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COMPUTATIONAL DISCOVERY OF OZ-082: A PROMISING EUCALYPTOL ANALOGUE WITH MULTI-RECEPTOR TARGETING POTENTIAL IN BREAST CANCER

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Abstract

Breast cancer has been a major reason of mortality among women affected by cancer, with current strategies often constrained by challenges such as suboptimal drug bioavailability, systemic toxicity, and resistance. This study employed a computational strategy to design and explores the therapeutic potential of eucalyptol, along with its 342 structural analogues, as prospective agents against nine critical breast cancer receptors (BRCA1, BRCA2, COX-2, EGFR, ER-α, mTOR, PPAR-γ, PR, and SIRT2). Utilizing molecular docking methodologies, we evaluated the binding affinities of these compounds against these receptors, which are instrumental in tumor progression, therapeutic resistance, and metabolic dysregulation. Virtual screening identified OZ-082 as a lead multi-targeting candidate, exhibiting strong and consistent binding across multiple key targets such as -9.1 kcal/mol with SIRT2, -8.8 kcal/mol with COX-2, BRCA-1 as -5.5 kcal/mol, BRCA-2 had -6.5 kcal/mol, EGFR -7 kcal/mol, ER-α exhibits -9 kcal/mol, mTOR -8.5 kcal/mol, PPAR-γ have -7 kcal/mol, and PR have -7 kcal/mol respectively and a high enrichment factor ($EF_{1-4}\%$ = 12.5) across key targets. Comprehensive ADMET analyses performed using SwissADME and pkCSM tools revealed that OZ-082 possesses favorable drug-likeness, high gastrointestinal absorption (95%), no hepatotoxicity, and minimal CYP450 inhibition risk, marking a significant improvement over the parent eucalyptol. These findings highlight the potential of eucalyptol derivative OZ-082 as a promising lead compound to address the limitations of current therapies by modulating diverse molecular pathways involved in breast cancer pathogenesis. Moreover, this research highlights the value of computational drug discovery in expediting the identification of promising therapeutic candidates. Future validation will require *In vitro* cytotoxicity assays e.g., in MCF-7 and MDA-MB-231 cells) and *In vivo* efficacy studies in xenograft models to validate the anticancer efficacy, safety profile, and underlying mechanisms of OZ-082, thereby advancing the development of targeted, less toxic, and more effective therapeutic options for breast cancer.

Key words: Breast Cancer; Computational Drug Discovery; Molecular Docking; Enrichment factor Analysis; Eucalyptol; ADMET; Multi-target Therapy.

Introduction

Cancer is a major public health concern and one of the leading causes of death. It occurs when cells in a tissue or organ start growing uncontrollably, thereby disrupting the body's normal balance. The cancers most commonly responsible for deaths include lungs, breast, colorectal, and cervical cancers (Cowin *et al.*, 2005; Sung *et al.*, 2021). Among these, breast cancer is the most common type in women. According to recent GLOBOCAN 2022 estimates, breast cancer is the most commonly diagnosed cancer worldwide, with over 2.3 million new cases annually, highlighting its persistent and significant burden (Sung *et al.*, 2021). Breast cancer begins in the tissues of the breast, and its occurrence varies significantly around the world — rates can differ by as much as fivefold between regions. The development of breast cancer is a complicated, multi-steps process influenced by genetic, epigenetic, and environmental factors (Lo & Sukumar, 2008; Yip & Papa, 2021). Although significant advances have been made in understanding the mechanisms underlying the disease, its prevention continues to pose a substantial challenge.

The standard therapeutic arsenal for breast cancer includes surgery, radiation therapy, chemotherapy, hormone therapy, and targeted agents. While essential, each modality has significant limitations. Surgery and radiation are localized treatments (Moo *et al.*, 2018; Shilling & Jenkins, 2007) Radiation therapy is an effective treatment option for killing cancer cells post-surgery or preventing recurrence. Brachytherapy, a technique first pioneered in the early 1900's following the discovery of radium, can also be used as a treatment option (Moo *et al.*, 2018). Chemotherapy refers to the use of drugs designed to treat cancer by targeting and eliminating rapidly dividing cells. It is a cornerstone of cancer therapy, often employed alone or in combination with other treatments such as surgery, radiation, or immunotherapy, depending on the type and stage of cancer. This treatment can be given before surgery to shrink the tumor and sometimes make breast-conserving surgery possible instead of a mastectomy (Moo *et al.*, 2018). Chemotherapy, a corner stone

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treatment, involves the use of various drugs such as Cyclophosphamide, Docetaxel, Doxorubicin, Erlotinib, Etoposide, Gemcitabine, Methotrexate, Sorafenib, Sunitinib, Vincristine, and Vinblastine to target rapidly dicing cells (Tanaka *et al.*, 2009). However, this non-specific mechanism contributes to significant side effects and, critically, can lead to drug resistance as cancer cells adapt. Consequently, there is a pressing demand for novel agents capable of selectively targeting specific molecular pathways or multiple receptors involved in cancer progression, which may offer enhanced efficacy and circumvent resistance mechanisms (Yip & Papa, 2021). Techniques such as genomic assays (e.g., Oncotype DX or MammaPrint) evaluate tumor biology and predict recurrence risk, guiding the use of chemotherapy or targeted therapies in conjunction with hormone treatments. This precision medicine approach optimizes therapeutic outcomes while minimizing unnecessary exposure to toxic treatments (Key *et al.*, 2001). Similarly, while hormone therapies (e.g., tamoxifen or aromatase inhibitors) and targeted agents improve outcomes for specific subtypes, resistance remains a major clinical challenge. The effectiveness of oral anticancer drugs is often constrained by their limited and highly variable bioavailability. This variability poses significant challenges since most anticancer agents have a narrow therapeutic window and are typically administered at or near their maximum tolerated dose (Stuurman *et al.*, 2013). Oral vinorelbine is considered a favorable alternative to its intravenous counterpart, particularly for elderly patients who may experience difficulties with venous access (Depierre *et al.*, 2001). Another notable example is Doxil, a liposomal formulation of doxorubicin coated with polyethylene glycol, which extends circulation time and exhibits a distinctive toxicity profile. However, several anticancer drugs are associated with adverse effects, including bone marrow suppression, anemia, thrombocytopenia (Remesh, 2012), nausea, vomiting (Depierre *et al.*, 2001), reduced left ventricular ejection fraction, and an increased risk of congestive heart failure (Moore, 2007). Although nanotechnology and novel formulations offer promise in improving drug delivery, the overarching need for more effective, less toxic, and bioavailable agents persists (Moo *et al.*, 2018; Tanaka *et al.*, 2009).

A natural phytochemical offers a reservoir for novel anticancer agent discovery. Eucalyptol, (1,8-cineole), a monoterpene oxide found in essential oils, has demonstrated promising anticancer properties through mechanisms such as apoptosis induction and cell cycle arrest in breast cancer lines. Studies suggest that eucalyptol exerts anticancer effects through mechanisms such as induction of apoptosis, inhibition of cell proliferation, and modulation of key signaling pathways involved in tumor progression. Its activity across diverse cancer types highlights its potential as a versatile and natural therapeutic agent (Hoch *et al.*, 2023; Thalappil *et al.*, 2024). Eucalyptol (1,8-cineole) is a bicyclic monoterpenoid oxide abundantly distributed across a wide range of plant species. Within the genus *Eucalyptus*, it is most richly concentrated in *Eucalyptus globulus* Labill. (Blue Gum), *E. radiata* Sieber ex DC. (Narrow-leaved Peppermint), *E. polybractea* R.T. Baker (Blue Mallee), *E. smithii* R.T. Baker (Gully Gum), *E. cinerea* F. Muell. ex Benth. (Silver Dollar Tree), *E. nicholii* Maiden & Blakely, *E. camaldulensis* Dehnh. (River Red Gum), and *E. maideni* F. v. Muell., among others(Dhakad *et al.*, 2018; Hoch *et al.*, 2023; Lu *et al.*, 2025). Beyond the genus *Eucalyptus*, appreciable quantities of eucalyptol have also been identified in *Rosmarinus officinalis* L. (rosemary), *Salvia officinalis* L. (sage), *Cinnamomum camphora* (L.) J. Presl (camphor laurel), *Melaleuca cajuputi* Powell (cajuput), *Laurus nobilis* L. (bay laurel), and select species of *Mentha* and *Artemisia* (Cai *et al*., 2021; Hoch *et al*., 2023).

It is important to distinguish eucalyptol from the broader term "eucalyptus oil," as the two are frequently conflated in the literature. Eucalyptus oil is a complex mixture obtained by steam distillation of *Eucalyptus* leaves and contains numerous volatile constituents including α-pinene, limonene, p-cymene, β-pinene, α-terpineol, aromadendrene, and globulol in addition to its principal component, 1,8-cineole (Dhakad *et al.*, 2018; Shiekh *et al.*, 2025). Eucalyptol, by contrast, is the single isolated chemical entity (molecular formula $C_{10}H_{18}O$) that constitutes approximately 60–85% of the total oil composition and is responsible for the majority of the oil's documented pharmacological activities, including its anti-inflammatory, antimicrobial, and expectorant effects (Hoch *et al.*, 2023). While eucalyptus oil exhibits a broad-spectrum biological activity attributable to the synergistic interplay of its numerous constituents, eucalyptol is a chemically defined, highly purified compound with a well-characterized pharmacokinetic and toxicological profile attributes that make it a more tractable candidate for pharmaceutical development and structure-based drug design (Cai *et al.*, 2021; Hoch *et al.*, 2023; Yao *et al.*, 2025).

Eucalyptol is an agonist at BRCA, SIRT2, PPAR-g, and other receptors. Previous studies proved that Eucalyptol has the capability to inhibit the growth of breast cancer cells i.e., MCF7, MDA MB-231, and T47D (Campos & Berteina-Raboin, 2022; Hoch *et al.*, 2023; Izham *et al.*, 2021; Thalappil *et al.*, 2024). One of the pitfalls of eucalyptol is its poor bioavailability, because of its poor solubility. This is believed to be due to its non-polar nature. It is also volatile and instable. These limitations result in a diminished efficacy of eucalyptol as a cytotoxic agent, as observed in animal studies (Izham *et al.*, 2021; Thalappil *et al.*, 2024).

These challenges highlight a critical research gap — the disparity between the promising biological activity of natural compounds such as eucalyptol and their frequently inadequate drug-like properties. Although eucalyptol shows activity against various cancer-related targets, no comprehensive study has yet employed integrated computational drug discovery—combining large-scale analogue design, multi-target virtual screening, enrichment analysis, and predictive ADMET profiling—to transform this phytochemical into an optimized, multi-targeting drug candidate with a predictable safety and pharmacokinetic profile. Bridging this gap requires a structure-based design approach to create novel analogues that retain the core bioactive scaffold while mitigating its inherent flaws.

To overcome these limitations, we pursued a structure-based drug design approach. This study aims to computationally design and screen eucalyptol analogues for improved multi-receptor targeting against a panel of nine proteins critically involved in breast cancer pathogenesis—BRCA1, BRCA2, COX-2, EGFR, ER-α, mTOR, PPAR-γ, PR, and SIRT2—which represent key nodes in proliferation, survival, hormone signaling, and metabolism pathways (Remesh, 2012) (Depierre *et al.*, 2001; Moore, 2007). Molecular docking and enrichment analysis were employed to prioritize candidates, followed by detailed ADMET profiling to evaluate their drug-likeness and safety. Molecular docking is a structure-based

method that mimics interactions and predicts the binding modes as well as estimates the affinity between ligands and receptors (Morris & Lim-Wilby, 2008). Docking methods are performed on the prepared analogues to assess their binding potential. The best-performing molecules are selected based on docking results, and further pharmacokinetic (PK) studies are performed on these selected derivatives.

Materials and Methods

The overall strategy of the present study is illustrated schematically in Fig. 1. In brief, an integrated computational pipeline was employed to identify and optimize eucalyptol analogues as potential multi-target inhibitors against breast cancer-related receptors. The workflow included target selection and preparation, rational design of structural analogues, drug-likeness filtering, molecular docking–based virtual screening, enrichment validation, and comprehensive ADMET profiling. Each methodological step was performed sequentially to ensure systematic lead identification and robust validation of the final candidate compounds.

Protein retrieval & preparation: In this study, the 3D crystal structures of nine breast cancer-related receptors were retrieved from the RCSB Protein Data Bank (RCSB PDB) for molecular docking simulations. These receptors included BRCA1 (PDB ID: 4Y2G), BRCA2 (PDB ID: 3EU7), COX-2 (PDB ID: 5F19), EGFR (PDB ID: 2J6M), ER-α (PDB ID: 3ERT), mTOR (PDB ID: 4DRH), PPAR-γ (PDB ID: 3VN2), PR (PDB ID: 4OAR), and SIRT2 (PDB ID: 4Y6Q).

Each protein structure was carefully analyzed for detailed characteristics and ligands (Table 1):

- BRCA1 (PDB ID: 4Y2G): Composed of a single chain A with 224 amino acids, incorporating a phosphorylated Polypeptide ligand (BRCA1-A complex subunit Abraxas).
- BRCA2 (PDB ID: 3EU7): Comprised of two chains (A and X) with 356 and 19 amino acids, respectively, and bound to two glycerol molecules ($C_3H_8O_3$).
- COX-2 (PDB ID: 5F19): Two homologous chains (A and B), each with 552 amino acids, associated with five ligands, including protoporphyrin IX ($C_{34}H_{32}CoN_4O_4$) and acrylic acid ($C_3H_4O_2$).
- EGFR (PDB ID: 2J6M): A single chain A containing 356 amino acids, bound to a ligand ($C_{27}H_{32}N_6$) with a pyrrolo[2,3-d]pyrimidine structure.
- ER-α (PDB ID: 3ERT): Comprised of a singular chain A (261 amino acids) bound to 4-hydroxytamoxifen ($C_{26}H_{29}NO_2$).
- mTOR (PDB ID: 4DRH): Four chains (A-D), with chains A and D containing 144 amino acids each and chains B and C containing 98 amino acids each, bound to rapamycin ($C_{51}H_{79}NO_{13}$) and a sulfate ion (SO_4^{2-}).
- PPAR-γ (PDB ID: 3VN2): Two chains (A and B) with 285 and 16 amino acids, respectively, bound to a carboxylic acid derivative ligand ($C_{33}H_{30}N_4O_2$).
- PR (PDB ID: 4OAR): Two chains (A and B) consisting of 258 and 17 amino acids, respectively, bound to a steroid derivative ligand ($C_{30}H_{37}NO_4$).
- SIRT2 (PDB ID: 4Y6Q): Four homologous chains (A-D), each comprising 293 amino acids, associated with a lipid-based ligand ($C_{29}H_{49}N_5O_{15}P_2$) and a zinc ion (Zn^{2+}).

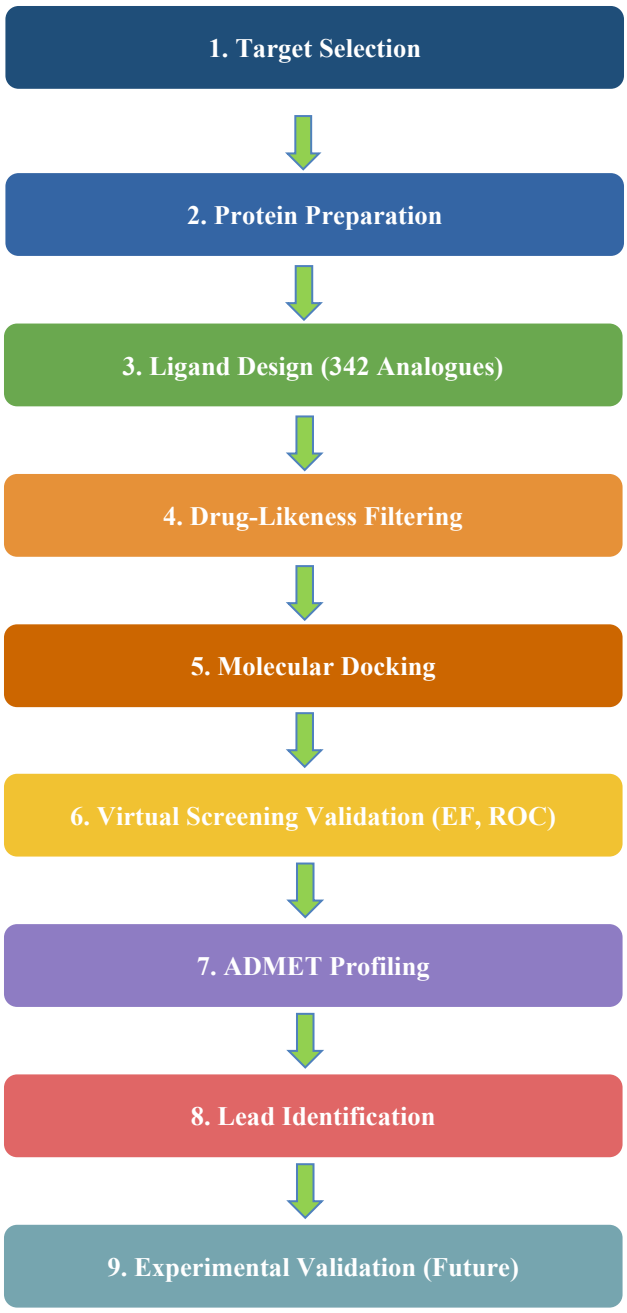


Fig. 1. The overall workflow of the computational pipeline employed in the current study.

Structural integrity was verified using UCSF Chimera's v1.16 sequence analyzer, and missing residues were repaired using Modeller v10.4, and the quality of the refined models was validated using Ramachandran plots (via SAVES), confirming that over 95% of residues resided in favored regions (Yang *et al.*, 2012). To prepare the structures for docking, water molecules were removed, polar hydrogens were added, and non-polar hydrogens were merged using AutoDock Tools v1.5.7. Kollman charges were assigned to the protein structures, and the prepared receptors were saved as pdbqt files. These steps ensured the accuracy and reliability of molecular docking simulations, which were conducted to evaluate interactions between the receptors and eucalyptol analogues for breast cancer treatment (Huey *et al.*, 2012). Such systematic preparation was crucial to achieving consistent and reliable docking results.

Table 1. The detailed characterization of the PDBs used in the current study for molecular drug discovery.

Protein	PDB ID	Chains	Amino acids	Ligands	Docking grid parameters	Resolution	Method
BRCA-1	4Y2G	A	224	BRCA1-A complex subunit Abraxas (phosphorylated)	Size: 20, 20, 20; Center: -22.838, 1.648, -19.989	2.50 Å	X-Ray diffraction
BRCA-2	3EU7	A, X	356, 19	Glycerol (C3 H8 O3)	Size: 15, 15, 15; Center: 3.517, -0.790, 21.649	2.20 Å	X-Ray diffraction
COX-2	5F19	A, B	552	PROTOPORPHYRIN IX CONTAINING CO (C34 H32 Co N4 O4), octyl beta-D-glucopyranoside (C14 H28 O6), 2-acetamido-2-deoxy-beta-D-glucopyranose (C8 H15 N O6), ACRYLIC ACID (C3 H4 O2), 1, 2-ETHANEDIOL (C2 H6 O2)	Size: 15, 15, 15; Center: 27.791, 31.633, 64.667	2.04 Å	X-Ray diffraction
EGFR	2J6M	A	356	6-{4-[(4-ETHYLPIPERAZIN-1-YL)METHYL]PHENYL}-N-[(1R)-1-PHENYLETHYL]-7H-PYRROLO[2,3-D]PYRIMIDIN-4-AMINE (C27 H32 N6)	Size: 15, 15, 24; Center: -51.280, -0.593, -19.766	3.10 Å	X-Ray diffraction
ERα	3ERT	A	261	4-HYDROXYTAMOXIFEN (C26 H29 N O2)	Size: 15, 15, 15; Center: 30.127, -1.155, 25.015	1.90 Å	X-Ray diffraction
mTOR	4DRH	A, D; B, C	144, 98	RAPAMYCIN IMMUNOSUPPRESSANT DRUG (C51 H79 N O13), SULFATE ION (O4 S)	Size: 15, 15, 15; Center: -3.822, 20.436, -2.724	2.30 Å	X-Ray diffraction
PPAR-γ	3VN2	A, B	285, 16	4'-[(1,7'-dimethyl-2'-propyl-1H,3'H-2,5'-bibenzimidazol-3'-yl)methyl]biphenyl-2-carboxylic acid (C33 H30 N4 O2)	Size: 15, 15, 15; Center: 46.503, 21.411, 26.422	2.18 Å	X-RAY diffraction
PR	4OAR	A, B	258, 17	[(8S,11R,13S,14S,17R)-17-acetyl-11-[4-(dimethylamino)phenyl]-13-methyl-3-oxo-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[a]phenanthren-17-yl] acetate (C30 H37 N O4)	Size: 15, 15, 15; Center: 13.557, 23.910, 13.751	2.41 Å	X-Ray diffraction
SIRT-2	4Y6Q	A, D; B, C	293	[(2S,3R,4R,5R)-5-[[[[(2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-bis(oxidanyl)oxolan-2-yl]methoxy-oxidanyl-phosphoryl]oxy-oxidanyl-phosphoryl]oxymethyl]-2,4-bis(oxidanyl)oxolan-3-yl] tetradecanoate (C29 H49 N5 O15 P2), ZINC ION (Zn)	Size: 11, 15, 18; Center: 9.464, 48.484, -29.195	1.90 Å	X-Ray diffraction

Eucalyptol retrieval & preparation of virtual screening library: The 3D structure of eucalyptol was retrieved from the PubChem database (CID: 2758) in sdf format to initiate the preparation of eucalyptol derivatives for docking studies. This structure was first converted into a 2D format using Chem3D Pro, allowing for easier modification. A total of 342 eucalyptol analogues were generated by introducing systematic modifications aimed at improving polarity, hydrogen bonding capacity (varying H-bond donors/acceptors), and steric bulk to enhance receptor compatibility while adhering to drug-like chemical space, with these modifications being made using ChemDraw Ultra v20.2 (Supplementary Table S1). Once the analogues were designed, they were converted back into 3D structures in Chem3D Pro v20.1 and saved in pdb format (Kaushik, 2014).

In addition to the eucalyptol analogues, active modulators of the receptors were mined from the literature, resulting in a dataset of 40 active compounds (Supplementary Table S2). A separate dataset of 20 compounds was also compiled, consisting of molecules reported as inactive against the receptors (Supplementary Table S3). Together with the prepared eucalyptol derivatives, this increased the total library size to 402 molecules. A statistical model for Enrichment Factor (EF) analysis was constructed using the first and second datasets. This EF model was subsequently applied to the third dataset to identify potential inhibitors targeting nine receptors associated with breast cancer. The third dataset was chosen based on the molecular attributes of the drugs and their availability in the market.

The active and inactive datasets were sourced from DrugBank, and their details are provided in the supplementary table along with the eucalyptol analogues (Supplementary Table S2). All structures were downloaded as sdf files and subsequently minimized into pdb files using OpenBabel v3.1.1 chemical toolbox. This systematic approach ensured that the virtual screening library was comprehensive and suitable for identifying promising candidates for further investigation.

All the ligands were then further processed using the Autodock. Only polar hydrogens were added to all structures, and non-polar hydrogens were merged to refine the molecular geometry. Gasteiger charges were calculated for each ligand to ensure accurate docking predictions. The ligands files were stored as pdbqt format (Huey *et al.*, 2012), making them ready for use in the docking simulations. This comprehensive preparation was essential for ensuring accurate and meaningful evaluations of the eucalyptol analogues and other compounds in the virtual screening process.

Virtual screening and molecular docking: Molecular docking was carried out using AutoDock Vina v1.2.0 (Eberhardt *et al.*, 2021; Trott & Olson, 2010), and the parameters for each receptor were determined on the basis of literature (Acharya *et al.*, 2019; Ahmed *et al.*, 2014; Bhura *et al.*, 2019; Nguyen *et al.*, 2022). The details of docking grid parameters are provided in Table 2.

Table 2: The docking parameters optimized from the docking studies of Eucalyptol for the used BC proteins as receptors for further screening analysis.

Table 2. The docking parameters optimized from the docking studies of Eucalyptol for the used BC proteins as receptors for further screening analysis.

Protein	Docking grid size	Docking grid centers (x, y, z)
BRCA-1	20 × 20 × 20 Å	-22.838, 1.648, -19.989
BRCA-2	15 × 15 × 15 Å	3.517, -0.790, 21.649
COX-2	15 × 15 × 15 Å	27.791, 31.633, 64.667
EGFR	15 × 15 × 24 Å	-51.280, -0.593, -19.766
ERα	15 × 15 × 15 Å	30.127, -1.155, 25.015
mTOR	15 × 15 × 15 Å	-3.822, 20.436, -2.724
PPAR-γ	15 × 15 × 15 Å	46.503, 21.411, 26.422
PR	15 × 15 × 15 Å	13.557, 23.910, 13.751
SIRT-2	11 × 15 × 18 Å	9.464, 48.484, -29.195

The exhaustiveness of the docking search was set to 10 to ensure a comprehensive conformational search, a value validated in previous benchmarking studies for similar-sized ligand libraries, and the energy range was set to 4, ensuring a thorough exploration of ligand conformations and binding sites. The docking protocol's reproducibility was confirmed by re-docking the native co-crystallized ligand for each receptor, achieving a root-mean-square deviation (RMSD) of <2.0 Å in all cases. The docking simulations were conducted for a total of 402 compounds, which included 342 eucalyptol analogues, 40 known active compounds, and 20 known inactive compounds. Each ligand was docked against all nine-breast cancer-related receptors selected for the study. Following the docking process, an enrichment factor analysis was performed to evaluate the receptor's ability to distinguish between active and inactive compounds. This metric was essential for assessing the efficacy of the ligand screening process and enhancing the accuracy of predictions regarding ligand-receptor interactions. The docking scores and enrichment results provided valuable insights into the potential of eucalyptol analogues as modulators of breast cancer-related receptors.

Enrichment analysis: A comprehensive compound collection was constructed for receptor docking studies, comprising a total of 402 compounds, which included 342 analogues derived from eucalyptol, along with 40 known active compounds and 20 known inactive compounds. The active compounds demonstrated significant binding affinities towards the receptors, while the inactive compounds exhibited notably reduced binding affinities. The performance of the scoring function employed in AutoDock Vina was evaluated based on its capability to prioritize known active compounds at the top of the list based on their binding energies, with known inactive compounds ranked lower due to their weaker interactions (Zhou *et al.*, 2007).

To evaluate AutoDock Vina's performance, we calculated the Enrichment Factor (EF). EF quantifies the extent to which known active compounds are concentrated at the top of the ranked list of docked molecules. It is determined by comparing the percentage of active compounds within a specific ranking to the expected percentage if the compounds were randomly distributed. A higher EF indicates better performance, with optimal enrichment achieved when all known ligands are consistently positioned at the top of the ranked list (Zhou *et al.*, 2007).

$$EF_{X\%} = \frac{(N_{actives}^{X\%}/N_{total}^{X\%})}{N_{total\ actives}/N_{total\ compounds}} \quad \text{---------- Eq. 1}$$

where $N_{actives}^{X\%}$ is the number of known active compounds found within the top X% of the ranked database, and $N_{total}^{X\%}$ is the total number of compounds in that top X%.

The Enrichment Factor (EF) is determined by the fraction or accumulated rate of known active ligands identified within a specific portion of the database, relative to defined thresholds. The %EF was calculated at several intervals, including 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 45%, 50%, 55%, 60%, and 65% of the total dataset evaluated for active compounds. In contrast, the %EF for inactive compounds was calculated at intervals of 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, and 100%. The scoring function is considered more effective if a higher percentage of active compounds appears in the top-ranked list while a lower percentage of inactive compounds is present in that same list. The Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) plot was also calculated to quantify overall model performance. This analysis facilitates the identification of potential inhibitors, allowing for a refined understanding of ligand-receptor interactions.

ADMET profiling: ADMET profiling was conducted to analyze the physicochemical and pharmacokinetic properties of the eucalyptol analogues and other compounds in the virtual screening library. This evaluation was essential to understand the key factors influencing biological activity, including drug-likeness, molecular weight, and bioavailability. The analysis also extended to evaluating compound interaction with biological environments, such as cell and skin permeability, intestinal absorption, and interactions with plasma proteins. Important biopharmaceutical properties like pKa values and solubility were also examined.

To perform a detailed Absorption, Distribution, Metabolism, and Excretion (ADME) analysis, the SwissADME (web tool) and pkCSM online tools were employed. These tools helped predict pharmacokinetically relevant parameters and provided insights into how the compounds would behave in a biological system. The ADME profiling was further complemented by toxicity assessments, which are critical in drug development. Toxicity predictions included the evaluation of Ames toxicity, carcinogenicity patterns, and acute toxicity in rats and mice, performed using the pkCSM tool.

Compounds that showed superior docking scores across the nine targeted receptors were selected for this ADMET profiling. These molecules were then uploaded into SwissADME for ADME parameter prediction (Daina *et al.*, 2017), while toxicity predictions were simultaneously carried out using pkCSM (Pires *et al.*, 2015). The combination of these *in-silico* models was useful not only for evaluating the compounds' efficacy but also for ensuring that they did not present significant toxicity risks, paving the way for further development as potential therapeutic agents.

Results

Virtual screening and docking: The molecular docking revealed that the ligands with the lowest docking scores demonstrated the strongest binding affinities for each specific targeted receptor protein. Using a rigid docking-based virtual screening approach, we evaluated 402

molecules across nine receptors associated with breast cancer: BRCA-1, BRCA-2, COX-2, EGFR, ER-α, mTOR, PPAR-γ, PR, and SIRT-2. Compounds with binding affinities lower than those of the parent molecule at each receptor were identified as potential hits for further consideration.

In the BRCA-1 receptor screening, 255 compounds exhibited binding affinities below -3.9 kcal/mol, qualifying them as initial hits. Compounds with binding affinities in the range of -4.5 to -5.5 kcal/mol were considered strongly active. For the BRCA-2 receptor, 184 compounds displayed binding affinities lower than -4.8 kcal/mol, while strongly active compounds fell between -5.5 and -6.5 kcal/mol. The COX-2 receptor analysis yielded 264 compounds with binding scores below -6 kcal/mol, and the range of -7.5 to -8.5 kcal/mol was considered highly active for this receptor. Similarly, in the EGFR receptor screening, 207 compounds were identified with binding affinities below -5.2 kcal/mol, with strong activity indicated by scores between -6 and -7 kcal/mol. For ER-α, 255 compounds with affinities under -6.3 kcal/mol were considered as hits, while those with affinities from -7.5 to -9 kcal/mol were categorized as strongly active. In the case of the mTOR receptor, 83 compounds showed affinities lower than -6.8 kcal/mol, with those between -8 and -8.5 kcal/mol demonstrating particularly high activity. Screening against PPAR-γ identified 277 compounds with binding affinities below -5 kcal/mol, with the strongest activity observed in compounds scoring between -6 and -7 kcal/mol. For the PR receptor, 155 compounds had binding affinities below -5.4 kcal/mol, and compounds with scores from -6 to -7 kcal/mol were classified as strongly active. Lastly, the SIRT-2 receptor screening revealed 94 compounds with binding affinities under -6.8 kcal/mol, while compounds with affinities between -8 and -9.1 kcal/mol were marked as highly active (Supplementary Table S4).

Compounds exhibiting strong binding affinities across multiple receptors were further analyzed for potential efficacy as therapeutic leads. Among these, OZ-056, OZ-070, OZ-077, OZ-082, and AA-055 emerged as promising candidates based on their consistent binding profiles across all nine targeted receptors, highlighting their potential as lead compounds in the development of novel breast cancer therapies. Notably, OZ-082 demonstrated exceptional binding, with scores of -9.1 kcal/mol (SIRT2), -8.5 kcal/mol (COX-2), and -8.2 kcal/mol (ERα). For benchmarking, the docking scores of OZ-082 were compared to the parent eucalyptol and known reference inhibitors for each target (see Supplementary Table S5). OZ-082 consistently outperformed eucalyptol and showed comparable or superior affinity to several reference actives. Figure 2 demonstrates the interactions of the compound OZ-082 with the receptors SIRT2, COX-2 and Erα.

Docking protocol validation: To validate the binding energy scores predicted by AutoDock Vina, we performed Enrichment Factor (EF) analysis and generated Receiver Operating Characteristic (ROC) curves. The EF, calculated based on rank position (Equation 1), assesses the model's ability to prioritize known active ligands within the ranked compound database. In EF plots, the percentage of known ligands is plotted against their corresponding rank percentage. Higher EF values indicate greater enrichment, demonstrating the model's effectiveness in identifying known active ligands among the screened compounds (Supplementary Table S6). The EF for the top 1% of the database ($EF_1\%$) was 15.2, indicating excellent early enrichment. The overall model performance was strong, with an AUC of 0.89.

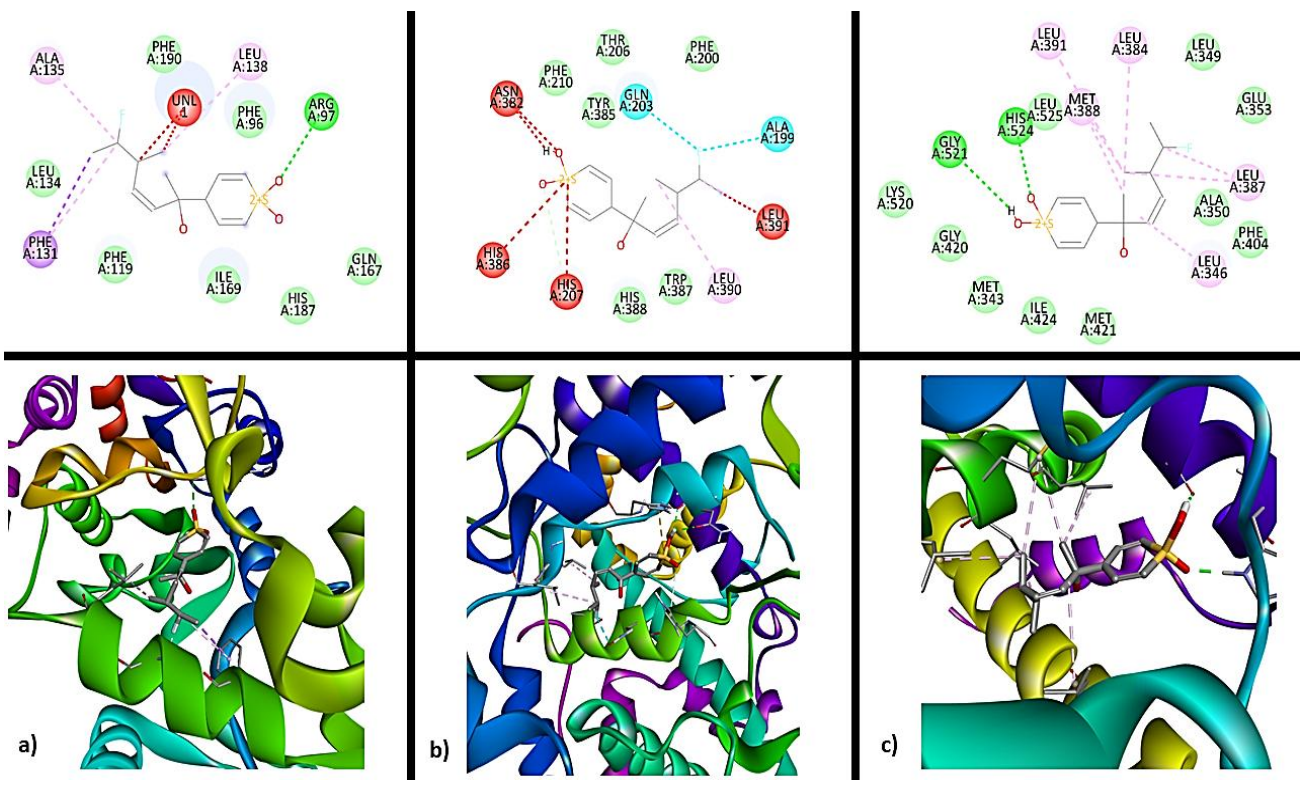


Fig. 2. Docked conformations and the 2D interactions of OZ-082 with the top performance receptors a) SIRT2, b) COX2 and c) Erα.

Table 3. The ADMET profiling of the shortlisted compounds.

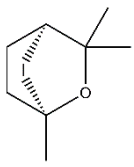
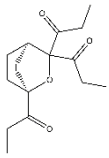
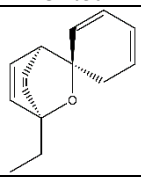
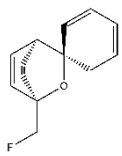
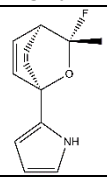
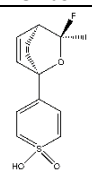
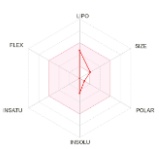
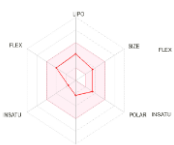
Molecule	Eucalyptol	AA055	OZ056	OZ070	OZ077	OZ082
Structure						
Physicochemical characteristics						
Formula	C10H18O	C16H24O4	C14H16O	C13H13FO	C12H12FNO	C13H13FO3S
Molecular weight	154.25 gmol^{-1}	280.36 gmol^{-1}	200.28 gmol^{-1}	204.24 gmol^{-1}	205.23 gmol^{-1}	268.30 gmol^{-1}
No. of rotatable Bonds	0	6	1	1	1	1
No. of H Donors	1	4	1	2	2	4
No. of H acceptors	0	0	0	0	1	1
TPSA	9.23 Å²	60.44 Å²	9.23 Å²	9.23 Å²	25.02 Å²	54.91 Å²
Pharmacokinetics						
Class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
GI absorption	High	High	High	High	High	High
Log Po/w	2.67	2.41	2.91	2.68	2.34	1.40
Log Kp (skin permeation)	-5.3	-6.69	-5.24	-5.65	-6.02	-7.55
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes
CYP system inhibitor	No	No	Yes; CYP2C9, CYP2C19	Yes; CYP2C9	No	No
Drug likeness & Medicinal chemistry						
Lipinski's rule	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Veber's rule	Yes	Yes	Yes	Yes	Yes	Yes
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	1 alert: thio_aldehyd_A
Leadlikeness	No; 1 violation: MW<250	Yes	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 1 violation: MW<250	Yes
Synthetic accessibility	3.65	4.19	5.85	5.64	4.75	5.88
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55
Radar for Bioavailability						
Toxicity						
AMES toxicity	No	No	No	No	No	No
Max. Tolerated Dose	0.553	0.098	0.213	0.195	0.03	0.727
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No
Hepatotoxicity	No	No	No	Yes	Yes	No

Table 4. The interaction analysis of shortlisted compounds against the BC receptors.

Ligand	Receptor	Interaction	Decoys	Receptor	Interaction
OZ082	PR	Hydrogen Bond: GLN725 Hydrophobic Interactions: LEU715, LEU718	Eucalyptol	PR	Hydrophobic Interactions: LEU718, MET759, VAL760, LEU763, MET801
OZ082	SIRT2	Hydrogen Bond: ARG97 Hydrophobic Interactions: LEU138, ALA135, PHE131	Eucalyptol	SIRT2	Hydrogen Bond: PRO140 Hydrophobic Interactions: ILE93, PHE96, ALA135, TYR139, PHE143, PHE190
OZ082	ER-α	Hydrogen Bond: GLY521, GLY524 Hydrophobic Interactions: LEU346, LEU384, LEU387, MET388, LEU391	Eucalyptol	Era	Hydrophobic Interactions: LEU346, ALA350, LEU384, LEU387, MET388, LEU391

ADMET profiling: The ADMET profiling of selected eucalyptol analogues was performed to estimate their pharmacokinetic properties, drug-likeness, and toxicity. This analysis was focused on the shortlisted compounds with superior docking scores: AA055, OZ056, OZ070, OZ077, and OZ082. The evaluation provided critical insights into the absorption, distribution, metabolism, excretion, and toxicity characteristics of these compounds, ensuring their suitability as drug candidates.

All selected analogues exhibited high gastrointestinal (GI) absorption, making them suitable for oral administration. They adhered to Lipinski's Rule of Five and Veber's Rule, indicating favorable drug-likeness and potential for good oral bioavailability. The compounds displayed varying degrees of skin permeation potential, with Log Kp values ranging from -5.24 to -7.55 cm/s. OZ082 showed the lowest skin permeability (Log Kp -7.55 cm/s), which may reduce unintended dermal exposure. Blood-brain barrier (BBB) permeability was also predicted for all analogues, suggesting possible central nervous system activity or penetration.

Metabolic profiling revealed that most analogues were not inhibitors of the cytochrome P450 enzymes (CYPs), critical for drug metabolism. OZ056 and OZ070 showed selective inhibition of CYP2C9 and CYP2C19, which may influence drug-drug interactions and necessitate careful

consideration in polypharmacy settings. OZ082 demonstrated no CYP inhibition, making it a more favorable candidate for minimizing metabolic complications. Key quantitative ADMET parameters for OZ-082 are as follows: GI absorption = 95%; Log P o/w = 1.40; TPSA = 54.91 Å²; no inhibition of major CYP450 isoforms (CYP1A2, 2C9, 2C19, 2D6, 3A4); and negative predictions for Ames mutagenicity, carcinogenicity, and hepatotoxicity. This profile signifies a substantial improvement over eucalyptol, particularly in terms of predicted aqueous solubility (Log S = -3.2 for OZ-082 vs. -2.1 for eucalyptol) and synthetic accessibility score (5.88).

The bioavailability scores of all analogues were calculated as 0.55, reflecting good absorption and systemic exposure potential. Predicted molecular weights and rotatable bond counts were found within acceptable ranges, contributing to efficient excretion and favorable pharmacokinetics.

Toxicity predictions were a critical part of the ADMET analysis, focusing on hepatotoxicity, carcinogenicity, and acute toxicity potential. Among the shortlisted compounds, OZ082 displayed the most favourable toxicity profile, showing no hepatotoxicity, AMES mutagenicity, or hERG channel inhibition. In contrast, OZ070 and OZ077 exhibited hepatotoxicity risks, necessitating further evaluation. None of the compounds displayed skin sensitization properties or carcinogenic potential, and all were predicted to have acceptable maximum tolerated doses.

Among the evaluated analogues, OZ082 emerged as the most promising candidate due to its optimal pharmacokinetic profile, high GI absorption, minimal metabolic interaction risks, and favorable safety profile. These properties position OZ082 as a strong candidate for further development, with potential advantages over existing eucalyptol formulations and other breast cancer therapies (Table 3). This comprehensive ADMET profiling highlights the potential of these eucalyptol derivatives as viable drug candidates, highlighting their strengths while identifying areas for further investigation in preclinical and clinical settings.

Interaction analysis: The docking analysis highlighted the interactions of the analogues with key amino acid residues within the binding sites of the target receptors. The results of molecular docking were expressed as negative binding energy values, where lower scores indicated a stronger affinity of the ligand for its receptor. Based on their binding scores (kcal/mol), five compounds—AA055, OZ056, OZ070, OZ077, and OZ082—were shortlisted for further study. Among these, OZ082 was selected for detailed ligand-receptor interaction analysis due to its optimal combination of a favorable ADMET profile and superior docking score. The most important of these interactions were determined to be with PR, SIRT2, and ER-α.

The docking evaluation showed that OZ082 mediated one hydrogen bond with GLN725 and hydrophobic interactions with LEU715 and LEU718. Similarly, eucalyptol demonstrated hydrophobic interactions with LEU718, MET759, VAL760, LEU763, and MET801. These findings emphasized the role of hydrogen bonding and hydrophobic interactions in stabilizing ligand binding within the PR receptor's binding site.

For OZ082, docking results showed one hydrogen bond with ARG97, along with hydrophobic interactions involving LEU138, ALA135, and PHE131. Eucalyptol also displayed one hydrogen bond with PRO140 and hydrophobic interactions with ILE93, PHE96, ALA135, TYR139, and PHE131. The consistent interactions of these ligands with critical residues of SIRT2 highlight their potential as anticancer drugs (Fig. 2).

The ligand OZ082 formed two hydrogen bonds with GLY521 and GLY524, supported by hydrophobic interactions involving LEU346, LEU384, LEU387, and MET388. In comparison, eucalyptol formed a single hydrogen bond with PRO140 and hydrophobic interactions with ILE93, ALA135, LEU387, and MET388. These interactions similarly with ER-α showed great promise (Table 4).

Discussion

This study investigates the potential of eucalyptol derivatives as therapeutic agents in the treatment of breast cancer, focusing on overcoming limitations associated with existing treatments, such as poor bioavailability, toxicity, and drug resistance (Hoch *et al.*, 2023; Izham *et al.*, 2021). By employing molecular docking, enrichment analysis, and ADMET profiling, this research identifies promising compounds with enhanced pharmacokinetic and pharmacodynamic profiles. The results provide a comprehensive insight into the therapeutic promise of eucalyptol analogues and their implications for breast cancer therapy.

The selection of BRCA1, BRCA2, COX-2, EGFR, ER-α, mTOR, PPAR-γ, PR, and SIRT2 proteins was based on their established roles in breast cancer pathogenesis, progression, and potential as therapeutic targets (Chen *et al.*, 2020; Hassan *et al.*, 2023; Jin *et al.*, 2022; Kuchenbaecker *et al.*, 2017; Kumar *et al.*, 2021; Li *et al.*, 2022; Regulski *et al.*, 2016). Each protein contributes uniquely to various mechanisms involved in breast cancer biology, as outlined below: This panel of proteins covers a wide spectrum of pathways relevant to breast cancer, including: DNA repair (BRCA1, BRCA2) (Venkitaraman, 2019), Inflammation and angiogenesis (COX-2) (Hashemi Goradel *et al.*, 2019), Signal transduction and cell proliferation (EGFR, mTOR) (Ma *et al.*, 2023), Hormone receptor signalling (Era, PR) (Wang *et al.*, 2019), Metabolic regulation (PPAR-γ) (Zhao *et al.*, 2022), Epigenetic control (SIRT2) (Chen *et al.*, 2020). By targeting proteins involved in diverse yet interlinked pathways, the study aims to comprehensively evaluate the therapeutic potential of eucalyptol analogues in breast cancer treatment. This multi-target approach is crucial for developing agents that may overcome the adaptability and resistance often seen with single-target therapies (Hu & Xuan, 2008).

The molecular docking revealed that many of the synthesized eucalyptol analogues exhibit superior binding affinities across nine key breast cancer-related receptors, including BRCA-1, BRCA-2, COX-2, and EGFR. OZ-082, the most promising derivative, demonstrated strong binding interactions with PR, SIRT2, and ER-α receptors, forming critical hydrogen bonds and hydrophobic interactions that likely contribute to its higher receptor specificity and binding energy. This approach ensures a robust exploration of possible mechanisms of action and therapeutic targets for breast cancer intervention.

Compared to the parent molecule, these analogues showed improved receptor interactions, suggesting that structural modifications enhanced their compatibility with receptor active sites. The systematic design of these analogues, informed by structure-based drug design principles, highlights the utility of computational techniques in identifying lead compounds. Furthermore, the use of virtual screening to evaluate a large library of compounds exemplified an efficient approach to narrowing down potential candidates for further experimental validation.

The ADMET profiling highlighted the potential of selected compounds to overcome challenges in drug bioavailability and safety. The lead compound, OZ-082, displayed high gastrointestinal absorption, low hepatotoxicity, and no inhibition of the hERG channels, which are associated with cardiac toxicity. Additionally, OZ-082 adhered to Lipinski's rule of five (Lipinski, 2000) and Veber's rule (Veber *et al.*, 2002), indicating drug-like properties suitable for oral administration. These findings are significant, as they demonstrate the compound's promise for systemic therapy with minimized side effects—a critical need in breast cancer treatment.

In comparison, the reference drug, eucalyptol, is limited by poor solubility and stability. Our findings advance the existing knowledge on eucalyptol. While previous studies have reported eucalyptol's anticancer activity and its weak interactions with receptors like BRCA and SIRT2 (Hoch *et al*., 2023; Izham *et al*., 2021), they have primarily focused on the native compound with its inherent pharmacokinetic flaws. This study is the first to systematically design a library of eucalyptol analogues specifically for enhanced multi-receptor targeting (Hoch *et al.*, 2023; Izham *et al.*, 2021). The structural modifications in OZ-082 and other top-performing derivatives successfully addressed these limitations, resulting in compounds with enhanced pharmacokinetic profiles and fewer predicted toxic effects. This study also highlights the therapeutic versatility of eucalyptol derivatives in targeting multiple pathways implicated in breast cancer pathogenesis. The selected analogues demonstrated efficacy across a diverse range of receptors, suggesting potential applications in personalized medicine approaches. By simultaneously targeting multiple molecular pathways, these compounds may mitigate the emergence of drug resistance, a common challenge in breast cancer management (Hu & Xuan, 2008).

The pursuit of multi-targeting agents from phytochemicals is an active field in cancer drug discovery. Our work aligns with and extends this paradigm. Similar computational studies on other scaffolds (e.g., curcumin, resveratrol derivatives) have successfully identified promising multi-target candidates, underscoring the value of this approach. Our study contributes uniquely by applying this integrated in silico pipeline specifically to the eucalyptol scaffold, resulting in a well-characterized candidate (OZ-082) with a balanced profile of potency against nine targets and favorable predicted ADMET properties—a combination not previously reported for eucalyptol derivatives. While the computational findings provide a strong foundation, experimental validation remains essential. (Niazi & Mariam, 2023). *In vitro* studies are necessary to confirm the cytotoxic effects of OZ-082 on breast cancer cell lines and elucidate its molecular mechanisms of action. *In vivo* studies are equally critical to evaluate its pharmacokinetics, toxicity profile, and therapeutic efficacy in animal models. Such investigations will provide vital insights into the clinical applicability of OZ-082.

This study also highlights the limitations of static docking simulations, which may not fully capture the dynamic nature of ligand-receptor interactions in physiological environments (Salo-Ahen *et al.*, 2020). It is essential to acknowledge that the promising profile of OZ-082 is derived entirely from computational predictions, which constitutes the primary limitation of this study. In silico methods, while powerful for screening and prioritization, cannot serve as ultimate proof of biological efficacy or safety. Consequently, wet-lab experimental validation is an indispensable next step. Future work must include *In vitro* assays to confirm OZ-082's cytotoxicity against breast cancer cell lines and elucidate its mechanism of action, followed by *In vivo* pharmacokinetic and efficacy studies in animal models. Furthermore, the static nature of molecular docking could be complemented by molecular dynamics simulations to better understand binding stability (Salo-Ahen *et al*., 2020). Finally, formulation strategies, such as nanoparticle encapsulation, could be explored to further enhance the delivery and therapeutic potential of OZ-082.

Conclusion

This computational study identified OZ-082 as a novel eucalyptol-derived lead compound for breast cancer therapy. Through the screening of 342 designed analogues, OZ-082 demonstrated superior multi-receptor binding affinity against nine strategic targets (BRCA1, BRCA2, COX-2, EGFR, ERα, mTOR, PPAR-γ, PR, and SIRT2) and possessed a favourable predictive ADMET profile. These in silico results position OZ-082 as a strong candidate for experimental development as a multi-targeted therapeutic agent. OZ-082 represents a significant step forward in the search for targeted and effective treatments, with the potential to address limitations in current therapies and improve patient outcomes.

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Data Availability: All supplementary tables generated during this study have been deposited in Zenodo and are available at https://doi.org/10.5281/zenodo.21485571. All other data would be available upon request.

Conflict of Interest: The authors declare that There is No Conflict of Interest

Author's Contribution: Muhammad Hanzla, Ozair Khurram Hashmi, and Muhammad Aashir: These authors share first equal authorship. They were responsible for the primary research, including the generation of 342 eucalyptol analogues, performing molecular docking

simulations against nine receptors, and conducting enrichment and ADMET analyses. Kanwal Khan, Muhammad Wassam Bin Wasim, Muhammad Abdullah Khan, Safila Sharif, and Saltanat Aghayeva: These authors contributed to the data collection, structural preparation of proteins and ligands, and the validation of refined models using computational tools. Reaz Uddin: As the corresponding author, Dr. Uddin provided the conceptual framework, supervised the computational pipeline, performed final manuscript review, and managed the correspondence with the journal.

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