., (Eds.) Manual of Clinical Microbiology, 6th edition, ASM Press, Washington/Main, 1385.

Received October 25th, 2001
Accepted January 17th, 2002