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Synthesis of some Mannich bases from sulphonamides and benzothiazole derivatives and evaluation of their anti tubercular activity and anti microbial activity

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ABSTRACT

Mannich bases of sulphadiazine, sulphamethoxazole, sulphacetamide with 2- amino -3 -methyl benzothiazole, 2 -amino chloro benzothiazole and 2 -amino 5 -chloro 6- fluro benzothiazole were synthesized. The structures of the various derivatives were characterized by various spectral data's and by elemental analysis. The synthesized compounds were screened for their anti tubercular activity useing Lowenstein-Jensen (L.J) medium against Mycobacterium tuberculosis H_{37} RV strains and the anti microbial activity against bacteria i.e. Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa Klebsiella pneumoniae and fungi Candida albicans and Aspergillus niger *. Compounds 6a, 6b, 6c and 6d were active against Mycobacterium tuberculosis H_{37} RV strains. The antimicrobial activity was evaluated by comparing the zone of inhibition of the tested compounds with the standard sulphamethaoxazole and Flucanazole. Very significant activity was exhibited from 6a, 6b, 6d and 6e against P aeruginosa, Significant activity was noted from 6a, 6d, 6e and 6h derivatives against Bacillus subtilis while 6a, 6b, 6d and 6e showed significant activity against E. coli. All other derivatives exhibited moderate activity against Bacillus subtilis and E. coli. However there was no activity against fungi.*

Key words: Anti- microbial, anti tubercular ,Mannich base, Sulphonamides, Benzothiazole.

INTRODUCTION

In the recent past there has been much progress in understanding of the various mechanisms by which drugs act and the resistance of the microbes towards anti bacterial drugs. Survey of literature has revealed that molecular manipulation is one of the sources for the designing of a new molecule. Mannich reaction is one such method for modification of the drug molecule. Many Mannich bases have been synthesized and have been reported to posses various activities that include anti tubercular[1], antidepressant[2] activity, anticonvulsant activity[3] and anti cancer properties[4]. The Mannich bases synthesized from various sulphonamides derivatives have been reported to posses anti bacterial activity[5,6,7] Literature survey revealed that benzothiazole is another important heterocyclic compound which posses anti fungal[8], anti tumour[9] and antibacterial activity[10]. Tuberculosis (TB) is one of the problems in the developing countries[1]. The major challenge to the world is the number of the people suffering from TB. The global resurgence of this disease and the resistance to these drugs by the bacteria has rekindled the need and an interest in the research for new drugs

The forgoing study focuses on the synthesis of Mannich bases by combining various sulpha drugs with the benzothiazole derivatives, characterization and their anti tubercular and antimicrobial activity to determine whether such Mannich bases showed an increase in activity.

MATERIALs AND METHODS

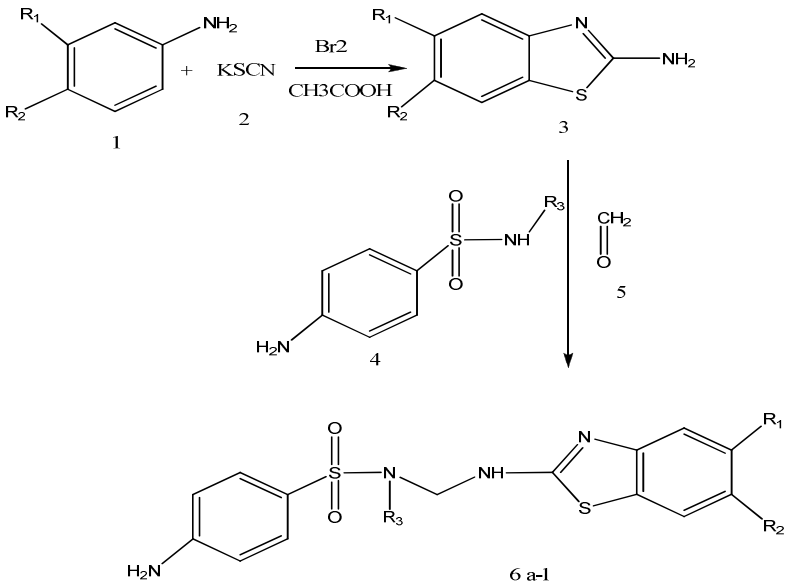
Synthesis of 2- amino benzothiazole

Potassium thiocyanate 0.08 moles and aniline 0.01 moles were added to 20 ml of glacial acetic acid and were cooled to 5º C; bromine 1.6 ml was added to reaction mixture dropwise with constant stirring. The reaction was stirred for 10 h at room temperature and was left overnight. The orange precipitate obtained was dissolved in water and glacial acetic acid and heated on steam bath. Further the reaction was cooled and P^H adjusted to 6 to obtain yellow precipitate which was re-crystallized from ethanol.

Mannich base of sulphanilamide with 2-amino benzothiazole (6 a-l)

A solution of 0.01 moles of substituted sulphanilamide in alcohol (QS) was prepared, to this 2-amino benzothiazole derivative and 0.01 moles formaldehyde was added and reaction was refluxed for 4 h and decomposed in crushed ice. The solid precipitate obtained was re-crystallized from ethanol.

Scheme of synthesis



Derivative	R_1	R_2	R_3
6a	H	H	H
6b	Cl	H	H
6c	Cl	F	H
6d	Cl	H	$C_4H_3N_2$
6e	H	H	$C_4H_3N_2$
6f	CH_3	H	$COCH_3$
6g	H	H	$COCH_3$
6h	Cl	H	$COCH_3$
6i	CH_3	H	H

Anti tubercular activity[12]:

The anti tubercular activity was performed at the National Tubercular Institute, Bangalore as follows: Lowenstein-Jensen (L.J) medium was prepared using beaten eggs, mineral salt solution and (0.2%) malachite green was autoclaved at 121^0 for 15 minute and used for the screening for anti tubercular activity.A concentration of 10 µg/ ml and 50 µg/ ml for the compound to tested were prepared in dimethyl formamide. Into each Mac cartney bottles 49 ml of L.J medium was added separately and 1ml of test compounds were added to it. The bottles were inspissated at $75\text{-}80^0$C for 2 h for sterilization and solidification. Similar concentrations for isonictonic acid hydrazide were prepared which served as standard drug. The control used was 49 ml of L.J medium and 1ml of N, N- dimethyl formamide. A loop full suspension of a *Mycobacterium tuberculosis* H_{37} RV strain culture was inoculated using nichrome wire loop on the surface of each of the L.J medium containing test compounds, standard and control. These inoculated mediums were incubated for 4 weeks at 37^0 C after which the reading was taken.

Anti microbial activity[11,12]:

The antibacterial activity of test compounds was evaluated against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* following agar diffusion method at concentration 10 µg /ml of the synthesized compounds. The anti fungal activity was evaluated against *Candida albicans* and *Aspergillus niger*.The zone of inhibition was compared with standard Sulphamethoxazole 10 µg/ml and control DMSO.

RESULTS AND DISCUSSION

Table 1.**Physical characterization of the compounds**:

Comp	Molecular formula	Mol wt	% yield	Rf value	M.P °C
6a	$C_{14}H_{14}N_4O_2S_2$	334	77.84	0.57	120
6b	$C_{14}H_{13}N_4O_2S_2Cl$	368	83.96	0.60	148
6c	$C_{14}H_{12}N_4O_2S_2ClF$	386	80.47	0.63	176
6d	$C_{18}H_{14}N_6O_2S_2Cl$	445	77.52	0.59	240
6e	$C_{18}H_{16}N_6O_2S_2$	412	82.52	0.76	248
6g	$C_{16}H_{16}N_4O_3S_2$	376	83.77	0.72	90
6h	$C_{16}H_{15}N_4O_3S_2Cl$	410	84.10	0.85	194
6i	$C_{15}H_{15}N_4O_2S_2$	386	72.82	0.84	108
6f	$C_{17}H_{18}N_4O_3S$	390	73.32	0.92	236

Table 2: Elemental analysis:

Derivative	Carbon		Nitrogen		Hydrogen	
	Calculated	Observed	Calculated	Observed	Calculated	Observed
6a	50.29	50.09	16.76	16.70	4.19	3.82
6b	45.65	44.82	15.21	14.82	3.53	3.75
6c	43.52	43.02	14.51	14.25	3.59	3.02
6d	48.53	48.10	18.87	18.24	3.59	3.49
6e	52.43	52.30	20.38	19.00	3.64	3.22
6f	52.30	51.90	14.35	14.98	4.61	4.24
6g	51.06	50.21	14.89	14.24	4.25	3.92
6h	46.82	46.16	13.65	12.98	3.65	3.30
6i	43.35	43.02	14.50	14.10	3.10	3.25

Table 1 depicts the physical characteristics of the synthesized Mannich bases. The structures of the synthesized title compounds were characterized from IR and ^{1}HNMR spectral data's.

N^1 – (2- aminomethylbenzololy)sulphanilamide 6a:-(KBr cms^{-1}) 3347 (N-H), 1701 (C=N), 1274 (Ar-NH_2), 672 (C-S), 1150 (S=O), 1310 (C-N) and 1595 (C=C). (^{1}HNMR δ ppm DMSO): 7.6-8.6 (m, 8H, Ar H), 6.2 (s, 2H, NH_2), 7.7(s, 1H, NH), 4.0(s, 1H, NH), 4.9 (s,2H, CH_2)

N^1-{2-(5'-chloro)-aminomethyl benzoyl} sulphanimalide 6b:- :-(KBr $cms^{-1)}$ 3365(N-H), 1306 (Ar-NH_2), 674 (C-S), 1149 (S=O), 1203 (C-N), 1595 (C=C)**.** ^{1}H NMR (δ ppm) 7.6-8.6 (m, 7H, Ar H), 6.2 (s, 2H, NH_2), 7.7(s, 1H, NH), 4.0(s, 1H, NH), 4.9 (s, 2H, CH_2)

N1-{2-(5'-chlor-6' - fluro)-aminomethyl benzoyl} Sulphanilamide (6c) :- (KBr $cms^{-1)}$ 3263N-H , 1701 C=N, 1309 NH_2, 674 C-S, 1150 S=O, 1377 C-N, 1596 C=C **,** ^{1}H N M R(δ ppm) 7.6-8.6 (m, 6H, Ar H), 6.2 (s, 2H, NH_2), 7.7(s, 1H, NH), 4.0(s, 1H, NH), 4.9 (s, 2H, CH_2)

N^1-{2-(5'-chloro)-aminomethyl benzoyl}-N^1-(2''-pyrimidyl) Sulphanilamide (6d) :-(KBr $cms^{-1)}$ 3364(N-H) ,1325 (Ar-NH_2), 1638 (C=N), 680 (C-S), 1149 (S=O), 639(C-Cl). NMR 7.6-8.2 (m, 10 H, ArH,) 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s,2H, CH_2)

N^1-{2-aminomethyl benzoyl}-N^1-(2''-pyrimidyl) Sulphanilamide (6e) :- (KBr $cms^{-1)}$ 3366 N-H, 1325(Ar- NH_2), 1643(C=N), 680 (C-S), 1152 (S=O), 1596 (C=C). ^{1}HNMR (δ ppm)**: -** 7.6-8.2 (m, 11 H, ArH), 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s, 2H, CH_2)

N^1 –(3-pyrimidyl)- N^1 – {2-(5'-chloro,6'-fluro)} amino methyl benzothiazoyl sulphanilamide (6f) :- (KBr $cms^{-1)}$ 3381(N-H) 1258 (Ar-NH_2), 721 (C-S), 1321 (C-N), 1595 (C=C). ^{1}HNMR (δ ppm): 7.6-8.2 (m,9 H, ArH,) 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s,2H, CH_2)

N^1-{2-aminomethyl benzothiazoyl}-N^1-acetyl sulphanilamide (6g) :-(KBr $cms^{-1)}$ 3326 (N-H), 1275 (Ar- NH_2), 1699 (C=N), 671(C-S), 1153(S=O), 1309 (C-N), 1593(C=C). ^{1}HNMR: 7.6-8.2 (m,8 H, ArH,) 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s,2H, CH_2) 2.0 (s, 3H, CH_3)

N^1 -2' – (5'-chloro)-aminomethyl benzothiazolyl}-N^1- acetyl Sulphanilamide (6h):-
(KBr $cms^{-1)}$ 3183(N-H), (1272 Ar-NH_2), 673 (C-S), 1135 (S=O), 1598(C=C). ^{1}HNMR (δ ppm) 6-8.2 (m, 7 H, ArH,) 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s,2H, CH_2) 2.0 (s, 3H, CH_3)

N^1 -2' – (5''-methyl)-aminomethyl benzothiazolyl}-N^1- acetyl Sulphanilamide (6i):- KBr cms^{-1}) 1707 (C=N), 1259 (Ar-NH_2), 666 (C-S), 1153 (S=O), 1594(C=C). ^{1}HNMR(δ ppm): 7.6-8.2 (m,7 H, ArH,) 6.27 (s, 2H, NH_2), 4.0 (s, 1H, NH) 4.9 (s,2H, CH_2) 2.0 (s, 3H, CH3),2.3 (s,3H,CH_3).

Table 3: Antimicrobial activity of the derivatives

Compound	Zone of inhibition (mm)					
	Escherichia coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Candida albicans*	*Aspergillus niger*
6a	20	25	25	22	-	-
6b	18	11	29	24	-	-
6d	17	22	25	21	-	-
6e	17	22	20	16	-	-
6g	15	15	14	15	-	-
6h	10	22	14	15	-	-
6i	11	10	12	10	-	-
6f	10	10	14	11	-	-
6c	10	12	14	13	-	-
Sulphamethoxazole 10 µg/ml Flucanazole	18	23	30	25	29	32

Table 3 shows the results of anti microbial activity of the Mannich bases prepared .The zone of inhibition of the tested compounds were compared with the standard sulphamethaoxazole .Very significant activity was exhibited from **6a, 6b, 6d and 6e** against *P aeruginosa*, while moderate activity was seen from **6c, 6f, 6g, 6h, 6i** against the same strain . Significant activity was noted from **6a, 6d, 6e and 6h** derivatives against *Bacillus subtilis* and derivatives **6a, 6b, 6d** and **6e** showed significant activity against *E. coli*. **6a, 6b** and **6c** showed significant activity against *K.pneumoniae.* All other derivatives exhibited moderate activity against *B.subtilis*, *E. coli* and *K.pneumoniae.* **6a** showed very mild activity against *A. niger* and all other compounds did not show any anti-fungal activity. None of the compounds showed any activity against *C. albicans.*

Sulphonamides have a strong structural resemblance with PABA. SO_2 is a strong electron withdrawing group that makes the hydrogen of NH_2 highly acidic. So replacing one of the hydrogens of NH_2 by a strong electron

withdrawing heteroaromatic ring increased the antimicrobial activity, acidifying the remaining hydrogen to a certain extent and in turn enhancing the potency. It also increased the water solubility in physiological conditions[13].

Anti tubercular activity:

Table 3: Antitubercular activity of the tested compounds:

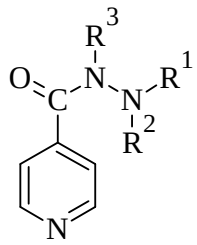
Compound	Dose 10 µg/ml	Dose 50 µg/ml
6a	+ + +	- - -
6b	+ + +	- - -
6c	+ + +	- - -
6i	+ + +	- - -
Control	+ + +	+ + +
INH (0.1 µg/ml)	- - -	- - -

+ + + = *growth of organism*
- - - = *No growth of organism*

Only four of the synthesized compounds (**6a, 6b, 6c and 6i)** were screened for antitubercular activity. The results are tabulated in table 3. All tested derivatives were potent at 50µg/ml and inhibited the growth of *Mycobacterium tuberculosis*, a similar result was seen with standard INH at 0.1 µg/ml.

In the case of Sulphonamides the aniline (N_4) amino group is very important for activity because any modification other than prodrug results in loss of activity. All N_4 acetylated metabolites of Sulphonamides are inactive.

In the case of the classic first generation Anti tubercular agents like Isoniazid, substitution of the hydrazine portion of INH with alkyl and aralkyl substituents resulted in active and inactive derivatives.



Substitution of R^1 and R^2 with alkyl groups at N_2 resulted in active compounds but substitution of R^3 with alkyl groups at N_1 with R^1 and R^2 as H destroyed the activity[14].

Keeping the above aspects in mind the synthesis was carried out and the results showed that the compounds with a similar substitution in their molecules exhibited similar activities.

CONCLUSION

From the results it can be established that some of the Mannich bases of sulpha drugs with substitued benzthioazole ie 6a, 6b, 6c and 6i derivatives showed inhibition of *Mycobacterium tuberculosis* at 50 µg/ml. Since only a few synthesized derivatives were screened for antitubercular activity it would be difficult to conclude whether the Mannich base formed or the substitution on the benzothiazole ring or the sulpha group was responsible for antitubercular activity. The newly synthesized Mannich bases appeared to have promising antimicrobial activity, but the potency of some compounds were moderate against *P. aeruginosa*, *E coli*, and *B subtilis*, *K.pnuemoniae.*Very significant activity was seen from 6a and 6b against *P aeruginosa*, *E coli*, *K.pnuemoniae.* However the compounds did not show any anti fungal activity.

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