Chapter 3

Plan of Work

The overall objective was achieved with the following work plan:

- **Preformulation**
  - Synergy analyses for determination of combination index of DOC and ERL combination
  - Screening of excipients for preparation of LNPs
  - Compatibility study between drugs and selected excipients
- **Preparation and optimization of LNPs**
  - Preparation of LNPs
  - Optimization of LNPs using Quality by Design (QbD) approach
  - Surface functionalization of LNPs using Folic acid as targeting ligand (FA–LNPs)
  - Lyophilization of optimized FA–LNPs
  - Stability study of FA–LNPs by following ICH Q1A (R2) guidelines
  - Characterization of drugs loaded FA–LNPs
    - Particle size, PDI, and zeta potential
    - Morphology analysis
    - % entrapment efficiency and % drug loading
    - *In vitro* drug release study and kinetic study in different release media
    - *In vitro* lipolysis study in intestinal condition
- Evaluations of drugs loaded FA–LNPs
  - *In vitro* cell culture studies in MDA–MB–231, and 4T1 cell lines
    - MTT assay
    - Qualitative and quantitative cellular uptake assay
    - Colony formation assay
    - Mitochondrial membrane potential (MMP) assay
    - Reactive oxygen species (ROS) assay
    - Annexin V apoptosis assay
    - Wound healing assay
    - Transwell migration assay
  - *In vivo* studies
    - Pharmacokinetic study after oral administration
    - Quantitative biodistribution study
    - *In vivo* anticancer efficacy study
    - Hematological parameter, biochemical parameter, and histopathology analysis

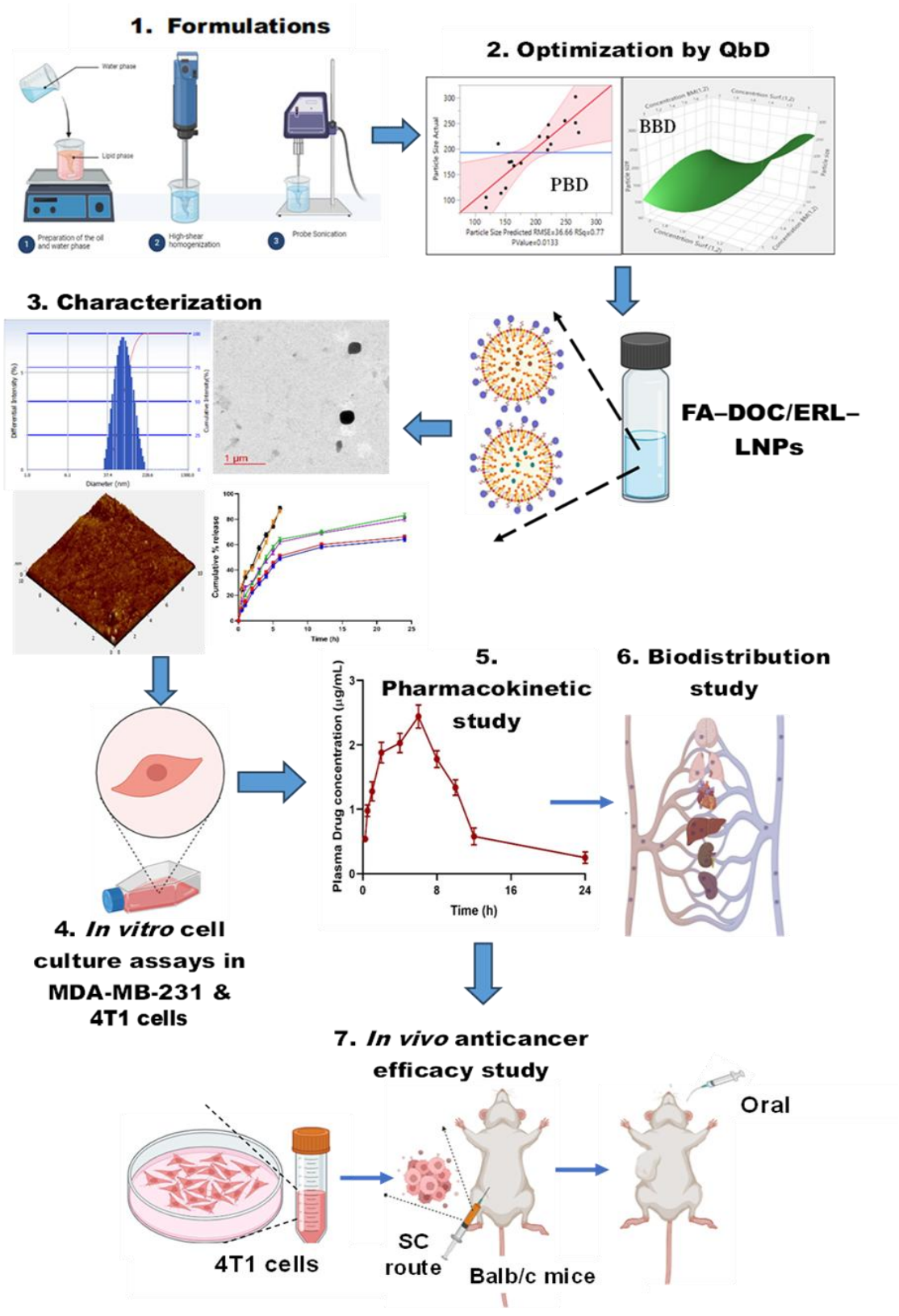

Figure 3. 1 : Graphical representation of the proposed work plan