Prisma Sains: Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram
https://e-journal.undikma.ac.id/index.php/prismasains/index
e-mail: prismasains.pkpsm@gmail.com

October 2026. Vol. 14, No. 4
p-ISSN: 2338-4530
e-ISSN: 2540-7899
pp. 2545-2556

In Silico Study of 4-Chloro-N-(Phenylcarbamothioyl)-benzamide as an Anticancer Candidate: Molecular Docking Analysis and ADMET Profiling

[1]Ida Bagus Oka Wisnu Andhika, [2]*Dini Kesuma, [3]Aguslina Kirtishanti

[1]Master's Program in Clinical Pharmacy, University of Surabaya, Indonesia
[2]Department of Pharmaceutical Chemistry, University of Surabaya, Indonesia
[3]Department of Drug Discovery and Development, University of Surabaya, Indonesia

*Corresponding author e-mail: dinikesuma015@gmail.com

Received: September 2026; Revised: October 2026; Published: October 2026

Abstract

Glutaminase (GLS) is an enzyme involved in cellular glutamine metabolism and has been investigated as a potential target in cancer research. This study aimed to conduct an exploratory in silico assessment of 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB) through molecular docking against GLS and prediction of its physicochemical and ADMET properties. The molecular structure of 4-Cl-PCTB was prepared using Chem3D version 16.0, while molecular docking was performed using Molegro Virtual Docker version 7.0 with the GLS structure obtained from the Protein Data Bank (PDB ID: 8JUB). The predicted physicochemical and pharmacokinetic properties were evaluated using SwissADME and pkCSM. The reported molecular docking analysis yielded a Rerank Score of −78.023 for 4-Cl-PCTB, compared with −85.728 for lapatinib. The predicted human intestinal absorption of 4-Cl-PCTB was 88.309%, while its molecular weight and consensus LogP were reported as 290.77 Da and 2.73, respectively. These results represent computational predictions and do not establish experimentally confirmed GLS binding affinity, enzyme inhibition, oral bioavailability, or safety. Furthermore, limitations in the available docking documentation and inconsistencies in some reported computational parameters restrict the interpretation of the findings. The comparison with lapatinib should also be interpreted cautiously because it was not established as a GLS-specific reference inhibitor. In conclusion, 4-Cl-PCTB warrants further investigation as a computationally assessed compound; however, its potential as an anticancer candidate remains unconfirmed. Reproducible docking studies, biochemical GLS inhibition assays, and appropriate cellular experiments are required to establish its biological relevance.

Keywords: 4-Cl-PCTB; ADMET prediction; Glutaminase; In silico study; Molecular docking

How to Cite: Andhika, I. B. O. W., Kesuma, D., & Kirtishanti, A. (2026). In Silico Study of 4-Chloro-N-(Phenylcarbamothioyl)-benzamide as an Anticancer Candidate: Molecular Docking Analysis and ADMET Profiling. *Prisma Sains: Jurnal Pengkajian Ilmu Dan Pembelajaran Matematika Dan IPA IKIP Mataram*, *14*(4), 2545–2556. https://doi.org/10.33394/j-ps.v14i4.22783

https://doi.org/10.33394/j-ps.v14i4.22783

Copyright© 2026, Andhika et al.
This is an open-access article under the CC-BY License

INTRODUCTION

Cancer remains a major global public health concern because of its substantial contribution to morbidity, mortality, and healthcare burden (Filho et al., 2025). Breast cancer is among the most frequently diagnosed malignancies worldwide, particularly among women, and continues to represent an important challenge in oncology (Freihat et al., 2025). Despite advances in diagnostic techniques, molecular characterization, and systemic treatment, the clinical management of breast cancer remains complex because of its biological heterogeneity and differences in therapeutic response (Xiong et al., 2025). These challenges emphasize the need to investigate new molecular targets and identify chemical compounds that may provide alternative approaches for cancer treatment.

Breast cancer is a heterogeneous disease commonly classified according to the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor

receptor 2 (HER2) (Tavčar Kunstič et al., 2023). This classification provides an important basis for selecting treatment strategies because each molecular subtype may exhibit different biological characteristics and therapeutic vulnerabilities. Triple-negative breast cancer (TNBC) is characterized by the absence of ER, PR, and HER2 expression (Bou Zerdan et al., 2022). Consequently, treatment strategies that specifically target these receptors are not applicable to this subtype. TNBC also encompasses biologically diverse tumors, and its clinical management remains challenging because of differences in disease progression, treatment response, and the availability of suitable molecularly targeted therapies (Bou Zerdan et al., 2022). These characteristics support continued investigation into molecular pathways that may contribute to TNBC cell survival and proliferation.

Cancer cells undergo metabolic adaptations that support their proliferation, survival, and ability to respond to changes in their microenvironment. One pathway receiving considerable attention is glutamine metabolism (Zhu et al., 2022). Glutamine serves as a metabolic substrate for the production of glutamate and downstream intermediates involved in energy metabolism, biosynthetic processes, and cellular redox regulation (Yoo et al., 2020). In some cancer models, increased dependence on glutamine metabolism may contribute to the maintenance of tumor growth and create potential metabolic vulnerabilities that can be explored for therapeutic development (Jin et al., 2023).

Glutaminase (GLS) is an enzyme that catalyzes the conversion of glutamine into glutamate, representing an important step in glutamine utilization. Through this reaction, GLS contributes to metabolic pathways that support cellular biosynthesis and energy production (Kumar et al., 2025; Matés et al., 2020). Previous research has demonstrated the importance of GLS-dependent glutamine metabolism in selected TNBC models and has suggested that suppressing this pathway may influence cancer cell growth (Lampa et al., 2017). These findings provide a scientific rationale for investigating GLS as a potential target in anticancer drug discovery. Nevertheless, the contribution of GLS may vary according to tumor biology, metabolic context, and experimental model. Therefore, the relevance of a compound as a potential GLS-targeting agent must be established through target-specific evidence rather than inferred solely from its predicted interaction with the protein.

The development of new anticancer compounds involves the exploration of chemical scaffolds that may interact with biologically relevant molecular targets. Thiourea derivatives have attracted interest in medicinal chemistry because this structural group has been associated with diverse biological activities, including antimicrobial and anticancer effects (Agili, 2024). The thiourea moiety contains nitrogen and sulfur atoms that can influence molecular polarity, hydrogen-bonding characteristics, and intermolecular interactions (Gumus et al., 2019). These properties may contribute to the recognition of particular molecular environments, although the resulting biological activity depends on the complete molecular structure and the characteristics of the target.

Within this chemical class, N-acylthiourea derivatives represent a group of compounds in which the thiourea functionality is incorporated into an acyl-containing structure. Differences in aromatic substitution and other structural features may alter physicochemical properties and potentially influence interactions with biological macromolecules (Agili, 2024). However, the biological activity reported for one thiourea derivative cannot automatically be generalized to another compound within the same class. Accordingly, compound-specific computational and experimental investigations are needed to determine whether a particular derivative exhibits a meaningful interaction with a proposed molecular target.

The compound 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB) is an N-acylthiourea derivative containing a chlorine substituent at the para position of a benzamide ring. The incorporation of a halogen substituent may influence molecular electronic properties, lipophilicity, and steric characteristics, potentially affecting the interaction of a compound with a protein-binding pocket. Previous research has examined the influence of chlorine substitution

on the biological properties of selected chemical scaffolds, demonstrating that substituent effects should be evaluated in the context of the complete molecular structure (Kadayat et al., 2016; Shinada et al., 2019).

For 4-Cl-PCTB, these structural characteristics provide a basis for exploring its predicted interaction with GLS. However, the presence of a para-chloro substituent does not, by itself, establish improved binding affinity, selectivity, or anticancer activity (Chiodi & Ishihara, 2023). Demonstrating the contribution of this substituent would require comparison with appropriate structural analogues and, ultimately, experimental evaluation. Therefore, the structural features of 4-Cl-PCTB are considered a rationale for computational investigation rather than direct evidence of its pharmacological potential.

Computational methods are frequently used in early-stage drug discovery to explore potential ligand–protein interactions and prioritize compounds for further investigation (Altharawi & Alqahtani, 2026). Molecular docking predicts plausible ligand orientations within a selected protein-binding region and estimates their relative favorability using a scoring function (X. Zhang et al., 2024). The resulting poses can be examined to identify predicted contacts with amino acid residues and to generate hypotheses regarding possible molecular recognition (Di Paola et al., 2013). However, docking scores are dependent on the scoring algorithm, receptor preparation, ligand conformation, and search parameters (Yang et al., 2022). They should not be interpreted as direct measurements of binding free energy or as conclusive evidence of enzyme inhibition.

In addition to docking, computational prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) can provide preliminary information about the physicochemical and pharmacokinetic characteristics of a compound (J. Zhang et al., 2025). These predictions can help identify properties that may warrant further evaluation during compound development. Nevertheless, model outputs are subject to prediction uncertainty and applicability-domain limitations. Predicted intestinal absorption, compliance with Lipinski's Rule of Five, and negative toxicity classifications do not independently establish oral bioavailability, pharmacological efficacy, or safety (Caminero Gomes Soares et al., 2023). Combining molecular docking with ADMET prediction can therefore provide an initial computational profile of a candidate compound. In the present context, this approach is intended to investigate whether 4-Cl-PCTB has a plausible predicted interaction with GLS and to characterize selected predicted physicochemical and ADMET properties. The results should be interpreted as computational evidence that may guide subsequent research, rather than as confirmation of biological activity or therapeutic suitability.

Although thiourea and N-acylthiourea derivatives have been investigated for various biological activities, the biological properties of one derivative cannot be directly extrapolated to another without compound-specific evidence. For 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB), the contribution of the para-chloro substituent to molecular recognition and biological activity remains undetermined because a direct comparison with structurally related analogues is not available in the present study. Therefore, its potential interaction with a cancer-related molecular target should be regarded as an exploratory question rather than an established mechanism.

Molecular docking and computational ADMET prediction can provide preliminary information for prioritising compounds for further investigation. However, docking scores are dependent on the computational protocol and do not establish experimental binding affinity or enzyme inhibition. Likewise, predicted pharmacokinetic and toxicity-related properties do not demonstrate actual bioavailability or safety. These limitations are particularly important when interpreting computational findings in the context of triple-negative breast cancer (TNBC), for which biological activity and target dependence require experimental confirmation.

Accordingly, this study aimed to conduct an exploratory in silico assessment of 4-Cl-PCTB by examining its predicted interaction with glutaminase (GLS), using the protein

structure identified as PDB ID 8JUB, and summarising its predicted physicochemical and ADMET properties. The study was intended to generate a preliminary hypothesis for further investigation, rather than to establish GLS inhibition, TNBC selectivity, or anticancer efficacy. The findings should therefore be interpreted within the limitations of the available computational data and require experimental validation.

METHOD

Computational Tools and Ligand Preparation

The computational analysis was performed using a computer equipped with an Intel Core i7 processor, 16 GB RAM, and Windows 10 Education 64-bit. The molecular structure of 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB) was obtained from PubChem in SMILES format. The structure was converted into a three-dimensional representation using Chem3D version 16.0 and saved in MOL2/SYBYL2 format for molecular docking using Molegro Virtual Docker (MVD) version 7.0.

Protein Structure and Molecular Docking

Molecular docking was performed using Molegro Virtual Docker version 7.0 with the protein structure identified as glutaminase (GLS), PDB ID 8JUB, obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). According to the original docking procedure, the crystallographic ligand was used as a reference for identifying the binding cavity. The native ligand was removed before docking the test compound.

The predicted docking poses were evaluated using the scoring functions available in MVD. The resulting Rerank Score was treated as a computational scoring output rather than an experimentally measured binding free energy. The docking results were interpreted as exploratory predictions of possible ligand accommodation within the selected protein-binding region and were not considered direct evidence of GLS inhibition or anticancer activity.

The available methodological records did not provide sufficient information to independently establish all receptor-preparation settings, search parameters, and docking-validation procedures. Accordingly, no unverified parameter values were assigned in the present analysis.

Analysis of Predicted Ligand–Protein Interactions

The predicted interactions between 4-Cl-PCTB and GLS were assessed from the available molecular docking outputs. Residue-level interactions were considered computational predictions and were interpreted qualitatively. Interactions for which the residue identity or geometry could not be verified were not treated as established findings.

The original report included a proposed water-mediated interaction. However, because the original preparation description stated that water molecules were removed, this interaction was not retained in the revised interpretation without independent confirmation that the relevant water molecule was present in the analyzed receptor structure.

The reported RMSD value of 0.693 Å was not used as definitive evidence of docking validation because the available records did not establish the reference ligand, redocking procedure, and pose comparison needed to verify the result.

Prediction of Physicochemical and ADMET Properties

The physicochemical properties and predicted absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of 4-Cl-PCTB were assessed using SwissADME and pkCSM, respectively. The compound structure in SMILES format was used as the input for computational prediction.

The reported descriptors included molecular weight, lipophilicity (LogP), hydrogen-bond donor and acceptor counts, and predicted human intestinal absorption, together with distribution, metabolism, and toxicity endpoints available from the respective platforms. The reported molecular weight, LogP, and predicted human intestinal absorption were 290.77 Da, 2.73, and 88.309%, respectively.

The computational predictions were interpreted as estimates rather than experimentally measured pharmacokinetic or toxicological properties. Results were not considered proof of oral bioavailability, metabolic stability, or safety.

Data Interpretation

The findings were summarized descriptively based on the available docking score and computational ADMET predictions. The interpretation was restricted to the results that could be supported by the available records. Because experimental binding, enzyme-inhibition, and cellular-assay data were not available, no definitive conclusions were drawn regarding GLS inhibition, selective activity against triple-negative breast cancer, or anticancer efficacy.

RESULTS AND DISCUSSION

Predicted Physicochemical Properties

The chemical structure of 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB) is shown in Figure 1, and its reported physicochemical descriptors are summarized in Table 1. The reported molecular weight (290.77 Da), LogP (2.73), and hydrogen-bond donor count (2) are below conventional Lipinski thresholds. Lipinski et al. (2001) developed these criteria as empirical indicators of potential absorption or permeation difficulties, rather than as sufficient evidence of oral drug performance. Molecular flexibility and polar surface area are also informative in compound prioritization (Veber et al., 2002); these descriptors were not presented in the available results.

The numerical descriptors are consistent with a preliminary drug-likeness screening profile, but this interpretation remains conditional. SwissADME integrates property estimation and medicinal-chemistry assessment, and different lipophilicity estimators can lead to distinct values (Daina et al., 2017). In particular, the iLOGP model illustrates that computed partitioning depends on the underlying estimation approach (Daina et al., 2014). The original narrative and Table 1 are inconsistent concerning the hydrogen-bond acceptor count, reported as one in the table; this value must be checked against the input structure and original output before any final structural inference.

A compound that satisfies rule-based thresholds does not necessarily exhibit useful pharmacokinetics or pharmacological activity. Bickerton et al. (2012) showed that drug-likeness assessment benefits from considering the balance of molecular attributes rather than treating binary rules as proof of development suitability. Structural-alert strategies have likewise been proposed as additional early-stage screens (Brenk et al., 2008; Baell & Holloway, 2010), although neither such analysis nor experimental assay-interference testing was performed here. The reported physicochemical profile therefore supports only further characterization of 4-Cl-PCTB, not a conclusion about its anticancer efficacy.

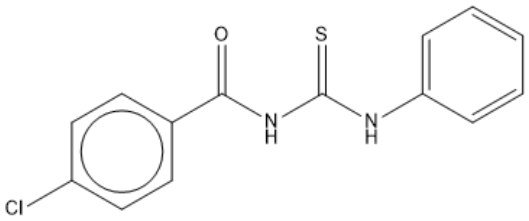


Figure 1. Chemical structure of 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB)

Table 1. Reported Physicochemical Descriptors of 4-Cl-PCTB

Compound Name	Molecular Weight (<500 Da)	Log P (< 5)	Hidrogen Bond		Lipinski's Rule of Five
			Donor (< 5)	Acceptor (< 10)	
4-Cl_PCTB	290.77	2.73	2	1	fullfill

Predicted ADMET Properties

Table 2 presents the reported pkCSM predictions for 4-Cl-PCTB, including water solubility of -4.39 log mol/L, Caco-2 permeability of 1.701 (reported log Papp), and human intestinal absorption of 88.309%. pkCSM is a structure-based prediction platform that estimates ADMET endpoints from molecular signatures, and its outputs should be treated as model-derived estimates rather than measured properties (Pires et al., 2015). The absorption prediction is compatible with a computational hypothesis of intestinal uptake, but it does not establish oral bioavailability. Passive permeability and oral absorption can depend on several molecular and physiological factors beyond one predicted value (Egan et al., 2000; Zhao et al., 2002).

Table 2. Reported Predicted ADMET Properties of 4-Cl-PCTB from The Original pkCSM analysis

Category	Predictor	Unit	Predicted Value
Absorption	Water solubility	log mol/L	-4.39
	Caco2 permeability	log Papp in 10^{-6} cm/s	1.701
	Intestinal absorption (human)	% Absorbed	88.309
	Skin Permeability	log Kp	-2.801
	P-glycoprotein substrate	Yes/No	Yes
	P-glycoprotein I inhibitor	Yes/No	No
	P-glycoprotein II inhibitor	Yes/No	No
Distribution	VDss (human)	log L/kg	-0.196
	Fraction unbound (human)	Was	0.016
	BBB permeability	log BB	0.415
	CNS permeability	log PS	-1.042
Metabolism	CYP2D6 substrate	Yes/No	No
	CYP3A4 substrate	Yes/No	Yes
	CYP1A2 inhibitor	Yes/No	Yes
	CYP2C19 inhibitor	Yes/No	Yes
	CYP2C9 inhibitor	Yes/No	No
	CYP2D6 inhibitor	Yes/No	No
	CYP3A4 inhibitor	Yes/No	No
Excretion	Total Clearance	log ml/min/kg	-0.419
	Renal OCT2 substrate	Yes/No	No
Toxicity	AMES toxicity	Yes/No	No
	Max. tolerated dose (human)	log mg/kg/day	0.197
	hERG I inhibitor	Yes/No	No
	hERG II inhibitor	Yes/No	No
	Oral Rat Acute Toxicity (LD50)	mol/kg	2.135
	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg_bw/day	1.886
	Hepatotoxicity	Yes/No	No
	Skin Sensitisation	Yes/No	No
	T.Pyriformis toxicity	log ug/L	1.305
	Minnow toxicity	log mM	0.533

The reported distribution outputs comprise VDss of -0.196 log L/kg, blood-brain barrier permeability (logBB) of 0.415, and central nervous system permeability (logPS) of -1.042. These describe different predicted endpoints and cannot be substituted for each other. Established computational frameworks distinguish gastrointestinal absorption predictions from estimates of central nervous system penetration (Daina & Zoete, 2016). Moreover, brain exposure is affected by a combination of physicochemical and transport processes (Hitchcock & Pennington, 2006). Consequently, these values should not be described as experimentally demonstrated tissue distribution or brain penetration.

The categorical outputs identify 4-Cl-PCTB as a predicted P-glycoprotein substrate and CYP3A4 substrate, with predicted inhibition of CYP1A2 and CYP2C19 but not CYP2D6,

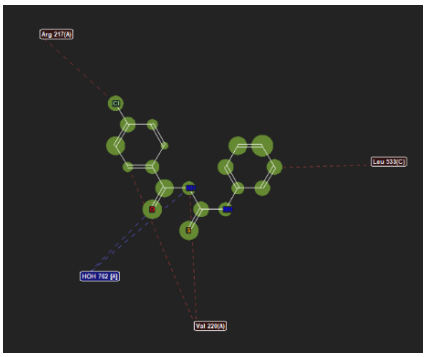
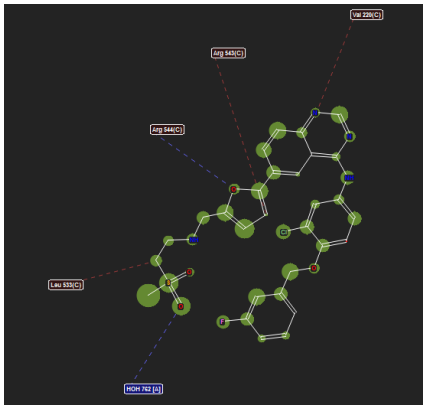
CYP2C9, or CYP3A4. These are screening alerts rather than confirmed mechanisms. CYP inhibition is important in assessing possible drug-drug interactions, but clinical implications require exposure information and appropriate experimental evaluation (Gajula et al., 2021). The original narrative reportedly conflicted with the tabulated CYP2D6 result; the table indicates no predicted CYP2D6 inhibition, and the unarchived pkCSM output prevents independent confirmation.

For toxicity, the reported negative AMES, hepatotoxicity, hERG I/II, and skin-sensitization predictions should not be interpreted as proof of safety. The importance and limitations of in silico hERG assessment have been demonstrated in structure-based benchmarking (Creanza et al., 2021) and integrated high-throughput prediction studies (Krishna et al., 2022). False-negative classifications remain possible, and experimental testing is essential for cardiac safety assessment. Additionally, the original units and definitions of the acute and chronic toxicity endpoints require verification before numerical interpretation. Overall, Table 2 provides a preliminary prioritization profile, not an experimentally validated pharmacokinetic or toxicological characterization.

Molecular Docking Results

The reported docking analysis against the protein identified as GLS (PDB ID: 8JUB) yielded Molegro Virtual Docker Rerank Scores of -78.023 for 4-Cl-PCTB and -85.728 for lapatinib (Table 3). The MolDock framework employs a docking scoring function and a subsequent reranking step to prioritize calculated poses (Thomsen & Christensen, 2006). These scores are software-dependent ranking outputs; without an established calibration they should not be expressed as measured binding free energies. Indeed, the table header reports kcal/mol whereas the narrative explicitly declines to assign an energy unit, an inconsistency that should be corrected.

Table 3. Reported MVD Rerank Scores for 4-Cl-PCTB and lapatinib in the original docking analysis

Name	Re-rank Score (kcal/mol)	Interaction
4-Cl-PCTB	-78.023	
Lapatinib	-85.728	

The more negative score obtained for lapatinib does not indicate greater GLS inhibition. Docking scores are sensitive to the scoring model, preparation choices, binding site, and search

implementation, and assessments have documented limits in the accuracy of protein-ligand ranking (Warren et al., 2006; Paggi et al., 2024). Distinct software architectures also generate scores that should not be compared without a shared validated benchmark; for example, AutoDock Vina was specifically designed with its own search and scoring framework (Trott & Olson, 2010). The present comparison is further limited because lapatinib was not established as a GLS-specific reference inhibitor.

The reported RMSD of 0.693 Å could be informative if it resulted from a correctly documented native-ligand redocking experiment, but the identity of the reference ligand, its crystallographic pose, and the alignment protocol have not been established. Therefore, this number cannot independently validate the docking procedure. Molecular docking is best treated as a hypothesis-generating structural method, with predictions evaluated against suitable reference structures and experiments (Ferreira et al., 2015). Interpretation of predicted residue contacts is also constrained because atom-level geometries were not independently verified, and a proposed water-mediated contact was not reconciled with the stated removal of waters during receptor preparation.

A biologically more relevant comparator would be a validated GLS-active reference compound accompanied by measured inhibition data. For example, Gross et al. (2014) reported biochemical and cellular evidence for the glutaminase inhibitor CB-839 in experimental triple-negative breast cancer models. Those findings establish a useful precedent for the types of experiments needed in this field, but they do not demonstrate that 4-Cl-PCTB binds or inhibits GLS. The present Rerank Scores therefore support further computational testing only, with no inference of GLS potency, TNBC selectivity, or anticancer efficacy.

Study Limitations and Implications

The physicochemical, ADMET, and docking findings describe an exploratory computational assessment of 4-Cl-PCTB, but several constraints limit their interpretation. The original SwissADME and pkCSM exports were unavailable for independent verification, including the hydrogen-bond acceptor discrepancy and selected ADMET categories. As predictive models depend on their training data, molecular descriptors, and applicability domains, agreement between model outputs and actual compound behavior should not be presumed (Daina et al., 2017; Pires et al., 2015).

The receptor preparation, search parameters, reference-ligand identity, and redocking procedure were not fully recoverable. This prevents a reproducible validation of the RMSD and limits interpretation of ligand poses and relative scores. General docking benchmarks underscore the importance of evaluating pose reproduction and compound ranking as distinct tasks (Warren et al., 2006; Paggi et al., 2024). Accordingly, the available computational evidence is insufficient to demonstrate a stable or specific GLS interaction.

No biochemical GLS inhibition, target-engagement, cellular viability, selectivity, or in vivo efficacy data are reported for 4-Cl-PCTB. Prior experimental work with a selective glutaminase inhibitor shows that target inhibition and anticancer phenotypes can be investigated with complementary metabolic, cellular, and animal assays (Gross et al., 2014); such evidence is absent for the test compound. Future work should first reconstruct a reproducible docking protocol with a justified GLS-active comparator, recheck the source ADMET exports and structural descriptors, then undertake GLS inhibition and appropriate cellular assays. The present findings should be described as computational hypotheses rather than evidence of anticancer activity.

CONCLUSION

This study provides an exploratory in silico assessment of 4-chloro-N-(phenylcarbamothioyl)-benzamide (4-Cl-PCTB) through molecular docking against glutaminase (GLS; PDB ID 8JUB) and prediction of its physicochemical and ADMET properties. The original analysis reported an MVD Rerank Score of −78.023 for 4-Cl-PCTB

and predicted human intestinal absorption of 88.309%. These findings represent computational outputs and should not be interpreted as evidence of experimentally confirmed binding affinity, GLS inhibition, oral bioavailability, or safety. The comparison with lapatinib does not establish relative GLS inhibitory activity because lapatinib was not used as a validated GLS-specific reference inhibitor. Furthermore, limitations in the available docking documentation, the unverified RMSD result, inconsistencies in the reported ADMET data, and the unresolved water-mediated interaction restrict the mechanistic interpretation of the findings. Therefore, the present results support only a preliminary computational hypothesis and do not establish anticancer efficacy or selectivity against triple-negative breast cancer. Further studies using a reproducible docking protocol, a justified GLS-active reference compound, biochemical enzyme-inhibition assays, and appropriate cellular experiments are required to determine the biological relevance of the predicted interactions.

RECOMMENDATION

Further studies should verify the computational predictions using a reproducible molecular docking protocol and a validated GLS inhibitor as a reference compound. Experimental GLS inhibition assays, followed by appropriate cellular, pharmacokinetic, and toxicity studies, are needed to determine the biological activity, selectivity, and potential therapeutic relevance of 4-Cl-PCTB as an anticancer candidate.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support and guidance of their academic supervisors and colleagues throughout this research. The authors also thank their institution for the support provided during the completion of this study.

FUNDING INFORMATION

This research received no external funding

AUTHOR CONTRIBUTIONS STATEMENT

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Ida Bagus Oka W. Andhika	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	
Dini Kesuma		✓				✓		✓	✓	✓	✓	✓		
Aguslina Kirtishanti	✓		✓	✓			✓			✓	✓		✓	✓

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest

ETHICAL APPROVAL

This study used established cell lines and did not involve human participants or live animals.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Agili, F. A. (2024). Biological applications of thiourea derivatives: Detailed review. *Chemistry*, *6*(3), 435–468. https://doi.org/10.3390/chemistry6030025

Altharawi, A., & Alqahtani, S. M. (2026). Integrative computational chemistry approaches in modern drug discovery: Advances in docking, pharmacophore modeling, molecular dynamics, and virtual screening. *Pharmaceutics*, *18*(5), 565. https://doi.org/10.3390/pharmaceutics18050565

Baell, J. B., & Holloway, G. A. (2010). New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *Journal of Medicinal Chemistry*, *53(7), 2719–2740*. https://doi.org/10.1021/jm901137j

Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S., & Hopkins, A. L. (2012). Quantifying the chemical beauty of drugs. *Nature Chemistry*, *4(2), 90–98*. https://doi.org/10.1038/nchem.1243

Bou Zerdan, M., Ghorayeb, T., Saliba, F., Allam, S., Bou Zerdan, M., Yaghi, M., Bilani, N., Jaafar, R., & Nahleh, Z. (2022). Triple negative breast cancer: Updates on classification and treatment in 2021. *Cancers*, *14*(5), 1–21. https://doi.org/10.3390/cancers14051253

Brenk, R., Schipani, A., James, D., Krasowski, A., Gilbert, I. H., Frearson, J., & Wyatt, P. G. (2008). Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. *ChemMedChem*, *3(3), 435–444*. https://doi.org/10.1002/cmdc.200700139

Caminero Gomes Soares, A., Marques Sousa, G. H., Calil, R. L., & Goulart Trossini, G. H. (2023). Absorption matters: A closer look at popular oral bioavailability rules for drug approvals. *Molecular Informatics*, *42*(11), 1–12. https://doi.org/10.1002/minf.202300115

Chiodi, D., & Ishihara, Y. (2023). "Magic chloro": Profound effects of the chlorine atom in drug discovery. *Journal of Medicinal Chemistry*, *66*(8), 5305–5331. https://doi.org/10.1021/acs.jmedchem.2c02015

Creanza, T. M., Delre, P., Ancona, N., Lentini, G., Saviano, M., & Mangiatordi, G. F. (2021). Structure-based prediction of hERG-related cardiotoxicity: A benchmark study. *Journal of Chemical Information and Modeling*, *61(9), 4758–4770*. https://doi.org/10.1021/acs.jcim.1c00744

Daina, A., & Zoete, V. (2016). A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, *11(11), 1117–1121*. https://doi.org/10.1002/cmdc.201600182

Daina, A., Michielin, O., & Zoete, V. (2014). iLOGP: A simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. *Journal of Chemical Information and Modeling*, *54(12), 3284–3301*. https://doi.org/10.1021/ci500467k

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7, Article 42717*. https://doi.org/10.1038/srep42717

Di Paola, L., De Ruvo, M., Paci, P., Santoni, D., & Giuliani, A. (2013). Protein contact networks: An emerging paradigm in chemistry. *Chemical Reviews*, *113*(3), 1598–1613. https://doi.org/10.1021/cr3002356

Egan, W. J., Merz, K. M., Jr., & Baldwin, J. J. (2000). Prediction of drug absorption using multivariate statistics. *Journal of Medicinal Chemistry*, *43(21), 3867–3877*. https://doi.org/10.1021/jm000292e

Ferreira, L. G., dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, *20(7), 13384–13421*. https://doi.org/10.3390/molecules200713384

Filho, A. M., Laversanne, M., Ferlay, J., Colombet, M., Piñeros, M., Znaor, A., Parkin, D. M., Soerjomataram, I., & Bray, F. (2025). The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. *International Journal of Cancer*, *156*(7), 1336–1346. https://doi.org/10.1002/ijc.35278

Freihat, O., Sipos, D., & Kovacs, A. (2025). Global burden and projections of breast cancer incidence and mortality to 2050: a comprehensive analysis of GLOBOCAN data. *Frontiers in Public Health*, *13*(October), 1–12. https://doi.org/10.3389/fpubh.2025.1622954

Gajula, S. N. R., Pillai, M. S., Samanthula, G., & Sonti, R. (2021). Cytochrome P450 enzymes: A review on drug metabolizing enzyme inhibition studies in drug discovery and development. *Bioanalysis*, *13(17), 1355–1378*. https://doi.org/10.4155/bio-2021-0132

Gross, M. I., Demo, S. D., Dennison, J. B., Chen, L., Chernov-Rogan, T., Goyal, B., Janes, J. R., Laidig, G. J., Lewis, E. R., Li, J., Mackinnon, A. L., Parlati, F., Rodriguez, M. L. M., Shwonek, P. J., Sjogren, E. B., Stanton, T. F., Wang, T., Yang, J., Zhao, F., & Bennett,

M. K. (2014). Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Molecular Cancer Therapeutics*, *13(4), 890–901*. https://doi.org/10.1158/1535-7163.MCT-13-0870

Gumus, I., Solmaz, U., Binzet, G., Keskin, E., Arslan, B., & Arslan, H. (2019). Supramolecular self-assembly of new thiourea derivatives directed by intermolecular hydrogen bonds and weak interactions: crystal structures and Hirshfeld surface analysis. *Research on Chemical Intermediates*, *45*(2), 169–198. https://doi.org/10.1007/s11164-018-3596-5

Hitchcock, S. A., & Pennington, L. D. (2006). Structure–brain exposure relationships. *Journal of Medicinal Chemistry*, *49(26), 7559–7583*. https://doi.org/10.1021/jm060642i

Jin, J., Byun, J. K., Choi, Y. K., & Park, K. G. (2023). Targeting glutamine metabolism as a therapeutic strategy for cancer. *Experimental and Molecular Medicine*, *55*(4), 706–715. https://doi.org/10.1038/s12276-023-00971-9

Kadayat, T. M., Park, S., Jun, K. Y., Magar, T. B. T., Bist, G., Shrestha, A., Na, Y., Kwon, Y., & Lee, E. S. (2016). Effect of chlorine substituent on cytotoxic activities: Design and synthesis of systematically modified 2,4-diphenyl-5H-indeno[1,2-b]pyridines. *Bioorganic and Medicinal Chemistry Letters*, *26*(7), 1726–1731. https://doi.org/10.1016/j.bmcl.2016.02.053

Krishna, S., Borrel, A., Huang, R., Zhao, J., Xia, M., & Kleinstreuer, N. (2022). High-throughput chemical screening and structure-based models to predict hERG inhibition. *Biology*, *11(2), Article 209*. https://doi.org/10.3390/biology11020209

Kumar, M. A., Baba, S. K., Khan, I. R., Khan, M. S., Husain, F. M., Ahmad, S., Haris, M., Singh, M., Akil, A. S. A. S., Macha, M. A., & Bhat, A. A. (2025). Glutamine metabolism: Molecular regulation, biological functions, and diseases. *MedComm*, *6*(7), 1–32. https://doi.org/10.1002/mco2.70120

Lampa, M., Arlt, H., He, T., Ospina, B., Reeves, J., Zhang, B., Murtie, J., Deng, G., Barberis, C., Hoffmann, D., Cheng, H., Pollard, J., Winter, C., Richon, V., Garcia-Escheverria, C., Adrian, F., Wiederschain, D., & Srinivasan, L. (2017). Glutaminase is essential for the growth of triple-negative breast cancer cells with a deregulated glutamine metabolism pathway and its suppression synergizes with mTOR inhibition. *PLoS ONE*, *12*(9). https://doi.org/10.1371/journal.pone.0185092

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *46(1–3), 3–26*. https://doi.org/10.1016/S0169-409X(00)00129-0

Matés, J. M., Campos-Sandoval, J. A., de los Santos-Jiménez, J., & Márquez, J. (2020). Glutaminases regulate glutathione and oxidative stress in cancer. *Archives of Toxicology*, *94*(8), 2603–2623. https://doi.org/10.1007/s00204-020-02838-8

Paggi, J. M., Pandit, A., & Dror, R. O. (2024). The art and science of molecular docking. *Annual Review of Biochemistry*, *93, 389–410*. https://doi.org/10.1146/annurev-biochem-030222-120000

Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, *58(9), 4066–4072*. https://doi.org/10.1021/acs.jmedchem.5b00104

Shinada, N. K., De Brevern, A. G., & Schmidtke, P. (2019). Halogens in protein-ligand binding mechanism: A structural perspective. *Journal of Medicinal Chemistry*, *62*(21), 9341–9356. https://doi.org/10.1021/acs.jmedchem.8b01453

Tavčar Kunstič, T., Debeljak, N., & Fon Tacer, K. (2023). Heterogeneity in hormone-dependent breast cancer and therapy: Steroid hormones, HER2, melanoma antigens, and cannabinoid receptors. *Advances in Cancer Biology - Metastasis*, *7*(December 2022). https://doi.org/10.1016/j.adcanc.2022.100086

Thomsen, R., & Christensen, M. H. (2006). MolDock: A new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry*, *49(11), 3315–3321*. https://doi.org/10.1021/jm051197e

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31(2), 455–461*. https://doi.org/10.1002/jcc.21334

Veber, D. F., Johnson, S. R., Cheng, H.-Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, *45(12), 2615–2623*. https://doi.org/10.1021/jm020017n

Warren, G. L., Andrews, C. W., Capelli, A.-M., Clarke, B., LaLonde, J., Lambert, M. H., Lindvall, M., Nevins, N., Semus, S. F., Senger, S., Tedesco, G., Wall, I. D., Woolven, J. M., Peishoff, C. E., & Head, M. S. (2006). A critical assessment of docking programs and scoring functions. *Journal of Medicinal Chemistry*, *49(20), 5912–5931*. https://doi.org/10.1021/jm050362n

Xiong, X., Zheng, L. W., Ding, Y., Chen, Y. F., Cai, Y. W., Wang, L. P., Huang, L., Liu, C. C., Shao, Z. M., & Yu, K. D. (2025). Breast cancer: Pathogenesis and treatments. *Signal Transduction and Targeted Therapy*, *10*(1). https://doi.org/10.1038/s41392-024-02108-4

Yang, C., Chen, E. A., & Zhang, Y. (2022). Protein–ligand docking in the machine-learning era. *Molecules*, *27*(14), 4568. https://doi.org/10.3390/molecules27144568

Yoo, H. C., Yu, Y. C., Sung, Y., & Han, J. M. (2020). Glutamine reliance in cell metabolism. *Experimental and Molecular Medicine*, *52*(9), 1496–1516. https://doi.org/10.1038/s12276-020-00504-8

Zhang, J., Li, H., Zhang, Y., Huang, J., Ren, L., Zhang, C., Zou, Q., & Zhang, Y. (2025). Computational toxicology in drug discovery: applications of artificial intelligence in ADMET and toxicity prediction. *Briefings in Bioinformatics*, *26*(5). https://doi.org/10.1093/bib/bbaf533

Zhang, X., Shen, C., Zhang, H., Kang, Y., Hsieh, C. Y., & Hou, T. (2024). Advancing ligand docking through deep learning: Challenges and prospects in virtual screening. *Accounts of Chemical Research*, *57*(10), 1500–1509. https://doi.org/10.1021/acs.accounts.4c00093

Zhao, Y. H., Abraham, M. H., Le, J., Hersey, A., Luscombe, C. N., Beck, G., Sherborne, B., & Cooper, I. (2002). Rate-limited steps of human oral absorption and QSAR studies. *Pharmaceutical Research*, *19(10), 1446–1457*. https://doi.org/10.1023/A:1020444330011

Zhu, L., Zhu, X., & Wu, Y. (2022). Effects of glucose metabolism, lipid metabolism, and glutamine metabolism on tumor microenvironment and clinical implications. *Biomolecules*, *12*(4). https://doi.org/10.3390/biom12040580