In Series 1, a total of fifteen 5,6-diphenyl-1,2,4-triazine-3(2*H*) ones bearing 5-substituted 1,3,4-oxadiazole **(S_14a-S_14o)** embedded within the same pharmacophore matrix were designed. The study intended to investigate the benefits of hybrid pharmacophore approach on the anticipated anti-inflammatory and analgesic activities devoid of undesirable gastric, hepatic and renal side effects associated with the traditional NSAID's. Compounds **S_14a-S_14o** were synthesised in appreciable yield (70-82%) and further characterised by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental (CHN) analysis.

Post-synthesis and structural characterisation, these derivatives were subjected to an initial anti-inflammatory screening using albumin denaturation assay. Based on its outcome, compounds **S_14d**; **S_14e**; **S_14g**; **S_14h; S_14j**; **S_14k; S_14l**; and **S_14o** averting a minimum of 65% protein denaturation were further advanced to *in vivo* acute, sub-chronic and chronic anti-inflammatory screening models.

Compounds **S_14d**; **S_14e**; **S_14g**; **S_14j** and **S_14l** elicited promising anti-inflammatory activity of 59.07%; 58.83%; 57.64%; 55.25% and 55.96% respectively as compared to standard indomethacin (62.64%) by the inhibition of carrageenan-induced oedema in the late phase (at 5 h). The phenyl group attached to the fifth position of the 1,3,4-oxadiazole ring with an electron-donating substituent such as a hydroxyl or amino group exhibited a significant increase in activity **(S_14d** and **S_14e)** compared to electron-withdrawing substituents such as chloro, and nitro groups **(S_14g,** and **S_14l)**. The outcome of this exercise implied their anti-inflammatory mechanism through the inhibition of prostaglandins (PGs).

Derivatives **S_14d** (19.19%); **S_14e** (16.45%); **S_14g** (17.58%); **S_14j** (13.71%); and **S_14l** (12.26) in arachidonic acid-induced oedema bioassay model whose results unveiled that unlike standard zileuton (67.42%), the evaluated compounds did not inhibit LOX biosynthesis. Further evaluation of derivatives **S_14d**, **S_14e, S_14g, S_14j** and **S_14l** on cotton pellet induced granuloma and FCA induced arthritis yielded promising anti-inflammatory activity comparable to standard indomethacin with negligible gastric, hepatic and renal toxic liability.

Further assessment of compounds **S14d**, **S14e, S14g, S14j** and **S14l** on *in vivo* analgesic models reiterated their analgesic potential [70.21-74.84% inhibition in abdominal writhes compared to indomethacin (77.28%)]. The evaluated derivatives exhibited analgesic effect through peripheral nociception by the inhibition of paw licking in phase II of formalin bioassay model ranging between 74.38-78.04% as compared to 10.59-13.68% inhibition in phase I which is attributed to the elevation in pain threshold by inhibition of COX-2.

Post evaluations of their *in vivo* anti-inflammatory and analgesic efficacies, derivatives **S14d**, **S14e, S14g, S14j** and **S14l**, were subjected to *in vitro* COX inhibition assay to gain insight on their relative inhibitory efficacy on the COX isozyme. The outcome of this exercise confirmed their preferential selectivity towards the inhibition of COX-2 (IC_{50}: 3.07-3.23 μM) over COX-1 (IC_{50}: 43.29-44.93 μM) with COX-2 selectivity index ranging between 13.91-14.16. The enzyme kinetics study of these potential derivatives helped to shed light on their competitive nature of COX-2 inhibition.

In silico docking study of the potential compounds, **S14d** and **S14e** contributed to shed light on their binding pose with different essential amino acids within the COX-2 active site of protein 1CX2. Further, the molecular dynamics (MD) simulation of the most potent compound **S14d** (Glide Score: -9.05) helped to adequately understand the role of active site hydration that prevails under normal physiological conditions which helped in better interpretation of its biological profile.

Finally, *in silico* estimation of drug-like properties was calculated using the online version of Molinspiration® software. Results indicated that none of the evaluated compounds violated the Lipinski's rule of five. The results also suggested that theoretically, the potential compounds do elicit a good oral absorption and could be promising candidates for further development.

In continuation with the "hybrid-pharmacophore" concept and pursuance in the development of newer and safer anti-inflammatory agents, the 5-substituted 1,3,4-oxadiazole nucleus clubbed with the 5,6-Diphenyl-1,2,4-triazin-3(2*H*) one were designed. Further, by invoking the philosophy of bioisosterism, this 1,3,4-oxadiazole nucleus was swapped with its bioisosteric

replacement of two different five-membered heterocycles such as 1,3,4-thiadiazole and the 1,2,4-triazole ring followed by the assessment of their relative effects on the selective COX-2 inhibitory profile of the compounds. The target compounds of Series 2 **($S_2$2a-$S_2$2e; $S_2$3a-$S_2$3e and $S_2$4a-$S_2$4e)** were synthesised from a common acetohydrazide precursor **($S_1$3)** which reacted with different aryl isothiocyanates to yield the corresponding aryl thiosemicarbazide derivatives **($S_2$1a-$S_2$1e)**. These thiosemicarbazide intermediates were cyclised under different conditions to afford the final fifteen compounds **($S_2$2a-$S_2$2e, $S_2$3a-$S_2$3e** and **$S_2$4a-$S_2$4e)**. The structure of the novel compounds was supported by FT-IR, ^{1}H-NMR, ^{13}C-NMR and elemental (C, H, N) analysis.

The derivatives were initially screened for *in vitro* anti-inflammatory potential by albumin denaturation assay. Compounds **($S_2$2c-$S_2$2e**, **$S_2$3c-$S_2$3e**, **$S_2$4d** and **$S_2$4e)** eliciting a minimum of 70% protection of protein denaturation comparable to standard drugs indomethacin (82.67%) and celecoxib (80.29%) were further assessed *in vivo* for anti-inflammatory, ulcerogenic and lipid peroxidation activities.

Of the fifteen synthesised derivatives, six compounds namely **$S_2$2c-$S_2$2e** (comprising of the substituted 1,3,4-oxadiazole nucleus) and **$S_2$3c-$S_2$3e** (comprising of the substituted 1,3,4-thiadiazole nucleus) exhibited comparable anti-inflammatory potential in the acute, sub-chronic and chronic models of inflammation together with negligible ulcerogenic potential (UI index 20-24) as compared to indomethacin (UI index 55) and celecoxib (UI index 26). These derivatives also showed reduced malondialdehyde (MDA) content (4.18-5.02 nmol for MDA content per 100 mg tissue) compared to indomethacin (8.38 nmol for MDA content per 100 mg tissue) and celecoxib (6.82 nmol for MDA content per 100 mg tissue). It suggested their protective effects to the inhibition of lipid peroxidation in the gastric mucosa. Additionally, these derivatives were also devoid of hepatic and renal side-effects as analysed by various serum biochemical parameters.

Evaluation of compounds **$S_2$2c-$S_2$2e** and **$S_2$3c-$S_2$3e** on *in vivo* analgesic models re-affirmed their analgesic potential [67.44-70.82% inhibition respectively in abdominal writhes as compared to indomethacin (75.67%) and

celecoxib (73.23%)]. Further evaluation reiterated their analgesic effect through peripheral nociception by inhibition of paw licking by 70.04-80.02% respectively in phase II of formalin bioassay model as compared to 11.87–14.42% inhibition in phase I. The analgesic effect of the evaluated compounds was attributed to the elevation in pain threshold by inhibition of COX-2.

In the *in vitro* COX inhibition assay and enzyme kinetics study, derivatives **$S_2$2c-$S_2$2e** and **$S_2$3c-$S_2$3e** were proved to be potent competitive COX-2 inhibitors with an IC_{50} range of 0.60-1.11 μM with significantly improved COX-2 selectivity index (56.03-104.97). Hybrids comprising of 5,6-diaryl-1,2,4-triazine ring bearing a 1,3,4-oxadiazole nucleus **($S_2$2c-$S_2$2e)** were marginally more efficient selective COX-2 inhibitors as compared to their 1,3,4-thiadiazole **($S_2$3c-$S_2$3e)** counterpart.

Based on their potent and selective nature of COX-2 inhibition, an additional study was performed to assess the cardiotoxic liability of the compounds **$S_2$2e** and **$S_2$3c** along with standard celecoxib. Cardiotoxic liability was evaluated by measuring the serum levels of biomarkers such as cardiac troponin I (cTnI) and Creatine kinase-MB (CK-MB) which are useful in establishing the diagnosis of myocardial infarction on isoproterenol induced myocardial infarcted rats. The outcome of the above study confirmed the cardiac safety of the promising derivatives **$S_2$2e** and **$S_2$3c**.

The binding modes of the compounds **$S_2$2c**; **$S_2$2d and $S_2$2e** into the COX-2 binding site of protein 3LN1 through docking studies followed by molecular dynamics (MD) simulation of the most potent compound **$S_2$2e** (Glide Score: -11.95) exemplified their consensual interaction and subsequent inhibition of enzyme thus corroborating with the outcomes of *in vitro* and *in vivo* biological evaluation. The *in silico* studies also helped to explain the geometric requisites of the ‘‘hybrid-pharmacophore” conducive for COX-2 inhibition which was absent in compounds **$S_2$4a-$S_2$4e**.

Prediction of ‘‘drug-likeliness” for the most active compounds was carried out using QikProp module of Schrödinger Maestro 10.5.014 wherein based on the evaluation of various *in silico* predicted parameters indicated that the

compounds **($S_2$2c-$S_2$2e** and **$S_2$3c-$S_2$3e)** did elicit "drug-like" characteristics and could be a potential candidate for future development.

In conclusion, compounds of a 1,3,4-oxadiazole ring and its bioisosteric 1,3,4-thiadiazole tethered to a 5,6-Diphenyl-1,2,4-triazin-3(2*H*)-one moiety can serve as a propitious scaffold for the development of newer and safer selective COX-2 inhibitory agents. The molecules have the potentiality for long-term management of inflammation and pain associated with the debilitating condition such as rheumatoid arthritis over the currently available treatments. Given the non-classical role played by COX-2 in the aetiology and progression of several diseases such as colon cancer and alzheimer's disease, further advancement in the design and development of selective COX-2 inhibitors with superior safety profile remains a viable avenue for future investigation.