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# Synthesis, Characterization and Antimicrobial Activities of Novel Heterocycles 8-(3-chloro-2-(2-hydroxy-3-nitrophenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one and 8-(4-(2-(3-bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one Derivatives

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***Abstract—*** *A convenient protocol for the synthesis of oxoazetidinyl-phenothiazinone derivatives has been initiated with the reaction of resorcinol and acetoacetic ester to yield coumarin. In another reaction, Schiff bases were prepared by the condensation of substituted-salicylaldehyde with benzidine and substituted-salicylaldehyde with benzene-1,4-diamine, subsequently cyclized with chloroacetyl chloride to form β-lactam-amine. Further, the amine reacted with coumarin in the presence of $ZnCl_2$ to form prefinal derivatives. The interaction of biphenylyl-azetidin-2-one or phenyl-azetidin-2-one with sulphur powder and iodine afforded the final phenothiazinone derivatives. The structures of the synthesized derivatives were determined by elemental analysis, UV-visible, FT-IR, $^1$H-NMR, and mass spectra. The obtained derivatives exhibited excellent to moderate antimicrobial activity.*

***Keywords— Phenothiazinone, acetoacetic ester, coumarin, benzidine, benzene-1,4-diamine, salicylaldehyde.***

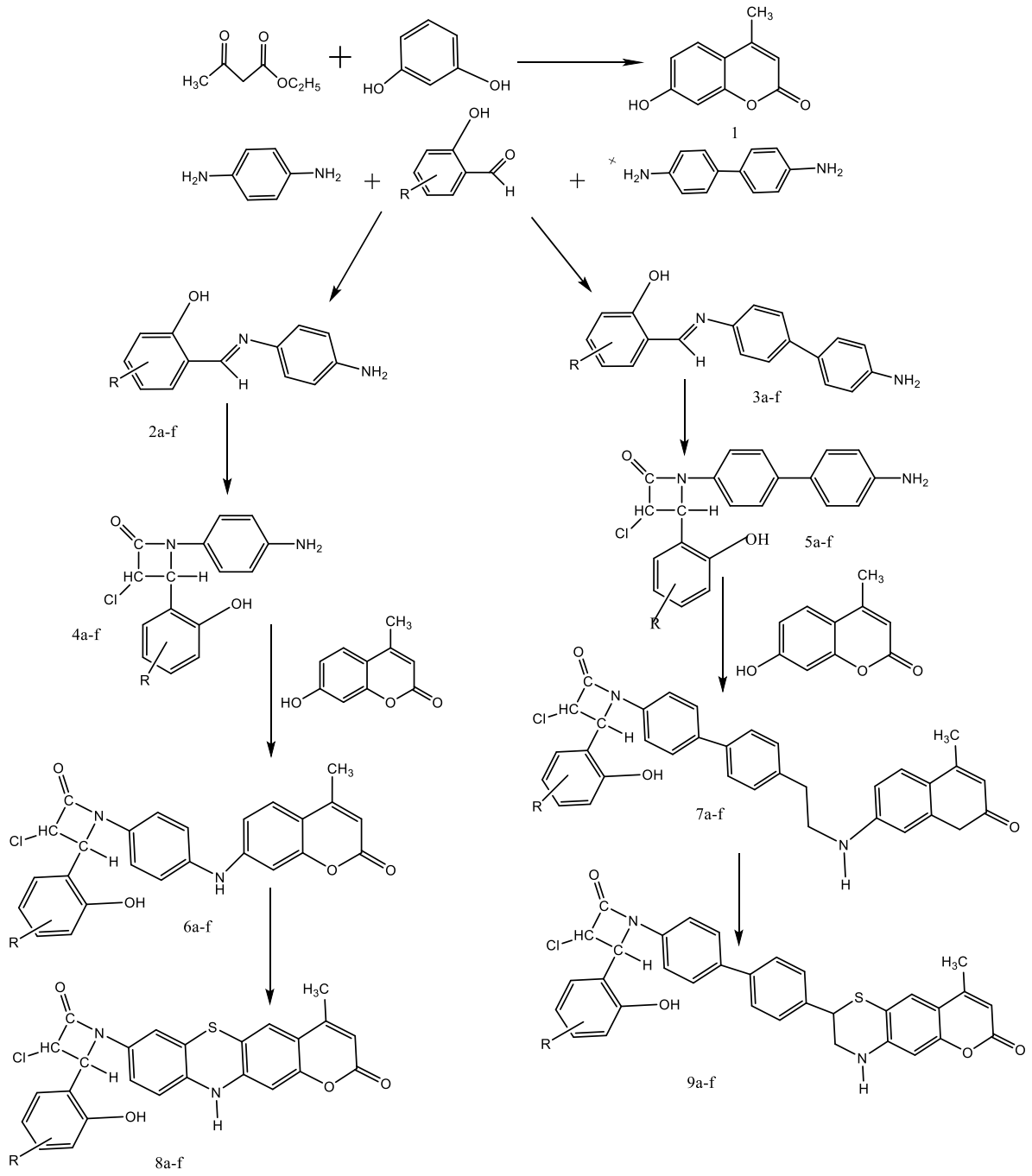
## I. INTRODUCTION

Phenothiazines are of major significance in heterocyclic chemistry. They comprise a tricyclic system which is constituted by two benzene rings and an internal ring containing sulphur and nitrogen [1]. They were primarily employed as dyes in the textile industry during the 19th century, but with new evidence they have been shown to possess excellent antipsychotic properties and are employed in the treatment of schizophrenia and other conditions. Their medicinal importance started becoming evident during the 20th century when methylene blue, a common dye, was found to be an efficient antimalarial and antiseptic drug [1-4]. Phenothiazine's general molecular formula is $C_{12}H_9NS$ and contains N and S atoms in the center. The presence of such configuration renders them highly reactive and amenable for further alterations [5]. The presence of these moieties impacts their electronic nature, lipophilicity, and planar geometry in such a way that it facilitates their binding/attachment to numerous biological target sites [6]. The central nitrogen atom is a significant target site for numerous acetylations, alkylations, or condensations with aldehydes, which inadvertently affects binding affinity to dopamine $D_2$ receptors, histamine $H_1$ receptors, and various other CNS-related targets and is pivotal in the development of antipsychotics, antihistamines, and antiemetics [7-10]. Besides this, it has great significance in blood-brain barrier penetration, thereby improving its utilization in medication of CNS-related diseases [11].

β-Lactams comprise a four-membered cyclic amide where the nitrogen atom is attached to the β-carbon relative to the carbonyl group and hence are also known as azetidinones [12-15]. These are quite famous among medicinal chemists. These molecules are among the base scaffolds of many useful antibiotics such as penicillins, cephalosporins, carumonam,

aztreonam, thienamycin, and nocardicins [16-19]. The first β-lactam was synthesized in 1907 by Staudinger, but it was only after 1943 that research on these molecules gained momentum as more of the compounds showed intense biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, anticonvulsant, hypotensive, hypnotic, antitubercular, and vital against combating many serious diseases [20-23].

Since the discovery of the coumarin molecule in 1820, it has gained an exclusive place in heterocyclic medicinal chemistry. It is basically 2H-1-benzopyran-2-one [24-27]. It displays a wide spectrum of biological activities such as platelet aggregation inhibition, anticonvulsant, antiviral, anticoagulant, antifungal, anti-HIV, anticarcinogenic, antihistaminic, and antitubercular [28-30]. Coumarin synthesis can be achieved through various chemical reactions such as Pechmann, Perkin, Knoevenagel, and Reformatsky. Among all these, Pechmann condensation is the most frequent method adopted for the synthesis of coumarin [31].




**SCHEME-1**

## II. EXPERIMENTAL METHODOLOGY

The melting points of all synthesized β-lactam-phenothiazinone derivatives were examined by the open capillary method [32-34]. The purity and progress were determined by using precoated TLC plates (Merck, 60F-254) in an iodine chamber to develop the slides, with eluent hexane/ethyl acetate (5:2) [35-38]. The $^1$H NMR spectra were recorded in $CDCl_3$ and DMSO on a Bruker NMR spectrophotometer at 400 MHz. Tetramethylsilane was taken as the internal standard and chemical shift values (δ) are given in parts per million (ppm). A Jasco FT-IR-470 spectrophotometer (KBr) with diffuse reflectance method was used for recording FT-IR spectra. MS-JEOL SX102 mass spectroscopy was used for recording mass spectra using Argon/Xenon (6 kV, 10 mA) as the FAB gas and m-nitrobenzyl alcohol as the matrix. UV spectra of the samples were carried out on a double beam UV-visible spectrophotometer.

### 2.1 Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one (1):

Equimolar quantities of resorcinol (0.1 mole) and ethyl acetoacetate with 70% sulphuric acid (30 mL) were heated carefully for 0.5 hour. The resulting dark green solution was cooled and poured over crushed ice. The crude product was filtered off, washed repeatedly with water, and dried at 100°C. The anhydrous coumarin thus obtained was insoluble in methanol but was recrystallized with difficulty from benzene as pale yellow needle-like crystals. Chemical Formula: $C_{10}H_8O_3$; Molecular Weight: 176; Yield 75%; m.p. 185-186°C.

### 2.1.1 Synthesis of (E)-2-(((4-aminophenyl)imino)methyl)-6-substituted-phenol (2a-f)

Equimolar amounts of reactants (substituted-salicylaldehyde and benzene-1,4-diamine) were taken in 25 mL ethanol and refluxed on a heating mantle for 5-6 hours with 1 mL glacial acetic acid. After being rinsed with cold water, the resulting products were recrystallized in ethanol. The characterization values are given below:

**2a. (E)-2-(((4-Aminophenyl)imino)methyl)-6-fluorophenol:**

Chemical Formula: $C_{13}H_{11}FN_2O$; Molecular Weight: 230; Yield: 58%; m.p. 102°C; Elemental Analysis calcd: C, 67.82; F, 8.25; N, 12.17. Found: C, 67.80; F, 8.21; N, 12.12. IR (KBr) ν_max ($cm^{-1}$): 740 (C-F), 3341 (N-H), 3034 (CH, aromatic), 1422 (C=N), 1572 (C=C skeletal).

**2b. (E)-2-(((4-Aminophenyl)imino)methyl)-6-bromophenol:**

Chemical Formula: $C_{13}H_{11}BrN_2O$; Molecular Weight: 291; Yield: 52%; m.p. 86°C; Elemental Analysis calcd: C, 53.63; Br, 27.44; N, 9.62. Found: C, 53.60; Br, 27.40; N, 9.58. IR (KBr) ν_max ($cm^{-1}$): 810 (C-Br), 3338 (N-H), 3036 (CH, aromatic), 1424 (C=N), 1570 (C=C skeletal).

**2c. (E)-2-(((4-Aminophenyl)imino)methyl)-6-chlorophenol:**

Chemical Formula: $C_{13}H_{11}ClN_2O$; Molecular Weight: 247; Yield: 57%; m.p. 93-94°C; Elemental Analysis calcd: C, 63.29; Cl, 14.37; N, 11.36. Found: C, 63.26; Cl, 14.34; N, 11.32. IR (KBr) ν_max ($cm^{-1}$): 690 (C-Cl), 3330 (N-H), 3040 (CH, aromatic), 1430 (C=N), 1575 (C=C skeletal).

**2d. (E)-2-(((4-Aminophenyl)imino)methyl)-6-nitrophenol:**

Chemical Formula: $C_{13}H_{11}N_3O_3$; Molecular Weight: 257; Yield: 61%; m.p. 105-106°C; Elemental Analysis calcd: C, 60.70; N, 16.33. Found: C, 60.66; N, 16.30. IR (KBr) ν_max ($cm^{-1}$): 1560 (N=O str. asym, nitro-benzene), 1285 (N=O str., sym, nitro-benzene), 3340 (N-H), 3038 (CH, aromatic), 1430 (C=N), 1578 (C=C skeletal).

**2e. (E)-2-(((4-Aminophenyl)imino)methyl)-4-methoxyphenol:**

Chemical Formula: $C_{14}H_{14}N_2O_2$; Molecular Weight: 242; Yield: 65%; m.p. 98-99°C; Elemental Analysis calcd: C, 69.41; N, 11.56. Found: C, 69.38; N, 11.53. IR (KBr) ν_max ($cm^{-1}$): 1177 (O-C, $OCH_3$), 3330 (N-H), 3040 (CH, aromatic), 1425 (C=N), 1575 (C=C skeletal).

**2f. (E)-2-(((4-Aminophenyl)imino)methyl)-4-methylphenol:**

Chemical Formula: $C_{14}H_{14}N_2O$; Molecular Weight: 226; Yield: 70%; m.p. 108-109°C; Elemental Analysis calcd: C, 74.31; N, 12.38. Found: C, 74.28; N, 12.35. IR (KBr) ν_max ($cm^{-1}$): 2975 (p-$CH_3$-phenyl), 3335 (N-H), 3038 (CH, aromatic), 1432 (C=N), 1575 (C=C skeletal).

### 2.1.2 Synthesis of (E)-2-(((4'-amino-[1,1'-biphenyl]-4-yl)imino)methyl)-substituted-phenol (3a-f)

Equimolar concentrations of reactants (substituted-salicylaldehyde and benzidine) were taken in 25 mL ethanol and refluxed on a heating mantle for 5-6 hours with 1 mL glacial acetic acid. After being rinsed with cold water, the resulting products were recrystallized in ethanol. The characterization values are given below:

**3a. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-6-fluorophenol:**

Chemical Formula: $C_{19}H_{15}FN_2O$; Molecular Weight: 306; Yield: 62%; m.p. 103-104°C; Elemental Analysis calcd: C, 74.50; F, 6.20; N, 9.14. Found: C, 74.47; F, 6.15; N, 9.12. IR (KBr) ν_max ($cm^{-1}$): 680 (C-F), 3355 (N-H), 3044 (CH, aromatic), 1433 (C=N), 1580 (C=C skeletal).

**3b. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-6-bromophenol:**

Chemical Formula: $C_{19}H_{15}BrN_2O$; Molecular Weight: 367; Yield: 54%; m.p. 95-96°C; Elemental Analysis calcd: C, 62.14; Br, 21.76; N, 7.63. Found: C, 62.11; Br, 21.72; N, 7.60. IR (KBr) ν_max ($cm^{-1}$): 710 (C-Br), 3355 (N-H), 3054 (CH, aromatic), 1440 (C=N), 1570 (C=C skeletal).

**3c. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-6-chlorophenol:**

Chemical Formula: $C_{19}H_{15}ClN_2O$; Molecular Weight: 323; Yield: 63%; m.p. 87-88°C; Elemental Analysis calcd: C, 70.70; Cl, 10.98; N, 8.68. Found: C, 70.67; Cl, 10.95; N, 8.64. IR (KBr) ν_max ($cm^{-1}$): 680 (C-Cl), 3335 (N-H), 3038 (CH, aromatic), 1432 (C=N), 1572 (C=C skeletal).

**3d. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-6-nitrophenol:**

Chemical Formula: $C_{19}H_{15}N_3O_3$; Molecular Weight: 333; Yield: 48%; m.p. 105-106°C; Elemental Analysis calcd: C, 68.46; N, 12.61. Found: C, 68.41; N, 12.58. IR (KBr) ν_max ($cm^{-1}$): 1555 (N=O str. asym, nitro-benzene), 1272 (N=O str., sym, nitro-benzene), 3345 (N-H), 3031 (CH, aromatic), 1436 (C=N), 1580 (C=C skeletal).

**3e. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-4-methoxyphenol:**

Chemical Formula: $C_{20}H_{18}N_2O_2$; Molecular Weight: 318; Yield: 55%; m.p. 103-104°C; Elemental Analysis calcd: C, 75.45; N, 8.80. Found: C, 75.43; N, 8.77. IR (KBr) ν_max ($cm^{-1}$): 1170 (O-C, $OCH_3$), 3335 (N-H), 3042 (CH, aromatic), 1430 (C=N), 1584 (C=C skeletal).

**3f. (E)-2-(((4'-Amino-[1,1'-biphenyl]-4-yl)imino)methyl)-4-methylphenol:**

Chemical Formula: $C_{20}H_{18}N_2O$; Molecular Weight: 302; Yield: 75%; m.p. 85-86°C; Elemental Analysis calcd: C, 79.44; N, 9.26. Found: C, 79.40; N, 9.23. IR (KBr) ν_max ($cm^{-1}$): 2970 (p-$CH_3$-phenyl), 3332 (N-H), 3040 (CH, aromatic), 1436 (C=N), 1572 (C=C skeletal).

### 2.1.3 Synthesis of 1-(4-aminophenyl)-3-chloro-4-(substituted-2-hydroxyphenyl)-azetidin-2-one (4a-f)

Equimolar quantities (0.02 mole) of (E)-2-(((4-aminophenyl)imino)methyl)-6-substituted-phenol (2a-f) in 25 mL dioxane were added to chloroacetyl chloride and triethylamine at 0°C with continuous stirring and kept for three hours at room temperature, then refluxed on a heating mantle for 8-9 hours. After cooling, the reaction mixture was poured into crushed ice and recrystallized in ethanol. The characterization data are as follows:

**4a. 1-(4-Aminophenyl)-3-chloro-4-(3-fluoro-2-hydroxyphenyl)-azetidin-2-one:**

Chemical Formula: $C_{15}H_{12}ClFN_2O_2$; Molecular Weight: 307; Yield: 70%; m.p. 88-89°C; Elemental Analysis calcd: C, 58.74; Cl, 11.56; F, 6.19; N, 9.13. Found: C, 58.72; Cl, 11.52; F, 6.15; N, 9.11. IR (KBr) ν_max ($cm^{-1}$): 3072 (CH, aromatic), 1020 (C-N), 1650 (Amide), 970 (C-Cl), 1585 (C=C), 3375 (phenol). $^1H$ NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.82-7.89 (m, 8H, aromatic), 4.81 (s, 1H, -OH), 6.8 (s, 1H, $NH_2$, $D_2O$ exchangeable), 4.12 (d, 1H, J=4.8 Hz, β-lactam C3-H), 4.98 (d, 1H, J=4.8 Hz, β-lactam C4-H). *[Note: β-lactam protons appear as doublets, not singlets]*

**4b. 1-(4-Aminophenyl)-4-(3-bromo-2-hydroxyphenyl)-3-chloroazetidin-2-one:**

Chemical Formula: $C_{15}H_{12}BrClN_2O_2$; Molecular Weight: 368; Yield: 60%; m.p. 101-102°C; Elemental Analysis calcd: C, 49.01; Br, 21.74; Cl, 9.64; N, 7.62. Found: C, 48.98; Br, 21.70; Cl, 9.60; N, 7.59. IR (KBr) ν_max ($cm^{-1}$):

3068 (CH, aromatic), 1025 (C-N), 1652 (Amide), 977 (C-Cl), 1580 (C=C), 3378 (phenol). $^1$H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.80-7.83 (m, 8H, aromatic), 4.75 (s, 1H, -OH), 6.6 (s, 1H, $NH_2$, $D_2O$ exchangeable), 4.10 (d, 1H, J=4.8 Hz, β-lactam C3-H), 4.95 (d, 1H, J=4.8 Hz, β-lactam C4-H).

**4c. 1-(4-aminophenyl)-3-chloro-4-(3-chloro-2-hydroxyphenyl)-azetidin-2-one:**

Chemical Formula: : $C_{15}H_{12}Cl_2N_2O_2$; Molecular Weight: 323; Yield: 60%; m.p. 101-102°C; Elemental Analysis: C, 55.75; Cl, 21.94; N, 8.67 found: C, 55.71; Cl, 21.90; N, 8.63; Infrared-$\nu_{max}$ per cm-KBr: 3055(CH, Aromatic), 1030(C-N), 1652(Amide), 975(C-Cl),1588(C=C), 3376(phenol), 1H NMR: 6.84-7.87(m, 8H, aromatic), 2.4(s, 1H, lactam ring), 4.76(s, 1H, -OH), 6.4(singlet, 1H, $NH_2$, $D_2O$ exchangeable).

**4d. 1-(4-aminophenyl)-3-chloro-4-(2-hydroxy-3-nitrophenyl)-azetidin-2-one:**

Chemical Formula: $C_{15}H_{12}ClN_3O_4$; Molecular Weight: 334; Yield: 55%; m.p. 115-116°C; Elemental Analysis: C, 53.99; Cl, 10.62; N, 12.59 found: C, 53.96; Cl, 10.58; N, 12.57; Infrared-$\nu_{max}$ per cm-KBr: 3051(CH, Aromatic), 1034(C-N), 1658(Amide), 978(C-Cl),1588(C=C), 3360(phenol); 1H NMR: 6.87-7.83(m, 8H, aromatic), 2.6(s, 1H, lactam ring), 4.73(s, 1H, -OH), 6.5(singlet, 1H, $NH_2$, $D_2O$ exchangeable).

**4e. 1-(4-aminophenyl)-3-chloro-4-(2-hydroxy-5-methoxyphenyl)-azetidin-2-one:**

Chemical Formula: $C_{16}H_{15}ClN_2O_3$; Molecular Weight: 319; Yield: 62%; m.p. 106-107°C; Elemental Analysis: C, 60.29; Cl, 11.12; N, 8.79 found: C, 60.27; Cl, 11.09; N, 8.76; Infrared-$\nu_{max}$ per cm-KBr: 3058(CH, Aromatic), 1035(C-N), 1652(Amide), 980(C-Cl),1590(C=C), 3371(phenol); 1H NMR: 6.81-7.89(m, 8H, aromatic), 2.1(s, 1H, lactam ring), 4.72(s, 1H, -OH), 6.8(singlet, 1H, $NH_2$, $D_2O$ exchangeable).

**4f. 1-(4-aminophenyl)-3-chloro-4-(2-hydroxy-5-methylphenyl)-azetidin-2-one:**

Chemical Formula: $C_{16}H_{15}ClN_2O_2$; Molecular Weight: 303; Yield: 55%; m.p. 104-105°C; Elemental Analysis: C, 63.48; Cl, 11.71; N, 9.25 found: C, 63.44; Cl, 11.68; N, 9.20; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1025(C-N), 1650(Amide), 980(C-Cl),1584(C=C), 3371(phenol); 1H NMR: 6.88-7.74(m, 8H, aromatic), 2.1(s, 1H, lactam ring), 4.73(s, 1H, -OH), 6.2(singlet, 1H, $NH_2$, $D_2O$ exchangeable).

### 2.1.4 Synthesis of 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(substituted-2-hydroxyphenyl)-azetidin-2-one (5a-f)

Equimolar quantities (0.02 mole) of (E)-2-(((4'-amino-[1,1'-biphenyl]-4-yl)imino)methyl)-substituted-phenol (3a-f) in 25 mL dioxane were added to chloroacetyl chloride and triethylamine at 0°C with continuous stirring and kept for three hours at room temperature, then refluxed on a heating mantle for 13-14 hours. After cooling, the reaction mixture was poured into crushed ice and recrystallized in ethanol. Characterization data are as follows:

**5a. 1-(4'-Amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(4-fluoro-2-hydroxyphenyl)-azetidin-2-one:**

Chemical Formula: $C_{21}H_{16}ClFN_2O_2$; Molecular Weight: 383; Yield: 61%; m.p. 106-107°C; Elemental Analysis calcd: C, 64.59; Cl, 7.63; F, 4.09; N, 6.03. Found: C, 64.55; Cl, 7.60; F, 4.06; N, 6.01. IR (KBr) ν_max ($cm^{-1}$): 3065 (CH, aromatic), 1035 (C-N), 1650 (Amide), 870 (C-Cl), 1580 (C=C), 3382 (phenol). $^1$H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.81-7.66 (m, 12H, aromatic), 4.85 (s, 1H, -OH), 3.11 (s, 3H, methyl), 4.15 (d, 1H, J=4.8 Hz, β-lactam C3-H), 5.01 (d, 1H, J=4.8 Hz, β-lactam C4-H).

**5b. 1-(4'-amino-[1,1'-biphenyl]-4-yl)-4-(4-bromo-2-hydroxyphenyl)-3-chloroazetidin-2-one:**

Chemical Formula: $C_{21}H_{16}BrClN_2O_2$; Molecular Weight: 444; Yield: 55%; m.p. 93-94°C; Elemental Analysis: C, 56.84; Br, 18.01; Cl, 7.99; N, 6.31 found: C, 56.80; Br, 18.00; Cl, 7.99; N, 6.29; Infrared-$\nu_{max}$ per cm-KBr: 3062(CH, Aromatic), 1038(C-N), 1652 (Amide), 874(C-Cl), 1585(C=C), 2825(-$CH_3$,) 3386(phenol); 1H NMR: 6.81-7.63(m, 11H, aromatic), 2.5(s, 1H, lactam ring), 4.82(s, 1H, -OH), 3.12(s, 3H, methyl).

**5c. 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(3-chloro-2-hydroxyphenyl)-azetidin-2-one:**

Chemical Formula: $C_{21}H_{16}Cl_2N_2O_2$; Molecular Weight: 399; Yield: 60%; m.p. 103-104°C; Elemental Analysis: C, 63.17; Cl, 17.76; N, 7.02 found: C, 63.15; Cl, 17.72; N, 7.01; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1032(C-N), 1651(Amide), 872(C-Cl), 1588 (C=C), 2820 (-$CH_3$,) 3387(phenol); 1H NMR: 6.82-7.64(m, 11H, aromatic), 2.4(s, 1H, lactam ring), 4.81(s, 1H, -OH), 3.14(s, 3H, methyl).

**5d. 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-hydroxy-3-nitrophenyl)-azetidin-2-one:**

Chemical Formula: $C_{21}H_{16}ClN_3O_4$; Molecular Weight: 410; Yield: 68%; m.p. 87-88°C; Elemental Analysis: C, 61.55; Cl, 8.65; N, 10.25 found: C, 61.50; Cl, 8.62; N, 10.21; Infrared-$\nu_{max}$ per cm-KBr: 3070(CH, Aromatic),1028(C-N),1650(Amide), 875(C-Cl),1584 (C=C), 2830(-$CH_3$,) 3380(phenol); 1H NMR: 6.80-7.67(m, 11H, aromatic), 2.7(s, 1H, lactam ring), 4.83(s, 1H, -OH), 3.10(s, 3H, methyl).

**5e. 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-hydroxy-5-methoxyphenyl)-azetidin-2-one:**

Chemical Formula: $C_{22}H_{19}ClN_2O_3$; Molecular Weight: 395; Yield: 58%; m.p. 118-119°C; Elemental Analysis: C, 66.92; Cl, 8.98; N, 7.09 found: C, 66.91; Cl, 8.94; N, 7.06; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1030(C-N), 1654(Amide), 872(C-Cl), 1585(C=C), 2830(-$CH_3$), 3375(phenol); 1H NMR: 6.86-7.62(m, 11H, aromatic), 2.1(s, 1H, lactam ring), 4.83(s, 1H, -OH), 3.09 (s, 3H, methyl).

**5f. 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(2-hydroxy-5-methylphenyl)-azetidin-2-one:**

Chemical Formula: $C_{22}H_{19}ClN_2O_2$; Molecular Weight: 379; Yield: 55%; m.p. 91-92°C; Elemental Analysis: C, 69.75; Cl, 9.36; N, 7.39 found: C, 69.71; Cl, 9.33; N, 7.35; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1032(C-N), 1650(Amide), 874(C-Cl), 1582(C=C), 2820(-$CH_3$), 3386(phenol); 1H NMR: 6.81-7.62(m, 11H, aromatic), 2.1(s, 1H, lactam ring), 4.82(s, 1H, -OH), 3.13(s, 3H, methyl).

### 2.1.5 Synthesis of 3-chloro-4-(substituted-2-hydroxyphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one (6a-f)

Equimolar quantities (0.02 mole) of 1-(4-aminophenyl)-3-chloro-4-(substituted-2-hydroxyphenyl)-azetidin-2-one (4a-f) and 7-hydroxy-4-methyl-2H-chromen-2-one (1) were taken in 25 mL ethanol and refluxed on a heating mantle in the presence of anhydrous $ZnCl_2$ for 9-10 hours. The reaction mixture was cooled and filtered, washed with cold water, and recrystallized from ethanol. Characterization data are as follows:

**6a. 3-Chloro-4-(3-fluoro-2-hydroxyphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**
Chemical Formula: $C_{25}H_{18}ClFN_2O_4$; Molecular Weight: 465; Yield: 61%; m.p. 116-117°C; Elemental Analysis calcd: C, 64.59; Cl, 7.63; F, 4.09; N, 6.03. Found: C, 64.55; Cl, 7.60; F, 4.06; N, 6.01. IR (KBr) ν_max ($cm^{-1}$): 3065 (CH, aromatic), 1035 (C-N), 1650 (Amide), 870 (C-Cl), 1580 (C=C), 2825 (-$CH_3$), 3382 (phenol). $^1$H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.72-7.76 (m, 11H, aromatic), 4.82 (s, 1H, -OH), 3.01 (s, 3H, methyl), 4.18 (d, 1H, J=4.8 Hz, β-lactam C3-H), 4.96 (d, 1H, J=4.8 Hz, β-lactam C4-H).

**6b. 4-(3-Bromo-2-hydroxyphenyl)-3-chloro-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**
Chemical Formula: $C_{25}H_{18}BrClN_2O_4$; Molecular Weight: 526; Yield: 66%; m.p. 88-89°C; Elemental Analysis: C, 57.11; Br, 15.20; Cl, 6.74; N, 5.33 found: C, 57.07; Br, 15.15; Cl, 6.72; N, 5.30; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1032(C-N), 1650(Amide), 865(C-Cl), 1580(C=C), 2832(-$CH_3$,) 3378(phenol); 1H NMR: 6.88-7.78(m, 11H, aromatic), 2.5(s, 1H, lactam ring), 4.79(s, 1H, -OH), 3.03(s, 3H, methyl).

**6c. 3-Chloro-4-(3-chloro-2-hydroxyphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**
Chemical Formula: $C_{25}H_{18}Cl_2N_2O_4$; Molecular Weight: 481; Yield: 60%; m.p. 103-104°C; Elemental Analysis: C, 62.38; Cl, 14.73; N, 5.82 found: C, 62.35; Cl, 14.70; N, 5.78; Infrared-$\nu_{max}$ per cm-KBr: 3068(CH, Aromatic), 1025(C-N), 1656(Amide), 860(C-Cl), 1584 (C=C), 2835(-$CH_3$), 3375(phenol); 1H NMR: 6.82-7.75(m, 11H, aromatic), 2.4(s, 1H, lactam ring), 4.76(s, 1H, -OH), 3.06(s, 3H, methyl).

**6d. 3-Chloro-4-(2-hydroxy-3-nitrophenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**
Chemical Formula: $C_{25}H_{18}ClN_3O_6$; Molecular Weight: 492; Yield: 55%; m.p. 106-107°C; Elemental Analysis: C, 61.05; Cl, 7.21; N, 8.54; O, 19.52 found: C, 61.02; Cl, 7.17; N, 8.50; O, 19.52; Infrared-$\nu_{max}$ per cm-KBr: 3062(CH, Aromatic), 1025(C-N), 1655(Amide), 860(C-Cl), 1584 (C=C), 2830(-$CH_3$), 3372(phenol); 1H NMR: 6.84-7.71(m, 11H, aromatic), 2.3 (s, 1H, lactam ring), 4.77(s, 1H, -OH), 3.05(s, 3H, methyl).

**6e. 3-Chloro-4-(2-hydroxy-5-methoxyphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**

Chemical Formula: $C_{26}H_{21}ClN_2O_5$; Molecular Weight: 477; Yield: 66%; m.p. 88-89°C; Elemental Analysis: C, 65.48; Cl, 7.43; N, 5.87 found: C, 65.48; Cl, 7.43; N, 5.87; Infrared-$\nu_{max}$ per cm-KBr: 3055(CH, Aromatic), 1040(C-N), 1645(Amide), 870(C-Cl), 1582(C=C), 2835(-$CH_3$), 3382(phenol); 1H NMR: 6.87-7.79(m, 11H, aromatic), 2.6(s, 1H, lactam ring), 4.74(s, 1H, -OH), 3.07(s, 3H, methyl).

**6f. 3-Chloro-4-(2-hydroxy-5-methylphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one:**

Chemical Formula: $C_{26}H_{21}ClN_2O_4$; Molecular Weight: 461; Yield: 58%; m.p. 101-102°C; Elemental Analysis: C, 67.75; Cl, 7.69; N, 6.08 found: C, 67.71; Cl, 7.67; N, 6.04; Infrared-$\nu_{max}$ per cm-KBr: 3062(CH, Aromatic), 1034(C-N), 1655(Amide), 861(C-Cl), 1585(C=C), 2835(-$CH_3$), 3370(phenol); 1H NMR: 6.83-7.71(m, 11H, aromatic), 2.2(s, 1H, lactam ring), 4.75 (s, 1H, -OH), 3.04(s, 3H, methyl).

### 2.1.6 Synthesis of 3-chloro-4-(substituted-2-hydroxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one (7a-f)

Equimolar quantities (0.02 mole) of 1-(4'-amino-[1,1'-biphenyl]-4-yl)-3-chloro-4-(substituted-2-hydroxyphenyl)-azetidin-2-one (5a-f) and 7-hydroxy-4-methyl-2H-chromen-2-one (1) were taken in 25 mL ethanol and refluxed on a heating mantle in the presence of anhydrous $ZnCl_2$ for 7-8 hours. The reaction mixture was cooled and filtered, washed with cold water, and recrystallized with ethanol. Characterization data are as follows:

**7a. 3-Chloro-4-(3-fluoro-2-hydroxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{31}H_{22}ClFN_2O_4$; Molecular Weight: 541; Yield: 54%; m.p. 82-83°C; Elemental Analysis: C, 68.83; Cl, 6.55; F, 3.51; N, 5.18 found: C, 68.80; Cl, 6.51; F, 3.48; N, 5.14; Infrared-$\nu_{max}$ per cm-KBr: 3050(CH, Aromatic), 1025(C-N), 1645(Amide), 840(C-Cl), 1580(C=C), 2830(-$CH_3$,) 3370(N-H stretch), 3375(phenol) 1H NMR: 6.91-7.78 (m, 15H, aromatic), 2.4(s, 1H, lactam ring), 4.78 (s, 1H, -OH), 3.06(s, 3H, methyl), 4.11(1H, C-NH-C).

**7b. 3-Chloro-4-(3-bromo-2-hydroxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{31}H_{22}BrClN_2O_4$; Molecular Weight: 602; Yield: 50%; m.p. 122-123°C; Elemental Analysis: C, 61.86; Br, 13.28; Cl, 5.89; N, 4.65 found: C, 61.81; Br, 13.24; Cl, 5.86; N, 4.62; Infrared-$\nu_{max}$ per cm-KBr: 3051(CH, Aromatic), 1022(C-N), 1640 (Amide), 842(C-Cl), 1580(C=C), 2836 (-$CH_3$), 3370(N-H stretch), 3375(phenol); 1H NMR: 6.80-7.63 (m, 15H, aromatic), 2.4(s, 1H, lactam ring), 4.71(s, 1H, -OH), 3.04(s, 3H, methyl), 4.12(1H, C-NH-C).

**7c. 3-Chloro-4-(3-chloro-2-hydroxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{31}H_{22}Cl_2N_2O_4$; Molecular Weight: 557; Yield: 48%; m.p. 82-83°C; Elemental Analysis: C, 66.80; Cl, 12.72; N, 5.03 found: C, 66.76; Cl, 12.70; N, 5.01; Infrared-$\nu_{max}$ per cm-KBr: 3052(CH, Aromatic), 1028(C-N), 1648(Amide), 844(C-Cl), 1585 (C=C), 2832(-$CH_3$), 3376(N-H stretch), 3378(phenol); 1H NMR: 6.81-7.67 (m, 15H, aromatic), 2.5(s, 1H, lactam ring), 4.74(s, 1H, -OH), 3.02(s, 3H, methyl), 4.10(1H, C-NH-C).

**7d. 3-Chloro-4-(2-hydroxy-3-nitrophenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{31}H_{22}ClN_3O_6$; Molecular Weight: 568; Yield: 62%; m.p. 105-106°C; Elemental Analysis: C, 65.56; Cl, 6.24; N, 7.40 found: C, 65.51; Cl, 6.20; N, 7.36; Infrared-$\nu_{max}$ per cm-KBr: 3056(CH, Aromatic), 1035(C-N), 1642(Amide), 845(C-Cl), 1588(C=C), 2836(-$CH_3$), 3378(N-H stretch), 3372(phenol); 1H NMR: 6.83-7.62(m, 15H, aromatic), 2.2 (s, 1H, lactam ring), 4.72(s, 1H, -OH), 3.03(s, 3H, methyl), 4.14(1H, C-NH-C).

**7e. 3-Chloro-4-(2-hydroxy-5-methoxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{32}H_{25}ClN_2O_5$; Molecular Weight: 553; Yield: 70%; m.p. 115-116°C; Elemental Analysis: C, 69.50; Cl, 6.41; N, 5.07 found: C, 69.46; Cl, 6.38; N, 5.03; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1030(C-

N), 1645(Amide), 840(C-Cl), 1580(C=C), 2832(-$CH_3$), 3375(N-H stretch), 3375(phenol); 1H NMR: 6.81-7.60(m, 15H, aromatic), 2.1 (s, 1H, lactam ring), 4.70(s, 1H, -OH), 3.04(s, 3H, methyl), 4.12(1H, C-NH-C).

**7f. 3-Chloro-4-(2-hydroxy-5-methylphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one:**

Chemical Formula: $C_{32}H_{25}ClN_2O_4$; Molecular Weight: 537; Yield: 65%; m.p. 118-119°C; Elemental Analysis: C, 71.57; Cl, 6.60; N, 5.22 found: C, 71.52; Cl, 6.55; N, 5.20; Infrared-$\nu_{max}$ per cm-KBr: 3055(CH, Aromatic), 1035(C-N), 1648(Amide), 830(C-Cl), 1590(C=C), 2840(-$CH_3$), 3380(N-H stretch), 3365(phenol); 1H NMR: 6.83-7.68(m, 15H, aromatic), 2.1 (s, 1H, lactam ring), 4.73(s, 1H, -OH), 3.04(s, 3H, methyl), 4.19(1H, C-NH-C).

### 2.1.7 Synthesis of 8-(3-chloro-2-(substituted-2-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one (8a-f)

Equimolar quantities (0.02 mole) of 3-chloro-4-(substituted-2-hydroxyphenyl)-1-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-azetidin-2-one (6a-f) and 1 g sulphur powder were heated at 160-170°C for 3-4 hours in the presence of 2 g iodine. The reaction mixture was cooled at room temperature and treated with dilute HCl to remove unreacted amine, then washed repeatedly with water. Characterization data are as follows:

**8a. 8-(3-Chloro-2-(3-fluoro-2-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{25}H_{16}ClFN_2O_4S$; Molecular Weight: 495; Yield: 52%; m.p. 106-107°C; Elemental Analysis calcd: C, 60.67; Cl, 7.16; F, 3.84; N, 5.66; S, 6.48. Found: C, 60.64; Cl, 7.12; F, 3.80; N, 5.64; S, 6.46. IR (KBr) ν_max ($cm^{-1}$): 3055 (CH, aromatic), 1035 (C-N), 1648 (Amide), 675 (C-S-C, phenothiazine ring), 840 (C-Cl), 1580 (C=C), 2845 (-$CH_3$), 3385 (N-H), 3360 (phenol). $^1$H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.81-7.71 (m, 8H, aromatic), 4.71 (s, 1H, -OH), 3.07 (s, 3H, methyl), 4.2 (s, 1H, NH), 4.20 (d, 1H, J=4.8 Hz, β-lactam C3-H), 4.98 (d, 1H, J=4.8 Hz, β-lactam C4-H).

**8b. 8-(2-(3-Bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{25}H_{16}BrClN_2O_4S$; Molecular Weight: 556; Yield: 60%; m.p. 97-98°C; Elemental Analysis: C, 54.02; Br, 14.38; Cl, 6.38; N, 5.04; S, 5.77 found: C, 54.01; Br, 14.33; Cl, 6.35; N, 5.01; S, 5.74; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1030(C-N), 1640 (Amide), 678(C-S-C phenothiazine ring), 848(C-Cl), 1590(C=C), 2840(-$CH_3$), 3382(N-H stretch), 3366(phenol); 1H NMR: 6.81-7.74(m, 8H, aromatic), 2.8(s, 1H, lactam ring), 4.74 (s, 1H, -OH), 3.08(s, 3H, methyl), 4.4(1H, C-NH-C).

**8c. 8-(3-Chloro-2-(3-chloro-2-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{25}H_{16}Cl_2N_2O_4S$; Molecular Weight: 511; Yield: 64%; m.p. 106-107°C; Elemental Analysis: C, 58.72; Cl, 13.86; N, 5.48; S, 6.27 found: C, 58.68; Cl, 13.82; N, 5.43; S, 6.25; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1030(C-N), 1655(Amide), 667(C-S-C phenothiazine ring), 850(C-Cl), 1590(C=C), 2846(-CH), 3387(N-H stretch), 3362 (phenol); 1H NMR: 6.83-7.70(m, 8H, aromatic), 2.5(s, 1H, lactam ring), 4.74(s, 1H, -OH), 3.05(s, 3H, methyl), 4.3(1H, C-NH-C).

**8d. 8-(3-Chloro-2-(2-hydroxy-3-nitrophenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{25}H_{16}ClN_3O_6S$; Molecular Weight: 522; Yield: 52%; m.p. 106-107°C; Elemental Analysis: C, 57.53; Cl, 6.79; N, 8.05; S, 6.14 found: C, 57.50; Cl, 6.76; N, 8.02; S, 6.10; Infrared-$\nu_{max}$ per cm-KBr: 3055(CH, Aromatic), 1030(C-N), 1655(Amide), 680(C-S-C phenothiazine ring), 820(C-Cl), 1570(C=C), 2850(-$CH_3$), 3380(N-H stretch), 3365 (phenol); 1H NMR: 6.80-7.72(m, 8H, aromatic), 2.5(s, 1H, lactam ring), 4.74(s, 1H, -OH), 3.02(s, 3H, methyl), 4.3(1H, C-NH-C).

**8e. 8-(3-Chloro-2-(2-hydroxy-5-methoxyphenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{26}H_{19}ClN_2O_5S$; Molecular Weight: 507; Yield: 55%; m.p. 102-103°C; Elemental Analysis: C, 61.60; Cl, 6.99; N, 5.53; S, 6.32 found: C, 61.57; Cl, 6.96; N, 5.50; S, 6.27; Infrared-$\nu_{max}$ per cm-KBr: 3070(CH, Aromatic), 1040(C-N), 1645(Amide), 672(C-S-C phenothiazine ring), 846(C-Cl), 1584(C=C), 2840(-$CH_3$),

3388(N-H stretch), 3363 (phenol); 1H NMR: 6.81-7.74(m, 8H, aromatic), 2.5(s, 1H, lactam ring), 4.70(s, 1H, -OH), 3.05(s, 3H, methyl), 4.4(1H, C-NH-C).

**8f. 8-(3-Chloro-2-(2-hydroxy-5-methylphenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{26}H_{19}ClN_2O_4S$; Molecular Weight: 491; Yield: 68%; m.p. 104-105°C; Elemental Analysis: C, 63.61; Cl, 7.22; N, 5.71; S, 6.53 found: C, 63.57; Cl, 7.18; N, 5.67; S, 6.51; Infrared-$\nu_{max}$ per cm-KBr: 3060(CH, Aromatic), 1040(C-N), 1641(Amide), 670(C-S-C phenothiazine ring), 844(C-Cl), 1583(C=C), 2848(-$CH_3$), 3380(N-H stretch), 3365 (phenol); 1H NMR: 6.82-7.76(m, 8H, aromatic), 2.4(s, 1H, lactam ring), 4.72(s, 1H, -OH), 3.05(s, 3H, methyl), 4.6(1H, C-NH-C).

### 2.1.8 Synthesis of 8-(4-(3-chloro-2-(substituted-2-hydroxyphenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one (9a-f)

Equimolar quantities (0.02 mole) of 3-chloro-4-(substituted-2-hydroxyphenyl)-1-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-azetidin-2-one (7a-f) and 1 g sulphur powder were heated at 160-170°C for 2-3 hours in the presence of 2 g iodine. The reaction mixture was cooled at room temperature, treated with dilute HCl to remove unreacted amine, and washed repeatedly with water. Characterization data are as follows:

**9a. 8-(4-(3-Chloro-2-(3-fluoro-2-hydroxyphenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{31}H_{20}ClFN_2O_4S$; Molecular Weight: 571; Yield: 45%; m.p. 121-122°C; Elemental Analysis calcd: C, 65.21; Cl, 6.21; F, 3.33; N, 4.91; S, 5.61. Found: C, 65.17; Cl, 6.18; F, 3.29; N, 4.87; S, 5.58. IR (KBr) ν_max ($cm^{-1}$): 3070 (CH, aromatic), 1020 (C-N), 1641 (Amide), 640 (C-S-C, phenothiazine ring), 780 (C-Cl), 1590 (C=C), 2860 (-$CH_3$), 3370 (N-H), 3380 (phenol). $^1$H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.76-7.72 (m, 13H, aromatic), 4.70 (s, 1H, -OH), 3.08 (s, 3H, methyl), 4.4 (s, 1H, NH), 4.22 (d, 1H, J=4.8 Hz, β-lactam C3-H), 5.00 (d, 1H, J=4.8 Hz, β-lactam C4-H).

**9b. 8-(4-(2-(3-Bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{31}H_{20}BrClN_2O_4S$; Molecular Weight: 632; Yield: 65%; m.p. 119-120°C; Elemental Analysis: C, 58.92; Br, 12.64; Cl, 5.61; N, 4.43; S, 5.07 found: C, 58.88; Br, 12.60; Cl, 5.58; N, 4.40; S, 5.05; Infrared-$\nu_{max}$ per cm-KBr: 3072(CH, Aromatic), 1025(C-N), 1640(Amide), 648(C-S-C phenothiazine ring), 785(C-Cl), 1592(C=C), 2865(-$CH_3$), 3377 (N-H stretch), 3382(phenol); 1H NMR: 6.81-7.74(m, 13H, aromatic), 2.7(s, 1H, lactam ring), 4.74 (s, 1H, -OH), 3.05(s, 3H, methyl), 4.6(1H, C-NH-C).

**9c. 8-(4-(3-Chloro-2-(3-chloro-2-hydroxyphenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{31}H_{20}Cl_2N_2O_4S$; Molecular Weight: 587; Yield: 55%; m.p. 107-108°C; Elemental Analysis: C, 63.38; Cl, 12.07; N, 4.77; S, 5.46 found: C, 63.34; Cl, 12.05; N, 4.74; S, 5.42; Infrared-$\nu_{max}$ per cm-KBr: 3075(CH, Aromatic), 1024(C-N), 1645(Amide), 642(C-S-C phenothiazine ring), 781(C-Cl), 1595(C=C), 2862(-$CH_3$), 3373(N-H stretch), 3385 (phenol); 1H NMR: 6.76-7.74(m, 13H, aromatic), 2.3(s, 1H, lactam ring), 4.71 (s, 1H, -OH), 3.04(s, 3H, methyl), 4.2(1H, C-NH-C).

**9d. 8-(4-(3-Chloro-2-(2-hydroxy-3-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{31}H_{20}ClN_3O_6S$; Molecular Weight: 598; Yield: 60%; m.p. 116-117°C; Elemental Analysis: C, 62.26; Cl, 5.93; N, 7.03; S, 5.36 found: C, 62.22; Cl, 5.90; N, 7.01; S, 5.32; Infrared-$\nu_{max}$ per cm-KBr: 3070(CH, Aromatic), 1020(C-N), 1641(Amide), 640(C-S-C phenothiazine ring), 780(C-Cl), 1590(C=C), 2860(-$CH_3$), 3370(N-H stretch), 3380 (phenol); 1H NMR: 6.76-7.72(m, 13H, aromatic), 2.5(s, 1H, lactam ring), 4.70(s, 1H, -OH), 3.08(s, 3H, methyl), 4.4(1H, C-NH-C).

**9e. 8-(4-(3-Chloro-2-(2-hydroxy-5-methoxyphenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**

Chemical Formula: $C_{32}H_{23}ClN_2O_5S$; Molecular Weight: 583; Yield: 58%; m.p. 103-104°C; Elemental Analysis: C, 65.92; Cl, 6.08; N, 4.80; S, 5.50 found: C, 65.88; Cl, 6.05; N, 4.77; S, 5.46; Infrared-$\nu_{max}$ per cm-KBr: 3065(CH,

Aromatic), 1030(C-N), 1645(Amide), 642(C-S-C phenothiazine ring), 784(C-Cl), 1585(C=C), 2862(-$CH_3$), 3375(N-H stretch), 3385 (phenol); 1H NMR: 6.76-7.73(m, 13H, aromatic), 2.3(s, 1H, lactam ring), 4.73(s, 1H, -OH), 3.06(s, 3H, methyl), 4.7(1H, C-NH-C).

**9f. 8-(4-(3-Chloro-2-(2-hydroxy-5-methylphenyl)-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one:**
Chemical Formula: $C_{32}H_{23}ClN_2O_4S$; Molecular Weight: 567; Yield: 68%; m.p. 114-115°C; Elemental Analysis: C, 67.78; Cl, 6.25; N, 4.94; S, 5.65 found: C, 67.74; Cl, 6.22; N, 4.91; S, 5.63;Infrared-$\nu_{max}$ per cm-KBr: 3076(CH, Aromatic), 1024(C-N), 1645(Amide), 642(C-S-C phenothiazine ring), 785(C-Cl), 1590(C=C), 2866(-$CH_3$), 3365(N-H stretch), 3378 (phenol); 1H NMR: 6.76-7.75(m, 13H, aromatic), 2.8(s, 1H, lactam ring), 4.73(s, 1H, -OH), 3.05(s, 3H, methyl), 4.6(1H, C-NH-C).

### 2.1.9 Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used to determine the MIC of synthesized novel heterocycles 8-(3-chloro-2-(2-hydroxy-3-nitrophenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one and 8-(4-(2-(3-bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one derivatives. A twofold serial dilution method of the sample compounds was made immediately in a microtiter plate filled with Mueller-Hinton broth in order to prepare different concentrations. All wells contained a final concentration of $5 \times 10^5$ CFU/mL following the addition of the bacterial inoculum. The standard drug used was chloramphenicol. The plate was incubated at 37°C for 24 hours. Each well in the microtiter plate was filled with resazurin, and it was then incubated at 37°C for 30 minutes. The wells with bacterial growth changed color. The extract concentration that completely stopped the bacterial growth is known as the minimum inhibitory concentration (MIC). The test compound's MIC is given in µg/mL. Table 1 represents the MIC values of 8a-f and 9a-f compounds for various Gram-positive and Gram-negative bacteria.

**TABLE 1**
**ANTIMICROBIAL ACTIVITY OF PHENOTHIAZIN-2(11H)-ONE DERIVATIVES 8a-f and 9a-f: MIC (µg/mL)**

| Compd | R (phenolic substituent) | *S. aureus* | *B. subtilis* | *E. coli* | *K. pneumoniae* |
|---|---|---|---|---|---|
| **8a** | m-Fluoro | 25 | 100 | 100 | 50 |
| **8b** | m-Bromo | 100 | 50 | 100 | 50 |
| **8c** | m-Chloro | 100 | 50 | 50 | 50 |
| **8d** | m-Nitro | 50 | 50 | 50 | **6.25** |
| **8e** | p-Methoxy | 50 | 25 | 12.5 | 100 |
| **8f** | p-Methyl | 100 | 50 | 50 | 100 |
| **9a** | m-Fluoro | **12.5** | 50 | 100 | 50 |
| **9b** | m-Bromo | 25 | 100 | 50 | 100 |
| **9c** | m-Chloro | 100 | 25 | 25 | 25 |
| **9d** | m-Nitro | 100 | **6.25** | 50 | 25 |
| **9e** | p-Methoxy | 100 | 100 | 50 | 100 |
| **9f** | p-Methyl | 100 | 100 | 100 | 50 |
| **Chloramphenicol** (standard) | | 25 | 50 | 25 | 25 |

***Note: MIC values are presented in µg/mL. All tests were performed in duplicate; variation was within ±1 dilution. For K. pneumoniae, chloramphenicol MIC was determined as 25 µg/mL.***

## III. RESULTS AND DISCUSSION

Novel synthesized heterocyclic 8-(3-chloro-2-(2-hydroxy-3-nitrophenyl)-4-oxoazetidin-1-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one and 8-(4-(2-(3-bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one derivatives (twelve derivatives, 8a-f and 9a-f) were checked for their antimicrobial activity against Gram-positive bacteria *B. subtilis*, *S. aureus* and Gram-negative bacteria *E. coli*, *K. pneumoniae*.

When the synthesized derivatives were evaluated against ***S. aureus***, compound **9a** (R = m-fluoro) showed excellent activity with an MIC value of 12.5 µg/mL, and compound **9b** (R = m-bromo) showed good activity with an MIC value of 25 µg/mL.

Against ***B. subtilis***, compound **9d** (R = m-nitro) displayed superior activity with an MIC value of **6.25 µg/mL**. Compounds **8e** (R = p-methoxy) and **9c** (R = m-chloro) exhibited MIC values of 25 µg/mL. The remaining compounds reflected only satisfactory activity.

Against ***E. coli***, compound **8e** (R = p-methoxy) displayed an MIC value of 12.5 µg/mL, and compound **9c** (R = m-chloro) exhibited an MIC value of 25 µg/mL. Ten compounds against *E. coli* showed good to moderate activity.

Against ***K. pneumoniae***, compound **8d** (R = m-nitro) was found to be the most lethal, achieving an MIC value of **6.25 µg/mL** with superior quality. Compounds **9c** (R = m-chloro) and **9d** (R = m-nitro) showed MIC values of 25 µg/mL. The remaining nine derivatives exhibited moderate activity against the *K. pneumoniae* strain.

From this microbial result analysis, **meta-positioned electronegative groups** (especially nitro, fluoro, and chloro) were responsible for achieving excellent MIC values.

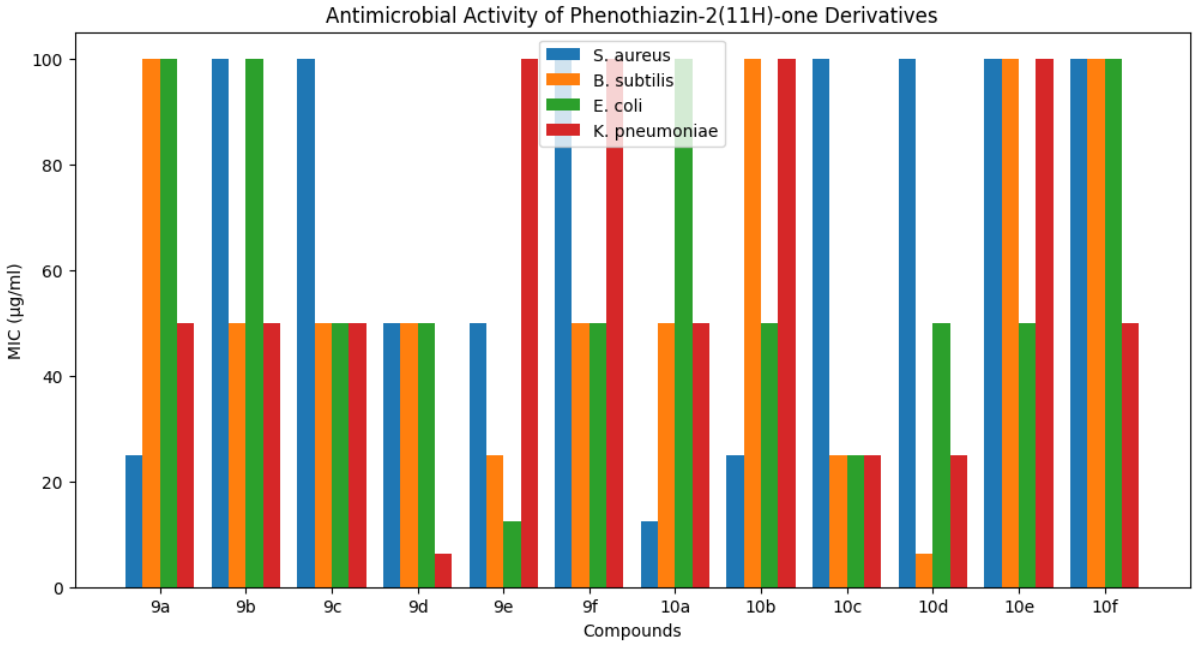



**FIGURE 1: Showing antimicrobial activity of Phenothiazine-2(11H)-one derivatives**

## IV. CONCLUSION

The current study demonstrated that the synthesized novel heterocyclic phenothiazin-2(11H)-one derivatives possessed significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains. The biological evaluation revealed that the antimicrobial activity was strongly influenced by the nature and position of substituents present on the phenolic ring. Among all the tested compounds, **nitro-substituted derivatives** displayed excellent antibacterial activity. Compound **8d** showed excellent activity against *K. pneumoniae* with an MIC value of 6.25 µg/mL, while compound **9d** displayed remarkable inhibition against *B. subtilis* with the same MIC value. Methoxy-substituted derivative **8e** showed appreciable activity against *E. coli*, whereas methyl-substituted compounds exhibited comparatively lower activity. Overall, the synthesized compounds showed comparable activity and, in some cases, superior activity to the

standard drug chloramphenicol against specific bacterial strains. It is suggested that phenothiazin-2(11H)-one derivatives represent promising scaffolds for the development of new antimicrobial agents.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

[1] Sandhu S, Bansal Y, Silakari O, Bansal G. Coumarin hybrids as novel therapeutic agents. Bioorg Med Chem. 2014;22:3806.

[2] Godge RK, Nalawade AK, Kolhe PV. Exploring the antifungal potential of 1, 2, 4-triazole derivatives: a comprehensive study on design and synthesis, Eur J Chem. 2023;4:4.

[3] Amaral L, Viveiros M, Molnar J, Kristiansen JE. Haloperidol as a known non-psychiatric drug with a potential for the management of multidrug-resistant tuberculosis. Expert Opin Drug Metab Toxicol. 2008;4:1337.

[4] Malmakova AE, Jones AM. Synthetic Routes and Bioactivity Profiles of the Phenothiazine Privileged Scaffold. Organics. 2025;6:46.

[5] Gershon S, Sakalis G, Bowers PA. Mesoridazine—a pharmacodynamic and pharmacokinetic profile. J Clin Psychiatry. 1981;42:463.

[6] Kalkanidis M, Klonis N, Tilley L, Deady LW. Novel phenothiazine antimalarials: synthesis, antimalarial activity, and inhibition of the formation of β-hematin. Biochem Pharmacol. 2002;63:833.

[7] Kaatz GW, Moudgal VV, Seo SM, Kristiansen JE. Kaatz, Glenn W., et al. "Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in Staphylococcus aureus. Antimicrob Agents Chemother. 2003;47:719.

[8] Amaral L, Viveiros M, Kristiansen JE. Amaral, Leonard, Miguel Viveiros, and Jette Elisabeth Kristiansen. "Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries. Trop Med Int Health. 2001;6:1016.

[9] Al Zahrani NA, El-Shishtawy RM, Elaasser MM, Asiri AM. Synthesis of Novel Chalcone-Based Phenothiazine Derivatives as Antioxidant and Anticancer Agents. Molecules. 2020;25:4566.

[10] Sun-Waterhouse D, Chen J, Chuah C, Wibisono R, Melton LD, Laing WA, Ferguson LR, Skinner M. Kiwifruit-based polyphenols and related antioxidants for functional foods: Kiwifruit extract-enhanced gluten-free bread. Int J Food Sci Nutr. 2009;60:251.

[11] Gwaram NS, Ali HM, Abdulla MA, Buckle MJC, Sukumaran SD, Chung LY, Othman R, Alhadi AA, Yehye WA, Hadi AHA. Synthesis, Characterization, X-ray Crystallography, Acetyl Cholinesterase Inhibition and Antioxidant Activities of Some Novel Ketone Derivatives of Gallic Hydrazide-Derived Schiff Bases. Molecules. 2012;17:2408.

[12] Gao S, Hu M. Bioavailability challenges associated with development of anti-cancer phenolics. Mini Rev Med Chem. 2010;10:550-567.

[13] Teixeira J, Silva T, Benfeito S, Gaspar A, Garrido J, Borges F. Teixeira, José, et al. "Exploring nature profits: Development of novel and potent lipophilic antioxidants based on galloyl–cinnamic hybrids. Eur J Med Chem. 2013;62:289.

[14] Liu N, Jin Z, Zhang J, Jin J. Antitumor evaluation of novel phenothiazine derivatives that inhibit migration and tubulin polymerization against gastric cancer MGC-803 cells. Invest New Drugs. 2019;37:188.

[15] Gao Y, Sun TY, Bai WF, Bai CG. Design, synthesis and evaluation of novel phenothiazine derivatives as inhibitors of breast cancer stem cells. Eur J Med Chem. 2019;183:111692.

[16] Luan Y, Liu J, Gao J, Wang J. The design and synthesis of novel phenothiazine derivatives as potential cytotoxic agents. Lett Drug Des Discov. 2020;17:57.

[17] Tlhapi D, Ramaite ID, Anokwuru CP, Van Ree T, Hoppe HC. In Vitro Studies on Antioxidant and Anti-Parasitic Activities of Compounds Isolated from *Rauvolfia caffra* Sond. Molecules. 2020;25:3781.

[18] Abramovič H, Grobin B, Ulrih NP, Cigić B. Relevance and Standardization of In vitro Antioxidant Assays: ABTS, DPPH, and Folin–Ciocalteu. J Chem. 2018;2018:1.

[19] Pai Mangalore R, Peel TN, Udy AA, Peleg AY. The clinical application of beta-lactam antibiotic therapeutic drug monitoring in the critical care setting. J Antimicrob Chemother. 2023;78:2395.

[20] Wi YM, Choi JY, Lee DE, et al. Antimicrobial activity of cephamycins and β-lactam/β-lactamase inhibitors against ESBL-producing Escherichia coli and Klebsiella pneumoniae under standard and high bacterial inocula. Sci Rep. 2025;15:9785.

[21] Neu HC. **Contribution of beta-lactamase to bacterial resistance and mechanisms to inhibit beta-lactamases.** Am J Med. 1985;79:2.

[22] Tooke CL, Hinchliffe P, Bragginton EC, et al. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J Mol Biol. 2019;431:3472.

[23] Frère JM, Page MGP. Penicillin-binding proteins: evergreen drug targets. Curr Opin Pharmacol. 2014;18:112.

[24] Pandey N, Cascella M. Beta-lactam antibiotics. StatPearls. 2023.

[25] Bush K. Past and present perspectives on β-lactamases. Antimicrob Agents Chemother. 2018;62.

[26] Livermore DM. beta-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev. 1995;8:557.

[27] Karaiskos I, Galani I, Daikos GL, Giamarellou H. Breaking Through Resistance: A Comparative Review of New Beta-Lactamase Inhibitors (Avibactam, Vaborbactam, Relebactam) Against Multidrug-Resistant Superbugs. Antibiotics. 2025;14:528.

[28] Tayeb SM, Alharbi JA, Alattas BB, Alotaibi DM, Althibaiti NM, Alharbi JF et al. Promising Future of Novel Beta-Lactam Antibiotics Against Bacterial Resistance. Drug Des Devel Ther 2025;19:9185–9197.

[29] Srivastava K, Prakash R, Singh RB, Srivastava A, Vishnoi RK. Synthesis, characterization and antibacterial evaluation of novel β-lactam and thiazolidin-4-one derivatives having thiadiazinyl ring. Bull Pharm Sci Assiut Univ. 2023;46(1):203-216.

[30] Drăgan M, Stan CD, Iacob AT, Dragostin OM, Boancă M, Lupușoru CE, Zamfir CL, Profire L. Biological evaluation of azetidine-2-one derivatives of ferulic acid as promising anti-inflammatoryagents. Processes. 2020;8(11):1-19.

[31] Drawz SM, Bonomo R. Three decades of b-lactamase inhibitors. Clin Microbiol Rev. 2010;23(1):160-201.

[32] Fedorchenko TG, Lipunova GN, Shchepochkin AV, Tsmokalyuk AN, Slepukhin PA, Chupakhin ON. Synthesis and properties of 1,3-diphenyl-5-(benzothiazol-2-yl)-6-R-verdazyls. Mendeleev Commun. 2018;28(3):297-299.

[33] Mishra CB, Shalini S, Gusain S, Prakash A, Kumari J, Kumari S, Yadav AK, Lynn AM, Tiwari M. Development of novel N-(6-methanesulfonyl-benzothiazol-2-yl)-3-(4-substituted-piperazin-1-yl)-propionamides with cholinesterase inhibition, anti-β-amyloid aggregation, neuroprotection and cognition enhancing properties for the therapy of Alzheimer's disease. RSC Adv. 2020;10(30):17602-17619.

[34] Pang B, Wang M, Liu W. Cyclization of polyketides and non-ribosomal peptides on and off their assembly lines. Nat Prod Rep. 2016;33(2):162-173.

[35] Agarwal N. Synthetic and therapeutic potential of 4-thiazolidinone and its analogs. Curr Chem Lett. 2021:119-138.

[36] Mishra I, Mishra R, Mujwar S, Chandra P, Sachan N. A retrospect on antimicrobial potential of thiazole scaffold. J Heterocyclic Chem. 2020;57:2304-2329.

[37] Vaidya A, Pathak D, Shah K. 1,3,4-oxadiazole and its derivatives, A review on recent progress in anticancer activities. Chem Biol Drug Des. 2021;97:572-591.

[38] Singh PP, Bansal S, Rawat K, Pullabhotla VSR. Synthesis of novel 1,4-bis-isoxazol/pyrazole and phenylpyrazole scaffolds by efficient green protocol and evaluation of biological properties thereof. Res J Chem Environ. 2021;25(5):48-61.