

INVESTIGATING METHYL AND NITRO SUBSTITUENTS AFFECT IN PARA POSITION ON N-BENZOYL-N'-PHENYLTHIOUREA COMPOUNDS AS POTENTIAL TREATMENTS FOR BREAST CANCER

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ABSTRACT

The primary challenge in chemotherapy for breast cancer stems from the limited effectiveness of existing drugs due to the diverse mutations, each requiring specific treatment. Consequently, it is essential to focus on the development of drugs specifically designed for anti-breast cancer therapy. In this investigation, we synthesized and evaluated compounds with methyl and nitro substituents on the para position in N-benzoyl-N'-phenylthiourea to examine their efficacy against breast cancer cells (MCF-7). Computational analyses demonstrated favorable interactions with EGFR, and experimental tests revealed IC₅₀ values of 0.42 mM for the first compound and 0.07 mM for the second compound. Remarkably, the second molecule showed enhanced selectivity and cytotoxicity (SI value of 937.57) in comparison to the first compound, suggesting that it could be a promising drug for the treatment of breast cancer.

Keywords: Molecular Docking, Breast Cancer, EGFR, Phenylthiourea, Cytotoxic Activity.

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INTRODUCTION

Breast cancer is a prominent cancer type in Indonesia, ranking first in prevalence according to Pathological Based Registration data, with a relative frequency of 18.6%. Projections suggest a potential increase to 25.7% by 2030. Despite the effectiveness of current treatments like breast-conserving therapy and mastectomy, the importance of chemotherapy remains. However, the complexity of addressing diverse mutations in breast cancer requires specific drugs, emphasizing the urgent need for drug development to identify potential candidates as raw materials for anti-breast cancer therapy.¹⁻⁴ An essential function of the Epidermal Growth Factor Receptor (EGFR) is to relay signals required for cellular growth. Changes or mutations in the EGFR regulatory system can result in increased EGFR expression, leading to uncontrolled cell growth, a phenomenon notably observed in several cancers, including breast cancer. Approximately 25%-30% of individuals with breast cancer demonstrate overexpression of EGFR, a characteristic linked to unfavorable clinical outcomes.⁵⁻⁸ Chemotherapeutic agents developed specifically for breast cancer treatment encompass derivatives such as thiourea and phenylthiourea. Li synthesized several phenylthiourea derivatives, and one of these showed good action against HER-2 and EGFR enzymes. Additionally, these derivatives demonstrated inhibitory effects on MCF-7 cell proliferation. In Kesuma et al.'s study, two phenylthiourea derivatives synthesized displayed cytotoxic activity against MCF-7 cells, surpassing that of hydroxyurea and erlotinib. Topliss modifications of the aromatic ring of the molecule can be used to create derivatives that affect physicochemical factors (lipophilic, electronic, and steric) in the Hansch model. Drug solubility in distribution is influenced by electronic parameters, drug penetration into cell membranes is improved by lipophilic parameters, and the strength of drug interactions with receptors is linked to steric parameters.⁹⁻¹² The research focuses on derivatives of phenylthiourea, specifically N-(4-Methyl)-Benzoyl-N'-Phenylthiourea (first compound) and N-(4-Nitro)-Benzoyl-N'-Phenylthiourea (second compound), emphasizing their interaction with the Epidermal Growth Factor Receptor (EGFR). Before synthesis, computational predictions were utilized to evaluate the cytotoxic potential of these compounds. Then each synthesized compound is analyzed to identify its structure

elucidation. Using MCF-7 cells, in vitro cytotoxicity was assessed using the tetrazolium microculture (MTT) method. The resultant IC₅₀ values were compared to reference chemicals hydroxyurea and erlotinib, where erlotinib was used as a clinical reference to suppress the proliferation of cancer cells. Examining normal Vero cells allowed for the determination of selectivity for cancer cells. This study aimed to identify a novel phenylthiourea-derived compound with potential as an anti-breast cancer candidate by predicting its ability to inhibit EGFR.

EXPERIMENTAL

Materials

EGFR receptor with PDB code 1M17 and a set of computers with programs for in silico testing. MCF-7 cancer cells, Vero cells, first compound and second compound compounds, Erlotinib, HU, and in vitro cytotoxic test reagents purchased from local suppliers, and also a set of tools for in vitro cytotoxic testing.

Docking Using Molegro Virtual Docker (MVD)

EGFR (1M17) with erlotinib standard ligand was selected. Docking was performed using MVD, generating rerank scores for compounds. Environmental conditions of compounds and receptors were analyzed (lipophilic, electronic, and hydrogen bonding properties). Amino acids and rerank score values in the interaction process were compared.

Cytotoxicity Against MCF-7 Cells

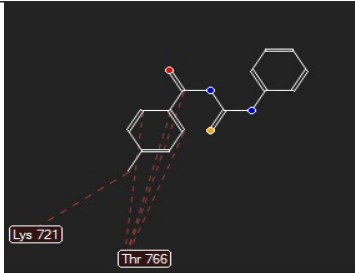
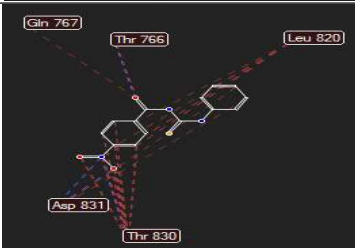
MCF-7 and Vero cells were cultured in 96-well plates and incubated for a full day. Subsequently, various concentrations of HU, erlotinib, and the experimental compounds were added to the wells, while control wells contained a cell-free culture medium. After the 24-hour incubation period, PBS was used to wash the wells. 500 ppm of MTT reagent (100 μ L) was added, and the mixture was incubated for four hours. Ten percent SDS in 0.01 n HCl (100 μ L) was added to dissolve the formazan crystals and eliminate the MTT response. Following established protocols, the absorption was measured using an ELISA reader at 595 nms. IC₅₀ values were determined through probit analysis. The ratio of IC₅₀ for normal cells to IC₅₀ for cancer cells was used to determine the selectivity for cancer cells.^{19,20}

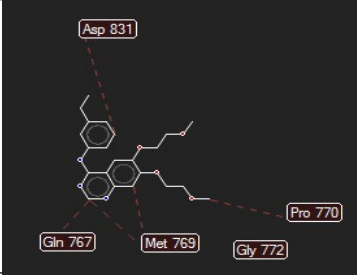
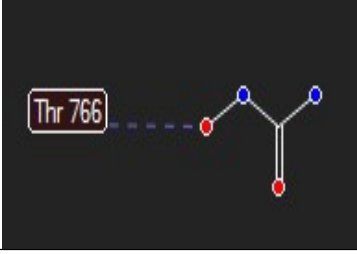
RESULTS AND DISCUSSION

In Silico Cytotoxicity Assessment

The in-silico test results for the first compound, second compound, and reference compound are presented in Table-1. Molecular docking results revealed distinct interactions between the test compounds and reference compounds on EGFR, potentially elucidating variations in in vitro activity tests.

Table-1: In Silico Test Results: Ligands' In Silico Docking Value at the EGFR Receptor: (PDB Code: 1M17)

Ligand	Rerank Score (kcal/mol)	Interactions
First compound	-78.0707	
Second compound	-90.1688	

Erlotinib	-92.0274	
Hydroxyurea	-38.4495	

RMSD = 1.95361

Table-2: Amino Acids and Chemical Bonds Involved in the Interaction Process of BPTU Compounds and the Derivatives and Comparison Compounds to EGFR Receptors

No.	Compound Name	Amino Acid							
		Lys 721	Thr 766	Gln 767	Met 769	Pro 770	Leu 820	Thr 830	Asp 831
1.	4-CH ₃ -BFTU	1S	4S	-	-	-	-	-	-
2.	4-NO ₂ -BFTU	-	1H /1S	1S	-	-	6S	4H/ 9S	2H /4S
3.	HU	-	1H/1S	-	-	-	-	-	-
4.	Erlotinib	-	-	1S	2S	1S	-	-	1S

Notes: H: hydrogen bond interaction; S: Van der Waals and hydrophobic interaction

Based on the results of molecular docking, the rerank score for the second compound was smaller than that for the first compound but not much different from the rerank score for erlotinib. This shows that the second compound is predicted to have better activity than the first compound, but is close to erlotinib as a comparison. Further analysis showed an interaction between the amino acids Gln767, Met769, and Asp831 with erlotinib. In contrast, the second compound showed an interaction of hydrogen bonds with Thr830 and Thr766 which allowed the test compound to have better in vitro activity than erlotinib.

Characteristics of First Compound and Second Compound

The structures of the first compound and second compound were identified through IR spectroscopy, NMR, and HRMS spectroscopy. The infrared spectrum of the modified chemical first compound produced with KBr pellets is illustrated in Fig.-1.

Figure-2 shows the 1H-NMR spectra of the first chemical compound in the DMSO-d₆ solvent.

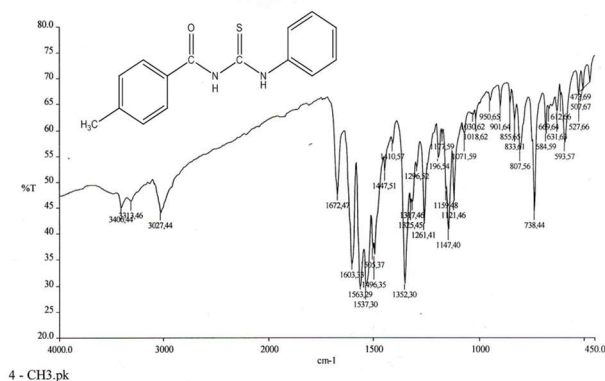


Fig.-1: First Compound Infrared Spectrum

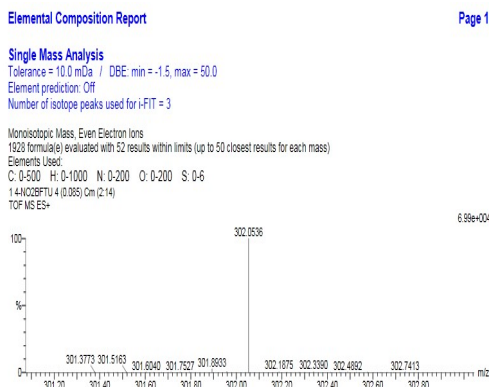


Fig.-8: Mass Spectrometry Spectrum (ESI-HRMS) of the Second Compound using DMSO-d₆ Solvent

CONCLUSION

The second and first compounds exhibited greater cytotoxic activity in vitro and in silico than the reference compounds. When compared to the first compound, the second compound exhibited more cytotoxic activity and selectivity against MCF-7 cancer cells.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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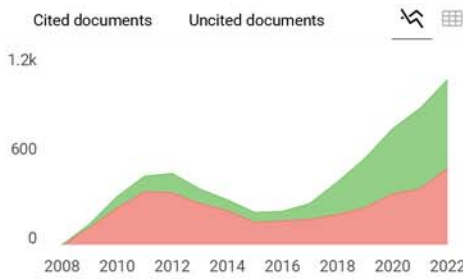
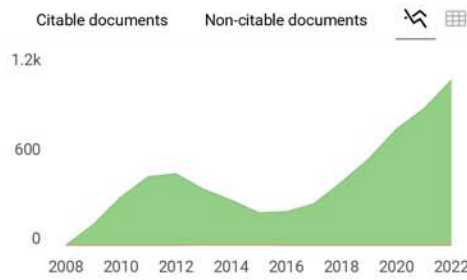
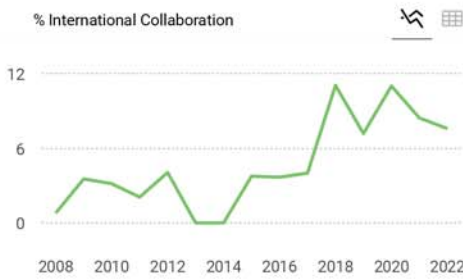
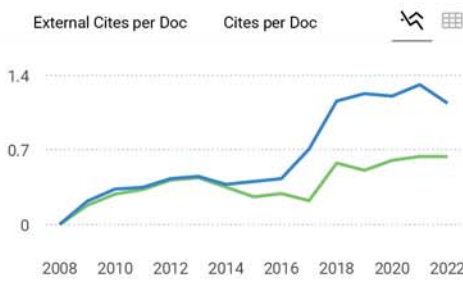
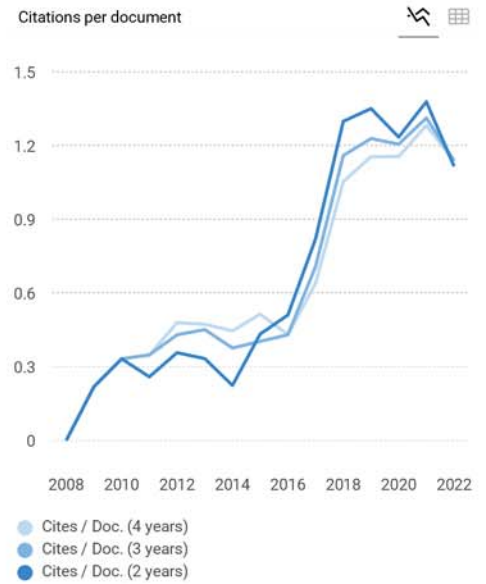
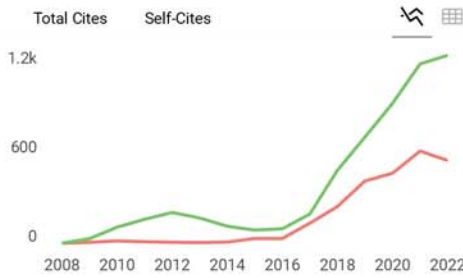
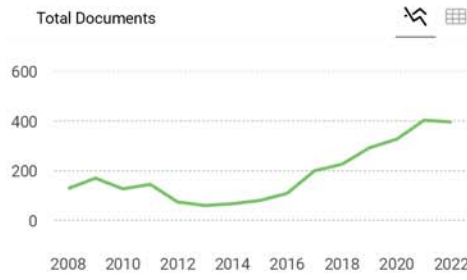
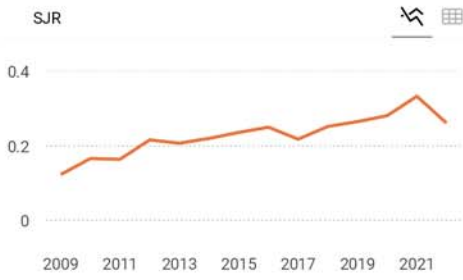
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