www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 209-213, Jan-Mar 2010

# Antimicrobial Evaluation of Some novel Schiff and Mannich bases of Isatin and its derivatives with quinolin

***Chhajed S.S.[1],Padwal M. S.[2]**

**[1]S.M.B.T. College of Pharmacy, Dhamangaon, Dist-Nasik, (MS), India**
**[2]Enem Nostrum Remedies Ltd. Mumbai, India.**

****Corres.author: santosh_chhajed@rediffmail.com***

**Abstract:** Isatin and its chloro derivative have been reacted with 5-amino, 8-Hydroxy-quinoline 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. The compounds are significantly active against bacteria and fungi.
**Keywords** Schiff bases; *N*-Mannich bases; Isatin; Antimicrobial activity.

## Introduction

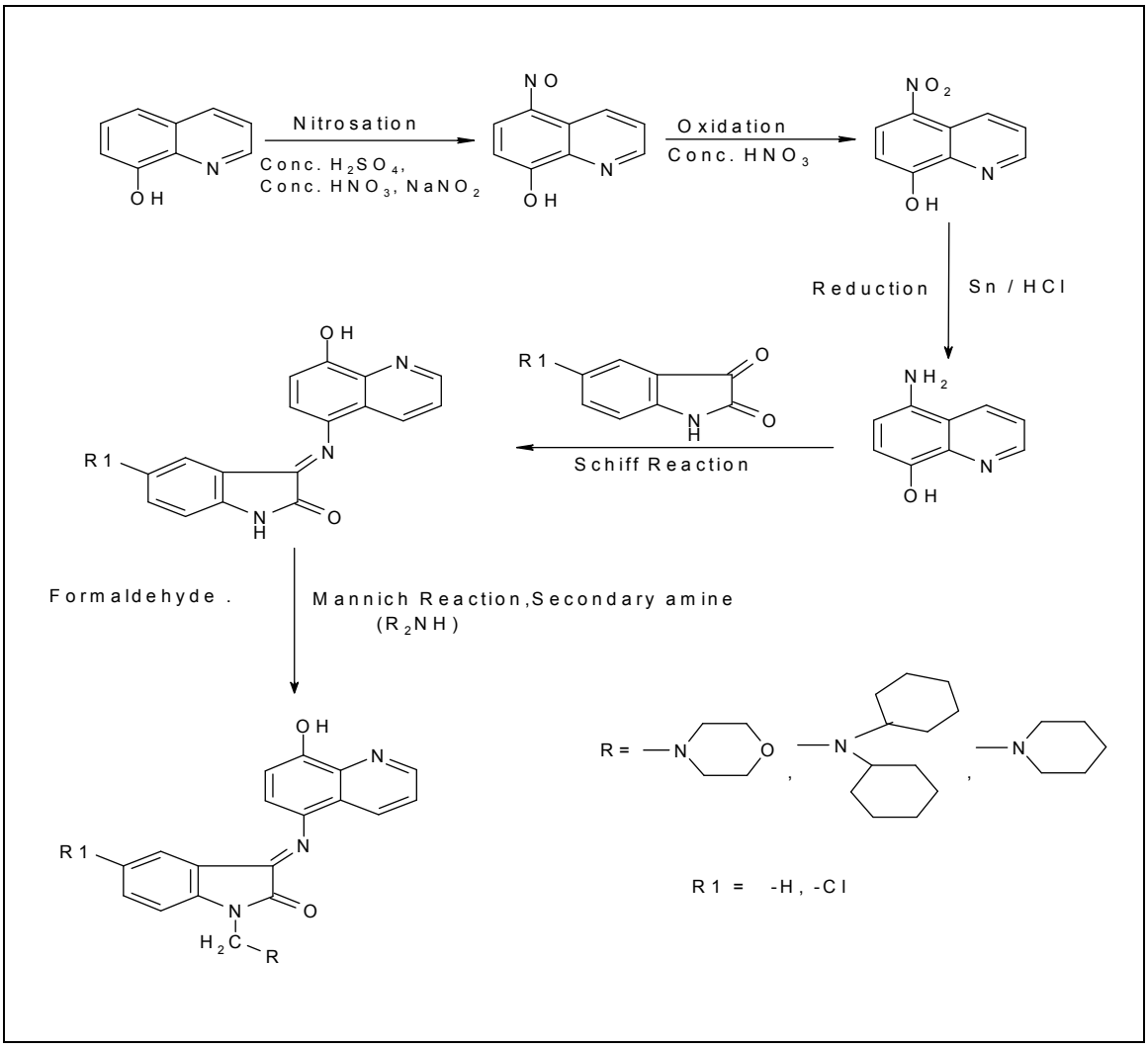
Isatin (Indole-2, 3-dione), its Schiff and Mannich bases are reported to show a variety of biological activities, such as antibacterial [1], antifungal [2] activities. Quinolines are reported to have antibacterial [3], antifungal [4] activities. In view of these facts and as a continuation of search of newer antimicrobial agents, Schiff and Mannich bases of Isatin and its derivatives with Quinoline are synthesized in the present work. 5-amino, 8-Hydroxy-quinoline was synthesized from 8-Hydroxy-quinoline [5]. It was condensed with isatin and its derivative 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). Purity of the compounds was ascertained by the thin layer chromatography (TLC), all compounds (Table 1) gave satisfactory elemental analysis. IR and $^1$H NMR spectra were consistent with the assigned structures. The synthesized scaffold was screened for antibacterial, antifungal activity by the agar dilution method.

## 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 was recorded using a (Brucker Avance II 400) spectrophotometer at a frequency of 400.13 MHz. Chemical shifts are given in ppm relative to tetramethylsilane (TMS) as internal standard. The electrospray mass spectra were recorded on MICROMASS QUATRO II triple quadrapole mass spectrophotometer. Analytical thin layer chromatography (TLC) was performed by using adsorbent silica gel G, Visualilization of the developed chromatogram was performed with iodine vapors. Solvents and reagents obtained from commercial sources were used without purification, unless noted.

**I]Synthesis of 5-Nitroso-8-hydroxyquinoline:** 8-hydroxyquinoline (7.34 g, 0.05mol) was dissolved in a continuously stirred solution of 66.7 mL of distilled water and 3 mL of concentrated sulfuric acid at a temperature of 15-18$^0$C. Sodium nitrite (3.67 g) in distilled water (6.78 mL) was added drop wise to the reaction mixture over a period of 30-40 min at reaction temperature of 15-18$^0$C. The mixture was maintained at this temperature for 3 hr. The completeness of nitrosation was tested by checking the $P^H$ of the reaction mixture ($P^H$ 1.0-2.0). The reaction mixture was cooled, and at a temperature not exceeding 25$^0$C neutralized with 42% sodium hydroxide solution to $P^H$ 7.5-8.0 and then acidified with glacial acetic acid to $P^H$ 3.0-4.0. The resulting precipitate (pale yellow) was filtered, washed with distilled water, and dried, yield, 89.5 % melting point 235-236$^0$C (dc).

**Scheme: Synthetic protocol of the compounds.**




**II] Synthesis of 5-Nitro-8-hydroxyquinoline**: After addition of 0.3 mL concentrated nitric acid drop wise with stirring over 20 min to a homogenous suspension of compound 5-Nitroso-8-hydroxyquinoline (2.5 g, 0.0144mol) in acetic acid (6.25mL) at 20-30$^0$C, the reaction mixture was held at this temperature for 2 hr. It was then cooled to 5-10$^0$C, and at temperature not exceeding 25$^0$C neutralized with 24% aqueous sodium hydroxide ($P^H$ 10-11). The reaction mixture was then cooled to 10-15$^0$C for 30 min and then acidified with glacial acetic acid to $P^H$ 5-6 and again cooled for 30 min to 10-15$^0$C. The technical product was recrystalized from acetone, yield: 84 % melting point 176-178$^0$C.

**III] Synthesis of 5-amino-8-hydroxyquinoline:** 0.190 g (0.01mol) of 5-Nitro-8-hydroxyquinoline in 25 mL of concentrated hydrochloric acid was allowed to warm. To it was added slowly, in small portions tin (Sn) metal (2.3 g, 0.02mol) it was heated at reflux for 5 hr in boiling water bath. The reaction mixture was allowed to cool down to room temperature. The yellow brown solution was then cooled with an ice bath, and to the solution was slowly added 20% solution of sodium hydroxide to get the precipitate of 5-amino-8-hydroxyquinoline. Technical product was extracted out with ether. Ether layer evaporated, product was air dried and recrystallized from ethyl acetate, yield 79.8 %, melting point 186-188$^0$C.

**IV] Synthesis of Schiff Bases:** Equimolar quantities (0.01mol) of (Substituted) Isatin and 5-amino-8-hydroxyquinoline (0.01mol) (1.6 g) were dissolved in 20 mL of dry ethanol. To it was added 1-2 drops of concentrated sulfuric acid and heated at reflux for 2-3 hrs. After standing for approximately 24 hrs at room temperature, the er product were separated by filtration, dried and recrystallized

**V] Synthesis of Mannich Bases:** Equimolar quantity (0.02 mol) of secondary amine was added in to slurry containing the appropriate Schiff base and (37%) formalin (1mL) solution dissolved in 10 mL of DMSO (Dimethyl sulphoxide). The reaction mixture was stirred for 1 hr at room temperature and refrigerated for 24 hrs. The products were separated, dried and recrystallized.

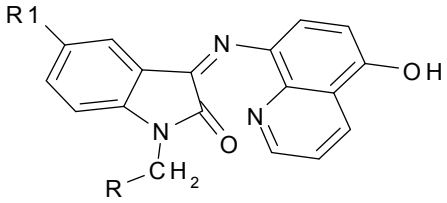
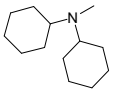
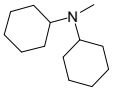



**Table1. Physicochemical and Spectral data of the compounds**

| Compd | R1 | R | M.P. ($^0$C) | Rf | % yield | Spectral data<br>IR (cm$^{-1}$) : $^1$H NMR δ (ppm) : m/z |
|---|---|---|---|---|---|---|
| **(I)** | -H |  | 178-180 | 0.88 | 64 | IR (cm$^{-1}$): 3451.12(O-H str),3056.42 (C-H str. Ar), 2922.09 (C-H str. CH2 As), 2865.81 (C-H str. CH2 sy), 1315.39 (C-N str)<br>$^1$H NMR δ (ppm) : 9.1 (s Ar-OH); 6.69 to8.1(m, Ar-H); 1.5 to 2.37 (m, 10H, piperidine); 2.2 (d, 1H, CH); 2.78 (s, 1H, CH)<br>m/z:- 386 |
| **(II)** | -H |  | 144-146 | 0.84 | 60 | IR (cm$^{-1}$): 3446.72 (O-H str.), 3122.24(C-H str. Ar), 2939.04 (C-H str. CH2 As), 2854.29(C-H str. CH2 sy) 1740.37 (C=O str.), 1571.62(C=C str), 1353.27(C-N str.).<br>$^1$H NMR δ (ppm) : 9.1 (s, Ar-OH); 1.4 to 1.45 (m, 20H, dicyclohexane); 2.2 to 2.3 (d, 1H, CH); 2.5 to 2.7 (d, 1H, CH); 6.69 to 8.1 (m, Ar-H).<br>m/z: - 483 |
| **(III)** | -H |  | 130-134 | 0.77 | 65 | IR (cm$^{-1}$): 3461.38 (O-H str.), 3042.65 (C-H str Ar), 2918.38 (C-H str. CH2 Asy.), 2856.62 (C-H str. CH2 sy.), 1315.24 (C-N str.), 1110.92 (C-O-C str).<br>$^1$H NMR δ (ppm) : 9.1 (s, Ar-OH); 2.27 (s, 1H, CH); 2.78 (s, 1H, CH); 6.69 to 8.12 (m, Ar-H); 2.37 (d, 4H, morpholine); 3.67 (d, 4H, morpholine).<br>m/z: 423 |
| **(IV)** | -Cl |  | 90-94 | 0.56 | 72 % | IR (cm$^{-1}$): - 3449.29 (O-H str.), 3051.15 (C-H str. Ar), 2920.21 (C-H str. CH2 As), 2859 .23(C-H str. CH2 sy), 1312.34 (C-N str.)<br>$^1$H NMR δ (ppm) : 9.0 (s Ar-OH); 6.71 to 8.2 (m, Ar-H); 1.5 to 2.37 (m, 10H, piperidine); 2.2 (d, 1H, CH); 2.78 (s, 1H, CH)<br>m/z:- 420 |
| **(V)** | -Cl |  | 156-158 | 0.79 | 79 % | IR (cm$^{-1}$): 3395.34 (O-H str.), 3056.54(C-H str. Ar), 2934.34 (C-H str. CH2 As), 2843.65 (C-H str. CH2 sy) 1722.60 (C=O str), 1588.43 (C=C str), 1353.12(C-N str.).<br>$^1$H NMR δ (ppm) : 9.7 (s, Ar-OH); 1.7 to 2.5 (m, 20H, dicyclohexane); 2.1 to 2.5 (d, 1H, CH); 2.1 to 2.4 (d, 1H, CH); 6.8 to 8.9 (m, Ar-H).<br>m/z: - 517 |
| **(VI)** | -Cl |  | 214-218 | 0.82 | 70 % | IR (cm$^{-1}$) : 3451.31(O-H str), 3052.19 (C-H str Ar), 2906.51 (C-H str CH2 Asy), 2848.40 (C-H str. CH2 sy), 1311.29 (C-N str.), 1109.32 (C-O-C str Six membered ring).<br>$^1$H NMR δ (ppm) : 8.2 (s, Ar-OH); 2.27 (s, 1H, CH); 2.78 (s, 1H, CH); 7.1 to 8.4 (m, Ar-H); 2.37 (d, 4H, morpholine); 3.67 (d, 4H, morpholine)<br>m/z : 457 |

***In-vitro* antimicrobial activity** [6]
Evaluation of antibacterial and antifungal activity is done by the agar dilution method. 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 synthesized compounds were subjected to antimicrobial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various gram positive and Gram negative bacteria and anti fungal activity against various fungal stains compared with standard drug (Sulphamethaxole and Ketoconazole) using solvent control.
The results were described in the table no. 2.

## Results and Discussion

All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. The purity of the synthesized compounds was checked by
performing thin layer chromatography and determining melting points. Then the synthesized compounds were subjected to spectral analysis such as IR, NMR and Mass Spectra to confirm the structures. All the analytical details show satisfactory results (Table.1) All the mass spectras showed the molecular ion peaks for their respective molecular weights apart from fragmentation profile. Since our titled compounds are known to possess antimicrobial activity, the compounds were screened for their antibacterial and antifungal activity by cup-plate method. Gram positive bacteria such as Staphylococcus aureus, Streptococcus faecalis and bacillus Subtilis, two gram negative bacteria such as Escherichia coli and Pseudomonas aeruginosa and two fungal species such as Aspergillus Niger and Candida albicans are tested for the activities. The concentration of 250, 500 and 750 µg/ml of our titled compounds has been used. Sulphomethaxozole and Ketoconazole have been used as standards. DMSO is used as solvent control. All the compounds have shown mild to moderate activities. Among these IV(3-(8-hydroxyquinlin-5-ylimino)-5-choloro-1-[(piperidin-1-yl)-methyl]-1H-inden-2 (3H)-one and VI ((3-(8-hydroxyquinlin-5-ylimino)-5-choloro-1-(morpholinomethyl)-1H-inden-2(3H)-one. have shown good activity in all the species.

**Table 2. Antimicrobilal screening of the synthesize scaffold**

| Compounds / Micro Organisms | Zone of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacteria | | | | | Fungi | |
| | B. substilis | S. aureus | S. faecalis | E. Coli | P. aeruginosa | C. albicans | A. niger |
| Sulphamethoxazole | 26 | 28 | 27 | 30 | 23 | NA | NA |
| Ketoconazole | NA | NA | NA | NA | NA | 24 | 20 |
| DMSO | - | - | - | - | - | - | - |
| I | 03 | 09 | 03 | 10 | 08 | 03 | 05 |
| II | 15 | 16 | 06 | 15 | 09 | 05 | 08 |
| III | 04 | 08 | 07 | 10 | 12 | 14 | 11 |
| IV | 19 | 21 | 10 | 24 | 19 | 18 | 18 |
| V | 09 | 11 | 13 | 12 | 03 | 10 | 11 |
| VI | 21 | 19 | 18 | 24 | 20 | 18 | 16 |

## References

1] Pandeya, S. and Sriram, D. Synthesis and screening of antibacterial activity of Schiff and Mannich bases of isatin derivatives, ***Acta. Pharm. Turc.* 40** (1998) 33.
2] Varma, R. and Nobles, W.ng Antimicrobial Screening of Substituted *N*-amino methyl isatins, ***J. Med. Chem*. 10** (1967) 510.
3] Wlson Gisovold's., Textbook of Organic Medicinal and Pharmaceutical Chemistry,Eleventh Edition, Lipincott Williams &Wilkins, 250.
4] Rigler, N. E., Greathouse, G. A., "Fungicidal Potency of Quinoline Homologs and Derivatives", Industrial and Engineering Chemistry Vol**.** 33, 5.
5]Voronin, V.,Petrova, V.,Leksin, C., and Shemeryankin, D.Methods for Synthesis of 5- nitro 8-HydroxyQuinoline, ***J.Chem.Heter.Comp.,*** *1976,* 21, 5, 1217.
6] Pandey V.K, Gupta V.D, Mridula Upadhyway, Singh V.K and Meenal Tandon., Synthesis, characterization and biological activity of 1, 3, 4-substituted 2-azetidinones,
**Ind.J. Chem.**, 2005, 44(B), 158.

*****