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SYNTHESIS AND BIOLOGICAL EVALUTION OF MEDICINALLY IMPORTANT MANNICH BASES OF 5-NITRO–2-FURFURALDEHYDE SEMICARBAZONE DERIVED FROM SECONDARY AMINES

SHEELA JOSHI[*a], ANJU DAS MANIKPURI and PRAPTI TIWARI

School of Chemical Sciences, Devi Ahilya University, Takshashila Campus, Khandwa Road, INDORE – 452001 (M. P.) INDIA

ABSTRACT

A series of Mannich bases was prepared from 5-nitro-2-furfuraldehyde semicarbazone by aminomethylation with formaldehyde and secondary amines. All newly synthesized compounds were screened for their antimicrobial activity. Compounds **4a** and **4e** exhibited promising antibacterial activity. Structures of all the newly formed compounds were established on the basis of elemental analysis and spectroscopic data (UV, IR and ^{1}H NMR). The results were analyzed statistically and the synthesized compounds were found to be low lethal as ascertained by LD_{50} test.

Key words : 5-Nitro-2-furfuraldehyde semicarbazone, Secondary amines, Mannich bases, Antimicrobial activity, Toxicity, Statistical analysis.

INTRODUCTION

Mannich bases have gained much importance in recent years due to their diverse biological activities, which is used to produce compounds with pharmaceutical[1,2] and industrial importance[3,4]. Also, a considerable number of secondary amines like morpholine, piperazine, dimethyl amine, diethanol amine and diphenylamine were reported to elicit forms a stable chloramine and generate enamines[5,6], anthelmintic[7] raw material in the production of many pharmaceuticals[8] and FGF-R2 autophosphorylation inhibitors[9]. Semicarbazone has proven to be better selective Kv1.5 blockers[10], antitubercular agents[11] and antioxidant property[12]. 5-Nitro 2- furfuraldehyde semicarbazone was the first nitro heterocyclic compounds, introduced into chemotherapy[13]. It is employed topically to

[*] Author for correspondence; Email: spjoshi11@rediffmail.com (S. Joshi); anju.dm@rediffmail.com (A. D. Manikpuri) Tel.: 091-731-247-8204; Fax: 091-731-247-0372;
[a]M-55, Khatiwala Tank, INDORE – 452001 (M. P.) INDIA

prevent bacterial infections associated with skin graft[14]. Therefore, pharmacological importance of secondary amine nucleus and semicarbazone is well established in pharmaceutical chemistry. This has given an impetus to work with such vibrant moieties using Mannich reaction, a convenient method for introduction of the basic amino alkyl chain, which alters the biological profile and physicochemical characteristics. Various drugs obtained from Mannich reaction have proved to be more effective and less toxic than their parent compounds [15]. With the aim to construct such Mannich bases of versatile utility, We turned our attention to synthesize a series of amino methyl derivatives with secondary amines that would be an important synthon for the evaluation of their biological significance and toxicity.

In view of the above and in continuation of our earlier study[16]. We report herein antibacterial activity of 5-Nitro--2-furfuraldehyde semicarbazone Mannich bases.

EXPERIMENTAL

Materials and methods

All the m. p. are uncorrected and were determined using Thomas Hoover capillary melting point apparatus. The ^{1}H NMR spectra in DMSO and $CDCl_3$ solvent were recorded on Bruker DRX-300 FT NMR Spectrometer. The IR spectra were recorded on Schimadzu 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 [17]. Mullar Hinton agar was taken as media for cultivation of bacteria. The inhibitory effect of the samples were measured against the bacteria after incubation for 24 hours at 37ºC. The experiments were run in triplicate and the mean of readings were recorded. The results were statistically analyzed[18].

Synthesis of Mannich bases from secondary amines

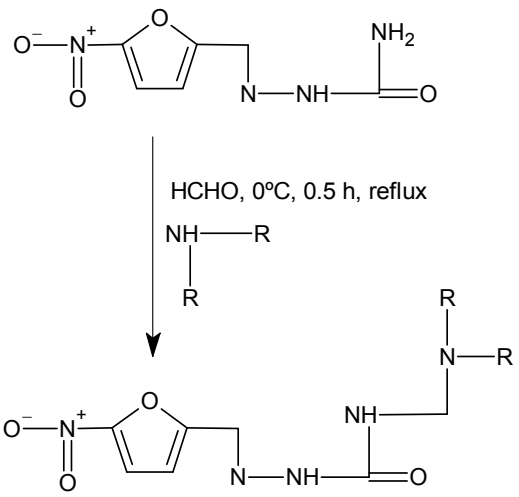
Secondary amine (0.01 mol) was added to an ethanolic solution (50 mL) of 5-Nitro-- 2-furfuraldehyde semicarbazone (0.01 mol) in a flat bottom flask. Amount of 0.4 mL (0.015 mol) of formaldehyde solution (37%, v/v) was added slowly with constant stirring. The reaction mixture was stirred at 70-75 ^{0}C 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 with their analytical data are presented in Tables 1 and 2.

RESULTS AND DISCUSSION

Spectral studies

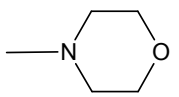
Synthesis of Mannich bases from secondary amines is represented in **Scheme 1.**



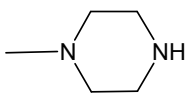
R = —CH_3 3a - 4a

—C_6H_5 3b - 4b

—C_2H_4OH 3c - 4c



3d - 4d



3e - 4e

Scheme 1

The newly formed Mannich bases were characterized by elemental analysis and

spectral data (UV, IR and ^{1}H NMR). The absorption bands of Mannich bases of 5-Nitro--2-furfuraldehyde semicarbazone methylamines totally agree with the anticipated structure. The physical characterization and spectral data are presented in Tables 1 and 2.

Table 1 : Physical characterization data of synthesized compounds

Comp.	Compounds	Molecular formula	M. P. (°C)	Elemental analysis [Found (Calcd.) %]		
				C	H	N
4a	5-Nitro-2-furaldehyde *N*-[(dimethylamino) methyl]semicarbazone	$C_9H_{13}N_5O_4$	195-196	42.95 (42.35)	5.32 (5.09)	27.62 (27.45)
4b	5-Nitro-2-furaldehyde *N*-[(diphenylamino) methyl]semicarbazone	$C_{19}H_{17}N_5O_4$	198-199	60.45 (60.15)	4.88 (4.48)	18.79 (18.46)
4c	5-Nitro-2-furaldehyde *N*-{[bis(2-hydroxyethyl) amino]methyl}semicarbazone	$C_{11}H_{17}N_5O_6$	193	41.39 (41.90)	5.25 (5.39)	22.13 (22.22)
4d	5-Nitro-2-furaldehyde *N*-[(morphilino) methyl]semicarbazone	$C_{11}H_{15}N_5O_5$	183-184	44.65 (44.44)	5.30 (5.05)	23.25 (23.56)
4e	5-Nitro-2-furaldehyde *N*-[(piperazino) methyl]semicarbazone	$C_{11}H_{16}N_6O_4$	138-140	44.51 (44.54)	5.39 (5.40)	28.49 (28.37)

Table 2 : Spectral data of prepared compounds

Comp.	UV (λ_{max} in nm)	IR (cm^{-1})	^{1}H NMR (δ in ppm)
4a	208 (C=O), 230 (C=N=N), 250 (Ar. ring), 305 (5-Nitro-furan derivatives).	3456 ν_{as} (NH) in sec. amide, , 3058 ν (=C-H) of hetero-aromatic ring, 2911 ν_{as} C-H in CH_2, 2729 ν >CH_2N<, 1680 ν (C=O), 1540 ν_{as} N⁻O, 1249 ν(C-H)	2.70 (d, 2H, *J* = 8.94, CH_2); 5.40 (s, 1H, NH); 6.50 (d, =CH-CH ring protons, *J* = 9.2); 6.70 – 7.2 (m, ArH); 7.10 (s, 1H, CONH); 7.80 (s, 1H,

Comp.	UV (λ_{max} in nm)	IR (cm^{-1})	^{1}H NMR (δ in ppm)
		in 1, 4 disubstituted benzene, 1087, ν_{as} C-O-C, 943 out of plane δ C-H in trisubstituted heteroaromatic ring, 867 ν C-N in $ArNO_2$	=CH-N); 7.90 (s, 1H, =N-NH)
4b	210 (C=O), 230 (azomethine), 252 (Ar. rRing), 307 (5-Nitro-furan derivatives).	3400 ν_{as} (NH) in sec amide, 2950 ν_{as} C-H in CH_2, 2805 ν >CH_2N<, 1660 ν (C=O) of sec. amide, 1540 ν_{as} N –O in $ArNO_2$, 1160 δ C-H in heteroaromatic ring, 1240 δ C-H in 1 : 4 disubstituted benzene, 875 ν C-N in $ArNO_2$	2.90 (d, 2H, *J* = 8.94, CH_2); 5.30 (s, 1H, NH); 6.50 (d, =CH-CH ring protons, *J* = 9.2); 6.80 – 8.01 (m, ArH); 7.10 (s, 1H, CONH); 7.80 (s, 1H, =CH-N); 7.90 (s, 1H, =N-NH)
4c	208 (C=O), 232 (azomethine), 251 (Ar. Ring), 306 (5-Nitro-furan derivative).	3400 ν_{as} (NH) in sec. amide, 2910 ν C-H in CH_2, 2790 -CH_2N <, 1680 ν (C=O) in sec. amide, 1580 δ NH, 1540 ν_{as} N –O in $ArNO_2$, 1250 in plane δ C-H in 1 : 4 disubstituted benzene, 870 ν C-N in $ArNO_2$	2.55 (d, 2H, *J* = 8.94, CH_2); 5.40 (s, 1H, NH); 6.40 (d, =CH-CH ring protons, *J* = 9.2); 6.70 – 7.8 (m, ArH); 7.08 (s, 1H, CONH); 7.80 (s, 1H, =CH-N); 7.90 (s, 1H, =N-NH)
4d	209 (C=O), 235 (azomethine), 250 (Ar. ring), 310 (5-Nitro-furan derivative)	3450 ν_{as} (NH) in sec. amide, 2905 ν_{as} C-H in CH_2, 2805 vib. due to -CH_2N<, 1680 ν (C=O) in sec. amide, 1540 ν_{as} N –O in $ArNO_2$, 1255 ν C-H in 1 : 4 disubstituted benzene, 1200 C-H in plane bending in heteroatomic ring, 845 ν C-N in $ArNO_2$	3.05 (d, 2H, *J* = 8.94, CH_2); 5.40 (s, 1H, NH); 6.40 (d, =CH-CH ring protons, *J* = 9.2); 6.65 – 8.0 (m, ArH); 7.20 (s, 1H, CONH); 7.80 (s, 1H, =CH-N); 8.10 (s, 1H, =N-NH) SO_2NH)

Comp.	UV (λ_{max} in nm)	IR (cm^{-1})	^{1}H NMR (δ in ppm)
4e	210 (C=O), 234 (azomethine), 251 (Ar. Ring), 305(5-Nitro-furan derivative)	3400 ν_{as} (NH) in sec. amide, 2900 ν_{as} C-H in CH_2, 2750 vib. due to -$CH_2N<$, 1685, 1660 ν (C=O) in sec. amide, 1540 ν N–O in $ArNO_2$, 1250 δ C-H in 1 : 4 disubstituted benzene, 1210 in plane bending vib. of C-H in heteroaromatic ring, 880 ν C-N in $ArNO_2$	2.60 (d, 2H, J = 8.94, CH_2); 5.30 (s, 1H, NH); 6.40 (d, =CH-CH ring protons, J = 9.2); 6.80 – 8.20 (m, ArH); 7.10 (s, 1H, CONH); 7.70 (s, 1H, =CH-N); 7.90 (s, 1H, =N-NH)

UV spectra of compounds **4a-4e** showed absorption bands at 208 ± 2 nm is observed due to amido moiety, whereas absorption band at 230 ± 5 nm is observed due to azomethine group. Another absorption maxima at 250 ± 2 nm correspond to benzene chromophore present in molecule. However, the band at 305 ± 5 nm, assigned to 5-Nitro--furan derivatives, is also observed in Mannich bases.

IR spectra further confirmed the anticipated structure. IR spectra of compounds showed peaks at 3450 ± 50 cm^{-1}, which is characterized as the NH due to secondary amide. Absorption bands at 2940 ± 30 cm^{-1} and 2850 ± 10 cm^{-1} correspond to C-H methylene group. The CH_2 scissoring vibrations at 1460 ± 10 cm^{-1} confirm the presence of methylene bridge between amido and amino moiety in the compounds under study. Band obtained at 2790 ± 25 cm^{-1} is assigned to –$CH_2N<$ group.

The ^{1}H NMR spectra of 5-Nitro- 2-furfuraldehyde semicarbazone methylamines showed resonance absorption due to aryl protons, NH protons, alkyl protons and heteroaryl protons. All the compounds show signal due to CH_2 protons as a doublet in the region δ 2.7 – 2.73 ppm (J = 8.94 Hz). The signal at δ 6.56 – 6.59 ppm (J = 9.20 Hz) as a doublet is observed due to heteroatomic ring protons.

Table 3. Antibacterial screening of prepared Mannich bases (zone of inhibition in mm)

Comp.	*S. enteritidis* (Conc. in mg/mL)				*P. multocida* (Conc. in mg/mL)				*B. anthracis* (Conc. in mg/mL)				*S. aureus* (Conc. in mg/mL)			
	20	**30**	**40**	**Avg**	**20**	**30**	**40**	**Avg**	**20**	**30**	**40**	**Avg**	**20**	**30**	**40**	**Avg.**
4a	18.20	18.40	19.00	18.53	12.60	13.55	14.02	13.39	13.00	13.46	14.06	13.50	16.04	16.24	16.80	18.36
4b	13.06	15.00	17.60	15.22	10.06	10.60	12.60	11.08	-	-	-	-	16.00	17.00	18.00	17.00
4c	15.08	15.46	19.40	16.64	11.04	11.56	12.30	11.63	-	-	-	-	-	-	-	-
4d	12.62	15.48	15.02	14.04	8.00	10.55	11.20	9.91	26.00	26.60	28.00	26.86	17.00	17.60	17.80	17.46
4e	23.62	24.06	25.60	23.75	25.00	25.40	26.00	25.40	25.88	26.20	27.00	26.36	20.00	22.66	24.00	22.88
Avg. of conc.	16.51	17.68	19.32		13.34	14.33	15.22		21.62	22.08	23.02		17.26	18.37	19.15	

	S.Ed.	**CD at 5%**	**S.Ed.**	**CD at 5%**	**S.Ed.**	**CD at 5%**	**S.Ed.**	**CD at 5%**
Compd	0.118	0.246	0.082	0.171	0.068	0.147	0.091	0.19
Conc.	0.053	0.107	0.016	0.032	0.036	0.073	0.022	0.044
Inter-action	0.184	0.382	0.053	0.11	0.102	0.219	0.073	0.153

S.Ed-standard error difference; CD-critical difference

Antimicrobial activity

The Mannich bases were screened for the biological significance. The antibacterial screening of duly characterized Mannich bases was performed using paper disc method against some pathogenic strains of *Salmonella entritidis, Pasturella multocida, Bacillus anthracis and Staphylococcus aureus*. Table 3 revealed significant results of Mannich bases against S. *enteritidis*. The interaction of concentration levels on zone of inhibition in each compound revealed that Mannich bases **4a** and **4e** were significantly active at 40 mg/mL against this pathogen rather at 30 mg/mL. The Mannich base **4e** is significantly superior to other Mannich bases in exhibiting their inhibition against *P. multocida*. Compound **4d** and **4e** were found to be highly active against *B. anthracis* and *S. aureus*, respectively. All the novel Mannich bases gave excellent response against chosen pathogens.

Toxicity

The Mannich bases were also screened for their toxicity by preliminary LD_{50} test. Mannich bases were found to show no adverse effects even at an oral dose of 6.4 g/kg of body mass 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.

CONCLUSION

The obtained Mannich bases appeared to be very potent outstanding antibacterial agents with promising activity and found safer. We, therefore, conclude that the obtained novel Mannich bases could be used as useful drug. Our findings will prove helpful to those who are engrossed in the synthesis of potential Mannich bases as drugs with minimum side effects and also having comparatively low cost. Thus, the manner represented in this paper is valuable in constructing pharmacologically imperative heterocycles as a new exotic drug. Efforts are continuing to synthesize new amino methyl derivatives of 5-Nitro-2-furfuraldehyde semicarbazones so that the derived compounds may have enhanced pharmacological activity.

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