## 3.1. RATIONALE & OBJECTIVE

Taking a cue from the reported biological profile as potential anti-inflammatory and analgesic agent including selective COX-2 inhibitory potential, the 1,2,4-triazine and 1,3,4-oxadiazole nucleus were initially selected as the framework for designing the new hybrids. Moreover, accumulating lines of evidence suggested that hybridisation of two or more diverse bioactive molecules with corresponding pharmacophore functions or different mechanisms of action rendered synergistic effects contributing to the overall activity profile of the molecule. Using the above-mentioned approach of molecular hybridisation, it was decided to swap the 1,3,4-oxadiazole nucleus with its bioisosteric compliment ring system of 1,3,4-thiadiazole and 1,2,4-triazole followed by the assessment of the effects of such substitution on the selective COX-2 inhibition profile of the compounds.

Based on the above considerations, the design and synthesis of some new 5,6-diphenyl-1,2,4-triazine-3(2*H*)-ones assembled into a structural hybrid with the 5-substituted 1,3,4-oxadiazole/thiadiazole or 1,2,4-triazole nucleus were envisaged so as to exploit their plausible synergistic COX-2 inhibitory activities. The hybrids were designed in conformation with the structural prerequisites for selective COX-2 inhibitory activity.

The fundamental feature consisted of a diaryl triazine moiety which is reported to play a crucial role in anchoring the molecule within the COX-2 active site through hydrophobic interaction. The diaryl moiety was attached to the fifth, and the sixth position of a central 1,2,4-triazine ring which resulted into a diaryl heterocyclic skeleton as observed in selective COX-2 inhibitors from the diaryl heterocycle series (e.g. celecoxib, rofecoxib, valdecoxib, and etoricoxib). This basic skeleton was then hybridised *via* a methylene linker with substituted five-membered heterocycles with the aim of imparting flexibility to the overall hybridised structure (**Fig. 3.1**).

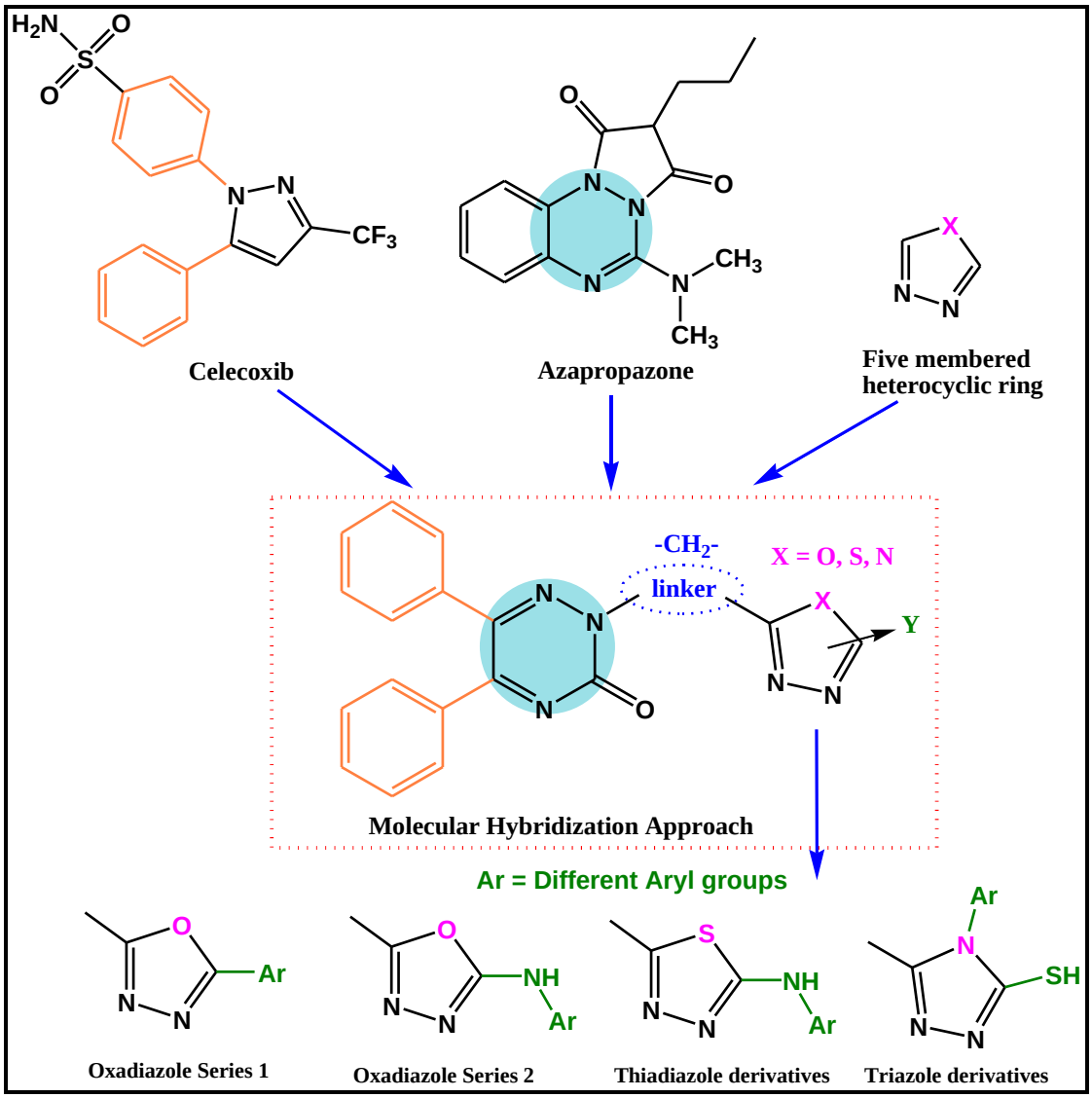


**Figure 3.1.** The design strategy for the proposed compounds under Series 1 and Series 2 using molecular hybridisation approach.

## 3.2. PLAN OF WORK

A brief outline of the research work carried out is summarised as follows:

### 3.2.1. SYNTHESIS

- **SERIES 1:** Synthesis of 5,6–diphenyl–1,2,4–triazine–3(2*H*)–ones bearing 5–substituted 1,3,4–oxadiazole.
- **SERIES 2:** Synthesis of 5,6-diphenyl-1,2,4-triazine-3(2*H*)-ones bearing five membered (1,3,4-oxadiazole/thiadiazole, 1,2,4-triazole) heterocyclic moieties.

### 3.2.2. Characterisation of the Synthesised Compounds

- Physicochemical characterisation including melting point, TLC analysis ($R_f$ value) and partition coefficient (Log P).
- Structural confirmation by FTIR, $^{1}H$ NMR, $^{13}C$ NMR and Elemental (CHN) analysis.

### 3.2.3. BIOLOGICAL ACTIVITY

**A) Evaluation of anti-inflammatory activity**

- Albumin denaturation assay
- Acute oral toxicity studies
- Carrageenan-induced rat paw oedema
- Arachidonic acid-induced rat paw oedema
- Cotton pellet-induced granuloma in rats
- Freund complete adjuvant-induced arthritis in rats
- Evaluation of ulcerogenic liability
- Assessment of gastric, renal and hepatic toxicity

**B) Evaluation of analgesic activity**

- Acetic acid-induced writhing in mice
- Formalin-induced paw licking in mice

**C) *In vitro* COX enzymatic studies**

**D) Evaluation of cardiotoxic liability**

### 3.2.4. COMPUTATIONAL STUDIES

- Molecular docking studies
- Molecular dynamics (MD) studies
- Prediction of drug likeliness and *in silico* ADME properties