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Synthesis, Characterization and Antimicrobial Activities of Novel 8-(4-(2-(Substituted/Unsubstituted-Phenyl)-4-oxothiazolidin-3-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one and 4-Methyl-8-(2-methyl-2-(Substituted/Unsubstituted-Phenyl)-4-oxothiazolidin-3-yl)pyrano[2,3-b]phenothiazin-2(11H)-one Heterocycles Derivatives

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Abstract— *The synthesis of oxothiazolidinyl-phenothiazinone derivatives was carried out starting from the condensation of resorcinol and acetoacetic ester, which gives coumarin. Simultaneously, synthesis of cyclic thiazolidin-4-one was done by condensation of aromatic substituted/unsubstituted aldehyde-benzidine and aromatic substituted/unsubstituted aldehyde-benzene-1,4-diamine with thioglycollic acid in the presence of $ZnCl_2$ in trace amounts. Subsequently, substituted-amine reacted with coumarin in the presence of $ZnCl_2$ to yield amino-thiazolidin-4-one, and further cyclization took place. The final phenothiazinone heterocycles were synthesized in the presence of sulfur powder and iodine. The structures of the novel synthesized derivatives were established by elemental analysis, UV, FT-IR, ^{1}H-NMR, and mass spectra. The obtained derivatives exhibited excellent to moderate antibacterial and antifungal activity.*

Keywords— Acetoacetic ester, coumarin, benzidine, benzene-1,4-diamine, thioglycollic acid, aromatic substituted/unsubstituted aldehyde.

I. INTRODUCTION

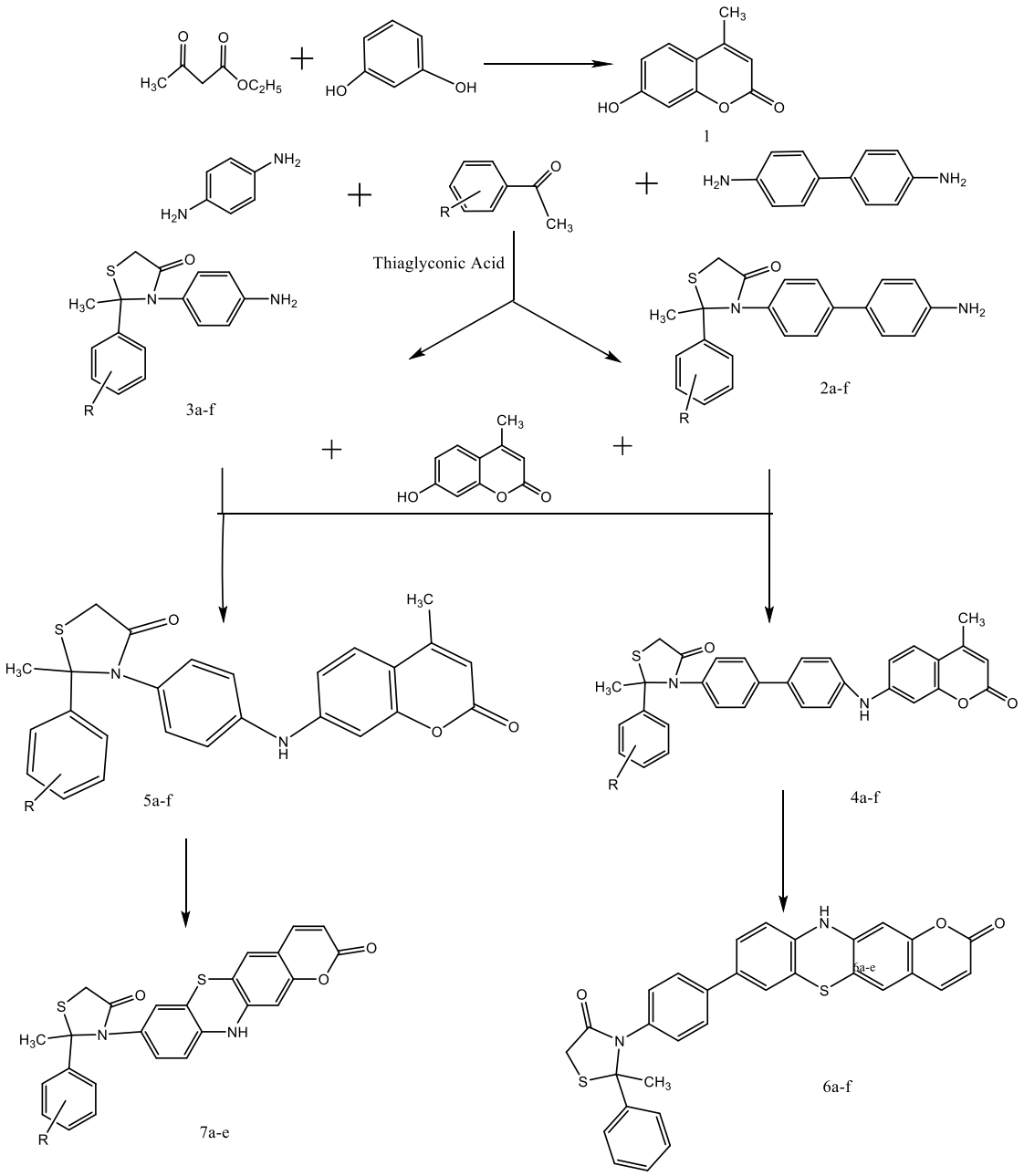
Phenothiazine is amongst the primary molecular synthons involved in the synthesis of antipsychotic drugs. It is a tricyclic compound of immense usage [1-3] in the manufacturing of emetics, antihistamines, anticancer [4-5], antifungal, anti-inflammatory, antitubercular, antioxidant, antileishmanial, and antimalarial treatments. These compounds exhibit extensive antimicrobial activities apart from their usage in psychiatric treatments. These compounds were synthesized in 1883 by Bernthsen. He was evaluating the proof of the structural studies on Lauth's Violet and methylene blue. These compounds [6-9] are attributed with optoelectrochemical and photophysical properties. Substituted phenothiazines have a desirable market in the pharmaceutical industry owing to their widespread capabilities to address a large number of chronic illnesses.

Phenothiazines work by modulating efflux pumps, acting as human cholinesterase inhibitors, characterized as multidrug-resistant reversal in many instances, and causing membrane disruption. Targeted alteration in the phenothiazine core is evaluated for the different biological properties [10-16] of the molecule. A vast body of documents highlights 10H-phenothiazine and its derivatives as playing crucial roles in biological, medicinal, and industrial applications. They are of immense value in CNS activity and thus are major parts of antipsychotic drugs. They possess two active sites at positions 2 and 10 which are hugely responsible for the various kinds of substitutions and chemical rearrangements undergone by the molecules. Chlorpromazine, which is a substituted phenothiazine, possesses extensive biological activities [17-18] against

viruses, bacteria, fungi, and other cancer cells; however, the usage is restricted because the effective antimicrobial dosage is far more than the safe clinical dose.

Thiazolidine derivatives [19-24], especially 4-oxothiazolidin-5-ylidene derivatives, have gained significant interest due to their broad spectrum of biological activities. This versatile class of compounds exhibits a broad spectrum of biological activities [25-30], including antifungal, antidiabetic, anti-inflammatory, antiviral, antiproliferative, antimicrobial [20], antioxidant, and antitumor activity. The 4-thiazolidinone core represents one of the fortunate design fragments widely utilized as a promising "building block" in modern medicinal chemistry for rational drug development. 4-Thiazolidinone-based compounds are characterized by various effective biological activities [31-36] such as anti-inflammatory, analgesic, trypanocidal, anticonvulsant, antiviral, antifungal, antibacterial, anticancer [37], etc. Considerable attention has been devoted to thiazolidinedione derivatives both from a synthetic point of view and biological applications. In this regard, thiazolidinedione derivatives [38-39] have been used as antibacterial and antifungal agents and as antiplasmodial inhibitors [40].

Since the current time demands novel pharmaceuticals to combat the ever-evolving microbes and their related diseases, we undertook the synthesis of phenothiazine-substituted compounds and the related biological efficiency.



Scheme 1

II. EXPERIMENTAL SECTION

To check the melting points of thiazol-phenothiazinone heterocycles, the open capillary method was used. The progress, purity, and structure of the prepared derivatives were determined by using precoated TLC plates (Merck, 60F-254) with iodine vapor as visualizing agent and eluent 5:2 hexane/ethyl acetate. 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) were given in parts per million. The Jasco FT-IR-470 spectrophotometer with KBr using diffuse reflectance method [38,39] was used. Mass spectra were recorded on MS-JEOL SX102 Mass spectrometer using Argon/Xenon (6 kV, 10 mA) as the FAB gas and m-nitrobenzyl alcohol as the matrix.

2.1 Synthesis of Coumarin

Equimolar amounts (0.01 mole) of resorcinol and ethyl acetoacetate were added to 20 ml conc. H_2SO_4; then the reaction was stirred for 3-4 hours. It was poured into ice added with 10 ml of pyridine when the crude product separated out, which was then recrystallized from ethanol.

Molecular formula: $C_{11}H_8O_3$, **Mol. Wt.:** 188.2, **Yield:** 72%, **m.p.:** 187-188 °C.

2.2 Synthesis of 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-(substituted/unsubstituted-phenyl)-2-methylthiazolidin-4-one (2a-f)

Equimolar amounts (0.01 mole) of substituted/unsubstituted acetophenone, benzidine, and thioglycolic acid, using $ZnCl_2$ in trace amount in 20 ml DMF, were refluxed on a heating mantle for 10-11 hours. The progress and purity of the novel heterocyclic cyclized compounds were monitored by TLC. The reaction mixture was poured into crushed ice. Solidification occurred, which was filtered off and washed with cold water. A pure sample was obtained after recrystallization from ethanol. The characterization data of the novel heterocyclic derivatives thus synthesized are given below:

2a. 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-methyl-2-phenylthiazolidin-4-one

- **Chemical Formula:** $C_{22}H_{20}N_2OS$, **m.p.:** 91-92 °C, **Molecular Weight:** 360, **Yield:** 62%
- **Elemental Analysis:** Calculated: C-73.30, N-7.77, S-8.89; Found: C-73.26, N-7.73, S-8.84
- **Infrared (ν_max per cm, KBr):** 3150 (N-H), 1625 (C=O, str. thiazol ring), 1540 (C=C skeletal), 1090 (C-S-C, thiazol ring), 3050 (Aromatic C-H str.), 1290 (C-N-C, thiazol ring)

2b. 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-(4-chlorophenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{22}H_{19}ClN_2OS$, **m.p.:** 85-86 °C, **Molecular Weight:** 395, **Yield:** 62%
- **Elemental Analysis:** Calculated: C-66.91, Cl-8.98, N-7.09, S-8.12; Found: C-66.87, Cl-8.96, N-7.06, S-8.10
- **Infrared (ν_max per cm, KBr):** 3152 (N-H), 1630 (C=O, str. thiazol ring), 1542 (C=C skeletal), 1095 (C-S-C, thiazol ring), 3055 (Aromatic C-H str.), 1292 (C-N-C, thiazol ring), 760 (C-Cl)

2c 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-(4-hydroxyphenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{22}H_{20}N_2O_2S$, **m.p.:** 102-103 °C, **Molecular Weight:** 376, **Yield:** 57%
- **Elemental Analysis:** Calculated: C-70.19, N-7.44, S-8.52; Found: C-70.16, N-7.41, S-8.48
- **Infrared (ν_max per cm, KBr):** 3155 (N-H), 1626 (C=O, str. thiazol ring), 1544 (C=C skeletal), 1097 (C-S-C, thiazol ring), 3054 (Aromatic C-H str.), 1290 (C-N-C, thiazol ring), 3607 (Ar-OH)

2d 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-(4-bromophenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{22}H_{19}BrN_2OS$, **m.p.:** 109-110 °C, **Molecular Weight:** 439, **Yield:** 65%
- **Elemental Analysis:** Calculated: C-60.14, Br-18.19, N-6.38, S-7.30; Found: C-60.11, Br-18.15, N-6.35, S-7.26
- **Infrared (ν_max per cm, KBr):** 3158 (N-H), 1620 (C=O, str. thiazol ring), 1540 (C=C skeletal), 1090 (C-S-C, thiazol ring), 3050 (Aromatic C-H str.), 1291 (C-N-C, thiazol ring), 630 (Ar-Br)

2e 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-methyl-2-(2-nitrophenyl)thiazolidin-4-one

- **Chemical Formula:** $C_{22}H_{19}N_3O_3S$, **m.p.:** 106-107 °C, **Molecular Weight:** 405, **Yield:** 68%
- **Elemental Analysis:** Calculated: C-65.17, N-10.36, S-7.91; Found: C-65.16, N-10.31, S-7.88
- **Infrared (ν_max per cm, KBr):** 3158 (N-H), 1627 (C=O, str. thiazol ring), 1540 (C=C skeletal), 1090 (C-S-C, thiazol ring), 3050 (Aromatic C-H str.), 1291 (C-N-C, thiazol ring), 1281 (-N=O, str. symmetric), 1681 (C=O, str. thiazolidinone ring), 1738 (-N=O, str. asymmetric)

2f 3-(4'-Amino-[1,1'-biphenyl]-4-yl)-2-(3-hydroxy-4-methoxyphenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{23}H_{22}N_2O_3S$, **m.p.:** 118-119 °C, **Molecular Weight:** 407, **Yield:** 62%
- **Elemental Analysis:** Calculated: C-67.96, N-6.89, S-7.89; Found: C-67.92, N-6.86, S-7.84
- **Infrared (ν_max per cm, KBr):** 3155 (N-H), 1630 (C=O, str. thiazol ring), 1545 (C=C skeletal), 1095 (C-S-C, thiazol ring), 3055 (Aromatic C-H str.), 1295 (C-N-C, thiazol ring), 1685 (C=O, str. thiazolidinone ring), 1170 (O-C, str. 4-OCH_3-phenyl), 3455 (O-H, str. 4-OH-phenyl)

2.3 Synthesis of 3-(4-Aminophenyl)-2-(substituted/unsubstituted-phenyl)-2-methylthiazolidin-4-one (3a-f)

Equimolar amounts (0.01 mole) of substituted/unsubstituted acetophenone, benzene-1,4-diamine, and thioglycollic acid, using $ZnCl_2$ in trace amount in 20 ml DMF, were refluxed on a heating mantle for 07-09 hours. The progress and purity of the novel heterocyclic cyclized compounds were monitored by TLC. The reaction mixture was poured into crushed ice. Solidification occurred, which was filtered off and washed with cold water. A pure sample was obtained after recrystallization from ethanol. The characterization data of the novel heterocyclic derivatives thus synthesized are given below:

3a 3-(4-Aminophenyl)-2-methyl-2-phenylthiazolidin-4-one

- **Chemical Formula:** $C_{16}H_{16}N_2OS$, **m.p.:** 97-98 °C, **Molecular Weight:** 284, **Yield:** 65%
- **Elemental Analysis:** Calculated: C-67.58, N-9.85, S-11.27; Found: C-67.54, N-9.81, S-11.26
- **Infrared (ν_max per cm, KBr):** 3120 (N-H), 1650 (C=O, str. thiazol ring), 1550 (C=C skeletal), 1080 (C-S-C, thiazol ring), 3015 (Aromatic C-H str.), 1280 (C-N-C, thiazol ring)

3b 3-(4-Aminophenyl)-2-(4-chlorophenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{16}H_{15}ClN_2OS$, **m.p.:** 130-131 °C, **Molecular Weight:** 318.5, **Yield:** 70%
- **Elemental Analysis:** Calculated: C-60.28, Cl-11.14, N-8.79, S-10.06; Found: C-60.25, Cl-11.10, N-8.76, S-10.03
- **Infrared (ν_max per cm, KBr):** 3150 (N-H), 1634 (C=O, str. thiazol ring), 1540 (C=C skeletal), 1090 (C-S-C, thiazol ring), 3050 (Aromatic C-H str.), 1295 (C-N-C, thiazol ring), 680 (C-Cl)

3c 3-(4-Aminophenyl)-2-(4-hydroxyphenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{16}H_{16}N_2O_2S$, **m.p.:** 104-105 °C, **Molecular Weight:** 300, **Yield:** 65%
- **Elemental Analysis:** Calculated: C-63.98, N-9.33, S-10.67; Found: C-63.95, N-9.32, S-10.64
- **Infrared (ν_max per cm, KBr):** 3158 (N-H), 1630 (C=O, str. thiazol ring), 1540 (C=C skeletal), 1095 (C-S-C, thiazol ring), 3050 (Aromatic C-H str.), 1295 (C-N-C, thiazol ring), 3620 (Ar-OH)

3d 3-(4-Aminophenyl)-2-(4-bromophenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{16}H_{15}BrN_2OS$, **m.p.:** 115-116 °C, **Molecular Weight:** 363, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-52.90, Br-22.00, N-7.71, S-8.83; Found: C-52.87, Br-21.96, N-7.68, S-8.80
- **Infrared (ν_max per cm, KBr):** 3160 (N-H), 1625 (C=O, str. thiazol ring), 1542 (C=C skeletal), 1098 (C-S-C, thiazol ring), 3055 (Aromatic C-H str.), 1285 (C-N-C, thiazol ring), 710 (Ar-Br)

3e 3-(4-Aminophenyl)-2-methyl-2-(2-nitrophenyl)thiazolidin-4-one

- **Chemical Formula:** $C_{16}H_{15}N_3O_3S$, **m.p.:** 102-103 °C, **Molecular Weight:** 329, **Yield:** 70%
- **Elemental Analysis:** Calculated: C-58.35, N-12.76, S-9.73; Found: C-58.32, N-12.72, S-9.70
- **Infrared (ν_max per cm, KBr):** 3156 (N-H), 1625 (C=O, str. thiazol ring), 1542 (C=C skeletal), 1095 (C-S-C, thiazol ring), 3045 (Aromatic C-H str.), 1288 (C-N-C, thiazol ring), 1270 (-N=O, str. symmetric), 1690 (C=O, str. thiazolidinone ring), 1730 (-N=O, str. asymmetric)

3f 3-(4-Aminophenyl)-2-(3-hydroxy-4-methoxyphenyl)-2-methylthiazolidin-4-one

- **Chemical Formula:** $C_{17}H_{18}N_2O_3S$, **m.p.:** 106-107 °C, **Molecular Weight:** 330, **Yield:** 55%
- **Elemental Analysis:** Calculated: C-61.80, N-8.48, S-9.70; Found: C-61.75, N-8.45, S-9.66
- **Infrared (ν_max per cm, KBr):** 3160 (N-H), 1632 (C=O, str. thiazol ring), 1541 (C=C skeletal), 1098 (C-S-C, thiazol ring), 3057 (Aromatic C-H str.), 1290 (C-N-C, thiazol ring), 1680 (C=O, str. thiazolidinone ring), 1174 (O-C, str. 4-OCH_3-phenyl), 3450 (O-H, str. 4-OH-phenyl)

2.4 Synthesis of 2-(Substituted/Unsubstituted-Phenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one (4a-f)

Equimolar quantities (0.02 mole) of reactants 3-(4'-amino-[1,1'-biphenyl]-4-yl)-2-(substituted/unsubstituted-phenyl)-2-methylthiazolidin-4-one (2a-f) and coumarin in 25 ml absolute ethanol were refluxed on a heating mantle in the presence of anhydrous $ZnCl_2$ for 05-06 hours. The mixture was cooled and the separated solid mass was filtered, washed with cold water, and recrystallized from ethanol. The characterization data of the novel heterocyclic derivatives thus synthesized are given below:

4a 2-Methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-2-phenylthiazolidin-4-one

- **Chemical Formula:** $C_{32}H_{26}N_2O_3S$, **m.p.:** 82-83 °C, **Molecular Weight:** 519, **Yield:** 65%
- **Elemental Analysis:** Calculated: C-74.11, N-5.40, S-6.18; Found: C-74.08, N-5.36, S-6.14
- **Infrared (ν_max per cm, KBr):** 3130 (N-H), 1640 (C=O, str. thiazol ring), 1560 (C=C skeletal), 1070 (C-S-C, thiazol ring), 3060 (Aromatic C-H str.), 1230 (C-N-C, thiazol ring), 1020 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.82-7.71 (m, 17H, aromatic), 3.62 (s, 3H, methyl), 4.12 (s, 3H, methyl), 3.82 (dd, 2H, O=C-CH_2-S-), 6.11 (s, 1H, -N-CH-S-)

4b 2-(4-Chlorophenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one

- **Chemical Formula:** $C_{32}H_{25}ClN_2O_3S$, **m.p.:** 123-124 °C, **Molecular Weight:** 553, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-69.49, Cl-6.41, N-5.07, S-5.80; Found: C-69.44, Cl-6.38, N-5.03, S-5.77
- **Infrared (ν_max per cm, KBr):** 3132 (N-H), 640 (C-Cl), 1645 (C=O, str. thiazol ring), 1562 (C=C skeletal), 1075 (C-S-C, thiazol ring), 3063 (Aromatic C-H str.), 1231 (C-N-C, thiazol ring), 1025 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.80-7.67 (m, 17H, aromatic), 3.64 (s, 3H, methyl), 4.14 (s, 3H, methyl), 3.80 (dd, 2H, O=C-CH_2-S-), 6.12 (s, 1H, -N-CH-S-)

4c 2-(4-Hydroxyphenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one

- **Chemical Formula:** $C_{32}H_{26}N_2O_4S$, **m.p.:** 114-115 °C, **Molecular Weight:** 535, **Yield:** 55%
- **Elemental Analysis:** Calculated: C-71.89, N-5.24, S-6.00; Found: C-71.86, N-5.20, S-5.96
- **Infrared (ν_max per cm, KBr):** 3130 (N-H), 3540 (Ar-OH), 1642 (C=O, str. thiazol ring), 1560 (C=C skeletal), 1072 (C-S-C, thiazol ring), 3060 (Aromatic C-H str.), 1230 (C-N-C, thiazol ring), 1030 (C-N)

- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.80-7.64 (m, 17H, aromatic), 3.66 (s, 3H, methyl), 4.15 (s, 3H, methyl), 3.83 (dd, 2H, O=C-CH_2-S-), 6.11 (s, 1H, -N-CH-S-), 5.2 (brs, 1H, exchangeable -OH)

4d **2-(4-Bromophenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one**

- **Chemical Formula:** $C_{32}H_{25}BrN_2O_3S$, **m.p.:** 103-104 °C, **Molecular Weight:** 598, **Yield:** 45%
- **Elemental Analysis:** Calculated: C-64.32, Br-13.37, N-4.69, S-5.37; Found: C-64.29, Br-13.33, N-4.65, S-5.34
- **Infrared (ν_max per cm, KBr):** 3135 (N-H), 720 (C-Br), 1645 (C=O, str. thiazol ring), 1565 (C=C skeletal), 1075 (C-S-C, thiazol ring), 3065 (Aromatic C-H str.), 1234 (C-N-C, thiazol ring), 1032 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.84-7.58 (m, 17H, aromatic), 3.61 (s, 3H, methyl), 4.10 (s, 3H, methyl), 3.81 (dd, 2H, O=C-CH_2-S-), 6.12 (s, 1H, -N-CH-S-)

4e **2-Methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)-2-(4-nitrophenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{32}H_{25}N_3O_5S$, **m.p.:** 116-117 °C, **Molecular Weight:** 564, **Yield:** 45%
- **Elemental Analysis:** Calculated: C-68.19, N-7.46, S-5.69; Found: C-68.15, N-7.42, S-5.66
- **Infrared (ν_max per cm, KBr):** 3133 (N-H), 1648 (C=O, str. thiazol ring), 1562 (C=C skeletal), 1070 (C-S-C, thiazol ring), 3067 (Aromatic C-H str.), 1235 (C-N-C, thiazol ring), 1038 (C-N), 1260 (-N=O, str. symmetric), 1762 (-N=O, str. asymmetric)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.84-7.53 (m, 17H, aromatic), 3.64 (s, 3H, methyl), 4.12 (s, 3H, methyl), 3.84 (dd, 2H, O=C-CH_2-S-), 6.10 (s, 1H, -N-CH-S-)

4f **2-(3-Hydroxy-4-methoxyphenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one**

- **Chemical Formula:** $C_{33}H_{28}N_2O_5S$, **m.p.:** 97-98 °C, **Molecular Weight:** 565, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-70.20, N-4.96, S-5.68; Found: C-70.17, N-4.92, S-5.67
- **Infrared (ν_max per cm, KBr):** 3140 (N-H), 1650 (C=O, str. thiazol ring), 1555 (C=C skeletal), 1075 (C-S-C, thiazol ring), 3070 (Aromatic C-H str.), 1240 (C-N-C, thiazol ring)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.81-7.57 (m, 17H, aromatic), 3.62 (s, 3H, methyl), 4.14 (s, 3H, methyl), 3.82 (dd, 2H, O=C-CH_2-S-), 6.12 (s, 1H, -N-CH-S-), 3.55 (s, 3H, Ar-OCH_3)

2.5 Synthesis of 2-(Substituted/Unsubstituted-Phenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one (5a-f)

Equimolar quantities (0.02 mole) of reactants 3-(4-aminophenyl)-2-(substituted/unsubstituted-phenyl)-2-methylthiazolidin-4-one (3a-f) and coumarin in 25 ml absolute ethanol were refluxed on a heating mantle in the presence of anhydrous $ZnCl_2$ for 06-07 hours. The mixture was cooled and the separated solid mass was filtered, washed with cold water, and recrystallized from ethanol. The characterization data of the novel heterocyclic derivatives thus synthesized are given below:

5a **2-Methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-2-phenylthiazolidin-4-one**

- **Chemical Formula:** $C_{26}H_{22}N_2O_3S$, **m.p.:** 131-132 °C, **Molecular Weight:** 443, **Yield:** 52%
- **Elemental Analysis:** Calculated: C-70.57, N-6.33, S-7.24; Found: C-70.54, N-6.30, S-7.21
- **Infrared (ν_max per cm, KBr):** 3150 (N-H), 1670 (C=O, str. thiazol ring), 1580 (C=C skeletal), 980 (C-S-C, thiazol ring), 3065 (Aromatic C-H str.), 1220 (C-N-C, thiazol ring), 1010 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.74-7.66 (m, 13H, aromatic), 3.52 (s, 3H, methyl), 4.14 (s, 3H, methyl), 3.77 (dd, 2H, O=C-CH_2-S-), 6.15 (s, 1H, -N-H)

5b **2-(4-Chlorophenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{26}H_{21}ClN_2O_3S$, **m.p.:** 128-129 °C, **Molecular Weight:** 477, **Yield:** 58%
- **Elemental Analysis:** Calculated: C-65.47, Cl-7.44, N-5.87, S-6.72; Found: C-65.44, Cl-7.41, N-5.84, S-6.69
- **Infrared (ν_max per cm, KBr):** 3155 (N-H), 1672 (C=O, str. thiazol ring), 1581 (C=C skeletal), 650 (C-Cl), 982 (C-S-C, thiazol ring), 3062 (Aromatic C-H str.), 1225 (C-N-C, thiazol ring), 1012 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.74-7.63 (m, 13H, aromatic), 3.54 (s, 3H, methyl), 4.15 (s, 3H, methyl), 3.78 (dd, 2H, O=C-CH_2-S-), 6.12 (s, 1H, -N-CH-S-)

5c **2-(4-Hydroxyphenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{26}H_{22}N_2O_4S$, **m.p.:** 106-107 °C, **Molecular Weight:** 459, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-68.11, N-6.11, S-6.99; Found: C-68.08, N-6.07, S-6.96
- **Infrared (ν_max per cm, KBr):** 3156 (N-H), 1674 (C=O, str. thiazol ring), 1585 (C=C skeletal), 3495 (Ar-OH), 988 (C-S-C, thiazol ring), 3060 (Aromatic C-H str.), 1230 (C-N-C, thiazol ring), 1015 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.76-7.68 (m, 13H, aromatic), 3.56 (s, 3H, methyl), 4.17 (s, 3H, methyl), 3.75 (dd, 2H, O=C-CH_2-S-), 6.14 (s, 1H, -N-CH-S-), 5.8 (brs, 1H, exchangeable -OH)

5d **2-(4-Bromophenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{26}H_{21}BrN_2O_3S$, **m.p.:** 101-102 °C, **Molecular Weight:** 521, **Yield:** 68%
- **Elemental Analysis:** Calculated: C-59.89, Br-15.32, N-5.37, S-6.15; Found: C-59.86, Br-15.28, N-5.34, S-6.11
- **Infrared (ν_max per cm, KBr):** 3152 (N-H), 1678 (C=O, str. thiazol ring), 1581 (C=C skeletal), 830 (Ar-Br), 982 (C-S-C, thiazol ring), 3065 (Aromatic C-H str.), 1232 (C-N-C, thiazol ring), 1012 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.76-7.63 (m, 13H, aromatic), 3.52 (s, 3H, methyl), 4.19 (s, 3H, methyl), 3.73 (dd, 2H, O=C-CH_2-S-), 6.16 (s, 1H, -N-CH-S-)

5e **2-Methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)-2-(4-nitrophenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{26}H_{21}N_3O_5S$, **m.p.:** 122-123 °C, **Molecular Weight:** 488, **Yield:** 70%
- **Elemental Analysis:** Calculated: C-64.05, N-8.62, S-6.58; Found: C-64.03, N-8.58, S-6.55
- **Infrared (ν_max per cm, KBr):** 3150 (N-H), 1672 (C=O, str. thiazol ring), 1260 (-N=O, str. symmetric), 1730 (-N=O, str. asymmetric), 1575 (C=C skeletal), 980 (C-S-C, thiazol ring), 3068 (Aromatic C-H str.), 1236 (C-N-C, thiazol ring), 1020 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.78-7.63 (m, 13H, aromatic), 3.53 (s, 3H, methyl), 4.12 (s, 3H, methyl), 3.71 (dd, 2H, O=C-CH_2-S-), 6.12 (s, 1H, -N-CH-S-)

5f **2-(3-Hydroxy-4-methoxyphenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one**

- **Chemical Formula:** $C_{27}H_{24}N_2O_5S$, **m.p.:** 117-118 °C, **Molecular Weight:** 488, **Yield:** 55%
- **Elemental Analysis:** Calculated: C-66.38, N-5.73, S-6.56; Found: C-66.35, N-5.70, S-6.53
- **Infrared (ν_max per cm, KBr):** 3158 (N-H), 1670 (C=O, str. thiazol ring), 1570 (C=C skeletal), 3492 (Ar-OH), 985 (C-S-C, thiazol ring), 3060 (Aromatic C-H str.), 1240 (C-N-C, thiazol ring), 1015 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.72-7.61 (m, 12H, aromatic), 3.50 (s, 3H, methyl), 4.15 (s, 3H, methyl), 3.73 (dd, 2H, O=C-CH_2-S-), 6.14 (s, 1H, -N-CH-S-), 3.62 (s, 3H, Ar-OCH_3), 5.8 (brs, 1H, exchangeable -OH)

2.6 Synthesis of 8-(4-(2-(Substituted/Unsubstituted-Phenyl)-4-oxothiazolidin-3-yl)phenyl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one (6a-f)

Equimolar quantities (0.02 mole) of reactants 2-(substituted/unsubstituted-phenyl)-2-methyl-3-(4'-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-[1,1'-biphenyl]-4-yl)thiazolidin-4-one (4a-f) and sulfur powder (1.0 g) were heated at 160-170 °C for 04-05 hours in the presence of iodine (2.0 g). The reaction was cooled to room temperature, treated with dilute HCl to remove unreacted amine, and washed with water. The solid obtained was dried in vacuum to yield novel thiazolidin-phenothiazine derivatives. The characterization data of the novel heterocyclic thiazol-phenothiazine derivatives thus synthesized are given below:

6a 8-(4-(2-Methyl-4-oxo-2-phenylthiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{31}H_{22}N_2O_3S_2$, **m.p.:** 103-104 °C, **Molecular Weight:** 535, **Yield:** 52%
- **Elemental Analysis:** Calculated: C-69.64, N-5.24, S-11.99; Found: C-69.60, N-5.22, S-11.96
- **Infrared (ν_max per cm, KBr):** 3090 (N-H), 1670 (C=O, str. thiazol ring), 1595 (C=C skeletal), 970 (C-S-C, thiazol ring), 3090 (Aromatic C-H str.), 1260 (C-N-C, thiazol ring), 1020 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.81-7.64 (m, 13H, aromatic), 3.25 (s, 3H, methyl), 4.61 (s, 3H, methyl), 3.36 (dd, 2H, O=C-CH_2-S-), 4.77 (s, 1H, -N-H phenothiazine ring)

6b 8-(4-(2-(4-Chlorophenyl)-2-methyl-4-oxothiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{31}H_{21}ClN_2O_3S_2$, **m.p.:** 83-84 °C, **Molecular Weight:** 569, **Yield:** 63%
- **Elemental Analysis:** Calculated: C-65.43, Cl-6.23, N-4.92, S-11.27; Found: C-65.41, Cl-6.20, N-4.90, S-11.24
- **Infrared (ν_max per cm, KBr):** 3095 (N-H), 1672 (C=O, str. thiazol ring), 660 (Ar-Cl), 1598 (C=C skeletal), 975 (C-S-C, thiazol ring), 3095 (Aromatic C-H str.), 1265 (C-N-C, thiazol ring), 1028 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.81-7.61 (m, 13H, aromatic), 3.27 (s, 3H, methyl), 4.66 (s, 3H, methyl), 3.34 (dd, 2H, O=C-CH_2-S-), 4.72 (s, 1H, -N-H phenothiazine ring)

6c 8-(4-(2-(4-Hydroxyphenyl)-2-methyl-4-oxothiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{31}H_{22}N_2O_4S_2$, **m.p.:** 114-115 °C, **Molecular Weight:** 551, **Yield:** 63%
- **Elemental Analysis:** Calculated: C-67.62, N-5.09, S-11.64; Found: C-67.58, N-5.06, S-11.60
- **Infrared (ν_max per cm, KBr):** 3085 (N-H), 1692 (C=O, str. thiazol ring), 3435 (Ar-OH), 1598 (C=C skeletal), 972 (C-S-C, thiazol ring), 3095 (Aromatic C-H str.), 1268 (C-N-C, thiazol ring), 1030 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.81-7.55 (m, 13H, aromatic), 3.24 (s, 3H, methyl), 4.64 (s, 3H, methyl), 3.36 (dd, 2H, O=C-CH_2-S-), 4.74 (s, 1H, -N-H phenothiazine ring), 5.8 (brs, 1H, exchangeable -OH)

6d 8-(4-(2-(4-Bromophenyl)-2-methyl-4-oxothiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{31}H_{21}BrN_2O_3S_2$, **m.p.:** 122-123 °C, **Molecular Weight:** 614, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-60.69, Br-13.02, N-4.57, S-10.45; Found: C-60.66, Br-13.01, N-4.54, S-10.42
- **Infrared (ν_max per cm, KBr):** 3082 (N-H), 1693 (C=O, str. thiazol ring), 715 (Ar-Br), 1590 (C=C skeletal), 975 (C-S-C, thiazol ring), 3096 (Aromatic C-H str.), 1265 (C-N-C, thiazol ring), 1035 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.81-7.58 (m, 13H, aromatic), 3.26 (s, 3H, methyl), 4.67 (s, 3H, methyl), 3.32 (dd, 2H, O=C-CH_2-S-), 4.72 (s, 1H, -N-H phenothiazine ring)

6e 8-(4-(2-Methyl-2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{31}H_{21}N_3O_5S_2$, **m.p.:** 107-108 °C, **Molecular Weight:** 580, **Yield:** 65%
- **Elemental Analysis:** Calculated: C-64.24, N-7.25, S-11.06; Found: C-64.21, N-7.21, S-11.02

- **Infrared (ν_max per cm, KBr):** 3085 (N-H), 1680 (C=O, str. thiazol ring), 1230 (-N=O, str. symmetric), 1770 (-N=O, str. asymmetric), 1592 (C=C skeletal), 988 (C-S-C, thiazol ring), 3077 (Aromatic C-H str.), 1255 (C-N-C, thiazol ring), 1045 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.71-7.68 (m, 12H, aromatic), 3.30 (s, 3H, methyl), 4.28 (s, 3H, methyl), 3.71 (dd, 2H, O=C-CH_2-S-), 4.87 (s, 1H, -N-H phenothiazine ring)

6f 8-(4-(2-(3-Hydroxy-4-methoxyphenyl)-2-methyl-4-oxothiazolidin-3-yl)phenyl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{32}H_{24}N_2O_5S_2$, **m.p.:** 92-93 °C, **Molecular Weight:** 581, **Yield:** 72%
- **Elemental Analysis:** Calculated: C-66.19, N-4.82, S-11.04; Found: C-66.16, N-4.78, S-11.01
- **Infrared (ν_max per cm, KBr):** 3090 (N-H), 1672 (C=O, str. thiazol ring), 1590 (C=C skeletal), 985 (C-S-C, thiazol ring), 3080 (Aromatic C-H str.), 1260 (C-N-C, thiazol ring), 1050 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.72-7.64 (m, 10H, aromatic), 3.40 (s, 3H, methyl), 4.24 (s, 3H, methyl), 3.74 (dd, 2H, O=C-CH_2-S-), 4.82 (s, 1H, -N-H phenothiazine ring), 3.52 (s, 3H, Ar-OCH_3), 5.21 (brs, 1H, exchangeable -OH)

2.7 Synthesis of 8-(2-(Substituted/Unsubstituted-Phenyl)-2-methyl-4-oxothiazolidin-3-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one (7a-f)

Equimolar quantities (0.02 mole) of reactants 2-(substituted/unsubstituted-phenyl)-2-methyl-3-(4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)phenyl)thiazolidin-4-one (5a-f) and sulfur powder (1.0 g) were heated at 160-170 °C for 03-04 hours in the presence of iodine (2.0 g). The reaction was cooled to room temperature, treated with dilute HCl to remove unreacted amine, and washed with water. The solid obtained was dried in vacuum to yield novel thiazolidin-phenothiazine derivatives. The characterization data of the novel heterocyclic thiazol-phenothiazine derivatives thus synthesized are given below:

7a 4-Methyl-8-(2-methyl-4-oxo-2-phenylthiazolidin-3-yl)pyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{26}H_{20}N_2O_3S_2$, **m.p.:** 103-104 °C, **Molecular Weight:** 473, **Yield:** 48%
- **Elemental Analysis:** Calculated: C-66.08, N-5.93, S-13.57; Found: C-66.05, N-5.90, S-13.54
- **Infrared (ν_max per cm, KBr):** 3095 (N-H), 1660 (C=O, str. thiazol ring), 1590 (C=C skeletal), 985 (C-S-C, thiazol ring), 3080 (Aromatic C-H str.), 1240 (C-N-C, thiazol ring), 1030 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.71-7.72 (m, 11H, aromatic), 3.38 (s, 3H, methyl), 4.22 (s, 3H, methyl), 3.72 (dd, 2H, O=C-CH_2-S-), 4.84 (s, 1H, -N-H phenothiazine ring)

7b 8-(2-(4-Chlorophenyl)-2-methyl-4-oxothiazolidin-3-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{26}H_{19}ClN_2O_3S_2$, **m.p.:** 97-98 °C, **Molecular Weight:** 507, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-61.59, Cl-6.99, N-5.53, S-12.65; Found: C-61.54, Cl-6.96, N-5.48, S-12.61
- **Infrared (ν_max per cm, KBr):** 3091 (N-H), 1665 (C=O, str. thiazol ring), 640 (C-Cl), 1595 (C=C skeletal), 988 (C-S-C, thiazol ring), 3075 (Aromatic C-H str.), 1245 (C-N-C, thiazol ring), 1032 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.71-7.68 (m, 11H, aromatic), 3.34 (s, 3H, methyl), 4.25 (s, 3H, methyl), 3.74 (dd, 2H, O=C-CH_2-S-), 4.81 (s, 1H, -N-H phenothiazine ring)

7c 8-(2-(4-Hydroxyphenyl)-2-methyl-4-oxothiazolidin-3-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one

- **Chemical Formula:** $C_{26}H_{20}N_2O_4S_2$, **m.p.:** 103-104 °C, **Molecular Weight:** 489, **Yield:** 64%
- **Elemental Analysis:** Calculated: C-63.92, N-5.73, S-13.12; Found: C-63.88, N-5.71, S-13.10
- **Infrared (ν_max per cm, KBr):** 3091 (N-H), 1668 (C=O, str. thiazol ring), 3480 (Ar-OH), 1592 (C=C skeletal), 980 (C-S-C, thiazol ring), 3078 (Aromatic C-H str.), 1240 (C-N-C, thiazol ring), 1040 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.71-7.64 (m, 11H, aromatic), 3.37 (s, 3H, methyl), 4.21 (s, 3H, methyl), 3.72 (dd, 2H, O=C-CH_2-S-), 4.84 (s, 1H, -N-H phenothiazine ring), 5.3 (brs, 1H, exchangeable -OH)

7d **8-(2-(4-Bromophenyl)-2-methyl-4-oxothiazolidin-3-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one**

- **Chemical Formula:** $C_{26}H_{19}BrN_2O_3S_2$, **m.p.:** 115-116 °C, **Molecular Weight:** 551, **Yield:** 55%
- **Elemental Analysis:** Calculated: C-56.63, Br-14.49, N-5.08, S-11.63; Found: C-56.58, Br-14.47, N-5.06, S-11.60
- **Infrared (ν_max per cm, KBr):** 3095 (N-H), 1670 (C=O, str. thiazol ring), 860 (Ar-Br), 1590 (C=C skeletal), 986 (C-S-C, thiazol ring), 3075 (Aromatic C-H str.), 1245 (C-N-C, thiazol ring), 1048 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.70-7.61 (m, 11H, aromatic), 3.33 (s, 3H, methyl), 4.24 (s, 3H, methyl), 3.76 (dd, 2H, O=C-CH_2-S-), 4.83 (s, 1H, -N-H phenothiazine ring)

7e **4-Methyl-8-(2-methyl-2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)pyrano[2,3-b]phenothiazin-2(11H)-one**

- **Chemical Formula:** $C_{26}H_{19}N_3O_5S_2$, **m.p.:** 118-119 °C, **Molecular Weight:** 518, **Yield:** 48%
- **Elemental Analysis:** Calculated: C-60.34, N-8.12, S-12.39; Found: C-60.30, N-8.10, S-12.36
- **Infrared (ν_max per cm, KBr):** 3092 (N-H), 1676 (C=O, str. thiazol ring), 1268 (-N=O, str. symmetric), 1740 (-N=O, str. asymmetric), 1592 (C=C skeletal), 985 (C-S-C, thiazol ring), 3070 (Aromatic C-H str.), 1250 (C-N-C, thiazol ring), 1042 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.72-7.63 (m, 12H, aromatic), 3.36 (s, 3H, methyl), 4.25 (s, 3H, methyl), 3.72 (dd, 2H, O=C-CH_2-S-), 4.84 (s, 1H, -N-H phenothiazine ring)

7f **8-(2-(3-Hydroxy-4-methoxyphenyl)-2-methyl-4-oxothiazolidin-3-yl)-4-methylpyrano[2,3-b]phenothiazin-2(11H)-one**

- **Chemical Formula:** $C_{27}H_{22}N_2O_5S_2$, **m.p.:** 102-103 °C, **Molecular Weight:** 519, **Yield:** 60%
- **Elemental Analysis:** Calculated: C-62.53, N-5.40, S-12.36; Found: C-62.50, N-5.37, S-12.32
- **Infrared (ν_max per cm, KBr):** 3095 (N-H), 1670 (C=O, str. thiazol ring), 1585 (C=C skeletal), 982 (C-S-C, thiazol ring), 3075 (Aromatic C-H str.), 1255 (C-N-C, thiazol ring), 1040 (C-N)
- **^{1}H NMR ($CDCl_3$) (δ ppm):** 6.70-7.67 (m, 10H, aromatic), 3.36 (s, 3H, methyl), 4.28 (s, 3H, methyl), 3.71 (dd, 2H, O=C-CH_2-S-), 4.82 (s, 1H, -N-H phenothiazine ring), 3.54 (s, 3H, Ar-OCH_3), 5.23 (brs, 1H, exchangeable -OH).

III. ANTIMICROBIAL ACTIVITY

TABLE 1
ANTIBACTERIAL ACTIVITY OF SYNTHESIZED PYRANO[2,3-b]PHENOTHIAZIN-2(11H)-ONE (6a-f and 7a-f) COMPOUNDS AGAINST GRAM-POSITIVE, GRAM-NEGATIVE BACTERIA AND FUNGI

S.No.	R-phenyl	MIC (µg/ml) against Gram +ve Bacteria	MIC (µg/ml) against Gram -ve Bacteria		MIC (µg/ml) against Fungus	
		B. subtilis	*E. coli*	*K. pneum*	*C. albicans*	*A. niger*
6a	H	100	50	50	100	50
6b	p-Cl-phenyl	100	50	25	100	25
6c	p-OH-phenyl	12.5	100	25	25	50
6d	p-Br-phenyl	100	25	100	25	100
6e	p-NO_2-phenyl	25	6.25	100	50	25
6f	3-OH,4-OCH_3-phenyl	50	100	50	50	12.5
7a	H	100	100	12.5	100	100
7b	p-Cl-phenyl	50	25	25	100	50
7c	p-OH-phenyl	50	25	100	12.5	50
7d	p-Br-phenyl	50	6.25	100	6.25	25
7e	p-NO_2-phenyl	100	50	25	100	50
7f	3-OH,4-OCH_3-phenyl	25	50	50	100	50
	Ciprofloxacin	50	50	50	-	-
	Clotrimazole	-	-	-	50	50

IV. In Vitro Anti-Bacterial Susceptibility Test

The newly designed pyrano[2,3-b]phenothiazin-2(11H)-one heterocyclic derivatives were screened for their antibacterial and antifungal activity against various bacterial species viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and fungi *Candida albicans*, *Aspergillus niger* in vitro. The test bacterial species' pure isolates were acquired from KGMU Lucknow's Department of Microbiology. The working strains' identities were verified using a documented process that included colony morphology and gram staining. The culture was prepared by mixing 20 ml of plain Luria-Bertani medium with 1 ml of anti-bacterial growth broth in order to check the microbial activity. Six separate sterile tubes were filled with 1.0 ml of the culture from the prepared mixture, while one sterile tube held 1.8 ml. The tube holding 1.8 ml of culture was then inoculated with 0.2 ml of sample solution (6a-f and 7a-f) in ethanol (1 mg/ml). One milliliter of the culture was taken from this tube and transferred into the second tube. The concentration of the sample was then halved in each subsequent tube by taking 1 ml of the culture from the second tube and transferring it into the third. Finally, one control tube using ciprofloxacin was prepared. Every prepared tube sample was incubated at 37 °C for a full day. After 24 hours, each conical tube's bacterial growth was assessed by measuring the absorbance value at 600 nm. The MIC (related to the decrease in optical density) of the specific derivative was determined by plotting the compound concentration and absorbance value. The MIC values of these derivatives were found between 6.25 to 100 µg/ml.

V. Antibacterial and Antifungal Activity Analysis of Synthesized Pyrano[2,3-b]phenothiazin-2(11H)-one Derivatives (6a-f and 7a-f)

The antimicrobial activity of 6a-f and 7a-f was tested against *B. subtilis*, *E. coli*, *K. pneumoniae*, and fungi *C. albicans* and *A. niger* using MIC values. Lower MIC values indicate higher antimicrobial potency.

- **Against *B. subtilis*:** Derivative 6c (p-OH-phenyl) exhibited the highest activity with MIC 12.5 µg/ml, while 6e (p-NO_2-phenyl) and 7f (3-OH,4-OCH_3-phenyl) derivatives showed good activity with MIC values of 25 µg/ml. The remaining derivatives displayed moderate to weak activity (50-100 µg/ml).
- **Against *E. coli*:** Derivatives 6e (p-NO_2-phenyl) and 7d (p-Br-phenyl) were the most potent with MIC values of 6.25 µg/ml, showing excellent antibacterial activity. The derivatives 6d, 7b, and 7c also demonstrated significant activity (MIC = 25 µg/ml). Hydroxyl- and halogen-substituted derivatives generally showed enhanced activity against *E. coli*.
- **Against *K. pneumoniae*:** Derivative 7a (R=H) showed the highest activity with an MIC of 12.5 µg/ml. Derivatives 6b, 6c, 7b, and 7e exhibited good activity with MIC values of 25 µg/ml. Bromo-substituted derivatives (6d and 7d) were less effective against this organism.
- **Antifungal Activity Against *Candida albicans*:** Derivatives 7c (p-OH-phenyl) and 7d (p-Br-phenyl) showed excellent antifungal activity with MIC values of 12.5 µg/ml and 6.25 µg/ml respectively. Derivatives 6c and 6d also exhibited strong activity (MIC = 25 µg/ml). These activities are comparable or superior to the reference drug clotrimazole (MIC = 50 µg/ml).
- **Against *Aspergillus niger*:** Derivative 6f (3-OH,4-OCH_3-phenyl) was the most active derivative with an MIC of 12.5 µg/ml. Derivatives 6b, 6e, and 7d showed good activity (MIC = 25 µg/ml).

VI. Conclusion

The synthesized pyrano[2,3-b]phenothiazin-2(11H)-one derivatives exhibited significant antimicrobial activity. The derivatives 6e (p-NO_2-phenyl) and 7d (p-Br-phenyl) were the most potent antibacterial agents against *E. coli* with MIC = 6.25 µg/ml, whereas 7c (p-OH-phenyl) and 7d (p-Br-phenyl) exhibited excellent antifungal activity against *C. albicans* (MIC = 12.5 µg/ml and 6.25 µg/ml respectively). Compound 6f (3-OH,4-OCH_3-phenyl) was the most active derivative against *A. niger* (MIC = 12.5 µg/ml). These results indicate that incorporation of OH, NO_2, Br, and OCH_3 substituents on the phenyl ring substantially influences antimicrobial potency and provides valuable leads for the development of new antimicrobial agents.

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Conflict of Interest

The authors declare no conflict of interest.

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