STUDIES OF BIOLOGICALLY POTENTIAL MANNICH BASES OF 3, 5-DINITROBENZOYL-4-AMINOBENZAMIDE

Sheela Joshi*[1] and Navita Khosla[2]

1. School of Chemical Sciences, D.A.V.V.,Takshila Campus, Khandwa road, Indore (INDIA)
2. Mathuradevi Institute of Technology and Management,Gari pipliya, near Hansakhedi, Nemawar road, Indore (INDIA)

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ABSTRACT

A series of novel Mannich bases of 3,5-dinitrobenzoyl-4-aminobenzamide were synthesized using Mannich reaction- a biosynthetic route to several drugs. They were characterized and subjected to acute antibacterial screening studies against several positive and gram negative bacteria at varying concentrations. All the derivatives belonging to this series delineated remarkable biological activity. They are nontoxic as ascertained by LD_{50} Test.

Key Words : Mannich Reaction, Mannich Bases, Antibacterial Screening, Statistical analysis, LD_{50} test, Toxicity

INTRODUCTION

Mannich base is a result of the condensation of a compound capable of supplying active hydrogen atom with aldehyde and amine. Several biologically active and medicinally useful Mannich bases have been critically reviewed by Tramontini and Angiolini[1].

Mannich bases are found to contain aminomethyl group which enhances the biological activity[2]. The versatile utility of the Mannich bases in the field of polymers, dispersants in lubricating oil and in pharmaceutical and medicinal chemistry[3-7] promoted us to synthesize and evaluate Mannich bases. The Mannich bases were synthesized from 3,5-dinitrobenzoyl-4-amino benzamide and several secondary amines. They were screened for their antibacterial activity against pathogenic strains of *E. coli*, *K. pneumonae, S. typhosa, S. aureus*. Their toxicity was ascertained by LD_{50} test.

MATERIAL AND METHODS

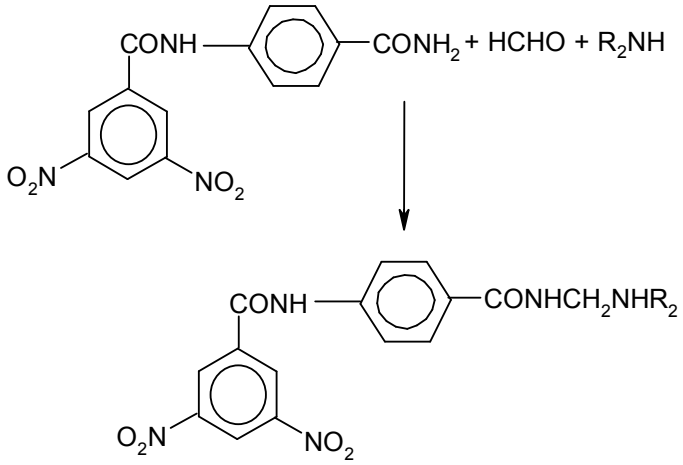
Synthesis of Mannich Bases from Secondary Amine

The ethanolic solution of 0.01 mol of 3,5-dinitrobenzoyl-4-amino benzamide was taken in a flat bottom flask fitted with a condenser. To this 0.01 mol of secondary amine and one half of 0.015 mol of HCHO solution (37%) were added. The reaction mixture was stirred on a magnetic stirrer and refluxed simultaneously at a temperature of 70-75°C. Reflux time varied with secondary amine used. The remaining portion of HCHO solution was added in two portions at an interval of one hour

*Author for correspondence

i.e. first and second hour from the start of the reaction respectively. Reaction mixture was kept overnight in the refrigerator. The excess of solvent was removed from mixture under pressure next day and kept for crystallization in refrigerator.

The crystallized product was recrystallized with dry methanol.



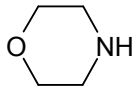
3,5-dinitrobenzoyl-4-aminobenzamidomethyl amine

$(CH_3)_2NH$; dimethyl amine (I)

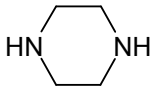
$(C_2H_5)_2NH$; diethylamine (II)

$(C_6H_5)_2NH$; diphenylamine (III)

$(C_2H_4OH)_2NH$; diethanolamine (IV)



morpholine (V)



piperazene (VI)

Antibacterial Screening

Mannich bases were screened against pathogenic bacteria of gram positive and negative strains. The screening is done by cup plate method[8]. Suitable media was prepared for the particular pathogen. All the test were performed in triplicate. The antibacterial activity was ascertained as zone of inhibition in mm. The results were statistically analysed by test of variance[9].

RESULTS AND DISCUSSION

The Mannich bases were synthesized and characterized by elemental analysis, UV, IR and NMR spectroscopy (**Table 1** and **Table 2**). Mannich bases had potential pharmaceutical activity[10].

They were screened for their antibacterial activity at arbitrarily chosen concentrations 40, 80, 120, 160 μg/ml. The activity was ascertained by zone of inhibition

Table 1 : Analytical Data of Mannich Bases (I-VI)

S. No.	Name	Mol. Formula	M.P. °C	Elemental Analysis Found/(Calcd)		
				C	H	N
I	3,5-dinitrobenzoyl-4-aminobenzamido methyl dimethylamine	$C_{17}H_{17}N_5O_6$	100-102	52.36 (52.71)	3.96 (4.39)	17.72 (18.08)
II	3,5-dinitrobenzoyl-4-aminobenzamido methyl diethylamine	$C_{19}H_{21}N_5O_6$	65	54.58 (54.93)	4.71 (5.06)	17.01 (16.86)
III	3,5-dinitrobenzoyl-4-aminobenzamido methyl diphenylamine	$C_{27}H_{21}N_5O_6$	89-90	62.98 (63.40)	4.46 (4.10)	14.00 (13.69)
IV	3,5-dinitrobenzoyl-4-aminobenzamido methyl diethanolamine	$C_{19}H_{21}N_5O_6$	110-112	50.68 (51.00)	4.27 (4.69)	15.19 (15.65)
V	3,5-dinitrobenzoyl-4-aminobenzamido methyl morpholine	$C_{19}H_{19}N_5O_7$	85	53.48 (53.14)	4.90 (4.42)	16.81 (16.31)
VI	3,5-dinitrobenzoyl-4-aminobenzamido methyl piperazene	$C_{34}H_{30}N_{10}O_{12}$	132-133	52.60 (52.98)	3.40 (3.89)	17.65 (18.18)

in mm. The compounds were screened against pathogenic strains of bacteria, *E. coli, K. pneumonae, S. aureus* and *S. typhosa*. The results were statistically analysed.

The Mannich bases I-VI were screened against these micro-organisms and showed good response. The results were statistically analysed and concluded that Mannich base IV and III were highly active in inhibiting the growth of *K. pneumonae* followed by Mannich base I **(Table 3)**. The activity against the pathogen was highest at 80 μg/ml.

Against *E. coli*, Mannich bases I and III showed maximum activity **(Table 3)**. Some compounds also showed some response against *S. aureus* and *S. typhosa*. 3,5-dinitrobenzoyl-4-aminobenzamidomethyl diethylamine gave remarkable response against *S. aureus* at 160 μg/ml concentration (19.8 mm).

Similarly, 3,5-dinitrobenzoyl-4-amino-benzamidomethyl diethanol-amine gave inhibitory effect against *S. typhosa* at 80 μg/ml (19.6 mm).[11]

CONCLUSION

The synthesized Mannich bases were screened for their toxicity. It was ascertained by LD_{50} test. The test was performed on white mice weighing 25 gms. The doses were given orally through catheter tube as well as intraperitoneally. Six mice were kept under observation for 72 hours for each trial[11]. Mice were given normal feed.

The results showed that Mannich bases are non toxic even at oral dose of 6400 mg/kg of the intraperitonially, they proved to be lethal at dose level of 1000 mg/kg of the body weight of mice.

Table 2 : Spectral Data of Mannich Bases (I-IV)

S. No.	UV (λ_{max} in nm)	IR(in cm^{-1})	NMR(δ in ppm)					
			δ CH_2 (d)	NH(S) of Ring I	CONH (S) of Ring I	Aryl Protons (d)	Aryl Protons (d) between NO_2 groups	CONH(t) of Ring II
I	208 (amido moiety), 245 (benzene chromophore)	3499 (νNH of $CONH_2$), 2940 (νCH_2), 2770 (νCH_2), 1680 (> C = O in $CONH_2$), 1619 (δNH), 1535 (νN=O in $ArNO_2$), 1190 (νC-N in $\{CH_2\text{-}N\text{-}(CH_3)_2\}$; 885 (δC-H in 1,3,5-trisubsti- tuted benzene).	2.50	6.0	7.5	7.80	9.00	8.2
II	210 (amido moiety), 250 (benzene chromophore)	3420 (νNH of $CONH_2$), 2995 (νC-H in CH_3), 2940 (νCH_2-N), 2840 (νCH_2), 1680 (> C = O in $CONH_2$), 1538 (νN=O in $ArNO_2$), 1075 (νC-N in amine), 918 (δC-H in 1,3,5-trisubstituted benzene).	2.52	6.0	7.5	7.75	9.00	8.4
III	208 (amido moiety), 245 (benzene chromophore)	3450 (νNH of $CONH_2$), 2940 (νCH_2), 1650 (> C = O in $CONH_2$), 1601 (δNH), 1535 (νN=O in $ArNO_2$), 920 (δC-H in 1,3,5-trisubstituted benzene).	2.55	6.1	7.5	7.80	9.00	8.4
IV	209 (amido moiety), 245 (benzene chromophore)	3350 (νNH of $CONH_2$), 1660 (>C=0 in $CONH_2$), 1601 (δNH), 1535 (νN=O in $ArNO_2$), 920 (δC-H in 1,3,5-trisubstituted benzene).	2.50	6.0	7.6	7.70	9.02	8.4
V	209 (amido moiety), 250 (benzene chromophore)	3401 (νNH of $CONH_2$), 2902 (νCH_2), 1665 (>C=0 in $CONH_2$), 1605 (δNH), 1530 (νN=O in $ArNO_2$), 920 (δC-H in 1,3,5-trisubstituted benzene).	2.54	6.0	7.5	7.89	9.00	8.2
VI	210 (amido moiety), 250 (benzene chromophore)	3250 (νNH of secondary amide), 2900 (νCH_2-N), 1650 (>C=0 in $CONH_2$), 1600 (δNH), 1535 (νN=O in $ArNO_2$), 920 (δC-H in 1,3,5-trisubstituted benzene).	2.52	6.1	7.6	7.80	9.02	8.2

Table 3 : Antibacterial Screening of 3,5-dinitrobenzoyl-4-amino benzamidomethyl amines

S. No.	Compound	*K. pneumonae* 40	80	120	160	*E. coli* 40	80	120	160
I	3,5-dinitrobenzoyl-4-aminobenzamido methyl dimethylamine	23.66	29.66	25.83	26.70	14.76	16.23	14.76	20.30
II	3,5-dinitrobenzoyl-4-aminobenzamido methyl diethylamine	19.16	7.03	7.33	29.76				
III	3,5-dinitrobenzoyl-4-aminobenzamido methyl diphenylamine	27.16	27.00	28.53	26.40				
IV	3,5-dinitrobenzoyl-4-aminobenzamido methyl diethanolamine	26.40	29.96	29.13	27.96	6.10	10.20	13.66	15.43
V	3,5-dinitrobenzoyl-4-aminobenzamido methyl morpholine	19.56	26.00	6.10	7.03				
VI	3,5-dinitrobenzoyl-4-aminobenzamido methyl piperazene	6.00	6.13	8.96	9.46				

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