



# **SYNTHETIC, SPECTROSCOPIC AND ANTIMICROBIAL STUDIES OF MANNICH BASES THROUGH ACTIVE HYDROGEN COMPOUNDS**

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## **ABSTRACT**

The purpose of this study was to prepare a targeted series of Mannich bases by introducing the 2-chloro 4-nitrobenzamide and 2-methylbenzamide pharmacophore into secondary amines for study of their biological effects. The structures of the synthesized compounds were assigned on the basis of elemental analyses, UV, IR and <sup>1</sup>H NMR spectral studies. The biological screening of these synthesized compounds were done against various Gram- positive and Gram-negative bacteria with a view to explore their antimicrobial action by disk diffusion method at 40, 80 and 160 mg/mL, respectively. The results demonstrate the potential and importance of mounting new Mannich bases against pathogens under investigation and found to be low toxic as ascertained by LD<sub>50</sub> test.

**Key words :** 2-Chloro-4-nitrobenzamide, 2-Methylbenzamide, Secondary amines, Mannich bases, Antimicrobial activity, Toxicity, Statistical analysis.

## **INTRODUCTION**

Besides highly promising antibacterial properties exhibited by benzamide nucleus<sup>1</sup>, it has become an attractive target for the development of new synthetic methodology to derive new compounds of medicinal importance. 2-Chloro-4-nitrobenzamide and 2-methylbenzamide is the derivative of substituted benzoic acid<sup>2</sup> that possesses a therapeutic category as coccidiostat and used versus infections caused by protozoa and bacteria. The chemistry of the aminoalkylation of aromatic substrates by the Mannich reaction is of great interest for the synthesis and modification of biologically active compound having physical<sup>3, 4</sup> and chemical importance<sup>5</sup> as well as physiological properties<sup>6-9</sup> because the amino group can be easily converted into a variety of other functionalities<sup>10</sup>. Moreover,

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some secondary amines are building blocker of Mannich bases. Its nucleus has well known pharmacological properties<sup>11-15</sup>.

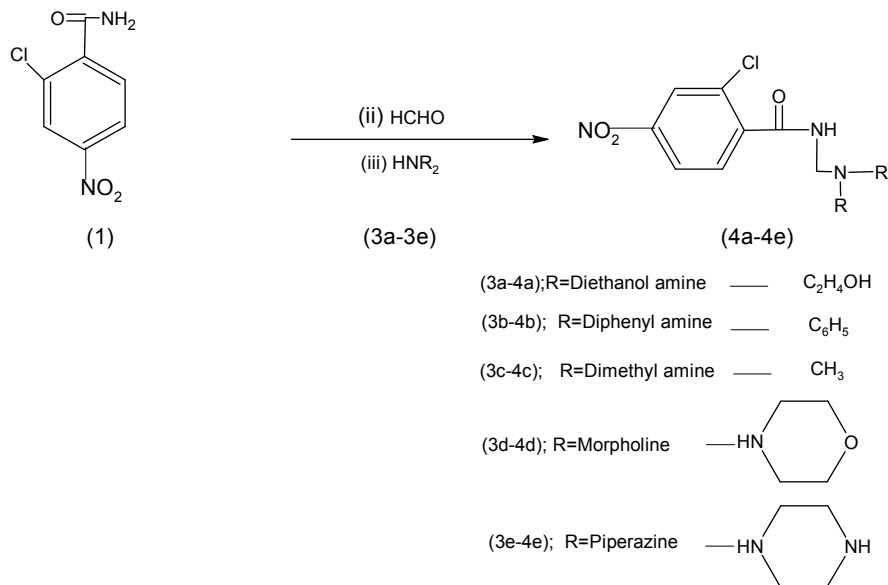
Hence, it was considered of attention to determine whether the compounds resulting from the aminomethylation of 2-chloro-4-nitrobenzamide and 2-methylbenzamide moiety would possess significant biological potency at various concentrations. In continuation of our work on antimicrobial activity of Mannich bases<sup>16</sup>, herein we describe the Mannich bases of above active hydrogen compounds, keeping this view that they are found to be potent, less toxic and claimed to have a broad spectrum of biological activities against pathogenic strains of chosen bacteria. From the above outlook and the present study, this paper reports the antimicrobial evaluation of Mannich bases **4a-4j**.

## EXPERIMENTAL

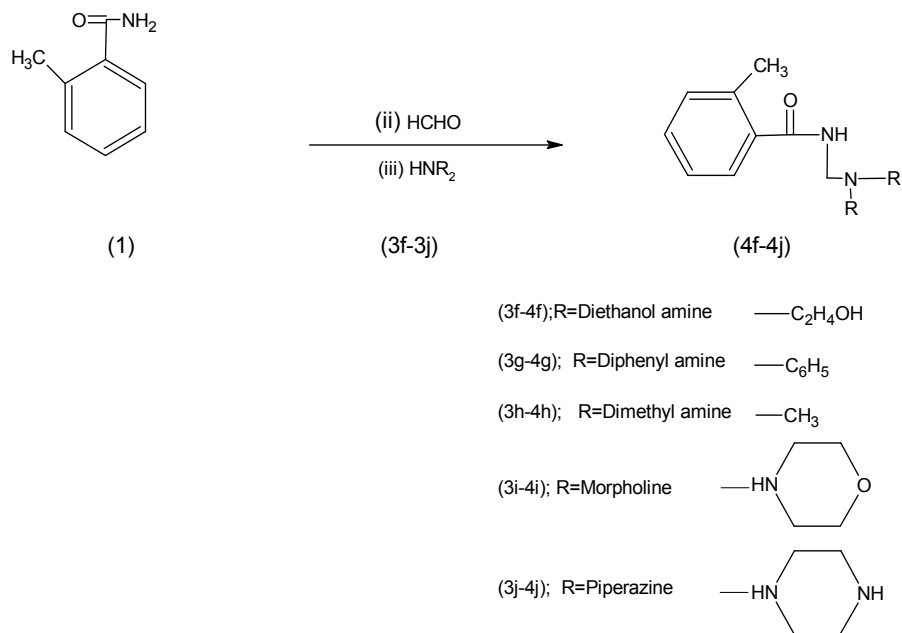
All the m. p. are uncorrected and were determined using Thomas Hoover capillary melting point apparatus. The <sup>1</sup>H NMR spectra in DMSO and CDCl<sub>3</sub> solvent were recorded on Bruker DRX-300 FT NMR Spectrometer. The IR spectra were recorded on Shimadzu 820 IPC FTIR spectrophotometer using KBr pellets. The UV spectra were recorded on Shimadzu UV-160A, UV-visible spectrophotometer. Single spot ascertained the purity of the compounds during TLC where mobile phase was chloroform/methanol mixture (90 : 10) and stationary phase was silica gel-G (chromatographic grade). The antimicrobial screening was performed using paper disc method and the results were statistically evaluated by analysis of variance. All substituted secondary amines were obtained as pure samples from the reputed pharmaceutical concern. Solvents used were distilled before use.

### General procedure for synthesis of Mannich bases **4a-4j**

Active hydrogen compound (0.01 mol) was dissolved in 50 mL ethanol. This was followed by equimolar addition of secondary amines (0.01 mol) in small installments. 0.4 mL (0.015 mol) of formaldehyde solution was added slowly with constant stirring at 70-75°C for 3-8.5 hours, depending upon the secondary amines. 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. The excess of solvent was distilled off under reduced pressure on next day. The Mannich base formed was recrystallized with dry distilled ethanol. Analogous members were prepared by the same procedure. Tables 1 and 2 reflect the physical and spectral data of novel Mannich bases. Schemes 1 and 2 scanned the synthesis of Mannich bases (**4a-4j**).



**Scheme 1**



**Scheme 2**

**Schemes 1 and 2 : Synthesis of Mannich bases of 2-chloro-4-nitro benzamide and 2-methyl benzamide from secondary amines**

**Table 1. Physical and spectral characteristics of compound (4a-4j)**

S. No.	Compound	Molecular formula	M. P. (C°)	Elemental analysis%		
				Calcd.	(Found)	
				C	H	N
<b>4a</b>	N- {[Bis(2-hydroxyethyl) amino]methyl}-2-chloro-4-nitrobenzamide	C <sub>12</sub> H <sub>16</sub> N <sub>3</sub> O <sub>5</sub> Cl	105	45.28 (45.35)	5.30 (5.04)	13.07 (13.22)
<b>4b</b>	2-Chloro-N- [(diphenylamino) methyl]-4-nitrobenzamide	C <sub>20</sub> H <sub>16</sub> N <sub>3</sub> O <sub>3</sub> Cl	140	62.76 (62.91)	4.08 (4.19)	10.98 (11.01)
<b>4c</b>	2-Chloro-N- [(dimethylamino) methyl]-4-nitrobenzamide	C <sub>10</sub> H <sub>12</sub> N <sub>3</sub> O <sub>3</sub> Cl	150- 152	46.24 (46.60)	4.15 (4.66)	16.03 (16.31)
<b>4d</b>	2-Chloro-N- [(morpholin-4-ylamino)methyl]-4-nitrobenzamide	C <sub>12</sub> H <sub>14</sub> N <sub>3</sub> O <sub>4</sub> Cl	125	48.02 (48.08)	4.59 (4.67)	13.99 (14.02)
<b>4e</b>	2-Chloro-4-nitro-N-[(piperazin-1-ylamino)methyl] benzamide	C <sub>12</sub> H <sub>15</sub> N <sub>4</sub> O <sub>3</sub> Cl	145- 150	48.17 (48.24)	5.26 (5.02)	18.13 (18.76)
<b>4f</b>	N- {[Bis(2-hydroxyethyl) amino]methyl}-2-methylbenzamide	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub> O	85	48.17 (48.24)	7.91 (7.93)	11.16 (11.11)
<b>4g</b>	N- [(Diphenylamino) methyl]-2-methylbenzamide-	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O	90	79.10 (79.74)	6.01 (6.33)	8.16 (8.86)

Cont...

S. No.	Compound	Molecular formula	M. P. (C°)	Elemental analysis% Calcd. (Found)		
				C	H	N
4h	N-[(Dimethylamino)methyl]-2-methylbenzamide	C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O	95	68.40 (68.75)	8.84 (8.33)	14.15 (14.58)
4i	2-Methyl-N-[(morpholin-4-ylamino)methyl]benzamide	C <sub>13</sub> H <sub>18</sub> N <sub>3</sub> O	55	66.47 (66.66)	7.52 (7.69)	7.52 (7.69)
4j	2-Methyl-N-[(piperazin-1-ylamino)methyl]benzamide	C <sub>13</sub> H <sub>19</sub> N <sub>3</sub> O	65	66.72 (66.95)	8.09 (8.15)	17.86 (18.02)

**Table 2. Spectral characteristics of compound (4a-4j)**

S. N.	UV	IR	<sup>1</sup> H NMR
4a	204 (secondary amines), 210 (chloro group in benzene ring), 212 (amido moiety), 240 (benzene nucleus containing NO <sub>2</sub> group), 255 (benzene chromophore)	3750 νas (NH) in secondary amides, 1730 ν(C=O) of secondary amide group, 740 ν(N-H), 2910 νas bridged methylene group between an amide and an amino group, 1520 ν(NO <sub>2</sub> ), 2320 ν >CH <sub>2</sub> N<, 2750 ν (C-H) in N-C-H group of N-CH <sub>3</sub> , 3080 ν (=C-H)	2.59(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.42(t, 1H, J= 24.01, CONH), 6.78-7.67(d, aryl ring proton), 3.05-3.07 (s, chloro and nitro substituted aryl proton), 6.53-6.56(d, J=8.32, aryl proton due to H ortho to NH), 8.5-8.7 (d, J = 16.11, aryl protons ortho to NO <sub>2</sub> )
4b	206 (secondary amines), 212 (chloro group in benzene ring), 214 (amido moiety), 242 (benzene nucleus containing NO <sub>2</sub> group), 256 (benzene	3760 νas (NH) in secondary amides, 1732 ν(C=O) of secondary amide group, 748 ν(N-H), 2910 νas bridged methylene group between an amide and an amino group, 1520 ν(NO <sub>2</sub> ), 2328 ν >CH <sub>2</sub> N<, 2752 ν	2.58(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.41(t, 1H, J = 24.01, CONH), 6.90-8.10 (d, aryl ring proton), 3.05-3.07 (s, chloro and nitro substituted aryl proton), 6.53-6.56 (d, J = 8.32,

S. N.	UV	IR	<sup>1</sup> H NMR
	chromophore)	(C-H ) in N-C-H group of N-CH <sub>3</sub> , 3086 v (=C-H)	aryl proton due to H ortho to NH), 8.5-8.7(d, J = 16.11, aryl protons ortho to NO <sub>2</sub> )
4c	204 (secondary amines), 210 (chloro group in benzene ring), 212 (amido moiety), 240 (benzene nucleus containing NO <sub>2</sub> group), 258(benzene chromophore)	3768 v <sub>as</sub> (NH) in secondary amides, 1730 v(C=O) of secondary amide group, 746 v(N-H), 2920 v <sub>as</sub> bridged methylene group between an amide and an amino group, 1528 v(NO <sub>2</sub> ), 2338 v >CH <sub>2</sub> N<, 2762 v (C-H ) in N-C-H group of N-CH <sub>3</sub> , 3096 v (=C-H)	2.64(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.43(t, 1H, J = 24.01, CONH), 6.92-7.6(d, Aryl ring proton), 3.05-3.07 (s, chloro and nitro substituted aryl proton), 6.53-6.56(d, J = 8.32, aryl proton due to H ortho to NH), 8.5-8.7 (d, J=16.11, aryl protons ortho to NO <sub>2</sub> )
4d	206 (secondary amines), 208 (chloro group in benzene ring), 212 (amido moiety), 240 (benzene nucleus containing NO <sub>2</sub> group), 258 (Benzene chromophore)	3764 v <sub>as</sub> (NH) in secondary amides, 1726 v(C=O) of secondary amide group, 740 v(N-H), 2910 v <sub>as</sub> bridged methylene group between an amide and an amino group, 1526 v(NO <sub>2</sub> ), 2338 v >CH <sub>2</sub> N<, 2762 v (C-H ) in N-C-H group of N-CH <sub>3</sub> , 3096 v (=C-H)	2.59 (d, 2H, J = 8.65, CH <sub>2</sub> ), 8.45(t, 1H, J = 24.01, CONH), 6.82-7.92(d, Aryl ring proton), 3.05-3.07 (s, chloro and nitro substituted aryl proton), 6.53-6.56(d, J=8.32, aryl proton due to H ortho to NH), 8.5-8.7 (d, J=16.11, aryl protons ortho to NO <sub>2</sub> )
4e	204 (secondary amines), 210 (chloro group in benzene ring), 212 (amido moiety), 240 (benzene nucleus containing NO <sub>2</sub> group), 255 (benzene chromophore)	3750 v <sub>as</sub> (NH) in secondary amides, 1730 v(C=O) of secondary amide group, 740 v(N-H), 2910 v <sub>as</sub> bridged methylene group between an amide and an amino group, 1520 v(NO <sub>2</sub> ), 2320 v >CH <sub>2</sub> N<, 2750 v (C-H ) in N-C-H group of N-CH <sub>3</sub> , 3080 v (=C-H)	2.58(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.41(t, 1H, J= 24.01, CONH), 6.82-7.92(d, Aryl ring proton), 3.05-3.07 (s, chloro and nitro substituted aryl proton), 6.53-6.56(d, J = 8.32, aryl proton due to H ortho to NH), 8.5-8.7 (d, J = 16.11, aryl protons

S. N.	UV	IR	<sup>1</sup> H NMR
			ortho to NO <sub>2</sub> )
4f	205 (secondary amines), 212 (amido moiety), 234 (benzene nucleus containing CH <sub>3</sub> group), 255 (benzene chromophore)	3800 $\nu$ as (NH) in secondary amides, 3100 $\nu$ (=C-H), 2720 $\nu$ >CH <sub>2</sub> N<, 1710 $\nu$ (C=O) of secondary amide group, 1060 $\nu$ (N-C-N ) linkage, 780 $\nu$ (N-H), 580 $\nu$ (CH <sub>3</sub> ),	2.62(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.10 (t, 1H, J= 24.01, CONH), 7.38-7.48(d, aryl ring proton)
4g	205 (secondary amines), 212 (amido moiety), 234 (benzene nucleus containing CH <sub>3</sub> group), 255 (benzene chromophore)	3680 $\nu$ as (NH) in secondary amides, 3063 $\nu$ (=C-H), 2700 $\nu$ >CH <sub>2</sub> N<, 1684 $\nu$ (C=O) of secondary amide group, 1088 $\nu$ (N-C-N ) linkage, 770 $\nu$ (N-H), 568 $\nu$ (CH <sub>3</sub> )	2.64(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.01(t, 1H, J= 24.01, CONH), 7.39-7.44 (d, aryl ring proton)
4h	206 (secondary amines), 210 (amido moiety), 236 (benzene nucleus containing CH <sub>3</sub> group), 257 (benzene chromophore)	3740 $\nu$ as (NH) in secondary amides, 3070 $\nu$ (=C-H), 2750 $\nu$ >CH <sub>2</sub> N<, 1700 $\nu$ (C=O) of secondary amide group, 1105 $\nu$ (N-C-N ) linkage, 780 $\nu$ (N-H), 560 $\nu$ (CH <sub>3</sub> )	2.63(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.02(t, 1H, J= 24.01, CONH), 7.40-7.56(d, Aryl ring proton), 2.85(s, 6H, dimethyl)
4i	208 (secondary amines), 212 (amido moiety), 238 (benzene nucleus containing CH <sub>3</sub> group), 258 (benzene chromophore)	3620 $\nu$ as (NH) in secondary amides, 3060 $\nu$ (=C-H), 2689 $\nu$ >CH <sub>2</sub> N<, 1682 $\nu$ (C=O) of secondary amide group, 1087 $\nu$ (N-C-N ) linkage, 760 $\nu$ (N-H), 567 $\nu$ (CH <sub>3</sub> )	2.62(d, 2H, J= 8.65), 8.20(t, 1H, J= 24.01), 7.43-7.59(d, Aryl ring proton), 3.1(t, H ortho to N of morpholine ring), 3.76 (t, H ortho to O of morpholine ring)
4j	206 (secondary amines), 214 (amido moiety), 238 (benzene nucleus containing CH <sub>3</sub> group), 255 (Benzene chromophore)	3750 $\nu$ as (NH) in secondary amides, 3080 $\nu$ (=C-H), 2950 $\nu$ >CH <sub>2</sub> N<, 1700 $\nu$ (C=O) of secondary amide group, 1080 $\nu$ (N-C-N ) linkage, 780 $\nu$ (N-H), 560 $\nu$ (CH <sub>3</sub> )	2.65(d, 2H, J= 8.65, CH <sub>2</sub> ), 8.04 (t, 1H, J= 24.01, CONH), 7.45-7.60 (d, aryl ring proton)

Table 3. Antibacterial activity of newly synthesized Mannich bases (4a-4j) (Zone of inhibition in mm)

Compd.	<i>E. coli</i> (Conc. in mg/mL)				<i>K. pneumoniae</i> (Conc. in mg/mL)				<i>B. subtilis</i> (Conc. in mg/mL)			
	40	80	160	Avg.	40	80	160	Avg.	40	80	160	Avg.
	4a	7.00	7.50	8.00	7.50	7.50	8.50	10.00	8.67	8.50	9.50	11.50
4b	7.50	8.50	9.00	8.33	7.50	9.50	10.50	9.17	10.50	12.00	13.50	12.00
4c	7.00	7.50	7.50	7.33	7.50	8.50	9.00	8.33	7.50	7.50	8.50	7.83
4d	-	-	-	-	-	-	-	-	-	-	-	-
4e	7.50	8.00	8.50	8.00	8.00	9.50	10.00	9.17	12.50	13.50	14.50	13.50
4f	-	-	-	-	-	-	-	-	-	-	-	-
4g	-	-	-	-	7.50	8.00	9.50	8.33	-	-	-	-
4h	7.00	7.00	7.50	7.17	7.50	7.50	8.50	7.83	7.00	7.50	8.50	7.67
4i	7.50	7.50	8.50	7.83	7.00	7.50	8.00	7.50	-	-	-	-
4j	7.50	8.50	14.00	10.00	7.50	8.50	15.00	10.33	8.00	8.50	9.50	8.67
Compound	S.Ed.	0.666	1.429	CD at 5%	S.Ed.	0.516	1.094	CD at 5%	S.Ed.	0.385	0.858	CD at 5%
Concentration	0.154	0.314	0.14	0.284	0.152	0.313	0.152	0.313	0.152	0.313	0.152	0.313
Interaction	0.462	0.943	0.421	0.851	0.303	0.626	0.303	0.626	0.303	0.626	0.303	0.626
S.Ed. - Standard error of difference, CD - Critical difference												



Table 3. Antibacterial activity of newly synthesized Mannich bases (4a-4j) (Zone of inhibition in mm)

Compd.	<i>S. aureus</i> (Conc. in mg/mL)				<i>S. typhi</i> (Conc. in mg/mL)				<i>P. aeruginosa</i> (Conc. in mg/mL)			
	40	80	160	Avg.	40	80	160	Avg.	40	80	160	Avg.
4a	7.50	9.50	15.50	10.83	9.50	11.50	14.50	11.83	-	-	-	-
4b	7.50	8.50	9.50	8.50	-	-	-	-	7.50	8.50	9.50	8.50
4c	7.00	8.50	13.00	9.50	7.50	8.00	8.50	8.00	7.00	7.50	7.50	7.33
4d	-	-	-	-	-	-	-	-	-	-	-	-
4e	7.50	8.50	10.50	8.83	7.50	8.50	9.50	8.50	7.50	8.00	8.50	8.00
4f	-	-	-	-	-	-	-	-	-	-	-	-
4g	7.00	7.50	7.50	7.33	-	-	-	-	7.50	7.50	8.50	7.83
4h	7.50	8.50	9.00	8.33	7.50	7.50	8.50	8.33	7.50	7.50	8.50	7.83
4i	7.00	8.00	8.50	7.83	7.00	7.50	8.00	7.83	7.00	7.50	8.00	7.50
4j	8.50	9.00	14.00	10.50	8.50	9.50	18.50	10.50	7.50	8.50	14.50	10.17
Compound	S.Ed.	0.435	0.923	0.357	S.Ed.	0.357	0.780	0.303	S.Ed.	0.303	0.641	0.641
Concentration	0.140	0.283	0.095	0.194	0.126	0.255	0.766	0.255	0.126	0.255	0.255	0.255
Interaction	0.420	0.849	0.284	0.583	0.379	0.766	0.766	0.766	0.379	0.766	0.766	0.766
S.Ed. - Standard error of difference, CD - Critical difference												

### Antimicrobial activity and toxicity

The antimicrobial screening was performed using paper disk method on pathogenic strains of *E. coli*, *S. aureus*, *K. pneumoniae*, *B. subtilis*, *S. typhi* and *P. aeruginosa*. Cultures of each bacterium was kept in Mullar Hinton Agar at 37°C for 24 hrs and then examined. The Mannich bases were studied for their antibacterial property at concentration of 40-160 mg mL<sup>-1</sup> using methanol as solvent. The solvent did not exhibit any activity at the concentrations used. Most of the compound were found to be effective against the tested microorganism by measuring the diameter of the growth inhibition zone according to Bauer et al.<sup>17</sup> The antimicrobial screening data are recorded in Table 3. Antibacterial screening of **4a-4j** against *E. coli* shows significant results. All the Mannich bases show antimicrobial activity against this bacterium except **4d**, **4f** and **4g**. Table 3 reflects that the compound **4j** is statistically superior to other Mannich bases in checking the growth of *E. coli*. In case of *S. aureus* all Mannich bases show antimicrobial activity against this bacterium except **4d** and **4f**. It further reflects that compound **4a** is statically superior to all arbitrarily chosen concentrations in checking the growth of *S. aureus*. On screening the antimicrobial activity of novel synthesized Mannich bases, it was found that all the compounds show activity against *K. pneumoniae* except **4d** and **4f** where as **4b** and **4e** are significantly active at all chosen concentrations.

Table 3 further reveals that except **4d**, **4f**, **4g** and **4i** all Mannich bases show antimicrobial activity against *B. subtilis* with higher zone of activity by **4b** and **4e**. It is further demonstrated by table that except **4b**, **4d**, **4f** and **4g** all Mannich bases show better zone of inhibition with significant antimicrobial activity by **4a** and **4j** against *S. typhi* where as highest zone of inhibition is shown by **4j** against *P. aeruginosa* with better activity by all Mannich bases except **4a**, **4d** and **4f**.

The concentration of 160 mg mL<sup>-1</sup> is found significantly superior to concentrations 80 mg mL<sup>-1</sup> and 40 mg mL<sup>-1</sup> in checking the growth of all microorganisms. Compound **4j** is found to be significantly active against all the pathogens under study.

The results were statistically evaluated by analysis of variance<sup>18</sup>. The Mannich bases were also screened for their toxicity by preliminary LD<sub>50</sub> test. The test was performed on white mice weighing 25 g. Doses were given orally as well as intraperitoneally and mice were kept under observation for 72 h for each trial<sup>19</sup>. The Mannich bases showed no adverse toxic effect even at an oral dose of 1600 mg/kg of the body weight of mice. However, when dose was administered intraperitoneally, they proved to be lethal at the dose level of 1000 mg/kg of the body weight of mice.

These conclusions suggest that the ten Mannich base compounds possess a very noticeable and prolonged antibacterial activity and that further studies should be performed to determine their value as antimicrobial agent. This work showed that Mannich bases are a potential source of compounds for inhibition of bacteria. We next focused our attention on antimicrobial analysis of Mannich bases of 2-chloro-4-nitrobenzamide and 2-methylbenzamide towards various Gram-positive and Gram-negative pathogens.

### ACKNOWLEDGEMENTS

Supports from Dr. K. P. Madhusudanan, R. S. I. C., CDRI, Lucknow for recording <sup>1</sup>H NMR spectra, are indebted.

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*Accepted* : 26.09.2008