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SYNTHESIS OF NEW ACETAMIDE-CONJUGATED MONOBACTAM ANTIBIOTICS

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ABSTRACT: In the present work, we have synthesized a new analoges of monocyclic *β*-lactam (2-(3-(2, 4-dichlorophenoxy)-2-(substituted aryl) - 4-oxoazetidin – 1 - ylamino) – N - (pyridin-2-yl) acetamide) derivatives in the presence of triethyl amine (TEA) and phosphorus oxychloride ($POCl_3$) under classical method by using Dichloromethane (DCM) as a solvent. The designed compounds 4(a-l) were prepared by Staudinger reaction ([2+2] ketene-imine cycloaddition reactions). In which an azetidin-2-one motif connects with pyridine-2-acetamide nucleus with two aromatic rings. The target compounds were screened for *in vitro* antibacterial activity against clinically relevant Gram-negative (*Escherichia coli and Klebsiella pneumonia*) and Gram-positive species (*Bacillus subtilis, Proteus vulgaris and Staphylococcus aureus*). The obtained results have demonstrated that all the synthesized imidazole-conjugated monocyclic *β*-lactam derivatives showed good antibacterial activity. Particularly the compounds 4e and 4l found to be effective in *P.vulgaris* as equal to reference ampicillin and other compounds showed moderate to good activity against five human bacterial pathogens. All these compounds have been characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectrometry and Elemental data.

INTRODUCTION: The *β*-lactam nucleus has fascinated synthetic and medicinal chemists worldwide because of its biological significance and synthetic potential.[1-2] *β*-lactam form a class of antibiotics characterized by the presence of an azetidine-2-one ring, which is the core structure responsible for biological activity.[3] The *β*-lactam ring is a common structural feature of a number of broad spectrum *β*-lactam antibiotics, including penicillins, cephalosporins, carbapenems. It also exhibit some other biological activities, for which they are considered as enzyme inhibitors,[4] potential chemo and neurotherapeutic drugs,[5] Penicillin Binding Protein,[6] human cytomegalovirus protease inhibitors,[7] anti-hyperglycemic,[8] antimalarial,[9] anti-tumor,[10] cholesterol absorption inhibitors,[11] anti-HIV,[12] protozoal,[13] anti-inflammatory, antimicrobial, [14-16] cytotoxic[17] and anticancer.[18]

The *β*-lactams have also been employed in the preparation of bis-*β*-lactams, pyrrolizidines, indolizidines, pyrrolidines, piperidines, cyclic enaminones, pyridones, oxazinones, and complex natural products through N1–C2 bond cleavage coupled with rearrangement reactions. However,

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microorganisms have built up resistance against the most traditional *β*-lactam antibiotics due to excess use of antibiotics.

Therefore there arises need to modify the structure of known active compounds and the development of new ones. Our research group has been largely involved in the synthesis of monocyclic *β*-lactam derivatives[19-21] through Staudinger reaction ([2+2] ketene-imine cycloaddition reaction).

MATERIALS AND METHODS:

Materials: All the chemicals and solvents were used AR grade without further purification. Melting points were taken in an open capillary tube. IR spectra were recorded on a Shimadzu Dr-8031 instrument. ^{1}H NMR spectra of the titled compounds were recorded on a Bruker-Avance (300 MHz) spectrophotometer using DMSO solvent and TMS as the internal standard. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source. The reactions were monitored and the purity of products was checked out on pre-coated TLC plates (Silica gel 60 F254, Merck), visualized the spots under ultraviolet light and iodine chamber.

Biology:

The synthesized compounds were screened by agar diffusion method. All human pathogenic bacteria *viz. Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, were obtained from the Osmania University, Hyderabad, India. Stock solutions of compounds were diluted in dimethyl sulfoxide (DMSO) to give a final concentration for determining the Minimum inhibitory concentration (MIC) value. About 9 ml of nutrient agar media were poured into petri plates (9cmin diameter) and inoculated with respective test organism. Wells were made with cork borer on the solid agar and loaded with 100 mg/ml of the test compound with Ampicillin as control. Petri dishes were incubated at 37 ^{0}C for 24 h and the average diameter of the inhibition zone surrounding the wells was measured after specified incubation period.

METHOD:

General procedure for the synthesis of Schiff base 3(a-l): A quantity of 0.02 mol of aryl-aldehyde, 0.02 mol of 2-hydrazinyl-N-arylacetamide (2) and 2-3 drops of glacial acetic acid in 20 ml of ethanol was refluxed for ~1h. The reaction was monitored by TLC. After completion of the reaction, the residue was stirred with ice cold water, filtered and dried. The crude product obtained was purified by *n*-hexane and EtOAc.

(3a): Yield 75%; m.p. 162 ^{0}C; Chemical formula: $C_{14}H_{14}N_4O$; IR (KBr, cm-^{1}): 3125 (NH), 1589 (CONH), 1545 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.55 (s, 1H, NH), 3.6 (s, 2H, CH_2), 6.7-7.7 (m, 9H, Ar-H), 8.48 (s, 1H, CONH), 8.71 (s, 1H, -CH=N-); ^{13}C NMR: δ 54.2 (-CH_2), 143.2 (N=CH), 167.8 (C=O), Aromatic carbons: 115.6, 119.3, 128.1, 130.5, 138.1, 146.2, 150.4; Elemental analysis: Calcd (found): C, 66.13 (66.22); H, 5.55 (5.47); N, 22.03 (22.11); Mass spectra, m/z = 254 (100%).

(3b): Yield 80%; m.p. 175 ^{0}C; Chemical formula: $C_{14}H_{14}N_4O_2$; IR (KBr, cm-^{1}): 3118 (NH), 1608 (CONH), 1552 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.51 (s, 1H, NH), 3.65 (s, 2H, CH_2), 6.5-7.8 (m, 8H, Ar-H), 8.51 (s, 1H, CONH), 8.75 (s, 1H, -CH=N-), 11.7(s, 1H, OH); ^{13}C NMR: δ 143.5 (N=CH), 161.5 (OH-C), 53.8 (-CH_2), 168.9 (C=O), Aromatic carbons: 116.1, 119.7, 128.5, 129.9, 138.4, 146.5,150; Elemental analysis: Calcd (found): C, 62.21 (62.15); H, 5.22 (5.29); N, 20.73 (20.64); Mass spectra, m/z = 270 (100%).

(3c): Yield 82%; m.p. 192 ^{0}C; Chemical formula: $C_{16}H_{19}N_5O$; IR (KBr, cm-^{1}): 3084 (NH), 1650 (CONH), 1554 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.50 (s, 1H, NH), 4.21 (s, 2H, CH_2), 2.92 (s, 6H, -N(CH_3)$_2$), 6.6-7.9 (m, 8H, Ar-H), 8.49 (s, 1H, CONH), 8.19 (s, 1H, -CH=N-); ^{13}C NMR: δ 41.5 (-CH_3), 55.0 (-CH_2), 147.7 (N=CH), 164.8 (C=O), Aromatic carbons: 114.5, 121.5, 125.7, 127.8, 129.7, 130.0, 136.3, 138.5; Elemental analysis: Calcd (found): C, 64.63 (64.71); H, 6.44 (6.36); N, 23.55 (23.62); Mass spectra, m/z = 297 (100%).

(3d): Yield 65%; m.p. 187 ^{0}C; Chemical formula: $C_{15}H_{16}N_4O_2$; IR (KBr, cm-^{1}): 3097 (NH), 1625

(CONH), 1531 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.55 (s, 1H, NH), 3.69 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.5-7.7 (m, 8H, Ar-H), 8.37 (s, 1H, CONH), 8.64 (s, 1H, -CH=N-); ^{13}C NMR: δ 54.5 (-CH_2), 55.3 (-OCH_3), 143.6 (N=CH), 168.5 (C=O), Aromatic carbons: 114.2, 115.3, 119.7, 126.5, 130, 138.2, 146.6, 150.3, 163.4; Elemental analysis: Calcd (found): C, 63.37 (63.29); H, 5.67 (5.62); N, 19.71 (19.80); Mass spectra, m/z = 284 (100%).

(3e): Yield 75%; m.p. 196 ^{0}C; Chemical formula: $C_{12}H_{12}N_4O_2$; IR (KBr, cm-^{1}): 3138 (NH), 1605 (CONH), 1550 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.49 (s, 1H, NH), 3.65 (s, 2H, CH_2), 6.5-7.9 (m, 7H, Ar-H), 8.52 (s, 1H, CONH), 8.51 (s, 1H, -CH=N-); ^{13}C NMR: δ 53.3 (-CH_2), 134.2 (N=CH), 168.7(C=O), Aromatic carbons: 109.3, 110.1, 115.4, 120.3, 138.7, 143.4, 146.8, 149.1, 150.4; Elemental analysis: Calcd (found): C, 59.01 (59.07); H, 4.95 (4.85); N, 22.94 (23.03); Mass spectra, m/z = 244 (100%).

(3f): Yield 80%; m.p. 182 ^{0}C; Chemical formula: $C_{15}H_{16}N_4O$; IR (KBr, cm-^{1}): 3172 (NH), 1636 (CONH), 1539 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.56 (s, 1H, NH), 3.53 (s, 2H, CH_2), 2.54 (s, 3H, CH_3), 6.6-7.8 (m, 8H, Ar-H), 8.61 (s, 1H, CONH), 8.57 (s, 1H, -CH=N-); ^{13}C NMR: δ 24.5 (-CH_3), 54.7 (-CH_2), 140.9 (N=CH), 168.7 (C=O), Aromatic carbons: 115.3, 119.2, 128, 129.5, 131.4, 138.5, 140.7, 146.6, 150.8; Elemental analysis: Calcd (found): C, 67.15 (67.07); H, 6.01 (6.09); N, 20.88 (20.74); Mass spectra, m/z = 268 (100%).

(3g): Yield 74%; m.p. 177 ^{0}C; Chemical formula: $C_{14}H_{13}N_5O_3$; IR (KBr, cm-^{1}): 3193 (NH), 1662 (CONH), 1559 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.55 (s, 1H, NH), 3.7 (s, 2H, CH_2), 6.6-7.8 (m, 8H, Ar-H), 8.62 (s, 1H, CONH), 8.8 (s, 1H, -CH=N-); ^{13}C NMR: δ 143.8 (N=CH), 54.5 (-CH_2), 168.2 (C=O), Aromatic carbons: 115.4, 119.4, 121.2, 130.5, 138, 140.4, 146.6, 150.2; Elemental analysis: Calcd (found): C, 56.18 (56.28); H, 4.38 (4.24); N, 23.40 (23.48); Mass spectra, m/z = 299 (100%).

(3h): Yield 82%; m.p. 180 ^{0}C; Chemical formula: $C_{14}H_{14}N_4O_2$; IR (KBr, cm-^{1}): 3145 (NH), 1653 (CONH), 1522 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.47 (s, 1H, NH), 3.75 (s, 2H, CH_2), 6.5-7.8 (m, 8H, Ar-H), 8.55 (s, 1H, CONH), 8.71 (s, 1H, -CH=N-), 11.5(s, 1H, OH); ^{13}C NMR: δ 143.1 (N=CH), 161.9 (OH-C), 53.4 (-CH_2), 168.3 (C=O), Aromatic carbons: 116.4, 119.2, 128.9, 130, 138.1, 146.9,150.5; Elemental analysis: Calcd (found): C, 62.21 (62.3); H, 5.22 (5.15); N, 20.73 (20.77); Mass spectra, m/z = 270 (100%).

(3i): Yield 71%; m.p. 189 ^{0}C: Chemical formula: $C_{14}H_{13}ClN_4O$; IR (KBr, cm-^{1}): 3167 (NH), 1668 (CONH), 1565 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.5 (s, 1H, NH), 3.7 (s, 2H, CH_2), 6.5-7.7 (m, 8H, Ar-H), 8.59 (s, 1H, CONH), 8.88 (s, 1H, -CH=N-); ^{13}C NMR: δ 54.7 (-CH_2), 143.5 (N=CH), 168.8 (C=O), Aromatic carbons: 115.9, 119.4, 120, 130.7, 151.5, 136.2, 138.9, 146.6, 151.3; Elemental analysis: Calcd (found): C, 58.24 (58.17); H, 4.54 (4.45); N, 19.40 (19.54); Mass spectra, m/z = 288 (100%).

(3j): Yield 74%; m.p. 181 ^{0}C; Chemical formula: $C_{14}H_{13}N_5O_3$; IR (KBr, cm-^{1}): 3134 (NH), 1634 (CONH), 1572 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.59 (s, 1H, NH), 3.64 (s, 2H, CH_2), 6.5-7.9 (m, 8H, Ar-H), 8.66 (s, 1H, CONH), 8.74 (s, 1H, -CH=N-); ^{13}C NMR: δ 53.7 (-CH_2), 142.8 (N=CH), 167.6 (C=O), Aromatic carbons: 114.7, 119.5, 123.6, 125.4, 129.4, 134.3, 135.6, 138.4, 146.7, 148.5, 150; Elemental analysis: Calcd (found): C, 56.18 (56.14); H, 4.38 (4.46); N, 23.40 (23.33); Mass spectra, m/z = 299 (100%).

(3k): Yield 72%; m.p. 185 ^{0}C;Chemical formula: $C_{15}H_{16}N_4O$; IR (KBr, cm-^{1}): 3142 (NH), 1668 (CONH), 1532 (-CH=N-); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.46 (s, 1H, NH), 3.57 (s, 2H, CH_2), 2.5 (s, 3H, CH_3), 6.5-7.7 (m, 8H, Ar-H), 8.67 (s, 1H, CONH), 8.52 (s, 1H, -CH=N-); ^{13}C NMR: δ 24.1 (-CH_3), 54.9 (-CH_2), 141.5 (N=CH), 168.2 (C=O), Aromatic carbons: 114.9, 118.8, 128.5, 129.2, 131.9, 138.1, 140.9, 145.3, 151.2; Elemental analysis: Calcd (found): C, 67.15 (67.23); H, 6.01 (6.05); N, 20.88 (20.77); Mass spectra, m/z = 268 (100%).

(3l): Yield 78%; m.p. 177 ^{0}C; Chemical formula: $C_{16}H_{18}N_4O_3$;; IR (KBr, cm-^{1}): 3158 (NH), 1691 (CONH), 1561 (-CH=N-); ^{1}H NMR (300 MHz,

$CDCl_3$) δ (ppm) = 2.49 (s, 1H, NH), 3.7 (s, 2H, CH_2), 3.82 (s, 6H, OCH_3), 6.5-7.8 (m, 7H, Ar-H), 8.59 (s, 1H, CONH), 8.65 (s, 1H, -CH=N-); ^{13}C NMR: δ 54.4 (-CH_2), 56.3 (-OCH_3), 142.5 (N=CH), 169.1 (C=O), Aromatic carbons: 100.4, 106.3, 109.8, 115.2, 119.6, 131.2, 138.8, 146.6, 150.4, 161.5, 164.7; Elemental analysis: Calcd (found): C, 61.13 (61.05); H, 5.77 (5.85); N, 17.82 (17.76); Mass spectra, m/z = 314 (100%).

General Procedure for the synthesis of *β*-lactam 4(a-l):

The appropriate Schiff base (0.02 mol), 2,4-dichlorophenoxy aceticacid (0.02 mol) and triethylamine (0.05 mol) was stirred in anhydrous dichloromethane (DCM), while a solution of $POCl_3$ (0.02 mol) in dry dichloromethane was added drop wise. The reaction mixture was stirred for ~14h. The completion of the reaction was monitored by TLC. The reaction mixture was washed with water and dried over sodium sulphate. The products obtained **4(a-l)** after removing the solvent was purified from ethyl acetate and n-hexane.

(4a): Yield 72%; m.p. 188 ^{0}C; Chemical formula: $C_{22}H_{18}Cl_2N_4O_3$; IR (KBr, cm-1): 3092 (NH), 1602 (CONH), 1732 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.21 (s, 1H, NH), 3.62 (s, 2H, CH_2), 5.35 (d, 1H, CH-Ar, *β*-lactam), 5.85 (d, 1H, CH-CO, *β*-lactam), 6.5-7.7 (m, 12H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 52.7 (-CH_2), 59.8, 89.5, 168.9 (C=O), 173.2 (C=O), Aromatic carbons: 115.4, 117.6, 119.8, 124.2, 127.3, 126.6, 131, 138.5, 143.1, 146.8, 150.2; Elemental analysis: Calcd (found): C, 57.78 (57.69); H, 3.97 (3.90); N, 12.25 (12.33); Mass spectra, m/z = 456 (100%).

(4b): Yield 77%; m.p. 195 ^{0}C; Chemical formula: $C_{22}H_{18}Cl_2N_4O_4$; IR (KBr, cm-1): 3060 (NH), 1612 (CONH), 1725 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.32 (s, 1H, NH), 3.56 (s, 2H, CH_2), 5.30 (d, 1H, CH-Ar, *β*-lactam), 5.75 (d, 1H, CH-CO, *β*-lactam), 6.5-7.6 (m, 11H, Ar-H), 8.57 (s, 1H, CONH), 11.45 (s, 1H, -OH); ^{13}C NMR: δ 52.3 (-CH_2), 60.1, 90.4, 168.2 (C=O), 173.8 (C=O), 154.2 (OH), Aromatic carbons: 115.6, 117.2, 119.9, 121.2, 124.4, 127.7, 130.5, 131.1, 138.7, 146.4, 149.2; Elemental analysis: Calcd (found): C, 55.83 (55.69); H, 3.83 (3.95); N, 11.84 (11.73); Mass spectra, m/z = 472 (100%).

(4c): Yield 76%; m.p. 202 ^{0}C; Chemical formula: $C_{24}H_{23}Cl_2N_5O_3$; IR (KBr, cm-1): 3080 (NH), 1624 (CONH), 1747 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.05 (s, 6H, -$N(CH_3)_2$), 3.41 (s, 1H, NH), 4.12 (s, 2H, CH_2), 5.19 (d, 1H, CH-Ar, *β*-lactam), 4.91 (d, 1H, CH-CO, *β*-lactam), 6.55-7.83 (m, 11H, Ar-H), 8.45 (s, 1H, CONH); ^{13}C NMR: δ 41.1 (CH_3), 49.3 (-CH_2), 53.4, 64.8, 166.9 (C=O), 170.1 (C=O), Aromatic carbons: 113.0, 115.5, 122.3, 123.3, 124.8, 125.2, 126.2, 127.1, 128.8, 146.7, 147.2, 152.1; Elemental analysis: Calcd (found): C, 57.61 (57.68); H, 4.63 (4.52); N, 14.0 (14.11); Mass spectra, m/z = 499 (100%).

(4d): Yield 67%; m.p. 192 ^{0}C; Chemical formula: $C_{23}H_{20}Cl_2N_4O_4$; IR (KBr, cm-1): 3072 (NH), 1656 (CONH), 1754 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.25 (s, 1H, NH), 3.59 (s, 2H, CH_2), 5.4 (d, 1H, CH-Ar, *β*-lactam), 5.72 (d, 1H, CH-CO, *β*-lactam), 3.83 (s, 3H, -OCH_3), 6.5-7.7 (m, 11H, Ar-H), 8.5 (s, 1H, CONH); ^{13}C NMR: δ 52.6 (-CH_2), 55.4 (-OCH_3), 59.5, 89.4, 168.1 (C=O), 174.1 (C=O), Aromatic carbons: 114.4, 115.5, 117.2, 124.1, 128.4, 131, 135.3, 146.6, 149.6, 159.2; Elemental analysis: Calcd (found): C, 56.69 (56.54); H, 4.14 (4.22); N, 11.50 (11.57); Mass spectra, m/z = 486 (100%).

(4e): Yield 65%; m.p. 210 ^{0}C; Chemical formula: $C_{20}H_{16}Cl_2N_4O_4$; IR (KBr, cm-1): 3089 (NH), 1636 (CONH), 1755 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.28 (s, 1H, NH), 3.45 (s, 2H, CH_2), 5.51 (d, 1H, CH-Ar, *β*-lactam), 5.76 (d, 1H, CH-CO, *β*-lactam), 6.6-7.9 (m, 10H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 51.7 (-CH_2), 57.2, 87.4, 168.5 (C=O), 173.9 (C=O); Aromatic carbons: , 105.5, 110.2, 115.7, 119.4, 124.4, 127.3, 131.6, 138.9, 141.5, 146.3, 149.3, 151.1; Elemental analysis: Calcd (found): C, 53.71 (53.78); H, 3.61 (3.73); N, 12.53 (12.46); Mass spectra, m/z = 446 (100%).

(4f): Yield 75%; m.p. 197 ^{0}C; Chemical formula: $C_{23}H_{20}Cl_2N_4O_3$; IR (KBr, cm-1): 3094 (NH), 1625 (CONH), 1761 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.32 (s, 1H, NH), 3.66 (s,

2H, CH_2), 5.41 (d, 1H, CH-Ar, *β*-lactam), 5.79 (d, 1H, CH-CO, *β*-lactam), 6.6-7.94 (m, 11H, Ar-H), 8.51 (s, 1H, CONH), 2.50 (s, 3H, CH_3); ^{13}C NMR: δ 25.1 (-CH_3), 52.7 (-CH_2), 59.4, 90.2, 169.2 (C=O), 174.1 (C=O), Aromatic carbons: 115.6, 117.2, 119.8, 124.6, 126.1, 131.2, 136.7, 138.4, 140.4, 146.2, 149.7; Elemental analysis: Calcd (found): C, 58.61 (58.55); H, 4.28 (4.22); N, 11.89 (11.96); Mass spectra, m/z = 470 (100%).

(4g): Yield 64%; m.p. 202 ^{0}C; Chemical formula: $C_{22}H_{17}Cl_2N_5O_5$; IR (KBr, cm-1): 3086 (NH), 1636 (CONH), 1755 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.28 (s, 1H, NH), 3.59 (s, 2H, CH_2), 5.41 (d, 1H, CH-Ar, *β*-lactam), 5.76 (d, 1H, CH-CO, *β*-lactam), 6.6-7.9 (m, 11H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 51.7 (-CH_2), 59.9, 89.5, 168.4 (C=O), 173.9 (C=O), Aromatic carbons: 115.5, 117.6, 119.2, 121.2, 124.5, 127.4, 131, 138.2, 149.6; Elemental analysis: Calcd (found): C, 52.60 (52.66); H, 3.41 (3.35); N, 13.94 (13.86); Mass spectra, m/z = 501 (100%).

(4h) Yield 62%; m.p. 189 ^{0}C; Chemical formula: $C_{22}H_{18}Cl_2N_4O_4$; IR (KBr, cm-1): 3034 (NH), 1615 (CONH), 1739 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.27 (s, 1H, NH), 3.63 (s, 2H, CH_2), 5.46 (d, 1H, CH-Ar, *β*-lactam), 5.75 (d, 1H, CH-CO, *β*-lactam), 6.53-7.78 (m, 11H, Ar-H), 8.56 (s, 1H, CONH), 11.56 (s, 1H, -OH); ^{13}C NMR: δ 51.8 (-CH_2), 59.9, 89.4, 168.7 (C=O), 174.1 (C=O), 156.6 (OH), Aromatic carbons: 115.8, 117.4, 119.5, 124.2, 127.4, 127.3, 128.4, 130.1, 131.5, 136.3, 138.6, 146.1, 149.5; Elemental analysis: Calcd (found): C, 55.83 (55.90); H, 3.83 (3.76); N, 11.84 (11.93); Mass spectra, m/z = 472 (100%).

(4i): Yield 70%; m.p. 199 ^{0}C; Chemical formula: $C_{22}H_{17}Cl_3N_4O_3$; IR (KBr, cm-1): 3027 (NH), 1663 (CONH), 1758 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.36 (s, 1H, NH), 3.69 (s, 2H, CH_2), 5.39 (d, 1H, CH-Ar, *β*-lactam), 5.84 (d, 1H, CH-CO, *β*-lactam), 6.5-8.0 (m, 11H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 52.4 (-CH_2), 59.8, 89.3, 168.1 (C=O), 180 (C=O), Aromatic carbons: 115.4, 117.2, 119.6, 124.1, 127.8, 131, 132.4, 138.7, 141.5, 146.2, 150; Elemental analysis: Calcd (found): C, 53.73 (53.65); H, 3.48 (3.54); N, 11.39 (11.45); Mass spectra, m/z = 490 (100%).

(4j): Yield 67%; m.p. 208 ^{0}C; Chemical formula: $C_{22}H_{17}Cl_2N_5O_5$; IR (KBr, cm-1): 3109 (NH), 1651 (CONH), 1766 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.25 (s, 1H, NH), 3.56 (s, 2H, CH_2), 5.43 (d, 1H, CH-Ar, *β*-lactam), 5.81 (d, 1H, CH-CO, *β*-lactam), 6.6-7.8 (m, 11H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 51.8 (-CH_2), 58.5, 89.6, 167.5 (C=O), 172.3 (C=O), Aromatic carbons: 115.2, 117.8, 119.8, 122.2, 124.4, 127.3, 131.3, 133.1, 138.6, 144.2, 146.5, 149.3; Elemental analysis: Calcd (found): C, 52.60 (52.53); H, 3.41 (3.50); N, 13.94 (13.99); Mass spectra, m/z = 501 (100%).

(4k): Yield 65%; m.p. 210 ^{0}C; Chemical formula: $C_{23}H_{20}Cl_2N_4O_3$; IR (KBr, cm-1): 3057 (NH), 1678 (CONH), 1772 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.21 (s, 1H, NH), 3.71 (s, 2H, CH_2), 2.36 (s, 3H, CH_3), 5.39 (d, 1H, CH-Ar, *β*-lactam), 5.8 (d, 1H, CH-CO, *β*-lactam), 6.65-8.27 (m, 11H, Ar-H), 8.51 (s, 1H, CONH); ^{13}C NMR: δ 24.7 (CH_3), 52.5 (-CH_2), 60.1, 89.2, 168.6 (C=O), 173.9 (C=O), Aromatic carbons: 115.2, 117.2, 119.8, 124.2, 127.5, 128.5, 131.1, 143.5, 146.7, 149.6; Elemental analysis: Calcd (found): C, 58.61 (58.67); H, 4.28 (4.33); N, 11.89 (11.82); Mass spectra, m/z = 470 (100%).

(4l): Yield 63%; m.p. 197 ^{0}C; Chemical formula: $C_{24}H_{22}Cl_2N_4O_5$; IR (KBr, cm-1): 3115 (NH), 1652 (CONH), 1748 (CO, *β*-lactam); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.28 (s, 1H, NH), 3.67 (s, 2H, CH_2), 3.82 (s, 6H, OCH_3), 5.32 (d, 1H, CH-Ar, *β*-lactam), 5.83 (d, 1H, CH-CO, *β*-lactam), 6.73-8.25 (m, 10H, Ar-H), 8.63 (s, 1H, CONH); ^{13}C NMR: δ 50.2, 52.1 (-CH_2), 55.9, 56.5 (OCH_3), 90.2, 167.4 (C=O), 172.6 (C=O), Aromatic carbons: 102.2, 106.5, 115.8, 117.4, 119.8, 121.7, 124.3, 127.7, 129, 138.4, 146.6, 149.8, 157.6, 159.4; Elemental analysis: Calcd (found): C, 55.72 (55.78); H, 4.29 (4.23); N, 10.83 (10.92); Mass spectra, m/z = 516 (100%).

RESULTS AND DISCUSSION:

Chemistry:

Assembling N-heterocycles is important in synthetic organic chemistry. The development of

highly efficient scaffolds for the preparation of pyridine-2-acetamide conjugated monobactam derivatives is of considerable interest. In the present work, we prepared a new series of monocyclic *β*-lactam derivatives in which an azetidin-2-one motif connects with pyridine-2-acetamide nucleus with two aromatic rings. The designed compounds **4(a-l)** were prepared by the following Staudinger reaction ([2+2] cycloaddition reactions).[22-23]

The Preparation of new compounds **4(a-l)** is depicted on **Scheme 1.** 2-chloro-N-(pyridin-2-yl) acetamide **(1)** was synthesized by pyridin-2-amine with chloroacetyl chloride. The model reaction was carried out simply treatment of 2-amino pyridine with chloroacetyl chloride in the presence of K_2CO_3 yielded 2-chloro-N-(pyridin-2-yl)acetamide **(1)**. These compounds, on amination with hydrazine hydrate afforded 2-hydrazinyl-N-(pyridin-2-yl) acetamide (**2**).

The condensation reaction of compound **2** with aromatic aldehydes yielded 2-(2-(substituted arylidene)hydrazinyl)-N-(pyridine-2-yl)acetamide **3(a-l)**. The compounds **3(a-l**), on reaction with 2,4-dichlorophenoxy acetic acid in the presence of $POCl_3$ and triethylamine (TEA) afforded azetidinones **4(a-l)**. These reactions are summarized in **Scheme 1**. The progress of the reaction was monitored by TLC.

TABLE 1: ANTIMICROBIAL ACTIVITIES OF SYNTHESIZED COMPOUNDS 4(a-l)

	Concentration of compounds 100 µg/ml Zone of inhibition (mm)				
	Gram +Ve			Gram -Ve	
Compd	*Bacillus subtilis*	*Proteus vulgaris*	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumonia*
4a	15	-	17	20	21
4b	-	18	-	14	-
4c	19	24	15	28	25
4d	29	32	33	32	33
4e	35	>40	36	29	31
4f	-	25	26	30	28
4g	-	4	9	-	3
4h	25	24	-	25	23
4i	32	25	33	31	34
4j	-	12	-	-	15
4k	6	14	22	25	14
4l	35	>40	36	35	28
Ampicillin	>40	>40	>40	>40	>40

Key to symbols: Inactive = (inhibition zone - mm); slightly active = (inhibition zone (1 to 20 mm); moderately active = (inhibition zone 21 to 30 mm); highly active = (inhibition zone >31 mm).

Biological activity:

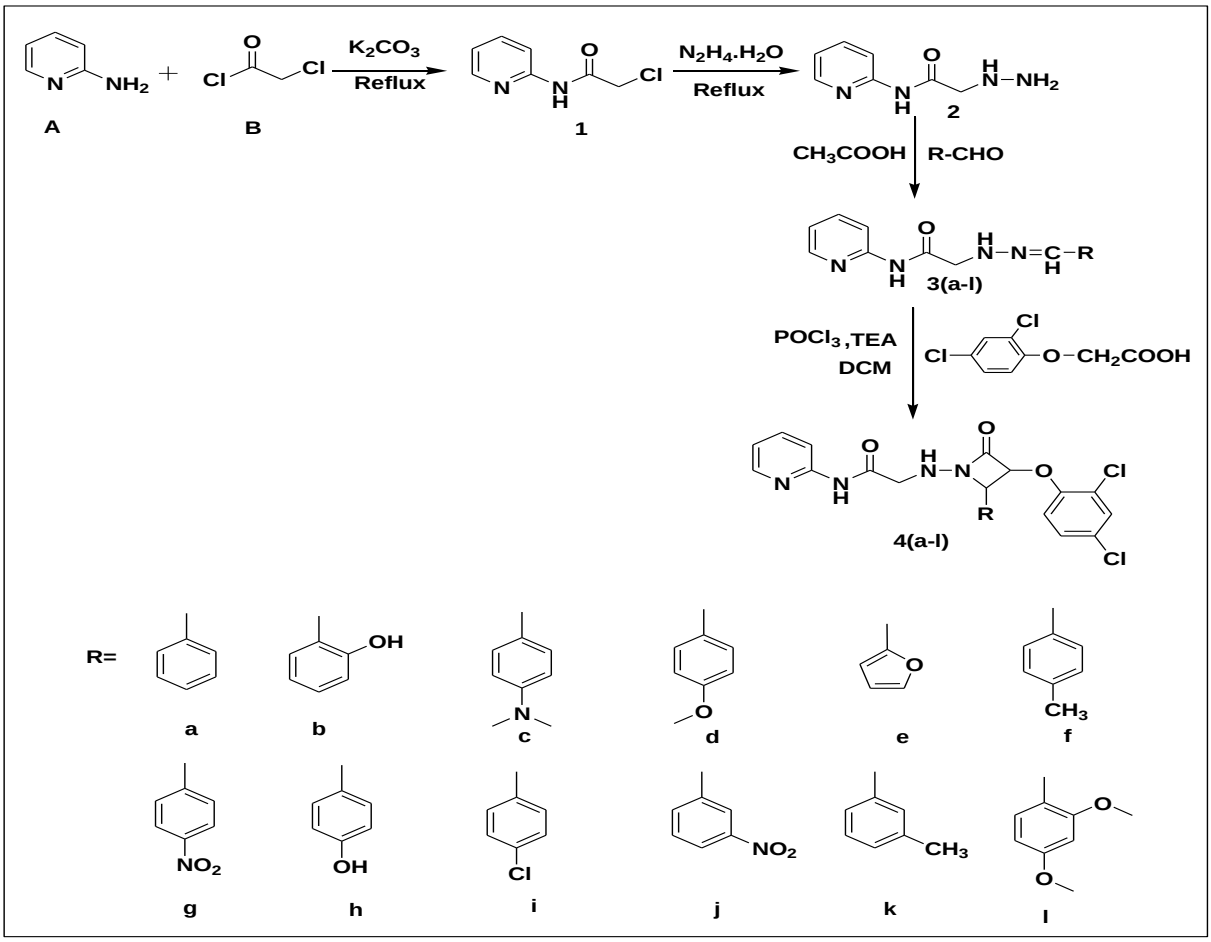
Antibacterial activity of acetamide conjugated *β*-lactams (agar diffusion assay):

The antibacterial activity of the synthesized twelve *β*-lactam compounds against human bacterial (Gram +ve and Gram -ve) pathogens as determined by agar diffusion method with Ampicillin as reference control was investigated the maximum antimicrobial activity and inhibition zone were observed for compounds **4e, 4i** and **4l** against *B. subtilis* while compounds **4d** and **4h** showed moderate activity and all other compounds showed low activity against this pathogen. For *P. vulgaris* the compounds **4d, 4e** and **4l** showed good antibacterial activity as that of the reference compound Ampicillin while **4c, 4f, 4h** and **4i** showed moderate activity the other compounds showed low activity against this pathogen. The compounds **4d, 4e, 4i** and **4l** showed very good activity against the bacteria *S. aures* while compounds **4f** and **4k** showed moderate activity.

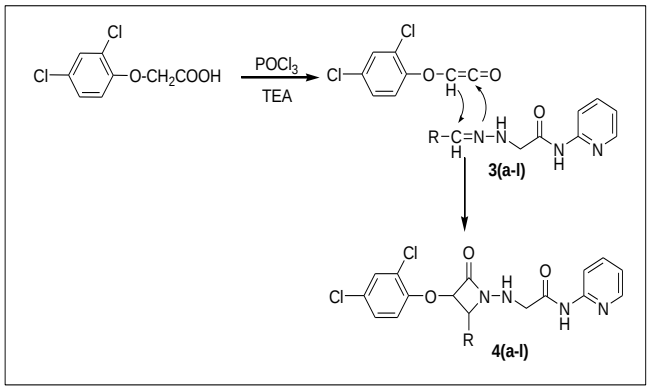
For the pathogen *E. coli* the compounds **4d, 4i,** and **4l** showed good inhibitory activity, while **4c, 4e, 4f, 4h** and **4k** showed moderate activity and all other compounds showed low activity against this pathogen. For the pathogen *K. pneumonia* the compounds **4d**, **4e** and **4i** showed good inhibitory activity, while **4e, 4f, 4h** and **4l** showed moderate activity and all other compounds showed low

activity against this pathogen. The compounds containing methoxy, chloro and furon groups showed good activity against all the pathogens in given concentration, which is comparable to the reference control.

The derived compounds **4d, 4e, 4i** and **4l** were found to be effective in controlling all the test pathogens and particularly the compounds **4e** and **4l** found to be effective in *P. vulgaris*. The activity is very much comparable to the reference control. Further biological studies are required to validate the effective compounds of the present study as an antimicrobial agent. The results are summarized in **Table 1** antibacterial activity against five human bacterial pathogens. The overall antibacterial activity of the synthesized compounds attributed in the presence of acetamide conjugated *β*-lactam substituted compounds.



SCHEME 1: SYNTHETIC ROUTE FOR 2-(3-(2, 4-DICHLOROPHENOXL)-2-(SUBSTITUTED ARYL)-4-OXOAZETIDIN-1-YLAMINO)-N-(PYRIDIN-2-YL) ACETAMIDE



SCHEME 2: MECHANISM FOR THE SYNTHESIS OF *β* - LACTAM DERIVATIVES 4(a-l).

CONCLUSION: In conclusion, we have successfully synthesized a series of novel acetamide-conjugated *β*-lactam derivatives **4(a-l)** of Staudinger [2 + 2] cycloaddition reaction. The obtained results have established that all the synthesized imidazole-conjugated monocyclic *β*-lactams showed good antibacterial activity. Particularly the compounds **4e** and **4l** found to be effective in *P.vulgaris* as equal to reference ampicillin and other compounds showed moderate to good activity against five human bacterial pathogens. The overall antibacterial activity of the

synthesized compounds attributed to the presence of *β*-lactam substituent in all the compounds. Their antimicrobial activity study revealed that all the compounds tested showed moderate to very good antibacterial activity and some compounds are inactive against pathogenic strains. Consequently N-pyridine acetamide substituted monobactam derivatives represent a class that needs further investigation with the hope of finding new antimicrobial agents.

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