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Molecular Docking Analysis of Soft Coral (*Rhytisma* sp.) Derived Compounds as EGFR Inhibitors in Non-Small Cell Lung Cancer (NSCLC)

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Abstract: The epidermal growth factor receptor (EGFR) is a crucial target in the treatment of non-small cell lung cancer (NSCLC) due to its significant role in tumor cell proliferation and survival. This study investigates the potential of marine-derived compounds from soft coral (Rhytisma sp.) as EGFR inhibitors through molecular docking and pharmacokinetic predictions. Eight compounds identified via GC-MS were docked against the EGFR tyrosine kinase domain (PDB ID: 2ITY) using AutoDock Vina, with Gefitinib serving as the reference drug. The top-performing compounds exhibited binding affinities ranging from –7.0 to –7.4 kcal/mol, closely aligning with Gefitinib's affinity of –7.6 kcal/mol. Notably, 4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.0^{4,6}]dodecane demonstrated the strongest interactions, involving critical residues such as MET793 and LYS745. Pharmacokinetic profiling conducted with SwissADME and pkCSM confirmed favorable drug-likeness and high absorption potential. Toxicity analysis using ProTox-3.0 indicated low toxicity (Class IV) and no predicted hepatotoxicity, carcinogenicity, or mutagenicity. These findings suggest that phytocompounds derived from Rhytisma sp., particularly terpenoid-based structures, present a promising scaffold for the development of EGFR-targeted anticancer drugs.
Keywords: EGFR, *Rhytisma* sp., molecular docking, NSCLC, marine natural products

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INTRODUCTION

Cancer is one of the leading causes of death worldwide, affecting individuals of all sexes, age groups, and income levels (Torre et al., 2016). Among various types of cancer, lung cancer remains the leading cause of cancer-related mortality globally. From a histological perspective, lung cancer is classified into two main categories: non-small cell lung cancer (NSCLC), which accounts for approximately 85% of cases, and small cell lung cancer (SCLC), representing about 15% (Giaccone & He, 2023). The five-year survival rate for lung cancer patients remains low, at around 15%. Each year, lung cancer is responsible for approximately 1.6 million deaths worldwide. Advanced age and tobacco use are the predominant risk factors, with smoking contributing to nearly 90% of cases. Other risk factors include environmental exposures, prior radiation therapy, racial and ethnic background, and genetic predisposition, as indicated by a family history of the disease (Thandra et al., 2021). Despite advances

in treatment, lung cancer continues to pose a significant global health challenge; therefore, more effective therapeutic strategies are urgently needed.

The epidermal growth factor receptor (EGFR) is one of the most well-established therapeutic targets in cancer treatment. It is a receptor tyrosine kinase that plays a crucial role in regulating cell proliferation, survival, and differentiation. Aberrant activation of EGFR signaling has been strongly associated with the development and progression of several cancers, particularly non-small cell lung cancer (NSCLC). In NSCLC, specific mutations in the EGFR kinase domain, such as L858R and G719S, lead to constitutive activation of the receptor, thereby promoting uncontrolled cell growth. These mutations also influence the sensitivity of cancer cells to EGFR-targeted therapies, making EGFR a critical target for the development of targeted treatments in NSCLC (Yun et al., 2007; Zubair & Bandyopadhyay, 2023)

Gefitinib (Iressa) is a cancer drug that works by blocking the epidermal growth factor receptor (EGFR). This prevents the EGFR from signaling, which stops tumor growth and cell proliferation. Gefitinib is used to treat non-small cell lung cancer (NSCLC) that has spread into the surrounding tissues or to other parts of the body. It's only effective in cancers with mutated and overactive EGFR (Cui et al., 2023; Liu & Seen, 2003). Gefitinib (PubChem CID: 123631). Several studies have demonstrated the potential of natural compounds as effective EGFR inhibitors using in-silico approaches. A study by Yousaf et al. (2024) employed molecular docking and dynamic simulations to evaluate bioactive compounds derived from Moringa oleifera against EGFR. Among the tested phytochemicals, quercetin and kaempferol exhibited strong binding affinities to the EGFR tyrosine kinase domain, comparable to that of known inhibitors such as erlotinib. The results were further supported by molecular dynamics simulations and ADMET predictions, which confirmed the stability and drug-like properties of these compounds. This highlights the promising role of natural products in the development of alternative EGFR-targeted therapies for lung cancer (Yousaf et al., 2024).

Targeting EGFR has become a cornerstone in the treatment of NSCLC, particularly among patients harboring EGFR mutations (Rasyid et al., 2021). Natural products have long been recognized as an important source of bioactive compounds for drug discovery. In particular, marine organisms represent a rich and relatively underexplored reservoir of unique chemical structures with potential pharmacological activities, including anticancer properties (Carroll et al., 2025; Nurisyah et al., 2024; Roney et al., 2025; Erdogan & Erdogan, 2025). Previous studies have reported that several natural compounds demonstrate promising inhibitory activity against EGFR through molecular docking and computational approaches (Saini et al., 2022; Yousaf et al., 2024). These findings suggest that marine-derived compounds may serve as potential candidates for the development of new EGFR-targeted therapies (Li et al., 2024). Therefore, this study aims to evaluate the potential inhibitory activity of bioactive compounds derived from *Lobophyllia* sp. against EGFR mutations (L858R and G719S) using an in silico molecular docking approach. By analyzing the binding affinity and interaction profiles of these compounds with the EGFR receptor, this study seeks to identify promising natural compounds that may serve as potential candidates for future development of anti-lung cancer agents.

METHOD

The method used in this research is molecular docking. First, we bound the ligand to the target protein, and then we observed the amount of energy and the interactions

formed between the ligand and the protein. Before carrying out molecular docking, compounds and proteins must be prepared first.

Protein/Macromolecule

The targeted protein in this research is EGFR (Epidermal Growth Factor Receptor) Receptor tyrosine kinase (PDB: 2ITY). The 3D structure was obtained from the Protein Data Bank (PDB) database (www.rcsb.org) using the X-ray crystallography method to determine the structure of 2ITY and has a resolution of 2.6 Å. Protein Preparation of 2ITY Using Discovery Studio Visualizer (DSV) 2025 version v25.1.0.24284, the water molecules and heteroatoms (HETATM), including the ligand IRE (Gefitinib), were removed from the structure. Hydrogen atoms were added to satisfy the valency of polar and heteroatoms.

Ligand Compound

The ligand compounds used in this research were extracted from *Rhytisma* sp. collected from Kolo Beach, Bima Regency, West Nusa Tenggara (NTB), Indonesia. To validate the results of the GC–MS analysis, we utilized the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) by cross-referencing both the CAS number and IUPAC name to identify the corresponding SMILES notation. In addition, the molecular weight and molecular formula were also synchronized with the data obtained from PubChem to ensure the accuracy of the compound identification. Swiss ADME (http://www.swissadme.ch/) used to analyze Lipinski's Rule of Five of ligand compounds. The human small intestine absorption analysis is carried out by pkCSM predictions (https://biosig.lab.uq.edu.au/pkcsm/) to determine the ability of a compound to be absorbed.

Figure 1. *Rhytisma* sp. used for extraction and GC–MS analysis, collected from Kolo Beach, Bima Regency, NTB, Indonesia

Validation of Docking Protocol

The docking protocol was validated using a redocking approach. The native ligand Gefitinib (IRE) was separated from the EGFR protein structure and then redocked into the original binding pocket. The docking protocol was considered valid if the root mean square deviation (RMSD) between the redocked ligand and the original crystallographic ligand position was less than 2.0 Å. The RMSD value obtained in this study was 1.62 Å, indicating that the docking method was reliable and capable of accurately reproducing the binding pose of the native ligand.

Molecular Docking

Molecular docking of 8 types of *Rhytisma* sp. compounds and Gefitinib as control to the 2ITY protein was carried out using PyRx v.0.8 software. The molecular binding target areas are center_x = -54.0; center_y = -5.0; center_z = -22.0 with exhaustiveness = 105 to ensure the accuracy. Coordinates for the docking grid box were defined based on the crystallized complex of EGFR with gefitinib (PDB ID: 2ITY), focusing on the active site where the ligand IRE (Gefitinib) binds. This approach ensures the docking simulations target a biologically relevant binding pocket, as previously described by Yun et al.(2008). Molecular docking was performed using PyRx 0.8, which integrates Open Babel 2.3.1 for ligand preparation and AutoDock Vina 1.1.2 for docking. Ligands were energy-minimized using the Universal Force Field (UFF) and converted to PDBQT format and binding affinities were recorded in kcal/mol. The results were visualized and analyzed using PyMol 2.5 and Discovery Studio v25. The visualization results then identify the amino acid residues that bind to the ligand compound.

Prediction of Biological Activities

Prediction of biological activities was conducted by retrieving the data and submitting it to the PASS server (http://www.way2drug.com/PASSonline). Only biological activities with Pa > 0.7 were considered relevant for further analysis, as this threshold indicates a high confidence of biological plausibility. Activities with Pa > Pi were retained and tabulated. The predicted functions were interpreted in the context of anticancer potential, particularly focusing on antineoplastic, apoptosis-related, antimetastatic, and MMP9 expression inhibition activities relevant to non-small cell lung cancer (NSCLC) pathogenesis.

Toxicity Prediction

In silico toxicity prediction of the isolated compounds from Rhytisma sp. was performed using ProTox-3.0, an updated web-based platform developed by Charité–Universitätsmedizin Berlin. This tool predicts chemical toxicity based on structural similarity, physicochemical properties, and machine learning algorithms. The evaluated parameters included oral LD_{50} (median lethal dose), toxicity class according to the Globally Harmonized System (GHS), and specific toxicity endpoints such as hepatotoxicity, carcinogenicity, and mutagenicity, using canonical SMILES as input.

The predicted oral LD_{50} values were used to estimate acute toxicity levels, where lower LD_{50} values indicate higher toxicity. Compounds were further categorized into toxicity classes (Class I–VI) based on GHS classification, ranging from highly toxic (Class I) to non-toxic (Class VI). This classification provides a systematic framework for assessing compound safety. In addition, predictions of hepatotoxicity, carcinogenicity, and mutagenicity were used to evaluate potential long-term adverse effects. These toxicity parameters are essential for early-stage drug discovery, as they help identify compounds with acceptable safety profiles and support the selection of promising candidates for further development as anticancer agents.

RESULTS AND DISCUSSION

The molecular docking analysis of compounds isolated from *Rhytisma* sp. against the 2ITY protein target (Table 1) revealed varying binding affinities. The control compound Gefitinib exhibited the highest binding affinity with a score of -7.6 kcal/mol. The tested compounds displayed binding affinities ranging from -4.6 to -7.4 kcal/mol. Following Gefitinib, the top three compounds with the highest binding affinities were 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane at -7.4 kcal/mol,

4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene Caryophyllene at -7.1 kcal/mol, and 4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol at -7.0 kcal/mol. The pharmacokinetic properties, assessed according to Lipinski's Rule of Five, indicated that some compounds had a single violation, primarily due to logP values exceeding 4.15. Nevertheless, all compounds maintained molecular weights below 500 Da, and most exhibited high Human Intestinal Absorption (HIA ≥ 80%).

Table 1. Lipinski's rule of five and docking analysis results

IUPAC Name	Molecular Formula	Mol. Weight (Da) (< 500 g/mol)	HIA (%)-(≥80%)	LogP (< 5)	H-Bond Donor (< 5)	H-Bond Acceptor (< 10)	Lipinski Violation	Binding Affinity (kcal/mol)
Gefitinib	C22H24C LFN404	446.9	High (90.992)	3.92	1	7	No	-7.6
1,5-dimethyl-8-propan-2-ylidenecyclodeca-1,5-diene	C15H24	204.35	Low (94.629)	4.6	0	0	Yes; 1 violation: MLOGP>4.15	-6.4
Pentadecan-1-ol	C15H32O	228.41	High (90.147)	5.06	1	1	Yes; 1 violation: MLOGP>4.15	-4.6
3-dodec-1-enyloxolane-2,5-dione	C16H26O3	266.38	High (94.326)	4.25	0	3	No	-5.8
4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene Caryophyllene	C15H24	204.35	Low (94.845)	4.24	0	0	Yes; 1 violation: MLOGP>4.15	-7.1
Octadeca-9,12-dienoic acid	C18H32O2	280.4	High (92.329)	5.45	1	2	Yes; 1 violation: MLOGP>4.15	-5.5
4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane	C15H24O	220.35	High (95.669)	3.68	0	1	No	-7.4
Hexadecanoic acid	C16H32O2	256.42	High (92.004)	5.2	1	2	Yes; 1 violation: MLOGP>4.15	-5.1
4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol	C15H26O	222.37	High (91.951)	3.72	1	1	No	-7

*Noted: Despite pkCSM predicted a high HIA percentage, SwissADME classified the compound with low GI absorption, suggesting potential limitations in permeability due to physicochemical properties

The molecular docking simulation was conducted to evaluate the binding affinity and interaction profiles of selected compounds derived from *Rhytisma* sp. against the EGFR tyrosine kinase domain. Gefitinib served as the reference inhibitor. Although RMSD values were not calculated, visual alignment of docked poses with the co-crystallized ligand (Gefitinib) showed similar orientation within the active site, supporting the validity of docking protocol.

The visualizations of ligand-receptor interactions are illustrated in Figure 3. Gefitinib (Control, Figure 3a) formed conventional hydrogen bonds with MET793 and ASP855, as well as π-cation interactions with LYS745, and π-alkyl interactions that contributed to binding stability. Compound b (4,12,12-trimethyl-9-methylidene-5-oxatricyclo dodecane, Figure 3b) established hydrogen bonds with MET793 and exhibited π-cation and π-alkyl interactions with residues in the active site. Compound c (4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene Caryophyllene, Figure 3c) demonstrated π-cation and π-alkyl interactions but lacked strong hydrogen bonding. Compound d (4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol) displayed a combination of conventional hydrogen bonding, π-alkyl, and π-sigma interactions, suggesting potential affinity for the EGFR active site. Each compound

exhibited unique interactions with residues within the EGFR binding pocket, indicating varying levels of binding potential.

The molecular docking simulation revealed significant interactions between the EGFR tyrosine kinase domain and the tested compounds, including the control ligand, Gefitinib. Gefitinib demonstrated a variety of interactions, such as conventional hydrogen bonds with LYS745, π-cation and π-sigma interactions with LYS745 and VAL726 respectively, π-sulfur interactions with MET766, and hydrophobic interactions involving VAL726, LEU718, and LYS745, resulting in a binding affinity of –7.6 kcal/mol. Among the tested compounds, 4,12,12-trimethyl-9-methylidene-5-oxatricyclo dodecane exhibited the highest binding affinity of –7.4 kcal/mol and engaged in alkyl interactions with VAL726, ALA743, LEU844, and LYS745, with bond distances ranging from 3.93 Å to 4.99 Å. The compound 4,11,11-trimethyl-8-methylidenebicyclo undec-4-ene formed multiple alkyl interactions involving VAL726, ALA743, LYS745, and an unidentified residue, with a slightly lower affinity of –7.1 kcal/mol. The third compound, 4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo annulen-7-ol, also interacted with VAL726, ALA743, LYS745, and LEU844, but exhibited fewer and less diverse contact types, with a binding affinity of –7.0 kcal/mol. The 3D and 2D visualizations further confirmed that all three test compounds occupied the ATP-binding pocket of EGFR and formed non-covalent interactions, primarily alkyl and π-alkyl contacts, similar in orientation to Gefitinib.

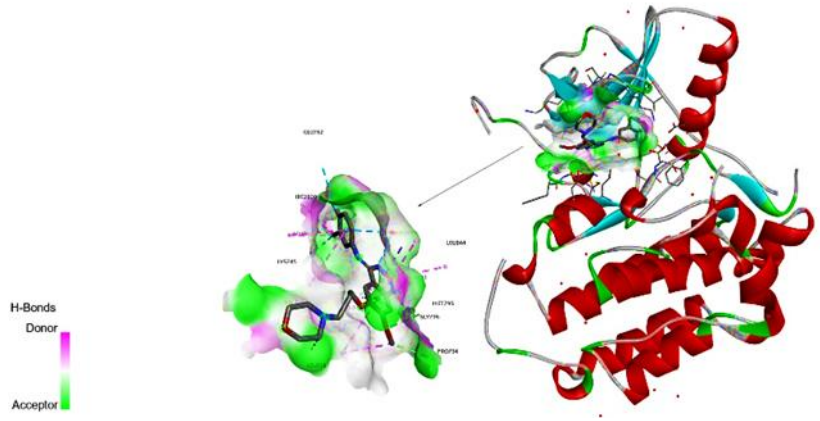

Figure 2. The visualization of EGFR Receptor tyrosine kinase (PDB: 2ITY)

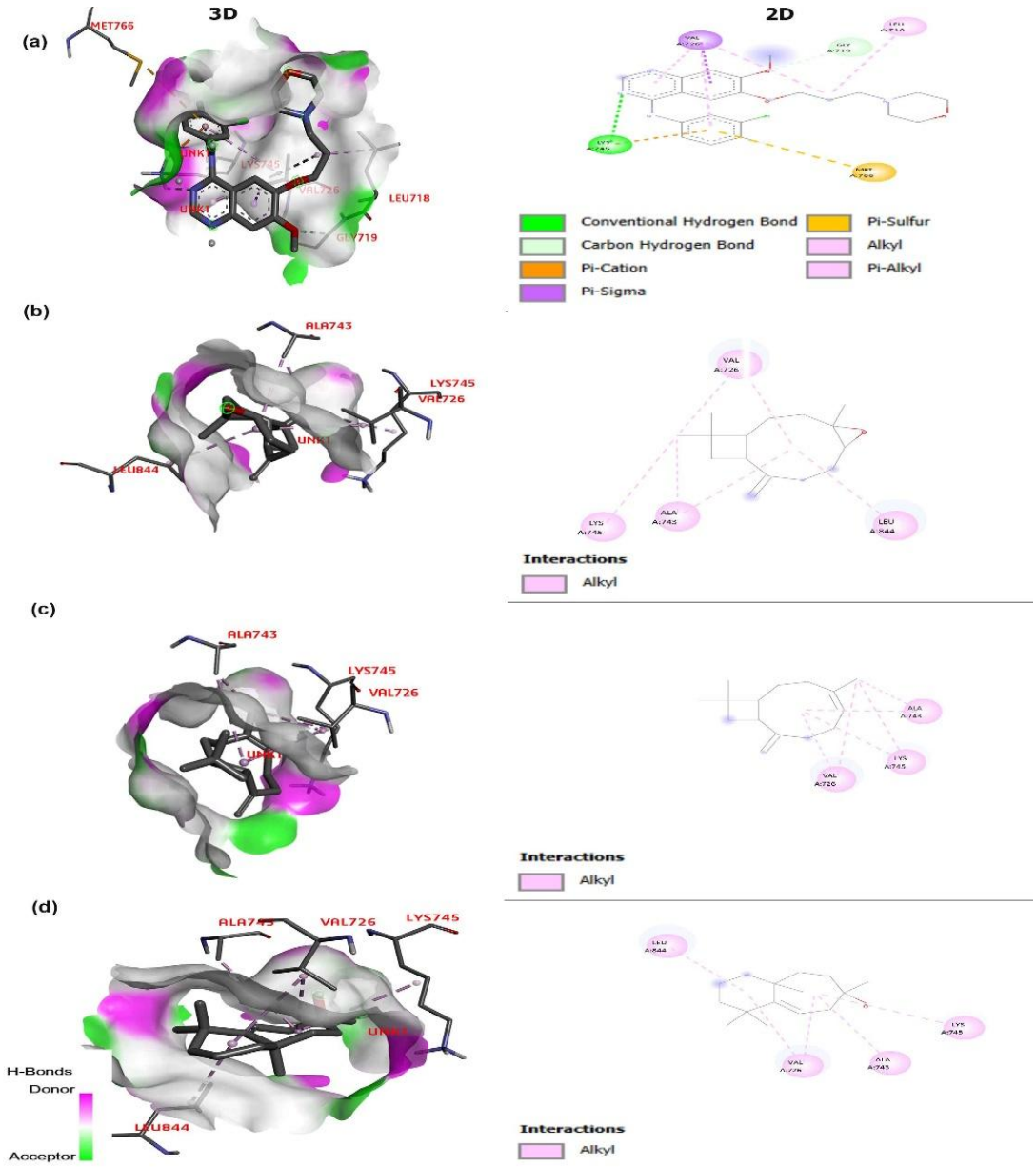

Figure 3. The 3D and 2D Visualization of molecular docking of EGFR Receptor tyrosine kinase (PDB: 2ITY). (a) Gefitinib (Control); (b) 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane; (c) 4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene Caryophyllene; and (d) 4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol.

PASS prediction analysis of the selected *Rhytisma* sp.-derived compounds revealed multiple biological activities relevant to anticancer properties (Table 3). Compound 1 (4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane) showed the highest predicted antineoplastic activity with Pa = 0.950 and apoptosis agonist activity (Pa=0.836). The compound also displayed antimetastatic potential (Pa=0.729). Compound 2 (4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene) exhibited high Pa values for general antineoplastic activity (0.915) and lung cancer-specific activity (0.763). Additionally, it showed apoptosis agonism (Pa = 0.847) and MMP9 inhibition (Pa = 0.709). Compound 3 (4,4,7,9a-tetramethyl-1,2,3,6,8,9-

hexahydrobenzo [7] annulen-7-ol) demonstrated a narrower profile with MMP9 expression inhibition as the most prominent activity (Pa = 0.733).

Table 2. Receptor-Ligand interaction validation with 3D (distances and types interaction shown and 2D Amino Acid Residue

Ligand	Type of Interaction	Amino Acid Residue	Bond Distance (Å)	Binding Affinity (kcal/mol)
Gefitinib (Kontrol)	H-Bond (Conv.)	LYS745	2.47	-7.6
	H-Bond (C–H)	GLY719	3.55	
	Pi–Cation	LYS745	3.02	
	Pi–Sigma	VAL726	3.71	
	Pi–Sulfur	MET766	5.95	
	Alkyl	**VAL726**, LEU718	4.45; 4.39	
	Pi–Alkyl	VAL726, LYS745	5.09; 5.24; 4.18	
4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane	Alkyl	**VAL726**, ALA743, LEU844, LYS745	4.52; 4.73; 3.93; 4.99; 4.09; 4.40	-7.4
4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene	Alkyl	**VAL726**, ALA743 (×2), LYS745, UNK1 (×2)	4.24; 5.30; 3.54; 5.34; 4.16; 4.30	-7.1
4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol	Alkyl	**VAL726** (×2), ALA743, LYS745, LEU844	4.61; 4.58; 5.14; 5.12; 4.52	-7

Table 4 presents the summary of predicted toxicological profiles for the three major compounds. All compounds were predicted to have an oral LD_{50} of 3700 mg/kg and were classified under toxicity class IV, indicating low acute toxicity. Additionally, none of the tested compounds showed predicted hepatotoxic, carcinogenic, or mutagenic effects. The molecular weights of the compounds ranged from 206.36 to 234.36 g/mol, suggesting that these molecules are within the drug-likeness range for small molecule.

The docking results indicate that several compounds derived from *Rhytisma* sp. exhibit potential as ligands for the 2ITY protein target, which is associated with the EGFR tyrosine kinase receptor. Although none of the tested compounds exceeded the binding affinity of Gefitinib (-7.6 kcal/mol), some demonstrated comparable binding strength, suggesting promising interaction potential. Notably, 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane emerged as a strong candidate with a binding affinity of -7.4 kcal/mol, no Lipinski violations, and high HIA, warranting further development.

Table 3. Biological activity confounds of *Rhytisma* sp. Derived Compounds as EGFR Inhibitors for Non-Small Cell Lung Cancer (NSCLC)

No	Compound	Pa	Pi	Activity Prediction	Relevance to EGFR/NSCLC
1	4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane	0.950	0.004	Antineoplastic	General anticancer
		0.836	0.006	Apoptosis agonist	Apoptosis via EGFR inhibition
		0.729	0.001	Antimetastatic	Inhibition of tumor spread
2	4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene	0.915	0.005	Antineoplastic	General anticancer
		0.763	0.005	Antineoplastic (lung cancer)	Target-specific (NSCLC)

No	Compound	Pa	Pi	Activity Prediction	Relevance to EGFR/NSCLC
		0.847	0.005	Apoptosis agonist	Apoptosis pathway activator
		0.709	0.011	MMP9 expression inhibitor	Invasion/metastasis modulation
3	4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol	0.733	0.005	MMP9 expression inhibitor	Indirect EGFR pathway modulation

Table 4. Toxicity prediction of selected compounds derived from *Rhytisma* sp.

No	Compound	BM (g/mol)	LD50 (mg/kg)	Toxicity Class	Hepato toxicity	Carcino genicity	Mutage nicity	Source
1	4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane	220.35	3700	IV	No	No	No	Essential Oil
2	4,11,11-trimethyl-8 methylidenebicyclo [7.2.0] undec-4-ene	206.36	3700	IV	No	No	No	Essential Oil
3	4,4,7,9a-tetramethyl-1,2,3,6,8,9-hexahydrobenzo [7] annulen-7-ol	234.36	3700	IV	No	No	No	Essential Oil

Conversely, compounds such as Pentadecan-1-ol and Hexadecanoic acid exhibited lower binding scores and logP violations, indicating a need for structural optimization to enhance their drug-likeness. Compounds with elevated logP values may experience reduced solubility in physiological environments; however, this issue can potentially be mitigated through appropriate pharmaceutical formulations. On the other hand, compounds free from Lipinski violations, including 3-dodec-1-enyloxolane-2,5-dione and 1,1,4a,7-Tetramethyl-benzo[a]cyclohepten-7-ol, present advantages in terms of drug-likeness and absorption potential.

3D and 2D interaction analyses reveal that Gefitinib, as anticipated, establishes strong and specific interactions within the EGFR active site, thereby validating the docking protocol. Its capacity to form hydrogen bonds with key residues MET793 and ASP855 is crucial for its high binding affinity and inhibitory function. Among the tested ligands, compound b exhibited the most comparable interaction pattern to Gefitinib. The presence of hydrogen bonds and π-interactions suggests it may replicate the binding mechanism of the control, implying potential for competitive inhibition of EGFR, making it a promising candidate for further evaluation. Compound c, while lacking hydrogen bonds, maintained hydrophobic interactions through π-cation and π-alkyl contacts. This may lead to a lower binding affinity but could still contribute to partial receptor modulation. Compound d displayed various interaction types, including hydrogen bonding and aromatic stacking (π-alkyl, π-sigma), supporting its potential as a moderate EGFR binder. The hydroxyl group in its structure likely facilitates hydrogen bonding, which is advantageous for receptor binding.

These findings suggest that marine-derived compounds from *Rhytisma* sp. particularly compound 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.04,6] dodecane may serve as promising scaffolds for the development of novel EGFR inhibitors. Further optimization and validation through in vitro assays are recommended. Additionally, the diversity of interaction types observed across the tested compounds underscores the structural versatility of *Rhytisma* sp.-derived metabolites, which could be harnessed for the rational design of EGFR-targeted

therapeutics. Structure-activity relationship (SAR) studies would be instrumental in elucidating the specific molecular features that enhance binding affinity and selectivity. Furthermore, the pharmacokinetic profiles of lead candidates should be assessed to ensure favorable ADME (Absorption, Distribution, Metabolism, and Excretion) properties, which are critical for clinical success.

The observed binding interactions within the ATP-binding pocket of EGFR suggest that the tested compounds may exert their inhibitory effects through a competitive inhibition mechanism, similar to that of known tyrosine kinase inhibitors such as gefitinib. The involvement of key residues, particularly LYS745, VAL726, and MET793, indicates that these ligands are positioned within a critical region responsible for ATP binding and kinase activation. Interactions with LYS745 are especially important, as this residue plays a central role in stabilizing ATP and facilitating phosphoryl transfer during signal transduction. Therefore, the presence of hydrophobic and π-alkyl interactions with LYS745 and surrounding residues may disrupt ATP binding, leading to inhibition of EGFR phosphorylation activity. Although hydrogen bonding was less prominent in some compounds, the dominance of hydrophobic interactions suggests that ligand binding stability may still be maintained within the hydrophobic pocket of the kinase domain. This is consistent with previous studies indicating that effective EGFR inhibitors do not solely rely on hydrogen bonding but also on optimal hydrophobic complementarity within the binding site. Consequently, compounds such as 4,12,12-trimethyl-9-methylidene-5-oxatricyclo dodecane, which exhibit strong hydrophobic interactions and favorable binding affinity, may act as potential competitive inhibitors of EGFR, thereby interfering with downstream signaling pathways involved in cell proliferation and survival in NSCLC.

CONCLUSION

This study demonstrates the potential of *Rhytisma* sp.-derived terpenoid compounds as novel EGFR inhibitors for the treatment of non-small cell lung cancer. Among the tested molecules, 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.0^{4,6}] dodecane exhibited the most promising profile, with strong EGFR binding affinity, favorable pharmacokinetics, and low predicted toxicity. The integration of docking analysis, ADME profiling, and toxicity prediction provides a comprehensive in silico framework that supports the advancement of marine natural products in targeted cancer therapy.

RECOMMENDATION

Further studies are recommended to validate the therapeutic potential of Rhytisma sp.-derived terpenoid compounds, particularly 4,12,12-trimethyl-9-methylidene-5-oxatricyclo [8.2.0.0^{4,6}] dodecane, through in vitro and in vivo approaches. Additional investigations should focus on confirming its EGFR inhibitory activity, anticancer efficacy against non-small cell lung cancer, and safety profile. Molecular dynamics simulations and structural optimization are also suggested to support its development as a potential targeted therapeutic agent.

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