International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073 (Online)
An Online International Journal Available at http://www.cibtech.org/jcs.htm
2012 Vol. 2 (1) January-March, pp. 37-42/Khare and Mandloi
Research Article

SYNTHESIS AND ANTIMICROBIAL STUDIES OF SOME NOVEL MANNICH BASES DERIVED FROM SECONDARY AMINES

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ABSTRACT
The amino alkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds. The Mannich derivatives of 2-Chloro 4-nitrobenzamide with formaldehyde and various secondary amines were synthesized for study of their biological effects. The structures of the synthesized compounds were assigned on the basis of elemental analyses, UV, IR and ^{1}HNMR spectral studies. The biological screenings of these synthesized compounds were done against some Gram-positive and Gram-negative bacteria with a view to explore their antimicrobial action by disc diffusion method at 40, 80 and 160 mg/ml respectively. The results reveal the potential and importance of mounting new Mannich bases against pathogens under investigation and found to be low toxic as ascertained by LD_{50} test.

***Key Words**: 2-chloro-4-nitro Benzamide, Secondary Amines, Mannich Reaction, Mannich Bases, Antibacterial Activity, Statistical Analysis And Toxicity*

INTRODUCTION
The aminoalkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds (Tramontini et al., 1990, 1994). 2-Chloro 4-Nitrobenzamide is the derivative of substituted benzoic acid (Windholz, 1984 and Joshi et al., 2008) that possesses a therapeutic category (veterinary), as coccidiostat. It is used in various infections caused by protozoa and bacteria especially in the treatment of coccidiosis caused by *E.tenella*, *E.necatrix* and *E.acervulina*. Moreover, some secondary amines are building blocker of Mannich bases (Anderson et al., 1995, Joshi et al., 2002 and 2004). Its nucleus has well known pharmacological properties. In continuation of our work on antimicrobial activity of Mannich bases (Khosla et al., 2003, Joshi et al., 2007 and 2009), herein we describe the Mannich bases **(3a-3e)** of 2-chloro 4-nitrobenzamide, keeping this view that they are found to more potent, less toxic and claimed to have a wide spectrum of biological activities than their parent compound.
Hence, it was considered of attention to determine whether the compounds resulting from the aminomethylation of 2-Chloro 4-nitrobenzamide moiety would possess significant biological potency at various concentrations.

MATERIALS AND METHODS
Melting points were determined and are uncorrected. IR spectra in KBr were recorded on spectrometer Shimadzu IR 460, UV spectra in ethanol and dioxane-water (1:1) were recorded on spectrophotometer Shimadzu UV 160 A and NMR spectra (Silverstein, 1998) in DMSO and $CDCl_3$ on FT NMR Bruker WM-400 (Bruker, Switzerland), using TMS as an internal standard (chemical shift in δppm). Purity of compounds were checked by TLC using chromatographic plates coated with silica gel (Merck, Germany). The solvent system used was methanol-chloroform mixture (1:9).

Synthesis of Mannich bases from secondary amines:

Secondary amine (0.01 mol) was added to an ethanolic solution (50 mL) of 2-chloro-4-nitrobenzamide (0.01 mol) in a flat bottom flask. Amount of 0.4 mL (0.015 mol) of formaldehyde solution (37%, v/v) was added slowly with constant stirring. The reaction mixture was stirred at 70-75 ^{0}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 was again kept for crystallization in the refrigerator. The products obtained were purified by recrystallization from dry distilled ethanol. The compounds thus synthesized and their analytical data are presented in Table 1.

ANTIMICROBIAL SCREENING OF MANNICH BASES (3a-3e)

Mannich bases of benzamide prepared from piperidine and diethylamine by Einhorn *et al.* (1905) and those of piperazine, piperidine and morpholine by Bindal *et al.* (Agrawal et al., 1985) show CNS depressant and cardiac stimulant nature. Checheleska (1956) reported Mannich bases from p-nitrobenzamide with diethylamine and dimethylamine to be active against *M. smegmatis*-279 *in vitro.* Thus, it was thought worthy that our compounds derived from various secondary amines may also possess such activity.

In the present study, we have synthesized Mannich bases through condensation of some aromatic and heteroaromatic amides with various secondary amines. They were screened for their antibacterial properties. The antibacterial activities (USP, 2002) of Mannich bases were tested against some pathogenic bacteria as follows.

Gram positive:

Bacillus subtilis (B. subtilis)
Staphylococcus aureus (S. aureus)

Gram negative:

Escherichia coli (E. coli)
Salmonella typhosa (*S. typhosa*)
Pseudomonad aeruginosa (P. aeruginosa)
Klebsiella pneumoniae (K. pneumoniae)

Result and Discussion of Antibacterial Screening of Mannich Bases:

2-chloro-4-nitrobenzamidomethyl amines were screened against pathogenic bacteria of gram positive and gram negative strain. The sensitivity of these compounds against bacteria was established by zone of inhibition in mm (in triplicate). The results were statistically analysed to justify the significant activity. These Mannich bases showed significant response against *E. coli, S. aureus, S. typhi, B. subtilis, P. aeruginosa* and *K. pneumonae.*

Antibacterial screening of 2-chloro-4-nitrobenzamidomethyl amines against *E. coli* show significant results. All the Mannich bases show antimicrobial activity against this bacterium except 3d. The Table 2a reflects that the compound 3b is significantly superior to other Mannich bases 3a, 3c, 3d and 3e are statistically at par in checking the growth of *E. coli*. The concentration 160 mg mL^{-1} is found significantly superior to concentrations 40 mg mL^{-1} and 80 mg mL^{-1} in checking the growth of *E. coli*. Statistically significant relationship has been studied between concentrations and compounds on zone of inhibition.

2-chloro-4-nitrobenzamidomethyl amines show significant antibacterial activity against *K. pneumoniae*. All Mannich bases show antimicrobial activity against this bacterium except 3d. The data in **Table 2a** reveals that the compound 3b is significantly superior to the compounds 3a, 3c, 3d and 3e inhibiting the growth of *K. pneumoniae*. However, Mannich bases 3b and 3e show statistically similar antibacterial activity. The concentration of 160 mg mL^{-1} is found significantly superior to concentrations 40 mg mL^{-1} and 80 mg mL^{-1} in checking the growth of *K. pneumoniae*. Interaction studies between the compounds and concentration have been studied on zone of inhibition. Data revealed that for the compounds 3a, 3b,

3c, 3d and 3e 160 mg mL^{-1} concentration is significantly superior to all arbitrarily chosen concentrations in checking the growth of *K. pneumoniae*.

Reaction mechanism

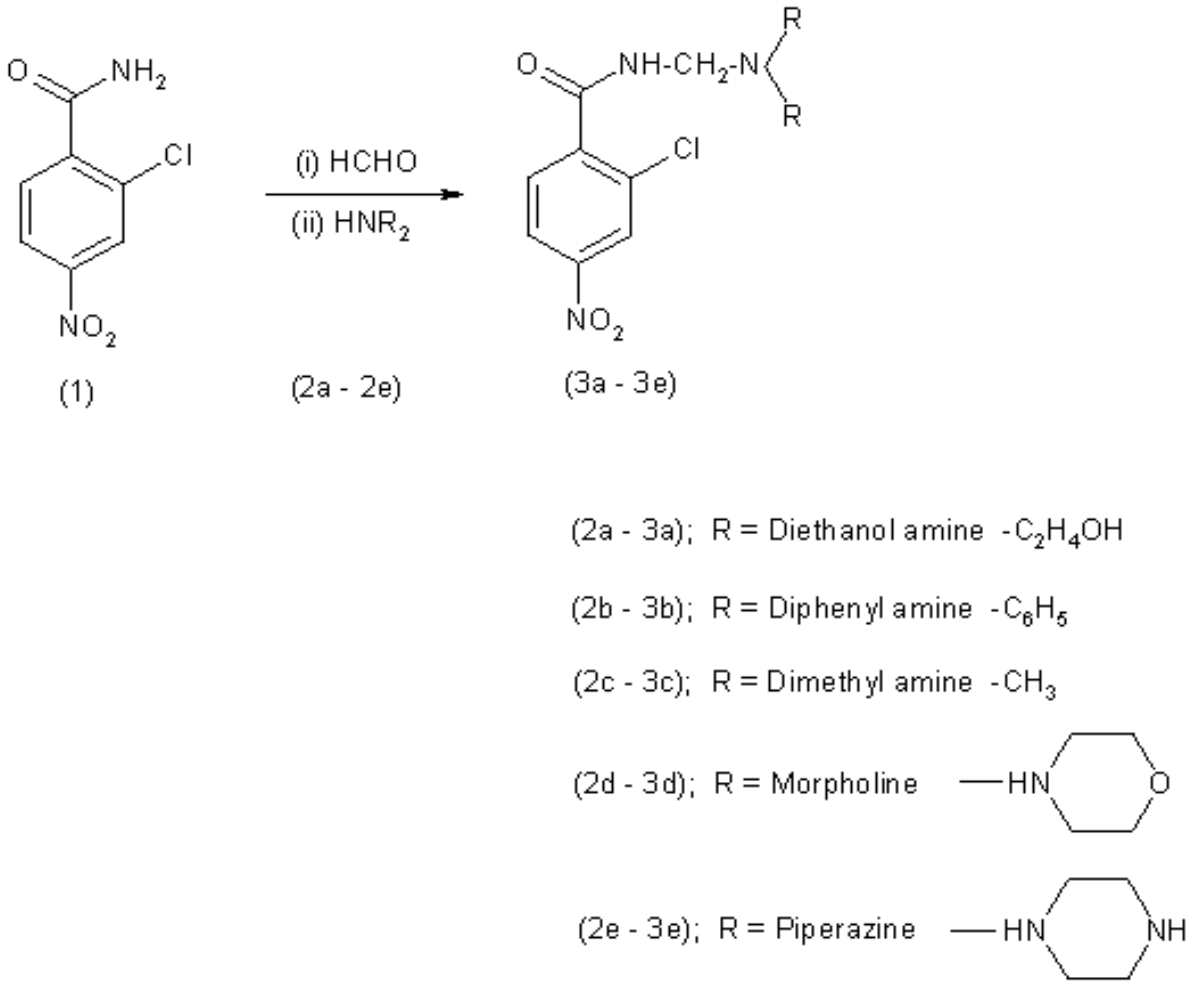

FIGURE - 1

Synthesis of Mannich Bases of 2-chloro-4-nitrobenzamide from Secondary Amines

Antibacterial screening of 2-chloro-4-nitrobenzamidomethyl amines against *B. subtilis* show significant results. All Mannich bases show antimicrobial activity against this bacterium except 3d. The Table 2a reflects that the compound 3e is significantly superior to all the compounds followed by the compounds 3a, 3b, 3c and 3d while rest of the compounds (3a, 3b and 3c) are statistically at par in showing their activity of inhibition in *B. subtilis*. The statistically significant relationship has been studied between concentrations and compounds on zone of inhibition. For the compound 3c, all the concentrations (40, 80 and 160 mg mL^{-1}) are statistically at par in inhibiting the growth of this bacterium.

Table 1: Characterization Data of Mannich Bases (3a-3e) of 2-Chloro-4-nitrobenzamide with Secondary Amines

S. No.	Compounds	Molecular Formula	M.p. (°c)	Elemental analysis(%) Found(calcd.)		
				(C)	(H)	(N)
3a.	2-chloro-4-nitrobenzamido-methyl diethanolamine.	$C_{12}H_{16}N_3O_5Cl$	105	45.28 (45.35)	5.30 (5.04)	13.07 (13.22)
3b.	2-chloro-4-nitrobenzamido-methyl diphenylamine.	$C_{20}H_{16}N_3O_3Cl$	140	62.76 (62.91)	4.08 (4.19)	10.98 (11.01)
3c.	2-chloro-4-nitrobenzamido-methyl dimethylamine	$C_{10}H_{12}N_3O_3Cl$	150-152	46.24 (46.60)	4.15 (4.66)	16.03 (16.31)
3d.	2-chloro-4-nitrobenzamido-methyl morpholine	$C_{12}H_{14}N_3O_4Cl$	125	48.02 (48.08)	4.59 (4.67)	13.99 (14.02)
3e.	2-chloro-4-nitrobenzamido-methyl piperazine	$C_{12}H_{15}N_4O_3Cl$	145-147	48.17 (48.24)	5.26 (5.02)	18.13 (18.76)

Antibacterial screening of 2-chloro-4-nitrobenzamidomethyl amines against *S. aureus* show significant results. All Mannich bases show antimicrobial activity against this bacterium. The **Table 2b** reflects that compound 3d is statistically superior in showing the antibacterial activity to other Mannich bases (3a, 3b, 3c and 3e) in checking the growth of *S. aureus*. However, Mannich bases 3a, 3b, 3c and 3e show statistically similar antibacterial activity against this bacterium. The concentration 160 mg mL^{-1} is found significantly superior to concentrations 80 mg mL^{-1} and 40 mg mL^{-1} in checking the growth of *S. aureus*. The statistically significant relationship has been studied between concentrations and compounds on zone of inhibition. Data revealed that compound 3c and 3e show statistically equal antibacterial activity at concentrations 40, 80 and 160 mg mL^{-1}. That is to say all concentrations are at par in inhibiting the growth of *S. aureus*.

Antibacterial screening of 2-chloro-4-nitrobenzamidomethyl amines against *S. typhi* show significant results. Mannich bases 3a, 3b, 3c, 3d and 3e show antimicrobial activity against this bacterium. The **Table 2b** reflects that the compound 3b and 3d are significantly superior to the compounds 3a, 3c and 3e. However, Mannich bases 3a and 3c show statistically similar antibacterial activity against this bacterium. The concentration of 160 mg mL^{-1} is found significantly superior to concentrations 80 mg mL^{-1} and 40 mg mL^{-1} in checking the growth of *S. typhi*. The significant statistical relationship has been studied between concentrations and compounds on zone of inhibition. For the compound 3a, 3b, 3c and 3d, 160 mg mL^{-1} concentration is significantly superior to 40 and 80 mg mL^{-1} concentrations, whereas concentrations 40 and 80 mg mL^{-1} are statistically at par in indicating zone of inhibition of *S. typhi*.

Table 2a: Antibacterial activity of synthesized Mannich bases (3a-3e) (zone of inhibition in mm)

Compounds	*E. coli* Conc. (mg mL^{-1})				*K. pneumoniae* Conc (mg mL^{-1})				*B. subtilis* Conc (mg mL^{-1})			
	40	**80**	**160**	**Avg.**	**40**	**80**	**160**	**Avg.**	**40**	**80**	**160**	**Avg.**
3a	7.0	7.5	8.0	7.50	7.5	8.5	10.0	8.67	8.5	9.5	11.5	9.83
3b	7.5	8.5	9.0	8.33	7.5	9.5	10.5	9.17	10.5	12.0	13.5	12.00
3c	7.0	7.5	7.5	7.33	7.5	8.5	9.0	8.33	7.5	7.5	8.5	7.83
3d	----	----	----	----	----	----	----	----	----	----	----	----
3e	7.5	8.0	8.5	8.00	8.0	9.5	10.0	9.17	12.5	13.5	14.5	13.50

		E. coli	*K. pneumoniae*	*B. subtilis*
Compound	SE	0.294	0.358	0.375
	CD at 5%	0.654	0.769	0.804
Concentration	SE	0.104	0.117	0.109
	CD at 5%	0.215	0.238	0.224
Interaction	SE	0.256	0.330	0.290
	CD at 5%	0.528	0.675	0.593

SE = standard error of difference, CD = Critical Difference

Antibacterial screening of 2-chloro-4-nitrobenzamidomethyl amines against *P. aeruginosa* show significant results. All Mannich bases show antimicrobial activity against this bacterium. The **Table 2b** reflects that the compound 3d is statistically superior to the compounds 3a, 3b, 3c and 3e in checking the growth of *P. aeruginosa*. However, Mannich bases 3a, 3b, 3c and 3e show statistically similar antibacterial activity. That is to say that they are statistically at par in checking the growth of *P. aeruginosa*. The concentration 160 mg mL^{-1} is found significantly superior to concentrations 80 mg mL^{-1} and 40 mg mL^{-1} in checking the growth of *P. aeruginosa*. The statistically significant relationship has been studied between concentrations and compounds on zone of inhibition. Data revealed that compound 3b and 3e show statistically equal antibacterial activity at concentrations 40, 80 and 160 mg mL^{-1}. That is to say all the concentrations are at par in inhibiting the growth of *P. aeruginosa*

Table 2b: Antibacterial activity of synthesized Mannich bases (3a-3e) (zone of inhibition in mm)

Compounds	*S. aureus* Conc. (mg mL^{-1})				*S. typhi* Conc (mg mL^{-1})				*P. aeruginosa* Conc (mg mL^{-1})			
	40	**80**	**160**	**Avg.**	**40**	**80**	**160**	**Avg.**	**40**	**80**	**160**	**Avg.**
3a	8.0	8.5	9.5	8.67	7.5	8.0	8.5	8.00	7.0	7.0	7.5	7.17
3b	7.0	7.5	7.5	7.33	9.5	11.5	12.5	11.17	7.5	7.5	8.0	7.67
3c	7.0	7.5	8.5	7.67	7.0	7.5	9.5	8.00	7.0	7.5	11.5	8.67
3d	9.5	10.5	13.0	11.00	10.5	12.5	15.5	12.67	10.5	12.5	16.5	13.17
3e	7.0	7.5	8.5	7.67	7.5	7.5	7.5	7.33	7.0	7.5	8.5	7.67

		E. coli	*K. pneumoniae*	*B. subtilis*
Compound	SE	0.294	0.358	0.375
	CD at 5%	0.654	0.769	0.804
Concentration	SE	0.104	0.117	0.109
	CD at 5%	0.215	0.238	0.224
Interaction	SE	0.256	0.330	0.290
	CD at 5%	0.528	0.675	0.593

SE = standard error of difference, CD = Critical Difference

ACKNOWLEDGEMENTS
The authors are indebted to Dr. K.P. Madhusudan, R.S.I.C., CDRI, Lucknow for recording C, H and N analysis data and ^{1}H NMR spectra. We are also grateful to Dr. Rakesh Sharda, College of veterinary sciences & A.H., Mhow, for providing facilities to do antimicrobial studies of our compounds.

REFERENCES
Tramontini M and Angiolini L (1990). Synthesis of Mannich Base, *Tetrahedron*, **46,** 1791.
Tramontini M., Angiolini L. (1994) Mannich Base: Chemistry and Uses; CRC Press: Boca Raton, **7**-20.
Windholz M (1983). The Merch index 10th ed., an Encyclopedia of chemicals, *Drugs and Biologicals*, 242.
Joshi S, Manikpuri AD and Khare D (2008). Synthesis, characterization and antimicrobial activity of Mannich bases of 2-chloro-4-nitro benzamide derived from sulphonamides, *Journal of Indian Chemical Society*, **85**, 508-512.
Li JJ, Anderson QD, Burton EG, Cogbour JN, Collins JT and Garland DJ (1995). *Journal of Medicinal Chemistry***, 38,** 4570
Joshi S, Khosla N, Khare D and Tiwari P (2002). Synthesis and antibacterial screening of novel sulphonamide Mannich bases, *Acta Pharmaceutica*, **52,** 197.
Joshi S, Khosla N and Tiwari P (2004). Synthesis and studies of some new antibacterial Mannich bases, *Bioorganic and Medicinal Chemistry*, **12,** 571-576
Joshi S, Khosla N and Khare D (2003). QSAR study on antibacterial activity of sulphonamides and derived Mannich bases, *Bioorganic and Medicinal Chemistry Letters***, 13,** 3747-51.
Joshi S, Manikpuri AD and Tiwari P (2007). Synthesis of a variety of Mannich bases from hetrocyclic aromatic substituted amide and secondary amines, *Bioorganic and Medicinal Chemistry Letters*, **17**, 645
Joshi S, Khare D and Manikpuri AD (2009). Synthetic, spectroscopic and antimicrobial studies of Mannich bases through active hydrogen compounds, *International Journal of Chemical Sciences***, 7** (2), 825-836.
Agarwal S, Pande A and Saxena VK (1985). *Acta Pharmaceutica Jugoslavica*, **35**, 31-39.
Silverstein RM, Bassler GC and Morrill TC (1998). Spectrometric Identification of Organic Compounds, 4th ed., (John Willey & Sons), Inc., New York.
United States Pharmacoepia (1882). 25th ed., By Authority of United States Pharmacoepial Convention Inc., Vol. II, 2002.