

# IJOER

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# ENGINEERING JOURNAL

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## Preface

We would like to present, with great pleasure, the volume-12, Issue-6, June 2026, of a scholarly journal, *International Journal of Engineering Research & Science*. This journal is part of the AD Publications series in the field of Engineering, Mathematics, Physics, Chemistry and science Research Development, and is devoted to the gamut of Engineering and Science issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Engineering and Science as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Engineering and Science community, addressing researchers and practitioners in below areas:

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Neural Networks	Plastic Engineering

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with IJOER. We are certain that this issue will be followed by many others, reporting new developments in the Engineering and Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOER* readers and will stimulate further research into the vibrant area of Engineering and Science Research.



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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<sub>2</sub> in trace amounts. Subsequently, substituted-amine reacted with coumarin in the presence of ZnCl<sub>2</sub> 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, <sup>1</sup>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

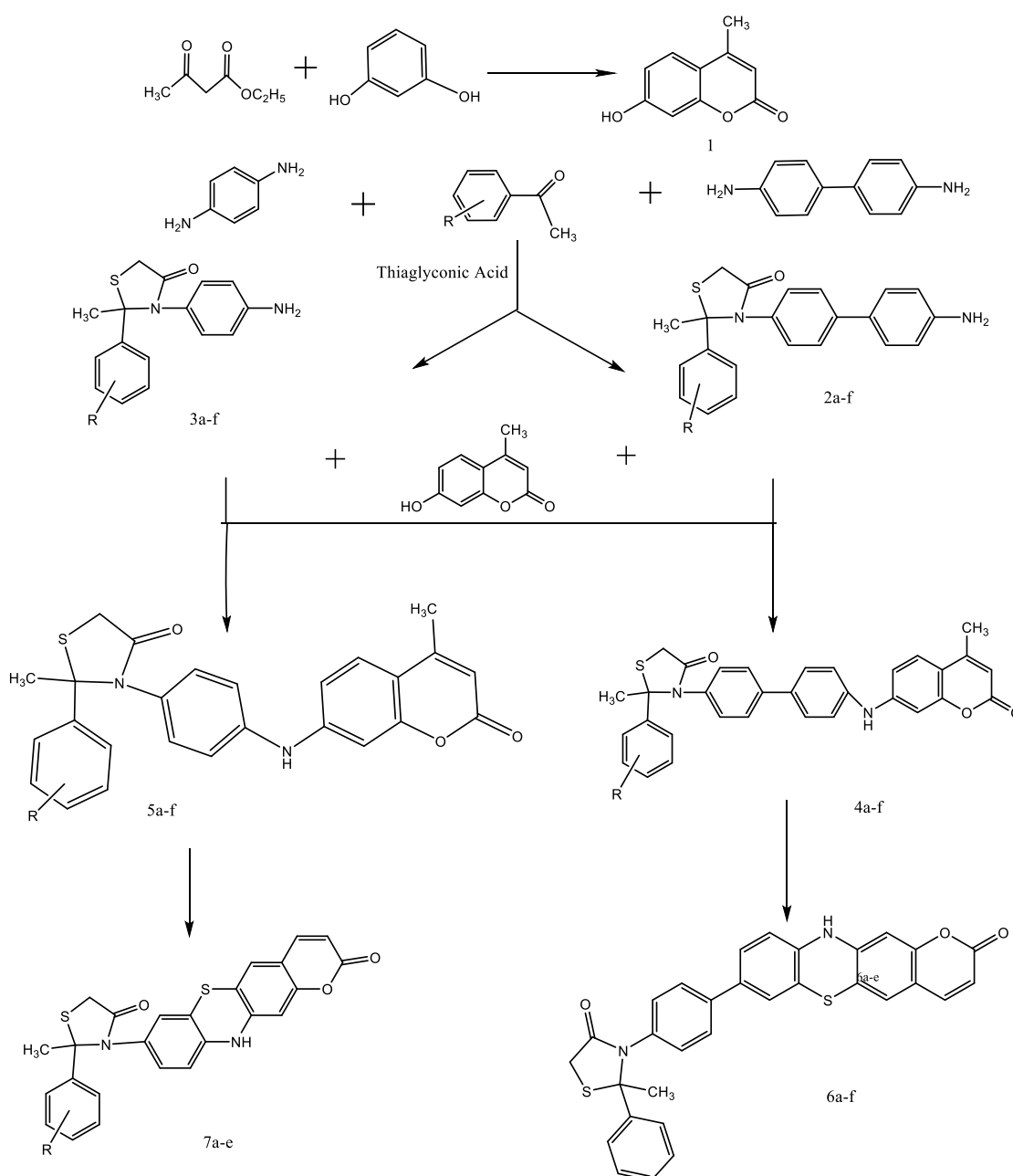
Phenothiazine is amongst the primary molecular synthons involved in the synthesis of antipsychotic drugs. It is a tricyclic compound of immense usage [1-3] in the manufacturing of emetics, antihistamines, anticancer [4-5], antifungal, anti-inflammatory, antitubercular, antioxidant, antileishmanial, and 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  $^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$  and DMSO on a Bruker NMR spectrophotometer at 400 MHz. Tetramethylsilane was taken as the internal standard and chemical shift values ( $\delta$ ) 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.  $\text{H}_2\text{SO}_4$ ; 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:**  $\text{C}_{11}\text{H}_8\text{O}_3$ , **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  $\text{ZnCl}_2$  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:**  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{OS}$ , **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 ( $\nu_{\text{max}}$  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:**  $\text{C}_{22}\text{H}_{19}\text{ClN}_2\text{OS}$ , **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 ( $\nu_{\text{max}}$  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:**  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$ , **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 ( $\nu_{\text{max}}$  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:**  $\text{C}_{22}\text{H}_{19}\text{BrN}_2\text{OS}$ , **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 ( $\nu_{\text{max}}$  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<sub>22</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>S, **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 (v<sub>max</sub> 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<sub>23</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub>S, **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 (v<sub>max</sub> 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<sub>3</sub>-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 thioglycolic acid, using ZnCl<sub>2</sub> 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<sub>16</sub>H<sub>16</sub>N<sub>2</sub>OS, **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 (v<sub>max</sub> 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<sub>16</sub>H<sub>15</sub>ClN<sub>2</sub>OS, **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 (v<sub>max</sub> 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<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>S, **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 (v<sub>max</sub> 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<sub>16</sub>H<sub>15</sub>BrN<sub>2</sub>OS, **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 (v<sub>max</sub> 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<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>S, **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<sub>max</sub> 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<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>S, **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<sub>max</sub> 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<sub>3</sub>-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<sub>2</sub> 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<sub>32</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>S, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>32</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub>S, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>32</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>S, **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<sub>max</sub> 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)

- <sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>32</sub>H<sub>25</sub>BrN<sub>2</sub>O<sub>3</sub>S, **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<sub>max</sub> 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)
  - <sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>32</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>S, **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<sub>max</sub> 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)
  - <sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>33</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>S, **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<sub>max</sub> 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)
  - <sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-S-), 6.12 (s, 1H, -N-CH-S-), 3.55 (s, 3H, Ar-OCH<sub>3</sub>)
- 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<sub>2</sub> 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<sub>26</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>S, **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<sub>max</sub> 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)
  - <sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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 ( $\nu_{\max}$  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)
- **$^1H$  NMR ( $CDCl_3$ ) ( $\delta$  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<sub>2</sub>-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 ( $\nu_{\max}$  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)
- **$^1H$  NMR ( $CDCl_3$ ) ( $\delta$  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<sub>2</sub>-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 ( $\nu_{\max}$  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)
- **$^1H$  NMR ( $CDCl_3$ ) ( $\delta$  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<sub>2</sub>-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 ( $\nu_{\max}$  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)
- **$^1H$  NMR ( $CDCl_3$ ) ( $\delta$  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<sub>2</sub>-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 ( $\nu_{\max}$  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)
- **$^1H$  NMR ( $CDCl_3$ ) ( $\delta$  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<sub>2</sub>-S-), 6.14 (s, 1H, -N-CH-S-), 3.62 (s, 3H, Ar-OCH<sub>3</sub>), 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<sub>31</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>31</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>31</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>31</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>31</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>, **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 (v\_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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>32</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>, **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 (v\_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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-S-), 4.82 (s, 1H, -N-H phenothiazine ring), 3.52 (s, 3H, Ar-OCH<sub>3</sub>), 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<sub>26</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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 (v\_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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>26</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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 (v\_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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>26</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>, **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 (v\_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)
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>26</sub>H<sub>19</sub>BrN<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
  - **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>26</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
  - **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-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<sub>27</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>, **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<sub>max</sub> 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)
  - **<sup>1</sup>H NMR (CDCl<sub>3</sub>) (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<sub>2</sub>-S-), 4.82 (s, 1H, -N-H phenothiazine ring), 3.54 (s, 3H, Ar-OCH<sub>3</sub>), 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	
		<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneum</i>	<i>C. albicans</i>	<i>A. niger</i>		
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 <sub>2</sub> -phenyl	25	6.25	100	50	25		
6f	3-OH,4-OCH <sub>3</sub> -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 <sub>2</sub> -phenyl	100	50	25	100	50		
7f	3-OH,4-OCH <sub>3</sub> -phenyl	25	50	50	100	50		
	<b>Ciprofloxacin</b>	50	50	50	-	-		
	<b>Clotrimazole</b>	-	-	-	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<sub>2</sub>-phenyl) and 7f (3-OH,4-OCH<sub>3</sub>-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<sub>2</sub>-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<sub>3</sub>-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<sub>2</sub>-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<sub>3</sub>-phenyl) was the most active derivative against *A. niger* (MIC = 12.5 µg/ml). These results indicate that incorporation of OH, NO<sub>2</sub>, Br, and OCH<sub>3</sub> 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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# Comprehensive Preformulation Analysis of FP-1 Peptide for Optimized Pharmaceutical Formulation

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**Abstract**— *Preformulation studies are essential for establishing the physicochemical and biopharmaceutical characteristics of novel therapeutic candidates before formulation development. The present investigation focuses on the systematic preformulation evaluation of FP-1 peptide, a promising bioactive compound with potential neuroprotective applications. The study aimed to assess key parameters influencing its stability, compatibility, and suitability for drug delivery.*

*FP-1 peptide was characterized for its organoleptic properties, solubility profile, and pH-dependent stability. The peptide exhibited good solubility in aqueous media with optimal stability observed within a neutral to slightly acidic pH range. Partition coefficient analysis indicated its hydrophilic nature, suggesting challenges in passive membrane permeability. Thermal behavior and crystallinity were evaluated using differential scanning calorimetry (DSC) and X-ray diffraction (XRD), which confirmed its amorphous-to-semicrystalline nature. Fourier-transform infrared spectroscopy (FTIR) analysis demonstrated the presence of characteristic functional groups without evidence of structural degradation.*

*Compatibility studies with selected excipients revealed no significant physicochemical interactions, supporting its suitability for formulation development. Additionally, preliminary stability testing under varying environmental conditions indicated that FP-1 peptide is sensitive to elevated temperature and oxidative stress, necessitating protective formulation strategies such as encapsulation.*

*Overall, the findings provide critical insights into the physicochemical attributes of FP-1 peptide and highlight considerations for its successful incorporation into advanced drug delivery systems. These results provide a strong foundation for further formulation development, particularly to enhance stability, bioavailability, and therapeutic efficacy*

**Keywords**— *FP-1 peptide, Preformulation studies, Physicochemical characterization, Stability analysis, Drug–excipient compatibility.*

## I. INTRODUCTION

Preformulation studies represent a fundamental and indispensable phase in the development of pharmaceutical dosage forms, serving as the scientific foundation upon which successful formulation strategies are built [1,2]. These studies involve the systematic investigation of the physicochemical, mechanical, and biopharmaceutical properties of a drug candidate before its incorporation into a dosage form. By providing detailed insights into parameters such as solubility, stability, partition behavior, crystallinity, and compatibility with excipients, preformulation analysis enables researchers to design formulations that are both effective and stable throughout their shelf life. In addition, these studies assist in identifying potential challenges early in the development process, thereby reducing formulation failures, minimizing cost, and accelerating the overall drug development timeline.

One of the primary objectives of preformulation studies is to determine the intrinsic properties of a drug substance that influence its performance in a delivery system. For instance, solubility plays a crucial role in drug absorption and bioavailability, particularly for compounds administered via oral or parenteral routes. Similarly, stability studies help in understanding how environmental factors such as temperature, pH, light, and oxidative conditions affect the integrity of the drug molecule [3,4]. Knowledge of these parameters allows formulation scientists to select appropriate excipients, packaging conditions, and storage environments to ensure product quality. Furthermore, compatibility studies between the drug and excipients are essential to avoid undesirable interactions that may lead to degradation or reduced therapeutic efficacy.

Peptides have emerged as an important class of therapeutic agents due to their high specificity, potent biological activity, and relatively low toxicity compared to small-molecule drugs. However, despite their therapeutic advantages, peptide-based drugs present significant formulation challenges. These molecules are inherently sensitive to environmental conditions such as temperature fluctuations, enzymatic degradation, and oxidation [5,6,7]. Additionally, peptides often exhibit poor membrane permeability and limited stability in biological environments, which can hinder their effective delivery. As a result, the development of peptide-based formulations requires careful consideration of their physicochemical and biopharmaceutical characteristics.

The structural complexity of peptides contributes to their instability, as they can undergo processes such as hydrolysis, deamidation, aggregation, and denaturation [8]. These degradation pathways are influenced by factors including pH, ionic strength, and the presence of moisture. Consequently, it becomes essential to conduct detailed preformulation studies to evaluate these parameters and identify optimal conditions for maintaining peptide stability. Analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) are commonly employed to characterize peptide structure, thermal behavior, and crystallinity [9,10]. These methods provide valuable information regarding the physical state of the peptide and its potential interactions with formulation components.

Another critical aspect of peptide formulation is the selection of an appropriate drug delivery system that can protect the peptide from degradation while ensuring efficient delivery. Conventional dosage forms often fail to meet these requirements due to rapid degradation and poor bioavailability of peptides. In this context, advanced drug delivery systems such as liposomes have gained considerable attention. Liposomes are spherical vesicular structures composed of phospholipid bilayers that can encapsulate both hydrophilic and lipophilic drugs. Their unique structure allows them to protect sensitive drug molecules from environmental degradation, enhance drug stability, and provide controlled release characteristics [11,12].

Liposomal drug delivery systems offer several advantages for peptide formulation. They can encapsulate peptides within their aqueous core or lipid bilayer, thereby shielding them from enzymatic and chemical degradation. Additionally, liposomes can improve the solubility and bioavailability of poorly permeable drugs. The biocompatibility and biodegradability of phospholipids further contribute to their suitability as drug carriers. Moreover, liposomes can be engineered to achieve targeted delivery and controlled release, making them a versatile platform for pharmaceutical applications. The ability to modify liposomal properties such as size, surface charge, and composition allows for optimization based on the specific requirements of the drug molecule [13,14].

FP-1 peptide is a bioactive compound that requires comprehensive evaluation to determine its suitability for pharmaceutical development. Like many peptide-based molecules, FP-1 peptide may exhibit sensitivity to environmental conditions and may require protective formulation strategies to maintain its stability and efficacy. Therefore, systematic preformulation studies are necessary to investigate its physicochemical properties, including solubility behavior, thermal characteristics, and compatibility with excipients. These studies provide a scientific basis for selecting appropriate formulation approaches and ensuring the successful development of a stable dosage form [5,8].

In addition to preformulation analysis, the formulation of FP-1 peptide into a suitable delivery system is crucial for enhancing its pharmaceutical performance. Liposomal encapsulation represents a promising approach for addressing the challenges associated with peptide stability and delivery. By incorporating FP-1 peptide into liposomes, it is possible to improve its stability under various environmental conditions, reduce degradation, and enhance its overall formulation characteristics. The thin-film hydration method is commonly used for the preparation of liposomes, allowing for efficient encapsulation and reproducibility [15,16].

The present study is designed to perform a comprehensive preformulation evaluation of FP-1 peptide, focusing on key parameters that influence its formulation and stability. These include organoleptic properties, solubility profile, thermal behavior, and compatibility with commonly used excipients. In addition, a liposomal formulation of FP-1 peptide is developed

and evaluated for basic characteristics such as appearance, pH, and uniformity. The integration of preformulation and formulation studies provides a holistic understanding of the peptide's behavior and its potential for pharmaceutical application [5,6,14].

Overall, this investigation aims to establish a strong scientific foundation for the development of FP-1 peptide formulations. By combining detailed physicochemical characterization with advanced drug delivery strategies, the study seeks to optimize the formulation of FP-1 peptide and enhance its stability and performance. The findings of this work are expected to contribute to the advancement of peptide-based drug development and support future research in this area.

## II. MATERIALS AND METHODS

### 2.1 Materials

FP-1 peptide (FPS-ZM1) was procured from a certified supplier (ACME Scientific Solution, Lucknow, India). Phospholipids (soy lecithin) and cholesterol were used as key components for liposomal formulation. Organic solvents such as chloroform, methanol, and ethanol were of analytical grade and used without further purification. Distilled water was used for all aqueous preparations. All other chemicals and reagents were of analytical grade and obtained from standard commercial sources.

### 2.2 Preformulation Studies

#### 2.2.1 Organoleptic Evaluation

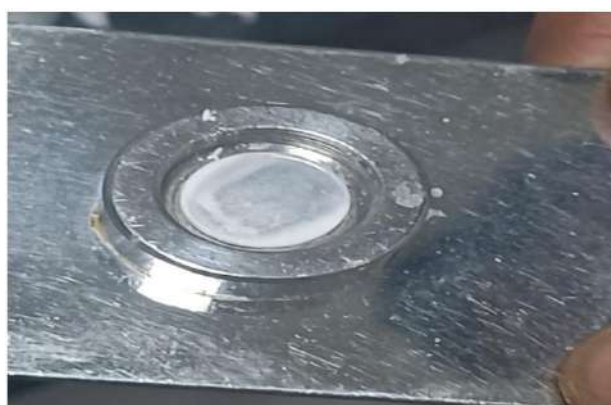
The FP-1 peptide was examined visually for its appearance, color, and physical state to establish its identity and purity. Observations were recorded under normal lighting conditions against a white background [17,18].

#### 2.2.2 Solubility Study

Solubility analysis was performed in various solvents including distilled water, phosphate buffer saline (pH 7.4), and organic media (methanol, ethanol, chloroform) to determine its dissolution behavior. An excess amount of FP-1 peptide was added to each solvent and equilibrated at  $25 \pm 1^\circ\text{C}$  for 24 hours with continuous shaking. After equilibration, samples were centrifuged, filtered, and the supernatant was analyzed using UV-visible spectrophotometry at appropriate wavelength. The study helps in selecting suitable solvents and formulation approaches [19,20,21].

#### 2.2.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was carried out to identify functional groups and evaluate drug–excipient compatibility. Samples were prepared using the potassium bromide (KBr) pellet method and scanned in the range of  $400\text{--}4000\text{ cm}^{-1}$  with a resolution of  $4\text{ cm}^{-1}$ . The spectra were recorded using a FTIR spectrophotometer, and the characteristic peaks were analyzed for functional group identification and potential interactions [22,23,24].



**FIGURE 1: FTIR spectrophotometer setup used for functional group identification and structural characterization of FP-1 peptide.**

#### 2.2.4 Thermal and Structural Characterization

**Differential Scanning Calorimetry (DSC):** DSC analysis was performed using a differential scanning calorimeter to determine the thermal properties of FP-1 peptide. Samples were accurately weighed and sealed in aluminum pans. The analysis

was conducted under nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 25–300°C. The thermograms were recorded and analyzed for melting point, glass transition temperature, and other thermal transitions [25,26].

**X-ray Diffraction (XRD):** XRD analysis was conducted to evaluate the crystallinity of FP-1 peptide formulations at different concentrations (5 mg, 10 mg, and 20 mg). The samples were scanned using a powder X-ray diffractometer with Cu-K $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) at a scanning rate of 2°/min over a  $2\theta$  range of 5–50°. The diffraction patterns were recorded and analyzed for crystalline or amorphous nature [25,26].

### 2.2.5 Scanning Electron Microscopy (SEM)

The surface morphology of FP-1 peptide formulations was examined using scanning electron microscopy. Samples were mounted on aluminum stubs using double-sided adhesive tape and coated with a thin layer of gold-palladium under vacuum to render them conductive. The coated samples were observed under the scanning electron microscope at appropriate magnification and accelerating voltage. Micrographs were captured at different magnifications to evaluate particle size, shape, surface characteristics, and aggregation behavior [27,28].

### 2.2.6 Drug-Excipient Compatibility Studies

Compatibility studies were conducted between FP-1 peptide and commonly used pharmaceutical excipients including phospholipids and cholesterol. Physical mixtures of drug and excipients were prepared in 1:1 ratio and stored under controlled conditions. FTIR spectroscopy was employed as the primary analytical technique to detect any potential interactions. The spectra of pure drug, pure excipients, and their physical mixtures were compared for any significant shifts in characteristic peaks or appearance of new peaks [22,23,24].

### 2.2.7 Preliminary Stability Testing

Stability testing was conducted under varying environmental conditions including elevated temperature (40°C and 60°C) and oxidative stress (exposure to hydrogen peroxide). Samples were stored under these conditions for specified time periods, and the degradation was monitored using appropriate analytical methods. The samples were periodically analyzed for any changes in physical appearance, color, and chemical integrity [29,30].

## 2.3 Liposomal Formulation Development

FP-1 peptide-loaded liposomes were prepared using the thin-film hydration method. Briefly, soy lecithin and cholesterol were dissolved in chloroform-methanol mixture in a round-bottom flask. The organic solvents were evaporated under reduced pressure using a rotary evaporator at 40°C to form a thin lipid film. The film was hydrated with phosphate buffer saline (pH 7.4) containing FP-1 peptide under continuous stirring. The resulting liposomal suspension was allowed to swell and was then sonicated to reduce vesicle size. The formulations were evaluated for appearance, pH, and uniformity [31,32].

## III. RESULTS

### 3.1 Organoleptic Properties

The FP-1 peptide appeared as a stable compound with consistent physical characteristics, confirming its suitability for further analysis. The peptide was observed as a white to off-white powder with a smooth texture and no visible impurities.

### 3.2 Solubility Profile

The peptide demonstrated good solubility in aqueous media (water and phosphate buffer saline), indicating its hydrophilic nature. Moderate solubility was observed in methanol and ethanol, while the peptide showed limited solubility in chloroform. This property supports its suitability for aqueous-based formulations but may require carrier systems for improved permeability.

### 3.3 FTIR Analysis

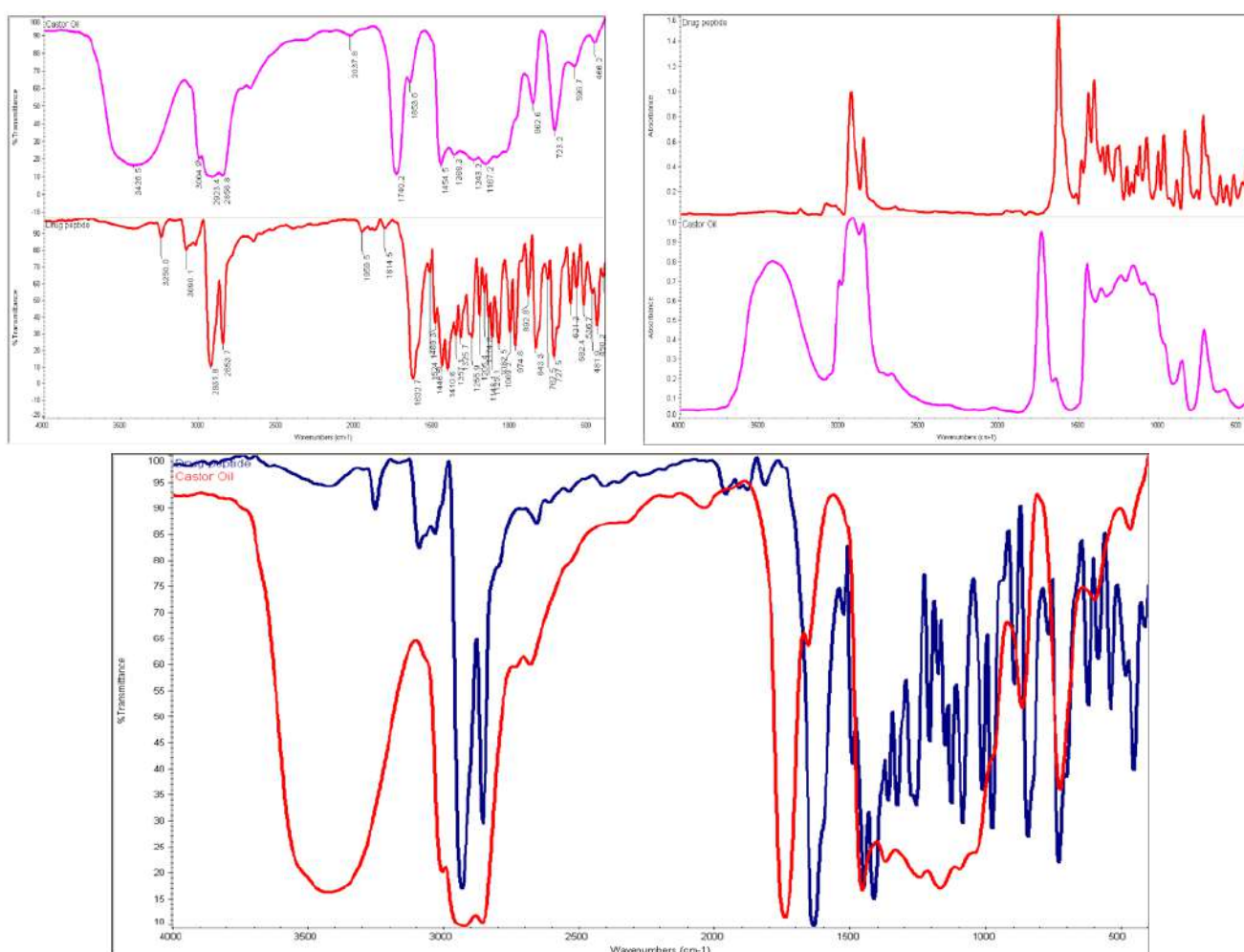
FTIR spectra of FP-1 peptide showed characteristic peaks corresponding to:

- O–H/N–H stretching ( $\sim 3400 \text{ cm}^{-1}$ )

- C–H stretching ( $\sim 2850\text{--}3000\text{ cm}^{-1}$ )
- Amide I band ( $\sim 1640\text{ cm}^{-1}$ )
- Amide II band ( $\sim 1540\text{ cm}^{-1}$ )

The FTIR spectra of drug-excipient mixtures (Figure 2) demonstrated that both drug and excipients maintained their distinct peaks, suggesting minimal interaction in this region. The broad O–H/N–H stretching region ( $3200\text{--}3600\text{ cm}^{-1}$ ) showed non-overlapping peaks, confirming structural uniqueness. The aliphatic C–H stretching region ( $2850\text{--}3000\text{ cm}^{-1}$ ) showed both peaks visible separately, indicating no significant shift or new bonding (e.g., amidation or esterification). Some overlap was observed in the carbonyl (C=O) and amide regions ( $1650\text{--}1750\text{ cm}^{-1}$ ), which is expected in organic molecule blends and does not suggest strong chemical interaction. The C–O and C–N stretching, fingerprint region ( $1000\text{--}1300\text{ cm}^{-1}$ ), showed intense, complex patterns; however, peaks remained distinguishable below  $1000\text{ cm}^{-1}$  in the fingerprint region.

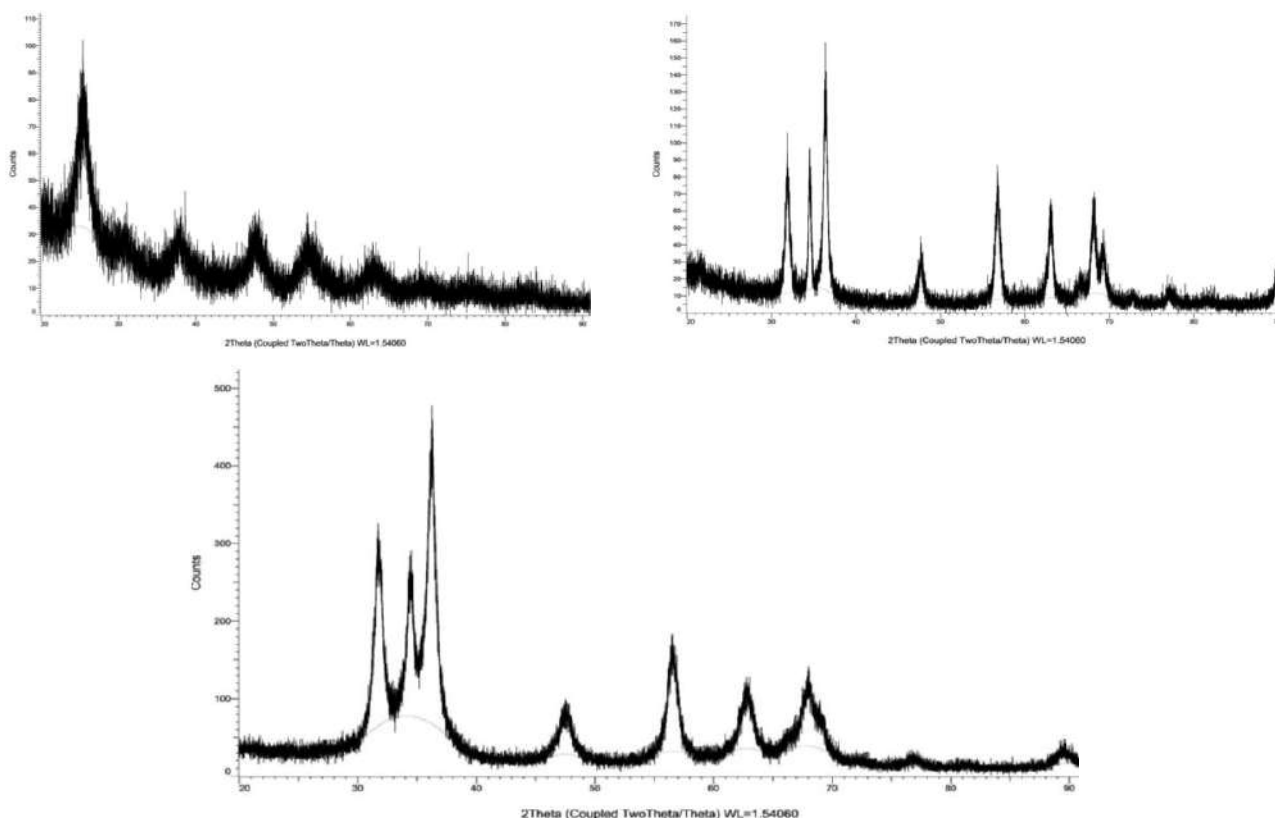
No significant peak shifts were observed in the presence of excipients, indicating compatibility between FP-1 peptide and the tested formulation components.



**FIGURE 2: Drug compatibility with common excipients: (A) Transmittance vs. wavelength; (B) Absorbance vs. wavelength.**

### 3.4 Thermal and Structural Properties

**XRD Analysis:** The XRD patterns of FP-1 peptide formulations at different concentrations (5 mg, 10 mg, and 20 mg) are presented in Figure 3. The patterns showed sharp peaks indicative of the crystalline nature of the peptide, with partial drug recrystallization observed at higher concentrations.



**FIGURE 3: XRD patterns of FPS-ZM1 peptide at (A) 5 mg, (B) 10 mg, and (C) 20 mg showing sharp peaks indicative of high crystallinity due to partial drug recrystallization.**

The comparative evaluation of encapsulation efficiency, release behavior, stability, and crystallinity trends at varying drug concentrations is summarized in Table 1.

**TABLE 1  
 COMPARATIVE EVALUATION OF ENCAPSULATION EFFICIENCY, RELEASE BEHAVIOR, STABILITY, AND CRYSTALLINITY TRENDS OF FPS-ZM1 PEPTIDE AT VARYING DRUG CONCENTRATIONS**

Objective	5 mg	10 mg	20 mg	Interpretation
Encapsulation/Solubility	Positive	Moderately positive	Negative (crystallization)	Indicates concentration-dependent behavior
Sustained Release/Stability	Moderate	Positive	Strongly positive	Better stability with optimal loading
Overall Crystallinity Trend	Low	Medium	High	Indicates recrystallization with increasing load

### 3.5 Scanning Electron Microscopy (SEM)

SEM micrographs of FPS-ZM1 pre-formulations at 5 mg, 10 mg, and 20 mg concentrations revealed concentration-dependent morphological changes (Figure 4). The formulations showed transition from heterogeneous irregular particles to more uniform, smooth, and compact nanosized vesicular structures with improved stability at higher concentration.



**FIGURE 4: SEM micrographs of FPS-ZM1 pre-formulations at (A) 5 mg, (B) 10 mg, and (C) 20 mg.**

The comparative SEM morphological analysis of FPS-ZM1 formulations is summarized in Table 2.

**TABLE 2  
 COMPARATIVE SEM MORPHOLOGY OF FPS-ZM1 FORMULATIONS**

Sample / Image Set	Particle Size (µm)	Morphology	Surface Features	Aggregation	Overall Remark
5 mg Sample	0.5–50 µm (Highly variable)	Irregular flakes, elongated sheets, ruptured vesicles	Smooth to wrinkled; lamellar folds observed	Moderate; presence of fused films	Heterogeneous, unstable morphology
10 mg Sample	0.2–3 µm (More controlled)	Mostly spherical small vesicles; intact structure	Smooth with slight wrinkles	Minimal; vesicles well-separated	Most uniform & stable morphology
20 mg Sample	0.3–12 µm (Moderate–large)	Multilamellar, fused aggregates; deformed spheres	Coarse, wrinkled, porous	High; strong fusion and coalescence	Aggregation and multilamellar collapse

### 3.6 Liposomal Formulation Evaluation

The prepared FP-1 peptide liposomal formulations were evaluated for basic characteristics. The formulations appeared as uniform, opalescent suspensions with no visible aggregates or precipitates. The pH of the formulations was found to be in the range of 6.8–7.2, which is suitable for parenteral administration. The formulations exhibited good uniformity and were stable under refrigerated conditions.

## IV. DISCUSSION

The present investigation provided a comprehensive preformulation and morphological characterization of FP-1 peptide (FPS-ZM1), emphasizing its physicochemical behavior, compatibility profile, crystallinity, and surface morphology at varying concentrations. Preformulation studies are considered a critical step in pharmaceutical development because they establish the foundation for designing stable, effective, and reproducible formulations [33,34,35]. The obtained findings demonstrated that FP-1 peptide possesses physicochemical characteristics favorable for pharmaceutical formulation, while also highlighting certain limitations associated with peptide-based therapeutics, including instability, aggregation tendency, and sensitivity to environmental conditions.

The solubility profile confirmed the hydrophilic nature of FP-1 peptide, which is consistent with the behavior of many therapeutic peptides reported in previous pharmaceutical studies. Hydrophilic peptides generally exhibit improved dissolution in aqueous systems but frequently demonstrate poor permeability across biological membranes due to their high molecular weight and polar functional groups [36,37,38]. Similar observations have been reported for peptide-based drug molecules where enhanced solubility is often accompanied by limited membrane transport and reduced bioavailability [39,40]. The

aqueous solubility observed in the present study suggests that FP-1 peptide may be suitable for parenteral and vesicular drug delivery systems; however, advanced delivery approaches may still be required to overcome permeability-related challenges.

FTIR analysis demonstrated the preservation of characteristic functional groups, including O–H/N–H stretching, aliphatic C–H stretching, and amide I and II bands. The absence of significant spectral shifts in the presence of excipients confirmed the peptide's compatibility with the formulation components [41,42]. Drug–excipient compatibility plays a major role in determining formulation stability because incompatible excipients can induce peptide degradation, denaturation, oxidation, or hydrolysis during storage. The present findings indicate that the selected excipients are suitable for peptide stabilization and can be safely used in subsequent formulation development. Similar compatibility profiles have been observed in peptide-loaded lipid systems and vesicular drug carriers where phospholipids and cholesterol provide structural support without causing chemical degradation [1,42,43].

The thermal and structural analyses revealed that FP-1 peptide exhibits amorphous to semi-crystalline characteristics. Amorphous systems are generally associated with enhanced dissolution and improved apparent solubility because of their higher free energy state compared with crystalline materials. However, amorphous systems may also exhibit reduced physical stability and increased susceptibility to moisture-induced degradation or recrystallization during storage. The XRD patterns observed in the present study demonstrated concentration-dependent crystallinity changes, where increasing peptide concentration resulted in greater crystallization and partial recrystallization behavior [44,45]. Such findings suggest that higher drug loading may influence molecular packing and crystal formation within the formulation matrix.

The comparative evaluation of encapsulation efficiency, release behavior, and crystallinity trends further supported the influence of peptide concentration on pre-formulation characteristics. The 5 mg formulation exhibited lower crystallinity and positive encapsulation behavior, suggesting efficient molecular dispersion of the peptide within the system. The 10 mg formulation displayed moderate crystallinity with improved sustained-release characteristics and greater structural stability, indicating balanced encapsulation and vesicle organization. In contrast, the 20 mg formulation showed strong crystallinity and evidence of recrystallization, which may negatively affect drug release and long-term stability. Similar concentration-dependent recrystallization phenomena have been reported in lipid-based delivery systems where excessive drug loading promotes phase separation and crystal formation [46,47].

SEM analysis provided important insights into the morphological characteristics of FPS-ZM1 formulations. The 5 mg formulation demonstrated highly heterogeneous morphology with irregular flakes, elongated sheets, ruptured vesicles, and fused films. Such irregularity indicates incomplete vesicle formation and limited structural stability, possibly due to insufficient lipid organization at lower peptide concentration [27,28]. The moderate aggregation observed in this formulation may also contribute to instability during storage and administration.

The 10 mg formulation displayed the most desirable morphology among all tested formulations. SEM micrographs revealed predominantly spherical vesicles with smooth surfaces, minimal aggregation, and well-separated structures. The vesicles appeared intact and uniformly distributed, suggesting optimal lipid packing and efficient peptide incorporation. Uniform particle morphology is particularly important for vesicular drug delivery systems because it contributes to improved stability, reproducibility, and controlled drug release behavior. The enhanced morphological stability observed in the 10 mg formulation may therefore provide advantages in pharmaceutical performance and therapeutic efficacy.

In contrast, the 20 mg formulation exhibited multilamellar fused aggregates with coarse, wrinkled, and porous surfaces. Extensive aggregation and coalescence were observed, indicating structural collapse and destabilization at higher peptide loading. The presence of deformed vesicles suggests that excessive peptide concentration may disrupt bilayer organization and increase intermolecular interactions, ultimately leading to vesicle fusion and aggregation. Similar findings have been reported in liposomal and nanoparticulate systems where excessive drug loading compromises vesicle integrity and reduces colloidal stability [46,48,49].

The overall SEM findings demonstrated a concentration-dependent transition in morphology from heterogeneous irregular particles toward more organized vesicular structures, followed by destabilization at excessive peptide concentration. These observations indicate that formulation concentration plays a decisive role in determining vesicle integrity, aggregation tendency, and structural stability. Among all formulations, the 10 mg system exhibited the most balanced characteristics with controlled particle size distribution, smooth morphology, and minimal aggregation.

The present findings collectively emphasize the importance of integrating preformulation characterization with morphological evaluation during peptide formulation development. Peptide-based therapeutics are inherently sensitive to temperature, oxidation, pH variations, and moisture, making detailed physicochemical analysis essential before dosage form development. The obtained data indicate that FP-1 peptide possesses suitable characteristics for advanced pharmaceutical formulation while also highlighting the need for optimized stabilization approaches.

Furthermore, the study supports the growing application of lipid-based and vesicular delivery systems for peptide therapeutics. Vesicular systems can enhance peptide stability, reduce enzymatic degradation, improve controlled release, and increase therapeutic efficiency. However, vesicle morphology, particle size distribution, aggregation behavior, and crystallinity must be carefully controlled to ensure formulation performance. The results obtained from the present investigation therefore provide valuable scientific insight for future optimization and large-scale formulation development of FP-1 peptide.

## V. CONCLUSION

The present study successfully established the physicochemical and morphological profile of FP-1 peptide (FPS-ZM1), demonstrating its favorable solubility, structural integrity, and compatibility for advanced pharmaceutical formulation development. SEM and structural analyses confirmed that peptide concentration critically influences vesicle morphology, crystallinity, aggregation behavior, and formulation stability, with the 10 mg formulation exhibiting the most optimal characteristics, including uniform spherical vesicles, minimal aggregation, and enhanced structural stability. Collectively, these findings provide strong scientific evidence supporting the potential of FPS-ZM1 as a promising candidate for stable peptide-based and neuroprotective drug delivery systems

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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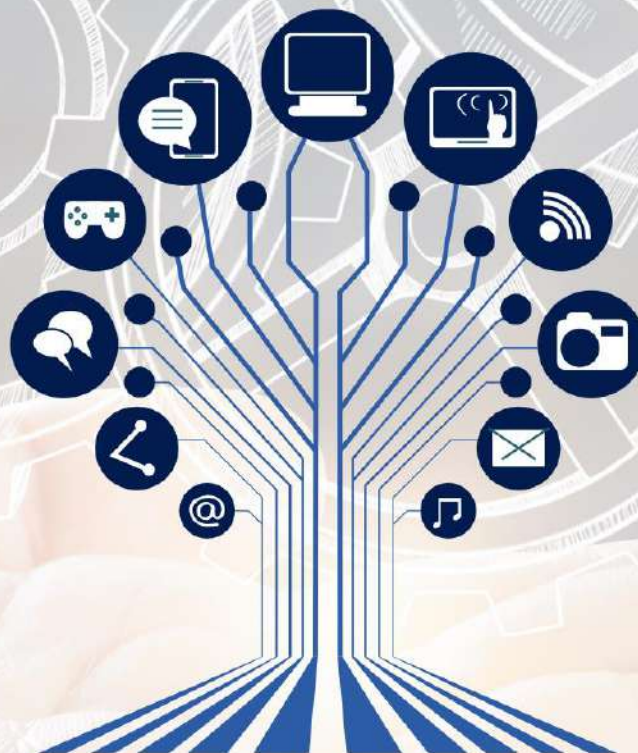
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