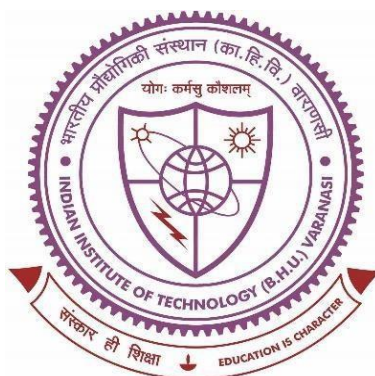


# **Design, Synthesis, and Biological Evaluation of Novel Heterocyclic Compounds as Dual COX-2/5-LOX Inhibitors**



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**By**

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## **Abstract:**

The study reports the design, synthesis, and biological evaluation of a novel series of heterocyclic compounds developed to achieve dual inhibition of COX-2 and 5-LOX enzymes. Computational methods, including molecular docking and dynamics simulations, were used to design the compounds and confirm their binding potential to both enzyme targets. The synthesized compounds were screened for their inhibitory activity, demonstrating moderate to excellent inhibition of both COX-2 and 5-LOX. *In vivo*, studies using rat paw edema models validated the anti-inflammatory efficacy of the lead compounds. These compounds also showed minimal gastrointestinal, liver, and kidney toxicity, and exhibited potential antioxidant properties. Importantly, they were devoid of cardiotoxicity in post-myocardial infarction models. These findings suggest that the novel heterocyclic compounds developed in this study are promising dual COX-2/5-LOX inhibitors, with potential applications in the treatment of inflammatory diseases and cancer, offering an effective and safer alternative to selective inhibitors.

A novel series of 5,6-diphenyl-1,2,4-triazine-3-thiol derivatives were designed, synthesized, and screened for their inhibitory potential against COX-2 and 5-LOX enzymes. The compounds from the series have shown moderate to excellent inhibitory potential against both targets. Compound **6k** showed the inhibitions against COX-2 ( $IC_{50} = 0.33 \pm 0.02 \mu M$ ) and 5-LOX inhibition ( $IC_{50} = 4.90 \pm 0.22 \mu M$ ) which was better than the standard celecoxib ( $IC_{50} = 1.81 \pm 0.13 \mu M$ ) for COX-2 and zileuton ( $IC_{50} = 15.04 \pm 0.18 \mu M$ ) for 5-LOX respectively. Further investigation on the selected derivative **6k** in rat paw edema models revealed significant anti-inflammatory efficacy. Compound **6k** has also shown negligible ulcerogenic liability as compared to indomethacin. Moreover, *in vivo* biochemical analysis also established the compound's antioxidant properties. Compounds **6c** and **6k** were also observed to be devoid of cardiotoxicity post-myocardial infarction in rats. The molecular docking and

dynamics simulation studies of the most active derivative **6k** affirmed their consentient binding interactions with COX-2 specific ravine and cleft of 5-LOX.

Furthermore, the piperazine derivatives were screened using the ChEMBL database, paving the way for the design, synthesis, and evaluation of a novel series of dual COX-2/5-LOX inhibitors and identifying their role in mitigating cancer cell proliferation. Compound **9d** with 4-Cl substitution at the terminal phenyl ring showed promising inhibition of COX-2 ( $IC_{50} = 0.25 \pm 0.03 \mu\text{M}$ ) and 5-LOX ( $IC_{50} = 7.87 \pm 0.33 \mu\text{M}$ ), outperforming the standards celecoxib ( $IC_{50} = 0.36 \pm 0.023 \mu\text{M}$ ) and zileuton ( $IC_{50} = 14.29 \pm 0.173 \mu\text{M}$ ), respectively. The two most active derivatives **9d** and **9g** indicated a significant anti-inflammatory response in the paw edema model by inhibiting PGE<sub>2</sub>, IL-6, and TNF- $\alpha$  and an increase in IL-10 concentrations. Interestingly, **9d** effectively reduced pain by 55.78%, closely comparable to the 59.09% exhibited by the standard indomethacin, and was also devoid of GI, liver, kidney, and cardiac toxicity. Furthermore, **9d** demonstrated anti-cancer potential against *in vitro* A549, COLO-205, and MIA-PA-CA-2 human cancer cell lines and *in vivo* *Drosophila* cancer model. The pharmacokinetic investigations revealed that **9d** has good oral absorption characteristics.

This study successfully developed and evaluated a novel series of heterocyclic compounds as dual COX-2/5-LOX inhibitors. Computational methods confirmed their strong binding potential, while biological assays demonstrated moderate to excellent enzyme inhibition. *In vivo* models validated their anti-inflammatory efficacy, revealing minimal toxicity and potential antioxidant properties, with no cardiotoxicity observed. These findings highlight the promising therapeutic potential of these compounds for treating inflammatory diseases and cancer, providing a safer and effective alternative to selective inhibitors.