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Synthesis and in vitro study of novel Mannich bases as antibacterial agents

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Abstract—A series of novel Mannich bases derived from 5-chloro-2-methoxybenzamide and sulfonamides/amines have been synthesised and the antibacterial activities were evaluated against various Gram positive and Gram negative strains of bacteria. Some of the synthesized compounds showed superior in vitro activities as compared to their parent sulfonamides.

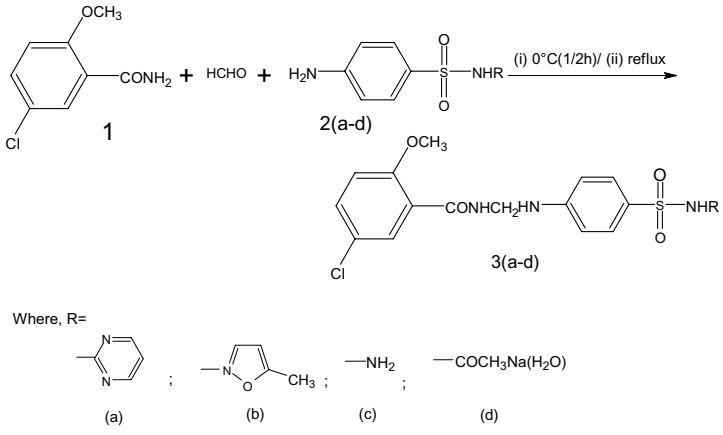
1. Introduction

The importance of sulfonamide nucleus is well established in pharmaceutical chemistry. A considerable number of sulfonamides are well known as antibacterial,[1] carbonic anhydrase inhibitor,[2] anticancerous,[3] anti-inflammatory agents.[4] This has given an impetus for the synthesis of Mannich bases from these compounds using Mannich reaction.[5] This reaction provides a method for the introduction of basic aminoalkyl chain. The various drugs obtained from Mannich reaction are proved to be more effective and less toxic than the parent antibiotic.[6–9]

The versatile utility of the Mannich bases in polymers,[10] dispersents in lubricating oil[11] and pharmaceutical chemistry,[12,13] prompted us to prepare a new series of aminomethyl derivatives and evaluate their biological significance and toxicity.

In view of the above and in continuation of our earlier study,[1,6] in the present study we report comparative study of antibacterial activity of sulfonamides and Mannich bases derived from them.

The Mannich bases under present study were prepared by us following the method described earlier[14,15] (Schemes 1



Scheme 1. Synthesis of Mannich bases from primary amines.

Keywords: Sulfonamides; 5-Chloro-2-methoxybenzamide; Mannich bases; Antibacterial activity; Statistical analysis; LD_{50} test.
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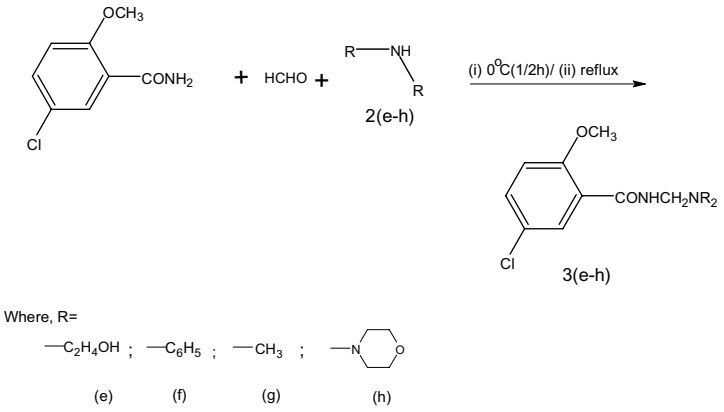

Scheme 2. Synthesis of Mannich bases from secondary amines.

Table 1. Characterisation data of Mannich bases of 5-chloro-2-methoxybenzamide with primary and secondary amines

S. no	Compounds	Molecular formula	Mp (°)	Elemental analysis (%) found (calcd)		
				(C)	(H)	(N)
3a	5-Chloro-2-methoxybenzamidomethyl sulfadiazine	$C_{19}H_{18}N_5O_4SCl$	160–165	50.47 (50.95)	4.13 (4.02)	15.58 (15.64)
3b	5-Chloro-2-methoxybenzamidomethyl sulfamethoxazole	$C_{19}H_{19}N_4O_5SCl$	110–112	50.78 (50.61)	4.32 (4.22)	12.22 (12.43)
3c	5-Chloro-2-methoxybenzamidomethyl sulfanilamide	$C_{15}H_{16}N_3O_4S$ Cl	165–170	48.59 (48.71)	4.19 (4.33)	11.29 (11.36)
3d	5-Chloro-2-methoxybenzamidomethyl sulfacetamidesodium	$C_{17}H_{17}N_3O_5SClNa$	210–215	47.52 (47.06)	3.76 (3.92)	9.19 (9.68)
3e	5-Chloro-2-methoxybenzamidomethyl diethanolamine	$C_{13}H_{19}N_2O_4Cl$	120	51.46 (51.57)	6.07 (6.28)	8.96 (9.25)
3f	5-Chloro-2-methoxybenzamidomethyl diphenylamine	$C_{21}H_{19}N_2O_2Cl$	155–160	68.37 (68.75)	5.03 (5.18)	7.26 (7.64)
3g	5-Chloro-2-methoxybenzamidomethyl dimethylamine	$C_{11}H_{15}N_2O_2Cl$	185–190	54.13 (54.43)	6.01 (6.18)	10.85 (11.54)
3h	5-Chloro-2-methoxybenzamidomethyl morpholine	$C_{13}H_{17}N_2O_3Cl$	110–115	54.23 (54.83)	5.63 (5.97)	9.02 (9.84)

and 2) and were characterised by elemental analysis (Table 1), UV, IR and ^{1}H NMR spectroscopy (Table 2). The comparative biological significance of sulfonamides as well as the Mannich bases derived from them was asserted by evaluation of their antibacterial activity. The compounds were active only against three bacteria (Table 3).

2. Results and discussion

The sulfonamides used were of BDH and/or equivalent grade. The Mannich bases were synthesised (Schemes 1 and 2) and characterised by elemental analysis, UV, IR and ^{1}H NMR spectral studies (Tables 1 and 2, Schemes 1 and 2). The characteristic UV bands with λ_{max}: 230, 210, 219, 235, 250, 260 were indicative of the presence of secondary amines, amido, sulfoxide, aromatic nucleus, benzene nucleus containing OCH_3 group and sulfonamide moiety. The presence of these moieties was further confirmed by IR spectral studies. The bands in cm^{-1} obtained at 3450, 3350, 2920, 2850, 2810, 1710, 1385, 1310, 1175 confirms the presence of NH of sulfonamide, CH_2 group, CH of OCH_3, C=O of secondary amide, NH bending and νCN, S=O of sulfonamide moiety.

The structural confirmation is further made using ^{1}H NMR spectra. It shows signals at δ, ppm: 2.50 (d, 2H, CH_2 proton $J = 5.08$ Hz), 5.850–6.1 (s, 1H, N^{4}H of sulfonamide), 6.09–6.12 (t, 1H of CONH of ring, $J = 13.54$ Hz), 7.16–7.19 (d, 1H *ortho* to OCH_3 $J = 27.08$ Hz), 10.68–11.06 (s, 1H, SO_2N^1H proton). Thus, confirming the proposed structure.

Biological significance of 5-chloro-2-methoxybenzamidomethyl amines was established by screening them against *Escherichia coli* (*E. coli*), *Salmonella enteritidis* (*S. enteritidis*), *Pasturella multocida* (*P. multocida*), *Bacillus anthracis* (*B. anthracis*), *Staphylococcus aureus* (*S. aureus*) (Table 3). Hence afterwards we have used abbreviated forms given in the bracket. The antibacterial activities of the parent sulfonamides were also determined and recorded in Table 4 for comparison. The results were statistically analysed.[16] The activities reported were mean of zone of inhibition in millimetre (in triplicate).

Table 3 reveals that the antibacterial potential of Mannich base (**3d**) is statistically similar to that of the Mannich base (**3h**) in checking the growth of *E. coli* but significantly superior to Mannich bases **3a**, **3b**, **3c** and **3e**. Moreover, all compounds, in general, showed significant activity at concentration 160 mg mL^{-1}. In Table 4, we observed that Mannich base (**3d**) shows more pronounced activity against *E. coli* as compared to sulfonamide.

The Mannich base **3d** and **3h** are statistically at par to those of Mannich base **3b**, which is significantly superior

Table 2. Spectral data of compounds **3a**–**h**

Compd	UV (λ_{max} values in mm)	IR (values in cm^{-1})	NMR (δ values in ppm)
3a	208 (C═O), 219 (S═O), 230 (Ar ring), 250 (benzene nucleus containing OCH_3 group), 261 (sulfonamide moiety)	3456 ν_{as} (NH) in sec. amide, 3323 ν_{NH} of SO_2NH, 3058 ν (═C–H) of hetero-aromatic ring, 2911 ν_{as} C–H in CH_2, 2729 ν >CH_2N<, 1680 ν (C═O), ν_{as} S═O, 1385 δ NH	2.50 (d, 2H, J = 5.08 Hz); 5.80 (s, 1H, N^4H of sulfonamide); 6.09 (t, 1H of CONH of ring, J = 13.54 Hz), 7.16 (d, 1H *ortho* to OCH_3, J = 27.08 Hz), 10.68 (s, 1H, SO_2N^1H)
3b	210 (C═O), 218 (S═O), 230 (Ar Ring), 252 (benzene nucleus containing OCH_3 group), 264 (sulfonamide moiety)	3400 ν_{as} (NH) in sec. amide, 3350 ν_{NH} of SO_2NH, 2950 ν_{as} C–H in CH_2, 2805 ν >CH_2N<, 1660 ν (C═O) of sec. amide, 1340, ν_{as} S═O, 1384 δ NH	2.49 (d, 2H, J = 5.08 Hz); 5.70 (s, 1H, N^4H of sulfonamide); 6.1 (t, 1H of CONH of ring, J = 13.54 Hz), 7.18 (d, 1H *ortho* to OCH_3, J = 27.08 Hz), 10.7 (s, 1H, SO_2N^1H)
3c	208 (C═O), 220 (S═O), 232 (Ar ring), 251 (benzene nucleus containing OCH_3 group), 261 (sulfonamide moiety)	3400 ν_{as} (NH) in sec. amide, 3360 ν_{NH} of SO_2NH, 2910 ν C–H in CH_2, 2790 –CH_2N<, 1680 ν (C═O) in sec. amide, 1580 δ NH, 1345 ν_{as} S═O	2.50 (d, 2H, J = 5.08 Hz); 5.8 (s, 1H, N^4H of sulfonamide); 6.11 (t, 1H of CONH of ring, J = 13.54 Hz), 7.19 (d, 1H *ortho* to OCH_3, J = 27.08 Hz), 11.0 (s, 1H, SO_2N^1H)
3d	209 (C═O), 219 (S═O), 235 (Ar ring), 250 (benzene nucleus containing OCH_3 group), 263 (sulfonamide moiety)	3450 ν_{as} (NH) in sec. amide, 3300 ν_{NH} of SO_2NH, 2905 ν_{as} C–H in CH_2, 2805 vib. due to –CH_2N<, 1680 ν (C═O) in sec. amide, 1340, ν_{as} S═O, 1380 δ NH	2.51 (d, 2H, J = 5.08 Hz); 6.0 (s, 1H, N^4H of sulfonamide); 6.12 (t, 1H of CONH of ring, J = 13.54 Hz), 7.16 (d, 1H *ortho* to OCH_3, J = 27.08 Hz), 10.9 (s, 1H, SO_2N^1H)
3e	210 (C═O), 231 (Ar ring), 252 (benzene nucleus containing OCH_3 group)	3400 ν_{as} (NH) in sec. amide, 3350 ν_{NH} of SO_2NH, 2900 ν_{as} C–H in CH_2, 2750 vib. due to –CH2N<, 1685, 1660 ν (C═O) in sec. amide, 1385 δ NH, 1194 ν CH in sec. amine	2.5 (d, 2H, J = 5.08 Hz); 6.10 (t, 1H of CONH of ring, J = 13.54 Hz), 7.18 (d, 1H *ortho* to OCH_3, J = 27.08 Hz)
3f	210 (C═O), 234 (Ar ring), 251 (benzene nucleus containing OCH_3 group)	3400 ν_{as} (NH) in sec. amide, 3350 ν_{NH} of SO_2NH, 2900 ν_{as} C–H in CH_2, 2750 vib. due to –CH2N<, 1685, 1660 ν (C═O) in sec. amide, 1380 δ NH, 1189 ν CH in sec. amine	2.5 (d, 2H, J = 5.08 Hz); 6.11 (t, 1H of CONH of ring, J = 13.54 Hz), 7.16 (d, 1H *ortho* to OCH_3, J = 27.08 Hz)
3g	210 (C═O), 232 (Ar ring), 252 (benzene nucleus containing OCH_3 group)	3400 ν_{as} (NH) in sec. amide, 3300 ν_{NH} in SO_2NH, 2900 ν_{as} C–H in CH_2, 2800 vib. due to –CH_2N<, 1660 ν (C═O) in sec. amide, 1384 δ NH, 1190 ν CH in sec. amine	2.51 (d, 2H, J = 5.08 Hz); 6.09 (t, 1H of CONH of ring, J = 13.54 Hz), 7.19 (d, 1H *ortho* to OCH_3, J = 27.08 Hz)
3h	208 (C═O), 235 (Ar ring), 250 (benzene nucleus containing OCH_3 group)	3400 ν_{as} (NH) in sec. amide, 3200 ν_{NH} of SO_2NH, 2950 ν_{as} C–H in CH_3, 2900 ν_{as} C–H in CH_2, 2750 vib. due to –CH_2N<, 1680 ν (C═O) in sec. amide, 1385 δ NH, 1195 ν C–H in sec. amines	2.51 (d, 2H, J = 5.08 Hz); 6.12 (t, 1H of CONH of ring, J = 13.54 Hz), 7.16 (d, 1H *ortho* to OCH_3, J = 27.08 Hz)

Table 3. Antibacterial activity of 5-chloro-2-methoxybenzamidomethyl amines

Compd	Zone of inhibition (mm)											
	E. coli				*K. pneumoniae*				*B. subtilis*			
	Concentrations (mg/mL)				Concentrations (mg/mL)				Concentrations (mg/mL)			
	40	80	160	Average	40	80	160	Average	40	80	160	Average
3a	8.0	8.5	9.5	8.67	7.5	8.0	8.5	8.0	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	10.0	12.5	15.5	12.67	10.5	12.5	16.5	13.17
3e	7.0	7.5	8.5	7.67	7.0	7.5	7.5	7.33	7.0	7.5	8.5	7.67
3f	—	—	—	—	7.0	7.0	8.0	7.33	7.5	7.5	8.0	7.67
3g	—	—	—	—	7.0	7.5	7.5	7.33	—	—	—	—
3h	9.5	10.5	12.5	10.67	10.5	12.5	14.5	12.50	8.5	9.0	10.5	9.33

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

Compd	*S. aureus*				*S. typhae*				*P. aeruginosa*			
	Concentrations (mg/mL)				Concentrations (mg/mL)				Concentrations (mg/mL)			
	40	80	160	Average	40	80	160	Average	40	80	160	Average
3a	9.5	11.5	15.0	12.00	7.0	7.0	7.5	7.17	7.5	7.5	8.5	7.83
3b	8.5	9.5	12.5	10.17	7.5	7.5	8.5	7.83	7.0	7.5	8.0	7.50
3c	7.5	8.5	11.0	9.00	7.0	7.5	8.0	7.50	7.5	8.0	8.5	8.00
3d	10.5	14.5	22.0	15.67	10.5	14.5	22.0	15.67	9.5	12.5	14.5	12.17
3e	—	—	—	—	—	—	—	—	—	—	—	—
3f	—	—	—	—	7.5	7.5	8.5	7.83	—	—	—	—
3g	—	—	—	—	—	—	—	—	7.0	7.5	7.5	7.33
3h	11.0	13.5	16.5	13.67	10.5	12.0	13.5	12.00	9.5	10.5	12.0	10.67

	Statistical data	*S. aureus*	*S. typhae*	*P. aeruginosa*
Compound	SEd	0.231	0.353	0.343
	CD at 5%	0.533	0.786	0.763
Concentration	SEd	0.175	0.274	0.193
	CD at 5%	0.364	0.566	0.398
Interaction	SEd CD at 5%	0.671	0.671	0.473
	CD at 5%	1.386	1.386	0.976

SEd = standard error of difference, CD = critical difference.

to **3a**,**c**,**e**–**g** in inhibiting the growth of *Klebsiella.* The concentration level of 160 mg/mL is superior in checking the growth of the bacterium. When the antibacterial activity of newly synthesised Mannich bases were compared to sulfonamides (Table 4), it was found that Mannich bases **3b**–**d** is significantly superior to sulfonamides against *K. pneumoniae*.

The Mannich base **3d** was significantly superior to other Mannich bases. However, Mannich bases **3c**, **3h** are statistically at par in their antimicrobial activity but superior to **3a**,**b**,**e**,**f**. Again significant inhibition occurred at concentration of 160 mg/mL in case of *B. subtilis*.

Table 3 reveals that Mannich base **3d** is significantly superior to all other Mannich bases in inhibiting *S. aureus*. Following **3d** is **3h**, which is superior to **3a** followed by **3b** and **3c**. Concentration 160 mg/mL is significantly superior to other concentrations in checking the growth of *S. aureus.* When we compared these Mannich bases with corresponding sulfonamides, we found Mannich base **3a** shows significantly superior antibacterial activity than its corresponding sulfonamide.

The Mannich base **3d** is statistically similar to Mannich base **3h** in checking the growth of *S. typhae* but significantly superior to Mannich bases **3a**–**c**,**f**. Moreover, all compounds showed in general significant activity at concentration 160 mg/mL. Compared to sulfonamides (Table 4) we observed that Mannich base **3c** and corresponding sulfonamide (sulfanilamide) are at par in their antibacterial activity. The Mannich base **3d** is significantly superior in their antibacterial activity than its corresponding sulfonamide, which fails to show any activity against *S. typhae* at these arbitrarily chosen concentrations.

Table 4. Antibacterial activity of 5-chloro-2-methoxybenzamidomethyl sulfonamides as compared to sulfonamides

Compd	Zone of inhibition (mm)											
	E. coli				*K. pneumoniae*				*B. subtilis*			
	Concentrations (mg/mL)				Concentrations (mg/mL)				Concentrations (mg/mL)			
	40	80	160	Average	40	80	160	Average	40	80	160	Average
3a	8.0	8.5	9.5	8.67	7.5	8.0	8.5	8.0	7.0	7.0	7.5	7.17
Sulfadiazine	8.5	9.5	12.5	10.17	12.5	13.5	15.0	13.67	8.5	9.5	13.5	10.50
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
Sulfamethoxazole	18.5	20.5	22.5	20.50	8.5	9.0	11.5	9.67	15.5	18.5	20.0	18.00
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
Sulfanilamide	16.5	18.0	20.5	18.33	—	—	—	—	10.5	11.5	13.0	11.67
3d	9.5	10.5	13.0	11.00	10.0	12.5	15.5	12.67	10.5	12.5	16.5	13.17
Sulfacetamide sodium	—	—	—	—	—	—	—	—	9.5	10.0	15.0	11.50

		E. coli	*K. pneumoniae*	*B. subtilis*
*MB & S	SEd	0.661	0.235	0.322
	CD at 5%	1.472	0.576	0.691
Concentration	SEd	0.139	0.191	0.131
	CD at 5%	0.288	0.405	0.267
Interaction	SEd	0.343	0.382	0.369
	CD at 5%	0.707	0.809	7.55

Compd	Zone of inhibition (mm)											
	S. aureus				*S. typhae*				*P. aeruginosa*			
	Concentrations (mg/mL)				Concentrations (mg/mL)				Concentrations (mg/mL)			
	40	80	160	Average	40	80	160	Average	40	80	160	Average
3a	9.5	11.5	15.0	12.00	7.0	7.0	7.5	7.17	7.5	7.5	8.5	7.83
Sulfadiazine	7.0	7.5	9.5	8.00	7.0	7.5	9.5	8.00	—	—	—	—
3b	8.5	9.5	12.5	10.17	7.5	7.5	8.5	7.83	7.0	7.5	8.0	7.50
Sulfamethoxazole	19.5	20.5	21.0	20.33	8.5	9.0	12.5	10.00	—	—	—	—
3c	7.5	8.5	11.0	9.00	7.0	7.5	8.0	7.50	7.5	8.0	8.5	8.00
Sulfanilamide	20.5	22.5	25.0	22.67	7.0	7.5	7.5	7.33	13.5	15.5	17.5	15.50
3d	10.5	14.5	22.0	15.67	10.5	14.5	22.0	15.67	9.5	12.5	14.5	12.17
Sulfacetamide sodium	19.5	21.0	24.0	21.50	—	—	—	—	—	—	—	—

		S. aureus	*S. typhae*	*P. aeruginosa*
*MB & S	SEd	0.293	0.143	0.173
	CD at 5%	0.628	0.317	0.745
Concentration	SEd	0.155	0.149	0.277
	CD at 5%	0.317	0.309	0.639
Interaction	SEd	0.439	0.367	0.392
	CD at 5%	0.896	0.756	0.904

*MB & S = Mannich bases and sulfonamides.
SEd = standard error of difference, CD = critical difference.

Table 3 shows that Mannich base **3d** is significantly superior to **3h** followed by **3a**, **3b**, **3c** and **3g** in exhibiting their antibacterial activity against *P. aeruginosa*. The concentration level 160 mg/mL, in general is significantly superior in checking the growth of this bacterium. It was found that Mannich bases **3a**, **3b** and **3d** are significantly superior in their antibacterial activity than their corresponding sulfonamides.

3. Conclusion

The 5-chloro-2-methoxybenzamidomethyl amines (Mannich bases) appeared to be better and more potent antibacterial agents than the sulfonamides themselves. We, therefore, conclude that the Mannich bases could be used as useful drug in preference to sulfonamides. Our findings will prove useful to those chemists, pharmacists, medicinal chemists who are interested in the synthesis of potential Mannich bases as drugs with minimum side effects and also having comparatively low cost.

4. Experimental

All the m.p. are uncorrected. 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 Schimadzu UV-160A, UV–visible spectrophotometer.

All substituted sulfonamides were obtained as pure samples from the reputed pharmaceutical concern. Solvents used were distilled before use. Schemes 1 and 2

represents the schematic sketch of the synthesis of Mannich bases (**3a**–**h**).

4.1. General procedure

4.1.1. Mannich bases derived from sulfonamides. To the ethanolic solution of 0.1 mol of amide was added to 0.1 mol of sulfonamide slowly with constant stirring under rigorous ice cooling. The reaction mixture was cooled well and 2.5 mL of formaldehyde solution (37% v/v) was added slowly with constant stirring. The reaction mixture was then adjusted to the pH of 3.5 with hydrochloric acid. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then refluxed on water bath. The reflux time was dependent upon the sulfonamide chosen. After refluxing, the refluxed mixture was cooled at 0 °C for 4 days, when crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF. Melting points were recorded and uncorrected (Table 1). The purity of the compounds was ascertained by single spot during TLC where mobile phase was chloroform/methanol mixture (90:10) and stationary phase was silica gel-G (chromatographic grade).

4.1.2. Mannich bases derived from secondary amines. Secondary amines (0.01 mol) were added to an ethanolic solution (50 mL) of 5-chloro-2-methoxybenzamide (0.01 mol) in a flat-bottomed flask. One half of 0.015 mol of formaldehyde solution (37%) was added slowly with constant stirring. The reaction mixture was stirred at 70–75 °C on a magnetic stirrer for 5.5 and 8.5 h, except for diethanolamine (3 h), depending upon the secondary amine taken. The remaining portion of formaldehyde solution was added in two installments at an interval of 1 h, that is first and second hour from the start of the reaction, respectively. The reaction mixture was kept overnight in the refrigerator. The excess of solvent was distilled off from the reaction mixture on vacuum pump, that is under reduced pressure next day. It was again kept for crystallization in the refrigerator. The product obtained was purified by recrystallization with dry distilled ethanol. Melting point was recorded and found uncorrected.

The compounds thus synthesized are presented in Schemes 1 and 2.

4.1.3. Antibacterial screening. The antimicrobial screening was performed using paper disc method on pathogenic strains of *E. coli*, *K. pneumoniae*, *B. subtilis*, *S. aureus*, *S. typhae* and *P. aerugionosa*. The Mannich bases (**3a**–**g**) were studied for their antibacterial property at concentration of 40–160 mg mL^{-1} using methanol as solvent. The solvent did not exhibit any activity at the concentrations used. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *F*-test. If the values of the calculated *F* are higher than the table value of *F* at 5% level, the character under study is said to be significantly influenced by the treatment. The significant or non-significant difference due to each of the treatments was judged under each character using standard error of difference (S.Ed) and critical difference (CD) values. The S.Ed between two treatments was calculated using error mean sum of squares (EMS). The CD were computed by multiplying the S.Ed value with the *t*-table (at 5%) for the error degree of freedom in order to judge the minimum difference in the means to qualify the treatment effects.

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References and notes

1. (a) Joshi, S.; Khosla, N. *Bioorg. Med. Chem. Lett.* **2003**, *52*, 197; (b) Joshi, S.; Khosla; Tiwari, P. *Bioorg. Med. Chem.* **2004**, *12*, 571.
2. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146.
3. Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.* **2003**, *10*, 925.
4. Weber, A.; Casini, A.; Heine, A.; Kuhn, D.; Supuran, C. T.; Scozzafava, A.; Kiebe, G. *J. Med. Chem.* **2004**, *47*, 550.
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.; Isakon, P. C.; Koboldt, C. M.; Logusch, E. W.; Norton, M. B.; Perkins, W. E.; Reinhard, E. J.; Seibert, K.; Veenhuizen, A. W.; Zang, Y.; Reitz, D. B. *J. Med. Chem.* **1995**, *38*, 4570.
6. Tapzergyar, E. G. *Austrian Pat.* **1996**, 246729.
7. Jain, S. M.; Dhaneshwar, S. R.; Trivedi, P. *Indian Drugs* **1991**, *29*, 64.
8. Joshi, S.; Khosla, N. *Acta. Pharm.* **1998**, *48*, 55.
9. Joshi, S.; Matkar, S.; Khosla, N.; Bhandari, V. *J. Indian Chem. Soc.* **1997**, *74*, 156.
10. Tramontini, M.; Angiolini, L.; Ghedini, N. *Polymer* **1998**, *29*, 771.
11. Goto, M.; Minoe, T. *Jpn. Kokai Tokkyo Koho, JP06* **1995**, *299*, 185.
12. Mitsch, A.; Wibner, P.; Sattler, I.; Schlitzer, M. *Arch. Pharm. Med. Chem.* **2001**, *334*, 40.
13. Sasse, A.; Ligneau, X.; Sadek, B.; Elz, S.; Pertz, H.; Ganelin, C. R.; Arrang, J. M.; Schwartz, J. C.; Schunack, W. *Arch. Pharm. Med. Chem.* **2001**, *334*, 45.
14. Botros, S.; Yousef, K. M.; Issac, Z. *Egypt J. Pharm. Sci.* **1989**, *30*, 419.
15. Khosla, N. Ph.D. Thesis, 1996.
16. Taylor, W. B. *Biometrics* **1957**, *13*, 1.