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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTIBACTERIAL SCREENING OF MEDICINALLY IMPORTANT MANNICH BASES DERIVED FROM 5H-DIBENZO [B, F] AZEPINE-5-CORBOXAMIDE

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Abstract
Biologically active mannich bases with heteroaromatic ring system of 5H-dibenzo [b, f]azepine-5-Corboxamide have been synthesized via mannich reaction. The aminomethylation of 5H-dibenzo [b,f]azepine-5-Corboxamide with various biologically potent sulphonamides and secondary amines was carried out. The synthesized mannich bases were characterized by elemental analysis and spectral studies –UV, IR and ^{1}HNMR. The compounds were screened for their antibacterial activity against pathogenic bacteria i.e. E.coli and B.subtilis at varying concentrations. The antibacterial activity of derived mannich bases was compare with parent sulphonamides where the results shows some of synthesized compounds shows prolongs activity against these pathogens.
***Key words**: 5H-dibenzo [b,f]azepine-5-Corboxamide; Sulphonamides; Mannich reaction; Mannich bases; Antibacterial activity; E.coli; B.subtilis*

1. Introduction

One of the key objectives of organic and medicinal chemistry is to design and synthesize molecules that possess potent therapeutic values. The rapid development of resistance to existing antimicrobial drugs generates a serious challenge to the scientific community. Consequently, there is a vital need for the development of new antimicrobial agents with potent activity against drug resistant microorganisms [1]. The chemistry of the amino alkylation of aromatic substrates by the mannich

reaction is of great interest for the synthesis and modification of biologically active compound having physical[2] and chemical importance[3] as well as physiological properties[4-5] because the amino group can be easily converted into a variety of other functionalities[6]. Mannich reaction offers a judicious method for introduction of basic amino alkyl chain in various drugs/compounds. Further a considerable amount pharmacological activity of various Mannich bases for analgesic, anti-inflammatory, anesthetics and antimicrobial activity as well as intermediates in drug synthesis [7-9]. In this context, literature survey has revealed a number of reports on antimicrobial activity of N-mannich bases. Pregnenolone-Carbamazepine complex shows inhibited activity against *Escherichia Coli* and *S. aureus* [10]. In addition to this the sulphonamide is well-known antibacterial [11-13], anti-tubercular [14], anti-inflammatory [15], carbonic inhibitory [16]. The Mannich bases incorporated with sulphonamides are reported to be potent antibacterial agents and less toxic than parent sulphonamide [17]. Keeping in view the unique features of these compounds 5H-dibenzo [b,f]azepine-5-Corboxamide as a substrate and sulphonamide as amine component were condensed via mannich reaction. A series of mannich bases were synthesized with different sulphonamides / secondary amines (Scheme 1 and 2). The synthesized mannich bases were characterized by elemental analysis and spectral studies- IR and ^{1}H NMR screened for in-vitro antibacterial activity gram-positive and gram-negative bacteria at arbitrarily chosen concentrations.

2. Experimental

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. The chemical reagents used in the synthesis were purchased from E. Merck and Aldrich.

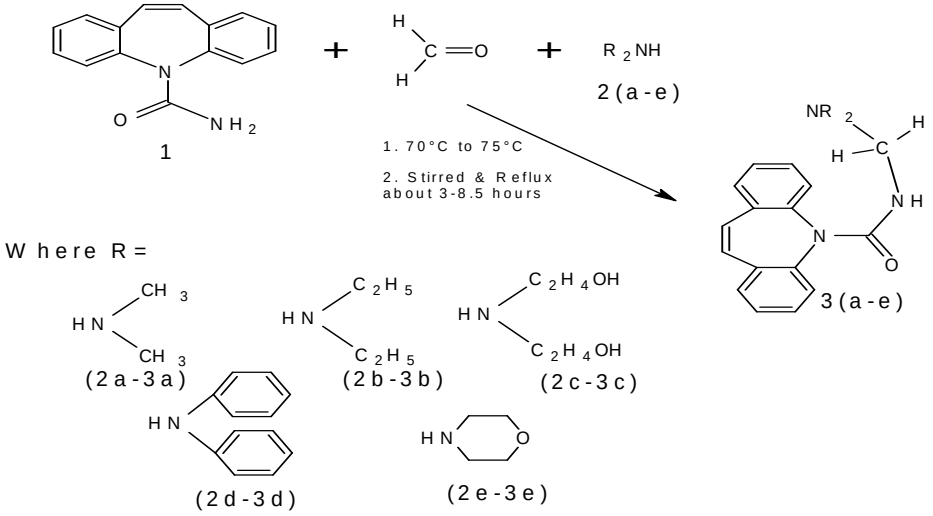
3. Chemistry

3.1 Synthesis of Mannich bases

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

3.1.1 Synthesis of Mannich bases (3a-3e)

Secondary amine 0.01 mol was added in an ethanolic solution 50 mL of Substrate (Comp. -1) 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.

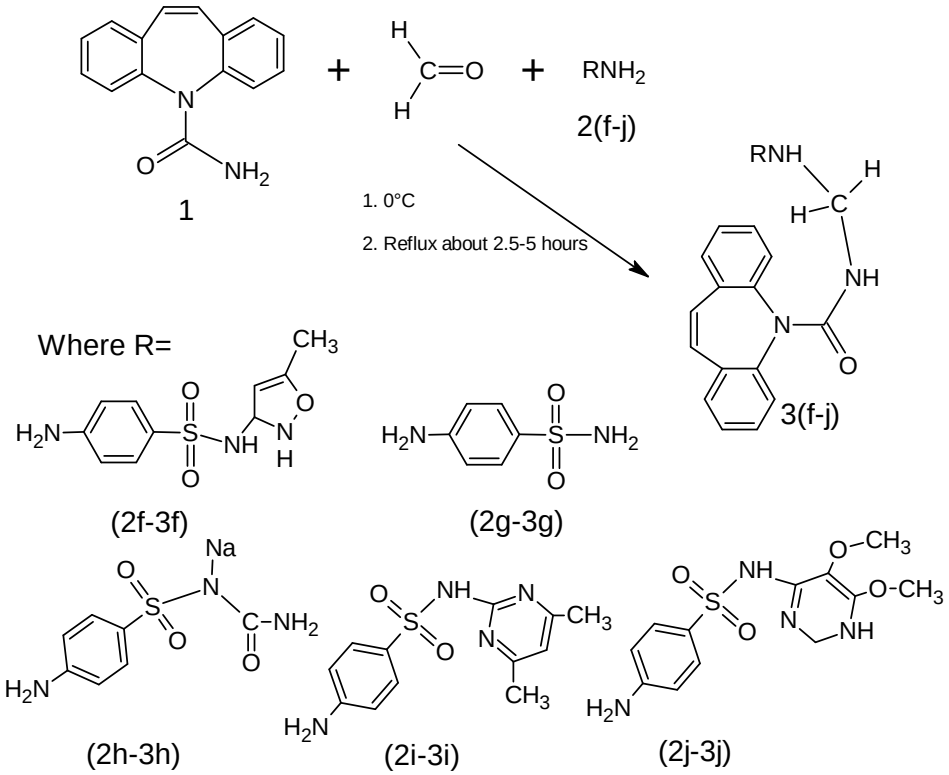


Scheme 1: Synthesis of mannich bases from secondary amines

The remaining portion of formaldehyde solution was added in two installments 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).

3.1.2 Synthesis of Mannich bases (3f-3j)

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^{-1}HCl. The mixture was kept in an efficient ice cooling for half an hour and then refluxed on water bath.



Scheme 2: Synthesis of Mannich bases from sulphonamide

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).

3.2 Physico-Chemical and Spectral Characterization of Synthesized Mannich Bases.

Compound 3a: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl dimethyl amine; $C_{18}H_{19}N_3O$; yield 79% , m.p. 190°C, Anal. Calcd. 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 streching, 3340 νas N-H, 3290 νs N-H, 2929 νas C-H in C-H Aliphatic, 2850 νs C-H in CH2, 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).

Compound 3b: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl diethyl amine; $C_{20}H_{23}N_3O$; yield 89% , m.p. 177°C, Anal. Calcd. 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 streching, 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).

Compound 3c: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl diethanol amine; $C_{20}H_{23}N_3O_3$; yield 84% , m.p. 190°C, Anal. Calcd. 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 streching, 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).

Compound 3d: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl diphenyl amine; $C_{28}H_{23}N_3O$; yield 83% , m.p. 177-180°C, Anal. Calcd. 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 streching, 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).

Compound 3e: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl morpholine; $C_{20}H_{21}N_3O_2$; yield 83% , m.p. 172°C, Anal. Calcd. 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 streching, 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).

Compound 3f: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl sulphamethoxazole; $C_{26}H_{25}N_5O_4S$; yield 69% , m.p. 142-145°C, Anal. Calcd. 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).

Compound 3g: 5H-dibenzo [b,f] azepine-5-Corboxamide methyl sulphadimidine; $C_{26}H_{26}N_6O_3S$; yield 86% , m.p. 168°C, Anal. Calcd. C, 63.86; H, 4.98; N, 15.96 Found C, 63.79; H, 4.93; N, 15.89. IR (KBr) νmax in cm-1 : 3463 O-H stretching, 3357 νas N-H in SO_2NH, 3319 νas N-H in NH_2, 3255 νs N-H in SO_2NH, 2979 νas C-H in C-H Aliphatic, 2861 νs C-H in CH_2, 1678 νs C=O in amide, 1599 νs C=N, 1604 N-H bending, 1340 νas of S=O, 1150 νas 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).

Compound 3h: 5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphaacetamide sodium; $C_{24}H_{21}N_2NaO_4S$; yield 76% , m.p. 190-192°C, Anal. Calcd. C, 59.50; H, 4.37; N, 11.56 Found C, 59.41; H, 4.33; N, 11.52. IR (KBr) νmax in cm-1 : 3462 O-H stretching, 3348 νas N-H in SO_2NH, 3419 νas N-H in NH_2, 3262 νs N-H in SO_2NH, 2931 νas C-H in C-H Aliphatic, 2850 νs C-H in CH_2, 1668 νs C=O in amide, 1600 νs C=N, 1593 N-H bending, 1356 νas of S=O, 1146 νas 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).

Compound 3i: 5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphanilamide; $C_{22}H_{20}N_4O_3S$; yield 87% , m.p. 191°C, Anal. Calcd. 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).

Compound 3j: 5H-dibenzo [b,f]azepine-5-Corboxamide methyl sulphadoxine; $C_{28}H_{26}N_6O_5S$; yield 72%, m.p. 173°C, Anal. Calcd. 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 vas N-H in SO_2NH, 3367 vas N-H in NH_2, 3231 vs N-H in SO_2NH, 2965 vas C-H in C-H Aliphatic, 2863 vs C-H in CH_2, 1677 vs C=O in amide, 1614 vs C=N, 1596 N-H bending, 1323 vas of S=O, 1151 vas of S=O, 643 bending in CONH.
^{1}H-NMR ($CDCl_3$) δ ppm: 3.58 (s,3H of O-CH3), 3.72 (s,3H of O-CH3),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).

4. Antimicrobial Activity

The newly synthesized mannich bases 3a-3j were screened for their antibacterial activity against pathogenic strains of *E.coli.and S.aureus* 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^{-1} using methanol as solvent. Cultures of each bacterium kept in Mullar Hinton Agar at 37^0C for 24 Hrs. and then examined. Antibacterial activity was ascertained by the zone of inhibition measured in mm as shown in table 1. 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 [18].

Compound No.	*E.coli*				*S.aureus*			
	Concentration in µg/ml				Concentration in µg/ml			
	80.0	160.0	320.0	Avg	80.0	160.0	320.0	Avg
3a	-	-	5.5	1.8	-	-	-	0.0
3b	4.7	5.3	5.4	5.1	-	3.2	5.6	2.9
3c	17.2	18.6	18.9	18.2	9.5	10.6	12.8	11.0
3d	6.9	7.6	8.4	7.6	14.2	16.2	16.6	15.7
3e	10.3	12.4	13.9	12.2	-	5.3	5.8	3.7
3f	17.7	20.3	23.6	20.5	12.5	15.2	17.6	15.1
3g	-	8.2	10.1	6.1	8.6	10.5	14.2	11.1
3h	10.2	11.6	14.9	12.2	14.6	15.3	15.7	15.2
3i	13.5	16.7	18.5	16.2	8.1	9.6	12.2	10.0
3j	16.2	18.3	19.9	18.1	15.4	17.6	19.4	17.5
2f	19.8	23.1	25.3	22.7	19.5	20.5	21.0	20.3
2g	-	-	-	-	15.3	16.9	18.5	16.9
2h	9.6	11.3	14.5	11.8	19.5	21.0	24.0	21.5
2i	12.3	14.1	17.2	14.5	14.0	15.3	16.2	15.2
2j	14.3	16.8	18.9	16.7	10.6	16.0	19.3	15.3

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

Table 1: Antibacterial screening of synthesized mannich bases and sulphonamides (Zone of inhibition in mm).

Antibacterial screening of 3a-3j against *E.coli* shows significant results. All the Mannich bases show antimicrobial activity against this bacterium. **Table 1** reflects that the compound 3f is statistically superior to other Mannich bases in checking the growth of *E.coli*. Compound 3g, 3h, 3i and 3j reflects more potent antibacterial activity compared to their parent sulfonamides. Compound 3c gives best activity in mannich bases derived from secondary amines. In case of *S.aureus* all Mannich bases show antimicrobial activity against this bacterium except compound 3a. Compound 3b and 3e are very less active against *S.aureus.* Compound 3j is statically superior against *S.aureus* and its also more potent in compare to parent sulphonamide. The concentration of 320 μg mL^{-1} is found significantly superior to concentrations 160 μg mL^{-1} and 80 μg mL^{-1} in checking the growth of all microorganisms.

5. Results and Discussion

The mannich bases synthesized by mannich reaction were obtained in good yield (≥79%). They were analyzed 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 *E.coli.* and *S.aureus* at varying concentrations–80, 160 and 320 μg/ml. These pathogens were subcultured on specific media. The Mannich bases and the standard compound (sulphonamide and secondary amines) were dissolved in methanol. The activities reported were mean of zone of inhibition in millimeter (in triplicate) All the reported compounds exhibit remarkable in vitro activity against these pathogens. Their activity was also compared with their parent sulphonamides. Table-1 reflects that most of the compounds had shown remarkable activity only at 320 μg/ml. Derived Mannich bases were shows activity against these pathogen but compound 3f was superior to other mannich bases and corresponding sulphonamides against *E.coli.* and compound 3j was superior to other mannich bases and corresponding sulphonamides against *S.aureus*. The Mannich bases 3c, 3e, 3h, 3i and 3j were significantly superior to other compounds in exhibiting antibacterial activity against *E.coli.* and Mannich bases 3d, 3f, 3h were significantly active against *S.aureus.* Moreover, concentration 320 μg/ml was superior for inhibiting the growth of the bacterium.

6. Conclusion

Conclusions suggest that the newly synthesized mannich bases of 5H-dibenzo [b,f]azepine-5-Corboxamide a very noticeable and prolonged antibacterial activity. Some derived mannich bases shows more potent antibacterial activity against their parent their parent sulphonamides. This work shows that mannich bases are a potential source of compounds for inhibition of bacteria and could be used as efficient drugs.

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