Chapter 2

Objective and Plan of Work

2 Objective and Plan of Work

2.1 Objective

The lactone-containing secondary metabolite produced by plant are one of the most abundant class presents in nature that exhibit diverse biological activities. Theses natural cyclic esters have gained specific interest amongst researchers as potential leads for drug discovery and development due to presence of many fragments like alkyl, lipophilic, and sulfhydryl groups. Also, ethnopharmacological, traditional, and various other studies have reported the potential of lactones as cytotoxic, anticancer, anti-tumor, and anti-inflamamtory. Owing to the rich structural and pharmacological potential of lactone as therapeutic agents various computational and medicinal chemistry approaches were planned to evaluate them as cytotoxic and anti-inflammatory agents.

2.2 Plan of Work

2.2.1 *In silico* identification of coumarin-based natural compounds as potential VEGFR-2 inhibitors

Objective: Utilize pharmacophore-based high throughput virtual screening approach to identify potential natural coumarins as VEGFR-2 inhibitors using a NP database.

Plan of Work:

- Build a pharmacophoric model of known VEGFR-2 inhibitor based on its important features
- Substructure filter the NP database for filtering only coumarin-containing compounds
- Pharmacophore-based virtual screening of filtered coumarins from database using the developed pharmacophore model to identify promising candidates
- High-throughput virtual screening of shortlisted candidates through docking studies to identify potential hits

- ADMET prediction, molecular dynamic simulation, and binding energy calculations of identified VEGFR-2 inhibitor leads

2.2.2 *In silico* analysis, isolation, and cytotoxicity evaluation of the coumestans from *Psoralea corylifolia* (L.)

Objective: Utilize molecular modeling approaches to screen the coumestan class of compounds from *Psoralea corylifolia* (L.) against Epidermal Growth Factor Receptor (EGFR) protein, followed by isolation and cytotoxic evaluation of identified hit.

Plant of Work:

- Screen the coumestans of *P. corylifolia* to identify the potential EGFR inhibitor through molecular docking studies
- Molecular dynamics simulation and binding free energy calculation studies of identified potential EGFR inhibitors
- Extraction, isolation and purification of the identified hit from the plant part of *P. corylifolia* extract
- Cytotoxicity evaluation of identified isolated hit through MTT assay

2.2.3 Isolation, cytotoxicity, and *in silico* screening of coumarins from *Psoralea corylifolia* (L.)

Objective: To prepare a library of coumarin compounds from *Psoralea corylifolia* (L.) and their cytotoxicity evaluation against different cancer cell lines, followed by the *in silico* validation of the coumarins with potent cytotoxicity against EGFR protein.

Plant of Work:

- Screen the coumestans of *P. corylifolia* to identify the potential EGFR inhibitor through molecular docking studies
- Molecular dynamics simulation and binding free energy calculation studies of identified potential EGFR inhibitors

- Extraction, isolation and purification of the identified hit from the plant part of *P. corylifolia* extract
- Cytotoxicity evaluation of identified isolated hit through MTT assay

2.2.4 Identifying inflammation-related targets of natural lactones using network pharmacology, molecular modeling, and *in vitro* approaches

Objective: Utilize network pharmacology-based approach to identify anti-inflammatory lactones from a NP database and their possible mechanism of action.

Plan of Work:

- Substructure filter of NP database for lactone-containing compounds and their further curation
- Target prediction of selected lactone candidates and construction of Compound-target network. Target matching of lactone candidates with inflammatory genes and construction of protein-protein interaction network
- Gene enrichment analysis of potential targets and selection of final inflammatory targets. Target matching with those of selected compounds
- Docking studies of final lactone with the inflammatory targets and identification of potential lactone hit and its potential targets. Molecular dynamic simulation of potential ligand-protein complex
- Extraction, isolation and purification of identified lactone hit and *in vitro* validation of its target inhibitory potential

2.2.5 Development of naproxen-like analogs from santonin, a sesquiterpene lactone

Objective: Semi-synthetic modifications on the potential anti-inflammatory lactone identified through network pharmacology-based approach. Determination of anti-inflammatory potential of synthesized analogs through *in vitro* enzyme inhibition assay followed by evaluation of *in vivo* anti-inflammatory potential of best analog.

Plan of Work:

- Semi-synthetic modification of identified sesquiterpene lactone and molecular docking studies of synthesized analogs with the target protein
- Molecular dynamic simulation of best analog and target protein complex
- Evaluation of enzyme inhibitory potential of synthesized analogs through *in vitro* assay
- Detailed evaluation of anti-inflammatory potential of best analog through *in vivo* studies

2.2.6 Phytochemical investigation of the *Vitex negundo* (L.) leaves

Objective: Phytochemical exploration of leaf extract of *Vitex negundo* for potential iridoid-lactone containing compound followed by subsequent isolation, purification, characterization, and pharmacological evaluation of phytoconstituents.

Plan of Work:

- Preparation and fractionation of extract of *V. negundo* leaves. Repeated purification of different fractions for isolation of different phytoconstituents
- LC-MS studies of the *V. negundo* leaf extract
- Molecular docking study to identify the potential interactions of ligands with the target proteins
- Detailed *in vitro* and *in vivo* pharmacological evaluation of isolated compound to determine its possible activity