CHAPTER 3

EXPERIMENTAL
Chapter 3. Experimental

MATERIALS AND METHODS

Chemicals

- 4-chloro aniline (S.D. Fine Chemicals Ltd., Mumbai).
- Absolute alcohol (Merck Ltd., Germany).
- Acetone (Merck Ltd., Mumbai).
- Amino antipyridine (Merck Ltd., Germany).
- Amino azo benzene (Merck Ltd., Germany).
- Amino benzthiazole (Sigma Aldrich Co., USA).
- Amino butyric acid (Sigma Aldrich Co., USA).
- Amino naphthalene (Sigma Aldrich Co., USA).
- Amino tetrazole (Sigma Aldrich Co., USA).
- Anesthetic ether (P.S.V. chemicals Ltd., Gujarat).
- Antipyrine (Merck Ltd., Mumbai).
- Benzene (S.D. fine chemicals Ltd., Mumbai).
- Benzene sulphonic acid (Merck Ltd., Germany).
- Benzene sulphonylhydrazide (Sigma Aldrich Co., USA).
- Benzoic acid (Merck Ltd., Mumbai).
- Benzoin (Merck Ltd., Mumbai).
- Calcium chloride (Fused) (Merck Ltd., Mumbai).
- Carrageenan (Sigma Aldrich Co., USA).
- Chlor benzene sulphonamide (Sigma Aldrich Co., USA).
- Chloroform (Merck Ltd., Mumbai).
- Formaldehyde (Merck Ltd., Mumbai).
- Furoic hydrazide (Sigma Aldrich Co., USA).
- Hydrochloric acid (Merck Ltd., Mumbai).
- Iodine (Merck Ltd., Germany).
- Isoniazid (Sigma Aldrich Co., USA).
- Lipopolysaccharide (Sigma Aldrich Co., USA).
- Methanol (Merck Ltd., Mumbai).
Chapter 3. Experimental

- o-Anisidine (Merck Ltd., Germany).
- o-nitro amine (Merck Ltd., Germany).
- p-Anisidine (Merck Ltd., Germany).
- para ethylene diamine (Sigma Aldrich Co., USA).
- Petroleum ether (S.D. fine chemicals Ltd., Mumbai).
- Phenyl hydrazide (Merck Ltd., Germany).
- p-nitro amine (Merck Ltd., Germany).
- Polyphosphoric acid (Merck Ltd., Mumbai).
- Potassium hydroxide (Merck Ltd., Mumbai).
- p-toluene sulphonyl hydrazide (Sigma Aldrich Co., USA).
- p-toluidine (Merck Ltd., Germany).
- Pyridine (Merck Ltd., Mumbai).
- Sodium hydroxide (Merck Ltd., Mumbai).
- Sulphuric acid (Merck Ltd., Mumbai).
- Xylene (Merck Ltd., Mumbai).
- Elisa Kit:TNF-alpha (Diclone, France).
- Elisa Kit-IL1-beta (RayBio, Georgia).
- The purity of the compounds was established by TLC (Thin Layer Chromatography) on silica gel-G using Benzene: Petroleum ether (9:1) and Chloroform: Methanol (9.8:0.2) as solvent system. Iodine chamber was used for the visualization of TLC spots.

Equipments

- The melting points were determined in open capillaries using LabIndia UV Vis., India melting point apparatus, and were uncorrected.
- The infrared spectra were recorded with Shimadzu 8400S-FTIR spectrophotometer, Japan in KBr discs.
- The $^1$H-NMR spectra in CDCl$_3$ were recorded on Bruker-DPX 300 NMR spectrophotometer, USA using tetra methyl silane (TMS) as an internal standard.
Chapter 3. Experimental

- The mass spectras were obtained on Shimadzu LC-MS 2010A, Japan and API 2000 Applied Biosystems, Canada.
- Elemental analysis (C, H, N) was performed on Elementar Vario EL III, elemental analyzer, Germany.
- ELISA reader.
- Plethysmometer (Ugo Basile, Italy).
- Vernier calipers

The work carried out has been discussed under the following main heads:

A. Chemical studies
B. Biological studies
C. QSAR studies
A. CHEMICAL STUDIES.

Synthesis of 3,4-Diphenyl-isochromen-1-one (J1)

An equimolar quantity of 2- Hydroxy-1, 2-diphenyl-ethanone (4.24 g, 0.02 mol) and benzoic acid (2.44 g, 0.02 mol) in polyphosphoric acid (10 mL) was heated on oil bath for 12 h. During heating the content were occasionally stirred. The reaction mixture was poured into ice water and left as such for half hour. The solid thus obtained was filtered, washed initially with 10% sodium bicarbonate and then washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield J1 (2.02 g, 67.71%), m. p. 164-166ºC, white solid.

Anal.: IR (KBr, νmax): 2955.59 cm⁻¹ (C-H str.), 1730.87 cm⁻¹ (C=O str.), 1521.9 cm⁻¹ (C=C str.), 1356.20 cm⁻¹ and 789.18 cm⁻¹ (C-H bend.), 1046.22 cm⁻¹ (C-O-C str.).

¹H-NMR (CDCl₃), δ ppm: 7.32 (2H, t, ar), 7.48 (4H, t, ar), 7.68 (4H, d, ar), 8.02 (3H, t, CH7 CH8 of isoq), 8.18 (2H, d, CH6 CH9 of isoq).

Anal. Calcd. for C₂₁H₁₄O₂: C 84.54, H 4.73. Found: C 84.56, H 4.79.

MS, m/z: 298.21 [M]+

Synthesis of 2-(4-Methoxy-phenyl)-3,4-diphenyl-2H-isquinolin-1-one (JS1)

An equimolar quantity of 3, 4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4 methoxy phenyl amine (0.12 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS1(0.32 g, 80.30%), m. p. 186-188ºC, light yellow solid.

Anal.: IR (KBr, νmax): 2949.58 cm⁻¹ (C-H str.), 1718.86 cm⁻¹ (C=O str.), 1359.12 cm⁻¹ (C=C str.), 1276.23 cm⁻¹ (C-N bend), 1043.22 cm⁻¹ (C-O-C str.), 715.05 cm⁻¹ (C=C ben.).

¹H-NMR (CDCl₃), δ ppm: 3.76 (3H, s, CH₃), 6.67 (2H, d, CH3 CH5 methoxy phenyl), 7.32 (6H, t, ar), 7.61 (2H, d, CH2 CH6 of methoxy phenyl), 7.63 (4H, d, ar), 7.69 (1H, t, CH8 of isoq), 7.73 (1H, t, CH7 of isoq), 7.92 (2H, d, CH6 CH9 of iso).


MS, m/z: 403.12 [M]+
Chapter 3. Experimental

Synthesis of 4-(Oxo-3,4-diphenyl-1H-isoquinolin-2-yl)-butyric acid (JS2)

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4 amino butyric acid (0.10 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS2 (0.22 g, 58.16%), m. p. 124-127ºC, white solid.

Anal.: IR (KBr, νmax): 3206.28 cm⁻¹ (O-H stretch), 2966.57 cm⁻¹ (C-H str.), 1727.28 cm⁻¹ (C=O stretch), 1479.38 cm⁻¹ (CH₂ bend), 1418.95 cm⁻¹ (O-H bend), 1372.29 cm⁻¹ (CH₃ bend), 1287.65 cm⁻¹ (C-N bend), 1235.19 cm⁻¹ (C=O stretch), 789.16 cm⁻¹ (C-H bend.), 11.0 (1H, s, OH).

1H-NMR (CDCl₃), δ ppm: 1.42 (2H, m, CH₂), 2.41 (2H, t, CH₂), 7.41 (2H, t, ar), 7.57 (1H, t, CH8 of isoq), 7.73 (4H, t, ar), 7.78 (4H, d, ar), 7.92 (1H, t, CH7 of isoq), 8.13 (2H, d, CH6 CH9 of isoq), 11.0 (1H, s, OH).


MS, m/z: 383.16 [M]+

Synthesis of 2-(2-Methoxy-phenyl)-3,4-diphenyl-2H-isoquinolin-1-one (JS3)

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.44 g, 0.001 mol) and 2-Methoxy Phenyl amine(0.12 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS3 (0.30 g, 73.16%), m. p. 185-188ºC, yellow solid.

Anal.: IR (KBr, νmax): 2950.55 cm⁻¹ (C-H str.), 1720.81 cm⁻¹ (C=O str.), 1382.22 cm⁻¹ (CH₃ bend), 1541.11 cm⁻¹ and 787.16 cm⁻¹ (C-H bend.), 1289.22 cm⁻¹(C-N bend), 1043.27 cm⁻¹ (C-O-C str.), 715.07 cm⁻¹ (C=C ben.).

1H-NMR (CDCl₃), δ ppm: 3.55 (3H, s, CH₃), 6.30 (1H, d, CH3 of methoxy phenyl), 6.65(2H, t, CH4 CH5 of methoxy phenyl), 7.69 (1H, t, CH8 of isoq), 7.73 (6H, t, ar), 7.79 (1H, t, CH7 of isoq), 7.81 (1H, d, CH6 of methoxy phenyl), 7.93 (4H, d, ar), 8.09 (2H, CH6 CH9 of isoq).


MS, m/z: 403.10 [M]+
**Synthesis of 2-(1,5-Diphenyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3,4-diphenyl-2H-isooquinolin-1-one (JS4)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4-Amino-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (0.20 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS4 (0.23 g, 47.98%), m.p. 175-178ºC, light brown solid.

**Anal.:**

IR (KBr, \( \nu_{\text{max}} \)): 2948.57 cm\(^{-1}\) (C-H str.), 1726.88 cm\(^{-1}\) (C=O str.), 1386.32 cm\(^{-1}\) (CH\(_3\) bend), 1345.05 cm\(^{-1}\) and 780.19 cm\(^{-1}\) (C-H bend.), 1289.22 cm\(^{-1}\) (C-N bend), 719.01 cm\(^{-1}\) (C=C bend).

\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 2.42 (3H, s, CH\(_3\)), 2.93 (3H, s, CH\(_3\)), 6.66 (2H, d, ar), 6.71 (1H, t, 4CH of 2 phenyl), 7.18 (2H, t, CH\(_3\) CH5 of 2 phenyl), 7.42 (2H, t, ar), 7.42 (4H, t, ar), 7.56 (4H, d, ar), 7.67 (1H, t, CH8 of isoq), 7.73 (1H, t, CH7 of isoq), 8.13 (2H, d, CH6 CH9 of isoq).

Anal. Calcd. for C\(_{32}\)H\(_{25}\)N\(_3\)O\(_2\): C 79.48, H 5.21, N 8.69. Found: C 79.53, H 5.27, N 8.73.

MS, \( m/z \): 483.23 [M]\(^+\)

**Synthesis of N-(1-Oxo-3,4-diphenyl-1H-isooquinolin-2-yl)-benzenesulphonamide (JS5)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and Benzene sulphonylhydrazide (0.17 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS5 (0.33 g, 71.82%), m.p. 187-189ºC, yellow solid.
**Chapter 3. Experimental**

**Anal.:**

IR (KBr, \( \nu_{\text{max}} \)): 2967.59 cm\(^{-1}\) (C-H str.), 1535.89 cm\(^{-1}\) (C=C str.), 1343.09 cm\(^{-1}\) and 1165.18 cm\(^{-1}\) (SO\(_2\) str.), 1086.56 cm\(^{-1}\) (C-N str.), 841.4 cm\(^{-1}\) (C-H bend.), 1350.08 and 1159.14 (SO\(_2\) str.).

\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 7.22 (2H, t, ar ), 7.32 (2H, d, ar), 7.42 (4H, t, ar), 7.49 (1H, t, ar), 7.62 (2H, t, ar), 7.74 (2H, t, CH7 CH8 of isoq), 7.95 (4H, d, ar), 8.13 (2H, d, CH6 CH9 of isoq).


MS, m/z: 452.15 [M]+.

**Synthesis of 2-Benzothiazole-2-yl-3,4-diphenyl-2H-isoquinolin-1-one (JS6)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4-Propenyl-thiazol-2-ylamine (0.15 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into water. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS6 (0.31 g, 72.01%), m.p. 160-162ºC, yellow solid.

**Anal.:**

IR (KBr, \( \nu_{\text{max}} \)): 2950.55 cm\(^{-1}\) (C-H str.), 1600.81 cm\(^{-1}\) (C=N str.), 1511.0 cm\(^{-1}\) (C=C str.), 1350.08 cm\(^{-1}\) and 1159.14 cm\(^{-1}\) (SO\(_2\) str.), 1095.49 cm\(^{-1}\) (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.).

\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 7.21 (2H, t, ar ), 7.35 (4H, t, ar), 7.51 (2H, t, CH7 CH8 of isoq), 7.59 (2H, t, CH4 CH5 benzthiazole), 7.62 (4H, d, ar), 7.95 (2H, d, CH6 CH9 of isoq), 8.22 (2H, d, CH3 CH6 of benzthiazole).


MS, m/z: 430.14 [M]+

**Synthesis of Furan-2-carboxylic acid(1-oxo-3,4-diphenyl-1H-isocoumarin-2-yl)amide (JS7)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and Furan-2-carboxylic acid hydrazide (0.13 mL, 0.001 mol) in anhydrous pyridine (10...
Chapter 3. Experimental

mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS7 (0.29 g, 70.61%), m.p. 181-183°C, yellow solid.

**Anal.:**

IR (KBr, ν_max): 2970.85 cm⁻¹ (C-H str.), 1519.20 cm⁻¹ (C=C str.), 1087.46 cm⁻¹ (C-N str.), 830.19 cm⁻¹ (C-H bend.)

¹H-NMR (CDCl₃), δ ppm: 6.70 (1H, t, CH₄ of furan), 7.30 (2H, d, CH₃ CH₅ of furan), 7.40 (2H, t, ar), 7.52 (5H, t, ar), 7.58 (4H, d, ar), 7.73 (1H, t, CH₇ of isoq), 7.95 (1H, d, CH-9 of isoq) 8.13 (1H, d, CH6 of isoq).


MS, m/z: 406.24 [M⁺]

**Synthesis of N-(1-oxo-3,4-diphenyl-1H-isocoumarin-2-yl)isonicotinamide (JS8)**

An equimolar quantity of 3, 4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and Isonicotinic acid hydrazide (0.14 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS8 (0.30 g, 71.86%), m.p. 187-190°C, light yellow solid.

**Anal.:**

IR (KBr, ν_max): 2930.59 cm⁻¹ (C-H str.), 1598.86 cm⁻¹ (C=N str.), 1524.17 cm⁻¹ (C=C str.), 1084.32 cm⁻¹ (C-N str.), 845.19 cm⁻¹ (C-H bend.)

H-NMR (CDCl₃), δ ppm: 7.30 (6H, t, ar), 7.59 (2H, t, CH7 CH8 of isoq), 7.68 (6H, d, CH3 CH5 of isonicotinic acid), 7.17 (2H, d, CH6 CH9 of isoq), 9.02 (2H, d, CH2 CH6 of isonicotinic acid). Anal. Calcd. for C₂₇H₁₉N₃O₃: C 77.68, H 4.59, N 10.07. Found: C 77.72, H 4.64, N 10.10.

MS, m/z: 417.12 [M⁺]

**Synthesis of 2-(2-Nitro-phenyl)-3,4-diphenyl-2H-isoquinolin-1-one (JS9)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 2-nitro phenylamine (0.14 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold
Chapter 3. Experimental

water and acidified with dil HCl. The solid thus obtained was filtered, washed several
times with water, dried and recrystallized from ethanol-dichloromethane mixture to
yield JS9 (0.30 g, 71.99%), m.p. 177-180°C, light yellow solid

Anal.:  
IR (KBr, ν_{max}): 3150.57 cm\(^{-1}\) (C-H str.), 1562.20 cm\(^{-1}\) (C=C str.), 1087.42 cm\(^{-1}\) (C-N str.), 855.16 cm\(^{-1}\) (C-H bend.), 1529 cm\(^{-1}\) (N=O str.), 1359 cm\(^{-1}\) (N-O str.).

\(^1\)H-NMR (CDCl\(_3\)), δ ppm: 7.39 (3H, t, ar), 7.45 (4H, t, ar), 7.60 (1H, d, ar), 7.70 (1H, t, CH8 of isoq), 7.89 (5H, d, ar), 8.15 (2H, d, isoq), 8.26 (1H, d, ar).

Anal. Calcd. for C\(_{27}\)H\(_{18}\)N\(_2\)O\(_3\): C 77.50, H 4.34, N 6.69. Found: C 77.54, H 4.39, N 6.73.

MS, m/z: 418.13 [M]\(^+\)

Synthesis of 2-(4-Nitro-phenyl)-3,4-diphenyl-2H-isooquinolin-1-one (JS10)
An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4-nitro phenylamine (0.14 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS10 (0.28 g, 66.49%), m.p. 182-184°C, light yellow solid

Anal.:  
IR (KBr, ν_{max}): 2959.58 cm\(^{-1}\) (C-H str.), 1606.89 cm\(^{-1}\) (C=N str.), 1531.6 cm\(^{-1}\) (C=C str.), 1356.07 cm\(^{-1}\) and 1164.17 cm\(^{-1}\) (SO\(_2\) str.), 1099.58 cm\(^{-1}\) (C-N str.), 841.6 cm\(^{-1}\) (C-H bend.).

\(^1\)H-NMR (CDCl\(_3\)), δ ppm: 7.44(2H, t, ar), 7.78 (1H, t, CH8 of isoq), 7.80(4H, t, ar), 7.89(4H, d, ar), 7.90(1H, t, CH7 of isoq), 8.21 (2H, d, CH3 CH5 of nitrophenyl).


MS, m/z: 418.16 [M]\(^+\)

Synthesis of 3,4 Diphenyl-2-(2-phenylamino-ethyl)--2H-isooquinolin-1-one (JS11)
An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and N-Phenyl-ethane-1,2-diamine (0.14 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed
Chapter 3. Experimental

several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS11 (0.36 g, 85.23%), m.p. 176-179°C, light yellow solid.

Anal.:  
IR (KBr, ν<sub>max</sub>): 2960.59 cm<sup>-1</sup> (C-H str.), 1539.19 cm<sup>-1</sup> (C=C str.), 1089.40 cm<sup>-1</sup> (C-N str.), 849.17 cm<sup>-1</sup> (C-H bend.)

<sup>1</sup>H-NMR (CDCl<sub>3</sub>), δ ppm: 3.02 (2H, t, ethyl), 3.52 (2H, t, ethyl), 6.66 (2H, d, CH2 CH6 of phenylamino), 6.83 (1H, t, 4CH of phenylamino), 7.12 (2H, t, CH3 CH5 of phenylamino), 7.37 (2H, t, ar), 7.40 (5H, t, CH8 of isoq), 7.70(1H, t, CH7 of isoq), 7.83 (2H, d, CH9 of isoq).

Anal. Calcd. for C<sub>29</sub>H<sub>24</sub>N<sub>2</sub>O: C 83.63, H 5.81, N 6.73. Found: C 83.66, H 5.86, N 6.75.

MS, m/z: 416.25 [M]<sup>+</sup>

Synthesis of 3,4-diphenyl-2-phenylamino-2H-isoquinolin-1-one (JS12)  
An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and phenyl hydrazine (0.11 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS12 (0.30 g, 76.97%), m.p. 132-135°C, yellow solid.

Anal.:  
IR (KBr, ν<sub>max</sub>): 2950.67 cm<sup>-1</sup> (C-H str.), 1517.19 cm<sup>-1</sup> (C=C str.), 1095.58 cm<sup>-1</sup> (C-N str.), 840.90 cm<sup>-1</sup> (C-H bend.).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>), δ ppm: 6.99 (2H, d, CH2 CH6 phenylamine), 7.10 (1H, t, CH4 of phenylamine), 7.19 (4H, t, ar ), 7.36 (5H, t, CH8 of isoq), 7.44 (4H, d, ar), 7.63 (1H, t, CH7 of isoq), 8.15 (1H, d, CH6 of isoq), 8.39 (1H, d, CH9 of isoq).

Anal. Calcd. for C<sub>27</sub>H<sub>20</sub>N<sub>2</sub>O: C 83.63, H 5.81, N 6.73. Found: C 83.66, H 5.86, N 6.75.

MS, m/z: 388.24 [M]<sup>+</sup>
ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS13 (0.34 g, 73.73%), m.p. 173-176°C, light yellow solid.

**Anal.:**

IR (KBr, \( \nu_{\text{max}} \)): 2961.69 cm\(^{-1}\) (C-H str.), 1520.34 cm\(^{-1}\) (C=C str.), 1359.17 cm\(^{-1}\) and 1165.49 cm\(^{-1}\) (SO\(_2\) str.), 1090.49 cm\(^{-1}\) (C-N str.), 840.19 cm\(^{-1}\) (C-H bend.), (CH\(_3\) str.).

\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm:

7.24 (3H, s, CH\(_3\)), 7.55 (2H, d, CH\(_3\) CH\(_5\) of benzenesulphonamide), 7.49 (4H, t, ar), 7.56 (3H, t, CH\(_8\) of isoq), 7.80 (1H, t, CH\(_7\) of isoq), 7.89 (2H, d, CH2 CH\(_6\) of benzenesulphonamide), 8.06 (4H, d, ar), 8.42 (1H, d, CH\(_6\) CH\(_9\) of isoq).

Anal. Calcd. for C\(_{28}\)H\(_{22}\)N\(_2\)O\(_3\)S: C 72.08, H 4.75, N 6.00. Found: C 71.3, H 4.82, N 6.03.

**Synthesis of 3,4-Diphenyl-2-(2-phenylazo-phenyl)2H-isoquinolin-1-one (JS14)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 2-Phenylazo-phenylamine (0.20 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS14 (0.25 g, 51.4%), m.p. 189-193°C, yellow solid.

**Anal.:**

IR (KBr, \( \nu_{\text{max}} \)): 2948.79 cm\(^{-1}\) (C-H str.), 1519.20 cm\(^{-1}\) (C=C str.), 1099.56 cm\(^{-1}\) (C-N str.), 836.25 cm\(^{-1}\) (C-H bend.).

\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm:

7.17 (1H, t, ar), 7.26 (2H, t, ar), 7.30 (3H, t, CH\(_3\) CH\(_5\) of azo phenyl), 7.43 (1H, t, ar), 7.52 (5H, t, ar), 7.62 (1H, t, CH\(_8\) of isoq), 7.79 (5H, t, CH\(_7\) of isoq), 8.11 (4H, m, ar), 8.19 (2H, d, CH\(_6\) CH\(_9\) of isoq).

Anal. Calcd. for C\(_{33}\)H\(_{22}\)N\(_3\)O: C 83.08, H 4.85, N 8.80. Found: C 83.09, H 4.92, N 8.83. MS, \( m/z \): 477.08 [M]+

**Synthesis of 2-Napthalen-1-yl-3,4-diphenyl-2H-isoquinolin-1-one (JS15)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and Napthalen-1-ylamine (0.14 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold
water and acidified with dil HCl. The solid thus obtained was filtered, washed several
times with water, dried and recrystallized from ethanol-dichloromethane mixture to
yield JS15 (0.37 g, 87.60%), m.p. 179-181ºC, light brown solid.

Anal.:  
IR (KBr, \( \nu_{\text{max}} \)): 2951.75 cm\(^{-1} \) (C-H str.), 1516.30 cm\(^{-1} \) (C=C str.), 1099.79 cm\(^{-1} \) (C-
N str.), 839.19 cm\(^{-1} \) (C-H bend.).  
\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 6.55 (1H, d, CH\(_2\) of nap), 7.04 (1H, m, ar), 7.25(4H, d, ar),
7.32 (6H, t, ar), 7.43 (2H, t, CH7 CH8 of isoq), 7.65 (2H, d, CH9 of isoq). 
Anal. Calcd. for C\(_{31}\)H\(_{21}\)NO: C 87.92, H 5.00, N 3.31. Found: C 87.95, H 5.04, N 3.35.  
MS, \( m/z \): 423.14 [M]+

Synthesis of 3,4-Diphenyl-2-p-tolyl-2H-isoquinolin-1-one (JS16)  
An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and p-
Tolylamine (0.11 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on
water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold
water and acidified with dil HCl. The solid thus obtained was filtered, washed several
times with water, dried and recrystallized from ethanol-dichloromethane mixture to
yield JS16 (0.22 g, 57.29%), m.p. 185-187ºC, light brown solid.

Anal.:  
IR (KBr, \( \nu_{\text{max}} \)): 2945.59 cm\(^{-1} \) (C-H str.), 1519.19 cm\(^{-1} \) (C=C str.), 1090.52 cm\(^{-1} \) (C-
N str.), 839.17 cm\(^{-1} \) (C-H bend.) 1370.22 cm\(^{-1} \) (CH\(_3\) str.).  
\(^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 2.34 (3H, s, CH\(_3\)), 7.11 (2H, d, ar), 7.21(6H, t, ar ), 7.34
(2H, d, ar), 7.79 (2H, t, CH7 CH8 of isoq), 7.84(6H, d, ar), 8.21(2H, d, CH6 CH9 of isoq)  
Anal. Calcd. for C\(_{28}\)H\(_{21}\)NO: C 86.79, H 5.46, N 3.61. Found: C 86.85, H 5.50, N 3.64.  
MS, \( m/z \): 387.05 [M]+

Synthesis of 3,4-Diphenyl-2-pyridin-2-yl-2H-isoquinolin-1-one (JS17)  
An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and
Pyridin-2-ylamine (0.09 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated
on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold
water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS17 (0.25 g, 67.84%), m.p. 192-194°C, light brown solid.

**Anal.:**

IR (KBr, $\nu_{\text{max}}$): 2952.55 cm$^{-1}$ (C-H str.), 1609.81 cm$^{-1}$ (C=N str.), 1509.10 cm$^{-1}$ (C=C str.), 1092.49 cm$^{-1}$ (C-N str.), 840.12 cm$^{-1}$ (C-H bend.).

$^1$H-NMR (CDCl$_3$), $\delta$ ppm: 6.77 (2H, t, ar), 7.30 (2H, t, ar), 7.45 (1H, d, CH$_5$ of pyridine), 7.77 (6H, t, CH$_8$ of isoq), 7.81 (1H, t, CH$_7$ of isoq), 7.89 (4H, d, ar), 8.09 (2H, d, CH$_6$ CH$_9$ of isoq), 8.21 (1H, d, CH$_2$ of pyridine).

Anal. Calcd. for C$_{26}$H$_{18}$N$_2$O: C 83.40, H 4.85, N 7.48. Found: C 83.46, H 4.91, N 7.44.

MS, $m/z$: 374.12 [M$^+$]

**Synthesis of 3,4-diphenyl-2-tetrazol-1-yl-2H-isooquinolin-1-one (JS18)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and Tetrazol-1-ylamine (0.09 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled, poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS18 (0.28 g, 76.63%), m.p. 173-175°C, white solid.

**Anal.:**

IR (KBr, $\nu_{\text{max}}$): 2954.55 cm$^{-1}$ (C-H str.), 1604.81 cm$^{-1}$ (C=N str.), 1519.10 cm$^{-1}$ (C=C str.), 1091.49 cm$^{-1}$ (C=N str.), 836.12 cm$^{-1}$ (C-H bend.).

$^1$H-NMR (CDCl$_3$), $\delta$ ppm: 7.77 (2H, t, CH$_7$ CH$_8$ isoq), 7.83 (6H, t, ar), 7.96 (4H, d, ar), 8.32 (2H, d, CH$_6$ CH$_9$ isoq).


MS, $m/z$: 365.14 [M$^+$]

**Synthesis of 2-(4-Chloro-phenyl)-3,4-diphenyl-2H-isooquinolin-1-one (JS19)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4-Chloro-phenylamine (0.13 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several
times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS19 (0.30 g, 72.32%), m.p. 167-170°C, light yellow solid.

**Anal.:**
IR (KBr, \(v_{\text{max}}\)): 2956.55 cm\(^{-1}\) (C-H str.), 1612.81 cm\(^{-1}\) (C=N str.), 1517.10 cm\(^{-1}\) (C=C str.), 1098.49 cm\(^{-1}\) (C-N str.), 831.12 cm\(^{-1}\) (C-H bend.), 1119.12 cm\(^{-1}\) (Ar-Cl str.).

\(^1\)H-NMR (CDCl\(_3\)), \(\delta\) ppm: 7.39 (6H, t, ar), 7.41 (2H, d, CH3 CH5 chlorophenyl), 7.56 (2H, d, CH2 CH6 chlorophenyl), 7.69 (1H, t, CH7 CH8), 7.85 (4H, d, ar), 8.22 (2H, d, CH6 CH9).

Anal. Calcd. for C\(_{27}\)H\(_{18}\)ClNO: C 79.50, H 4.45, N 3.43. Found: C 79.61, H 4.52, N 3.47.

MS, \(m/z\): 408.06 [M]\(^+\)

**Synthesis of 2-(4-Chloro-benzenesulfonyl)-3,4-diphenyl-2H-isouinanin-1-one (JS20)**

An equimolar quantity of 3,4-Diphenyl-isochromen-1-one (0.30 g, 0.001 mol) and 4-Chloro-benzene sulphonamide (0.19 mL, 0.001 mol) in anhydrous pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and then poured into ice cold water and acidified with dil HCl. The solid thus obtained was filtered, washed several times with water, dried and recrystallized from ethanol-dichloromethane mixture to yield JS20 (0.32 g, 68.23%), m.p. 192-195°C, light yellow solid.

**Anal.:**
IR (KBr, \(v_{\text{max}}\)): 2945.55 cm\(^{-1}\) (C-H str.), 1610.81 cm\(^{-1}\) (C=N str.), 1521.10 cm\(^{-1}\) (C=C str.), 1348.08 cm\(^{-1}\) and 1156.14 cm\(^{-1}\) (SO\(_2\) str.), 1092.49 cm\(^{-1}\) (C-N str.), 828.12 cm\(^{-1}\) (C-H bend.), 1110.12 cm\(^{-1}\) (Ar-Cl str.).

\(^1\)H-NMR (CDCl\(_3\)), \(\delta\) ppm: 7.31 (6H, t, ar), 7.65 (2H, d, CH3 CH5 of chlorobenene), 7.74 (2H, t, CH7 CH8 isoq), 7.82 (4H, d, ar), 7.99 (2H, d, CH2 CH6 of chlorobenene), 8.16 (2H, d, CH6 CH9 of isoq).

Anal. Calcd. for C\(_{27}\)H\(_{18}\)ClNO\(_3\)S: C 68.71, H 3.84, N 3.43. Found: C 68.78, H 3.88, N 3.47.

MS, \(m/z\): 473.15 [M]\(^+\)
Chapter 3. Experimental

Synthesis of 2-(4-Methoxy-phenyl)-3,4-diphenyl-2H-beno(e)(1,2)thiazine 1,1-dioxide (JS21)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and p-Anisidine (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS21 (0.31 g, 70.98%), m.p. 186-188ºC, yellow solid.

Anal.: 
IR (KBr, ν_{max}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_{2} str.), 1095.49 (C-N str.), 835.12 cm^{-1} (C-H bend.)

^{1}H-NMR (CDCl_{3}), δ ppm: 3.70 (3H, s, CH_{3}), 6.40 (2H, d, 2CH 6CH of methoxy phenyl), 6.55 (2H, d, 3CH 5CH of methoxy phenyl), 7.29 (6H, t, ar), 7.38 (1H, t, 7CH 8CH of benzothiazine), 7.45 (4H, d, ar), 7.83 (2H, d, 6CH 9CH of benzothiazine).

Anal. Calcd. for C_{27}H_{21}NO_{3}S: C 73.78, H 4.82, N 3.19. Found: C 73.82, H 4.88, N 3.17.

MS, m/z: 439.12 [M]+

Synthesis of 4-(4,4-dioxo-4,4-diphenyl-1H-1--benzo(e)(1,2)thiazine-2-yl)-butyric acid (JS22)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and 4 amino butyric acid (0.10 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS22 (0.32 g, 64.65%) m.p. 186-188ºC, yellow solid.

Anal.: 
IR (KBr, ν_{max}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_{2} str.), 1095.49 (C-N str.), 835.12 cm^{-1} (C-H bend.).

^{1}H-NMR (CDCl_{3}), δ ppm: 1.90 (2H, m, CH_{2}), 2.29 (2H, t, CH_{2}), 3.22 (2H, t, CH_{2}), 7.21 (2H, t, ar), 7.28 (4H, t, ar), 7.42 (2H, t, 7CH 8CH of benzothiazine), 7.49 (4H, d, ar), 7.81 (2H, d, 6CH 9CH of benzothiazine), 11.0 (1H, s, OH).

Anal. Calcd. for C_{27}H_{21}NO_{4}S: C 68.72, H 5.05, N 3.34. Found: C 68.74, H 5.07, N 3.32.

MS, m/z: 419.10 [M]^+
Chapter 3. Experimental

Synthesis of 2-(2-Methoxy-phenyl)-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS23)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and o-Anisidine (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS23. (0.30 g, 68.03%). m.p. 186-188°C, light yellow solid.

Anal.:  
IR (KBr, νmax): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO2 str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)  
¹H-NMR (CDCl₃), δ ppm: 3.56 (3H, s, CH₃), 6.30 (1H, d, 6CH of methoxy phenyl), 6.45 (2H, t, 4CH 5CH of methoxy phenyl), 6.54 (1H, d, 3CH of methoxy phenyl), 7.23 (2H, t, ar), 7.38 (4H, t, ar), 7.42 (2H, t, 7CH 8CH of benzothiazine), 7.46 (4H, d, ar), 7.91 (2H, d, 6CH 9CH of benzothiazine).

Anal. Calcd. for C_{27}H_{21}NO₃S: C 73.78, H 4.82, N 3.19. Found: C 73.80, H 4.87, N 3.18.

MS, m/z: 439.16[M]^+

---

Synthesis of 4-(1, 1- Dioxo- 3,4 –diphenyl -1H -1- benzo (e)(1,2) thiazine-2- yl) 1,5-dimethyl-2- phenyl -1,2-dihydro-pyrazol-3-one (JS24)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and amino antipyrine (0.20 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS24 (0.40 g, 76.08%), m.p. 186-188°C, brown solid.

Anal.:  
IR (KBr, νmax): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO2 str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)  
¹H-NMR (CDCl₃), δ ppm: 1.75(3H, s, CH3 of 1methyl pyrazol), 2.40 (3H, s, CH3 of 1 methyl pyrazol), 6.55 (2H, d, 2CH 6CH of 2 phenyl), 6.69 (1H, t, 4CH of 2 phenyl), 7.28(2H, t, 3CH 5CH of 2 phenyl), 7.39 (2H, t, ar), 7.42 (4H, t, ar), 7.54 (1H,
Chapter 3. Experimental

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Benzene Sulphonylhydrazide (0.17 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS25, (0.37 g, 74.91%), m.p. 186-188ºC, yellow solid.

Anal.: IR (KBr, ν_{max}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_{2} str.), 1095.49 (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.)

\(^{1}\)H-NMR (CDCl\(_{3}\)), δ ppm: 7.28 (6H, t, ar), 7.36 (1H, t, 4CH of benzene of sulphonamide ), 7.32 (1H, t, 7CH of benzothiazine), 7.36(1H, t, 8CH of benzothiazine), 7.42 (4H, d, ar), 7.60 (2H, t, 3CH 5CH of benzene of sulphonamide), 7.69 (1H, d, 6CH of benzothiazine), 7.85 (2H, d, 2CH 6CH of benzene of sulphonamide), 7.99 (1H, d, 9CH of benzothiazine).

Anal. Calcd. for C\(_{26}\)H\(_{20}\)N\(_{2}\)O\(_{4}\)S\(_{2}\) : C 63.92, H 4.13, N 5.73. Found: C 63.95, H 4.15, N 5.71.
MS, m/z: 488.48 [M]\(^{+}\)

Synthesis of 2-Benzothiazole-4-yl-3,4-diphenyl-2H-benzo(e)(1,2)thiazin-1,1-dioxide (JS26)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Amino benzothiazole (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS26 (0.32 g, 69.09%) m.p. 186-188ºC, brown solid.
Chapter 3. Experimental

Anal.: IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO$_2$ str.), 1095.49 (C-N str.), 835.12 cm$^{-1}$ (C-H bend.)

$^1$H-NMR (CDCl$_3$), $\delta$ ppm: 6.60 (1H, d, 5CH of benzthiazole), 7.05 (2H, t, ar), 7.26 (4H, t, 6CH of benzthiazole), 7.40 (2H, t, 7CH 8CH of benzothiazine), 7.46 (4H, d, ar), 7.50(1H, d, 7CH of benzthiazole), 7.69 (2H, d, 6CH 9CH of benzothiazine), 9.23 (1H, s, 2CH of benzthiazole).

Anal. Calcd. for C$_{27}$H$_{18}$N$_2$O$_2$S$_2$: C 69.50, H 3.89, N 6.00. Found: C 69.55, H 3.93, N 6.56.

MS, m/z: 466.08 [M]$^+$

**Synthesis of Furan-2-carboxylic acid(1,1-dioxo-3,4-diphenyl-1H-1 benzo(e)(1,2)thiazine-2-yl) amide(JS27)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathine 1,1-dioxide (0.33 g, 0.001 mol) and Furoic hydrazide (0.13 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS27 (0.30 g, 68.25%), m.p. 186-188$^\circ$C, white solid.

Anal.: IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO$_2$ str.), 1095.49 (C-N str.), 835.12 cm$^{-1}$ (C-H bend.)

$^1$H-NMR (CDCl$_3$), $\delta$ ppm: 6.55 (1H, t, 4CH of furan), 7.02(6H, t, ar), 7.27 (2H, t, 7CH 8CH of benzothiazine), 7.55 (4H, d, ar), 7.66 (1H, d, 5H of furan), 7.88 (1H, d, 3CH of furan), 7.91(2H, d, 6CH 9CH of benzothiazine), 9.21(2H, d, 6CH 9CH of benzothiazine), 9.23 (1H, s, 2CH of benzthiazole).


MS, m/z: 442.49 [M]$^+$

**Synthesis of N-(1,1-dioxo-3,4-diphenyl-1H-1-benzo(e)(1,2)thiazin-2-yl) isonicotinamide 2H- 1,1-dioxide(JS28)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Isoniazid (0.14 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The
solid thus obtained was filtered, washed with water and crystallized from methanol-
dichloromethane mixture to yield JS28 (0.39 g, 85.55%), m.p. 186-188°C, yellow solid.

Anal.:  
IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO$_2$ str.), 1095.49 (C-N str.), 835.12 cm$^{-1}$ (C-H bend.)  
$^1$H-NMR (CDCl$_3$), $\delta$ ppm: 7.21 (6H, t, ar), 7.30 (2H, t, 7CH 8CH of benzothiazine), 7.52 (4H, d, ar), 7.80 (2H, d, 6CH 9CH of benzothiazine), 7.95 (2H, d, 3CH 5CH of isonicotinic acid), 9.06 (2H, d, 2CH 6CH of isonicotinic acid).  
MS, $m/z$: 453.41 [M]$^+$  

**Synthesis of 2-(2-Nitro-phenyl)-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS29)**  
An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and o- nitro aniline (0.14 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-
dichloromethane mixture to yield JS29 (0.33 g, 73.05%). m.p. 186-188°C, brown solid.  

Anal.:  
IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO$_2$ str.), 1095.49 (C-N str.), 835.12 cm$^{-1}$ (C-H bend.)  
$^1$H-NMR (CDCl$_3$), $\delta$ ppm 6.66 (1H, d, 6CH of nitrophenyl), 6.75 (1H, t, 4CH of nitrophenyl), 7.11 (6H, t, ar), 7.29 (2H, t, 7CH 8CH of benzothiazine), 7.30 (1H, t, 5CH of nitrophenyl), 7.45 (4H, d, ar), 7.90 (2H, d, 6CH 9CH of benzothiazine), 8.21(1H, d, 3CH of nitrophenyl).  
MS, $m/z$: 454.40 [M]$^+$
Synthesis of 2-(4-Nitro-phenyl)-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS30)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2) oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and p-nitro aniline (0.14 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS30 (0.37 g, 80.31%), m.p. 186-188°C, light brown solid.

Anal.:  
IR (KBr, ν_{max}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_2 str.), 1095.49 (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.)
\(^1\)H-NMR (CDCl_3), δ ppm: 6.72 (2H, d, 2CH 6CH of nitrophenyl), 6.88 (6H, t, ar), 7.25 (2H, t, 7CH 8CH of benzothiazine), 7.40 (4H, d, ar), 7.85 (2H, d, 6CH 9CH of benzothiazine), 8.21 (2H, d, 3CH 5CH of nitrophenyl).


MS, m/z: 454.30 [M]^+  

Synthesis of (2-(1,1-Dioxo-3,4-diphenyl-1H-1-benzo(e)(1,2)thiazin-2-yl)-ethyl)-phenyl-amine (JS31)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and para ethylene diamine (0.14 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS31 (0.31 g, 68.50%), m.p. 186-188°C, brown solid.

Anal.:  
IR (KBr, ν_{max}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_2 str.), 1095.49 (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.)
\(^1\)H-NMR (CDCl_3), δ ppm: 3.30 (2H, t, CH2), 3.39 (2H, t, CH2), 6.22 (2H, d, 2CH 6CH of phenylamino), 6.58 (1H, t, 4CH of phenylamino), 7.04 (H, t, 3CH 5CH of phenylamino), 7.26 (6H, t, ar), 7.32 (2H, t, 7CH 8CH of benzothiazine), 7.42 (4H, d, ar), 7.85 (2H, d, 6CH 9CH of benzothiazine).
Chapter 3. Experimental

MS, $m/z$: 452.31 [M]⁺

**Synthesis of (1,1-Dioxo-3,4-diphenyl-1H-1-benzo(e)(1,2)thiazin-2-yl)-phenyl-amine (JS32)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and phenyl hydrazide (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS32 (0.33 g, 78.21%), m.p. 186-188°C, orange solid.

**Anal.:**
IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO₂ str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)
$^1$H-NMR (CDCl₃), δ ppm: 6.55 (2H, d, 2CH of phenylamine), 6.70 (1H, t, 4CH of phenylamine), 7.00 (2H, t, ar), 7.18 (2H, t, 3CH of phenylamine), 7.30 (4H, t, ar), 7.41 (2H, t, 7CH 8CH of benzothiazine), 7.50 (4H, d, ar), 7.99 (2H, d, 6CH 9CH of benzothiazine).
Anal. Calcd. for $C_{28}H_{20}N_{2}O_{2}$: C 73.56, H 4.75, N 6.60. Found C 73.59, H 4.79, N 6.57.
MS, $m/z$: 424.32 [M]⁺

**Synthesis of N-(1,1-Dioxo-3,4-diphenyl-1H-1--benzo(e)(1,2)thiazin -2-yl)-4 methyl-benzenesulphonamide (JS33)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and p-toluene sulphonyl hydrazide (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS33 (0.40 g, 79.98%), m.p. 186-188°C, white solid.

**Anal.:**
IR (KBr, $\nu_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO₂ str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)
Chapter 3. Experimental

1H-NMR (CDCl3), δ ppm: 2.39 (3H, s, CH3), 7.00 (6H, t, ar), 7.39 (2H, d, 3CH 5CH of benzenesulphonamide), 7.43 (2H, t, 7 CH 8CH of benzothiazine), 7.46 (4H, d, ar), 7.81 (2H, d, 2CH 6CH of benzenesulphonamide), 8.11 (2H, d, 6CH 9CH of benzothiazine).

Anal. Calcd. for C27H22N2O4S2: C 64.52, H 4.41, N 5.57. Found: C 64.57, H 4.46, N 5.54.

MS, m/z: 502.28 [M]+

Synthesis of (2-(1,1-Dioxo-3,4-Diphenyl-benzo(e)(1,2)thiazin-2yl)-phenyl-diazene (JS34)
An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and amino azo benzene (0.12 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS34 (0.39 g, 75.74%), m.p.186-188ºC, orange solid.

Anal:
IR (KBr, νmax): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO2 str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)
1H-NMR (CDCl3), δ ppm: 6.66 (1H, d, 2CH of N phenyl), 6.82 (1H, t, 4CH of N phenyl), 7.11 (6H, t, ar), 7.21 (1H, t, 3CH of N phenyl), 7.33 (2H, t, 7CH 8CH of benzothiazine), 7.42 (4H, d, ar), 7.55 (3H, t, 3CH, 4CH 5CH of azo phenyl) 7.68 (1H, d, 5CH of N phenyl), 7.80 (2H, d, 6 CH 9CH of benzothiazine), 7.93 (2H, d, 2CH 6CH of azo phenyl).

MS,m/z: 513.29 [M]+

Synthesis of 2-Naphthalen-1-yl-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS35)
An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Amino Napthalene (0.14 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil
HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS35 (0.32 g, 69.63%), m.p. 186-188°C, brown solid.

**Anal.:**

IR (KBr, \(\nu_{\text{max}}\)): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO\(_2\) str.), 1095.49 (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.)

\(^1\)H-NMR (CDCl\(_3\)), \(\delta\) ppm: 6.59 (2H, d, 2CH of napthalen), 7.16 (2H, t, ar), 7.15 (1H, t, 3CH of napthalen), 7.26 (4H, t, 3&5CH of 3&4 phenyl), 7.30 (1H, t, 7CH of benzothiazine), 7.30 (2H, t, 7CH 8CH of napthalen), 7.39 (1H, t, 8CH of benzothiazine), 7.42 (4H, d, 2&6CH of 3&4phenyl), 7.58 (2H, d, 6CH 9CH of Napthalen), 7.88 (2H, d, 6CH 9CH of benzothiazine).

Anal. Calcd. For: \(\text{C}_{30}\text{H}_{21}\text{NO}_2\text{S}\): C 78.41, H 4.61, N 3.05. Found: C 78.46, H 4.66, N 3.03.

MS, \(m/z\): 459.21 [M]\(^+\)

**Synthesis of 3,4-Diphenyl-2-p-tolyl-2H--benzo(e)(1,2)thiazine 1,1-dioxide (JS36)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and p-toluidine (0.11 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS36.

JS36 (0.30 g, 70.60%), m.p. 186-188°C, light yellow solid.

**Anal.:**

IR (KBr, \(\nu_{\text{max}}\)): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO\(_2\) str.), 1095.49 (C-N str.), 835.12 cm\(^{-1}\) (C-H bend.)

\(^1\)H-NMR (CDCl\(_3\)), \(\delta\) ppm: 2.35 (3H, s, CH\(_3\)), 6.34 (2H, d, 2CH 6CH of toyl), 6.81 (2H, d, 3CH 5CH of toyl), 7.26(6H, t, ar), 7.34 (2H, t, 7CH 8CH of benzothiazine), 7.42 (4H, d, ar), 7.91 (2H, d, 6CH 9CH of benzothiazine).

Anal. Calcd. for \(\text{C}_{27}\text{H}_{21}\text{NO}_2\text{S}\): C 76.57, H 5.00, N 3.31. Found: C 76.62, H 5.09, N 3.27.

MS, \(m/z\): 423.33 [M]\(^+\)
Chapter 3. Experimental

Synthesis of **3,4-Diphenyl-2-pyridin-2-yl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS37)**

An equimolar quantity of 3,4 Diphenyl - benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Amino pyridine (0.10 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS37 (0.28 g, 67.24%), m.p. 186-188ºC, light orange solid.

**Anal.:**

IR (KBr, ν max): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_2 str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)

¹H-NMR (CDCl₃), δ ppm: 6.65 (1H, t, 5CH of pyridin), 6.79 (1H, d, 3CH of pyridin), 7.30 (6H, t, ar), 7.39 (2H, t, 7CH 8CH of benzothiazine), 7.42 (4H, d, ar), 7.44 (1H, t, 4CH of pyridin), 7.85 (2H, d, 6CH 9CH of benzothiazine), 8.10 (1H, d, 6CH of pyridin).


MS, m/z: 410.41 [M]+

Synthesis of **3,4-diphenyl-2-tetrazol-1-yl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS38)**

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Amino tetrazole (0.09 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS38 (0.28 g, 70.50%), m.p. 186-188ºC, white solid.

**Anal.:**

IR (KBr, ν max): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_2 str.), 1095.49 (C-N str.), 835.12 cm⁻¹ (C-H bend.)

¹H-NMR (CDCl₃), δ ppm: 7.22 (6H, t, ar), 7.38 (1H, t, 7CH 8CH of benzothiazine), 7.42 (4H, d, ar), 7.80 (2H, d, 6CH 9CH of benzothiazine), 8.70 (1H, s, 5CH of tetrazol).
Chapter 3. Experimental


MS, m/z: 401.44 [M]^+

Synthesis of 2-(4-Chloro-phenyl)-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS39)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and 4- chloro aniline (0.13 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS39 (0.29 g, 64.65%), m.p. 186-188ºC, yellow solid.

Anal.: IR (KBr, ν_{\text{max}}): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO_{2} str.), 1095.49 (C-N str.), 835.12 cm^{-1} (C-H bend.)

^1H-NMR (CDCl$_3$), δ ppm 6.49 (2H, d, 2CH 6CH of chlorophenyl), 7.09 (2H, d, 3CH 5CH of chlorophenyl), 7.16 (6H, t, ar), 7.39 (1H, t, 7CH, 8CH of benzothiazine), 7.42 (4H, d, ar), 7.88 (2H, d, 6 CH 9CH of benzothiazine).

Anal. Calcd. for C_{26}H_{18}ClNO_{2}S: C 70.34, H 4.09, Cl 7.99, N 3.16. Found: C 70.39, H 4.15, Cl 7.97, N 3.13.

MS, m/z: 445.30 [M]^+

Synthesis of 2-(4-Chloro-benzenesulfonyl)-3,4-diphenyl-2H-benzo(e)(1,2)thiazine 1,1-dioxide (JS40)

An equimolar quantity of 3,4 Diphenyl-benzo(c)(1,2)oxathiine 1,1-dioxide (0.33 g, 0.001 mol) and Chlorbenzene sulphonamide (0.19 mL, 0.001 mol) in pyridine (10 mL) was heated on water-bath for 2 h. The reaction mixture was cooled and poured into dil HCl. The solid thus obtained was filtered, washed with water and crystallized from methanol-dichloromethane mixture to yield JS40 (0.31 g, 60.75%), m.p. 186-188ºC, yellow solid.
Chapter 3. Experimental

Anal.:  
IR (KBr, $v_{\text{max}}$): 2950.55 (C-H str.), 1600.81 (C=N str.), 1512.10 (C=C str.), 1350.08 and 1159.14 (SO$_2$ str.), 1095.49 (C-N str.), 835.12 cm$^{-1}$ (C-H bend.).

$^1$H-NMR (CDCl$_3$, $\delta$ ppm): $\delta$ ppm: 7.09 (2H, t, 4CH of 3&4 phenyl), 7.28 (4H, t, 3&5 CH of 3&4 phenyl), 7.36 (2H, t, 7CH, 8CH of benzothiazine), 7.40 (4H, d, 2CH, 6CH of 3&4 phenyl), 7.60 (2H, d, 3CH, 5CH of chlorsulphonyl), 7.87 (2H, d, 2CH, 6CH of chlorsulphonyl), 7.90 (1H, d, 9CH, 6CH of benzothiazine).

Anal. Calcd. for C$_{26}$H$_{18}$ClNO$_4$S$_2$: C 61.47, H 3.57, Cl 6.98, N 2.76. Found: C 61.52, H 3.61, Cl 6.97, N 2.74.

MS, $m/z$: 508.11[M]$^+$
Chapter 3. Experimental

B. BIOLOGICAL STUDIES

B.1. Pharmacological Evaluation

Experimental animals

The animals used for the experiments were breaded in Animal House, Delhi Institute of Pharmaceutical Science and Research, New Delhi. The experimental protocol was approved by Institutional Animal Ethics Committee (protocol No. I.A.E.C/2009-I/ProtNo 03, I.A.E.C/2009-I/ Prot. No 05 and I.A.E.C/2011-I/ Prot. No 11).

Laboratory conditions

The rats were housed in a group of three in a single clean plastic cage with a metal frame lid on its top. The animal house is maintained under normal controlled conditions. Pelleted food (Golden feed, Mehrauli, New Delhi) and filtered tap water was made available ad libitum.

Bedding

In a present study we provided clean paddy husk bedding. Bedding was changed every day to maintain proper hygienic conditions.

The pharmacological studies were done under the following heads:

B.1.a. Evaluation of anti-inflammatory activity
   i. Carrageenan induced rat hind paw edema method.
   ii. Xylene induced topical Anti-inflammatory activity.
   iii. Cotton pellet induced granuloma method.
   iv. Estimation of plasma level of proinflammatary cytokines on E.coli LPS treated rats by ELISA method
      a) Estimation of Tumor Necrosis Factor-alpha (TNF-α)
      b) Estimation of Intraleukin-1β (IL-1β)

B.1.b. Evaluation of analgesic activity
   i. Acetic acid induced writhing method

B.1.c. Evaluation of anti-arthritic activity
   i. Formaldehyde induced arthritic method

B.2. Evaluation of antimicrobial activity
   i. Antibacterial activity
   ii. Antifungal activity

B.3. Histopathological studies
Chapter 3. Experimental

B.1.a. EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

B.1.a.i. Carrageenan induced rat hind paw edema method

Selection of experimental animals

The experiment was carried out on healthy *Wistar* albino rats of either sex weighing between 150-200 g.

Drugs and dose

All the standard and test drugs were suspended with 0.5% w/v carboxy methyl cellulose (CMC).

Control group: 0.5% w/v CMC (10 mL/kg) p.o.

Standard group: Indomethacin (10 mg/kg) p.o.\textsuperscript{159-160}

Test group: Test drugs (100 mg/kg) p.o.

Phlogistic agent

A volume of 0.1 mL, 1% w/v freshly prepared suspension of carrageenan in 0.9% w/v sodium chloride solution was used as phlogistic agent to induce swelling or inflammation in the rat paw.

Route of administration

All the drugs or standard or vehicle were administered to rats by oral route while 0.1 mL of 1% w/v carrageenan solution was injected subcutaneously (s.c.) under the sub planter region of right hind paw of the rats.

Procedure

Healthy *Wistar* albino rats of either sex weighing between 150-200 g were divided into 23 groups comprised of 6 animals each. The animals were fasted over night before administering the drug but water was provided *ad libitum*. The rats were marked on the right hind paws just beyond tibiotarsal junction to ensure constant dipping in the saline solution upto fixed mark. The initial paw volume of each rat was noted by saline displacement method using Plethysmometer (Ugo Basile, Italy). Carboxy methyl cellulose (0.5% w/v, CMC) was administered orally at the dose of 10 mL/kg to animals in group I, which served as a control. The standard drug Diclofenac was administered orally at the dose of 10 mg/kg to standard group animals, which served as a standard. Test drugs at the dose of 10 mg/kg were administered orally to test group. One hour after the drug treatment, 0.1 mL of 1% w/v carrageenan suspension in saline was injected into subplantar region of the right hind paw of each rat. The paw volumes of the rats were measured at 0, 1, 2, 3, 4 and 6 h intervals after
the injection of the carrageenan. The % inhibition of edema was calculated using the following formula.\footnote{161}

\[
\% \text{ Inhibition of edema} = 1 - (V_t / V_c) \times 100
\]

Where, \( V_t = \) mean relative change in paw volume in test group

\( V_c = \) mean relative change in paw volume in control group

*Statistical analysis*

All the results were expressed as mean \( \pm \) standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.

**B.1.a.ii. Xylene induced Topical anti-inflammatory activity\footnote{162,163}**

*Selection of experimental animals* The experiment was carried out on healthy *Wistar* albino mice of either sex weighing between 18-24g.

*Drugs and dose* All the standard and test drugs were suspended in acetone

- **Control group**: 0.5% w/v CMC (10 mL/kg)
- **Standard group**: Indomethacine (10 mg/kg)
- **Test group**: Test drugs (10 mg/kg)

*Phlogistic agent*

A volume of 0.1 mL of freshly prepared 1% w/v xylene in acetone solution was used as phlogistic agent to induce swelling or inflammation in the rat ear

*Route of administration: Xylene and drug both* applied topically

*Procedure*

Healthy *Wistar* albino mice of either sex weighing between 18-24g were divided into group of 6 animals each. Carboxy methyl cellulose (0.5% w/v, CMC) was administered orally at the dose of 10 mL/kg to animals in group I, which served as a control. Inflammation is induced by topical application of 0.1 mL of 1% w/v xylene in acetone (10 per ear) on both surfaces of right ear. The synthesized compound 0.5mg dissolved in 20 acetone or 80% ethanol will be applied on each ear 30 minutes before the inflammatory agent xylene is applied. The thickness of the ear will be measured using a vernier caliper, prior the experiment and 30 minutes after the induction of inflammation. Percentage inhibition will be calculated using the following formula.

\[
\% \text{ Inhibition of edema} = 1 - (T_t / T_c) \times 100
\]

Where, \( T_t = \) mean relative change in ear thickness in test group

\( T_c = \) mean relative change in ear thickness in control group
Chapter 3. Experimental

Statistical analysis
All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.

B.1.a.iii. Cotton pellet induced granuloma method

Selection of experimental animals
The experiment was carried out on healthy male Wistar albino rats weighing between 150-200 g.

Drugs and dose
All the standard and test drugs were suspended with 0.5% w/v carboxymethyl cellulose (CMC).

Control group: 0.5% w/v CMC (10 mL/kg) p.o.
Standard group: Indomethacin (10 mg/kg) p.o.\textsuperscript{166}
Test group: Test drug (10 mg/kg) p.o.

Procedure
Healthy male Wistar albino rats weighing between 150-200 g were divided into 4 groups comprised of 6 animals each. The animals were fasted over night before administering the drug but water was provided ad libitum. The rats were anesthetized with anesthetic ether and sterile cotton pellets (10±1 mg) were inserted in the axilla region of each rat through a single needle incision. The test drug (10 mg/kg), Indomethacin (10 mg/kg) and vehicle 0.5% CMC (10 mL/kg) were administered orally for seven consecutive days from the next day of cotton pellet implantation. The animals were anesthetized on eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The moist pellets were weighed and dried at 60°C for 24 h, after that the dried pellets were weighed again. Increment in the dry weight of the pellet was taken as measure of granuloma formation. The percentage inhibition of granuloma formation was calculated using the following formula.

\[
\% \text{ Inhibition of granuloma} = 1- \left( \frac{W_t}{W_c} \right) \times 100
\]

Where, \( W_t \) = mean weight of the test cotton pellet
\( W_c \) = mean weight of the control cotton pellet

Statistical analysis
All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.
B.1.a.iv. Estimation of plasma level of proinflammatory cytokines on E.coli LPS treated rats by ELISA method. 167-169

Selection of experimental animals

The experiment was carried out on healthy Wistar albino rats of either sex weighing between 220-250 g.

Drugs and dose

All the standard and test drugs were suspended with 0.5% w/v carboxy methyl cellulose (CMC).

Control group: 0.5% w/v CMC (10 mL/kg) p.o.

Standard group: Indomethacin (10 mg/kg) p.o

Test group : Test drugs (10 mg/kg) p.o.

Route of administration

The control and test drugs were given by oral route while 500 μg/kg suspension of Escherichia coli Lipopolysaccharide (E-colii 0128:B12 LPS, Sigma Aldrich) in saline was injected intraperitoneally (i.p) to all rats.

Procedure

Healthy Wistar albino rats of either sex weighing between 220-250 g were divided into 4 groups comprised of 6 animals each. The animals were fasted over night before administering the drug but water was provided ad libitum. Carboxy methyl cellulose (0.5% w/v CMC) was administered orally at the dose of 10 mL/kg to animals in group I, which served as a control. The standard drug Indomethacin was administered orally at the dose of 10 mg/kg to group II animals, which served as standard. Test drugs at the dose of 10 mg/kg were administered orally to group III and IV. One hour after the drug treatment E-colii, LPS was injected intraperitoneally (i.p.) at a dose of 500 μg/kg in normal saline to all rats. Blood samples were collected at 0, 30, 60, 90 and 120 min after LPS administration into eppendorf tubes containing 0.1 mL of Acid citrate dextrose (ACD) solution. One hour after blood collection, the blood samples were subjected for cooling centrifugation at 4000 rpm for 15 min at 4°C. Plasma were separated and kept at -20 ℃ until TNF-α,IL-1β,IL-6 analysis was performed. The plasma levels of TNF-α, IL-1β IL-6 were estimated using rat enzyme linked immunosorbent assay (ELISA) kits; IL-TNF-α (ELISA) kit( (Diaclone, France), IL-1β (ELISA) kit(RayBio,Georgia),IL-6 (ELISA) kit(RayBio,Georgia) as per the protocol provided by the manufacturer.
B.1.a.iv(a) Estimation of Tumor Necrosis Factor-alpha (TNF-α)

Principle

Rat TNF-α kit is used and it is based on solid phase sandwich enzyme linked immunosorbent assay method. A polyclonal antibody specific for rat TNF-α has been coated onto the wells of the micro titer strips provided. Samples including standards of known rat TNF-α concentrations and unknowns are pipetted into these wells. During the first incubation, the rat TNF-α antigen and a biotinylated polyclonal antibody specific rat TNF-α were simultaneously incubated. After washing, the enzyme (Streptavidin–Peroxidase) was added. After incubation and washing to remove the unbound enzyme, a substrate solution, which was acting on the bound enzyme, was added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of rat TNF-α present in the samples.

Assay method for TNF-α

Reagents

- Washing buffer
- Standard diluent buffer
- TNF-α standard
- Preparation of biotinylated anti TNF-α
- Streptavidin-HRP
- TMB substrate
- H₂SO₄ Stop reagent

All the reagents were mixed thoroughly without foaming. Standard diluent was prepared at various concentrations and added to standard wells. 100 μL of appropriate standard diluent in duplicate was added to the blank wells. 100 μL of sample (plasma) in duplicate was added to the designed sample wells. Control vial was reconstituted with appropriate standard diluent and 100 μL in duplicate was added to control wells. 50 μL of dil biotinylated anti TNF-α was added to all wells. All the wells were covered with a plate cover and incubated at room temperature (18°C to 25°C) for 3 h. The cover was removed and the plate was washed as follows: a. The liquid was aspirated from each well and washed with 0.3 mL of washing buffer for two times. 100 μL of Streptavidin-HRP solution was added to all wells, including blanks. The plate was covered and incubated again at room temperature (18°C to
Chapter 3. Experimental

25°C for 3 h. The cover was removed and all the wells washed with 0.3 mL of washing buffer as mentioned earlier. 100 μL TMB substrate solution was added to all wells, including blank and incubated in dark place for 15 min at room temperature. The enzyme-substrate reaction was stopped by quickly adding 100 μL of H₂SO₄ stop reagent into each well, including the blank too completely and uniformly to inactive the enzyme. The results were red immediately after adding stop reagent on an ELISA reader (Awareness) using 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength.

The average absorbance values for each set of duplicate standards, samples and controls were calculated. Standard curve was plotted using the mean optical density against the TNF-α standard concentration. The mean absorbance values for each sample were used to determine the corresponding concentration of TNF-α in pg/mL from the standard curve.

Statistical analysis

All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.

B.1.a.iv (b) Estimation of plasma level of IL1-beta on E.coli LPS treated rats by ELISA method 170-171

Principle- Rat IL1-beta kit is used and it is based on solid phase sandwich enzyme linked immunosorbent assay method. A polyclonal antibody specific for rat IL1-beta has been coated onto the wells of the micro titer strips provided. Samples including standards of known rat IL1-beta concentrations and unknowns are pipetted into these wells. During the first incubation, the rat IL1-beta antigen and a biotinylated polyclonal antibody specific rat IL1-beta were simultaneously incubated. After washing, the enzyme (Streptavidin–Peroxidase) was added. After incubation and washing to remove the unbound enzyme, a substrate solution, which was acting on the bound enzyme, was added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of rat IL1-beta present in the samples.

Assay method for IL1-beta

Reagents

- IL-1 beta microplate coated with anti-rat IL-1 beta
Washing buffer
IL1-beta standard
Assay Diluant A
Assay Diluant B
Detection antibody IL-1beta - biotinylated anti IL-1beta
HRP-Streptavidin
TMB substrate
H₂SO₄ Stop reagent

All the reagents were mixed thoroughly without foaming.

**Standard preparation**: briefly spin the vial containing IL1-beta standard(Item C) given in kit and then add 400µl assay diluent A to prepare 50,000 pg/ml standard. Pipette 260 µl assay diluent A into each tube. 50,000pg/ml standard solution is used for dilution series. Mix each tube thoroughly before transfer by gentle vortex. Assay Diluent A serves as zero standard(0pg/ml)

**Biotenated anti IL1-beta**–Briefly spin the detection antibody vial before use. Add 100µlof Assay Diluent B into the vial to prepare a detection antibody concentrate

**HRP syreptavudine**–Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use.HRP-streptavudin concentrate should be dild to 20,000 fold with assay diluents B

**Assay Procedure**: All the reagents and samples are brought at room temp (18-25 C) before use. Add 100 µl of each sample and standard into appropirate walls. Cover well and incubate for 2.5 hours at room temp or overnight at 4ºC with gentle shaking. Discard the solution and wash 4 times with wash solution. Invert the plate and blot it against clean paper towels. Add 100 µl of biotinated antibody to each well. Incubate for 1 Hour at room temperature with gentle shaking. Discard the solution. Wash with wash buffer. Add 100 µl prepared Steptavudin solution to each well including blanks. The plate was covered and Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution and wash with washing buffer. Add 100 µl of TMB one step substrate reagent to each well. Incubate for 30 minutes at room temp in the dark. Add 50 µl of stop solution to each well to completely and uniformly to inactive the enzyme. Read at 450 nm immediately

The average absorbance values for each set of duplicate standards, samples and controls were calculated. Standard curve was plotted using the mean optical density against the IL1-beta standard concentration. The mean absorbance values for each
sample were used to determine the corresponding concentration of IL1-beta in pg/mL from the standard curve.

The average absorbance values for each set of duplicate standards, samples and controls were calculated. Standard curve was plotted using the mean optical density against the IL1-beta standard concentration. The mean absorbance values for each sample were used to determine the corresponding concentration of IL1-beta in pg/mL from the standard curve.

**Statistical analysis**

All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.

**B.1.b. Evaluation of analgesic activity**

Acetic acid induced writhing method.

The peripheral analgesic activity was assessed by acetic acid induced writhing method.  

**Selection of experimental animals**

The experiment was carried out on Swiss albino mice of either sex weighing between 25-35 g. Each mice was given 10 mL/kg animal body weight of 0.6% v/v acetic acid intraperitoneally (i.p.) and was settled singly into a cage made of transparent acrylic resin. Only mice showing writing syndromes within 5 min after acetic acid injection were used for the experiment.

**Drugs and dose**

All the doses of standard and test drugs were prepared in 0.5% w/v carboxy methylcellulose (CMC), which served as drug vehicle.

- **Control group:** 0.5% w/v CMC (10 mL/kg) p.o.
- **Standard group:** Aspirin (100 mg/kg) p.o.
- **Test group:** Test drug (100 mg/kg) p. o.

**Writhing inducing agent**

A 0.6% v/v solution of acetic acid in distilled water was used as writhing inducing agent at the dose of 10 mL/kg.

**Route of administration**

The control, standard and test drugs were given by oral route while 0.6% v/v acetic acid solution was injected intraperitoneally (i.p.) to all mice.
**Chapter 3. Experimental**

**Procedure**

Healthy *Swiss* albino mice of either sex weighing between 25-35 g were divided into 23 groups comprised of 6 animals each. All the animals were fasted over night prior to the test but water was provided *ad libitum*. Carboxy methyl cellulose (0.5% w/v CMC) was administered orally at the dose of 10 mL/kg to animals in group I, which served as a control. The standard drug Asprin was administered orally at the dose of 100 mg/kg to group II animals, which served as standard. Test drugs at the dose of 100 mg/kg were administered orally to group III-XXIII. One hour after the drug treatment writhing response was induced by an intraperitoneal injection (i.p.) of 0.6% v/v acetic acid solution. The mice were placed in separate boxes under observation immediately after acetic acid injection and the number of abdominal constrictions was counted over a period of 20 min. The mean writhing scores were calculated in control, standard and test groups. The percentage analgesic activity was calculated for standard and test compounds treated animals using the following formula.\(^{192}\)

\[
\% \text{ Analgesic activity} = \frac{(n-n')}{n} \times 100
\]

Where,
\(n\) = mean number of writhes of control group
\(n'\) = mean number of writhes of test group

**Statistical analysis**

All the results were expressed as mean ± SEM. The statistical evaluation was performed using ANOVA followed by Dunnett’s multiple comparisons test (Graph Pad Prism, Version 3.06, 32 bit for windows, Graph Pad software, Inc., California, and U.S.A.).

**B.1.c. Evaluation of anti arthritic activity**

**Formaldehyde induced arthritis method.**\(^{174}\)

**Selection of experimental animals**

The experiment was carried out on healthy male *Wistar* albino rats weighing between 150-200 g.

**Drugs and dose**

All the standard and test drugs were suspended with 0.5% w/v carboxy methyl cellulose (CMC).

Control group: 0.5% w/v CMC (10 mL/kg) p.o.
Chapter 3. Experimental

Standard group: Diclofenac (10 mg/kg) p.o.

Test group: Test drugs (10 mg/kg) p.o.

Phlogistic agent

A volume of 0.1 mL, 1% w/v freshly prepared suspension of formaldehyde in 0.9% w/v sodium chloride solution was used as phlogistic agent to induce swelling or inflammation in the rat paw.

Route of administration

All the drugs or standard or vehicle were administered to rats by oral route while 0.1 mL of 1% w/v formaldehyde solution was injected subcutaneously (s.c.) under the sub planter region of right hind paw of the rats.

Procedure

Healthy male Wistar albino rats weighing between 150-200 g were divided into groups comprised of 6 animals each. The animals were fasted over night before administering the drug but water was provided ad libitum. The rats were marked on the right hind paws just beyond tibiotarsal junction to ensure constant dipping in the saline solution upto fixed mark. The initial paw thickness of each rat was noted by using Vernier callipers. Carboxy methyl cellulose (0.5% w/v, CMC) was administered orally at the dose of 10 mL/kg to animals in group I, which served as a control. The standard drug Diclofenac was administered orally at the dose of 10 mg/kg to group II animals, which served as a standard. Test drugs at the dose of 10 mg/kg were administered orally to all test groups. One hour after the drug treatment, 0.1 mL of 1% w/v formaldehyde suspension in saline was injected into sub plantar region of the right hind paw of each rat. The paw volumes of the rats were measured at day 1 to day 10 after the injection of the formaldehyde. The % inhibition of edema was calculated using the following formula.

\[ \% \text{ Inhibition of edema} = 1 - \frac{V_t}{V_c} \times 100 \]

Where, \( V_t = \text{mean relative change in paw thickness in test group} \)

\( V_c = \text{mean relative change in paw thickness in control group} \)

Statistical analysis

All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test.
B.2. EVALUATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity was evaluated by tube dilution method, which depends upon the inhibition of growth of a microbial culture in a uniform solution of antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic. In this method minimum inhibitory concentration (MIC) of the antimicrobial agent is determined. The MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of the test organism.\textsuperscript{175-177}

B.3.i. Evaluation of antibacterial activity.

The synthesized compounds were evaluated for the \textit{invitro} antibacterial activity against \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, (Gram-positive) and \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} (Gram-negative).

\textbf{Preparation of standard and test solution}

Test compounds and standard drug (Ciprofloxacin) were dissolved in dimethyl sulfoxide to give a concentration of 10 $\mu$g/mL.

\textbf{Preparation of double strength nutrient media}

\textbf{Formula}

\begin{itemize}
  \item Peptone – 1 g
  \item Yeast - 0.3 g
  \item Sodium chloride – 0.5 g
  \item Distilled water – 50 mL
\end{itemize}

The solid ingredients were dissolved in water and the media was sterilized by autoclaving at 15-lb/Psi pressure for 15 min.

\textbf{Preparation of suspension of microorganisms}

Suspension of each microorganism was made by transferring the organism from culture to 10 mL of sterile normal saline solution.

\textbf{Determination of minimal inhibitory concentration}

One mL of 10 $\mu$g/mL test solution in dimethylsulfoxide (DMSO) was transferred to a sterile test tube containing 1ml of sterile nutrient media and serially diluted to give a concentration of 5, 2.5, 1.25, 0.625, 0.312 $\mu$g/mL. To all the tubes 0.1 mL of suspension of bacteria in saline was added and the tubes were incubated at 37
C for 24 h. The growth in the tube was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity. From the MIC values observed the intermediate concentrations between MIC values were prepared and the accurate MIC values were determined. The procedure was performed for four bacterial species for 40 test compounds and activity was compared with standard Ciprofloxacin.


The synthesized compounds were evaluated for the in vitro antifungal activity against Candida albicans and Aspergillus niger similar to antibacterial activity assay method by use of Sabouraud’s glucose broth as media. The inoculated tubes were incubated for 48 h for Candida albicans and 7 days for Aspergillus niger. The procedure was performed for both the fungal species for 40 test compounds and activity was compared with standard (Fluconazole).

Preparation of Sabouraud’s glucose broth

Formula

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>8 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>2 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

Glucose and peptone were dissolved in distilled water with aid of heating. Then the medium was cooled and filtered, pH was adjusted to 5.4 with 10% lactic acid. The media was sterilized by autoclaving at 15-lb/Psi pressure for 15 min.

B.3. Histopathological studies

After chronic inflammation studies the animals showing the most potent activity were sacrificed and the stomach, heart and liver of the animals were separated and were kept in 10% formaldehyde solution and in finely cut, stained and fixed. The histopathological slides were observed under under binocular microscope (10x and 40x).
C. QSAR STUDIES

Data set

In the present work, the anti-inflammatory and analgesic activity of benzothiazine derivatives (JS21-JS40) was subjected to MLR analysis with their physicochemical properties.

Descriptor generation

The numerical descriptors responsible for encoding structural features of the molecules can be categorized as hydrophobic, geometric, electronic and topological characters. The structures of JS21-JS40 were first pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in Hyperchem 6.0, Hypercube, Inc., Florida, 1993 and the resulting geometries are further refined by means of the semiempirical method PM3 (parametric Method-3). We chose a gradient norm limit of 0.01 kcal/A˚ for the geometry optimization. The different molecular descriptors (independent variables) like log of octanol–water partition coefficient (log P), molar refractivity (MR), Kier’s molecular connectivity (nΩ, nΩv), Randic topological index (R), Balaban topological index (J), Wiener topological index (W), Total energy (Te) and dipole moment (μ) calculated for the synthesized benzothiazine derivatives (JS21-JS40) using the software TSAR 3D Version 3.3, Oxford Molecular Limited, 2000. Since, there were a large number of descriptors for each compound, we used Pearson’s correlation matrix as a qualitative model, in order to select the suitable descriptors for MLR analysis. The stepwise multiple linear regression procedure was used for model generation. The stepwise addition method implemented in the SPSS software package (SPSS for windows, Version 10.05 1999) was used for choosing the descriptors contributing to the biological activities. Further, the regression analysis was performed using the SPSS software package, Version 10.05, SPSS Inc., Bangalore, India, 1999.

3.2. Cross validation

The predictive powers of the equation were validated by determination of cross-validated r2 (q2) using leave one out (LOO) cross-validation method [13], where a model is built with N- 1 compounds and the Nth compound is predicted. Each compound is left out of the model derivation and predicted in turn.