

Chapter-6

Summary & Conclusions

6.1 Summary and Conclusion

The study focuses on the development of a novel series of heterocyclic compounds designed to achieve dual inhibition of COX-2 and 5-LOX enzymes, a strategy aimed at addressing the limitations of conventional anti-inflammatory drugs. Using a molecular hybridization strategy combined with computer-aided drug design (CADD) approaches, the compounds were rationally designed to enhance their binding potential to both enzyme targets. Molecular docking and molecular dynamics simulations were employed to evaluate the molecular interactions and stability of the ligand-enzyme complexes. These computational studies provided valuable insights into the structural and energetic aspects of binding, guiding the design and synthesis of hybrid molecules with improved biological activity. The novel series of compounds were successfully synthesized and characterized for confirmation of the structures and purity of the compounds.

The synthesized compounds were systematically screened for their biological activity, with results showing moderate to excellent inhibition of both COX-2 and 5-LOX. This dual inhibition approach is particularly significant, as it targets two key pathways in inflammation, potentially offering greater therapeutic efficacy compared to single-target inhibitors. The *in vitro* findings were corroborated by *in vivo* studies using rat paw edema models, which confirmed the anti-inflammatory efficacy of the lead compounds.

Safety evaluation was a key component of the study, addressing common adverse effects associated with anti-inflammatory drugs. The lead compounds demonstrated minimal toxicity to the gastrointestinal tract, liver, and kidneys in preclinical assessments. Furthermore, they were found to be devoid of cardiotoxic effects, even in post-myocardial infarction models, a significant improvement over some selective COX-2 inhibitors known for their cardiovascular risks. Additional findings revealed that these compounds

also exhibited antioxidant properties, suggesting potential benefits in mitigating oxidative stress, which is often associated with chronic inflammatory conditions.

The dual COX-2/5-LOX inhibitory activity, combined with the favorable safety profile, positions these novel heterocyclic compounds as promising candidates for the treatment of inflammatory diseases. Moreover, the demonstrated safety and efficacy of the compounds suggest potential applications in cancer therapy and chronic inflammation in tumorigenesis. This study underscores the potential of multitarget therapeutic strategies to develop safer and more effective alternatives to current anti-inflammatory drugs, paving the way for further research and clinical development of these compounds.

The first study highlights the design, synthesis, and biological evaluation of a novel series of 5,6-diphenyl-1,2,4-triazine-3-thiol derivatives targeting dual inhibition of COX-2 and 5-LOX enzymes. This dual inhibition approach addresses the limitations of selective inhibitors by modulating key pathways involved in inflammatory processes. The synthesized compounds were systematically screened for their inhibitory potential, revealing moderate to excellent activity against both COX-2 and 5-LOX. Among the derivatives, compound **6k** emerged as the most potent, exhibiting superior inhibition compared to standard drugs, with IC₅₀ values significantly better than celecoxib for COX-2 and zileuton for 5-LOX.

Further, *in vivo* studies validated the therapeutic potential of compound **6k**. In rat paw edema models, it demonstrated significant anti-inflammatory efficacy, highlighting its effectiveness in mitigating acute inflammation. Importantly, compound **6k** showed negligible ulcerogenic liability compared to indomethacin, addressing one of the critical limitations of traditional NSAIDs. Biochemical analyses further established its antioxidant properties, which contribute to reducing oxidative stress associated with

chronic inflammation. Safety profiling of the compounds was also undertaken, with a focus on cardiotoxicity. Notably, compounds **6c** and **6k** were observed to be devoid of cardiotoxic effects in post-myocardial infarction models, a significant improvement over some existing anti-inflammatory drugs. This finding underscores their potential for safer therapeutic application, particularly for patients at risk of cardiovascular complications.

The molecular mechanisms underlying the efficacy of compound **6k** were elucidated through computational studies. Molecular docking demonstrated its stable and specific binding interactions within the COX-2 active site and the cleft of 5-LOX. Molecular dynamics simulations further confirmed the stability of these interactions, supporting its dual inhibitory activity. These computational insights provided a comprehensive understanding of the structure-activity relationship and guided the optimization of the compound. In conclusion, this study presents compound **6k** as a promising dual COX-2/5-LOX inhibitor with potent anti-inflammatory and antioxidant properties, minimal ulcerogenic liability, and a favorable safety profile. These findings establish a strong foundation for further development of this series as multifunctional therapeutic agents for inflammatory conditions.

This second study investigates the development of piperazine derivatives as dual COX-2/5-LOX inhibitors with additional potential for mitigating cancer cell proliferation. Leveraging data from the ChEMBL database, a series of compounds were designed, synthesized, and evaluated for their biological activity. Among these, compound **9d**, featuring a 4-Cl substitution on the terminal phenyl ring, demonstrated exceptional inhibitory activity against COX-2 and 5-LOX enzymes. Its IC₅₀ values surpassed those of standard drugs celecoxib and zileuton, marking it as a highly potent dual inhibitor.

The anti-inflammatory efficacy of the most active compounds, **9d** and **9g**, was validated through *in vivo* studies. In a paw edema model, these compounds significantly reduced inflammation by inhibiting pro-inflammatory mediators such as PGE₂, IL-6, and TNF- α while elevating the anti-inflammatory cytokine IL-10. Additionally, compound **9d** exhibited remarkable analgesic properties, reducing pain levels comparably to the standard drug indomethacin. A critical advantage of **9d** was its favorable safety profile, being devoid of gastrointestinal, liver, kidney, and cardiac toxicity, which are common drawbacks of traditional anti-inflammatory drugs.

Beyond its anti-inflammatory properties, compound **9d** also displayed promising anti-cancer activity. It effectively inhibited the proliferation of A549 (lung), COLO-205 (colon), and MIA-PA-CA-2 (pancreatic) human cancer cell lines *in vitro*. Its anticancer potential was further corroborated by *in vivo* studies using the *Drosophila* cancer model, highlighting its broad therapeutic applications. Pharmacokinetic investigations of compound **9d** revealed favorable oral absorption characteristics, further underscoring its potential as a drug candidate. The combination of potent dual COX-2/5-LOX inhibition, robust anti-inflammatory and analgesic effects, a strong safety profile, and anticancer properties positions compound **9d** as a promising lead for the development of multifunctional therapeutic agents. This study provides a strong foundation for further exploration of piperazine derivatives in the treatment of inflammatory disorders and cancer.

6.2 Scope and future directions

The following outlines the future directions of this research:

- 1. Optimization of Lead Compounds:**

2. Exploration of structural modifications to improve the potency, selectivity, and pharmacokinetic properties of lead compounds (e.g., **6k** and **9d**).

2. Detailed Pharmacokinetic and Toxicological Studies:

- Conduct comprehensive in vivo ADME and long-term toxicity studies to evaluate the drug-likeness and safety of the lead compounds.
- Assess the compounds for their chronic toxicity profiles, ensuring their suitability for prolonged therapeutic use.

3. Preclinical and Translational Studies:

- Advancement of promising compounds to preclinical testing in animal models of chronic inflammation and cancer.
- Exploring their potential in combination therapies to enhance efficacy and minimize resistance.

4. Application in Other Disease Models:

- Assess the efficacy of dual inhibitors in treating neurodegenerative disorders, cardiovascular diseases, and autoimmune conditions, where inflammation plays a critical role.
- Study their role in inhibiting cancer progression and metastasis.

5. Innovative Drug Delivery Approaches:

- Develop advanced drug delivery systems, such as nanoformulations or targeted delivery platforms, to improve bioavailability and minimize systemic side effects.

6. Clinical Development:

- Collaborate with clinical researchers to transition the lead compounds to early-phase clinical trials.

- Identify patient populations that may benefit most from these novel dual inhibitors, particularly those with comorbid inflammatory and oncological conditions.

This research establishes a strong foundation for the development of dual COX-2/5-LOX inhibitors as multitarget therapeutic agents. Future studies will aim to optimize these compounds and evaluate their potential in addressing unmet clinical needs, ultimately contributing to the advancement of safer and more effective treatments for inflammatory and oncological diseases.