The potential of Dayak tribal herbal leaves as an anti-breast cancer agent: In silico approach

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Abstract. Breast cancer is one of the leading causes of mortality among women worldwide, prompting the exploration of alternative, natural therapies. This study examines the anti-breast cancer potential of traditional herbal plants used by the Dayak tribe, particularly *Kleinhovia hospita* Linn. Through in silico approaches, the study investigates the cytotoxic effects and binding affinities of key compounds, such as Scopoletin, Quercetin, and Eleutherol, with breast cancer-related proteins. Molecular docking analysis demonstrated that Quercetin and Eleutherol exhibit high binding affinities (−9 and −8 kcal/mol, respectively) with target proteins, indicating significant potential for inhibiting cancer cell proliferation by targeting proteins like EGFR, JNK, and NUDT5. The drug-likeness analysis confirmed that Quercetin and Eleutherol meet criteria for further therapeutic exploration. These findings suggest that compounds from Dayak tribal plants could be viable anti-cancer agents, providing a scientific foundation for developing culturally relevant and effective treatments for breast cancer. Further research is recommended to isolate and evaluate the bioactive compounds in preclinical and clinical settings. This work supports the potential of traditional Dayak herbal medicine as a natural therapeutic strategy against breast cancer.

1 Introduction

Breast cancer represents a substantial health concern for women. The World Health Organization documented 2.3 million cases and 685,000 deaths in 2020. By 2023, it had become the predominant disease in 173 countries. Breast cancer is the most common cancer

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in Indonesia, in addition to cervical cancer, lung cancer, colorectal cancer, and liver cancer. In 2020 alone, according to Global Cancer Statistics (Globocan) data released by WHO, in Indonesia, there were 396,914 new cancer cases with 234,511 deaths caused by cancer[1]. The prevention and treatment of breast cancer are the subject of extensive investigation by scientists worldwide. The use of medicinal plants was long forgotten as synthetic medications were developed as a result of the rapid breakthroughs in technology [2], but over the past few decades, the promise of phytomedicine has drawn more attention due to the serious side effects that such synthetic medications induce [3].

Breast cancer is a type of cancer that affects many women. In molecular regulation, some proteins play a role in cancer cell activity [4]. One of the first significant targets for these new anticancer drugs to be discovered is the epidermal growth factor receptor (EGFR). According to recent research, EGFR and its downstream pathway control migration, tumor invasion, and the epithelial-mesenchymal transition. Additionally, high EGFR expression is a marker of a poor prognosis in Inflammatory breast cancer [5]. The amplification of the Human Epidermal Growth Factor (EGFR) in breast cancers has been regarded to accelerate tumor growth [6]. New evidence suggests that JNK promotes tumor development and is associated with a number of cancers, including breast cancer. The c-Jun N-terminal kinase (JNK) protein is involved in signaling and affects both the survival of cancer cells and the apoptotic pathway [7]. Than, NUDT5 (nucleotide diphosphate hydrolase type 5) is a crucial biomarker for cancer stratification and a target for drugs, acting as an upstream regulator of tumor drivers [8]. These proteins play an important role in the regulation and progression of breast cancer. With the potential of multi-target medications, scientists have moved from monotherapy to combination therapy and now to multi-targeted agents. Multi-target therapy, used sequentially or in combination, aims to overcome both inherent and acquired resistance to anti-cancer drugs [9].

The prevalence of breast cancer in Indonesia is notably high; however, it is comparatively lower in Kalimantan [10], where the indigenous Dayak tribe has traditionally used Tahongai (*Kleinhovia hospita* Linn) as a medicinal herb [11]. Tahongai is known for various pharmacological properties, with research suggesting its potential as an anticancer, antidiabetic, antioxidant, and hepatoprotective agent [12]. Despite these promising findings, the specific potential of compounds within Tahongai as an anti-breast cancer agent remains largely unexplored, underscoring the need for further research to investigate its efficacy and mechanisms in breast cancer prevention and treatment.

2 Materials and methods

2.1 Samples Preparation

Targets including C-Jun N-Terminal Kinase (JNK) (PDB ID: 6EMH), Human Epidermal Growth Factor (EFGR) (PDB ID: 1XKK), NUDT5 (Nucleotide diphosphate hydrolase type 5) (PDB ID: 5QTR) were downloaded via protein data bank (PDB). The proteins were preprocessed through Biovia Discovery studio 2021, then polar charge and hydrogen were added through autodocktools, then saved in pdbqt format. Compounds from the Tahongai (*Kleinhovia hospita* L.) leaves include scopoletin (CID_5280460), quercetin (CID_5280343), and Eleutherol (CID_120697) downloaded from PubChem.

2.2 Drug likeness

For each compound, drug-likeness properties were evaluated using the SwissADME online tool (http://www.swissadme.ch/). SwissADME predicts each compound's potential for oral

bioavailability, permeability, and adherence to rules such as Lipinski's Rule of Five, which is commonly used to determine drug-likeness [13]. These evaluations are essential to ensure the compounds possess characteristics suitable for further development as therapeutic agents.

2.3 Anti-breast cancer prediction

Anti-breast cancer potential of each compound was predicted using the Way2Drugs PASS online tool (https://www.way2drug.com). This tool requires the SMILES (Simplified Molecular Input Line Entry System) notation for each compound to carry out the predictions. The SMILES format encodes the molecular structure, allowing PASS online to evaluate the likelihood of each compound exhibiting anti-breast cancer activity by analyzing its pharmacological properties and biological activities.

2.4 Molecular docking

Molecular Docking analysis simulation was carried out using PyRx with different coordinate for each protein, which is customized from each of its native ligands. CJUN Center X:7.1386 Y:3.0296 Z :- 16.3675 Dimensions (Angstrom) X: 14.7041 Y: 12.8263 Z: 19.1591, EGFR Center X:17.1804 Y:33.9350 Z:38.4155 Dimensions (Angstrom) X: 21.0253 Y: 17.1490 Z:23.0959, and NUDT5 Center X :- 16.3654 Y :- 14.2164 Z :- 4.8240 Dimensions (Angstrom) X: 21.1624 Y: 14.0542 Z: 13.5638..

2.5 Interaction and visualization

The molecular docking results of ligands were integrated with protein complexes using Biovia Discovery Studio 2021 to visualize docking positions within each target protein. This allowed for detailed analysis of interactions, examining bonds like hydrogen bonding, hydrophobic contacts, and van der Waals forces between protein residues and ligands at the binding sites. This method provided insights into binding affinity and the potential efficacy of the ligands as therapeutic agents.

2 Results and discussion

Table 1. Druglikeness analysis

Compound	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score	Drug-like Molecule
Eleutherol	Yes	Yes	Yes	Yes	Yes	0.55	Yes
Quercetin	Yes	Yes	Yes	Yes	Yes	0.55	Yes
Scopoletin	Yes	Yes	Yes	Yes	No; 1 violation: MW<200	0.55	Yes

Table 1 presents a drug-likeness analysis of three compounds: Eleutherol, Quercetin, and Scopoletin. The drug-likeness was evaluated based on five commonly used rules: Lipinski, Ghose, Veber, Egan, and Muegge. Both Eleutherol and Quercetin satisfy all five rules, indicating they meet the structural and physicochemical criteria typically associated with drug-like compounds. Scopoletin, while meeting the Lipinski, Ghose, Veber, and Egan criteria, shows one violation in the Muegge rule due to a molecular weight (MW) below 200. Furthermore, the results of compound prediction using Way2Drugs are presented in Figure 1.

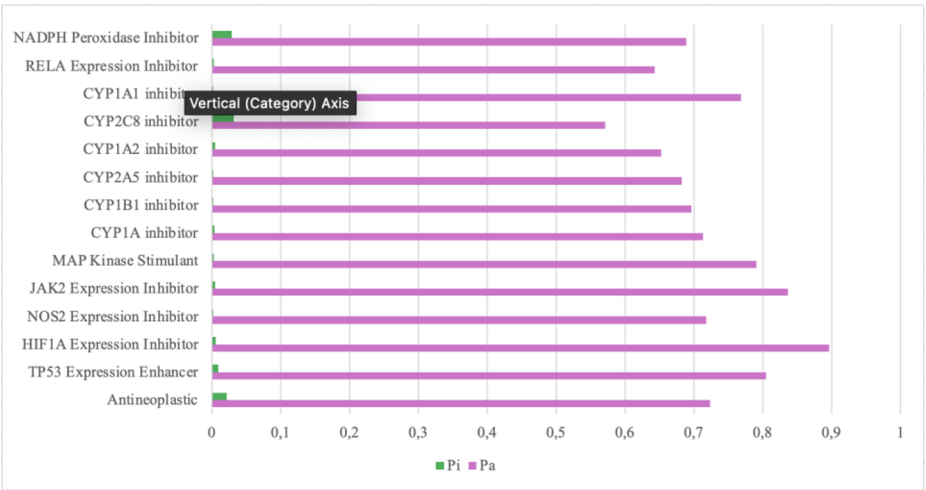

Fig 1. Predicted biological activities of compounds (probability of activity vs probability index) from Way2Drug.

Way2drug results indicate higher compound activity likelihood, with Pa surpassing Pi, Figure 1 shows the predicted biological activities of compounds, where *Pa* (Probability of Activity, purple) indicates the likelihood of the compound being active against a target, and *Pi* (Probability Index, green) represents the probability of inactivity. Higher *Pa* values suggest a greater chance of relevant activity [13]. NOXs promote breast cancer progression and treatment resistance via various pathways, such as Nox4-mediated lymphangiogenesis and metastasis through ROS/ERK/CCL21[14] and NOX1/NOX5-induced MMP-9 expression and cell invasion [15]. NOX inhibitors, including GKT831 and HBB, enhance the inhibition of ROS generation, cell proliferation, and tumor growth [16]. A naphthotriazolyl-4-oxoquinoline derivative selectively induces apoptosis by increasing ROS production and inhibiting glycolysis [17]. However, the dual role of ROS in cancer cell survival and death necessitates careful therapeutic consideration.

Inhibiting RELA expression regulates pathways in breast cancer progression and treatment resistance by promoting genes related to tumor growth, metastasis, and therapy resistance. RELA enhances CXCR5 and CXCL13 transcription, associated with EMT and lymph node metastasis [18]. RELA and NFATc2 upregulate Ets1, increasing invasiveness of breast cancer cells [19]. RELA activation is linked to resistance to ErbB2 tyrosine kinase inhibitors, and inhibiting RELA can enhance lapatinib-induced apoptosis in ErbB2(+) breast cancer cells [20]. High RELA levels contribute to cell survival and resistance to radiation and chemotherapy [21]. RELA inhibition could reduce invasiveness and overcome resistance, benefiting aggressive and metastatic cases [18-20]. However, careful consideration is needed due to NF-kappaB signaling's role in various cellular processes.

CYP inhibitors alter chemotherapeutic drug metabolism in breast cancer treatment, influencing efficacy and toxicity. While CYP2D6 inhibitors increase doxorubicin toxicity, leading to unplanned medical visits and dose reductions, CYP3A4 inhibitors do not significantly affect toxicity outcomes [22]. Inhibiting cytochrome P450 enzymes prevents rapid drug metabolism and improves efficacy [23]. Despite CYP2D6's role in tamoxifen metabolism, large studies show that CYP2D6 inhibitors do not significantly increase adverse outcomes [24]. The impact of CYP inhibitors varies by enzyme and drug, highlighting their importance in personalized therapy.

MAP Kinases, especially the ERK and p38 pathways, are crucial in breast cancer progression and treatment response by regulating cell proliferation, apoptosis, and invasion, making them vital therapeutic targets [25], [26] . The Ras/Raf/MEK/ERK pathway

significantly affects cell survival and tumor development in breast cancer [27]. Polyisoprenylated Cysteinyl Amide Inhibitors (PCAIs) target hyperactive RAS proteins in triple-negative breast cancer (TNBC), promoting apoptosis and reducing invasion [28]. Elevated p38 MAPK in ER+ tumors indicates potential for p38 MAPK inhibitors [26]. In MCF-7 cells, muscarinic cholinergic receptor activation triggers MAPK/ERK phosphorylation, enhancing proliferation and protein synthesis, showing pathway complexity [29]. MAPK pathway roles and therapeutic responses differ across subtypes, with TNBCs exhibiting lower p38 MAPK than ER+ tumors [26].

JAK2 inhibitors like pacritinib and ruxolitinib can suppress tumor growth and metastasis in HER2-positive and triple-negative breast cancers (TNBC) by targeting the JAK2/STAT3 pathway. However, resistance mechanisms and effects on immune cells can limit their efficacy. Co-targeting JAK2 and TrkA pathways synergistically inhibits growth and metastasis, reduces cancer stem cell populations, and induces apoptosis [30]. Resistance in TNBC cells to JAK2 inhibitors may occur via Serine/Threonine Kinase 16 (STK16), indicating the need for combination therapies [31]. JAK2 expression correlates with tumor-infiltrating lymphocytes and better breast cancer outcomes.

NOS2 substantially affects breast cancer progression, particularly in aggressive subtypes like TNBC, by promoting proliferation, metastasis, and immune suppression, frequently in conjunction with COX-2, and is associated with poor survival, especially in ER- subtypes [32-33]. NOS2 and COX-2 generate a pro-inflammatory tumor microenvironment that promotes cancer progression [32-33]. Pharmacological NOS2 inhibition decreases tumor growth and enhances survival in breast cancer models [34]. Inhibiting NOS2 and COX-2 increases CD8+ T cell tumor infiltration, potentially enhancing immune response and reducing tumor growth in TNBC models, with their spatial distribution influencing immune cell localization and activity [35-36].

HIF1A overexpression in breast cancer promotes tumor growth, metastasis, and chemoresistance by regulating genes involved in glycolysis, angiogenesis, metastasis, immune evasion, and drug resistance, leading to poor prognosis [37]. Elevated HIF1A expression reduces sensitivity to taxane-based chemotherapy through the IL-17 signaling pathway [38], while HNK inhibits HIF1A-mediated glycolysis, promoting apoptosis and inhibiting tumor growth [39]. Overexpression of p53 isoforms, such as Δ40p53, is associated with stemness and reduced chemotherapy sensitivity, suggesting that TP53 expression levels are more predictive of treatment outcomes than mutations. TP53 mutations, prevalent in breast cancer, correlate with specific p53 protein expression patterns, often indicating underlying mutations [40]. Genetic alterations affect TP53 expression and isoforms, contributing to cancer progression and the complexity of p53 regulation involves interactions between TP53 variants and other genes [41].

Antineoplastic drugs, including cytotoxic chemotherapies and targeted biological therapies, are essential in breast cancer treatment but can cause significant side effects, targeting rapidly dividing cells like hair follicles and hematopoietic cells, leading to alopecia and bone marrow suppression [42]. Targeted therapies aim to minimize non-cancerous cell damage but still have specific toxicities [42]. Chemotherapy increases oxidative stress markers and negatively impacts hematological profiles, causing anemia and leukopenia [43]. Adjuvant treatments are associated with weight gain [44]. In male breast cancer models, cisplatin reduces tumor volume by inducing apoptosis, while tamoxifen affects hormone receptor expression [45]. Commonly used drugs like methotrexate, 5-fluorouracil, and taxanes have unique cancer-inhibiting mechanisms [46]. While crucial for managing breast cancer, antineoplastic drugs present challenges, requiring personalized treatment strategies.

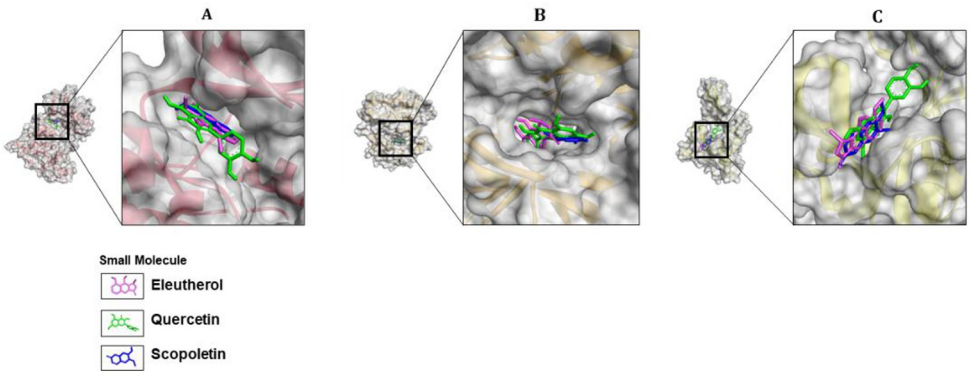

Fig 2. Binding site interaction between receptors and ligand; A) JNK and Tahongai compound; B) EGFR and Tahongai compound; C) NUDT5 and Tahongai compound.

Table 2. Binding score and chemical interaction between receptors and ligands

Compound	Binding Afinity (kkal/mol)			Chemical interaction		
	JNK	EGFR	NUDT5	JNK	EGFR	NUDT5
Eleutherol	-7.8	-8	-6.3	Hydrogen: MET149, LEU148, SER193 Hydrophobic: VAL78, ILE70, ALA91, VAL196, LEU206	Hydrogen: THR854 Hydrophobic: VAL726, ALA743, LYS745, MET766, THR790, LEU858	Hydrogen: TYR90 Hydrophobic: PRO85, PHE83, PHE167
Quercetin	-7.4	-9	-5,8	Hydrogen: GLU147, LEU148, MET149 Hydrophobic: ILE70, VAL78, ALA91, VAL196	Hydrogen: ALA743, MET793, ASP855 Hydrophobic: LEU718, VAL726, LYS745, THR790, LEU844	Hydrogen: GLN82, PHE83, PHE167 Hydrophobic: PRO85, PHE167
Scopoletin	-6.8	-6,5	-5.4	Hydrogen: GLU147, MET149 Hydrophobic: MET149, ILE70, ALA91, VAL78, VAL196, LEU206	Hydrogen: GLN791, MET793, GLY796 Hydrophobic: LEU718, VAL726, ALA743, LEU844	Hydrogen: GLN82, TYR90 Hydrophobic: PRO85, PHE167

Note: The similar color show similarity interaction between receptors and compound with native ligands

The table highlights the binding interactions of Eleutherol with the JNK, EGFR, and NUDT5 proteins. Eleutherol shows the strongest binding affinity with EGFR (−8 kcal/mol), suggesting a favorable interaction. Hydrogen bonds are observed with residues such as MET149 and LEU148 in JNK and THR854 in EGFR. Additionally, *Eleutherol* forms hydrophobic interactions with residues like VAL78 and ILE70 in JNK, as well as VAL726 and LEU858 in EGFR, indicating a stable interaction with these target sites.

Quercetin exhibits the highest binding affinity among the compounds, particularly with EGFR (−9 kcal/mol), indicating a strong interaction with this protein. Key hydrogen bond interactions include residues GLU147 and LEU148 in JNK, and ALA743 and ASP855 in EGFR. Hydrophobic contacts with residues such as VAL78 and VAL726 further stabilize its binding to both JNK and EGFR, suggesting *Quercetin* as a potent candidate for interactions

with these proteins. Quercetin enhances the effectiveness of anti-cancer agents, reduces required dosages, and resensitizes resistant cancer cells. Improved delivery systems can further boost its potential. More research is needed to assess its pharmaco-toxicological profile and its use in adjuvant and chemoprevention therapy [47], [48].

Scopoletin shows moderate binding affinities with JNK, EGFR, and NUDT5, with values ranging from -6.8, -6.5, and -5.4 kcal/mol. This compound interacts via hydrogen bonds with residues such as GLU147 and MET149 in JNK, and GLN82 and TYR90 in NUDT5, with additional hydrophobic interactions involving ILE70, VAL78, and LEU844. Despite its notable antitumor and anticancer activities demonstrated in *in vitro* studies, Scopoletin has limited representation in preclinical and clinical research. Previous studies indicate that Scopoletin exerts anticancer effects across various types of cancer cells, highlighting its potential as a therapeutic candidate [49]. Although the binding affinities of *Scopoletin* are lower than those of Quercetin and Eleutherol, its consistent interaction across the three proteins indicates its potential for versatile applications.

3 Conclusion

This study demonstrates that Dayak tribal herbal leaves, particularly *Kleinhovia hospita Linn*, show promising potential as anti-breast cancer agents based on in silico analysis. Key compounds, including Quercetin, Eleutherol, and Scopoletin, exhibited interactions with breast cancer-related proteins such as EGFR, JNK, and NUDT5, displaying significant binding affinities. Notably, Quercetin and Eleutherol achieved the highest binding affinities with EGFR at −9 kcal/mol and −8 kcal/mol, respectively, suggesting strong potential to inhibit cancer cell proliferation through these targets. Drug-likeness analysis also confirmed that Quercetin and Eleutherol meet essential criteria for further therapeutic development. These findings support the traditional use of Dayak herbal plants and provide a scientific foundation for developing culturally relevant natural therapies against breast cancer. Further research is essential to isolate and evaluate these bioactive compounds in preclinical and clinical studies to better understand their mechanisms and efficacy in breast cancer treatment.

References

1 KEMENKES, 'Kanker Masih Membebani Dunia'. https://sehatnegeriku.kemkes.go.id/baca/blog/20240506/3045408/kanker-masih membebani-dunia/ (2024).
2 V. Shrihastini et al, Plant derived bioactive compounds, their anti-cancer effects and in silico approaches as an alternative target treatment strategy for breast cancer: An updated overview, Cancers (Basel). **13**, 24 (2021). https://doi.org/10.3390/cancers13246222.
3 S.Y. Pan et al, New perspectives on how to discover drugs from herbal medicines: CAM'S outstanding contribution to modern therapeutics, Evidence-based Complementary and Alternative Medicine. **2013** (2013). https://doi.org/10.1155/2013/627375.
4 A. F. Dibha et al, utilization of secondary metabolites in algae kappaphycus alvarezii as a breast cancer drug with a computational method, Pharmacognosy J. **14,** 3 (2022). https://doi.org/10.5530/pj.2022.14.68.
5 H. Masuda, D. Zhang, C. Bartholomeus et al, Role of epidermal growth factor receptor in breast cancer, Breast Cancer Res. Treat. **136**, 331-345 (2012), https://doi.org/10.1007/s10549-012-2289-9.Role.

6 F. Ye et al, Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer, BioMed Central Ltd. **01** (2023). https://doi.org/10.1186/s12943-023-01805-y.
7 N.A. Ramadanti, L. Erlina, R.I. Paramita, A. Tedjo, F. Fadillah, and S. Dwira, Pharmacophore modeling, molecular docking, and ADMET approach for identification of anti-cancer agents targeting the C-Jun N-Terminal Kinase (JNK) protein, **24,** 1 (2023) https://doi.org/10.24036/eksakta/vol24-iss01/391.
8 R. Sultana, Molecular docking based virtual screening of the breast cancer target NUDT5, Bioinformation. **15** (11) (2019), https://doi.org/10.6026/97320630015784.
9 A. Doostmohammadi, H. Jooya, K. Ghorbanian, S. Gohari, and M. Dadashpour, Potentials and future perspectives of multi-target drugs in cancer treatment: the next generation anti-cancer agents, Cell Com. Sig. **22** (1) (2024). https://doi.org/10.1186/s12964-024-01607-9.
10 Peringati Hari Kesehatan Nasional, Dinkes Kaltim Gelar Pemeriksaan Kanker Gratis'. https://diskominfo.kaltimprov.go.id/kesehatan/peringati-hari-kesehatan-nasional-dinkes-kaltim-gelar-pemeriksaan-kanker -gratis (2022).
11 A. Najihudin, D. Rahmat, and S. Evani, A. Rizki, Formulation of instant granules from ethanol extract of tahongai (*Kleinhovia hospita* L .) leaves as antioxidant, Jurnal Ilmiah Farmako Bahari. **10,** 91–112, (2019). https://doi.org/10.52434/jfb.v10i1.651
12 S. Paramita, Tahongai (*Kleinhovia hospita* L.): Review sebuah tumbuhan obat dari Kalimantan Timur, Jurnal Tumbuhan Obat Indonesia. **9**, 1 (2016) https://doi.org/10.22435/toi.v9i1.6390.29-36.
13 D. S. Druzhilovskiy et al, Computational platform Way2Drug: from the prediction of biological activity to drug repurposing, Russian Chemical Bulletin, **66**, (2017).
14 X. Wang et al, Inhibition of NADPH oxidase 4 attenuates lymphangiogenesis and tumor metastasis in breast cancer, The FASEB Journal. **35**, 4 (2021). https://doi.org/10.1096/fj.202002533R.
15 H.K. Song, J.M. Kim, E.M. Noh, H.J. Youn, and Y.R. Lee, Role of NOX1 and NOX5 in protein kinase C/reactive oxygen species-mediated MMP-9 activation and invasion in MCF-7 breast cancer cells, Mol. Med. Rep. **30**, 4 (2024). https://doi.org/10.3892/mmr.2024.13312.
16 L. Zhu et al, Inhibition of NADPH oxidase-ROS signal using hyaluronic acid nanoparticles for overcoming radioresistance in cancer therapy, ACS Nano. **16**, 11 (2022). https://doi.org/10.1021/acsnano.2c07440.
17 J. R. Branco et al, A novel naphthotriazolyl-4-oxoquinoline derivative that selectively controls breast cancer cells survival through the induction of apoptosis, Curr. Top. Med. Chem. **18,** 17 (2018).
18 S.R. Chowdhury, S. Biswas, G. Mandal, and A. Bhattacharyya, Abstract 943: RelA regulates CXCR5/CXCL13 transcription and associated immune response in breast cancer, Cancer Res. **77**, 13 (2017). https://doi.org/10.1158/1538-7445.AM2017-943.
19 G.C. Kim et al, Upregulation of Ets1 expression by NFATc2 and NFKB1/RELA promotes breast cancer cell invasiveness, Oncogenesis. **7**, 11 (2018). https://doi.org/10.1038/s41389-018-0101-3.
20 W. Xia et al, Resistance to ErbB2 tyrosine kinase inhibitors in breast cancer is mediated by calcium-dependent activation of RelA, Mol. Cancer Ther. **9**, 2 (2010). https://doi.org/10.1158/1535-7163.MCT-09-1041.
21 Z.-X. Nora D. Mineva1, W. Xiaobo, Y. Sanghwa, Y. Haoqiang, and G.E.S.J. Xiao, F. Michael, Inhibition of RelB by 1,25-Dihydroxyvitamin D3 promotes sensitivity of breast cancer cells to radiation, Neuron. **61,** 1 (2009). https://doi.org/10.1002/jcp.21765.Inhibition.

22 A.P. Dawson, C.D. Frick, M.Burd, and B. Conliffe, Clinical significance of coadministration of moderate to strong CYP enzyme inhibitors with doxorubicin in breast cancer patients receiving AC chemotherapy. J. Oncol. Pharm. **0**, 10781552231223124. https://doi.org/10.1177/10781552231223125.
23 B. Kawahara and P. Mascharak, Inhibition of cytochrome P450 by carbon monoxide: relevance to drug resistance in human breast cancer therapy. Med. Res. Arch. **11**, 4 (2023). https://doi.org/10.18103/mra.v11i4.3732.
24 S.E. Mayer, N.S. Weiss, J. Chubak, D.R. Doody, C.S. Carlson, K.W. Makar, M.A. Wurscher, K.E. Malone, CYP2D6-inhibiting medication use and inherited CYP2D6 variation in relation to adverse breast cancer outcomes after tamoxifen therapy. Cancer Causes and Control. **30**, 1 (2019). https://doi.org/10.1007/s10552-018-1117-x.
25 D.A. Ahmad, O.H. Negm, M.L. Alabdullah, S. Mirza, M.R. Hamed, V. Band, A.R. Green, I.O. Ellis, E.A. Rakha, Clinicopathological and prognostic significance of mitogen- activated protein kinases (MAPK) in breast cancers. Breast Cancer Res. Treat. **159**, 3 (2016). https://doi.org/10.1007/s10549-016-3967-9.
26 J. Merlin, A. Harlé, M. Lion, C. Ramacci, and A. Leroux, Expression and activation of P38 MAP kinase in invasive ductal breast cancers : Correlation with expression of the estrogen receptor , HER2 and downstream signaling phosphorylated proteins. **30**, 4, 1943–1948 (2013). https://doi.org/10.3892/or.(2013).2645.
27 H. Lu, Y. Guo, G. Gupta, and X. Tian, Mitogen-activated protein kinase (MAPK): New insights in breast cancer. J. Environ. Pathol. Toxicol. Oncol. **38**, 1 (2019). htts://doi.org/10.1615/JEnvironPatholToxico lOncol.2018028386.
28 J. M. S. Lazarte and N. S. Lamango, Activation of MAP kinase pathway by polyisoprenylated cysteinyl amide inhibitors causes apoptosis and disrupts breast cancer cell invasion. Biomedicines. **12**, 3 (2024). https://doi.org/10.3390/biomedicines12030470.
29 E. Jiménez and M. Montiel, Activation of MAP kinase by muscarinic cholinergic receptors induces cell proliferation and protein synthesis in human breast cancer cells. J. Cell. Physiol. **204**, 2 (2005). https://doi.org/10.1002/jcp.20326.
30 A.T. Regua, S. Bindal, M.K. Najjar, C. Zhuang, M. Khan, A.B.J. Arrigo, A.O Gonzalez, X.R. Zhang, J.J. Zhu, K. Watabe, H.W. Lo, Dual inhibition of the TrkA and JAK2 pathways using entrectinib and pacritinib suppresses the growth and metastasis of HER2-positive and triple-negative breast cancers. Cancer Lett. **10**, 597 (2024). https://doi.org/10.1016/j.canlet.2024.217023.
31 Z. Zhu, Q. Xiang, S. Li, C. Chen, and J. Shi, Serine/Threonine kinase 16 phosphorylates STAT3 and confers a JAK2-Inhibition resistance phenotype in triple-negative breast cancer. Biochem. Pharmacol. **225**, 116268 (2024). https://doi.org/10.1016/j.bcp.2024.116268.
32 L. L. Coutinho, L.L Femino, A.L. Gonzalez, R.L. Moffat, W.F. Heinz, R.Y.S. Cheng, S.J. Lockett, M.C. Rangel, L.A. Ridnour, D.A. Wink, NOS2 and COX-2 Co-expression promotes cancer progression : a potential target for developing agents to prevent or treat highly aggressive breast cancer. Int. J. Mol. Sci. **25**, 11 (2024).
33 R.Y.S. Cheng, L.A. Ridnour, A.L. Wink, A.L. Gonzalez, E.L. Femino, H. Rittscher, V. Somasundaram, W.F. Heinz, L. Coutinho, M.C. Rangel, E.F. Edmondson, D. Butcher, R.J. Kinders, X. Li, S.T.C. Wong, D.W. McVicar, S.K. Anderson, S.M. Pore M, Hewitt, T.R. Billiar, S.A. Glynn, J.C. Chang, S.J. Lockett, S. Ambs, D.A. Wink, Interferon-gamma induces NOS2 and COX2 in ER-breast cancer that drives increase metastatic potential. Cancer Res. **83,** 7 (2023). https://doi.org/10.1038/s41419-023-05834-9
34 N. Avtandilyan, H. Javrushyan, M. Ginovyan, A. Karapetyan, and A. Trchounian, Anti-cancer effect of in vivo inhibition of nitric oxide synthase in a rat model of breast cancer. Mol. Cell. Biochem., **478,** 2 (2023).

35 V. Somasundaram *et al.*, NOS2 and COX2 Blockade Limits TNBC Disease Progression and Alters CD8+ T Cell Spatial Orientation and Density (2022).
36 V. Somasundaram, L.A. Ridnour, R.Y. Cheng, A.J. Walke, N. Kedei, D.D. Bhattacharyya, A.L. Wink, E.F. Edmondson, D. Butcher, A.C. Warner, T.H. Dorsey, D.A. Scheiblin, W. Heinz, R.J. Bryant, R.J. Kinders, S. Lipkowitz, S.T. Wong, M. Pore, S.M. Hewitt, D.W. McVicar, S.K. Anderson, J. Chang, S.A. Glynn, S. Ambs, S.J. Lockett, D.A. Wink, Systemic Nos2 Depletion and Cox inhibition limits TNBC disease progression and alters lymphoid cell spatial orientation and density. Redox. Biol., **58,** 102529 (2022). https://doi.org/10.1016/j.redox.2022.102529.
37 S. Zhi, C. Chen, H. Huang, Z. Zhang, F. Zeng, and S. Zhang, Hypoxia-inducible factor in breast cancer: role and target for breast cancer treatment. Front. Immunol. **15** (2024). https://doi.org/10.3389/fimmu.2024.1370800.
38 H. Dai, X. Sheng, Y. Wang, L. Zhou, Y. Lin, Y. Du, F. Yang, R. Sha, J. Peng, L. Yao, W. Yin, L. Lu, HIF1α regulates IL17 signaling pathway influencing sensitivity of taxane-based chemotherapy for breast cancer. Front. Cell. Dev. Biol. **9** (2021). https://doi.org/10.3389/fcell.2021.729965.
39 X. Yi, M. Qi, M. Huang, S. Zhou, and J. Xiong, Honokiol inhibits HIF-1α-mediated glycolysis to halt breast cancer growth. Front. Pharmacol**.** **13** (2022). https://doi.org/10.3389/fphar.2022.796763.
40 S.A. Anderson, B.B. Bartow, S. Harada, G.P. Siegal, S. Wei, V.L. Dal Zotto, X. Huang, p53 protein expression patterns associated with TP53 mutations in breast carcinoma. Breast Cancer Res. Treat., **207**, 1 (2024), https://doi.org/10.1007/s10549-024-07357-z.
41 L. S. Reinhardt, K. Groen, A. Xavier, and K.A. Avery-Kiejda, p53 dysregulation in breast cancer: insights on mutations in the TP53 network and p53 isoform expression. Int. J. Mol. Sci. **24**, 12 (2023), https://doi.org/10.3390/ijms241210078.
42 T. Haddad and Y. Douglas, Basic principles of antineoplastic therapies, Springer New York, 707–715 (2011).
43 S. P. T. Pullakanam, M. Mannangatti, R. Nekkala, V. B. Madhavi, and R. S. Bellala, Effects of antineoplastic drugs on oxidative stress and prognosis of hematological and various biochemical parameters in the treatment of breast cancer, Int. J. Health Sci. (Qassim), **6** (2022), https://doi.org/10.53730/ijhs.v6ns6.12464.
44 C. Ambrosi *et al.*, Effect of adjuvant antineoplastic treatment on body weight change in women with breast cancer. Sci. Med. (Porto Alegre). **22**, 3, 124–130, (2012), https://doi.org/10.15448/1980-6108.2012.3.10738.
45 S. N. Shishido, E. B. Faulkner, A. Beck, and T. A. Nguyen, The effect of antineoplastic drugs in a male spontaneous mammary tumor model. PLoS One, **8**, 6 (2013). https://doi.org/10.1371/journal.pone.0064866.
46 Steelman. James, A. McCubrey, Richard, A. Franklin, Patrick, M. Navolanic, Alberto, M. Martelli, S. Linda, Chemotherapy for breast cancer, Br. Med. J., **1**, 6009, 583 (1976), https://doi.org/10.1136/bmj.1.6009.583-a.
47 A. Maugeri et al., Targets Involved in the Anti-Cancer Activity of Quercetin in Breast, Colorectal and Liver Neoplasms', Int J Mol Sci, **24**, 3 (2023), https://doi.org/10.3390/ijms24032952.
48 M. Ezzati, B. Yousefi, K. Velaei, and A. Safa, A review on anti-cancer properties of Quercetin in breast cancer. Life Sci. **248** (2020)..
49 L. Meilawati, R. Dewi, A. Tasfiyati, A. Septama, and L. D. Antika, Scopoletin: anticancer potential and mechanism of action. Asian Pac. J. Trop. Biomed. **13**, 1, 1–8 (2023), https://doi.org/10.4103/2221-1691.367685.