Chapter 9

Summary & Conclusion

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NPs inspire the discovery of many therapeutic drugs, offering a broad range of biological activities. Over the past 40 years, about two-thirds of FDA-approved small-molecule drugs have origins related to NPs or their derivatives. Among these, plant-derived cyclic esters, or lactones, stand out due to their abundant presence and diverse activities. Researchers are interested in lactones, especially γ and δ types, for their potential as drugs because of their varied structures. Coumarins, a type of benzenoid δ-lactone from plants, are chemically diverse with different bioactivities. Their modifiable core makes coumarins valuable for drug development. Similarly, γ-lactones from plants offer diverse structures and biological potential, making them important for discovering new drug leads.

The objective of the thesis was to explore the medicinal value of plant-derived naturally-occurring lactones. The focus was to find novel lactone containing-lead compounds from plants with cytotoxic and anti-inflammatory properties. Various methods were used to explore the biological potential of natural lactones, such as computational screenings, phytochemical analysis, semi-synthetic modifications, and pharmacological screening.

Pharmacophore-based virtual screening of coumarins from the COCONUT NP database revealed three promising VEGFR-2 inhibitors: CNP0056360, CNP0340213, and CNP0366287. These hits showed good potential in inhibiting VEGFR-2, a key target in cancer angiogenesis, compared to lenvatinib, an FDA-approved multikinase inhibitor. *In silico* studies indicated that the hits exhibited strong stability and binding affinity with the VEGFR-2 receptor. These results highlight the effectiveness of the identified leads as VEGFR-2 inhibitors, providing a solid basis for developing novel coumarin-based therapies for cancer. Further investigation through detailed *in vitro* and *in vivo* studies could advance the development of these identified leads as potential VEGFR-2 inhibitors.

Molecular modeling of coumestans from *P. corylifolia* against the EGFR protein revealed two promising leads: psoralidin (**1**) and isopsoralidin (**5**), showing strong EGFR inhibitory potential compared to Gefitinib and Erlotinib, known EGFR inhibitors. The ligand-protein complex demonstrated stability and favorable energy profiles. Psoralidin (**1**) also exhibited significant *in vitro* cytotoxicity against MDA-MB-231 and A549 cell lines, with IC_{50} values of 22.21 µM and 23.64 µM, respectively. Given EGFR's high expression in these cell lines, the proposed mechanism of action for Psoralidin's cytotoxicity could likely be EGFR inhibition. However, further detailed studies are needed to confirm this postulation. The identified coumestan lead from *P. corylifolia* could serve as a starting point for developing more effective EGFR inhibitors. Further modifications may lead to potent and efficient EGFR inhibitors for cancer treatment.

Phytochemical analysis of *P. corylifolia* seeds and leaves yielded library of fourteen coumarins, including six previously unreported coumarins (Marmin, Marmesin, Seselin, Heratomine, Heratomol benzoate, and Oxypeucedanin). The cytotoxicity screening on MDA-MB-231 and A549 cell lines identified four potent cytotoxic coumarins i.e., Marmesin, Seselin, Xanthotoxin, and Xanthotoxol with $IC_{50} < 1$ µM. Xanthotoxol was most effective against A549, while Marmesin, Seselin, and Xanthotoxin showed efficacy against MDA-MB-231 cells. Molecular docking and MD simulations demonstrated strong binding and good stability with EGFR, suggesting EGFR inhibition as a potential mechanism. *In silico* results indicate EGFR inhibition as the likely mode of action, yet thorough *in vitro* and *in vivo* studies are required for validation. The findings highlight the potential of coumarins of *P. corylifolia* as promising leads for developing novel cancer therapies, especially targeting EGFR-driven cancer therapies. The potent cytotoxicity of these coumarins prompts further investigation into their efficacy against other cancer types and potential modifications for enhanced effectiveness.

The application of network pharmacology approach for exploring the anti-inflammatory lactones of Sistemat X database of plant secondary metabolite resulted in identification of STX 12273 i.e., santonin as lead anti-inflammatory lactone with COX-2 inhibition as its possible mechanism of action. The computational studies of STX 12273 with COX-2 protein also revealed the stability and energetic favourability of their complex within a simulated biological environment. STX 12273 displayed good *in vitro* COX-2 inhibitory potential with IC_{50} value of 622 nM that also validated the *in silico* findings. The study provided a valuable insight into the putative mechanism of action of five-membered lactone STX 12273 in inflammation and offers a promising lead for further investigation in inflammation treatment.

Semi-synthetic modifications of the lead compound santonin, identified through network pharmacology, resulted in twenty derivatives. These santonin analogs, synthesized with molecular iodine as a catalyst, closely resembled naproxen, a NSAID. *In vitro* COX inhibition assay of the synthesized derivatives revealed analog **12** as a potent COX-2 inhibitor with COX-2 selectivity. Further investigation into its anti-inflammatory potential using *in vivo* studies, showed that **12** significantly reduced provoked and spontaneous pain, attenuated the oxido-nitrosative stress markers without affecting locomotor activity and motor coordination in rats. The findings suggest that developing new NSAID pain relievers targeting selective inhibition could offer safer and more effective treatments for chronic pain. The analog **12** represent a promising and safer lead, alternative to naproxen for patients with chronic inflammation.

The phytochemical investigation of *Vitex negundo* leaves in search of finding novel anti-inflammatory iridoid containing compounds resulted in unexpected isolation of 2,3-Dehydrosilychristin (**7**), a silychristin-derivative and minor silymarin flavonolignan. The *in vitro* and *in vivo* studies suggested the antioxidant, anti-inflammatory and

hepatoprotective potential of **7**, suggesting its role in analgesic properties of Nirgundi leaves. The identification and isolation of silychristin and 2,3-Dehydrosilychristin, respectively from *V. negundo* opens new avenues to explore it as an alternative source of silymarin flavonolignans and could serve as economical option for their large-scale extraction. The study presents *V. negundo* as potential plant species to investigate for diverse biological potentials apart from antioxidant and anti-inflammatory activities.

The work successfully identified natural lactones as potential lead compounds with cytotoxic and anti-inflammatory activities through amalgamation of computational, phytochemical, medicinal, and pharmacological approaches. The identified leads could serve as good starting point for carrying out detailed studies into their biological potential. They could also be utilized as leads for developing more effective and potent derivatives. It also presents, the well explored medicinal plants as a continuous source of novel phytoconstituents.