SYNTHESIS AND *IN*-VITRO STUDY OF SOME MEDICINALLY IMPORTANT MANNICH BASES DERIVED FROM 2-AMINO-9 [{(1, 3 DIHYDROXY PROPANE-2YL) OXY} METHYL] 6-9 DIHYDRO-3H-PURIN-6-ONE

SHEELA JOSHI[A], PURTI BILGAIYAN[A] AND ANJU PATHAK[A]*

[a] *School of Chemical Sciences, D.A.V.V., Indore 452001,M.P., India*
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ABSTRACT

A series of biologically active Mannich bases with heteroaromatic ring system have been synthesized employing Mannich reaction of 2-amino-9 [{(1,3 dihydroxy propane-2yl) oxy}methyl] 6-9 dihydro-3H-purin-6-one (potent drug) with biologically active sulphonamides and secondary amines. They were analyzed by elemental analysis and characterized by spectral studies- UV, IR, ^{1}H NMR, Powder X-ray diffraction and Scanning Electron Microscopy. The Mannich bases were screened for their antibacterial activity against various gram + and gram - bacteria. The results had shown that the Mannich bases are quiet active against pathogens under study at varying concentrations. The toxicity of synthesized Mannich bases was ascertained by LD_{50} test.

Key words: 2-amino-9 [{(1,3 di hydroxy propane-2yl) oxy} methyl] 6-9 dihydro-3H-purin-6-one (Ganciclovir), Sulphonamides, Mannich reaction, Mannich bases, Antibacterial activity , LD_{50} test.

1. INTRODUCTION

Heterocyclic compounds are of immense biological and industrial importance, Majority of pharmaceuticals are heterocyclic[1]. 2-amino-9 [{(1,3 di hydroxy propane-2yl) oxy} methyl] 6-9 dihydro-3H-purin-6-one, an antiviral agent derived from guanine (heterocyclic compound) are used in the prevention and treatment of opportunistic cytomegalovirus(CMV) infection in HIV infected patients and inhibits replication of herpes virus in-vitro. It helps in keeping CMV infection under control[2]. The sulphonamide nucleus has well known pharmacological properties: antibacterial[3], anticancer[4], anti-inflammatory[5], carbonic inhibitory[6], insecticidal[7]. Even N-morpholino, piperidino, dimethylamino, diethyl amino Mannich bases show local anesthetic[8] and hydrophilic property[9].

These considerations provoked us to synthesize Mannich bases of 2-amino-9 [{(1,3 di hydroxy propane-2yl) oxy} methyl] 6-9 dihydro-3H-purin-6-one using sulphonamides and secondary amines by Mannich reaction, which may have biological significance. This reaction offers a convenient route to introduce amino methyl chain, which alters the biological profile and physico-chemical characteristics of the drug[10-12].

Various drugs obtained from Mannich reaction have proved to be less toxic and more effective than their parent drug[13]. The versatile utility of Mannich bases in polymers[14], dispersants[15] and pharmaceutical chemistry[16] prompted us to prepare aminomethyl derivatives and evaluate their biological significance. Here we reports antibacterial activity of Mannich bases of 2-amino-9 [{(1,3 di hydroxyl propane-2yl) oxy} methyl] 6-9 dihydro-3H-purin-6-one including sulphonamide and secondary amines and their comparative study (Table 1).

In the present work, Mannich bases of 2-amino-9 [{(1, 3 di hydroxy propane-2yl) oxy} methyl] 6-9 dihydro-3H-purin-6-one were synthesized by condensation of different primary/secondary amines with formaldehyde. The substrate first reacts with formaldehyde to form an intermediate, which then react with sulphonamides/secondary amines in the presence of acid. The synthesized Mannich bases contains amino methyl group (as justified from the spectral data), which changes the physiochemical characteristic of the drugs and makes it less toxic[17-18]. The synthesized Mannich bases were analyzed for elemental analysis and characterized by spectral studies- UV, IR, ^{1}H NMR, Powder X-ray diffraction and Scanning Electron Microscopy.

All the synthesized compounds were evaluated for antibacterial activity against the pathogenic strains of *S.typhi, K.pneumoniae, and P.aeruginosa* at varying concentrations 80, 160, 320 mg/ml. All the Mannich bases had shown significant activity.

2. EXPERIMENTAL

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 XRD measurements were carried out on Bruker D8 Advance X-ray diffractometer using CuKα at a wavelength of 1.54 A°. SEM studies were performed with a Jeol JSM 5600 instrument having magnification range × 18 to × 300,000 and at an accelerating voltage of 0.5 to 30kV.The chemical reagents used in the synthesis were purchased from E. Merck and Aldrich.

2.1 Synthesis

2.1.1. 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 0ºC 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.

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

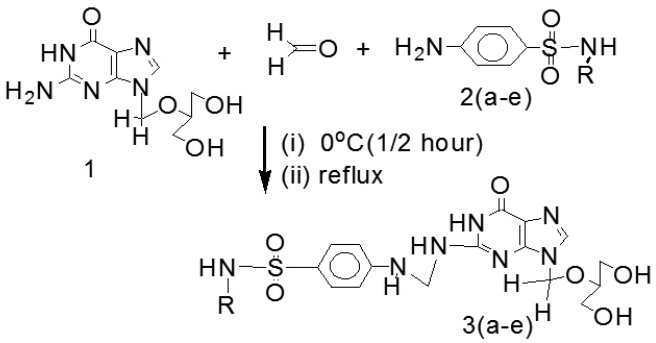
Synthetic pathway for the synthesis of compounds **3f-3j** is represented in **Scheme-2**. Secondary amine 0.01 mol was added in an ethanolic solution 50 mL of Substrate (Comp. -1) 0.01 mol in a flat bottom flask. Amount of 0.4 mL of formaldehyde solution (37%) was added slowly with constant stirring. The reaction mixture was stirred at 70-75ºC 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 over night in the refrigerator. Next day, the excess of solvent was distilled off from the reaction mixture under reduced pressure. It 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.

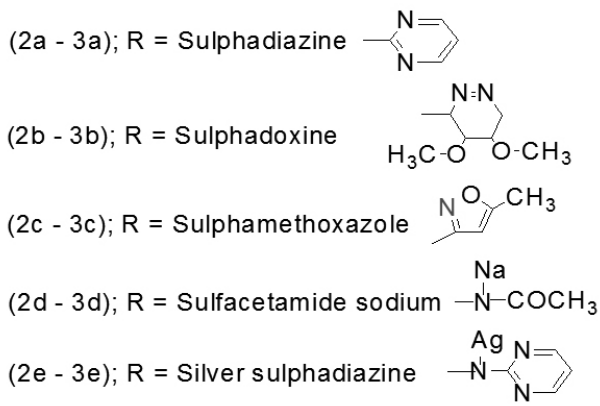
2.2 Spectral Studies

Compound 3a: $C_{20}H_{23}N_9O_6S$, m.p. 160-163ºC.Anal. Calcd C,46.40; H,4.58; N,24.36 Found C,46.16;H,4.42; N,24.23. UV (λ max) nm: 208 (C=O), 190 (C=N), 205 (sulfonamide group), 186, 207, 251 (for benzene chromophore), 254 (sulphonamide moiety). IR (KBr) ν max in cm^{-1}: 3442 u_s N-H, 3398 u_{as} N-H in SO_2NH, 3302 u_s O-H, 2940 u_{as} CH_2, 1688 u_s C=O, 1345 u_s S=O, 1130 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H-NMR (DMSO) δ ppm: 5.04 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine

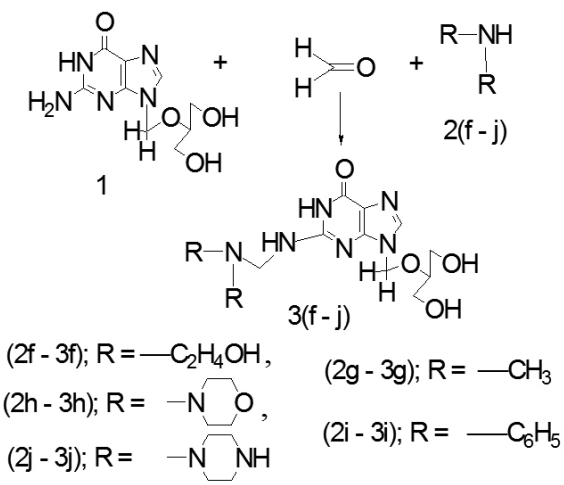
e-mail- sheelajoshi2010@gmail.com

ring), 6.36 (S, 1H, NH of sulphonamide), 7.93 (s, 1H, CH of purine ring), 7.84 (s, 1H, free OH of Comp.1), 3.72(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring), 9.03 (s, 1H of SO_2NH), 6.6 – 7.2 (m, ring proton of sulphonamide,





Scheme 1. Synthesis of Mannich bases from primary amines.



Scheme 2. Synthesis of Mannich bases from secondary amines.

Compound 3b: $C_{20}H_{22}N_9O_8S$, m.p. 105-107°C. Anal. Calcd. C, 45.74; H, 4.71; N, 21.82; Found C, 45.61; H, 4.46; N, 21.64. UV (λ max) nm: 210 (C=O), 197 (C=N), 208 S=O, 182, 205, 250 for benzene chromophore; 258 sulphonamide moiety. IR (KBr) ν max in cm^{-1} : 3440 u_s N-H, 3380 u_{as} N-H in SO_2NH, 3302 u_s O-H, 2910 u_{as} C-H in CH_2, 1653 u_s C=O, 1380 u_s S=O, 1135 C-H in plane bending vibration of 1:4 disubstituted benzene; ^{1}H NMR (DMSO) δ ppm: 3.43 (s, 3H of OCH_3) 5.04 (s,2H,CH_2), 5.5 (s,2H,CH_2 attached to purine ring), 6.4 (s, 1H, NH of sulphonamide), 7.9 (s, 1H, CH of purine ring), 7.4 (s, 1H, free OH of Comp.1), 3.74(2CH_2 attached with purine ring), 8.66 (N^1H of purine ring) 9.2 (s, 1H of SO_2NH), 6.6 – 7.07 (m, ring proton of sulphonamide).

Compound 3c: $C_{20}H_{24}N_8O_7S$; m.p.180-181°C, Anal. Calcd. C, 46.13; H, 4.65; N, 21.53; Found C, 46.13; H, 4.25; N, 21.33. UV (λ max) nm: 201 (C=O), 192 (C=N), 208 (S=O), 186, 207, 250 for benzene chromophore; 255(sulphonamide moiety). IR (KBr) ν max in cm^{-1} : 3475 u_s N-H, 3350 u_{as} N-H in SO_2NH, 3318 u_s O-H, 2915 u_{as} C-H in CH_2, 1685 u_s C=O, 1349 u_s S=O, 1110 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H NMR (DMSO) δ ppm: 2.71(s, 3H of CH_3)5.04 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 6.3 (s, 1H, NH of sulphonamide), 7.93 (s, 1H, CH of purine ring), 7.84 (s, 1H, free OH of Comp.1), 3.73(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring), 9.03 (s, 1H of SO_2NH), 6.6 – 7.2 (m, ring proton of sulphonamide,

Compound 3d: $C_{18}H_{22}N_7O_7NaS$; m.p. 90°C, Anal. Calcd. C, 42.93; H, 4.41; N, 19.48 Found C, 42.52; H, 4.21; N, 19.22. UV (λ max) nm: 208 (C=O), 190 (C=N), 205 S=O, 186, 207, 251 for benzene chromophore; 254 sulphonamide moiety. IR (KBr) ν max in cm^{-1} : 3482 u_s N-H, 3351 u_{as} N-H in SO_2NH, 3295 u_s O-H, 2950 u_{as} C-H in CH_2, 1647 u_s C=O, 1385 u_s S=O, 1109 disubstituted benzene. ^{1}H NMR (DMSO) δ ppm: 2.16 (s, 3H acetamide CH_3) 5.04 (s, 2H, CH_2), 5.5 (s,2H,CH_2 attached to purine ring), 6.3 (s, 1H, NH of sulphonamide), 7.9 (s, 1H, CH of purine ring), 6.7 (s, 1H, OH of Comp.1), 3.72(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring) 9.42 (s, 1H of SO_2NH), 6.6 – 7.07 (m, ring proton of sulphonamide).

Compound 3e: $C_{20}H_{22}N_9O_6SAg$: m.p. 222°C. Anal. Calcd. C, 38.46; H, 3.55; N, 20.19 Found C, 38.32; H, 3.51; N, 20.24. UV (λ max) nm: 203 (C=O), 194 (C=N), 208 S=O, 180, 203, 250 for benzene chromophore; 257 sulphonamide moiety. IR (KBr) ν max in cm^{-1} : 3496 u_s N-H, 3348 u_{as} N-H in SO_2NH, 3300 u_s O-H, 2952 u_{as} C-H in CH_2, 1638 u_s C=O, 1355 u_s S=O, 1100 C-H in plane bending vibration of 1:4 disubstituted benzene. ^{1}H NMR (DMSO) δ ppm: 5.04 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 6.1 (s, 1H, NH of sulphonamide), 7.9 (s, 1H, CH of purine ring), 6.72 (s, 1H, OH of Comp.1), 3.70(2CH_2 attached with purine ring), 8.66 (N^1H of purine ring), 9.03 (s, 1H of SO_2NH) 6.6 – 7.2 (m, ring proton of sulphonamide).

Compound 3f: $C_{14}H_{24}N_6O_6$: m.p. 160-162°C. Anal. Calcd. C, 45.14; H, 6.49; N, 22.56 Found C, 45.13; H, 6.51; N, 22.50. UV (λ max) nm: 208 (C=O), 191 (C=N), 185 205, 250 for benzene chromophore. IR (KBr) ν max in cm^{-1} : 3482 u_s N-H, 3352 u_s O-H, 2944 u_{as} C-H in CH_2, 1680 u_s C=O. ^{1}H NMR (DMSO) δ ppm : 4.51 (s,2H,CH_2), 5.60 (s,2H,CH_2 attached to purine ring), 7.9 (s, 1H, CH of purine ring), 4.92 (s, 1H, OH of Comp.1), 3.72(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring) .

Compound 3g : $C_{12}H_{20}N_6O_4$: m.p. 225-227°C, Anal. Calcd. C, 46.13; H, 6.46; N, 26.90 Found C, 46.4; H, 6.51; N, 26.82. UV (λ max) nm: 210 (C=O), 195 (C=N), 185, 205, 255 for benzene chromophore. IR (KBr) ν max in cm^{-1}: 3412 u_s N-H, 3310 u_s O-H, 2909 u_{as} C-H in CH_2, 1655 u_s C=O. ^{1}H NMR (DMSO) δ ppm: 2.23(s, 3H of methyl group), 4.48 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 7.9 (s, 1H, CH of purine ring), 6.12 (s, 1H, OH of Comp.1) 3.72(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring) .

Compound 3h : $C_{14}H_{22}N_6O_5$; m.p. 215-218°C., Anal. Calcd. C, 47.43; H, 6.26; N, 23.71 Found C, 47.32; H, 6.30; N, 23.52. UV (λ max) nm: 204 (C=O), 190 (C=N), 184, 209, 254 for benzene chromophore. IR (KBr) ν max in cm^{-1}: 3477 u_s N-H, 3361 u_s O-H, 2947 u_{as} C-H in CH_2, 1630 u_s C=O, 1244 u_s C-O. ^{1}H NMR (DMSO) δ ppm: 4.4 (s, 2H, CH_2), 5.5 (s,2H,CH_2 attached to purine ring), 7.93 (s, 1H, NH of purine ring), 6.21 (s, 1H, OH of Comp.1) 3.72(2CH_2 attached with purine ring), 8.65 (N^1H of purine ring), .

Compound 3i : $C_{22}H_{24}N_6O_4$: m.p. 235-236°C, Anal. Calcd. C, 60.52; H, 5.55; N, 19.25 Found C, 60.61; H, 5.32; N, 19.10. UV (λ max) nm: 209 (C=O), 190 (C=N), 184, 206, 260 for benzene chromophore. IR (KBr) ν max in cm^{-1}: 3406 u_s N-H, 3318 u_s O-H, 2932 u_{as} C-H in CH_2, 1643 u_s C=O. ^{1}H NMR (DMSO) δ ppm: 4.5 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 7.9 (s, 1H, CH of purine ring), 6.17 (s, 1H, OH of Comp.1), 3.72(2CH_2 attached with purine ring), 8.60 (N^1H of purine ring).

Compound 3j : $C_{14}H_{23}N_7O_4$: m.p. 220°C. Anal. Calcd C, 47.57; H, 6.56; N, 27.74 Found C, 47.80; H, 6.32; N, 27.62. UV (λ max) nm: 205 (C=O), 190 (C=N), 184, 206, 260 for benzene chromophore. IR (KBr) ν max in cm^{-1}: 3458 u_s N-H, 3342 u_s O-H, 2937 u_{as} C-H in CH_2, 1639 u_s C=O. ^{1}H NMR (DMSO) δ ppm: 4.5 (s, 2H, CH_2), 5.5 (s, 2H, CH_2 attached to purine ring), 7.9 (s, 1H, CH of purine ring), 5.5 (s, 1H, OH of Comp.1) 3.72(2CH_2 attached with purine ring), 8.67 (N^1H of purine ring),.

Powder X-ray diffraction studies

Powder X-ray diffraction patterns of three of the synthesized compounds namely ganciclovir methyl silver sulphadiazine (3e), ganciclovir methyl diphenylamine (3i) and ganciclovir methyl Piperazine (3j) (Fig 1,2 and 3 respectively) have been reported and important structural information have been shown in table 1,2,and 3 respectively.

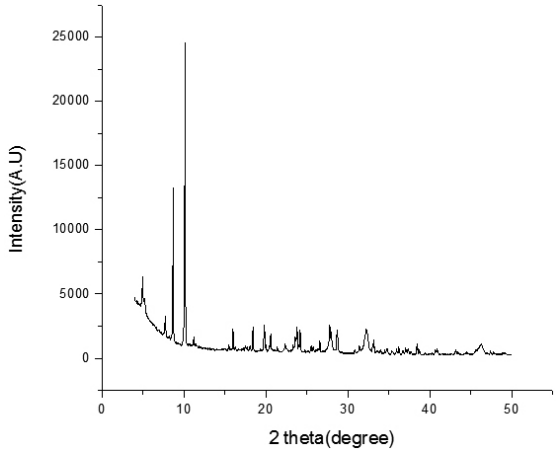


Figure 1. Powder X-ray diffraction pattern of Ganciclovir methyl Silver Sulphadiazine (3e).

Average particle size of the synthesized compound was determined with the help of the Scherrer formula, in which particle size D is defined as $0.9\lambda / B \cos\theta$,

Where 0.9 = constant, λ = wavelength, B = angular width

And θ = diffraction angle. Average particle size of the compound determined were 94.18, 32.03 and 64.13 nm respectively. Diffraction data of the compounds are listed in table 1,2 and 3. All of our synthesized compounds had a

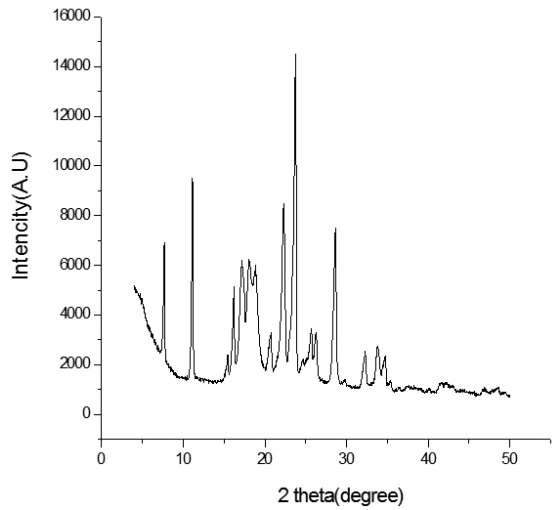


Figure 2. Powder X- Ray diffraction pattern of Ganciclovir methyl diphenylamine (3i).

Orthorhombic crystal system and XRD patterns showed that all are polycrystalline in nature. The largest relative deviation between the calculated and experimental d value was nearly equal to one, which indicates that all the synthesized compounds are multiphase compounds. Interplaner d spacing and unit cell volume of the synthesized compound were calculated by the formulae:

$$1/d^2 = h^2/a^2 + k^2/b^2 + l^2/c^2$$

$$V = abc$$

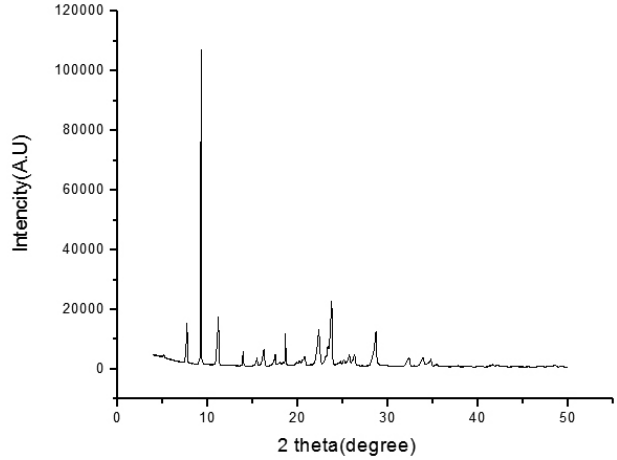


Figure 3. Powder X-ray diffraction pattern of Ganciclovir methyl Piperazine (3j).

Table1 . The experimental data and the calculated results for powder X-ray pattern of the ganciclovir methyl Silver Suldhadizine(3e).

2θ	d spacing(A°) Observed	d spacing(A°) Calculated	Relative intensity (%)	h k l
19.79	4.48	4.46	98.96	210
20.58	4.31	4.14	73.17	023
22.37	3.96	4.00	43.61	220
24.19	3.67	3.67	84.83	004
25.53	3.48	3.48	37.33	141
26.59	3.34	3.04	50.69	043
27.79	3.20	3.32	100	133
29.17	3.05	3.31	19.97	114
30.81	2.89	2.73	22.32	152
31.98	2.79	2.85	46.99	134
33.09	2.70	2.46	55.81	134
34.50	2.59	2.59	25.63	060
35.94	2.49	2.40	27.90	045
37.08	2.42	2.42	32.71	340
38.43	2.33	2.33	42.30	400
39.45	2.28	2.26	15.31	260
40.89	2.20	2.36	29.36	106
43.17	2.09	2.46	24.98	161
45.99	1.97	1.98	31.87	360
47.78	1.90	2.20	19.51	421
48.89	1.86	2.13	15.74	171
49.21	1.84	1.81	17.32	520

Lattice parameters and angle calculated are: a = 9.32 A° or 0.93nm, b = 15.54 A° or 1.55nm, C = 14.68 A°or 1.46nm, β = 94.18°. Unit cell volume of the complex: $2{,}126.14\times10^{-8}$ cm

Peaks having important characteristics information have been identified with the help of standard diffraction card JCPDS 31-1859, 38-1709, 49-2499 and 35-1687 and indicate formation of nanosized, polycrystalline, two phase and low symmetry compounds.

Table2. The experimental data and the calculated results for powder X-ray pattern of the ganciclovir methyl biphenyl amine(3i).

2θ	d spacing (A°) Observed	d spacing (A°) Calculated	Relative intensity (%)	h k l
10.97	8.05	8.00	27.38	110
14.87	5.94	5.94	10.07	120
20.76	4.27	4.24	22.82	102
21.99	4.03	4.00	31.25	220
23.75	3.74	4.20	100	201
24.74	3.59	3.56	15.36	140
27.97	3.18	3.18	10.12	003
29.05	3.06	3.34	10.54	202
30.46	2.14	3.01	8.14	103
32.30	2.76	3.28	17.48	212
36.46	2.46	2.61	7.52	302
37.61	2.38	2.96	7.94	301
38.40	2.34	2.34	7.63	400
40.04	2.24	2.25	7.28	260
44.00	2.05	2.40	7.03	161
45.02	2.01	1.98	6.48	360
46.81	1.93	1.93	7.01	080
47.04	1.92	2.18	6.98	421
48.54	1.87	2.10	7.49	171
49.15	1.85	1.81	6.18	520

Lattice parameters and angle calculated are: a = 9.36 A° or 0.93nm, b = 15.44 A° or 1.54nm, C = 9.54A°or 0.954nm, β = 32.03°.Unit cell volume of the complex: $1{,}378.70\times10^{-8}$ cm.

Table3. The experimental data and the calculated results for powder X-ray pattern of the ganciclovir methyl Piperazine(3j).

2θ	d spacing(A°) Observed	d spacing(A°) Calculated	Relative intensity (%)	h k l
19.85	4.53	4.53	10.43	200
22.25	4.06	4.12	46.49	113
23.78	3.81	3.81	100	040
25.71	3.54	3.51	21.29	140
27.97	3.28	3.38	6.57	042
28.67	3.20	3.27	55.90	133
30.08	3.07	3.51	4.90	140
31.98	2.90	2.80	7.92	320
34.76	2.70	2.72	14.79	025
39.16	2.43	2.43	4.59	006
40.16	2.38	2.95	4.99	301
41.68	2.31	2.83	6.86	151
43.06	2.25	2.21	4.76	260
44.26	2.20	2.52	4.55	144
46.81	2.11	2.14	4.96	206
47.89	2.07	2.41	6.18	161
49.39	2.02	1.94	4.30	360

Lattice parameters and angle calculated are: a = 9.06 A° or 0.90 nm, b = 15.24 A° or 1.52nm, C = 14.58 A°or 1.45nm, β = 64.13°.Unit cell volume of the complex: $2{,}013.12\times10^{-8}$ cm.

Scanning Electron Microscopy (SEM) Studies

Scanning Electron Microscopy Studies of two of the synthesized compound namely ganciclovir methyl silver sulphadiazine (3e), and ganciclovir methyl Piperazine (3j) have been carried out at a magnification of ×1,000 - 10 µm and × 600 - 2µm. SEM uses a focused beam of high energy electrons to generate a variety of signals from the surface of a signals reveal information about the sample, including external morphology, topography, chemical composition crystalline structure, and orientation of materials making up the sample.

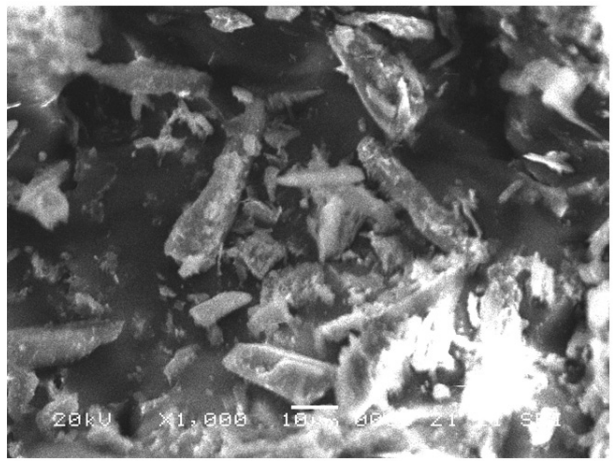


Figure 4

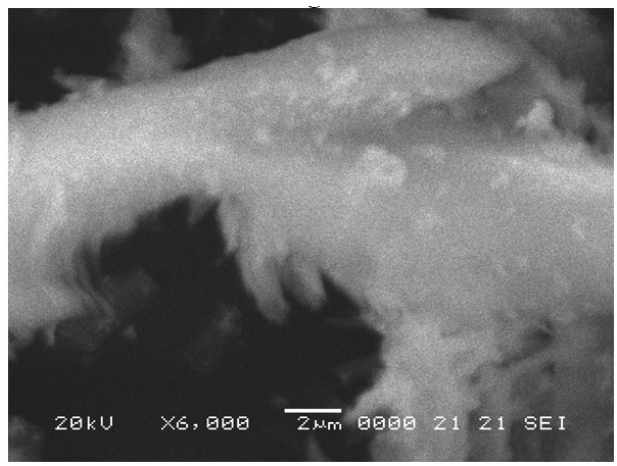


Figure 5

We recorded different magnifications, as described above. Compound ganciclover methyl silver sulphadiazine (3e) (Fig 4 and 5) has a different topography and morphology at the two different magnifications. In the images, the particles exhibit different sizes and shapes, and are present in the form of closed polygonal structure hand, the compound ganciclover methyl Piperazine (3j) (Fig 6 and 7) shows at different magnifications a finer morphology as compared to the other compound, appears in form of simple polygonal structures.

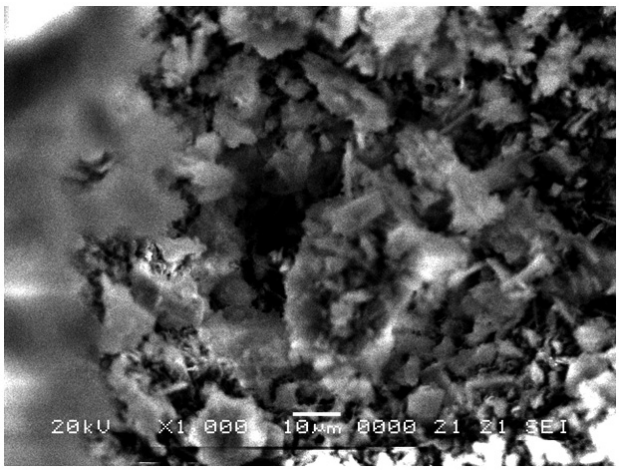


Figure 6

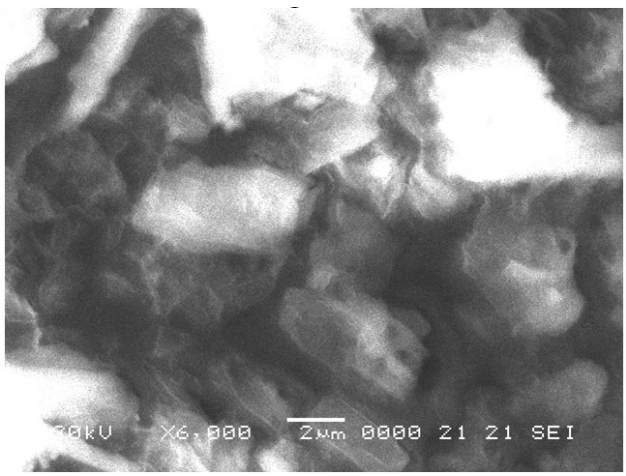


Figure 7

SEM studies show microstructure of the compounds, which mainly include surface morphology.

2.3. Antimicrobial Activity

The study was performed by the cup plate method[19] on pathogenic strains of *S.typhi, P.aeruginosa and K.pneumoniae*. The media used for antibacterial screening were from hi-media. The media were prepared in triple distilled water and autoclaved at 15 lb pressure. The culture of bacterium was mixed with autoclaved media, poured in plates, and bored. The solution of Mannich bases were poured in these cups in triplicate and incubated at 37°c for 24 hours. The Mannich bases (3a-3j) were studied for their antibacterial property at concentration of 80, 160, 320 mg/mL.

2.4 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 hr for each trial. The Mannich bases showed no adverse toxic effect even of an oral dose of 1400mg/kg of the body weight of mice. However when dose was administered intraperitoneally they proved be lethal at the does level of 800mg/kg of the body weight of mice.

3. RESULTS AND DISCUSSION

The Mannich bases synthesized by Mannich reaction were obtained in good yield (≥85%). 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 cm-^{1} and 1442-1450 cm-^{1}. This shows the presence of amino methyl linkage in the synthesized Mannich bases. The ^{1}H NMR also confirms aminomethyl linkage (-CH_2) between amine and active hydrogen (5.04-5.20 ppm). Some of the synthesized compounds are of nano size. The use of nano particle in medicine offers some exciting possibilities. Some techniques are only imagined, while others are at various stage of testing, or actually being used today.The Mannich bases were screened for their biological significance. They were evaluated for antibacterial activity against pathogenic strains of *S.typhi, P.aeruginosa and K.pneumoniae*. at varying concentrations–80mg/ml, 160mg/ml and 320 mg/ml. All the reported compounds exhibit remarkable *in vitro* activity against these pathogens. Their activity was also compared with their parent sulphonamides.

Table 4 Antibacterial screening of synthesized Mannich Bases and sulphonamides against *S.typhi*, *K.pneumoniae* and *P.aeruginosa* (Zone of inhibition in mm*)*.

Comp. No	***S.typhi*** (Concn. in µg/mL)				***K.pneumoniae*** (Concn. in µg/mL)				***P.aeruginosa*** (Concn. in µg/mL)			
	80	160	320	Av	80	160	320	Av	80	160	320	Av
3a	7.3	9.3	11.0	9.2	7	7.3	10.3	8.2	-	7.3	8.3	5.2
3b	7	7.6	9.3	7.9	9.6	13.6	18.3	13.8	-	-	7.0	2.3
3c	-	-	7.3	2.4	10.6	17.6	21.6	16.6	9.6	12.6	20.3	14.2
3d	7.3	9.3	11.6	9.4	-	-	-	-14.0	7.0	8.6	10.3	8.6
3e	-	-	8.6	2.8	18.3	20.3	17.5		7.3	8.3	12.3	9.3
3f	7.0	8.3	10.6	8.6	7.6	10.6	16.3	11.5	-	7.3	9.6	5.6
3g	-	-	9.3	3.1	-	-	7.0	2.3	-	-	8.6	2.8
3h	-	-	-	-	7.3	8.0	11.6	8.9	-	-	-	-
3i	7.3	8.6	10.0	8.6	8.3	8.6	10.3	9.0	7.3	9.0	11.6	9.3
3j	-	-	8.6	2.8	-	-	-	-	-	-	9.0	3.0
1	-	7.0	8.3	5.1	-	-	10.6	3.5	-	-	8.0	2.6
2a	-	-	7.3	2.4	13.6	21.0	24.3	9.5	-	7.3	13.3	6.8
2b	-	-	-	-	10.3	14.3	17.6	14.1	7	8.3	9.3	8.2
2c	-	7.3	9.3	5.5	10.3	14.3	11.6	12.1	9.6	13.3	19.6	14.1
2d	-	-	-	-	12.3	17	21.3	16.8	-	-	9.6	3.2
2e	-	-	-	-	9.3	11.6	16.6	2.3	-	9.6	13.3	7.6

Table-4 reflects that most of the compounds had shown remarkable activity only at 320 mg/ml.

Antibacterial screening of Mannich bases against *S. typhi,* showed interesting results (Table 4). That reflects, Mannich bases 3a and 3d were at par in their antibacterial activity followed by 3f and 3i were superior to other Mannich bases in case of *S. typhi*. All the Mannich bases were found to be significantly superior to their corresponding sulphonamide except 3c.

Mannich base 3e followed by 3c was found to superior in inhibitory effect against *K. pneumoniae* over other compounds. The antibacterial activity was compared with parent sulphonamides. It was found that sulphonamides were equally potential against *K. pneumoniae* as the synthesized Mannich bases. Table 1 reflects that except 3h all the synthesized Mannich bases had proven to be potent against *P.aeruginosa*, 3c are significantly superior to others in inhibiting the growth of this pathogen. Mannich bases 3e and 3i are at par their antibacterial activity followed by 3d in inhibiting the growth of this pathogen.

4. CONCLUSION

Conclusion suggest that the newly synthesized Mannich bases of 2-amino-9 [{(1,3 di hydroxy propane-2yl) oxy} methyl] 6-9 dihydro-3H-purine-6-one possess a very noticeable and prolonged antibacterial activity. These derived Mannich bases are less toxic in comparison of their parent sulphonamides. This work shows that Mannich bases are a potential source of compounds for inhibition of bacteria and could be used as efficient drugs with minimum side effects.

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REFERENCES

1. Katritzky, A. R. *Chem. Rev.* **2004**, 104, 2125.
2. Sean, S. D.; Essy, M.; Eric, J.S.; Richard, W. *Clinical Therapeutics* **1996**, 18, 546.
3. Joshi, S.; Khosla, N.; Tiwari, P. *Bioorg. Med. Chem.* **2004**, 12, 571.
4. Sondhi, S. M.; Johar, M.; Singhal, N.; Dastidar, S.G.; Shukla, R.; Raghubir, R. *Monatshefte Furchem*, **2000**, 13, 511.
5. LI, J. J.; Anderson, Q. D.; Burton, E.G.; Cogburn, J. N.; Collins, J.T.; Garland, D. J.; Gregory, S. A.; Haung, H. C.; Isakson, P. C.; Koboldt, C. M.; Logush, E. W.; Norton, M. B.; Perkns, W. E.; Reinhard, E. J.; Seibert, K.; Veenhuizen, A. W.; Zang, Y.; Reitz, D.B. *J. Med. Chem.* **1995**, 38, 4570.
6. Supuran, C. T.; Scozzafava, A.; Jurca, B. C.; Ilies, M. A. *Eur. J. Med. Chem.* **1998**, 33, 83.
7. Singh, B. J. *J. Indian Chem. Soc.* **1976**, 56, 720.
8. Moore, M. B.; Repla, R. T. *J. Am. Chem. Soc.* **1946**, 68, 1675.
9. Tramontini, M.; Angiolini, L. *Mannich Bases: Chemistry and Uses*; CRC Press, BOCA Raton, **1994**, Ch.1, pp 07.
10. Saab, A. N.; Slowan, K. B.; Beall, H. D.; Villanueva, R. *J. Pharm. Sci.*, **1990**, 79, 1099.
11. Bonati, A.; Bombardelli, E.; Gabetto, B. *British Patent*, **1975**, 1383, 053, *Chem. Abstr,* **1975**, 83, 43342.
12. Lepetit, S. A. *Netherlands Patent Appl.*, **1965**, 6144012, *Chem. Abstr.* **1965**, 63, 13180.
13. Mandloi, D.; Joshi, S.; Khadikar, P.V.; Khosla, N. *Bioorg. Med. Chem. Lett.* **2005**, 17, 15405.
14. Tramontini, M.; Angiolini, L.; Ghedini, N. Polymer 1998, 29, 771.
15. Goto, M.; Minoe, T. Jpn. Kokai Tokkyo Koho. **1995**, JP06, 185.
16. Mitsch, A.; Wibner, P.; Sattler, I.; Schlitzer, M. *Arch. Pharm. Med. Chem.* **2001**, 334, 40.
17. Joshi, S.; Manikpuri, A.; Tiwari, P. Bioorg. Med. Chem lett**. 2006,** 17, 645.
18. Manikpuri, A.; Joshi, S.; Kadikar, P.V. J. Chil. Chem. Soc, 55, N° 3 2010 283.
19. *United States Pharmacopoeia*, 25th ed., Vol. II, *United States Pharmacoepial Convention*, Inc., Rockville**, 2002**, 1882.