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Synthesis, spectral characterization and *in vitro* antibacterial activity of amino methylated derivatives of cefuroxime axetil

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ABSTRACT

Biologically active compounds with heteroaromatic ring system of Cefuroxime axetil have been synthesized via aminomethylation reaction. The aminomethylation of Cefuroxime axetil with various biologically potent sulphonamides / secondary amines was carried out and then characterized by elemental analysis and spectral studies – IR, ^{1}H-NMR and ^{13}C-NMR. The compounds were screened for their antibacterial activity against various pathogenic bacteria at varying concentrations. The antibacterial activity of Cefuroxime axetil derivatives was compare with parent sulphonamides. The toxicity of synthesized Cefuroxime axetil derivatives was ascertained by LD_{50} test.

Key words: Cefuroxime axetil, Sulphonamides, Antibacterial activity.

INTRODUCTION

Since the introduction of the first antibiotic (penicillin, 1942) into medical practice, to date, there has been an ongoing “race” between scientists creating new drugs and pathogenic bacteria. This specific “arms race” causes that thousands of potentially active chemicals are synthesized in laboratories around the world every day. The chemistry of the aminoalkylation of aromatic substrates by the Mannich reaction is of great interest for the synthesis and modification of biologically active compound [1-3]. Mannich reaction offers a judicious method for introduction of basic aminoalkyl chain in various drugs/compounds. In this context, literature survey has revealed a number of reports on antimicrobial activity of N-Mannich bases. Cefuroxime axetil (CA) (1-acetoxyethyl ester of a β-lactamase-stable cephalosporin), an orally absorbed pro-drug of cefuroxime is used in the treatment of common community acquired infections because of its in-vitro antibacterial activity against several gram-positive and gram-negative organisms[4]. It has a carbamoyl group, which gives it a considerable metabolic stability and it has a methoxy-imino group, which makes it more stable against β-lactamase attack. Together with the furyl ring, these groups contribute to the antibacterial properties of the molecule by enhancing its activity against gram-negative bacteria[5].

In addition to this, the sulphonamide is well-known antimicrobial agents[6], anti-inflammatory[7], antiproliferative[8], Carbonic anhydrase inhibitors [9], anti-tumor[10] and radiosensitizing agents[11].

The Mannich bases incorporated with sulphonamides were reported to be potent antibacterial agents and less toxic than parent sulphonamide [12, 13]. Keeping in view, the unique features of Pyridine-3-carboxamide and

sulphonamide were condensed via Mannich reaction. A series of Pyridine-3-carboxamide derivatives Niacinamide were synthesized with different sulphonamides / secondary amines (scheme 1 and scheme 2). The synthesized compounds were characterized by elemental analysis and spectral studies-IR, ^{1}H- NMR, ^{13}C-NMR and screened for in-vitro antibacterial activity gram-positive and gram-negative bacteria at arbitrarily chosen concentrations.

The aminometylation incorporated with sulphonamides are reported to be potent antibacterial agents and less toxic than parent sulphonamide. Keeping in view the unique features of Cefuroxime axetil and sulphonamide were condensed via aminometyhylation reaction. A series of Cefuroxime axetil derivatives were synthesized with different sulphonamides / secondary amines (scheme 1 and scheme 2). The synthesized compounds were characterized by elemental analysis and spectral studies- IR, ^{1}H- NMR, ^{13}C-NMR and screened for in-vitro antibacterial activity gram-positive and gram-negative bacteria at arbitrarily chosen concentrations.

MATERIALS AND METHODS

All the melting points were determined in open capillary tubes and were uncorrected. Thin layer chromatography was used for monitoring the reaction and to check purity. IR spectra (KBr) were recorded as potassium bromide pellets on Schimadzu 820 IPC FTIR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO on Avance-II (Bruker) FT NMR Spectrometer. Its ^{1}H frequency is 400 MHz, while for ^{13}C-NMR the frequency is 100 MHz and 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. All substituted sulphonamide were obtained as pure samples from reputed pharmaceutical establishment.

2.1 Chemistry

The reaction routes for synthesis of Cefuroxime axetil derivatives were described as shown in scheme 1 and scheme 2.

2.1.1 Synthesis of Cefuroxime axetil methyl sulphonamide (3a-3f) (Scheme 1)

To the ethanolic solution of 0.1 mol of Cefuroxime axetil 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 in refrigerator for 4 days, till crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF (1:1). Melting points were recorded and uncorrected. 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 (chromatographic grade).

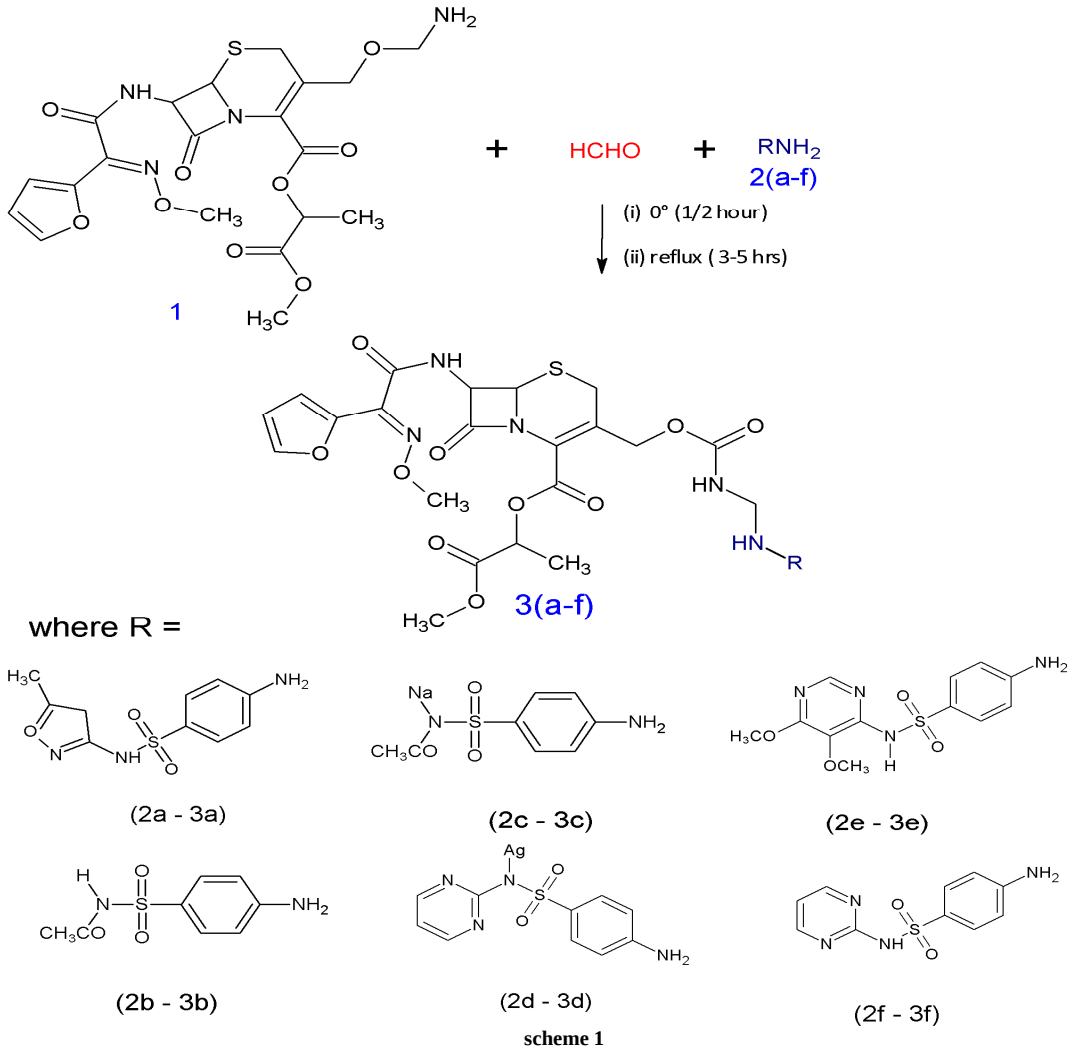
2.1.2 Synthesis of Cefuroxime axetil methyl amines (3g-3k) (Scheme 2)

Secondary amines (0.01 mol) were added to an ethanolic solution (50 mL) of Cefuroxime axetil (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 installments at an interval of 1 h, where first installment was added immediately and second was added after one hour from start of experiment. The reaction mixture was kept overnight in the refrigerator. Next day excess of solvent was distilled off from the reaction mixture through vacuum pump which 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 scheme 1 and scheme 2.

2.2 Spectral Studies

Compound 3a: Cefuroxime axetil methyl sulpha methoxazole; $C_{31}H_{33}N_7O_{13}S_2$; yield 70%, m.p. 200-201°C. Anal. Calcd C, 48.00; H, 4.29; N, 12.64 Found C, 48.02; H, 4.22; N, 12.63. IR (KBr) ν_{max} in cm^{-1}: 3442 (ν_s N-H); 3398 (ν_{as} N-H in SO_2NH); 3080 (Ar. CH– str. in hetero.); 2872 (ν_s C-H in CH_2); 2920 (ν_{as} C-H in CH_2); 1779 (ν_s C=O in beta lactam); 1661 (ν_s C=O in amide); 1620 (ν_s C=O in COONH); 1646 (ν_s C=N); 1538 (N-H bending); 1606 (N-H bending in CO-NH); 1345 (ν_s S=O); 1130 (C-H in plane bending vibration of 1:4 disubstituted benzene); 1033 (ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 2.28 (s, 3H, CH_3 attached to oxazole); 6.38 (s, 1H, ring proton's of

oxazole); 3.74 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 and 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s ,1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 9.03 (s, 1H , SO2NH); 6.6 – 7.2 (m, ring proton of sulphonamide). ^{13}C-NMR (DMSO), δ ppm: 13.22 (CH3 attached to oxazole); 25.78 (CH2 attached to thiazine); 37.11 (CH_2-COO); 52.06 (CH3-COO); 52.32 (-N-CH2-N'-), 57.72 (CH attached to thiazine); 58.64 (CH attached to azetidine); 62.44 (N-O-CH3); 168.22 (COO-CH3); 157.15 (COO-NH); 148.59, 143.42, 111.79 & 111.74 (ring carbon's of furan ring); 113.47 & 128.88 (ring carbon's of sulphonamide); 167.45, 160.16 & 96.84 (ring carbon's of oxazole).

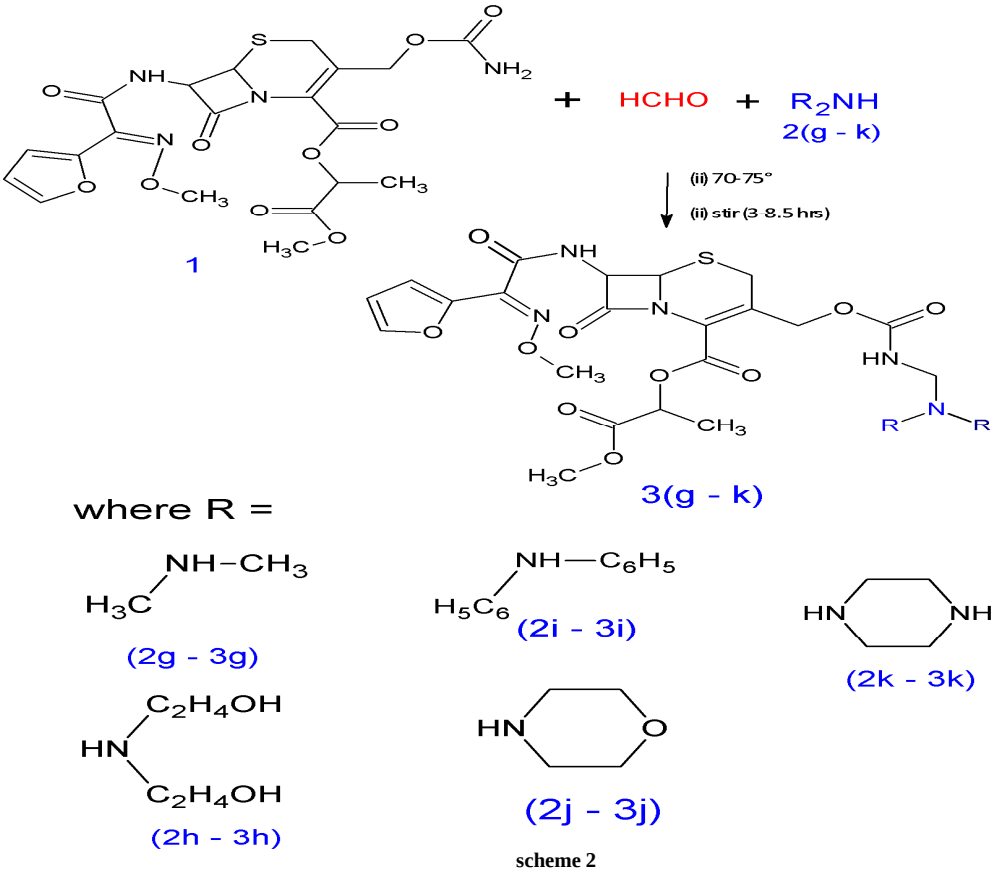


scheme 1

Compound 3b: Cefuroxime axetil methyl Sulphacetamide; $C_{29}H_{32}N_6O_{13}S_2$; yield 77%, m.p. 168 °C. Anal. Calcd. C, 47.28; H, 4.38; N, 11.41; Found C, 47.21; H, 4.36; N, 11.38. IR (KBr) ν_{max} in cm^{-1}: 3436 (ν_s N-H); 3390 (ν_{as} N-H in SO_2NH); 3098 (Ar. CH– str. in hetero.); 2852 (ν_s C-H in CH_2); 2926 (ν_{as} C-H in CH_2); 1778 (ν_s C=O in beta lactam); 1668 (ν_s C=O in amide); 1620 (ν_s C=O in COONH); 1643 (ν_s C=N); 1538 (N-H bending) 1606 (N-H bending in CO-NH); 1345 (ν_s S=O); 1130 (C-H in plane bending vibration of 1:4 disubstituted benzene); 1046(ν_s N-O).^{1}H-NMR (DMSO) δ ppm : 1.89 (s, 3H, CO-CH_3); 3.70 (s, 3H, CH_3-COO); 2.99 (s, 2H, CH_2-COO); 3.54 and 3.48 (ABq, 2H, CH_2 attached to thiazine ring); 5.08 (d, 1H, CH attached to thiazine ring); 5.47 (dd, 1H, CH attached to azetidine ring); 8.20 (s, 1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.83 (s, 3H, NO-CH3); 8.84 (s, 1H, COO-NH); 7.88 (s, 1H, NH-CH_2-NH'); 9.05 (s, 1H , SO_2NH); 6.7–7.5 (m, ring proton of sulphonamide).^{13}C NMR (DMSO), δ ppm: 23.20 (CH_3); 25.90 (CH_2 attached to thiazine); 37.31 (CH_2-COO); 52.80 (CH_3-COO); 52.32 (-N-CH_2-N'-); 57.75 (CH attached to thiazine); 58.68 (CH attached to azetidine); 62.49 (N-O-

CH_3); 168.20 ($COO-CH_3$); 157.80 (COO-NH); 148.99, 143.72, 111.76 & 111.73 (ring carbon's of furan ring); 113.44 & 128.80 (ring carbon's of sulphonamide); 168.84 ($COCH_3$).

Compound 3c: Cefuroxime axetil methyl Sulphacetamide sodium; $C_{29}H_{31}N_6NaO_{13}S_2$; yield 80%, m.p. 190-191°C, Anal. Calcd. C, 45.91; H, 4.12; N, 11.08; Found C, 45.83; H, 4.15; N, 11.03. IR (KBr) ν_{max} in cm^{-1}: 3430 (ν_s N-H); 3377 (ν_{as} N-H in SO_2NH); 3100 (Ar. CH– str. in hetero.); 2859 (ν_s C-H in CH_2); 2922 (ν_{as} C-H in CH_2); 1778 (ν_s C=O in beta lactam); 1671 (ν_s C=O in amide); 1626 (ν_s C=O in COONH); 1645 (ν_s C=N); 1532 (N-H bending); 1611 (N-H bending in CO-NH); 1340 (ν_s S=O); 1131 (C-H in plane bending vibration of 1:4 disubstituted benzene); 1042(ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 1.85 (s, 3H, $CO-CH_3$); 3.73 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 & 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s, 1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 6.8 – 7.3 (m, ring proton of sulphonamide). ^{13}C NMR (DMSO), δ ppm: 22.43(CH_3); 167.50 ($COCH_3$); 25.92 (CH_2 attached to thiazine); 37.10 (CH_2-COO); 52.12 (CH_3-COO); 52.32 (-N-CH_2-N'-); 57.76 (CH attached to thiazine); 58.63 (CH attached to azetidine); 62.46 (N-O-CH_3); 168.29 ($COO-CH_3$); 157.45 (COO-NH); 148.19, 143.22, 111.83 & 111.80 (ring carbon's of furon ring); 113.50 & 128.82 (ring carbon's of sulphonamide).



scheme 2

Compound 3d: Cefuroxime axetil methyl silver sulphadiazine; $C_{31}H_{31}AgN_8O_{12}S_2$; yield 78%, m.p. 170-171°C, Anal. Calcd. C, 42.33; H, 3.55; N, 12.26 Found C, 42.30; H, 3.52; N, 12.22. IR (KBr) ν_{max} in cm^{-1}: 3449 (ν_s N-H); 3383 (ν_{as} N-H in SO_2NH); 3100 (Ar. CH– str. in hetero.); 2850 (ν_s C-H in CH_2); 2918 (ν_{as} C-H in CH_2); 1775 (ν_s C=O in beta lactam); 1663 (ν_s C=O in amide); 1625 (ν_s C=O in COONH); 1644 (ν_s C=N); 1531 (N-H bending) 1613 (N-H bending in CO-NH); 1347 (ν_s, S=O); 1132 (C-H in plane bending vibration of 1:4 di substituted benzene); 1047(ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 3.79 (s, 3H, CH_3-COO); 2.91 (s, 2H, CH_2-COO); 3.61 & 3.54 (ABq, 2H, CH_2 attached to thiazine ring); 5.09 (d, 1H, CH attached to thiazine ring); 5.48 (dd, 1H, CH attached to azetidine ring); 8.28 (s ,1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 9.03 (s, 1H , SO_2NH); 6.6 – 7.2 (m, ring proton of sulphonamide); 8.43

(d, 2H ring proton's of diazine); and 6.82 (t, 1H, ring proton of diazine). ^{13}C-NMR (DMSO), δ ppm: 25.73 (CH_2 attached to thiazine); 37.13 (CH_2-COO), 52.01 (CH_3-COO); 52.22 (-N-CH_2-N'-); 57.72 (CH attached to thiazine); 58.62 (CH attached to azetidine); 62.44 (N-O-CH_3); 168.34 (COO-CH_3); 157.15 (COO-NH); 148.59, 143.42, 111.39 & 111.44 (ring carbon's of furan ring); 113.43 & 128.80 (ring carbon's of sulphonamide); 163.78, 157.23 & 112.86 (ring carbon's of diazine).

Compound 3e: Cefuroxime axetil methyl Sulphadoxine; $C_{35}H_{36}N_8O_{14}S_2$; yield 84%, m.p. 203-202°C. Anal. Calcd. C, 49.06; H, 4.23; N, 13.08 Found C, 49.02; H, 4.21; N, 1304. IR (KBr) ν_{max} in cm^{-1}: 3.77 (s, 3H, CH_3-COO), 2.93 (s, 2H, CH_2-COO), 3.63 and 3.59 (ABq, 2H, CH_2 attached to thiazine ring), 5.05 (d, 1H, CH attached to thiazine ring), 5.40 (dd, 1H, CH attached to azetidine ring), 8.29 (s ,1H, CO-NH), 9.58 (d, 1H, NH-C=0 of Cefuroxime axetil), 3.80 (s, 3H, NO-CH_3), 8.83 (s, 1H, COO-NH), 7.86 (s, 1H, NH-CH_2-NH'), 9.09 (s, 1H , SO_2NH), 6.8 – 7.4 (m, ring proton of sulphonamide), 2.38 (s, 3H, $COCH_3$); 9.66 (s, 1H, ring proton of diazine). ^{1}H-NMR (DMSO) δ ppm: 3.77 (s, 3H, CH_3-COO), 2.93 (s, 2H, CH_2-COO), 3.63 and 3.59 (ABq, 2H, CH_2 attached to thiazine ring), 5.05 (d, 1H, CH attached to thiazine ring), 5.40 (dd, 1H, CH attached to azetidine ring), 8.29 (s, 1H, CO-NH), 9.58 (d, 1H, NH-C=0 of Cefuroxime axetil), 3.80 (s, 3H, NO-CH_3), 8.83 (s, 1H, COO-NH), 7.86 (s, 1H, NH-CH_2-NH'), 9.09 (s, 1H , SO_2NH), 6.8 – 7.4 (m, ring proton of sulphonamide), 2.38 (s, 3H, $COCH_3$); 9.66 (s, 1H, ring proton of diazine). ^{13}C-NMR (DMSO), δ ppm: 13.22 (CH_3 attached to oxazole); 25.98 (CH_2 attached to thiazine); 37.21 (CH_2-COO); 52.10 (CH_3-COO); 52.32 (-N-CH_2-N'-); 57.72 (CH attached to thiazine); 58.64 (CH attached to azetidine); 62.44 (N-O-CH_3); 168.22 (COO-CH_3); 157.11 (COO-NH); 148.55, 143.43, 111.80 & 111.79 (ring carbon's of furan ring); 113.43 & 128.73 (ring carbon's of sulphonamide); 159.67, 157.76, 149.43 & 110.66 (ring carbon's of diazine); 30.45 (CH_3-CO); 30.45 (CH_3-CO-CH-N); 199.0 (CH_3-CO).

Compound 3f: Cefuroxime axetil methyl sulphadiazine; $C_{31}H_{32}N_8O_{12}S_2$; yield 80%, m.p. 180-181°C. Anal. Calcd. C, 48.18; H, 4.17; N, 14.50 Found C, 48.13; H, 4.13; N, 14.47. IR (KBr) ν_{max} in cm^{-1}: 3432 (ν_s N-H); 3393 (ν_{as} N-H in SO_2NH); 3050 (ν_s Ar. C-H in hetero.); 2850 (ν_s C-H in CH_2); 2953 (ν_{as} C-H in CH_2); 1774 (ν_s C=O in beta lactam); 1669 (ν_s C=O in amide); 1623 (ν_s C=O in COONH); 1645 (ν_s C=N); 1540 (N-H bending) 1609 (N-H bending in CO-NH); 1351 (ν_s S=O); 1139 (C-H in plane bending vibration of 1:4 disubstituted benzene); 1044 (ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 3.78 (s, 3H, CH_3-COO), 2.92 (s, 2H, CH_2-COO), 3.53 & 3.46 (ABq, 2H, CH_2 attached to thiazine ring), 5.02 (d, 1H, CH attached to thiazine ring), 5.49 (dd, 1H, CH attached to azetidine ring), 8.38 (s ,1H, CO-NH), 9.57 (d, 1H, NH-C=0 of Cefuroxime axetil), 3.81 (s, 3H, NO-CH_3), 8.79 (s, 1H, COO-NH), 7.81 (s, 1H, NH-CH_2-NH'), 9.01 (s, 1H , SO_2NH), 6.3 – 7.0 (m, ring proton of sulphonamide), 8.26-7.80 (m, 3H ring proton's of diazine).^{13}C-NMR (DMSO), δ ppm: 13.22 (CH_3 attached to oxazole); 25.78 (CH_2 attached to thiazine); 37.11 (CH_2-COO); 52.06 (CH_3-COO); 52.32 (-N-CH_2-N'-), 57.72 (CH attached to thiazine); 58.64 (CH attached to azetidine); 62.44 (N-O-CH_3); 168.22 (COO-CH_3); 157.15 (COO-NH); 148.59, 143.42, 111.80 & 111.73 (ring carbon's of furan ring); 113.42 & 128.77 (ring carbon's of sulphonamide); 159.69, 156.66, 149.43 & 110.66 (ring carbon of diazine).

Compound 3g: Cefuroxime axetil methyl dimethyl amine; $C_{23}H_{29}N_5O_{10}S$; yield 67 %, m.p. 96°C, Anal. Calcd. C, 48.67; H, 5.15; N, 12.34 Found C, 48.41; H, 5.20; N, 12.43. IR (KBr) ν_{max} in cm^{-1}: 3469 (ν_s N-H); 2877 (ν_s C-H in C-H in CH_2); 2937 (ν_{as} C-H in CH_2), 1765 (ν_s C=O in beta lactam); 1650 (ν_s C=O in amide) 1620 (ν_s C=O in COONH); 1584 (ν_s C=N); 1531 (N-H bending); 1032 (ν_s N-O).^{1}H-NMR (DMSO) δ ppm: 3.70 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.53 & 3.47 (ABq, 2H, CH_2 attached to thiazine ring); 5.02 (d, 1H, CH attached to thiazine ring); 5.33 (dd, 1H, CH attached to azetidine ring); 8.26 (s, 1H, CO-NH); 9.50 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.83 (s, 3H, NO-CH_3); 8.81 (s, 1H, COO-NH); 7.82 (s, 1H, NH-CH_2-NH'); 2.55 (3H, N'-CH_3).^{13}C-NMR (DMSO), δ ppm: 40.18 (N-CH_3); 25.72 (CH_2 attached to thiazine); 37.13 (CH_2-COO); 52.09 (CH_3-COO); 52.30 (-N-CH_2-N'-); 57.70 (CH attached to thiazine); 58.62 (CH attached to azetidine); 62.40 (N-O-CH_3); 168.20 (COO-CH_3); 157.11 (COO-NH); 148.57, 143.41, 111.70 & 111.75 (ring carbon's of furon ring); 113.49 & 128.80 (ring carbon's of sulphonamide).

Compound 3h: Cefuroxime axetil methyl diethanol amine; $C_{25}H_{33}N_5O_{12}S$; yield 74 % , m.p. 110-113°C, Anal. Calcd. . C, 47.84; H, 5.30; N, 11.16 Found C, 42.41; H, 4.51; N, 14.10. IR (KBr) ν_{max} in cm^{-1}: 3465 (ν_s N-H); 3342 (ν_s O-H); 2870 (ν_s C-H in C-H in CH_2); 2947 (ν_{as} C-H in CH_2); 1764 (ν_s C=O in beta lactam); 1643 (ν_s C=O in amide); 1624 (ν_s C=O in COONH); 1583 (ν_s C=N); 1536 (N-H bending); 1030 (ν_s N-O).^{1}H-NMR (DMSO) δ ppm: 3.73 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 & 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s ,1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH). ^{13}C-NMR (DMSO), δ ppm: 25.81 (CH_2 attached to thiazine); 37.13 (CH_2-COO); 52.13 (CH_3-COO); 52.04 (N'-CH_2 of ethanol amine); 52.37 (-N-CH_2-

N'-); 57.72 (CH attached to thiazine); 57.33 (CH_2-OH); 58.62 (CH attached to azetidine); 62.40 (N-O-CH_3); 168.33 (COO-CH_3); 157.18 (COO-NH); 148.57, 143.43, 111.75 & 111.74 (ring carbon's of furon ring).

Compound 3i: Cefuroxime axetil methyl morpholine; $C_{25}H_{31}N_5O_{11}S$; yield 85%, m.p. 210-212°C, Anal. Calcd. C, 49.26; H, 5.13; N, 11.49 Found C, 49.22; H, 5.10; N, 11.45. IR (KBr) ν_{max} in cm^{-1}: 3458 (ν_s N-H); 2864 (ν_s C-H in CH_2); 2952 (ν_{as} C-H in CH_2); 1763 (C=O in beta lactam); 1652 (ν_s C=O in amide); 1621 (ν_s C=O in COONH); 1582 (ν_s C=N); 1513 (N-H bending); 1030 (ν_s N-O).^{1}H-NMR (DMSO) δ ppm: 3.73 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 & 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s, 1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 9.03 (s, 1H, SO_2NH); 6.6 – 7.2 (m, ring proton of sulphonamide); 2.56 (t, 2H, N'-CH_2 of morpholine); 3.49 (t, 2H, O-CH_2 of morpholine).^{13}C-NMR (DMSO), δ ppm: 50.67 (N-CH_2 of morpholine); 67.88 (O-CH_2 of morpholine); 25.78 (CH_2 attached to thiazine); 37.11 (CH_2-COO); 52.06 (CH_3-COO); 52.32 (-N-CH_2-N'-); 57.76 (CH attached to thiazine); 58.68 (CH attached to azetidine); 62.50 (N-O-CH_3); 168.25 (COO-CH_3); 157.19 (COO-NH); 148.55, 143.49, 111.89 & 111.70 (ring carbon's of furan ring).

Compound 3j: **Cefuroxime axetil methyl piperazine;** $C_{25}H_{32}N_6O_{10}S$; yield 82%, m.p. 198°C, Anal. Calcd. C, 49.34; H, 5.30; N, 13.81 Found C, 49.30; H, 5.32; N, 13.75. IR (KBr) ν_{max} in cm^{-1}: 3457 (ν_s N-H); 2884 (ν_s C-H in CH_2); 2968 (ν_{as} C-H in CH_2); 1768 (C=O in beta lactam); 1657 (ν_s C=O in amide); 1620 (ν_s C=O in COONH); 1587 (ν_s C=N); 1520 (N-H bending); 1038 (ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 3.73 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 & 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s ,1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 2.18 (t, 2H, N'-CH_2 of piperazine); 2.64 (t, 2H, NH-CH_2 of piperazine). ^{13}C-NMR (DMSO), δ ppm: 52.78 (N'-CH_2 of piperazine); 45.90 (NH-CH_2 of piperazine); 25.78 (CH_2 attached to thiazine); 37.11 (CH_2-COO); 52.06 (CH_3-COO); 52.32 (-N-CH_2-N'-); 57.72 (CH attached to thiazine); 58.64 (CH attached to azetidine); 62.44 (N-O-CH_3); 168.22 (COO-CH_3); 157.33 (COO-NH); 148.61, 143.62, 111.59 & 111.84 (ring carbon's of furan ring).

Compound 3k: Cefuroxime axetil methyl diphenyl amine; $C_{33}H_{33}N_5O_{10}S$; yield 80%, m.p. 165-167°C. Anal. Calcd. C, 52.88; H, 4.13; N, 12.76. Found C, 52.80; H, 4.10; N, 12.72. IR (KBr) ν_{max} in cm^{-1}: 3450 (ν_s N-H); 2877 (ν_s C-H in CH_2); 2941 (ν_{as} C-H in CH_2); 1760 (C=O in beta lactam); 1651 (ν_s C=O in amide); 1614 (ν_s C=O in COONH); 1579 (ν_s C=N); 1520 (N-H bending); 1039 (ν_s N-O). ^{1}H-NMR (DMSO) δ ppm: 3.74 (s, 3H, CH_3-COO); 2.94 (s, 2H, CH_2-COO); 3.57 & 3.41 (ABq, 2H, CH_2 attached to thiazine ring); 5.05 (d, 1H, CH attached to thiazine ring); 5.43 (dd, 1H, CH attached to azetidine ring); 8.28 (s, 1H, CO-NH); 9.53 (d, 1H, NH-C=0 of Cefuroxime axetil); 3.84 (s, 3H, NO-CH_3); 8.88 (s, 1H, COO-NH); 7.84 (s, 1H, NH-CH_2-NH'); 7.60-7.10 (m, 5H, proton's of phenyl ring). ^{13}C-NMR (DMSO), δ ppm: 25.78 (CH_2 attached to thiazine); 37.14 (CH_2-COO); 52.10 (CH_3-COO); 52.30 (-N-CH_2-N'-); 57.74 (CH attached to thiazine); 58.64 (CH attached to azetidine); 62.45 (N-O-CH_3); 168.21 (COO-CH_3); 157.23 (COO-NH); 148.59, 143.42, 111.80 & 111.74 (ring carbon's of furan ring); 124.40 & 122.52 (Carbon's of phenyl ring).

2.3. Antimicrobial Activity and LD_{50} Test

The newly synthesized Cefuroxime axetil derivatives (3a-3k) were screened for their antibacterial activity against pathogenic strains of *S.typhi* and *B.subtilis* at varying concentrations-80µg/ml, 160µg/ml and 320µg/ml using corresponding sulphonamide as their standards by cup plate 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 and bored. The solution of Cefuroxime axetil derivatives were poured in these cups in triplicate and incubated at 37°C for 24 hours. 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 sulphonamide.

The toxicity of synthesized Cefuroxime axetil derivatives was ascertained by LD_{50} test. The test was performed on white mice weighing 25g. Doses were given orally as well as intraperitoneally and mice were kept under observation for 72 hr for each trial. The Cefuroxime axetil derivatives showed no adverse toxic effect even of an oral dose of 1400 mg/kg of the body weight of mice. However, when dose was administered intraperitoneally they proved to be lethal at the dose level of 750 mg/kg of the body weight of mice.

Table1. Antibacterial screening of synthesized Mannich Bases and sulphonamides against *S.typhi* and *B.subtilis* (Zone of inhibition in mm)

Comp. No.	*S.typhi* Concentration in µg/ml				*B. subtilis* Concentration in µg/ml			
	80	160	320	Avg	80	160	320	Avg
3a	20.34	26.5	29.9	18.93	-	13.4	15.9	9.76
3b	-	-	-	-	7.0	11.9	12.1	10.33
3c	-	-	-	-	8.0	10.8	13.3	10.7
3d	6.0	6.9	8.7	7.2	-	-	-	-
3e	20.1	21.8	28.3	23.4	11.0	12.7	14.8	12.83
3f	20.8	22.3	25.6	22.9	10.4	13.9	16.6	13.63
3g	6.6	11.7	16.0	11.43	5.5	8.6	10.9	8.33
3h	14.8	16.1	17.3	16.06	-	11.3	15.0	8.76
3i	6.0	9.5	12.1	9.1	12.4	16.0	20.6	16.33
3j	-	-	-	-	-	5.9	11.4	5.76
3k	-	-	-	-	5.0	8.3	10.5	7.93
2a	-	7.3	9.5	5.6	25.6	28.0	29.6	27.6
2b	8.0	8.5	9.5	8.67	7.5	8.5	9.0	8.33
2c	-	-	-	-	9.6	17.6	21.6	16.2
2d	-	-	-	-	8.5	9.5	11.0	9.67
2e	-	-	7.3	2.4	8.6	10.3	12.6	10.5
2f	-	-	7.5	2.5	17.0	19.6	27.6	21.4

RESULTS AND DISCUSSION

The Cefuroxime axetil derivatives synthesized by Aminomethylation reaction were obtained in good yield (≥85%). 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 of IR and NMR. 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 using. The spectral studies have shown characteristic band of methylene group incorporated between Cefuroxime axetil and the amine component due to aminomethylation. This shows the presence of amino methyl linkage in the synthesized Cefuroxime axetil derivatives. The NMR also confirms amino methyl linkage ($-CH_2$) between amine and active hydrogen. The Cefuroxime axetil derivatives 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 subcultured on specific media. The Cefuroxime axetil derivatives and the standard compound (sulphonamide and secondary amines) were dissolved in DMF. The reported activities 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 sulphonamide.

Table-1 reflects that most of the compounds had shown remarkable activity only at 320 µg/ml. Antibacterial screening of Cefuroxime axetil derivatives against *S.typhi* shows interesting results. 3e was superior to other followed by 3f and 3a in inhibiting the growth of this pathogen. On Comparison with parent sulphonamide shows that compound 3a, 3d, 3e, and 3f were superior to the corresponding sulphonamide.
Cefuroxime axetil derivatives had shown significant activity against *B.subtilis* The compound 3i, 3f, 3e were significantly superior to other compounds in exhibiting antibacterial activity against *S.aureus*. Comparative study with sulphonamides indicates that compound 3b and 3d are superior to the corresponding sulphonamides. Moreover, concentration 320 µg/ml was superior for inhibiting the growth of the bacterium.

Comparison of Cefuroxime axetil derivatives with sulphonamides shows that, some Cefuroxime axetil derivatives are having more antibacterial activity, but former is less toxic than latter as revealed by LD_{50} test on white mice of weight 25gm. the newly synthesized compounds seems to be really promising compounds for their antibacterial activity. In the light of those finding we will undertake further synthetic studies on the new compounds in the future.

CONCLUSION

This work shows that Cefuroxime axetil derivatives are a potential source of compounds for inhibition of bacteria and could be used as efficient drugs with minimum side effects.

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