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SYNTHESIS AND ANTIBACTERIAL SCREENING OF NOVEL MANNICH BASES OF 2,4, DIAMINO-6-PIPERIDINO PYRIMIDINE-3-OXIDE (MINOXIDILL)

Purti Bilgaiyan
Department of Chemistry, SAGE University, Indore (M.P.) India

ABSTRACT

2,4, diamino-6-piperidino pyrimidine-3-oxide (minoxidill) with various biologically potent sulphonamides was carried out and then characterized by elemental analysis and spectral studies – UV, IR and ^{1}HNMR. The compounds were screened for their antibacterial activity against various pathogenic bacteria E.coli, S.aureus, B.subtilis, at varying concentrations. The antibacterial activity of derived Mannish bases was compare with parent sulphonamides. The toxicity of synthesized Mannish bases was ascertained by LD_{50} test.

Keywords: *2,4, diamino-6-piperidino pyrimidine-3-oxide (minoxidill), Sulphonamides, Mannich reaction, Mannich bases, Antibacterial activity, LD_{50} test.*

I. INTRODUCTION

Development of efficient and environmentally friendly chemical process for preparation of new biologically active molecules constitutes a major assignment for chemists in organic synthesis[(1)] the amino alkylation of aromatic substrates by the Mannish reaction is of great interest for the synthesis and modification of biologically active compound having physical[1-3] and chemical importance[4] as well as physiological properties[5-8], because the amino group can be easily converted into a variety of other functionalities[9]. Mannich reaction offers a judicious method for introduction of basic amino alkyl chain in various drugs/compounds. Further a considerable amount of work has been reported on synthesis and pharmacological activity of various Mannich bases for analgesic, anti-inflammatory, anesthetic and antimicrobial activity as well as intermediates in drug synthesis[10-13] . 2,4, diamino-6-piperidino pyrimidine-3-oxide (minoxidill) is a drug that was initially developed as an antihypertensive agent by Upjohn Company it is also effective topically as a hair growth stimulant and is indicated for the treatment of alopecia androgenetica[(14)] used as a active hydrogen compound. In addition to this the sulphonamide is well-known antibacterial[15-18], antitubercular[19], anti-inflammatory[20], carbonic inhibitory[21], insecticidal[22].

The Mannich bases incorporated with sulphonamides are reported to be potent antibacterial agents and less toxic than parent sulphonamide[23-26]. Keeping in view the unique features of these compounds 2,4, diamino-6-piperidino pyrimidine-3-oxide (minoxidill) as a substrate and sulphonamide as amine component were condensed via Mannich reaction. A series of Mannich bases were synthesized with different sulphonamides/ secondary amines **(Scheme 1&2)**. The synthesized Mannich bases were characterized by elemental analysis and spectral studies-UV, IR and ^{1}H NMR and screened for *in-vitro* antibacterial activity gram-positive and gram-negative bacteria at arbitrarily chosen concentrations.

II. MATERIAL AND METHODS

All the melting points were determined in open capillary tubes and are uncorrected. Thin layer chromatography was used for monitoring the reaction and to check purity. UV spectra were studied on Schimadzu UV-160A, UV-visible spectrophotometer; IR spectra (KBr) were recorded as potassium bromide pellets on Schimadzu 820 IPC FTIR spectrometer and ^{1}HNMR spectra on Bruker DRX-300 FT NMR Spectrometer and chemical shifts were expressed as (ppm) values against tetramethylsilane (TMS) as internal reference. The chemical reagents used in the synthesis were purchased from E. Merck and Aldrich.

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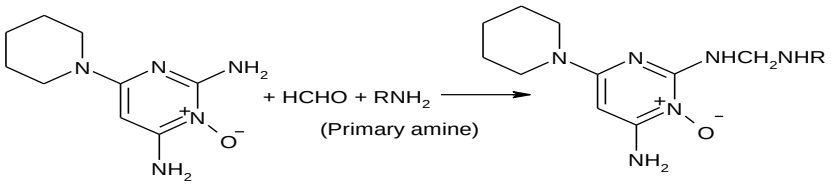
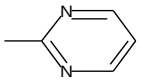
Synthesis of Mannich bases from primary amines (3a-3e)

Synthetic pathway for the synthesis of compounds **3a-3e** is represented in **Scheme-1**. In ethanolic solution of 0.01 mol of Substrate (Comp.-1), 0.01 mol of sulfonamide and 2.5 mL of formaldehyde solution (37% v/v) were added. The mixture was kept in an efficient ice cooling for half an hour and then refluxed on water bath. The refluxed mixture was kept at 0°C for four days when crystalline product was obtained. The obtained product was recrystallized with dry distilled ethanol and DMF (1:1). The Mannich bases (**3a-3e**) were thus obtained in (≥85%) yield.

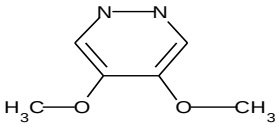


1 2(a-e) 3(a-e)

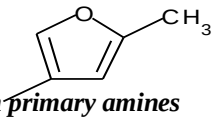
(2a – 3a); R = Sulphadiazine



(2b – 3b); R = Sulphadoxine

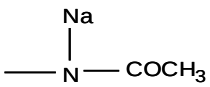


(2c – 3c); R = Sulphamethoxazole

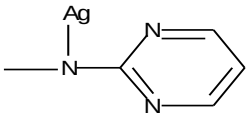


Scheme 1.Synthesis of Mannich bases from primary amines

(2d – 3d); R = Sulfacetamide sodium



(2e – 3e); R = Silver sulphadiazine



Synthesis of Mannich bases from secondary amines (3f-3j)

Synthetic pathway for the synthesis of compounds **3f-3j** is represented in **Scheme-2**. Secondary amine 0.01 mol was added in an ethanolic solution 50 ml of Substrate (Comp. -1) 0.01 mol in a flat bottom flask. Amount of 0.4 ml of formaldehyde solution (37%) was added slowly with constant stirring. The reaction mixture was stirred at 70-75°C for 3.0 to 8.5 hours, depending upon the secondary amine. The remaining portion of formaldehyde solution was added in two installments after 1 and 2 hours, respectively. The reaction mixture was kept overnight in the refrigerator. Next day, the excess of solvent was distilled off from the reaction mixture under reduced pressure. It

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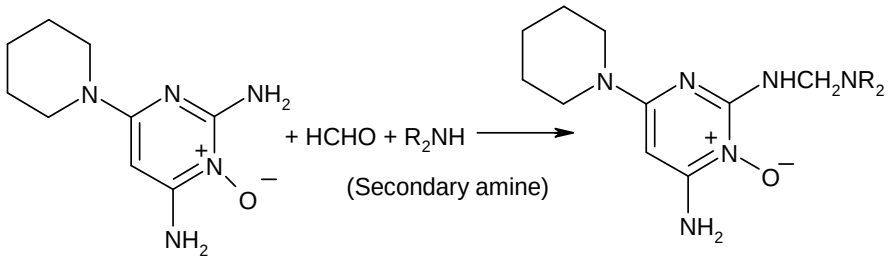
was again kept for crystallization in the refrigerator. The product obtained was purified by recrystallization from dry distilled ethanol and DMF (1:1). The Mannich bases (**3f-3j**) were thus obtained in (≥85%) yield.

Spectral Studies

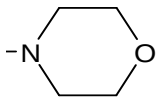
Compound 3a: $C_{20}H_{25}N_9O_3S$; yield 80%, m.p. 200-203°C. Anal.Calcd C, 50.9; H, 5.30; N, 26.7 Found C, 50.5; H, 5.6; N, 27. 1UV (λ max) nm : 190 (C=N), 205 (sulfonamide group), 186, 207, 251 (for benzene chromophore), 254 (sulphonamide moiety). IR (KBr) v max in cm^{-1}: 3442 υ_s N-H, 3398 υ_{as} N-H in SO_2NH, , 2940 υ_{as} CH_2,1345 υ_s S=O, 1130 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H-NMR (DMF) δ ppm: 4.80 (s, 2H, CH_2), 6.36 (S, 1H of sulphonamide), 9.03 (s, 1H of SO_2NH), 6.6 – 7.2 (m, ring proton of sulphonamide), 7.48-8.47 (m, 3H of sulphadiazine ring),5.24(s, NH_2 of pyrimidine ring).



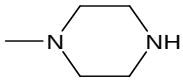
1 **2(f-j)** **3(f-j)**

(2f – 3f); R = **- C_2H_4OH**

(2g – 3g); R = **- CH_3**

(2h – 3h); R  **=**

(2i – 3i); R = **- C_6H_5**

(2j – 3j); R = 

Scheme 2. Synthesis of Mannich bases from secondary amines.

Compound 3b: $C_{22}H_{29}N_9O_5S$; yield 72%, m.p. 158°C. Anal.Calcd. C, 49.2; H, 5.4; N, 23.2; Found C, 49.7; H, 5.5; N, 23.7. UV (λ max) nm: 197 (C=N), 208 S=O, 182, 205, 250 for benzene chromophore; 258 sulphonamide moiety. IR (KBr) v max in cm^{-1} : 3440 υ_s N-H, 3380 υ_{as} N-H in SO_2NH, 2910 υ_{as} C-H in CH_2 ,1380 υ_s S=O, 1135 C-H in plane bending vibration of 1:4 disubstituted benzene; ^{1}H NMR (DMF) δ ppm: 3.43 (s, 3H of OCH_3) 4.80 (s, 2H, CH_2), 6.4 (s, 1H of sulphonamide), 9.2 (s, 1H of SO_2NH), 6.6 – 7.07 (m, ring proton of sulphonamide),5.67 (s, NH_2 of pyrimidine ring). .

Compound 3c: $C_{20}H_{26}N_8O_4S$; yield 84%, m.p. 220-222°C, Anal. Calcd. C, 50.5; H, 5.5; N, 23.4; Found C, 50.6; H, 5.5; N, 23.6. UV (λ max) nm: 192 (C=N), 208 (S=O), 186, 207, 250 for benzene chromophore; 255 (sulphonamide moiety). IR (KBr) v max in cm^{-1} : 3475 υ_s N-H, 3350 υ_{as} N-H in SO_2NH, 2915 υ_{as} C-H in CH_2, 1349 υ_s S=O, 1110

C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H NMR (DMF) δ ppm: 2.71(s, 3H of CH_3) 5.04 (s, 2H, CH_2), 6.3 (s, 1H o sulphonamide) 9.2 (s, 1H of SO_2NH), 5.67 (s, NH_2 of pyrimidine ring).

Compound 3d: $C_{18}H_{24}N_4O_4NaS$; yield 78%, m.p. 180-183^{o}C, Anal. Calcd. C, 47.9; H, .54; N, 21.3 Found C, 47.2; H, 5.3; N, 21.4 UV (λ max) nm: 190 (C=N), 205 S=O, 186, 207, 251 for benzene chromophore; 254 sulphonamide moiety. IR (KBr) v max in cm^{-1} : 3482 υ_s N-H, 3351 υ_{as} N-H in SO_2NH, 2950 υ_{as} C-H in CH_2, 1385 υ_s S=O, 1109 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H NMR (DMF) δ ppm: 2.16 (s, 3H acetamide CH_3) 4.80 (s, 2H, CH_2), 6.3 (s, 1H, NH of sulphonamide), 9.42 (s, 1H of SO_2NH), 5.94 (s, NH_2 of pyrimidine ring).

Compound 3e: $C_{20}H_{24}N_9O_3SAg$; yield 85%, m.p. -200-202^{o}C. Anal.Calcd.C, 41.8.46; H, 4.2; N, 21.2 Found C, 41.5; H, 4.20; N, 21.8. UV (λ max) nm: 194 (C=N), 208 S=O, 180, 203, 250 for benzene chromophore; 257 sulphonamide moiety. IR (KBr) v max in cm^{-1}: 3496 υ_s N-H, 3348 υ_{as} N-H in SO_2NH, 2952 υ_{as} C-H in CH_2, 1355 υ_s S=O, 1100 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H NMR (DMF) δ ppm: 5.04 (s, 2H, CH_2), 6.1 (s, 1H, NH of sulphonamide), 9.42 (s, 1H of SO_2NH), 5.94 (s, NH_2 of pyrimidine ring).
Compound 3f: $C_{14}H_{26}N_6O_3$; yield 80%, m.p. 100-102^{o}C. Anal.Calcd.C, 51.4; H, 8.3; N, 25.2 Found C, 51.5; H, 8.0; N, 25.7. UV (λ max) nm: 191 (C=N), 185 205, 250 for benzene chromophore. IR (KBr) v max in cm^{-1} : 3482 υ_s N-H, 2944 υ_{as} C-H in CH_2,. ^{1}H NMR (DMF) δ ppm: 4.51 (s, 2H,CH2), 5.45 (s, NH_2 of pyrimidine ring).

Compound 3g: $C_{12}H_{22}N_6O$; yield 71%, m.p. 110-113^{o}C, Anal. Calcd.C, 28.1; H, 4.1; N, 16.1Found C, 28.1; H, 4.1; N, 16.1. UV (λ max) nm: 195 (C=N), 185, 205, and 255 for benzene chromophore. IR (KBr) v max in cm^{-1}: 3412 υ_s N-H, 2909 υ_{as} C-H in CH_2,.^{1}H NMR (DMF) δ ppm: 4.48 (s, 2H, CH_2), 5.45 (s, NH_2 of pyrimidine ring).

Compound 3h: $C_{14}H_{24}N_6O_2$; yield 79%, m.p.70-72^{o}C, Anal. Calcd. C, 54.0; H, 7.6; N, 27.0 Found C, 54.5; H,7.8; N, 27.2. UV (λ max) nm: 190 (C=N), 184, 209, and 254 for benzene chromophore. IR (KBr) v max in cm^{-1}: 3477 υ_s N-H, 2947 υ_{as} C-H in CH_2, 1244 υ_s C-O. ^{1}H NMR (DMF) δ ppm: 4.4 (s, 2H, CH_2), 5.65 (s, NH_2 of pyrimidine ring).

Compound 3i:$C_{22}H_{26}N_6O_4$ yield 82%, m.p. 155-157^{o}C, Anal. Calcd.C, 67.5; H, 6.5; N, 21.2 Found C, 67.6; H, 6.72; N, 21.5. UV (λ max) nm: 190 (C=N), 184, 206, and 260 for benzene chromophore. IR (KBr) v max in cm^{-1}: 3406 υ_s N-H, 2932 υ_{as} C-H in CH_2, 1643 υ_s C=O. ^{1}H NMR (DMF) δ ppm: 4.5 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 5.41 (s, NH_2 of pyrimidine ring).

Compound 3j: $C_{14}H_{25}N_7O$; yield 80%, m.p. 230-232^{o}C. Anal.Calcd C, 54.1; H, 8.1; N, 32.1Found C, 54.7; H, 8.2; N, 31.9. UV (λ max) nm: 190 (C=N), 184, 206, and 260 for benzene chromophore. IR (KBr) v max in cm^{-1}: 3458 υ_s N-H, 3342 υ_s O-H, 2937 υ_{as} C-H in CH_2, 1639 υ_s C=O. ^{1}H NMR (DMF) δ ppm: 4.5 (s, 2H, CH_2), 5.45 (s, NH_2 of pyrimidine ring).

LD_{50} Test
The toxicity of synthesized Mannich bases was ascertained by LD_{50} test. The test performed on white mice weighing 25g. Doses were given orally as well as intraperitoneally and mice were kept under observation for 72 h for each trial. The Mannich bases showed no adverse toxic effect even of an oral dose of 1400mg/kg. However when dose was administered intraperitoneally they proved to be lethal at the dose level of 800mg/kg.

III. RESULTS AND DISCUSSION

The Mannich bases synthesized by Mannish reaction were obtained in good yield. They were analyzed for elemental analysis and results were found to be in full agreement with the calculated values. The anticipated structure was in agreement with the spectral data– UV, IR and ^{1}HNMR. The spectral studies have shown characteristic band due to methylene group incorporated between active hydrogen substrate and the amine component as a result of Mannich reaction at (2940-2950) and (1442-1450). This shows the presence of amino methyl linkage in the synthesized Mannich bases. The ^{1}H NMR also confirms amino methyl linkage (-CH_2) between amine and active hydrogen (5.04-

5.20). The Mannich bases were screened for their biological significance. They were evaluated for antibacterial activity against pathogenic strains of *E.coli, S.aureus* and *B.subtilis* at varying concentrations–80, 160 and 320 µg/ml. All the reported compounds exhibit remarkable *in vitro* activity against these pathogens. Their activity was also compared with their parent sulphonamides. Table-1 reflects that most of the compounds had shown remarkable activity only at 320 µg/ml. compound 3a and 3h shows remarkable activity in case of E.Coli. on comparison 3e shows similar activity to the corresponding sulphonamide . Compound 3c, 3d & 3e are shows superior activity than corresponding sulphonamide against S.aureus . In reference to B.subtilis only 3e shows supieror activity A comparative study between Mannich bases and sulphonamides shows that, Mannich bases are potent antibacterial agents having less toxicity than their parent sulphonamide, as revealed by LD_{50} test on white mice of weight 25gm.

Table – 1 : Antibacterial Screening of Mannich Bases 3a – 3j

Compound No.	E.Coli Conc. in µg/ml			S.aureus Conc. in µg/ml			B.Subtilis Conc. in µg/ml		
	80	160	320	80	160	320	80	160	320
3a	7.6	11.6	16.0	-	-	-	8.0	8.6	10.3
3b	7.3	8.0	10.3	7.3	8.0	8.6	7.6	8.3	10.3
3c	9.0	11.6	13.6	14.3	20.6	22.3	12.0	18.3	22.3
3d	-	-	-	9.6	12.3	15.3	-	-	-
3e	9.0	11.6	15.0	7.6	11.0	13.6	9.3	12.3	15
3f	-	9.6	13.6	-	8.0	12.0	8.0	9.6	12.3
3g	-	9.6	13.6	-	8.6	13.0	-	-	8.6
3h	14.6	17.6	19.0	7.6	11.0	17.3	7.0	8.6	12.0
3i	-	-	11.6	-	9.3	12.6	7.3	9.0	10.3
3j	-	-	11.0	-	-	12.6	-	-	-
2a	12.0	16.6	20.0	15.6	18.6	21.3	17.0	19.6	27.6
2b	14.6	17.0	19.0	15.3	19.0	20.0	17.3	21.6	27.3
2c	14.0	16.3	20.0	10.6	16.0	19.3	15.6	18.0	19.6
2d	9.6	11.6	17.6	9.0	11.0	14.6	9.6	17.6	21.6
2e	9.6	10.3	16.0	7.3	8.3	11.0	8.6	10.3	12.6

IV. CONCLUSION

The results show that the Mannich bases of 2,4, diamino-6-piperidino pyrimidine-3-oxide possess antibacterial activity. Moreover, these derived Mannich bases are less toxic in comparison with their parent sulphonamides. This work shows that Mannich bases are a potential source of new compounds for inhibition of bacteria infections.

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