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Original Research Article

Synthesis of (3E,5E)-1-benzil-3,5 bis (3 (benziloksi)benziliden)piperidin-4-on curcumin analogues and their potential as breast anticancer agents: Assessment using MTT test and molecular docking

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ABSTRACT

Breast cancer is a serious disease that occurs in women and contributes to the highest mortality compared to other types of cancer. This study aims to synthesize curcumin analog compounds ((3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidin-4-one), test them in vitro against various breast cancer cells (T47D, HER-2, MCF-7, and 4T1) and normal cells (vero cells), and study their molecular docking. Synthesis was carried out by reacting 3-benzyloxybenzaldehyde with N-benzyl-4-piperidone catalyzed by 5% KOH at room temperature; in vitro testing was carried out using the Microculture Tetrazolium Technique Assay method, ADMET analysis with an online database server, and molecular docking studies in Autodoc Vina. The synthesis results obtained yellow solid powder with a yield of 65.85%, characterization with TLC gave black fluorescence (Rf 0.63), melting point 114-116oC, TLC scanner one peak (100%), retention time 0.65 minutes, 1H &13C-NMR analysis showed the molecular formula $C_{40}H_{35}NO_3$, moderate activity against 4T1 breast cancer cells and inactivity on T47D, HER-2, and MCF-7 cancer cells, and did not show cytotoxicity to normal cells (vero cells). ADMET predictions from Lipinski's five rules contained two parameters that did not meet, namely molecular weight and log P value. Molecular docking studies were carried out on estrogen receptor protein (ER)-α (PDB ID: 2ERT), which showed a binding affinity energy of -8.7 kcal/mol and -7.1 kcal/mol of the native ligand. Further research and development is needed on synthetic curcumin analog compounds to increase their activity value against breast cancer by paying attention to Lipinski's five rules to obtain compounds with better potential activity and ADMET.

Introduction

Globally, breast cancer is the most common type of cancer and contributes to the highest mortality in women compared to other types of cancer (Flint et al., 2022). Factors for a woman to be diagnosed with breast cancer occur at an advanced age, are monophause, have dense breast tissue, are not breastfeeding, have a history of hormone therapy and radiation therapy in the chest, and have BRCA 1/2 gene mutations (Trayes & Cokenakes, 2021; Hong & Xu, 2022). Breast cancer treatment strategies are highly dependent on the molecular subtype and involve multidisciplinary (Smolarz et al., 2022). Some

treatments for breast cancer include surgery, radiation, chemotherapy, or a combination of these. However, these actions can cause side effects such as nausea, drug resistance, cytotoxicity, recurrence, and decreased quality of life, which are considered suboptimal for treating it (Praseetha et al., 2022). Although there is continuous progress in detection and treatment, it has not yet provided a significant increase in reducing mortality rates, so it is necessary to find new therapy methods as well as predictive and prognostic factors (Smolarz et al., 2022).

Breast cancer is characterized by pathological heterogeneity, gene expression, genomic changes, and tumor microenvironment, which affect clinical and treatment responses. Classic parameters are histopathology, tumor size and grade, nodal involvement, and marker expression used for treatment and are generally imperfect, especially in advanced breast cancer conditions resulting in resistance and worsening health conditions. Therefore, potential active compounds are needed that provide a positive therapeutic response and provide better treatment optimization (Nolan et al., 2023). The acquisition of potential active compounds in breast cancer treatment can be sourced from natural materials (Baraya et al., 2016; Wang et al., 2019; Gurning et al., 2024; Haryadi et al., 2024) and synthetic results (Mbese et al., 2019; Barbosa and Martel, 2020; Song et al., 2021). One of the active compounds sourced from natural materials and potential is curcumin (Song et al., 2019) and various synthesized analogs to increase treatment activity and study the side effects caused (Mbese et al., 2019; Fabianowska-Majewska et al., 2021;). Based on the description above, this study aims to synthesize N-benzyl-4-piperidone analog curcumin and test its potential activity as an anti-breast cancer agent using the MTT test method and molecular docking studies.

Materials and Methods

Synthesis of (3E,5E)-1-benzyl-3,5 bis(3 (benzyloxy)benzylidene)piperidine-4-one) curcumin analog

15.4 mmol of 3-benzyloxybenzaldehyde was reacted with 7 mmol of N-benzyl-4-piperidone derivative. Ethanol as much as 10 mL was added to the mixture and then stirred at room temperature for 20 minutes. 5% KOH catalyst was added as much as 2 mL dropwise. The mixture was stirred with a magnetic stirrer or sonicated at room temperature until the product was formed. The reaction was monitored using TLC. The solid obtained was filtered using a Buchner funnel, then washed with cold ethanol and cold distilled water. The solid was dried and stored in a desiccator. The synthesis results were analyzed for purity by TLC, and melting point tests were carried out. The synthesis products were characterized using FTIR, GC-MS, and 1H-NMR and 13C-NMR instruments.

ADMET prediction

Prediction of adsorption, distribution, metabolism, excretion, and toxicity (ADMET) was carried out through the SwissADME online site (http://www.swissadme.ch/index.php), and ProTox-3.0: Prediction of TOXicity of Chemicals (https://tox.charite.de/protox3/) by entering the SMILES (Simplified Molecular Input Line Entry System) code of the synthesized curcumin analogues drawn using ChemDraw 15.0 (Chunaifah et al., 2024).

Molecular docking study

The protein used was obtained from the results of the approach by referring to previous research with PDB ID code 3ERT (Shah et al., 2022). The protein structure and standard ligand were prepared using Autodock tools. Curcumin analog compounds and curcumin guide compounds were modeled and optimized using GaussView 5.0 software. Molecular docking simulations were performed using Autodock Vina. The parameters reviewed in molecular docking were affinity energy (increasingly negative values indicate higher stability), RMSD value (<2.00 Å), and interaction between ligand and receptor protein. Interaction analysis of the most stable conformation was visualized using BIOVIA Discovery Studio 2019 Client software (Puspitasari et al., 2020; Astuti et al., 2021; Haryadi and Pranowo, 2023).

Cytotoxicity test using the MTT method

The cytotoxicity test was carried out using the MTT method. The test solution of 100 µL was suspended with 100 µL of cells in RPMI 1640 medium. 10% FBS and 1% penicillin-streptomycin were added to the mixture and then put into a 96-well microplate. T47D, HER-2, MCF-7 and 4T1 cancer cells were incubated for 24 hours in a 5% CO_2 incubator at 37°C. Samples were put into the plate with varying concentrations of 500; 250; 125; 62.5; 31.25; and 15.625 µg/mL. The samples were then incubated in the incubator for 24 hours. After incubation, the cell media was discarded and washed with PBS, and then 110 µL of MTT reagent was added to each well, including the media control. The cells were incubated again for 4 hours in a CO_2 incubator. After the formation of formazan marked by the appearance of purple color, 10% SDS solution in 0.01 N HCl in as much as 100 µL was added and incubated again in a dark place at room temperature for 24 hours. The absorbance in each well was read with an ELISA reader at a wavelength of 595 nm. The IC_{50} value and selectivity index of the compound can be calculated based on the absorbance obtained (Sismindari et al., 2004; Puspitasari et al., 2020).

Results and Discussion

Synthesis of (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analog

Curcumin analogue (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) was formed by reacting N-benzyl-4-piperidone and 3-benzyloxybenzaldehyde compounds through electrophilic substitution reaction under alcoholic base conditions. Electrophilic substitution reaction was carried out on two moles of aldehyde groups sourced from 3-benzyloxybenzaldehyde compound and nucleophilic source from N-benzyl-4-piperidone. The reaction took place with the formation of two moles of water (H_2O) as a by-product (Fig-1). The compound in the form of yellow solid powder was characterized using a TLC scanner and obtained one peak (100%), retention time 0.65 minutes, and molecular formula

weight $C_{40}H_{35}NO_3$ (577.71 g/mol). The resulting melting point of 114-116°C with black fluorescence was observed under UV light 254 nm and 366 (Rf 0.63) with eluent n-hexane: ethyl acetate (12:5) (Fig-2). The synthesis product of curcumin analog was further analyzed by structure using FT-IR and NMR.

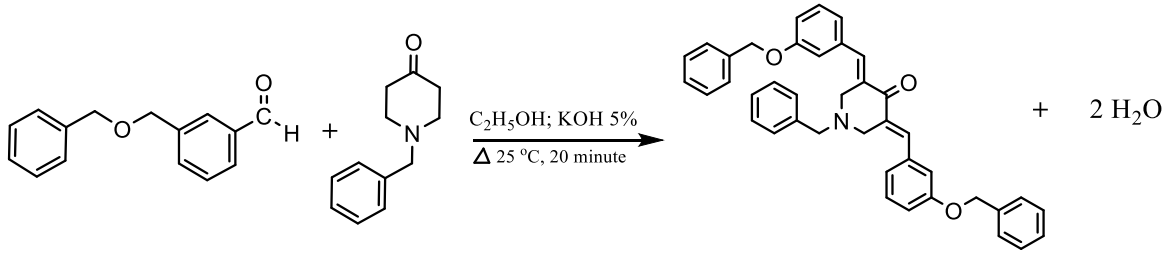

Fig-1. Synthesis reaction of (3E,5E) -1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analogue

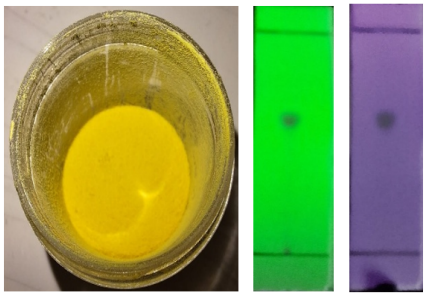

Fig-2. Compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene)piperidine-4-one) curcumin analogue; (a) synthesis product and (b) observation by TLC.

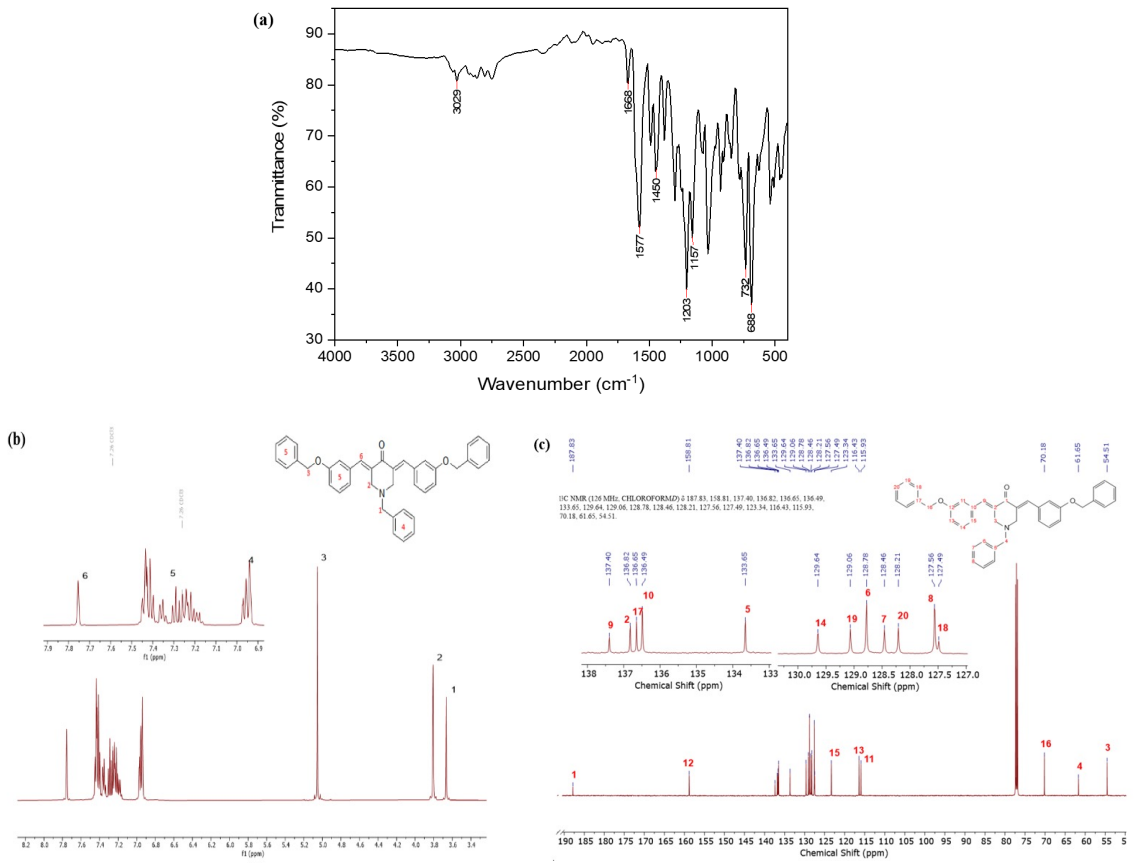

Fig-3. Structural analysis of the compound (3E,5E)-1-benzyl-3,5 bis(3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analogue; (a) FT-IRKBr, (b) 1H-NMR, and (c) 13C-NMR.

FT-IRKBr analysis using KBr plate showed absorption of aliphatic C-H functional groups (3029 cm-1), C=O ketone (1668 cm^{-1}), C=C alkene (1577 cm^{-1}), aromatic C=C (1450 cm^{-1}), C-O methoxy (1157 cm^{-1}), aliphatic C-N (1203 cm^{-1}), and the pinprint area of 732 cm^{-1} and 688 cm^{-1} confirmed the presence of absorption of aromatic compound functional groups (Fig-3

(a)). 1H-NMR analysis (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -CH3), δ 3.81 (s, 4H, -CH2-), δ 5.05 (s, 4H, Ar-CH_2-O-), δ 6.97-6.94 (m, 18H, Ar-H), δ 7.45-7.16 (m, J = 2 Hz, 5H, Ar-H), δ 7.75 (s, J = 2 Hz, 2H, Ar-CH=C-) (Fig-3 (b)). 13C-NMR (100 MHz, CDCl3) δ 54.55 (2 C), δ 61.69 (1C), δ 70.22 (2C), δ 115.97 (2C), δ 116.47 (2C), δ 123.38 (2C), δ 127.53 (1C), δ 127.60 (4C), δ 128.25 (2C), δ 128.50 (2C), δ 128.82 (4C), δ 129.10 (2C), δ 129.68 (2C), δ 133.70 (2C), δ 136, 53 (2C), 136.69 (2C), 136.86 (2C), δ 137.44 (1C), δ 158.85 (2C), δ 187.87 (1C) (Fig-3 (c)).

Cytotoxic testing of breast cancer cells

The curcumin analog compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) formed was tested for anti-breast cancer activity in vitro against T47D, HER-2, MCF-7, 4T1 cancer cells, and vero cells (normal cells). The type of HER2 breast cancer cells is a type of triple-negative breast cancer (TNBC) that overexpresses receptors in the cell nucleus (Núñez et al., 2016), including the subtitle of breast cancer that over-amplifies genes, causing aggressive proliferation, increased cancer cell invasion, and metastasis (Meiyanto et al., 2021). The type of 4T1 cancer cells is a model of breast cancer cells with primary tumors in the breast but have experienced metastasis (Subramanian et al., 2015; Yang et al., 2020). The type of MCF-7 cancer cells that express estrogen receptors (p53+ and ER+), while the type of T47D cancer cells that express receptors (mutant p53 and ER+) (El-Masry et al., 2019). NCI groups the category of anticancer activity based on the IC_{50} value into three groups, namely IC_{50} < 20 µg/mL, the high activity category; IC_{50} values between 200 and 100 µg/mL, including the moderate category; and IC_{50} values > 100 µg/mL, including inactive as an anticancer (Widiandani et al., 2023; Zulkipli et al., 2024). The anticancer activity test of the compound showed better activity with a moderate category against 4T1 cells (IC_{50} 203.05 ± 0.76 µg/mL) and did not show toxicity to normal cells (vero cells) (IC_{50} 190648.40 ± 1.45 µg/mL), while against other breast cancer cells (HER2, MCF-7, and T47D) did not show anticancer activity (inactive category) and against normal cells did not show toxicity (inactive category). The selectivity index of the synthesized compound against all types of breast cancer cells showed high selectivity because all had a selectivity index value > 6 (Sancha et al., 2023). However, this curcumin analog compound has more potential for use in 4T1 cancer cells because it shows anticancer activity in a moderate category. When compared with drugs commonly used as anticancer (doxorubicin), it does not show equivalent activity where doxorubicin has very potential as an anticancer, as shown by the IC_{50} value, which is relatively much smaller than the IC_{50} of the synthetic curcumin analog compound. However, the selectivity value of doxorubicin is better on T47D and HER-2 cancer cells when compared to the selectivity against MCF-7 and 4T1 cells; this also confirms that doxorubicin also shows poor toxicity to normal cells. Testing the activity of the synthetic curcumin analog compound against various breast cancer cells is shown in Table 1.

Table 1. Testing the activity of the compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analog against various breast cancer cells.

Samples	VERO cell		T47D cell			HER-2 cell			MCF-7 cell			4T1 cell		
	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2	SI	IC_{50} (µg/mL)	R^2	SI	IC_{50} (µg/mL)	R^2	SI	IC_{50} (µg/mL)	R^2	SI
Compound	190648.40 ± 1.45	0.9577	603.50 ± 0.57	0.9499	316.08	2527.67 ± 0.58	0.4542	75.43	819.46 ± 0.68	0.9574	232.58	203.05 ± 0.76	0.9951	938.13
Doxorubicin	17.97 ± 0.01	0.9962	0.35 ± 0.04	0.781	51.34	0.02 ± 0.01	0.9868	898.5	28.63 ± 0.02	0.9876	0.63	6.51 ± 0.01	0.8868	2.76

ADMET predictions

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) are important parts that cannot be ignored before being tested in the clinical phase. A good and quality drug candidate must have efficacy against the therapeutic target and show the appropriate ADMET properties at good therapeutic doses (Guan et al., 2019). ADMET has an important role in studying and determining its pharmacokinetic and toxicity properties. Drug similarity is used in predicting the pharmacokinetics and safety of drugs before clinical trials. This prediction was first proposed in 1997 with an oral absorption system and must meet Lipinski's five rules (Jia et al., 2020). ADMET prediction of compound (3E,5E)-1-benzyl-3,5 bis(3 (benzyloxy)benzylidene)piperidine-4-one) curcumin analogue using available database sites as follows: molecular formula $C_{40}H_{35}NO_3$, molecular weight 577.71 g/mol, H-bond acceptor 4, H-bond donor 0, TPSA 38.77Å2, log Po/w 6.91, log S -8.73, BBB permeant no, Lipinski no, bioavailability score 0.17, LD50 1000 mg/kg, and predicted toxicity class 4. The ADME rules set by Lipinski are that large drug-like molecules have log P ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤5 (Rashid, 2020). Based on these rules, it shows that the curcumin analog compound has two parameters that do not meet, namely molecular weight and log P value. Curcumin analog compounds may have poor bioavailability. Based on the toxicity value given, it shows good and does not penetrate the permeable layer of the brain barrier, a relatively low lethal dose, and is included in class 4.

Molecular docking study

The interaction of the compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analog was carried out on the estrogen receptor protein (ER)-α (PDB ID: 3ERT). The estrogen receptor protein (ER)-α is mediated by genomic estrogen signals, has an important role, and is responsible for the proliferation of estrogen-stimulated cells in the development of ER-positive breast cancer. In addition, estrogen proteins and their receptors (ER) play an important role in the development of malignant breast cancer, regulating various transcription genes that bind estrogen response elements (EREs) upstream of target genes. The molecular docking interaction of curcumin analog compounds with estrogen receptor (ER)-α protein and native ligand is shown in Fig-4. Curcumin analog compounds have a binding affinity energy of -8.7 kcal/mol with van der Waals interactions with amino acid residue. arbon hydrogen bonds with amino acid residue Leu82,

carbon hydrogen bonds with amino acid residue Gly85, Pi-Pi T-shaped with amino acid residue Trp55, Pi-Pro101, andith amino acid residues Pro19, Met52, Prthe prosetine21, while the native ligand against prosetin receptor has a binding affinity energy of -7.2 kcal/mol and Arg89;en bond interactions with acid residue Arg89, van der Waals bonds Gly85, Glu48, Pi-Anion Glu18, Pi-Sigma Ile21, and Pi-Alkyl with Trp88, Arg89.

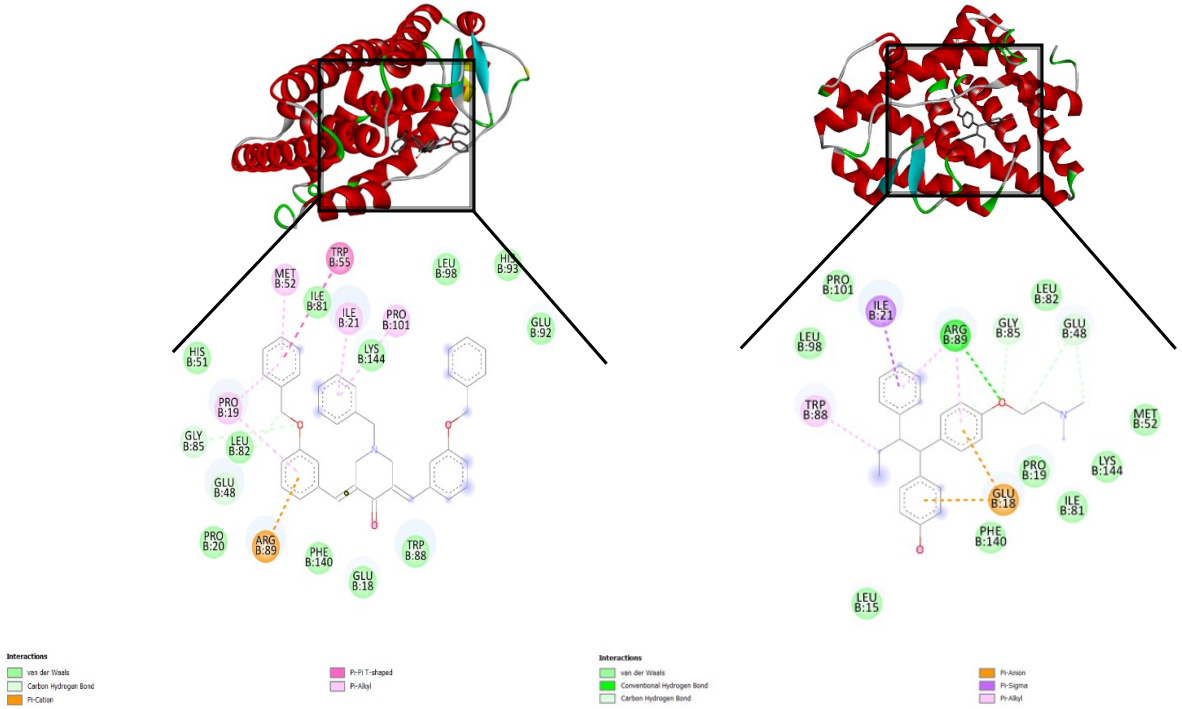

Fig-4. 2D Molecular docking interaction of estrogen receptor (ER)-α protein with (a) compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analogue, and (b) compound 4-hydroxytamoxifen (native ligand).

The 4-hydroxytamoxifen compound is a common nonsteroidal antiestrogen used for the treatment and prevention of breast cancer in estrogen receptor positive (ER+) patients (Lin et al., 2020). This compound interacts with certain selective serotonin reuptake inhibitors (SSRIs) used to prevent hot flashes formed by the CYP2D6 enzyme (Jordan, 2007; Siqueira et al., 2021). However, long-term use of this drug can cause drug resistance, recurrence, and death, so its long-term use is not recommended and does not provide any effect on breast cancer in estrogen receptor negative (ER-) patients (Lin et al., 2020). Interestingly, the results of the molecular docking study of curcumin analog compounds with native ligands showed that the synthetic curcumin analog compounds showed more negative binding affinity energy than native ligands, indicating better structural stability. However, the activity given to breast cancer cells is still relatively weak (4T1 cancer cells) or even inactive in other cells. This indicates that structural modification is needed to increase its anticancer activity. The results of the synthesis of this curcumin analogue require further research and development in modifying the structure to increase activity against breast cancer.

Conclusion

The compound (3E,5E)-1-benzyl-3,5 bis (3 (benzyloxy)benzylidene) piperidine-4-one) curcumin analogue was successfully synthesized and tested in vitro against various breast cancer cells and provided low activity against 4T1 cells, was inactive against MCF-7 T47D and HER-2 cancer cells, and did not show cytotoxicity against normal cells (Vero cells). The activity provided has not been able to overcome the commonly used drug compound (doxorubicin) as a breast cancer drug. Molecular docking studies of estrogen receptor (ER)-α protein showed lower binding affinity energy compared to the original ligand (4-hydroxytamoxifen), and this indicates that the synthetic curcumin analog compound shows better molecular bond stability compared to the original ligand. Further development is needed to improve better activity against breast cancer treatment.

Conflict of Interests

The author declares that there is no conflict of interest in this research and manuscript.

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References

Astuti, E., Raharjo, T.J., Boangmanalu, P.M., Putra, I.S.R., Waskitha, S.S.W., Solin, J. 2021. Synthesis, molecular docking, and evaluation of some new curcumin analogs as antimalarial agents. *Indonesian Journal of Chemistry* 21, 452–461. https://doi.org/10.22146/IJC.57646

Baraya, Y.S.B., Wong, K.K., & Yaacob, N.S. 2016. The immunomodulatory potential of selected bioactive plant-based compounds in breast cancer: A review. *Anticancer Agents Med Chem* 17. https://doi.org/10.2174/1871520616666160817111242

Barbosa, A.M., Martel, F., 2020. Targeting glucose transporters for breast cancer therapy: The effect of natural and synthetic compounds. *Cancers* 12, 154. https://doi.org/10.3390/CANCERS12010154

Chunaifah, I., Venilita, R.E., Tjitda, P.J.P., Astuti, E., Wahyuningsih, T.D. 2024. Thiophene-based N-phenyl pyrazolines: synthesis, anticancer activity, molecular docking and ADME study. *J Appl Pharm Sci* 14, 063–071. https://doi.org/10.7324/JAPS.2024.146832

Dwi Puspitasari, A., Dwi Pranowo, H., Astuti, E., Dwi Wahyuningsih, T. 2020. Design of new chlorochalcone derivatives as potential breast anticancer compound based on QSAR analysis and molecular docking study. *CMUJ. Nat. Sci*. 2021 20, 2021070–2021071. https://doi.org/10.12982/CMUJNS.2021.070

El-Masry, O.S., Brown, B.L., Dobson, P.R.M. 2019. AMPK activation of apoptotic markers in human breast cancer cell lines with different p53 backgrounds: MCF-7, MDA-MB-231 and T47D Cells. *Asian Pac J Cancer Prev* 20, 3763. https://doi.org/10.31557/APJCP.2019.20.12.3763

Fabianowska-Majewska, K., Kaufman-Szymczyk, A., Szymanska-Kolba, A., Jakubik, J., Majewski, G., Lubecka, K. 2021. Curcumin from turmeric rhizome: A potential modulator of DNA methylation machinery in breast cancer inhibition. *Nutrients* 13, 332. https://doi.org/10.3390/NU13020332

Flint, A.L., Hansen, D.W., Brown, L.V.D., Stewart, L.E., Ortiz, E., Panda, S.S. 2022. Modified curcumins as potential drug candidates for breast cancer: An overview. *Molecules* 27. https://doi.org/10.3390/molecules27248891

Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., Liu, G., Tang, Y., 2019. ADMET-score – a comprehensive scoring function for evaluation of chemical drug-likeness. *Medchemcomm* 10, 148–157. https://doi.org/10.1039/C8MD00472B

Gurning, K., Suratno, S., Astuti, E., Haryadi, W. 2024. Untargeted LC/HRMS Metabolomics Analysis and Anticancer Activity Assay on MCF-7 and A549 Cells from Coleus amboinicus Lour Leaf Extract. *IJ Pharmaceutical Research* 23. https://doi.org/10.5812/IJPR-143494

Haryadi, W., Gurning, K., Astuti, E. 2024. Molecular target identification of two Coleus amboinicus leaf isolates toward lung cancer using a bioinformatic approach and molecular docking-based assessment. *J Appl Pharm Sci* 14, 203–210. https://doi.org/10.7324/japs.2024.164753

Haryadi, W., Pranowo, H.D., 2023. Molecular docking and dynamics analysis of halogenated imidazole chalcone as anticancer compounds. *Pharmacia* 70, 323-329. https://doi.org/10.3897/PHARMACIA.70.E101989

Hong, R., Xu, B. 2022. Breast cancer: an up-to-date review and future perspectives. *Cancer Commun* 42, 913-936. https://doi.org/10.1002/cac2.12358

Jia, C.Y., Li, J.Y., Hao, G.F., Yang, G.F. 2020. A drug-likeness toolbox facilitates ADMET study in drug discovery. *Drug Discov Today* 25, 248–258. https://doi.org/10.1016/J.DRUDIS.2019.10.014

Jordan, V.C. 2007. New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer. *Steroids* 72, 829–842. https://doi.org/10.1016/J.STEROIDS.2007.07.009

Lin, H.Y., Han, H.W., Wang, Y.S., He, D.L., Sun, W.X., Feng, L., Wen, Z.L., Yang, M.K., Lu, G.H., Wang, X.M., Qi, J.L., Yang, Y.H. 2020. Shikonin and 4-hydroxytamoxifen synergistically inhibit the proliferation of breast cancer cells through activating apoptosis signaling pathway in vitro and in vivo. *Chinese Medicine* 15, 1–14. https://doi.org/10.1186/S13020-020-00305-1/FIGURES/7

Mbese, Z., Khwaza, V., Aderibigbe, B.A. 2019a. Curcumin and its derivatives as potential therapeutic agents in prostate, colon and breast cancers. *Molecules* 24. https://doi.org/10.3390/molecules24234386

Meiyanto, E., Husnaa, U., Kastian, R.F., Putri, H., Larasati, Y.A., Khumaira, A., Pamungkas, D.D.P., Jenie, R.I., Kawaichi, M., Lestari, B., Yokoyama, T., Kato, J.Y. 2021. The target differences of anti-tumorigenesis potential of curcumin and its analogues against HER-2 positive and triple-negative breast cancer cells. *Adv Pharm Bull* 11, 188–196. https://doi.org/10.34172/apb.2021.020

Nolan, E., Lindeman, G.J., Visvader, J.E. 2023. Deciphering breast cancer: from biology to the clinic. *Cell* 186, 1708–1728. https://doi.org/10.1016/J.CELL.2023.01.040

Núñez, C., Capelo, J.L., Igrejas, G., Alfonso, A., Botana, L.M., Lodeiro, C. 2016. An overview of the effective combination therapies for the treatment of breast cancer. *Biomaterials* 97, 34–50. https://doi.org/10.1016/J.BIOMATERIALS.2016.04.027

Praseetha, N.G., Divya, U.K., Nair, S. 2022. Identifying the potential role of curcumin analogues as anti-breast cancer agents; an in silico approach. *Egyptian Journal of Medical Human Genetics* 23. https://doi.org/10.1186/s43042-022-00312-x

Rashid, M., 2020. Design, synthesis and ADMET prediction of bis-benzimidazole as anticancer agent. *Bioorg Chem* 96, 103576. https://doi.org/10.1016/J.BIOORG.2020.103576

Sancha, S.A.R., Szemerédi, N., Spengler, G., Ferreira, M.J.U. 2023. Lycorine carbamate derivatives for reversing P-glycoprotein-mediated multidrug resistance in human colon adenocarcinoma cells. *Int J Mol Sci* 24, 2061. https://doi.org/10.3390/IJMS24032061/S1

Shah, V., Bhaliya, J., Patel, G.M. 2022. In silico docking and ADME study of deketene curcumin derivatives (DKC) as an aromatase inhibitor or antagonist to the estrogen-alpha positive receptor (Erα+): potent application of breast cancer. *Struct Chem* 33, 571–600. https://doi.org/10.1007/S11224-021-01871-2/FIGURES/12

Siqueira, M.L.S., Andrade, S.M.V., Vieira, J.L.F., Monteiro, M.C. 2021. A preliminary study on the association of tamoxifen, endoxifen, and 4-hydroxytamoxifen with blood lipids in patients with breast cancer. *Biomedicine & Pharmacotherapy* 142, 111972. https://doi.org/10.1016/J.BIOPHA.2021.111972

Sismindari, Sudibyo, R.S., Astuti, E. 2004. Cytotoxic effects of protein fraction isolated from Curcuma mangga Val rhizomes and containing ribosome-inactivating proteins on cancer cell-lines and normal cell. *Indonesian Journal of Chemistry* 4, 206–211.

Smolarz, B., Zadrożna Nowak, A., Romanowicz, H. 2022. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). *Cancers* 14, 2569. https://doi.org/10.3390/CANCERS14102569

Song, F., Huo, X., Guo, Z. 2021. Anti-breast cancer potential of natural and synthetic coumarin derivatives. *Curr Top Med Chem* 21, 1692–1709. https://doi.org/10.2174/1568026621666210303145430/CITE/REFWORKS

Song, X., Dai, M.Z., Luo, Y. 2019. Molecular targets of curcumin in breast cancer (Review). *Molecular Medicine report* 23–29. https://doi.org/10.3892/mmr.2018.9665

Subramanian, A., Manigandan, A., P.R., S., Sethuraman, S. 2015. Development of nanotheranostics against metastatic breast cancer — A focus on the biology & mechanistic approaches. *Biotechnol Adv* 33, 1897–1911. https://doi.org/10.1016/J.BIOTECHADV.2015.10.002

Sun, D., Chen, G., Dellinger, R.W., Duncan, K., Fang, J.L., Lazarus, P. 2006. Characterization of tamoxifen and 4-hydroxytamoxifen glucuronidation by human UGT1A4 variants. *Breast Cancer Research* 8, 1–11. https://doi.org/10.1186/BCR1539/TABLES/1

Trayes, K., Cokenakes, S. 2021. Breast cancer treatment. *Am Fam Physician* 104, 171–178.

Wang, X., Yang, Y., An, Y., Fang, G. 2019. The mechanism of anticancer action and potential clinical use of kaempferol in the treatment of breast cancer. *Biomedicine & Pharmacotherapy* 117, 109086. https://doi.org/10.1016/J.BIOPHA.2019.109086

Widiandani, T., Tandian, T., Zufar, B.D., Suryadi, A., Purwanto, B.T., Hardjono, S., Siswandono, S. 2023. In vitro study of pinostrobin propionate and pinostrobin butyrate: Cytotoxic activity against breast cancer cell T47D and its selectivity index. *J Public Health Afr* 14, 2516. https://doi.org/10.4081/JPHIA.2023.2516

Yang, L., Yong, L., Zhu, X., Feng, Y., Fu, Y., Kong, D., Lu, W., Zhou, T-Y. 2020. Disease progression model of 4T1 metastatic breast cancer. *J Pharmacokinet Pharmacodyn* 47, 105–116. https://doi.org/10.1007/S10928-020-09673-5/FIGURES/5

Zulkipli, N.N., Rahman, S.A., Taib, W.R.W., Razali, R.M., Ismail, I., Ahmad, W.A.N.W., Daud, C.K.D.C.K. 2024. The cytotoxicity effect and identification of bioactive compounds of Prismatomeris glabra crude leaf extracts against breast cancer cells. *Beni Suef Univ J Basic Appl Sci* 13, 1–18. https://doi.org/10.1186/S43088-024-00490-0/FIGURES/7