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# Synthesis, Antibacterial and Antifungal Activities of N-Mannich Bases of 3-[N²-Pyrimethaminylimino] isatin

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**Schiff base of isatin with pyrimethamine and its N-Mannich bases were synthesized. Their chemical structures have been confirmed by UV, IR, $^1$H-NMR data and by elemental analyses. Antimicrobial evaluation was done by agar dilution method against 25 pathogenic bacteria and 6 pathogenic fungi. Among the new derivatives evaluated, 2-[1"-(morpholinomethyl)-3"-isatinimino]-5-(4'-chlorophenyl)-6-ethyl-4-aminopyrimidine (PY4) exhibited higher potency compared to the standard drugs, against all bacteria. All the compounds exhibited antifungal activity.**

Recently we have reported that several Schiff and N-Mannich bases of isatin derivatives possessed marked antibacterial, antifungal and antiHIV activities[1-5]. In continuation of our work on the N-Mannich bases of isatin-3-imines, the synthesis and antimicrobial evaluation of some new compounds are reported herein. The Schiff base, 3-[N²-pyrimethaminylimino] isatin (**PY1**) was accomplished by the reaction of isatin with pyrimethamine in presence of glacial acetic acid. The confirmation for the reaction of 2-amino group of the pyrimethamine with the ketone group of isatin at position -3 comes from the observation of a group of amidinobenzylpyrimidines and their Schiff bases with different ketones[6]. The N-Mannich bases (**PY2-PY7**) of the above Schiff base were prepared by condensing with formaldehyde and secondary amines. The structures of all the new compounds were confirmed by their UV, IR and $^1$H-NMR spectra and elemental analyses. All the synthesized compounds were screened for antibacterial and antifungal activity by agar dilution method.

## EXPERIMENTAL

The melting points were determined by using Thomas Hoover melting point apparatus and are uncorrected. UV, IR and $^1$H-NMR spectra were recorded for the compounds on a Jasco J-0063 model, Jasco IR Report-100 (KBr) and JEOL Fx 90Q FT-NMR (90 MHz) instruments, respectively.

### Synthesis of 2-[3"-isatinimino]-5-(4'-chlorophenyl)-6-ethyl-4-aminopyrimidine (PY1):

Equimolar quantities (0.06 mol) of isatin (8.82 g) and pyrimethamine (14.92 g) were dissolved in ethanol (75 ml) containing 2-3 drops of glacial acetic acid. The reaction mixture was refluxed for 4 h and set aside. The resultant solid was filtered, washed with ethanol and dried. Recrystallised from ethanol:chloroform mixture. Yield 78.34%; m.p. 185-190°; UV ($CH_3OH$), $\lambda_{max}$: 270.5 nm; IR (KBr): 3300 (NH), 1659 (C=O), 1578 (C=N) $cm^{-1}$; $^1$H-NMR ($CDCl_3$) $\delta$ ppm: 0.97 (t, 3H, $CH_3$ of $C_2H_5$), 2.1(q, 2H7 $CH_2$ of $C_2H_5$), 5.4 (bs, 2H, $NH_2$), 6.8-7.2 (m, 8H, Ar-H), 10.4 (s, 1H, NH); Mol. Formula ($C_{20}H_{16}N_5OCl$).

### Synthesis of 2-[1"-(N,N-dimethylamino)methyl-3"-isatinimino]-5-(4'-chlorophenyl)-6-ethyl-4-aminopyrimidine (PY2):

To a slurry containing **PY1** (0.003 mol), ethanol (5 ml) and 37% formalin (1 ml), was added N,N-dimethylamine (0.003 mol) drop wise with good stirring. The reaction mixture was cooled and allowed to stand at room temperature for 1 h with occasional shaking. It was warmed on a steam bath for 15 mm, cooled and the product was recovered.

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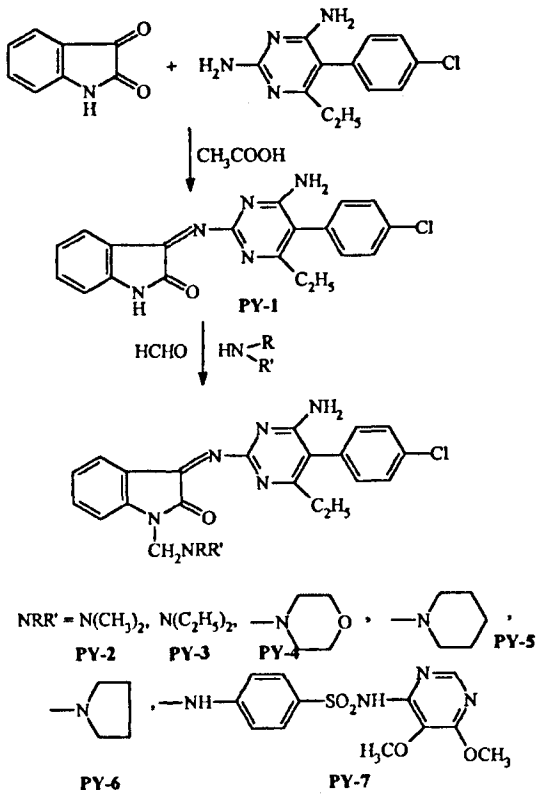


**Scheme: Synthetic protocol of the title compounds.**

Recrystallised from chloroform-petroleum ether (1:1) mixture. Yield 62.11%; m.p. 120-124°; UV ($CHCl_3$), $\lambda_{max}$: 273.5 nm; IR (KBr): 2970, 2800 (CH of $CH_3$), 2860 (CH of $CH_2$), 1644 (C=O), 1576 (C=N); $^1$H-NMR ($CDCl_3$) δ ppm: 0.97 (t, 3H, $CH_3$ of $C_2H_5$), 2.1 (q, 2H, $CH_2$ of $C_2H_5$), 2.2 (s, 6H, $N(CH_3)_2$), 4.9 (s, 2H, -N-$CH_2$-N-) 5.4 (bs, 2H, $NH_2$), 6.8-7.2 (m, 8H, Ar-H); Mol. Formula ($C_{23}H_{23}N_6OCl$). The physical data of the title compounds are represented in Table 1.

**Antibacterial activity:**

The antibacterial activity was determined by agar dilution technique[7] against 25 pathogenic bacteria, procured from the department of microbiology, IMS, BHU. The medium was prepared as per the instructions of the manufacturer of dry Mueller Hinton agar powder (Hi-Media). The concentrations 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 the lowest concentration (higher dilution) without visible growth. The study was simultaneously performed for the pure standard drugs (pyrimethamine and sulphadoxine) also. The MICs are reported in Table 2.

**Antifungal activity:**

Five compounds (**PY3-PY7**) were screened for antifungal sensitivity by agar dilution method at a concentration of 100 µg/ml against 6 pathogenic fungi. The compounds were solubilized in DMF.

## RESULTS AND DISCUSSION

All the compounds showed marked activity against a variety of microorganisms. The MICs of the compounds against 25 pathogenic bacteria are presented in Table 2. Also included are the activity of pyrimethamine and sulphadoxine. All the N-Mannich bases (**PY2-PY7**) were more potent than pyrimethamine. The compounds **PY2** and **PY5** were more potent than sulphadoxine against *Staphylococcus aureus, Escherichia coli* MCTC10418, *Vibrio cholerae* nonO$_1$, *Pseudomonas aucroginosa* MCTC10662, *Salmonella typhimurium* and *Morganella morgani*. The compounds **PY1**

TABLE 1: PHYSICAL CONSTANTS OF THE SYNTHESIZED COMPOUNDS.

| Compound code | Yield (%) | M.P. (°) | Molecular Formula | Molecular Weight | $R_f$* |
|---|---|---|---|---|---|
| PY-1 | 78 | 188 | $C_{20}H_{16}ON_5Cl$ | 377 | 0.77 |
| PY-2 | 62 | 122 | $C_{23}H_{23}ON_6Cl$ | 434 | 0.68 |
| PY-3 | 63 | 127 | $C_{25}H_{27}ON_6Cl$ | 462 | 0.62 |
| PY-4 | 75 | 123 | $C_{25}H_{25}O_2N_6Cl$ | 476 | 0.72 |
| PY-5 | 52 | 138 | $C_{26}H_{27}ON_6Cl$ | 474 | 0.52 |
| PY-6 | 76 | 124 | $C_{25}H_{25}ON_6Cl$ | 460 | 0.75 |
| PY-7 | 54 | 108 | $C_{33}H_{30}O_5N_9SCl$ | 699 | 0.53 |

*Eluant used in TLC was $CHCl_3$ : MeOH : Ammonia in 9:1:1-2drops.

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS.

| Microorganism/Drug | PY1 | PY2 | PY3 | PY4 | PY5 | PY6 | PY7 | PYRI | SULD |
|---|---|---|---|---|---|---|---|---|---|
| *Bacillus subtilis* | 39 | 19.5 | 2.44 | 2.44 | 19.5 | 312.5 | 39 | 1250 | 0.15 |
| *Staph. aureus* | 4.88 | 39 | 2.44 | 0.075 | 2.44 | 78 | 9.76 | 2500 | 156.25 |
| *Staph. albus* | 2.44 | 39 | 2.44 | 0.075 | 2.44 | 19.5 | 2.44 | 2500 | 0.3 |
| *E. coli* | 1250 | 78 | 2.44 | 2.44 | 625 | 78 | 625 | 2500 | 156.25 |
| *E. coli MCTC10418* | 156.25 | 625 | 78 | 2.44 | 1250 | 312.5 | 78 | >5000 | >5000 |
| *V. chol.* $O_1$ | 312.5 | 78 | 156.25 | 2.44 | 9.76 | 78 | 19.5 | >5000 | 0.61 |
| *V. chol. Non* $O_1$ | 625 | 625 | 39 | 0.3 | 78 | 19.5 | 39 | >5000 | 2500 |
| *V. parahaemolyticus* | 156.25 | 625 | 2.44 | 0.075 | 5000 | 19.5 | 9.76 | >5000 | 19.5 |
| *Shigella boydii* | 312.5 | 78 | 156.25 | 0.3 | 625 | 2.44 | 19.5 | 2500 | 19.5 |
| *Sh. dysentriae* | 156.25 | 625 | 78 | 0.075 | 625 | 19.5 | 78 | 2500 | 312.5 |
| *Sh. enteriditis* | 625 | 625 | 2.44 | 0.15 | 625 | 312.5 | 78 | >5000 | 19.5 |
| *Sh. flexneri* | 4.88 | 625 | 9.76 | 0.3 | 4.88 | 312.5 | 9.76 | 2500 | 625 |
| *Sh. sonneii* | 39 | 78 | 39 | 0.3 | 625 | 312.5 | 78 | 2500 | 39 |
| *Klb. pneumoneae* | 1250 | 625 | 156.25 | 2.44 | 625 | 156.25 | 78 | >5000 | 39 |
| *Enterobacter* | 1250 | 78 | 156.25 | 2.44 | 625 | 78 | 78 | 1250 | 19.5 |
| *P. vulgaris* | 1250 | 625 | 2.44 | 2.44 | 625 | 19.5 | 625 | 2500 | 19.5 |
| *P. aeruginosa* | >5000 | 625 | 2.44 | 0.075 | 625 | 2.44 | 156.25 | >5000 | 1.22 |
| *P. aeruginosa MCTC10662* | >5000 | 625 | 39 | 0.075 | 625 | 312.5 | 156.25 | >5000 | >5000 |
| *Sal. typhimurium* | 312.5 | 312.5 | 39 | 0.3 | 625 | 312.5 | 78 | >5000 | >5000 |
| *Sal. paratyphi A* | 2.44 | 625 | 2.44 | 0.075 | 625 | 19.5 | 9.76 | 2500 | 19.5 |
| *Sal. paratyphi B* | 156.25 | 625 | 78 | 0.3 | 625 | 19.5 | 2.44 | 2500 | 19.5 |
| *A. hydrophile* | 9.76 | 625 | 2.44 | 0.3 | 625 | 19.5 | 9.76 | >5000 | 1.22 |
| *M. morganii* | 625 | 78 | 39 | 0.3 | 1250 | 312.5 | 156.25 | 2500 | >5000 |
| *S. murscesonse* | 1250 | 78 | 39 | 2.44 | 625 | 312.5 | 78 | >5000 | >5000 |
| *C. freundii* | 625 | 19.5 | 39 | 2.44 | 625 | 312.5 | 78 | 625 | 1.22 |

MIC's of the compound in µg/ml.

and **PY6** were more potent than sulphadoxine against *Staphylococcus aureus* and *Vibrio parahaemolyticus* and **PY1** was found to be inactive against *Pseudomonas* species. The most active compound of this series was morpholinomethyl derivative (**PY4**) with MIC of 0.075 µg/ml against *Staphylococcus aureus, Staphylococcus albus, Vibrio parahaemolyticus, Shigella dysenteriae, Pseudomonas* and *Salmonella paratyphi* A. The MIC against *V. chlorae* non $O_1$, *Shigella, Salmonella typhimurium, Aeromonas hydrophile* and *Morganella* species was 0.3 µg/ml and the MIC was less than 10 µg/ml against all other bacterial strains.

In the antifungal testing, the compounds **PY3-PY7** showed activity at 100 µg/ml against *Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, Microsporum audonil, Trichophyton menagophytes* and *As-*

*pergillus niger.*


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