Review

Proving the Absence of Correlation Between Molecular Docking Predictions and In Vitro Cytotoxicity Study in Anti Breast Cancer Drugs Development

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Simple Summary: In silico methods have revolutionized drug development by enabling quick and cost-effective assessments, but they face limitations in predicting complex quantum-level relationships, requiring validation with empirical data. This review examines the correlation between computational Gibbs energy (ΔG) predictions and in vitro IC50 values in MCF7 breast cancer cells. Contrary to theoretical expectations of a direct relationship, findings reveal no consistent linear correlation due to biological variability, target-specific responses, and differing experimental conditions. To improve predictive accuracy and drug development, integrated approaches combining computational and experimental data, considering factors like chemical metabolism, cellular permeability, and target selectivity, are recommended.

Abstract: Background: In silico methods have transformed the field of drug development by allowing quick and cost-efficient assessment of prospective therapeutic substance. Nevertheless, these computational algorithms encounter constraints when it comes to predict the complex relationships existing at the quantum level, therefore requiring thorough validation using actual data. **Aims of Study:** The aim of this review was to investigate the correlation between in silico predictions of Gibbs energy (ΔG) and in vitro IC50 values throughout studies performed on the MCF7 breast cancer cell line. **Methodology:** This review provides a systematic approach to collect relevant literature by employing the inclusion and exclusion criteria. **Results:** Experimental proof obtained from investigations on MCF7 cells indicate that, contrary to theoretical predictions of a direct relationship between ΔG and IC50 values, there is an absence of a consistent linear association among all the chemicals and protein targets that were examined. These contradictions arise due to factors such as variations in biology, particular responses to the target, and differences in conditions for experimentation. To enhance the accuracy of predictions and optimize drug development processes, it is crucial to employ integrated methodologies that combine computational predictions with empirical data. These approaches should take into account chemical metabolism, cellular permeability, and target selectivity. **Conclusion:** In conclusion, this review demonstrates no linear correlation between Gibbs energy values and IC50 values in MCF7 cell studies. Future studies should focus on specific cell lines and receptors, under well-defined experimental conditions and parameters, to enhance predictive accuracy and reliability in drug discovery.

Keywords: molecular docking; In-vitro cytotoxic study; anti breast cancer

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1. Introduction

Computational chemistry has become powerful tools in the field of drug discovery and development, fundamentally transforming the process by which researchers find and enhance prospective medicinal medicines. Computational techniques enable the simulation and study of drug-receptor interactions, making it possible to screen large chemical libraries quickly and efficiently. In silico techniques greatly accelerate the discovery of new drugs by accurately predicting the binding affinity and stability of drug candidates, hence reducing the time and money required for traditional experimental procedures. [1–3]

In silico approaches possess impressive capabilities, yet they are inherently limited in terms of their accuracy in predicting. The complicated principles of quantum physics, which drug molecules work inside due to their small dimensions, are not fully captured by most computational tools. Despite the use of modern techniques to make predictions at the quantum level, the quantum realm remains inherently unpredictable, which introduces a level of uncertainty to the simulation findings. The unpredictability of drug candidates' behavior in biological systems poses a barrier to the reliability of in silico approaches in accurately predicting their outcomes. [4,5]

In order to improve the reliability and strength of in silico approaches, it is crucial to conduct thorough data verification and validation. The integration of empirical data with computational predictions is essential for enhancing these models and ensuring their predictive capability. Through a thorough comparison of computer-generated results with actual experimental results, researchers can detect inconsistencies, enhance algorithms, and ultimately create more precise and dependable computational tools for the process of discovering new drugs. [6,7]

Several published research have examined the relationship between in silico (computer-based) and in vitro (laboratory-based) outcomes, although they frequently report a lack of consistent correlation. These findings emphasize the complex relationships and difficulties involved in converting computer predictions into biological actualities. This discrepancy highlights the need for further focused research to better comprehend the processes that contribute to the observed differences and to improve the accuracy of prediction of in silico approaches. [5,6,8,9]

This work is driven by the necessity for more accurate and dependable associations, and it specifically focuses on the development of drugs that combat breast cancer. Our study intends to reduce variations by focusing primarily on the MCF-7 cell line in laboratory conditions and precisely investigating protein targets that are expressed or dysregulated in breast cancer. This approach is important due to the widespread usage of in silico technologies in breast cancer research. We utilize IC50 values obtained from in vitro experiments to directly quantify the cytotoxic doses of the test compounds and examine their relationship with Gibbs energy values derived from in silico investigations. This approach is founded on the theoretical assumption that there should be a direct link between IC50 and Gibbs energy values. This correlation allows for a more nuanced comprehension of the connection between computational predictions and experimental results in the development of drugs for breast cancer.

2. Methodology

This review article employs a systematic methodology to gather relevant literature, ensuring a focused discussion on the correlation between IC50 values and Gibbs energy (ΔG) in MCF7 cell line studies. A structured search strategy using specific keywords such as "In Silico," "In Vitro," "MCF7," and "breast cancer" was applied to identify pertinent studies. Inclusion criteria mandated that studies provide both in vitro and in silico data, perform cytotoxicity assays on MCF7 cell lines, and conduct molecular docking studies targeting proteins relevant to breast cancer. Only studies focusing on single compounds were included, excluding those on multi-compound extracts. This approach ensured the collection of high-quality, relevant research papers, all of which provided comprehensive in vitro cytotoxicity data and in silico molecular docking studies. The consistent use of MCF7 cell lines across the selected studies facilitated a detailed analysis of the correlation between IC50 values and ΔG, providing a robust foundation for this review.

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3. Molecular Docking Simulation in Drug Discovery

3.1. Molecular Docking Simulations and Their Role in Drug Design

Molecular docking simulations have emerged as a fundamental methodology in drug design, providing a computational approach to forecast the atomic-level interactions between tiny chemicals (ligands) and biological molecules, typically proteins. This enables researchers to virtually evaluate the manner in which a prospective medication candidate could attach itself to a particular target protein that is linked to a certain disease. Important factors involve the prediction of binding affinity, where docking software calculates the strength of the contact between a ligand and a protein, aiding in the identification of molecules with strong affinity that are essential for the efficiency of drugs. In addition, docking simulations offer valuable information about the binding modes, which unveil the precise orientation and conformation of the ligand when it is attached to the protein. This knowledge is helpful in the process of lead optimization. Virtual screening relies on this methodology to efficiently screen large collections of prospective drug candidates using computer simulations, resulting in a substantial reduction in time and resources compared to traditional high-throughput screening methods. After identifying promising drug candidates, docking simulations can be used to enhance their structure in order to improve their ability to bind to specific target proteins. This process involves iteratively refining the ligand's fit for the target protein, hence increasing its binding affinity and selectivity. In addition, docking simulations provide valuable insights into the mechanism of action by illustrating the precise atomic-level interactions between a drug candidate and the target protein. This understanding is essential for comprehending the medication's mechanism and possible adverse effects. Nevertheless, there are certain constraints to consider. One such limitation is the issue of accuracy, as the reliability of predictions relies heavily on the quality of both the protein structure and the docking software employed. Additionally, there is a limitation in terms of dynamics, as the process of docking often portrays an interaction in a fixed state, when in reality, proteins and ligands are capable of flexibility and shape alteration. Although there are limitations, molecular docking simulations are a potent tool that has transformed the field of drug discovery. They provide a rapid and cost-efficient method to evaluate potential drug candidates, thereby expediting the development of novel therapeutics for diverse ailments. [10–12].

3.2. Overview of How Simulations Provide Insights into Molecular Behavior

The realm of molecules, characterized by complicated interplay of forces and interactions, is frequently too little and delicate to be directly observed. Computer simulations are effective instruments for revealing the intricacies of molecular behavior. They function as virtual laboratories, enabling researchers to modify and examine molecules at the atomic scale. There are two main categories of simulations: Molecular Dynamics (MD) simulations and Monte Carlo (MC) simulations. Molecular dynamics (MD) simulations are akin to microscopic films, as they trace the paths of individual atoms throughout time, unveiling alterations in molecular structure, protein folding routes, and the kinetics of chemical reactions. On the other hand, Monte Carlo simulations employ a statistical methodology by randomly selecting various arrangements and determining their energies in order to investigate thermodynamic characteristics, phase changes, and the binding of ligands. Simulations surpass the constraints of experiments by investigating severe situations, offering intricate information at the atomic level, and demonstrating the ability to forecast material design and drug development. Nevertheless, these models have several drawbacks such as substantial computing expenses, reliance on model precision, and frequently shorter timeframes in comparison to real-world phenomena. Simulations have the ability to alter our comprehension of molecular behavior, providing distinctive perspectives on the dynamics, interactions, and characteristics of molecules. They are highly valuable tools in various domains such as medicine, materials science, chemistry, and physics. [13].

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3.3. The Use of Molecular Docking Simulation on Anti Breast Cancer Drug Design and Development

Molecular docking simulations have emerged as a helpful tool in the battle against breast cancer, expediting the process of designing and developing novel anti-cancer medications. The progression of breast cancer is frequently dependent on the presence of the estrogen receptor (ER) protein. Molecular docking is a vital technique used to identify prospective ER ligands by virtually screening collections of candidate molecules. This process aims to identify compounds that are anticipated to have a strong affinity for the ER binding pocket. This enables the rapid identification of potential medication candidates that have the ability to disrupt estrogen signaling and hinder the proliferation of cancer cells. In addition, docking simulations have the ability to specifically target different subtypes of the estrogen receptor, such as ERα and ERβ. This enables the development of more precise medicines that have fewer adverse effects. In addition to ER-positive breast cancer, docking simulations can be used to target other signaling pathways that are utilized by cancer cells and to block enzymes that are essential for cancer growth. These simulations enhance drug design by improving the structure of drug candidates to better match the binding pocket of the target protein. This results in more powerful and specific medications. Additionally, the simulations predict any unintended side effects to prevent unpleasant consequences. Nevertheless, docking simulations are subject to some constraints, including their ability to only provide a fixed representation of interactions and their reliance on the accuracy of protein structures and docking software. Molecular docking simulations are transforming the field of anti-breast cancer drug design by facilitating virtual screening, identifying targets, and optimizing leads. This provides a potent tool in the continuous fight against breast cancer, despite the obstacles faced. [14–17].

4. In-Vitro Studies in Drug Discovery

4.1. Explanation of In-Vitro Experiments and Their Significance

In vitro investigations are essential in the initial stages of drug discovery as they provide a controlled setting to investigate the interaction between prospective therapeutic compounds and biological targets. These studies include a wide variety of tests aimed at evaluating the effectiveness, strength, specificity, harmfulness, and pharmacokinetic characteristics of a potential medicine. Enzyme inhibition assays can be used to quantify a compound's capacity to bind to and hinder the activity of an enzyme linked with a disease. On the other hand, receptor binding assays assess the compound's attraction to a particular cellular receptor. Utilizing cultured cells or isolated tissues in in vitro models allows researchers to examine the effects of a medicine on cellular viability, signal transduction pathways, and specific disease characteristics. Moreover, in vitro ADME (absorption, distribution, metabolism, and excretion) studies have the ability to forecast a drug's bioavailability, likelihood of drug-drug interactions, and elimination from the body. The importance of in vitro research rests in their capacity to efficiently evaluate a large number of therapeutic candidates, pick promising leads for further advancement, and detect potential safety issues before moving on to more intricate in vivo trials. Although in vitro models may not possess the complete intricacy of a living organism, they offer essential insights that direct the drug discovery process and facilitate the creation of safe and effective treatments. [18–20].

4.2. Common Methodologies Used in In-Vitro Studies

Drug molecules are analyzed using a wide range of methods in laboratory investigations to examine their potential therapeutic effects. Cell-based assays are commonly used to evaluate several aspects of drug activity by exploiting cultivated cells. The methods used to assess the effectiveness of drugs include: (1) Cytotoxicity assays, which measure cell death or proliferation when exposed to the drug; (2) Enzyme activity assays, which quantify the drug's ability to inhibit or activate a specific enzyme target; (3) Receptor binding assays, which determine the drug's affinity and selectivity for a specific cellular receptor; (4) Reporter gene assays, which monitor changes in gene expression caused by the drug; and (5) Cellular signaling assays, which evaluate the drug's impact on signal transduction pathways. Surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) are

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biophysical techniques that accurately determine the binding affinity of medicines and their targets. In addition, cell-free assays that utilize pure enzymes or separated proteins provide a quick evaluation of a drug's interaction with its specific molecular target. Moreover, in vitro ADME (absorption, distribution, metabolism, and excretion) experiments employ specialized methodologies to forecast the pharmacokinetic characteristics of a medicine. The choice of these approaches relies on the particular drug target, intended result, and stage of drug discovery, collectively offering a thorough assessment of a drug molecule's potential as a therapeutic agent. [21–23].

4.3. Importance of Reliable In-Vitro Data for Predicting Drug Behavior

Accurate in vitro data plays a crucial role in forecasting how drugs will behave in living organisms, serving as a necessary link between initial discovery and successful clinical trials. Firstly, the use of strong and reliable in vitro data enables the rapid screening of large collections of prospective drug candidates, effectively identifying those that have the desired ability to bind to and selectively interact with the target molecule. This greatly decreases the amount of effort and money allocated to compounds that are unlikely to be successful. Furthermore, in vitro experiments offer significant insights into the mechanism of action of a drug, clarifying its contact with the target and the subsequent cellular response. An essential aspect of this process is to comprehend the underlying mechanisms, which is vital for enhancing the effectiveness of primary chemicals and anticipating any possible unintended consequences. Moreover, obtaining dependable in vitro data regarding the ADME (absorption, distribution, metabolism, and excretion) properties of a drug aids in evaluating its bioavailability, potential for drug-drug interactions, and elimination from the body. This information is crucial for making informed decisions about the drug's formulation and dosage strategies. Although in vitro models cannot completely mimic the intricacies of a living organism, they provide a regulated and repeatable setting to produce statistically meaningful data. This data may then be used to make more confident predictions about how drugs would behave in a living organism. This ultimately enhances the rate of success in drug discovery, resulting in the creation of safer and more effective treatments. [24–26].

4.4. In-Vitro Cytotoxicity Study in Anti Breast Cancer Drug Discovery and Development

In the relentless quest for novel weapons against breast cancer, in-vitro cytotoxicity assays play a crucial role in the initial phases of drug discovery and development. These assays provide a controlled setting to evaluate the potential efficacy of new drug candidates by examining their ability to kill breast cancer cells. These assays assess the capacity of a potential treatment to hinder the development or survival of breast cancer cells by employing laboratory-grown cultures of human breast cancer cell lines that reflect several subtypes of the disease. The medication candidate is administered to the grown cells at different concentrations. Following incubation, cell viability is assessed using techniques such as the MTT test, XTT assay, cell counting, and Annexin V/PI staining. The advantages of these assays encompass their capacity for high-throughput analysis, cost-effectiveness, and ability to generate consistent outcomes in a controlled setting, enabling the early detection of potential drug candidates. Nevertheless, there are certain constraints such as the restricted intricacy and physiological significance of cell lines, as well as apprehensions regarding the drug's specificity towards cancer cells as opposed to healthy cells. Although there are difficulties, in-vitro cytotoxicity assays play a vital role in drug development by offering initial information on a medication's capacity to specifically attack and destroy cancer cells. This, in turn, facilitates subsequent in-vivo investigations and clinical trials. In addition to their role in initial screening, these assays are valuable for investigating pharmacological mechanisms of action, assessing the effectiveness of combination therapy, and finding potential candidates capable of overcoming drug resistance. In-vitro cytotoxicity tests play a crucial role in the process of discovering drugs to combat breast cancer, making a substantial contribution to the battle against this destructive illness. [27–30].

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5. Basic Theory of the Correlation Between In-Vitro and In-Silico Study

5.1. Theoretical Correlation Between Gibbs Energy and IC50 Values

In the field of drug development, the Gibbs free energy (ΔG) derived from molecular docking simulations is frequently employed as a measure of the strength of interaction between a ligand (such as a drug molecule) and its target protein. The fundamental concept states that a decrease in ΔG value is indicative of a higher binding affinity. The reason for this is that the Gibbs free energy shift indicates the spontaneity of the binding process. A greater negative ΔG value indicates a more favorable interaction.

Conversely, the half-maximal inhibitory concentration (IC50) quantifies the effectiveness of a molecule in suppressing a certain biological or metabolic activity. The IC50 value denotes the concentration of the drug necessary to hinder 50% of the target activity. In theory, a drug that has a high binding affinity (shown by a larger negative ΔG) should need a smaller dose to accomplish the same degree of inhibition, leading to a lower IC50 number.

The correlation between ΔG and IC50 can be estimated using the following equation: The change in Gibbs free energy (ΔG) can be calculated using the equation $\Delta G = RT \ln(IC50)$, where R is the gas constant and T is the temperature. R represents the gas constant, whereas T represents the temperature measured in Kelvin. This equation demonstrates a negative correlation: if the value of ΔG decreases, the IC50 values should also fall, indicating a higher level of potency. [31–35].

6. Development of Anti Breast Cancer Agent: In Silio and In Vitro Studies

Table 1. In vitro cytotoxic studies of several compounds on the MCF-7 cell line and molecular docking studies on several proteins, receptors, and enzymes with up-down regulation on breast cancer.

No	Compound test	In vitro target	In silico target	IC50 (μM)	Gibbs Energy/Internal Energy (Kcal/mol)	References
1	5-Pentylresorcinol	MCF7	BRCA1	919,44	-3,24	[36]
2	1,3-diynyl-noscapinoids (Derivat 20)	MCF7	Tubulin	27,30	-6,70	[37]
3	1,3-diynyl-noscapinoids (Derivat 21)	MCF7	Tubulin	18,70	-7,29	[37]
4	1,3-diynyl-noscapinoids (Derivat 22)	MCF7	Tubulin	12,70	-7,47	[37]
5	spirooxindoles (Derivat 9A)	MCF7	EGFR	6,47	-10,72	[38]
6	Disogenin	MCF7	IGF1R	29,06	-8,60	[27]
7	1-Formyl-2-Pyrazolines	MCF7	EGFR-TK	82,87	-7,90	[39]
8	2-(5,6-dicyano-1H-imidazo[4,5-b]pyrazin-2-yl)-N-phenylbenzamides	MCF7	Aurora Kinase	9,70	-10,50	[40]
9	Adapalen	MCF7	ARPBCC	12,00	-10,20	[41]
10	(2R)-2-((S)-sec-butyl)-5-oxo-4-(2-oxochroman-4-yl)-2,5-dihydro-1H-pyrrol-3-olate	MCF7	NUDT5	163,74	-6,57	[42]
11	6,8-dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazoline	MCF7	ER Alpha	20,56	-25,30	[15]
12	Quinolone	MCF7	TNFRSF5	0,05	-6,60	[43]
13	Quinolone	MCF7	MK167	0,05	-6,90	[43]
14	Nitidine	MCF7	Tubulin	0,28	-14,45	[44]
15	DHNP	MCF7	Exemestane	209,52	-8,33	[45]
16	HEHP	MCF7	Exemestane	30,67	-8,51	[45]
17	4-nitrobenzoyl-3-allylthiourea	MCF7/HER 2	HER2	225,00	-91,04	[46]
18	4-nitrobenzoyl-3-allylthiourea	MCF7	EGFR	85,00	-90,64	[46]
19	bis(1,4-dihydropyridine	MCF7	cIAP1	46,30	-21,34	[47]
20	bis(1,4-dihydropyridine	MCF7	xIAP	46,30	-22,04	[47]
21	Azomethine	MCF7	6NLV-4BRTH	140,46	-18,63	[48]
22	Azomethine	MCF7	6NLV-APTH	140,46	-19,84	[48]
23	Pterostilbene	MCF7	Telomerase	49,07	-7,10	[49]
24	1-(4-Bromophenyl)-3-(1,3-dioxoisoindolin-2-yl)urea (7c)	MCF7	EGFR	5,99	-7,56	[50]
25	1,3,5-triazine (Derivat A)	MCF7	topoisomerase-IIβ	12,40	-6,27	[51]

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26	1,3,5-triazine (Derivat B)	MCF7	topoisomerase-IIβ	0,01	-7,52	[51]
27	3-[(4-hydroxyphenyl)methyl]-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	MCF7	HER2	72,90	-9,40	[52]
28	11-oxo-11H-pyrido [2, 1-b] quinazoline-6-carboxylic acid 3 (deriivat B)	MCF7	Hexamer-DNA	2,07	-11,70	[53]
29	11-oxo-11H-pyrido [2, 1-b] quinazoline-6-carboxylic acid 3 (Derivat A)	MCF7	Hexamer-DNA	2,07	-8,32	[53]
30	amide enriched 2-(1H)- quinazolinone (Derivative A)	MCF7	EGFR	10,80	-9,00	[54]
31	amide enriched 2-(1H)- quinazolinone (Derivative b)	MCF7	EGFR	0,07	-9,67	[54]
32	Deoxybenzoins (1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone) (Derivat A)	MCF7	ER Alpha	12,00	-6,50	[55]
33	Deoxybenzoins (1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone) (Derivat B)	MCF7	ER Betha	5,00	-8,50	[55]
34	1,2,3-triazole-benzofuran	MCF7	BCL, Tubulin, C-ABL, CLK-3	0,01	-8,02	[56]
35	1,2,3-triazole-benzofuran	MCF7	BCL, Tubulin, C-ABL, CLK-2	21,80	-2,74	[56]

7. Discussion

7.1. MCF-7 Cell Line: A Key Model for Anti-Breast Cancer Drug Development

The MCF7 cell line, which first emerged in the 1970s, is a crucial resource in breast cancer research, playing a fundamental role in the study of prospective anti-cancer substances. MCF7 cells are derived from a pleural effusion of a woman with metastatic breast cancer and they represent an invasive ductal carcinoma, which is the most prevalent form of breast cancer. These cells display the traits of specialized mammary epithelium, including the presence of specific markers like E-cadherin, β-catenin, and cytokeratin 18 (CK18), while not showing markers associated with mesenchymal cells such vimentin and smooth muscle actin (SMA). Significantly, MCF7 cells have the ability to generate dome-like structures in laboratory conditions, closely resembling the structures found in mammary glands. [57–61]

MCF7 cells have strong expression of estrogen receptor (ER) and progesterone receptor (PR) at the molecular level. This characteristic makes them well-suited for investigating estrogen-dependent breast cancers, which are the most common form of the disease. The tumors do not have HER2 gene amplification, indicating that they belong to the luminal A subtype of breast cancer. This subtype is known to have a favorable prognosis. In addition, MCF7 cells exhibit characteristics similar to stem cells, allowing them to generate mammospheres (3D clusters) in a laboratory setting and develop tumors in mice. In addition, they also exhibit the presence of additional receptors, such as the androgen receptor, which may serve as prospective targets for innovative treatments. [57–61]

The MCF7 cell line has numerous benefits for cytotoxicity investigations. Their proliferation and accessibility from commercial sources render them suitable for extensive-scale experimentation. The fact that they are ER positive enables researchers to examine the impact of anti-estrogen treatments on the proliferation and viability of cells. MCF7 cells serve as a typical model for luminal A breast cancer, enabling the evaluation of medication effectiveness against this prevalent subtype. The biology of MCF7 cells has been widely studied for many decades, establishing a strong basis for future research.

Nevertheless, it is crucial to acknowledge the constraints of MCF7 cells. They do not cover all types of breast cancer, so other cell lines must be used to provide a more complete understanding. Furthermore, the restricted ability of these malignancies to spread to other parts of the body may not accurately represent the very aggressive nature of metastatic cancers. Although MCF7 cells have the ability to generate tumors in mice, this poses ethical concerns in the context of animal experimentation.

To summarize, the MCF7 cell line is an effective tool for early drug development and comprehending the mechanisms of anti-cancer drugs, namely those that target estrogen receptor-

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positive breast tumors. Researchers must recognize the constraints of this cell line and employ it in conjunction with other cell lines and research methodologies to get a comprehensive comprehension of breast cancer biology and formulate efficacious treatments. MCF7 cells are commonly used in cytotoxic studies due to their convenient proliferation, wide availability, and their ability to represent luminal A breast cancer. These cells have been extensively studied and characterized, making them highly valuable for breast cancer research.

7.2. Protein, Receptor and Enzymes Used as Target for Development of Anti Breast Cancer Agent

The subsequent targets are frequently employed in molecular docking investigations in this study owing to their pivotal functions in cancer advancement and therapy:

7.2.1. Maintenance of DNA Integrity and Preservation of Genomic Stability

BRCA1, also known as Breast Cancer Type 1 Susceptibility Protein, plays a vital role in the process of DNA repair and the preservation of genomic stability. BRCA1 mutations greatly enhance the probability of developing breast and ovarian cancer, making it a crucial focus for medicines aimed at leveraging its involvement in DNA repair processes to specifically eliminate cancer cells. [62,63]

7.2.2. The Process of Cell Division and the Movement of Microtubules

Tubulin, an essential protein for the production of microtubules and cell division, represents another crucial target. By impeding the process of tubulin polymerization, the normal functioning of microtubules is disrupted, resulting in the halting of the cell cycle and the initiation of programmed cell death. This property makes it a viable approach in the treatment of cancer. Aurora Kinase, which regulates chromosome segregation and cytokinesis during cell division, is a potential therapeutic target for triggering mitotic arrest and death in cancer cells. [64]

7.2.3. Receptor Tyrosine Kinases

Receptor tyrosine kinase a type of protein that play a role in cell signaling by transferring phosphate groups to tyrosine residues. EGFR, also known as Epidermal Growth Factor Receptor, and its tyrosine kinase domain, referred to as EGFR-TK, have significant functions in the processes of cell proliferation, survival, and differentiation. EGFR is frequently overexpressed or mutated in various types of cancer, such as breast cancer. By specifically targeting EGFR, it is possible to impede the growth and advancement of tumors. Likewise, HER2 (Human Epidermal Growth Factor Receptor 2), a different type of protein that triggers cell growth, is excessively produced in highly aggressive breast tumors. By specifically focusing on HER2, it is possible to greatly enhance the prognosis and overall well-being of patients. The Insulin-like Growth Factor 1 Receptor (IGF1R) is responsible for regulating cell growth and promoting cell survival. By blocking IGF1R, cancer cell proliferation can be reduced and the effectiveness of other treatments can be enhanced. [65,66]

7.2.4. Estrogen Receptors

Estrogen Receptor Alpha (ER Alpha) plays a vital role in controlling the activation of genes in response to estrogen. A significant number of breast malignancies have ER-positive characteristics, and the inhibition of ER Alpha can effectively suppress the development and proliferation of cancer cells driven by estrogen. Exemestane, an aromatase inhibitor, reduces the levels of estrogen and is employed in the treatment of estrogen-dependent breast cancer. ER Beta (Estrogen Receptor Beta) provides alternate approaches for the treatment of estrogen-dependent breast tumors. [67,68]

7.2.5. Immune Response and Cellular Proliferation

TNFRSF5 (CD40) and Ki-67 (MK167) play crucial roles in immune responses and cellular proliferation, respectively. Therefore, they are significant targets for manipulating immune responses and evaluating the efficacy of anti-cancer therapies. [69]

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7.2.6. Regulation of Apoptosis

Proteins like cIAP1 (Cellular Inhibitor of Apoptosis Protein 1) and xIAP (X-linked Inhibitor of Apoptosis Protein) prevent cell death by attaching to and deactivating caspases, which are enzymes involved in the process of apoptosis. By specifically targeting these inhibitors, it is possible to induce apoptosis (cell death) in cancer cells, hence improving the effectiveness of cancer treatments. BCL proteins, which control programmed cell death (apoptosis), are important targets for cancer treatment. [70,71]

7.2.7. Cellular Metabolism and Telomere Maintenance

NUDT5, a Nudix Hydrolase 5 enzyme, is involved in cellular metabolism. Inhibiting NUDT5 can interfere with crucial metabolic processes in cancer cells, resulting in decreased cell growth and survival. Telomerase, an enzyme that preserves the length of telomeres and safeguards chromosomes from deterioration, is frequently overexpressed in cancer cells, making it a possible target for restricting their ability to replicate. [72]

7.2.8. DNA Transcription and RNA Processing

Topoisomerase-IIβ, which plays a role in DNA transcription, is specifically targeted to inhibit DNA replication in cancer cells, ultimately resulting in cell death. CLK-3, also known as CDC-like Kinase 3, plays a role in controlling RNA splicing. If CLK-3 is inhibited, it can interfere with the processing of RNA in cancer cells. This disruption has the potential to cause the cells to stop dividing and undergo programmed cell death, known as apoptosis. [73,74]

7.2.9. The Cytoskeleton and Cellular Movement

ARPBCC, also known as Actin-Related Protein Binding Complex Component, controls the structure of the actin cytoskeleton and the shape of cells. Directing efforts against ARPBCC can impact the movement of cancer cells and potentially hinder the spread of cancer to other parts of the body.

7.2.10. Signal Transduction

Signal transduction refers to the process by which cells communicate and transmit signals inside the body. C-ABL, also known as ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase, plays a crucial role in many communication pathways. As a result, it is an important target for cancer treatment. [75].

7.3. Correlation Between In-Vitro Cytotoxicity Study (IC50) and Molecular Docking Study (Gibs Energy)

Several important insights are revealed when examining the dynamics of IC50 values and Gibbs energy (ΔG) in MCF7 cell investigations. IC50 values vary greatly, ranging from very strong (e.g., 0.01 μM) to weaker (e.g., 82.87 μM) (Table 1). These values represent the different abilities of drugs to inhibit specific protein targets in MCF7 cells. These values are crucial in clinical contexts, providing guidance for determining appropriate dosage strategies and assessing the effectiveness of treatments. [76–79]

Theoretical frameworks of ΔG emphasize its function in assessing the thermodynamic favorability of compound-protein interactions. Lower ΔG values (-14.45 kcal/mol to -2.74 kcal/mol) suggest higher binding affinities (Table 1), which are essential for optimal therapeutic activity. ΔG predictions are used as prognostic markers in the field of drug discovery, helping to prioritize compounds for further research based on their capacity to bind.

Although theoretical predictions indicate a clear relationship between ΔG and IC50 values, where a lower ΔG corresponds to better potency (lower IC50) [80–82], findings in this review from MCF7 cell research reveal a more intricate situation. Although many compounds display predictable patterns, the dataset demonstrates a degree of unpredictability (Figure 1). Main Discovery in this

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study revealed an important finding regarding the correlation. While theoretical expectations indicate a direct correlation between ΔG and IC50 values, the dataset shows that there is no consistent linear correlation across all compounds and protein targets tested (Figure 1). Simply Despite attempting a thorough analysis by categorizing the target receptor, we have not discovered any linear correlation between the Gibbs energy value and the IC50 value. This disparity highlights the intricacies involved in transforming theoretical predictions into practical results in the field of pharmaceutical research. Biological intricacies inside cellular contexts have a significant impact, affecting the effectiveness of compounds beyond what can be predicted by ΔG. Divergent IC50 values might arise due to several factors, including drug absorption, metabolism, and unique interactions with physiological pathways, despite the indication of great binding affinity based on ΔG. [80–82]

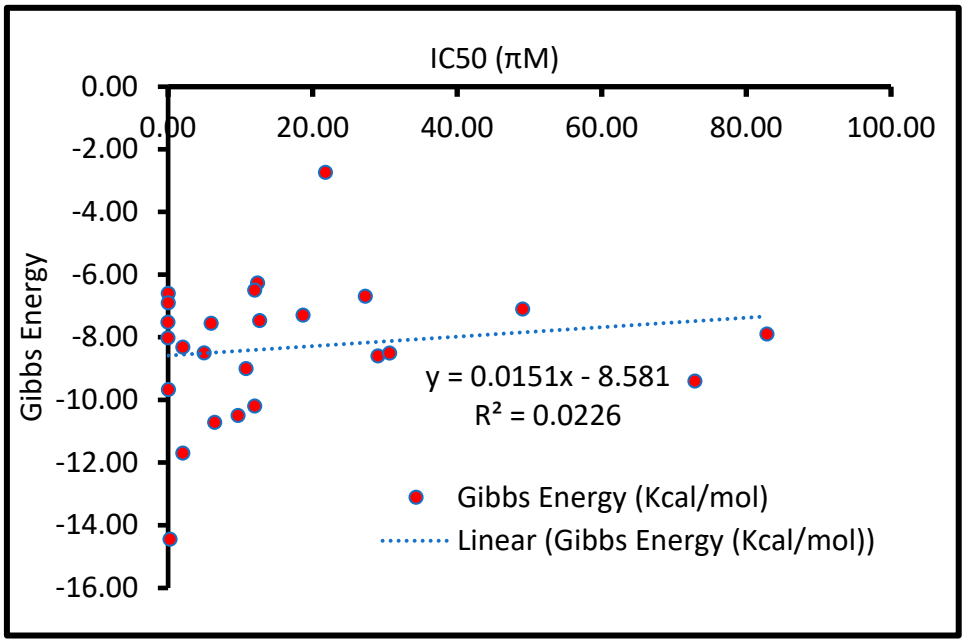

Figure 1. Correlation of cytotoxic results of the experiments of several compounds on the MCF-7 cell line in an in-vitro study, as well as the molecular docking investigations on various proteins/receptors/enzymes that exhibit up-regulation or down-regulation in breast cancer.

In addition, the presence of target-specific variability adds more complexity to the interaction. Protein targets have distinct interactions with substances because of differences in their structure and function [83,84]. Differences in the amounts of target expression, alterations that occur after protein synthesis, and the specific location inside the cell all have a major effect on the measured IC50 values, regardless of the predictions of thermodynamic free energy change (ΔG). Various factors, such as different methods used in experiments, specific details of cell culture, and the sensitivity of the assay, can cause variations in IC50 results. These variations may hide any connections between ΔG and IC50 values that are seen in real-world situations.

In the future, the combination of computational ΔG estimates with empirical IC50 data shows potential when used together. Nevertheless, comprehensive assessments that span the metabolism of compounds, the permeability of cells, and the selectivity of targets are essential in order to improve the accuracy of predictions and optimize tactics for discovering drugs. Precision medicine strategies, customized for individual protein targets and biological circumstances, provide chances to discover potent and selective treatment options.

To summarize, the complex connection between IC50 values and ΔG in MCF7 cell research emphasizes the difficulties of converting theoretical projections into practical results in the field of drug development. Researchers can improve the reliability of preclinical assessments and advance personalized medicine by addressing biological complexities and experimental factors.

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8. Limitation and Challenge

There are various inherent limits and obstacles in comprehending IC50 numbers and Gibbs energy (ΔG) in MCF7 cell investigations. The presence of biological complexity, such as variations in cellular surroundings and interactions with pathways, has an independent impact on IC50 values, which makes precise measurements more challenging, regardless of ΔG predictions. Moreover, the presence of various structural and functional traits in protein targets, such as Tubulin, EGFR, and HER2, leads to different reactions to chemicals. This variability makes it difficult to establish general correlations between ΔG-IC50. The presence of many experimental elements, such as different assay techniques and settings, leads to variations in measurements. Therefore, it is necessary to establish standardized protocols in order to provide reliable validation. The ability of ΔG to accurately predict IC50 values is still a difficult task, and it requires the development of better computational models and empirical validations. To connect preclinical discoveries with clinical uses, it is necessary to tackle translational challenges such as the metabolism of compounds and the individualized responses of patients. The integration of extensive datasets and the construction of complete models are essential for enhancing drug discovery and personalized medicine techniques. These approaches help overcome hurdles and improve therapeutic development.

9. Conclusion

In conclusion, the examination of IC50 values and Gibbs energy (ΔG) in MCF7 cell research highlights the complex nature of drug discovery and development. This review establishes that there is no immediate correlation between Gibbs energy and IC50 values. To improve the accuracy of predictions and the possibility for practical application, it is important to combine advanced computational models with empirical validations and foster cooperation across different disciplines. To enhance the accuracy and dependability of future studies, it is recommended to concentrate on certain cell lines in in vitro cytotoxic investigations and to target specific receptors in molecular docking studies, all conducted under clearly specified experimental parameters. Furthermore, the utilization of precise parameters in computational models, such as binding affinities and molecular dynamics, can enhance the accuracy of predictions and strengthen the effectiveness of drug development procedures.

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