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In vitro study of some medicinally important Mannich bases derived from antitubercular agent

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Abstract—Biologically active Mannich bases with heteroaromatic ring system have been synthesised employing Mannich reaction of isonicotinyl hydrazide with various sulphonamides/secondary amines. They were analysed by elemental analysis and characterized by uv, ir, ^{1}H nmr spectroscopic studies. The Mannich bases were screened for their antibacterial activity against various gram positive and gram negative bacteria and were analyzed statistically. The results have shown that the compounds are quiet active against pathogens under study and were nontoxic.

1. Introduction

Isonicotinyl hydrazide is the hydrazide of isonicotinic acid which is keystone of modern treatment of tuberculosis.[1] Isoniazid is bacteriostatic in resting bacilli but bactericidal for actively dividing *Mycobacterium tuberculosis*. It is suggested that it inhibits biosynthesis of mycotic acids, which are important constituents of the mycobacterial cell wall.[2] The sulphonamide nucleus has well known pharmacological properties: antibacterial,[3] anticancer,[4] antiinflammatory,[5] carbonic inhibitory,[6] insecticidal.[7] Even *N*-morpholino, piperidino, dimethylamino, diethylamino Mannich bases show local anesthetic property.[8] The sulphonamide is a known patent drug and isoniazid is a well known antitubercular drug. These considerations have provoked Mannich bases of secondary amine by Mannich reaction. This reaction offers a convenient method for introduction of the basic aminoalkyl chain, which alters the biological profile and physicochemical characteristics.[9] Various drugs obtained from Mannich reaction have proved more effective and less toxic than their parent antibiotics.[10] The versatile utility of the Mannich bases in polymers,[11] dispersants in the lubricating oil[12] and pharmaceutical chemistry,[13] prompted us to prepare a series of aminomethyl derivatives and evaluate their biological significance and toxicity. In the present study, we report antibacterial activity of Isoniazid Mannich bases and their comparative study with sulphonamides. Statistics is used for final comparison (Table 1).

2. Results and discussion

The Mannich bases were synthesized, analysed by elemental analysis (Table 2) and characterized by uv, ir and ^{1}HNMR spectral studies (Table 3). The characteristic uv bands with λ_{max}: 208±3, 251±5, and 255±1, 257±2, 262±3, 270±5 were indicative of the presence of amido, benzene chromophore, pyridine nucleus, sulphonamide moiety in (**3a**–**3g** compounds), C=O chromophore, respectively.

IR spectra further confirmed the structural features of the Mannich bases (**3a**–**3l**). The ir bands at 3450, 3500 cm^{-1} (υ_{as} NH of s. amide), 3400–3420 cm^{-1} (hydrazine group), 3550, 3360 cm^{-1} (υ_{as} NH of sulphonamide group), 3100, 3140 cm^{-1} (υC-H in N containing heterocyclic ring), 2850, 2940 cm^{-1}(υ C-H of CH_2 group), 2810, 2750 (υ_sC-H of CH_2N), 1655–1670 cm^{-1}(υ C=O of secondary amide group), 1460 cm^{-1} (CH_2 scissoring), 1150–1160 cm^{-1} (υS=O group), 820–825 cm^{-1} (out of plane C–H bending in 1:4 disubstituted benzene), 660, 670 cm^{-1} (out of plane CH of 1:4 disubstituted pyridine) confirm the assigned structures.

The structural confirmation is further made using ^{1}H nmr spectra. It shows signals at δ ppm: 2.42 (d, 2H, CH_2

Keywords: Isonicotinylhydrazide; Sulphonamides; Mannich bases; Antibacterial activity; Toxicity; Statistical analysis.
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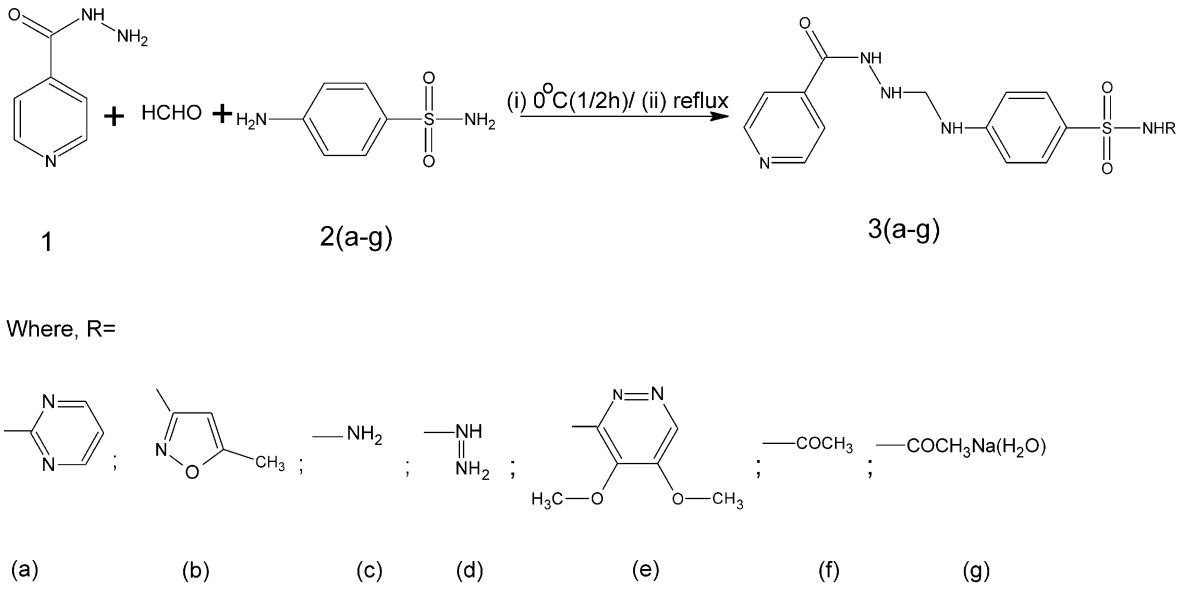
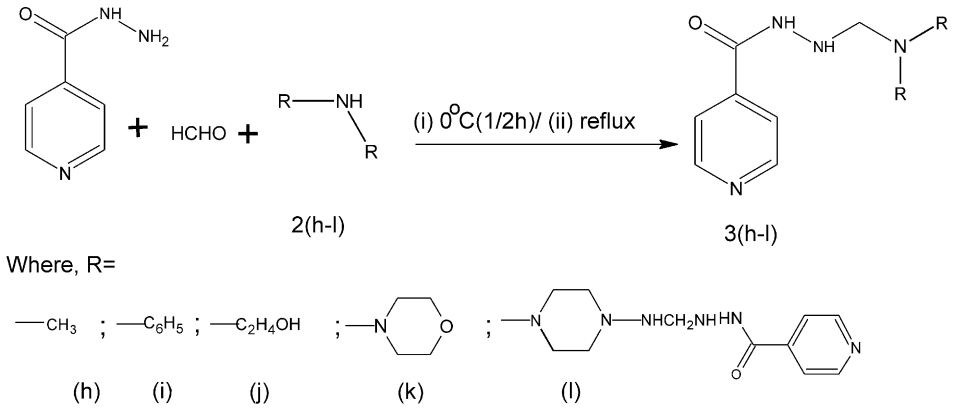
Table 1. Schemes for synthesis of Mannich bases



Scheme 1



Scheme 2

Table 2. Characterization data of isonicotinyl hydrazide methyl amines

S. no.	Compds	Molecular formula	Mp (°C)	Elemental analysis [found (calcd.) %]		
				C	H	N
3a	Isonicotinyl hydrazide methyl N′-2-pyrimidinyl p-aminobenzene sulphonamide	$C_{17}H_{17}N_7O_3S$	145–146	50.98 (51.12)	4.36 (4.26)	24.86 (24.56)
3b	Isonicotinyl hydrazide methyl N′-[5(methyl-3-isoxazolyl) p-aminobenzene sulphonamide]	$C_{17}H_{18}N_6O_4S$	118–120	50.84 (50.74)	4.51 (4.47)	20.13 (20.89)
3c	Isonicotinyl hydrazide methyl p-aminobenzene sulphonamide	$C_{13}H_{15}N_5O_3S$	134–135	48.65 (48.59)	4.77 (4.67)	21.92 (21.80)
3d	Isonicotinyl hydrazide methyl N′-(diaminomethylene) p-aminobenzene sulphonamide	$C_{14}H_{17}N_7O_3S$	173–175	46.02 (46.28)	4.87 (4.68)	26.51 (26.99)
3e	Isonicotinyl hydrazide methyl *N*′-(5,6-dimethoxy-4-pyrimidinyl)-*p*-aminobenzene sulphonamide	$C_{19}H_{21}N_7O_5S$	153–154	49.34 (49.67)	4.78 (4.57)	21.66 (21.35)
3f	Isonicotinyl hydrazide methyl sulphacetamide	$C_{15}H_{17}N_5O_4S$	183–184	49.36 (49.58)	4.68 (4.68)	19.33 (19.28)
3g	Isonicotinyl hydrazide methyl sodium sulphacetamide	$C_{15}H_{16}N_5O_4Sna$	157–159	46.33 (46.75)	4.86 (4.15)	18.43 (18.18)
3h	Isonicotinyl hydrazide methyl dimethylamine	$C_9H_{14}N_4O$	155–156	55.83 (55.67)	7.36 (7.21)	28.46 (28.86)
3i	Isonicotinyl hydrazide methyl diphenylamine	$C_{19}H_{18}N_4O$	162–163	71.16 (71.69)	5.82 (5.66)	17.18 (17.61)
3j	Isonicotinyl hydrazide methyl diethanolamine	$C_{11}H_{18}N_4O_3$	150–152	51.38 (51.96)	6.98 (7.08)	22.39 (22.04)
3k	Isonicotinyl hydrazide methyl morpholine	$C_{11}H_{16}N_4O_2$	165–166	55.68 (55.93)	6.19 (6.77)	23.82 (27.72)
3l	Isonicotinyl hydrazide methyl piperazine	$C_{11}H_{17}N_5O$	169–170	56.41 (56.17)	7.84 (7.23)	29.78 (29.78)

Table 3. Spectral data of compounds **3a–l**

Compd	uv (λ_{max} values in nm)	ir (values in cm^{-1})	NMr (δ values in ppm)
3a	208 (amido moiety), 217 (S=O), 257 (Py. ring), 261 (sulphonamide moiety)	3450 (NH, sec. amide); 3411 (hydrazine-group); 3350 (NH, sulphonamide); 3100 (Ar. CH– str. in hetero.) 2941, 2850 (C–H, CH_2); 1655 (C=O); 1350, 1145 (S=O, SO_2NH)	2.49 (**d**, 2H, $J=8.86$, CH_2); 6.2 (**s**, 1H, NH); 6.3–7.0 (**m**, Ar. H,); 7.1 (**s**, 1H, CONH); 7.6 (**d**, 2H, –NH–NH–); 11.8 (**s**, 1H, SO_2NH)
3b	207 (amido moiety), 215 (S=O), 259 (Py. ring), 262 (sulphonamide moiety)	3400 (NH, sec. amide); 3420 (hydrazine-group); 3360 (NH, sulphonamide); 3150 (Ar. CH– str. in hetero.) 2950, 2850 (C–H, CH_2); 1660 (C=O); 1374, 1150 (S=O, SO_2NH)	2.82 (**d**, 2H, $J=8.86$, CH_2); 6.1 (**s**, 1H, NH); 6.6–7.2 (**m**, Ar. H,); 7.4 (**s**, 1H, CONH); 7.5 (**d**, 2H, –NH–NH–); 11.0 (**s**, 1H, SO_2NH)
3c	208 (amido moiety), 217 (S=O), 258 (Py. ring), 261 (sulphonamide moiety)	3500 (NH, sec. amide); 3400 (hydrazine-group); 3350 (NH, sulphonamide); 3050 (Ar. CH– str. in hetero.) 2910, 2850 (C–H, CH_2); 1660 (C=O); 1380–1340, 1150 (S=O, SO_2NH)	2.5 (**d**, 2H, $J=8.86$, CH_2); 6.2 (**s**, 1H, NH); 6.5–7.0 (**m**, Ar. H,); 7.1 (**s**, 1H, CONH); 7.6 (**d**, 2H, –NH–NH–); 10.9 (**s**, 1H, SO_2NH)
3d	210 (amido moiety), 218 (S=O), 260 (Py. ring), 260 (sulphonamide moiety)	3505 (NH, sec. amide); 3405 (hydrazine-group); 3360 (NH, sulphonamide); 3150 (Ar. CH– str. in hetero.) 2930, 2850 (C–H, CH_2); 1655 (C=O); 1380, 1160 (S=O, SO_2NH)	2.53 (**d**, 2H, $J=8.86$, CH_2); 6.1 (**s**, 1H, NH); 6.6–7.2 (**m**, Ar. H,); 7.4 (**s**, 1H, CONH); 7.6 (**d**, 2H, –NH–NH–); 10.8 (**s**, 1H, SO_2NH)
3e	210 (amido moiety), 220 (S=O), 256 (Py. ring), 260 (sulphonamide moiety)	3500 (NH, sec. amide); 3400 (hydrazine-group); 3350 (NH, sulphonamide); 3140 (Ar. CH– str. in hetero.) 2950, 2850 (C–H, CH_2); 1665 (C=O); 1340, 1170 (S=O, SO_2NH)	2.5 (**d**, 2H, $J=8.86$, CH_2); 5.86 (**s**, 1H, NH); 6.55–7.6 (**m**, Ar. H,); 7.1 (**s**, 1H, CONH); 7.8 (**d**, 2H, –NH–NH–); 10.8 (**s**, 1H, SO_2NH)
3f	209 (amido moiety), 217 (S=O), 257 (Py. ring), 260 (sulphonamide moiety)	3500 (NH, sec. amide); 3405 (hydrazine-group); 3350 (NH, sulphonamide); 3100 (Ar. CH– str. in hetero.) 2950, 2850 (C–H, CH_2); 1660 (C=O); 1370, 1150 (S=O, SO_2NH)	2.6 (**d**, 2H, $J=8.86$, CH_2); 5.82 (**s**, 1H, NH); 6.6–7.3 (**m**, Ar. H,); 7.4 (**s**, 1H, CONH); 7.6 (**d**, 2H, –NH–NH–); 11.6 (**s**, 1H, SO_2NH)
3g	208 (amido moiety), 217 (S=O), 258 (py. ring), 261 (sulphonamide moiety)	3500 (NH, s. amide); 3410 (hydrazine-group); 3350 (NH, sulphonamide); 3100 (Ar. CH– str. in hetero.) 2920, 2850 (C–H, CH_2); 1660 (C=O); 1340, 1145 (S=O, SO_2NH)	2.4 (**d**, 2H, $J=8.86$, CH_2); 6.2 (**s**, 1H, NH); 6.6–7.8 (**m**, Ar. H,); 7.1 (**s**, 1H, CONH); 7.6 (**d**, 2H, –NH–NH–); 11.8 (**s**, 1H, SO_2NH)
3h	210 (amido moiety), 255 (py. ring)	3450 (NH, s. amide); 3380 (hydrazine-group); 3050 (Ar. CH– str. in hetero.) 2910, 2850 (C–H, CH_2); 1655 (C=O)	2.7 (**d**, 2H, $J=8.86$, CH_2); 7.1–7.6 (**m**, Ar. H,); 7.7 (**s**, 1H, CONH); 7.9 (**d**,2H, –NH–NH–);
3i	208 (amido moiety), 259 (py. ring)	3445 (NH, s. amide); 3370 (hydrazine-group); 3100 (Ar. CH– str. in hetero.) 2940, 2846 (C-H, CH_2); 1660 (C=O)	2.4 (**d**, 2H, $J=8.86$, CH_2); 6.6–8.7 (**m**, Ar. H,); 7.9 (**s**, 1H, CONH); 8.2 (**d**,2H, –NH–NH–);
3j	207 (amido moiety), 258 (Py. Ring)	3450 (NH, s. amide); 3375 (hydrazine-group); 3150 (Ar. CH– str. in hetero.) 2940, 2850 (C–H, CH_2); 1658 (C=O)	2.6 (**d**, 2H, $J=8.86$, CH_2); 6.9–7.5 (**m**, Ar. H,); 7.7 (**s**, 1H, CONH); 8.1 (**d**, 2H, –NH–NH–);
3k	212 (amido moiety), 259 (Py. Ring)	3450 (NH, s. amide); 3375 (hydrazine-group); 3050 (Ar. CH- str. in hetero.) 2920, 2850 (C–H, CH_2); 1660 (C=O)	2.7 (**d**, 2H, $J=8.86$, CH_2); 6.6–7.4 (**m**, Ar. H,); 7.5 (**s**, 1H, CONH); 8.2 (**d**,2H, –NH–NH–);
3l	210 (amido moiety), 258 (Py. Ring)	3450 (NH, s. amide); 3380 (hydrazine-group); 3140 (Ar. CH– str. in hetero.) 2940, 2850 (C–H, CH_2); 1658 (C=O)	2.5 (**d**, 2H, $J=8.86$, CH_2); 6.6-7.4 (**m**, Ar. H,); 7.5 (**s**, 1H, CONH); 8.0 (**d**, 2H, –NH–NH–);

proton $J=7.46$ Hz), 2.5(d, 2H, CH_2, $J=10.36$ Hz), 6.5 (m, ArH protons), 6.9–7.0 (m, ArH in diphenylamine), 6.9 (t, 3H, pyrimidine ring), 7.9(s, 1H, CONH), 8.62–8.63 (d, 2H at the 2,6 position of pyridine ring, $J=9.00$ Hz), 8.68–8.70 (d, 2H at 3,5 position of pyridine ring), 11.7 (s,1H, SO_2N^1H proton). Thus, confirming the proposed structure.

Biological significance of isonicotinyl hydrazide methyl amines was established by screening them against *Escherichia coli*, *Salmonella enteritidis*, *Pasturella multocida*, *Bacillus anthracis*, *Staphylococcus aureus* (Table 4). The antibacterial activities of the parent sulphonamides were also obtained and recorded in Table 5 for comparison. The results were statistically analysed.[14] The activities reported were mean of zone of inhibition in mm (in triplicate).

Table 4 reveals that compound **3e** followed by **3d** are statistically superior in inhibiting the growth of *E.coli*. However, **3c**, **3g**, **3i** are statistically equivalent in antibacterial activity and better than remaining Mannich bases. All compounds in general showed statistically significant activity at 40 mg/mL. We have compared the antibacterial activities of Mannich bases with the sulphonamides (Table 5). Results show Mannich bases **3a**, **3d**, **3e** and **3g** to have significantly better inhibitory aptitude against *E. coli* as compared to sulphonamides. Mannich bases **3b** and **3d** and sulphonamides are statistically equivalent in inhibiting the growth of this bacterium.

Antibacterial screening of isonicotinylhydrazidemethylamines against *S. enteritidis* shows (Table 4) that compounds **3d** and **3l** show statistically significant activity over **3a**, **3c**, **3e**, **3g**, **3h**, **3i** and **3j**. The concentration of 40 mg/mL is found to be superior to other arbitrarily chosen concentrations in checking the growth of this pathogen. Comparative study reveals that Mannich base **3g** shows pronounced activity against *S. enteritidis* as compared to sulphonamides.

When antibacterial study was done against *P. multocida*, we found that Mannich bases **3a** and **3b** are statistically equivalent in inhibiting the growth of the pathogen. Also they are superior in activity over other

Table 4. Antibacterial activity of isonicotinyl hydrazide methyl amines

S.no.	Zone of inhibition in mm											
	Concentration in mg/mL											
	E. coli				*S. enteritidis*				*P. multocida*			
	20	30	40	Average	20	30	40	Average	20	30	40	Average
3a	10.40	12.80	15.45	12.88	8.45	10.06	10.93	9.81	25.00	30.02	30.46	28.49
3b	10.46	11.23	12.00	11.23	—	—	—	—	26.00	29.02	30.00	28.34
3c	15.00	20.00	30.00	21.67	10.20	10.46	12.00	10.88	20.00	22.00	24.00	22.00
3d	20.80	25.90	30.23	25.64	—	—	—	—	18.00	20.23	20.90	19.71
3e	30.46	31.42	35.00	32.29	10.00	10.90	15.76	12.22	20.00	22.20	22.86	21.68
3f	15.93	18.23	20.46	18.21	10.00	15.30	16.40	13.90	20.23	20.90	25.00	22.04
3g	20.00	21.00	25.00	22.00	7.80	10.23	12.86	10.29	12.00	12.96	15.00	13.32
3h	17.00	18.00	20.00	18.33	8.36	10.90	12.00	10.42	20.00	20.86	25.00	21.95
3i	15.00	16.00	18.00	16.33	8.20	15.20	15.62	13.00	—	—	—	—
3j	18.00	19.00	22.00	19.66	8.42	10.63	15.63	11.57	18.00	20.00	20.76	19.58
3k	20.00	22.00	24.00	22.00	—	—	—	—	—	—	—	—
3l	20.00	21.00	22.00	21.00	12.00	13.00	15.00	13.33	22.06	22.80	24.00	22.95
Average of concentration	17.75	19.71	22.84		9.27	11.85	14.02		20.12	22.09	23.79	

Statistical data	S.Ed	CD at 5%	S.Ed	CD at 5%	S.Ed	CD at 5%
Compounds	0.306953	0.63662	0.3740	0.79288	0.354164	0.744098
Concentrations	0.073756	0.148545	0.26958	0.5472	0.045277	0.091505
Interaction	0.255496	0.529898	0.80874	1.714	0.143178	0.300817

	S. aureus				*B. anthracis*			
	20	30	40	Average	20	30	40	Average
3a	10.00	12.00	13.00	11.66	14.20	16.26	18.00	16.15
3b	—	—	—	—	15.00	16.00	16.20	15.73
3c	14.00	15.00	16.00	15.00	13.00	13.40	13.60	13.33
3d	—	—	—	—	16.00	16.20	16.40	16.20
3e	—	—	—	—	12.00	12.80	13.00	12.60
3f	15.00	16.00	17.00	16.00	—	—	—	—
3g	—	—	—	—	—	—	—	—
3h	16.40	18.00	20.28	18.23	20.00	22.00	24.00	22.00
3i	—	—	—	—	15.00	15.40	16.00	15.46
3j	—	—	—	—	—	—	—	—
3k	—	—	—	—	—	—	—	—
3l	10.40	10.80	12.00	11.06	12.00	12.60	14.00	12.86
Average of concentration	13.16	14.36	15.65		14.65	15.58	16.40	

Statistical data	S.Ed	CD at 5%	S.Ed	CD at 5%
Compounds	0.450068	1.03785	0.05655	0.121526
Concentrations	0.09685	0.20203	0.047261	0.096507
Interaction	0.216564	0.499397	0.133674	0.286731

S.Ed, standard error of difference; CD, critical difference.

Mannich bases. Further, Table 4 also points out that compounds **3c**, **3e**, **3f**, **3h** are statistically similar in inhibiting the growth of *Pastueralla*. Again, in this case, concentration 40 mg/mL significantly inhibits the growth of the pathogen.

Comparing antibacterial activity of newly synthesized Mannich bases with sulphonamides we observed that Mannich bases **3g** is significantly better than its corresponding sulphonamide in checking the growth of *P. multocida*. Sulphonamide failed to show any activity at these arbitrarily chosen concentrations.

Table 4 reveals that compounds **3h** is superior (statistically) to all the Mannich bases in their antibacterial activity against *S. aureus*. Compared to sulphonamides, Mannich bases show statistically significant activity and are found superior to sulphonamides. Sulphonamides fail to show any activity against this bacterium (Table 5).

Antibacterial screening of newly synthesized Mannich bases against *B. anthracis* shows compound **3h** to be superior to other Mannich bases in their antibacterial activity against *B. anthracis*.

None of the sulphonamides, which are known for their antibacterial activity, showed any activity against this pathogen while corresponding Mannich bases showed statistically significant activity against *B. anthracis* (Table 5).

The Mannich bases were also screened for their toxicity by preliminary LD_{50} 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

Table 5. Antibacterial activity of isonicotinyl hydrazide methyl amines as compared to sulphonamides

S.no.	Zone of inhibition in mm											
	Concentration in mg/mL											
	E. coli				*S. enteritidis*				*P. multocida*			
	20	30	40	Average	20	30	40	Average	20	30	40	Average
3a	10.40	12.80	15.45	12.88	8.45	10.06	10.93	9.81	25.00	30.02	30.46	28.49
3b	10.46	11.23	12.00	11.23	—	—	—	—	26.00	29.02	30.00	28.34
3c	15.00	20.00	30.00	21.67	10.20	10.46	12.00	10.88	20.00	22.00	24.00	22.00
3d	20.80	25.90	30.23	25.64	—	—	—	—	18.00	20.23	20.90	19.71
3e	30.46	31.42	35.00	32.29	10.00	10.90	15.76	12.22	20.00	22.20	22.86	21.68
3f	15.93	18.23	20.46	18.21	10.00	15.30	16.40	13.90	20.23	20.90	25.00	22.04
3g	20.00	21.00	25.00	22.00	7.80	10.23	12.86	10.29	12.00	12.96	15.00	13.32
3h	17.00	18.00	20.00	18.33	8.36	10.90	12.00	10.42	20.00	20.86	25.00	21.95
3i	15.00	16.00	18.00	16.33	8.20	15.20	15.62	13.00	—	—	—	—
3j	18.00	19.00	22.00	19.66	8.42	10.63	15.63	11.57	18.00	20.00	20.76	19.58
3k	20.00	22.00	24.00	22.00	—	—	—	—	—	—	—	—
3l	20.00	21.00	22.00	21.00	12.00	13.00	15.00	13.33	22.06	22.80	24.00	22.95
Average of concentration	17.75	19.71	22.84		9.27	11.85	14.02		20.12	22.09	23.79	

Statistical Data	S.Ed	CD at 5%	S.Ed	CD at 5%	S.Ed	CD at 5%
Compounds	0.306953	0.63662	0.3740	0.79288	0.354164	0.744098
Concentrations	0.073756	0.148545	0.26958	0.5472	0.045277	0.091505
Interaction	0.255496	0.529898	0.80874	1.714	0.143178	0.300817

	S. aureus				*B. anthracis*			
	20	30	40	Average	20	30	40	Average
3a	10.00	12.00	13.00	11.66	14.20	16.26	18.00	16.15
(a)	—	—	—	—	—	—	—	—
3b	—	—	—	—	15.00	16.00	16.20	15.73
(b)	—	—	—	—	—	—	—	—
3c	14.00	15.00	16.00	15.00	13.00	13.40	13.60	13.33
(c)	—	—	—	—	—	—	—	—
3d	—	—	—	—	16.00	16.20	16.40	16.20
(d)	—	—	—	—	—	—	—	—
3e	—	—	—	—	12.00	12.80	13.00	12.60
(e)	—	—	—	—	—	—	—	—
3f	15.00	16.00	17.00	16.00	—	—	—	—
(f)	—	—	—	—	—	—	—	—
3g	—	—	—	—	—	—	—	—
(g)	—	—	—	—	—	—	—	—

S.Ed, standard error of difference; CD, critical difference.

for 72 h for each trial.[15] 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.

3. Conclusion

The newly synthesized Mannich bases are found better and more potent antibacterial agents than sulphonamides themselves. They can prove to be potent drugs having minimum side effects and also having comparatively low cost.

4. Experimental

All melting points are uncorrected. The ^{1}H nmr spectra in DMSO and $CDCl_3$ solvent were recorded on Brucker-DRX-300 FT NMR. The IR spectra were recorded on Shimadzu 820 IPC FTIR spectrometre using KBr pellets. The uv spectra were recorded in methanol using Shimadzu UV-160 uv–visible spectrophotometer.

All substituted sulphonamides were obtained as pure samples from reputed pharmaceutical establishment. Solvents were distilled before use.

4.1. Synthesis of isonicotinyl hydrazide methyl sulphonamide (Scheme 1)

To the ethanolic solution of 0.1 mol of amide was added to 0.1 mol of sulphonamide 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 sulphonamide

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 2). 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 (chromatograhic grade).

4.2. Synthesis of isonicotinyl hydrazide methyl amines (Scheme 2)

Secondary amines (0.01 mol) were added to an ethanolic solution (50 mL) of 5-chloro-2-methoxybenzamide (0.01 mol) in a flat-bottom 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 instalments 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 Table 1 (Schemes 1 and 2).

5. Antibacterial screening

The antimicrobial screening was performed using paper disc method[16] on pathogenic strains of *E. coli*, *P. multocida*, *S. entertiidis*, *S. aureus* and *B. anthracis*. The Mannich bases (**3a–l**) were studied for their antibacterial property at concentration of 20–40 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.[14] 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.

6. Toxicity

The toxicity was ascertained by LD_{50} test. The test was performed on swiss strain white male mice weighing 25 g, ±1.5 months old. The compounds were dissolved in methanol and given orally (through catheter tube) as well as intraperitoneally. Six were kept under observation for 72 h for each trial.[15] Toxicity of methanol was checked and was found that upto 4 mL of methanol was harmless and non-toxic.

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