applied sciences

MDPI

Review

Integration of Molecular Docking and In Vitro Studies: A Powerful Approach for Drug Discovery in Breast Cancer

Claudia Cava [1,*] and Isabella Castiglioni [2]

[1] Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR), Via F.Cervi 93, 20090 Segrate-Milan, Italy

[2] Department of Physics "Giuseppe Occhialini", University of Milan-Bicocca Piazza dell'Ateneo Nuovo, 20126 Milan, Italy; isabella.castiglioni@unimib.it

* Correspondence: claudia.cava@ibfm.cnr.it

Received: 29 August 2020; Accepted: 2 October 2020; Published: 6 October 2020

Abstract: Molecular docking in the pharmaceutical industry is a powerful in silico approach for discovering novel therapies for unmet medical needs predicting drug–target interactions. It not only provides binding affinity between drugs and targets at the atomic level, but also elucidates the fundamental pharmacological properties of specific drugs. The purpose of this review was to illustrate newer and emergent uses of docking when combined with in vitro techniques for drug discovery in metastatic breast cancer. We grouped the selected articles into five main categories; namely, systematic repositioning of drugs, natural drugs, new synthesized molecules, combinations of drugs, and drug latentiation. We focused on new promising drugs that have a good affinity with their targets, thus inducing a favorable biological response. This review suggests that the integration of molecular docking and in vitro studies can accelerate cancer drug discovery showing a good consistency of the results between the two approaches.

Keywords: molecular docking; in vitro; metastatic breast cancer; drug discovery

1. Introduction

Breast cancer (BC) is the most common type of tumor in women, but metastases are the main cause of death. Metastasis is a complex process where cancer cells move into the blood vessels, invade other tissues, and determine a colony in secondary sites. Indeed, BC initiates as a local disease but can spread with metastases to distant sites, such as the lymph nodes and different organs [1]. This process involves the expression of a series of genes that regulate the survival and invasion of cancer cells. Therefore, drugs that modulate the genes/proteins that regulate cancer cell survival, metastasis, apoptosis, and invasion are of great importance as potential drug targets in the drug discovery process [2,3]. However, although the development of new therapies has significantly reduced mortality for metastatic BC, the resistance to anticancer agents can lead to treatment failure [4].

A drug discovery process originates because there is a clinical condition without a suitable therapy. The first step of research often begins in academia, where a hypothesis is generated; for example, the inhibition or induction of a protein or pathway as a therapeutic effect in a disease condition [5]. Indeed, a crucial point of the research process is the selection of a target, which can be a range of biological entities such as proteins, RNA, and genes that can be selected via bioinformatics analyses [6,7]. An optimal target must be accessible to the putative drug molecule and the binding drug–target complex should induce a biological response [5], which can be quantified with in vitro models. The most used in vitro BC models are cell lines, as they share many molecular and genomic features of BC. The binding affinity between the drug and the target can be calculated in silico with

molecular docking. Thus, in silico and in vitro screenings may help to quickly identify the toxicity of the tested drugs/molecules, thus avoiding further steps such as in vivo and preclinical studies (in case of unfavorable results from in silico and in vitro methods) [5].

In silico approaches with docking studies require at least two elements: a protein/drug database and a molecular docking algorithm. Protein and drug databases are a collection of the structures of proteins and drugs. The rapidly increasing number of structures has created big data, which offer a wide range of biological and chemical information and are a recent opportunity to develop better knowledge of the relationships between drugs and targets (usually proteins), drugs and diseases, and targets and diseases. However, although the available data are often heterogeneous and incomplete, computational methods can exploit this knowledge to deepen these interactions [8]. Given the cost and time consumption of experimental methods, high-performing computational algorithms for drug discovery processes are needed. The computational technique known as "docking" can predict the binding of drug–target complexes, as well as the conformation of the ligand upon binding to a protein target. The binding free energy of target–drug interactions establishes the affinity of an association and the conditions for forming a complex. Ranked binding free energies are not always precise, but they can be used to select new drugs such as small molecules to be experimentally tested in a virtual screening approach [9–11]. Small molecules are promising new drugs with a low molecular weight, which allows them to penetrate cells easily [12]. In addition, molecular docking can be also used for predicting the effects of a drug; for example, the identification of an undesired interaction between a compound and off-targets. To date, 57,000 abstracts/papers have been published on molecular docking, indicating the importance of this computational method in drug development [13–15].

Despite encouraging results, the real condition of the cellular environment, such as the pH and temperature, cannot be fully replicated in a docking study. Each docking algorithm has its limitations and advantages. Therefore, it has been reported that a binding free energy that integrates the results from different docking algorithms can lead to a higher performance in a virtual screening process [16]. Moreover, molecular docking, being a structure-based method, is limited to receptors and ligands with a known stable structure. Thus, the integration of in vitro and in vivo studies as a validation step of in silico methods is an indispensable part of the drug discovery process. These techniques can study different aspects of potential drugs, such as absorption, regulation of targets, metabolic stability, and toxicity [17].

The goal of this review was to describe recent studies in metastatic BC that used molecular docking and in vitro studies for drug development.

2. Materials and Methods

Papers published on the PubMed platform on the use of in vitro and molecular docking strategies in metastatic BC were included in this review. Papers were included in the review only if: (1) they were published in the last five years (from 2015 to the end of the search on 27 August 2020); (2) they were published in full-text English language in a peer-reviewed journal (excluding short communications and abstracts); (3) they included in vitro and docking analyses in metastatic BC. Papers were excluded if: (1) only one in vitro or docking analysis was performed; (2) they did not provide in vitro studies on BC cell lines.

3. Results

We categorized the selected papers into five main groups based on the characteristics of the drugs and approaches used: systematic repositioning of drugs/molecules, natural molecules, new synthesized molecules, combinations of drugs, and drug latentiation.

3.1. Systematic Repositioning of Drugs/Molecules

Drug discovery is a time-consuming and labor-intensive work process. On average, the development of a new drug takes 10–15 years. Drug repositioning, namely, the use of old drugs for new diseases, is an efficient strategy for its low-cost and riskless characteristics [15]. Several studies have performed systematic approaches, using a combination of in silico and experimental methods, to reposition known drugs/molecules. Table 1 presents the works in the last five years that have implemented in silico and in vitro models for drug discovery using a systematic repositioning of drugs for metastatic BC.

In particular, the study of Rymbai et al. [18] focused on the similarity between the side effects of two different drugs. Indeed, the assumption of the study was that if the two drugs have common side effects, then they can also have common gene targets and clinical indications. In vitro cell line and molecular docking studies have shown that ropinirole shares many side effects with letrozole (used in the management of advanced and metastatic BC) and is efficient in the treatment of breast cancer. An in vitro study of ropinirole on MCF-7 cells by a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (MTT) was performed to test the ability of ropinirole to inhibit cell growth. Molecular docking showed a good interaction with a good binding affinity of −7.8 kcal/mol between ropinirole and aromatase, a well-known target of letrozole. The three-dimensional structure of aromatase (Protein Data Bank (PDB) ID: 3EQM) is available for computational docking analysis in the Protein Data Bank (PDB).

In a recent study, Liu et al. screened more than 1000 known small molecular compounds and focused on 15 compounds [19], studying their effect on breast cancer cell viability and migration with in vitro studies on MCF-7, MDA-MB-231, and BT-474 BC cell lines. Molecular docking was performed to demonstrate the ability of the binding of the 15 compounds with chemokine ligand 18 (CCL18), as previous studies suggested that CCL18 is a chemokine derived from tumor-associated macrophages (TAMs) to induce BC metastasis [20]. Therefore, CCL18 is considered a potential drug target (PDB ID: 4MHE). Narrowing the selection of ligands from more than 1000 compounds to 15 was performed by evaluating the binding energy with CCL18 obtained via molecular docking studies. In this way, 15 compounds were selected as potential drugs targeting CCL18, and the toxicity of these 15 compounds was evaluated by using cell counting kit-8 (CCK-8) assays, which indicated that most of the compounds did not influence cell viability. A total of 6 of the 15 compounds inhibited CCL18-induced cell migration; this anticancer activity was also confirmed by adherence and invasion assays [19].

In vitro, in silico, in vivo, and ex vivo analyses have been performed to evaluate the cytotoxic action of etoposide (ET), doxorubicin (DOX), pifithrin-α (PIF), and dexamethasone (DEX) in triple-negative BC (TNBC) [21]. TNBC is a molecular subtype of BC that is negative for three hormone receptors—namely, estrogen receptors (ERs), progesterone receptors (PRs), and human epidermal receptor 2 (HER2) [21]. ET, a podophyllotoxin derivative, is a chemotherapy medication used against a wide range of cancers (e.g., lung cancer, lymphoma, lung cancer, leukemia, and glioblastoma multiforme), but its efficacy against TNBC is still unknown [22]. DOX, alone or in combination with other drugs, is an effective therapy for numerous cancers, including breast cancer [23]. The antiapoptotic and non-topoisomerase inhibitors PIF and DEX were considered in a previous study as a negative control [21]. In this study, the authors identified tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and death receptor 5 (DR5) as potential drug targets (PDB ID: 4N90) [21]. Molecular docking and molecular dynamics studies have shown the ability of ET, DOX, PIF, and DEX to stabilize the TRAIL–DR5 complex. In vitro technologies have demonstrated that ET and DOX increase apoptosis, demonstrating their synergistic effect with TRAIL. These results were confirmed with the binding energy for the ternary complexes TRAIL–DR5–ET and TRAIL–DR5–DOX [21].

Thionine (TH), an organic dye, and its derivatives have been proposed as promising drugs for the photodynamic therapy of cancer [24]. Previous studies reported that TH shows possible genotoxic and cytotoxic activity in prokaryotic cells [25]. Since there is a wide range of applications of TH, it is important to evaluate the site-specific interaction of TH with human serum albumin (HSA), the main

protein in plasma, which is responsible for the maintenance and regulation of the colloidal osmotic pressure of blood [24]. HSA has several binding sites to which various ligands can bind. The binding of ligands to this protein can influence drug distribution, as HSA plays a crucial role in the transport of endogenous/exogenous compounds. Therefore, HSA is widely used in clinical applications as a drug delivery system. Manivel et al. revealed that TH interacts with the hydrophobic cavity of subdomain IIA of HSA (PDB ID:1AO6) and that the complex shows a good level of cytotoxicity in cancer cells through in vitro studies [24].

Previous studies have shown the anticancer and antimetastatic effects of the microbial polyketide 2,4-diacetylphloroglucinol (DAPG) through the regulation of NF-κB activity [26]. The induction of NF-κB also mediates the expression of other antiapoptotic proteins and protein kinases in cancer cells. However, the mechanism of action of DAPG acting on metastatic proteins such as Matrix metalloproteinase 9 (MMP9), MMP2, NF-κB, and the antiapoptotic Bcl-2 family proteins are not yet known. In a recent study [27], binding energies, computed by molecular docking, revealed that MMP2, MMP9, and NF-κB achieved a higher interaction with DAPG. In vitro studies have confirmed that DAPG compounds are able to inhibit cancer cells in several cancer cell lines, including MDA-MB-231 [27].

Drug repositioning offers new clinical indications for known drugs/molecules using an efficient, low-cost and riskless strategy. However, despite encouraging results, drug repositioning is a complex process that involves different elements, such as the use of big medical data, to develop an appropriate approach for drug repositioning and a new framework for the integration of available resources.

3.2. Natural Molecules

Historically, natural products, which contain a wide range of compounds for drug discovery, have been considered in multiple clinical trials, especially as anticancer and antimicrobial agents [28]. The advantage of natural products with respect to synthetic compounds is that they are also metabolites. Therefore, they are biologically active and can also be substrates for transporter systems [28]. Table 2 shows the works in the last five years that have implemented in silico and in vitro models for drug discovery using natural ligands as drugs for metastatic BC.

Hypercholesterolemia has been reported to play a role in the progression of BC and resistance to hormonal therapy. High low-density lipoprotein (LDL) levels, primarily caused by familial hypercholesterolemia, are also one of the risk factors of the initiation and promotion of BC [29]. Proprotein convertase subtilisin/kexin type-9 (PCSK9) binds to LDL receptors (avoiding binding with LDL) and regulates the cholesterol metabolism, targeting the receptor for lysosomal degradation and thus leading to the degradation of LDL. Pseurotin A (PS) is a microbial secondary metabolite originally isolated from the fungal culture of *Pseudeurotium ovalis* in 1976 [30]. PS shows different biological activities, including the inhibition of the fungal chitin synthase [31], the activation of cell differentiation [32], and apomorphine antagonist activity [33]. Abdelwahed et al. showed that PS reduces PCSK9 secretion, suggesting its potential as a drug [34]. In particular, they performed predictive molecular modeling to evaluate the binding of PS to PCSK9 (PDB ID: 4NE9, 4NMX, and 3GCW). Docking analysis has revealed that PS is able to successfully bind to the PCSK9 domain, thereby disrupting PCSK9–LDL receptor interactions. Furthermore, this finding has been validated in vitro by surface plasmon resonance, confirming the capacity of PS to interfere with the PCSK9–LDL complex at their binding interface.

Table 1. Characteristics of the studies reported herein categorized as "systematic repositioning of drugs" used in metastatic breast cancer. The table reports the drug, its target with Protein Data Bank (PDB) ID, the in silico/in vitro/in vivo methods used to test the drug, clinical trials, the original use, and the reference.

Drug	Target	In Silico	In Vitro	In Vivo	Clinical Trials	Original Use	Ref.
Ropinirole	aromatase enzyme (PDB [1] ID: 3EQM)	Docking studies	MTT [2] assay	-	-	antiparkinsonism	[19]
15 small molecular compounds	CCL18 (PDB ID: 4MHE)	Docking studies	Cell viability, Boyden chamber, adherence assay	Tumor xenografts	-	CCL18 antagonist	[20]
Topoisomerase inhibitor etoposide (ET) and doxorubicin (DOX)	TRAIL-DR5 (PDB ID: 4N90)	Docking, mutational and dynamics studies	MTT assay, FACS [3]	Tumor xenografts	NCT00004 906	against a wide range of cancers	[21]
Thionine	human serum albumin (HSA) (PDB ID:1AO6)	Dockingstudies	MTT assay and Fluorescence microscopic	-	-	against bacteria, viruses and yeasts	[24]
2,4-diacetylphloroglucinol	Bcl-2 (PDB ID: 4AQ3), Bcl-xL (PDB ID: 2YQ6), Bcl-w (PDB ID: 2Y6W), MMP2 (PDB ID: 1HOV), MMP9 (PDB ID: 1GKC), NF-jB p65 (PDB ID: 1VKX)	Docking studies	MTT and invasion assay	-	-	antimicrobial, antiviral, and anticancer	[27]

[1] PDB: Protein Data Bank, [2] MTT: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, [3] FACS: Fluorescence-activated cell sorting.

In an another study, the interactions of atranorin (ATR), a metabolite of many lichens, with the target proteins that are overexpressed in BC, such as AKT, BCL-2, BAX, BCL-W, and BCL-XL, were studied with docking and in vitro analyses [35]. Commonly, ATR is metabolite present in numerous lichens such as *Stereocaulon cacspitorim*, *Everniastrum vexans*, *Parmatrema* species, and others [35]. The complex formed by ATR and AKT shows a better binding energy, but interacting residues show minor affinities to inhibiting the overexpression of BC biomarkers. Interestingly, previous studies have reported the activation of AKT in drug resistance [36] and in vitro studies have been performed on MDA-MB-231 and MCF-7. The inhibitory activity of ATR has been tested with MTT assays, showing a downregulation of oncoproteins. Furthermore, gene expression analysis of the ATR–AKT model has shown the induction of apoptosis in BC cell lines [35].

Another natural compound, eugenol, a phenylpropanoid obtainable from honey and some essential oils with antioxidant and anticancer properties, has been tested with in silico and in vitro approaches [37]. In vivo and in vitro studies have demonstrated that eugenol promotes the inhibition of β-catenin, a biomarker associated with the progression of cancer and the development of lymph node metastasis. β-catenin accumulation in the nucleus is evident in BC because of aberrant wnt signaling. Western blot analysis has confirmed a significant modification in the expression level of total β-catenin in in vivo and in vitro models. In addition, eugenol demonstrates a downregulation of the expression of cancer stem cell biomarkers. Docking studies have revealed many binding sites of the complex and the data support a good interaction between the ligand and the β-catenin protein (PDB ID: 3BCT) [37].

Pharmacological research in recent years has also proposed the plant *Astragalus membranaceus* (AM) as a natural product for cancer treatment. A recent study using a multidisciplinary approach constituted by gene expression analysis, pharmacokinetic screening, biological network analysis, and in vitro approaches investigated the possible and novel mechanism of AM in TNBC [38]. All of the ingredients of AM were collected for a total of 87 compounds and 16 active components and *Astragalus polysaccharides* (APS) was proposed as a potential compound against BC. Indeed, docking analysis showed good results between APS and the proteins AKT, BCL2, and PIK3CG. Indeed, in vitro experiments confirmed that the compound can inhibit migration and invasion and can induce apoptosis [38].

Natural compounds can be also administrated to avoid the side effects of drugs. In a recent study, drug design methods were performed to investigate plant-derived inhibitors against sirtuin (SIRT) proteins [39]. As SIRT, which comprises seven human isoforms, is associated with the metastatic and oncogenic progression of advanced BC, its inhibition is a promising approach against tumorigenesis. A previous study considered 21 plant-derived inhibitors as ligands and the seven human isoforms of SIRT as targets [39], and molecular docking with the binding energies of the ligand–receptor complexes showed that sulforaphane, kaempferol, and apigenin can achieve the highest binding energies against SIRT1, 3, and 6, respectively. To validate these in silico results, they explored the role of these potential small molecules against BC cell lines on cellular viability using MTT assays [39].

Noscapine (NOS) is a phthalide isoquinoline alkaloid derived from the *Papaver somniferum* plant. As several studies have reported the ability of NOS to inhibit the growth of tumor cells and to activate apoptosis, there are ongoing phase I/II clinical trials for cancer management, but not for BC [40]. Maurya et al. [41] studied the interaction between NOS and carrier protein HSA using different techniques, including in vitro approaches and computational methods. The cytotoxicity findings by MTT assay indicated that NOS has good potential in cancer. Meanwhile, the in silico results showed that the main binding site for NOS was site I (subdomain II A) of HSA [41].

Shikonin (SK), a phytochemical derived from the medicinal plant *Lithospermum erythrorhizon*, has been demonstrated to induce tumor immunogenicity [42]. However, the molecular mechanisms of action and the pharmacological processes are still unknown. First, in a recent study [43] the authors performed a computation prediction analysis, applying molecular docking and a virtual screening system. They screened 27,317 human protein structures deposited in the Protein Data Bank in order to search for the molecular targets of SK. Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) obtained the highest binding energy with SK (−15.3 kcal/mol), and this binding leads to the suppression

of post-transcriptional mRNA processing in vitro [43]. In addition, the SK– hnRNPA1 complex inhibits the splicing and suppresses the nuclear export activities of specific inflammation-associated genes, resulting in a reduction in acute cytokine storms [43].

Matrix metalloproteinase 9 (MMP9) and matrix metalloproteinase 2 (MMP2) regulate the tumor microenvironment and tumor metastasis. Therefore, the inhibition of MMP9 and MMP2 could reduce invasion and tumor metastasis [44]. Currently, traditional Chinese medicines (TCMs) are proposed in the prevention and treatment of several diseases, including cancer. In a recent study, plantamajoside (PMS), a Chinese herbal medicine, was proposed as an inhibitor of MMP9 and MMP2. Indeed, molecular docking and in vitro analyses confirmed the good interaction of the molecules with the proteins and the inhibition of the proliferation, migration, and invasion of BC cell lines [44]. Specifically, in vitro studies on the MDA-MB-231 and 4T1 BC cell lines have demonstrated that PMS reduces the activity of MMP9 and MMP2. In addition, after treatment with PMS, BC cell lines show a decrease in cell proliferation.

The aryl hydrocarbon receptor (Ahr), a helix–loop–helix transcription factor, is a promising regulator of the invasiveness and metastasis of BC cells. It is regulated by a wide variety of natural molecules such as flavonoids—among which, flavipin is the least studied. Hanieh et al. [45] applied in silico and in vitro methods to investigate the relationships between flavipin and Ahr. Docking analysis revealed eight hydrogen bonds involving the Phe115, Leu116, and Ala119 residues of the Ahr molecule. The results of the in vitro analysis showed that flavipin has inhibitory effects on the migration of MDA-MB-231 and T47D cells [45].

The studies reported above show that the growing application of in silico and in vitro techniques may contribute to the recovery of interest in natural products for drug discovery.

Table 2. Characteristics of the studies reported herein categorized as "natural drugs" used in metastatic breast cancer. The table reports the drug, its target with PDB ID, the in silico/in vitro/in vivo methods used to test the drug, clinical trials, the mechanism of action, and the reference.

Drug	Target	In Silico	In Vitro	In Vivo	Clinical Trials	Mechanism of Action	Ref.
Pseurotin A	PCSK9 (PDB [1] ID: 4NE9, 4NMX, and 3GCW)	Docking studies	MTT [2] assay	Tumor xenografts	-	cholesterol metabolism	[34]
Atranorin	AKT, BCL-2, BAX, BCL-W and BCL-XL (PDB ID: NA)	Docking studies	MTT assay	-	-	apoptosis	[35]
Eugenol	β-catenin (PDB ID: 3BCT)	Docking studies	MTT assay	Tumor xenografts	-	Cancer Stem Cell	[37]
Astragalus membranaceus	AKT (PDB ID:3QKK), BCL2 (PDB ID: 4AQ3), and PIK3CG (PDB ID: CHX)	Differential expression analysis Docking, dynamics studies	CCK-8 [3], Chamber, FITC [4] assay	-	NCT03314805, NCT03634150	apoptosis	[38]
21 plant-derived inhibitors	human sirtuin (SIRTs 1-7). SIRT1 (PDB ID: 4I5I), SIRT2 (PDB ID: IJ8F), SIRT3 (PDB ID: 5D7N), SIRT5 (PDB ID: 2B4Y), SIRT6 (PDB ID: 3K35) and SIRT7 (PDB ID: 5IQZ)	Docking and dynamics studies	MTT, trypan blue, sirtuin, Anchorage-dependent clonogenic assay	-	-	sirtuin inhibitors	[39]
Noscapine	human serum albumin (HSA) (PDB ID: 1AO6)	Docking and dynamics studies	MTT assay	-	-	inhibition of cell growth	[41]
Shikonin	27,317 human protein structures	Docking studies	calorimetry analysis and electrophoretic mobility shift assay	Tumor xenografts	-	suppression of post-transcriptional mRNA processing	[42]
Plantamajoside	Matrix metalloproteinase 2 and 9 (PDB ID: NA)	Docking studies	CCK-8, chamber wound assay	Tumor allografts	-	inhibition of cell growth	[44]
Flavipin	Aryl hydrocarbon receptor (Ahr) (PDB ID: 4M4X)	Docking studies	CCK-8 and Boyden chamber	-	-	cancer cell motility	[45]

[1] PDB: Protein Data Bank, [2] MTT: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, [3] CCK-8 cell counting kit-8, [4] FITC Annexin-V fluorescein isothiocyanate.

3.3. New Synthesized Molecules

Since there is not yet a definite solution for the treatment of cancer, there is a clear need to research new molecules with anticancer properties. The combination of in vitro techniques and molecular docking can also be used also to test new synthesized molecules. Table 3 shows the works in the last five years that have implemented in silico and in vitro models for drug discovery using new synthesized molecules as drugs for metastatic BC.

For example, Nashaat et al. [46] synthesized a new series of compounds; i.e., new benzimidazole derivatives against BC-targeting peptidylprolyl cis-trans isomerase NIMA-interacting 1 (PIN1). PIN1 proteins play a role in cell cycle regulation, and their overexpression is correlated with human cancer [46]. It has also been described that PIN1 increases DNA binding to estrogen receptors [47]. Anticancer effects have been tested in vitro with MTT assays using the MCF-7 cell line: Several synthesized compounds have shown a very strong efficacy against this BC cell line. Furthermore, the interactions of three synthesized compounds with PIN1 crystal structures (PDB: 4TYO) have been validated using molecular docking, and the formed complexes show some essential interactions for PIN1 inhibition [46]. Specifically, the interaction with Lys63 is crucial for PIN1 inhibition.

In another study, Vaz et al. [48] synthesized the new hybrid dihydroquinoline derivative (M-CNP) compound. They designed this hybrid compound composed of sulfonamide, quinoline, and chalcone. The idea behind the study was that molecules containing sulfonamide, quinoline, and chalcone could originate new hybrid architectures with novel anticancer properties. The aim is that compounds derived from more molecules can enhance the pharmacological effectiveness. Anticancer activity of the novel compound was demonstrated by regulating aldehyde dehydrogenase 1 family member A1 (ALDH1A1), which, by converting retinal (retinaldehyde) to retinoic acid (RA), plays a role in the differentiation of cells and signaling events. Interestingly, an in vivo test on a metastatic BC cell lines (i.e., MDA-MB-231) has confirmed the promising role of ALDH1A1. Indeed, cytotoxicity assays have demonstrated the specific activity of the compounds against tumor cells. Furthermore, molecular docking analyses have demonstrated that the M-CNP derivative plays a role as an anticancer drug because of its good affinity with ALDH1A1 [48].

Previous studies have shown that compounds can bind to DNA with covalent and non-covalent interactions; for example, cisplatin, a well-known anticancer drug, interacts with DNA via a covalent link. However, covalent interactions can induce serious side effects, highlighting the importance of non-covalent interactions between DNA and drugs [49]. Among metal complexes, nickel aroylhydrazone Schiff base complexes show non-covalent interactions. Following this assumption, Li et al. [49] synthesized two nickel-derived complexes, and molecular docking revealed that both compounds could bind to DNA through the interaction of the phenyl rings with the double helix. In addition, the association between DNA and bovine serum albumin (BSA) is able to modify the secondary structure of BSA. The anticancer activity of individual complexes was also evaluated with in vitro cytotoxicity assays on the A549, MCF-7, and L-02 cell lines [49]. Both complexes obtained a lower cytotoxic effect than cisplatin against normal cell lines. Thus, in vitro analysis suggests more selective effects of the compounds against cancer cell lines.

Ruarene complexes have been observed to be possible agents against cisplatin resistance with fewer side effects, demonstrating a different mechanism of action. Acharya et al. synthesized four ruarene complexes and characterized them using X-ray crystallography [50], and molecular docking was performed to demonstrate their ability to bind tubulin proteins (PDB: 1SA0). The cytotoxicity of the molecules was tested in vitro by MTT assays in three different cancer cell lines, including MDA-MB-231. The complexes that demonstrated a lower toxicity were selected to test their effect on the inhibition of the microtubule network in the MDA-MB-231 cell line. In silico and in vitro studies demonstrated good binding between the compounds and the tubulin, as well as antiproliferative action against advanced subtypes of cancer, such as triple-negative metastatic BC [50].

Glycyrrhiza glabra, an Indian therapeutic herb, contains a diglucopyranosiduronic acid of glycyrrhetinic acid (GA). GA plays a role in immune responses, cell cycle, apoptosis, and autophagy [51].

Shukla et al. [52] designed five glycyrrhetinic acid (GA) derivatives and analyzed their in vitro action in a metastatic breast cancer cell line (i.e., MDA-MB-231). Molecular docking studies have been carried out to investigate the action of compounds on BC targets such as glyoxalase-I (GLO-I). BC receives energy from glycolysis based on the Warburg effect, and GLO-1 is able to inhibit and inactivate methyl glyoxalases, a compound formed during glycolysis, making GLO-I inhibitors potential anticancer agents. It has been shown that GA-1 increases cytotoxic action, and a study using molecular docking confirmed the binding of GA derivatives with GLO-I [52].

As previous studies have described that epidermal growth factor receptor tyrosine kinase (EGFR-TK) is upregulated in BC, it has been suggested as a potential drug target for novel therapy agents. The EGFR-TK inhibitory activity of known 1,3,5-triazines derivatives against BC has been examined [53]. In addition, the effects of the compounds on several BC cell lines have been estimated, including MDA-MB-231, a metastatic BC cell line. The consequence of the compounds on β-catenin expression has also been evaluated: all designed compounds achieved a good binding energy against the target protein. Furthermore, in vitro experiments have confirmed the docking analysis since the synthesized derivatives demonstrated an inhibitory effect on EGFR-TK [53].

Rac family small GTPase 1 (RAC1) is involved in the migration and invasion of BC cells. Therefore, therapeutic strategies that silence RAC1 could be a new challenge [54]. A new series of carbazole derivatives have been designed for their antitumor properties. Vlaar et al. evaluated the role of the compounds via interactions with RAC1 through molecular docking: the molecular docking results indicated a favorable conformation of the receptor–ligand complex. The compounds also demonstrated moderate antiproliferative activity using in vitro techniques [54].

The main site of BC metastasis is bone, and bone metastases often lead to complications, including fractures, bone pain, and hypercalcemia. However, to date, no biomarkers have been identified that are able to predict bone metastases. A first circulating fragment of parathyroid hormone-related protein (PTHrP), PTHrP(12-48), has been proposed as a biomarker associated with the presence of bone metastases. Kamalakar et al. [55] investigated the biological processes and mechanisms of action of PTHrP(12-48). First of all, they predicted the tertiary structure of PTHrP(12-48) through bioinformatics analyses, and the molecular modeling found that PTHrP(12-48) interacts via a weak binding with the PTH1 receptor (PTHR1). In vitro analysis supported this model: PTHrP(12-48) treatment does not promote an increase in cAMP in PTHR1-expressing SaOS2 cells. In conclusion, these data indicate that PTHrP(12-48) acts in the regulation of the differentiation of hematopoietic cells and regulates the osteoclasts within the tumor–bone marrow microenvironment, possibly to induce bone metastasis [55].

Angiogenesis, a physiological process that produces new blood vessels from pre-existing blood cells, consists of a series of steps such as the production of protease, endothelial cell migration and proliferation, vascular tube formation, and the maturation of cells [56]. Accelerated angiogenesis is correlated with several diseases, including cancer. The inhibitors of angiogenesis that target several proteins, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), that are involved in the regulation of the process can be used as a therapeutic strategy. The main factor in the angiogenesis process is VEGF [56]. Aboul-Enein et al. [57] designed 7-chloro-4-(piperazin-1-yl)quinoline derivatives as VEGFR-II inhibitors, and the anticancer abilities of these compounds were tested in vitro using breast cancer and prostate cancer cell lines. These analyses demonstrated that compound **4q** is the most active on both cell lines, and molecular modeling revealed that **4q** has similar sites of binding as sorafenib and lenavatinib at the ATP binding site of VEGFR-II. Furthermore, the binding energy of **4q** is slightly better than that of lenavatinib but lower than that of sorafenib [57].

Apoptosis is one of the key hallmarks of cancer. The BCL-2 family is a group of proteins that have a fundamental role in the regulation of apoptosis. For this reason, BCL-2 has become one of the most studied drug targets over recent years. Ziedan et al. [58] designed a 3D pharmacophore model to act as an inhibitor of the antiapoptotic BCL-2 protein. The 15 oxadiazole derivative compounds were tested to demonstrate their inhibitory activity in two cancer cell lines (i.e., cervical HeLa and breast

MDA-MB-231). Molecular docking was carried out to evaluate the interaction scores between the compounds and the BCL-2 protein. Some of the proposed compounds obtained good antiproliferative activity and good binding energy with the BCL-2 protein (compounds **1** and **16j**) [58]. The simplicity of the synthesis of these compounds and their low molecular weight is promising; therefore, additional studies should be employed based on this novel class of BCL-2 inhibitors.

Oncogenic proteins such as tyrosine kinases, cell cycle regulators, and transcriptional factors are implicated in metastatic pathways in cancer. Many of them interact with heat shock protein 90 (Hsp90). Therefore, Hsp90 inhibitors have been proposed as novel cancer treatment methods, although they can lead to adverse effects in clinical trials. To overcome these disadvantages, Koca et al. [59] designed novel molecular Hsp90 inhibitors. Specifically, they designed novel pyrimidinyl acyl thiourea derivatives as Hsp90 inhibitors, and in vitro analyses revealed that these compounds can inhibit cell proliferation and demonstrate cytotoxic effects in BC and human bone osteosarcoma cell lines. Molecular docking confirmed the interaction of these compounds with the Hsp90 domain [59].

Previous studies have suggested KDM5A and KDM5B as oncogenic regulators [60]. The catalytic domain of KDM5 proteins has an unusual inclusion of an ARID and PHD1 domain that divides the catalytic domain into two subdomains—namely, JmjN and JmjC. Horton et al. demonstrated that a deletion of the ARID and PHD1 domains has a negative impact on the in vitro enzymatic kinetics of the KDM5 family. Thus, the challenge is finding inhibitors that act on the catalytic domain of the KDM5 family; to this end, the authors proposed GSK-J1 as an inhibitor of the KDM family through in silico studies [61].

EPH receptor A2 (EphA2) is a receptor tyrosine kinase that is involved in drug resistance and metastatic processes [62]. Gambini et al. designed agonistic peptides that target the ligand-binding domain of the EphA2 receptor, called 135H11 and 135H12 [63]. In vitro approaches and computational methods have suggested that both are effective agonistic EphA2 agents and are effective in inhibiting cell migration and invasion [63]. In addition, both dimeric agents are able to induce EphA2 receptor degradation.

The use of drugs to treat or manage the progression of BC is the best strategy. However, the efficacy of traditional drugs has been seriously compromised due to the phenomenon of resistance. Therefore, it is essential to discover new synthesized drugs that target novel sites and regulate biological processes involved in the progression of cancer.

Table 3. Characteristics of studies reported in the review categorized as "new synthesized molecules" used in metastatic breast cancer. The table reports the drug, its target with PDB ID, in silico/in vitro/in vivo methods used to test the drug, clinical trials, mechanism of action and reference.

Drug	Target	In Silico	In Vitro	In Vivo	Clinical Trials	Mechanism of Action	Ref.
new benzimidazole derivatives	PIN1 (PDB [1]: 4TYO)	Docking studies	MTT [2] and apoptosis assay	-	-	apoptosis	[46]
Dihydroquinoline derivate, M-CNP	ALDH1A1 (PDB ID: NA)	Docking studies	MTT assay	-	-	cell viability	[48]
Two new nickel (II) triphenylphosphine complexes	DNA (PDB ID:1Z3F), BSA (PDB ID: 4F5S)	Docking studies	CCK-8 [3] assay	-	-	antioxidant activity	[49]
Ruarene complexes	Tubulin (PDB ID: 1SA0)	Docking studies	MTT, Annexin-V/PE assays	-	-	proliferation	[50]
5 glycyrrhetinic acid (GA) derivates	GLO-I (PDB: 4PV5)	Docking studies	cytotoxicity assay	-	-	metabolism	[52]
1,3,5-triazine derivatives	epidermal growth factorreceptor-tyrosine kinase (EGFR-TK) (PDB ID: 1M17)	Docking studies	MTT and apoptosis assay	-	-	apoptosis	[53]
carbazole derivatives	RAC1 (PDB ID: NA)	Docking studies	Wound healing	-	-	migration	[54]
PTHrP(12-48)	PTH1 receptor (PDB ID: NA)	Docking studies	Immunofluorescence assays	-	NCT00051779	activity of osteoclasts	[55]
Certain 7-Chloro-4-(piperazin-1-yl)quinoline Derivatives	VEGFR-II (PDB ID: NA)	Docking studies	SRB [4] assay	-	-	proliferation	[57]
Oxadiazole derivates	BCL-2 protein (PDB ID: 1YSW)	Docking studies	MTT assay	-	-	apoptosis	[58]
novel pyrimidinyl acyl thiourea derivatives	Heat Shock Protein 90(Hsp90) (PDB ID: 1UYM)	Docking studies	XTT [5] assay	-	-	ATPase function	[59]
crystalstructure of the linked JmjN-JmjC domain	KDM5A and KDM5B (PDB ID: NA)	Docking studies	SRB assay	-	-	cell growth	[61]
135H11 and 135H12	EphA2 (PDB ID: 6B9L)	Docking studies	Wound healing	-	-	migration	[63]

[1] PDB: Protein Data Bank, [2] MTT: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, [3] CCK-8 cell counting kit-8, [4] SRB: SulfoRhodamine-B stain, [5] XTT: 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide.

3.4. Combination of Drugs

Current chemotherapeutic agents can lead to many adverse effects and can be toxic to healthy cells. For this reason, the identification of new agents that can effectively eradicate tumorigenic cells without damaging normal cells is necessary. A possible solution could be the use of combinations of drugs. Indeed, the efficacy of treatment could be improved, as individual drugs can target different biological pathways. Moreover, combinations of drugs could also potentially reduce drug resistance [64]. In silico and in vitro approaches have been used to test combinations of small molecules against cancer. Table 4 shows the studies in the last five years that have implemented in silico and in vitro models for drug discovery using combinations of drugs/molecules for metastatic BC.

For example, Nayak et al. [65] showed the ability of quinacrine and curcumin to regulate the apoptosis of cancer stem cells with an in vitro model. Curcumin is a diarylheptanoid, which is a natural phenol isolated from the *Curcuma longa* plant, and has multiple pleiotropic effects, such as the suppression of multiple signaling pathways, the inhibition of cell proliferation, and antimetastatic properties [66]. Quinacrine, a 9-aminoacridine (9-AA) derivative, shows anticancer properties against several cancers, such as breast, pancreatic, and lung cancers. Its antiapoptotic activity is shown by its ability to arrest the cell cycle in the S-phase via the inactivation of topoisomerase activity, the activation of p53 and p21, and the inhibition of NF-kβ [67]. The study of Nayak et al. [65] analyzed the anticancer effects of curcumin and quinacrine, as well as their combination, using in vitro and molecular modeling. Multiple BC cells were used to characterize a metastatic model that demonstrated the effects of the combination of molecules on decreasing the migration and invasion and inducing apoptosis. The cytotoxic and antiproliferative activity results showed the synergistic action of the drugs, and molecular docking showed a good affinity of the molecules with ABCG2, a biomarker of BC. Specifically, the binding site is in the transmembrane domain of ABCG2 [65].

Table 4. Characteristics of studies reported in the review categorized as "combination of drugs" used in metastatic breast cancer. The table reports the drug, its target with PDB ID, in silico/in vitro/in vivo methods used to test the drug, clinical trials, mechanism of action and reference.

Drug	Target	In Silico	In Vitro	In Vivo	Clinical Trials	Mechanism of Action	Ref.
Quinacrine and curcumin	ABCG2 (PDB [1] ID: NA)	Docking studies	MTT [2] assay	-	-	DNA damage and repair	[65]
ITH-47 and ESE-15-ol	bromodomain-containing protein 4 32(BRD4) (PDB ID: NA)	Docking and dynamics Studies	Annexin V-FITC [3] and caspase activation assays	-	-	apoptosis	[68]
Vitamin E and Paclitaxel	Bovine serum albumin: (PDB ID: 4OR0)	Docking studies	MTT assay	Tumor xenografts	-	proliferation	[69]

[1] PDB: Protein Data Bank, [2] MTT: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide [3] FITC Annexin-V fluorescein isothiocyanate.

In another study [68], the authors investigated the combination of two novel compounds, namely, ITH-47 (a BRD4 inhibitor) and ESE-15-ol (an antimitotic agent). The in vitro study revealed that the combination of these two compounds inhibits the growth of MDA-MB-231. To compare the binding energy of the two molecules, they performed molecular docking with a known drug—i.e., JQ1—which revealed that, compared to JQ1, the molecules can achieve similar binding energies and sites as bromodomain-containing protein 4 (BRD4). BRD4 plays a role in regulating c-Myc, a key regulator of cell growth and apoptosis [68].

Paclitaxel (PTX), also known as Taxol, is used as a drug in clinical treatment against different cancers. However, as it causes different side effects, Tang et al. [69] suggested vitamin E (VE)–albumin core–shell nanoparticles (NPs) to improve the efficacy of PTX in BC models. They also investigated the cytotoxicity with in vitro approaches on MCF-7 BC cell lines. Docking studies were performed to analyze the interaction between PTX or VE and BSA, and the results demonstrated a strong receptor–ligand interaction and PTX–VE NPs exhibited better cytotoxic effects than PTX NPs [69].

The antitumor effect was also studied using the xenograft model, showing that treatment with PTX–VE NPs is more effective and lowers the toxicity of the molecules.

3.5. Drug Latentiation

Drug latentiation is a procedure where a compound is chemically modified to improve its binding affinity with a target in order to increase its therapeutic activity.

Gefitinib is one of the more effective and specific epidermal growth factor receptor (EGFR) inhibitors, which interacts with the adenosine triphosphate (ATP)-binding site of the EGFR tyrosine kinase enzyme. Sharma et al. designed three gefitinib-based derivatives to improve the ligand–receptor interaction [70]. Molecular docking studies were also presented for the study of the interactions of the gefitinib derivatives with EGFR, DNA, and BSA. Synthesized compounds were further screened in different cancer cell lines, including MDA-MB-231, to evaluate the cytotoxicity of the new compounds. The results demonstrated a similar effect between experimental and molecular docking analyses, suggesting the important role of gefitinib-based derivatives [70]. Indeed, the in vitro cytotoxicity and antiproliferative activity demonstrated that the derivatives are more potent than gefitinib.

4. Conclusions

In this review, we reported recent studies that have used molecular docking and in vitro studies in metastatic BC for drug discovery. We divided the studies into five main categories: "Systematic repositioning of drugs/molecules", "natural drugs", "new synthesized molecules", "combinations of drugs", and "drug latentiation".

The studies in the systematic repositioning of drugs/molecules category generated new clinical indications for old known drugs or molecules, such as ropinirole, small molecular compound SYSU-21598, etoposide, thionine, and 2,4-diacetylphloroglucinol, reporting a new possible application in metastatic BC. In addition, natural products such as pseurotin A, atranorin, eugenol, astragalus membranaceus, 21 plant-derived inhibitors, noscapine, shikonin, plantamajoside, flavipin, and 13 new synthesized molecules were analyzed and proposed as effective drugs.

Another possible application of molecular docking is studying combinations of drugs and drug latentiation in metastatic BC. We proposed, as promising combinations of drugs, quinacrine and curcumin, ITH-47 and ESE-15-ol, and vitamin E and paclitaxel. The drug latentiation procedures demonstrated that the three gefitinib-based derivatives are more potent than gefitinib.

Overall, the computational and experimental results included herein reported a good consistency and demonstrated that molecular docking and in vitro studies should be used as complementary methods, which, together, can increase the knowledge for drug discovery and development. Indeed, molecular docking generates a binding score that shows the affinity between drugs and targets and in vitro studies investigate the biological responses. However, the binding affinity energy obtained by a single docking algorithm could be inaccurate due to incorrect ligand poses. A better strategy could be the use of combined scores obtained by two or more docking algorithms and/or the use of molecular dynamics. Molecular dynamics is a computational method that describes the dynamic behavior of a biological complex as a function of time. These methods could be more powerful approaches to investigate the novel biological aspects of disease mechanisms, providing a combined procedure to increase innovation in the pharmaceutical industry and to discovery novel therapies for unmet medical needs.

Author Contributions: Conceptualization, C.C.; methodology, C.C.; writing—original draft preparation, C.C.; supervision, I.C.; project administration, I.C.; funding acquisition, I.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Weigelt, B.; Peterse, J.L.; Veer, L.J.V. Breast cancer metastasis: Markers and models. *Nat. Rev. Cancer* **2005**, *5*, 591–602. [CrossRef]
2. Cava, C.; Pini, S.; Taramelli, D.; Castiglioni, I. Perturbations of pathway co-expression network identify a core network in metastatic breast cancer. *Comput. Biol. Chem.* **2020**, *87*, 107313. [CrossRef] [PubMed]
3. Bravatà, V.; Cava, C.; Minafra, L.; Cammarata, F.P.; Russo, G.; Gilardi, M.C.; Castiglioni, I.; Forte, G.I. Radiation-Induced Gene Expression Changes in High and Low Grade Breast Cancer Cell Types. *Int. J. Mol. Sci.* **2018**, *19*, 1084. [CrossRef] [PubMed]
4. Ponnusankar, S.; Mohan, A. Newer therapies for the treatment of metastatic breast cancer: A clinical update. *Indian J. Pharm. Sci.* **2013**, *75*, 251–261. [CrossRef] [PubMed]
5. Hughes, J.; Rees, S.; Kalindjian, S.; Philpott, K. Principles of early drug discovery. *Br. J. Pharmacol.* **2011**, *162*, 1239–1249. [CrossRef]
6. Cava, C.; Colaprico, A.; Bertoli, G.; Bontempi, G.; Mauri, G.; Castiglioni, I. How interacting pathways are regulated by miRNAs in breast cancer subtypes. *BMC Bioinform.* **2016**, *17*, 111–133. [CrossRef]
7. Cava, C.; Novello, C.; Martelli, C.; Lodico, A.; Ottobrini, L.; Piccotti, F.; Truffi, M.; Corsi, F.; Bertoli, G.; Castiglioni, I. Theranostic application of miR-429 in HER2+ breast cancer. *Theranostics* **2020**, *10*, 50–61. [CrossRef]
8. Yang, Y.; Adelstein, S.J.; Kassis, I.A. Target discovery from data mining approaches. *Drug Discov. Today* **2009**, *14*, 147–154. [CrossRef]
9. Meng, X.-Y.; Zhang, H.-X.; Mezei, M.; Cui, M. Molecular docking: A powerful approach for structure-based drug discovery. *Curr. Comput. Drug Des.* **2011**, *7*, 146–157. [CrossRef]
10. McConkey, B.J.; Sobolev, V.; Edelman, M. The performance of current methods in ligand-protein docking. *Curr. Sci.* **2002**, *83*, 845–855.
11. Csermely, P.; Korcsmáros, T.; Kiss, H.J.; London, G.; Nussinov, R. Structure and dynamics of molecular networks: A novel paradigm of drug discovery: A comprehensive review. *Pharmacol. Ther.* **2013**, *138*, 333–408. [CrossRef] [PubMed]
12. Ngo, H.X.; Garneau-Tsodikova, S. What are the drugs of the future? *MedChemComm* **2018**, *9*, 757–758. [CrossRef] [PubMed]
13. Lin, X.; Li, X.; Lin, X. A Review on Applications of Computational Methods in Drug Screening and Design. *Molecules* **2020**, *25*, 1375. [CrossRef] [PubMed]
14. Bafna, D.; Ban, F.; Rennie, P.S.; Singh, K.; Cherkasov, A. Computer-Aided Ligand Discovery for Estrogen Receptor Alpha. *Int. J. Mol. Sci.* **2020**, *21*, 4193. [CrossRef]
15. Xue, H.; Xie, H.; Xie, H.; Wang, Y. Review of Drug Repositioning Approaches and Resources. *Int. J. Biol. Sci.* **2018**, *14*, 1232–1244. [CrossRef]
16. Palacio-Rodríguez, K.; Lans, I.; Cavasotto, C.N.; Cossio, P. Exponential consensus ranking improves the outcome in docking and receptor ensemble docking. *Sci. Rep.* **2019**, *9*, 5142. [CrossRef]
17. Bajpai, M.; Esmay, J.D. In Vitro Studies in Drug Discovery and Development: An Analysis of Study Objectives and Application of Good Laboratory Practices (GLP). *Drug Metab. Rev.* **2002**, *34*, 679–689. [CrossRef]
18. Rymbai, E.; Sugumar, D.; Saravanan, J.; Divakar, S. Ropinirole, a potential drug for systematic repositioning based on side effect profile for management and treatment of Breast Cancer. *Med. Hypotheses* **2020**, *144*, 110156. [CrossRef]
19. Liu, Y.; Zheng, H.; Li, Q.; Li, S.; Lai, H.; Song, E.; Li, D.; Chen, J. Discovery of CCL18 antagonist blocking breast cancer metastasis. *Clin. Exp. Metastasis* **2019**, *36*, 243–255. [CrossRef]
20. Chen, J.; Yao, Y.; Gong, C.; Yu, F.; Su, S.; Chen, J.; Liu, B.; Deng, H.; Wang, F.; Lin, L.; et al. CCL18 from Tumor-Associated Macrophages Promotes Breast Cancer Metastasis via PITPNM3. *Cancer Cell* **2011**, *19*, 541–555. [CrossRef]
21. Das, S.; Tripathi, N.; Siddharth, S.; Nayak, A.; Nayak, D.; Sethy, C.; Bharatam, P.V.; Kundu, C.N. Etoposide and doxorubicin enhance the sensitivity of triple negative breast cancers through modulation of TRAIL-DR5 axis. *Apoptosis* **2017**, *22*, 1205–1224. [CrossRef] [PubMed]
22. Baldwin, E.L.; Osheroff, N. Etoposide, Topoisomerase II and Cancer. *Curr. Med. Chem. Anti Cancer Agents* **2005**, *5*, 363–372. [CrossRef] [PubMed]

23. Zhao, N.; Woodle, M.C.; Mixson, A.J. Advances in Delivery Systems for Doxorubicin. *J. Nanomed. Nanotechnol.* **2018**, *9*, 1–9. [CrossRef] [PubMed]
24. Manivel, P.; Paulpandi, M.; Murugan, K.; Benelli, G.; Ilanchelian, M. Probing the interaction of thionine with human serum albumin by multispectroscopic studies and its in vitro cytotoxic activity toward MCF-7 breast cancer cells. *J. Biomol. Struct. Dyn.* **2016**, *35*, 3012–3031. [CrossRef]
25. Dohno, C.; Stemp, E.D.A.; Barton, J.K. Fast Back Electron Transfer Prevents Guanine Damage by Photoexcited Thionine Bound to DNA. *J. Am. Chem. Soc.* **2003**, *125*, 9586–9587. [CrossRef]
26. Veena, V.K.; Popavath, R.N.; Kennedy, K.; Sakthivel, N. In vitro antiproliferative, pro-apoptotic, antimetastatic and anti-inflammatory potential of 2,4-diacteylphloroglucinol (DAPG) by Pseudomonas aeruginosa strain FP10. *Apoptosis* **2015**, *20*, 1281–1295. [CrossRef]
27. Veena, V.K.; Kennedy, K.; Lakshmi, P.; Krishna, R.; Sakthivel, N. Anti-leukemic, anti-lung, and anti-breast cancer potential of the microbial polyketide 2, 4-diacetylphloroglucinol (DAPG) and its interaction with the metastatic proteins than the antiapoptotic Bcl-2 proteins. *Mol. Cell. Biochem.* **2016**, *414*, 47–56. [CrossRef]
28. Harvey, A.L.; Edrada-Ebel, R.; Quinn, R.J. The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.* **2015**, *14*, 111–129. [CrossRef]
29. Nelson, E.R.; Chang, C.-Y.; McDonnell, D.P. Cholesterol and breast cancer pathophysiology. *Trends Endocrinol. Metab.* **2014**, *25*, 649–655. [CrossRef]
30. Bloch, P.; Tamm, C.; Bollinger, P.; Petcher, T.J.; Weber, H.P. Pseurotin, a new metabolite of Pseudeurotium ovalis Stolk having an unusual hetero-spirocyclic system. *Helv. Chim. Acta* **1976**, *7*, 133–137. [CrossRef]
31. Wenke, J.; Anke, H.; Sterner, O. Pseurotin A and 8-Odemethylpseurotin A from Aspergillus fumigatus and their inhibitory activities on chitin synthase. *Biosci. Biotech. Biochem.* **1993**, *57*, 961–964. [CrossRef]
32. Komagata, D.; Fujita, S.; Yamashita, N.; Saito, S.; Morino, T. Novel Neuritogenic Activities of Pseurotin A and Penicillic Acid. *J. Antibiot.* **1996**, *49*, 958–959. [CrossRef] [PubMed]
33. Wink, J.; Grabley, S.; Gareis, M.; Zeeck, A.; Phillips, S. Biologically active pseurotin A and D, new metabolites from Aspergillus fumigatus, process for their preparation and their use as apomorphine antagonists. *Eur. Pat. Appl.* **1993**, ep546475.
34. Abdelwahed, K.S.; Siddique, A.B.; Mohyeldin, M.M.; Qusa, M.H.; Goda, A.A.; Singh, S.S.; Ayoub, N.M.; King, J.A.; Jois, S.D.; El Sayed, K.A. Pseurotin A as a novel suppressor of hormone dependent breast cancer progression and recurrence by inhibiting PCSK9 secretion and interaction with LDL receptor. *Pharmacol. Res.* **2020**, *158*, 104847. [CrossRef] [PubMed]
35. Harikrishnan, A.; Veena, V.; Lakshmi, B.; Shanmugavalli, R.; Theres, S.; Prashantha, C.N.; Shah, T.; Oshin, K.; Togam, R.; Nandi, S.; et al. Atranorin, an antimicrobial metabolite from lichen Parmotrema rampoddense exhibited in vitro anti-breast cancer activity through interaction with Akt activity. *J. Biomol. Struct. Dyn.* **2020**, *9*, 1–11. [CrossRef]
36. Kaboli, P.J.; Salimian, F.; Aghapour, S.; Xiang, S.; Zhao, Q.; Li, M.; Wu, X.; Du, F.; Zhao, Y.; Shen, J.; et al. Akt-targeted therapy as a promising strategy to overcome drug resistance in breast cancer—A comprehensive review from chemotherapy to immunotherapy. *Pharmacol. Res.* **2020**, *156*, 104806. [CrossRef]
37. Choudhury, P.; Barua, A.; Roy, A.; Pattanayak, R.; Bhattacharyya, M.; Saha, P. Eugenol restricts Cancer Stem Cell population by degradation of β-catenin via N-terminal Ser37 phosphorylation-an in vivo and in vitro experimental evaluation. *Chem. Biol. Interact.* **2020**, *316*, 108938. [CrossRef]
38. Liu, C.; Wang, K.; Zhuang, J.; Gao, C.; Li, H.; Liu, L.; Feng, F.; Zhou, C.; Yao, K.; Deng, L.; et al. The Modulatory Properties of Astragalus membranaceus Treatment on Triple-Negative Breast Cancer: An Integrated Pharmacological Method. *Front. Pharmacol.* **2019**, *10*. [CrossRef]
39. Sinha, S.; Patel, S.; Athar, M.; Vora, J.; Chhabria, M.T.; Jha, P.C.; Shrivastava, N. Structure-based identification of novel sirtuin inhibitors against triple negative breast cancer: An in silico and in vitro study. *Int. J. Biol. Macromol.* **2019**, *140*, 454–468. [CrossRef]
40. Stanton, R.A.; Gernert, K.M.; Nettles, J.H.; Aneja, R. Drugs that target dynamic microtubules: A new molecular perspective. *Med. Res. Rev.* **2011**, *31*, 443–481. [CrossRef]
41. Maurya, N.; Maurya, J.K.; Singh, U.K.; Dohare, R.; Yab, Z.; Alam Rizvi, M.M.; Kumari, M.; Patel, R. In Vitro Cytotoxicity and Interaction of Noscapine with Human Serum Albumin: Effect on Structure and Esterase Activity of HSA. *Mol. Pharm.* **2019**, *16*, 952–966. [CrossRef] [PubMed]

42. Chen, H.M.; Wang, P.H.; Chen, S.S.; Wen, C.C.; Chen, Y.H.; Yang, W.C.; Yang, N.S. Shikonin induces immunogenic cell death in tumor cells and enhances dendritic cell-based cancer vaccine. *Cancer Immunol. Immunother.* **2012**, *61*, 1989–2002. [CrossRef] [PubMed]
43. Yin, S.-Y.; Efferth, T.; Jian, F.-Y.; Chen, Y.-H.; Liu, C.-I.; Wang, A.H.; Chen, Y.-R.; Hsiao, P.-W.; Yang, N.S. Immunogenicity of mammary tumor cells can be induced by shikonin via direct binding-interference with hnRNPA1. *Oncotarget* **2016**, *7*, 43629–43653. [CrossRef] [PubMed]
44. Pei, S.; Yang, X.; Wang, H.; Zhang, H.; Zhou, B.; Zhang, D.; Lin, D. Plantamajoside, a potential anti-tumor herbal medicine inhibits breast cancer growth and pulmonary metastasis by decreasing the activity of matrix metalloproteinase-9 and-2. *BMC Cancer* **2015**, *15*, 965. [CrossRef]
45. Hanieh, H.; Mohafez, O.; Hairul-Islam, V.I.; Alzahrani, A.; Ismail, M.B.; Thirugnanasambantham, K. Novel Aryl Hydrocarbon Receptor Agonist Suppresses Migration and Invasion of Breast Cancer Cells. *PLoS ONE* **2016**, *11*, e0167650. [CrossRef]
46. Nashaat, S.; Henen, M.A.; El-Messery, S.M.; Eisa, H. Synthesis, state-of-the-art NMR-binding and molecular modeling study of new benzimidazole core derivatives as Pin1 inhibitors: Targeting breast cancer. *Bioorganic Med. Chem.* **2020**, *28*, 115495. [CrossRef]
47. Bacharaju, K.; Jambula, S.R.; Sivan, S.; JyostnaTangeda, S.; Manga, V. Design, synthesis, molecular docking and biological evaluation of new dithiocarbamates substituted benzimidazole and chalcones as possible chemotherapeutic agents. *Bioorganic Med. Chem. Lett.* **2012**, *22*, 3274–3277. [CrossRef]
48. Vaz, W.F.; Custodio, J.M.F.; D'Oliveira, G.D.C.; Neves, B.J.; Junior, P.S.C.; Filho, J.T.M.; Andrade, C.H.; Perez, C.N.; Silveira-Lacerda, E.P.; Napolitano, H.B. Dihydroquinoline derivative as a potential anticancer agent: Synthesis, crystal structure, and molecular modeling studies. *Mol. Divers.* **2020**, 1–12. [CrossRef]
49. Li, Y.; Li, Y.; Wang, N.; Lin, D.; Liu, X.; Yang, Y.; Gao, Q. Synthesis, DNA/BSA binding studies and in vitro biological assay of nickel(II) complexes incorporating tridentate aroylhydrazone and triphenylphosphine ligands. *J. Biomol. Struct. Dyn.* **2019**, 1–20. [CrossRef]
50. Acharya, S.; Maji, M.; Ruturaj; Purkait, K.; Gupta, A.; Mukherjee, A. Synthesis, Structure, Stability, and Inhibition of Tubulin Polymerization by RuII–p-Cymene Complexes of Trimethoxyaniline-Based Schiff Bases. *Inorg. Chem.* **2019**, *58*, 9213–9224. [CrossRef]
51. Cai, Y.; Zhao, B.; Liang, Q.-Y.; Zhang, Y.; Cai, J.; Li, G. The selective effect of glycyrrhizin and glycyrrhetinic acid on topoisomerase IIα and apoptosis in combination with etoposide on triple negative breast cancer MDA-MB-231 cells. *Eur. J. Pharmacol.* **2017**, *809*, 87–97. [CrossRef] [PubMed]
52. Shukla, A.; Tyagi, R.; Meena, S.; Datta, D.; Srivastava, S.K.; Khan, F. 2D- and 3D-QSAR modelling, molecular docking and in vitro evaluation studies on 18β-glycyrrhetinic acid derivatives against triple-negative breast cancer cell line. *J. Biomol. Struct. Dyn.* **2019**, *38*, 168–185. [CrossRef] [PubMed]
53. Yan, W.; Zhao, Y.; He, J. Anti-breast cancer activity of selected 1,3,5-triazines via modulation of EGFR-TK. *Mol. Med. Rep.* **2018**, *18*, 4175–4184. [CrossRef] [PubMed]
54. Vlaar, C.P.; Castillo-Pichardo, L.; Medina, J.I.; Marrero-Serra, C.M.; Velez, E.; Ramos, Z.; Hernández, E. Design, synthesis and biological evaluation of new carbazole derivatives as anti-cancer and anti-migratory agents. *Bioorganic Med. Chem.* **2018**, *26*, 884–890. [CrossRef] [PubMed]
55. Kamalakar, A.; Washam, C.L.; Akel, N.S.; Allen, B.J.; Williams, D.K.; Swain, F.L.; Leitzel, K.; Lipton, A.; Gaddy, D.; Suva, L. PTHrP(12-48) Modulates the Bone Marrow Microenvironment and Suppresses Human Osteoclast Differentiation and Lifespan. *J. Bone Miner. Res.* **2017**, *32*, 1421–1431. [CrossRef]
56. Rajabi, M.; Mousa, S.A. The Role of Angiogenesis in Cancer Treatment. *Biomedicines* **2017**, *5*, 34. [CrossRef]
57. Aboul-Enein, M.N.; El Azzouny, A.; Ragab, F.A.-F.; Hamissa, M.F. Design, Synthesis, and Cytotoxic Evaluation of Certain 7-Chloro-4-(piperazin-1-yl)quinoline Derivatives as VEGFR-II Inhibitors. *Arch. Pharm.* **2017**, *350*, 1600377. [CrossRef]
58. Ziedan, N.I.; Hamdy, R.; Cavaliere, A.; Kourti, M.; Prencipe, F.; Brancale, A.; Jones, A.T.; Westwell, A.D. Virtual screening, SAR, and discovery of 5-(indole-3-yl)-2-[(2-nitrophenyl)amino] [1,3,4]-oxadiazole as a novel Bcl-2 inhibitor. *Chem. Biol. Drug Des.* **2017**, *90*, 147–155. [CrossRef]
59. Koca, I.; Özgür, A.; Er, M.; Gümüş, M.; Coşkun, K.A.; Tutar, Y. Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis. *Eur. J. Med. Chem.* **2016**, *122*, 280–290. [CrossRef]

60. Nie, Z.; Shi, L.; Lai, C.; O'Connell, S.M.; Xu, J.; Stansfield, R.K.; Hosfield, D.J.; Veal, J.M.; Stafford, J.A. Structure-based design and discovery of potent and selective KDM5 inhibitors. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 1490–1494. [CrossRef]
61. Horton, J.R.; Engstrom, A.; Zoeller, E.L.; Liu, X.; Shanks, J.R.; Zhang, X.; Johns, M.A.; Vertino, P.M.; Fu, H.; Cheng, X. Characterization of a Linked Jumonji Domain of the KDM5/JARID1 Family of Histone H3 Lysine 4 Demethylases. *J. Biol. Chem.* **2015**, *291*, 2631–2646. [CrossRef] [PubMed]
62. Salem, A.F.; Wang, S.; Billet, S.; Chen, J.-F.; Udompholkul, P.; Gambini, L.; Baggio, C.; Tseng, H.-R.; Posadas, E.M.; Bhowmick, N.A.; et al. Reduction of Circulating Cancer Cells and Metastases in Breast-Cancer Models by a Potent EphA2-Agonistic Peptide–Drug Conjugate. *J. Med. Chem.* **2018**, *61*, 2052–2061. [CrossRef] [PubMed]
63. Gambini, L.; Salem, A.F.; Udompholkul, P.; Tan, X.-F.; Baggio, C.; Shah, N.; Aronson, A.; Song, J.; Pellecchia, M. Structure-Based Design of Novel EphA2 Agonistic Agents with Nanomolar Affinity in Vitro and in Cell. *ACS Chem. Biol.* **2018**, *13*, 2633–2644. [CrossRef]
64. Cava, C.; Bertoli, G.; Castiglioni, I. In silico identification of drug target pathways in breast cancer subtypes using pathway cross-talk inhibition. *J. Transl. Med.* **2018**, *16*, 154. [CrossRef]
65. Nayak, D.; Tripathi, N.; Kathuria, D.; Siddharth, S.; Nayak, A.; Bharatam, P.V.; Kundu, C.N. Quinacrine and curcumin synergistically increased the breast cancer stem cells death by inhibiting ABCG2 and modulating DNA damage repair pathway. *Int. J. Biochem. Cell Biol.* **2020**, *119*, 105682. [CrossRef]
66. Minafra, L.; Porcino, N.; Bravatà, V.; Gaglio, D.; Bonanomi, M.; Amore, E.; Cammarata, F.P.; Russo, G.; Militello, C.; Savoca, G.; et al. Radiosensitizing effect of curcumin-loaded lipid nanoparticles in breast cancer cells. *Sci. Rep.* **2019**, *9*, 11134. [CrossRef] [PubMed]
67. Gurova, K.V.; Hill, J.E.; Guo, C.; Prokvolit, A.; Burdelya, L.G.; Samoylova, E.; Khodyakova, A.V.; Ganapathi, R.; Tararova, N.D.; Bosykh, D.; et al. Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-B-dependent mechanism of p53 suppression in tumors. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17448–17453. [CrossRef] [PubMed]
68. Mqoco, T.; Stander, A.; Engelbrecht, A.-M.; Joubert, A.M. A Combination of an Antimitotic and a Bromodomain 4 Inhibitor Synergistically Inhibits the Metastatic MDA-MB-231 Breast Cancer Cell Line. *BioMed Res. Int.* **2019**, *2019*, 1850462. [CrossRef]
69. Tang, B.; Qian, Y.; Gou, Y.; Cheng, G.; Fang, G. VE-Albumin Core-Shell Nanoparticles for Paclitaxel Delivery to Treat MDR Breast Cancer. *Molecules* **2018**, *23*, 2760. [CrossRef]
70. Sharma, M.J.; Kumar, M.S.; Murahari, M.; Mayur, Y.C. Synthesis of novel gefitinib-based derivatives and their anticancer activity. *Arch. Pharm.* **2019**, *352*, e1800381. [CrossRef]