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Synthesis and antibacterial activity of novel substituted azetidinones bearing 3,4,5-trimethoxy phenyl ring

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ABSTRACT

Azetidinone is a saturated form of nitrogen containing cyclobutane having carbonyl group. It has been considered as a versatile nucleus which posses almost all types of biological activities mainly antibacterial and antifungal activity. In view of the above biological importance of azetidinone motif's, author's have undertaken the synthesis of some novel azetidinone ring bearing 3,4,5-trimethoxy phenyl ring. Treatment of gallic acid with dimethylsulphate yielded methyl 3,4,5-trimethoxybenzoate ***1,*** *which was treated with hydrazine hydrate to obtain 3,4,5-trimethoxy benzohydrazide* ***2.*** *Condensation of* ***2*** *with selected aldehydes resulted in hydrazone derivatives* ***3a-k*** *which upon reaction with 2-(4-chlorophenoxy)acetylchloride in presence of triethylamine yielded substituted N-(3-(4-chlorophenoxy)-2-oxo-4-heteroaryl aryl azetidin-1-yl)-3,4,5-trimethoxybenzamides* ***4a-k****. All the compounds were characterized by IR,* ^{1}*HNMR and mass spectroscopic techniques and were tested for their anti bacterial activity.*

Key words: Anti bacterial activity, Azetidinone, Hydrazide and 3,4,5-trimethoxy benzoic acid

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INTRODUCTION

Azetidin-2-one, a four-membered cyclic lactam (β-lactam) skeleton has been recognized as a useful building block for synthesis of a large number of organic molecules by exploiting the strain energy associated with it. They are the carbonyl derivatives of azetidines containing carbonyl group at the position-2. These are also known as 2-azetidinones. Azetidinones which are part of antibiotics structure are known to exhibit interesting biological activities. A large number of 3-chloro-2-azetidinones possesses powerful antimicrobial [1-3], anti-inflammatory [4-5], analgesic [5], anticonvulsant [6,7], antitubercular [8,9], antioxidant [10], antihyperglycemic [11]. The Staudinger reaction (ketene- imines cycloaddition reaction) is regarded as one of the most fundamental and versatile methods for the synthesis of structurally diverse 2-azetidinone derivatives. Azetidin-2-ones can also be synthesized by enolate–imines condensations and cyclization reactions. Also it is used in the synthesis of a variety of β-lactam antibiotics.

Bacterial and fungal infection is most common problem of the world. Some serious and life threatening diseases also caused by bacteria or fungal infection. In addition, in organ transplantation or surgery microbial infection is also common problem. From the last decade, researchers made a continuous effort to fight these diseases. Several new classes of chemotherapeutic agents have been introduced in the last decade. Several azole or azetidine constitute containing drugs displayed promising results.

This diversity in the biological response profile has attracted the attention of many researchers to explore this skeleton to its multiple potential against several activities. These biological activities of both has fascinated our attention to synthesize some new compounds which contains 3,4,5-trimethoxy phenyl ring and azetidine in single frame work. In the present study, we report herein the synthesis and antibacterial activity of new series of N-(3-(4-chlorophenoxy)-2-(substituted-phenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide, compounds **4(a-k)** from gallic acid.

MATERIALS AND METHODS

The dry solvents and the chemicals available commercially are employed for the chemical process. Silica gel 60 F24 of Merck pre-coated plates are employed for their thin layer chromatography (TLC) analysis and the spots formed are visualized by UV-light. Merck silica gel (230-400) mesh was employed for flash column chromatography and the eluting solvents are mentioned in the procedures. Melting point (mp) determined by Mel-temp apparatus. ^{1}H NMR spectra was recorded using Varian MR-400 MHz NMR devise. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals are reported as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiple) and coupling constants in Hz. The data related to mass spectra was recorded on Agilent ion trap MS. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer.

Preparation of methyl 3,4,5-trimethoxybenzoate (1)
To a stirred solution of gallic acid (5g, 29.40 mmol) in acetone (150 mL) was added dimethylsulphate (18 g, 141.80 mmol), K_2CO_3 (18 g, 129.30 mmol), tetrabutyl ammonium bromide (TBAB) (100 mg) and the mixture was stirred at room temperature for 3h. After completion of the reaction (monitored by TLC), the reaction mixture was passed through celite bed, concentrated and diluted with EtOAc (150 mL) and washed with water (2 x 100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to obtain methyl 3,4,5-trimethoxybenzoate **(1)**. White solid; Yield: 4g, 62%; m.p.: 78-82 °C; ESI-MS, *m/z*: 227.6 $[M+H]^+$.

Preparation of 3,4,5-trimethoxybenzohydrazide (2)
To a solution of methyl 3,4,5-trimethoxybenzoate (**1**) (2 g, 8.84 mmol) in 1,4-dioxane (20 mL) was added hydrazine hydrate (2.8 mL, 88.50 mmol) and stirred at 100 °C for 24h. After completion of reaction (monitored by TLC), the reaction mixture was minimized to 10% volume by evaporation under reduced pressure, cooled, filtered the solid precipitated out and dried under vacuum to obtain 4-(furan-2-yl)-1H-pyrrole-3-carbohydrazide (**2**). White solid; Yield: 1.3g, 65%; m.p.: 158-160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (brs, 1H, -NH-C=O-), 6.96 (s, 2H, 3,4,5-trimethoxy phenyl ring), 4.1 (brs, 2H, -NH-NH_2), 3.88 (s, 6H, -$(OCH_3)_2$), 3.83 (s, 3H, -OCH_3). ESI-MS, *m/z*: 227.2 $[M+H]^+$.

General procedure for the preparation of 3a-k
To a mixture of 3,4,5-trimethoxybenzohydrazide (**2**) (10 mmol), appropriate aldehyde from the list **a-k** (10 mmol) in ethanol (25 mL) was added catalytic amount of conc. HCl and stirred at 85 °C for 3-4 h**.** After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the solid precipitated was filtered, washed with n-pentane and dried to obtain crude **3a-k**. The obtained crude compounds were recrystallized from ethanol to afford pure **3a-k**.

(*E*)-N'-benzylidene-3,4,5-trimethoxybenzohydrazide (3a)
Off white solid; Yield: 95mg, 68%; IR (KBr): ν_{max} 3412, 3110, 2944, 1709, 1681, 1585, 1494, 1417, 1332, 1222, 1131, 995, 821, 793, 724 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (brs, 1H, -NH-C=O-), 8.47 (s, 1H, -N=CH), 7.72 (d, *J* = 6 Hz, 2H, Ar-H), 7.46-7.44 (m, 3H, Ar-H), 7.23 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, O-CH_3); ESI-MS, *m/z*: 314.9 $[M+H]^+$.

(*E*)-N'-(4-fluorobenzylidene)-3,4,5-trimethoxybenzohydrazide (3b)
Off white solid; Yield: 92%; IR (KBr): ν_{max} 3126, 2919, 1730, 1690, 1598, 1494, 1427, 1336, 1174, 1133, 1085, 1077, 1048, 991, 941, 881, 861, 840, 824, 766, 729 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (brs, 1H, -NH-C=O-), 8.46 (s, 1H, -N=CH), 7.78 (t, *J* = 7.1 Hz, 2H, Ar-H), 7.29 (t, *J* = 8.7 Hz, 2H, Ar-H), 7.22 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, O-CH_3); ESI-MS, *m/z*: 333.3 $[M+H]^+$.

(*E*)-3,4,5-trimethoxy-N'-(4-(trifluoromethyl)benzylidene)benzohydrazide (3c)
Off white solid; Yield: 73%; IR (KBr): ν_{max} 2941, 2840, 1712, 1586, 1492, 1417, 1325, 1217, 1170, 1132, 1066, 991, 933, 838, 726 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.88 (brs, 1H, -NH-C=O-), 8.53 (s, 1H, -N=CH), 7.94 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.82 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.23 (s, 2H, Ar-H), 3.86 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, O-CH_3); ESI-MS, *m/z*: 383.35 $[M+H]^+$.

(*E*)-N'-(3,4-difluorobenzylidene)-3,4,5-trimethoxybenzohydrazide (3d)
Off white solid; Yield: 90%; IR (KBr): ν_{max} 3180, 3031, 2838, 1647, 1586, 1551, 1502, 1461, 1437, 1413, 1333, 1281, 1235, 1182, 1127, 1069, 998, 955, 822, 756 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.81 (brs, 1H, -NH-C=O-), 8.44 (s, 1H, -N=CH), 7.75 (t, *J* = 9.8 Hz, 1H, Ar-H), 7.59-7.49 (m, 2H, Ar-H), 7.22 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, O-CH_3); ESI-MS, *m/z*: 351.32 $[M+H]^+$.

(E)-N'-(3,5-difluorobenzylidene)-3,4,5-trimethoxybenzohydrazide (3e)
Off white solid; Yield: 85%; IR (KBr): ν_{max} 3156, 3075, 2842, 1651, 1583, 1500, 1443, 1335, 1268, 1230, 1180, 1121, 983, 860, 770, 731 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.90 (brs, 1H, -NH-C=O-), 8.45 (s, 1H, -N=CH), 7.42 (d, *J* = 6 Hz, 2H, Ar-H), 7.31 (t, *J* = 9.1 Hz, 1H, Ar-H), 7.23 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.83 (s, 3H, O-CH_3); ESI-MS, *m/z*: 351.32 $[M+H]^+$.

(*E*)-N'-(4-hydroxybenzylidene)-3,4,5-trimethoxybenzohydrazide (3f)
Off white solid; Yield: 89%; IR (KBr): ν_{max} 3219, 3082, 2940, 2836, 1647, 1605, 1581, 1500, 1413, 1333, 1125, 1065, 836 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.49 (brs, 1H, -NH-C=O-), 9.90 (s, Ar-OH), 8.35 (s, 1H, -N=CH), 7.55 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.21 (s, 2H, Ar-H), 6.83 (d, *J* = 8.2 Hz, 2H, Ar-H), 3.84 (s, 6H, -$(OCH_3)_2$), 3.71 (s, 3H, O-CH_3); ESI-MS, *m/z*: 331.34 $[M+H]^+$.

(*E*)-3,4,5-trimethoxy-N'-(4-(methylthio)benzylidene)benzohydrazide (3g)
Pale yellow solid; Yield: 65%; IR (KBr): ν_{max} 3235, 2937, 1709, 1682, 1585, 1493, 1415, 1382, 1237, 1218, 1133, 1076, 995, 936, 844, 825, 734, 706 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.64 (brs, 1H, -NH-C=O-), 8.40 (s, 1H, -N=CH), 7.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.32 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.22 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, O-CH_3), 2.49 (s, 3H, S-CH_3); ESI-MS, *m/z*: 361.35 $[M+H]^+$.

(*E*)-N'-(4-fluoro-3-nitrobenzylidene)-3,4,5-trimethoxybenzohydrazide (3h)
Light yellow solid; Yield: 93%; IR (KBr): ν_{max} 3216, 3186, 3013, 2976, 2942, 2842, 1952, 1652, 1584, 1546, 1332, 1233, 1123, 835, 677 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.93 (brs, 1H, -NH-C=O-), 8.52 (s, 1H, -N=CH), 8.46 (d, *J* = 6 Hz, 1H, Ar-H), 8.13 (brs, 1H, Ar-H), 7.68 (t, *J* = 2.1 Hz, 1H, Ar-H), 7.23 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.83 (s, 3H, O-CH_3); ESI-MS, *m/z*: 378.34 $[M+H]^+$.

(*E*)-3,4,5-trimethoxy-N'-(thiophen-2-ylmethylene)benzohydrazide (3i)
Off white solid; Yield: 81%; IR (KBr): ν_{max} 2925, 1697, 1588, 1492, 1456, 1414, 1247, 1221, 1168, 1131, 1092, 1076, 998, 937, 876, 822, 727 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.63 (brs, 1H, -NH-C=O-), 8.68 (s, 1H, -N=CH), 7.66 (d, *J* = 2.9 Hz, 1H, Ar-H), 7.46 (d, *J* = 2.9 Hz, 1H, Ar-H), 7.20 (s, 2H, Ar-H), 7.14 (t, *J* = 3.0 Hz, 1H, Ar-H), 3.84 (s, 6H, -$(OCH_3)_2$), 3.71 (s, 3H, O-CH_3); ESI-MS, *m/z*: 321.24 $[M+H]^+$.

(*E*)-N'-(furan-3-ylmethylene)-3,4,5-trimethoxybenzohydrazide (3j)
Off white solid; Yield: 83%; IR (KBr): ν_{max} 3412, 1709, 1681, 1585, 1493, 1417, 1332, 1222, 1131, 1075, 995, 821, 793, 724 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.53 (brs, 1H, -NH-C=O-), 8.41 (s, 1H, -N=CH), 8.15 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.20 (s, 2H, Ar-H), 6.8 (s, 1H, Ar-H), 3.84 (s, 6H, -$(OCH_3)_2$), 3.71 (s, 3H, O-CH_3); ESI-MS, *m/z*: 305.26 $[M+H]^+$.

(*E*)-3,4,5-trimethoxy-N'-((6-methylpyridin-3-yl)methylene)benzohydrazide (3k)
Off white solid; Yield: 65%; IR (KBr): ν_{max} 3460, 1585, 1553, 1500, 1413, 1387, 1327, 1231, 1180, 1124, 1003, 945, 820, 748 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.76 (brs, 1H, -NH-C=O-), 8.74 (d, *J* = 14.5 Hz, 1H, Ar-H), 8.48 (s, -N=CH), 8.03 (d, *J* = 7.1 Hz, 1H, Ar-H), 7.32 (dd, *J* =8, 15.2 Hz, 1H, Ar-H), 7.22 (s, 2H, Ar-H), 3.85 (s, 6H, -$(OCH_3)_2$), 3.75 (s, 3H, O-CH_3), 2.50 (s, 3H, Ar-CH_3); ESI-MS, *m/z*: 330.34 $[M+H]^+$.

General Experimental Procedure for the Synthesis of Azetidinone derivatives 4a-k

To a stirred solution of hydrazone derivatives **3a-k** (1.0 mmol) in toluene was added triethylamine (3.0 mmol) followed by 4-chlorophenoxyacetyl chloride (2.0 mmol) stirred at 60 ^{o}C for 3h. The reaction mixture was cooled to room temperature, diluted with ethylacetate and washed with water. The organic layer was separated, dried over Na_2SO_4, filtered, concentrated and recrystalized in acetonitrile and diethylether to obtain the desired compounds **4a-k**.

N-(3-(4-chlorophenoxy)-2-oxo-4-phenylazetidin-1-yl)-3,4,5-trimethoxybenzamide (4a)

White solid; Yield: 72mg, 23%; m.p.: 212-214 ^{o}C; IR (KBr): ν_{max} 3399, 3078, 2998, 2938, 2838, 1694, 1621, 1585, 1507, 1490, 1465, 1416, 1375, 1286, 1244, 1134, 1085, 1008, 994, 839 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52-7.42 (m, 5H, Ar-H), 7.32 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.20 (s, 1H, -NH-C=O-), 7.10 (s, 2H, Ar-H), 6.91 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.23 (d, *J* = 16.1 Hz, 1H, -N-CH-Phenyl-), 5.12 (d, *J* = 16.1 Hz, -CH-O-Phenyl), 3.74 (s, 6H, -$(OCH_3)_2$), 3.86 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164 (1C), 156.8 (1C), 155.2 (1C), 153.1 (2C), 140.8 (1C135.9 (1C), 130 (2C), 129 (1C),128.7 (2C), 126.6 (2C), 124.6 (1C),118.7 (1C), 116.3 (2C), 104.1 (2C), 92.2 (1C), 65.3 (1C), 60.1 (1C), 56 (2C); ESI-MS, *m/z*: 483.15 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(4-fluorophenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4b)

Pale yellow solid; Yield: 16%; m.p.: 158-162 ^{o}C; IR (KBr): ν_{max} 3462, 3065, 2934, 1690, 1598, 1494, 1427, 1336, 1174, 1133, 1085, 1077, 1048, 991, 941, 881, 861, 840 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.27 (d, *J* = 9.3 Hz, 2H, Ar-H), 7.24 (s, 1H, -NH-C=O-), 7.19 (d, *J* = 19.4 Hz, 2H, Ar-H), 7.17 (s, 2H, Ar-H), 7.03 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.27 (d, *J* = 14.2 Hz, 1H, -N-CH-Phenyl-), 5.13 (d, *J* = 12.5 Hz, 1H, -CH-O-Phenyl), 3.79 (s, 6H, -$(OCH_3)_2$), 3.76 (s, 3H, O-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3 (1C), 158.6 (1C), 154.5 (1C), 153.2 (2C), 142.6 (1C), 139.7 (1C), 133.7 (1C), 130.5 (2C), 128.6 (2C), 128.2 (1C), 125.9 (1C), 116.5 (2C), 115.3 (1C), 115.2 (1C), 106.6 (2C), 91.4 (1C), 64.9 (1C), 60.8 (1C), 56.1 (2C); ESI-MS, *m/z*: 500.95 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-oxo-4-(4-(trifluoromethyl)phenyl)azetidin-1-yl)-3,4,5-trimethoxybenzamide (4c)

Off white solid; Yield: 21%; m.p.: 166-170 ^{o}C; IR (KBr): ν_{max} 3464, 3089, 2984, 2931, 1692, 1661, 1629, 1603, 1548, 1462, 1417, 1295, 1234, 1133, 1018, 823, 651 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (d, *J* = 11.8 Hz, 2H, Ar-H) , 7.34 (s, 1H, -NH-C=O-), 7.26 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.32 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.16 (s, 2H, Ar-H), 7.02 (d, *J* = 9.8 Hz, 2H, Ar-H), 5.31 (d, *J* = 15.3 Hz, 1H, -N-CH-Phenyl-), 5.19 (d, *J* = 14.9 Hz, 1H, -CH-O-Phenyl), 3.83 (s, 6H, -$(OCH_3)_2$), 3.81 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2 (1C), 163.7 (1C), 155.5 (1C), 153.2 (1C), 153.1 (1C), 146.8 (1C), 142.6 (1C), 133.7 (1C), 131.5 (2C), 129 (1C), 125.9 (1C), 125.7 (2C), 124.9 (2C), 124 (1C), 117.5(2C), 105.6 (2C), 91.3 (1C), 63.9 (1C), 60.7 (1C), 54.9 (2C); ESI-MS, *m/z*: 551.13 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(3,4-difluorophenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4d)

Grey solid; Yield: 18%; m.p.: 174-178 ^{o}C; IR (KBr): ν_{max} 3433, 3038, 2997, 2942, 2850, 1689, 1583, 1492, 1466, 1419, 1297, 1244, 1084, 1014, 992, 832, 753 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67-7.61 (m, 1H, Ar-H), 7.53 (q, *J* = 8.4 Hz, 1H, Ar-H), 7.42 (t, *J* = 29.4 Hz, 1H, Ar-H), 7.02 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.22 (s, 1H, -NH-C=O-), 7.10 (s, 2H, Ar-H), 6.87 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.22 (d, *J* = 16.2 Hz, 1H, -N-CH-Phenyl-), 5.10 (d, *J* = 16.2 Hz, 1H, -CH-O-Phenyl), 3.86 (s, 6H, -$(OCH_3)_2$), 3.82 (s, 3H, O-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3 (1C), 156.8 (1C), 155 (1C), 153.1 (2C), 140.9 (1C), 133.5 (2C), 129 (1C), 124.6 (1C), 123.9 (1C), 118.5 (2C), 118.1 (1C), 117.9 (1C), 116.3 (2C), 116.2 (1C), 104.2 (2C), 90.8 (1C), 65.3 (1C), 60.1 (1C), 46.1 (2C); ESI-MS, *m/z*: 517.7 [M-H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(3,5-difluorophenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4e)

Off white solid: Yield: 19%; m.p.: 156-160 ^{o}C; IR (KBr): ν_{max} 3448, 2942, 2841, 1695, 1686, 1602, 1584, 1493, 1459, 1446, 1417, 1334, 1236, 1133, 1003, 983, 855 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (d, *J* = 6.3 Hz, 1H, Ar-H), 7.38-7.30 (m, 1H, Ar-H), 7.28 (d, *J* = 1.8 Hz, 2H, Ar-H), 7.20 (s, 1H, -NH-C=O-), 7.10 (s, 2H, Ar-H), 7.01 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.96 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.30 (d, *J* = 16.3 Hz, 1H, -N-CH-Phenyl-), 5.10 (d, *J* = 16.1 Hz, 1H, -CH-O-Phenyl), 3.81 (s, 6H, -$(OCH_3)_2$), 3.71 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.7 (1C), 170.1 (1C), 163.8 (1C), 161.3 (1C), 156.7 (1C), 152.8 (2C), 148.5 (1C), 143.1 (1C), 137.1 (1C), 128.9 (1C), 127.9 (2C), 124.5 (1C), 116.1 (2C), 110.8 (1C), 107.5 (1C), 106.4 (2C), 105.9 (1C), 66.1 (1C), 60.2 (1C), 56.3 (2C); ESI-MS, *m/z*: 519.13 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4f)
Off white solid: Yield: 22%; m.p.: 186-190 ^{o}C; IR (KBr): $\nu_{max:}$ 3436, 3063, 2938, 1680, 1572, 1667, 1579, 1456, 1436, 1414, 1323, 1291, 1268, 1161, 1092, 1006, 948, 937, 837, 727 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H, Ar-OH), 7.38 (d, *J* = 13.5 Hz, 2H, Ar-H), 7.19 (s, 2H, Ar-H), 7.16 (d, *J* = 13.2 Hz, 2H, Ar-H), 7.14 (s, 1H, -NH-C=O-), 7.02 (d, *J* = 11.3 Hz, 2H, Ar-H), 6.87 (d, *J* = 3.9 Hz, 2H Ar-H), 5.21 (d, *J* = 14.3 Hz, 1H, -N-CH-Phenyl-), 5.11 (d, *J* = 14.1 Hz, 1H, -CH-O-Phenyl), 3.81 (s, 6H, -$(OCH_3)_2$), 3.79 (s, 3H, O-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8 (1C), 162.7 (1C), 155.6 (1C), 155.4 (1C), 153.9 (1C), 153.7 (1C), 143.6 (1C), 136.7 (1C), 133.7 (1C), 131.4 (2C), 129.1 (1C), 127.8 (2C), 119.3 (2C), 116.8 (2C), 108.1 (2C), 91.8 (1C), 63.9 (1C), 61.1 (1C), 56.3 (2C); ESI-MS, *m/z*: 499.25 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(4-(methylthio)phenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4g)
Grey solid; Yield: 16 %; m.p.: 154-158 ^{o}C; IR (KBr): ν_{max} 3423, 3056, 2952, 1681, 1553, 1695, 1579, 1459, 1424, 1402, 1319, 1283, 1264, 1261, 1053, 1046, 948, 917, 837 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (d, *J* = 5.9 Hz, 2H, Ar-H), 7.38 (d, *J* = 11.5 Hz, 2H, Ar-H) , 7.34 (s, 1H, -NH-C=O-), 7.26 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.16 (s, 2H, Ar-H), 6.99 (d, *J* = 7.2 Hz, 2H, Ar-H) 5.23 (d, *J* = 15.5 Hz, 1H, -N-CH-Phenyl-), 5.13 (d, *J* = 14.9 Hz, 1H, -CH-O-Phenyl), 3.81 (s, 6H, -$(OCH_3)_2$), 3.79 (s, 3H, -OCH_3), 2.64 (s, 3H, -SCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1(1C), 163.1 (1C), 155.3 (1C), 153.4 (1C), 153.3 (1C), 143.6 (1C), 140.9 (1C), 139.2 (1C), 131.6 (2C), 129.8 (2C), 128.3 (1C), 127.9 (2C), 126.4 (1C), 118.1 (2C), 107.2 (2C), 91.4 (1C), 63.2 (1C), 59.9 (1C), 56.5 (2C), 14.6 (1C): ESI-MS, *m/z*: 528.99 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(4-fluoro-3-nitrophenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4h)
Light brown solid; Yield: 26 %; m.p.: 154-158 ^{o}C; IR (KBr): ν_{max} 3435, 3050, 2938, 2845, 1681, 1620, 1538, 1492, 1417, 1220, 1134, 1008, 819, 640 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (dd, *J* = 2.0, 7.2 Hz, 1H, Ar-H), 8.0-7.97 (m, 1H, Ar-H), 7.69 (dd, *J* = 8.8 Hz, 1H, Ar-H), 7.36 (s, 1H, -NH-C=O-), 7.28 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.11 (s, 2H, Ar-H), 6.98 (d, *J* = 9.2 Hz, 2H, Ar-H), 5.23 (d, *J* = 16.2Hz, 1H, -N-CH-Phenyl-), 5.12 (d, *J* = 9.9Hz, 1H, -CH-O-Phenyl), 3.83 (s, 6H, -$(OCH_3)_2$), 3.72 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5 (1C), 156.5 (1C), 155.1 (1C), 153.1 (2C), 140.9 (1C), 137 (1C), 134.5 (1C), 133.2 (1C), 129 (2C), 124.8 (1C), 124.6 (1C), 119.4 (1C), 119.2 (1C), 118.4 (1C), 116.3 (2C), 104.2 (2C), 90.3 (1C), 65.3 (1C), 60.2 (1C), 56.1 (2C); ESI-MS, *m/z*: 546.1 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-oxo-4-(thiophen-2-yl)azetidin-1-yl)-3,4,5-trimethoxybenzamide (4i)
Off white solid; Yield: 19 %; m.p.: 194-200 ^{o}C; IR (KBr): ν_{max} 3436, 3095, 2927, 1685, 1583, 1645, 1567, 1428, 1464, 1423, 1364, 1239, 1164, 1182, 1023, 998, 937, 877, 727 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.42 (d, *J* = 5.7 Hz, 2H, Ar-H), 7.27 (s, 1H, -NH-C=O-), 7.19 (s, 2H, Ar-H), 7.04 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.01 (dd, *J* = 4.1, 8.5 Hz, 1H, Ar-H), 6.92 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.24 (d, *J* = 13.4 Hz, 1H, -N-CH-Phenyl-), 5.15 (d, *J* = 11.2 Hz, 1H, -CH-O-Phenyl), 3.79 (s, 6H, -$(OCH_3)_2$), 3.74 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2 (1C), 164.3 (1C), 156.5 (1C), 154.2 (2C), 143.6 (1C), 133.5 (1C), 131.4 (1C), 131.3 (1C), 130.9 (1C), 129.4 (1C), 128.1 (1C), 127.4 (1C), 126.3 (1C), 118.4 (2C), 109.3 (2C), 92.4 (1C), 63.1 (1C), 59.9 (1C), 56.4 (2C); ESI-MS, *m/z*: 488.95 [M+H]$^{+}$.

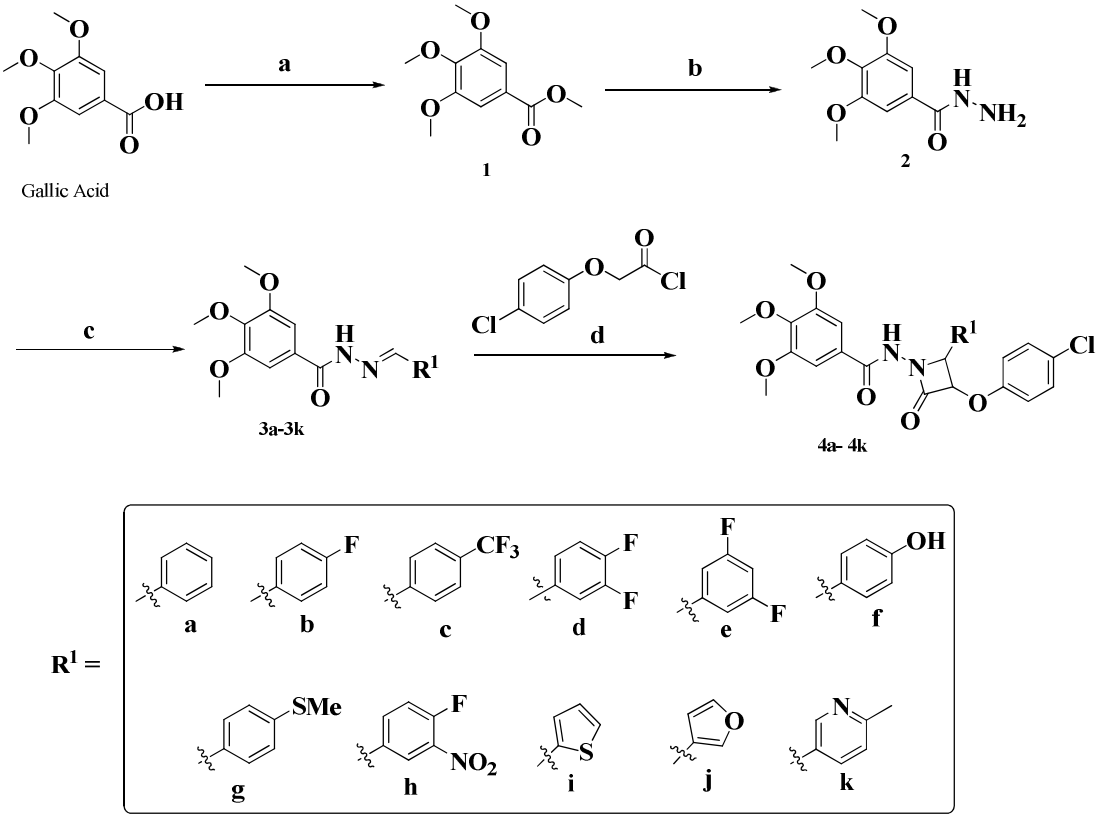
N-(3-(4-chlorophenoxy)-2-(furan-3-yl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4j)
Off white solid; Yield: 21 %; m.p.: 178-184 ^{o}C; IR (KBr): ν_{max} 3464, 3054, 2934, 1687, 1629, 1602, 1564, 1465, 1401, 1364, 1285, 1148, 1169, 1049, 982, 937, 834, 727 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, *J* = 6.7 Hz, 2H, Ar-H), 7.27 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.24 (d, *J* = 3.1 Hz, 1H, Ar-H), 7.22 (s, 1H, -NH-C=O-), 7.18 (s, 2H, Ar-H), 7.02 (d, *J* = 11 Hz, 2H, Ar-H), 6.34 (d, *J* = 2.4, Hz 1H, Ar-H), 5.21 (d, *J* = 12.4 Hz, 1H, -N-CH-Phenyl-), 5.12 (d, *J* = 11.9 Hz, 1H, -CH-O-Phenyl), 3.74 (s, 6H, -$(OCH_3)_2$), 3.71 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2 (1C), 164.8 (1C), 154.9 (1C), 153.6 (2C), 142.8 (1C), 142.6 (1C), 140.1 (1C), 131.1(2C), 128.9 (1C), 128.6 (1C), 126.1 (1C), 118.2 (2C), 109.2 (1C), 108.7 (2C), 91.3 (1C), 60.8 (1C), 58.1 (1C), 56.3 (2C); ESI-MS, *m/z*: 472.96 [M+H]$^{+}$.

N-(3-(4-chlorophenoxy)-2-(6-methylpyridin-3-yl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide (4k)
Brown solid; Yield: 29.2%; m.p.: 192-194 ^{o}C; IR (KBr): ν_{max} 3425, 3060, 2935, 1682, 1583, 1697, 1589, 1492, 1456, 1414, 1333, 1221, 1168, 1131, 1092, 1076, 998, 937, 877, 727 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.81 (dd, *J* = 5.9, 8 Hz, 1H, Ar-H), 7.34 (dd, *J* = 8.1, 15.4 Hz, 1H, Ar-H), 7.30 (s, 1H, -NH-C=O-), 7.27 (d, *J* = 22.8 Hz, 2H, Ar-H), 7.10 (s, 2H, Ar-H), 6.91 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.12 (d, *J* = 8.9 Hz, 1H, -N-CH-Phenyl-), 5.09 (t, *J* = 13.9 Hz, -CH-O-Phenyl), 3.82 (s, 6H, O-CH_3), 3.69 (s, 3H, -OCH_3), 2.49 (s, 3H,

Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6 (1C), 160.4 (1C), 156.8 (1C), 156.6 (1C), 153.5 (2C), 147.2 (1C), 141.8 (1C), 135 (1C), 129.3 (2C), 128.7 (1C), 126.5 (1C), 123.4 (1C), 118.5 (2C), 116.2 (1C), 104.6 (2C), 90.8 (1C), 66 (1C), 61 (1C), 56.3 (2C), 29.6 (1C); ESI-MS, *m/z*: 498.32 $[M+H]^+$.

Antibacterial Screening

The antibacterial activity was determined using disc diffusion method by measuring zone of inhibition in mm [12]. All the compounds, **4a-k** was screened *in-vitro* at a concentration of 250µg/mL for antibacterial activity against two Gram-positive pathogenic organisms: *Staphylococcus aureus* and *Staphylococcus pyogenes*, two Gram-negative organisms: *Escherichia coli* and *Pseudomonas aeruginosa* (**Table-1**). Standard antibacterial drug ciprofloxacin (250 µg/disc) was also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken. Growth inhibition was calculated with reference to positive control. Compounds (**4a-k)** were dissolved in dimethyl sulphoxide at 250 µg/mL concentration. The inhibition zones were measured in millimeters at the end of an incubation period of 48 hours at (35±2) °C. DMSO alone showed no inhibition. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4.



SCHEME-1: Synthesis of novel azetidinone derivative 4a-k

EXPERIMENTAL CONDITIONS: *a) Dimethyl sulphate, K_2CO_3, TBAB, Acetone, reflux, 3h; b) Hydrazine-hydrate, 1,4-dioxane, 100 °C, 24h; c) aldehydes* ***a-k****, conc. HCl, Ethanol, 80 °C, 3h; d) 4-chlorophenoxyacetyl chloride, Et_3N, Toulene, r.t. to 60 °C, 3h*

RESULTS AND DISCUSSION

N-(3-(4-chlorophenoxy)-2-(substituted-phenyl)-4-oxoazetidin-1-yl)-3,4,5-trimethoxybenzamide compounds **4a-k** were prepared in four steps from commercially available gallic acid (**Scheme 1**). Gallic acid was treated with dimethyl sulphate in presence of TBAB in acetone at reflux to obtain methylated compound **1**. Compound **1** on reaction with hydrazine hydrate in dioxane at reflux temperature yielded compound **2**. Condensation of hydrazide **2** with appropriate aldehyde (**a-k)** and catalytic amount of conc. HCl in ethanol at reflux resulted in hydrazide-hydrazone derivatives **3a-k**. These compounds on treatment with 2-(4-chlorophenoxy)acetyl chloride in presence of

triethyl amine in toluene at reflux for 12 h furnished final compounds **4a-k**. The structures of all synthesized compounds were confirmed by chemical and spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, and mass.

^{1}H NMR spectra of compounds **4a-k** showed two doublet for (-N-CH) and (-CH-O-) in the range δ 5.25-5.21 and 5.13-5.11 ppm respectively. In ^{13}C NMR spectra, compound **4h** showed three characteristic signals appeared for (-N-CH-), (-CH-O-) and (CO cyclic) at δ 65.8, 90.3 and 164.53 ppm respectively. In the IR spectra of compounds **4a-k** carbonyl group of β-lactam ring showed characteristic absorption in the range of 1680-1695 cm^{-1}. The disappearance of -N=CH- functionality in IR absorption, ^{1}H and ^{13}C NMR signals indicates the formation of azetidinone ring , thus confirming the formation of compounds **4a-k**.

Antibacterial Activity

The results of the antibacterial data of novel azetidinone derivatives **4a-k** is given in **Table 1**. The antibacterial activity was measured in terms of zone of inhibition (ZI, in mm). In case of *E.coli* and *P.aeruginosa*, the ZI is classified as, good activity (ZI: 13-18 mm) and weak activity (ZI: 8-10 mm), while in case of *S.aureus* and *S.pyogenes*, ZI with 11-16 mm, and 6-8 mm is considered as good and weak activity respectively. From table-1, it is observed that, in general, compounds **4h** having 3-NO_2-4-F-phenyl group and compound **4k** having 2-methyl-pyridine group exhibited good antibacterial activity when tested against all the bacterial strains. Compounds **4b** (R = 4-fluorophenyl), **4c** (R = 4-trifluoromethyl phenyl), **4d** (R = 3,4-di-fluro phenyl) and **4e** (R = 3,5-di-fluoro phenyl), **4g** (R = 4-thiomethyl phenyl) and **4j** (R = furan) displayed weak antibacterial activity while compounds **4a** (R = phenyl)**, 4f** (R = 4-hydroxy) and **4i** (R = 2-thiophene) showed nil antibacterial activity.

Table 1: Antibacterial activity data of novel hydrazo-hydrazide derivatives 4a-k

Compound no.	*Gram negative bacteria*		*Gram positive bacteria*	
	E. coli MTCC 443	***P. aeruginosa MTCC 424***	***S.aureus MTCC 96***	***S.poygenes MTCC 442***
	Diameter of Zone of inhibition in mm			
4a	--	--	--	--
4b	7	9	5	4
4c	7	5	6	5
4d	9	9	5	6
4e	10	8	6	9
4f	--	--	--	--
4g	7	8	6	5
4h	17	13	11	12
4i	--	--	--	--
4j	8	9	9	6
4k	18	15	16	14
SD* Ciprofloxacin (Conc. 250 µg/mL)	28	27	22	22

CONCLUSION

In conclusion, we have reported the synthesis of novel substituted N-(3-(4-chlorophenoxy)-2-oxo-4-heteroaryl aryl azetidin-1-yl)-3,4,5-trimethoxybenzamides compounds **4 a-k**. All the compounds were characterized by IR, ^{1}HNMR and mass spectroscopic techniques and were tested for their anti bacterial activity. The antibacterial test results revealed that compound **4h** having 3-NO_2-4-F-phenyl group and compound **4k** having 2-methyl-pyridine group exhibited good antibacterial activity, while the remaining compounds displayed weak antibacterial activity.

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