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ANTIBACTERIAL SCREENING OF NEWLY SYNTHESIZED MANNICH BASES DERIVED FROM 5H-DIBENZO[B,F]AZEPINE-5-CORBOXAMIDE AGAINST *GRAM POSITIVE AND GRAM NEGATIVE PATHOGENS*

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ABSTRACT

Mannich bases are well known for their antimicrobial activities, In this study, Mannich bases 3a-3j (5H-dibenzo[b,f]azepine-5-Corboxamide methyl amines), were designed, synthesized, characterized and evaluated for antimicrobial screening.A series of Mannich bases of 5H-dibenzo [b,f]azepine-5-Corboxamidewere synthesised via reacting 5H-dibenzo [b,f]azepine-5-Corboxamide with secondary amines and sulphonamides. Structureevaluation of synthesized compounds wasdone on the basis of elemental analyses, UV, IR and ^{1}HNMR spectral studies. The biological screening of these synthesized compounds was done against pathogenic microorganism *Bacillus subtilis*(Gram-positive bacteria) and *Salmonella typhi* (Gram-negativebacteria) with a view to explore their antimicrobial action by paper disk method at 80, 160 and 320µg/ml respectively. The antibacterial activity of derived Mannich bases was compared against their parent sulphonamides. The results demonstrate the potential and importance of mounting new Mannich bases against pathogens.

KEYWORDS: 5H-dibenzo[b,f]azepine-5-corboxamide, Sulphonamides, Amines, Mannich reaction, Mannich bases andAntimicrobial activity.

INTRODUCTION

Designing of new drug using natural or synthetic ingredients is one of the most common methodologies adopted by pharmaceutical scientists. In 1912 Germen chemist Carl Mannich

did amino alkylation of Salicylantipyrine and Urotropin with acid,[1] this amino alkylation is formally known as Mannich reaction. The chemistry of Mannich reaction offers a simple synthetic methodology for the development of large variety of compounds shows medicinal, pharmaceutical, biological and commercial importance.[2] Mannich bases are used as antibacterial,[3-5] antifungal,[6-7] anticonvulsant,[8] anticancer[9] and anti-inflammatory[10] agents.

5H-dibenzo[b,f]azepine-5-corboxamide(trade name Carbamazepine) molecule shows nucleophilicity, it have ability to react with formaldehyde molecule to give aminomethyl derivatives. 5H-dibenzo[b,f]azepine-5-corboxamide has great pharmaceutical importance and it is used mainly as an anticonvulsant agent.[11] It is also used to treat resistant schizophrenia,[12] post-traumatic stress disorder, alcohol withdrawal and restless leg syndrome. Sulfonamides have been widely used for the treatment of microbial disease[13] however development of more potent drugs limited the medicinal uses of sulfonamides. The sulfonamides functional group is an important pharmacophore and in three components Mannich reaction sulfonamides play role of amine moiety.[14]

Our past research work shows the great importance of Mannich bases as pharmacophore and in continuation of our studies for the development of cheap antibacterial agents with green chemistry environment, we synthesized a series of Mannich bases derived from 5H-dibenzo[b,f]azepine-5-corboxamide with sulfonamides (as primary amine) and secondary amines. The structure was confirmed using spectral data UV, IR, ^{1}H NMR. Antimicrobial screening of newly synthesized compounds were performed against pathogenic bacteria i.e. *Bacillus subtilis* and *Salmonella typhi*. The results obtained were compared with antibacterial activity of parent compound and sulfonamides.

MATERIALS AND METHODS

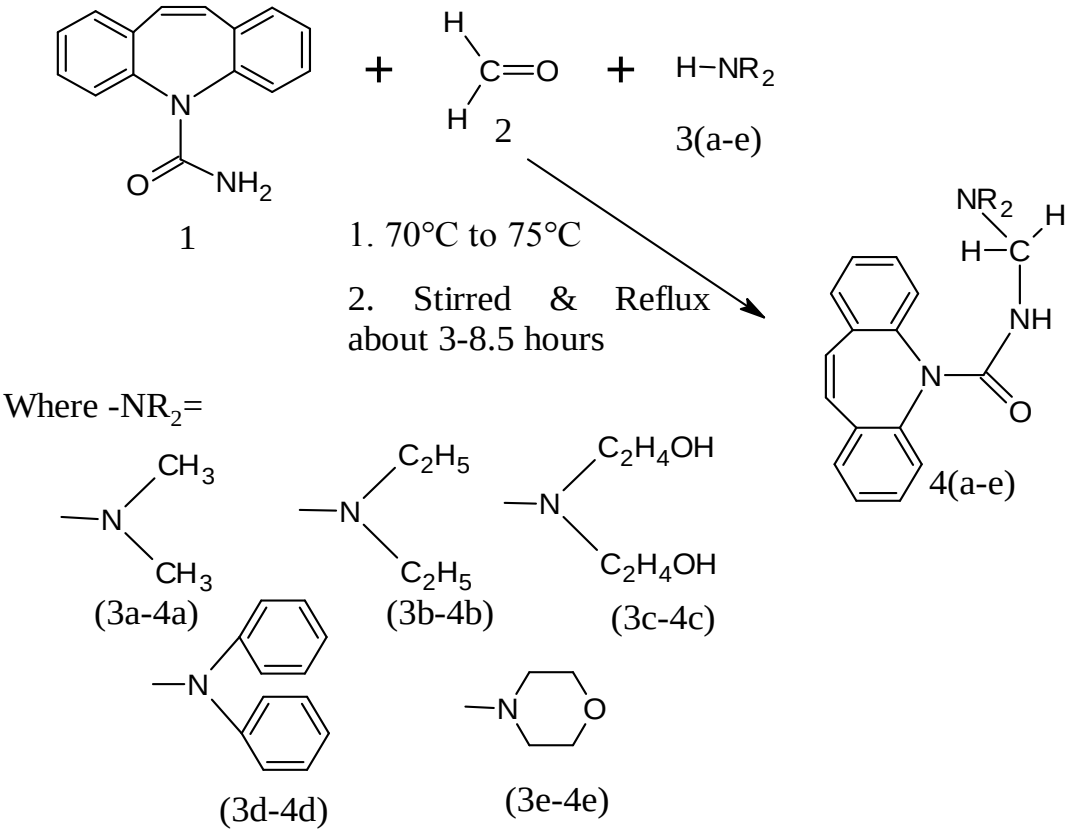
The chemical reagents used in the synthesis were purchased from E. Merck and Aldrich. All the melting points were determined in open capillary tubes and are uncorrected. Thin layer chromatography was used for monitoring the reaction and to check purity. IR spectra (KBr) were recorded as potassium bromide pellets on Perkin Elmer SP 10 FTIR spectrometer. ECX-JEOL 400 MH high resolution multinuclear NMR Spectrometer were used to record ^{1}HNMR spectra chemical shifts were expressed as (ppm) values against tetramethylsilane (TMS) as internal reference.

Synthesis of Mannich bases

The reaction routes for synthesis of the title compound were described as shown in Scheme 1 and Scheme 2. The synthesized mannich bases (4a-4j) were obtained thus in (≥79%) yield.

Synthesis of Mannich bases (4a-4e)

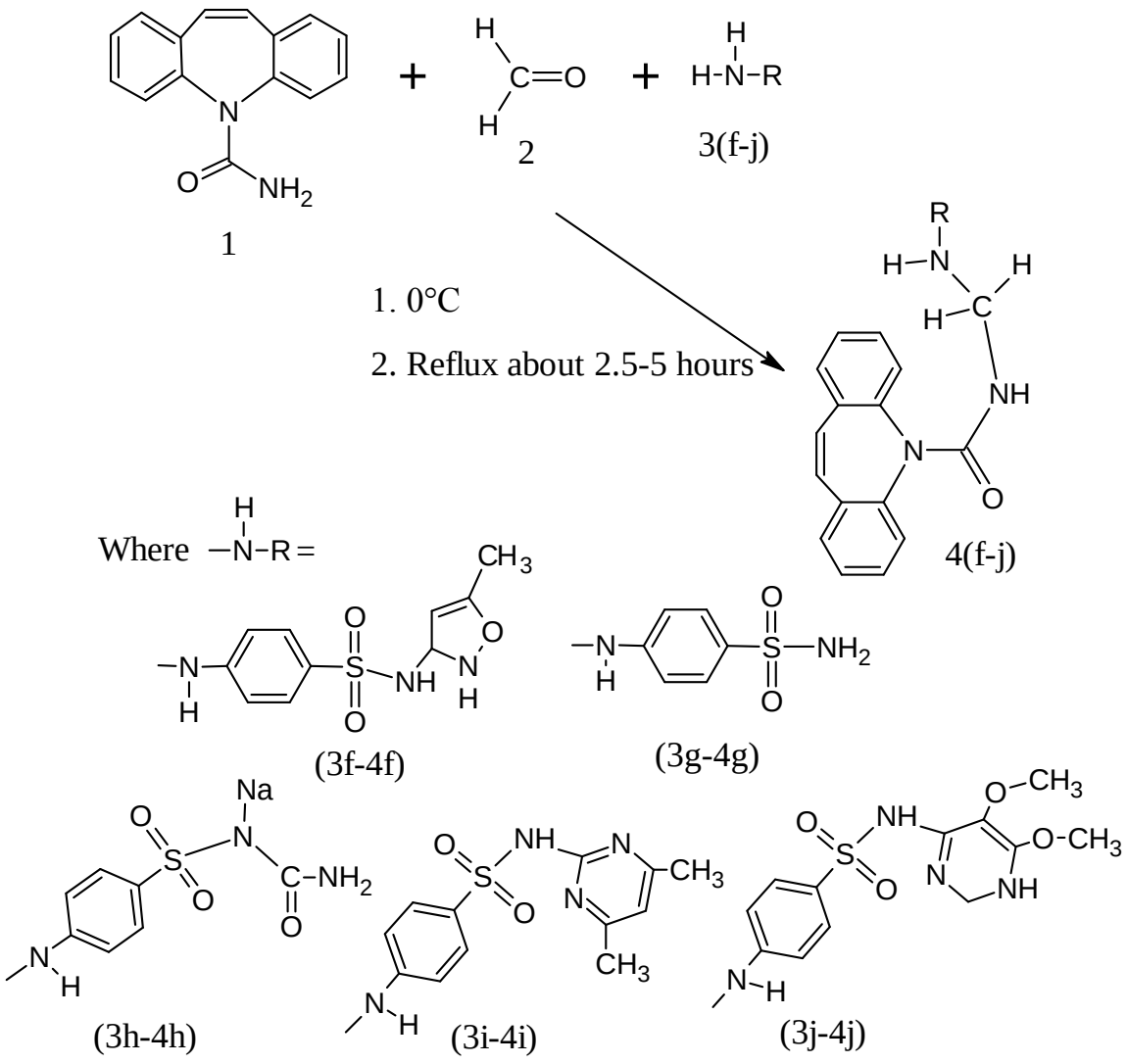
Secondary amine 0.01 mol was added in an ethanolic solution 50 ml of substrate (5H-dibenzo [b,f]azepine-5-corboxamide) 0.01 mol in a flat bottom flask. Amount of 0.4 ml of formaldehyde solution (37%) was added slowly with constant stirring. The reaction mixture was stirred at 70-75°C for 3.0 to 8.5 hours, depending upon the secondary amine. The remaining portion of formaldehyde solution was added in two instalments after 1 and 2 hours, respectively. The reaction mixture was kept over-night 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 product obtained was purified by recrystallization from dry distilled ethanol and DMF (1:1).



Scheme 1: Synthesis of Mannich bases from secondary amines.

Synthesis of Mannich bases (4f-4j)

In ethanolic solution of 0.01 mol of substrate (5H-dibenzo [b,f]azepine-5-corboxamide), 0.01 mol of sulfonamide and 2.5 ml of formaldehyde solution (37% v/v) were added. The pH of mixture was adjusted to 3.5 by adding 0.5ml of 1 mol HCl. The mixture was kept in an efficient ice cooling for half an hour and then refluxed on water bath. The reflux time is varied with the sulphonamide used. Refluxed mixture was kept at 0°C for four days when crystalline product was obtained. The obtained product was recrystallized with dry distilled ethanol and DMF (1:1).



Scheme 2: Synthesis of Mannich bases from sulphonamide (primary amines).

Antimicrobial Activity

The newly synthesized mannich bases 4a-4j were screened for their antibacterial activity against pathogenic strains of *Bacillus subtilis* (Gram-positive bacteria) and *Salmonella typhi* (Gram-negative bacteria) at varying concentrations- 80µg/ml, 160µg/ml and 320µg/ml using

corresponding sulphonamides as their standards by paper disk method. Nutrient agar media were prepared for bacterial growth. The media was autoclaved at 15 lbs pressure (121.6°C) for 30 minutes. The culture of bacterium was mixed with autoclaved media and poured in plates.

The Mannich bases were studied in triplicate for their antibacterial property at concentration of 80-320 µg/ml using methanol as solvent. Cultures of each bacterium kept in Mullar Hinton Agar at 37°C for 24 Hrs. and then examined. Antibacterial activity was ascertained by the zone of inhibition measured in mm as shown in table I. The similar procedure was followed for the parent sulphonamides. The solvent did not exhibit any activity at the concentrations used. Most of the compounds were found to be effective against the tested microorganism by measuring the diameter of the growth inhibition zone according to Bauer et al.[15]

RESULTS

Characterization of Mannich Bases (4a-4j) by Physico-Chemical and Spectral data

5H-dibenzo [b,f] azepine-5-Corboxamide methyl dimethyl amine (4a)

$C_{18}H_{19}N_3O$: yield 79%, m.p. 190°C, Anal. Calculated C, 73.69; H, 6.53; N, 14.32 Found C, 73.36; H, 5.49; N, 14.26. IR (KBr) νmax in cm^{-1}: 3466 O-H stretching, 3340 νas N-H, 3290 νs N-H, 2929 νas C-H in C-H Aliphatic, 2850 νs C-H in CH_2, 1672 νs C=O in amide, 1603 νs C=N, 1607 N-H bending, 615 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 1.23 (s, 6H, CH_3 attached with N), 2.93-2.97 (d, 2H, NH-CH_2-N), 6.93 (d, 2H, olefinic protons), 7.36, 7.49-7.6, 7.73 (protons of phenyl ring).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl diethyl amine (4b)

$C_{20}H_{23}N_3O$: yield 89%, m.p. 177°C, Anal. Calculated C, 74.74; H, 7.21; N, 13.07 Found C, 74.63; H, 7.14; N, 13.01. IR (KBr) νmax in cm^{-1}: 3463 O-H stretching, 3319 νas N-H, 3245 νs N-H, 2979 νas C-H in C-H Aliphatic, 2861 νs C-H in CH_2, 1678 νs C=O in amide, 1604 νs C=N, 1599 N-H bending, 635 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 0.99 (t, 6H, CH_3 of ethylamine), 2.64 (q,4H, CH_2 of ethylamine), 2.90 (d, 2H, NH-CH_2-N), 6.94 (d, 2H, olefinic protons), 7.36, 7.49-7.60, 7.71 (protons of phenyl ring).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl diethanol amine (4c)

$C_{20}H_{23}N_3O_3$: yield 84%, m.p. 190°C, Anal. Calculated C, 67.97; H, 6.56; N, 11.89 Found C, 67.86; H, 6.52; N, 11.62. IR (KBr) νmax in cm^{-1}: 3466 O-H stretching, 3412 νas N-H, 3362 νs N-H, 2931 νas C-H in C-H Aliphatic, 2850 νs C-H in CH_2, 1668 νs C=O in amide, 1605 νs C=N, 1600 N-H bending, 628 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 2.88 (t, 4H, CH_2 of CH_2OH), 3.57 (t, 4H, CH_2 of ethanolamine), 4.17 (d, 2H, NH-CH_2-N), 6.65 (d, 2H, olefinic protons), 7.36, 7.44-7.53, 7.72 (protons of phenyl ring).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl diphenyl amine (4d)

$C_{28}H_{23}N_3O$: yield 83%, m.p. 177-180°C, Anal. Calculated C, 80.55; H, 5.55; N, 10.06 Found C, 80.47; H, 5.49; N, 10.02. IR (KBr) νmax in cm^{-1}: 3468 O-H stretching, 3340 νas N-H, 3288 νs N-H, 2940 νas C-H in C-H Aliphatic, 2872 νs C-H in CH_2, 1672 νs C=O in amide, 1614 νs C=N, 1600 N-H bending, 623 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 4.65 (d, 2H, NH-CH_2-N), 6.44 (d, 2H, olefinic protons), 6.9-7.1 (protons of diphenylamine) 7.37, 7.44-7.53, 7.72 (protons of phenyl ring).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl morpholine (4e)

$C_{20}H_{21}N_3O_2$: yield 83%, m.p. 172°C, Anal. Calculated C, 71.62; H, 6.31; N, 12.53 Found C, 71.57; H, 6.27; N, 12.49. IR (KBr) νmax in cm^{-1}: 3464 O-H stretching, 3397 νas N-H, 3351 νs N-H, 2965 νas C-H in C-H Aliphatic, 2863 νs C-H in CH_2, 1677 νs C=O in amide, 1614 νs C=N, 1609 N-H bending, 643 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 4.65 (d, 2H, NH-CH_2-N), 6.44 (d, 2H, olefinic protons), 6.9-7.1 (protons of diphenylamine) 7.37, 7.44-7.53, 7.72 (protons of phenyl ring).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl sulphamethoxazole (4f)

$C_{26}H_{25}N_5O_4S$: yield 69%, m.p. 142-145°C, Anal. Calculated C, 62.01; H, 5.00; N, 13.91 Found C, 61.93; H, 4.97; N, 13.87. IR (KBr) νmax in cm^{-1}: 3468 O-H stretching, 3350 νas N-H in SO_2NH, 3342 νas N-H in NH_2, 3240 νs N-H in SO_2NH, 2929 νas C-H in C-H Aliphatic, 2850 νs C-H in CH_2, 1672 νs C=O in amide, 1607 νs C=N, 1600 N-H bending, 1350 νas of S=O, 1220 stretching vibration of N-O, 1150 νas of S=O, 631 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 1.96 (s,3H,CH_3), 4.58 (d, 2H, NH-CH_2-NH), 5.22 & 5.90 (d, 1H, 1H of oxazole ring), 6.45 (d, 2H, olefinic protons), 7.37, 7.44-7.53, 7.72 (protons of phenyl ring of carbamazepine).7.69-7.80 (ring protons of sulphonamide).

5H-dibenzo [b,f] azepine-5-Corboxamide methyl sulphadimidine (4g)

$C_{26}H_{26}N_6O_3S$: yield 86%, m.p. 168°C, Anal. Calculated C, 63.86; H, 4.98; N, 15.96 Found C, 63.79; H, 4.93; N, 15.89. IR (KBr) vmax in cm^{-1}: 3463 O-H stretching, 3357 vas N-H in SO_2NH, 3319 vas N-H in NH_2, 3255 vs N-H in SO_2NH, 2979 vas C-H in C-H Aliphatic, 2861 vs C-H in CH_2, 1678 vs C=O in amide, 1599 vs C=N, 1604 N-H bending, 1340 vas of S=O, 1150 vas of S=O, 635 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 2.58 (s, 6H, CH_3 of pyrimidine ring)), 4.57 (d, 2H, NH-CH_2-NH), 6.35 (d, 2H, H of olefinic protons), 6.56 (s, 1H, of pyrimidine ring), 7.24, 7.33, 7.49-7.61 (protons of phenyl ring of carbamazepine), 7.06-7.08 & 7.52-7.54 (protons of sulphonamide ring).

5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphaacetamide sodium (4h)

$C_{24}H_{21}N_2NaO_4S$: yield 76%, m.p. 190-192°C, Anal. Calculated C, 59.50; H, 4.37; N, 11.56 Found C, 59.41; H, 4.33; N, 11.52. IR (KBr) vmax in cm^{-1}: 3462 O-H stretching, 3348 vas N-H in SO_2NH, 3419 vas N-H in NH_2, 3262 vs N-H in SO_2NH, 2931 vas C-H in C-H Aliphatic, 2850 vs C-H in CH_2, 1668 vs C=O in amide, 1600 vs C=N, 1593 N-H bending, 1356 vas of S=O, 1146 vas of S=O, 628 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 2.18 (s,3H,of $COCH_3$), 4.57 (d, 2H, NH-CH_2-NH), 6.35 (d, 2H, H of olefinic protons), 6.56 (s, 1H, of pyrimidine ring),, 7.06, 7.33, 7.52-7.57 (protons of phenyl ring of carbamazepine), 7.06-7.07 & 7.52-7.57 (protons of sulphonamide ring).

5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphanilamide (4i)

$C_{22}H_{20}N_4O_3S$: yield 87%, m.p. 191°C, Anal. Calculated C, 62.84; H, 4.79; N, 13.32 Found C, 62.76; H, 4.75; N, 13.29. IR (KBr) vmax in cm^{-1}: 3466 O-H stretching, 3360 vas N-H in SO_2NH, 3332 vas N-H in NH_2, 3248 vs N-H in SO_2NH, 2940 vas C-H in C-H Aliphatic, 2872 vs C-H in CH_2, 1672 vs C=O in amide, 1614 vs C=N, 1591 N-H bending, 1304 vas of S=O, 1135 vas of S=O, 623 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 4.58 (2H, NH-CH_2-NH), 6.35 (d, 2H, H of olefinic protons), 6.56 (s, 1H, of pyrimidine ring),, 7.01-7.11, 7.33, 7.49-7.59 (protons of phenyl ring of carbamazepine), 7.01-7.11 & 7.49-7.59 (protons of sulphonamide ring).

5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphadoxine (4j)

$C_{28}H_{26}N_6O_5S$: yield 72%, m.p. 173°C, Anal.Calculated C, 60.20; H, 4.69; N, 15.04 Found C, 60.12; H, 4.65; N, 15.01. IR (KBr) vmax in cm^{-1}: 3466 O-H stretching, 3382 νas N-H in SO_2NH, 3367 νas N-H in NH_2, 3231 νs N-H in SO_2NH, 2965 νas C-H in C-H Aliphatic, 2863 νs C-H in CH_2, 1677 νs C=O in amide, 1614 νs C=N, 1596 N-H bending, 1323 νas of S=O, 1151 νas of S=O, 643 bending in CONH.

^{1}H-NMR ($CDCl_3$) δ ppm: 3.58 (s,3H of O-CH_3), 3.72 (s,3H of O-CH_3),4.59 (2H, NH-CH_2-NH), 6.35 (d, 2H, H of olefinic protons), 6.56 (s, 1H, of pyrimidine ring),, 7.01-7.11, 7.33, 7.49-7.61 (protons of phenyl ring of carbamazepine), 7.01-7.11 & 7.49-7.61 (protons of sulphonamide ring), 8.27 (s,1H of pyrimidine ring).

Antibacterial Screening

Antibacterial screening of compounds (4a-4j) and sulphonamides (3f-3j) against *S.typhi* and *B.subtilis* is tabulated in Table-I.

Table I: Antibacterial screening of synthesized Mannich bases and sulphonamides (Zone of inhibition in mm).

Compound No.	*S.typhi*				*B.subtilis*			
	Concentration in µg/ml				Concentration in µg/ml			
	80.0	**160.0**	**320.0**	**AVG**	**80.0**	**160.0**	**320.0**	**AVG**
4a	7.5	8.3	8.5	**8.1**	5.6	7.2	9.3	**7.4**
4b	-	5.4	7.6	**4.3**	5.3	6.8	8.6	**6.9**
4c	9.6	11.2	13.4	**11.4**	-	2.8	3.1	**2.0**
4d	-	4.5	4.9	**3.1**	5.8	6.8	7.4	**6.7**
4e	6.2	8.3	4.8	**6.4**	7.8	8.3	9.4	**8.5**
4f	-	-	-	**0.0**	22.1	25.3	26.7	**24.7**
4g	-	-	-	**0.0**	18.6	21.2	24.3	**21.4**
4h	8.3	9.3	9.8	**9.1**	16.3	18.2	20.3	**18.3**
4i	6.7	7.5	9.4	**7.9**	12.5	13.1	13.7	**13.1**
4j	-	-	-	**0.0**	18.6	23.5	31.2	**24.4**
3f	5.6	7.2	8.7	**7.2**	24.7	27.8	29.4	**27.3**
3g	-	6.8	8.6	**5.1**	20.3	22.4	26.9	**23.2**
3h	-	-	-	**0.0**	10.6	11.3	12.4	**11.4**
3i	5.9	7.3	9.4	**7.5**	11.4	12.8	13.6	**12.6**
3j	-	-	4.7	**1.6**	16.8	20.9	26.7	**21.5**

**Avg: Average value of Antibacterial Activity (for 80,160 and 320µg/ml).*

DISCUSSION

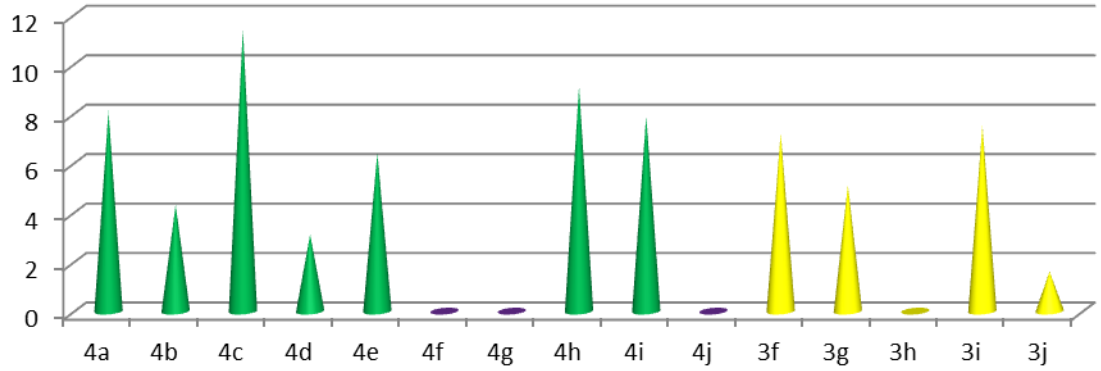
The mannich bases synthesized by mannich reaction were obtained in good yield (≥79%). They were analysed for elemental analysis and results were found to be in full agreement with the calculated values. The anticipated structure was in agreement with the spectral data– IR and ^{1}HNMR. The purity of synthesized compounds was assured with aid of chromatographic technique.

The stationary phase was silica gel-G. It was of chromatographic grade. The solvent used for mobile phase were methanol and chloroform. They were distilled before use. The spectral studies have shown characteristic band due to methylene group incorporated between active hydrogen substrate and the amine component as a result of mannich reaction at (2940-2950) and (1442-1450). This shows the presence of amino methyl linkage in the synthesized Mannich bases.The ^{1}H NMR also confirms aminomethyl linkage (-CH_2) between amine and active hydrogen (2.9-4.6).

The Mannich bases were screened for their biological significance. They were evaluated for antibacterial activity against pathogenic strains of *S.typhi* and *B.subtilis* at varying concentrations– 80, 160 and 320 μg/ml. These pathogens were subculture on specific media. The Mannich bases and the standard compound (sulphonamide and secondary amines) were dissolved in methanol. The activities were reported as mean of zone of inhibition in millimetre (in triplicate). All the reported compounds exhibit remarkable in vitro activity against these pathogens. Their activity was also compared with their parent sulphonamides.

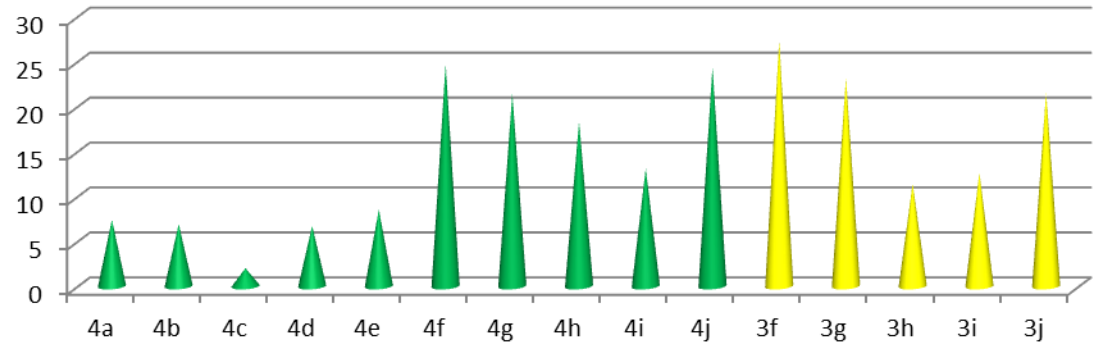
Table-I reflects that most of the compounds had shown remarkable activity only at 320 μg/ml. Out of these ten synthesized compounds seven compounds (4a, 4b, 4c, 4d, 4e, 4h and 4i) shows antibacterial activity against S.typhi. Fig.1 reflects that compound 4c is statistically more superior against S.typhi in compare to other compound of this series. Compound 4h and 4i shows more potent antibacterial activity against parent sulphonamides.

Fig.1: Antibacterial activity of synthesized compounds and sulphonamides against *S.typhi*



In case of *B.subtilis* all synthesized Mannich bases show antimicrobial activity against this bacterium (Fig.2). Compound 4c is very less active against B.subtilis. Compound 4f and 4j are statistically superior against B.subtilis and compound 4g, 4h, 4i, 4j are more potent in compare to parent sulphonamide. However compound 4f shows comparatives less potent against parent sulphonamide.

Fig.2: Antibacterial activity of synthesized compounds and sulfonamides against *B.subtilis*



The concentration of 320 µg/ml is found significantly superior to concentrations 160 µg/ml and 80 µg/ml in checking the growth of all microorganisms.

CONCLUSION

Work reported shows synthesis and characterization of a series (4a-4j) of Mannich bases synthesized from 5H-dibenzo[b,f]azepine-5-corboxamide with primary and secondary amines. Some newly synthesised compounds shows very noticeable and prolonged antibacterial activity. Some derived mannich bases shows more potent antibacterial activity

against their parent their parent sulphonamides. Obtained results show that Mannich bases are important pharmacophore for inhibition of bacteria and work could be more investigated to determine the possibility of more potent drug with less side effects.

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