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SYNTHESIS AND CHARACTERIZATION OF N–MANNICH BASES WITH PYRIMETHAMINE FOR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Schiff bases of isatin with pyrimethamine and its N–Mannich bases were synthesized. Their chemical structures have been confirmed by UV, IR and ^{1}H NMR data. Antimicrobial evaluation was done by agar dilution method against 10 pathogenic bacteria and 4 pathogenic fungi. The new derivatives exhibited higher potency compared to the standard drugs against all organisms (against all bacteria). All the compounds exhibited antifungal acitivity.

Key word : Antimicrobial activity, N-Mannich bases, Pyrimethamine

INTRODUCTION

Several Schiff and N– Mannich bases of isatin derivatives possessed marked antibacterial, antifungal and anit – HIV activities[1–5]. Synthesis of some new compounds of N–Mannich bases using isatin with pyrimethamine for antibacterial and antifungal activities are reported herein. The Schiff base, (3– pyrimethyminylimino isatin) was prepared by the reaction of isatin with pyrimethamine in presence of glacial acetic acid. The N– Mannich bases (PM_2–PM_6) of the above Schiff bases were prepared by condensing with formaldehyde and secondary amines. The structure of all the new compounds were confirmed by their UV, IR and ^{1}H NMR spectra and elemental analysis. All the synthesized compounds have screened for antibacterial and antifungal activity by agar dilution method.

EXPERIMENTAL

The melting points were determined using capillary tube method and are uncorrected. The UV, IR and NMR spectra were recorded on a Shimadzu 1601 Model Shimadzu, FTIR (KB) and JEOL FX 90 QFT – NMR (90 MHz) instruments, respectively.

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Synthesis of 2–(3''–isatinimino)–5–(4'–chlorophenyl)–6–ethyl–4–minopyrimidine (PM_1)

Equimolar quantities (0.05 mol) of isatin (7.55 g) and pyrimethamine (12.55 g) were dissolved in ethanol (75 mL) and 2–3 drops of glacial acetic acid. The reaction mixture was refluxed for 4 hours and set aside. The precipitate was filtered, washed with ethanol and dried. Recrystallisation using ethanol: chloroform mixture yield 71.86%; m.p. 186 – 190°C; UV (CH_3OH), λ_{max} 270.5 nm ; IR (KBr): 3300 (NH), 1659 (C=O), 1578 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm: 0.98 (3H, CH_3 of C_2H_5), 2.2 (2H of CH_2 of C_2H_5) 5.4 (2H, NH_2), 6.8 – 7.2 (m, 8H, Ar – H), 10.4 (s, 1H, NH); Mol. formula is C_{20} H_{16} N_5 OCl.

Synthesis of 2–(1''–N, N–diphenylamino) methyl–3''–isatinimino)–5–(4'–chlorophenyl)–6–ethyl–4–aminopyrimidine (PM_2)

The PM_1 (0.003 mol.) step product was dissolved in ethanol (5 mL), 37% Fromaldehyde (1.5 mL). To this mixture, 5 mL of N, N–diphenylamine was added dropwise with rigorous stirring and allowed to stand at room temperature for 1 hour with occasional shaking. It was warmed on a water bath for 20 minutes, cooled and the product was filtered. Recrystalization using chloroform – petroleum ether (1:1) mixture yield : 54.90%; m.p: 131 – 135°C; UV ($CHCl_3$), λ_{max} 273.5 nm; IR (KBr) 2970, 2800 (CH of CH_3), 2860 (CH of CH_2), 1659 (C=O), 1578 (C=N) ^1H NMR ($CDCl_3$) δ ppm: 0.98 (3H, CH_3 of C_2H_5), 2.1 (2H, CH_2 of C_2H_5), 2.2 (10H, s, N $(C_6H_5)_2$; 4.89 (2H, N– CH_2–N) 6.8– 7.2 (m. 6H, Ar– H); Mol formula : C_{33} H_{27} N_6 OCl. The physical constants of the title compounds are represented in Table –1.

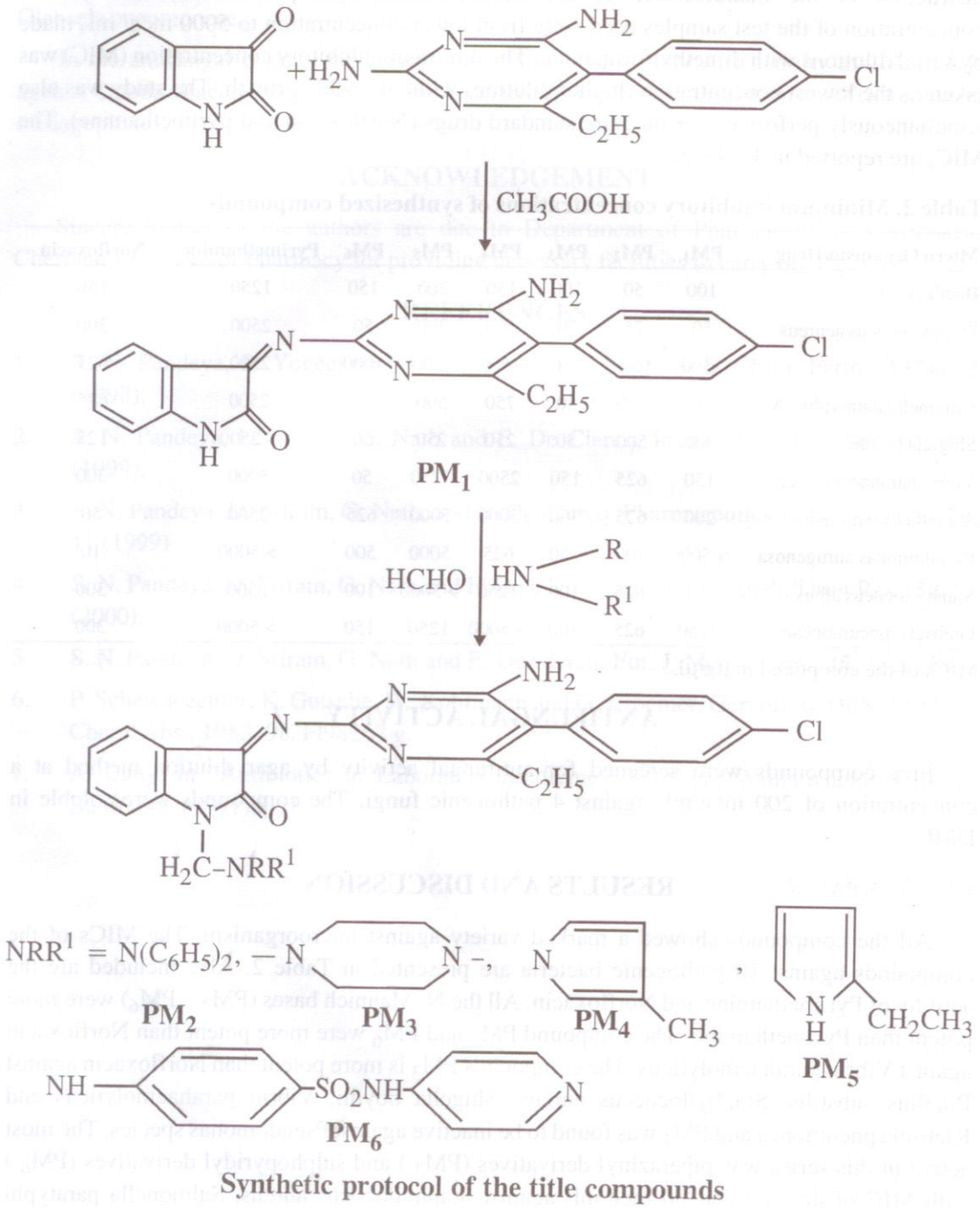
Table 1. Physical constant of the synthesized compounds

Compound	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight	R_f value
PM_1	71	188	C_{20} H_{16} N_5OCl	377	0.77
PM_2	54	133	C_{33} H_{27} N_6OCl	558	0.67
PM_3	61	137	C_{25} H_{25} N_7OCl	474	0.59
PM_4	59	128	C_{26} H_{23} N_6OCl	470	0.59
PM_5	60	127	C_{26} H_{25} N_7OCl	486	0.63
PM_6	52	112	C_{32} H_{27} N_8O_3Cl	606	0.54

*Eluent used in TLC was $CHCl_3$: CH_3OH : Ammonia in 7:2:1 ratio.

ANTIBACTERIAL ACTIVITY

The antibacterial activity was determined by agar dilution method against 10 pathogenic bacteria, procured from microbiological laboratory. The medium was prepared as per the



Synthetic protocol of the title compounds

instruction of the manufacturer of dry Muller–Hinton agar powder (Hi Media). The concentration of the test samples used were from lower concentration to 5000 mcg/ mL made by serial dilutions with dimethylformamide. The minimum inhibitory concentration (MIC) was taken as the lowest concentration (higher dilution) without visible growth. The study was also simultaneously performed for the pure standard drugs (Norfloxacin and pyrimethamine). The MIC_s are reported in Table–2.

Table 2. Minimum inhibitory concentrations of synthesized compounds

Micro Organism/Drug	PM_1	PM_2	PM_3	PM_4	PM_5	PM_6	Pyrimethamine	Norfloxacin
Bacillus subtilis	100	50	100	150	300	150	1250	150
Staphyllococus aeureus	50	75	50	100	300	50	2500	300
E. coli	1500	1000	150	1500	2500	1000	2500	625
Samonella paratyphi - A	50	500	50	750	500	40	2500	625
Shigella boydii	300	500	50	250	250	50	> 2500	125
Vibrio parahaemolyticus	150	625	150	2500	1250	50	5000	300
Shigella dysenteriae	200	625	300	5000	5000	625	2500	50
Pseudomonas aurugenosa	> 5000	100	150	625	5000	500	> 5000	10
Staphyllococus albus	50	625	100	1250	> 5000	100	2500	300
Glebsela. pneumoneae	1250	625	100	> 5000	1250	150	> 5000	300

MICs of the compound in μg/μL.

ANTIFUNGAL ACTIVITY

Five compounds were screened for antifungal activity by agar dilution method at a concentration of 200 mcg/mL against 4 pathogenic fungi. The compounds were soluble in DMF.

RESULTS AND DISCUSSION

All the compounds showed a marked variety against microorganism. The MICs of the compounds against 10 pathogenic bacteria are presented in Table 2. Also included are the activity of Pyrimethamine and Norfloxacin. All the N–Mannich bases (PM_2 – PM_6) were more potent than Pyrimethamine. The compound PM_1 and PM_6 were more potent than Norfloxacin against Vibrio parahaemolyticus. The compounds PM_3 is more potent than Norfloxacin against Bacillus substilis, Staphyllococcus aureus, Shigella boydii, Vibrio parahaemolyticus and Klebsela pneumonea and PM_1 was found to be inactive against Pseudomonas species. The most active in this series was piperazinyl derivatives (PM_3) and sulphopyridyl derivatives (PM_6) with MIC of 40 mcg and 50 mcg/mL against Staphyllococus aureus, Salmonella paratyphi

Shigella boydii, Vibrio parahaemolyticus, Shigella dysentriae, Staphylococcus albus and Glebsela pneumoneae.

In the antifungal testing, the compounds (PM_3 – PM_6) showed activity at 100 µg/µL against Canida albicans, Cryptococcus neoformans, Histoplasma capsulatum and Trichophyton menagophytes.

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