Edited Book

Drug Repurposing : Novel Therapeutic Innovations & Challenges

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About it

Drug repurposing—the strategy of identifying new therapeutic uses for existing or investigational drugs—represents a paradigm shift in pharmaceutical innovation. Unlike *de novo* drug development, which requires over a decade and costs billions, repurposing leverages established safety profiles, known pharmacokinetics, and existing manufacturing pathways. This approach offers a faster, more cost-effective route to clinical impact, particularly for rare diseases, emerging pandemics, and conditions with limited treatment options. However, the path to repurposing is fraught with challenges. The primary obstacle is the lack of a clear commercial incentive; because repurposed drugs are often off-patent, the financial return on investment is limited, creating a "translational gap" where academic discoveries fail to secure industry backing. Furthermore, navigating intellectual property for new formulations, securing funding for indication-specific Phase II/III trials, and overcoming regulatory ambiguities remain significant hurdles. Overcoming these barriers will require novel public-private partnerships, adaptive trial designs, and policy reforms to fully unlock the therapeutic potential hidden within existing pharmacopoeias.

Dr. Priya Sharma

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Chapter- One

Holistic Drug Design: Siddha Concepts in the Era of Systems Medicine

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Abstract

In contemporary drug design, the concept of prodrugs is a method to enhance the pharmacokinetic and pharmacodynamic constraints of active drug molecules. Through biotransformation, metabolism, and synergistic activation, natural products—especially those derived from traditional medical systems like Siddha medicine—represent prodrug-like principles. Herbo-mineral formulations and siddha medicinal plants frequently contain inactive or less active ingredients that the body metabolically converts into therapeutically active metabolites. With a focus on Siddha medicinal plants and medicines, this chapter discusses the conceptual relationship between prodrug design and natural products. Traditional knowledge, contemporary scientific interpretation, and the applicability of Siddha formulations in current drug discovery paradigms are discussed.

Keywords: *medicinal plants*

Introduction

Pharmacologically inactive or partially active substances that undergo in vivo conversion to release the active drug are known as prodrugs. To increase bioavailability, lower toxicity, improve solubility, and accomplish site-specific drug delivery, the prodrug approach is frequently used in contemporary drug discovery. [1] It's interesting to note that the fundamental ideas of prodrug activation are very similar to traditional medical practices, especially in Siddha medicine, where metabolic transformation, purification (suddhi), and synergistic formulation are frequently necessary for therapeutic efficacy.

The Siddha system of medicine is among the oldest systems practiced in South India. Its early flourishing in this region aligns with the belief that the southern peninsula is the cradle of the human race. Siddha medicine is a traditional system that emphasizes the use of plant-based, mineral-based, and herbo-mineral drugs. Many medicinal plants in the Siddha system contain glycosides, alkaloid precursors, tannins, and conjugated phytochemicals that become biologically active after administration. This inherent prodrug-like feature makes Siddha medicine a valuable source for lead identification and drug design. Medicines are classified as either internal or external, with each group further divided into 32 types. Every preparation requires specific basic steps.[2]

Material and Methods

Definition of medicine:

The objective of the Siddha system differs profoundly from that of other systems in one respect. The prevention and cure of illness are aims of all systems of medicine, which

have, in addition, a transcendental motivation, a concern for what might have been called the immortality of the body. Saint Thirumoolar quotes as,

"Maruppathu udal noyai marunthanalhum
Maruppathu ula noyai marunthanalahum
Maruppathu Ininoi varamaikku
Maruppathu savai marunthenalamae" –Thirumanthiram

The medicine that cures the physical ailments of the body, the medicine that cures mental ailments of the mind, the medicines that prevent the disease, the medicine that prevents death. This is the unique feature of the system.

Medicine preparation

Basic concepts in Medicine preparation are as follows, [3]

1. *Egamooligai prayogam*(Single drug usage)
2. *Marana Prayogam*
3. *Diravaga Prayogam*
4. *Cheyaneer Prayogam*
5. *Muppu chunna Prayogam*

1. *Egamooligai prayogam* means application or using herbal products only. Here, selecting a single drug or a combination of many, based on the *boothas* that are present in the drug to neutralize the affected *thodam* (Humors)
2. *Marana Prayogam* (based on the synergetic and antagonistic properties)

Marana is the technical term used to denote the disintegration of the drug substance. Here it means to destroy the toxic effect of the drug. The drugs are selected in such a way that the toxins are removed by the antagonistic drug, and its potency is increased by a synergetic drug.

Ex. Copper and sulphur- antagonistic property Mercury and sulphur - synergetic property

3. *Dravagam* means the process in which raw drugs are distilled in water or some salts are distilled in a specialized distillery pot.

Ex. Sanga dravagam (distilling conch water)

4. *Cheyaneer Prayogam*

Cheyaneer means a fluid prepared by exposing various salts and alkalis to dews. The *cheyaneer* is used for preparing higher medicines, which are difficult to prepare, and also increases the potency of the drug.

Ex. Vediyuppu chyaneer

5. *Muppu chunna Prayogam*

The tradition of this *muppu* differs from region to region, but *muppu* merely means a combination of three salts, which are composed of *Vayu*, *Theyu* and *Appu boothas*. Muppu is a catalytic agent used to simplify the elaborate process of higher medicines. It also increases the potency of the drug.

Purification of Raw Drug

- All the ingredients for the formulae need purification methods. The herbal ingredients undergo various purification methods. Some of them need to remove soil, stones, dust, and some pieces of packing materials,
- The next step is to undergo Heat application to dried raw drugs:
- Mild roasting/ heating of raw drugs before going to prepare *Chooranam* (powder form)

- Then, steaming the powdered product using milk for some preparations.
- Many purification methods are applying/adjust the heat.
- Some ingredients, like *Vasambu* (Acorus calamus) to be burnt a little before the ash stage. These are some of the purification methods.
- By using controlled thermal processing, traditional Siddha medicine exhibits a sophisticated and methodical approach to drug preparation. Ancient practitioners used exact heat regulation techniques to ensure accurate, repeatable, and timely preparation of medicinal formulations long before thermodynamic principles were codified. These techniques demonstrate a thorough empirical grasp of material transformation, heat transfer, and pharmaceutical optimization.
- A variety of fuel sources, including sand, cow dung cakes, firewood, and controlled exposure times, were used to carefully regulate the amount of heat applied during the preparation of medicines. Extended heating protocols, sometimes lasting up to 36 hours, are described in classical Siddha texts. The length and intensity of the heat were modified based on the type of raw materials and the intended formulation. In order to accomplish desired physicochemical transformations, such practices suggest intentional temperature modulation and prolonged heat delivery.
- Siddha pharmaceutical procedures heavily rely on sand-roasting techniques (*Valuka Iyanthiram*). Because these characteristics affect heat distribution and retention, literature highlights the importance of choosing sand based on particle size and composition.

Prodrug Concept in Siddha Medicinal Plants

Numerous Siddha medicinal plants act as prodrugs in their natural state. Glycosides found in plants like Cassia angustifolia (Senna) and Terminalia chebula are either inactive or only slightly active until they are metabolized in the intestines, releasing therapeutically effective aglycones. This process of metabolic activation resembles the function of carrier-linked prodrugs used in contemporary pharmacology. Likewise, plants that contain alkaloids, such as Rauwolfia serpentina, have precursor compounds that are converted through enzymatic processes to produce antihypertensive and neuropharmacological effects. [4, 5]

Table 1. Siddha Medicinal Plants Exhibiting Prodrug-Like Behavior [5]

Siddha Plant	Major Constituent	Prodrug Nature	Active Metabolite	Therapeutic Use
Cassia angustifolia	Sennosides	Glycosidic prodrug	Rhein anthrone	Laxative
Terminalia chebula	Chebulinic acid	Hydrolysable tannin	Gallic acid	Digestive, detoxifier
Rauwolfia serpentina	Alkaloid precursors	Bioprecursor	Reserpine metabolites	Antihypertensive
Glycyrrhiza glabra	Glycyrrhizin	Prodrug glycoside	Glycyrrhetinic acid	Anti-inflammatory
Aloe vera	Aloin	Glycoside	Aloe-emodin	Purgative

Some important natural products derived from plant sources

Types	Botanical	Ethno	Therapeutics application

	source	botanical compounds	
Single molecules	Piper nigrum	Piperine	Nanot theragnostic agent for cancer Treatment [7]
Phyto Pharmaceutical drug	Berberis vulgaris L	Berberine, Jatrorrhizine, Palmatine, ceptisine	Antidiabetic, Anticancer, Antibacterial, Analgesic, Anti-inflammatory, and Cardiovascular [8]
	Salvia divinorum	Salvinorin A	Neuro-psycho-pharmacological therapeutic plant-based drugs [9]
	Cleome	Pinocembrin, Kaempferol, Kaempferitrin	Anti-cancer [10]
	Curcuma longa L. (Turmeric)	Curcumin	Antioxidant, anti-inflammatory, metabolic syndrome and pain [11]
	Azadirachta indica A. Juss	*Azadirachtin*	Insecticidal and antimicrobial [12]
	Punica granatum (Pomegranate)	Extract	Antidiarrheal activity [13]
	Trigonella foenum-graceum L	Trigonelline, Diaszhenin	Antidiabetic, Anti-conception [14]

Siddha Formulations as Multi-Component Prodrug Systems

Complex Siddha formulations frequently function as multi-component prodrug systems. The inclusion of bioenhancers, adjuvants, and detoxified minerals promotes gradual activation, precise delivery, and minimized toxicity. Traditional Siddha pharmaceutical methods like suddhi (purification) and marana (calcination) correspond to contemporary prodrug optimization strategies designed to enhance bioavailability and safety. [15,16]

Table 2. Siddha Dosage Forms and Prodrug-Like Characteristics

Siddha Formulation	Processing Principle	Prodrug Relevance	Pharmacological Advantage
Kudineer	Decoction	Enzymatic activation	Rapid bioavailability
Chooranam	Powdered form	Gradual metabolism	Sustained release
Ilagam	Polyherbal matrix	Synergistic activation	Reduced toxicity
Nei	Lipid-based	Enhanced absorption	Improved bioavailability
Parpam	Herbo-mineral	Biotransformation	Target specificity

Correlation with Modern Prodrug Design

The Siddha approach highlights a comprehensive method, engagement with multiple targets, and harmony within metabolic processes. These concepts closely correspond

with current strategies in drug development, including polypharmacology, network pharmacology, and systems biology. When considering Siddha medicines from the perspective of prodrug research, they offer a significant understanding of regulated drug release, targeted activation in specific tissues, and the minimization of side effects. [17-19]

Table 3. Comparison of Modern Prodrugs and Siddha Concepts

Modern Prodrug Principle	**Siddha Concept**	**Scientific Interpretation**
In vivo activation	*Yakkai illakkanam*	Metabolic bioconversion
Reduced toxicity	*Suddhi*	Detoxification
Targeted delivery	*Naadi*-based therapy	Personalized medicine
Improved absorption	*Anupanam*	Bio enhancement

Numerous secondary metabolites exhibiting a range of structures and medicinal attributes have already been discovered in plants. The identification of compounds from Papaver somniferum in 1803 created the modern age of drug discovery.[20] Progress in fields such as genomics, proteomics, transcriptomics, and metabolomics has significantly improved the role of natural substances in this domain. Plants generate various signaling compounds—such as auxin, abscisic acid, cytokinins, gibberellic acid, salicylic acid, and brassinosteroids—along with secondary metabolites like alkaloids, terpenoids, and phenylpropanoids, which are essential for numerous developmental and defense mechanisms.[20] These compounds are critical in regulating the plant life cycle and are frequently considered small molecules that are produced in response to stress, helping the plant fend off pathogens, adapt to climate variations, and combat radiation. They are also important in traditional medicinal systems and are increasingly recognized as valuable components in therapeutic applications.

Phytopharmaceutical Drug

Phyto pharmaceutical drugs represent an emerging category of herbal medications formulated in accordance with the guidelines established by Ayush and the CDSCO in India. These drugs are characterized as standardized and purified portions of medicinal plant extracts that contain at least four bioactive phytoconstituents, aimed at treating and preventing illnesses.[17] PPDs are concentrated extracts composed of phytonutrients, including flavonoids, carotenoids, polyphenols, lycopene, anthocyanidins, omega-3 fatty acids, phytoestrogens, and glucosinolates, each with unique pharmacological effects.
The production of Phyto (PPD) presents several challenges, including: [21]

1. A limited supply of plant materials.
2. Plant extracts are soluble in water and various solvents.
3. The presence of cytotoxic elements within the extract.
4. Restricted bioavailability.
5. Misapplication of available phytomedicines leading to toxic incidents.
6. Mistakes in plant identification and their application.
7. Improper use of popular herbal remedies/OCS.

8. Domestic incidents resulting from consuming ornamental plants that contain cardiotonic substances.
9. Hemorrhagic and hypertensive incidents caused by coumarin derivatives found in certain plants.
10. The existence of estrogen-like components in plants.
11. The use of certain plants may trigger allergic reactions due to pollen or other substances.

Through traditional purification (suddhi) and pharmaceutical processing techniques that detoxify plant materials, minimize cytotoxicity, and enhance bioavailability, siddha medicine successfully tackles these issues. By improving absorption and focused efficacy, techniques such as regulated heating, grinding, fermentation, and the incorporation of specific adjuvants work similarly to contemporary prodrug strategies. While contemporary authentication methods minimize adulteration, ongoing plant exposure and conventional identification procedures reduce misidentification. The Siddha literature's emphasis on appropriate dosage and indication helps to avoid the toxicity and abuse of plant constituents that are estrogenic, cardiotonic, and allergenic. All things considered, Siddha medicine provides a logical, conventional framework for overcoming significant obstacles in the development of plant-based prodrugs.

Conclusion

The prodrug concept paves an important scientific foundation for comprehending the therapeutic effectiveness of Siddha medicinal plants and formulations. Siddha medicine naturally incorporates concepts of metabolic activation, synergistic effects, and controlled release, which are fundamental to current prodrug development. Merging traditional Siddha wisdom with modern drug discovery methods can expedite the process of finding safe, efficient, and sustainable therapeutics derived from natural products.

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Chapter- Two

Drug Discovery and Design: From Target Identification to Preclinical Evaluation

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Abstract

From the first scientific idea to the commercial release of a completed pharmaceutical product, the development of a novel therapeutic agent is a very complex, time-consuming, and resource-intensive process that usually takes 12 to 15 years and requires financial investments exceeding one billion US dollars. Numerous sources, such as scholarly research, clinical observations, translational studies, and industrial innovation, can lead to the discovery of a promising therapeutic target. Before starting a high-risk drug discovery program, establishing a strong scientific justification for target selection frequently necessitates rigorous experimental validation and the gathering of supporting biological, genetic, and pharmacological information. Following target selection, early-stage drug discovery procedures in the pharmaceutical industry and, increasingly, in academic research settings, have been significantly streamlined thanks to developments in molecular biology, medicinal chemistry, and screening technology. The creation of trustworthy and predictive biological assays that allow for the methodical assessment of target modification complements target identification and validation. Large and chemically diverse compound libraries can be quickly examined using high-throughput screening (HTS) and in silico techniques to find active compounds, or "hits." In order to improve potency, selectivity, pharmacokinetic characteristics, and safety profiles, subsequent hit-to-lead and lead optimisation stages entail iterative cycles of chemical modification directed by structure–activity relationship studies. In order to get into clinical development, a candidate molecule is ultimately chosen based on a thorough evaluation of efficacy, drug-likeness, and preclinical safety. This paper offers a thorough summary of the major preclinical phases of drug discovery, emphasising current tactics, technical developments, and difficulties faced from target identification to candidate selection.

Keywords: *Drug discovery, Novel therapeutic agent, experimental validation, high-throughput screening.*

Introduction

The goal of the multidisciplinary, methodical process of drug development is to find novel therapeutic molecules that can prevent, treat, or manage illnesses. Combining concepts from medicinal chemistry, pharmacology, biology, biotechnology, and computational sciences, it

serves as the cornerstone of pharmaceutical research and development. The need for safer, more efficient, and more reasonably priced medications is still being driven by the rising prevalence of chronic illnesses, new infections, drug resistance, and unmet clinical demands, despite tremendous advancements in medical science.

In the past, traditional knowledge systems, such as treatments based on plants and minerals, empirical observations, and serendipity were all major factors in drug discovery. Numerous traditional medications, like morphine, quinine, and aspirin, came from natural sources. Nevertheless, this method of trial and error was laborious and lacked mechanical comprehension. Drug discovery has developed into a logical, target-driven process because to developments in molecular biology and analytical methods, allowing researchers to create and refine compounds with increased efficacy and specificity(1).

A thorough understanding of disease biology is usually the first step in modern drug discovery, which is followed by the identification and validation of appropriate molecular targets. These targets could be enzymes, receptors, ion channels, transporters, or nucleic acids that are essential for the development of a disease. Potential chemical entities, or "hits," are found via computational methods or experimental screening after a target has been verified. Through methodical optimisation to improve potency, selectivity, pharmacokinetic characteristics, and safety, these hits are further developed into "lead" molecules.

Drug discovery is still a difficult, expensive, and dangerous process despite advances in technology. Only a small percentage of candidate compounds are successfully brought to market, and the process typically takes ten to fifteen years with a high attrition rate. Late-stage failures are largely caused by issues including toxicity, ineffectiveness, poor bioavailability, and regulatory barriers. Improving early-stage discovery techniques is therefore becoming more and more important in order to lower costs and boost success rates(2).

New technologies have changed the landscape of drug research in recent years. Large biological datasets are being analysed, lead compounds are being optimised, and drug–target interactions are being predicted using computational techniques, artificial intelligence (AI), and machine learning (ML). Furthermore, a better knowledge of disease mechanisms has been made possible by omics technologies, such as proteomics, metabolomics, and genomes, which have made it easier to find precise and customised medications. In addition to synthetic methods, there has been a resurgence of interest in natural compounds and herbal drug development, especially when paired with contemporary analytical and computational methods (3).

This chapter aims to provide a comprehensive overview of the drug discovery process, from target identification to preclinical evaluation, highlighting both conventional and emerging approaches. Special emphasis is placed on rational drug design strategies, the role of artificial intelligence, and the integration of natural products into modern drug discovery pipelines. By presenting current trends, challenges, and future perspectives, this chapter seeks to offer valuable insights for students, researchers, and professionals in pharmaceutical and biomedical sciences.

Overview of the Drug Discovery Pipeline

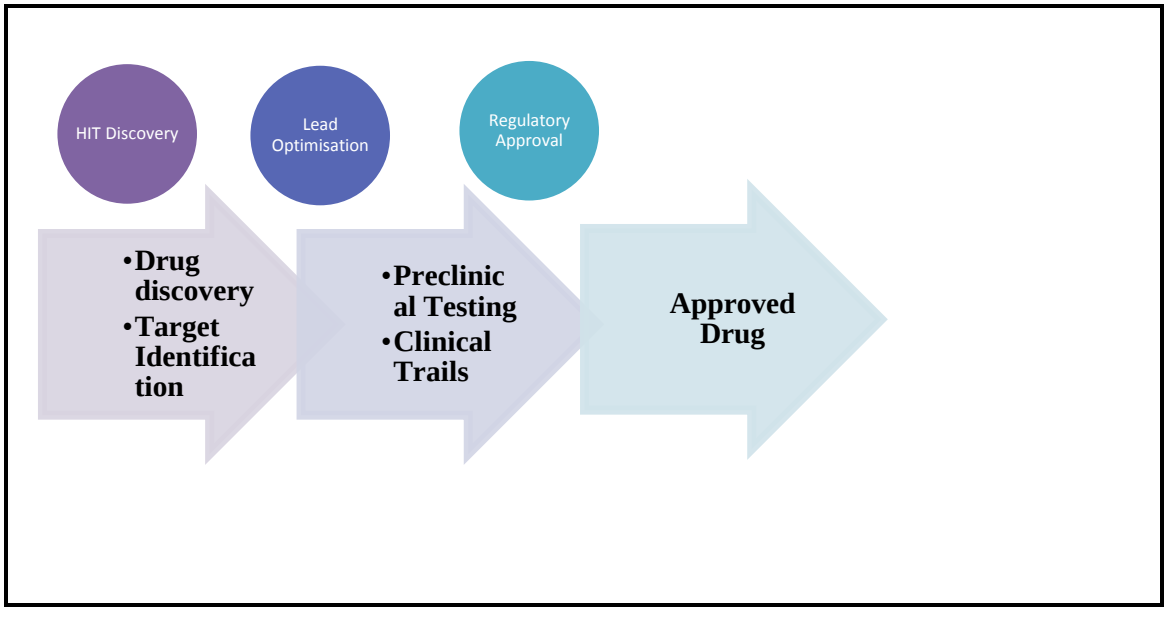

Figure 1: Overview of the Drug Discovery Pipeline

A scientific concept is transformed into a possible therapeutic candidate through a methodical, sequential procedure known as the drug discovery pipeline. It consists of several interrelated steps, all of which are intended to reduce risk and increase the possibility of finding safe and efficient medication compounds. In reality, the pipeline is very iterative with constant feedback between phases, despite the fact that it is frequently depicted linearly. Understanding diseases and identifying targets, where the molecular pathways behind a disease are investigated, is the first step in the process. Advances in systems biology, bioinformatics, and molecular biology are crucial to this stage. Following the identification and validation of a target, the focus switches to finding chemical entities that can alter the target's activity.

The next critical step is hit identification, which involves screening vast chemical libraries using computational or experimental techniques to identify molecules that exhibit quantifiable biological activity against the target. These hits usually have low selectivity and mild potency, which calls for more improvement. Enhancing the chemical and biological characteristics of hit compounds is the main goal of the lead discovery and optimization phase. Medicinal chemists systematically modify molecular structures to enhance potency, selectivity, pharmacokinetics, and safety while reducing toxicity. Structure–activity relationship (SAR) studies play a central role during this phase, supported by computational modelling and in vitro assays(4).

Promising compounds go through preclinical investigation, which includes pharmacokinetic, pharmacodynamic, and toxicological studies, after lead optimization. These evaluations aid in determining whether a chemical has sufficient safety profiles and appropriate absorption, distribution, metabolism, and excretion (ADME) characteristics for advancement into clinical trials. Differentiating between medication development and

discovery is crucial. While medication development entails clinical trials, regulatory approval, and post-marketing surveillance, drug discovery concentrates on finding and refining candidate compounds. Failures in the early stages of discovery can greatly increase development costs and timetables, even though both phases are essential to bringing a medication to market.

Academic institutions, pharmaceutical companies, biotechnology firms, and contract research organizations (CROs) work together in the drug discovery pipeline. While industry provides the infrastructure and resources needed for extensive screening, optimization, and development, academic research frequently drives target discovery and early innovation. Overall, a well-structured drug discovery pipeline enhances efficiency, reduces attrition rates, and increases the probability of clinical success. Continuous integration of emerging technologies such as artificial intelligence, high-throughput screening, and omics-based approaches has further strengthened this pipeline, making drug discovery more precise and data-driven(5).

Target Identification

Target identification is a fundamental step in the drug discovery process, as the success of a therapeutic agent largely depends on the selection of an appropriate biological target. A drug target is typically a biomolecule whose modulation produces a desirable therapeutic effect in a disease condition. Common drug targets include enzymes, receptors, ion channels, transporters, and nucleic acids involved in key pathological pathways.

The process of target identification begins with a comprehensive understanding of **disease pathophysiology**. Advances in molecular biology and biomedical research have enabled scientists to elucidate the complex cellular and molecular mechanisms underlying various diseases. By identifying critical pathways and molecular nodes involved in disease progression, researchers can pinpoint potential targets for therapeutic intervention.

Modern target identification heavily relies on **omics technologies**, such as genomics, transcriptomics, proteomics, and metabolomics. Genomic studies, including genome-wide association studies (GWAS), help identify disease-associated genes and mutations. Proteomic approaches provide insights into protein expression, post-translational modifications, and protein–protein interactions, while metabolomics reveals altered metabolic pathways associated with disease states. Together, these technologies generate large datasets that facilitate systematic target discovery(6).

Bioinformatics and systems biology tools play a crucial role in analyzing and integrating omics data. Network-based analyses help identify key regulatory proteins and signalling hubs that are more likely to serve as effective drug targets. Computational models also assist in predicting target–disease associations and assessing drug ability.

An essential aspect of target identification is the concept of **drug ability**, which refers to the likelihood that a target can be modulated by a small molecule or biological agent. Druggable targets typically possess well-defined binding sites and exhibit a strong correlation between target modulation and therapeutic outcome. Factors such as target accessibility, expression pattern, and safety profile are also carefully evaluated(7).

In addition to synthetic targets, **natural and herbal sources** continue to be valuable in target identification. Ethnopharmacological knowledge and traditional medicine systems often provide clues about biological pathways involved in disease treatment. Modern analytical and computational techniques enable the systematic identification of molecular targets affected by bioactive phytochemicals.

Effective target identification reduces the risk of late-stage drug failure and improves overall efficiency in the drug discovery pipeline. As scientific understanding of disease biology continues to expand, target identification has evolved into a data-driven and multidisciplinary process, forming the cornerstone of successful drug discovery programs(8).

Target Validation

A crucial stage in the drug discovery process is target validation, which verifies whether modifying a chosen biological target will result in the intended therapeutic effect without producing intolerable side effects. Target validation offers experimental proof of the target's applicability and viability for drug development, whereas target identification suggests possible targets based on illness connection.In vitro, in vivo, and in silico methods are typically combined to validate targets. To evaluate the functional role of the target, in vitro studies employ molecular methods, biochemical tests, and cell-based assays. RNA interference (RNAi) and CRISPR-Cas9 are two examples of gene editing or gene silencing technologies that are frequently used to suppress or alter target expression and track the ensuing biological effects.

To assess the physiological significance of the target, in vivo validation uses animal models that replicate human illness circumstances. Because they provide a clear connection between target modulation and illness outcome, transgenic and knockout animal models are very useful. Early information about toxicity, safety, and off-target effects is also provided by these research.Using established ligands, inhibitors, or antibodies to alter the target's function is known as pharmacological validation. Confidence in the goal is increased when dose-dependent responses and therapeutic effects are observed. Furthermore, biomarkers linked to target engagement are shown to aid in translational research and upcoming clinical investigations.

In vivo validation employs animal models that mimic human disease conditions to evaluate the physiological importance of the target. Transgenic and knockout animal models are highly helpful because they offer a clear link between target modulation and sickness outcome. These studies also offer early data regarding toxicity, safety, and off-target effects.Pharmacological validation is the process of changing the target's function using recognized ligands, inhibitors, or antibodies. Observing dose-dependent reactions and treatment outcomes boosts confidence in the objective. Additionally, biomarkers associated with target engagement have been demonstrated to support future clinical studies and translational research(10).

Hit Identification

Hit identification is the process of identifying chemical entities, or "hits," that exhibit measurable biological activity against a confirmed therapeutic target. These hit compounds serve as the foundation for further optimization in the drug discovery process. The primary

objective of this stage is to identify a range of reproducible molecules with sufficient potency and selectivity.

One of the most often used techniques for hit identification is high-throughput screening (HTS). HTS enables the rapid evaluation of thousands to millions of compounds against a specific target through the use of automated assays and detection devices. Advances in robotics, downsizing, and data analytics have significantly improved the efficacy and reliability of HTS approaches. Due to the high resource requirements of HTS, Tests for confirmation and secondary screening are necessary. Due to HTS's high resource requirements and potential for false positives, secondary screening and confirmation tests are required.

Virtual screening is a more cost-effective alternative or addition to experimental screening. To find potential target binders, large virtual chemical libraries can be screened utilizing in silico techniques like molecular docking and pharmacophore modeling. While reducing time and money, virtual screening raises the possibility of discovering high-quality hits. Fragment-based drug discovery (FBDD) is another innovative method of hit detection. This approach is used to screen for low-molecular-weight fragments that bind to the target weakly. Fragment hits provide valuable structural information and can be logically engineered to become potent lead compounds despite their low potency.

An additional or more affordable option to experimental screening is virtual screening. Large virtual chemical libraries can be screened using in silico methods such as pharmacophore modeling and molecular docking to identify possible target binders. Virtual screening increases the likelihood of finding high-quality hits while saving time and money.
Another cutting-edge approach to hit identification is fragment-based drug discovery (FBDD). Low-molecular-weight fragments that bind to the target weakly are screened for using this method. Despite their low potency, fragment hits can be logically developed to become potent lead compounds and offer useful structural information.

The success of drug discovery programs is greatly impacted by effective hit identification, which lays the groundwork for successful lead optimization. Combining computational and experimental techniques has increased hit quality and decreased. The experimental & computational methods are improved hit quality and reduced attrition in subsequent stages(11).

Lead Discovery and Optimization

Lead identification and optimization, which transforms confirmed hit compounds into enhanced lead prospects with improved drug-like properties, is a crucial step in the drug discovery process. Increasing biological activity while lowering toxicity and improving pharmacokinetic characteristics is the main objective of this stage.
As part of the hit-to-lead process, hit compounds are methodically chemically altered to improve potency, selectivity, and target specificity. Medicinal chemists design and synthesize analogues based on structural data obtained from computer modeling and biological testing. These recurrent cycles of design, synthesis, and testing provide promising lead molecules.

Structure–activity relationship (SAR) The foundation of lead optimization is research. Researchers can pinpoint important functional groups in charge of target binding and

effectiveness by examining how modifications in chemical structure affect biological activity. Rational optimization is guided by both qualitative and quantitative SAR techniques. Enhancing pharmacokinetic characteristics, such as absorption, distribution, metabolism, and excretion (ADME), is another goal of optimization. Lead failure is frequently caused by poor solubility, low bioavailability, and fast metabolism. Early assessment of ADME parameters lowers the chance of late-stage attrition and aids in the removal of inappropriate drugs(12).

Safety and toxicity assessment is yet another essential element of lead optimization. To find any safety issues, in vitro toxicity tests like genotoxicity and cytotoxicity are carried out. Structure-based approaches are employed to lessen toxicity and limit off-target effects. By forecasting binding affinity and drug-likeness, computational methods like molecular docking, molecular dynamics simulations, and QSAR modeling aid in lead optimization. Lead optimization has recently been expedited by artificial intelligence and machine learning techniques, which allow for the quick prediction of ideal chemical changes. After this phase, a balanced evaluation of efficacy, safety, and developability is used to choose one or more optimized lead candidates. After that, these candidates move on to preclinical assessment(13).

Drug Design Strategies

Because they make it possible to rationally create and optimize molecules with desired biological activity, drug design methodologies are essential to current drug discovery. These approaches employ biological, chemical, and structural data to direct the creation of therapeutic candidates with enhanced safety, efficacy, and selectivity.

Structure-based drug design (SBDD) is among the most used methods for rational drug design. It depends on the biological target's three-dimensional structure, which is usually acquired via methods like cryo-electron microscopy, nuclear magnetic resonance (NMR) spectroscopy, or X-ray crystallography. Researchers can improve binding affinity and specificity by designing compounds that perfectly fit into the active site when they are aware of the target's binding location. In SBDD, molecular docking and molecular dynamics simulations are often employed tools.

Ligand-based drug design (LBDD)
is used when knowledge on active ligands is available but the target's structure is unknown. This method creates new molecules with comparable or enhanced activity by utilizing the chemical and biological characteristics of existing substances. Ligand-based tactics rely heavily on methods like pharmacophore modeling and quantitative structure–activity relationship (QSAR) analysis.

Computational drug design combines ligand-based and structure-based methods, greatly cutting down on drug discovery time and expense. Prioritizing compounds prior to experimental testing is aided by virtual screening, molecular modeling, and predictive simulations. These techniques increase productivity and lessen reliance on extensive experimental screening(14).

Artificial intelligence (AI) and machine learning (ML) in drug design
Recent developments in machine learning (ML) and artificial intelligence (AI) have greatly changed medication design by making data-driven and predictive methods possible. Early in

the discovery process, AI-based models can evaluate drug-likeness and toxicity, optimize chemical structures, and predict drug–target interactions with high accuracy. Through the quick investigation of a large chemical space made possible by these technologies, new molecular scaffolds with enhanced safety and efficacy profiles can be found. Additionally, machine learning methods allow rational lead optimization and improve structure-activity connection analysis. AI-assisted drug design increases the overall effectiveness of drug discovery programs by lowering trial expenses, time, and attrition rates. Drug design is becoming a potent, effective, and essential part of contemporary pharmaceutical research thanks to the combination of computational tools, AI technology, and medicinal chemistry.

Preclinical Evaluation

Prior to optimized lead compounds entering clinical trials, preclinical review is a crucial stage in the drug development process that evaluates their safety, effectiveness, and pharmacokinetic characteristics. Determining if a medication candidate is appropriate for human testing while reducing potential dangers is the main goal of this stage.The study of pharmacokinetics (PK) and pharmacodynamics (PD) is a crucial part of preclinical evaluation. While pharmacodynamic studies concentrate on the connection between drug concentration and its biological action, pharmacokinetic studies look at how the drug is absorbed, distributed, metabolized, and eliminated in the body. These investigations aid in determining suitable treatment windows and dosage schedules.

ADME studies are conducted early in the preclinical review process to identify potential limitations related to drug-drug interactions, metabolic stability, and bioavailability. In vitro experiments using liver microsomes and cell-based models provide significant insights into metabolic pathways and clearance mechanisms in conjunction with in vivo animal research.Another crucial component of preclinical testing is toxicological evaluation. To assess possible negative consequences, animal models are used for acute, subacute, and chronic toxicity assessments. Depending on the drug's intended clinical usage, specific toxicity studies, such as genotoxicity, reproductive toxicity, and carcinogenicity, may be necessary. By identifying toxic liabilities early on, expensive failures in later stages can be avoided.

Preclinical studies also involve the evaluation of efficacy in relevant disease models. Animal models that closely mimic human disease conditions are used to confirm therapeutic effectiveness and mechanism of action. Biomarkers are often employed to assess target engagement and disease modulation.In addition to scientific considerations, preclinical evaluation must comply with regulatory and ethical guidelines. Good Laboratory Practice (GLP) standards ensure data reliability and reproducibility, while ethical regulations govern the humane use of animals in research.Successful completion of preclinical evaluation leads to the selection of a candidate suitable for clinical development. Robust and well-designed preclinical studies significantly increase the likelihood of clinical success and regulatory approval(15).

Role of Artificial Intelligence and Machine Learning in Drug Discovery

In the field of drug discovery, artificial intelligence (AI) and machine learning (ML) have become revolutionary tools that facilitate quicker, more effective, and data-driven decision-making. Large biological and chemical datasets are used by these technologies to find trends,

forecast results, and streamline drug discovery procedures at various pipeline stages. AI algorithms examine genomes, proteomics, and clinical data in target discovery and validation to find new targets linked to diseases. By forecasting druggability, safety, and clinical relevance, machine learning models aid in target prioritization and lower uncertainty throughout the early stages of discovery.
AI-driven methods have greatly improved virtual screening and hit recognition. Deep learning methods are capable of accurately predicting drug-target interactions and quickly screening large chemical libraries. AI-based screening lowers processing costs and enhances hit quality as compared to conventional techniques.

Machine learning methods forecast pharmacokinetic characteristics, toxicity profiles, and structure–activity connections during lead optimization. These prediction techniques help medicinal chemists create molecules with the best possible potency and drug-like qualities. De novo drug design is made possible by AI, which creates new molecular structures with desired characteristics.
By forecasting ADME and toxicity outcomes, lowering the need for animal testing, and eliminating late-stage failures, AI and ML support preclinical research. Candidate selection is accelerated and risk assessment is improved by integrating AI with experimental data. AI-based drug discovery methods have benefits, but they also have drawbacks, such as issues with data quality, model interpretability, and regulatory acceptability. However, the importance of AI in pharmaceutical research is anticipated to be further strengthened by ongoing improvements in computer power, data accessibility, and algorithm development.
Overall, AI and ML are reshaping the drug discovery landscape by improving efficiency, reducing cost, and enabling the discovery of innovative therapeutic agents(16).

Drug Discovery from Natural and Herbal Sources

For ages, natural goods and herbal remedies have been essential in the search for new drugs because they offer a wealth of bioactive molecules with a variety of chemical structures. Plants, microbes, and marine sources have been the source of numerous clinically effective medications, such as anticancer, antibacterial, and cardiovascular medicines. Because of their long history of traditional use, chemical diversity, and biological significance, natural and herbal sources continue to be of interest.

An essential foundation for the development of herbal medicines is ethnopharmacology. Ayurveda, Traditional Chinese Medicine, and Unani medicine are examples of traditional medical systems that provide important insights into the therapeutic potential of medicinal plants. The choice of plants for scientific study is frequently influenced by these conventional assertions.

Traditional knowledge is combined with cutting-edge analytical and experimental methods in modern herbal medicinal discovery. To separate and identify active ingredients, phytochemical screening and bioassay-guided fractionation are frequently employed. The structural characterization of bioactive chemicals is made easier by methods like nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and high-performance liquid chromatography (HPLC) (17).

Standardization is one of the main obstacles in the search for herbal drugs. The quality and consistency of herbal products can be impacted by variations in plant sources, growth conditions, and extraction techniques. Modern methods include metabolomics, chemometric analysis, and quality-by-design principles to overcome this problem and guarantee efficacy and reproducibility.
Herbal medicine research is increasingly using AI-based techniques and computational tools. Molecular docking, network pharmacology, and in silico target prediction all aid in identifying possible molecular targets of phytochemicals and clarifying their methods of action. These methods help close the gap between contemporary drug development and conventional therapy.

Despite challenges related to complexity, intellectual property, and regulatory approval, natural and herbal sources remain invaluable in the search for new therapeutic agents. Integrating traditional knowledge with modern drug discovery technologies offers promising opportunities for developing safe, effective, and affordable medicines. Despite significant technological advances, drug discovery remains a highly complex, time-consuming, and costly process. Several challenges and bottlenecks affect the efficiency and success rate of bringing new drugs to the market(18).

Challenges and Bottlenecks in Drug Discovery

1. **High Risk, Cost, and Time Constraints:**
   Drug discovery is a lengthy, costly, and risky process that can take up to 10-15 years. Fewer than 10% of clinical trial candidates make it to market owing to effectiveness, safety, or pharmacokinetic issues. Extensive screening, optimization, and clinical testing increase attrition and financial burden.

2. **Scientific and Technical Challenges**
   Scientific and technical challenges include selecting the appropriate target, validating it, and comprehending complex disease biology. Unexpected toxicity, poor druggability, and data complexity from high-throughput and omics research all enhance the likelihood of failure. Reproducibility difficulties in experiments hamper the discovery process.

3. **Regulatory, Ethical, and Source-Specific Challenges:**
   Development may be delayed by ethical, legal, and source-specific issues. Batch variability, standardization, and ambiguous source-specific, ethical, and regulatory challengesProgress can be delayed by strict legal restrictions, moral guidelines, and intellectual property issues. The range of applications for natural and herbal products is restricted by standardization, batch variability, and unclear mechanisms of action. Integrated methods based on systems pharmacology, artificial intelligence, and creative preclinical models are required to overcome these limitations(19).

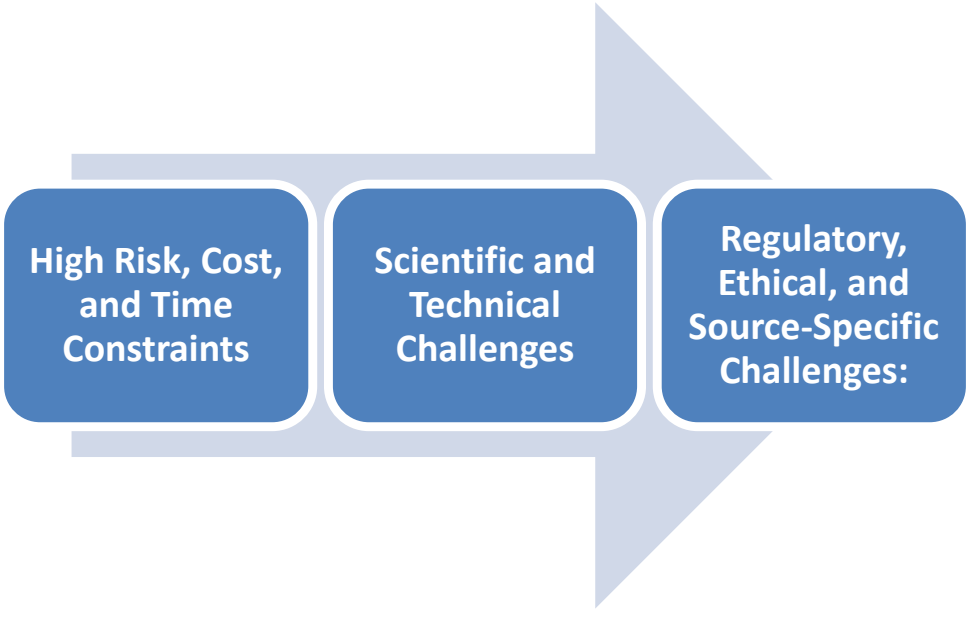

Figure 2:Challenges and Bottlenecks in Drug Discovery

Future Perspectives

The future of drug discovery is being shaped by technological innovation, data-driven approaches, and a growing understanding of human disease at the molecular level. Emerging strategies promise to accelerate the discovery of safer, more effective, and personalized therapeutics.

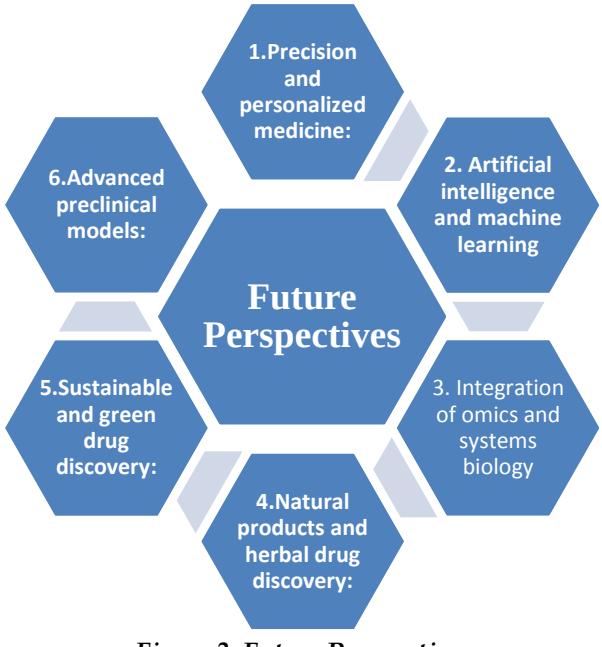

Figure 3: Future Perspectives

1. Precision and personalised medicine
The creation of medications customized to each patient's unique genetic and molecular profile is made possible by developments in proteomics, genomics, and patient-derived data. The goal of precision medicine is to lessen side effects while increasing therapeutic results.

2. Artificial intelligence and machine learning
From target identification to lead optimization and prediction toxicity, AI and ML are anticipated to have an even greater impact on drug research. Predictive modeling can lower experimental costs, increase hit quality, and speed up discovery times.

3. Integration of omics and systems biology
A thorough understanding of disease pathways is made possible by combining transcriptomics, proteomics, metabolomics, and genetics. Multi-target drug development is made possible by systems pharmacology techniques, particularly for complicated disorders.

4. Natural products and herbal drug discovery
The identification and standardization of bioactive chemicals from natural sources are being improved by cutting-edge analytical, computational, and AI-driven methods. Novel medicines could result from integration with contemporary discovery strategies.

5. Sustainable and green drug discovery
The identification and standardization of bioactive chemicals from natural sources are being improved by cutting-edge analytical, computational, and AI-driven methods. Novel medicines could result from integration with contemporary discovery strategies.

6. Advanced preclinical models
It is anticipated that organ-on-chip, 3D cell culture, and patient-derived organoids will increase translational relevance, decrease the need for animals, and enhance clinical outcome prediction(20).

7. Collaborative and open innovation
Open-access databases, crowdsourced projects, and collaborations between academia, business, and CROs are promoting innovation and speeding up drug discovery worldwide. In general, faster, safer, more economical, and patient-centred methods are becoming the norm in drug discovery. The future generation of therapies will be defined by the fusion of conventional wisdom, contemporary technology, and interdisciplinary cooperation.

Conclusion

The foundation of contemporary pharmaceutical research is the intricate, diverse, and dynamic process of drug discovery. Every step of the process, from the first identification of disease-associated targets to the optimisation of lead molecules and preclinical evaluation, calls for meticulous preparation, rigorous science, and creative thinking. The scope and effectiveness of drug discovery have been increased by combining traditional knowledge—such as the sources of herbal and natural products—with contemporary methods—such as medicinal chemistry, computational modelling, and artificial intelligence.
Drug discovery still faces several obstacles despite the impressive advancements, such as high attrition rates, safety issues, regulatory barriers, and the growing complexity of diseases. Addressing these challenges requires a combination of experimental, computational, and

translational approaches, supported by interdisciplinary collaboration between academia, industry, and regulatory bodies.

Precision medicine, AI-driven drug design, sustainable practices, and data-integrated methods are the ways of the future for drug discovery. Organ-on-chip models, multi-omics integration, and AI-assisted lead optimization are examples of emerging technologies that have the potential to speed up discovery, lower costs, and enhance patient outcomes. By balancing innovation with scientific validation and ethical responsibility, drug discovery will continue to deliver novel and effective therapeutics to meet the evolving healthcare needs of society.

In summary, drug discovery is not just the identification of molecules but a strategic, systematic, and multidisciplinary journey toward improving human health. Continued research, technological integration, and global collaboration will shape the next generation of drugs and transform the landscape of pharmaceutical science.

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Chapter- Three

Reinventing Lipid Nanotechnology: Next-Generation Nanostructured Lipid Carriers for Smarter Drug Delivery

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Abstract

Drug delivery technologies have undergone remarkable progress with the advent of nanostructured lipid carriers (NLCs), NLCs creates a distinctively disordered matrix by combining liquid and solid lipids, which improves drug-loading capabilities, preserves stability and permit a more regulated release profile. In contrast to traditional solid lipid nanoparticles (SLNs), which can suffer from premature drug expulsion and limited encapsulation efficiency, NLCs greatly minimize these drawbacks-allowing for better retention and higher loading of active pharmaceutical ingredients. The fabrication of NLCs can be achieved through several methods, such as high-pressure homogenization, solvent diffusion, or micro fluidization, each permitting precise tailoring of the carrier system. Structurally, NLCs may be categorized into imperfect crystalline, multiple, or amorphous types, with each variant offering specific advantages for encapsulating drugs with varying physicochemical characteristics. NLCs have demonstrated considerable versatility in their routes of administration, showing promise in pulmonary, topical and ocular applications. Such flexibility not only enhances bioavailability but also reduces undesired systemic side effects an essential consideration in modern pharmaceutical design. Furthermore, their biocompatibility, along with ease of large-scale production, underscores the potential of NLCs as robust platforms for targeted therapies and innovative pharmaceutical development.

Keywords: *Targeted therapy, Controlled release, Liquid lipid incorporation, Bioavailability, Therapeutic targeting.*

Introduction

In modern decades, considerable advancement has been achieved in the development of various drug delivery platforms have been developed to enhance therapeutic efficacy of API as well as enable site-specific delivery. Among these systems, nanotechnology has evolved into groundbreaking strategy, especially within the field of nanomedicine. Nanoparticles, owing to their distinctive physicochemical including a high surface-to-volume ratio, enhanced permeability distinct optical and electrical characteristics have been extensively investigated for their potential to improve drug delivery. Their nano-scale size allows better penetration through biological barriers and more precise targeting of diseased tissues[1]. These innovations have contributed to the development of nanocarriers, which are now widely recognized for their ability to increase bioavailability, control drug release and reduce

systemic toxicity. lipid-based nanocarriers particularly solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have gained significant attention for their able to incorporate both hydrophilic and lipophilic drugs[2]. This approach offers several advantages, including biocompatibility, biodegradability and scalability, making them promising potential solutions to address the limitations of conventional drug administration strategy. As a result, nanotechnology is now seen as a crucial bridge between physical and biological sciences in advancing targeted and efficient drug therapies[3].

Classes of Nanocarriers

Nanocarriers is able to broadly classified according to the materials used for the purpose of construct them this includes lipid-based nanocarriers, polymer-based nanocarriers, dendritic nanocarriers, inorganic nanoparticles and nano emulsions[4-5]. Lipid-based nanocarriers are biocompatible, biodegradable systems capable of encapsulating both hydrophilic and lipophilic drugs. Liposomes are spherical vesicles composed of phospholipid bilayers surrounding an aqueous core, enabling dual drug loading[6-7]. They exhibit excellent biocompatibility and low toxicity, with variants such as conventional, PEGylated, ligand-targeted, and theranostic liposomes. Common preparation methods include thin-film hydration, solvent injection, surfactant solubilization and microfluidics, though limited internal space can restrict hydrophobic drug loading[8-9]. Solid lipid nanoparticles (SLNs) are submicron carriers made from lipids that remain solid at physiological temperatures, offering stability, controlled release, and suitability for diverse drugs; however, their ordered matrix may limit drug encapsulation[10-11]. Nanostructured lipid carriers (NLCs), composed of mixed solid and liquid lipids, feature a less ordered structure that enhances drug loading, minimizes leakage, and improves stability, representing an advanced alternative to SLNs.

Polymer-based nanocarriers are versatile systems constructed from natural or synthetic polymers, enabling efficient encapsulation of diverse therapeutic agents[12-13]. Polymeric micelles form through the self-assembly of amphiphilic block copolymers above the critical micelle concentration, producing core–shell structures (5–100 nm). The hydrophobic core solubilizes poorly water-soluble drugs, while the hydrophilic shell enhances stability, circulation time, and biocompatibility[14-15]. They are typically prepared by direct dissolution, solvent evaporation, or dialysis. Polymer vesicles (polymersomes) are bilayered structures formed from amphiphilic block copolymers that mimic liposomes, with sizes ranging from nanometers to micrometers. Their responsiveness to pH and temperature allows customizable drug loading and controlled release. Dendrimers are highly branched, monodisperse macromolecules (5–20 nm) with tunable surface functionalities[16-17]. They enable high drug loading via encapsulation, electrostatic interactions, or covalent conjugation and release drugs through enzymatic or environmental triggers. Nanoemulsions are kinetically stable dispersions with droplet sizes ≤100 nm, available as O/W, W/O, and multiple emulsions. Prepared using high-energy (ultrasonication, high-pressure homogenization) or low-energy (phase inversion) methods, they enhance solubility and bioavailability[18-19]. Inorganic nanoparticles—including gold, silver, iron oxide, and silica—offer unique optical, magnetic, and functionalization properties, broadening their application in imaging, diagnostics, and drug delivery[20-21]. Together, these polymer-based and hybrid systems provide adaptable platforms for targeted, controlled, and efficient therapeutic delivery.

Methods of Preparation

One method for creating nanostructured lipid carriers (NLCs) is to use a variety of techniques, each with distinct advantages and limitations depending on the drug properties, scalability, thermal sensitivity and desired particle characteristics.

High-Pressure Homogenization (HPH)

High-pressure homogenization is one of the most widely used top-down method suitable for both laboratory and industrial-scale production. Its breakdown larger particles into nanosized carriers through high shear forces. There are two main types of HPH: Hot HPH and Cold HPH.

Hot high-pressure homogenization (HPH) involves melting the drug and lipid above the lipid's melting point and blending the mixture with a hot surfactant solution to form a pre-emulsion. This pre-emulsion is processed through a high-pressure homogenizer, where intense shear reduces droplet size. Upon cooling, the lipid solidifies, forming nanostructured lipid carriers (NLCs). The method offers advantages such as small particle sizes, scalability, and the absence of organic solvents. However, it is unsuitable for thermolabile drugs, as high temperature and mechanical stress may degrade sensitive compounds.

Cold HPH was developed to overcome these limitations. In this technique, the drug–lipid mixture is melted, rapidly cooled, and solidified, then ground into fine microparticles. These solid particles are dispersed in a cold surfactant solution and homogenized under high pressure. This approach enhances entrapment efficiency and preserves heat-sensitive drugs, although it generally produces broader particle size distributions compared to the hot method.

High-Speed/Shear Homogenization (HSH)

High-speed homogenization involves melting the lipids and emulsifying them with a surfactant at high speed. The mixture is then subjected to ultrasonication and cooling to form NLCs. This method is fast, energy-efficient and relatively simple to perform. However, it has limitations such as the risk of particle aggregation, potential metal contamination from ultrasonic probes and reduced storage stability of the final product.

Microemulsion Technique

This method involves on forming a hot oil-in-water (o/w) microemulsion using lipid, surfactant and water at elevated temperatures. This hot microemulsion is then rapidly dispersed into cold water (0–4°C), which causes rapid recrystallization and formation of NLCs. The technique is simple and does not require high-pressure equipment, making it accessible. However, it requires high amounts of surfactants and water and careful optimization of the system is needed for consistent results[23].

Solvent Evaporation & Diffusion Techniques

Solvent-based bottom-up methods are advantageous for formulating heat-sensitive drugs. In solvent evaporation, lipids and drugs are dissolved in a water-immiscible organic solvent and emulsified in an aqueous surfactant solution, often using ultrasonication. As the solvent evaporates, NLCs form, though concerns include residual solvent toxicity and low lipid content. In solvent diffusion, a lipid–solvent mixture is emulsified into a surfactant solution and diluted with water, allowing the solvent to diffuse out and nanoparticles to precipitate. This technique is suitable for thermolabile drugs and avoids high temperatures but still carries risks associated with organic solvent use.

Hot Melt Extrusion (HME)
Hot melt extrusion involves feeding a mixture of solid lipid and drug into an extruder, where they are blended under high temperature and pressure. The material is then forced through form nanoparticles. HME is a continuous, solvent-free procedure that enhances the solubility of hydrophobic drugs. However, the high temperatures involved are not suitable for heat-sensitive compounds and the equipment cost is relatively high[25].

Solvent Injection Technique
By dissolving the lipid in a water-miscible organic solvent (such as ethanol), the lipid is injected into an aqueous surfactant solution while being continuously stirred. Lipid precipitation and the development of NLC are caused by rapid solvent diffusion. This method doesn't require high-pressure equipment and is quick and simple to use. On the down side, problems could arise from remaining solvent and comparatively higher particle sizes[26].

Phase Inversion Temperature (PIT) Method
The PIT technique based on non-ionic surfactants temperature-dependent hydrophilic-lipophilic balance (HLB). The emulsion is subjected to controlled temperature cycles (e.g., 85–60–85°C), during which it alternates between water-in-oil and oil-in-water phases. Final dilution with cold water result in NLCs formation. This method is solvent-free, energy-efficient and ideal for thermolabile drugs. It can achieve very small particle sizes (<25 nm), although it requires precise temperature control.

Micro fluidization Method
Micro fluidization employs a specialized device that forces liquids through microchannels at extremely high speeds (up to 400 m/s), this generates intense shear, cavitation and impact forces resulting in nanosized particles. This method produces highly uniform NLCs and is scalable for industrial applications. It is especially useful when narrow particle size distribution is required[27].

Membrane Contactor Method
This process involves applying pressure to force the molten lipid phase through a membrane's pores and into a cool aqueous surfactant solution. The lipid droplets are immediately cooled and solidified to form NLCs. This technique offers good control over particle size and is scalable. However, membrane clogging is a potential challenge, especially with viscous lipid mixtures.

Supercritical Fluid (SCF) Method
This technique involves dissolving the lipid and drug in a supercritical fluid, typically supercritical CO_2, followed by rapid expansion or atomization into a chamber where pressure reduction leads to precipitation and NLC formation. It is a solvent-free, environmentally friendly method that produces dry particles. Despite its promise, it remains under development and requires tight control over operating parameters.

Double Emulsion Technique (w/o/w)
The double emulsion (w/o/w) technique is particularly effective for encapsulating hydrophilic drugs. In this method, the aqueous drug solution is first dispersed into a molten lipid phase to form a primary water-in-oil (w/o) emulsion. This is then re-emulsified into an external aqueous surfactant solution to create the final water-in-oil-in-water (w/o/w) system.

Following solvent removal or cooling, nanostructured lipid carriers (NLCs) are produced. Although this method enables high encapsulation efficiency, its multistep nature makes it complex and susceptible to stability challenges[28].

Different nanocarrier production methods vary in solvent use, scalability, heat suitability, and particle-size control. Hot HPH is solvent-free and scalable but unsuitable for thermolabile drugs. Cold HPH, high shear homogenization, microemulsion methods, solvent injection, PIT, microfluidization, membrane contactors, and supercritical fluid technology generally support heat-sensitive drugs and offer good to excellent size control. Solvent-based techniques and double emulsion methods suit thermolabile drugs but carry concerns regarding residual solvents[29].

TYPES OF NANOSTRUCTURED LIPID CARRIERS (NLCS)

Nanostructured lipid carriers (NLCs) are categorized into three main types based on the arrangement of solid and liquid lipids within their matrix. Type I, the Imperfect Crystal Type, is produced by blending structurally different solid and liquid lipids, creating a disordered matrix with voids that enhance drug loading[30]. These imperfections improve entrapment efficiency and accommodate moderately lipophilic drugs, though slight leakage may occur. Type II, the Multiple Type, contains small oily compartments dispersed within a solid lipid matrix. This structure forms when excess liquid lipid is added, resulting in internal oil pools that greatly enhance the solubility and loading of highly lipophilic drugs while providing controlled release and excellent stability[31]. Type III, the Amorphous Type, is designed to prevent crystallization by incorporating special lipids that keep the matrix in a non-crystalline amorphous state. This eliminates drug expulsion during cooling and offers superior long-term stability, making it ideal for drugs susceptible to leakage from crystalline structures[32].

Table-2 types of Nanostructured Lipid Carriers (NLCs)

Type	Structure	Liquid Lipid Content	Main Features	Suitable For
Type I	Imperfect Crystal (Disordered Matrix)	Low–Moderate	Contains voids and lattice imperfections; enhanced drug loading; partially crystalline	Moderately lipophilic drugs
Type II	Multiple Type (Oil-in-Fat-in-Water)	High	Internal oil compartments; improved solubility; controlled and sustained release	Highly lipophilic drugs
Type III	Amorphous (Non-Crystalline Matrix)	Variable	Completely amorphous; prevents crystallization and drug expulsion; excellent stability	Thermolabile or crystallization-sensitive drugs

MECHANISM OF NANOSTRUCTURED LIPID CARRIER (NLC) SYSTEM

Disordered Lipid Matrix

A defining feature of NLCs is the incorporation of liquid lipids into a solid lipid matrix, which results in a structurally disordered and less crystalline structure. This irregularity increases imperfections within the lipid matrix, creating additional space for drug molecules

to be incorporated and retained. Unlike highly crystalline structures, these imperfections help reduce drug expulsion during storage and improve encapsulation efficiency. Drugs in NLCs can localize within amorphous regions, lipid bilayers, or even in structural voids formed between fatty acid chains. This facilitates both sustained release and higher drug loading capacity[33].

Effect of Lipid Purity

The purity and crystallinity of lipids used in NLCs significantly influence drug release kinetics. Lipid matrices composed of highly pure and ordered lipids (e.g., tristearin) tend to form tightly packed structures with minimal imperfections. As a result, these systems usually exhibit low drug-loading capacity and faster drug release, often occurring within a few hours to a couple of days. In contrast, NLCs with less ordered lipid matrices provide a more sustained and controlled release profile due to their ability to trap drugs more effectively.

Biphasic Drug Release

Many NLC systems demonstrate a biphasic release pattern, characterized by two distinct phases:

Initial Burst Release: This phase occurs quickly after administration and is attributed to drug molecules that are loosely associated with or adsorbed onto the exterior or surface of the NLCs.

Sustained Release: Following the initial burst, the remaining drug is released gradually as it diffuses through the dense lipid core. This allows for extended therapeutic action over time.

For example, melatonin-loaded NLCs have shown a sustained release of approximately 92% over a 48-hour period, highlighting their potential for prolonged drug delivery[34].

Stimuli-Triggered Release

In some applications, external triggers such as temperature changes or water evaporation can induce controlled or targeted drug release. This mechanism is particularly useful in topical therapies, such as delivering cyclosporine for psoriasis treatment. When heat is applied to the NLC system: Water loss from the formulation occurs. This leads to reordering of the lipid matrix due to thermal motion. This leads to drug expulsion and a burst release, enhancing localized therapeutic effect. This controlled activation enables on-demand drug release in response to physiological or environmental conditions[35].

Polymer-Coated NLCs

To further refine and control drug release, polymer coatings can be applied to NLCs. These coatings act as semi-permeable barriers, slowing the release of the drug by regulating diffusion through the polymer layer. This approach provides additional control over release kinetics, protects the encapsulated drug from premature degradation and may enhance the stability and targeting capabilities of the formulation[36].

Table-3: Factors influencing drug release

Factor	Effect on Drug Release
Particle Size	Smaller particles release drugs faster due to higher surface area; also improve physical stability.
Lipid Matrix	Lipid type affects melting point, HLB, and drug binding; e.g.,

	Precirol ATO 5 shows higher drug entrapment than Compritol 888.
Surfactant Type & Concentration	Surfactants reduce surface tension, improve particle size and uniformity; Gelucire combined with Tween 80 or Pluronic F68 gives smallest particles.
Drug Localization	Drugs in the shell cause burst release; drugs in the core provide sustained release.
Auxiliary Ingredients	Can form complexes with drugs, altering solubility and release kinetics (e.g., sustained-only release).
Stirring Time	Insufficient stirring produces larger particles and inconsistent release.
Temperature During Preparation	Optimal 5–10°C above lipid melting point; too low causes incomplete melting, too high may degrade drug/lipid.
Preparation Method	Hot HPH may cause burst release due to redistribution; Cold HPH avoids heat degradation, giving sustained release.

SOURCES OF COMPONENTS IN NANOSTRUCTURED LIPID CARRIERS (NLCs)

The formulation and performance of Nanostructured Lipid Carriers (NLCs) depend heavily on the selection of lipids, surfactants, and other excipients, which collectively influence drug loading, particle size, stability, crystallinity, release profile, and biocompatibility.

Lipids serve as the core structural component, determining drug encapsulation, release, and physical stability. Solid lipids like fatty acids, triglycerides, steroids, and waxes provide structural integrity, while liquid lipids (oils) disrupt crystalline packing, increasing drug accommodation. Ideal lipids are non-toxic, physiologically compatible, and GRAS-listed. Lipid selection is influenced by drug solubility, crystallinity, hydrophilicity/hydrophobicity, melting point, and crystal morphology, all affecting encapsulation efficiency, particle size, and stability.

Surfactants stabilize the lipid–aqueous interface, prevent aggregation, and impact particle size, drug permeability, and release kinetics. They reduce interfacial tension, stabilize nanoparticles during crystallization, and improve dissolution and bioavailability. The required HLB (rHLB) identifies the optimal surfactant type and concentration for stable NLC formation. Common surfactants include Tween 80, Poloxamers, Gelucire, or combination systems, chosen to ensure stability and controlled drug release. Other components like organic salts, ionic polymers, and surface modifiers (PEG, poloxamers) enhance drug entrapment, stability, biocompatibility, circulation time, targeted delivery, and epithelial transport40.

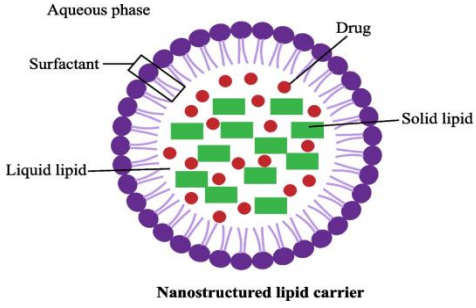

Figure 1. Nanostructured Lipid Carrier

Targeted Drug Delivery System Using Lipid Nanocarriers

Lipid-based nanocarriers, including Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), offer targeted drug delivery by enhancing therapeutic efficacy, reducing systemic side effects, and enabling controlled release. Drug release occurs primarily via diffusion through the lipid matrix and erosion of the lipid core, influenced by external factors such as temperature and pH. Pulmonary delivery is a notable non-invasive route, leveraging the lungs' large surface area, rich blood supply, and avoidance of first-pass metabolism, suitable for local and systemic treatment of cancer, infections, immune deficiencies, and inflammatory diseases. NLCs promote uniform alveolar distribution, improved bioavailability, and reduced drug degradation. Topical delivery benefits from bypassing first-pass metabolism and maintaining local drug levels, with lipid nanocarriers enhancing skin penetration, providing sustained release, and reducing irritation. In ocular delivery, physiological barriers such as tear drainage and low corneal permeability limit drug availability; lipid nanocarriers improve corneal penetration, prolong residence time, and increase bioavailability. These carriers are effective for glaucoma, ocular infections, inflammation, and posterior eye diseases, demonstrating versatility across multiple targeted delivery routes[40].

Targeted Drug Delivery System

Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) are two examples of lipid nanocarriers that offer a sophisticated platform for targeted drug administration because of their controlled release characteristics, biocompatibility and capacity to enhance therapeutic efficacy. The primary drug release mechanism in these carriers involves diffusion via breakdown and the lipid matrix or erosion of the lipid structure. External factors such as pH changes and temperature fluctuations can also impact the stability of the lipid membrane, thereby influencing drug release. These systems can be engineered for delivery that is site-specific, reducing systemic side effects and improving treatment outcomes.[41]

Pulmonary Delivery

Pulmonary delivery is a non-invasive route ideal for achieving both local and systemic therapeutic effects. The lungs provide a large surface area, extensive blood supply and limited first-pass metabolism, allowing for rapid and efficient drug absorption. Lipid nanocarriers administered via inhalation distribute drugs uniformly in the alveolar region, enhancing bioavailability and reducing drug degradation. This method has been explored for treating diseases such as cancer, autoimmune disorders, infections and acute pain. Additionally, NLCs promote patient compliance because of their minimal side effects and non-invasive nature[42].

Topical Delivery

Topical drug delivery is widely preferred for treating skin conditions because of its localized effect and minimal systemic absorption. This route bypasses first-pass metabolism and allows for prolonged drug retention at the application site. However, drug penetration often limited by the skin's outer barrier—the stratum corneum. Lipid nanoparticles such as SLNs and NLCs overcome this limitation by enhancing permeability, offering sustained drug release and minimizing skin irritation. These advantages make them superior to traditional topical formulations like creams and emulsions[43].

Ocular Delivery

Delivering drugs to the eye, partially the posterior segment, is challenging due to various anatomical and physiological barriers. After conventional topical administration, only about 5% of the drug remains at the site of action due to rapid tear turnover and drainage. Invasive methods such as intraocular injections are sometimes used, but carry risks including infection, bleeding, and potential vision damage. NLCs and SLNs provides a non-invasive, safe and effective alternative with improved corneal permeability, longer retention time and enhanced bioavailability. These formulations are being extensively investigated for the treatment of ocular disorders such as glaucoma, infections, inflammation and diseases affecting the posterior segment, offering better patient compliance and minimal local side effects[44].

Conclusions

Nanostructured Lipid Carriers (NLCs) represent a notable advancement in drug delivery systems, largely owing to their superior drug encapsulation, stability and ability to provide controlled release. By leveraging a blend of solid and liquid lipids, NLCs successfully mitigate several challenges faced by earlier lipid-based drug carriers. Their adaptability across multiple administration routes such as pulmonary, topical and ocular applications-positions them as promising platforms for targeted therapy, potentially enhancing bioavailability while minimizing adverse effects. As progress continues in nanotechnology and pharmaceutical formulation, NLCs are poised to play an increasingly important role in the development of personalized and highly effective drug delivery strategies.

Future Prospects

Nanotechnology plays a pivotal role in modern cosmetics, enhancing skin penetration, protection, moisturization, and anti-aging effects. With advancements in omics sciences, big data and machine learning, it is now possible to evaluate cellular responses to cosmetic ingredients. The rise of "cosmeceuticals" highlights products that not only improves appearance but also treat skin conditions. Nanoparticles such as liposomes, dendrimers and nano emulsions enable efficient delivery through the skin barrier. Nanobiotechnology aids in developing biomimetic carriers that transport natural compounds to targeted cells. Additionally, it supports sustainable cosmetic formulation by enabling structural customization and improving excipients for better safety, efficacy and consumer satisfaction.

Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviations

Solid Lipid Nanoparticles (SLNs), Polyethylene Glycol (PEG), Critical Micelle Concentration, (CMC), Phase Inversion Temperature (PIT), Hydrophilic-Lipophilic Balance (HLB)

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Chapter- Four

Synthesis Pathways of Anticancer Drugs: Integrating Conventional, Non-Conventional Approaches with DFT Insights and Mechanistic Understanding

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Abstract

Synthesis and development of Anticancer Agents comes into prominence, offering potential pathways for cancer treatment. Continuous search for effective, selective, less or non toxic anti-cancer agents is a critical challenge because of the complexity of cancer as a disease and its increasing global health burden. This review investigates the synthesis schemes of anticancer agents; examining both conventional and non-conventional pathways such as classical organic synthesis and semi-synthetic pathways that incorporate green chemistry principle, bio-catalysis and nanotechnology assisted strategies along with some highlighting insights from Density Functional Theory (DFT) studies based on available literature. DFT studies provides understanding of molecular reactivity, stability and interaction mechanism that support the informed and systematic design of a molecule. Anticancer agents are examined from their roots in traditional chemotherapy to their role in today's cutting-edge treatments in terms of method of synthesis, Mechanism of action, targeted therapies, potency, toxicity, etc. This review provides an overview of the synthesis of anticancer agents and their variety of attributes.

Keywords: *Synthesis of Anticancer Agents, conventional and non-conventional pathways, DFT studies.*

Introduction

Cancer- a second leading cause of death after cardiovascular diseases; concisely distinguished by uncontrolled growth and spread of cells caused by both environmental (tobacco, chemicals, radiations infections) and biological factor (inherited or metabolic mutations, hormones, immune conditions)[1].According to the World Health Organization, cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018 (WHO, 2023). Among three major therapies, Drug Therapy remains a key cancer treatment on account of advances in science and technology, molecular oncology and biology; driving emergence and development of novel anti-cancer agents marking new stage in anti-cancer drug development[2].

Anticancer agents are the entities having chemical and biological framework used to prevent, control or eradicate the malignant cells encompassing different working mechanism. Some may damage the DNA, blocking signaling pathways that assist tumour growth, strengthening of immune system to target and eliminate infectious cells,etc.[3,4,5]. The intrinsic

or acquired drug resistance arising from tolerance of cancer cells to pharmaceutical treatment and toxicity[6,7] are the major problems in cancer therapies. Drug development, discovery and manufacturing have faced major challenges over the last two decades because of low success rate and limited progress in cancer treatment despite spending a lot of money[8].

Rather than former trial and error method for the screening of chemical compounds, the advancement in science and technology makes the research more coherent and controlled[9]. Targeted therapies have shown that cancer drugs aimed at specific molecular target can yield excellent outcomes. For example the imatinib for chronic myeloid leukemia[10]. Immunotherapies like checkpoint inhibitors involves activation of body's immune system against tumour Even though these traditional synthetic approaches are essential[11], but modern synthesis approaches like microwave assisted synthesis[12], flow chemistry[13], click chemistry[14] and green chemistry[15] helps to synthesize drug candidate more efficiently.

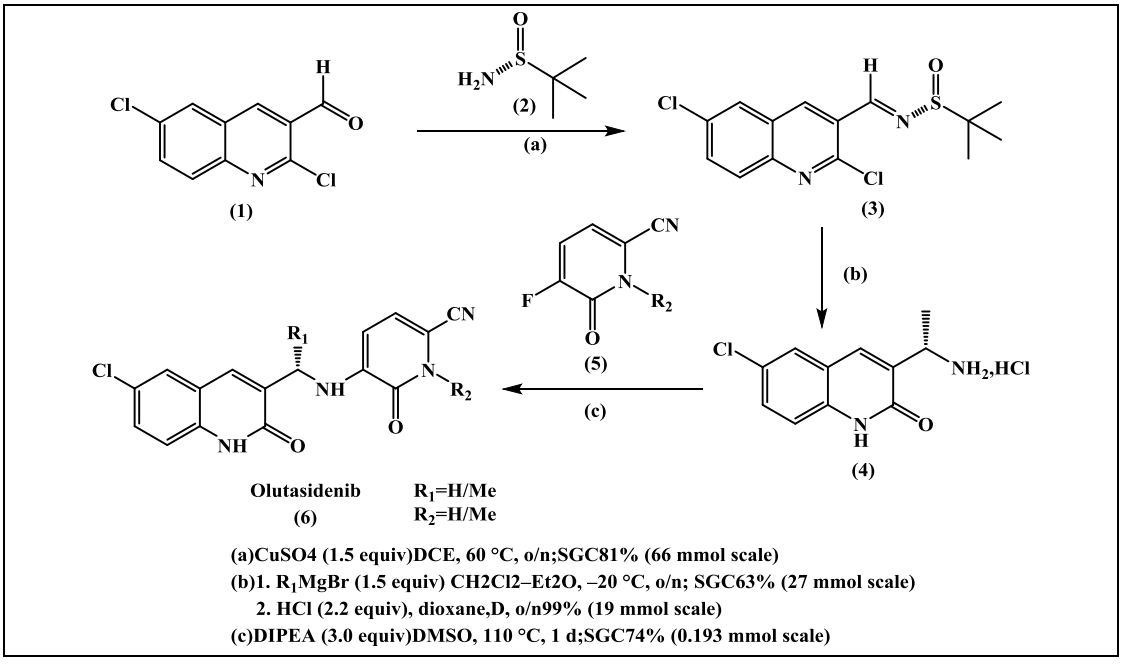
Along with the experimental study, computational chemistry has become vital tool in drug development. Of which Density Functional Theory (DFT) being notably beneficial; as it describes electronic molecular structure allowing scientist to study drug interactions at atomic level- more accurately and with reasonable computational efforts[16].the basis of DFT was developed by Kohn and Sham; who introduced the self consistent equations accounting electronic interaction through exchange and correlation effect[17].DFT is widely employed to study binding interaction between drug and biological target[18]; activation of pro-drug[19] and electronic features contributing to activity or toxicity[20].It provides detailed insights about electron distribution, frontier orbitals and reaction pathways which are often difficult to observe directly in experiments.This information provides the understanding of why certain drug modifications increase potency and reduce side effects. For example, potential toxicity due to reactive metabolites can be predicted by analyzing electronic properties and redox behaviour of compounds[21]. This DFT model supplements to the other modelling approaches like molecular docking, molecular dynamics and hybrid (QM/MM) methods and has predictive ability that helps chemist to focus on molecules with better safety before costly experiments which makes it highly valuable[22].

High efficiency can be achieved in drug discovery thorough the assimilation of synthetic chemistry-allowing rapid exploration of new chemical structure and DFT-providing mechanistic understanding that guides further modification together these techniques reduces the risk of failure, saves significant amount of time, and improves the probability of synthesizing clinically useful anticancer agents[23]. The persistent problem like drug resistance arising from specific mutations in targets or altered metabolism in tumour cells can be overcome through this combined approach[24].

Highlighting the recent progress in the development of anticancer agents with focusing on synthesis methods (conventional and non-conventional), computational studies using DFT, and their role in understanding drug reactivity, mechanism and toxicity is the main purpose of this comprehensive review. By examining the literature we aim to provide a clearer picture of how experimental and theoretical methods work together and ti identify research gaps that need further exploration.

CONVENTIONAL SYNTHESES

1. Olutasidenib:



Scheme 1: Synthesis of Olutasidenib by Ellman Davis Addition.

Ellman–Davis diastereoselective addition of alkylmagnesium bromide to tert-butanesulfinimine (3) that delivered the sulfinamine adduct in 63% yield and dr = 98:2 is pivotal step in the synthesis of olutasidenib (FT-2102) [25]. The (S)-methyl group is responsible for improvement in solubility and potency (IC_{50} = 21 nM). The in vivo and in vitro preclinical study results confirms that the Olutasidenib has good balance of potency and desirable drug like properties inhibiting (R)-2-hydroxyglutarate (2-HG) production by multiple IDH1-R132 mutants, making it effective in treating tumors that have specific IDH1-R132 mutants.It is highly selective for IDH1 isoforms, showing no appreciable inhibition against wild-type IDH1 and IDH2 mutants. The Structure Activity Relationship (SAR) study validates that the substitution of methyl group at R_1 and R_2 position shows excellent potency, solubility and human liver microsomes (HLM) stability[26].

Table 1: Structure−Activity Relationship of Quinoline Inhibitors of mIDH1-R132H (Mutant selective isocitrate dehydrogenase 1)

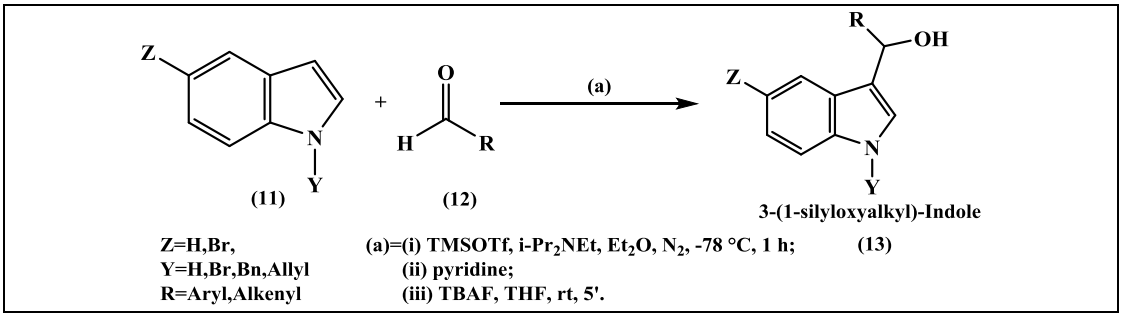
R_1	R_2	IDH1-R132H IC_{50} (µM)	HLM/MLM(% left at 30 min)	Solubility(µM)
H	Me	0.0403	99/73	0.295
H	H	0.0647	100/86	2.21
Me	Me	0.0212	100/92	34.5

Olutasidenib which has potential utility as a brain penetrant therapeutic agent for treatment of isocitrate dehydrogenase [NADP(+)] 1 (mIDH1)-driven CNS tumors including

hematologic malignancies, solid tumors, and gliomas, is currently undergoing clinical evaluation [Error! Bookmark not defined.]. IDH1 mutations cause an increase in 2-hydroxyglutarate, which impairs normal cell differentiation. Olutasidenib inhibits mutant forms of IDH1, leading to decreased levels of 2-hydroxyglutarate, which in turn, restores normal differentiation of hematopoietic cells[27].

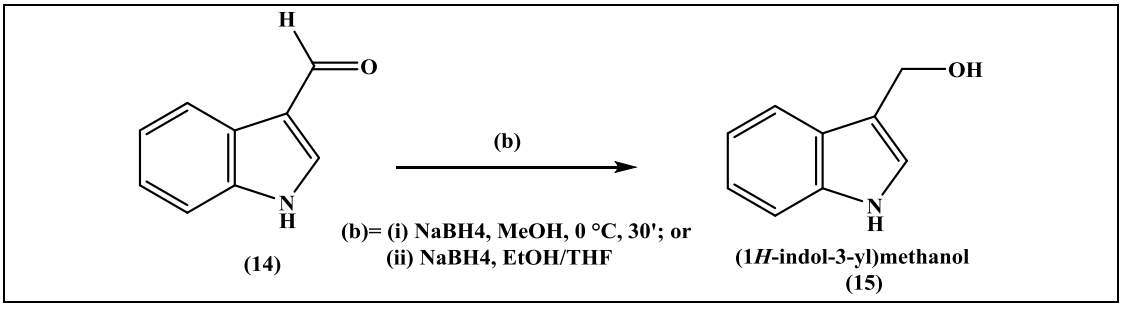
2. Indole-3-Carbinol (I3C) :

Amongst the large number of methods to synthesize Indole-3-Carbinol **(I3C)**, two characteristic methods **(Scheme 2 & 3)**, one that gives higher yield **(Scheme 2)** and another that works well for large scale production **(Scheme 3)** are chosen. Friedel-Crafts addition of indoles**(11)** to aldehydes**(12)** yield 3-(1-silyloxyalkyl)-Indoles**(13)** using trimethylsilyl trifluoro methanesulfonate along with trialkylamine. Reaction is arrested by the addition of pyridine followed by deprotection with tetrabutylammonium fluoride (TBAF) under basic condition and yields the desired product **(13)(Scheme 2)** [28].



Scheme 2: Synthesis of I3C through Friedel Craft Addition of Indole and Aldehyde

Another method for the synthesis of I3C is reduction of corresponding aldehydes**(14)** using sodium boron hydride ($NaBH_4$) under basic condition as follows **(scheme 3)**[29].



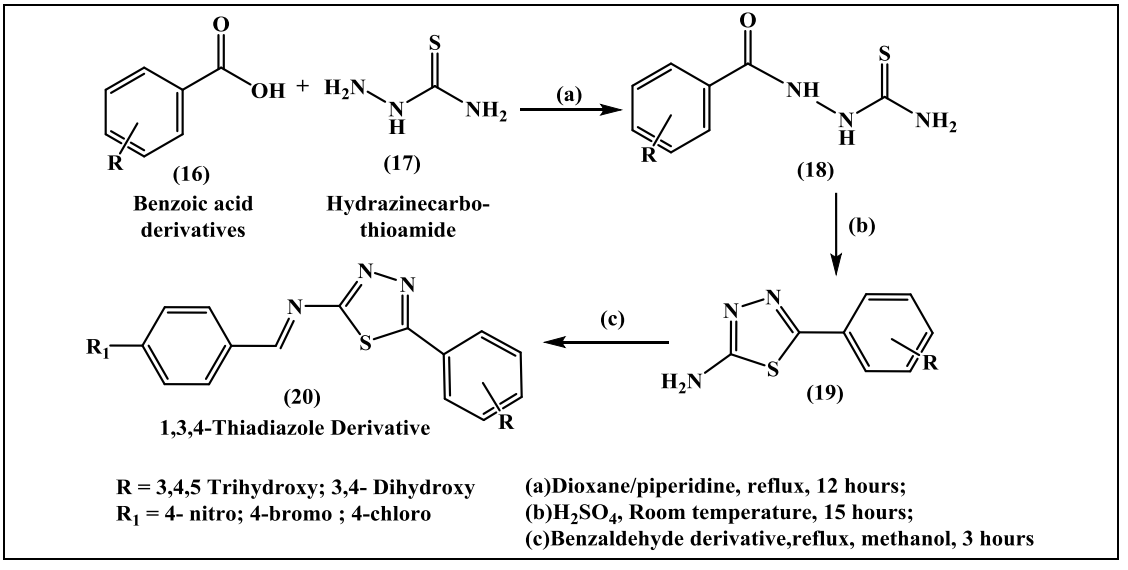
Scheme 3: Synthesis of Indole-3-Carbinol through Reduction of Aldehydes

Cruciferous vegetables containing I3C- A natural compound having cytostatic and apoptosis inducing effect in neoplastic cells through various mechanism[30]. Estrogen promotes the growth and survival of tumors, I3C mitigate the estrogen's effect by enhancing the rate of apoptosis and arresting cell growth. I3C triggers cytochrome P-450 1A1 expression causing the shifting of estrogen metabolic pathway to C-2 hydroxlation instead of formation of 16-hydroxyestrone a suspected endogenous carcinogen[31]. It acts as a Aryl Hydrocarbon Receptor (AhR) agonist which are transcription factor involved in regulating the expressions of genes. It bring about suppression in cell division in prostate and cervical cell line (IC_{50}= 55

μM). Also suppresses the induction of multi-drug resistance (MDR) in cancer cells[32]. Along with prostate and cervical cancer cell line it seems to be effective in human lung, liver cancer The dose range required for indole-3-carbinol to suppress cancer cell proliferation is 50-100 μM, which is difficult achieve in tumour site. Poor cellular uptake, metabolic instability, multifunctional activity of its metabolites and hepatotoxicity are the factors impeding its clinical application [34].

3. 1,3,4-Thiadiazole Derivatives:

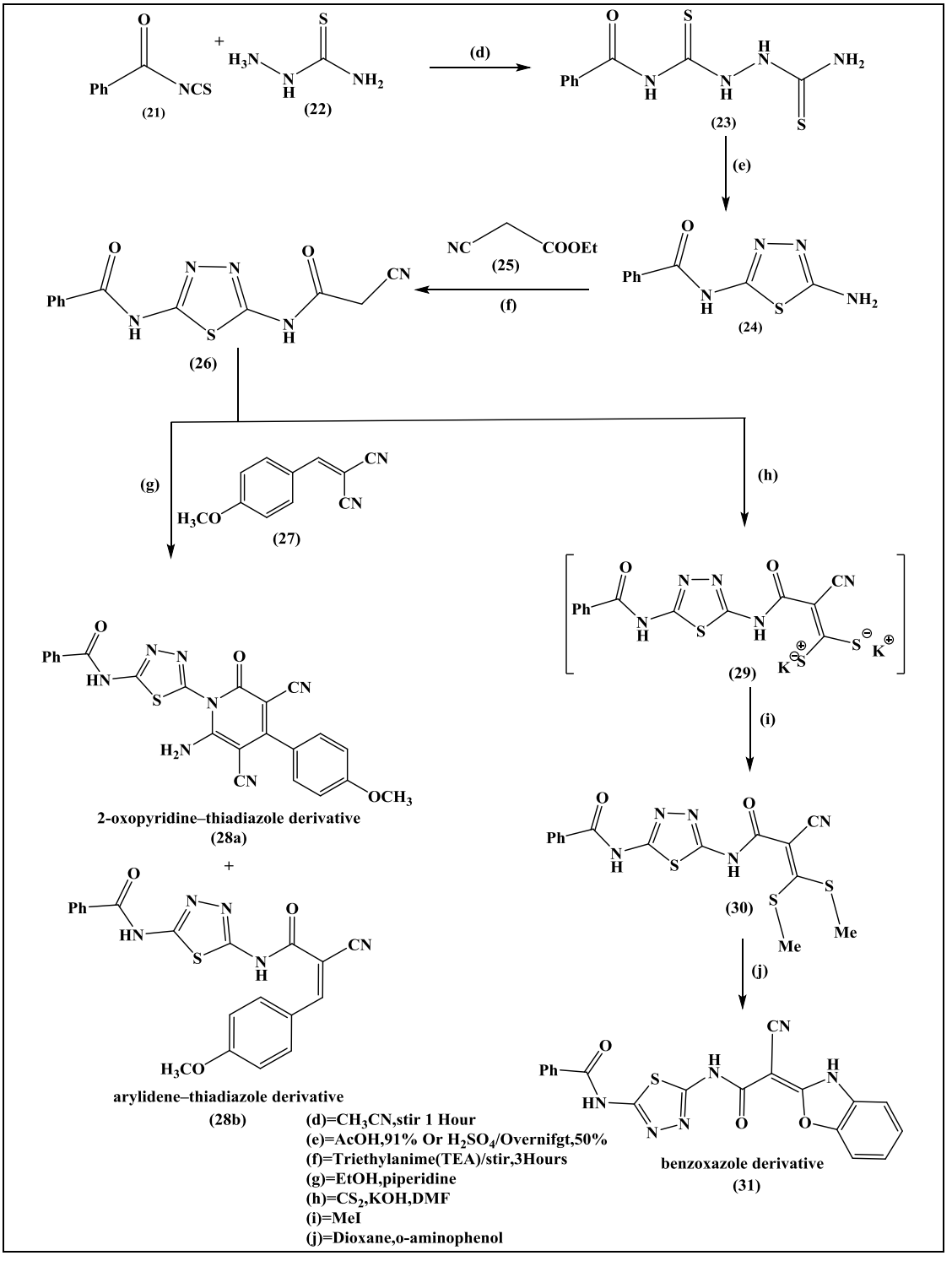
1,3,4-Thiadiazole Derivatives**(20)** were synthesized from benzoic acid derivatives**(16)** and hydrazinecarbothioamide**(17)** in presence of piperidine catalyst in dioxane solvent under the reflux for 12 hours yielding intermediate compound**(18)** which is mixed with conc. H_2SO_4 and deionized water, stirred at room temperature for 15 hours. The precipitate obtained**(19)** and appropriately substituted benzaldehyde is mixed in ethanol and heated on a reflux for 3 hours**(scheme 4)**. Derivative with R= 3,4,5-Trihydroxy and R_1=4-Nitro5-(5-{(Z)-[(4-nitrophenyl)methylidene]amino}-1,3,4-thiadiazol-2-yl)benzene -1,2,3-triol is highly effective against breast cancer with high binding and coordination with its target[35].



Scheme 4:Synthesis of 1,3,4-Thiadiazole Derivatives from Benzoic acid derivatives and Hydrazinecarbothioamide.

N-(5-Amino-1,3,4-thiadiazol-2-yl)benzamide**(24)** was obtained from benzoylisothio-cyanate**(21)** and thiosemicarbazide**(22)** in dry acetonitrile, followed by cyclization. N-(5-Amino-1,3,4-thiadiazol-2-yl)benzamide**(24)** reacted with ethyl cyanoacetate**(25)** using a catalytic amount of triethylamine to yield N-(5-(2-cyanoacetamido)-1,3,4-thiadiazol-2-yl)benzamide**(26)** in high yield, serving as a key intermediate. Compound**(26)** was refluxed with 2-(4-methoxybenzylidene)-malono-nitrile**(27)** to give a grey crystalline product identified as the 2-oxopyridine–thiadiazole derivative**(28a)** (major product). Acidification of the filtrate with cold dilute HCl produced an orange precipitate, identified as the arylidene derivative**(28b)** (minor product). The reaction of **(26)** with carbon disulfide in DMF containing potassium hydroxide at room temperature produced a dipotassium dithiocarbazate intermediate**(29)**. This intermediate reacted in situ with methyl iodide to

form ketene S,S-dithioacetal**(30)**, which was then refluxed with bidentate nucleophiles like o-aminophenol to yield the benzoxazole derivative**(31)**.

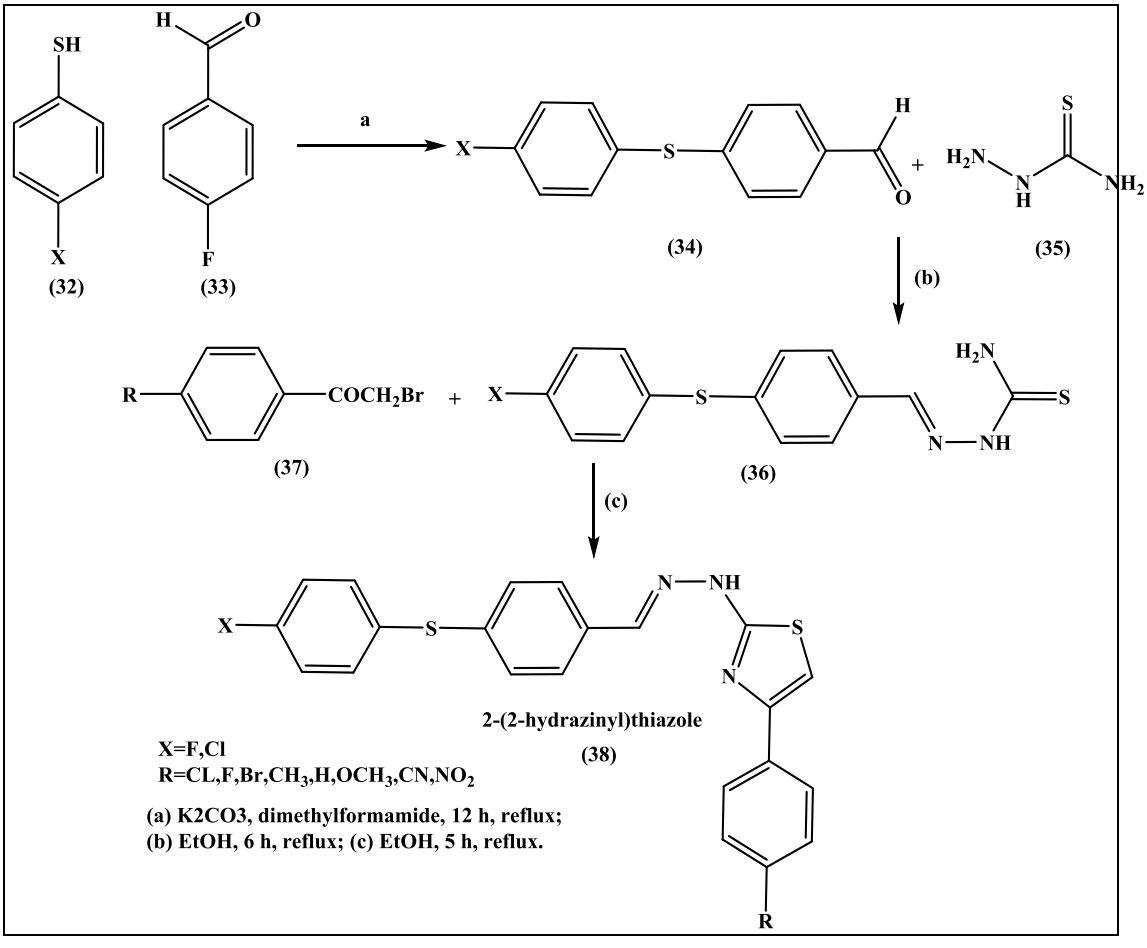


Scheme 5:Synthesis of 1,3,4-Thiadiazole derivatives via N-(5-(2-cyanoacetamido)-1,3,4-thiadiazol-2-yl)benzamide Intermediate.

DFT calculations suggest high polarizability and strong bio-activity because of low HOMO-LUMO gap. Compound **(28b)** and **(31)** shows (IC_{50}<10μM) anti-tumour activity by inhibiting CDK1 to arrest G_2-M and by inducing necrosis respectively[36].

4. Hydrazinyl thiazole:

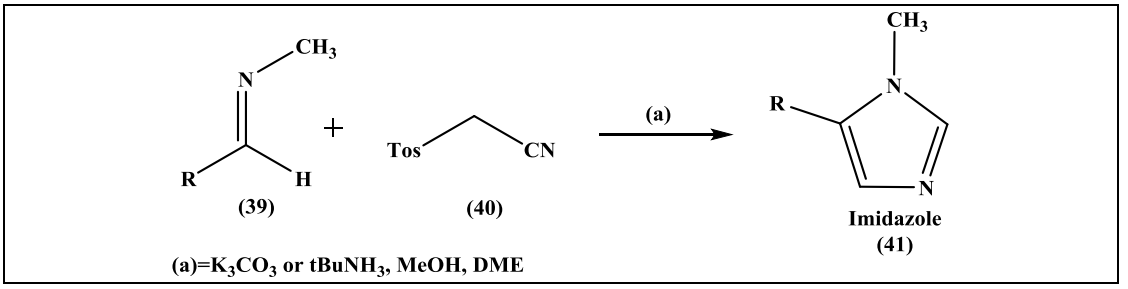
Hydrazinyl thiazole shows broad spectrum of biological effects and is used as anti-inflammatory[37], anticonvulsant[38], antidepressant[39] and anti-microbial[40] agent. Thiazole-containing heterocycles are regarded as highly important targets in drug discovery due to their wide range of pharmacological activities[41,42]. First 4-chloro/fluorothiophenol**(32)** was refluxed with 4-fluorobenzaldyde**(33)** in dimethylformamide followed by the reaction with thiosemicarbazide**(35)** in ethanol to yield hydrazine-1-carbothioamide intermediate**(36)**. This intermediate on reaction with phenacyl bromide**(37)** yields final product**(38)(scheme 6)**[43].



Scheme 6: Synthesis of 2-(2-hydrazinyl)thiazole from 4-Chloro/Fluorothiophenol and 4-Fluorobenzaldyde

Thiophene derivatives have been reported to act as epidermal growth factor receptor (EGFR) and caspase-9 inhibitors, inducing apoptosis [44].

5. Imidazole:

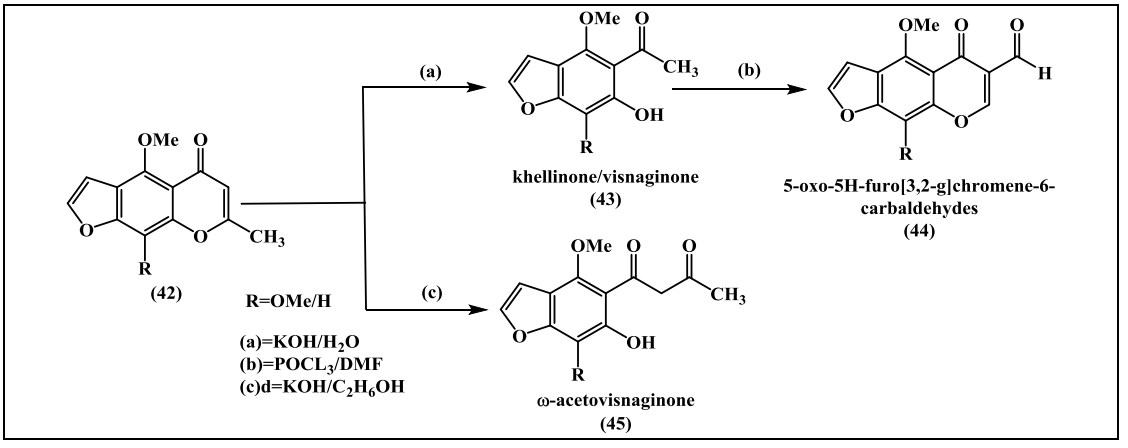


Scheme 7:Synthesis of Imidazole through Base Induced Cycloaddition

Reaction of aldimines**(39)** with tosylmethylisocinide**(40)** undergo base induced cycloaddition to yield 1,5 disubstituted imidazole **(scheme 7) (41)**[45]. Imidazole scaffolds act on multiple oncogenic targets.Some of the derivatives have shown to inhibit topoisomerase catalytic activity, extensively high anti-cancer activity than etoposide and 5-fluorouracil in kidney and breast cancer cell lines having lower toxicity towards non cancerous cells while some block CYP26A1 (IC_{50}=0.22–1.11 μM), rapid accelerated fibrosarcoma (RAF)-kinase enzyme demonstrating broad anticancer potential[46].

6. Benzofuran derivatives:

Alkaline hydrolysis of khellin and visnagin**(42)** undergo alkaline hydrolysis to yield khellinone and visnaginone**(43)**; while alcoholic hydrolysis yield ω-acetokhellinone and ω-acetovisnaginone **(45)**. Khellinone and visnaginone**(43)** yields 5-oxo-5H-furo[3,2-g]chromene-6-carbaldehydes**(44)** via Vielsmeier-Haack reaction **(scheme 8)**[47].



Scheme 8: Synthesis of Benzofuran derivatives via Vielsmeier-Haack reaction

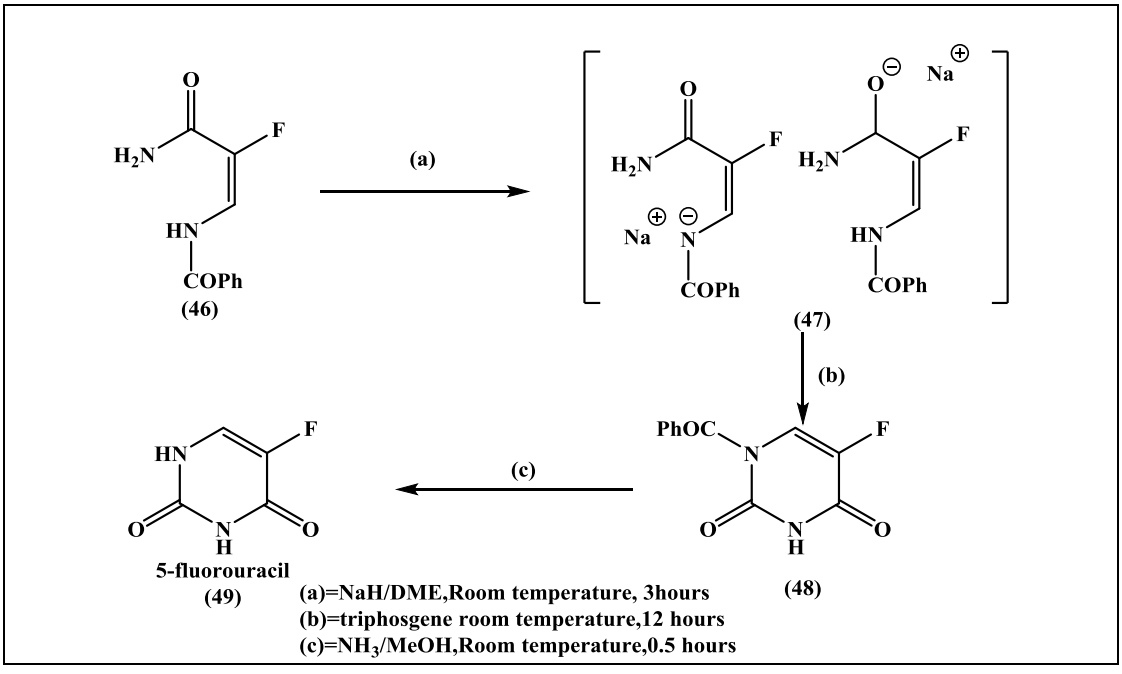
Some benzofuran derivatives like N-(5-(2-bromobenzyl)thiazole-2-yl) benzofuran-2-carboxamide supresses the growth of a human heptacellular carcinoma (HCC) cells, induced apoptosis, restricts cell growth and angiogenesis[48], benzofuran-4,5-dione selectively inhibit human peptide deformylase[49], Trimethoxybenzoyl benzofuran disrupts tumour vasculature[50],benzofuran lignan as an apoptosis inducer in p-53 positive cells[51] and

effective chemotherapeutic agent against malignant T cells[52], the 3-aminobenzofuran derivatives contributes to neurological tumour growth inhibition[53].

Benzofuran-5-carbonyl heterocyclic derivatives were found to be more potent than the standard chemotherapeutic agents like fluorouracil and doxorubicin and cytarabine when tested against 12 human cancer cell line. Some derivatives were reported as a novel pro-drug with excellent anti-tumour activity against human tumour xenografts compared ti cis-platin and carzelesin[54].DFT calculations on bromobenzofuran oxadiazole hybrids reveal a small HOMO–LUMO gap (~2.0 eV) and high electrophilicity, predicting strong interaction with kinase ATP sites[55]; some of the derivatives inhibits Pin1 (peptidyl-prolyl cis–trans isomerase NIMAinteracting 1)- an oncogenic agent, effective therapeutic agent for human non-small cell lung carcinoma (NSCLC) cells, PC9 and A549, induced significant apoptosis in the leukemia HL-60 cell line and effectively inhibited lung tumor growth. Moreover, they exhibited superior anticancer activity compared to several standard reference drugs, demonstrating IC_{50} values in the nanomolar range against panels of more than 40 human cancer cell lines at low concentrations[56].

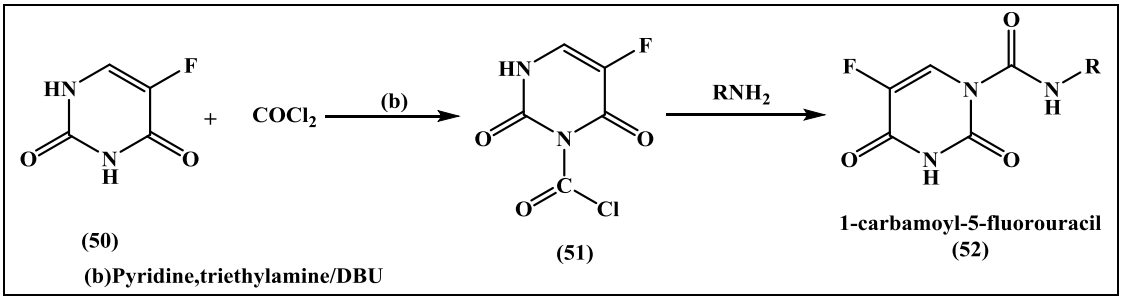
6. Fluorouracil and Fluorouracil derivatives:

Cycloaddition of sodium salt of (E)-N-(3-amino-2-fluoro-3-oxoprop-1-en-1-yl)benzamide**(46)** with phosgene yields 1-benzoyl-5-fluoropyrimidine-2,4(1H,3H)-dione**(48)** which on hydrolysis with ammonia (NH_3) in methanol yield higher percentage of 5-fluorouracil**(49)**[57].



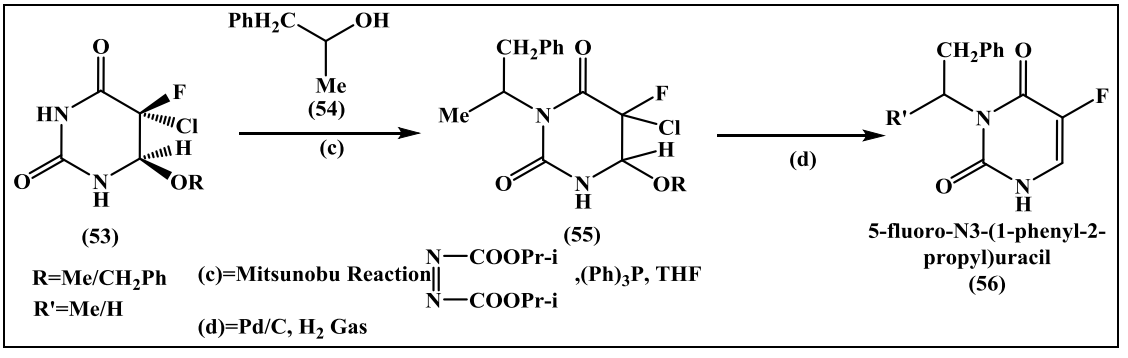
Scheme 9: Synthesis of Fluorouracil via Cycloaddition

The condensation of phosgene and 5-fluorouracil**(50)** results in a 1-chloroformyl-5-uracil**(51)** which when treated with aliphatic amine in-situ yields a potent 5-fluorouracil derivative 1-carbamoyl-5-fluorouracil**(52)**[58].



Scheme 10: Synthesis of Fluorouracil derivative through condensation of phosgene and 5-fluorouracil

5-chloro-5-fluoro-6-methoxy-5,6-dihydro uracil**(53)** with 1-phenyl-2-propanol**(54)** in presence of di-isopropyl azodicarboxylate and triphenylphosphine in dry THF undergo Mitsunobu reaction and yield to give the adduct**(55)** undergoing reduction by palladium on activated carbon(5%) in H_2 environment yields 5-fluoro-N^3-(1-phenyl-2-propyl)uracil**(56)**[59].



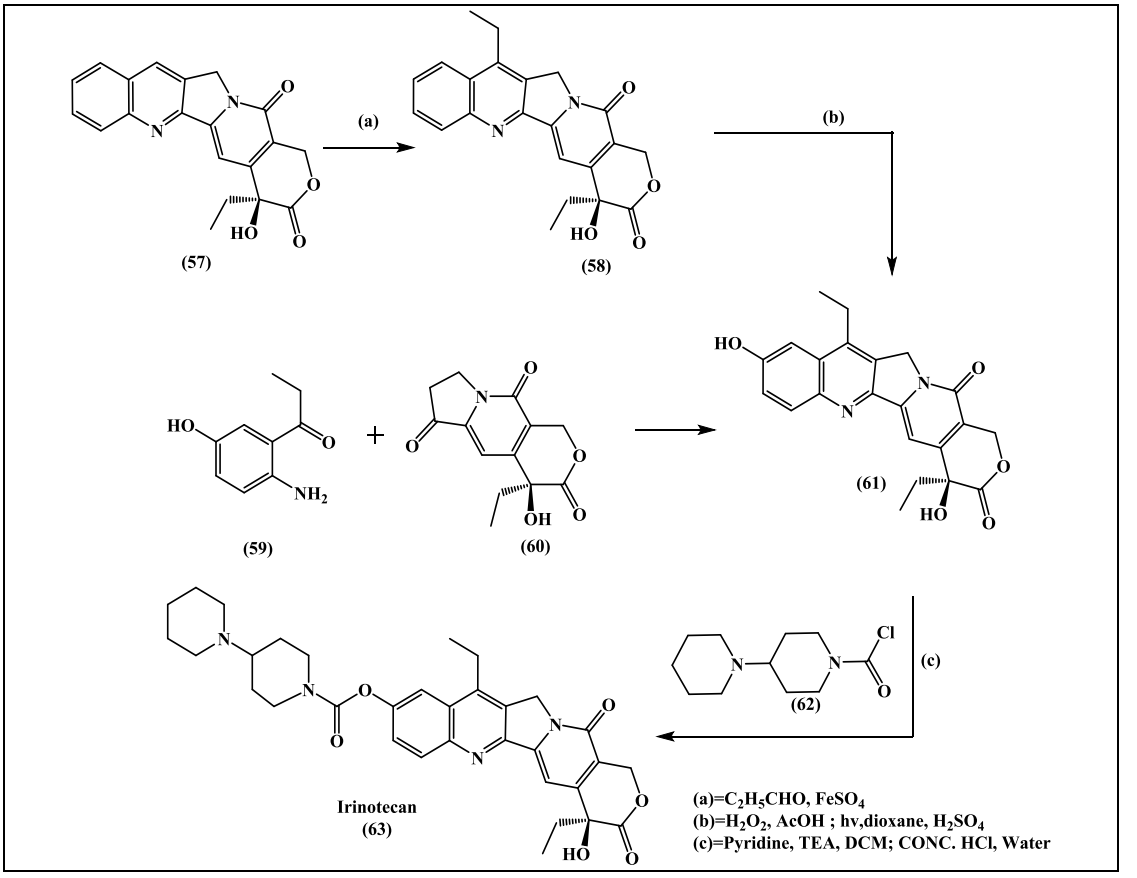
Scheme 11: Synthesis of Fluorouracil derivative by Mitsunobu Reaction

Clinically it shows activity against solid tumours such as colorectal, gastric, pancreatic, breast, head and neck and basal cell carcinomas[60].DFT studies revealed that 5-FU strongly adsorbs onto C_6N_6 with an energy of −28 kcal mol^{-1}, transferring −0.16 e^- and reducing the HOMO–LUMO gap, which indicates activation for release under acidic tumor pH[61]. The cage forms a B–O covalent bond with 5-FU (−11 kcal mol^{-1}), allowing pH-responsive drug release and improved anticancer efficacy [62].

7. Irinotecan:

Irinotecan**(63)** is widely used in the treatment of colorectal cancer. It is the derivatised form of natural product camptothecin**(57)**[63].7-ethyl-10-hydroxy camptothecin **(61)** which is hydrolyzed form of Irinotecan is a topoisomerase 1 inhibitor[64]. A solution containing Camptothecin**(57)** dissolved in H_2SO_4 along with $FeSO_4$ and freshly distilled propionaldehyde was cooled. Hydrogen peroxide was added to this ice cold solution dropwise to yield 7-Ethylcamptothecin**(58)** which is then converted to its N-Oxide form followed by the dissolution in equimolar mixture of dioxane and sulfuric acid; The mixture is then irradiated to obtain 10-hydroxy derivative**(61)**[65]. With the help of the Friendlander condensation approach, Rama, V, et al. reported the convenient method with comparatively higher yield.Reaction between (S)-trione**(60)** and 2-amino-5-hydroxypropiophenone (AHP)

(59) affords 7-ethyl-10 -hydroxy camptothecin **(61)**; followed by the treatment with 4-piperidinopiperidinecarbonyl chloride**(53)** and HCl gives Irinotecan**(63)**[66].

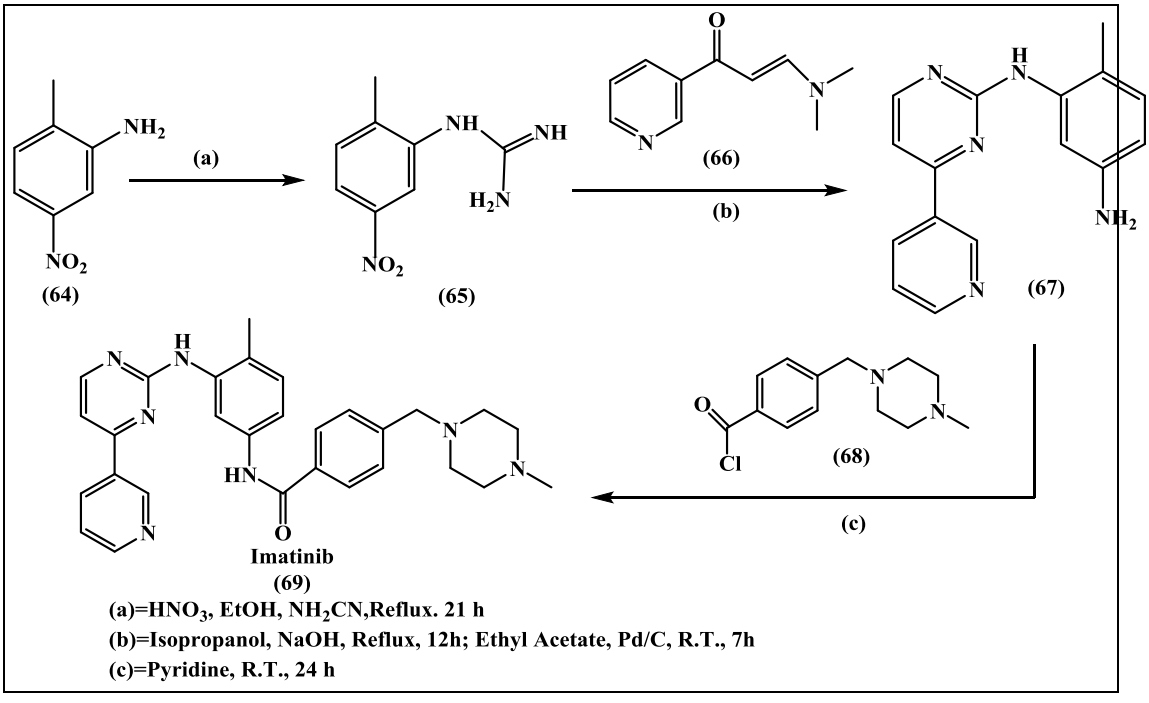


Scheme 12: Synthesis of Irinotecan from Camptothecin and Friendlander Condensation

DFT optimization of Irinotecan (B3LYP/6-31G(d)) reproduces experimental IR/Raman frequencies ($R^2 \approx 0.98$) and yields a HOMO-LUMO gap of ~3.2 eV; Molecular Electrostatic Potential (MEP) mapping shows electron-rich quinoline N and carbonyl O as sites for DNA intercalation, supporting its topoisomerase-I inhibition[67].Irinotecan (CPT‑11) is a prodrug converted to SN‑38, a potent topoisomerase I inhibitor that stabilizes the Topo I DNA cleavage complex, causing replication fork arrest, double strand breaks and apoptosis; also it is found to be effective against Colorectal, Pancreatic, lung, ovarian cancer[68]. SN38 is 100–1000‑fold more cytotoxic than irinotecan, driving tumor cell death[69].

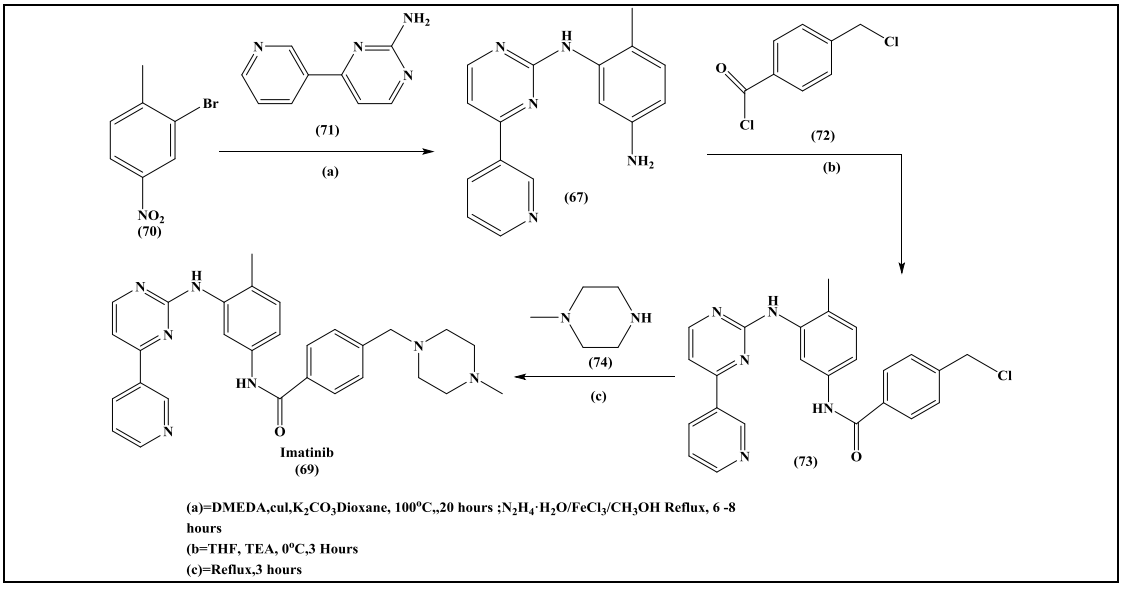
8. Imatinib:

The Zimmermann's route of synthesis for imatinib**(69)** is carried out by the conversion of methyl-nitro-aniline**(64)** into guanidino containing intermediate**(65)** which subsequently undergoes two step transformation involving hydrazine treatment and enone amine**(66)** formation yielding pyrimidine amine derivative **(67)** which react with acyl chloride**(68)** to afford imatinib**(69)** as final product[70].



Scheme 13:Synthesis of Imatinib via Zimmermann's route

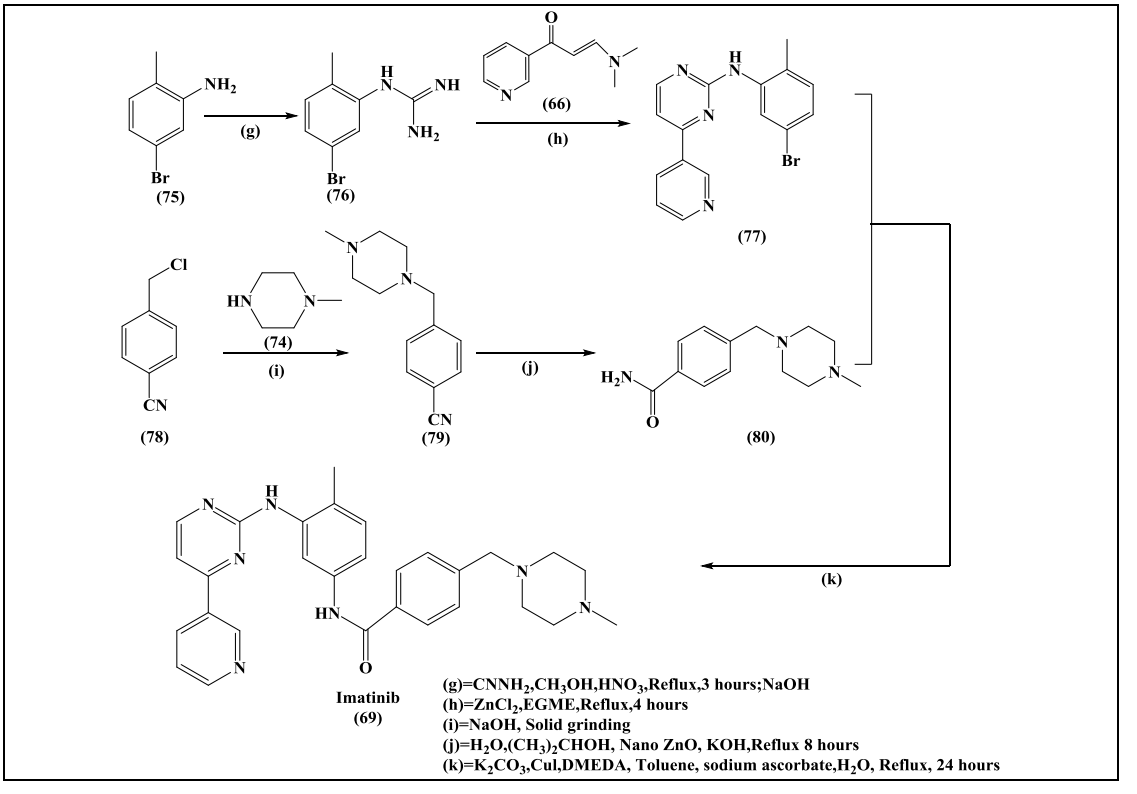
Another synthesis begins with 2-bromo-4-nitrotoluene**(70)**, which undergoes a copper-catalyzed C–N coupling reaction. The resulting product is then subjected to reduction of the nitro group using the $N_2H_4{\cdot}H_2O/FeCl_3/CH_3OH$ system, yielding pyrimidine amine derivative **(67)** . Subsequently, the target molecule is obtained. This modified synthetic approach for imatinib preparation is safer, more environmentally benign, and exhibits promising potential for large-scale industrial application[71].



Scheme14: Synthesis of Imatinib via Copper catalyzed C-N Coupling Reaction

Solid-phase synthesis was used to prepare 4-(4-methylpiperazine-1-methyl)benzonitrile **(79)** via on-resin formation of N-methylpiperazine **(74)** followed by coupling with 4-chloromethylbenzonitrile **(78)** under solvent-free conditions which is then hydrolyzed to 4-(4-Methylpiperazine-1-methyl)-benzamide**(80)**.N-(5-bromo-2-methylphenyl)-4 (pyri-din-3-yl)pyrimidin-2-amine**(77)** is obtained through enone amine**(66)** and 1-(5-bromo-2-methylphenyl)guanidine**(76)** which is obtained from 5-bromo-2-methylaniline**(75)**.4-(4-Methylpiperazine-1-methyl)-benzamide**(80)** and N-(5-bromo-2-methylphenyl)-4-(pyridin-3-yl)pyrimidin-2-amine**(77)** undergo C-N coupling reaction using antioxidant sodium ascorbate in non polar solvent[72].

B3LYP/631G(d) optimization of imatinib reveals a planar quinazoline core; the HOMO (−5.8 eV) resides on the pyridine nitrogen and the LUMO (−2.3 eV) on the amide carbonyl, giving a ~3.5 eV gap that favors strong H‑bonding to the ATP pocket of BCRABL[73]. Imatinib inhibits the BCRABL tyrosine kinase and also blocks PDGFR and cKIT, preventing downstream signaling and inducing apoptosis; in K562 chronic myeloid leukemia cells it shows IC_{50} ≈0.1 μM, confirming potent cytotoxicity



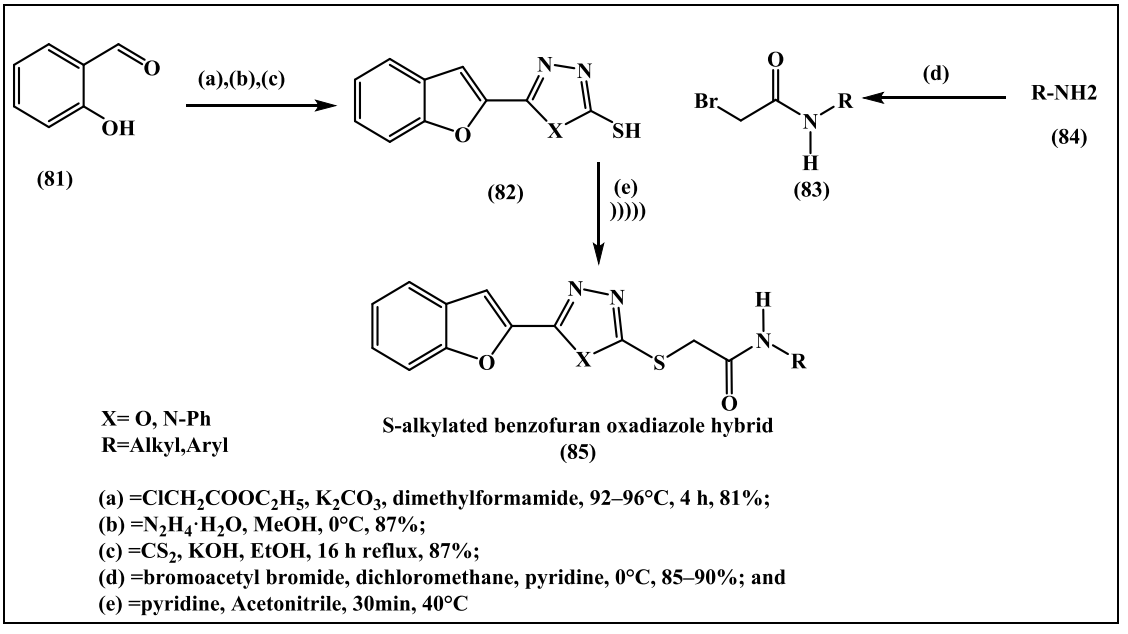
Scheme 15: Synthesis of Imatinib using Solid Phase Synthesis of 4-(4-methylpiperazine-1-methyl)benzonitrile.

NON CONVENTIONAL SYNTHESES

1. S-alkylated benzofuran oxadiazole hybrids :

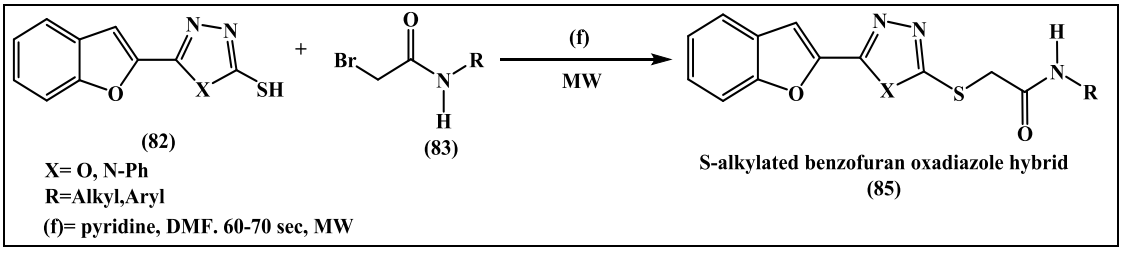
Heating salicyldehyde**(81)** and ethyl chloroacetate with K_2CO_3 using dimethylformamide giving benzofuran-2-carboxylate which on further treatment with hydrazine monohydrate in

menthol to form benzofuran-2-carbohydrazide. This produces benzofuran oxadiazole hybrid**(82)** upon treating with CS_2/KOH[75,76]. Treatment of aliphatic or aromatic amine**(84)** with bromoacetyl bromide mediated by pyridine/ dichloromethane (DCM) yields substituted bromoacetanilide derivative**(83)**[77]. Oxidiazole and triazole based hybrid was dissolved in acetonitrile, pyridine is added and this mixture is stirred for about 15 min at 0℃, after the addition of substituted bromoacetanilide, mixture is allowed to sonicate for about 30 minutes at 40℃ to obtain S-alkylated benzofuran oxadiazole hybrids**(85)**[78] .



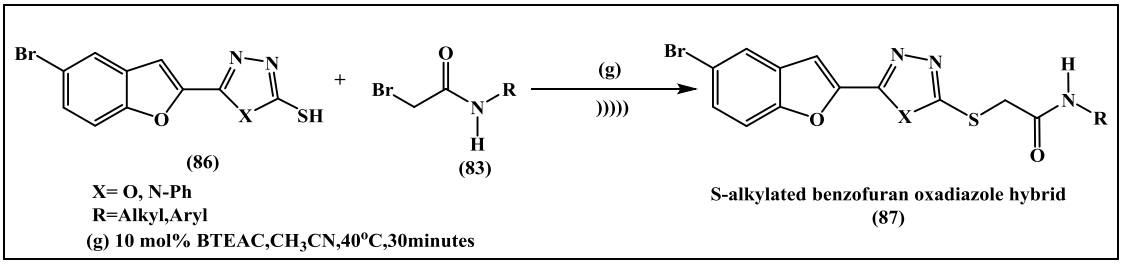
Scheme 16:Ultrasound-Assisted Synthesis of benzofuran oxidiazole hybrids.

In method for the synthesis S-alkylated benzofuran oxadiazole hybrids the initial steps are same as illustrated in (**scheme 16)** but instead of pyridine, dimethylformamide (DMF) is used as a solvent to dissolve the Oxidiazole and triazole based hybrid, and reaction mixture is irradiated in microwave oven for 60-70 seconds**(Scheme 17)**



Scheme 17:Microwave-Assisted Synthesis of benzofuran oxidiazole hybrids.

A mixture of bromobenzofuran-oxadiazole-2-thiol and substituted bromoacetanilide derivatives using CH_3CN as a solvent was sonicated at 40°C for 30 minutes using BTEAC (10 mol%) as a catalyst**(Scheme 18)**[79].



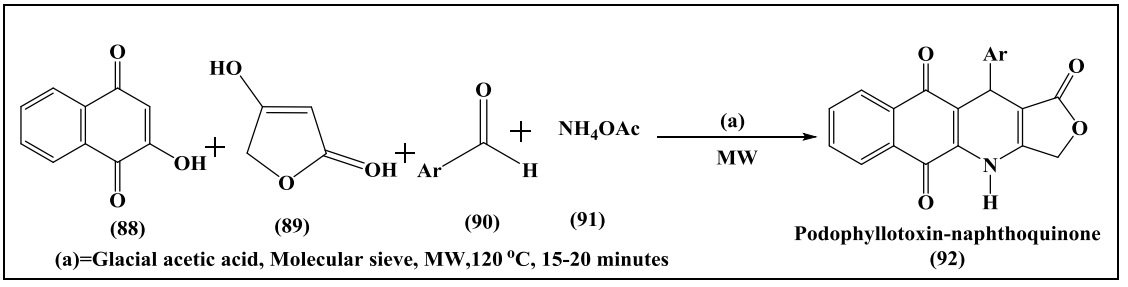
Scheme 18: BTEAC catalyzed Ultrasonic assisted Synthesis of benzofuran oxidiazole hybrids.

A cytotoxic activity of a derivatives was tested using MTT assay against the lung cancer cell line with standards like crizotinib and cis-platin; among them benzofuran oxidiazole derivative with R= anisole (methoxy group being ortho to the substitution) showed the strongest effect with cell viability of 27.49 ± 1.9 and IC_{50} being 6.3 ± 0.7 µM outperforming than other derivatives. Docking study shows that this derivative has strong binding affinity (-9.925 kcal/mol) than the crizotinib (-8.985 kcal/mol) to the anaplastic lymphoma kinase (ALK). It exhibited excellent interaction within the receptor binding pockets supporting its superior anticancer potential. DFT reveals a small HOMO LUMO gap (~2.4 eV) and a pronounced electron‑rich MEP over the S alkyl moiety, promoting binding to EGFR/PI3K targets. Based on the cytotoxicity and in silico studies, it is an promising anticancer agent[Error! Bookmark not defined.]. In silico ADMET predicts moderate low mutagenicity

2.Podophyllotoxin-naphthoquinone

:

Microwave assisted reaction of 2-hydroxy-1,4-napthaquinone**(88)**,tetronic acid**(89)** and aromatic aldehydes**(90)** and ammonium acetate yields**(91)** 11-phenyl-4,11-dihydrobenzo[g]furo[3,4-b]quinoline-1,5,10(3H)-trione**(92)(Scheme 19)**. In the temperature range of 80-130 different organic solvents like ethanol, t-butanol and glacial acetic acid were used to carry out this four component reaction using molecular sieve for water absorption; out of which the reaction involving glacial acetic acid as a solvent at 120^0 gives higher yield (82%)[80].

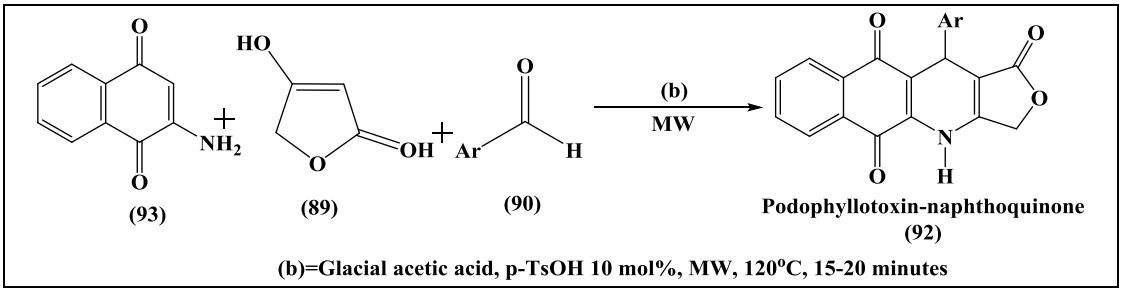


Scheme 19:Microwave assisted synthesis of Podophyllotoxin-naphthoquinone.

Nguyen, Ha Thanh, et al.[81] reported three component synthesis in presence of para-toluenesulfonic acid as a catalyst and glacial acetic acid as a solvent giving higher yield in less reaction time**(Scheme 20)** as compared to previous scheme**(Scheme 19)**.

The investigation of cytotoxicity profile against four cancer cell line (KB, HepG2, MCF7, A549) and non-cancerous human embryonic kidney (Hek-293) cell line signifies compound

with Ar = 3- Methoxyphenol/3-Bromophenol having high toxicity towards human cancer cell line and less toxic to the non cancerous Hek-293 cell line with IC_{50} values 9.35µM and 7.20µM respectively.

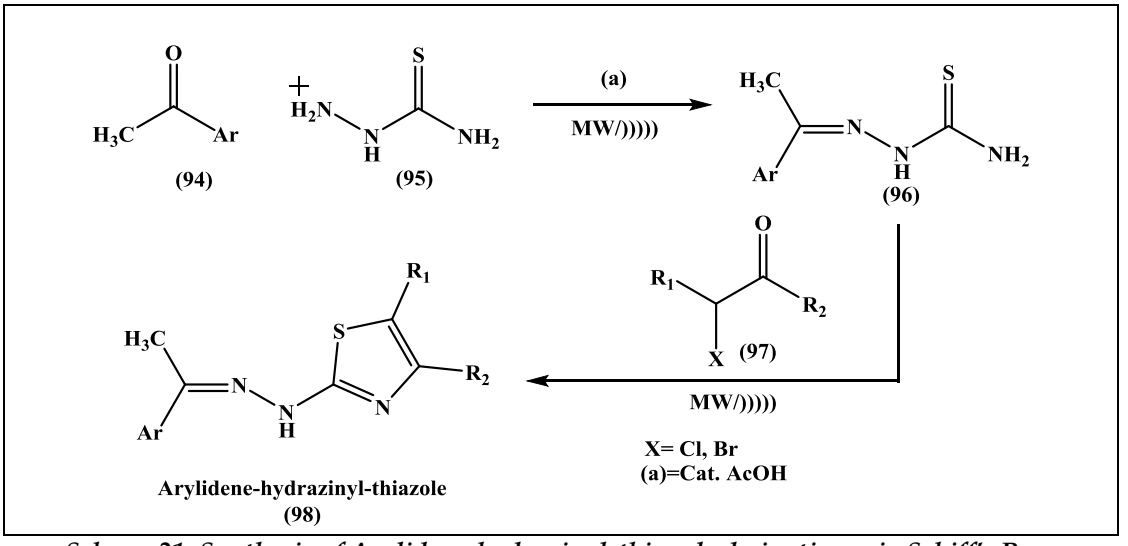


Scheme 20: Microwave assisted synthesis of Podophyllotoxin-naphthoquinone catalyzed by para-toluenesulfonic acid.

ADMET 2.0 evaluation indicate that these naphthaquinone derivatives satisfy most of the physicochemical criteria (i.e. Molecular weight, Hydrogen bond counts) exhibiting low risk of cardiotoxicity and multi drug-resistance issue but they are overly lipophilic, poor gastrointestinal permeability and oral viability

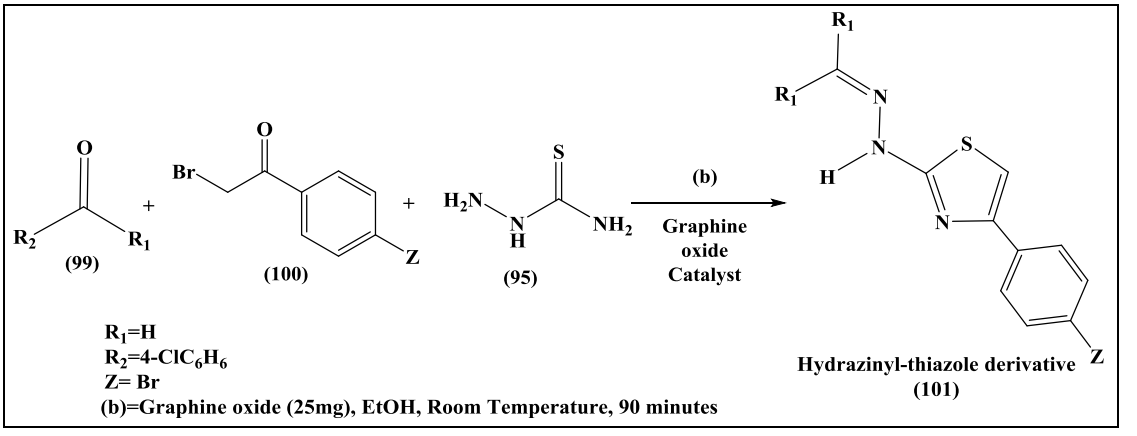
3.Hydrazinyl-thiazole derivatives :

The first step in the synthesis of the arylidene-hydrazinyl-thiazole derivatives is the condensation of aryl amine**(94)** with thiosemicarbazide**(95)** in presence of acetic acid yielding arylidene-thiosemicarbazones-a Shiff's base**(96)**. This schiff's base undergoes cyclisation with α-halocarbonyl derivatives**(97)** to afford corresponding arylidene-hydrazinyl-thiazole derivatives**(Scheme 21)(98)**,both the steps are carried out in Hantzsch protocol (ultrasonic and microwave)[82]



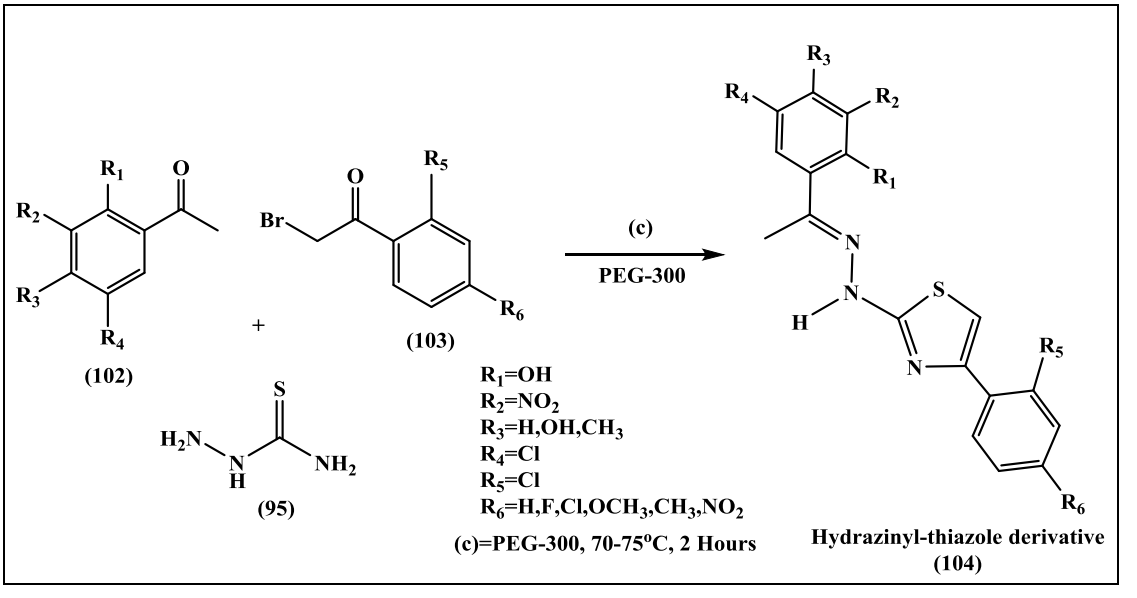
Scheme 21: Synthesis of Arylidene-hydrazinyl-thiazole derivatives via Schiff's Base Cyclisation

At room temperature using ethanol as a solvent, acetophenone derivatives**(99)**, phenacyl bromide derivatives**(100)** and thiosemicarbazide**(95)** undergo reaction catalyzed by graphene oxide yields hydrazinyl-thiazole derivatives**(Scheme 22)(101)**[83].



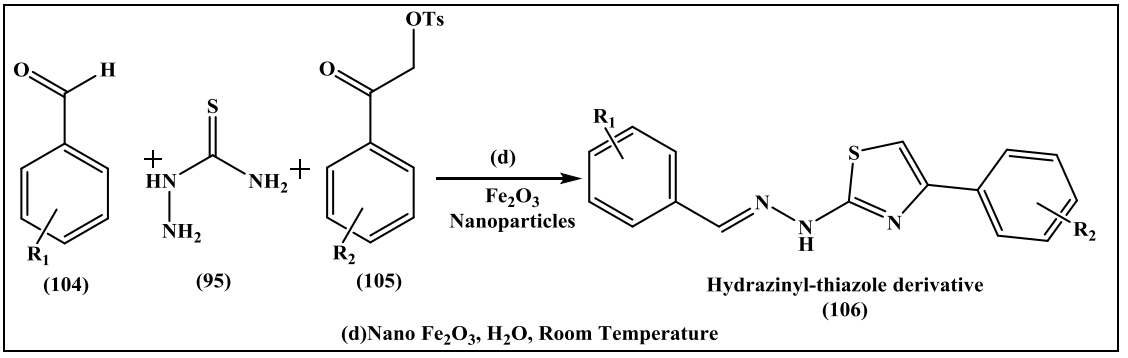
Scheme 22: Synthesis of Hydrazinyl-thiazole derivatives Using Graphine Oxide Catalyst

Another method involves the use of cheap and easily available PEG-300 as a supportive catalyst. Condensation reaction of acetophenone derivatives**(102),** with thiosemicarbazide**(95)** yields reactive intermediate- thiosemicarbazone in presence of glacial acetic acid which on further reaction with α-halo ketone**(103)** yields the hydrazinyl-thiozole derivative**(Scheme 23)(104)**[84].



Scheme 23: Synthesis of Hydrazinyl-thiazole derivatives Using PEG-300 as supportive catalyst

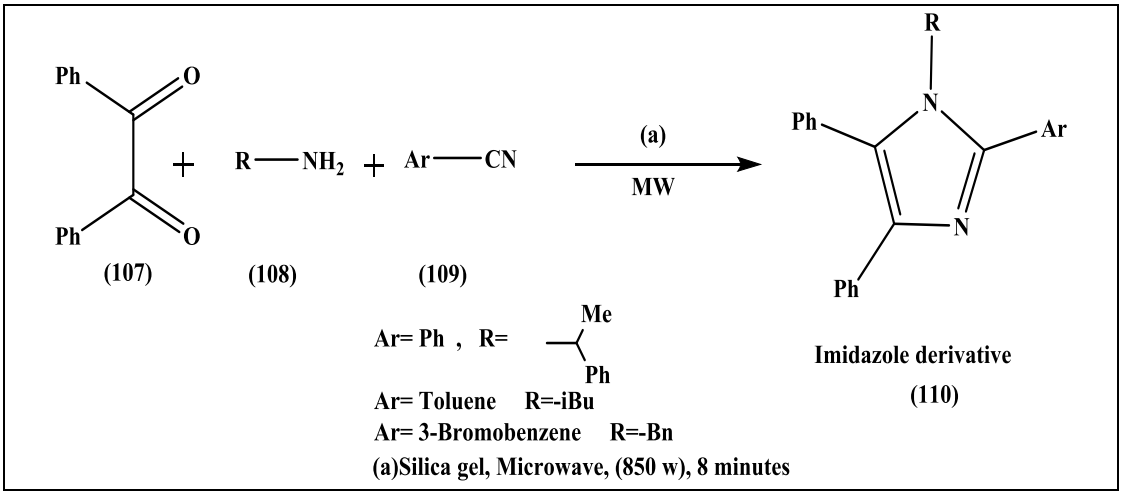
One pot multi-component reaction involving rust derived Fe_2O_3 nanoparticles as a green catalyst for the synthesis of hydrazinyl-thiazole derivatives**(Scheme 24)**[85].



Scheme 24: Synthesis of Hydrazinyl-thiazole derivatives Using rust derived Fe_2O_3 Nanoparticles as a Green Catalyst

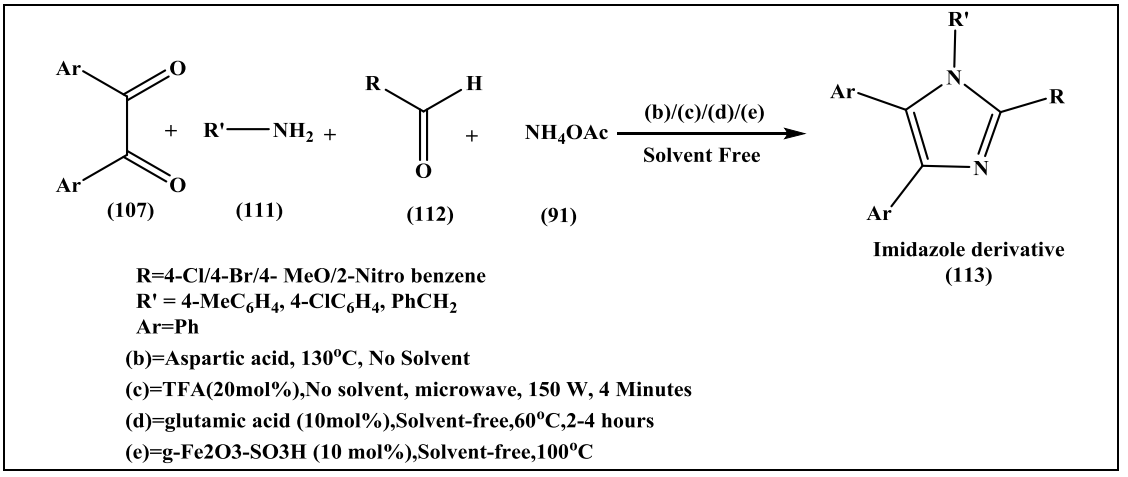
4. Imidazole derivatives:

Balalaie, Saeed, et al. Reported one pot three component synthesis of benzil**(107)**, primary amine**(108)** and aromatic nitrile**(109)** via condensation using silica gel to obtain imidazole derivatives**(110)** with higher yield**(Scheme 25)**[86].



Scheme 25: Microwave Assisted Synthesis of Imidazole derivatives using Silica Gel

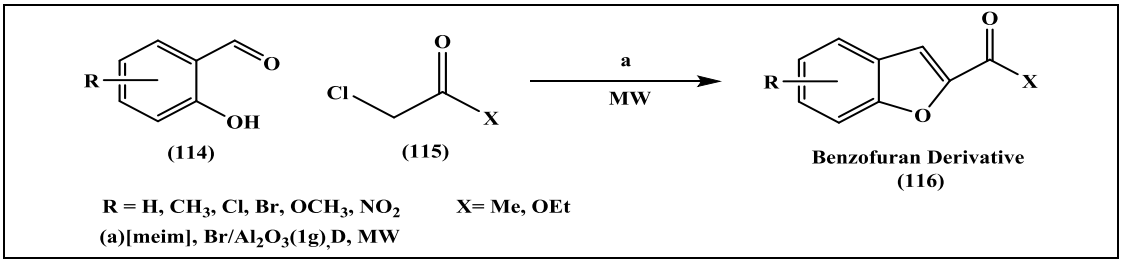
The simple and efficient method for the synthesis of 1,3,4,5-tetrasubstituted imidazole**(113)**; catalyzed by inexpensive and non-toxic organocatalyst- Aspartic acid by the condensation of 4-chlorobenzaldehyde**(112)** with 4-chloroaniline or benzylamine**(111)**, benzil and ammonium acetate**(91)** in molar ratio 1:1:1:1.2:0.4 gives optimum yield**(Scheme 26)**[87].
Mohammadizadeh, Mohammad R., et al. Reported microwave assisted one pot synthesis of these derivatives by using Trifluoroacetic Acid (TFA) as an Efficient Catalyst which is cost effective and safe avoiding problem associated with pollution[88].Glutamic acid(10 mol%)[89], magnetic nanoparticles supported sulfonic acid γ-Fe_2O_3-SO_3H (10 mol%)[90] were also found to be a non hazardous, green, efficient catalyst under mild,thermal conditions.



Scheme 26: Microwave Assisted Synthesis of Imidazole derivatives using Aspartic Acid as a Catalyst

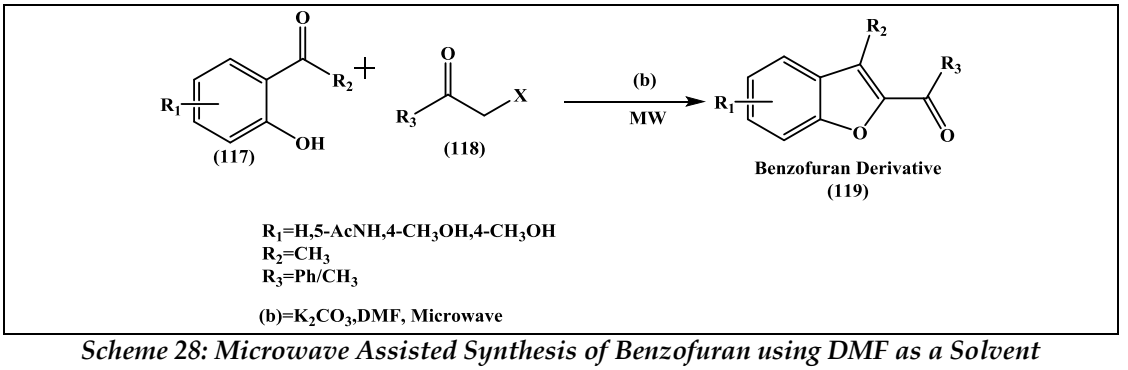
5.Benzofuran :

1-Methyl-3-ethyl imidazolium bromide [meim](2 mmol) Br/basic alumina (Al_2O_3)(1g) was found to facilitate the cyclocondensation of salicylaldehyde**(114)** (2 mmol), chloroacetone**(115)** (or chloroethyl acetate, 2 mmol) which are mixed in a borosil beaker with glass rod.The homogeneous blend was then subjected to microwave irradiation (360 W) in 10 second intervals**(Scheme 27)**[91].



Scheme 27: Microwave Assisted Synthesis of Benzofuran Using Ionic Solution

The microwave assisted synthesis of substituted benzofuran derivatives from salicylaldehyde and α-haloacetate derivatives with DMF as an efficient solvent**(Scheme 28)** was reported by Liu, Junqiang, et al.**(Scheme 28)**[92] .



Scheme 28: Microwave Assisted Synthesis of Benzofuran using DMF as a Solvent

The higher yield of derivatives with different substitutions and different microwave irradiation conditions are listed below **(table 2)** :

Table 2: Yield of Benzofuran derivatives obtained at different Microwave Irradiation Condition and with different Substitution

Entry	R_1	R_2	R_3	X	Irradiation condition	%yield
1.	H	CH_3	Ph	Br	500W,3Minutes	91
2.	5-AcNH	CH_3	Ph	Br	550W,5Minutes	89
3.	4-CH_3O	CH_3	Ph	Br	550W,2Minutes	90
4.	4-CH_3O	CH_3	CH_3	Cl	350W,3Minutes	85
5.	H	H	Ph	Br	550W,3Minutes	96

These benzofuran derivatives exhibit potent anticancer activity achieving low micro-molar to sub-nanomolar IC_{50}'s against breast, lung, colon and leukemia cells without damaging to normal cells. Halogen substitution and hybridization with piperazine, imidazole and sulfonamides were observed to boost potency [93].

Conclusion

In conclusion, this comprehensive literature review has systematically examined the Specific Anticancer Agent or Class of Agents, integrating data on its chemical synthesis, theoretical properties, and biological activity. The findings highlight that the synthetic methodologies are becoming increasingly refined, offering pathways to produce with high purity and structural diversity, a crucial step for clinical translation.

The application of Density Functional Theory (DFT) has proven to be an indispensable tool in understanding the fundamental electronic and geometric structure of these agent. Specifically, the computed parameters, such as the HOMO-LUMO gap and molecular electrostatic potential maps, provide critical insights into stability, reactivity, and predicted interaction sites with biomolecules. This theoretical foundation has guided rational design efforts aimed at optimizing the pharmacophore.

The extensive literature on in vitro cytotoxicity clearly establishes that these agents exhibits significant potency and cytotoxicity against a range of cancer cell lines, having IC_{50} values in the nanomolar or low micromolar range, which is comparable to or superior to established clinical drugs e.g., cis-platin or doxorubicin. Key pathways identified include induction of apoptosis via the intrinsic mitochondrial pathway, G2/M cell cycle arrest, and inhibition of topoisomerase activity. This potency directly correlates with specific structural modification.

While the existing literature presents a highly promising profile for Specific Anticancer Agent or Class of Agents, future research must focus on transitioning from in vitro to in vivo models to fully assess pharmacokinetics, bioavailability, and overall therapeutic window. Establishing a clear link between the theoretically predicted properties, the observed cytotoxicity, and the precise molecular targets will be essential for advancement toward preclinical development and, ultimately, to expand the scope of effective anticancer therapeutics.

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Chapter- Five

Drug Repurposing: Novel Therapeutic Avenues and Innovations: Natural products

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Abstract

Drug repurposing**,** a strategy for finding new therapeutic uses for existing drugs and compounds**,** has emerged as a transformative approach in modern healthcare. This book presents a comprehensive exploration of repurposing paradigms with particular emphasis on natural products, including plant-derived bio actives and traditional medicinal agents. Unlike new chemical entity development, repurposing leverages known safety profiles and pharmacological data to accelerate therapeutic discovery while reducing time and cost burdens. Natural compounds have been used for centuries to treat a wide array of human ailments with minimal side effects, and many such agents are now investigated for novel indications beyond their traditional uses. Clinically approved natural products — including phytochemicals, marine derivatives, and microbial metabolites — are revisited through the lens of repurposing for diseases such as diabetes, neurological disorders, cancer, infectious diseases, and organ-specific pathologies**.** The volume integrates experimental, computational, and translational perspectives, addressing mechanisms of action, regulatory considerations, and the role of *in silico* screening and bioinformatics in identifying repurposing candidates. Challenges related to safety, quality control, and intellectual property are examined alongside case studies demonstrating successful repurposing endeavors. Ultimately, this work underscores the innovative therapeutic potential held by natural products**,** advocating for their continued integration into drug repurposing pipelines to meet unmet clinical needs across diverse disease domains.

Keywords: *Drug repurposing; Natural products; Bioactive compounds; Traditional medicine; Molecular targets; In silico screening; Network pharmacology; Translational medicine; Phytopharmaceuticals; Multi-target drugs; Safety and efficacy.*

Introduction

Drug repurposing (or repositioning) identifies new therapeutic uses for approved or investigational drugs, accelerating development, slashing costs, leveraging established safety data, and mitigating resistance-outpacing traditional discovery. For centuries, natural compounds have safely treated diseases with minimal side effects; their structural diversity and potency make them ideal for this strategy, including for challenges like COVID-19, with many clinically approved natural products now repurposed for novel indications.

History of repurposing

Drug repurposing, or repositioning, entails discovering novel therapeutic uses for approved drugs or bioactive compounds, slashing development timelines, expenses, and safety concerns relative to traditional de novo discovery [1]. Initially fueled by chance clinical findings-like those behind aspirin, sildenafil, and minoxidil—it has matured into a deliberate approach bolstered by molecular pharmacology, bioinformatics, and translational studies [1].

Role of traditional medicine and natural sources

Natural products and traditional medicine have long shaped drug discovery and repurposing, with medicinal plants and remedies central to systems like Ayurveda and Traditional Chinese Medicine (TCM) for millennia [2]. Many contemporary pharmaceuticals trace back to ethnomedicinal roots, such as aspirin from *Salix alba*, quinine from *Cinchona* species, and artemisinin from *Artemisia annua*—the latter drawn from ancient antimalarial uses and subsequently repurposed for expanded applications [2]. Their chemical diversity, polypharmacology, and proven safety profiles position natural products as ideal for modern repurposing, especially against cancer, metabolic conditions, and neurodegeneration [3, 4]

Natural Products as Candidates for Repurposing

Natural products offer a rich source for identifying diverse molecular scaffolds and bioactivities, many of which can be readily repurposed as novel drugs for both new and established diseases. This approach to drug repositioning based on natural products provides several key advantages.

Phytochemicals and bioactives with repurposing potential

Phytomedicine serves as a vast reservoir of bioactive molecules with medicinal value and pharmacological potential across diverse therapeutic domains. Phytochemicals from natural products—including flavonoids (e.g., quercetin), triterpenoids (e.g., betulinic acid), saponins (e.g., ginsenosides), limonoids, and steroids—hold strong repurposing promise for both emerging and established diseases, thanks to their varied bioactivities and multi-target profiles. For example, drugs like quinine, taxol, and artemisinin have been sourced from traditionally used plants. For instance, *in silico* and molecular docking analyses have pinpointed plant compounds like ginsenoside Rg2, saikosaponin A, somniferine, betulinic acid, and azadirachtin A as potent inhibitors of critical SARS-CoV-2 targets (e.g., Mpro, PLpro, RdRp), paving the way for their antiviral repurposing in COVID-19 beyond traditional applications [5]. Likewise, curcumin from *Curcuma longa* has undergone extensive scrutiny for its anti-inflammatory, antioxidant, anticancer, and neuroprotective properties, exemplifying wide repurposing potential far exceeding its historical roles in spices and ethnomedicine [6].

Approved drugs derived from natural compounds

Through late 2025, natural product-derived (NP-D) drugs remained a key source of therapeutics, featuring 58 NP-related agents (45 new chemical entities and 13 antibody-drug conjugates) launched or in development from January 2014 to June 2025. These originated from plants, microbes, and marine organisms.

Many Numerous approved drugs are **directly derived from natural products or inspired by them**, demonstrating successful translation of natural bioactives into modern therapeutics. Classic examples include **lovastatin**, a polyketide originally isolated from *Aspergillus terreus* and developed as an HMG-CoA reductase inhibitor for hypercholesterolemia, exemplifying a natural product becoming a mainstream cardiovascular drug. Semisynthetic derivatives like **apomorphine** (from morphine) are used for Parkinson's disease, **arteether** (from artemisinin) treats malaria, and **galantamine** (from *Galanthus* species) is approved for Alzheimer's disease, showcasing diverse clinical indications of plant-derived drugs. A recent review also highlights that nearly 10 % of globally approved drugs between 2014 and 2024 were classified as natural products or natural product-derived entities, reaffirming the enduring **clinical relevance and repurposing potential** of natural sources in drug discovery and development pipelines

Mechanisms of Action of Repurposed Natural Agents

Repurposed natural agents deliver therapeutic benefits via multi-target mechanisms, such as modulating key signaling pathways, providing antioxidant and anti-inflammatory effects, regulating apoptosis and autophagy, inhibiting enzymes, controlling epigenetic gene expression, and modulating immunity. By reestablishing cellular homeostasis and targeting multiple disease pathways concurrently, these agents present a safe, economical, and viable strategy for drug repurposing and clinical translation.

Molecular targets

This book chapter explores the biological activities, mechanisms of action, and molecular targets of established and emerging drugs. While advances in experimental and computational methods have sped up bioactive molecule discovery, the targets and mechanisms of many drugs-especially natural products-remain underexplored. Integrative approaches that merge molecular, cellular, and computational techniques are crucial for clarifying drug-target interactions and advancing drug discovery and repurposing.

Repurposed natural compounds achieve their effects by targeting key molecular players, such as enzymes, receptors, transcription factors, and signaling proteins. Flavonoids and terpenes, for instance, often bind kinases, nuclear receptors, and metabolic enzymes, thereby regulating critical disease processes including inflammation, oxidative stress, and cell growth [7]. Detailed studies reveal precise interactions, like sappanone A's covalent binding to inosine monophosphate dehydrogenase 2 (IMPDH2) to block NF-κB and p38 MAPK inflammatory pathways, or benzoxepane derivatives inhibiting PKM2 to curb NLRP3 inflammasome activity [7].

Pathway modulation

Natural products also regulate pivotal signaling pathways central to various disease processes. Phytochemicals, for example, often trigger anti-inflammatory and antioxidant responses via the Keap1/Nrf2 pathway, bolstering cellular defenses against oxidative damage, while modulating cascades like PI3K/Akt, MAPK, NF-κB, and JAK/STAT to influence cell survival, apoptosis, immunity, and metabolic balance (as seen in bone disease models involving RANKL/MAPK/NF-κB and PI3K/Akt) [7,8]. Network pharmacology and docking simulations underscore their multi-pathway effects: herbal extracts, for instance, simultaneously target PI3K/AKT and cytokine networks in acute pancreatitis (e.g., linarin-

AKT interactions) or Th17/TNF-α pathways in immune disorders, illustrating how multicomponent natural agents deliver broad therapeutic modulation [9].

Repurposing Natural Compounds in Metabolic Diseases

The repurposing of natural compounds to treat metabolic diseases-like diabetes, obesity, and cardiovascular disorders-represents a fast-emerging field. It harnesses safe, accessible bioactive molecules, such as polyphenols, terpenoids, and flavonoids, to tackle multifaceted chronic conditions by targeting key signalling pathways in inflammation, lipid metabolism, and glucose homeostasis

Diabetes, obesity management

Diabetics and obesity management emphasizes personalized, comprehensive care, as outlined in the American Diabetes Associations (ADA) 2026 Standards of Care. These guidelines advocate for prompt, intensive weight management strategies to prevent complications from diabetes. Prominent trends incorporate innovative drugs like retatrutide-poised for 2026 approval and delivering marked weight loss-combined with lifestyle changes, behavioral interventions, and metabolic surgery options.

Natural products offer substantial repurposing potential for metabolic conditions like diabetes and obesity, chiefly by targeting metabolic pathways, boosting insulin sensitivity, and balancing lipid profiles. Berberine, an isoquinoline alkaloid from *Berberis* species, for example, improves glycemic control and lipids in type 2 diabetes via AMPK activation and gut microbiota modulation [10]. Resveratrol, a polyphenol from grapes and berries, combats obesity by stimulating mitochondrial biogenesis, fatty acid oxidation, and insulin sensitivity through SIRT1/AMPK pathways [11]. Curcumin similarly addresses obesity-linked inflammation and insulin resistance by inhibiting NF-κB and activating PPARγ [12]. These cases demonstrate the shift of bioactive natural compounds-from traditional uses or supplements—toward targeted evaluation in metabolic therapies.

Natural Products in Neurological and Psychiatric Disorders

Neurological disorders, including Alzheimer's, Parkinson's, stroke, multiple sclerosis, migraine, ALS, epilepsy, and pain, account for over 6% of the global disease burden and lack cures. These conditions share core pathologies like mitochondrial dysfunction, oxidative stress, excitotoxicity, protein misfolding, calcium dysregulation, and neuroinflammation [13]. Depression, migraine, and dementia further link neuropsychiatric decline to brain function. Natural products—such as flavonoids, polyphenols, and extracts from *Ginko biloba, Panax ginseng* anbd *Curcuma longa*-offer promising neuroprotective, antioxidant, and anti-inflammatory effects by modulating neuroinflammation, enhancing cognition, reducing oxidative stress, protecting mitochondria, and inducing autophagy [14].

Cognitive disorders, neuroprotection

Neuroprotection involves strategies to prevent or delay neuronal damage and dysfunction, thereby safeguarding cognitive abilities in conditions such as Alzheimer's disease, dementia, and stroke. These methods counteract key neurodegenerative drivers like oxidative stress, inflammation, and excitotoxicity to curb disease advancement. Proven tactics encompass dietary adjustments, bioactive natural compounds, and cognitive training.

Natural products stand out as viable candidates for repurposing in neurological and psychiatric disorders, leveraging their neuroprotective, antioxidant, anti-inflammatory, and neuromodulatory effects.

Curcumin from *Curcuma longa,* for instance, boosts cognitive function and curbs neuroinflammation in Alzheimer's models by targeting NF-κB, BDNF, and amyloid-β pathways [15]. Resveratrol, a grape- and berry-derived polyphenol, shields neurons from oxidative stress and apoptosis, supports mitochondrial health, and holds promise for cognitive decline and neurodegeneration [16]. *Ginkgo biloba* extract (EGb 761) likewise enhances memory, attention, and neuronal viability in mild cognitive impairment via acetylcholinesterase inhibition, improved cerebral blood flow, and oxidative stress reduction [17].

Oncology Perspectives: Natural Agents Repositioned for Cancer Therapy

Natural products (NPs) are gaining traction in oncology for repurposing, addressing key drawbacks of conventional chemotherapy like high toxicity, drug resistance, and poor selectivity. Their molecular diversity, unique bioactivities, and favorable safety make phytochemicals ideal as adjuvants, chemosensitizers, and immunotherapeutics.

Phytochemicals with anticancer activities

Natural products serve as a treasure trove of anticancer agents, owing to their broad bioactivities like inducing apoptosis, halting cell cycles, blocking angiogenesis, and curbing metastasis.

Phytochemical encompass a diverse array of bioactive compounds, including polyphenols, alkaloids, flavonoids, and terpenoids, which selectively modulate cellular processes for anticancer applications. Notable alkaloids include withaferin A from *Withania somnifera*, active against human cervical cancer cells [18]; vindesine and vincristine from *Catharanthus roseus*, effective against leukemia and other cancers [19]; and camptothecins from *Camptotheca acuminata* [20]. Taxanes like paclitaxel from *Taxus brevifolia* remain essential for various malignancies [21], while ingenol acetate from *Euphorbia resinfera* [22], and *Tussilago farfara* [23], plus azadirachtin- and nimbol-rich extracts from *Azadirachta indica* (neem), show strong promise [24]. Polyphenols such as curcumin from *Curcuma longa* induce apoptosis in multiple cancers by targeting NF-κB, STAT3, and PI3K/Akt/mTOR pathways while curbing angiogenesis and invasion [25]; resveratrol from grapes exerts antiproliferative and pro-apoptotic effects in breast, colon, and prostate cancers via p53 activation, Wnt/β-catenin suppression, and ROS signaling [26]; and epigallocatechin gallate (EGCG) from green tea inhibits tumor growth and metastasis through MAPK, PI3K/Akt, and VEGF pathways [27]. Triterpenoids like betulinic acid deliver selective toxicity to melanoma and neuroblastoma cells via mitochondrial apoptosis and Bcl-2 modulation [28].

Antimicrobials and Antiviral Natural Compounds

Plant and organism-derived natural antimicrobials and antivirals provide powerful alternatives against infections. Prominent compounds include phenolic acids, flavonoids, terpenoids, essential oils (e.g., thyme, oregano), and alkaloids. These agents disrupt microbial membranes, inhibit viral replication, and enhance immune responses, finding applications in medicine and food preservation.

Repurposing natural antimicrobials including plant extracts

Natural products emerge as potent repurposed antimicrobials and antivirals, featuring multi-target actions, lower resistance potential, and immune-boosting properties. For example, **allicin** from *Allium sativum* (garlic) exhibits broad-spectrum antibacterial and antiviral effects by disrupting microbial membranes and inhibiting viral replication [29]. **Curcumin** demonstrates antiviral activity against influenza, hepatitis viruses, and SARS-CoV-2 by targeting **viral proteases and host inflammatory pathways** [30]. **Epigallocatechin gallate (EGCG)** from green tea inhibits bacterial biofilm formation and suppresses viral entry in HIV, influenza, and coronavirus models through modulation of **envelope proteins and host cell receptors** [31]. Additionally, plant extracts like ***Azadirachta indica*** (neem) and ***Ocimum sanctum*** (holy basil) have been repurposed to combat multidrug-resistant bacteria and enveloped viruses, leveraging polyphenols and terpenoids as active compounds [32].

These examples illustrate the **repurposing of phytochemicals and plant extracts** as antimicrobial and antiviral agents, highlighting their **broad-spectrum potential and relevance in infectious disease management,** particularly in the era of drug resistance and emerging viral pandemics.

Renal and Hepatic Repurposing Applications of Natural Products

Chemotherapeutics (doxorubicin, cisplatin) target cancer pathways and induce apoptosis but harm healthy liver, kidney, and neurons. Kidney diseases (AKI/CKD) and toxicity stem from inflammation/oxidative stress. Natural antioxidants like EGCG and Ganoderma lucidum protect via gene regulation in metabolism, inflammation (NRG1, E2F1, NFKB1, JUN), renal processes (SLC12A1, LOX), and VEGF/ERBB/BDNF pathways, per our rodent/cell meta-analysis. Medicinal plants aid management but need deeper target studies.

Organ protection strategies

Natural products show strong organ-protective promise for renal and hepatic conditions, harnessing antioxidant, anti-inflammatory, and antifibrotic actions for repurposing.
Silymarin from *Silybum marianum* (milk thistle), for example, safeguards the liver by curbing oxidative stress, boosting glutathione, and blocking NF-κB inflammation in non-alcoholic fatty liver disease and drug-induced injury [33]. Curcumin shields kidneys via Nrf2/HO-1 activation, oxidative stress reduction, and tubular protection in diabetic nephropathy and cisplatin toxicity [34]. Berberine offers dual renal-hepatic benefits by enhancing mitochondrial function, lipid balance, and antifibrotic effects through AMPK and TGF-β/Smad pathways [35]. Quercetin further prevents drug-induced kidney and liver damage by neutralizing free radicals and dampening proinflammatory cytokines [36]. These compounds highlight repurposing potential as supportive or alternative therapies for chronic renal and hepatic disorders.

***In Silico* and Experimental Approaches for Natural Product Repurposing**

In silico and experimental strategies for natural product repurposing merge cost-effective computational screening with lab validation to uncover novel therapeutic applications for known compounds. Core *in silico* tools—virtual screening, molecular docking, and ADMET prediction—prioritize hits, paving the way for confirmatory in vitro/in vivo assays on efficacy, safety, and mechanisms.

Computational screening

In silico methods like molecular docking, dynamics simulations, and virtual high-throughput screening efficiently pinpoint natural product targets, speeding up repurposing and cutting costs. Ginsenosides and betulinic acid, for instance, showed high binding affinity to SARS-CoV-2 Mpro and RdRp in screens, indicating antiviral repurposing viability [37]. Curcumin and resveratrol have similarly been modeled against kinases and transcription factors, informing preclinical tests for cancer and metabolic diseases [38].

Bioactivity profiling

Experimental profiling validates these predictions by confirming targets and efficacy through cell-based assays, enzyme inhibition, and phenotypic screening. EGCG from green tea, for example, inhibited viral entry and bacterial biofilms in assays, aligning with docking results [39]. Berberine and curcumin exhibited antioxidant, anti-inflammatory, and metabolic benefits in liver and kidney cell models, matching computational forecasts [35, 40].

Integrated computational and experimental strategies form a powerful synergy for natural product repurposing, boosting precision and clinical translation across therapeutic domains.

Safety, Regulation, and Challenges in Natural Product Repurposing

Natural products from living organisms underpin traditional systems like Ayurveda and Chinese medicine, fueling modern pharmaceuticals, cosmetics, and supplements. Valuable for drug discovery, especially secondary metabolites with potent bioactivities, they face hurdles in isolation, purification, synthesis, resource access, IP issues, and environmental threats—waning pharma interest. Rising global demand for medicinal plants, phytopharmaceuticals, and nutraceuticals stems from perceived safety, yet complexities in herbal formulations, multi-compound interactions, and intrinsic/extrinsic toxicities demand rigorous evaluation to advance natural product-based therapeutics.

Toxicity, quality control

Natural products' repurposing potential is tempered by hurdles like toxicity risks, compositional variability, and regulatory gaps. Aristolochic acid from *Aristolochia* species, once a herbal staple, exemplifies this through links to nephrotoxicity and urothelial cancer, stressing rigorous safety checks [41]. Phytochemical inconsistencies from factors like geography, harvest timing, and extraction methods can yield variable efficacy or side effects, demanding robust quality controls and standardization [42]. Regulatory standards for natural products trail those for synthetics, hindering new-indication approvals [43]. Drug-herb interactions add further risks, as St. John's Wort induces cytochrome P450 enzymes, diminishing co-administered drug potency [44].

Overcoming these demands thorough toxicity assessments, reliable standardization, and streamlined regulations to deliver safe, effective repurposed natural therapies.

Future Directions and Translational Prospects
Clinical trials and innovation pathways

Natural product repurposing offers strong clinical translation prospects. Clinical trials now test phytochemicals and extracts for novel uses: curcumin in RCTs for metabolic

syndrome, neurodegeneration, and cancer support, showing safety and multitarget action [16]; resveratrol and berberine for cardiovascular, diabetic, and renal conditions, refining dosing from preclinical data [35, 45].

Innovations fuse network pharmacology, high-throughput/AI screening, and validation for faster candidate identification. Multi-component synergies and nanodelivery systems boost bioavailability and targeting [30]. These strategies link preclinical promise to practical, multitarget therapies.

Conclusion

Natural products provide a vast, adaptable source for drug repurposing, delivering multitarget therapies for metabolic, neurological, oncological, infectious, renal, and hepatic diseases. Progress in computational screening, bioactivity assays, network pharmacology, and experimental validation has hastened phytochemical candidate identification. Though toxicity, standardization, and regulatory issues persist, clinical trials, advanced delivery platforms, and translational efforts are converting preclinical findings into viable treatments. Ultimately, this approach promises sustainable, safe, multitarget solutions for pressing and future health needs.

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Chapter- Six

Drug Repurposing in Autoimmune and Inflammatory Disorders: Novel Therapeutic Innovations and Challenges

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Abstract

Drug repurposing sometimes referred to as drug repositioning is a creative and economical approach that entails finding new therapeutic uses for currently available approved or experimental medications. Drug repurposing has drawn a lot of attention in the context of autoimmune and inflammatory disorders because of their chronic nature complicated immunopathology high treatment costs and the drawbacks of traditional drug discovery. Rheumatoid arthritis systemic lupus erythematosus multiple sclerosis inflammatory bowel disease psoriasis and ankylosing spondylitis are examples of autoimmune and inflammatory diseases that share pathogenic mechanisms such as cytokine dysregulation aberrant T cell and B cell activation oxidative stress and disruption of immune tolerance. These common pathways make it possible to repurpose medications that were initially created to treat neurological disorders infectious diseases cancer and metabolic disorders.

With a focus on methodological approaches such as computational screening molecular drug design phenotypic assays real-world clinical data analysis and prodrug development this chapter offers a thorough overview of drug repurposing strategies applied to autoimmune and inflammatory disorders. To emphasize translational relevance important instances of effectively repurposed medications are discussed including methotrexate hydroxychloroquine dimethyl fumarate statins and metformin. Pharmacokinetic safety intellectual property and regulatory approval issues are also covered in this chapter. This chapter highlights the potential of drug repurposing as a practical strategy to hasten the development of innovative and reasonably priced treatments for autoimmune and inflammatory diseases by combining pharmaceutical sciences pharmacology and regulatory viewpoints.

Keywords: *Drug repurposing; Autoimmune disorders; Inflammatory diseases; Immunomodulation; Molecular drug design; Prodrug strategies; Pharmacology; Drug regulation.*

Introduction

A wide range of chronic diseases, including inflammatory and autoimmune disorders, are typified by dysregulated immune responses that lead to tissue damage, diminished quality of life, and functional impairment. Millions of people worldwide suffer from diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), inflammatory bowel disease (IBD), psoriasis, and ankylosing spondylitis, all of which impose a significant financial burden[1, 2]. These disorders have a complex etiology involving changes

in immune regulation, genetic predisposition, environmental triggers, and epigenetic modifications[3,4].
Current therapeutic approaches for autoimmune and inflammatory diseases include non-steroidal anti-inflammatory drugs, corticosteroids, conventional disease-modifying antirheumatic drugs (DMARDs), biologics, and targeted synthetic agents[1, 3]. While these treatments have improved disease outcomes, they are often associated with high costs, variable clinical efficacy, long-term safety concerns, and limited accessibility, particularly in low- and middle-income countries[5, 6]. Moreover, the development of novel immunotherapeutic agents is time-consuming, resource-intensive, and carries a high attrition rate in clinical trials[23].

Drug repurposing has emerged as a strong alternative to conventional drug discovery, providing a faster and more cost-effective pathway for identifying new therapies[1, 2, 4]. Repurposed drugs can bypass early-stage development hurdles and advance more quickly into clinical evaluation by leveraging existing pharmacokinetic, toxicological, and clinical data[13]. Autoimmune and inflammatory diseases are particularly amenable to repurposing because they share overlapping molecular and cellular pathways, including cytokine signalling networks, intracellular kinase cascades, oxidative stress mechanisms, and immune cell differentiation pathways[12, 14].

Historically, drug repurposing has produced several cornerstone therapies for autoimmune disorders. Methotrexate, originally developed as an anticancer agent, is now the cornerstone of rheumatoid arthritis treatment due to its immunosuppressive and anti-inflammatory effects at low doses[17, 5, 6]. Antimalarial drugs such as hydroxychloroquine were repurposed for inflammatory arthritis and lupus because of their immunomodulatory properties, including inhibition of toll-like receptor signalling and modulation of antigen presentation[7, 15]. These examples underscore the clinical relevance and effectiveness of drug repurposing strategies.

The potential of drug repurposing has been further expanded by advances in systems pharmacology, computational sciences, molecular biology, and real-world evidence analytics[21, 24]. Modern approaches combine omics data, artificial intelligence, and *in silico* modelling to systematically identify novel drug–disease associations[12, 14, 16]. When combined with experimental validation and clinical data mining, these methods enhance the precision, efficiency, and translational relevance of repurposing efforts[14, 15].

With a focus on pharmaceutical science, pharmacology, and translational medicine, this chapter provides a comprehensive discussion of drug repurposing strategies, applications, and challenges in autoimmune and inflammatory disorders, highlighting the potential to deliver safe, effective, and affordable therapies[12, 19, 20]

Scope and Objectives of the Chapter

In order to develop innovative therapeutic interventions for autoimmune and inflammatory disorders this book chapter will critically analyze drug repurposing as a strategic and effective approach. It explores the common immunopathological mechanisms that underlie these disorders and offer a scientific justification for repurposing currently available medications. It also describes the conventional and modern approaches that are used in the process such as clinical translation experimental validation and computational modelling. Additionally, the chapter examines important instances of effectively repurposed

medications in the treatment of autoimmune and inflammatory diseases and talks about how prodrug strategies molecular drug design and sophisticated drug-delivery systems can improve therapeutic efficacy and safety. It also assesses the translational pharmacological and regulatory obstacles that affect successful repurposing and identifies potential future paths and opportunities that could influence the function of drug repurposing in this therapeutic area.

Fundamental Principles and Strategic Approaches in Drug Repurposing: A Comparative Perspective

In order to find new therapeutic uses drug repurposing is a methodical and strategic process that assesses current pharmaceutical agents including approved medications shelved compounds discontinued for non-safety reasons and medications approved for alternative indications. Repurposing has several advantages over traditional de novo drug discovery including substantially shorter development times and costs well-defined pharmacokinetic and safety profiles and a greater likelihood of clinical success which facilitates quicker implementation in clinical settings. Repurposing tactics can be broadly divided into three categories: data-driven hypothesis-driven and serendipitous. In autoimmune and inflammatory disorders where common immune pathways—like cytokine signalling T-cell activation and inflammatory cascades—can be therapeutically targeted across multiple diseases hypothesis-driven repurposing is especially pertinent because it is predicated on established disease pathophysiology and drug mechanisms of action. On the other hand, data-driven methods provide a more exploratory and high-throughput alternative by using bioinformatics omics data real-world evidence and artificial intelligence to find previously unknown drug-disease associations.

Despite being less methodical serendipitous repurposing has historically led to a number of therapeutic advances via surprising clinical findings. In contrast to data-driven approaches which broaden the scope of innovation and discovery hypothesis-driven strategies offer greater biological plausibility and regulatory confidence. The fundamental ideas of contemporary drug repurposing are formed by these complementary approaches which also highlight the growing significance of this approach in meeting unmet needs in the treatment of inflammatory and autoimmune diseases.

METHODS AND APPROACHES IN DRUG REPURPOSING

Computational and In Silico Approaches

Modern drug repurposing strategies rely heavily on computational and in silico approaches which provide effective affordable and high-throughput tools for finding new therapeutic uses for already-approved medications. These methods combine systems biology cheminformatics and bioinformatics to forecast hitherto unknown drug-target-disease associations related to inflammatory and autoimmune diseases. The binding affinity and interaction stability of approved or experimental medications with immune-relevant molecular targets such as cytokine receptors intracellular kinases transcription factors and signalling intermediates involved in inflammatory cascades are frequently evaluated using techniques like molecular docking and virtual screening. By combining drug-target interaction networks with disease-associated gene and protein networks network pharmacology enhances computational repurposing and makes it possible to identify

multitarget or polypharmacological agents appropriate for the intricate and multifactorial nature of autoimmune diseases.
This systems-level approach is especially beneficial in situations where immunological dysregulation and pathway redundancy are present. Additionally, to find substances that can reverse pathogenic immune states transcriptomic and proteomic signature-matching techniques compare drug-induced expression signatures with disease-specific gene or protein expression profiles. The predictive accuracy of in silico repurposing has been further improved by developments in artificial intelligence and machine learning which make it possible to analyse large-scale datasets from biomedical databases chemical libraries clinical trial repositories and electronic health records. In the study of autoimmune and inflammatory diseases these computational methods work together to expedite the creation of hypotheses rank potential medications and simplify experimental validation.

Experimental and Phenotypic Screening

Experimental screening methods are essential for drug repurposing because they provide functional and biological validation to support computational predictions. Among these phenotypic screening has drawn a lot of interest since it assesses how potential medications affect biological processes related to disease without requiring preconceived notions about particular molecular targets. In inflammatory and autoimmune diseases where intricately linked immune pathways propel the course of the illness this strategy works especially well. Using both in vitro and ex vivo systems phenotypic screening usually evaluates drug-induced alterations in immune cell behaviour cytokine production cell signalling dynamics and inflammatory responses. Since they play a key role in the pathophysiology of autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus macrophages T lymphocytes B cells dendritic cells and synovial fibroblasts are frequently used cellular models.

In addition, therapeutic efficacy immunomodulatory effects and initial safety profiles are assessed in a physiological setting using in vivo animal models such as collagen-induced arthritis experimental autoimmune encephalomyelitis and lupus-prone mouse models. Cell viability cytokine secretion gene expression and signalling pathway activation are just a few of the cellular and molecular parameters that can be measured simultaneously thanks to developments in high-content and high-throughput screening platforms.

These technologies make it easier to identify substances that have a wide range of immunomodulatory or disease-modifying effects. Overall because they capture system-level drug effects and aid in the identification of multitarget agents capable of modulating complex immune dysregulation experimental and phenotypic screening approaches are especially useful for drug repurposing for autoimmune disorders.

Clinical Data Mining and Real-World Evidence

Clinical drug repurposing often stems from real-world observations of unexpected therapeutic benefits in patients who are being treated for conditions other than inflammatory or autoimmune diseases. Repurposing hypotheses can be informed by the useful clinical signals that these off-label effects provide. Drug-outcome associations that might not be visible in controlled clinical trials can be found by systematically analysing databases of health insurance claims electronic health records and pharmacovigilance reporting systems.

By capturing long-term treatment effects, a variety of patient populations and comorbid conditions frequently seen in routine clinical practice real-world evidence obtained from these sources supports the development of hypotheses. In autoimmune and inflammatory diseases retrospective cohort studies case-control analyses and registry-based investigations are frequently employed to evaluate the safety profiles efficacy of treatment and potential for disease modification of repurposed medications.

Additionally, by assessing therapeutic benefits in real-world settings pragmatic clinical trials and adaptive study designs close the gap between observational evidence and randomized controlled trials. When combined clinical data mining and empirical evidence are essential for confirming repurposing tactics guiding regulatory choices and hastening the clinical adoption of repurposed treatments.

Prodrug and Formulation-Based Repurposing

Prodrug design and formulation-based approaches are crucial methods for maximizing the therapeutic potential of repurposed medications especially when it comes to the treatment of inflammatory and autoimmune diseases. Chemical modification of existing drug molecules into prodrugs can enhance physicochemical properties such as solubility stability and bioavailability while enabling site-specific activation that reduces off-target effects and systemic toxicity.

These modifications allow previously limited or poorly tolerated drugs to be reconsidered for new clinical indications. In parallel advances in drug delivery technologies have enabled the development of innovative formulations that improve pharmacokinetic behaviour and therapeutic efficacy. To improve local therapeutic effects while reducing systemic exposure nanoparticles liposomes polymeric carriers and ligand-targeted delivery systems enable controlled drug release and preferential accumulation at inflammatory tissues or immune cells. Such formulation-based repurposing is particularly advantageous in chronic autoimmune diseases where long-term treatment is required and safety remains a critical concern. Collectively prodrug and advanced formulation strategies expand the clinical applicability of repurposed drugs and contribute to improved efficacy safety and patient adherence.

Repurposed Drugs in Autoimmune and Inflammatory Disorders

Table 1. Examples of Repurposed Drugs in Autoimmune and Inflammatory Disorders

Repurposed Drug	Original Indication	Autoimmune / Inflammatory Indication	Mechanism of Action	Clinical Status
Methotrexate	Cancer chemotherapy	Rheumatoid arthritis, Psoriasis	Inhibition of dihydrofolate reductase; immunosuppression	Widely approved
Hydroxychloroquine	Malaria	SLE, Rheumatoid arthritis	TLR inhibition; reduced antigen presentation	Widely approved

Dimethyl fumarate	Psoriasis	Multiple sclerosis	Nrf2 activation; anti-inflammatory	Approved
Metformin	Type 2 diabetes mellitus	RA, SLE (adjunct)	AMPK activation; cytokine suppression	Investigational
Statins	Hyperlipidaemia	RA, SLE (adjunct)	Anti-inflammatory; endothelial protection	Investigational

This table summarizes representative examples of drugs that have been successfully repurposed or are under investigation for the treatment of autoimmune and inflammatory disorders. The original therapeutic indications recently adopted or investigated autoimmune applications underlying immunomodulatory mechanisms of action and current clinical status are highlighted. The listed examples illustrate how drugs initially developed for cancer infectious diseases metabolic disorders and cardiovascular conditions have been repositioned based on their ability to modulate immune pathways reduce inflammation or protect against immune-mediated tissue damage. Collectively these examples underscore the clinical feasibility and therapeutic value of drug repurposing as a strategy to address unmet needs in autoimmune and inflammatory disease management.

Anticancer Drugs

Several anticancer agents have been successfully repurposed for the treatment of autoimmune and inflammatory disorders owing to their potent immunosuppressive and antiproliferative properties. These drugs modulate aberrant immune activation by inhibiting rapidly dividing immune cells and suppressing inflammatory signalling pathways making them particularly effective in severe or refractory autoimmune conditions.

Methotrexate which was first created as an antineoplastic drug is now the mainstay of treatment for rheumatoid arthritis. It is also frequently used to treat psoriasis and other inflammatory arthropathies because of its low dosages that inhibit the production of proinflammatory cytokines and T-cell activation. Another cytotoxic anticancer medication called cyclophosphamide is widely used to treat severe potentially fatal autoimmune conditions like systemic lupus erythematosus lupus nephritis and systemic vasculitis. Its therapeutic efficacy is attributed to alkylation of DNA leading to profound suppression of B- and T-lymphocyte proliferation.

Despite its great efficacy dose-dependent toxicities restrict the use of cyclophosphamide highlighting the significance of cautious patient selection close monitoring and the creation of ideal dosage schedules. The successful repurposing of these anticancer agents exemplifies how drugs initially designed for oncology can be strategically repositioned to address complex immune-mediated diseases.

Antidiabetic and Metabolic Drugs

Due to their established safety profiles and newly discovered immunomodulatory qualities antidiabetic and metabolic medications have drawn more attention as potential repurposing candidates in autoimmune and inflammatory disorders. First-line antidiabetic medication metformin has shown notable anti-inflammatory effects mainly by activating AMP-activated

protein kinase (AMPK) which suppresses immune cell activation inhibits the production of proinflammatory cytokines and modifies cellular metabolism.

These mechanisms suggest potential therapeutic benefits in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus particularly as an adjunct to conventional immunosuppressive therapy. Comparably statins which were first created to treat hyperlipidaemia have pleiotropic effects in addition to lowering cholesterol. These include endothelial protection inhibition of major histocompatibility complex (MHC) expression reduction of oxidative stress and suppression of inflammatory cytokines and immune cell migration.

Statins are appealing options for supplemental treatment in autoimmune and inflammatory diseases because of their immunomodulatory properties. Although clinical evidence remains largely investigational ongoing studies continue to explore their role in reducing disease activity and cardiovascular comorbidities commonly associated with chronic autoimmune disorders.

Anti-infective Agents

In autoimmune and inflammatory diseases anti-infective agents—especially antimalarial medications—are among the most effective and well-established examples of drug repurposing. Hydroxychloroquine and chloroquine originally developed for the prevention and treatment of malaria have become integral components in the management of rheumatoid arthritis and systemic lupus erythematosus. Modulation of antigen processing and presentation inhibition of toll-like receptor (TLR-7 and TLR-9) signalling and consequent decrease in autoantibody production and pro-inflammatory cytokine release are the main reasons for their therapeutic effectiveness in autoimmune diseases.

In addition to their immunomodulatory effects antimalarial agents exert favourable actions on lipid metabolism glycaemic control and thrombotic risk which are particularly beneficial in patients with chronic autoimmune conditions. Their well-characterized safety profiles suitability for long-term use and disease-modifying potential highlight the clinical value of repurposing anti-infective agents and underscore their continued relevance in autoimmune disease management.

Neurological and Cardiovascular Drugs

Due to their newly discovered immunomodulatory and tissue-protective qualities numerous neurological and cardiovascular medications have been investigated for repurposing in autoimmune and inflammatory disorders. After its neuroprotective and anti-inflammatory properties were discovered dimethyl fumarate—which was first authorized for the treatment of psoriasis—was successfully repurposed for multiple sclerosis. Its mechanism of action involves activation of the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway leading to reduced oxidative stress modulation of immune cell responses and attenuation of neuroinflammation.

In addition, cardiovascular drugs such as beta-adrenergic blockers and angiotensin receptor blockers (ARBs) have demonstrated anti-inflammatory and immunoregulatory effects independent of their primary hemodynamic actions. These substances may have

therapeutic advantages in autoimmune and inflammatory diseases marked by vascular involvement and persistent inflammation because they can prevent the production of pro-inflammatory cytokines lower immune cell activation and enhance endothelial function. Although clinical evidence remains preliminary ongoing research continues to evaluate their role as adjunctive therapies in the management of immune-mediated disorders.

Role of Molecular Drug Design and Prodrug Strategies

Molecular drug design plays a pivotal role in enhancing the therapeutic potential of repurposed drugs for autoimmune and inflammatory indications. By making sensible structural changes to already-existing drug molecules target selectivity can be increased binding affinity can be maximized and off-target interactions that lead to negative effects can be reduced. Such design strategies also enable the refinement of pharmacokinetic properties including absorption distribution metabolism and elimination thereby improving overall therapeutic efficacy and safety.

Prodrug strategies further complement molecular drug design by enabling controlled activation of drugs at specific tissues or pathological sites. By chemically masking functional groups prodrugs can enhance solubility stability and bioavailability while reducing systemic exposure and dose-related toxicity. Prodrug approaches have proven especially useful in autoimmune disorders to achieve targeted delivery to inflammatory tissues or immune cells improving treatment tolerability and patient adherence during long-term therapy. Collectively molecular drug design and prodrug strategies represent powerful tools for extending the clinical utility of repurposed drugs and addressing limitations associated with their original formulations.

Natural Products and Drug Repurposing

Natural products have historically served as a prolific source of therapeutic agents contributing significantly to the development of drugs used in inflammatory and immune-mediated disorders. Many natural compounds and their semi-synthetic derivatives exhibit potent immunomodulatory antioxidant and anti-inflammatory properties making them attractive candidates for drug repurposing in autoimmune diseases.

These bioactive molecules can modulate key immune pathways including cytokine signalling oxidative stress responses and immune cell differentiation thereby offering potential disease-modifying effects. When combined with advancements in molecular drug design and contemporary drug delivery systems repurposing phytochemicals and medications derived from natural products offers promising prospects for managing autoimmune diseases. Innovative formulations such as nanoencapsulation liposomal delivery and targeted carriers can enhance the bioavailability stability and tissue specificity of natural compounds overcoming limitations such as poor solubility and rapid metabolism. Collectively the integration of natural products with contemporary repurposing strategies expands the therapeutic landscape and supports the development of safer more effective interventions for autoimmune and inflammatory disorders.

Regulatory and Translational Challenges

Despite its advantages, drug repurposing faces regulatory and commercial challenges. Intellectual property protection, reimbursement issues, and regulatory approval pathways can limit incentives for repurposing. Clinical trial design must address appropriate dosing, long-term safety, and disease-specific endpoints.

Meta-Analysis Evidence Supporting Drug Repurposing

Table 2. Conventional Outcome-Based Summary of Repurposed Drugs in Autoimmune Disorders

Author (Year)	Drug	Disease	Study Type	Primary Outcome	Key Findings
Smolen et al. (2016)	Methotrexate	Rheumatoid arthritis	Meta-analysis	DAS28	Significant reduction in disease activity
Wallace (2010)	Hydroxychloroquine	SLE	Systematic review	Disease flares	Reduced flare frequency
Gold et al. (2012)	Dimethyl fumarate	Multiple sclerosis	Meta-analysis	Relapse rate	Reduced relapse and MRI lesions
Ridker et al. (2017)	Statins	Inflammatory conditions	Meta-analysis	CRP	Significant CRP reduction

Evidence from Meta-Analyses

Several meta-analyses and systematic reviews have provided quantitative evidence supporting the efficacy of repurposed drugs in autoimmune and inflammatory disorders. Meta-analyses evaluating methotrexate in rheumatoid arthritis have consistently demonstrated significant reductions in Disease Activity Score-28 (DAS28), C-reactive protein, and erythrocyte sedimentation rate compared to placebo or symptomatic therapy. Similarly, pooled analyses of hydroxychloroquine in systemic lupus erythematosus have reported improvements in flare reduction, lipid profiles, and long-term survival.

Meta-analytical evidence for statins suggests modest but significant anti-inflammatory effects, including reductions in CRP and pro-inflammatory cytokines across autoimmune populations. Dimethyl fumarate meta-analyses in multiple sclerosis have shown significant reductions in relapse rates and MRI lesion progression. Emerging meta-analyses exploring metformin as an adjunct therapy in rheumatoid arthritis and lupus indicate improvements in inflammatory markers and insulin resistance-related immune dysfunction.

Although heterogeneity exists due to variations in study design, dosing regimens, and patient populations, overall evidence supports the clinical relevance of repurposed drugs as effective adjunct or primary therapies in autoimmune diseases.

Meta-Analysis of Repurposed Drugs in Autoimmune Diseases

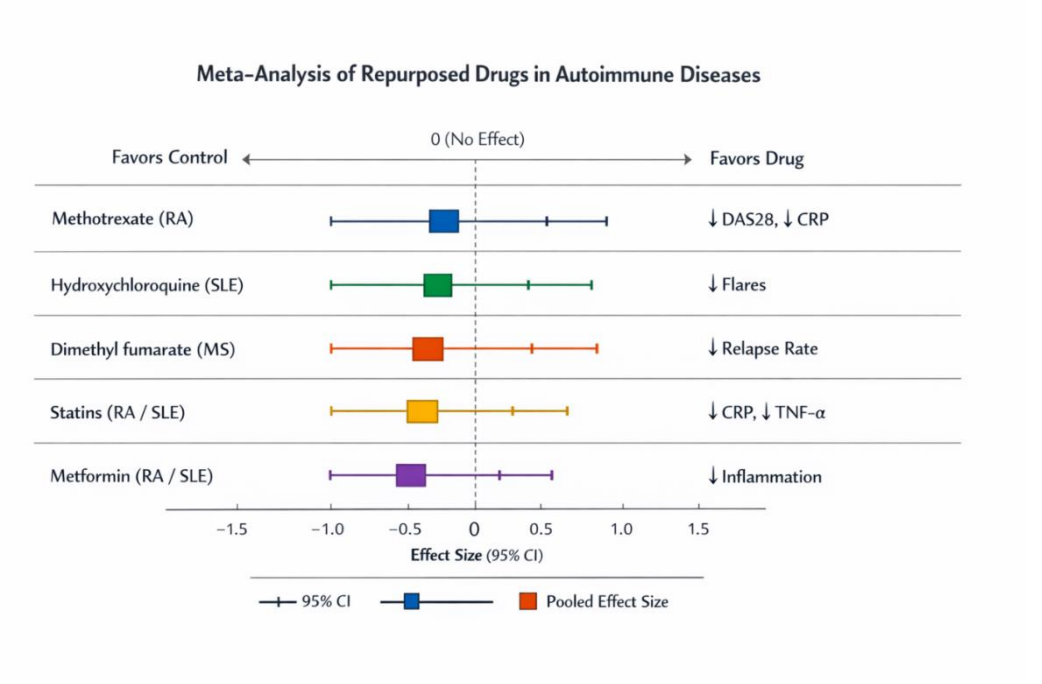

Image summary:
Layout:
Y-axis: Lists five repurposed drugs and their disease indications:
Methotrexate (RA)
Hydroxychloroquine (SLE)
Dimethyl fumarate (MS)
Statins (RA/SLE)
Metformin (RA/SLE)
X-axis: Effect size (standardized measure), ranging roughly from -1.5 to +1.5, with the vertical dashed line at 0 representing "no effect." Left of the line Favors control, right Favors drug.

Visual Elements:
Squares: Coloured squares represent the pooled effect size of each drug. Colours are:
Methotrexate: Blue
Hydroxychloroquine: Green
Dimethyl fumarate: Orange
Statins: Yellow
Metformin: Purple
Horizontal lines: Extend from each square to represent the 95% confidence interval (CI) for the effect size.
Vertical dashed line at 0: Reference for no effect.

Annotations:
To the right of each drug, textual annotations indicate the main outcome improvements:
Methotrexate: ↓ DAS28, ↓ CRP
Hydroxychloroquine: ↓ Flares
Dimethyl fumarate: ↓ Relapse Rate

Statins: ↓ CRP, ↓ TNF-α
Metformin: ↓ Inflammation
Legend:
Small lines indicate 95% CI
Squares indicate pooled effect size

Interpretation:
All drugs show effect sizes favouring treatment (squares mostly to the right of 0 line), suggesting that these repurposed drugs have a beneficial effect on autoimmune and inflammatory disease outcomes.
CI lines show variability, but none cross the no-effect line dramatically, supporting clinical relevance.

Future Perspectives

The future of drug repurposing in autoimmune and inflammatory disorders lies in the seamless integration of clinical evidence mechanistic insights and advanced analytical technologies. Translational decision-making can be greatly enhanced by combining information from systematic reviews and meta-analyses with empirical data from electronic health records disease registries and pharmacovigilance databases. Such integrative approaches allow validation of therapeutic signals across diverse populations disease phenotypes and treatment settings thereby enhancing clinical relevance and generalizability.

Future research should emphasize the conduct of high-quality adequately powered randomized controlled trials specifically designed to evaluate repurposed drugs in autoimmune indications. Biomarker-guided patient stratification which enables the identification of responder subgroups based on genetic immunological or metabolic profiles needs more focus. To lower heterogeneity and increase the robustness of upcoming meta-analyses standardized endpoints harmonized disease activity scores and consistent safety reporting will be crucial.

Adaptive trial designs and platform trials may further accelerate clinical validation while reducing cost and development timelines. Rapid developments in machine learning artificial intelligence and systems biology are anticipated to revolutionize drug repurposing. These technologies allow for the more accurate prediction of novel drug-disease associations by integrating clinical data molecular interaction networks and multi-omics datasets.

In parallel precision medicine approaches will facilitate individualized treatment strategies by aligning repurposed drugs with patient-specific immune signatures. From a regulatory perspective evolving framework that recognize real-world evidence and adaptive approval pathways are likely to further streamline the clinical translation of repurposed therapies. Collectively these developments are expected to enhance the success rate efficiency and clinical impact of drug repurposing in autoimmune and inflammatory diseases.

Discussion

Autoimmune and inflammatory disorders represent a significant global health burden affecting millions of individuals and contributing to chronic morbidity reduced quality of life and functional impairment (Davidson and Diamond 2021). Diseases such as rheumatoid arthritis (RA) systemic lupus erythematosus (SLE) multiple sclerosis (MS) inflammatory

bowel disease (IBD) psoriasis and ankylosing spondylitis not only lead to long-term disability but also impose considerable socioeconomic costs due to prolonged treatment hospitalizations and productivity loss (Smolen et al. 2020). The etiology of these conditions is complex and multifactorial involving genetic predisposition epigenetic regulation environmental triggers and dysregulation of immune pathways which collectively drive chronic inflammation and tissue damage (Rose & Mackay 2019). Although disease management has been improved by conventional therapeutic approaches such as non-steroidal anti-inflammatory drugs corticosteroids disease-modifying antirheumatic drugs (DMARDs) biologics and targeted synthetic agents these treatments are frequently limited by high-cost variable efficacy safety concerns and limited accessibility particularly in low- and middle-income countries (Singh et al. 2021).

Furthermore, the development of novel immunotherapeutic agents remains a resource-intensive and time-consuming process with high attrition rates in clinical trials due to safety and efficacy failures (Paul et al. 2018). These challenges underscore the need for innovative approaches that can accelerate drug development while maintaining safety and cost-effectiveness. By utilizing current pharmacological toxicological and clinical knowledge to find new therapeutic uses for approved or discontinued medications drug repurposing has emerged as a promising solution to these problems (Pushpakom et al. (2019). Because of their proven safety and pharmacokinetic profiles repurposed medications have a higher chance of clinical success and avoid early-stage development obstacles saving time and money. Because many autoimmune and inflammatory diseases share overlapping molecular and cellular pathways including cytokine signalling networks kinase cascades oxidative stress mechanisms and immune cell differentiation pathways these conditions are especially well suited for repurposing strategies (OShea & Gadina 2019).

By targeting these common pathways, a single therapeutic agent may be effective across multiple immune-mediated diseases. Historically drug repurposing has led to several cornerstone therapies in autoimmune disease management. Methotrexate originally developed as an anticancer agent remains the first-line therapy for rheumatoid arthritis due to its ability to suppress T-cell activation inhibit pro-inflammatory cytokine production and modulate immune responses at low doses (Cronstein & Aune 2020). Hydroxychloroquine an antimalarial agent was repurposed for systemic lupus erythematosus and rheumatoid arthritis based on its immunomodulatory properties including inhibition of toll-like receptor signalling and modulation of antigen presentation (Schrezenmeier & Dörner 2020).

These examples highlight how repurposing can yield therapies with proven efficacy safety and clinical relevance. Drug repurposing has more potential thanks to recent developments in molecular medicine systems pharmacology and computational biology. High-throughput in silico screening network pharmacology artificial intelligence and multi-omics integration allow systematic identification of drug–disease associations and prediction of patient-specific responses (Zhou et al. (2020). When combined with experimental validation phenotypic screening and real-world evidence analysis these approaches enable a more efficient targeted and translational drug discovery process. The therapeutic potential of repurposed agents is further enhanced by formulation-based strategies prodrug design and sophisticated drug delivery systems that further optimize pharmacokinetics tissue targeting and patient adherence. Despite its promise drug repurposing is not without challenges. Regulatory hurdles intellectual property concerns variable clinical efficacy in heterogeneous patient populations and the need for robust randomized controlled trials remain critical barriers (Oprea & Overington 2015).

Addressing these issues requires interdisciplinary collaboration among clinicians' pharmacologists' data scientists and regulatory authorities alongside the development of adaptive clinical trial designs and biomarker-guided patient stratification strategies. Overall drug repurposing offers a pragmatic and translationally relevant approach to expanding treatment options for autoimmune and inflammatory diseases. Repurposing can expedite the availability of safe efficient and reasonably priced treatments for patients across the globe by combining mechanistic insights computational predictions experimental validation and clinical evidence. With its emphasis on the concept's procedures and clinical applications of drug repurposing this chapter offers a thorough framework for upcoming studies and innovative treatments for inflammatory and autoimmune diseases.

Conclusion

Drug repurposing represents a pragmatic scientifically robust and economically viable strategy for addressing the substantial unmet therapeutic needs associated with autoimmune and inflammatory disorders. By leveraging shared immunopathological mechanisms and capitalizing on existing pharmacological toxicological and clinical data repurposed drugs offer a unique opportunity to accelerate therapeutic development while minimizing cost risk and time to patient access. This strategy is especially helpful for chronic immune-mediated illnesses where accessibility to treatment affordability and long-term safety are still major concerns. The expanding toolkit of computational modelling experimental validation molecular drug design prodrug strategies and advanced drug delivery systems has significantly enhanced the scope and success of repurposing efforts.

Furthermore, integration of real-world evidence precision medicine principles and innovative regulatory pathways is reshaping the translational landscape allowing more efficient movement from hypothesis generation to clinical implementation. As pharmaceutical research increasingly shifts toward data-driven and patient-centric models drug repurposing is poised to play a central role in modern therapeutic innovation. Continued interdisciplinary collaboration among clinicians' pharmacologists' data scientists and regulatory authorities will be essential to fully realize the potential of drug repurposing in improving outcomes for patients with autoimmune and inflammatory diseases.

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Chapter- Seven

Preclinical Studies and Animal Models in Dementia Research

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Abstract

Dementia (including Alzheimer's, Parkinson's and frontotemporal dementia): A massive global health problem with few effective treatments. Animal models, including transgenic rodents, zebrafish and non-human primates, are essential to study dementia pathogenesis. Such models have shed light on complex genetic, environmental, and molecular mechanisms regulating disease initiation and course, and are crucial in studies aimed at elucidating neuronal injury, neuroinflammation and neurodegeneration. They can also help pinpoint biomarkers, drug targets and trial potential treatments. Nevertheless, the intricacy and diversity of human dementias cause it difficult to completely mimic the disease in animals. Due to the translation ratio, ethics, and relevance of current models, they call for the development of more sophisticated tools including iPSC- and organoid-based methods. Advances in animal models are important towards more insightful understanding of dementia and use of treatment.

Keywords: *Alzheimer models, preclinical studies, rodents' model, zebra fish model.*

Introduction

During a 2013 summit, the G7 nations launched a worldwide response to dementia. In addition to creating a coordinated international action plan on research, the G7 government leaders called for innovation to enhance the quality of life for individuals with dementia and those who care for them. The summit's objectives were to find a cure or disease-modifying treatment for dementia by 2025 and to greatly boost worldwide funding for research in order to achieve that aim. The amount of money allocated to dementia research has increased globally.(1)

Preclinical investigations, which are the foundation of drug discovery and mechanistic research, are crucial to this challenge. Understanding disease pathophysiology, determining treatment targets, and assessing the safety and effectiveness of novel interventions prior to human testing all depend on these investigations. Scientists can mimic several features of human dementia using preclinical models, such as the buildup of tau protein tangles, amyloid-beta plaques, neuroinflammation, and synaptic loss.(2) Despite a great deal of study, effective dementia disease-modifying treatments are still hard to come by. The majority of approved medications, including galantamine, donepezil, rivastigmine, and memantine, simply relieve symptoms; they do not stop or reverse the course of the disease. In this regard, preclinical research is essential to the identification and creation of new therapeutic agents and diagnostic instruments. Preclinical research serves as a link between fundamental scientific findings and clinical implementation.(3) In order to explore the intricate pathophysiological mechanisms that underlie dementia, such as amyloid-beta

(Aβ) plaque deposition, tau hyperphosphorylation, neuroinflammation, oxidative stress, synaptic dysfunction, and neuronal loss, these studies mainly rely on in vitro systems and in vivo animal models. Before starting human trials, these models also make it easier to assess the pharmacokinetics, safety, toxicity, and effectiveness of potential compounds.(4)(Figure 1)

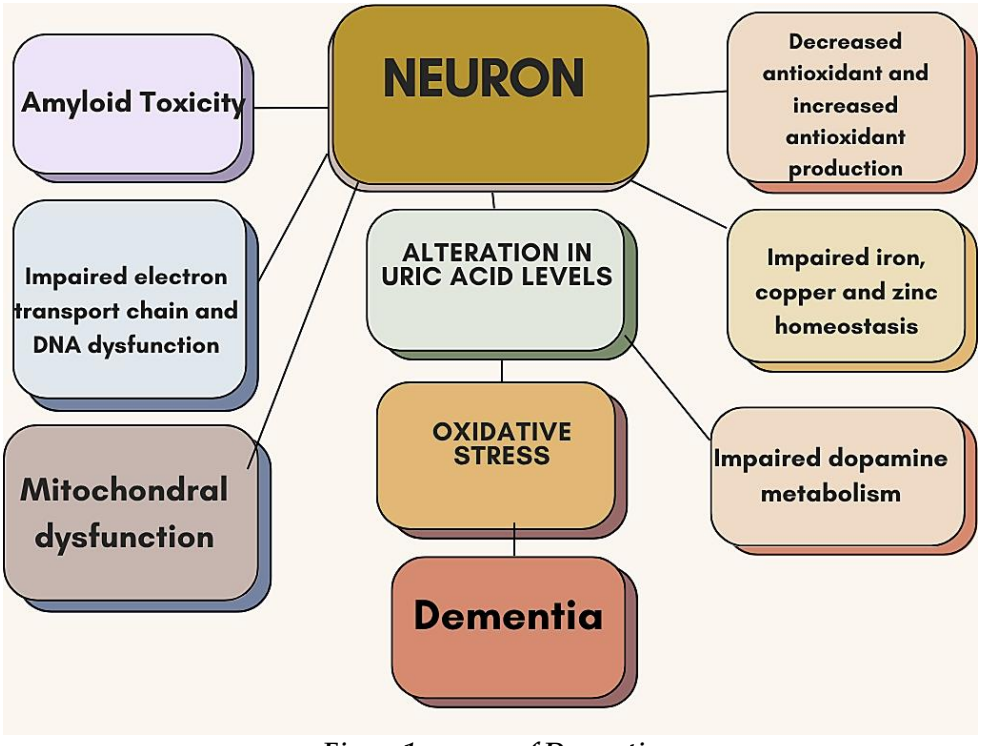

Figure 1: causes of Dementia

Mechanism of Dementia

At the cellular and neural network levels, a number of pathways have been proposed; nevertheless, the precise molecular mechanisms causing dementia and other neurodegenerative illnesses remain unclear. It is well recognised that protein deposits or misfolding's are frequently linked to neurodegenerative illnesses. These conditions ultimately result in chemical alterations and gradual neuronal death in the brain and spinal cord, which impairs function.(1,5) The amyloid-β, tau, α-synuclein, and prion proteins are the most often found protein types linked to the development of these aggregates. The **figure 2** shows all the mechanism. Memory loss, altered behaviour, and judgemental difficulties are the hallmarks of Alzheimer's disease (AD), which is the cause of nearly all dementia cases.(5,6) The two most well-known neuropathological indicators of AD are extracellular neuroneuritic plaques and intercellular neurofibrillary tangles (NFTs), which are comprised of aggregated β amyloid (Aβ) and hyperphosphorylated tau protein. Furthermore, there is growing evidence that the pattern of tau deposition in AD and other tau-related diseases progresses stereotypically, in a "prion-like" fashion, between anatomically connected brain regions, and that the seeding and spread of pathological tau is what causes progressive neurodegeneration. (7)

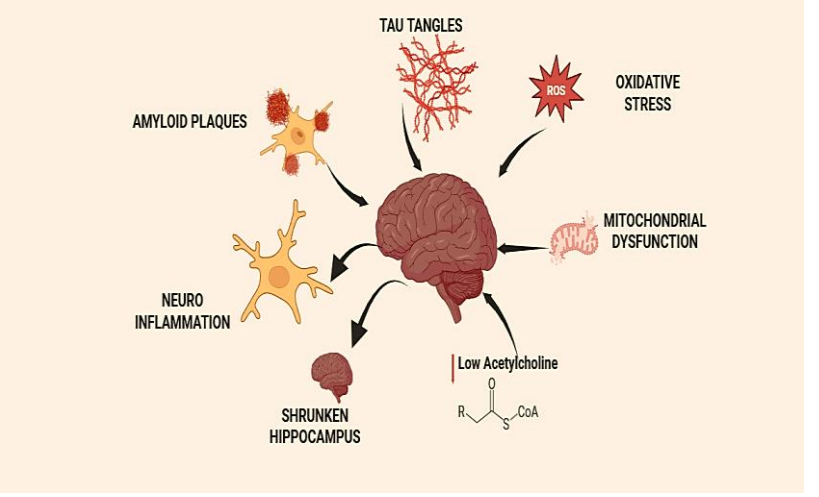

Figure 2:Mechanism of dementia

In Vivo model of Dementia

Research on dementia relies heavily on animal models since they provide a comprehensive grasp of the disease's intricate aetiology. By simulating several facets of human dementia, these models allow scientists to track and examine alterations at the molecular, cellular, and systemic levels. Crucially, they make it possible to evaluate behavioural symptoms that resemble clinical presentations in human patients, such as anxiety, memory loss, and altered locomotion. A variety of behavioural tests designed for rodents or other species can be used to assess cognitive abilities like learning, attention, and executive function. Additionally, animal models offer important insights into neuropathological characteristics such as neuroinflammation, tau hyperphosphorylation, amyloid plaque formation, neuronal loss, and synaptic dysfunction. In order to test the safety and effectiveness of novel therapeutic medicines in a living biological setting and to research the course of diseases, such thorough examination is essential.(8)

Animal models of Dementia:(9)

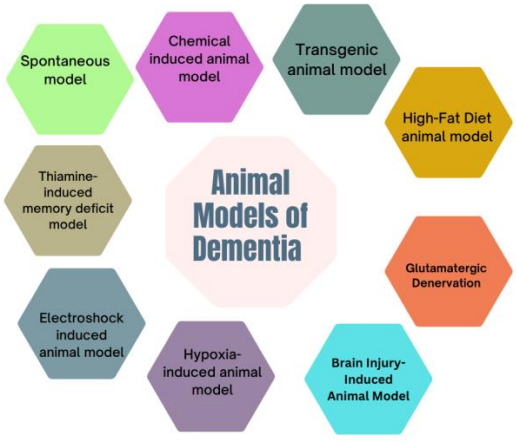

Figure 3: Different animal model for Dementia

Intracerebroventricular (ICV) Aβ Injection Model (Rodent model)

Amyloid-β (Aβ) is a key pathological mediator of Alzheimer's disease (AD), which can be sporadic or familial. Aβ oligomers and plaques are seen to gradually accumulate in the brains of AD patients. It is thought that these Aβ anomalies cause cognitive impairments, disrupt synaptic function, and prevent long-term potentiation.(10) It is well established injection of amyloid-β (Aβ) peptides intracerebroventricular (ICV) in rodent as a widely used approach to trigger Alzheimer like pathology in vivo. The first step is the production of Aβ peptides, which can be chemically synthesized or obtained commercially. Aβ is solubilized and disaggregated using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) by sonication for 5 min at RT, followed by vortexed and incubated for 30 min and maintained at homogeneity. HFIP is fully evaporated with nitrogen gas, and the peptide film is frozen at −80°C.Aβ monomers for injection are prepared by dissolving Aβ in DMSO to a concentration of 1 mM and 10×dilution to get a 100 μM working solution in PBS (10% DMSO, 90% PBS). For oligomer formation, the monomer solution is incubated at 37°C for 3–7 days (depending on the Aβ variant) in a sealed tube inside a humidity-controlled bag to avoid liquid evaporation. The formation of oligomers can be verified by SDS-PAGE and photo-induced crosslinking methods.(11) For the injection, a 26 G stainless-steel micro syringe is used. The syringe and all surgical instruments are autoclave sterilized followed by exposure to ultraviolet light for 20 min. Before final UV sterilization, the fume hood is dried for 30 minutes, rinsing with distilled water, and running washes with 70% ethanol twice, sonicating for 30 minutes dissolves the inner spaces of the syringe. The surgical area and all the instruments are disinfected using 70% ethanol and UV light. Mice are anesthetized via intraperitoneal injection of a standard cocktail of xylazine 20 mg/kg and zolazepam-tiletamine 80 mg/kg and are placed on a warming mat. Anaesthesia adequacy is assessed via foot-pinch reflex along with the application of sterile PBS eye drops to prevent corneal desiccation. The forehead of the mouse is disinfected with 70% ethanol and 2% chlorhexidine solution alternately for a total of three cycles.(12)The bregma can be found by making a triangular shape with the two eyes and the intersection of the thumb and index finger clasping the forehead. Injection coordinates from the bregma are usually at posterior 1.0 mm, 1.8 mm lateral, and 2.4 mm deep for general mice (or B6 mice at −0.9 mm posterior, 1.7 mm lateral, and 2.2 mm deep). To aid in perpendicular alignment of the syringe and mouse, mirrors with drawn vertical lines can be placed parallel and perpendicular to the mouse's head and body.(13)

The syringe is filled with 10 μL of Aβ solution (or vehicle control in PBS and 10%DMSO), taking care not to introduce air bubbles. The needle is inserted perpendicular to the skull surface and pushed in so the Parafilm is at the skin. A volume of 5 μL is injected slowly over 5 seconds, then the needle remaining in situ for a further 3 to 5 seconds before withdrawal without tilting. The animal is replaced on the warming mat for recovery and observed until conscious. Mice are then individually housed from non-surgical mice and closely observed for signs of infection, distress, or abnormality for the next 5 to 7 days. The syringe is cleaned after surgery using the same ethanol and sonication process. Cognitive impairment induced by Aβ injection can be quantified 3 days post-surgery by behavioral testing in the Y-maze. The Y-maze has three identical 120° arms. Each mouse is placed in one arm and allowed free exploration during 8 minutes while alternations and arm entries are counted to assess spatial working memory.(14)Postmortem histological examination of brain tissue is employed to confirm successful targeting of the ICV site. Mice are anesthetized and killed, and their brains are extracted. The injection site is identified by the fact that a trace of the needle reaches or passes through the lateral ventricles; animals with misplaced injections

are discarded. This assay provides a low-cost, fast model for the investigation of acute Aβ effects (shown in figure 4) on cognition and neuropathology in rodents.(15,16)

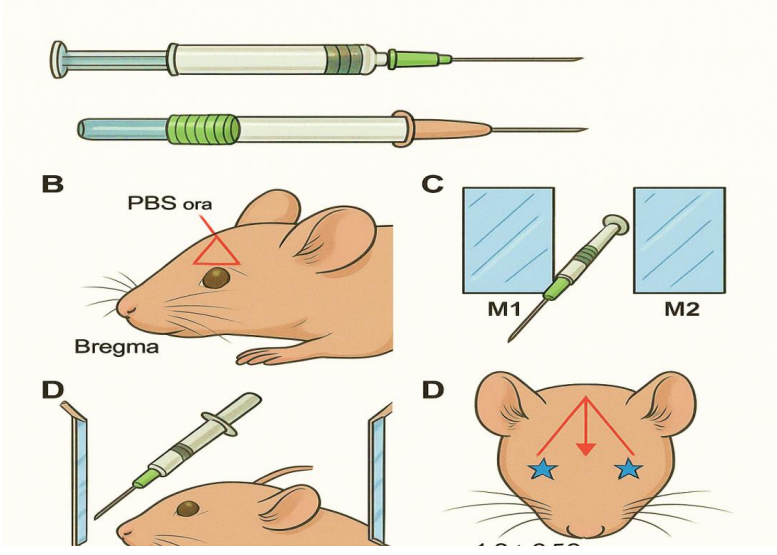

Figure 4:procedure of Aβ induction on rodents

Amyloid-β Microinjection Procedure in Zebrafish

Researchers widely use zebrafish (Danio rerio) as a vertebrate model for studying neurodegenerative disorders, including Alzheimer's disease, due to their genetic homology, transparent embryos, and ease of genetic manipulation. In zebrafish models, amyloid-β (Aβ) peptides are typically introduced via intracerebral microinjection into the telencephalon of adult fish or the brain ventricle of larvae. This procedure induces Alzheimer-like pathologies including cognitive deficits, neuroinflammation, and oxidative stress.(17)To begin, Aβ peptides—commonly Aβ1–42—are pretreated to avoid aggregation. The preparation involves solubilizing lyophilized Aβ in hexafluoroisopropanol (HFIP) to disaggregate existing fibrils, followed by evaporation of HFIP and resolubilization in dimethyl sulfoxide (DMSO). The peptide is then diluted in phosphate-buffered saline (PBS) to obtain monomers or oligomers. For oligomer formation, the peptide solution is incubated at 4°C for 24–72 hours depending on the desired species. Confirmation of oligomerization is performed using SDS-PAGE and Western blotting adult zebrafish are anesthetized with buffered tricaine methane sulfonate (MS-222; 0.02%) before injection. The fish are then placed laterally on a moist sponge under a stereomicroscope. Using a fine glass micropipette (tip diameter ~10–20 µm) connected to a microinjector, researchers inject 1–2 µL of the prepared Aβ solution into the dorsal telencephalon. Care is taken to avoid damaging major vasculature or penetrating too deep into the brain tissue. After injection, the fish are immediately transferred to fresh water and monitored until full recovery. The survival rate is high when aseptic conditions and correct stereotactic landmarks are maintained.(18)

In larvae (typically 2–4 days post-fertilization), microinjections are performed into the hindbrain ventricle under a high-resolution microscope. The larvae are immobilized in low-melting-point agarose and injected using a pulled capillary needle. Only nanolitre volumes are used to prevent intracranial pressure damage. Post-injection, larvae are washed and incubated in embryo media for behavioural and molecular analysis.(19)

Streptozotocin (STZ)-Induced Alzheimer's Disease Model in Animals

The streptozotocin (STZ)-induced model is a well-established and widely accepted method to simulate insulin-resistant brain states that mirror the pathophysiology of sporadic Alzheimer's disease (AD), particularly in non-transgenic animals. Researchers initiate the model by administering STZ directly into the lateral ventricles of rodents via intracerebroventricular (ICV) injection. Typically, a low dose of STZ (3 mg/kg) is injected bilaterally on two consecutive days to impair insulin signalling in the brain without affecting peripheral glucose levels.(20)Before injection, animals are anesthetized with an appropriate agent such as ketamine-xylazine and placed in a stereotaxic frame to ensure accurate delivery of STZ into the ventricular space. A micro syringe fitted with a fine needle is used to inject the STZ solution, freshly prepared in citrate buffer (pH 4.5), in a volume of 10 µL per side. Researchers avoid systemic hyperglycaemia by maintaining the dose and ensuring CNS-specific delivery. Following the injection, animals are returned to their cages and monitored for behaviour and recovery.(21) Over the subsequent days to weeks, the animals develop key features of sporadic AD, including impaired learning and memory, oxidative stress, cholinergic dysfunction, neuroinflammation, mitochondrial deficits, and tau phosphorylation. Researchers frequently assess cognitive function using behavioural paradigms like the Morris water maze, novel object recognition, or passive avoidance tests. Postmortem analysis of brain tissue often confirms neuronal damage in the hippocampus and cortex, mimicking the neurodegeneration observed in human AD. (22)
This STZ-based model is particularly valuable for evaluating insulin signalling pathways in the brain and for testing potential anti-diabetic and neuroprotective agents. Its ability to induce Alzheimer-like pathology in a non-genetic, cost-effective, and reproducible manner makes it a robust tool for preclinical research.(23)

Transgenic Animal Models of Alzheimer's Disease

Transgenic mouse models have revolutionized Alzheimer's disease (AD) research by enabling the investigation of specific gene mutations associated with familial forms of AD and their corresponding neuropathological features. These models replicate several hallmarks of AD, including amyloid plaque accumulation, tau hyperphosphorylation, synaptic loss, and progressive cognitive decline, thereby serving as indispensable tools in preclinical drug development and mechanistic studies.(24)One of the most extensively utilized models is the APP/PS1 transgenic mouse, which co-expresses human amyloid precursor protein (APP) with the Swedish mutation and presenilin-1 (PSEN1) with a deltaE9 mutation. These mice begin to exhibit amyloid-β (Aβ) plaque deposition as early as 4–6 months of age, accompanied by neuroinflammatory responses, gliosis, and spatial memory deficits detectable in tasks such as the Morris water maze.[19] The robust amyloid pathology and early onset of symptoms make APP/PS1 mice a valuable model for studying the role of Aβ accumulation in AD pathogenesis.(25)

The triple-transgenic mouse model (3xTg-AD), which harbours mutations in APP (Swedish), PS1 (M146V), and tau (P301L), provides a more comprehensive representation of AD pathology. These mice develop both extracellular Aβ plaques and intracellular neurofibrillary tangles, mimicking the dual-pathology found in human AD. Pathological features emerge in a spatiotemporal manner, with Aβ pathology appearing in the cortex and hippocampus followed by tau pathology, particularly in the hippocampal CA1 region and amygdala. This model is uniquely suited for exploring the interplay between amyloid and

tau pathologies and for evaluating multi-target therapeutic approaches.(26)Additional models such as the 5xFAD mouse, which expresses five familial AD mutations, are gaining popularity for their extremely rapid development of amyloid pathology and severe cognitive impairment by 4 months of age. These models exhibit significant neuronal loss and gliosis and are useful for high-throughput screening of anti-amyloid therapies.(27)Transgenic models remain a cornerstone of AD research due to their ability to replicate many facets of the disease. However, limitations such as overexpression artifacts and lack of full recapitulation of sporadic AD highlight the importance of integrating these models with others like toxin-induced or metabolic models for a holistic understanding. Transgenic mouse models have played a pivotal role in advancing our understanding of Alzheimer's disease by allowing the study of genetic mutations associated with familial AD and their impact on neuropathology.(28)The APP/PS1 model is one of the most frequently used. These mice co-express mutated forms of the human amyloid precursor protein (APP) and presenilin-1 (PS1), both of which are linked to early-onset familial AD. As a result, APP/PS1 mice begin to develop extracellular amyloid-β (Aβ) plaques as early as 6 months of age. These plaques progressively accumulate in the cortex and hippocampus, leading to synaptic loss, memory impairment, and neuroinflammation.(29)

Another widely used model is the triple-transgenic mouse model (3xTg-AD), which harbours three mutations: APP (Swedish), PS1 (M146V), and tau (P301L). These mice recapitulate both amyloid plaque deposition and neurofibrillary tangle formation, providing a more comprehensive representation of AD pathology. The model demonstrates progressive cognitive decline along with intracellular Aβ and hyperphosphorylated tau pathology, particularly in the hippocampus and amygdala. The 3xTg-AD model is especially valuable for studying interactions between Aβ and tau pathologies and for testing multi-target therapeutic approaches.(30)

Glutamatergic denervation models

Recent studies have highlighted the pivotal role of glutamatergic dysfunction in the pathogenesis of dementia, particularly Alzheimer's disease (AD). Research by Bi et al. (2025) demonstrated that AD is associated with glucose hypometabolism in temporoparietal and frontal regions, correlating with alterations in serotonergic, GABAergic, dopaminergic, and glutamatergic systems.(31) These findings underscore the complex interplay between metabolic disturbances and neurotransmitter system vulnerabilities in AD. Furthermore, cortical denervation, characterized by the loss of cholinergic and GABAergic inputs, disrupts the excitatory/inhibitory (E/I) balance of glutamatergic pyramidal cells, leading to neuronal hyperexcitability and cognitive impairments. This imbalance is exacerbated by amyloid-beta (Aβ) accumulation, which impairs GABAergic interneuron function and further promotes excitotoxicity. Collectively, these studies emphasize the critical role of glutamatergic dysfunction in dementia and suggest that targeting glutamate signaling pathways may offer therapeutic potential for mitigating cognitive decline in AD. (32)

Brain injury-induced animal models

Brain injury-induced animal models, particularly those involving traumatic brain injury (TBI), ischemic stroke, and focal brain lesions, are essential tools for studying the mechanisms of dementia, especially Alzheimer's disease (AD), and other cognitive disorders.(33) These models, which typically induce damage to key brain regions involved in memory and

cognitive function, such as the hippocampus, temporal cortex, and prefrontal cortex, replicate the neurodegenerative processes observed in dementia patients. Traumatic brain injury (TBI), for example, is often induced through controlled cortical impact (CCI) or weight drop methods, which lead to neuroinflammation, neuronal loss, and cognitive deficits, mimicking the pathophysiology of AD. Ischemic stroke models, particularly the middle cerebral artery occlusion (MCAO) model, induce brain ischemia that results in neuronal death and cognitive impairments, similar to those seen in human stroke patients and AD.(34) Focal brain lesions, such as hippocampal or prefrontal cortex lesions, provide insights into how localized damage in these regions can lead to memory deficits, executive dysfunction, and behavioural changes typical of dementia. Recent research has shown that these models are useful for understanding the role of neuroinflammation, excitotoxicity, and neuronal repair in dementia, as well as for testing potential therapies aimed at reducing neurodegeneration and improving cognitive function. The use of these brain injury models has provided valuable insights into the mechanisms of cognitive decline and the therapeutic potential of neuroprotective strategies targeting neuroinflammation, synaptic plasticity, and neuronal regeneration.(35)

Hypoxia induced animal model

Hypoxia-induced animal models have become increasingly valuable in studying the pathophysiology of dementia and other neurodegenerative diseases, as they replicate the effects of reduced oxygen supply to brain tissues, a condition that occurs in various forms of brain injury and neurodegeneration. These models typically involve the exposure of animals, particularly rodents, to controlled levels of hypoxia, leading to neuronal damage, neuroinflammation, and cognitive deficits that are commonly observed in Alzheimer's disease (AD), stroke, and other dementias. (36)Hypoxia is known to induce metabolic disturbances, oxidative stress, and alterations in neurotransmitter signaling, particularly in glutamatergic and GABAergic systems, which are critical in memory and cognitive function. For instance, exposure to chronic hypoxia can impair hippocampal function, resulting in memory deficits and neurodegeneration similar to those seen in dementia. Recent studies have shown that hypoxia-induced damage in the hippocampus and cortex leads to reductions in synaptic plasticity and neurogenesis, contributing to cognitive decline. Additionally, these models are used to investigate the role of hypoxia-inducible factors (HIFs), which regulate cellular responses to low oxygen conditions, and their potential therapeutic role in neuroprotection. (37)The use of hypoxia models in dementia research has provided crucial insights into how reduced oxygen levels can exacerbate neurodegenerative processes and has led to the development of potential therapeutic strategies aimed at restoring oxygen homeostasis or reducing hypoxia-induced damage. These models are also instrumental in testing neuroprotective agents and exploring the therapeutic potential of targeting hypoxia-related pathways in dementia.(38)

Electroshock Induced animal model

These models are generally employed by using electroconvulsive shock (ECS) or electroshock (ES) in order to produce a condition of brain dysfunction or damage that simulates the pathological symptoms giving rise to dementia. A widely used technique for this is the electroshock-induced seizures, hippocampus damage and behavioural alterations to help to interpret pathogenesis of dementia, especially Alzheimer's disease (AD) as well as other cognitive impairment. In such models, electroshock is delivered to the brain (in a generalized

or focal manner), and animals are then assessed for cognitive impairments which may include amnestic features, learning disturbances and/or impaired motor behaviour. This model reproduces the brain injury and neuron loss seen in a dementia sufferer.(39)Memory and learning are often the focus of these studies, with researchers looking at the hippocampus in particular as it is particularly vulnerable to damage in dementia. Some electroshock-derived animal models are used to investigate the pathogenesis of neurodegeneration, particularly oxidative stress, inflammation or amyloid-β deposition. Furthermore, these models may be particularly helpful in screening the effectiveness of putative therapeutic drugs of dementia, given that treatments or pharmacological agents can be administrated subsequent to the electric shock for the evaluation of protective effects. Studies have demonstrated that electroshock models lead to marked phenomena of brain response, such as the enhancement of neuroinflammation markers and neuronal apoptosis. Moreover, electroshock-induced models have demonstrated the relevance of the glutamatergic system because ECS suppresses activation of glutamate receptors, a mechanism which is essential to synaptic plasticity and cognitive function.(40) Electroshock-induced models are another means by which to decipher the involvement of neurogenesis in dementia. Inhibition of hippocampal neurogenesis, related to cognitive function and memory consolidation that are impaired in dementia, has been demonstrated with ECS, although models induced electroshock have a utility, there are some drawbacks. The induced stress by electroshock may cause variability in the generated results and the degree of brain damage may vary according to the intensity and duration for which the electric shock is administered. Furthermore, electroshock-induced models can produce some dementia-like features, but do not generally parallel the chronicity of neurodegenerative diseases such as Alzheimer's. Vitamin B1 (thiamine) is a very important factor in brain metabolism, mainly as a cofactor for the generation of energy through pentose phosphate pathway and to keep neuronal function.(41)

Thiamine Induced Dementia Model

Thiamine leads to impaired cellular metabolism and thus neuronal dysfunction, oxidative stress and inflammation which ultimately translate into cognitive impairment, particularly in the domain of memory functions and learning. In thiamine-induced memory deficit models, animals (most often rodents) become thiamine deficient as a result of dietary manipulation or treatment with a thiamine antagonist, such as pyrithiamine (that inhibits the uptake of thiamine in the brain). These models have been widely used to investigate memory loss and cognitive decline, which are typical symptoms of thiamine deficiency in patients or subjects suffering from alcohol-induced dementia. Model systems may be useful to investigate neurobiological mechanisms of cognitive dysfunction and the evaluation of candidate therapeutic agents for the treatment of thiamine-deficiency-associated memory impairment.(42)The hippocampus, the region that is essential for regulation of learning and memory at fundamental level, and the thalamus are the main brain regions affected by TD since they contain structural and functional disturbances secondarily to decreased amounts of thiamine. In such models, animals demonstrate classic showed symptoms of memory deficits, behavioural anomalies and some motor dysfunction which are analogous to the cognitive and motor deficits seen in dementia. Memory assessment in these animals is commonly evaluated through behavioural tests such as Morris water maze, passive avoidance and Y-maze that address the spatial memory, learning activity and exploration. It has been demonstrated in thiamine-induced models that oxidative stress is involved in the pathogenesis of cognitive deficits due to thiamine deficiency. Enhancement of the neurotoxicity is explained by increased ROS (reactive oxygen species) generation with antioxidant defence diminish in the brain which results in the damage and degeneration of

neurons. (43)Moreover, the reduction of neurotransmitter systems has been attributed to thiamine deficiency, including systems such as acetylcholine that is important in memory and cognitive functions. Thiamine induced models also demonstrate neuroinflammatory pathways that contribute to the neuronal injury and cognitive deterioration noted in these animals. These models are invaluable for elucidating dementia pathogenesis and as experimental tools in future drugs discovery program. For instance, thiamine supplement was capable of improving cognitive functions and ameliorating the oxidative stress and neuroinflammation injury in TD animals. Furthermore, since antioxidant agents, cholinergic drugs, and anti-inflammatory therapies have been employed in these models, it has also offered potential opportunities for recovery of memory/cognitive functions. But this model regards some restrictions, since it does not mimic the chronic and progressive form of common dementias such as Alzheimer's disease.(44)

Behavioural Assay in Zebrafish for Cognitive Assessment

Zebrafish The zebrafish has an elaborated nervous system that allows for more sophisticated behaviours, and that can be sensitive to seizures. Adult zebrafish can display a full spectrum of mature behaviour that is therefore particularly suitable for model development. (45)Over the past recent years, zebrafish have been gaining growth as an alternative animal model for the study of molecular mechanisms involved in cognitive deficits and screening for potential lignostilbenesEND bioactive compounds.(46)There is abundant scientific literature demonstrating the advantages of animals like zebrafish as alternative and superior animal model for drug discovery (Figure 4). Genomic organization of zebrafish resembles human. About 70% of genes are shared with humans, and around 84% of the genes associated with human disease have a zebrafish counterpart.(47) In life sciences, modeling can also refer to experimental methods and using nonhuman organisms as models. For example, a neuroscientist might investigate working memory by testing rats in a radial-arm maze or anxiety by testing mice using an open-field test. (48)The quality of a model is, most times, directly related to its fidelity for theoretical models, it can be measured by the resolution on generated results and, in case of biological models (who we will use as examples from now on), its validity. In this chapter we concentrate on biological models in neuroscience, for which the problem is especially hard, as both the behavior and brain processes are highly complex. The idea is to find species that do the things we want them to do and whose neural mechanisms are tractable.(49)

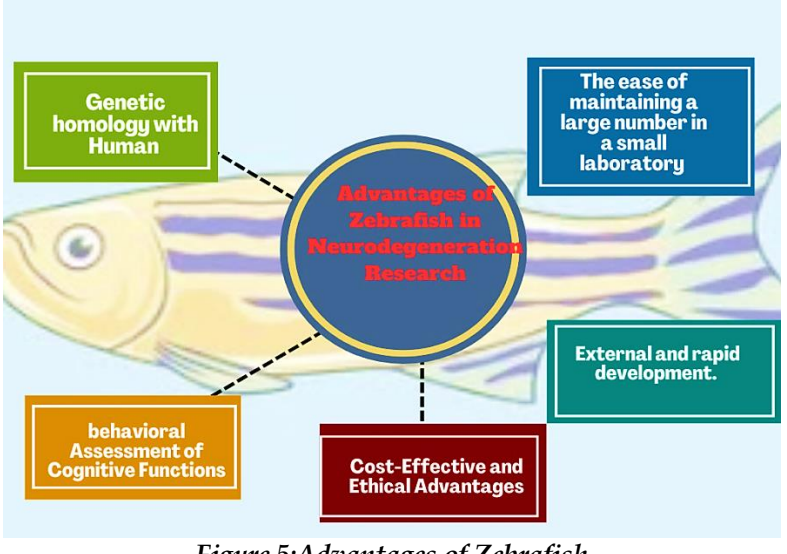

Figure 5:Advantages of Zebrafish

Novel tank assay analysis

The Novel Tank Diving (NTD) test is widely used to model anxiety in zebrafish, characterized by the behavioural observation of a single fish placed in a novel tank. Normally an adult zebrafish will remain on the bottom in a new tank, showing stress. Eventually, the fish begins to investigate the aquarium, and degree of exploration is a measure of anxiety.(50) The Novel Tank Diving test (NTD) is a common behavioural assay utilized to investigate piscine anxiety-like and exploratory behaviour of zebrafish, De Vos et al. It offers an easy and reliable method for measuring anxiety level of fish according to their natural respondence to novelty. A zebrafish was subjected to the following test in which it is placed into a novel, plastic tank, with clear walls (29 cm long×24 cm wide×19.5 cm high) that has both shallow and deep areas. Being instinctively afraid of open space, the fish will tend to hug the bottom or sides when newly introduced which is a high sign of stress. This negative reaction in the bottom part of the tank, which is known as thigmotaxis, is believed to be a natural stress coping mechanism caused by environmental novelty. The zebrafish start to swim in the bottom area of tank as the assay progresses, indicating a transition from anxiety-like behaviour to more exploratory behaviour. The magnitude of this shift is proportional to degree of anxiety and was considered as the time spent at the bottom of tank showing high anxiety, while percentage range in top zone observed low anxiety. (51)This straightforward test has been commonly employed to evaluate the effects of several pharmacological interventions such as anxiolytic and anxiogenic agents. For example, drugs such as fluoxetine, nicotine and caffeine were reported to impact behaviour of zebrafish in the NTD paradigm (either decreasing anxiety-related or increasing exploratory behaviour; The NTD test has not only heuristic applicability to increase the knowledge of anxiety but also its importance in preclinical studies shown in figure 4, especially regarding drug discovery and validation of therapeutic agents for neuropsychiatric disorders.(52)

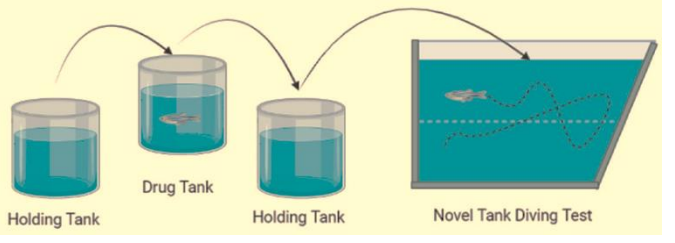

Figure 6:Novel diving test

Y- Maze and T-Maze

The Y-and T-Mazes are frequently applied behavioral paradigms for evaluating spatial learning and memory as well as decision making in zebrafish (Danio rerio). The mazes were developed to test how well zebrafish can use environmental cues to get around and make decisions, providing researchers with important information about their cognitive capabilities. In the Y-Maze, the fish were located in and given time to explore a "Y" shaped maze. Following an exploratory phase, fish are given the choice between two arms - one is one that they have already explored (familiar) and one is a new arm for them to explore (novel). The preference for the novelty arm is suggested to reflect spatial memory of the fish, and a strong preference for the novel arm indicates that the memory is intact and exploratory behavior toward.(53)This test is especially valuable for investigating the fish's capacity to

remember and distinguish between familiar and unfamiliar areas, an essential component of spatial learning and memory. Also a simpler version of this apparatus is the T-Maze where the fish has two options: one leading to a positive reinforcement (like food) and another to an un-rewarded or neutral zone. This test is sensitive for decision-making where the fish can correctly discriminate the rewarded path is reflective of functional spatial memory and cognitive flexibility.(54) The choice behavior, i.e., whether the fish prefers the novel or correct arm, and latency to decide (indicating how long it takes before a decision is made by the animal) are two key measurements extracted from both the Y-Maze and T-Maze. Shorter latency is often, but not always, a sign of better memory or problem-solving skills.These mazes are valuable tools to neurobehavioral researchers, as they can be used to quantify the impact of pharmacological interventions, genetic modifications or disease models like Alzheimer's Disease that have been shown to produce deficits in spatial memory and decision-making.(55)Both Y-maze and T-maze tests are of considerable value in the field of behavioral neuroscience to assess spatial memory, cognitive flexibility, and decision-making skills. All of these capabilities are frequently compromised in neurodegenerative diseases, so these mazes function as useful tools to investigate potential effects of medications or genetic manipulations affecting brain functions and learning capacity on Cog health in preclinical animal models.(56)

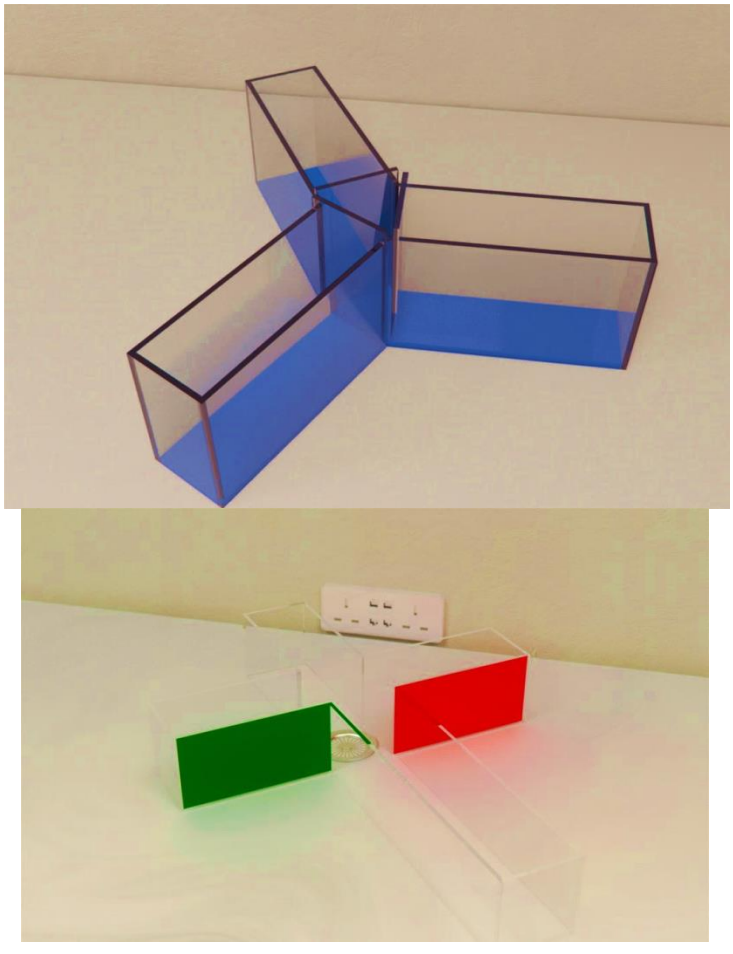

Figure 7: Y maze and T Maze

Conditioned Avoidance Responses in the Zebrafish

Conditioned avoidance responses (CAR) in zebrafish are a useful model to investigate mechanisms of associative learning and memory. In such paradigms, zebrafish are conditioned to connect a neutral stimulus in their environment, like a visual cue or environmental contextrelated to an aversive outcome that is generally accompanied with an electric shock.(57) Upon presentation of the conditioned stimulus (CS) alone, fish show avoidance responses like reduced exploratory activity or increased freezing, which reflects that a memory trace has established associating the CS with the aversive unconditioned stimulus (US). More recently it has been shown that zebrafish can also generate strong aversive memories from a single training session. For example, Moreira et al. (2025) reported that zebrafish could remember a CR 2–48 h after 10 trials were applied, although they did not report maintenance of memory at later points.(58) This long-term memory retention further highlights the value of zebrafish as a model in long-term memory studies. Brain circuits involved in CAR have been the focus of research in zebrafish. Recent progress in imaging and molecular methods has allowed us to identify some of the brain regions that are necessary for associative learning. For instance, research has linked the dorsolateral habenula to the regulation of learned avoidance behavior, demonstrating that it plays a key role in aversive processing as well as decision-making. Moreover, role of neurotransmitter systems like serotonin and dopamine in modulation of CAR has been demonstrated, further enlightening the biochemical pathways through which memory gets consolidated.(59)Also, variation between zebrafish in learning and memory performance has been found related to personality traits. A study by Corcoran et al. (2025) found that bold zebrafish learned more quickly than shy fish in a classical associative learning task, indicating that individual differences in personality may influence learning abilities. (60)This range highlights the need for individual differences to be included in behavioral assays and their potential impact on the study of cognitive processes. In conclusion, the conditioned avoidance response in zebrafish represents a potent model to study molecular and neural mechanisms of associative learning and memory. The capacity to associate and remember aversive events, and the discovery of underlying neural circuits associated with these processes as well as an understanding of their modulation by individual variation make zebrafish a potentially influential model organism for cognitive neuroscience research.(61)

The Novel Object Recognition (NOR)

The Novel Object Recognition (NOR) test is a commonly used paradigm to measure recognition memory in different species, zebrafish (Danio rerio) included. This assay is based on the natural tendency of zebrafish to explore and as a consequence they tend to prefer an unfamiliar stimulus rather than a familiar one. In the classical NOR task fish were habituated to an arena and then subjected to a familiarization phase, in which they are presented with two identical objects. (62)One of the two familiar objects is replaced by a novel object following a retention interval, and then the exploration time of fish is measured. A longer exploration time to the novel object compared with that of the familiar one reflects recognition memory in order findings.(63)Ye olde goldfish According to recent studies, the zebrafish has a short-term and long-term recognition memory. For example, studies have demonstrated that zebrafish are able to remember a novel object up to 24 h after familiarization, and for this type of memory it has been found that protein synthesis is important in the consolidation processes underlying long-term memory. In addition, the NOR test has been implemented in zebrafish larvae to study developing memory

formation.(64) The zebrafish NOR test is a useful tool in toxicological and pharmacological studies. It has been used in the study of the effects of diverse pharmacological agents on memory function, such as measuring scopolamine-induced deficits or testing potential memory-enhancing compounds. Additionally, the task has been used to investigate the effect of environmental manipulations [e.g. enrichment]) on recognition memory. (53)

Figure 8: Novel Object Recognition

Cognitive bias testing in zebrafish

Cognitive bias testing in zebrafish (Danio rerio) has become an increasingly popular tool to study the emotional states of these animals by measuring their responses to ambiguous stimuli. This approach is based on the idea that one's affective state may impact judgment and decision making. Cognitive biases tests in zebrafish usually train subjects with cues associated either to positive or negative outcomes, and later present ambiguous cues to test their responses.(65)comparable findings of robust individual differences in emotional-type states have been observed in zebrafish investigating the behavioral responses to ambiguous stimuli. For example, it has been found that from subjective judgement of ambiguous cues zebrafish can be divided into "optimists" and "pessimists"; the optimists making a positive/strong/ interpretation of these cues and the pessimists viewing them more negatively. Such biases are not only stable through time, but can be modulated by environmental conditions, personality traits and genetic predispositions. For instance, zebrafish with shorter telomeres— an indicator of cellular aging — tend to have more negative biases, which may indicate a connection between physical health and emotional outlook.(66)Cognitive bias tests have also been used to evaluate the well-being of zebrafish in laboratory conditions. Zebrafish maintained in an enriched environment exhibit greater bias towards optimistic judgment, which is associated with better welfare, whereas those maintained in a barren environment have shown more pessimistic judgment. This indicates that cognitive bias testing might be used as an approach to assess and improve welfare for

laboratory zebrafish. Caveats and future directions Although screening tests for cognitive bias in zebrafish have promising applications, there are limitations and obstacles. Methodological differences, including different experimental protocols and environmental conditions may cause this contradiction. Furthermore, disambiguating the responses to ambiguous stimuli is not straightforward since they may be modulated by factors that are unrelated to an emotional state, such as motivation or past experience. Accordingly, it is important to consider confounding variables and to pre-establish testing procedure standards for the assessment of cognitive bias in zebrafish.(67)

The Light/Dark Preference Test

It is a widely accepted behavioral test for zebrafish (Danio rerio) used to measure anxiety-like behavior. It's a setup in which zebrafish are put into a two-sided arena, one light and the other dark. The test exploits the natural photophobic behavior of fish, since zebrafish show a preference for the dark area, and an increase in preference for the light zone is interpreted as diminished anxiety. (68)This test has been validated pharmacologically in the sense that anxiolytics, such as clonazepam and buspirone, decrease the time spent in dark booth and anxiogenics like imipramine increase preference for the dark compartment representing high anxiety.(69)Nevertheless, the sensitivity of the test may fluctuate as several studies have reported its susceptibility to be inferior when compared to other assays such as the Novel Tank Dive Test. Notwithstanding these limitations, the L/DPT is considered a reliable method to assess anxiety-like behaviour in adult zebrafish, and it is widely utilized for pharmacological and toxicological studies.(70)

Conclusion

Preclinical dementia models the development of dementia is a complex process. These models, including genetic, pharmacological and environmental paradigms have contributed to our current understanding of the pathophysiology underlying dementia such as Alzheimer's disease (AD), vascular dementia and other cognitive disorders. However, none of the preclinical models is seamlessly complementary to all haplotypes, suggesting that a multimodal approach will be needed to fully model the complexities of dementia. Indeed, the use of animal models including transgenic mice, chemically induced models and neurodegenerative injury models has been crucial to test potential therapeutic interventions as well as deciphering molecular pathways of dementia. However, model validation, species variation, and the repetition of findings from animal models to human patients are still major obstacles.

Discussions

Diseases, though one needs to carefully consider their weaknesses when interpreting results. For example, transgenic models can provide valuable information on genetic components of dementia, but often do not recapitulate the entire landscape of human disease, including intricate neuroinflammatory and environmental influences. Likewise, chemical-induced ones that use manipulation of amyloid-β or tau protein can model some aspects of dementia but not necessarily the chronic, progressive condition observed in humans. One particularly relevant aspect is the exploitation of polypharmacology models, where a spectrum of underlying mechanisms (i.e., oxidative stress, inflammation and protein aggregation) is co-analysed. These models can offer insight as to how different processes interact during the development of dementia that may in turn identify multiple therapeutic targets. The latter is

the most crucial item on the agenda, because it is still a long road from practical APAP-dosimetry to animal studies although cost-benefit of new model animals regarding such diagnostic tools has been proven to be acceptable more than once before. New technologies, such as CRISPR/Cas9 gene editing and improved imaging technology bring new opportunities for more accurate, human-relevant models. Targeting individual genes or pathways in preclinical models may enable us to approach dementia-associated processes with increased precision, which can improve the power of therapeutic interventions. The development of an 'in-between' animal, as non-rodents for example primates or at least another rodent species than the one in which vaccine was tested but also not man, could be helpful to translate findings from rodents to human. In the long run, better validation and translation of the experimental model is the only way forward for dementia research

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Chapter- Eight

Rational Molecular Drug Design: Structural Methodologies And Computational Technique Workflow For Drug Repurposing

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Abstract

Drug repurposing is a technique for identifying new therapeutic actions of already existing drugs of known safety profile. For example, a well-known drug, Aspirin, originally an analgesic, is repurposed to be used as an antiplatelet medication. The emergence of this technique has advanced the therapeutic development and has proven to be cost-effective. The advancement of computational technologies and their integration in rational molecular design have transformed modern drug development by optimising drug-target interactions at the fundamental level. This study deals with an overview of such applied techniques used for drug repurposing. Insilico methodologies like, molecular dynamic simulations, molecular docking, ligand-based approaches, fragment-based drug development, molecular mechanics/quantum mechanics approaches, and deep learning or AI-operated drug-target interaction predictions are discussed in detail. Critical challenges such as binding affinity optimisation, controlled poly-pharmacology, etc are addressed in this study. Altogether, this study highlights the recent advancements, limitations and future of integrative computational approaches in modern drug repurposing.

Keywords: *Drug repurposing; Rational molecular design; Molecular docking; Molecular dynamics simulation; Structure-based virtual screening; Pharmacophore modeling; Fragment-based drug discovery; Artificial intelligence; ADMET prediction; Polypharmacology.*

Introduction

Drug discovery is a complex, time-consuming, and resource-intensive process that is often associated with high attrition rates, particularly during late-stage clinical development. Drug repurposing offers a promising alternative to de novo drug discovery by identifying new therapeutic indications for approved or clinical-stage compounds. Advances in computational chemistry, structural biology, and bioinformatics have enabled rational molecular design approaches that systematically analyse and optimise drug target interactions at the molecular level. Structure-based and ligand-based computational strategies provide critical insights into binding mechanisms, selectivity, and safety, thereby reducing experimental burden and development timelines.

Recent advancements in computational biology have reshaped the drug discovery and development landscape by reducing both time and financial obstacles. Conventional drug discovery is a lengthy and labour-intensive process, often requiring more than a decade for a drug candidate successfully complete clinical trials and reach the market. With increasing global health care demands, such traditional approaches are no longer sustainable on their own. So computational biology has emerged as pivotal; component of modern

pharmaceutical research, enabling the integration of silico techniques throughout multiple stages of drug discovery.

Drug repurposing, which involves identifying new therapeutic indications for existing developed drugs, offers a promising alternative to traditional drug discovery. By leveraging established safety, pharmacokinetic, and formulation data, repurposing strategies substantially reduce development time, cost, and risk compared to de novo drug discovery.
Computational drug repurposing pipelines combine both structure-based and ligand-based strategies to systematically identify promising therapeutic candidates. Structure-based methods rely on experimentally resolved protein structures, homology-modelled targets, which are analysed using molecular docking and structure-based virtual screening to predict binding interactions and rank compounds for larger chem libraries. These predictions are refined again through molecular dynamics (MD) simulations.

Despite advances in structure and ligand-based methods, high failure rates in drug development persist due to unexpected toxicity, off-targets, and limited efficacy. Artificial intelligence (AI) and machine learning (ML) based approaches have therefore arrived as powerful tools for predicting drug target interactions (DTIs) and improving early decision-making in drug repurposing. Although drug repurposing reduces cost and development time, its success is often limited by insufficient target affinity, off-targets and unfavourable pharmacokinetic profiles. These challenges are addressed through rational affinity optimisation, controlled polypharmacology, and in-silico ADMET evaluation to ensure safety and drug-affinities. Recent advances in AI-based drug–target interaction prediction complement docking and virtual screening by enabling scalable, target-agnostic analysis across chemical space. Together, these computational strategies, as mentioned above, provide an integrated framework for rational molecular design, significantly improving the efficiency and reliability of drug repurposing pipelines.
This chapter outlines key computational workflows and structural strategies that reinforce rational drug repurposing and highlight their role in accelerating translational drug discovery.

Methods

Rational molecular design workflows for drug repurposing integrate multiple computational methodologies to identify, optimize, and validate drug candidates. Protein structures are obtained from experimental databases or constructed through homology modelling, followed by structural preparation including protonation, energy minimization, and binding site identification. Molecular docking is employed to predict ligand binding poses and estimate binding affinity, while structure-based virtual screening enables rapid evaluation of large compound libraries. Molecular dynamics simulations are conducted to incorporate protein and ligand flexibility, solvent effects, and time-dependent conformational changes. Free-energy calculation methods such as MM-PBSA and MM-GBSA are applied to refine binding affinity predictions. Ligand-based pharmacophore modelling and fragment-based drug discovery strategies support lead identification and optimization in the absence of high-resolution target structures.

1.1. Core Computational Methodologies

- **Molecular Mechanics (MM) and Force Fields:** MM models systems with a large number of atoms by ignoring explicit electrons and treating molecules as balls (atoms) connected by springs (bonds). The total energy is computed using force

fields (e.g., AMBER, CHARMM, OPLS) which are sets of parameters describing electrostatic, van der Waals, stretching, bending, and torsional interactions.

- **Quantum Mechanics (QM):** Used for higher precision in smaller systems, QM solves the Schrödinger equation to describe electronic structures. Density Functional Theory (DFT) is a commonly used QM method that approximates electron density to study chemical reactivity and reaction mechanisms.
- **Hybrid QM/MM:** These methods partition complex systems—treating the active site (e.g., in an enzyme) with quantum accuracy while treating the surrounding environment with computationally efficient molecular mechanics.
- **Molecular Dynamics (MD) Simulation:** MD simulations predict the time-dependent behavior of atoms and molecules. It acts as a "molecular microscope," allowing researchers to inspect the stability of ligands within receptor pockets. Advanced sampling techniques like Replica Exchange Molecular Dynamics (REMD) are used to explore rugged energy landscapes.
- **Coarse-Grained (CG) Models:** CG models, such as the MARTINI force field, simplify molecular structures by grouping multiple atoms into "beads". This reduces computational degrees of freedom, enabling simulations of large biomolecular complexes over extended periods
- **Structure-Based Virtual Screening (SBVS):**
  Structure-based virtual screening (SBVS) is a computational approach used in the early-stage drug discovery campaign to search a chemical compound library for novel bioactive molecules against a certain drug target. It utilizes the three-dimensional (3D) structure of the biological target, obtained from X-ray, NMR, or computational modeling, to dock a collection of chemical compounds into the binding site and select a subset of these compounds based on the predicted binding scores for further biological evaluation. In the present work, we illustrate the basic process of conducting a SBVS with examples using freely accessible tools and resources.(1)

1.2. Ligand-Based Pharmacophore Mapping (LBPM)

Ligand-Based Pharmacophore Mapping (LBPM) is a cost-effective *in silico* approach in drug discovery that uses the **3D chemical features of known active ligands** to identify new targets or lead compounds. It is particularly valuable when the crystal structure of the target protein is unavailable. LBPM defines the essential spatial arrangement of pharmacophoric features such as hydrogen bond donors and acceptors, aromatic rings, and hydrophobic regions required for biological activity, enabling virtual screening and lead discovery without protein structural information.

1.3. Molecular Dynamics (MD) Simulation

Molecular Dynamics (MD) simulations enable the study of biomolecular motions in realistic environments, including water, ions, lipid membranes, and cofactors. By accounting for molecular flexibility, MD provides deeper insights into binding mechanisms, conformational stability, and free-energy changes, thereby improving the reliability of drug design strategies.(2)

Principles of Molecular Dynamics Simulations

MD simulations are based on **classical Newtonian mechanics**, where atoms are treated as particles whose motions are computed by solving Newton's equations of motion over time. The resulting trajectory describes the evolution of atomic positions and energies.

Atomic interactions are described using **force fields**, which include:

- **Bonded interactions:** bond stretching, angle bending, torsions
- **Non-bonded interactions:** van der Waals and electrostatic forces

Although force fields neglect explicit electronic effects, they effectively reproduce many experimental observations. Common force fields include **AMBER, CHARMM, GROMOS, and OPLS**. MD simulations capture motions ranging from picoseconds to milliseconds, making them valuable for studying protein–ligand recognition, conformational flexibility, and structural stability.

Software, Force Fields, and Solvent Models
Widely used MD software packages include **AMBER, GROMACS, CHARMM, NAMD, DESMOND, and ACEMD**, each differing in performance and hardware compatibility. The adoption of **GPU acceleration** has dramatically increased simulation speed, enabling longer simulations and improved conformational sampling.

Solvent Representation
Solvent effects are modelled using two main approaches:

- **Explicit solvent models**, which include individual water molecules and provide high accuracy.
- **Implicit solvent models** (e.g., Generalized Born), which approximate solvent effects and reduce computational cost but may sacrifice accuracy.

General Workflow of MD Simulations
A typical MD workflow includes:

1. **Target selection and preparation** from experimental structures or modeling methods
2. **Structure validation and refinement**
3. **System setup**, including solvent, ions, and protonation states
4. **Energy minimization**
5. **Equilibration** under controlled temperature and pressure
6. **Production MD simulation**
7. **Analysis and application** for docking or free-energy calculations

Extending Simulation Time and Sampling Efficiency
Adequate sampling remains a major challenge in MD. Advances in GPU computing and specialized hardware have enabled microsecond- to millisecond-scale simulations. Enhanced sampling techniques such as **umbrella sampling, accelerated MD (aMD), and hyper dynamics** help overcome energy barriers and access rare but biologically relevant conformations.

Analysis of MD Simulation Data
MD trajectories generate large datasets requiring specialized analysis. Common analyses include:

Structural Stability and Flexibility

- **RMSD:** overall structural deviation
- **RMSF:** residue-level flexibility
- **Radius of Gyration (Rg):** protein compactness

Clustering Analysis
Clustering identifies representative conformations for docking and virtual screening, helping account for protein flexibility.

Secondary Structure Analysis
Monitoring changes in helices, sheets, and loops reveals folding, unfolding, or ligand-induced structural transitions.

1.4. Structure-Based Virtual Screening (SBVS) in Drug Discovery:

Early drug discovery traditionally relied on high-throughput screening, which is costly and time-consuming. These limitations have led to the adoption of **Structure-Based Drug Discovery (SBDD)**, which integrates computational tools with structural biology.
SBVS is a key component of SBDD, using the 3D structure of a target protein to screen large compound libraries and prioritize molecules with favorable binding potential. This approach accelerates lead identification while reducing experimental costs.

Overview of Structure-Based Virtual Screening
SBVS involves docking small molecules into protein binding sites and estimating binding affinity using scoring functions. Target structures may originate from experimental data or computational modeling. The typical workflow includes protein preparation, binding site identification, library preparation, docking, scoring, and post-processing.

Protein Preparation for SBVS
Accurate protein preparation is critical for reliable docking. This process includes adding hydrogens, assigning protonation states, optimizing hydrogen-bond networks, correcting residue orientations, handling metal ions, and evaluating crystallographic water molecules. Proper preparation significantly improves screening performance.

Binding Site Identification
When binding sites are unknown, computational methods are used to identify cavities with favorable geometric and physicochemical properties. These methods include static, dynamic (MD-based), mixed, and water-based approaches, enabling identification of both orthosteric and allosteric sites.

Compound Library Preparation and Design
Compound libraries are designed to contain drug-like or lead-like molecules based on physicochemical criteria such as molecular weight, lipophilicity, and hydrogen-bond capacity. Libraries undergo preprocessing steps including structure cleanup, protonation state assignment, 3D conformation generation, and toxicity filtering.

Docking and Scoring Methods
Docking algorithms predict ligand binding poses using systematic, stochastic, or MD-based approaches. Scoring functions—force-field-based, empirical, knowledge-based, or hybrid—estimate binding affinity. While docking reliably predicts poses, accurate affinity ranking remains challenging.

Post-Processing and Hit Selection
Post-processing filters false positives through visual inspection, rescoring, physicochemical and ADMET filtering, and clustering for chemical diversity. These steps improve the quality of compounds selected for experimental validation.

Advanced Strategies to Improve SBVS Performance

Ensemble Docking

Multiple protein conformations derived from experiments or MD simulations are used to account for protein flexibility.

Induced-Fit and Consensus Docking

Induced-fit docking allows receptor adaptation, while consensus docking combines multiple scoring approaches to reduce bias and improve accuracy.

Advantages and Limitations of SBVS

SBVS reduces time and cost while enabling rational exploration of chemical space. Limitations include imperfect treatment of protein flexibility and scoring inaccuracies. Despite these challenges, SBVS remains an essential tool in modern drug discovery.(3)

1.5. Ligand-Based Pharmacophore Mapping: Alignment-Free Approaches:

When protein structures are unavailable, ligand-based pharmacophore modeling identifies the spatial arrangement of chemical features responsible for activity. Traditional methods rely on molecular alignment, which can introduce bias.

To address this, **alignment-free 3D pharmacophore signatures** were developed, enabling rapid comparison of pharmacophoric patterns without explicit superposition.

Concept of 3D Pharmacophore Signatures

This approach encodes pharmacophoric features—such as donors, acceptors, hydrophobic and charged centers—into numerical 3D signatures that capture spatial relationships. These signatures allow fast, alignment-independent comparison across large datasets.

Methodology Overview

Data Preparation

Active and inactive compounds are collected to identify activity-specific pharmacophore patterns.

3D Pharmacophore Encoding

Pharmacophoric features are extracted and encoded into 3D signatures that preserve spatial relationships without molecular alignment.

Model Generation and Selection

Candidate signatures are evaluated to identify models that best discriminate active compounds from inactive ones.

Advantages of the 3D Signature Approach

- **High computational efficiency**
- **Reduced alignment bias**
- **Applicability to targets without structural data**

These advantages make the method well suited for early-stage virtual screening.

Tools and Implementation

This approach is implemented in open-source tools such as **PMapper** for generating pharmacophore hashes and **PSearch** for pharmacophore-based virtual screening, enabling practical application in drug discovery workflows.(4)

2. Cutting-edge technologies for drug design:

2.1. AI and Machine learning based methodologies for predicting drug-target interactions (DTI)

The complications like unexpected problems with efficacy, toxic effects or off-target interactions are the reasons most of the drug candidates that enter preclinical and clinical phases fail, which results in high developmental costs and discontinuation rates.(5)(6)

In recent years, the pattern of drug discovery has evolved due to the introduction of artificial intelligence (AI) and machine learning (ML) as revolutionary tools. These tools have opened the gates to process large biomedical datasets and pick up hidden arrangements to make drug development more feasible and reliable. (7)

Improved outcomes of clinical trials are one of the evident proofs that AI is compelling. An 80% to 90% success rate in phase 1 in recent studies is a demonstration that AI-discovered drugs are more reliable than the 40-65% industry average. This improvement means higher outreach and fewer costly failures, promising a healthier future.(8)

Early computational technologies included molecular docking and ligand-based approaches, where molecular docking includes 3D representative structures of drug molecules and targets through multiple docking arrangement simulations to determine the suitable binding sites. Other ligand-based approaches to study drug-target interactions involve identifying the molecules and their targets by evaluating the similarities between a query molecule and a dataset of known molecules. (8,9)

These methods were successful in generating promising prediction but they only demand high-quality datasets of proteins or drug molecules, making the drug-target-interaction prediction involving tasks less suitable.(10)

Drug-target-interactions predictions carried by AI models work on three basic steps:

1. Data processing
2. Model training
3. Final prediction
4. Validation

The direct usage of raw data and the extraction of important features and parameters from selected drug and target data are involved in data processing. The data sets are divided into three: - The training set, the test set and the validation set. The training set facilitates the AI learning processes, the test datasets are used to evaluate the AI's performance on unseen data sets, and the validation set is used to refine the AI models' parameters. Following this, the trained AI model is used to perform DTI predictions on test sets and on other data that were not observed during the training process.(11)

Some of the principles depicting the intricacy of AI models are listed below:

• Fingerprint models: These approaches convert molecules into numerical fingerprints that highlight their chemical features. Dimensionality‐reduction tools help simplify this information before it enters learning algorithms. They are efficient for smaller datasets but depend heavily on careful feature selection.

• Similarity models: These use the idea that chemically related molecules or biologically related targets often behave similarly. They create similarity matrices and use them to guide interaction predictions. Their performance depends strongly on how meaningful the underlying similarity measurements are.

• Sequence‐based models: These methods treat molecular strings and protein sequences as structured text. Early models used systems that processed information sequentially, while more recent versions use attention‐based architectures capable of capturing long‐range

relationships. Although computationally efficient, they may miss important three-dimensional cues.

• Graph and network models: In these systems, drugs, targets, and related biological concepts are represented as connected nodes within large networks. These models are particularly well-suited for discovering repurposing opportunities. However, they can become computationally heavy as the network grows.

• Image-inspired models: Here, molecules are represented as two-dimensional images. Convolution-based networks analyse structural patterns and arrangements that may not appear in textual descriptions.

• Structure-based models: These incorporate three-dimensional information about molecules and proteins. Some modern variations also include changes over time. They offer richer detail but require accurate structural inputs.(12)

ML-based approaches can use chemical and genomic features to train models to detect and predict drug-target interactions.

Molecules are the input data of machine learning-based drug discovery, and drugs and protein targets are all small or large molecules. In describing these drug and protein molecules, we offer a description of different molecular representations widely used in today's studies. The data representation is further spitted into the drug molecule representation and protein molecule representation, as a model diagram of the representations of drug molecules. Data representation is inherently associated with the knowledge induced by the learning model and plays an important role in precondition, thus selecting an appropriate representation can strongly affect the performance of predictive models.(13)

4.2. Fragment Library Design and Interpretation

Fragment collections are intentionally small but chemically diverse. Guidelines known informally as the 'Rule of 3' help keep fragment features manageable and suitable for screening. After screening, evaluating both the strength and the relevance of binding is important. Additional structural work often clarifies exactly how a fragment interacts with the target, allowing chemists to determine how best to evolve it. (14)

Techniques for Detecting Fragment Binding

• NMR: Sensitive methods reveal whether fragments interact with proteins and help identify the binding region.

• X-ray crystallography: When suitable crystals can be obtained, it offers detailed insights into how a fragment fits within the target site.

• SPR: Provides real-time confirmation of fragment binding and helps assess dose-dependent behaviour.

• Other tools: Mass spectrometry, calorimetry, and computational screening techniques can complement the main biophysical approaches.

From Fragment Hit to Optimized Lead

• Merging: Combines overlapping chemical features from different fragments into a single, more effective structure.

• Linking: Joins fragments that bind in neighbouring locations using an appropriately rigid connector.

• Growing: Extends a single fragment step-by-step into adjacent pockets to strengthen interactions.

Challenges in Fragment Progression

False positives can arise in certain screening conditions, making it necessary to validate hits across multiple approaches. Some small molecules tend to form aggregates that mimic

binding activity, while impurities or reactive species can also distort results. In addition, fragments must remain soluble at the concentrations required for screening. A complete understanding of structure–activity relationships must rely on measured changes in affinity rather than structural intuition alone.(15)

4.3. Hybrid QM/MM Approaches

Hybrid computational frameworks allow a chemically active region of a system to be modelled using quantum mechanics while the surrounding environment is handled through classical mechanics. This approach balances detail and efficiency, making it suitable for studying enzyme reactions, transition states, and fine-grained binding behaviours.

By concentrating quantum-level calculations on the specific area where chemistry occurs, researchers can study complex systems more efficiently while still capturing essential electronic details.(16)

Molecular Mechanics Concepts

Molecular mechanics represents atoms as units connected by classical forces resembling springs. It estimates total energy through factors such as bond lengths, angles, torsions, and nonbonded interactions. While not concerned with electronic structures, MM is extremely useful for analysing relative conformational energies and exploring molecular flexibility.

Quantum Mechanical Methods

Quantum-level modelling treats electrons and nuclei explicitly and calculates properties from approximations of the Schrödinger equation. These methods, while computationally demanding, can describe structures, energies, and reaction pathways with high accuracy. For larger systems, simplified or hybrid methods are often used.

Role of Computer-Aided Design in Modern Discovery

A broad suite of in-silico tools—including docking, virtual screening, QSAR modeling, and molecular simulations—allows researchers to explore chemical space more efficiently. By integrating computational predictions with experimental work, teams can progress from initial hits to refined leads with greater speed and precision.(17,18)

4.4. Addressing Molecular Challenges in Repositioning

Drug repositioning, or repurposing, involves finding new medical uses for existing medications. This approach offers advantages such as faster development times, lower costs, and improved success rates in clinical trials due to existing safety data. However, challenges arise when a drug designed for one target is applied to a different disease, particularly in terms of binding affinity. This can result from changes in binding site structures or molecular interactions.

4.5. Impact of Structural Modifications on ADME Properties:

Structural optimization aimed at enhancing binding affinity or polypharmacology often alters key physicochemical properties such as lipophilicity, molecular weight, and polarity, impacting absorption and distribution. While increased lipophilicity can enhance membrane permeability, it may also lead to higher plasma protein binding and metabolic clearance, whereas excessive polarity can reduce oral bioavailability.

In silico models help evaluate the effects of chemical changes on ADME (Absorption, Distribution, Metabolism, and Excretion). A significant challenge in drug repositioning is the binding affinity gap, which occurs when existing drugs do not engage robustly with new targets, often due to their original design for different binding conditions. Addressing this

gap is vital, especially when increasing dosages is limited by pharmacokinetic factors or toxicity concerns.

4.6. Structural Basis of the Binding Affinity Gap:

At the molecular level, variations in binding affinity often stem from incomplete complementarity between a ligand and its target binding site. Differences in size, shape, residue composition, and flexibility can hinder effective hydrogen bonding and other interactions. Additionally, entropic costs from ligand flexibility and negative desolvation effects can weaken binding strength. These issues highlight the importance of rational optimization to improve ligand-target complementarity while retaining key drug-like characteristics.

4.7. Structure-Based Optimization Approaches:

Structure-based drug design (SBDD) effectively enhances binding affinity when detailed structural data about a new target becomes available. Molecular docking is often the first step to identify binding configurations, but docking scores alone may not predict actual binding strength. Thus, methods like molecular dynamics (MD) simulations and free energy assessments are increasingly employed to account for protein flexibility and solvent effects.

Fragment-based optimization helps bridge gaps in binding affinity by breaking down the parent drug into smaller fragments or identifying favorable fragment-sized components. Medicinal chemists can use strategies such as fragment growing, merging, or linking to improve affinity systematically and explore the chemical space around the initial scaffold.(19)

4.8. Ligand-Centric and Medicinal Chemistry Strategies:

When structural data for a target is limited, ligand-based approaches are vital. Techniques like pharmacophore modeling, QSAR analysis, and similarity-based screening can help design analogues with improved affinity. Bioisosteric replacement is often used to enhance interactions, reduce steric clashes, or improve electronic complementarity. Additionally, conformational restriction—limiting rotatable bonds or locking flexible parts into bioactive shapes—can reduce entropic penalties during binding. However, these modifications should be balanced to avoid compromising solubility or permeability.(20)

4.9. Role of Machine Learning in Affinity Optimization:

Recent advances in machine learning and deep learning have enhanced drug-target binding affinity predictions. Models like graph neural networks and attention-based architectures help prioritize chemical modifications that improve binding. These data-driven methods are especially useful in drug repositioning where experimental data may be limited. Combining ML with physics-based methods creates hybrid pipelines that efficiently screen large libraries of analogues, while molecular simulations provide mechanistic insights and validate top candidates.(21)

Experimental Validation and Iterative Optimization:

Computational predictions must be validated experimentally using biophysical techniques like surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC), which measure binding affinity and thermodynamic parameters. This data informs optimization efforts and clarifies enthalpic and entropic contributions to binding. An iterative cycle of design, testing, and analysis, combining computational modeling with medicinal chemistry and experimental assays, is essential for enhancing binding affinity. Improvements should also consider pharmacokinetic and safety aspects for practical application.(22)

Implications for Drug Re-positioning Success:
Bridging the binding affinity gap turns weak off-target interactions into actionable mechanisms. By combining structural biology, computational chemistry, machine learning, and experimental validation, repositioned drugs can reach first-in-class potency levels. This approach enhances therapeutic efficacy and expands the diseases that can benefit from drug repositioning strategies.(23)

Polypharmacology by Design: Engineering "Promiscuous" Molecules to Hit Multiple Disease Pathways:

Concept and Rationale of Polypharmacology in Drug Re-positioning:
Polypharmacology refers to the capability of a single medication to interact with various molecular targets. In the context of drug repositioning, this is considered advantageous for addressing complex illnesses such as cancer and neurodegenerative diseases. Numerous repurposed medications prove effective by influencing multiple signaling pathways rather than focusing on just one. Since diseases frequently have compensatory mechanisms that can diminish the effectiveness of selective medications, polypharmacological agents can target several points within a disease network, thereby increasing effectiveness and lessening resistance. The challenge lies in achieving controlled promiscuity—optimizing target engagement while limiting negative effects.(24)

Network Pharmacology and Systems-Level Target Identification:
Network pharmacology serves as the foundation for rational polypharmacology by conceptualizing diseases as interconnected biological networks that include proteins, genes, metabolites, and signaling pathways. In the context of drug repositioning, network analysis helps identify disease modules and key hubs, facilitating synergistic therapeutic outcomes through the concurrent modulation of multiple targets. By employing computational tools, the relationships among drugs, targets, and diseases are illustrated, enabling the assessment of repurposed drugs for their effects on disease-specific subnetworks rather than individual targets, thus supporting the rationale for multi-target activity.(25)

Rational Design Strategies for Multi-Target Ligands:
The field of engineering polypharmacology focuses on creating multi-target ligands (MTDLs) by altering chemical structures to engage multiple therapeutic sites. Typical approaches involve combining pharmacophores, linking them, and hybridizing scaffolds. Researchers frequently utilize privileged scaffolds that interact with different families of proteins to reposition existing compounds. It is essential to fine-tune the molecular size, flexibility, and characteristics to ensure a balanced affinity across the various targets. Nevertheless, overly complicated
molecules may experience issues with poor bioavailability and metabolic stability, which emphasizes the importance of iterative optimization.(26)

4.10. In Silico ADMET Re-evaluation: Predicting Consequences of Structural Changes for Safety Profiles:

Importance of ADMET Re-evaluation in Drug Re-positioning:
Drug repositioning leverages the safety data from existing clinical studies, but molecular optimization frequently necessitates structural changes that can influence pharmacokinetics and toxicity. Even minor chemical alterations can have an impact on solubility, permeability, metabolic stability, or off-target toxicity. As a result, in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluations are essential for addressing

these issues, ensuring that modified molecules uphold safety while improving efficacy. These computational evaluations facilitate the early detection of safety concerns, thereby decreasing the need for expensive late-stage testing and lowering attrition rates in the development process.(27)

Impact of Structural Modifications on ADME Properties:
Optimizing structure for enhanced binding affinity or polypharmacology frequently modifies essential physicochemical attributes such as lipophilicity, molecular weight, polarity, and conformational flexibility. These alterations influence absorption and distribution characteristics, including intestinal permeability and tissue distribution. Elevated lipophilicity can enhance membrane permeability but might also increase plasma protein binding and metabolic clearance, whereas high polarity can potentially decrease oral bioavailability. In silico modeling aids in assessing how chemical modifications affect ADME (Absorption, Distribution, Metabolism, and Excretion) properties, helping medicinal chemistry achieve a balanced optimization.(28)

Computational Prediction of Drug Metabolism and Clearance:
Metabolism-related liabilities lead to considerable drug attrition, particularly for repurposed compounds that have undergone structural changes. In silico predictions of cytochrome P450 (CYP) inhibition, induction, and metabolic sites are crucial for evaluating the risks of drug-drug interactions and for pinpointing metabolic "soft spots" that are vulnerable to rapid clearance or the formation of toxic metabolites. Machine learning and rule-based methods effectively forecast metabolic stability and steer structural alterations to enhance drug half-life while maintaining safety.(29)

In Silico Toxicity Prediction and Safety Profiling:
Changes in compound structures can lead to new toxicity hazards, such as damage to the heart, liver, genetic material, and impacts on the central nervous system. In silico models for predicting toxicity utilize chemical structures, physicochemical characteristics, and past data to detect these hazards early in the development process, which is crucial for drug repurposing. Important predictions include the inhibition of hERG channels, the generation of reactive metabolites, and liver damage. By integrating various toxicity endpoints into a single computational framework, researchers can more effectively prioritize safer analogues for further testing.(30)

Conclusion

Rational molecular drug design has redefined the concept of drug repurposing, assimilating structural biology, computational chemistry, and artificial intelligence into one cohesive, efficient workflow. In striking contrast to conventional trial-and-error strategies, the identification, optimization, and validation in state-of-the-art repurposing approaches are based on mechanistic insights at the molecular level. Structure-based methodologies comprise molecular docking, structure-based virtual screening, molecular dynamics simulations, and free-energy calculations that allow detailed insight into binding mechanisms, conformational flexibility, and thermodynamic stability. Complementary ligand-based and fragment-based strategies allow for lead identification even without high-resolution structural data, while hybrid QM/MM approaches offer electronic-level precision for studies of catalytic and binding events. Integration of machine learning and AI-driven drug-target interaction prediction further enhances the scalability and predictive power of

repurposing pipelines. These approaches enable rapid screening across large chemical and biological spaces, improve binding affinity optimization, and enable rational polypharmacology design. The network pharmacology concepts extend this paradigm to address complex diseases by multibinding, while in silico ADMET evaluation ensures that structural modifications maintain acceptable pharmacokinetic and safety profiles. These multidisciplinary tools put together create an iterative design-test-refine cycle that limits attrition rates and speeds up translational outcomes.

Although much has been achieved, there remain areas to tackle, such as developing predictive binding affinity, dealing with flexibility, data limitations within AI models, and how to optimize potency, selectivity, and safety at the same time. Addressing these limitations would need to be achieved by improving scoring function development, sampling, data standardization, and interactions between computational models and experimental data.

In conclusion, with the advent of structurally convergent methodologies and the use of highly sophisticated computational methods, repurposing of drugs has now moved from being an opportunistic approach towards becoming a more rational discipline. The integrating potential that combines molecular findings with whole-systems understanding promises to yield considerable potential for accelerating therapy discovery, reducing development costs, and improving therapy options for unmet medical needs.

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Chapter- Nine

Toxicological Evaluation of Natural Product-Derived Molecules in Drug Repurposing: Safety Challenges and Therapeutic Opportunities

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Abstract

Natural products have traditionally been a rich source of bioactive molecules and continue to dominate modern drug discovery and rediscovery initiatives. Many compounds of natural origin based on traditional indications are being repurposed in new therapeutic uses in oncology, infectious diseases, metabolic diseases, and neurodegenerative diseases. These molecules, although considered safe because of their natural origin, frequently demonstrate complex toxicological profiles that are due to pleiotropic actions, bioactivation as well as fluctuating pharmacokinetics. The chapter offers a profound toxicological understanding of the drug repurposing of natural product-based molecules. Among them are mechanistic toxicity, dose-dependent toxicity, organ toxicity and metabolic liabilities that are linked with phytochemicals, alkaloids, terpenoids, and polyphenols. Issues of chronic exposure, herb-drug interactions and differences in safety profiles of natural molecules when used as new targets or with alternative routes are also considered in the chapter. The regulatory toxicology issues are looked into such as safety re-assessment, non-clinical bridging studies, and translational risk assessment which is very critical. The chosen case studies demonstrate the successful and the unsuccessful repurposing attempts and focus on the lessons of toxicological reassessment. Lastly, novel methods, including computational toxicology, systems toxicology, and omics-based safety analysis, are discussed as ways of de-risking natural product-based drug repurposing. This chapter highlights the need to have strict toxicological paradigms that would guarantee safety and efficacy of repurposed therapeutic products derived by means of natural products.

Keywords: *Natural products; Drug repurposing; Toxicology; Phytochemicals; Organ toxicity; Herb–drug interactions; Safety assessment.*

Introduction

Natural Products as a Source of Repurposable Drugs

Natural products (NPs) have been a foundational source of therapeutic innovation that has made significant contributions in the field of modern pharmacotherapy in oncology, infectious diseases, cardiovascular disorders and neurological diseases (1). The recent years have seen a renewed enthusiasm in the development of drug repurposing, which suggests that NP-derived molecules and their semi-synthetic analogues still represent a large share of

approved medicines, due to their outstanding structural diversity, biological activity, and evolutionary selection of protein interactions (1,2). The concept of drug repurposing has brought back the interest in natural products in the last several years and has turned them into promising candidates in the process of therapeutic repositioning (as opposed to the idea of de novo drug discovery) (3).

Drug repurposing includes the discovery of new clinical uses of an existing pharmacologically characterized bioactive compound or drug. The molecules derived in a natural product are of particular interest to the present approach as many have a history of human exposure by use in traditional medicine, dietary supplementation or previous clinical development, providing partial safety and pharmacokinetic information (4). The mechanistic deconvolution of NP bioactivity has also been made possible due to the advances in the field of analytical chemistry, metabolomics, chemoproteomics, and systems biology, allowing the identification of previously unknown molecular targets and disease-relevant pathways to support repurposing approaches (5,6).

More current NP repurposing activities are becoming more ingrained with omics-based methods such as transcriptomics and proteomics to align molecular signatures induced by compounds with disease phenotypes. The strategies have been effective in the identification of new signs of alkaloids, polyphenols, terpenoids, and glycosides especially in cancer, inflammation, metabolic illnesses and neurodegeneration (7). Also, computational drug repositioning systems and artificial intelligence-based target prediction systems have further broadened the NP repurposing repertoire by facilitating in silico screening of large natural compounds libraries on a variety of disease models (8).

Nevertheless, pleiotropy of most NP-derived molecules is inherent which gives it a drawback and a strength at the same time. Although multi-target activity might be used to improve the efficacy of therapy in complex diseases, there have been increased chances of off-target activity and non-predictable toxicity profiles, particularly when drugs are being reused in long-term therapy or exposed to higher systemic concentration than usually anticipated (9). Furthermore, natural molecules often exhibit complex pharmacokinetic behavior, including low oral bioavailability, extensive first-pass metabolism, and interaction with drug-metabolizing enzymes and transporters, which may be altered when used in new clinical contexts (10).

Rationale for Toxicological Reassessment in Drug Repurposing

The most widespread misconception about natural products is that they are inherently safe due to the fact of their natural origin or historic use. As a matter of fact, most natural compounds have very limited therapeutic indices, bioactivation-related hepatotoxicity, or cumulative adverse effects, which only manifest themselves in certain circumstances of exposure (11).

Dose and duration dependency is one of the main reasons behind reinstated toxicological assessment. Recycled indications might need prolonged exposure which is higher than the usual or previously recommended dosing schedule and thus raising the chances of long-term organ toxicity, such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and neurotoxicity (12). Indicatively, phytochemicals that are safe in the short term may cause oxidative stress, mitochondrial damage or immune mediated damage during the long term administration (13). Bioactivation and metabolic liability is another important point of concern. A large percentage of NP-derived molecules are highly biotransformed by the cytochrome P450 enzymes to form reactive intermediates that can bind covalently to proteins and cause cell damage. These mechanisms have been observed to cause idiosyncratic drug-

induced liver injury and other uncommon but severe adverse events (14). From a regulatory perspective, repurposing does not eliminate the need for non-clinical safety evaluation. Regulatory agencies increasingly emphasize risk-based toxicology, requiring bridging studies to address gaps between existing safety data and the new intended clinical use (15).

Classes of Natural Product-Derived Molecules

The unparalleled chemical diversity and biological interest have kept natural products as one of the key pillars in pharmaceutical research over the decades. Natural products have been and continue to serve as inspiration in current drug discovery due to many clinically useful drugs either directly derived or inspired by natural product scaffolds [19,20]. Plants, microorganisms, and marine organisms produce these compounds as biosynthetic products to aid ecological roles, which include defense and signaling among others and are usually classified as secondary metabolites. Natural products are generally differentiated into alkaloids, terpenoids and steroids, polyphenols and flavonoids, and glycosides among other secondary metabolites based on their biosynthetic origin and chemical structure (16).

Alkaloids

Alkaloids are one of the most pharmacologically important groups of natural products that are characterized by the presence of no less than one nitrogen atom that is typically integrated into a heterocyclic structure (17). They are majorly made out of amino acid precursors like tryptophan, tyrosine, phenylalanine and ornithine. This nitrogen group allows its basicity and allows it to interact with a large variety of biological targets, such as enzymes, receptors, and nucleic acids (18).

It is due to such properties that alkaloids have a wide range of biological activity, including analgesic, antimalarial, anticancer, antihypertensive, and neuroactive effects (19). Their pharmacological effects are commonly the regulation of the neurotransmitter system, the blockage of key enzymes, or the disruption of the DNA replication and protein production (16). Although alkaloids have a therapeutic value, a number of them have dose-limiting toxicity, and many of them have narrow therapeutic indices. This has led to the recent studies that have concentrated on semi-synthetic modification, structure-activity relationship studies and biosynthetic engineering strategies in order to enhance safety and selectivity without affecting the biological efficacy (20).

Terpenoids and Steroids

The largest and the most structurally diverse group of natural products is terpenoids. The following compounds are constructed out of five carbon isoprene units through the mevalonate and methylerythritol phosphate pathways (21). Terpenoids are further divided into monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, tetraterpenoids and polyterpenoids (22). based on their carbon skeletons. Steroids are a separate group of triterpenoids; they are defined by a stiff tetracyclic cyclopentanoperhydrophenanthrene structure.

Terpenoids and steroids have been linked to many biological processes and pharmacological processes such as anti-inflammatory, antiviral, antimicrobial, anticancer, and immunomodulatory (23,24). The steroidal compounds and especially have a crucial role in clinical practice because of their use as hormones, anti-inflammatory agents and immunosuppressive drugs (25). The recent progress in the field of metabolic engineering,

synthetic biology, and microbial biotransformation has contributed greatly to increasing the chemical space of terpenoids and steroids, enabling the production of new derivatives with greater bioactivity and better pharmacokinetic properties (26).

Polyphenols and Flavonoids

Polyphenols are a large class of plant-derived compounds characterized by multiple phenolic hydroxyl groups and are widely distributed in medicinal plants and dietary sources. Flavonoids, which represent a major subclass of polyphenols, share a common C6–C3–C6 carbon framework and include flavones, flavonols, flavanones, isoflavones, anthocyanins, and catechins (27,28).

Extensive research has demonstrated that polyphenols and flavonoids exert diverse biological effects, including antioxidant, anti-inflammatory, cardioprotective, antidiabetic, and neuroprotective activities (29,30). Although their antioxidant ability has remained a major focus, it is now understood that these compounds also regulate intracellular signaling cascades, transcription factors and enzyme systems that mediate oxidative stress and inflammatory responses. Moreover, there is emerging evidence that microbiota-mediated metabolism of gut microbiota are highly important in regulating the bioavailability, systemic actions of dietary polyphenols, and that they frequently do so by generating metabolites with specific biological actions. (31,32).

Glycosides and Other Secondary Metabolites

Glycosoids consist of a sugar moiety (glycone) that is covalently bound to a non-sugar aglycone which can be an alkaloid, terpenoid, steroid or phenolic compounds [38]. This glycosylation process has a significant effect on the physicochemical and pharmacokinetic characteristics of natural products, changing such parameters as solubility, stability, membrane permeability, and tissue distribution (33).

A number of glycoside classes have established pharmacology. Examples of cardiac glycosoids, such as exert a potent positive inotropic activity by inhibiting Na + /K +-ATPase, and remain at clinical use despite their narrow therapeutic indices (34). Saponin glycosides have been reported to have immunomodulatory, antifungal, and cytotoxic effects (35). In addition to glycosides, other classes of secondary metabolites, including coumarins, lignans, iridoids and tannins also add to the chemical complexity and therapeutic potential of natural products (36). The structural characterization and quantitative analysis of such compounds has been highly enabled by the advances of analytical methods, especially, high resolution LC- MS/MS and multidimensional NMR spectroscopy (37).

Mechanistic Toxicology of Natural Product Molecules

Although natural product–derived molecules are widely used for therapeutic purposes, their complex chemistry and biological activity can also lead to toxic effects. Toxicity often arises from defined molecular mechanisms rather than nonspecific cellular damage. Mechanistic toxicology focuses on identifying these pathways to support safety assessment and rational drug development. Among the recognized mechanisms, target-mediated toxicity, metabolic bioactivation, and oxidative stress–associated mitochondrial dysfunction represent the principal contributors to natural product–induced toxicity (38–42).

Table 1: Major mechanisms involved in the toxicological effects of natural product–derived molecules

Toxicological mechanism	Primary biological target/process	Representative natural product classes	Key toxicological outcomes	Mechanistic characteristics
Target-mediated toxicity	Receptors, enzymes, ion channels, transporters	Alkaloids, cardiac glycosides, steroids	Neurotoxicity, cardiotoxicity, endocrine disruption	Excessive or prolonged target modulation leading to loss of physiological homeostasis
Metabolic bioactivation	Phase I metabolic enzymes (e.g., cytochrome P450)	Alkaloids, flavonoids, terpenoids	Hepatotoxicity, immune-mediated injury	Formation of electrophilic intermediates capable of covalent protein and DNA binding
Reactive metabolite formation	Cellular macromolecules (proteins, lipids, nucleic acids)	Pyrrolizidine alkaloids, phenolic compounds	Enzyme inactivation, cellular stress responses	Covalent adduct formation triggering toxicity and immune activation
Oxidative stress	Redox homeostasis and antioxidant systems	Polyphenols (high dose), quinone-forming compounds	Lipid peroxidation, DNA damage	Excess reactive oxygen species generation exceeding antioxidant capacity
Mitochondrial dysfunction	Electron transport chain, ATP synthesis	Terpenoids, alkaloids, flavonoids	Energy depletion, apoptosis, organ injury	Impaired mitochondrial respiration and membrane integrity
Inflammatory amplification	Cytokine and immune signaling pathways	Diverse natural product classes	Chronic tissue damage, fibrosis	Secondary inflammatory responses following cellular injury

Target-Mediated Toxicity

Target-mediated toxicity results from excessive or prolonged interaction of a natural product with a physiological target essential for normal function. Since many natural products act on receptors, enzymes, or ion channels, overactivation or sustained inhibition of these targets can disrupt cellular and systemic homeostasis This form of toxicity is closely linked to the pharmacological mechanism of action and is typically dose-dependent. As a result,

therapeutic and toxic effects may overlap, complicating safety margins and dose optimization (1)

Bioactivation and Reactive Metabolite Formation

Bioactivation is a critical mechanism underlying the toxicity of several natural products. During metabolic processing, particularly in the liver, certain compounds are converted into reactive intermediates. These electrophilic metabolites can form covalent adducts with cellular macromolecules, leading to enzyme inactivation, cellular stress, and tissue injury. Cytochrome P450–mediated oxidation is a major contributor to reactive metabolite formation. The extent of toxicity depends on the balance between bioactivation and detoxification pathways, as well as individual variability in metabolic capacity (43–45).

Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress is often associated with the toxicity caused by natural products, and could be caused by the formation of reactive metabolites or by direct interference with redox mechanisms. The overproduction of reactive oxygen species may overcome the antioxidant defenses leading to lipid peroxidation, protein oxidation, and DNA damage. Such injury is especially prone to mitochondria because they have a central position in the metabolism of energy. Disturbance of mitochondrial electron transfer, membrane potential or ATP generation may result in the depletion of energy and the subsequent stimulation of cell death pathways. Prolonged mitochondrial malfunction may also increase the oxidative stress and lead to organ specific toxicity (29,30).

Organ-Specific Toxicity Concerns

Natural product–derived molecules are often perceived as inherently safe; however, substantial experimental and clinical evidence indicates that they may produce organ-specific toxic effects under certain conditions (29,30). Such toxicities typically arise from selective tissue exposure, metabolic activation within specific organs, or heightened vulnerability of particular cell types to oxidative and mitochondrial injury (1).

Table 2 Organ-specific toxicity associated with natural product–derived molecules

Target organ	Primary toxic mechanism	Natural product classes involved	Major toxic outcomes
Liver	Bioactivation, oxidative stress, mitochondrial dysfunction	Alkaloids, flavonoids, terpenoids	Hepatitis, cholestasis, liver failure
Kidney	Oxidative stress, tubular accumulation	Alkaloids, phenolics	Acute kidney injury, fibrosis
Heart	Ion channel interference, mitochondrial dysfunction	Cardiac glycosides, alkaloids	Arrhythmia, cardiomyopathy
Nervous system	Neurotransmitter modulation, oxidative stress	Alkaloids, neuroactive terpenoids	Neurotoxicity, cognitive impairment

Hepatotoxicity

The liver is especially susceptible to natural product–induced toxicity due to its central role in xenobiotic metabolism (14). The liver-related side effects include both temporary increase in serum transaminases and acute hepatitis, cholestatic liver injury, and liver failure in extreme cases (46).

At the mechanistic stage, hepatotoxicity is usually linked to bioactivation by cytochrome P450 which leads to the formation of electrophilic metabolites that form covalent bonds with the hepatic proteins (47). These processes favor the occurrence of oxidative stress, glutathione loss, and the dysfunction of mitochondria as well as the stimulation of inflammatory signaling pathway activation (48–50). These mechanisms have been reiterated in implicating alkaloids, flavonoids and terpenoids in herb- and natural product-induced liver injury (49).

Nephrotoxicity

Another important organ of natural product toxicity is the kidneys due to their concentration and excretion functions of xenobiotics and their metabolism (51). Depending on the duration of exposure and the properties of the compound, renal toxicity can be clinically reflected as acute kidney damage, tubular dysfunction or a progression of interstitial nephritis (52). Some natural products or their metabolites have the predilection to deposit in the tissues of the kidneys, which poses the risk of focal damage.

Mechanistic evidence has shown that oxidative stress, inflammatory reactions, and mitochondrial dysfunction in renal tubular epithelial cells are common in nephrotoxicity (53). Moreover, the disruption of the renal transport systems can cause intracellular retention of toxic substances, which will increase the cell damage (17). Long-term exposure to some herbal constituents has been linked to irreversible renal fibrosis, particularly in individuals with pre-existing kidney disease or compromised hydration status (51,52).

Cardiotoxicity and Neurotoxicity

The heart and nervous system exhibit heightened sensitivity to toxic insults due to their dependence on finely regulated electrical and biochemical signaling processes and their limited regenerative capacity (54). Cardiotoxic effects associated with natural products are frequently attributed to disruption of ion channel function, altered calcium homeostasis, or impairment of myocardial energy metabolism, leading to arrhythmias and contractile dysfunction (55,56). Mitochondrial injury and oxidative stress further contribute to compromised cardiac performance (57).

Neurotoxicity arises primarily from excessive modulation of neurotransmitter receptors, inhibition of synaptic enzymes, or disruption of neuronal ion channel activity. Several bioactive plant constituents, particularly alkaloids, have been shown to induce oxidative stress, mitochondrial dysfunction, and excitotoxic damage in neuronal cells Because elimination of many compounds from the central nervous system is limited by the blood–brain barrier, neurotoxic effects may persist and worsen with chronic exposure. Clinically, such toxicity may present as cognitive impairment, motor disturbances, or peripheral neuropathy [73–75].

Table 3 Mechanistic pathways involved in natural product–induced organ toxicity

Mechanistic pathway	Primary cellular target	Affected organ(s)	Toxicological outcome
Target-mediated toxicity	Receptors, ion channels	Heart, brain	Arrhythmia, neurotoxicity
Reactive metabolite formation	Proteins, DNA	Liver	Hepatocellular injury
Oxidative stress	Antioxidant systems	Liver, kidney	Cellular damage
Mitochondrial dysfunction	Electron transport chain	Heart, kidney, brain	Energy depletion, cell death

Dose, Exposure, and Duration-Related Toxicity

The toxicological effects of natural product–derived molecules are closely dependent on dose, frequency of exposure, and duration of use. Although many natural products are considered safe at low or therapeutic doses, adverse effects may arise when exposure exceeds the capacity of metabolic and detoxification systems. In contrast to conventional pharmaceuticals, natural products are often consumed without standardised dosing, increasing the likelihood of dose- and duration-related toxicity (58). Therefore, evaluation of acute, sub-chronic, and chronic exposure patterns is essential for accurate safety assessment.

Table 4 Dose-, exposure-, and duration-dependent toxicity of natural product–derived molecules

Exposure type	Duration	Primary toxic mechanisms	Typical toxic outcomes	Relevance to natural products
Acute toxicity	Single or short-term exposure	Target overload, rapid bioactivation	GI irritation, neurotoxicity, acute organ injury	Accidental overdose, concentrated extracts
Sub-chronic toxicity	Repeated exposure (weeks–months)	Enzyme modulation, oxidative stress	Reversible organ injury, inflammation	Habitual herbal use
Chronic toxicity	Long-term exposure (months–years)	Cumulative mitochondrial damage, oxidative stress	Irreversible organ damage	Long-term supplementation

Acute and Sub-chronic Toxicity

Acute toxicity is the occurrence of adverse effects following one dose or in the short-term usually with a period of 24 hours (59). Acute toxicity in the case of natural products is most commonly associated with large doses, incorrect formulation, or concentrated active components (1) The acute manifestations are usually characterized by gastrointestinal irritation, neurological, cardiovascular disruptions, or acute organ damage, and they vary based on the chemical properties of the compound and biological targets (60).

At the mechanistic level, acute toxicity is often linked to fast target interaction or abrupt metabolic bioactivation, which results in the development of oxidative stress, mitochondrial dysfunction and cellular homeostasis disruption (61,62).

Sub-chronic toxicity occurs due to the chronic exposure over weeks and months and is specifically useful with products of natural origin used regularly (58). Consistent exposure can also change the causes of enzyme activity in the metabolism, lead to a change in pharmacokinetic conduct, and contribute to an increase in the concentration of parent substances or metabolites in particular organs. The sub-chronic toxicity is usually defined as persistent oxidative stress, low-grade inflammatory reactions, and premature mitochondrial damage, which can initially be reversible but can lead to further development over time with prolonged exposure limit is necessary in anticipating long-term safety (63,64).

Chronic Toxicity and Cumulative Effects

Chronic toxicity arises from long-term exposure to natural products over months or years and represents a major concern in prolonged therapeutic or preventive use. Unlike acute toxicity, chronic toxic effects often develop gradually and may remain undetected until significant cumulative damage has occurred. Continuous exposure can result in bioaccumulation of lipophilic compounds, persistent mitochondrial dysfunction, and prolonged oxidative stress (65,66).

Cumulative toxicity is especially relevant for natural products that generate reactive metabolites or chronically impair antioxidant defense mechanisms. Repeated low-dose exposure may progressively compromise cellular repair systems, leading to irreversible damage in organs such as the liver, kidneys, heart, and nervous system (1) At the mechanistic level, chronic toxicity is associated with sustained mitochondrial injury, DNA damage, epigenetic alterations, and dysregulation of immune and inflammatory pathways (67).
Assessment of chronic toxicity is essential for establishing acceptable daily intake levels and defining safe duration of use for natural product-based interventions (51).

Herb–Drug Interactions and Safety Implications

Combination of herbal products and conventional medications has been on the rise especially in patients with chronic illnesses. Despite the presumptions of harmlessness, considerable evidence suggested that, despite their perceived safety, herbal preparations may change the pharmacokinetics of drugs co-administered with them, resulting in lower therapeutic or greater toxicity (58). The mechanisms that lead to the emergence of herb-drug interactions are mostly the process of drug-metabolizing enzymes and membrane transporter modulations which are central in the process of drug absorption, metabolism and elimination (9). Such interactions are a serious safety issue particularly among patients being given long-term pharmacotherapy or those undergoing administration of drugs with a narrow therapeutic index.

Table 5 Major mechanisms involved in herb–drug interactions and their safety implications

Interaction mechanism	Primary target	Representative herbal constituents	Effect on co-administered drugs	Safety implication
CYP inhibition	CYP3A4, CYP2C9	Flavonoids, alkaloids	Reduced drug clearance	Increased toxicity
CYP induction	CYP3A4	Certain	Accelerated	Reduced

		phytochemicals	metabolism	efficacy
Efflux transporter inhibition	P-glycoprotein	Polyphenols, saponins	Increased drug absorption	Toxic accumulation
Uptake transporter modulation	OATPs, OCTs	Alkaloids, phenolics	Altered drug distribution	Variable drug exposure

Cytochrome P450 Modulation

Cytochrome P450 (CYP) enzymes are responsible for the oxidative metabolism of the majority of clinically used drugs. Herbal constituents capable of inhibiting or inducing CYP enzymes can therefore produce clinically meaningful changes in drug exposure (69). Inhibition of CYP activity may decrease drug clearance, resulting in elevated plasma concentrations and an increased risk of adverse effects, whereas enzyme induction can accelerate metabolism and lead to subtherapeutic drug levels (70).
Numerous phytochemicals, including flavonoids, alkaloids, and terpenoids, have been shown to inhibit major CYP isoforms such as CYP3A4, CYP2C9, CYP2D6, and CYP1A2 through competitive or mechanism-based mechanisms (71). In contrast, prolonged exposure to certain herbal products can induce CYP expression via activation of nuclear receptors, including the pregnane X receptor and constitutive androstane receptor, thereby enhancing drug metabolism (72,73).

Transporter-Mediated Interactions

In addition to metabolic enzymes, drug transporters play a key role in determining drug disposition. Transport proteins such as P-glycoprotein (P-gp), organic anion-transporting polypeptides (OATPs), organic cation transporters (OCTs), and multidrug resistance-associated proteins (MRPs) regulate drug absorption, tissue distribution, and excretion (74). Herbal constituents capable of modulating these transporters can significantly alter systemic drug exposure.

Inhibition of efflux transporters, particularly P-gp, may increase intestinal absorption and tissue accumulation of drugs, thereby elevating the risk of toxicity (75). Conversely, induction of efflux transporters can reduce drug bioavailability and compromise therapeutic effectiveness. Modulation of uptake transporters such as OATPs may also affect hepatic drug clearance and plasma drug concentrations (51). These interactions are especially relevant for drugs that rely heavily on transporter-mediated disposition (76).

Safety Implications

Herb–drug interactions remain underrecognized in clinical practice due to limited standardization of herbal products, variability in phytochemical content, and inadequate patient disclosure. Many interactions are not identified during preclinical testing and only become evident after widespread use. Healthcare professionals should therefore routinely inquire about herbal product use and consider potential enzyme- and transporter-mediated interactions when prescribing medications. Systematic evaluation and improved awareness of herb–drug interactions are essential to minimize adverse outcomes and ensure safe and effective therapy (77,78).

Regulatory Toxicology and Translational Challenges

The clinical transition of molecules based on naturally derived products of preclinical research to clinical practice is associated with unique regulatory and toxicological issues. Though lots of natural products were traditionally used for a long time, this usage cannot guarantee safety according to new standards of the regulations. Regulatory toxicology focuses on generating robust, reproducible safety data to support clinical development, while translational toxicology aims to bridge findings from non-clinical studies to human risk assessment. For natural products, this process is complicated by chemical complexity, variability in composition, and limited standardization, which collectively challenge conventional regulatory frameworks (79–82).

Non-clinical Safety Bridging Studies

Non-clinical safety bridging-studies are important to connect the current toxicological information with new clinical response, formulations, or administration route. In the case of natural product derived molecules, this type of study is frequently necessary when a reformulated, repurposed, or exceeded dose is being tested [109].

Translationally non-clinical bridging studies of safety assist in dose determination in first-in-human trials and aid in establishing safety margins. The incorporation of toxicokinetic data is especially crucial when there is the need to correlate the levels of systemic exposure in animals and with the levels of exposure expected in humans. Regulatory advice tends more to better use mechanistic biomarkers and exposure-based methods to enhance the predictive utility of non-clinical safety data. In the case of natural products, such strategies are necessary to solve the inter-batch variability and guarantee the reproducible safety evaluation (80,83).

Risk–Benefit Assessment in Repurposed Indications

Risk–benefit assessment is a central component of regulatory decision-making, particularly when natural product–derived molecules are repurposed for new therapeutic indications. Repurposing often involves changes in dose, treatment duration, or patient population, all of which can alter the balance between therapeutic benefit and toxic risk. Regulatory agencies therefore require a systematic evaluation of whether anticipated clinical benefits justify potential safety concerns (84,85).

In repurposed indications, toxicological risk may increase due to prolonged exposure, higher systemic concentrations, or use in vulnerable populations such as elderly patients or individuals with comorbidities. Chronic toxicity, cumulative organ damage, and herb–drug interactions become particularly relevant under these conditions. Therefore, risk-benefit evaluation should examine conventional toxicological outcomes in addition to the new safety issues during translational development (3,87).

Case Studies of Repurposed Natural Product-Derived Molecules

Repurposing of natural product-derived molecules has also become a promising approach to expedite the therapeutic development process based on the pharmacological and safety experience. In a number of instances, substances initially created and applied to a single indication be useful in completely new clinical settings. Simultaneously, repurposing activities have also shown the safety restrictions that could not be observed during previous

use. The analysis of success stories and failure cases gives a good understanding of the role toxicological factors play in translational activity and regulation decision-making (1)

Therapeutic Successes and Toxicological Lessons

Certain molecules derived out of natural products have been repurposed successfully due to the fact that their toxic effects could be well managed and safety profiles were adequately known. A good example is the artemisinin and its derivatives. These compounds were originally designed to be used as antimalarial agents, and subsequently to be used as anticancer and antiviral agents. Although preclinical trials provided evidence of issues in terms of neurotoxicity and embryotoxicity at high doses or during long-term exposure, by attentive dosing schedules and duration of exposure, further clinical research was enabled (88,89).

Clinical Failures Due to Safety Limitations

Contrastingly, some of the repurposing efforts on natural product-based molecules have been unsuccessful because the safety issues are unacceptable. A good example is kava (Piper methysticum). Kava was traditionally utilized as an anxiolytic but it was developed as a standardized herbal medication product. Although clinical efficacy was demonstrated, some severe cases of hepatotoxicity were reported and regulatory restrictions and market recall were imposed in several countries. Later studies implied that metabolic bioactivation of herbs, disparities in extraction, and herb-drug interactions were also the cause of liver-damage. This case proved that historical use does not ensure safety in the current patterns of exposure or formulation (90,91).

Emerging Tools in Toxicological Risk Assessment

Some of the recent developments in the field of toxicological science have resulted in the creation of novel tools aimed at enhancing the prediction, interpretation, and translation of natural product-derived molecule-related safety hazards. To improve risk assessment and minimize uncertainty in regulatory decisions, emerging technologies, such as computational and systems toxicology and omics-based safety profiling, provide mechanistic and data-driven models that improve risk assessment (92–94).

Computational and Systems Toxicology

Computational toxicology is a branch of cheminformatics and bioinformatics that uses in silico modeling in prediction of toxicological effects in the basis of chemical structure and biological interaction patterns. The methods are especially useful in the case of natural products since these are commonly characterized by structural complexity and multi-target action. Toxicity potential, metabolic liability and systemic exposures are often estimated by quantitative structureactivity relationship (QSAR) models, molecular docking, and physiologically based pharmacokinetic (PBPK) modelling (1).

Omics-Based Safety Profiling

Toxicological research has been revolutionized by omics based technologies which allow the thorough evaluation of the effects of chemical exposure on the cell at the molecular level.

Transcriptomics, proteomics, metabolomics, and epigenomics are high-resolution techniques to study cellular responses and pathways of toxicity at an initial stage, usually before the apparent tissue injury can be detected (95,96). The methods are especially effective in detecting low or cumulative toxicity related to long-term exposure to natural products.

Challenges and Future Perspectives

Although notable progress has been achieved in toxicological testing of natural product–derived molecules, several challenges continue to limit their safe translation into clinical use. The inherent chemical and biological complexity of natural products, often comprising multiple bioactive constituents, complicates dose attribution, standardization, and inter-study reproducibility, and restricts reliable extrapolation of non-clinical data to human risk. Long-term and cumulative toxicity prediction remains another major concern, as conventional toxicological models are poorly suited to detect delayed effects, low-dose chronic exposure, or mixture-related toxicities common to natural products used over extended periods. These issues are further compounded by inter-individual variability in metabolism, genetic background, and gut microbiota, leading to inconsistent safety outcomes and uncertainty in risk assessment.

In addition, inadequate awareness and systematic evaluation of herb–drug interactions pose significant safety concerns, since many natural products modulate drug-metabolizing enzymes and transporters but are not routinely assessed or reported in clinical settings. Regulatory challenges persist due to the lack of harmonized frameworks tailored to natural product–derived molecules, with variability in raw materials, extraction processes, and formulations complicating safety evaluation and regulatory decision-making. While emerging tools such as computational and systems toxicology and omics-based approaches show promise, their routine regulatory application requires validation and standardization. Future progress will depend on integrating mechanistic toxicology with translational and regulatory science, emphasizing exposure-based risk assessment, early safety evaluation, and human-relevant models, supported by stronger interdisciplinary collaboration to fully and safely realize the therapeutic potential of natural products.

Conclusion

Natural product–derived molecules remain an important source of therapeutics due to their structural diversity and multi-target activity, but their clinical use and repurposing present distinct toxicological challenges. Toxicity is often mechanism-driven, involving target-mediated effects, metabolic bioactivation, oxidative stress, and mitochondrial dysfunction, leading to organ-specific toxicities influenced by dose, exposure pattern, and duration of use. Chronic and cumulative toxicities, herb–drug interactions, and the misconception that natural products are inherently safe further complicate risk management. While repurposing can be successful when toxicological liabilities are well understood and dose-dependent, failures underscore the need for early and systematic safety re-evaluation, exposure-based risk assessment, and regulatory standardization. Integrating mechanistic toxicology, advanced predictive tools, translational models, and regulatory science through a multidisciplinary approach is essential for the safe and responsible advancement of natural products in modern therapeutics.

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Chapter- Ten

A Review on Nanostructured Cochleate: Recent Drug Delivery System for Advanced Therapy

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Abstract

Nanocochleates is a novel particulate drug delivery method that can be used orally, topically or systemically. It can encapsulate both hydrophobic and hydrophilic as well as water-soluble cationic API. Using nanotechnology, medications with poor oral bioavailability and cationic and anionic natures can be encapsulated. Cochleates with nanostructures are employed as a vehicle to deliver hydrophobic or water-insoluble medications with antiviral, anti-inflammatory, and anti-cancer and much more properties. The active agents are encapsulated using nanocochleates technology, which has low permeability, oral bioavailability and solubility. This review article focuses on several cochleate preparation techniques and their mechanism of action, which involves a drug's release into the systemic circulation by macrophagic endocytosis and interactions with receptors, phospholipids, and membrane proteins. Because of nanocochleates have many fewer restrictions than conventional dosage forms and delivery systems, they are a more universally applicable and promising medication for advanced delivery method. So, NC are one of the promising delivery methods in the future to reduces the problems with liposomal stability by encapsulation efficiency of cochleate. Lipids are employed as carriers for the administration of water-insoluble or hydrophobic medications with variety of anti-inflammatory, antiviral, and anti-cancer properties in order to get around the drawbacks of nano-polymeric carriers, such as their non-biodegradability and variable release kinetics.

Keywords: *Cochleate, Cancer therapy, liposomes, phospholipid, peptides.*

Introduction

The second extreme collective cause of death globally is cancer. According to the International Agency for Research on Cancer, around 10.0 million people died from various types of cancer in 2020 [1]. The American Cancer Society states that there will be about 609,360 deaths in the US in 2022, or 1700 deaths every day. Surprisingly, they forecasted the highest mortality rates for females from breast, lung, and colorectal cancer and males from lung, prostate, and colorectal cancer [2].

In both high-income and low- and middle-income countries (LMIC), female breast cancer (BC) is a cancer that is highly prevalent worldwide, with an estimated 2.3 million new cases

and 6.9% fatality rate [1]. Three of the four types of BC are triple-negative. BC is a malignancy that has spread widely and has a poor patient survival rate [3].

Statistics in India about Breast Cancer (BC)

Cancer that starts and spreads in the breast tissues is known as breast cancer. Over 99 percent of all incidences of breast cancer are in women [4]. Rendering to a World-Health Organization(WHO) study, ultimate communal malignancy in women is breast cancer, which is also on the rise in practically every region. In nations with poor and moderate incomes, location, according to World Health Organization (WHO) research. In low- and middle-income countries Breast cancer is the most common cancer among women, and its prevalence is rising in almost every, breast cancer is identified quite late, despite the fact that some risks can be decreased through preventive measures [5]. BC is a significant global public health issue that accounts for about 30% of new cases in women [6] and in the United States alone, there will be approximately 1,735,350 new cases and 2018 American Cancer Society data study found 609,640 deaths, including 266,120 new cases of invasive BC. [7] In addition to women, transsexuals and men are also impacted by BC [8].

Causes of Cancer in India

Around the world, the causes of cancer are similar. The primary variables that contribute to the progression of cancer are various biological, chemical, and environmental factors.[9]

Treatment options for breast cancer and complications

Numerous therapeutic techniques, such as surgery, radiation, immunotherapy, and chemotherapy, are employed to treat BC [10]. Furthermore, certain BCs have become radiation resistant. Although immunotherapeutics are quite expensive, it has been shown that immunotherapy is effective in boosting immunity. Chemotherapy has also been crucial in the treatment of BC. Systemic treatment and different forms of chemotherapy have been approved for almost all forms of BC. Systemic chemotherapy is highly wanted to prevent metastatic BC because there is no specific endocrine treatment that targets the receptors for BC hormones. Many breast tumors, nevertheless, have demonstrated resistance to radiation and chemotherapy. Surprisingly, almost 30% of BC recurred following treatment.

Additionally, several anti-neoplastic medications approved for BC have serious side effects, high prices, and poor anticancer efficacy. The formation and resistance of clones to therapy, the unique heterogeneity of breast tumors, the presence of cancer stem cells, other significant issues with BC treatment include rapid angiogenesis, metastasis, and recurrence after treatment [11].

Oral Drug Delivery System

Several modes of administration, including oral, intravenous (iv), and even subcutaneous (sc.) injection, have helped to mitigate the side effects of anti-cancer medications to a certain degree. The therapeutic modality usually dictates the route of administration. Therefore, for simple compounds, oral administration is favored, but for more complex molecules, such biologics, intravenous administration is preferred. Subcutaneous administration is underrepresented in the development and approval of late-stage cancer medications, despite the fact that it is attractive and inspires continuous preclinical research. Intravenous injections are less variable across and within patients and have a higher bioavailability than the oral method. Nevertheless, there are still issues with this method of drug delivery for cancer treatments, such as the following: Prolonged infusion times are often necessary because of the poor pharmacokinetic and biodistribution properties of the prescription compounds,

such as quick metabolism, rapid excretion, rapid plasmatic clearance, or a short residence time at the tumor site. Targeted therapy is necessary to increase therapeutic efficacy because conventional chemotherapeutic drugs are typically nonspecific and equally dispersed in malignant and normal tissue, leading to serious side effects and off-target damage. [12].
It has been demonstrated that some APIs are not very bioavailable or soluble. Furthermore, they might have low intestinal epithelial permeability and GI tract stability, which would make oral administration impossible [13].

The effectiveness of active substances is limited by their low therapeutic index and potential negative effects on healthy tissues. The main physicochemical characteristic that controls dosage penetration across the barrier's biological membrane is the drug's solubility. Medication penetration and absorption are impacted by drug solubility. The challenges of creating medications with reduced solubility and administering them orally have made drug solubility a problem for formulation scientists. Solubility problems have been found in both new and existing medication candidates, even as pharmaceutical corporations keep growing their clinical and drug development capacities. Nearly 40% of drugs are insoluble in water, according to numerous studies [14]. Similar solubility constraints apply to nearly one-third of all medications listed in the pharmacopoeia. Low water solubility medications hinder proper absorption, which reduces their therapeutic effectiveness [15]. The solubility of novel drug candidates is the main barrier to creating dose delivery systems that are therapeutically efficacious.

Potential benefits of oral anticancer drug delivery

Parenteral administration accounted for a large percentage of cancer treatment until the last several decades. Conversely, the oral route is straightforward, practical, and secure [16]. Some benefits of oral anticancer therapy include the following [17]. Anticancer drugs can continue to affect cancerous cells for a longer amount of time if the drug concentration in the plasma is maintained. There are now additional long-term treatment options for certain cell cycle inhibitors that block signal transduction and angiogenesis. To satisfy requirements, the way the medication is released from the dosage form may also be changed.

The most effective way to administer medications is through co-administration, which either gets beyond biological barriers or changes the physicochemical characteristics of the drug to make it easier to absorb. High solubility, encapsulation effectiveness, modified absorption pathways, prevention of metabolic degradation, and the abundance of materials available for drug preparation, design, and targeting, as well as surface functionalization, are the main advantages of nanoparticulate delivery systems. [18]

Nanoparticulate DDS

Drug delivery research has made significant strides in the creation of nano drug delivery devices over the last century. The new establishment has been made possible in large part by advancements in a variety of drug delivery techniques both in the scientific and educational domains as well as in the industrial and commercial administrations. The creation of data was primarily the result of a groundbreaking innovation that integrated several patented studies and academic publications from all around the world. Nanoparticle drug delivery systems are technologically advanced devices that use nanoparticles to precisely and precisely administer medicinal drugs.

A contemporary medication delivery system should minimize adverse effects while lowering frequency and dosage. Because of their potential for drug delivery, nanoparticles are very valuable [19]. The goal of drug delivery using nanoparticles is to reduce cytotoxicity and increase pharmacological efficacy. When modifying nanoparticle properties for effective

medication delivery, the following problems must be fixed. The nanoparticles' surface-area-to-volume ratio can be altered to encourage better ligand binding to the surface[20]. A higher ligand binding efficiency can reduce the toxicity of nanoparticles and lower dosage. Efficiency is increased when the mass of nanoparticles per mass of drug lowers due to a drop in dosage or frequency.

The short duration of action, poor biodistribution, limited efficacy, and lack of selectivity are some of the disadvantages of traditional pharmacological therapy. [21].

Advantages of nanoparticulate drug delivery system

Nanotechnology-based controlled and targeted drug delivery technologies get around the limitations of conventional medicine therapy by precisely delivering an active component via nanocarriers to spot of action, exploiting therapeutic effectiveness also removing adversative side effects.

Additionally, by hindering rapid clearance or degradation, nanocarriers can improve the API's solubility plus pharmacokinetic outline, prolong the medication's residence time, prevent dosage dumping by controlling the drug release profile, and increase its bioavailability. As the medication becomes more effective, patient compliance increases. [22]

Types of nanoparticulate drug delivery system

As possible drug delivery systems for the management of particular illnesses, specific nanoparticulate delivery techniques have been extensively researched. These delivery systems include, but are not limited to, Nanogels, micelles, liposomes, dendrimers, nanocapsules, nanofibers, nanotubes, solid lipid nanoparticles, metal nanoparticles, polymeric nanoparticles, and nanogels [23].

One significant application of nanoparticles is their use as carriers for anticancer therapies. Following intravenous injection, nanoparticles showed a notable propensity to assemble in a range of malignancies. This behavior has been connected to the frequently seen increase in endocytic activity in cancer cells. Additionally, it has been demonstrated that certain malignancies exhibit elevated vascular permeability, which may boost the accessibility of nanoparticles to tumor cells outside of the bloodstream.[24]

API in its free form was less effective against tumors than anticancer medications like 5-fluorouracil, doxorubicin, methotrexate, and dactinomycin; immonomodulators (muramyl dipeptide-L-analylcholesterol); and antisense oligonucleotides attached to polyalkylcyanoacrylaete (PACA) or albumin nanoparticles. Doxorubicin was chemically bonded to the PLGA terminal end group to create nanoparticles that released continuously for one month. Furthermore, that was discovered that the released doxorubicin fraction of exhibited cytotoxicity equivalent to that of free API [25]. One of the best anticancer medications is paclitaxel, which is present in nature. It was challenging to maintain the drug's effectiveness due to its poor permeability and solubility, which are connected to the efflux of mucosal P-glycoprotein.

A PLGA nanoparticle formulation with an emulsifier, TPGS was one such vitamin E-developed in order to remedy this medication's shortcoming [26]. To enhance the medication's size distribution, encapsulation, and percentage release, tocopheryl polyethylene glycol 1000 succinate was added [27]. Treatment of hepatic metastases is a major use of drug-loaded nanoparticles in cancer therapy. Kuppfer cells are primarily responsible for the uptake of intravenous nanoparticles. These cells then serve as a drug reservoir, enabling widespread and sustained drug diffusion into nearby neoplastic cells. In addition to passive targeting, it has been demonstrated that adding magnetic particles to albumin nanoparticles and then using electromagnetic guidance to guide them increases the

antimetastatic capability of the anticancer drug carrier by making extravascular tumors more accessible [28].

Liposomes/Nanoliposomes

A drug delivery method that employs a range of biomaterials, including biodegradable nanoparticles, is necessary in this situation. Thus, liposomes are considered a breakthrough method for targeted drug administration. Liposomes can be prepared using a variety of methods[29]. Either the drug's molecular structure is altered or it is incorporated into the carrier network employing cutting-edge drug delivery technology to achieve distribution efficiency. The words "liposome" are derived from the Greek words "soma" (body) and "lipos" (fat)[30]. The vesicle known as a liposome is composed of the same substance as a cell membrane. Among these, head-and-tail molecules called phospholipids are common. Tail, which was prepared by extended chain of hydro-carbons, is hydrophobic, whereas the head is hydrophilic. Phospholipids are typically found in bilayers.

The main factor propelling liposome research forward is its capacity to replicate biological cells. Because of their high biocompatibility, liposomes are an ideal choice for a drug delivery method. Adjuvants, enzymes, fungicides, antiviral, antibacterial, and antiparasite drugs, as well as transdermal vaccination carriers and diagnostic instruments, can all be made from them. Nowadays, antifungal and anticancer medications based on liposomes are sold commercially. [31].

Structural Components of Liposome:

Phospholipids and cholesterol make up the majority of liposomes. The behavior of liposomes is determined by the chemical characteristics of these lipids.

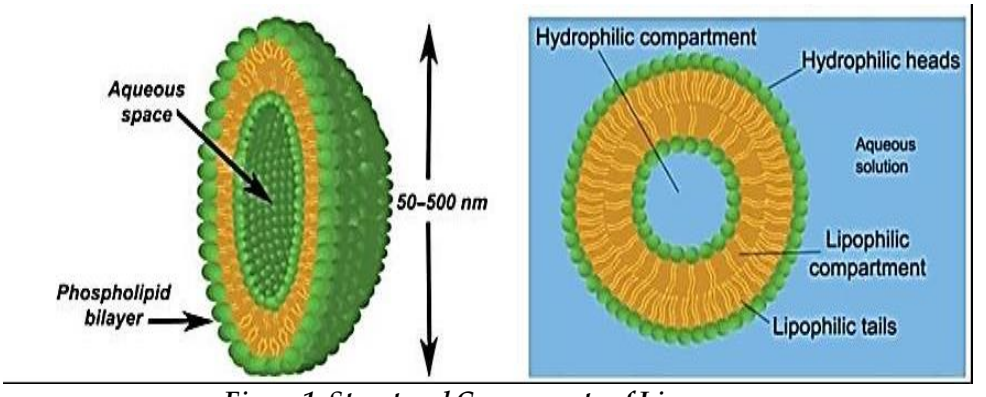

Figure 1: Structural Components of Liposome

Phospholipids

Synthetic phosphatidylcholine (PC) and natural phosphatidylcholine (egg or soy) are the two phospholipids most frequently used for liposome production. The large levels of polyunsaturated fatty acids in natural phospholipids, like those in eggs or soybeans, make them less stable than their synthetic equivalents.

2-Distearoyl-sn-glycerophosphocholine (DSPS) is the phospholipid most frequently used in liposomes. This molecule consists of a hydrophobic portion composed of hydrocarbon chains and a polar phosphate head group.The polar head comprises the outer layer of the liposome bilayer, while the hydrocarbon chains comprise the interior layer. A functional group can be attached to change the head section.[32]

Cholesterol

The primary ingredient added to liposomal formulations to stabilize the liposome bilayer is cholesterol. Since liposomes' membrane fluidity, elasticity, permeability, and stability are all facilitated by cholesterol, its molar percentage of the total components of liposomes fluctuates. Between 30 and 45 percent, contingent on the bilayer's fluidity and stiffness. A lipid bilayer contains the polar heads of cholesterol and phospholipids. Because it is hydrophobic, cholesterol fills in the gaps created by improperly packed phospholipid molecules and works well inside lipid bilayers. Transport across membranes and the flip-flop of membrane components are shielded by cholesterol packing in phospholipid bilayers.[33]

Method of Preparation

A. MECHANICAL DISPERSION METHODS

1. Thin Film Hydration:

Although it is the easier method of making liposomes, its low encapsulation efficiency limits its use. By shaking thin lipid films composed of a glass wall covered with an organic solution at temperatures above the transition temperature (Tc), this technique creates liposomes. Evaporation completely removes the solvent in a rotating evaporator while reducing pressure. A dry layer of lipids forms on the circular bottom of the flask, and a buffer is added to hydrate it.

Solution containing a water-soluble marker. Only a tiny portion of the solute is trapped when the lipid gets hydrated and begins to form closed vesicles. A population of MLVs with a diameter larger than 1μm and a range of sizes was generated using this technique. A sonicator is then used to shape the generated liposome into an SUV.[34]

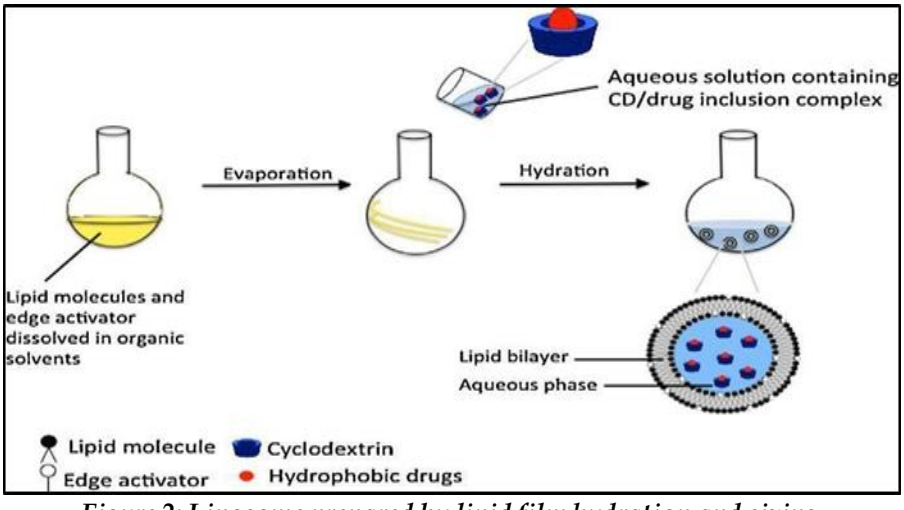

Figure 2: Liposome prepared by lipid film hydration and sizing.

Sonication:

This action reduces the size of the vesicles while providing energy to the lipid solution. This can occur by exposing the MLV to ultrasonic radiation. There are two sonication techniques. The sonicators for the bath (a) and the probe (b). The majority of suspensions that need a lot of energy in a small area are treated with probe sonication. Large volumes of diluted lipids

are the primary use for the bath sonicator. The probe sonicator's drawback is that metal from the probe tip may contaminate preparation.
Tiny unilamellar vesicles are produced by this method and subsequently purified by ultra-centrifugation.[35]

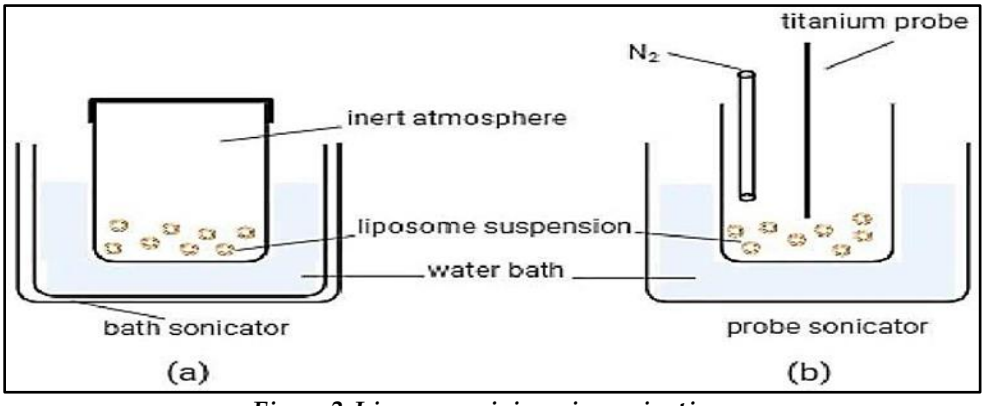

Figure 3: Liposome sizing via sonication.

French Pressure Cell Method:
MLV is extruded via a small hole at 40°C and 20,000 pressure in this procedure. The method offers many advantages over the sonication approach. In addition to being quick, easy, and repeatable, this process also involves treating unstable materials gently. Compared to sonicated SUVs, the produced liposomes are a little bigger. The relatively modest working volumes (limit of 50 mL) and the challenge of reaching the temperature are the method's shortcomings. rapid, reproducible, and entails delicately managing unstable materials. Compared to sonicated SUVs, the resultant liposomes are a little bigger. The difficult temperature control and extremely tiny working volumes (maximum of 50 mL) of this approach are its disadvantages.[36]

Micro-emulsification of liposome:
A device called a micro fluidizer is primarily used to create small vesicles from concentrated lipid suspension. It is possible to add the lipids as a large MLV suspension to the fluidizer. This device uses very high pressure to pump the fluid via five-micron screens. Following that, two streams of fluids guided by long microchannels collide at right angles at a very high speed.[37]

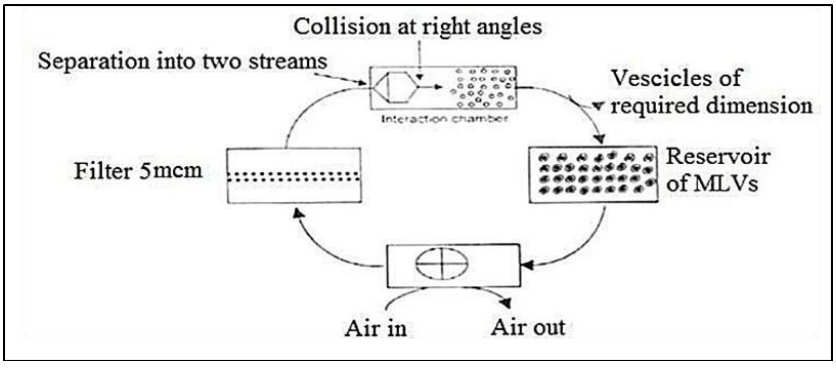

Figure 4: Liposome prepared by Micro-emulsification method.

Membrane Extrusion Liposome:

By passing them via a membrane filter that has a predetermined pore size, this procedure shrinks the size. Membrane filters come in two varieties as the twisting path nature and the nucleation way type. Sterile filtering is done with the former. There are cross fibers in this random path.

The fiber density within the matrix establishes the fibers' average diameter. Liposomes that try to pass through such a membrane but are bigger than the channel diameter are affected. A thin, continuous polycarbonate sheet makes up this kind of nucleation track. They will provide less of a barrier to liposome passage since their pore holes are straight-sided, exactly the same width, and bored from one side to the other. [38]

Freeze Thawed Liposome:

SUVs freeze quickly and then gradually defrost. The collected components are dispersed to LUV by the brief sonication. Unilamellar vesicles are created when SUVs unite during the freezing and thawing processes. Increasing the medium's ionic strength and phospholipid concentration are the primary methods for preventing this kind of development. 20% to 30% encapsulation efficacies were discovered.[39]

B. **Solvent-Dispersion Scheme**

1. Ether Inoculation (Solvent Evaporate)

At temperatures between 550°C and 650°C, or at lower pressure, a lipid solution diluted with an aque mixture of material to be captured is progressively mixed by means of ether (diethyl) or an ether & methanol blend.

Liposomes are formed once the ether was completely detached beneath vacuum. The populace's heterogeneity (from 70 to 200 nm) as well as the compounds' exposure to organic solvents at high temperatures are the procedure's primary drawbacks. [40]

2. Ethanol Injects

Significant extent of buffer is speedily mixed through a solution of lipid-ethanol. The MLV's were produced in real time.

The method's shortcomings include the high dilution of the liposomes and the heterogeneity of the population (30–110 nm). Since ethanol and water mix to form an azeotrope, it is difficult to completely eliminate it, and even trace amounts of ethanol are likely to render the various physiologically active macromolecules inactive.[41]

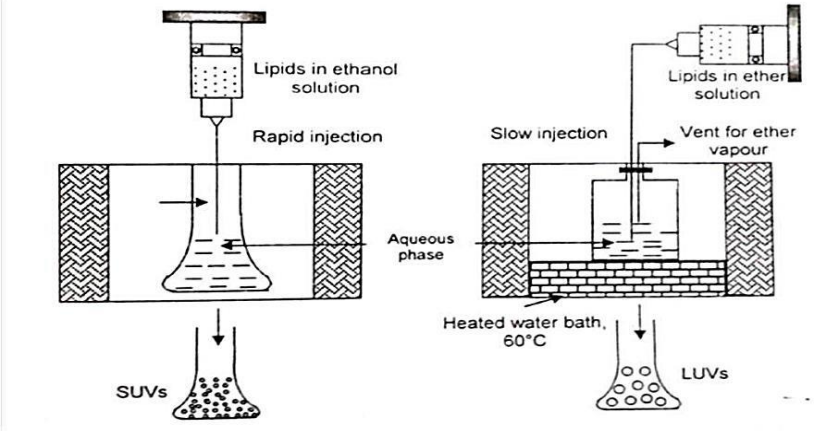

Figure 5: Liposome by i) Ethanol inject way. ii) Ether injecting way.

3. Reverse phase evaporating system:
A significant extent of buffer was speedily mixed through solution of ethanol as a lipid. The MLV's were produced in real time. The method's shortcomings include the high dilution of the liposomes and the heterogeneity of the population (30–110 nm). Since ethanol and water mix to form an azeotrope, it is difficult to completely eliminate it, and even trace amounts of ethanol are likely to render the various physiologically active macromolecules inactive. Up to 65% encapsulation efficacy can be achieved using this technique in medium by a small ionic strength, such as Nacl (0.01 M).

This way can be taken to capture macro-molecules of any size. The main disadvantages of this approach are those the constituents are encapsulated which was exposed toward organic solvents plus short-term sonication times.[42]

Detergent removal Technique:
Detergent dissolves lipids into crucial micelles conc. As the detergent is gradually removed, the micelles increase in phospholipid eventually combine to create large unilamellar vesicles (LUVs). The detergent can be effectively eliminated through dialysis. High reproducibility and the production of liposome populations with consistent sizes are two advantages of the detergent dialysis technique. The persistence of detergent residues within liposomes is the primary drawback of this strategy. In order to eradicate detergents, a Commercial dialysis equipment, called LIPOPREP, is available. Additional methods for eliminating Among the detergents are: (a) using a Sephadex G-25 column in gel chromatography; (b) binding or absorbing and (c) affixing the detergent octyl glucoside beads.[43]

NANOCOCHLEATES DDS

Nanocochleate, a drug delivery system derived from liposomes, is a novel technological approach that involves entrapment or microencapsulation of the desired drug molecule into multilayered assemblies made of divalent cations without an internal aqueous phase and negatively charged phospholipid sheets rolled up in a spiral shape. Unlike liposomes, cochleates are stiff and water-free. [44] This makes it appropriate for the delivery of mostly hydrophobic medications with low bioavailability, such as clofazimine, amphotericin B, and doxorubicin.[45] Consequently, it offers a possible way to provide a broad range of medicines, as like peptides and proteins, anti-inflammatory preparations (steroidal), anesthetics, antivirals, and antitumor immunosuppressive, and tranquilizers, among others. [46] In 1975, Dr. Dimitrious Papahadjoupoulos made the discovery of cochleates. This was called "cochleate" because of its cylindrical shape, which means it was rolled up.[47] Cochleates are composed of a number of solid layers and are water-free, thus when they are exposed to hostile environments or enzymes, the encapsulated drug molecules are unaffected and remain intact inside.[48]

Components of Nanocochleate Drug Delivery System[49]

Lipids:
The study utilized various phospholipids, including Di-Myristoyl Phosphatidyl Choline (DMPC), Di-Oleoyl Phosphatidyl Serine (DOPS), Di-Stearoyl Phosphatidyl Serine (DSPS), Di-Phosphatidyl Glycerol (DPG), Dioleoyl Phosphatidyl Glycerol (DOPA), and Di-Palmitoyl Phosphatidyl Glycerol (DPPG).

Cations:
The divalent cations employed were calcium (Ca^{2+}), zinc (Zn^{2+}), magnesium (Mg^{2+}), and barium (Ba^{2+}).

Techniques of Preparation
The nanocochleates are usually prepared by following methods

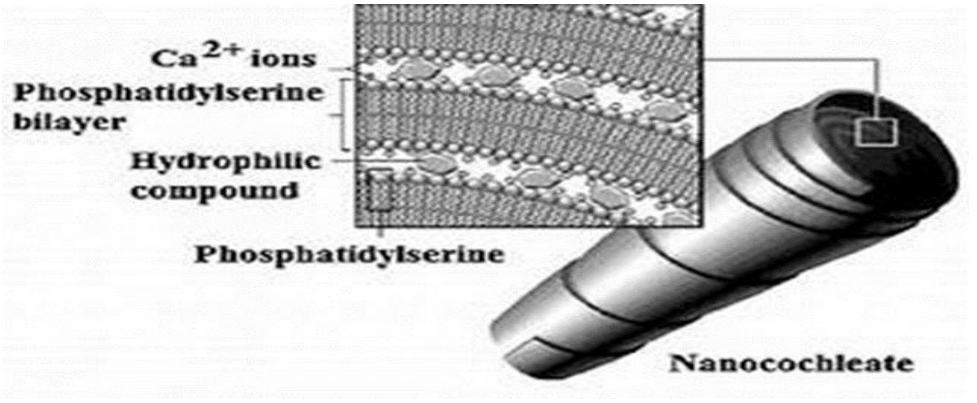

Figure 6: Schematic representation of nanocochleate formulation Trapping Method

1. Trapping method
To create an aggregate of cochleates, a liposome suspension is prepared with either a hydrophilic drug in the liposome's aqueous space or a hydrophobic drug intercalated between the bilayers. A solution of CaCl2 is then added dropwise. The cochleates produced using the trapping method show greater aggregation than those produced using earlier techniques.[50]

2. Hydrogel Method
Initially, minor unilamellar liposomes containing the drug are prepared through the hydrogel method, which involves processes such as sonication, microfluidization, or other suitable approaches. Those liposomes subsequently combined with polymer-A, as like phosphatidylserine, polyethylene glycol, or dextran (mol. wt. -200,000-500,000). Polyvinyl alcohol, polyvinyl pyrrolidone, and polyvinyl methyl ether are examples of polymer-B. These are combined with the liposome/polymer that was previously created. a combination that is produced by the mutual immiscibility of the polymers to form a watery two-phase polymer system. In order to create more small-size cochleate, a cation salt solution is added to a 2-phase scheme. This causes cationic cross-linkage of the polymer, which allows the cation to enter the second polymer and subsequently the polymer particles. Re-suspending the polymer in physiological buffer will eliminate it. Cochleate precipitates are frequently cleaned. [51]

3. The System of Binary Aqueous-Aqueous Emulsion
This method involves employing a film approach or a high pH to create microscopic liposomes, which are then combined with a polymer, such as dextran. Dextran can progressively move from one phase to another by combining this system with a second polymer, resulting in nanocochleates with particles smaller than 1000 nm.[52]

4. Solvent Drip Method

This technique uses a hydrophobic or amphipathic cargo moiety solution combined with soy PS to produce a liposomal suspension. DMSO or DMF are the solvents chosen for hydrophobic drugs. It is noteworthy that after adding the solution to the liposomal suspension, the cargo moiety's solubility decreases since the solvent is miscible in water. Calcium is then added to produce the cochleates, and any excess solvent is subsequently washed away.[53]

5. Liposomes before Cochleates Dialysis Method

This method starts with a lipid and detergent mixture, and the detergent is then eliminated via double dialysis.

Detergent plays a role in liposome disruption. Polymer A is treated with a mixture of lipid and detergent, same like in the hydrogel approach. Polymer B is then treated with cationic salt to create cochleates, and the polymer is removed by washing.[54]

6. Direct Calcium Dialysis Method

This technique produces larger cochleates and eliminates the requirement for intermediary liposome synthesis, in contrast to the liposome-pre-cochleate dialysis strategy. This method involves dialyzing a mixture of lipids and detergents directly against a solution of calcium chloride. Calcium's condensation of bilayers and detergent's exclusion from the detergent/lipid/drug micelles compete to form large, needle-shaped structures.[55] In non-ionic extraction buffer, phosphotidylserine and cholesterol (9:1 wt/wt) are mixed together. After adding detergent and a preset concentration of polynucleotide, the mixture is vortexed for five minutes. Three buffer changes are then applied to the resultant transparent, colorless solution that is dialyzed at room temperature after that.

The last dialysis solution that is commonly used is 6 mM Ca2+. The dialyzed to buffer ratio needs to be at least 1:100 for each change. The white calcium-phospholipid precipitates that form are known as direct calcium cochleates.[56]

Benefits of the Nanocochleate DDS

- Since nanocochleates don't contain oxygen, their lipids are less prone to oxidation, making them more stable than liposomes.
- While lyophilization damages the architecture of liposomes, they retain their structure even after lyophilization.
- The release of biological molecules can be controlled or slowed by nanocochleates as they gradually unwind or dissolve in different ways.
- Additionally, they are able to incorporate biological molecules enters the cochleate structure's lipid bilayer, namely those with hydrophobic moieties.
- **It shields the active medication from deterioration by preventing direct exposure to extreme environmental factors like oxygen, water, sunshine, and temperature. [57]**

Disadvantages

- They require particular storage circumstances.
- Aggregation inhibitors can be used to stop aggregation, which can happen during storage over time. The cost of manufacturing is high. [58]

Cochleate Cell Interaction and Influence of Shape

Using laser scanning confocal fluorescence microscopy, a group of writers conducted a thorough investigation on the location of choline and phosphatidylcholine fluorescent derivatives in cancer cells. [59] In contrast to the cells incubated with the liposome, the cells

exposed to the cochleate exhibit bright luminous cell surfaces. This is shown when human fibroblast cells are cultivated under the identical conditions with liposomes containing the same fluorescently tagged lipid component and cochleates that are nanometers in size.This suggests that cochleates may combine with cell surfaces due to their edge, unlike edge-free liposomes.

The impact of nanoparticle shape on adhesion kinetics was also investigated by a few research groups. In this experiment, shear flow and Brownian forces are used to impact the free movement of nanoparticles across the simulated channel. The investigation found that during its passage through the channel, the spherical particle made no contact with the vessel wall. Small Brownian diffusion to initiate bonding is the cause of this. The non-spherical, rod-shaped particle's notable tumbling motion as it passes through the channel causes it to frequently attach to the vessel wall.[60]

Mechanism of Drug Release from Nanocochleates

After oral delivery, the intestines absorb the nanocochleates. The active medication molecule is transported into blood vessels by nanocochleates after they pass through the intestinal epithelium.

Delivery following Membrane Fusion and Phagocytosis

It is well known that membrane phosphatidylserine (PS) receptors are present in neutrophils and macrophages which cause nanocochleate to be phagocytosed. After that, nanocochleate closely resembles a lysosome membrane. A fusion event between the lysosome membrane and the outer layer of the nanocochleate results from additional lysosomal membrane disruption and reorganization. A small amount of the encochleated material is delivered into the target cell's cytoplasm as a result of this fusion.[61]

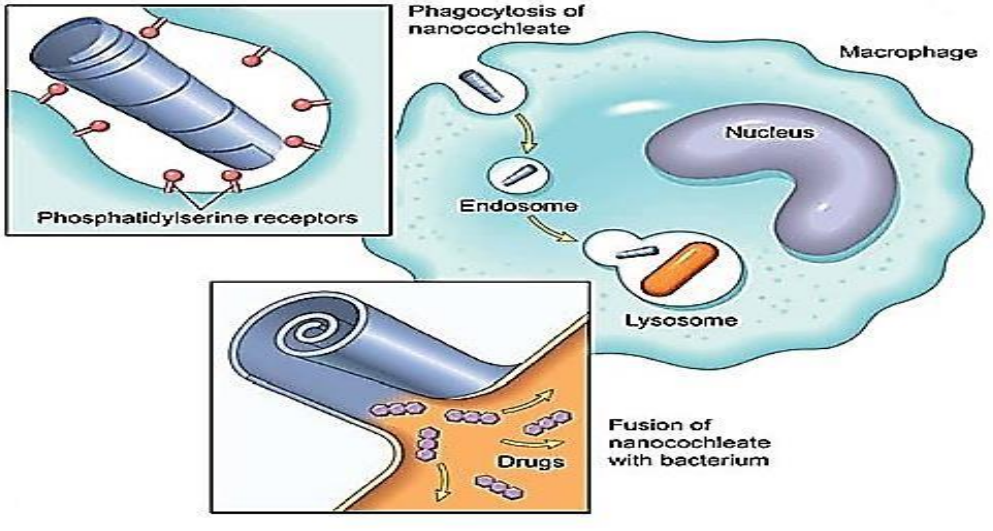

Figure 7: Membrane Fusion Applications of Nanocochleates

Applications of Nanocochleates

1. Nanocochleates based on Apo-A1 show promise in treating atherosclerosis and other cardiac conditions.

2. In addition to stabilizing and preserving a wide range of micronutrients, nanochleates can raise the nutritional value of processed foods.
3. Nutritional components like vitamins and omega fatty acids can be efficiently delivered by nanochleates.
4. The food business can also use it. It utilized to provide lycopene without changing the food's color or flavor.[62]

Conclusion

A Nanocochleates are novel drug delivery system approach broadly applicable to encapsulation of biologically important molecules such as genes, vaccine, antigen, and also encapsulate both hydrophilic and hydrophobic drugs. Encochleation helps to improve the efficiency of the product because of the accurate delivery increases the quality of the formulation which also enhances bioavailability and increase the therapeutic efficacy of therapeutically active agent. Nanocochleate are promising for oral, transdermal and dermal drug delivery. Many more drug candidates can be encapsulated into Nanocochleates for getting their effective delivery.

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Chapter- Eleven

Drug Repurposing: Strategies, Methodologies, and Emerging Therapeutic Innovations

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Abstract

Drug repurposing, also referred to as drug repositioning or therapeutic switching, is a strategic approach aimed at identifying novel therapeutic indications for existing drugs, including approved, investigational, or discontinued molecules. Conventional drug discovery is associated with prolonged development timelines, high research and development costs, and significant failure rates, particularly during late-stage clinical trials. Drug repurposing addresses these challenges by leveraging prior knowledge of pharmacokinetics, safety, toxicity, and manufacturing processes, thereby substantially reducing development time and economic burden. The present chapter provides a structured and comprehensive overview of drug repurposing based on well-established strategies and methodologies. The introduction outlines the rationale and growing importance of repurposing in modern pharmaceutical sciences. The methods section, derived from established conceptual frameworks, describes on-target and off-target repurposing strategies, experimental and in-silico approaches, methodological classifications, and key databases used in the repurposing process. Recent advances, including artificial intelligence, network pharmacology, and multi-omics integration, are incorporated to reflect current trends. This chapter emphasizes drug repurposing as a rational, efficient, and sustainable approach to accelerate therapeutic innovation and address unmet medical needs.

Keywords: *Drug repurposing; Drug repositioning; On-target repurposing; Off-target repurposing; In-silico drug discovery; Pharmaceutical sciences.*

Introduction

The process of drug discovery and development is widely recognized as one of the most complex, time-consuming, and resource-intensive undertakings in pharmaceutical sciences. Despite remarkable advances in molecular biology, medicinal chemistry, genomics, and high-throughput screening technologies, the overall productivity of conventional drug discovery pipelines has declined over the past few decades. On average, the development of a new chemical entity from target identification to market approval requires approximately 10–15 years and is associated with an estimated cost exceeding one billion US dollars [1,2]. Furthermore, only a small fraction of candidate molecules entering clinical trials ultimately receive regulatory approval, with the highest attrition rates observed during Phase II and Phase III clinical studies due to lack of efficacy or unexpected safety concerns.

These challenges have prompted both the pharmaceutical industry and academic research institutions to explore alternative strategies capable of reducing development

timelines, minimising financial risk, and improving success rates. Among these strategies, drug repurposing—also referred to as drug repositioning or therapeutic switching—has emerged as a particularly attractive and pragmatic approach. Drug repurposing involves the identification of new therapeutic indications for existing drugs, including approved drugs, investigational compounds, and molecules that were discontinued during development for reasons unrelated to safety [3]. By leveraging existing knowledge of pharmacokinetics, toxicology, formulation, and clinical safety, drug repurposing significantly reduces uncertainty in the development process.

The conceptual basis of drug repurposing aligns strongly with the principles of rational drug design and translational research. Unlike de novo drug discovery, which begins with limited biological and chemical information, repurposing efforts start with compounds that already possess well-characterized physicochemical properties and human exposure data. This advantage not only shortens development timelines but also enhances the probability of regulatory success [4]. Consequently, drug repurposing has gained increasing attention as a cost-effective and efficient strategy to address unmet medical needs, particularly in therapeutic areas characterised by high failure rates.

Historically, many successful examples of drug repurposing were discovered serendipitously through clinical observation rather than systematic investigation. A classic example is aspirin, initially developed as an analgesic and anti-inflammatory agent, which was later repurposed for cardiovascular disease prevention due to its antiplatelet activity and is currently being explored for potential anticancer applications [5]. Similarly, minoxidil, originally approved as an oral antihypertensive agent, was repositioned for the treatment of androgenic alopecia following observations of increased hair growth in patients receiving the drug [6]. These examples, which are also highlighted in foundational teaching frameworks, clearly demonstrate the translational potential of repurposing strategies.

While early repurposing discoveries relied largely on chance, contemporary drug repurposing has evolved into a structured and hypothesis-driven discipline. Advances in bioinformatics, cheminformatics, systems biology, and computational chemistry have enabled systematic identification of drug–target and drug–disease relationships [7]. The availability of large-scale biomedical databases containing chemical, biological, genomic, and clinical information has further facilitated data-driven repurposing efforts. Resources such as DrugBank, ChEMBL, PubChem, ClinicalTrials.gov, KEGG, and OMIM provide curated datasets that support comprehensive analysis of drug properties, molecular targets, biological pathways, and clinical outcomes [8–10].

The growing importance of drug repurposing is also driven by the increasing burden of complex and multifactorial diseases. Chronic conditions such as cancer, neurodegenerative disorders, metabolic diseases, and cardiovascular disorders involve multiple molecular pathways, making them particularly challenging targets for single-target drugs developed through traditional approaches. Drug repurposing offers the opportunity to identify multi-target drugs or drugs with pleiotropic effects that may be better suited for such complex disease states [11]. In addition, repurposing plays a critical role in the management of rare and neglected diseases, where limited commercial incentives often restrict investment in de novo drug development.

Global health emergencies have further highlighted the strategic value of drug repurposing. During outbreaks of emerging infectious diseases, including the COVID-19 pandemic, repurposing strategies enabled rapid evaluation of approved drugs and clinical candidates for antiviral and host-directed therapeutic effects [12]. Although not all repurposed candidates demonstrated clinical efficacy, these efforts underscored the ability of repurposing frameworks to generate rapid therapeutic hypotheses in crises. As a result,

regulatory agencies and funding bodies have increasingly recognised drug repurposing as a vital component of emergency preparedness and response strategies.

Recent advances in artificial intelligence (AI) and machine learning (ML) have further transformed the landscape of drug repurposing. AI-driven models integrate chemical structures, bioactivity data, transcriptomic signatures, and clinical records to predict novel drug–disease associations with improved accuracy [13–15]. Network pharmacology approaches complement these methods by modelling diseases as perturbations of complex biological networks rather than isolated targets. Together, these technologies have shifted drug repurposing from a largely empirical practice to a data-intensive and predictive science.
In addition to scientific and technological drivers, regulatory and economic factors have contributed to the growing adoption of drug repurposing. Regulatory pathways such as the United States Food and Drug Administration (FDA) 505(b)(2) application allow sponsors to rely partly on existing data for previously approved drugs, thereby reducing the scope of additional studies required for new indications [16]. Similar regulatory mechanisms exist within the European Medicines Agency framework. These pathways provide a structured route for the clinical development and approval of repurposed drugs, further enhancing their commercial and translational feasibility.

Taken together, these factors position drug repurposing as a central strategy in modern pharmaceutical sciences. The systematic integration of experimental validation, computational modelling, and regulatory support has transformed repurposing into a mature and impactful discipline. This chapter builds upon established conceptual frameworks—commonly used in pharmaceutical education and research—to present a structured overview of drug repurposing strategies, methodologies, and outcomes. By combining foundational principles with recent advances reported between 2020 and 2025, the chapter aims to provide a comprehensive understanding of drug repurposing as a sustainable and innovative pathway for drug development.

Methods

The methodology of drug repurposing is based on a structured and systematic framework designed to identify new therapeutic indications for existing drug molecules. Unlike conventional drug discovery, which begins with target identification followed by lead optimisation and extensive preclinical testing, drug repurposing methodologies leverage prior knowledge of drug safety, pharmacokinetics, pharmacodynamics, and clinical usage. This significantly reduces development time, cost, and uncertainty while improving translational success [17,18]. The methodological framework adopted in this chapter is derived from established educational and research models in pharmaceutical sciences and integrates strategic classification, experimental validation, in-silico prediction, methodological orientation, and data-driven integration.

Strategic Classification of Drug Repurposing

Drug repurposing strategies are broadly classified into on-target and off-target repurposing. This classification provides a mechanistic foundation for understanding how existing drugs can be redirected toward new therapeutic applications and is widely accepted in repurposing research frameworks [19,20].

On-target drug repurposing involves applying the known pharmacological mechanism of a drug to a new disease indication that involves the same molecular target. In this approach,

the biological target remains unchanged, while the clinical application differs. On-target repurposing is particularly effective when a target plays a role in multiple disease pathways or physiological processes. Because the mechanism of action is already well characterised, on-target repurposing generally requires fewer additional mechanistic studies and is associated with higher regulatory acceptance [21].

In contrast, off-target drug repurposing identifies novel therapeutic effects mediated through biological targets or pathways different from the drug's original mechanism of action. Off-target effects may arise from secondary binding interactions, modulation of signalling cascades, or pleiotropic biological responses. While off-target repurposing often leads to unexpected therapeutic opportunities, it typically requires more extensive validation to ensure safety and efficacy in the new indication [22].

Experimental Approaches in Drug Repurposing

Experimental or activity-based repurposing approaches rely on biological assays to identify new pharmacological activities of existing drugs without prior assumptions regarding molecular targets. These approaches are particularly useful for uncovering complex or multi-target effects and are well-suited for diseases with poorly understood molecular mechanisms [23].

Target-Based Screening

Target-based screening evaluates interactions between approved or investigational drugs and specific disease-relevant molecular targets, such as enzymes, receptors, ion channels, or transporters. This approach is hypothesis-driven and is most effective when the target has been well validated in disease pathophysiology. High-throughput biochemical assays are commonly employed to screen drug libraries against selected targets, enabling rapid identification of potential repurposing candidates [24].

Cell-Based and Phenotypic Screening

Cell-based and phenotypic screening approaches assess the effects of drugs on cellular functions without prior knowledge of specific molecular targets. These assays capture integrated biological responses, including alterations in cell morphology, proliferation, differentiation, apoptosis, and intracellular signaling. Phenotypic screening is particularly valuable for identifying off-target effects and drugs with pleiotropic activity, making it highly relevant for oncology, neurological disorders, and infectious diseases [25,26].

In Vivo Models

Animal models play a critical role in validating repurposing candidates identified through in vitro or computational approaches. In vivo studies provide essential information on pharmacokinetics, pharmacodynamics, tissue distribution, efficacy, and toxicity within a whole-organism context. Although resource-intensive, animal models remain indispensable for assessing translational relevance before clinical evaluation [27].

Clinical and Observational Studies

Clinical observations and retrospective analyses of patient data represent an important experimental approach in drug repurposing. Unexpected therapeutic benefits observed during routine clinical use often generate repurposing hypotheses. Increasing availability of real-world evidence from electronic health records, insurance databases, and patient registries has further strengthened the role of observational studies in repurposing research [28].

In-Silico Approaches in Drug Repurposing
In-silico drug repurposing employs computational tools to predict drug–target and drug–disease associations. These approaches offer significant advantages in terms of speed, scalability, and cost-effectiveness and have become central to modern repurposing pipelines [29].

Molecular Docking and Virtual Screening
Molecular docking predicts the binding orientation and affinity of drugs to molecular targets using three-dimensional structural information. Virtual screening enables rapid evaluation of large drug libraries against disease-associated targets, allowing prioritisation of candidates for experimental validation. These techniques are widely used in early-stage repurposing studies [30].

Ligand-Based Similarity Approaches
Ligand-based methods identify repurposing candidates based on chemical similarity to known active compounds. These approaches assume that structurally similar molecules may exhibit similar biological activities and are particularly useful when target structural data are unavailable [31].

Network Pharmacology
Network pharmacology models biological systems as interconnected networks of genes, proteins, pathways, and diseases. This approach recognises that drugs often exert therapeutic effects through the modulation of multiple targets rather than single molecular entities. Network-based analysis is especially relevant for complex and multifactorial diseases such as cancer, neurodegenerative disorders, and metabolic syndromes [32].

Artificial Intelligence and Machine Learning
Recent advances in artificial intelligence and machine learning have significantly enhanced in-silico drug repurposing. AI-driven models integrate chemical structures, bioactivity data, transcriptomic profiles, and clinical information to predict novel drug–disease associations. Deep learning and graph-based approaches have demonstrated improved predictive accuracy and translational relevance in repurposing studies published between 2020 and 2025 [33–35].

Methodological Orientation of Drug Repurposing
Drug repurposing methodologies can also be categorised based on their primary orientation, as given in Table 2:

Drug-Oriented Approaches
Drug-oriented approaches focus on intrinsic properties of drugs, including chemical structure, known pharmacological activities, adverse effect profiles, and pharmacokinetic characteristics. Side-effect similarity analysis and chemical fingerprinting are commonly employed techniques in this category [36].

Target-Oriented Approaches
Target-oriented approaches prioritise disease-relevant molecular targets and identify existing drugs capable of modulating these targets. These methods are particularly effective when disease biology is well understood, and suitable targets have been validated [37].

Disease-Oriented Approaches
Disease-oriented approaches integrate genomic, proteomic, metabolomic, and transcriptomic data to identify drugs capable of reversing disease-specific molecular signatures. Signature matching between disease states and drug-induced gene expression profiles is a widely used strategy within this framework [38,39].

Databases and Data Resources Supporting Drug Repurposing
The effectiveness of drug repurposing methodologies is strongly supported by the availability of comprehensive biomedical databases. Resources such as DrugBank, ChEMBL, PubChem, ClinicalTrials.gov, KEGG, and OMIM provide curated information on drug properties, molecular targets, biological pathways, and clinical outcomes [40–43]. Integration of these databases enables systematic data mining, hypothesis generation, and validation across both experimental and computational repurposing studies.

Integration of Hybrid Repurposing Approaches
Modern drug repurposing increasingly relies on hybrid approaches that combine in-silico prediction with experimental validation. Computational methods are used to prioritise candidates, which are subsequently evaluated through in vitro assays, animal models, and clinical studies. This iterative workflow enhances efficiency, reduces false-positive rates, and improves translational success [44,45].

Results

Comparative Outcomes of Drug Repurposing versus Conventional Drug Development
Comparative analysis of outcomes from traditional drug development and drug repurposing demonstrates clear advantages of repurposing strategies in terms of development timeline, cost efficiency, and probability of success. Conventional drug discovery follows a linear and resource-intensive process involving target identification, lead optimisation, preclinical testing, and multiple phases of clinical trials, with high attrition rates particularly during Phase II and Phase III clinical evaluation [46]. In contrast, drug repurposing leverages existing knowledge of drug safety, pharmacokinetics, formulation, and manufacturing, allowing several early-stage development steps to be bypassed.

Reported outcomes from academic and industrial repurposing programs indicate that development timelines can be reduced to approximately 3–6 years, compared with more than a decade for de novo drug discovery [47]. Financial investment is correspondingly lower, and the availability of prior clinical data reduces the likelihood of late-stage failure. These comparative outcomes strongly support the role of drug repurposing as an efficient and pragmatic alternative to traditional drug development pipelines, as per Table 2.

Outcomes of Experimental Drug Repurposing Approaches
Experimental repurposing approaches have produced significant outcomes across multiple therapeutic areas. Target-based screening has successfully identified existing drugs capable of modulating disease-relevant molecular targets, particularly in oncology, infectious diseases, and inflammatory disorders [48]. In several cases, approved drugs demonstrated inhibitory or modulatory effects on targets that were not initially associated with their original therapeutic indication.

Cell-based and phenotypic screening approaches have yielded particularly impactful results by identifying off-target and pleiotropic drug effects. These assays capture integrated biological responses and have led to the discovery of drugs capable of modulating complex

cellular processes such as apoptosis, autophagy, immune signalling, and metabolic regulation [49]. Phenotypic screening outcomes have been especially valuable in diseases with poorly understood molecular mechanisms, where target-centric approaches alone are insufficient.
In vivo validation studies further confirmed the translational relevance of repurposed drugs identified through in vitro screening. Animal models demonstrated improved efficacy and acceptable safety profiles for several repurposing candidates, supporting their progression toward clinical evaluation [50]. Collectively, these experimental outcomes highlight the robustness and translational potential of activity-based repurposing methodologies.

Outcomes of In-Silico Drug Repurposing Approaches
In-silico repurposing approaches have generated substantial outcomes by enabling rapid and cost-effective identification of candidate drugs for new indications. Molecular docking and virtual screening studies have successfully prioritised approved drugs with high binding affinity toward disease-associated targets, many of which were subsequently validated experimentally [51]. Ligand-based similarity approaches further supported the identification of drugs with shared pharmacological properties and potential therapeutic overlap.

Network pharmacology-based repurposing has produced outcomes of particular significance for complex and multifactorial diseases. By modelling diseases as perturbations of interconnected biological networks, network-based approaches identified drugs capable of modulating multiple targets and pathways simultaneously [52]. Such outcomes are especially relevant for cancer, neurodegenerative disorders, and metabolic diseases, where single-target interventions often prove insufficient.

Computational repurposing played a critical role during global health emergencies, including the COVID-19 pandemic. Large-scale virtual screening of approved drug libraries enabled rapid prioritisation of antiviral and host-directed therapeutic candidates, demonstrating the practical utility of in-silico repurposing as a rapid response strategy [53]. Although not all candidates demonstrated clinical efficacy, these outcomes underscored the speed and scalability of computational repurposing pipelines.

Case Study Outcomes: Aspirin
Aspirin represents one of the most well-documented and successful examples of drug repurposing, illustrating the impact of off-target therapeutic effects. Originally developed and marketed as an analgesic and anti-inflammatory agent, aspirin was later repurposed for cardiovascular disease prevention due to its antiplatelet activity mediated through irreversible inhibition of cyclooxygenase-1 in platelets [54].

Subsequent clinical and epidemiological studies revealed additional outcomes associated with long-term aspirin use, including reduced incidence and mortality of certain cancers, particularly colorectal cancer [55]. These findings positioned aspirin as a multi-indication therapeutic agent and demonstrated how off-target effects can lead to substantial clinical benefit. Aspirin's repurposing trajectory remains a benchmark example of successful therapeutic switching driven by mechanistic understanding and clinical evidence.

Case Study Outcomes: Minoxidil
Minoxidil exemplifies successful on-target drug repurposing. Initially approved as an oral antihypertensive agent due to its vasodilatory effects mediated through potassium channel activation, minoxidil was later repositioned as a topical therapy for androgenic alopecia following consistent observations of hair growth in treated patients [56].

Clinical outcomes demonstrated that topical minoxidil effectively promoted hair regrowth and slowed hair loss progression in both male and female patients. Importantly, the

repurposing outcome was achieved by exploiting the same pharmacological mechanism in a different physiological context, highlighting the efficiency and predictability of on-target repurposing strategies when supported by a clear mechanistic rationale.

Therapeutic Area-Specific Repurposing Outcomes

Oncology

Oncology represents one of the most active and productive areas of drug repurposing research. Several non-oncology drugs, including metformin, thalidomide, disulfiram, and beta-blockers, have demonstrated anticancer activity through mechanisms such as metabolic modulation, angiogenesis inhibition, immune regulation, and proteasome interference [57–59]. Preclinical and clinical outcomes suggest that repurposed drugs may enhance the efficacy of standard chemotherapeutic regimens while reducing toxicity.

Infectious Diseases

In infectious diseases, repurposing outcomes have been accelerated by the integration of computational screening and experimental validation. Approved drugs with antiviral, immunomodulatory, or host-directed effects were rapidly evaluated during recent viral outbreaks, demonstrating the strategic value of repurposing in emergency settings [53,60]. While clinical outcomes varied, these efforts highlighted the feasibility of rapidly generating and testing therapeutic hypotheses.

Neurological and Psychiatric Disorders

Repurposing outcomes in neurological and psychiatric disorders have identified neuroprotective and neuromodulator effects of drugs originally developed for cardiovascular and metabolic indications. Antidiabetic and antihypertensive drugs have demonstrated potential benefits in neurodegenerative conditions by modulating inflammation, oxidative stress, and mitochondrial function [61,62]. These outcomes are particularly important given the high failure rates associated with traditional neurogram development.

Metabolic and Cardiovascular Diseases

In metabolic and cardiovascular diseases, repurposing outcomes have revealed pleiotropic effects of existing drugs beyond their original indications. Lipid-lowering and antidiabetic agents have shown additional benefits in reducing inflammation, improving endothelial function, and lowering cardiovascular risk, thereby expanding their therapeutic utility [63].

Outcomes of Artificial Intelligence-Driven Drug Repurposing

Recent results demonstrate that artificial intelligence and machine learning significantly enhance the efficiency and predictive accuracy of drug repurposing. AI-driven models integrate chemical structures, bioactivity data, transcriptomic signatures, and clinical information to identify novel drug–disease associations with improved confidence [64]. Studies published between 2020 and 2025 consistently report superior performance of deep learning and graph-based models compared with traditional computational approaches.

AI-based repurposing outcomes have successfully identified high-priority candidates that were subsequently validated experimentally, underscoring the translational relevance of these approaches [65,66]. Integration of AI with network pharmacology and real-world evidence further strengthens the robustness of repurposing predictions and supports precision medicine initiatives.

Conclusion

Drug repurposing has emerged as a rational, efficient, and increasingly indispensable strategy in modern pharmaceutical research. By identifying new therapeutic indications for existing drugs, repurposing directly addresses the major limitations of traditional drug discovery, including prolonged development timelines, high research and development costs, and high attrition rates. The systematic frameworks discussed in this chapter demonstrate that drug repurposing is no longer a serendipitous process but a structured, methodology-driven discipline supported by experimental validation and advanced computational tools.

The methods outlined in this chapter—derived from established conceptual models—highlight the importance of on-target and off-target repurposing strategies, experimental screening, and in-silico approaches. The integration of drug-oriented, target-oriented, and disease-oriented methodologies provides a comprehensive and flexible framework for identifying repurposing candidates. The growing availability of curated biomedical databases and the application of artificial intelligence further enhance the precision and scalability of repurposing efforts.

The results presented clearly demonstrate the success of drug repurposing across multiple therapeutic areas, including oncology, infectious diseases, neurological disorders, metabolic diseases, and cardiovascular conditions. Classic examples such as aspirin and minoxidil illustrate the translational impact of repurposing, while recent advances underscore the expanding role of artificial intelligence, network pharmacology, and multi-omics integration in identifying novel drug–disease relationships. Despite certain challenges related to regulatory approval, intellectual property protection, and translational validation, drug repurposing remains a highly promising approach for accelerating therapeutic innovation.

In conclusion, drug repurposing represents a sustainable and cost-effective pathway for drug development that aligns with current scientific, regulatory, and societal needs. Continued interdisciplinary collaboration, methodological refinement, and responsible application of emerging technologies will further strengthen the role of drug repurposing in addressing unmet medical needs and advancing global healthcare.

Table 1: Classification of Drug Repurposing Strategies

S.No	Classification	Description	Key Advantage	Typical Examples
1.	On-target repurposing	Same target, new indication	Predictable mechanism	Minoxidil (hypertension → alopecia)
2.	Off-target repurposing	New target, new indication	Novel therapeutic effects	Aspirin (analgesic → antiplatelet)
3.	Drug-oriented approach	Based on drug properties	Rapid prioritization	Side-effect similarity
4.	Target-oriented approach	Based on disease targets	Mechanism-driven	Kinase inhibitors
5.	Disease-oriented approach	Based on disease signatures	Systems-level insight	Transcriptomics-based repurposing
6.	Hybrid approach	Experimental + in-silico	Higher success rate	AI-assisted repurposing

Table 2: Comparison between Traditional Drug Development and Drug Repurposing

S.No	Parameter	Traditional Drug Development	Drug Repurposing
1.	Starting point	Novel chemical entity	Approved / investigational / discontinued drug
2.	Knowledge of safety	Limited, unknown initially	Largely established
3.	Development timeline	10–15 years	3–6 years
4.	Development cost	Very high (>$1 billion)	Significantly reduced
5.	Attrition rate	High, especially in Phase II–III	Lower due to prior data
6.	Mechanistic understanding	Developed progressively	Often already available
7.	Risk of toxicity	High	Reduced
8.	Regulatory pathway	Full NDA / MAA	505(b)(2) or equivalent
9.	Suitability for rare diseases	Limited	Highly suitable
10.	Examples	New oncology molecules	Aspirin, Minoxidil, Metformin

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Chapter- Twelve

Clinicopathological correlation of Chemotherapy Response Score (CRS) in Advanced High-Grade Serous Ovarian Carcinoma after Neoadjuvant Chemotherapy

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Abstract

The Chemotherapy Response Score (CRS) is a standardized histopathological grading system designed to quantify tumor regression in High-Grade Serous Ovarian Carcinoma (HGSOC) following Neoadjuvant Chemotherapy (NACT). While radiologic and biochemical markers are standard for assessment, structured pathological evaluation provides superior insight into the biological response. This prospective study evaluated the distribution of CRS and its association with clinicopathologic parameters in 50 patients with advanced HGSOC treated with platinum-taxane NACT followed by Interval Debulking Surgery (IDS) between April 2023 and September 2024. Omental and adnexal specimens were graded using the three-tier CRS (Böhm) system. The mean patient age was 56.9 +/_ 10.9 years, with bilateral ovarian involvement in 84% of cases. Distribution of scores showed CRS1 in 44%, CRS2 in 18%, and CRS3 in 38% of patients. While significant radiologic tumor reduction was observed post-NACT ($p < 0.001$), imaging findings did not consistently predict microscopic residual disease, highlighting a discrepancy between macroscopic and cellular response. Higher CRS categories (complete/near-complete response) significantly correlated with prominent stromal regression patterns. Notably, no significant correlation was found between the number of NACT cycles administered and the final CRS category ($r = -0.105$; $p = 0.472$), suggesting that intrinsic tumor biology may outweigh treatment duration in determining response. In conclusion, CRS provides a reproducible and objective histopathologic assessment of treatment response in advanced HGSOC. These findings underscore the biological heterogeneity of the disease and suggest that integrating structured CRS reporting into routine pathology is essential for accurate postoperative risk stratification and multidisciplinary clinical decision-making.

Keywords: *High-Grade Serous Ovarian Carcinoma, Neoadjuvant Chemotherapy, Böhm criteria, Interval Debulking Surgery, Omental Assessment, Pathologic Tumor Regression, Residual Disease.*

Introduction

According to data from GLOBOCAN 2020, HGSOC represents 70- 80% of all epithelial ovarian cancers (EOC) and is the leading cause of gynaecological cancer mortality.[3] This subtype is characterized by rapid growth, extensive peritoneal dissemination, and frequent association with BRCA1 and BRCA2 mutation.[4] The majority of patients present with

disseminated peritoneal disease, where optimal cytoreductive surgery combined with platinum-based chemotherapy constitutes the cornerstone of management. In recent years, the therapeutic paradigm for advanced-stage disease has evolved to incorporate NACT followed by IDS as an alternative to primary cytoreductive surgery in carefully selected patients.[5] This approach is particularly beneficial for individuals with extensive tumor burden or poor performance status, as it aims to reduce disease volume, improve resectability, and decrease surgical morbidity without compromising overall survival outcomes.

While clinical examination, radiologic imaging, and serum biomarkers such as CA-125 are routinely used to evaluate response to NACT, these modalities provide indirect or surrogate assessments of tumor regression.[6] Histopathological evaluation of post-NACT surgical specimens offers a more definitive and biologically meaningful measure of therapeutic response. Unlike chemotherapy-naïve tumors, specimens obtained after NACT demonstrate a spectrum of morphological changes, including variable proportions of viable tumor cells, stromal fibrosis, necrosis, inflammatory infiltrates, and vascular alterations. These therapy-induced modifications reflect tumor chemosensitivity and the dynamic interaction between malignant cells and the tumor microenvironment.[7] Consequently, pathological assessment has emerged as a critical determinant of prognosis and an essential component of multidisciplinary management.

The need for standardized evaluation of post-NACT specimens led to the development of structured regression grading systems, most notably the Chemotherapy CRS system for HGSOC. This three-tiered scoring system, primarily applied to omental specimens, stratifies response into minimal (CRS1), partial (CRS2), and complete or near-complete response (CRS3). Validation studies have demonstrated strong interobserver reproducibility and significant correlation between higher CRS categories and improved progression-free and overall survival. As a result, the CRS system has been incorporated into international reporting guidelines and is increasingly utilized in routine pathological practice.

Despite these advances, several challenges persist. Tumor heterogeneity, both between and within anatomical sites, complicates uniform assessment of response.[8] Furthermore, non-serous histologic subtypes may exhibit distinct morphological and molecular patterns of regression, limiting the generalizability of existing scoring systems. Accurate staging after NACT also requires meticulous gross examination and extensive tissue sampling to avoid underestimation of residual disease. Ancillary techniques, including immunohistochemistry and emerging molecular assays, may enhance detection of residual tumor cells and provide additional prognostic insights.

In the era of personalized oncology—marked by the integration of targeted therapies such as PARP inhibitors and immune checkpoint inhibitors—the role of pathological response assessment continues to expand.[9] Standardized and reproducible histopathological evaluation not only informs individual patient management but also serves as a critical endpoint in clinical trials investigating novel therapeutic strategies. Accordingly, refining pathological assessment methods and understanding their prognostic implications remain essential for optimizing outcomes in ovarian carcinoma.

However data regarding CRS distribution and clinicopathologic corelation in Indian tertiary centre remain limited so this study aims to address the gap.

Methodology

1. Study Design

We conducted a prospective study to evaluate histopathological changes in ovarian carcinoma following NACT. The study focused exclusively on patients diagnosed with HGSOC who underwent NACT followed by IDS. The primary objective was to assess the three- tiered Chemotherapy Response Score and correlate it with prognostic outcomes.

2. Study Setting and Duration

The study was conducted in the Department of Radiation Oncology with collaboration with Department of Onco- Pathology at Government Medical College and State Cancer Institute, Chhatrapati Sambhajinagar, a tertiary care referral centre in India. The institution manages a high volume of advanced ovarian cancer cases and is equipped with comprehensive surgical, oncological, and histopathological facilities. The study was carried out over a period of 18 months, from April 2023 to September 2024. A total of 50 patients were included

3. Study Participants

This study was conducted in accordance with the Declaration of Helsinki, with approval obtained from Institutional Ethics Committee at Government Medical College & State Cancer Institute, Chhatrapati Sambhajinagar. Written Informed consent was obtained from all participants before enrolment

Inclusion Criteria

- Histologically confirmed HGSOC.
- Patients receiving platinum- taxane combination NACT followed by IDS.
- Surgically resected specimens with adequate tissue for complete histopathological evaluation.

Exclusion Criteria

- Non-serous or low-grade ovarian carcinomas.
- Specimens received for second opinion only.
- Patients undergoing primary debulking surgery without prior NACT.

4. Study Stratification

Patients were stratified post- operative based on their CRS into three categories: (Figure 1)

- **CRS 1:** Minimal or no response to chemotherapy
- **CRS 2:** Partial response
- **CRS 3:** Complete or near-complete response

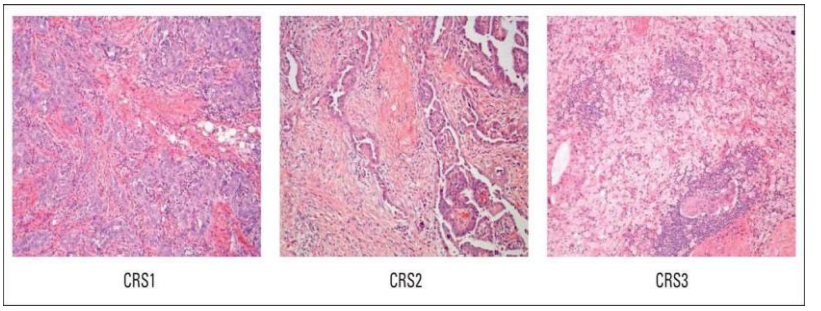


Figure 1: Chemotherapy Response Score (CRS) System

These categories were used for correlation with clinicopathological parameters.

5. Study Parameters

Primary Outcome

- Chemotherapy Response Score, assessed in omental and adnexal specimens.

Secondary Outcomes

1. Histopathological features including residual tumor burden, fibrosis, necrosis, inflammatory infiltrate, and psammoma bodies.

Clinical Variables

- Age at diagnosis
- Number of chemotherapy cycles
- Surgical outcomes (residual disease status)

6. Study Procedure

Pre-chemotherapy Assessment: Diagnosis was established through image-guided biopsy and radiological evaluation (CT/MRI). Clinical staging was performed according to standard guidelines.

NACT: Patients received chemotherapy with Inj. Paclitaxel 175mg/m^2 + Inj. Carboplatin AUC 5 in 3 weekly cycles and Inj. Paclitaxel 80mg/m^2 + Inj. Carboplatin AUC 2 in weekly cycles.

IDS: Following NACT, IDS was performed. Surgical specimens including omentum, adnexa, lymph nodes and peritoneal deposits were submitted for histopathological examination.

Histopathological Evaluation:

- Gross examination documented tumor size, areas of necrosis, fibrosis, and distribution of disease.
- Microscopic evaluation included assessment of viable tumor, regressive changes, and CRS scoring according to Böhm et al. criteria.
- Residual tumor quantification.

7. Data Analysis

- Descriptive Statistics: Mean, median, and percentages for clinical/pathological variables.
- Inferential Statistics: Chi-square/Fisher's exact test: Association between CRS and histopathological features.
- Statistical Analysis performed using SPSS version 29 (IBM Corporation, NY, USA)

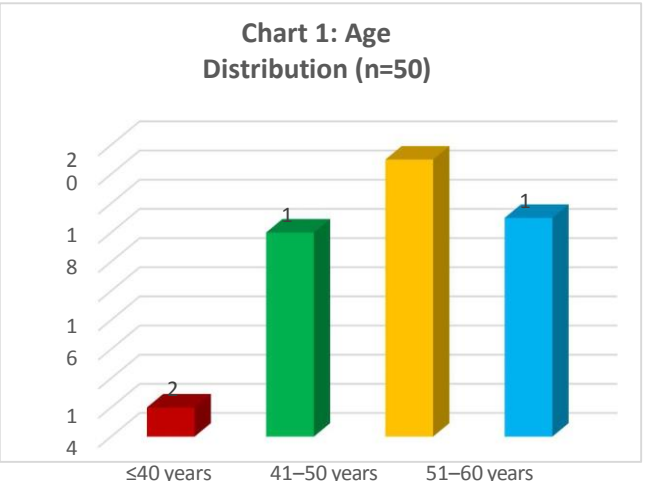
Results & Key Findings

Overview

This study included 50 patients with high-grade serous ovarian carcinoma treated with NACT followed by IDS. The key clinicopathological findings and response parameters are summarized below.

Age Distribution (Chart 1)

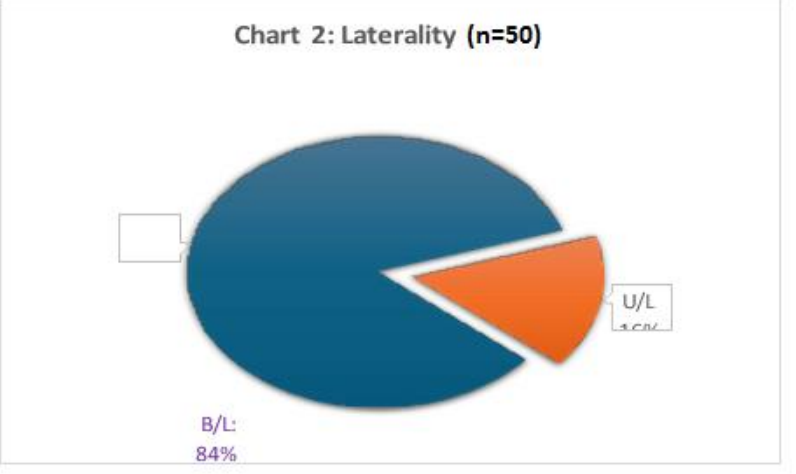
The mean age of the cohort was **56.98 ± 10.97 years**, with a median of 54.5 years (range: 33–79 years). Most patients were in the **51–60 years (38%)** and **>60 years (30%)** age groups, while 28% were aged 41–50 years and only 4% were ≤40 years. This distribution reflects the typical peri- and post-menopausal predominance of epithelial ovarian carcinoma.



Clinical Background

Laterality: (Chart 2)

- Bilateral ovarian involvement was observed in **84%** of cases & Unilateral disease accounted for **16%**.
- This finding is consistent with the known biological behaviour of advanced high-grade serous carcinoma.



Radiological Tumor Size Reduction (Table 1)

NACT resulted in a statistically significant reduction in tumor size bilaterally.

Table 1. Radiological Tumor Size (in centimetres)

Ovary	Pre-treatment (Mean ± SD)	Post-treatment (Mean ± SD)	Mean Difference (POST-PRE)	t-value	p-value
Right	8.53 ± 4.46	5.78 ± 4.35	-2.75	5.56	<0.001
Left	7.45 ± 3.30	5.09 ± 3.53	-2.36	4.04	<0.001

These findings confirm effective tumor burden reduction prior to interval debulking surgery Serum CA-125 Levels (Table 2): **All patients (100%) had elevated pre-treatment CA-125 levels, with a mean value of** 1872.86 ± 2036.37 U/mL**, reflecting advanced disease burden at presentation.**

Table 2. Serum CA-125 Levels

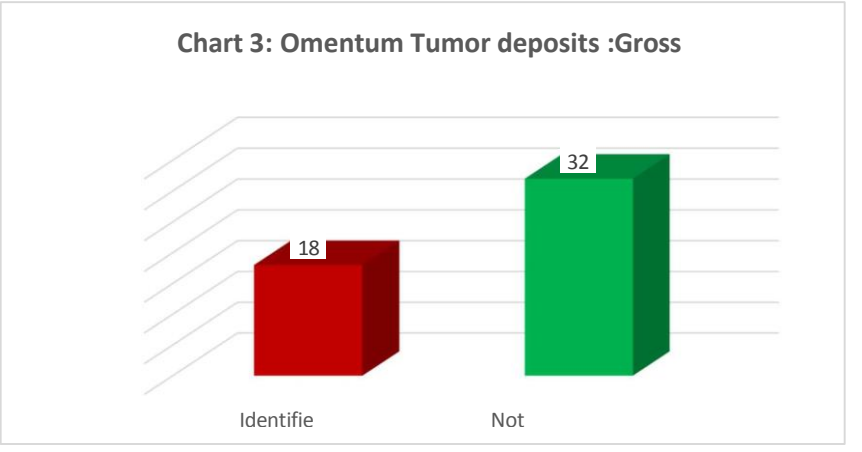
Parameter	Value
Mean ± SD	1872.86 ± 2036.37
Raised	50 (100.0%)

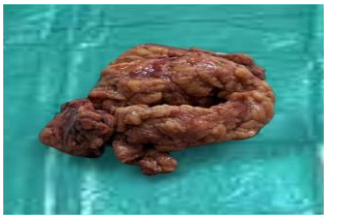
Number of NACT Cycles : The majority received 3- 6 cycles in 3 weekly doses and some received 9- 18 cycles in weekly doses consistent with standard oncologic protocols.
Correlation analysis between NACT cycles and Chemotherapy Response Score (CRS) showed a weak, non-significant negative relationship (r = –0.105, p = 0.472), indicating that increasing chemotherapy cycles did not significantly influence pathological response. (Table 3)

Table 3. Correlation between Number of NACT Cycles and CRS

Variable Pair	Correlation Coefficient (r)	95% Confidence Interval	p-value	Interpretation
NACT Cycles vs CRS	-0.105	-0.383 to 0.190	0.472	Not statistically significant

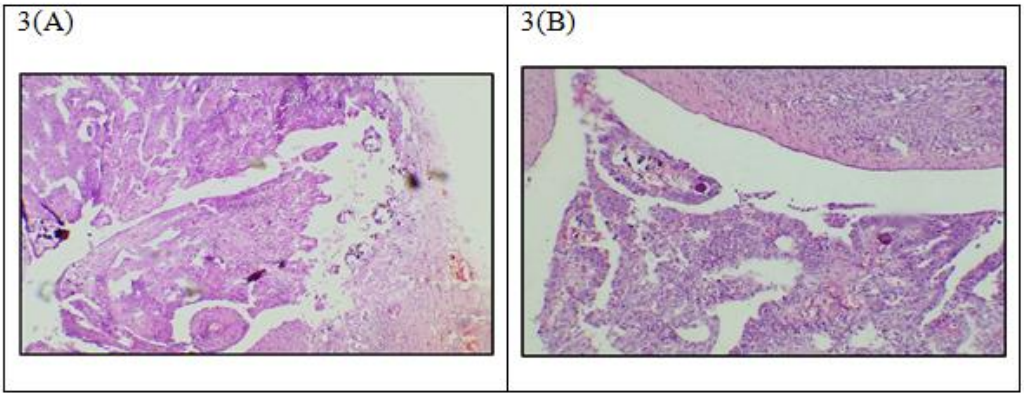
Omental Findings: Gross Findings: (**Chart 3 & Figure 2)**





Ovarian Microscopic Findings (Figure 3)

Figure 3 A&B: H&E,post NACT Ovary showing High Grade Serous Carcinoma: (A) 40X; (B) 100X



Right Ovary (n=48 evaluable): (Table 4)

Table 4. Right Ovary – Microscopic Findings (n = 48)

	n	% (out of 48)
High-grade serous ovarian carcinoma	40	83.4%
No tumor / free of tumor	8	16.6%

Left Ovary (n=48 evaluable): (Table 5)

Table 5. Left Ovary – Microscopic Findings (n = 48)

	n	% (out of 48)
High-grade serous ovarian carcinoma	39	81.3%
No tumor / free of tumor	9	18.7%

Nearly one-fifth of ovaries demonstrated complete pathological regression.

Chemotherapy-Related Histopathological Changes Lt/ Rt Ovary (**Table 6 & Figure 4)**

Table 6. LT/RT Ovary – Chemotherapy-Related Findings

Finding	N=50	%
Necrosis / Hyalinization (with hemosiderophages, fibrosis, calcification, foamy macrophages, sclerosis, psammoma bodies)	14	29.2%
Hemosiderophages with hyalinization/necrosis	6	12.5%
Fibrosis ± calcification	3	6.3%
Pigmented macrophages ± calcification	3	6.3%
Chronic inflammation with hemosiderin deposits	2	4.2%

Psammomatous calcification	2	4.2%
Massive necrosis (LT ovary)	1	2.1%
No changes seen	19	38%

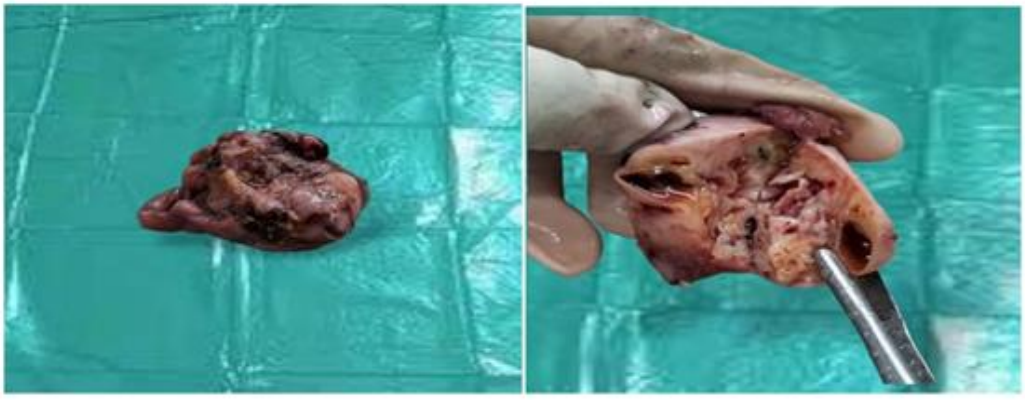
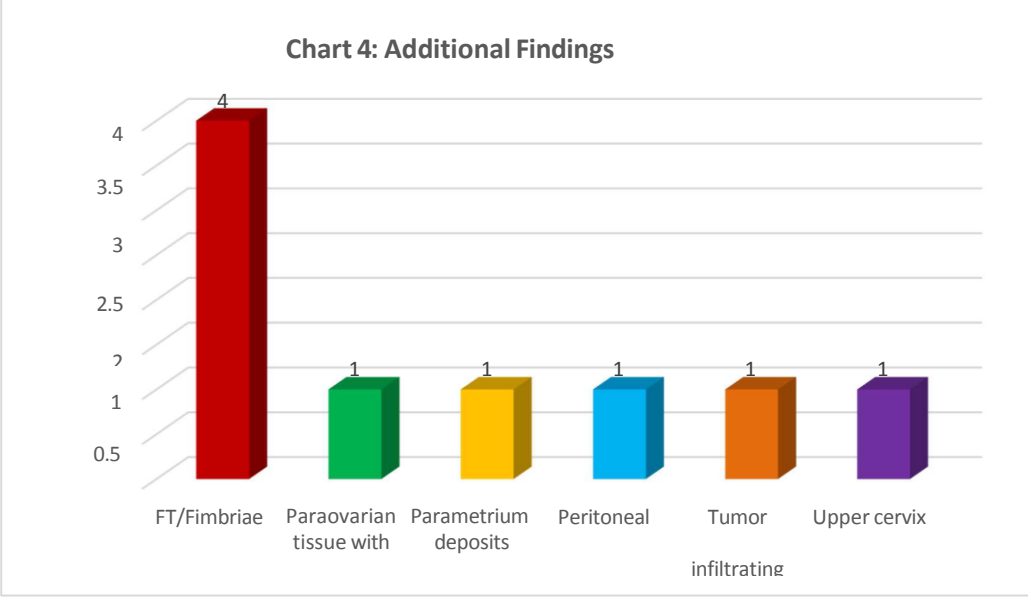


Figure 4: Post NACT Left Ovary, On cut section solid-cystic

Additional Sites of Involvement (Chart 4)
Extra-ovarian microscopic involvement was documented in: Fallopian tube/fimbriae (8%), Para- ovarian tissue (2.6%), Parametrium (2.6%), Peritoneum (2.6%), Ileum (2.6%), Upper cervix (2.6%)

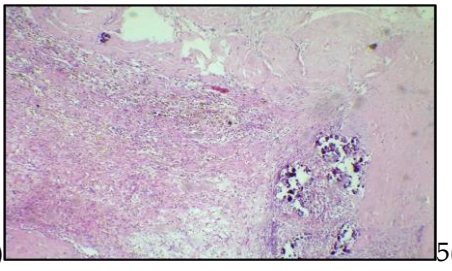
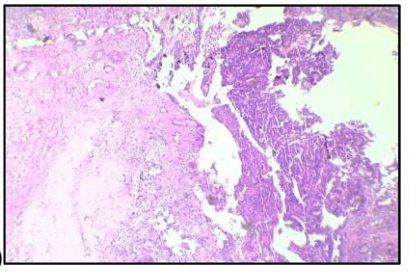


NACT Related Omentum Findings (Table 7 & Figure 5 A&B)

Table 7. NACT RELATED OMENTUM FINDINGS

Histological Feature	Present n (%)	Absent n (%)
Fibrosis	16 (32%)	34 (68%)
Necrosis	8 (16%)	42 (84%)
Inflammatory infiltrates	16 (32%)	34 (68%)
Foamy macrophages	8 (16%)	42 (84%)
Psammomatous calcification	9 (18%)	41 (82%)

Figure 5:A&B: H&E, Post NACT , Omentum showing (A) Collection of macrophages, Lymphocytes & Fibrosis. No Residual viable tumour (40x) & (B) Tumour necrosis (40x)

5(A)  5(B) 

Omentum – Residual Viable Tumor (**Table 8 & Figure 6 A& B)**

Table 8. Residual Viable Tumor – Omentum Findings

Finding	n	%
Deposits of tumor	30	60.00%
Free of tumor	20	40.00%

6(A) **6(B)**

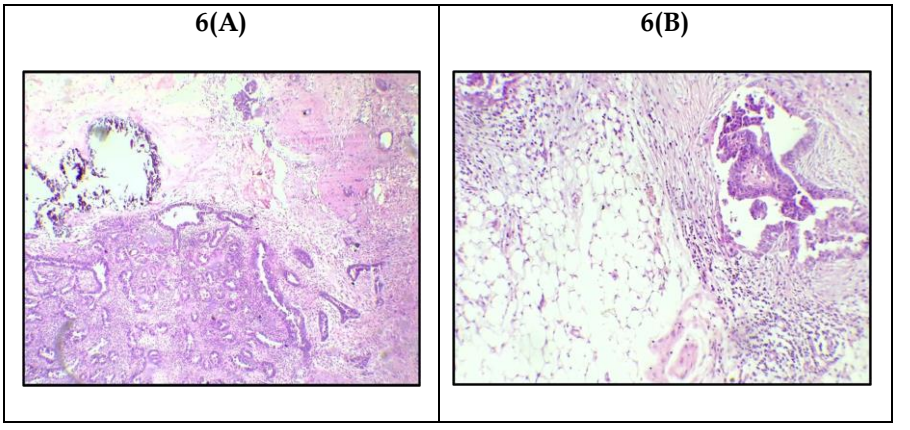


Figure 6: H&E, post NACT, Omentum Invasive deposits of tumour: (A) 40X & (B) 100X

Residual Viable Tumor vs Omentum Gross Findings (Table 9)

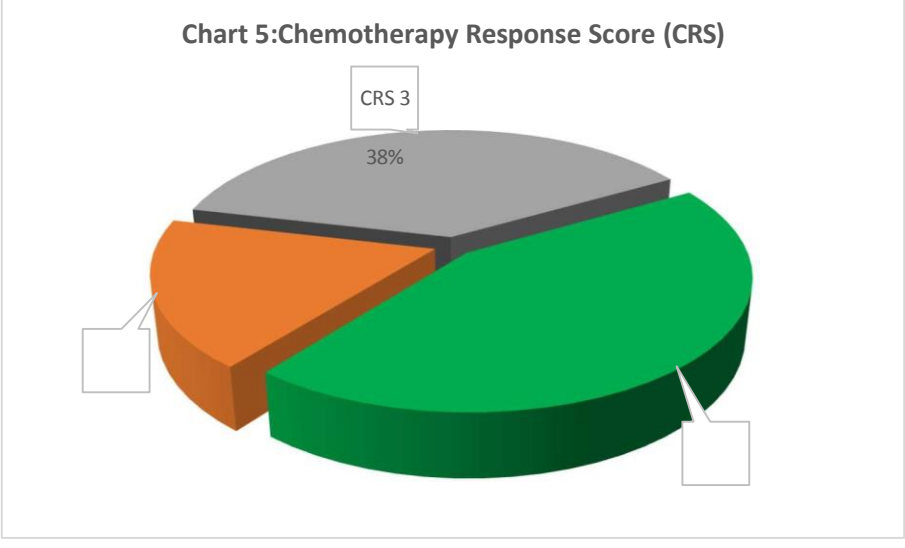
Table 9. Residual Viable Tumor in Relation to Omentum Gross Findings

Residual Viable Tumor	Omentum Gross Findings					
	Identified		Not identified		Total	
	n	%	n	%	n	%
Deposits of tumor	17	34.00%	13	26.00%	29	58.00%
Free of tumor	1	2.00%	19	38.00%	20	40.00%
Grand Total	**18**	**36.00%**	**32**	**64.00%**	**50**	**100.00%**

Chemotherapy Response Score (CRS) Distribution (Chart 5)

- **CRS 1:** 44%
- **CRS 2:** 18%
- **CRS 3:** 38%

Over one-third achieved complete or near-complete pathological response (CRS3), while 44% demonstrated minimal response.

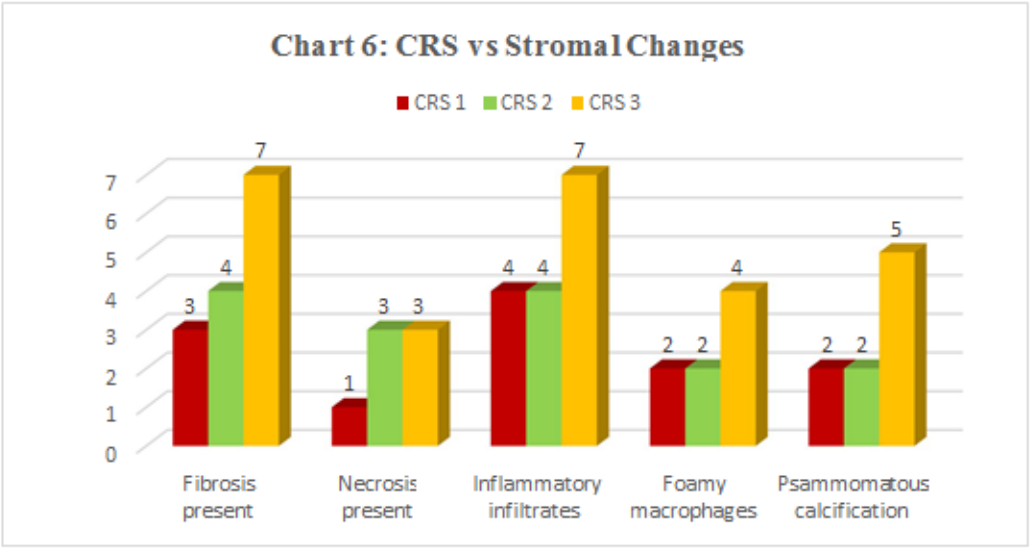


Lymph Node Status (Table 10)

Table 10. Lymph Node Status

Lymph Node Type	Negative n (%)	Positive n (%)	NA	Total
Pelvic LN	11 (22%)	1 (2%)	–	12
Para-aortic LN	21 (42%)	1 (2%)	–	22
Overall	32 (64%)	2 (4%)	16 (32%)	50

CRS and Stromal Changes **(Chart 6)**
Higher CRS categories were associated with increased fibrosis, inflammatory infiltrates, and psammomatous calcification, supporting the biological correlation between stromal regression and effective chemotherapy response.



Key Findings

- Significant radiological tumor reduction following NACT.
- 38% achieved CRS3 (near-complete/complete response).
- Omentum was the most reliable site for response assessment.
- Pathological response was independent of number of chemotherapy cycles.
- Stromal regressive features correlated with higher CRS.

These findings reinforce the prognostic and evaluative significance of standardized pathological response assessment following neoadjuvant chemotherapy in high-grade serous ovarian carcinoma.

Discussion

This study aimed to evaluate the clinicopathological correlation of CRS in advanced high-grade serous ovarian carcinoma after NACT, with emphasis on age distribution, biochemical markers, morphologic response, and prognostic stratification.

The mean patient age was 56.98 ± 10.97 years (median 54.5; range 33–79), with 68% of cases above 50 years. This confirms that advanced epithelial ovarian carcinoma treated with NACT predominantly affects middle-aged and older women. In contrast, Le T et al. reported improved survival in younger patients with early-stage disease undergoing comprehensive staging [11]. Petrillo et al. further demonstrated that younger age was associated with higher rates of pathological complete response (pCR) and improved progression-free survival (PFS) after NACT [12]. However, our findings indicate that meaningful chemosensitivity is achievable even within an older cohort, as nearly one-fifth of ovaries were tumor-free histologically after NACT. These observations suggest that biological tumor responsiveness

may supersede chronological age in determining pathological outcome in advanced-stage disease.

All patients exhibited elevated CA-125 levels (100%), with a mean of 1872.86 ± 2036.37 U/mL, reflecting high baseline tumor burden. Barlow et al. demonstrated that normalization of CA-125 during NACT correlates with optimal cytoreduction and enhanced chemosensitivity [15]. However, Lee JY et al. observed that CRS does not consistently parallel CA-125 kinetics or radiologic regression, emphasizing histopathologic assessment as the definitive endpoint . While CA-125 and imaging provide valuable biochemical and volumetric monitoring, they cannot reliably distinguish partial response from complete microscopic clearance. Böhm et al. further showed that CRS3 predicts survival independent of baseline biomarker levels. Thus, pathological regression graded by CRS remains superior to surrogate response indicators.

Most patients received either 3 cycles (38%) or 6 cycles (56%) of NACT, reflecting contemporary practice. Prolonged treatment beyond six cycles was rare. Nitecki et al. demonstrated comparable survival between primary debulking surgery (PDS) and NACT followed by IDS when complete cytoreduction (R0) was achieved, highlighting surgical completeness and tumor biology rather than chemotherapy duration as principal outcome determinants [16]. Our findings support limiting NACT to the optimal surgical window rather than extending cycles without demonstrable benefit.

The prognostic significance of histopathological response is well established. Böhm et al. validated the CRS system and demonstrated that CRS3 strongly correlates with improved survival, independent of demographic variables. Cohen et al., in a meta-analysis of 1,102 patients, confirmed CRS3 as an independent predictor of survival regardless of age [13]. External validation by Lee JY et al. reported CRS3 in 32% of a comparable cohort and similarly found that age did not preclude favourable response [14]. Our CRS distribution—CRS1 (44%), CRS2 (18%), CRS3 (38%)—reflects the biological heterogeneity of chemosensitivity in advanced HGSC and reinforces CRS3 as the most robust indicator of therapeutic success following NACT.

Morphologically, therapy-induced changes included fibrosis and inflammation (32% each), calcification (18%), and necrosis with foamy macrophages (16%). These features represent a biologically coherent response continuum: tumor cell death, macrophage-mediated clearance, and stromal remodelling. Rodolakis et al. emphasized that CRS remains the most validated and clinically actionable grading system in HGSC when omental sampling is adequate [17]. In our cohort, fibrosis, inflammatory infiltrates, and psammomatous calcification were enriched in CRS3 cases, whereas necrosis and foamy macrophages were more broadly distributed, suggesting that organized stromal remodelling is more discriminatory than isolated necrosis. Matsuo et al. similarly demonstrated that CRS3 frequently co-expresses fibrosis, calcification, and tumor-infiltrating lymphocytes, reinforcing these as hallmarks of deep pathological response [18].

Lymph node evaluation showed nodal negativity in 32/50 patients (64%), positivity in 2/50 (4%), and non-assessment in 16/50 (32%). Among the 34 patients who underwent sampling, the positivity rate was 5.9% (1/12 pelvic and 1/22 para-aortic nodes). The low positivity rate may reflect effective tumor downstaging following NACT; however, lack of nodal assessment in nearly one-third of cases introduces potential under-detection bias. Le T et al. emphasized the importance of systematic lymph node assessment for prognostic precision .

However, Melamed et al. demonstrated that absence of residual disease after cytoreduction is the strongest determinant of overall survival, with nodal status exerting comparatively less influence when complete macroscopic clearance is achieved [19].Furthermore, Böhm et al. and the meta-analysis by Cohen et al. confirmed that CRS3 independently predicts improved progression-free and overall survival irrespective of nodal status. Lee JY et al. similarly validated CRS as a superior prognostic indicator and cautioned that lymph node positivity should be interpreted within the broader context of overall pathological response. Overall, while nodal evaluation contributes to staging completeness, depth of histopathological regression—captured by CRS—appears to hold greater prognostic significance in the post-NACT setting.

In summary, this study reinforces that in advanced HGSOC treated with NACT, histopathological regression graded by CRS—particularly CRS3—serves as the most reliable and reproducible prognostic indicator. Age, CA-125 kinetics, chemotherapy duration, and nodal status contribute contextual information, but none supersede the prognostic centrality of structured pathological response assessment.

Limitations

Several limitations merit acknowledgment. First, the relatively small sample size (n = 50) limits statistical power and may reduce the generalizability of the findings. In addition, incomplete nodal assessment (non-assessed in 32% of cases) may have led to underestimation of true nodal positivity and reduced staging accuracy. Second, post-treatment CA-125 kinetics and formal radiologic–pathologic site mapping were not uniformly integrated, limiting the ability to correlate biochemical and imaging responses with site-specific histologic regression. Although the Chemotherapy Response Score (CRS) was systematically applied, interobserver variability—particularly at the CRS2 versus CRS3 threshold—remains possible in the absence of centralized or blinded dual review. Additionally, molecular stratifiers (e.g., BRCA mutation and homologous recombination deficiency [HRD] status) and immune microenvironment variables (e.g., tumor-infiltrating lymphocyte density) were not incorporated, despite their growing relevance to chemosensitivity and maintenance therapy selection. Finally, longer-term survival endpoints, including progression-free survival (PFS) and overall survival (OS), were not analysed in this dataset, precluding comprehensive prognostic modelling integrating CRS with clinical outcomes.

Future Directions

Future studies should prioritize prospective, adequately powered multicenter cohorts with standardized omental sampling schemas and predefined quality metrics for tissue handling. Blinded dual-pathologist CRS reads and reproducibility audits would strengthen grading consistency and external validity. Integration of CA-125 trajectories, RECIST-based radiologic changes, and site-matched histopathology could enable construction of composite response nomograms that better capture multidimensional treatment effect.

Embedding genomic stratifiers—particularly BRCA and broader homologous recombination deficiency (HRD) status—alongside microenvironmental markers (e.g., TIL density) would refine risk stratification within CRS1–3 categories. Such integration may help identify candidates for treatment de-escalation (e.g., selected CRS3 patients) or early therapeutic intensification/novel-agent trials (e.g., persistent CRS1). Further work should also explore

interactions among cycle number, dose intensity, and time-to-surgery to optimize timing rather than duration of NACT alone.

Importantly, evaluation of composite endpoints—such as R0 cytoreduction combined with CRS—should be compared against either metric alone for predicting PFS/OS, potentially establishing a dual-anchor prognostic framework. Finally, development of standardized operating procedures, structured training modules, and synoptic reporting templates for CRS application would enhance interinstitutional consistency and scalability, ensuring that pathology-centered response assessment remains reproducible and actionable across diverse practice settings.

Conclusion

Our study aimed to deliver a comprehensive, pathology-anchored evaluation of NACT response in epithelial ovarian carcinoma, positioning the omentum and ovaries as biological barometers of therapeutic effect and the CRS as the standardized histologic endpoint. Specifically, we sought to: (i) quantify pre- to post-NACT radiological change in ovarian tumor size; (ii) characterize baseline disease ecology (age distribution, laterality, CA-125 levels) to contextualize response patterns; (iii) map gross and microscopic omental status to determine whether absence of a grossly recognized omentum reliably excludes residual disease; (iv) catalogue therapy-induced morphologic changes—fibrosis, chronic inflammation, calcification, necrosis, and foamy macrophages—and correlate these with CRS strata; (v) assess lymph-node status for staging relevance; and (vi) evaluate whether extending NACT cycles translates into improved pathological response. The significance of this work lies in transforming routine clinical and histopathological data into decision-ready signals that directly inform interval debulking timing, omental sampling strategy, and postoperative treatment planning.

By synoptically integrating clinical, radiologic, biochemical, and histologic axes within a unified dataset, this study proposes a pragmatic response-assessment framework centered on pathology—particularly omental histology and CRS—as the definitive arbiter of therapeutic effect. In doing so, it advances a more precise and individualized approach to care for patients undergoing NACT for advanced epithelial ovarian carcinoma, emphasizing that true response is ultimately a microscopic, not merely radiologic, phenomenon.

Abbreviations

- NACT – Neoadjuvant Chemotherapy
- EOC – Epithelial Ovarian Carcinoma
- IDS – Interval Debulking Surgery
- CRS – Chemotherapy Response Score
- HGSOC – High Grade Serous Ovarian Carcinoma
- PARP – Poly ADP-Ribose Polymerase
- PCR – Pathological Complete Response

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Conflict of interest: None Declared

Ethical Approval: The study was conducted in accordance with the Declaration of Helsinki, with approval obtained from the Institutional Ethics Committee of the Government Medical College and State Cancer Institute, Chhatrapati Sambhajinagar. Written informed consent was obtained from all participants before enrolment.

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Chapter- Thirteen

Computerized System Validation in the Pharmaceutical Industry: Concepts and Applications

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Abstract

Computerized CSV, or system validation, is an essential regulatory requirement in industries such as pharmaceuticals, biotechnology, and medical devices where data integrity, patient safety, and product quality are paramount.[1] CSV ensures that software systems used in regulated environments consistently perform as intended, meeting both user needs and regulatory expectations. This process encompasses the planning, testing, and documentation of computerized systems to confirm their reliability and compliance throughout their lifecycle.[8,10] The validation process begins with a risk-based evaluation to ascertain the degree of verificationneeded, followed by the creation of essential documentation such as validation plans, user requirements specifications (URS),[12] functional specifications, and test protocols. Thorough testing—such as Performance Qualification (PQ), Operational Qualification (OQ), and Installation Qualification (IQ)—is conducted to verify system functionality. Any deviations identified during testing are carefully analysed and resolved, ensuring that the system operates without compromising data integrity or patient safety.[4,13]In the current digital era, computerized systems are becoming increasingly complex and integrated, necessitating a more robust and agile approach to validation. Regulatory guidelines from authorities like the EU Annex 11 and FDA [7](21 CFR Part 11) emphasize the importance of maintaining data accuracy, security, and traceability. Additionally, modern validation approaches such as Computer Software Assurance (CSA) promote critical thinking and focus on high-risk areas rather than exhaustive documentation.In conclusion, CSV is not merely a compliance exercise but a vital component of quality assurance. When implemented effectively, it enhances operational efficiency, safeguards data, and supports regulatory compliance, ultimately contributing to patient safety and product efficacy.

Keywords: *Regulatory Compliance, Validation Planning, System Risk Assessment, Requirements Traceability, Testing and Documentation.*

Introduction

Computerised System In regulated industries like pharmaceuticals, biotechnology, and medical devices, validation (CSV) is an essential procedure[8,20,22]. It guarantees that the computer systems and software utilizedin the production, testing, and distribution of products consistently perform as intended and comply with regulatory standards. Global regulatory agencies like the World Health Organization (WHO), European Medicines Agency (EMA), and U.S. Food and Drug Administration [7,5],(FDA) have mandated CSV.CSV involves a structured approach to documenting that a system of computers is suitable for its purpose,meets user requirements, and functions reliably under normal and abnormal conditions. This process typically includes phases such as planning, requirements definition, Performance qualification (PQ), operational qualification (OQ), installation qualification (IQ), and design qualification (DQ). To show traceability, accountability, and compliance, every stage is meticulously recorded.

The need for validation arises from the dangers connected to erroneous or unreliable data, which can compromise product quality and patient safety. Through computerized system validation, organizationsguarantee the integrity of the data,reduce compliance risks, and preserve the quality of the product throughout the lifecycle. CSV also supports Good Automated Manufacturing Practices (GAMP)[29][15],which provide guidelines to achieve compliance through risk-based approaches.

In today's digital era, organizations rely heavily on computerized systems for activities like data collection, laboratory testing, [23],manufacturing operations, and inventory management. Therefore, regulatory agencies emphasize CSV to prevent software malfunctions, unauthorized access, and data manipulation.
Overall, Computerised In addition to being required by law, validation is a best practice to increase system reliability. Ensure product quality, and protect patient safety. [26,24],As digital technologies continue to evolve, CSV remains an essential process in ensuring that these technologies serve their intended purpose without introducing risks to compliance or public health.

HISTORY
Computerized System Validation (CSV) is a systematic process of documenting and proving that a computerized system performs reliably and consistently according to its intended use and regulatory requirements[3].

Origins of Validation: this figure1 explainthe concept of validation predates computerized systems. It originated as a quality assurance methodology in engineering,[27,5,3] used to ensure that equipment and processes delivered reliable and expected performance. In the pharmaceutical industry, this evolved as regulators and manufacturers sought systematic ways to guarantee drug product quality.Early Foundations **(1970s–1980s) 1979:** The concept of formal validation in pharmaceutical manufacturing was first proposed by[21,9,12] FDA officials Ted Byers and Bud Loftus. Prior efforts focused on validating equipment and processes to improve product quality and consistency.Early 1980s: As computer systems became more prevalent in regulated environments, the need for Computerized System Validation emerged. In 1983, the U.S. FDA published a guide to inspecting computerized systems used in pharmaceutical processing — commonly known as the FDA

bluebook.Regulatory Milestones in CSVRegulatory requirements have been the primary drivers in shaping how companies approach CSV. Below are the major milestones:

FDA & Early CSV Guidelines1983 – FDA Bluebook: One of the first regulatory guides focused specifically on computerized systems in pharmaceutical contexts.1997 – 21 CFR Part 11: As long as systems are secure and validated, the U.S. FDA's[10,24,33] historic regulation officially acknowledged the use of electronic records and electronic signatures as being on par with paper records and handwritten signatures. This regulation established the foundation for contemporary CSV and reaffirmed the necessity of thoroughly documented validation procedures.21 CFR Part 11 became effective in August 1997 and significantly broadened expectations about data integrity, audit trails, and control of electronic systems.Regulators have continued revising and clarifying CSV expectations — moving toward risk-based approaches, aligning CSV with international standards (like ISO 13485), and incorporating new technology domains such as cloud computing and AI systems.In 1994, GAMP, or Good Automated Manufacturing Practice guidelines were introduced by the Pharmaceutical Industry Computer Systems Validation Forum (PICSVF) and later became associated with the International Society for Pharmaceutical Engineering (ISPE). GAMP provided structured models for CSV, including lifecycle approaches, qualification activities, and risk-based thinking.GAMP 5 A widely adopted version introduced more flexible, risk-based perspectives and lifecycle models for validating computerized systems. Over time, it has become a de facto industry standard for CSV methodology.From Paper to Digital: CSV also reflects broader transitions in information management and technology. Electronic Records EraBefore computerized systems, regulated data existed primarily in paper form. The shift to electronic records required validationnot

Just of outputs but of system behavior, security, data integrity, and auditability.[23] 21 CFR Part 11 formalized the legal framework for this transition.In recent years, regulators and industry have moved toward risk-based validation, where validation efforts are proportional to the system's impact on quality and safety — rather than a one-size-fits-all approach. This has been reflected in updated guidance documents and industry frameworks European and International Regulations

EU GMP Annex 11: [19]Guidelines for Good Manufacturing Practices from the European Union include Annex 11, which sets out expectations for Computerised Systems used in GMP environments. Although the exact publication date varied by revision, it became a significant regulatory requirement in the EU framework[18,5]. In recent years, regulators and industry have moved toward risk-based validation, where validation efforts are proportional to the system's impact on quality and safety — rather than a one-size-fits-all approach. This has been reflected in updated guidance documents and industry frameworks. Contemporary Developments and Future DirectionToday, CSV continues evolvingCloud, SaaS, and Mobile Systems: [6,10]These new architectures introduce challenges in validation scope, control, and security expectations. AI and Machine Learning Systems: Regulatory discussions now consider how advanced AI components should be validated, especially in quality systems or decision-support roles.

Table 1: History of computer system validation

Period / Year	Milestone / Event	Key Developments	Significance to CSV
Pre-1970s	Traditional quality control	Validation concepts applied mainly to equipment and manufacturing processes	Foundation of validation philosophy before computer usage
1970s	Introduction of process validation	FDA officials (Ted Byers & Bud Loftus, 1979) formalized process validation	Established validation as a regulatory expectation
Early 1980s	Introduction of computers in pharma	Computers used for manufacturing control, data handling, and analysis	Need identified to validate computerized systems
1983	FDA "Blue Book"	First FDA guidance for inspection of computerized systems in drug manufacturing	First formal recognition of CSV by regulators
Late 1980s	Expansion of automated systems	Use of PLCs, LIMS, SCADA, and manufacturing software	Increased regulatory focus on software reliability
1990–1993	Growing compliance concerns	Inconsistent validation practices across industry	Highlighted need for standardized CSV guidance
1994	Introduction of GAMP	In 1994, ISPE published GAMP (Good Automated Manufacturing Practice), which offered a structured lifecycle approach to CSV.	Provided structured lifecycle approach to CSV
1997	21 CFR Part 11 (USA)	Electronic records and electronic signatures legally accepted	Made system validation mandatory for electronic data
Late 1990s	Global regulatory alignment	Increased inspections focused on computerized systems	CSV became a core GMP requirement
2000–2003	Part 11 enforcement discretion	FDA clarified expectations due to industry challenges	Shift toward practical, risk-based compliance
2008	GAMP 5 released	Emphasized risk-based and lifecycle validation	Reduced over-documentation; improved efficiency
2011	EU GMP Annex 11 revision	Formalized requirements for computerized systems in EU	Strengthened CSV expectations in Europe
2010s	Data integrity focus	ALCOA/ALCOA+ principles introduced	Expanded CSV scope to data integrity assurance
2018–2020	Cloud & SaaS adoption	Validation of cloud-based and third-party systems	Introduced shared responsibility validation models
2020–2022	Computer Software	FDA promoted critical thinking over excessive documentation	Modernized CSV approach

	Assurance (CSA)		
2023–Present	**AI & advanced analytics**	**Validation challenges for AI/ML systems**	**CSV evolving toward continuous and adaptive validation**

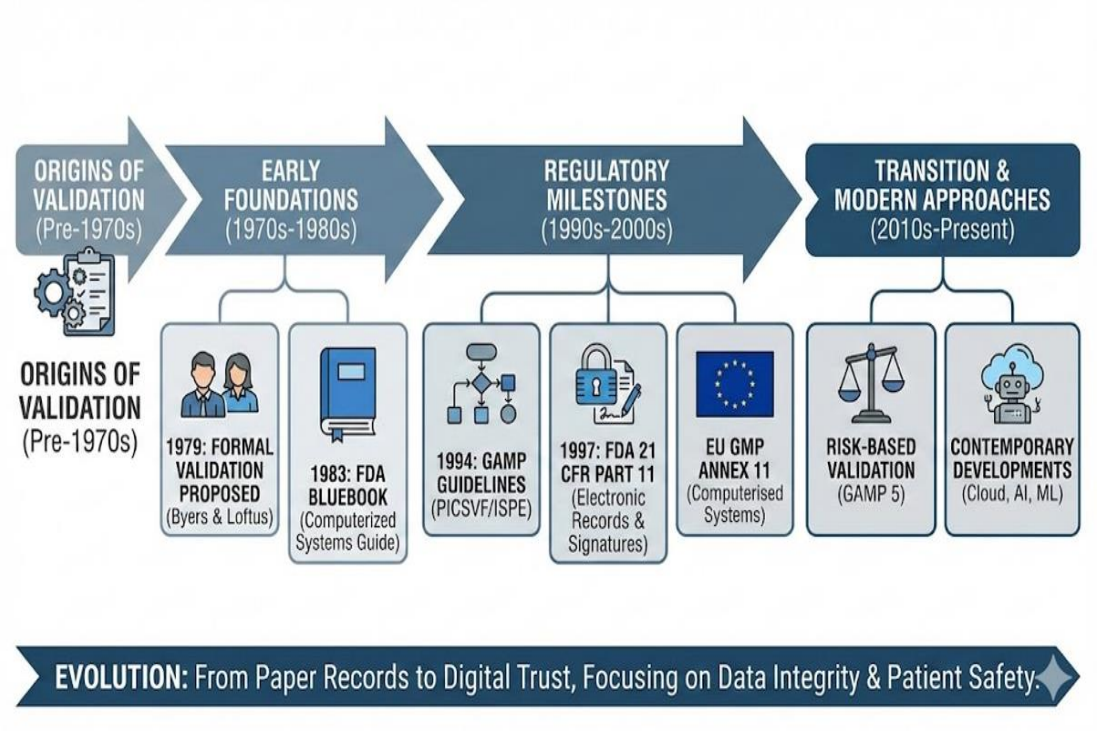


Figure 1: History of Computer System Validation

Life cycle of system development:
this figure2 explain The System Development Life Cycle, or SDLC,[22,20,27] is a structured process for developing software systems. It comprises stages like organizing, evaluating, and designing,developmenttesting,execution and upkeep. By offering a precise framework for ensuring high-quality software, lowering risks, and increasing project efficiency, SDLC managing each phase of the system's life span

NEED FOR VALIDATION QUALIFICATION AND SYSTEM VALIDATION:

In regulated sectors like biotechnology, pharmaceuticals, andhealthcare, validation and qualification are essential components of compliance and quality assurance. [1,8] Validation ensures that processes, systems, and software perform reliably and consistently, while qualification focuses on confirming that equipment and systems are installed and operating as intended.

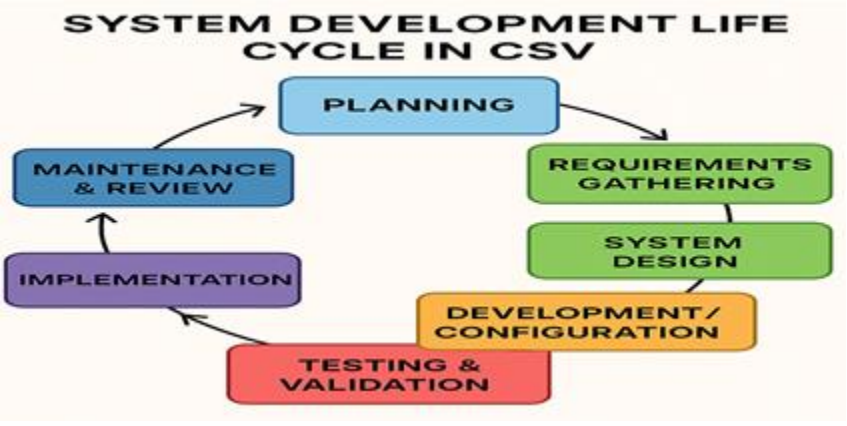


Figure 2: system development life cycle in csv

Computerised System Validation (CSV) specifically refers to the methodical procedure for making sure that computerised systemsfulfiltheir intended purpose and comply with applicable regulatory standards like EU Annex 11 and FDA 21 CFR Part 11[23,44]CSV is essential formaintaining data integrity, product quality, and patient safety. It ensures that all software and hardware used in regulated environments are thoroughly tested, properly documented, and remain under control throughout their lifecycle.

The CSV process is based on qualification activities, such as Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). These stages guarantee that systems are installed correctly, tested for functionality, and operate dependably in practical settings.[45,19]The need for validation and qualification in CSV arises from the growing reliance on automated systems to handle critical operations such as data recording, processing, and reporting. Without proper validation, organizations risk system failures, data breaches, regulatory non-compliance, and ultimately, harm to public health.

Therefore, validation and qualification are not only regulatory requirements but also business-critical practices that support trust, traceability, and transparency in digital systems. They form the backbone of robust computerized system validation, ensuring systems remain compliant, efficient, and audit-ready throughout their lifecycle.

TYPES OF VALIDATION IN COMPUTERISED SYSTEM VALIDATION (CSV):
In CSV, several types of validation are used to guarantee that computerized systems are appropriate for their intended use, abide by rules, and reliable throughout their lifecycle. Here are the main types:

1. Qualification for Installation (IQ)
confirms the proper installation of the system and its parts.[15]Includes checking hardware, software versions, network connections, and documentation.
Example: Verifying server specs match system requirements.
2.Qualification for Operations (OQ) Confirms that the system functions correctly under expected conditions.
Tests all operational controls, functions, and alarms.
Example: Validating login access controls, workflows, and error messages.

3. **PerformanceQualification** (**PQ**)
Ensures the system performs effectively in the live environment.
Conducted using real-world scenarios and data [11,18].
Example: Running a production batch and verifying system output matches expectations.
4. Qualification of Design (DQ)
confirms that the design of the system satisfies user and legal requirements.
carried out in the design stage.Example :Confirming that the software can generate audit trails as specified.
5. Process Validation
Validates that a business or manufacturing process, when executed via the system, yields consistent, expected results.
Example: Validating an electronic batch record system.
6. **CleaningValidation** (if applicable)
Ensures cleaning processes (often in lab/manufacturing software interfaces) do not affect product quality or system performance.
7.Re-Validation
Conducted after significant system changes, upgrades, or periodically to ensure ongoing
An organized procedure called the System Development Life Cycle (SDLC) is used toin Computerized System Validation (CSV) to guarantee the development, deployment, and upkeep of computerized systemsin a validated state. In regulated industries like pharmaceuticals and biotechnology, [40]SDLC helps ensure systems meet Gxp requirements and comply with regulatory standards like[33]FDA 21 CFR Part 11 and EU Annex 11.
V model :this figure3 explains the V-model approach in Computerized System Validation (CSV) is a structured methodology that emphasizes a clear relationship between development and testing phases. It maps each development stage (like user requirements, functional specs, and system design) to a corresponding validation activity (such as PQ, OQ, and IQ.The left sideofThe "V"represents system definition and design, while Verification and validation are displayed on the right side. This guarantees quality, traceability, and compliance with
regulatory standards such as FDA 21 CFR Part 11[28]. The V-model is perfect for validated systems in regulated industries because it encourages early testing and defect prevention[3].

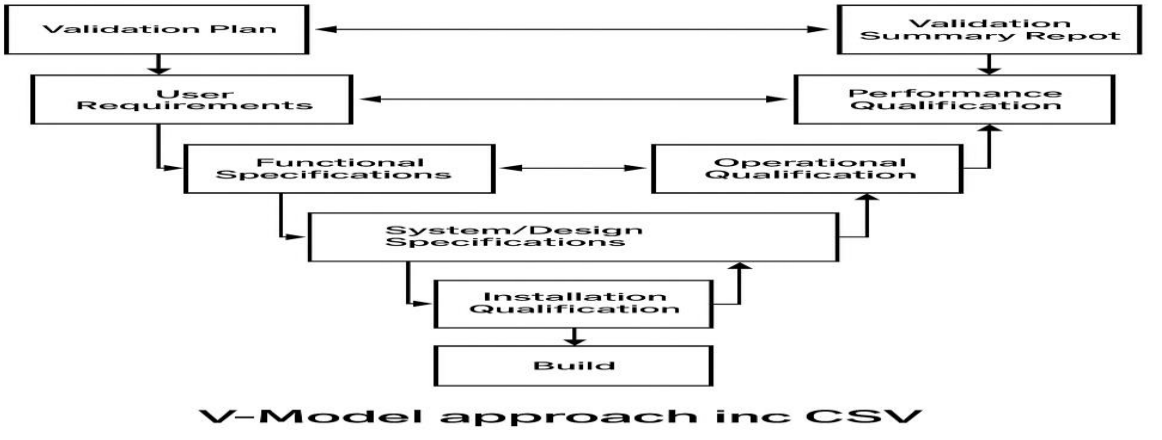


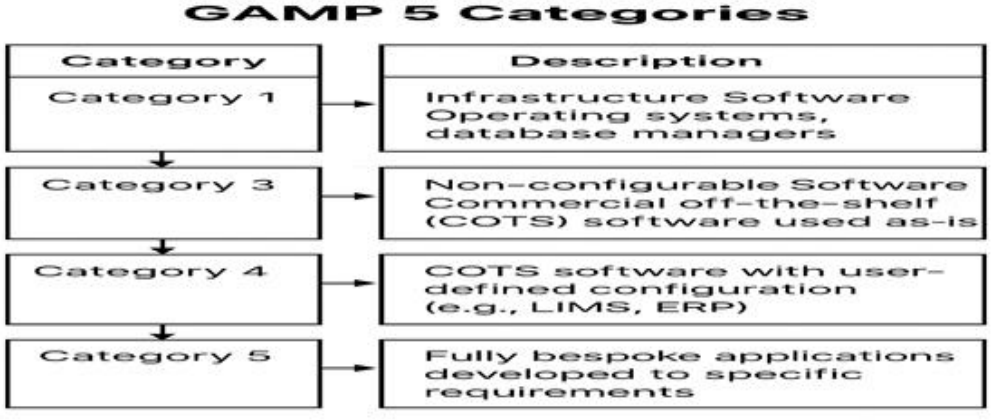
Figure 3: v model approach

Codeoffederalregulations(CSF)
21 CFR in Computerized A crucial regulatory framework known as System Validation (CSV) controls computerized systems' application in regulated industries like biotechnology, medical devices, and pharmaceuticals.[8]

Whatis21CFR
The United States is represented by 21 CFR. Title 21 of the Code of Federal Regulations was released by the Food and Drug Administration (FDA)[22]. includes regulations guaranteeing patient safety, data integrity, and product quality.

KeyPartsof21CFRRelevanttoCSV:
1. 21 CFR Part 11: Electronic Signatures and Records Definescriteria for the use of electronic signatures and documents as dependable and trustworthy.
Requires:
Computer system validation
Secure user access
Audit trails
Record retention
Electronic signature controls
1.21 FR Part 210 & 211 – [30,33]Good Manufacturing Practice (GMP) for Drugs
Ensures systems used in manufacturing, quality control, and documentation are validated for consistent, accurate performance.
21 CFR Part 820 [45,6,9,]–[Quality System Regulation (QSR)
Applies to medical devices and mandates validation of automated processes to ensure device quality and regulatory compliance.

PurposeinCSV:
CSV ensures that systems comply with 21 CFR requirements, safeguarding data integrity, product quality, and regulatory acceptance [7,22,16,12].
In short: 21 CFR is the foundation that CSV is built to support.



GAMP 5 aligns with

Figure 4: GAMP 5 guidelines

GAMP5

this figure 4 explains GAMP 5, or Good Automated Manufacturing Practice, provides a risk-based method forvalidating computerized systems in regulated industries. It emphasizes understanding product and process, applying scalable lifecycle activities, and focusing efforts based on system risk and complexity. GAMP 5 promotes critical thinking, categorizes software types, and encourages leveraging vendor documentation when appropriate. Core principles include proper planning, specification, verification, and maintaining data integrity

throughout the system lifecycle. Widely used in Computerized System Validation (CSV),

international laws such as FDA 21 CFR Part 11 and EU Annex 11. confirming that systems are suitable for their intended use and comply with regulations.

Software Used in Computerized System Validation (CSV)

figure5 explains to ensure compliance with FDA 21 CFR Part 11, EU GMP Annex 11, WHO, and GAMP 5 guidelines, computerized system validation uses a combination of specialized validation tools, quality systems, and regulated operational software. Based on their function in the CSV life cycle, these software tools can be broadly categorized**.** [45,22]1. Management of the Validation Lifecycle Programs These tools manage CSV documentation, traceability, and validation execution.Commonly Used SoftwareValGenesis VLMS, KneatGx,MasterControl ValidationVeeva Vault Validation Management, TrackWise Digital Validation

Purpose:URS, FS, DS authoring,[30,47]Risk assessment and traceability matrix,IQ/OQ/PQ protocol management, electronic approvals and audit trails. The Application in CSVWidely used for prospective and risk-based validation of GxP systems.2. Quality Management System (QMS) SoftwareQMS platforms support compliance and controlled processes around validated systems.ExamplesVeeva Vault QMSMasterControl QMSETQ RelianceTrackWise Digital QMSSparta SystemsRole in CSVChange controlDeviation and CAPA managementPeriodic review of validated systemsDocument control3. Electronic Document Management Systems (EDMS)EDMS software ensures controlled documentation, a critical requirement for CSV.ExamplesVeeva Vault DocsOpenText DocumentumSharePoint (validates
configuration)MasterControlDocsCSVRelevanceControlledSOPandvalidationdocumentsVersioncontrol
electronic signaturesCompliance with 21[9,23] CFR Part 14. Test Management and Automated Testing SoftwareUsed to execute and document validation testing, especially in agile or continuous validation models.ExamplesHP ALM / Quality CentreJira + X-ray / Zephyr,TestRail, qTestSelenium (regulated use)Application Functional testing (OQ)Regression testing after changesAutomated evidence generation5.Infrastructure and System Monitoring SoftwareEnsures validated state of IT infrastructure.ExamplesServiceNown Solar WindsNagiosBMC RemedyRole in CSV Incident managementChange trackingSystem availability monitoringInfrastructure qualification support[46,39,]6. Laboratory and Manufacturing GxP Systems (Subject to CSV)These systems themselves must be validated A. Laboratory SystemsLIMS (LabWare, STARLIMS)Chromatography Data Systems (CDS)EmpowerOpen LabChromeleonB. Manufacturing & Quality SystemsMES (WerumPAS-X, Syncade)ERP (SAP GxP modules, Oracle)SCADA / DCS (DeltaV, Siemens PCS7)7. Cloud-Based and SaaS Validation PlatformsIncreasingly used in modern CSV strategies.ExamplesVeeva Vault (SaaS)Salesforce

(validated configuration)AWS & Azure GxP[18,45,49]environmentsCSV Considerations Shared responsibility modelSupplier qualificationContinuous validationData integrity controls8. Electronic Records and Electronic Signature (ERES) SoftwareCritical for compliance with 21 CFR Part 11.Examples DocuSign (validated use)Adobe Acrobat Sign (regulated use)Veeva eSignature9. Risk Assessment and Data Integrity Tools Used to support ICH Q9 and ALCOA+ compliance[50].ExamplesRiskWatchFMEA software toolsCustom risk assessment spreadsheets (validated)

major global companies widely recognized for strong Creating Shared Value (CSV) and Corporate Social Responsibility (CSR) practices that you can use as examples in your article — including why they're noteworthy:

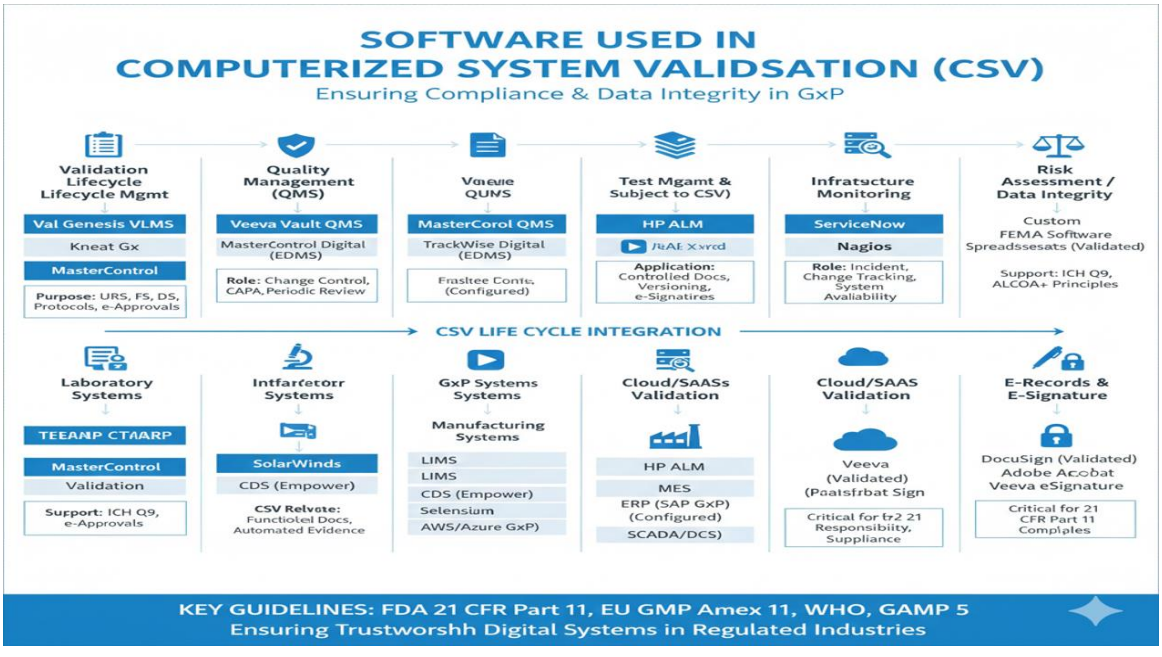


Figure 5: software in computerized system validation

Top Companies Known for CSV / CSR Worldwide

NestléKnown for embedding Creating Shared Value into its global strategy, with programs focused on nutrition, water stewardship, and rural development. Nestlé even created the Creating Shared Value Prize to encourage CSV globally[46,30,20]. 2.ItsSustainableLivingPlan,Unileverworkstodecouplegrowthfromenvironmentalimpact and enhance livelihoods. Examples include health campaigns (like rural hygiene education) that improve lives while strengthening markets for its products. 3. IBMUses initiatives like IBM's Corporate Service Corps to send experts to emerging markets for projects (e.g., smart cities,education) that benefit communities and create new business insights. 4. Google / Alphabet Offers global services (like Search and Maps) that create shared value by increasing access to information and helping small businesses grow. Projects like Project Loon aimed to bring connectivity to remote areas. 5. Novo NordiskA leader in healthcare CSV, especially in diabetes care programs that improve public health while expanding its markets (e.g., physician training and community outreach). 6. PatagoniaA pioneer in environmental sustainability, encouraging product longevity (e.g., Worn Wear program) and donating a

portion of sales to environmental causes building brand loyalty and environmental impact. 7.IKEA Committed torenewable and recycled materials by 2030, focusing on energy efficiency and sustainable products that help customers live sustainably. 8. Coca-Cola (CSV-Oriented Initiatives)Through initiatives like youth employment and water stewardship programs, Coca-Cola works to create shared opportunities for communities while reinforcing local business ecosystems.9. Adidas Works with Parley for the Oceans to use recycled ocean plastics in products, reducing waste and benefiting coastal communities — a clear example of CSV in action.

Case studies-Case Study 1: Enhanced Adherence via Successful CSV
Due to inconsistent validation procedures, a mid-sized pharmaceutical company frequently encountered problems with data integrity and regulatory findings. They started a thorough CSV improvement project, evaluating all important systems and giving top priority to those that pose the greatest risk to compliance. [50,19,6] Data integrity, security, and GxP compliance were the main topics of the development of Clear User Requirements Specifications (URS). A cross-functional team implemented new IQ/OQ/PQ procedures that complied with 21 CFR Part 11 requirements and other current standards.They put in place ongoing staff training and a strong change control procedure. Improved product release times, streamlined operations, and a notable decrease in data integrity problems were the results. [11,]Regulators observed the existence of audit trails, appropriate documentation, and controlled changes, indicating how proactive CSV improves efficiency and compliance. Subsequent audits revealed no significant CSV observations.

Case Study 2-Reacting to Regulatory Audit Findings through CSV The FDA sent a warning letter to a major pharmaceutical company, pointing out serious CSV flaws such as missing audit trails on vital systems, a lack of OQ/PQ evidence, and inadequate validation documentation. They established a remediation task force comprising IT, QA, and production to reduce the risk to product licenses and reputation. A gap analysis was carried out in comparison to GAMP 5 principles and FDA requirements[33,22]. Their Validation Master Plan was revised, completed IQ/OQ/PQ again with all the necessary paperwork, and made technical corrections like turning on and check),in audit trails in manufacturing and lab systems. All audit findings were successfully addressed, regulatory trust was rebuilt, and system reliability was strengthened by this comprehensive but reactive CSV overhaul.

Case Study 3 – Application of CSV in Clinical Research [48](CRO) To guarantee data integrity and regulatory compliance, a clinical research organization (CRO) deployed CSV for its Clinical Data Management System (CDMS). A risk-based evaluation, the creation of URS and Functional Specifications (FS), and the use of actual clinical data scenarios in the IQ/OQ/PQ testing were all part of the strategy.[10] Testing and documentation were standardized through the use of automation tools. Reduced errors, timely regulatory submissions, and accurate, auditable data collection were all made possible by the validated CDMS. The importance of CSV in preserving data integrity in intricate, high-stakes clinical settings is demonstrated by this case.

Case Study 4 – Integrating AI into the CSV lifecycle[6] A consulting firm collaborated with more than 20 clients to modernize CSV. For instance, a GPT-enabled solution improved standardization and cut down on drafting time by 40% by automating the creation of baseline scripts for reporting dashboards.In order to free up human reviewers to concentrate on context-specific inputs, the AI tool took care of navigation paths, filters, and data logic. Real-

time validation insights were made possible by this transition from manual, document-heavy processes to dynamic, AI-augmented validation, which showed how automation can improve efficiency without sacrificing compliance.

Conclusion

In regulated industries, especially pharmaceuticals, biotechnology, medical devices, and healthcare, computerized system validation (CSV)[1,8,9] has developed into a key component of quality assurance. As organizations increasingly rely on computerized systems to manage critical data, automate processes, and ensure regulatory compliance, the validation of these systems is no longer optional but a regulatory and operational necessity. CSV ensures that computerized systems consistently perform as intended, maintain data integrity, and support patient safety and product quality throughout their lifecycle[2,3,5].The review highlights that modern CSV practices have shifted from traditional document-heavy, rigid validation approaches to more risk-based, lifecycle-oriented methodologies, as emphasized in global regulatory guidance such as FDA 21 CFR Part 11, EU GMP Annex 11, ICH Q9, [14,13,19] and GAMP 5[22,23]. This evolution reflects the growing complexity of computerized systems, including enterprise resource planning (ERP), laboratory informatics (LIMS, ELN, SDMS), manufacturing execution systems (MES), cloud-based platforms, and automated quality management systems (QMS).By adopting risk-based validation, organizations can focus validation efforts on systems and functions that directly impact product quality, data integrity, and regulatory compliance, thereby improving efficiency while maintaining compliance.Furthermore, CSV plays a critical role in ensuring data integrity, which has become a central focus of regulatory inspections worldwide. [8,12] Validated systems support the ALCOA+ principles by guaranteeing that information is attributed, readable, current, unique, accurate, comprehensive, consistent, long-lasting, and accessible. Effective CSV implementation strengthens inspection readiness, reduces the risk of regulatory observations, and enhances organizational confidence in electronic records and electronic signatures[49,39,7]. The review also underscores the growing importance of vendor involvement, supplier assessment, and system configuration management in CSV. As businesses use cloud-based solutions and commercial off-the-shelf (COTS) software more frequently, cooperation between system owners,IT teams, quality units, and vendors is essential to ensure compliant system design, controlled changes, and continuous validated status. [36]Lifecycle activities such as change management, periodic review, deviation handling, and system retirement are now recognized as integral components of CSV, rather than post-validation obligations. Looking forward, the future of CSV is closely linked with digital transformation initiatives, including automation, artificial intelligence, machine learning, and cloud computing. These technologies present new validation challenges pertaining to system transparency, algorithm control, cybersecurity, and regulatory acceptance, even though they provide notable benefits in efficiency and data management[48,]. Consequently, CSV strategies must continue to evolve, integratingquality risk management principles, data governance frameworks, and regulatory intelligence to address emerging technologies.

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Chapter- Fourteen

Unlocking Hidden Therapeutic Potential: Strategic Approaches to Drug Repurposing In Complex Diseases

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Abstract

Drug repurposing — the systematic identification of new therapeutic applications for approved or investigational compounds — has emerged as a transformative strategy in modern pharmaceutical development, reducing development costs by 60–85% and timelines by half compared to de novo drug discovery. This chapter provides a comprehensive examination of the strategic landscape of drug repurposing in complex diseases, encompassing four major methodological pillars: computational approaches (network pharmacology, molecular docking, AI/machine learning, transcriptomics via the Connectivity Map, and phenome-wide association studies), experimental phenotypic screening, target-based strategies, and clinical epidemiology. Applications across oncology, neurodegeneration, cardiovascular disease, and rare and neglected diseases are analyzed in depth, with detailed case studies of landmark repurposing successes including thalidomide for multiple myeloma, sildenafil for Alzheimer's disease, colchicine for secondary cardiovascular prevention, baricitinib for COVID-19, and rituximab for autoimmune conditions. The chapter critically examines persistent structural barriers — intellectual property gaps, commercial disincentives, translational failures, and trial design challenges — and presents emerging frontiers in AI-driven discovery, multi-omics integration, patient-derived organoid screening, and open-science platforms. Drug repurposing represents one of the most efficient pathways to addressing the 90% of human diseases still lacking effective treatment options.

Keywords: *drug repurposing; drug repositioning; network pharmacology; artificial intelligence; machine learning; connectivity map; cancer; neurodegeneration; colchicine; baricitinib; thalidomide.*

Introduction

Conceptual Foundations and the Economic Imperative

Drug repurposing — also termed drug repositioning, drug reprofiling, drug re-tasking, or drug rescue — was formally defined by Ashburn and Thor in their landmark 2004 Nature Reviews Drug Discovery paper as the process of finding new uses outside the scope of the original medical indication for existing drugs.1 Pushpakom et al. refined this definition in their 2019 comprehensive review, describing it as a strategy for identifying new uses for approved or investigational drugs that are outside the scope of the original medical indication, emphasizing that the concept involves the use of de-risked compounds, with potentially lower overall development costs and shorter development timelines.2 Despite multiple synonymous terms circulating in the literature, the core concept remains consistent: leveraging existing pharmacological, toxicological, and clinical data to accelerate the development of drugs for new indications.

The economic rationale for drug repurposing is compelling and increasingly urgent. DiMasi, Grabowski, and Hansen estimated in their influential 2016 Journal of Health Economics study that the average capitalized cost of developing a new molecular entity reached $2.6 billion (in 2013 dollars), with out-of-pocket development costs of approximately $1.4 billion and a development timeline of 10–17 years. Repurposed drugs, by contrast, typically require only $200–$300 million and 3–12 years to reach approval, bypassing substantial preclinical toxicology, Phase I dose-escalation studies, and formulation development because existing pharmacokinetic, pharmacodynamic, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) data are already available from regulatory dossiers.4

The attrition advantage of drug repurposing is equally significant. Traditional drug development suffers approximately 90% attrition from clinical entry to approval, with Phase II failure (largely due to lack of efficacy) representing the highest-risk stage. Drug repurposing achieves an estimated 30% FDA approval rate — a threefold improvement over new molecular entities — largely because safety-related failures, which account for roughly one-third of clinical attrition for novel drugs, are substantially mitigated by pre-existing human safety and tolerability data.

A longitudinal analysis by Akodad et al. (2025, Communications Medicine) of all FDA-approved drugs from 1985 to 2024 confirmed that 451 drugs received subsequent approval for a new therapeutic use, with a mean interval of 7.2 years between original and repurposed approval — substantially shorter than de novo timelines.3 Historically, most successful repurposing arose through clinical serendipity: sildenafil's erectile dysfunction benefit discovered during angina trials, minoxidil's hair regrowth observed during antihypertensive therapy, and thalidomide's anti-inflammatory properties discovered by physician Jacob Sheskin in 1964 when treating erythema nodosum leprosum. The modern era, catalyzed by computational biology, artificial intelligence, and large-scale genomic and clinical datasets, has shifted the paradigm decisively toward systematic, rational repurposing strategies.

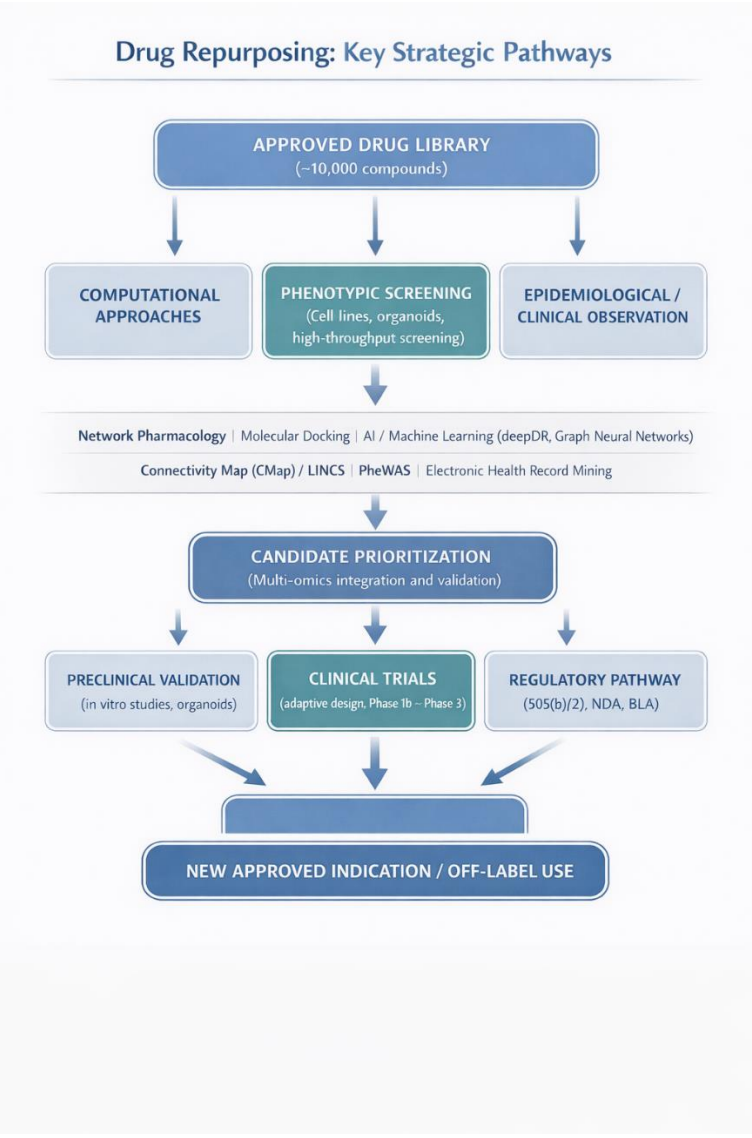

Figure 1. Schematic workflow of drug repurposing strategies. Approved drug libraries are screened through computational, phenotypic, and epidemiological approaches, followed by candidate prioritization, preclinical validation, adapted clinical trials, and regulatory approval. AI/ML = artificial intelligence/machine learning; CMap = Connectivity Map; GNN = graph neural network; HTS = high-throughput screening; PheWAS = phenome-wide association study.

Computational and Bioinformatics Approaches

2.1 Network Pharmacology: Mapping the Interactome

Network pharmacology, first articulated by Andrew Hopkins in his 2008 Nature Chemical Biology paper, challenges the reductive 'one drug, one target' paradigm by mapping drugs, targets, and diseases onto biological networks and exploiting the emergent properties of molecular interactions.5 The foundational insight — formalized mathematically by Guney, Menche, Vidal, and Barabási in Nature Communications (2016) — is that therapeutic effects

are localized within small network neighborhoods called disease modules.6 Drugs whose targets are proximal to disease-associated genes on the human protein-protein interactome are predicted to have therapeutic potential — the 'network proximity' hypothesis. Their analysis of 238 drugs across 78 diseases demonstrated this proximity metric reliably distinguishes effective from ineffective drug-disease pairs.

The approach reached clinical validation when Cheng et al. (2018, Nature Communications) used network-based proximity to predict drug repurposing candidates, then validated predictions against 220 million patient insurance records.7 They identified hydroxychloroquine as potentially protective against coronary artery disease (HR 0.76, 95% CI 0.59–0.97). During COVID-19, Morselli Gysi et al. (2021, PNAS) deployed network diffusion and proximity algorithms to rank 6,340 drugs for SARS-CoV-2 efficacy, providing a systematically derived prioritization list validated against emerging clinical evidence.8

2.2 AI and Machine Learning: The Prediction Revolution

Deep learning architectures have dramatically improved drug repurposing prediction accuracy and scalability. The deepDR framework (Zeng et al., 2019), a multi-modal deep autoencoder learning from 10 drug-related networks, achieved an AUROC of 0.908, outperforming traditional DTINet by 4.6%.11 deepDTnet (Zeng et al., 2020), combining deep autoencoder representations of drug-target networks, reached an AUC-ROC of 0.963, substantially outperforming random forest (0.911), SVM (0.869), and k-NN (0.839).12

Knowledge graph approaches represent a distinct and powerful paradigm. Himmelstein et al. (2017, eLife) built Hetionet, integrating 29 types of nodes and 24 types of edges from biomedical databases spanning 47,031 compounds, 28,274 genes, 137 diseases, and 4,137 anatomical features, to systematically prioritize drug repurposing candidates.38 BenevolentAI's knowledge graph platform — the same system that identified baricitinib for COVID-19 — operates at the scale of hundreds of millions of biomedical relationships. The global AI-in-drug-discovery market was valued at $1.72 billion in 2024 and is projected to reach $8.53 billion by 2030, driven by platforms including Recursion (which leveraged 65 petabytes of proprietary phenomics data to forge a $1.5 billion partnership with Bayer), Insilico Medicine, and Exscientia.

2.3 Transcriptomics and the Connectivity Map

The Connectivity Map (CMap), introduced by Lamb et al. in Science (2006), operationalized a conceptually elegant approach: if a drug-induced gene expression signature is anti-correlated with a disease expression signature, that drug may therapeutically reverse the disease phenotype.9 The next-generation LINCS L1000 platform (Subramanian et al., 2017, Cell) massively expanded this resource to over 3 million gene expression profiles from approximately 33,000 small molecules and 9,200 genetic perturbagens across more than 200 cell types.10 The L1000 assay measures 978 landmark genes sufficient to capture approximately 81% of genome-wide transcriptional variation.

Successful CMap-based predictions include topiramate for inflammatory bowel disease (Dudley et al., 2011, Science Translational Medicine) and cimetidine for lung adenocarcinoma (Sirota et al., 2011). However, a critical evaluation by Lim and Pavlidis (2021, Scientific Reports) found only a 17% cross-version reproducibility rate for CMap predictions, raising important concerns about platform reliability that the field must address through independent validation pipelines.13

2.4 PheWAS and Electronic Health Record Mining

Phenome-wide association studies (PheWAS), pioneered by Denny et al. (2010, Bioinformatics), reverse the GWAS paradigm by starting with a genetic variant and systematically testing associations across hundreds of clinical phenotypes derived from electronic health records.14 For drug repurposing, genetic variants in drug target genes that associate with multiple diseases via PheWAS identify those drugs as multi-indication repurposing candidates. Diogo et al. (2018, Nature Communications) conducted PheWAS of 25 SNPs across 19 drug targets in up to 697,815 individuals, replicating 75% of known GWAS associations and identifying 9 novel associations.15 Mendelian randomization — using genetic variants as instrumental variables to infer causality from observational data — adds a critical layer by predicting both therapeutic efficacy and potential adverse effects of target modulation, addressing the confounding that plagues conventional pharmacoepidemiology.

Table 1. Comparative Overview of Major Drug Repurposing Strategies

Strategy	Key Tools / Methods	Advantages	Limitations	Example Success
Network Pharmacology	Protein-protein interactome, STRING, Cytoscape, network proximity algorithms	Captures polypharmacology; validated computationally & clinically	Incomplete interactome data; high false-positive rates	Hydroxychloroquine for CAD (Cheng et al., 2018)
Molecular Docking / Virtual Screening	AutoDock Vina, Glide, DOCK, UCSF Chimera	Atomic-level structural insight; rapid screening of large libraries	Scoring inaccuracy; requires high-quality protein structures	Imatinib for GIST via c-kit off-target binding
AI / Machine Learning	deepDR, deepDTnet, GNNs, knowledge graphs, BenevolentAI platform	Integrates heterogeneous data; AUC up to 0.963; scalable	Black-box models; limited interpretability; risk of overfitting	Baricitinib for COVID-19 (Richardson et al., 2020)
Transcriptomics / CMap	LINCS L1000 (3M+ profiles), CLUE.io, gene set enrichment analysis	Phenotypic disease reversal logic; captures complex biology	Reproducibility concerns (17% across versions); cell-line limitations	Topiramate for IBD (Dudley et al., 2011)
PheWAS / EHR Mining	eMERGE network, UK Biobank, Mendelian	Real-world clinical validation; large sample sizes	Confounding by indication; selection bias; data	Sildenafil for AD (Fang et al., 2021)

	randomizatio n, ICD codes		heterogeneity	
Phenotypic Screening	PRISM (Broad), patient-derived organoids, high-content imaging	Unbiased target discovery; captures whole-cell responses	Low throughput; difficult to identify mechanism of action	Itraconazole anti-angiogenic activity (Chong et al., 2007)

Abbreviations: AUC = area under the curve; CAD = coronary artery disease; CMap = Connectivity Map; EHR = electronic health record; GNN = graph neural network; GIST = gastrointestinal stromal tumor; IBD = inflammatory bowel disease; NSCLC = non-small cell lung cancer; PheWAS = phenome-wide association study.

3. Applications in Complex Diseases

3.1 Oncology: The Largest and Most Active Arena

3.1.1 Metformin: Compelling Biology, Elusive Clinical Benefit

The metformin-cancer story began with Evans et al.'s landmark 2005 BMJ observational study, which reported substantially reduced cancer incidence among diabetic patients receiving metformin versus other antidiabetic therapies.16 Subsequent meta-analyses appeared to confirm the association: a large 2014 meta-analysis of 47 studies encompassing 65,540 cancer cases by Gandini et al. found overall cancer incidence reduced by 31% (SRR = 0.69, 95% CI 0.52–0.90), with site-specific reductions in colorectal, hepatocellular, and pancreatic cancers.17

The proposed mechanisms are mechanistically well-characterized. Metformin inhibits mitochondrial complex I, activating AMPK and subsequently suppressing mTORC1 signaling, reducing translation of oncogenic proteins including cyclin D1 and c-Myc. Indirect effects include substantial reductions in circulating insulin and IGF-1 levels, disrupting the PI3K/AKT/mTOR axis that drives proliferation in many tumor types. Despite enthusiasm, clinical translation has been profoundly disappointing. A 2023 British Journal of Cancer perspective raised the pointed question of whether metformin repurposing for cancer remains worthwhile, noting that large randomized trials across multiple tumor types have failed to demonstrate clear benefit.18 The critical problem: epidemiological studies compared metformin users to users of other diabetic drugs, some of which may themselves increase cancer risk, confounding the apparent benefit. Additionally, the supraphysiological concentrations required for anticancer effects in vitro far exceed achievable clinical plasma levels. Metformin's story now serves as a cautionary paradigm about the gap between epidemiological association and interventional efficacy.

3.1.2 Thalidomide: From Teratogen to Cornerstone Cancer Therapy

Thalidomide's trajectory from catastrophic teratogen to cornerstone cancer therapy remains the most dramatic repurposing narrative in pharmaceutical history. After withdrawal in 1961 following over 15,000 birth defects, D'Amato, Loughnan, Flynn, and Folkman demonstrated anti-angiogenic properties in 1994 using rabbit corneal micropocket assays, linking the drug to Folkman's seminal 1971 hypothesis that tumor angiogenesis is required for growth beyond 2mm diameter. Singhal et al.'s landmark 1999 New England Journal of Medicine paper

reported a 32% response rate in 84 patients with refractory multiple myeloma — a population with no remaining treatment options — including complete responses in patients resistant to all prior therapies.19

The mechanistic understanding deepened when Ito et al. (2010, Science) identified cereblon (CRBN) as thalidomide's primary molecular target, revealing that thalidomide and its analogs (immunomodulatory drugs, or IMiDs) function as molecular glues that recruit neo-substrates including Ikaros and Aiolos to the CRL4-CRBN E3 ubiquitin ligase complex for proteasomal degradation.20 This discovery — itself driven by understanding a repurposed drug — spawned lenalidomide (Revlimid) and pomalidomide, drugs that became cornerstones of myeloma therapy. Lenalidomide alone generated over $12 billion in annual sales at its commercial peak, illustrating the enormous commercial potential that a single repurposing success can unlock when novel analogs are pursued.

3.1.3 Additional Oncology Candidates: Itraconazole and Aspirin

Itraconazole, an oral antifungal used since the 1980s, was identified through systematic phenotypic screening as a potent angiogenesis inhibitor. Kim et al. demonstrated Hedgehog pathway inhibition, and Xu et al. showed mTOR suppression via disrupted cholesterol trafficking. In a Phase 2 trial, Rudin et al. (2013, Journal of Thoracic Oncology) reported that adding itraconazole to pemetrexed in metastatic non-squamous NSCLC produced a doubling of progression-free survival and a fourfold increase in overall survival, though the small sample size (23 patients) limits generalizability.22 The ReDO (Repurposing Drugs in Oncology) Project, founded in 2014 by Pantziarka, Bouche, and Sukhatme through the Anticancer Fund and GlobalCures, systematically curates evidence for non-cancer drugs with anticancer potential, cataloguing 268 candidate drugs in its ReDO_DB with published preclinical and clinical activity.24

Aspirin's anticancer evidence is strongest for colorectal cancer. Rothwell et al.'s meta-analyses of randomized trials with 20-year follow-up demonstrated that daily aspirin reduced colon cancer incidence (HR 0.76) and mortality (HR 0.65), with benefits appearing after five or more years of continuous use and persisting for decades.23 The mechanism involves permanent inactivation of platelet COX-1, reducing pro-tumorigenic thromboxane A2 and prostaglandin E2, plus COX-2 inhibition in tumors overexpressing this enzyme in 80–90% of colorectal cancers.

3.2 Neurodegeneration: Unlocking Novel Mechanisms

3.2.1 Sildenafil for Alzheimer's Disease

Sildenafil for Alzheimer's disease represents computational drug repurposing at its most sophisticated. Fang et al. (2021, Nature Aging) at Cleveland Clinic used endophenotype disease module-based methodology to screen 1,600+ FDA-approved drugs against Alzheimer's disease-associated gene networks spanning approximately 350,000 protein interactions.25 Sildenafil emerged as the top candidate, and retrospective analysis of 7.23 million insurance claims records revealed that sildenafil users were 69% less likely to develop Alzheimer's disease (HR 0.31, 95% CI 0.25–0.39, $P < 1.0 \times 10^{-8}$). A 2024 validation study confirmed findings across 11.52 million subjects, with protective effects ranging from HR 0.54 to HR 0.70 across two independent insurance databases. Mechanistically, PDE5 inhibition by sildenafil preserves cGMP and activates CREB-dependent synaptic plasticity pathways while reducing tau phosphorylation — targets directly relevant to both amyloid and tau pathology.

3.2.2 GLP-1 Receptor Agonists and Ambroxol

GLP-1 receptor agonists, originally developed as incretins for type 2 diabetes, show remarkable neuroprotective potential. The ELAD trial demonstrated that liraglutide reduced cognitive decline on ADASexec tests ($P < 0.001$) and significantly preserved temporal lobe volumes ($P < 0.001$) and total grey matter ($P = 0.002$) over one year in MCI/AD patients. Athauda et al. (2017, Lancet) demonstrated in a Phase 2 trial that exenatide significantly improved motor scores in Parkinson's disease, with benefits persisting for 12 months after drug discontinuation.27

Ambroxol, a mucolytic used clinically since the 1970s, was identified as a small-molecule chaperone for glucocerebrosidase (GCase) — the enzyme encoded by GBA, the most common genetic risk factor for Parkinson's disease. The AIM-PD Phase 2a open-label study in 17 Parkinson's patients demonstrated that ambroxol at 1,260 mg/day (10–40 times the standard mucolytic dose) crossed the blood-brain barrier (achieving CSF concentrations of 156 ng/mL) and increased CSF GCase protein by 35% ($P = 0.002$), establishing pharmacodynamic proof-of-concept.28 Multiple Phase 2 placebo-controlled trials are now underway. In contrast, nilotinib — a BCR-ABL kinase inhibitor tested for Parkinson's disease — represents the critical counterfactual: while the Georgetown single-center study reported promising biomarker changes, the independent NILO-PD trial (Simuni et al., 2020, JAMA Neurology) found negligible CSF penetration, no dopamine metabolite changes, and worsened motor scores versus placebo — underscoring the absolute necessity of rigorous independent validation.29

3.3 Cardiovascular and Anti-inflammatory Applications

Colchicine for cardiovascular disease represents one of the clearest recent repurposing successes in any disease area. The ancient gout remedy — used continuously in medicine since its isolation from autumn crocus in 1820 — inhibits tubulin polymerization and NLRP3 inflammasome assembly, reducing IL-1β and IL-6 production. This mechanism directly targets the inflammatory hypothesis of atherosclerosis, which holds that residual inflammatory risk — independent of lipid levels — drives cardiovascular events in treated patients.

Two pivotal trials established colchicine's cardiovascular efficacy. The COLCOT trial (Tardif et al., 2019, NEJM), enrolling 4,745 patients within 30 days of myocardial infarction, demonstrated a 23% reduction in the composite cardiovascular endpoint (HR 0.77, 95% CI 0.61–0.96, $P = 0.02$).30 The LoDoCo2 trial (Nidorf et al., 2020, NEJM), enrolling 5,522 patients with stable chronic coronary disease, demonstrated a 31% reduction in the primary composite endpoint (HR 0.69, 95% CI 0.57–0.83, $P < 0.001$), with consistent benefit maintained across years of follow-up.31 In June 2023, the FDA approved low-dose colchicine (Lodoco, 0.5 mg daily) as the first anti-inflammatory drug specifically indicated for secondary cardiovascular prevention — a landmark decision now reflected in the 2023 AHA/ACC guidelines.

3.4 Baricitinib: AI-Accelerated Repurposing for COVID-19

Baricitinib for COVID-19 stands as the paradigm case for AI-accelerated drug repurposing with documented global impact. In February 2020 — mere weeks into the pandemic — Richardson et al. at BenevolentAI used an AI-enhanced biomedical knowledge graph to identify this JAK1/2 inhibitor (approved for rheumatoid arthritis in 2018) as a dual-

mechanism COVID-19 candidate: anti-inflammatory via JAK inhibition of cytokine signaling, and potentially antiviral via inhibition of AAK1-mediated clathrin-mediated endocytosis.32

The ACTT-2 trial (Kalil et al., 2021, NEJM) in 1,033 hospitalized patients demonstrated that baricitinib plus remdesivir reduced median recovery time from 18 to 10 days in patients requiring high-flow oxygen or non-invasive ventilation.33 The COV-BARRIER trial (Marconi et al., 2021, Lancet Respiratory Medicine) in 1,585 hospitalized patients demonstrated a 38% relative reduction in 28-day all-cause mortality (8% vs. 13%; HR 0.57, P = 0.0018).34 Baricitinib received FDA Emergency Use Authorization in November 2020 and full approval in May 2022. The entire arc — from AI identification to regulatory approval — took approximately two years, an unprecedented timeline that validated the real-world translational potential of AI-driven repurposing.

3.5 Autoimmune Diseases and Rituximab

Rituximab, the anti-CD20 monoclonal antibody first approved for non-Hodgkin lymphoma in 1997, exemplifies progressive indication expansion driven by mechanistic understanding of B cells in autoimmunity. Following Edwards and Cambridge's 2001 hypothesis that B-cell depletion could treat rheumatoid arthritis, rituximab gained FDA approvals for RA (2006), granulomatosis with polyangiitis (2011), and pemphigus vulgaris (2018).35 Off-label use expanded dramatically, rising from 1.2% of all rituximab use in 2009 to 55.6% by 2017, spanning multiple sclerosis, membranous nephropathy, myasthenia gravis, and autoimmune hemolytic anemia.

3.6 Rare and Neglected Diseases

Sirolimus (rapamycin), an mTOR inhibitor originally developed as a transplant immunosuppressant, became the first approved therapy for lymphangioleiomyomatosis (LAM) — a rare progressive cystic lung disease affecting 3–5 per million people predominantly female patients — following the landmark MILES trial (McCormack et al., 2011, NEJM), which demonstrated stabilized FEV1 decline over 12 months ($P < 0.001$).36 For neglected tropical diseases, the Drugs for Neglected Diseases initiative (DNDi) operates a virtual biotechnology model developing treatments for sleeping sickness, Chagas disease, and leishmaniasis through systematic screening of existing compound libraries, though fewer than 5% of global R&D resources target the diseases affecting over 1.7 billion people predominantly in low-income countries.

Table 2. Landmark Drug Repurposing Successes in Complex Diseases

Drug (Original Use)	Repurposed Indication	Mechanism of Action	Key Evidence	Regulatory Status	Year Repurposed
Thalidomide (Sedative/antiemetic)	Multiple myeloma	Cereblon binding; IMiD-class immunomodulator; anti-angiogenic	Singhal et al., 1999, NEJM; 32% response in refractory MM	FDA approved (2006)	1999
Sildenafil	Pulmonary arterial	PDE5	Galie et	FDA	2005

(Angina)	hypertension (PAH)	inhibition; pulmonary vasodilation via cGMP elevation	al., 2005, NEJM; improved 6MWD vs. placebo	approved (2005)	
Colchicine (Gout)	Secondary cardiovascular prevention	NLRP3 inflammasome inhibition; IL-1β/IL-6 reduction	COLCOT (2019): 23% HR reduction; LoDoCo2 (2020): 31% reduction	FDA approved (Lodoco, 2023)	2019–2023
Baricitinib (Rheumatoid arthritis)	COVID-19 (hospitalized adults)	JAK1/2 inhibition (anti-inflammatory) + AAK1 inhibition (antiviral)	ACTT-2: recovery 10 vs. 18 days; COV-BARRIER: 38% mortality reduction	FDA approved (2022)	2020–2022
Rituximab (Non-Hodgkin lymphoma)	Rheumatoid arthritis, granulomatosis, pemphigus vulgaris	Anti-CD20 B-cell depletion	Multiple Phase III RCTs across indications	FDA approved (multiple indications)	2006–2018
Sirolimus/Rapamycin (Transplant immunosuppression)	Lymphangioleiomyomatosis (LAM)	mTOR inhibition; TSC protein target	MILES trial, 2011, NEJM; stabilized FEV1 decline	FDA approved (2015)	2015
Metformin (Type 2 diabetes)	Cancer prevention/treatment (investigational)	AMPK activation; mTORC1 suppression; insulin reduction	Evans et al. 2005 BMJ; 30-50% cancer risk reduction (observational only)	Off-label / multiple trials ongoing	2005 (observational)
Itraconazole (Antifungal)	NSCLC, prostate cancer (investigational)	Hedgehog pathway inhibition;	Rudin et al. 2013, J Thorac	Investigational (Phase 2)	2007–2013

		anti-angiogenesis; mTOR suppression	Oncol; doubled PFS in NSCLC Phase 2		

*Abbreviations: AD = Alzheimer's disease; CML = chronic myeloid leukemia; FEV1 = forced expiratory volume in 1 second; IMiD = immunomodulatory drug; LAM = lymphangioleiomyomatosis; MM = multiple myeloma; NSCLC = non-small cell lung cancer; PAH = pulmonary arterial hypertension; RA = rheumatoid arthritis. * Year of key pivotal evidence published or regulatory approval obtained.*

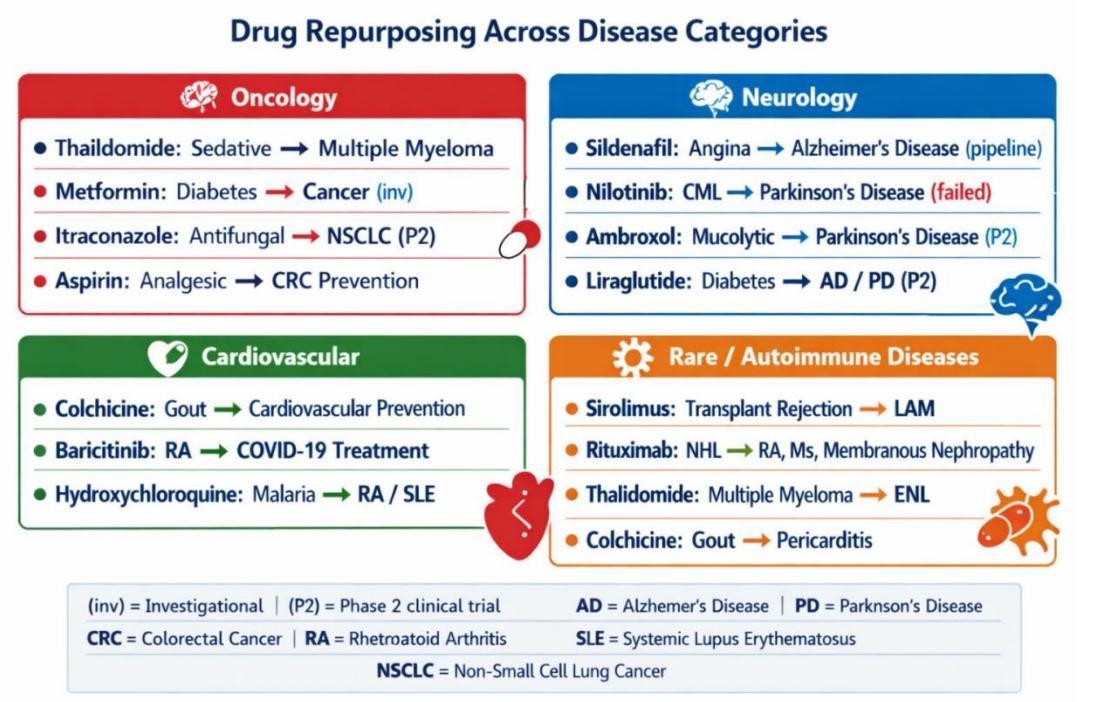

Figure 2. Summary of representative drug repurposing examples across oncology, neurology, cardiovascular medicine, and rare/autoimmune disease. Drugs are listed with their original approved indication (left) and their repurposed or investigational indication (right). Status annotations indicate regulatory approval stage.

4. Challenges and Structural Barriers

4.1 Intellectual Property and Commercial Disincentives

The most fundamental obstacle to drug repurposing is intellectual property. Composition-of-matter patents — the strongest form of pharmaceutical exclusivity — are typically expired for repurposing candidates, which by definition have been on the market for years or decades. Method-of-use patents can theoretically protect new indications, but are considered significantly more vulnerable to legal challenge than composition patents, are not patentable in Europe or many other jurisdictions, and are difficult to enforce in practice because pharmacists typically dispense generics by international nonproprietary name regardless of the prescribing physician's intended indication. The result is a structural funding gap: no pharmaceutical company can justify the $100–840 million minimum investment required for

repurposing trials if the resulting product will face immediate generic competition upon approval.

Between jurisdictions, regulatory exclusivity provisions offer partial mitigation. The US Orphan Drug Act provides 7 years of market exclusivity and substantial development incentives for indications affecting fewer than 200,000 patients — making rare disease repurposing the most commercially viable application. The FDA's 505(b)(2) pathway, which permits new drug applications to rely on the agency's prior findings of safety and effectiveness for the original indication, substantially reduces the data package required and accelerates approval timelines. Between 2010 and 2020, 570 of 1,001 NDAs were approved via this pathway.

4.2 Clinical Trial Design Challenges

Drug repurposing presents unique clinical trial design challenges that are commonly underappreciated. Dosing for a new indication may differ substantially from the original — metformin's in vitro anticancer concentrations far exceed achievable clinical plasma levels, while ambroxol's neuroprotective dose (1,260 mg/day) is 10–40 times its standard mucolytic dose. Trial endpoints, biomarker strategies, and patient selection criteria must all be reconsidered from first principles. Patient stratification is particularly challenging: complex diseases like cancer and Alzheimer's disease are biologically heterogeneous, and a repurposed drug may benefit only genomically or phenotypically defined subpopulations that standard eligibility criteria may not enrich for.

Adaptive trial designs — prospectively planned modifications based on accumulating data — offer partial solutions, enabling early termination for futility, dose optimization, and dynamic patient allocation. Basket trials (a single drug across multiple tumor types sharing a molecular feature) and umbrella trials (multiple drugs within a single disease) are increasingly employed in oncology. The FDA and EMA have issued adaptive design guidance documents, and platforms like NCATS provide regulatory science support for academic repurposing investigators.

4.3 Translational and Reproducibility Failures

Translational failures are disproportionately prevalent in drug repurposing precisely because the low apparent cost and rapid timeline can lead to underpowered or inadequately controlled studies entering the scientific literature and clinical practice. The 17% cross-version reproducibility rate for Connectivity Map signatures (Lim and Pavlidis, 2021) raises fundamental concerns about computational prediction reliability.13 Observational studies linking existing drug use to reduced disease risk are susceptible to confounding by indication, healthy user bias, selection bias, and publication bias — the metformin-cancer and sildenafil-Alzheimer's associations, while epidemiologically compelling, remain hypothesis-generating rather than confirmatory.

The nilotinib-Parkinson's disease experience provides the starkest cautionary tale: single-center pilot data from Georgetown University suggested meaningful clinical benefit and dopamine metabolite normalization, driving widespread academic interest and patient demand. The independent NILO-PD multicenter, placebo-controlled trial, however, found negligible CNS drug exposure, no dopaminergic biomarker changes, and numerically worse motor outcomes than placebo — a finding that would not have been discoverable without a rigorously designed independent validation study.29 This emphasizes that computational

predictions and promising pilot data must be validated through adequately powered, placebo-controlled, multi-center randomized trials before repurposed drugs reach routine clinical adoption.

Table 3. Key Challenges in Drug Repurposing and Proposed Mitigation Strategies

Challenge Category	Specific Barrier	Mitigation Strategies & Examples
Intellectual Property	Expired composition-of-matter patents; method-of-use patents difficult to enforce and not patentable in Europe	Orphan drug designation (7-yr exclusivity); 505(b)(2) pathway; public-private partnerships (NCATS, Cures Within Reach); academic-led trials
Regulatory	Complex data requirements for new indication; unclear regulatory pathway for academic sponsors; limited FDA guidance on repurposing-specific trials	EMA adaptive licensing; FDA 505(b)(2); breakthrough therapy designation; NCATS regulatory science support; orphan designation
Clinical Trial Design	Dose optimization for new indication; placebo group ethics for life-threatening disease; patient stratification for heterogeneous disease	Adaptive trial designs; basket/umbrella protocols; master protocols; biomarker-driven enrichment; Mendelian randomization for dose modeling
Funding & Commercial	Investment disincentives for off-patent drugs; high Phase III costs (~$200M+); lack of pharma interest in generics	NIH Common Fund; Wellcome Trust; EU REMEDi4ALL; philanthropic foundations (Every Cure, GlobalCures, Anticancer Fund); prize mechanisms
Translational Gaps	CMap reproducibility (~17% cross-version); animal models failing to predict human response; observational confounding in EHR-based studies	Patient-derived organoids; Mendelian randomization; independent replication studies; pre-registration of computational predictions; open data standards
Data & AI Limitations	Incomplete interactome data; black-box deep learning models; overfitting; selection bias in training data	Explainable AI methods; knowledge graph approaches; multi-omics integration; open-access databases (Open Targets, DrugBank, LINCS)

Abbreviations: AI = artificial intelligence; EHR = electronic health record; IP = intellectual property; NCATS = National Center for Advancing Translational Sciences; NDA = new drug application; NIH = National Institutes of Health; PheWAS = phenome-wide association study.

5. Emerging Frontiers and Future Directions

5.1 Next-Generation AI and Multi-Omics Integration

AI-driven drug repurposing has progressed from proof-of-concept demonstrations to clinical impact measured in lives saved. Graph neural networks now exploit the rich topological

structure of heterogeneous biological networks — integrating drugs, proteins, diseases, side effects, pathways, and phenotypes — to predict novel drug-disease interactions with increasing accuracy. The PRISM project at the Broad Institute screened 4,518 drugs against 578 cancer cell lines, discovering unexpected anticancer activity in numerous non-oncology drugs that would never have been tested through traditional target-based approaches.37

Multi-omics integration substantially improves prediction quality by capturing biology at multiple molecular layers simultaneously. Fang et al. (2020, Medicinal Research Reviews) demonstrated this approach for Alzheimer's disease, constructing endophenotype network modules spanning amyloidosis, tauopathy, and neuroinflammation to guide drug prioritization, resulting in the identification of sildenafil and other high-priority candidates.40 Patient-derived organoids represent a complementary frontier: three-dimensional tissue models derived from patient biopsies that recapitulate the molecular heterogeneity of individual tumors enabling personalized drug screening at the individual patient level. Karkampouna et al. (2021, Nature Communications) demonstrated organoid-based identification of effective drugs in treatment-resistant prostate cancer, establishing proof of principle for organoid-guided personalized repurposing.

Major AI-pharma partnerships are channeling unprecedented capital into this space. Novo Nordisk-Valo Health ($2.76 billion), Eli Lilly-Isomorphic Labs ($1.75 billion), and Bayer-Recursion ($1.5 billion) signal sector-wide conviction that AI-driven drug discovery — including systematic repurposing — will become the dominant paradigm within the next decade. Insilico Medicine's ISM001-055, a first-in-class TNIK inhibitor for idiopathic pulmonary fibrosis generated entirely by AI, showed positive Phase IIa results in 2023 — the first AI-designed drug to reach proof of concept — validating the generative potential of AI across de novo and repurposing applications.

5.2 Open Science Platforms and Global Initiatives

A growing ecosystem of open-access platforms is democratizing access to the data and tools needed for drug repurposing. The Drug Repurposing Hub at the Broad Institute (Corsello et al., 2017, Nature Medicine) provides a hand-curated collection of over 6,000 compounds annotated with targets and indications. Open Targets, a partnership between EMBL-EBI, the Wellcome Sanger Institute, and GSK, integrates GWAS, functional genomics, somatic mutations, and drug data to systematically score target-disease associations and prioritize candidates. DrugBank catalogs approximately 14,000 drug entries with comprehensive pharmacological information.

International collaborative initiatives are scaling rapidly. The EU's REMEDi4ALL project (Horizon Europe-funded, 24 partner organizations) aims to build a patient-centric drug repurposing platform addressing scientific, regulatory, intellectual property, and financial dimensions, with demonstration projects spanning pancreatic cancer, COVID-19, and rare diseases. NCATS' CURE ID platform enables crowdsourced reporting of novel drug uses by healthcare providers treating patients with difficult conditions. David Fajgenbaum's Every Cure organization uses AI to systematically identify repurposing opportunities, inspired by his own experience treating fatal idiopathic multicentric Castleman disease with sirolimus and achieving over ten years of remission. These platforms collectively represent a paradigm shift from proprietary, siloed pharmaceutical research toward open, collaborative science as the primary engine of repurposing discovery.

Figure 3. Converging Frontiers: Future of Drug Repurposing — Key Emerging Technologies and Translational Pathways.

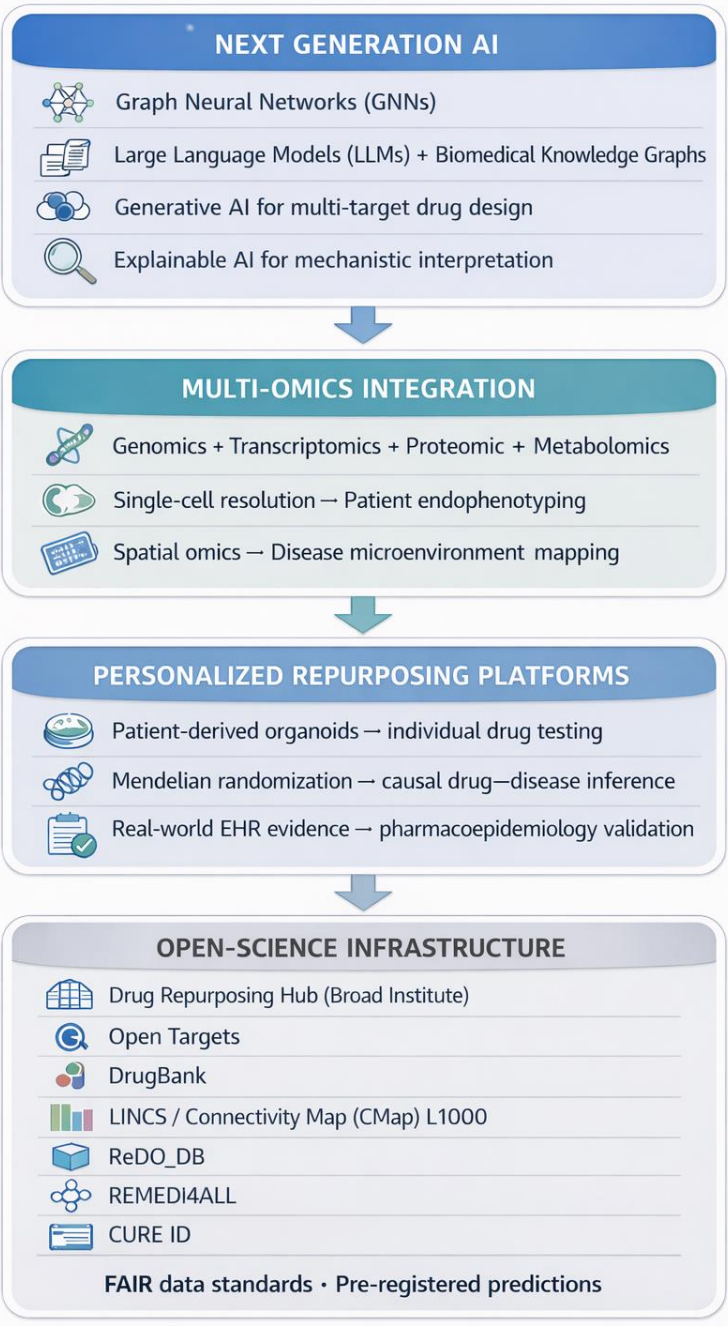

Figure 3. Converging frontiers that will define the next generation of drug repurposing. Advances in AI architecture, multi-omics data integration, personalized screening platforms, and open-science infrastructure are collectively transforming repurposing from an opportunistic practice into a systematic, data-driven discipline. EHR = electronic health record; FAIR = findable, accessible, interoperable, reusable; LLM = large language model.

Conclusion

Drug repurposing has evolved profoundly over the past two decades — from an opportunistic practice dependent on clinical serendipity and pharmacological intuition into a data-driven discipline deploying sophisticated computational, experimental, and epidemiological tools in systematic pursuit of latent therapeutic value within the existing pharmacopeia. The field's landmark successes underscore its transformative potential: thalidomide generating a multi-billion-dollar immunomodulatory drug class for multiple myeloma; colchicine becoming the first anti-inflammatory drug specifically approved for cardiovascular secondary prevention after 3,000 years of therapeutic use; baricitinib progressing from AI identification to COVID-19 regulatory approval in under two years; and sildenafil moving from cardiovascular medicine to Alzheimer's disease clinical trials.

Yet structural barriers persist and demand urgent attention. The intellectual property gap systematically discourages investment in off-patent drugs; translational failures like nilotinib in Parkinson's disease expose the critical distance between computational prediction and clinical reality; and the metformin-cancer experience reveals how epidemiological promise can persistently fail under the rigorous scrutiny of randomized controlled trials. These failures are not indictments of the repurposing strategy but rather roadmaps for improvement: they define the standards of evidence, the rigor of validation, and the clinical and mechanistic depth required before repurposed drugs can be responsibly adopted into routine care.

The path forward requires convergence across multiple dimensions simultaneously. AI architectures incorporating knowledge graphs, heterogeneous multi-omics data, and real-world evidence from hundreds of millions of electronic health records are reaching clinical-grade prediction accuracy. Patient-derived organoid systems enable personalized drug selection at the individual tumor level. Open-science platforms — the Drug Repurposing Hub, Open Targets, LINCS/CMap, ReDO_DB, REMEDi4ALL — are democratizing access to critical data and analytical tools that were previously restricted to well-resourced pharmaceutical companies.

The most urgent unmet need, however, is not technological but structural: creating sustainable funding mechanisms and regulatory incentives that make it economically rational — not merely scientifically justifiable — to pursue the hidden therapeutic potential locked within thousands of characterized, safe, affordable, and globally accessible drugs. With approximately 90% of known diseases still lacking fully effective treatments, and an estimated 10,000+ approved and investigational drugs harboring undiscovered pharmacological activities, the systematic exploitation of this vast latent therapeutic resource represents one of the most efficient and ethically urgent strategies available to modern medicine.

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