Use of In Silico and In Vitro Models to Assess the Pharmacokinetics and Toxicology of Natural Compounds Before Human Trials

Bridging Traditional Medicine with Modern Drug Development

INNO-SAFE-LIFE

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Introduction

Natural products remain a vital source of therapeutic agents.

However, natural compounds often have unknown or complex ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles.

Preclinical screening is essential for identifying safe and effective candidates.

In silico (computer-simulated) and **in vitro** (lab-based) models help evaluate these properties efficiently before moving into costly and ethically sensitive **in vivo** and human trials.

Objectives of the Presentation

- Introduce in silico and in vitro models used in early pharmacological research.
- Demonstrate how these models assess ADMET characteristics of natural compounds.
- Explore the advantages and limitations of both models.
- Provide examples of natural compounds evaluated using these approaches.
- Encourage integration of predictive tools in natural product research pipelines.

Why Focus on Natural Compounds?

Over 60% of approved drugs are derived from natural products.

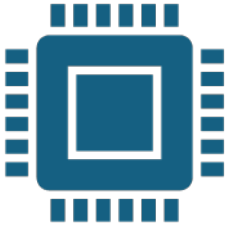
Found in plants, marine organisms, fungi, and microorganisms. Offer structural diversity not found in synthetic libraries.

Challenges:

- Poor bioavailability
- Metabolic instability
- Batch variability
- Potential toxicity due to plant or environmental contaminants

Early predictive testing helps to prioritize viable candidates.

What Are In Silico Models?



Definition: Computational simulations that model drug behavior in silico ("in silicon", i.e., on a computer).

Applications:

Predict physicochemical properties (e.g., lipophilicity, solubility)

Estimate ADMET characteristics

Model receptor-ligand interactions via molecular docking

Build QSAR (Quantitative Structure-Activity Relationship) models to relate structure to biological activity or toxicity

Reduce experimental load in early discovery stages

Common In Silico Tools

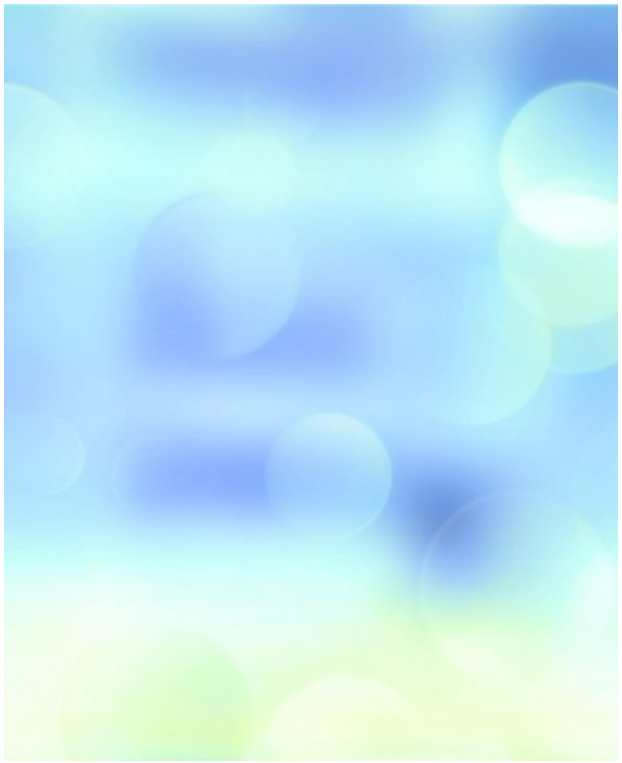
SwissADME: Predicts GI absorption, BBB penetration, Lipinski's Rule of 5

pkCSM & ADMETlab: Toxicity prediction (hERG inhibition, LD50, hepatotoxicity)

AutoDock / MOE / PyRx: Simulates molecular docking to predict receptor binding

PASS Online: Predicts possible biological activity spectra of compounds

Derek Nexus / VEGA: Rule-based toxicological prediction systems



What Are In Vitro Models?

- **Definition:** Laboratory-based experiments conducted on cells, enzymes, or isolated tissues in controlled environments.
- **Role in Pharmacokinetics and Toxicology:**
  - Provide mechanistic understanding
  - Test metabolism using liver microsomes or hepatocytes
  - Assess transport, permeability, protein binding
  - Evaluate cytotoxicity and organ-specific toxicity (e.g., liver, heart)
  - Offer human-relevant alternatives to animal testing

In Vitro Techniques for Pharmacokinetics

- **Caco-2 Cell Assay:** Mimics intestinal epithelium; predicts oral absorption and permeability
- **Liver Microsomes / Hepatocytes:** Simulate liver metabolism (Phase I/II)
- Used to identify metabolic stability, potential drug interactions
- **Plasma Protein Binding (PPB):** Predicts distribution and free drug concentration
- **PAMPA (Parallel Artificial Membrane Permeability Assay):** Models passive diffusion across membranes

In Vitro Toxicology Methods

Cytotoxicity Assays:	MTT, Trypan Blue, Alamar Blue for cell viability
Genotoxicity Assays:	Comet Assay: Detects DNA strand breaks Micronucleus Test: Identifies chromosomal damage
Hepatotoxicity Models:	HepG2 or primary hepatocytes for liver-specific toxicity
Cardiotoxicity Assays:	hERG channel inhibition tests predict arrhythmic potential
Oxidative Stress Indicators:	ROS generation, glutathione depletion

Understanding ADMET

Absorption:	How well does the compound get into the bloodstream? Evaluated by Caco-2, PAMPA, or in silico permeability models.
Distribution:	Where does the compound go in the body? Protein binding and tissue affinity models assess this.
Metabolism:	How is the compound broken down? Studied using liver microsomes, S9 fractions, and enzyme assays.
Excretion:	How is it eliminated (urine, bile, feces)?
Toxicity:	Acute, chronic, organ-specific, genotoxic, or mutagenic effects.

Advantages of In Silico & In Vitro Approaches

Rapid & Cost-Efficient: Screen hundreds of compounds before investing in animals/humans.

Ethical Compliance: Support the 3Rs principle (Replacement, Reduction, Refinement).

Human-Relevant Results: Some in vitro models use human cells, offering more accurate predictions.

Data-Rich Outputs: Generate pharmacokinetic and toxicological profiles early in development.

Limitations of the Models

In Silico:

Accuracy depends on algorithm and database quality.

May not account for all metabolic pathways or rare adverse effects.

In Vitro:

Lack systemic interactions (e.g., immune response, organ crosstalk).

Scalability and reproducibility can be challenges.

May not fully mimic in vivo drug metabolism or transport dynamics.

Case Study: Berberine

Source:

- Alkaloid from *Berberis* species

In Silico Findings:

- High predicted liver metabolism
- Low oral bioavailability

In Vitro Results:

- Low permeability in Caco-2
- Some cytotoxicity in HepG2 cells at high concentrations

Outcome:

- Prompted structural modification and use of nanoparticle delivery systems to enhance bioavailability and safety.

Integration in the Drug Development Pipeline

Discovery Phase: Use in silico models for early filtering.

Preclinical Phase: Validate leads using in vitro pharmacokinetics and toxicity tests.

Lead Optimization: Refine chemical structure or delivery based on results.

Preclinical In Vivo Testing: Only proceed with best-performing compounds.

Clinical Phase: Safer and more predictable outcomes due to robust early screening.

Regulatory Perspective

OECD Guidelines: Recognize standardized in vitro toxicity tests (e.g., OECD TG 431, 439)

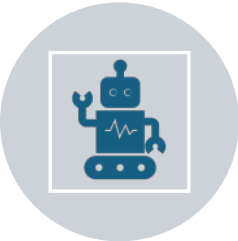
FDA & EMA Support: Encourage non-animal testing, especially for natural health products

ICH Guidelines (S6, M3, S4): Outline safety pharmacology studies, allow use of predictive models

Conclusions

- In silico and in vitro tools significantly streamline drug development.
- Particularly useful for screening natural compounds with diverse and complex profiles.
- Help avoid late-stage failures due to poor pharmacokinetics or toxicity.
- Integration of these models enhances safety, reduces costs, and promotes ethical research.

Future Directions



AI and Machine Learning Integration: Improve predictive accuracy of toxicity and metabolism.

Organs-on-Chips & 3D Cultures: Bridge gap between in vitro and in vivo.

Big Data for Natural Compounds: Build robust ADMET databases for herbal compounds.

Personalized Testing Models: Use patient-derived cells for individual response predictions.

Short test

Which of the following best describes the purpose of *in silico* models in pharmacokinetics?

A. Culturing cells to assess metabolism
B. *Simulating drug behavior using computer algorithms*
C. Testing drugs on human volunteers
D. Measuring protein expression levels

What does the Caco-2 cell assay primarily evaluate?

A. Blood-brain barrier penetration
B. Genetic mutations
C. *Intestinal drug absorption*
D. Liver enzyme activity

Short test

Which tool is commonly used to predict ADMET properties in silico?

A. ELISA
B. AutoDock
C. *SwissADME*
D. MTT Assay

What is a key advantage of using in vitro methods in early drug development?

A. They are more expensive than animal tests
B. They require no ethical approval
C. They can model full-body pharmacokinetics
D. *They reduce the need for animal testing*

Short test

Which of the following best describes ADMET?

A. A database for storing herbal formulas

B. A tool for measuring genetic similarity

C. *A profile describing drug absorption, distribution, metabolism, excretion, and toxicity*

D. A scoring system for herbal product purity