

# Computational and Medicinal Chemistry Approach to Explore the Pharmacological Potential of Naturally-Occurring Lactones



Thesis submitted in partial fulfilment for the  
Award of Degree

**Doctor of Philosophy**

By

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### List of Abbreviations

<b>Abbreviation</b>	<b>Full Form</b>
<b>2D</b>	Two-dimensional
<b>3D</b>	Three-dimensional
<b>A549</b>	Human lung adenocarcinoma cell line
<b>ACN</b>	Acetonitrile
<b>ADMET</b>	Absorption, Distribution, Metabolism, Excretion & Toxicity
<b>Akt</b>	Protein kinase B
<b>ALOX5</b>	Arachidonate-5 lipoxygenase
<b>ALT</b>	Alanine aminotransferase
<b>ANOVA</b>	Analysis of variance
<b>AP-1</b>	Activator protein-1
<b>ASC</b>	Apoptosis-associated speck-like protein
<b>AST</b>	Aspartate aminotransferase
<b>BBB</b>	Blood-brain barrier
<b>BIOS</b>	Biology-oriented synthesis
<b>BRD4</b>	Bromodomain-containing protein 4
<b>Calc.</b>	Calculated
<b>CASP1</b>	Caspase-1
<b>CDK9</b>	Cyclin-dependent kinase 9
<b>CDCl<sub>3</sub></b>	Deuterated chloroform
<b>CD<sub>3</sub>OD</b>	Deuterated methanol
<b>CDP</b>	Consensus diversity plot
<b>CFA</b>	Complete Freund's adjuvant
<b>CHCl<sub>3</sub></b>	Chloroform
<b>CMAUP</b>	Collective molecular activities of useful plants
<b>CNS</b>	Central nervous system
<b>COCONUT</b>	Collection of open natural products
<b>COSY</b>	Correlation spectroscopy
<b>COX</b>	Cyclooxygenase
<b>COX 1</b>	Cyclooxygenase 1
<b>COX-2</b>	Cyclooxygenase 2
<b>CTRC</b>	Chymotrypsin C
<b>CTSG</b>	Cathepsin G
<b>CYP2D6</b>	Cytochrome P2D6
<b>DCM</b>	Dichloromethane
<b>DMEM</b>	Dulbecco's modified eagle medium
<b>DMSO</b>	Dimethyl sulfoxide
<b>DMSO-d<sub>6</sub></b>	Deuterated dimethyl sulfoxide
<b>DNP</b>	Dictionary of natural products
<b>DOGS</b>	De novo design of generic structures
<b>DOS</b>	Diversity-oriented synthesis

<b>Abbreviation</b>	<b>Full Form</b>
<b>DPPH</b>	2, 2-diphenyl-1-picrylhydrazyl
<b>DTNB</b>	5,5'-dithiobis (2-nitrobenzoic acid)
<b>EGFR</b>	Epidermal growth factor receptor
<b>EIC</b>	Extracted ion chromatogram
<b>eNOS</b>	Endothelial nitric oxide synthase
<b>ERK</b>	Extracellular signal-regulated kinase
<b>ERBB2</b>	Erythroblastic oncogene B homolog 2
<b>ESI-MS</b>	Electrospray ionization-Mass spectrometry
<b>EtOAc</b>	Ethyl acetate
<b>F2</b>	Coagulation factor II
<b>FAME</b>	Fast metabolizer
<b>FASTA</b>	Fast adaptive shrinkage threshold algorithm
<b>FAX</b>	Fatty acid export
<b>FBS</b>	Foetal bovine serum
<b>FLT1</b>	Fms related receptor tyrosine kinase 1
<b>GMQE</b>	Global model quality estimate
<b>GO</b>	Gene ontology
<b>GSH</b>	Glutathione
<b>H-Bond</b>	Hydrogen bond
<b>HEPES</b>	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
<b>HeLa</b>	Human cervical cancer cell line
<b>hERG</b>	human ether-a-go-go-related gene
<b>HIA</b>	Human Intestinal Absorption
<b>HMBC</b>	Heteronuclear multiple bond correlation
<b>HPLC</b>	High-performance liquid chromatography
<b>HRMS</b>	High-resolution mass spectrometry
<b>HSQC</b>	Heteronuclear single quantum coherence spectroscopy
<b>HTS</b>	High-throughput screening
<b>HTVS</b>	High-throughput virtual screening
<b>iNOS</b>	Inducible nitric oxide synthase
<b>IC<sub>50</sub></b>	Half-maximal inhibitory concentration
<b>ID<sub>50</sub></b>	Half-maximal infectious dose
<b>IL-1</b>	Interleukin-1
<b>IL-1<math>\beta</math></b>	Interleukin-1 beta
<b>IL-6</b>	Interleukin-6
<b>IL1R1</b>	Interleukin-1 receptor
<b>IKKB</b>	Inhibitor of nuclear factor kappa B kinase subunit beta
<b>JAK1</b>	Janus kinase 1
<b>JNK</b>	c-Jun N-terminal kinase
<b>JNK3</b>	c-Jun N-terminal kinase 3
<b>KDR</b>	Kinase insert domain

<b>Abbreviation</b>	<b>Full Form</b>
<b>KEGG</b>	Kyoto encyclopedia of genes and genomes
<b>KNIME</b>	Konstanz Information Miner
<b>LC-MS</b>	Liquid chromatography-mass spectrometry
<b>LC-QTOF</b>	Liquid chromatography quadrupole time-of-flight
<b>LGA</b>	Lamarckian genetic algorithm
<b>LOX</b>	Lipoxygenase
<b>LPO</b>	Lipid peroxidation
<b>LPS</b>	Lipopolysaccharide
<b>LTA4H</b>	Leukotriene A4 hydrolase
<b>MAPK/ MEK</b>	Mitogen-activated protein kinase
<b>MAPK8</b>	Mitogen-activated protein kinase 8
<b>MD</b>	Molecular dynamic
<b>MDA</b>	Malondialdehyde
<b>MDA-MB-231</b>	Human metastatic breast adenocarcinoma cell line
<b>MEM</b>	Minimum essential medium
<b>MolSA</b>	Molecular surface area
<b>MMP2</b>	Matrix metalloproteinase 2
<b>MM-GBSA</b>	Molecular mechanics-Generalized born surface area
<b>MM-PBSA</b>	Molecular mechanics-Poisson boltzmann surface area
<b>MRSA</b>	Methicillin-resistant <i>Staphylococcus aureus</i>
<b>MS</b>	Mass spectrometry
<b>MTT</b>	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
<b>NCE</b>	New chemical entity
<b>NC-MFP</b>	Natural compound molecular fingerprint
<b>NF-<math>\kappa</math>B</b>	Nuclear factor-kappa B
<b>NLRP3</b>	NLR family pyrin domain containing 3
<b>NMR</b>	Nuclear magnetic resonance
<b>NO</b>	Nitric oxide
<b>NOESY</b>	Nuclear overhauser effect spectroscopy
<b>NOS2</b>	Nitric oxide synthase-2
<b>NP</b>	Natural product
<b>Nrf2</b>	Nuclear factor erythroid 2-related factor 2
<b>NSAID</b>	Non-steroidal anti-inflammatory drugs
<b>NSCLC</b>	Non-small cell lung cancer
<b>PAINS</b>	Pan-assay interference compounds
<b>PBS</b>	Phosphate buffer saline
<b>PDB</b>	Protein data bank
<b>PDBQT</b>	Protein data bank, partial charge (Q), & atom type (T)
<b>PCA</b>	Principal component analysis
<b>PGE2</b>	Prostaglandin E <sub>2</sub>
<b>PGH2</b>	Prostaglandin H <sub>2</sub>

<b>Abbreviation</b>	<b>Full Form</b>
<b>PI3K</b>	Phosphoinositide-3-kinase
<b>PKC</b>	Protein kinase C
<b>PLA2R1</b>	Phospholipase A2 receptor 1
<b>PLC<math>\gamma</math></b>	Phosphoinositide phospholipase C
<b>PMA</b>	Phorbol myristate acetate
<b>PME</b>	Particle mesh ewald
<b>PPB</b>	Plasma protein binding
<b>PPI</b>	Protein-protein interaction
<b>PRKCA</b>	Protein kinase C alpha
<b>PRKCBP1</b>	Protein kinase C binding protein 1
<b>PRKCG</b>	Protein kinase C gamma
<b>PRSS1</b>	Protease serine 1
<b>PSA</b>	Polar surface area
<b>PTGES</b>	Prostaglandin E synthase
<b>PTGES1</b>	Prostaglandin E synthase 1
<b>PTGES2</b>	Prostaglandin E synthase 2
<b>PWF</b>	Paw withdrawal frequency
<b>PWL</b>	Paw withdrawal latency
<b>PWT</b>	Paw withdrawal threshold
<b>Rg/RoG/rGyr</b>	Radius of gyration
<b>RMSD</b>	Root mean square deviation
<b>RMSF</b>	Root mean square fluctuation
<b>ROCS</b>	Rapid overlay of chemical structures
<b>Ro5</b>	Rule of five
<b>ROS</b>	Reactive oxygen species
<b>SAR</b>	Structure-activity relationship
<b>SASA</b>	Solvent-accessible surface area
<b>SB-DFP</b>	Statistical-based database fingerprint
<b>SCLC</b>	Small cell lung cancer
<b>SD</b>	Standard deviation
<b>SDS</b>	Sodium dodecyl sulphate
<b>SEM</b>	Standard error mean
<b>SHB</b>	SH2 domain containing adaptor protein B
<b>SL</b>	Sesquiterpene lactone
<b>SRC</b>	Proto-oncogene tyrosine-protein kinase
<b>SP</b>	Standard precision
<b>SPiDER</b>	Self-organizing map-based prediction of drug equivalence relationships
<b>STarFish</b>	Stacked ensemble target fishing
<b>STAT3</b>	Signal transducer and activator of transcription 3
<b>TAK1</b>	Transforming growth factor- $\beta$ (TGF- $\beta$ )-activated kinase 1
<b>TBA</b>	Thio barbituric acid

<b>Abbreviation</b>	<b>Full Form</b>
<b>TBARS</b>	Thiobarbituric acid reactive substances
<b>TBX2</b>	T-Box transcription factor 2
<b>TCM</b>	Traditional Chinese Medicine
<b>TIP3P</b>	Transferable intermolecular potential with 3 points
<b>TLC</b>	Thin-layer chromatography
<b>TMS</b>	Tetramethylsilane
<b>TNB</b>	Thionitro benzoic acid
<b>TNF-<math>\alpha</math></b>	Tumor necrosis factor- $\alpha$
<b>TNBC</b>	Triple-negative breast cancer
<b>t-SNE</b>	T-distributed stochastic neighbor embedding
<b>TIGER</b>	Target inference generator
<b>TSAd</b>	T cell-specific adapter protein
<b>UMAP</b>	Uniform manifold approximation and projection
<b>US FDA</b>	United States Food & Drug administration
<b>UV</b>	Ultraviolet
<b>VEGFs</b>	Vascular endothelial growth factors
<b>VEGFRs</b>	Vascular endothelial growth factor receptors
<b>VEGFR-2</b>	Vascular endothelial growth factor receptor- 2
<b>VISA</b>	Vancomycin-intermediate <i>Staphylococcus aureus</i>
<b>VRE</b>	Vancomycin-resistant <i>Enterococci</i>
<b>XP-</b>	Extra precision
<b>XRD</b>	X-ray diffraction
<b>YES1</b>	YES proto-oncogene 1

## List of Symbol

<b>Symbols</b>	<b>Meaning</b>
$\alpha$	Alpha
$\beta$	Beta
$\gamma$	Gamma
$\delta$	Delta
$\omega$	Omega
~	Approximately
Å	Angstrom
$\kappa$	Kappa
$\pm$	Plus or Minus
<	Less than
>	Greater than
$\geq$	Greater than equal to
$\leq$	Less than or equal to
%	Percent
$\mu\text{M}$	Micromolar
$\text{nM}$	Nanomolar
$\text{mM}$	Millimolar
$\mu\text{L}$	Microliter
$\text{mL}$	Milliliter
L	Liter
$\mu\text{g/mL}$	Micrograms per milliliter
pH	Potential of hydrogen
K	Kelvin
ns	Nanosecond
ps	Picosecond
$\text{kcal/mol}$	Kilocalories per mole
nm	Nanometer
sec	Second
min	Minute
hr	Hour
Hz	Hertz
MHz	Megahertz
J	Coupling constant
ppm/ $\delta$	Parts per million
s	Singlet
d	Doublet
t	Triplet
dd	Doublet of doublet
dt	Doublet of triplet
m	Multiplet

<b>Symbols</b>	<b>Meaning</b>
<b>m/z</b>	Mass-to-charge ratio
<b>mg</b>	Milligrams
<b>mg/dl</b>	Milligrams per deciliter
<b>g</b>	Grams
<b>kg</b>	Kilograms
<b>mg/kg</b>	Milligrams per kilogram
<b>rpm</b>	Revolutions per minute
<b>i.p.</b>	Intraperitoneal

## Preface

Natural products (NPs) are and have remained a rich source of inspiration in drug discovery and development. They exhibit a broad spectrum of structural diversity and complexity with vast pharmacological potential. A significant portion of approved anticancer and anti-infective drugs have natural origins. Till now, nearly one-third of the approved drugs and two-thirds of small-molecule drugs had some connection to NPs, whether in their original form, as derivatives, or mimetics. While successful drugs have historically emerged from NPs, there has recently been a noticeable decline in the number of new drugs derived from them, but despite the challenges, the significant impact of NPs on drug discovery remains evident.

Plant-derived cyclic esters, known as lactones, are abundant in nature's chemical reservoir and offer a wide range of structural diversity, thus serving as unique frameworks for lead compounds. Among naturally occurring lactones,  $\gamma$ - and  $\delta$ -lactones are the most abundant. The present thesis work was aimed at the discovery of new lead molecules with cytotoxic and anti-inflammatory potential derived from natural lactones. The thesis consists of ten chapters in which the natural lactones were explored for their biological potential using a multitude of approaches that included computational screenings, phytochemical investigations, semi-synthetic derivatization, and pharmacological screenings.

**Chapter 1** initiates a comprehensive exploration of NPs, encompassing their role in drug discovery, their precedence over synthetic molecules, their limitations and challenges, and their contribution toward approved drugs. It also discusses about natural lactones in drug discovery and their current therapeutic landscape. Additionally, the role of computational approaches in NP-based drug discovery and the advantages of amalgamating them are discussed.

In **Chapter 2**, the objectives of the study and plan of work are enumerated.

**Chapter 3** describes the identification of natural coumarin compounds from the COCONUT database as potential VEGFR-2 inhibitors (anti-angiogenic). By developing a pharmacophore of lenvatinib and applying pharmacophore-based virtual screening and molecular modeling, the chapter identifies promising candidates associated with VEGFR-2 inhibition, contributing to the development of inhibitors of cancer-related angiogenesis.

**Chapter 4** mentions the computational exploration of coumestans (type of lactone-containing phytoconstituents) of *Psoralea corylifolia* (L.) through molecular modeling studies for their EGFR (an important target for anticancer therapies) inhibitory potential and subsequent lead identification. The chapter then proceeds to extraction, isolation, and *in vitro* cytotoxicity screening of identified coumestan hit from *P. corylifolia*.

**Chapter 5** describes the phytochemical investigations of leaves and seed of *P. corylifolia* (L.) for the presence of potential coumarin (benzenoid  $\delta$ -lactones) phytoconstituents. The study proceeds through extraction of *P. corylifolia* leaves and seeds, followed by isolation and purification of various fractions for pure compounds. Later, the chapter mentions the evaluation of cytotoxic potential of isolated phytoconstituents and molecular modeling studies of the compounds that possessed potent cytotoxicity.

**Chapter 6** explores a network pharmacology-based approach to identify anti-inflammatory lactones from a NP database and their possible mechanism of action. The study then proceeds to docking studies of selected lactone candidates with the inflammatory targets and identification of potential lactone hit and its potential targets. Later, the section describes molecular dynamic studies, extraction, isolation and purification of lactone hit and *in vitro* validation of its target inhibitory potential.

**Chapter 7** mentions the semi-synthetic modification of lactone hit identified in Chapter 6. The chapter describes the development of naproxen-like analogs from santonin, a

sesquiterpene lactone identified through network pharmacology-based approach. The section then describes the evaluation of the anti-inflammatory potential of synthesized analogs through enzyme inhibition assay and later evaluation of the *in vivo* anti-inflammatory potential of the best analog.

**Chapter 8** describes the phytochemical exploration of *Vitex negundo* (L.) leaves for potential phytoconstituents. The chapter encompasses the extraction of *V. negundo* leaves, resin-based fractionation, and repeated purification of fractions to obtain pure phytoconstituents. The section mentions the isolation of 2,3-Dehydrosilychristin, a silymarin flavanolignan, from the leaf extract of *V. negundo* along with 12 other phytoconstituents. It also describes the pharmacological exploration of 2,3-Dehydrosilychristin through *in vitro* and *in vivo* studies.

**Chapter 9** outlines the summary and conclusions of the research work undertaken.

In **Chapter 10**, the references used to carry out the research work are enumerated.

An appendix containing additional supporting information, spectral data of representative compounds and a bibliography of publications during the course of the Ph.D. is included.

