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REVIEW ARTICLE

From laboratory to computer models: Enhancing coumarin discovery through interdisciplinary research

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ABSTRACT

Coumarin-derived compounds have garnered extensive interest due to their diverse applications in medicinal chemistry, pharmacology, food, and cosmetics. There are pros and cons to both in vitro and in silico methods used in coumarin-based research. The focus is on their roles, pros, and cons in finding out the biomedical properties of these compounds. In vitro studies, conducted in controlled environments, enable detailed investigations into cellular mechanisms, enzyme interactions, and cytotoxic effects. These studies are valuable for elucidating coumarin's biological activity and therapeutic potential. Although these studies are accurate, morally acceptable, and repeatable, their inability to fully replicate complex biological systems necessitates extrapolation to real-life situations. In contrast, in silico studies leverage computational tools to model molecular interactions, predict pharmacokinetic behaviors, and simulate biological pathways. These techniques are time- and cost-efficient, capable of high-throughput screening, and useful for hypothesis generation. However, their reliability depends on the accuracy of input data and assumptions, which can limit their predictive power in real-world scenarios. Integrating these two study types provides a synergistic framework that enhances coumarin-based research. In silico models can guide the design of experiments, while in vitro assays validate computational predictions. Emerging technologies, such as machine learning, organ-on-a-chip systems, and 3D cell cultures, promise to further refine this integration, enabling faster, more accurate, and ethical research. We conducted an investigation and a literature review, utilizing PubMed data and limiting the publication period from 2000 to mid-2024. This study demonstrates the effective combination of in vitro and in silico methods to advance coumarin-based research and unlock its full therapeutic potential.

Keywords: coumarins; in vitro, in silico; effective coupling

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1. Introduction

Coumarin-based compounds (CBCs) are a family of natural and synthetic compounds with a coumarin backbone in their frameworks[1]. Their wide use field includes medicinal chemistry, pharmacology, the food industry, spices, perfumes, and cosmetics. Due to the widespread use and potential applications of CBCs, the interest in these molecules has increased exponentially among many researchers[2]. In vitro and in silico studies have proven to be useful tools for understanding the properties of a wide range of CBCs. Furthermore, the convergence of in vitro and in silico studies in current research presents a promising alternative[3].

Considering all these factors, we concentrated on investigating the properties of CBCs through in vitro and in silico studies, discussing their potential as therapeutic candidates. We also attempted to compare the research methods, highlighting the advantages and disadvantages of both in silico and in vitro studies with respect to these properties.

Based on the above observation, the objectives of this study are to dissect these two study types to show the advantages and disadvantages of each study method in the investigation of the CBCs' bioactivity. The subject's complexity necessitates the documentation of each study in separate sections. We found no literature to compare the two studies conducted on CBCs, despite a literature review revealing the relationship between these two important studies from a variety of molecular perspectives.

2. Historical perspective of coumarin-based research

In 1830, Vogel of Göttingen obtained coumarin from tonka beans and established its melting point as 68°C, while in 1868, Petrou isolated this phytochemical from a Melilotus coerulea mill. In 1886, V. P. Krymberg determined the structure of coumarin, and in the same year, A. Pictet and F. Myer described the synthesis and chemical properties of "synthetic" or "artificial" coumarin[4,5]. On the other hand, Idris Davulcu was the first man who isolated coumarin from Thymus vulgaris[6,7]. Therefore, using currently developed ultramodern techniques, a wide range of promising and potentially beneficial activities are attributed to this single chemically heterocyclic compound. Historically, coumarin played a significant role in various cosmetics like bathing powders, toilet vinegar for hair washing, and Eau de Cologne[8,9]. Within a decade, Wittstein discovered coumarin and introduced it to the scientific world as a new perfumed compound. In retrospect, scientists appreciate that coumarin's field of action extends beyond this. Decidedly significant hostility was also shown in agricultural chemical activities when W. Brode assessed the moth-resistant property of coumarin[10,11].

Researchers have conducted extensive studies on the synthetic route for the coumarin backbone. Also, they have determined the overall percentage yield of coumarin based on these reactions. Many coumarin-related publications also discuss the use of synthetic coumarin as a rigid framework for the development and design of biological activities[12,13]. The plant families Asperulaceae and Rubiaceae largely distribute CBCs, and scientists have identified over 130 natural CBCs from sixty related plant species, primarily in the genera Galium and Asperula[14,15]. Interestingly, the use of coumarin has not decreased in traditional medicine, and there has been a growing fascination with CBCs due to the extensive number of research studies conducted[16,17]. In fact, the research on these compounds has broken all records in becoming a standard pharmacological search tool, with more than 18,000 published articles since 1978[18,19]. Today, the increasing availability of new data has led to the writing of several reviews on coumarin, CBCs, and sarcomeres[20–22]. Researchers are reviewing coumarin itself, not its derivatives, based on its combined applications of in vivo and in vitro investigations[23,24]. Furthermore, at the beginning of the second decade of the 21st century, research on a small, chemically heterocyclic compound like coumarin has become a necessity, and this may be one of the few results available based on both historical and backup data[25–27]. Regarding many aspects, **Table 1** provides a comparison between these two types of investigations.

Table 1. Comparison of in vitro and in silico studies: Key aspects, tools, and applications.

Aspect	In vitro studies	Ref.	In silico studies	Ref.
Definition	Experiments conducted outside a living organism, typically in controlled lab environments (e.g., petri dishes and test tubes)	[28]	Studies conducted using computer simulations, algorithms, or computational models	[29]
Primary tools	Cell cultures, biochemical assays, microfluidics, and laboratory equipment	[30]	Computational models, bioinformatics tools, machine learning, and databases	[31]
Applications	Drug efficacy testing, toxicity screening, molecular interactions, and biological pathway analysis	[32]	Virtual screening, predicting molecular properties, modeling biological systems	[33]
Accuracy	Provides direct biological relevance but may not fully mimic physiological conditions	[34]	Dependent on the quality of the data and algorithms; results may lack experimental realism	[35]

Aspect	In vitro studies	Ref.	In silico studies	Ref.
Speed	Slower due to manual experimental procedures and cell culture growth times	[36]	Faster, as simulations and analyses can often be automated and run on powerful computers	[37]
Cost	High, due to reagents, equipment, and labor costs	[38]	Typically lower, requiring computational infrastructure and software	[39]
Ethical concerns	May involve the use of animal or human-derived cells, raising ethical questions	[40]	No ethical concerns related to direct experimentation; relies on existing dataset	[41]
Scalability	Limited scalability due to physical constraints in lab setups		Highly scalable, as multiple simulations can run simultaneously	[42]
Limitations	- Limited ability to mimic whole-organism complexity - Time- and resource-intensive	[43]	- Results depend on the quality of input data - Simplifications may overlook key biological nuances	[44]
Complementary role	Provides validation of hypotheses generated by in silico studies	[45]	Generates hypotheses and narrows down targets for in vitro and in vivo validation	[46]

Table 1. (*Continued*)

We looked at every publication from 2000 to the middle of 2024 and found that 4854 papers talk about CBCs and their in vitro research, 505 articles talk about these compounds and their in silico research, and 258 articles link CBCs to both types of analysis. These figures demonstrate that when assessing the biomedical characteristics of CBCs, in vitro research takes precedence over in silico studies. **Figure 1** shows how few research studies have used CBCs and combined the findings of these two kinds of investigations. This is why we did this review study to highlight the importance of in vitro-in silico CBC evaluation and encourage more research.

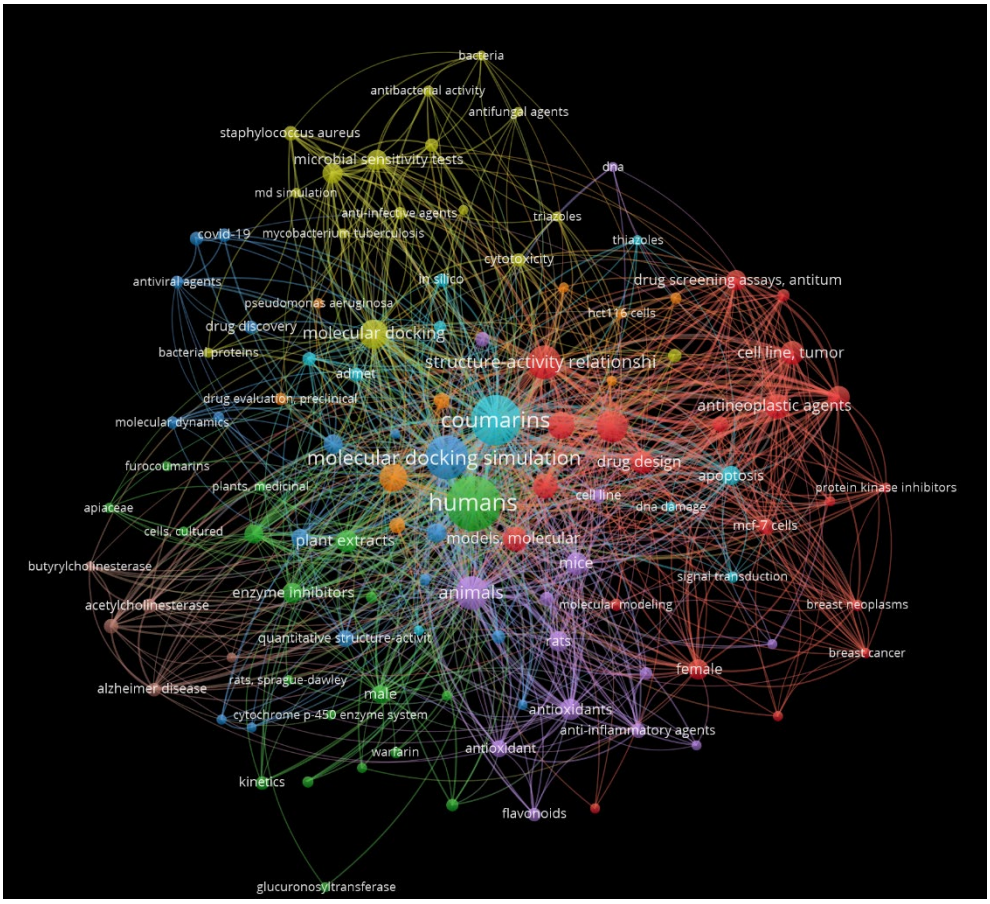

Figure 1. Co-occurrence network relationship between CBCs, in vitro, and in silico studies.

3. Fundamentals of in vitro studies

The study of biological phenomena resides on two principles: gaining insights reduces risks, and designing tests for prediction makes it possible to model the behavior of whole living beings[47,48]. In this case, researchers conduct an in vitro study using freshly excised tissue from a whole animal or organ[49,50]. They also design in vitro studies to thoroughly examine a single biological process, both empirically and statistically, while minimizing noise[51,52]. Basically, in vitro studies help us understand, at the cellular or tissue level, the mechanism by which that organism achieves a certain end[53,54]. One of the three most commonly used in vitro methods for research involves designing an experiment to extend data to tissues or the entire biological level, thereby enabling researchers to answer meaningful questions[55,56].

One of the advantages of using in vitro methods is that most biological agents can tolerate a wide range of culture conditions; therefore, it is possible to design experiments to confirm previous in vivo results. Research advancements can occasionally prompt a return to in vitro studies for the purpose of conducting these experiments[57,58]. Rather than discarding all the lessons learned from the in vitro study, we could save time and expense by using it to inform opinions or future research, potentially leading to different conclusions. Among the three areas of applications within coumarin-based research, most in vitro studies fall into one category that discussed above[59,60].

The next subheads report examples of these studies and their underlying methodologies, illustrating the current implementation of these methodologies in coumarin-based research. However, while the information provided is not exhaustive, it also illustrates the variety of methods and models employed in today's coumarin-based research. The methodologies used in the in vitro proof of concept and first exploratory studies in humans in phase I and phase II studies are presented as examples: these types of studies and their underlying methodologies are commonly employed in therapeutic research.

3.1. Types of in vitro studies

Several types of in vitro studies exist, including the use of established cell lines or primary cells, as well as organotypic, organ-on-a-chip, or organoid studies. These studies concentrate on organs with a breed-like structure and cells whose culture typically lasts no more than 100 days[61,62]. Biochemical tests also check the activity or selectivity of different P450 isoforms and other enzymes that break down xenobiotics[63,64]. Based on experimental phenomena, in vitro studies primarily focus on three areas of pharmaceutical-biological research related to CBCs. 1) Investigating the mechanisms of cytotoxic and tumor-suppressive effects[65,66]; 2) Examining the interactions between cytochrome oxidase[67,68]; and 3) Investigating the anti-adherent and anti-infectious effects[69,70].

We identified 23 in vitro studies in the field of coumarin metabolism. The most important research questions in this area are how CYP2A6, 2B6, 2C8, 2C9, 2E1, and 3A4 can prevent CBCs from functioning abroad. The other question is how well or selectively can enzymes demethylate a certain pure compound or its group of non-oxygenated, monomethoxylated, dimethoxylated, and trimethoxylated compounds?[71,72] The enzymes 7-ethoxyresorufin O-deethylase, 7-methoxyresorufin O-demethylase, 7-pentoxyresorufin O-dependentylation, and 7-benzyloxyresorufin O-debenzylation are CYP-specific and ultrapotent; they are reversible, with 1:1 competitive and mixed inhibition[73,74].

The versatility and variability of in vitro study types in coumarin-based research differ in certain aspects from one to the other. It can adopt the in vitro study in its most extreme form if it's feasible or strictly necessary[75,76]. Otherwise, it may choose the most suitable and/or available in vitro experiment. This experiment has pros and cons, with its lack of relevance being the most contentious[77,78]. It is true that no individual or meta-analysis of in vitro experiments provides statistically or practically sufficient evidence. However, multidirectional evidence from these experiment that adhere to strict methodological guidelines not

only enhances our understanding of the compound's individual in vitro properties, but also presents a potentially appealing approach for scientists to conduct in vivo experiments, thereby enhancing our understanding of in-phase experiments.

3.2. Techniques and methods

The in vitro assay systems utilized in the conduct of CBC studies require different techniques and methods. Spectrophotometric methods have been in use for some time, and there is also much application and knowledge about them[79,80]. Additionally, methods exist in the fields of chromatographic analyses and molecular assays. These techniques and methods employed hold equal importance to the accuracy and reliability of measurements and values, as analytical methods offer insights into the characteristics and effects of CBCs[81,82]. Molecular bioassays serve as a foundation in the field of chromatography, facilitating the preparation of CBCs in various areas and applications for both healthy and diseased individuals. In vitro studies dedicate a separate section to sample preparation techniques, the most crucial component of laboratory facilities[83,84]. The preparation processes of CBCs for molecular methods and systems are more difficult than chromatography. The main bottlenecks of spectrophotometric and chromatographic methods are their high minimum detectable levels[85,86].

The experimental section of all in vitro studies, including the aforementioned assays, includes major validation studies. In recent years, researchers have conducted all standard cell culture-related studies under sterile conditions, using tissue culture media at 37°C and 5% CO_2[87,88]. Laboratory studies primarily undergo processing due to the use of biologically and analytically validated tools in their environments[89,90]. In coumarin-based research, a comparative analysis of in vitro and in silico studies highlights the importance of standard and computable conditions.

4. Fundamentals of in silico studies

In silico studies, also known as computational ones, are becoming increasingly popular in pharmaceutical research. Computational modeling and simulation are among these studies, enabling the complex interactions at both the biological and chemical levels[91,92]. Additionally, these studies supplement traditional experimental methods by enhancing our understanding of molecular interactions, biological pathways, and the absorption, distribution, breakdown, excretion, and toxic effects of drugs and other chemicals[93,94]. One of the major advantages of in silico studies is their ability to provide a vast amount of information in a very short period, using a cost-effective and time-efficient approach[95,96]. Combinatorial chemistry studies design high-throughput experimental procedures to synthesize a large number of compounds. Implementing experimental investigations and assays to synthesize compounds can be both cost-inefficient and time-consuming[97,98]. In silico techniques could potentially alleviate these challenges by objectively selecting compounds that are more likely to have biological effects or exhibit adoptable behaviors[99,100].

In silico tools are computer-based and can simulate different types of compounds acting in biological pathways. For instance, it is possible to simulate compounds that act as agonists or antagonists, inhibiting or activating enzymes and other biological agents[101,102]. These tools are capable of performing the aforementioned functions using the structure of compounds as a foundation[103,104]. Currently, scientists model and simulate these biological effects primarily through statistical models known as quantitative structure-activity relationship (QSAR) models and, more recently, pharmacophore-based methods. Both approaches incorporate diverse methodologies, some of which have proven to yield authentic insights into biological interactions[105,106].

The development of a variety of in silico studies integrated into drug discovery and development—also known as supplementary material—has been made possible by the increasing complexity of computers and the improvement of software dedicated to a wide range of applications[107,108]. However, over the years,

numerous challenges and limitations have been established because of their complexity, contradictions in software, and availability for systems with different computational resources. It has problems like not being able to use accurate computational methods, using the wrong structural and molecular information to create the in silico approach, using the wrong software, the method being too complicated within the software, using the wrong software to predict and describe the effect, not having enough time, and using unethical methods, all of which are debated by researchers[109,110]. The implementation of more recent in silico approaches enhances traditional methodologies in foreign applications and significantly contributes to the scientific decision process in hypothesis generation and screening[111,112].

4.1. Types of in silico studies

The interest in CBCs has increased in the last 10 years due to their variety of biological activities. In the fields of medicinal and food chemistry, there is interest in identifying potential targets of these compounds[113,114]. In silico studies are useful because they allow for the rapid identification of molecular targets and the study of properties of interest in this context. Therefore, the scientific community accepts in silico studies as the primary procedural tools for discovering new potential biological CBC properties. Molecular docking, computational chemistry, and pharmacokinetic simulations are the three main methods used in these studies[115,116].

Computational chemistry is the largest field of in silico study. This encompasses two distinct types of predictions: the use of QSAR for studying biological activity and toxicity as well as the application of thermodynamic features to predict the contour of properties[117]. Finally, pharmacokinetic simulations help understand the fate of a drug within an organism and can predict potential biological activity[118]. As seen above, in silico studies range from a superficial level, such as predicting interactions using molecular docking or virtual screening, to a more in-depth level, evaluating pharmacokinetic and toxicity properties[119,120]. One key advantage of in silico studies is their speed since each in vitro and in vivo study takes at least one week. Furthermore, these studies can predict experimental results and validate them to some extent. They use current data and all of the model's assumptions, so use them with caution. When possible, analyze the results of in silico studies with biological activity as part of the overall research[121,122].

4.2. Techniques and tools

Today, scientists have access to a myriad of in silico software tools that can provide structural predictions and useful thermodynamic data comparable to in vitro and clinical studies[123,124]. The discipline of computational modeling is a versatile topic, combining multiple techniques that pertain to molecular modeling, virtual screening, quantum chemical calculations, and molecular dynamics simulations. Thorough knowledge and experience with the software or algorithm chosen are essential because they could determine how reliable the outcomes of molecular docking, virtual screening, quantum chemical calculations, and molecular dynamics simulations are in the end[125,126]. Investigational aims, user expertise, and anticipated outcomes regarding CBC interactions with proteins or macromolecules influence the choice of algorithms[127,128].

Molecular modeling and quantum chemical calculations are crucial in CBC-docking studies. Quantum chemical calculations can help us understand the energies between CBCs and their ligands[129]. Types of molecular modeling tools may include those for proteins, ligands, and drug design. On the other hand, molecular dynamics tools such as AMBER, GROMACS, CHARMM, NAMD, and LAMMPS can be utilized to perform molecular simulations[130]. The majority of these are free to use under certain conditions. To simulate molecular dynamics on a receptor structure, in silico-induced fit methods require initial preparation and format conversion tools[131]. As technology and algorithms get better, these methods will benefit the most. New tools are always coming out that give accurate pictures of how CBCs and proteins interact at the molecular level[132].

Over time, the expansion of available resources has led to the improvement of some commercial and quasi-commercial platforms, enabling the completion of necessary molecular studies in a single location or within a standardized computational workflow[133]. Over the short term, non-expert users, or those with resource and time constraints, might turn to platforms, software, or hardware that offer simpler and more accurate calculations of CBC interactions. Researchers have also developed newer protocols based on machine learning to create models that can understand how this chemical type interacts with a specific ligand or macromolecule as a drug target[134]. These models can guess how CBCs bind to small molecules or larger drug or protein structures, what kinds of bioactivity they have, how selective they are, or whether they interact with other proteins[135].

5. Study considerations

By dividing the benefits, drawbacks, and uses of in vitro and in silico research into two separate parts, **Figure 2** provides a visual comparison of the two. Symbolizing their controlled conditions, cost-effectiveness, and ethical simplicity, the in vitro studies section showcases a laboratory setup with test tubes and a microscope. The in silico studies section, featuring a computer with molecular models and algorithms, serves as an example of computational approaches, their efficiency, and their potential use in drug discovery. To highlight the interdependencies and complementary nature of the three sections' categories, icons and arrows graphically link them. Thanks to the well-organized layout, even the most technical details are simple to grasp.

Figure 2. Comparison of in vitro and in silico studies: Advantages, limitations, and applications.

5.1. Advantages of in vitro studies

Conducting in vitro assays in a controlled environment significantly increases their precision and the accuracy of the quantified results. In comparison to in vivo tests, in vitro studies are simple to perform and do not raise ethical concerns[136]. Researchers are increasingly focusing on precision medicine and in vitro methods, but they should exercise caution when applying the information from these studies to real-world scenarios[137]. This is the result of eliminating all complex reactions and processes occurring at the organ and whole-organism levels from the assay; extrapolating outcomes to humans requires direct or indirect in vivo

models[138]. The relatively low cost and ease of performance make in vitro assays especially applicable to high-throughput screening[139].

5.2. Limitations of in vitro studies

There are significant limitations in all in vitro assays related directly to the study environment. The microenvironment of the biological system could influence the results from in vitro assays[140]. These limitations also impact the application of experimental in vitro data to in vivo or clinical situations. Different cells and chemicals in the biological environment are known to react together. The behavior of cells and tissues can facilitate these interactions by modulating the chemical system[141]. However, in vitro assays typically lack or contain minimal cellular heterogeneity, making it possible to control all conditions at the microenvironment level, unlike in vivo biological settings[122].

5.3. Advantages of in silico studies

In terms of in silico studies, the use of computational methods broadened our horizon and expanded our capacity to explore the unknown. These methods have yielded benefits such as insightful data analysis and hypothesis generation, which enable the performance and validation of wet laboratory studies[142]. These methods also use computer programs and tools to make thousands of 3D structures at the atomic level, model the structures of compound-protein complexes, figure out the role of solvents, see how complex compounds behave in protein environments, create energy fields, and quickly check the movement of molecules[143]. Importantly, they are efficient and more affordable than wet laboratory studies, need no ethical approval for simulations, and simultaneously run in parallel with a few constraints[144].

5.4. Limitations of in silico studies

The limitations and criticisms surrounding in silico studies have hindered their acceptance and use. These constraints include reliance on input from laboratory or in vitro studies of an analogous target[145]. Studies also rely on the crystallographic structure, which could potentially lead to bias in the design[146]. Furthermore, they fundamentally assert that the predictions are undesirable, particularly in the context of ligand-fragment protein docking scenarios[147]. However, it is also argued that in today's biological world, fully comprehending the system is very hard, and sometimes a precise guess of the active site is the only way to go. Because of this, their reasoning often lacks experimental validation, leading to disagreements[148].

A major limitation is the accuracy of simulating phenotypes because we do not know everything about biological pathways and complexity. Structure-based functions and protein evolution complicate in silico systems, necessitating the use of appropriate in vitro and in vivo tests to determine their toxicity mechanisms[149]. Another drawback of these systems studies is the assumptions behind models, and the mechanism of prediction has limitations; it provides indications of the events but not the actual ones, and examination of predictions is also necessary[150]. Studies from the past have paved the way for efficient predictions of molecular drug design, toxicity studies, and extensions of life sciences with minimum laboratory tests in a short frame of time[151]. However, the use of computational-based methods escalates the timeline. In general, in silico studies are still useful additions to in vitro and in vivo studies for studying biological targets, molecular mechanisms, predicting ligand binding, and running many simulations[152].

5.5. Applications of in vitro studies in coumarin-based research

In vitro studies offer a wide range of possible topics for coumarin-based research, ranging from toxicity testing to investigating coumarin-related biomedical effects[153]. For instance, researchers can evaluate the anticoagulant effect of 4-hydroxycoumarin (**Figure 3**), one of the first CBCs used as a clinical anticoagulant since the 1950s. As a result, researchers introduced crucial in vitro assay systems on liver microsomes to investigate the metabolism of this CBC. Due to their increasing application, these assays are now commercially available not only for hepatocytes but also for endothelial cells and other cells relevant to coumarin-related

research topics[154]. Today's advancements in generating pluripotent stem cells from nearly every cell in an individual's body, and then producing the necessary cell entities through cell differentiation, have also led to the creation of the first few in vitro cell systems[155] that closely resemble the blood–brain barrier between humans and primates[156].

The incorporation of additional in vitro bioassays into the development of modern drugs and vaccines could potentially reduce the need for in vivo research in certain cases[157]. Conversely, enhancing the use of in vitro assay systems might necessitate tight collaboration with disciplinary regulatory offices to prevent financial and time wastage in the event that a drug or vaccine fails to receive approval due to toxic effects not detected by the in vitro tests[158]. Furthermore, studies on the reversibility in the central nervous system of an effect described in vitro studies for a drug such as a CBC may be pivotal. Disregard the naive expectation that in vitro activity in liver microsomes must entail a potential risk for a blood coagulation-related side effect; detailed in vivo studies must address this[159]. However, warfarin (**Figure 3**) serves as an excellent model for the necessary translation studies. Despite the racemic warfarin's marked in vivo anticoagulant effect, the S-enantiomer exhibits an antagonistic vitamin K action in vitro. This knowledge led to the development of an S-warfarin-based rodenticide, which counteracts the in vivo warfarin effect when combined with R-warfarin and sold in the market[160].

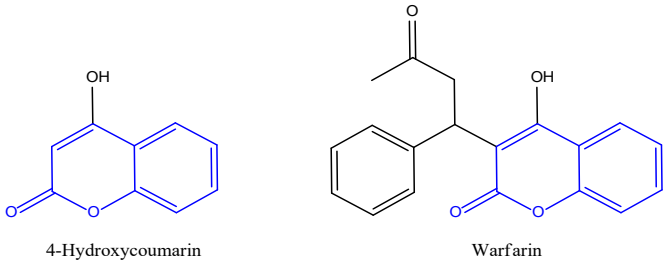

Figure 3. The chemical structures of 4-hydroxycoumarin and warfarin exhibit a blue-colored coumarin backbone.

Clearly, in vivo investigations are indispensable, and this applies not only to revealing pharmacokinetic profiles but also to the predictive quality of in vitro studies, provided results are available. Documenting the lack of predictive quality from in vitro-in vivo translation is necessary[161]. Similarly, despite extensive efforts, in vitro modeling systems are unable to resolve issues such as variations in drug metabolism and blood clotting patterns among individuals. Moreover, clinical practice can easily address issues of batch and patient heterogeneity, which go beyond influencing studies on the reversibility of an action[162]. We need to prove such activity both in vitro and in vivo, and currently, no such studies exist for CBCs. Regrettably, the literature often mistakenly publishes the poorly predictive in vitro studies as evidence-based, approved strategies, thereby contributing to its already vast volume.

5.6. Applications of in silico studies in coumarin-based research

These studies have strengthened current perceptions and advanced the knowledge of CBCs and their pharmacological potential. Numerous studies have employed the in silico approach to forecast the biological activity of these compounds[163]. Firstly, researchers have utilized numerous molecular docking simulations to identify potential targets for CBCs, with the results potentially aiding in pinpointing precise biological targets[164]. Not only has the in silico approach effectively identified potential targets for networks, but it has also paved the way for the CBC's progression to preclinical and clinical studies. Furthermore, it has blinded analysis of an already identified targets for the compounds under investigation[165].

Additionally, researchers conducted several in silico studies to enhance drug administration, and they used a drug metabolism study to identify the cytochrome P450 enzymes responsible for CBC metabolism[166]. Another study looked at how human serum albumin and human alpha-1-acid glycoproteins affect the

pharmacokinetic behavior of CBCs, with the goal of improving their bioavailability and effectiveness[167]. Similarly, scientists used molecular docking and molecular dynamics to study how nitric oxide-CBC prodrugs bind and interact with each other[168]. Using molecular dynamics in a computer study, researchers looked into the best way to find out how well CBCs fight gliomas by making them more bioavailable across the blood-brain barrier[169]. However, the computer-aided drug design process limits the use of new drug candidates. Consequently, it is critical to perform in vitro and in vivo studies to further verify the pharmacological activities of helper CBCs[170]. Additionally, the computer-aided drug design process has become a significant approach for drug development, with the expectation that it will save time and money, reduce side effects on the intended target, and enhance efficacy compared to existing drugs[171].

6. Integration of in vitro and in silico approaches

The integration of two complementary, highly valuable techniques, such as in vitro assays and in silico approaches, can provide synergies among them. In the case of CBCs, the possibility of integrating both research fields can provide useful information in order to have a comprehensive overview of their biomedical properties, as shown in **Figure 4**. In the early stages of drug discovery, in silico studies can predict pharmacokinetic parameters for ADMET characteristics like absorption, distribution, metabolism, excretion, and toxicity[172]. In vitro studies, on the other hand, use real mechanisms to look into one or more of these characteristics. Through collaborative frameworks, in silico and in vitro researchers have combined to produce pioneering and successful integrative studies[173]. In some of them, the obtained in vitro or in silico findings serve as a guide, exclusively supporting and suggesting the experimental or in silico approach[174,175]. Here, we have identified a few integrated research approaches that can optimize and deepen the level of detail, selecting them based on our vision and knowledge of the field. In these examples, the in silico approach serves as a guide for designing, predicting, and supporting in vitro studies, and vice versa[172,173,176].

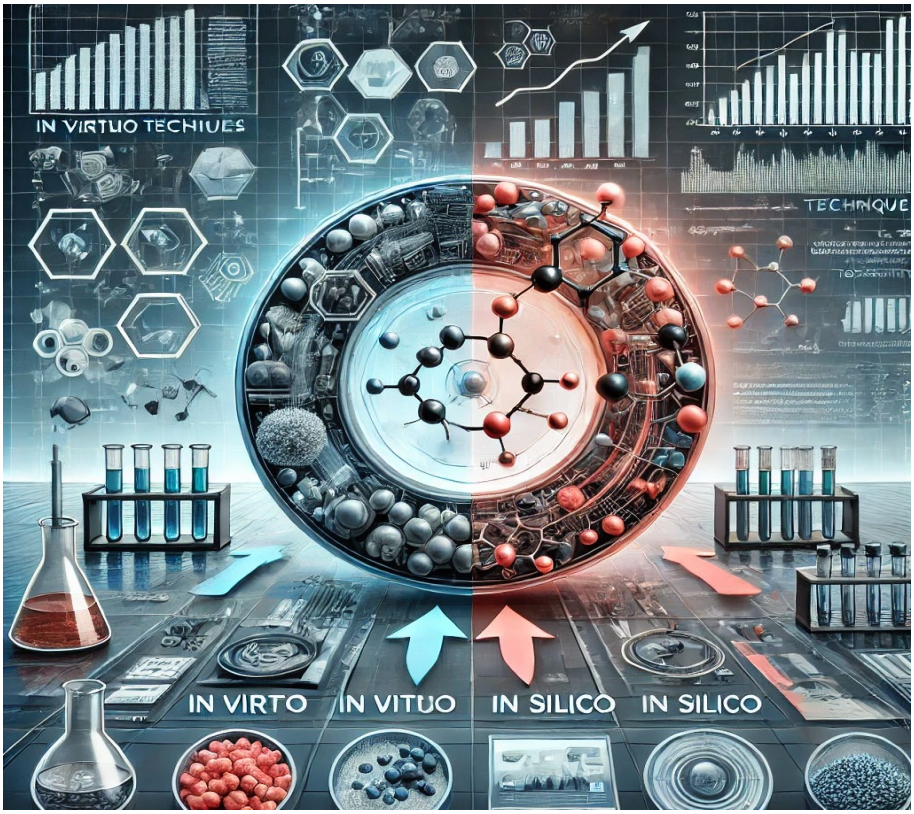

Figure 4. CBCs in the context of integrating in vitro and in silico techniques.

Interdisciplinary research primarily benefits from the design of new synthetic strategies and the active testing of novel (or already established) CBCs. Above all, the large chemical space under investigation necessitates the use of mechanistic and property predictive methods or tools to minimize costs and time[177]. Both in vitro and in silico studies have their limitations; the former approaches lead to potentially time-consuming, costly, and low-throughput research, whereas the predictive capability of in silico approaches is strongly dependent on the level of modeling and on the inter-predictability that can be high but not always[178]. So, combining and adapting new methods would let researchers make the most of the possibilities offered by in vitro research while also making toxicological research more reliable and repeatable[179]. This is why interdisciplinary research would be a new and possibly useful way to do research in the current system.

7. Future directions and emerging technologies

In vitro methods have combined with promising technologies like organ-on-a-chip, microphysiological systems, organoids, and other types of 3D cell cultures in the past few years. These technologies can help reduce the number of animals used in experiments. At the moment, in silico methods are also getting better. Quantum mechanics and molecular mechanics calculations let us study how molecules bind, taking into account both the electronic structure and the system's large-scale relaxation. In the future, we expect the integration of artificial intelligence tools, particularly machine learning and computationally driven drug design, to revolutionize in silico studies, particularly in virtual high-throughput screening.

Despite the recent technological advances, there are still numerous challenges in routine in vitro research that include increasing the efficiency of drug discovery and validation platforms, improving predictive capacity, and enhancing integration with in silico research. Integrating compound profiling with other omics data from both in vitro and in vivo systems could potentially yield more efficient outcomes. To reduce content generation times and costs, it is also beneficial to use other test systems to refine and potentially replace animal experiments. Examples of this could include enhancing the confidence in data to facilitate the creation of more accurate and efficient in vitro alternatives. Combining modern high-throughput equipment with automation, robotics, and homogeneous time-resolved fluorescence or immunofluorescence detection methods could have a big effect. Other things that could help are lentiviral transduction, which works with cells that are hard to transfect and can be titrated, and advanced cell cultures like 3D organoids or other 3D cell cultures to make the model more physiologically relevant and useful.

8. Conclusion and summary

This study provides a comparative analysis of coumarin based on both in vitro and in silico methodologies to inspire future research in this field. We observe that both methodologies have their advantages and limitations and applying them to better understand CBCs requires a clear and balanced perspective. The primary advantages of in vitro techniques are that they require minimal prior knowledge, and the results obtained reflect the whole-body effects of the applied dose. The major advantages of in silico techniques reside in their valuable time- and cost-saving capabilities and their potential to predict different ramifications, such as toxicity and efficacy. However, a significant drawback of in silico techniques is their need for a substantial amount of data and prior information. Furthermore, current these techniques are not capable of simulating the complex enzymatic reactions found in living organisms.

One of the most significant challenges in scientific research is to summarize each and every aspect of a subject matter. This study comprehensively summarizes scientific data related to in vitro and in silico approaches, revealing insights. Here, we advocate for the preference of new methodologies over in silico research or in vitro studies, as they offer a more effective and integrative approach. These new, easier, and more convenient ways make it easier for data to flow through many systems and parts of living things. But, computerized and artificial techniques, information technology, spotting opportunities, and better

pharmacokinetic/pharmacodynamic modeling should help us learn more about the pros and cons of CBCs in humans, as well as how safe they are. These new-generation in silico models should guide future in vitro or in vivo research. This study also provides a new comprehensive analysis that emphasizes the advantages and challenges of both in vitro and in silico methodologies in coumarin-based research. We conclude that both methodologies offer synergistic advantages and shortcomings in this research type.

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Conflict of interest

The authors declare that they no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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