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QSAR Study on Antibacterial Activity of Sulphonamides and Derived Mannich Bases

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Abstract—The paper describes synthesis and comparative study on antibacterial activities of sulphonamides and Mannich bases derived from them. The compounds 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.

Introduction

The importance of sulphonamide nucleus and nicotinamide is well established in the pharmaceutical chemistry. A considerable number of sulphonamides are well known as antibacterial,[1] carbonic anhydrase inhibitor,[2] anticancerous,[3] anti-inflammatory agents.[4] Moreover, nicotinamide moiety, which has well known biological significance, is a constituent of Vitamin B complex. This has given an impetus for the synthesis of Mannich bases (Table 1) from these compounds using Mannich reaction.[5] This reaction posses a judious method for the introduction of the basic aminoalkyl chain. The various drugs obtained from Mannich reaction are proved to be more effective and less toxic than the parent antibiotic.[6]

The versatile utility of the Mannich bases in polymers,[7] dispersents in lubricating oil[8] and the pharmaceutical chemistry,[9,10] 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 a comparative QSAR study of antibacterial activity of sulphonamides and Mannich bases derived from them.

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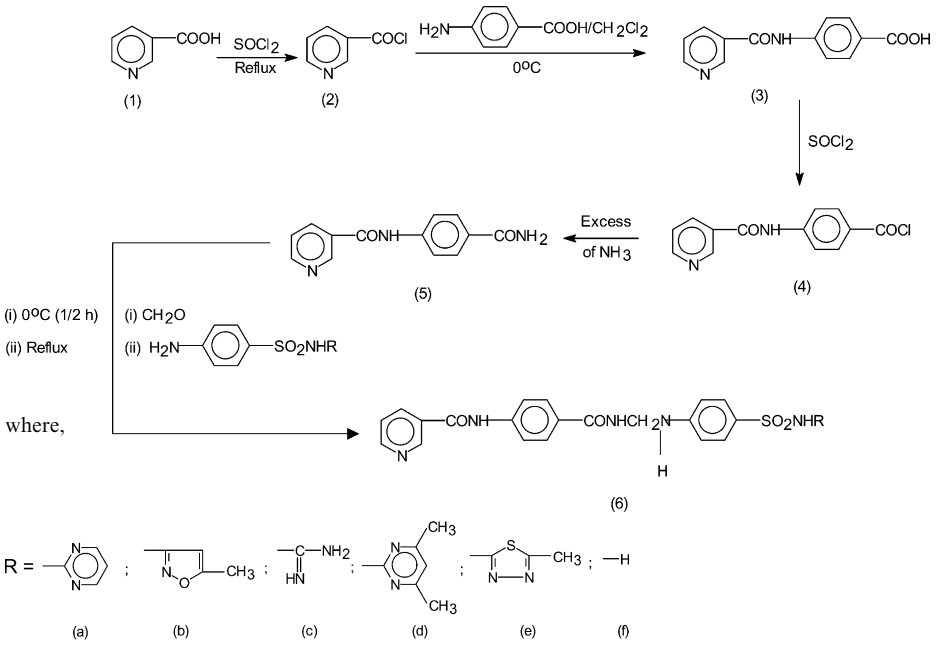
The Mannich bases under present study were prepared by us following the method described earlier[11,12] and were characterized by elemental analysis (Table 2), uv, ir and ^{1}H NMR spectroscopy (Table 3). The comparitive biological significance of sulphonamides as well as the Mannich bases derived from them was asserted by evaluation of their antibacterial activity. The compounds were active only against three bacteria (Table 4).

Result and discussion

The sulphonamides used were of BDH and/or equivalent grade.The Mannich bases were synthesised and charecterized by elemental analysis, uv, ir and ^{1}H NMR spectral studies (Tables 2 and 3) The characteritstic uv bands with λ_{max}: 210, 219, 250, 257 and 260 nm were indicative of the presence of amido, sulphoxide, aromatic nucleus, pyridine and sulphonamide moieties, respectively. The presence of these moieties were further confirmed by their spectral studies. The bands in cm^{-1} obtained at 3450, 3100, 1660, 1600, 1385 were indicative of the amino group of secondary amide, amino group of sulphonamide, C–H of pyridine nucleus, C=O group of amide, NH of CONH group, S=O group of sulphonamide.

The structural cofirmation is made from ^{1}H NMR spectra of Mannich bases (Table 3). The observed resonance absorptions at δ 2.5–2.75 ppm (d, 2H of CH, $J=8.57$ Hz), δ 5.85–6.10 ppm (s, 1H of N^4H of sulphonamides) δ 7.7–7.73 ppm (s, 1H of CONH of ring

Table 1. Scheme for the synthesis of Mannich bases



I), δ 8.25–8.3 ppm (t, 1H of CONH of ring II, $J = 9.64$ Hz), δ 9.2 ppm (d, 1H of pyridine nucleus at the second position) δ 10.9–11.0 ppm (s, 1H of SO_2N^1H of sulphonamide moiety). Thus confirm the proposed structure.

The aforementioned Mannich bases duly characterized by elemental and spectral techniques, were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The antibacterial activities of the parent sulphonamides were also obtained and recorded in Table 4 for comparison. The activities of these compounds were statistically significant against all the bacteria used. The activities reported were the mean of three measurements. We now discuss as below the results obtained against each of the bacteria used.

Table 4 reveals that the antibacterial potential of Mannich base (**6e**) is statistically similar to those of the Mannich bases **6a**, **6d**, **6f** in checking but significantly superior to Mannich bases **6b** and **6c**. Moreover, all compounds, in general, showed significant activity at concentration 80 μg mL^{-1}. In Table 4, we observed that Mannich base (**6c**) shows more pronounced activity against *E. coli* compared to sulphonamide.

Table 2. Characterization data of *N*-nicotinoyl-4-aminobenzamidomethyl sulphonamides

S. no.	Compd	Mol. formula	Yield (%)	Mp (°C)	Elemental analysis found (calcd) (%)		
					C	H	N
6a	*N*-Nicotinoyl-4-aminobenzamidomethyl-N^1-2-pyrimidnyl *p*-aminobenzene sulphonamide	$C_{24}H_{21}N_7O_4S$	85	165	56.85 (57.25	3.80 4.17	18.98 19.48)
6b	*N*-Nicotinoyl-4-aminobenzamidomethyl-N^1-(5-methyl-3-isoxazolyl) *p*-aminobenzene sulphonamide	$C_{24}H_{22}H_6O_5S$	90	140–142	56.60 (56.81	3.98 4.34	16.15 16.60)
6c	*N*-Nicotinoyl-4-aminobenzamidomethyl-N^1-(diaminomethylene) *p*-aminobenzene sulphonamide	$C_{21}H_{21}N_7O_4S$	87	125	53.45 (53.96	4.05 4.49	20.65 20.98)
6d	*N*-Nicotinoyl-4-aminobenzamidomethyl-N^1-(4,6-dimethyl) 2-pyrimidnyl *p*-aminobenzene sulphonamide	$C_{26}H_{25}N_7O_4S$	83	98–100	58.25 (58.75	5.00 4.70	19.00 18.45)
6e	*N*-Nicotinoyl-4-aminobenzamidomethyl-N^1-(5-methyl-1,3,4-thiadiazol-2yl) *p*-aminobenzene sulphonamide	$C_{23}H_{21}N_7O_4S_2$	78	140	52.33 (52.70	4.50 4.01	18.38 18.73)
6f	*N*-Nicotinoyl-4-aminobenzamidomethyl *p*-aminobenzene sulphonamide	$C_{20}H_{19}N_5O_4S$	85	138–140	56.88 (56.47	4.90 4.47	16.98 16.47)

Table 3. Spectral data of compounds **6a–6f**

Compd	Uv (λ_{max} values in nm)	ir (values in cm^{-1})	NMR (δ values in ppm)
6a	210 (C=O), 219 (S=O), 250 (Ar. ring), 257 (Py. ring), 260 (sulphonamide moiety)	3450 (NH, sec. amide); 3398, 3280 (NH, sulphonamide); 2950, 2885 (C–H, CH_2); 1655 (C=O); 1329, 1158 (S=O, SO_2NH)	2.71 (d, 2H, $J=8.56$, CH_2); 6.15 (s, 1H, NH); 6.5–7.9 (m, Ar. H,); 7.71 (s, 1H, CONH of ring I); 8.25 (t, 1H, $J=9.64$, CONH of ring II); 10.9 (s, 1H, SO_2NH)
6b	208 (C=O), 219 (S=O), 245 (Ar. ring), 261 (sulphonamide)	3500 (NH, sec. amide); 3380 (NH, sulphonamide); 2950, 2830 (C–H, CH_2); 1655 (C=O, CONH); 1389, 1320, 1160 (S=O, SO_2NH)	2.82 (d, 2H, $J=8.56$, CH_2); 6.10 (s, 1H, NH); 6.8–7.8 (m, Ar. H); 7.4 (s, 1H, CONH of ring I); 8.3 (t, 1H, $J=9.64$, CONH of ring II); 11.0 (s, 1H, SO_2NH)
6c	210 (C=O), 220 (S=O), 248 (Ar. ring), 260 (sulphonamide moiety)	3450 (NH, sec. amide); 3350 (NH, sulphonamide); 2950 (C–H, CH_2); 1670 (C=O, CONH); 1380, 1175 (S=O, SO_2NH)	2.5 (d, 2H, $J=8.56$, CH_2); 6.15 (s, 1H, NH); 6.5–7.9 (m, Ar. H); 7.7 (s, 1H, CONH of ring I); 8.25 (t, 1H, $J=9.64$, CONH of ring II); 10.8 (s, 1H, SO_2NH)
6d	212 (C=O), 220 (S=O), 259 (Py. ring), 250 (Ar. ring) 261 (sulphonamide moiety)	3500 (NH, sec. amide); 3350 (NH, sulphonamide); 2960, 2955 (C–H, CH_2); 1665 (C=O, CONH); 1385, 1349, 1150 (S=O, SO_2NH)	2.5 (d, 2H, $J=8.56$, CH_2); 6.10 (s, 1H, NH); 6.5–7.6 (m, Ar. H); 7.8 (s, 1H, CONH of ring I); 8.4 (t, 1H, $J=9.64$, CONH of ring II); 10.8 (s, 1H, SO_2NH)
6e	209 (C=O), 219 (S=O), 249 (Ar. ring), 260 (sulphonamide moiety)	3440 (NH, sec. amide); 3340 (NH, sulphonamide); 2930 (C–H, CH_2); 1660 (C=O, CONH); 1325, 1140 (S=O, SO_2NH)	2.75 (d, 2H, $J=8.56$, CH_2); 6.10 (s, 1H, NH); 6.5–7.8 (m, Ar. H); 7.4 (s, 1H, CONH of ring I); 8.3 (t, 1H, $J=9.64$, CONH of ring II); 10.9 (s, 1H, SO_2NH)
6f	208 (C=O), 220 (S=O), 245 (Ar. ring), 260 (sulphonamide moiety)	3420 (NH, sec. amide); 2920 (NH, sulphonamide); 1660 (C=O, CONH); 1315, 1149 (S=O, SO_2NH)	2.75 (d, 2H, $J=8.56$, CH_2); 5.85 (s, 1H, NH); 6.5–7.8 (m, Ar. H, m); 7.7 (s, 1H, CONH of ring I); 8.3 (t, 1H, $J=9.64$, CONH of ring II); 10.9 (s, 1H, SO_2NH)

Table 4. Antibacterial screening of Mannich bases and their corresponding sulphonamides Zone of inhibition in mm

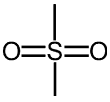
Compd	*E. coli* concentration in μg/mL					*K. pneumonae* concentration in μg/mL					*B. subtilis* concentration in μg/mL				
	10	20	40	80	Average	40	80	120	160	Average	40	80	120	160	Average
Mannich bases															
6a	10.4	10.6	13.7	18.7	13.38	18.9	18.3	19.2	20.9	19.35	15.4	20.4	22.9	25.3	21.03
6b	11.3	11.5	11.8	13.4	12.05	12.5	14.7	14.4	13.7	13.85	14.1	18.6	20.7	23.5	19.23
6c	10.9	11.4	11.0	11.7	11.29	—	—	—	—	—	—	—	—	—	—
6d	11.4	13.9	13.7	14.1	13.28	—	—	—	—	—	6.0	11.2	13.6	17.6	12.14
6e	12.4	14.9	14.4	15.0	14.26	—	—	—	—	—	—	—	—	—	—
6f	13.1	12.7	13.2	13.2	13.07	11.1	12.0	13.7	21.4	14.56	—	—	—	—	—
Sulphonamides															
a	9.7	17.2	22.2	25.5	18.65	27.0	29.2	29.5	25.6	27.85	20.8	27.2	26.3	26.9	25.10
b	19.6	22.9	23.2	23.6	22.35	11.6	22.4	26.4	6.0	16.63	22.8	25.4	27.4	27.9	25.90
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
d	15.1	16.9	18.8	18.5	17.34	—	—	—	—	—	15.4	19.9	21.7	22.0	19.80
e	16.2	17.4	22.2	25.5	20.33	—	—	—	—	—	—	—	—	—	—
f	20.4	22.3	22.8	25.4	22.75	—	—	—	—	—	—	—	—	—	—
Statistical data															
Compounds															
SED		0.543					0.287					0.371			
CD at 5%		1.064					0.587					0.773			
Concentration															
SED		0.343					0.287					0.303			
CD at 5%		0.672					0.587					0.631			
Interaction															
SED		1.087					0.576					0.742			
CD at 5%		2.131					1.176					1.492			

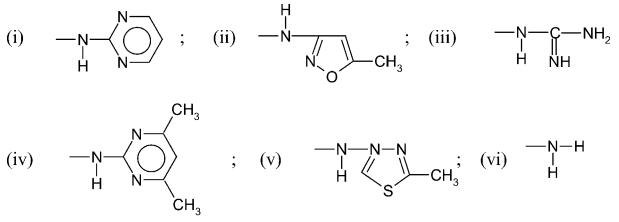
SED, standard error of difference; CD, critical difference.

The Mannich base (**6a**) is significantly superior to other Mannich bases in exhibiting their inhibition against *Klebsiella*. The concentration level of 160 μg mL^{-1} is superior in inhibiting the growth of *K. pneumoniae*. When the antibacterial activity of the newly synthesised Mannich bases were compared with their corresponding sulphonamides (Table 4), it was found that Mannich base (**6f**) was significantly superior to sulphonamide against *K. pneumoniae*.

The Table 4 reveals that Mannich base (**6a**) is significantly superior to Mannich bases (**6b**) and (**6d**). The concentration level in all cases is found 160 μg mL^{-1} which significantly inhibits the growth of *B. subtilis*.

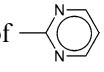
It is interesting to record that none of the Mannich bases exhibits inhibitory activity against *S. aureus* and *P. aeruginosa*.

With this background, it will be interesting to compare variation in antibacterial activity of Mannich bases with the changes in their structure. In all the cases change in structure occurred with the substitution of hydrogen(s) of the $-N^4H_2$ group attached at  position. These groups includes:



It is worth recording that substitution at N^4 nitrogen atom changes the orientation at N^1 nitrogen atom which in turn is responsible for changes in the activity of Mannich bases.[13]

A perusal of Table 4 shows that all Mannich bases are active against *E. coli* and that the Mannich bases **6a**, **6d**, **6e** and **6f** exhibit similar antimicrobial behaviour. It means that heterocyclic nitrogen(s) and methyl(s) have probably changed the orientation of N^1Hgroup which is responsible for the change of activity against *E. coli*.

Against *K. pneumonia,* Mannich base **6a** showed maximum inhibition. This may be attributed to the presence of  grouping.

In the case of *B. subtilis*, again the Mannich base **6a** shows pronounced activity compared to other Mannich bases under present investigation. Looking to this, we may say that the presence of  grouping is responsible for this enhancement in the antibacterial activity of Mannich base **6a**.

The synthesised 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 for 72 h for each trial.[14] The Mannich bases showed no adverse toxic effect even at an oral dose of 6400 mg kg^{-1} of the body weight of mice. However, when dose was administered intraperitonially, they proved to be lethal at a dose level of 1000 mg kg^{-1} of the body weight of mice.

Conclusion

The nicotinoyl-4-aminobenzamidomethylsulphonamides Mannich bases appeared to be better and more potent antibacterial agents than the sulphonamides themselves. We therefore conclude that the Mannich bases could be used as useful drugs in preference to the sulphonamides. Our findings will prove useful to those chemists, pharmacists, medicinal chemists who are interested in the synthesis of potential Mannich bases as drugs having minimum side effects and also having comparatively low cost.

Experimental

All melting points are uncorrected. The ^1H NMR spectra in DMSO and $CDCl_3$ solvent were recorded on Jeol FT NMR. The IR spectra were recorded on Perkin-Elmer spectrometre (model 377) using KBr pellets. The uv spectra were recorded in methanol using Beckman-26 spectrophotometre.

All substituted sulphonamides were obtained as pure samples from reputed pharmaceutical establishment. Solvents used were distilled before use. Compound **5** was obtained in several steps (Table 1).

General procedure

Mannich bases derived from sulphonamides: To the methanolic 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 methanol. 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).

Antibacterial screening

The antimicrobial screening was performed by cup plate method[15] on pathogenic strains of *E. coli*, *K. pneumo-*

niae, *S. typhosa*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*. The Mannich bases (**6a–f**) were studied for their antibacterial property at concentration of 10–160 μg 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.[16] The null hypothesis was tested using the *F* test. If the values of the calculated *F* are higher than the table value of *F* at the 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 (SED) and critical difference (CD) values. The SED between two treatments was calculated using error mean sum of squares (EMS). The CD were computed by multiplying the SED 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.

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.[14] Toxicity of methanol was checked and was found that upto 4 mL of methanol was harmless and non-toxic.

Uncited reference

Ref. 16 is not cited.

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