



### Original Article

## In Silico Study of the Quantitative Structure-Activity Relationship of Pelargonidin Compounds from Barley (*Hordeum vulgare*) as EGFR Inhibitors in Lung Cancer

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### Abstract

Mutations in the epidermal growth factor receptor (EGFR) contribute to the development of non-small cell lung cancer (NSCLC). Although EGFR tyrosine kinase inhibitors are effective, drug resistance remains a major challenge, highlighting the need for new inhibitors derived from natural compounds. This in silico study aimed to characterize the quantitative structure–activity relationship (QSAR) of pelargonidin derivatives and evaluate their potential as EGFR inhibitors. QSAR modelling was performed using multiple linear regression, followed by molecular docking against EGFR (Protein Data Bank ID: 5UG8) using AutoDockTools. Pharmacokinetic and toxicity profiles were predicted using pkCSM and ProTox. The best QSAR model showed an adjusted  $R^2$  value of 0.661. All selected derivatives subjected to molecular docking showed more negative predicted binding energies than the native ligand. Among the tested compounds, 3-Methoxy-5-Methyl Pelargonidin exhibits the strongest binding affinity of  $-9.73$  kcal/mol and an inhibition constant ( $K_i$ ) of  $74.17$  nM. This compound also satisfies Lipinski's Rule of Five and demonstrates favourable predicted absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. In conclusion, 3-Methoxy-5-Methyl Pelargonidin is a promising candidate for further investigation as an EGFR inhibitor through in vitro and in vivo studies.

**Keywords:** Anthocyanidin; Barley; EGFR Molecular docking; QSAR.

### INTRODUCTION

Lung cancer remains a major contributor to cancer-related mortality worldwide and continues to pose a substantial public health challenge in Indonesia. Data from the Global Cancer Observatory (GLOBOCAN) for 2022 indicated approximately 38,000–39,000 newly diagnosed lung cancer cases in Indonesia, accompanied by around 34,000 deaths during the same year (International Agency for Research on Cancer, 2022; Asmara et al., 2023). Lung cancer is classified into two major categories according to its histopathological features: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Among

these subtypes, NSCLC accounts for the majority of cases, comprising approximately 85% of all lung cancer diagnoses (Joshi & Sheikh, 2025).

Dysregulation of the epidermal growth factor receptor (EGFR) may promote the pathogenesis of NSCLC through the persistent activation of intracellular signalling cascades that drive excessive cell growth and proliferation. EGFR tyrosine kinase inhibitors (EGFR-TKIs), including osimertinib, have improved treatment outcomes among patients with EGFR-mutated NSCLC. Nevertheless, resistance to these agents frequently develops after approximately 8–12 months of treatment and remains an

important factor contributing to treatment failure. This challenge underscores the need to investigate alternative therapeutic candidates (Fu et al., 2022; Laface et al., 2023).

Bioactive compounds derived from natural sources have emerged as promising candidates for the discovery and development of cancer therapeutics. Anthocyanins and anthocyanidins are flavonoid-related compounds present in various plants, including barley (*Hordeum vulgare*). Previous studies have associated these compounds with antioxidant, antiproliferative, and anticancer activities (Suriano et al., 2019; ElShamey et al., 2025). Experimental investigations have also reported their ability to suppress cancer cell growth and promote apoptosis through different molecular mechanisms (Saini et al., 2024; Kowalczyk et al., 2024).

Despite this potential, the structural determinants underlying the inhibitory effects of barley-derived anthocyanidins on specific targets, including EGFR, remain inadequately elucidated. QSAR modelling and molecular docking serve as complementary computational strategies for elucidating the influence of molecular characteristics on biological activity and characterizing the potential binding interactions between candidate molecules and their target proteins. Therefore, this study integrates QSAR modelling, molecular docking, and ADMET prediction to investigate structurally modified pelargonidin derivatives as potential EGFR inhibitors.

This research sought to characterize the structure–activity relationships of barley (*Hordeum vulgare*)-derived anthocyanidins and explore their potential as EGFR-targeting agents for lung cancer treatment. Computational analyses were

conducted to determine the association between molecular structural features and predicted biological activity, while also examining the binding potential of these compounds toward EGFR.

## METHODS

### Materials

Pelargonidin and its structurally modified derivatives were investigated in this study using computational methods. The materials comprised the parent pelargonidin compound, ten designed derivatives, and a training dataset containing 19 anthocyanidin compounds for quantitative structure–activity relationship (QSAR) analysis. The EGFR crystal structure identified by Protein Data Bank (PDB) code 5UG8, chain A, was selected as the molecular docking target. The native ligand was used to validate the docking protocol, while osimertinib served as the reference compound. The 19-compound dataset was obtained from Prasetiawati et al. (2021). Molecular structures of pelargonidin and the anthocyanidin derivatives were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and the EGFR structure was downloaded from the Protein Data Bank (<https://www.rcsb.org/>).

Ten derivatives were designed by introducing methyl (–CH<sub>3</sub>), methoxy (–OCH<sub>3</sub>), acetoxy (–OCOCH<sub>3</sub>), fluoro (–F), or chloro (–Cl) substituents at selected positions on the pelargonidin scaffold. The resulting derivatives were 3,5-Dimethyl Pelargonidin, 3-Acetoxy Pelargonidin, 3-Chloro Pelargonidin, 3-Fluoro Pelargonidin, 3-Methoxy-5-Methyl Pelargonidin, 5-Acetoxy Pelargonidin, 5-Fluoro Pelargonidin, 3-Methyl Pelargonidin, 5-Methoxy Pelargonidin, and

5-Methyl Pelargonidin. Seven molecular descriptors were initially considered: HOMO and LUMO energy levels, dipole moment, solvent-accessible surface area (SAA), molecular volume, the octanol–water partition coefficient (LogP), and molecular weight (MW). Descriptors were then evaluated during model development to identify the variables included in the selected regression model.

### Research Methods

This investigation employed an in silico experimental design combining QSAR modelling, molecular docking, ligand–protein interaction assessment, and predictions of physicochemical, pharmacokinetic, and toxicity properties. The work was carried out at the Bachelor of Clinical and Community Pharmacy Study Programme, STIKes Widya Dharma Husada Tangerang, from March to June 2026.

Computational work was performed using two laptops: a Lenovo device with an AMD A9-9425 Radeon R5 processor, a 3.10 GHz dual-core CPU, 4 GB RAM, Windows 10 Pro 64-bit version 21H2, and 1.14 TB storage; and an Acer Swift 3 equipped with an Intel Core i5 processor, 8 GB RAM, a 512 GB SSD, and Windows 10 64-bit. The software used included ChemDraw, Chem3D, Gaussian 09, HyperChem, AutoDockTools version 1.5.7, AutoDock4, BIOVIA Discovery Studio 2025, and IBM SPSS Statistics. Pharmacokinetic characteristics were predicted using pkCSM (<https://biosig.lab.uq.edu.au/pkcsm/>), while toxicity was assessed computationally with ProTox 3.0 (<https://tox.charite.de/protox3/>).

### Compound Structure Preparation and Modification

The structure of pelargonidin was retrieved from PubChem and redrawn using ChemDraw while preserving the atomic arrangement and bonding pattern of the reference structure. Structural modifications were performed by substituting methyl, methoxy, acetoxy, fluoro, and chloro groups at specific positions on the core pelargonidin scaffold. The resulting ten derivative structures were converted into three-dimensional structures and optimised using Chem3D through energy minimisation. Further optimisation and molecular descriptor calculations were performed using Gaussian 09 and HyperChem. The resulting descriptors were used as variables in QSAR modelling and for predicting the activity of the modified compounds.

### Quantitative Structure–Activity Relationship Modelling

A total of 19 anthocyanidin compounds were used as the QSAR modelling dataset. Their binding affinity values were obtained from Prasetyawati et al. (2021) and used as the response variable in the regression analysis. These values were obtained from molecular docking simulations as reported in the original study. Multiple linear regression analysis was performed using IBM SPSS Statistics to determine the associations between molecular descriptors and biological activity. Variable selection was performed to identify the combination of descriptors that produced the best QSAR model.

Model adequacy was evaluated using statistical measures comprising R, R<sup>2</sup>, standard error, and the F-statistic, while its predictive validity was assessed through leave-one-out cross-validation (LOO-CV), the prediction residual error sum of squares

(PRESS), and the cross-validated determination coefficient ( $q^2$ ). The best model was selected by considering its explanatory power, validation results, and the number of descriptors included.

### Receptor Preparation and Molecular Docking Validation

The EGFR crystal structure with PDB identifier 5UG8, chain A, was used as the docking receptor. Preparation was performed in BIOVIA Discovery Studio 2025 by removing water molecules and the native ligand. The extracted native ligand was retained for validation of the docking procedure. Ten pelargonidin derivatives were designed and their predicted activities were evaluated using the developed QSAR model. Based on the predicted QSAR results, five derivatives were selected for subsequent molecular docking analysis.

The receptor and ligand structures were then processed using AutoDockTools version 1.5.7. Receptor preparation involved the incorporation of polar hydrogens and Kollman partial charges, while the ligand structure was modified by adding hydrogen atoms and assigning Gasteiger partial charges. Rotatable bonds were specified to accommodate ligand conformational flexibility during docking. Following preparation, both molecular structures were exported and stored in PDBQT file format for subsequent docking analysis.

The grid box was positioned according to the location of the native ligand within the receptor binding pocket. Its dimensions were set to  $40 \times 40 \times 40$ , with centre coordinates of  $X = -13.156$ ,  $Y = 15.093$ , and  $Z = -25.719$ . Validation of the docking procedure involved repositioning the native co-crystallized ligand within the receptor's binding pocket using AutoDock4 and the Lamarckian

Genetic Algorithm (LGA), with 100 independent runs conducted. The accuracy of the docking protocol was determined by comparing the redocked ligand conformation with its original crystallographic pose using root mean square deviation (RMSD).

### Molecular Docking of Test Compounds

Molecular docking was performed on pelargonidin as the parent compound, selected derivatives based on QSAR predictions, and osimertinib as the reference compound. Docking was conducted using AutoDock4 with the validated grid box parameters and algorithm. A total of 100 runs were performed to obtain optimal binding conformations. The analysed parameters included binding energy, inhibition constant ( $K_i$ ), and the interaction patterns between the compounds and amino acid residues of the EGFR receptor.

### Molecular Interaction Analysis

The molecular docking results were visualised in two- and three-dimensional formats using BIOVIA Discovery Studio 2025. The analysis identified conventional hydrogen bonds, carbon hydrogen bonds, hydrophobic interactions, van der Waals interactions, halogen interactions, and unfavourable interactions. The amino acid residues involved in these interactions were examined to compare the binding patterns of the pelargonidin derivatives with those of the native ligand and osimertinib.

### Prediction of Drug-Likeness, Pharmacokinetics, and Toxicity

Compound drug-likeness was assessed through the pkCSM platform by applying Lipinski's Rule of Five, focusing on four physicochemical descriptors: molecular weight (MW), the octanol–water partition coefficient (LogP), and the numbers of hydrogen bond donors (HBD)

and acceptors (HBA). Compounds were assessed against the following thresholds: MW not exceeding 500 Da, LogP  $\leq 5$ , a maximum of five HBD groups, and no more than ten HBA groups.

Pharmacokinetic predictions were performed using pkCSM based on the SMILES notation of each compound retrieved from PubChem. The evaluated parameters covered absorption, distribution, metabolism, and excretion. Toxicity predictions were performed using ProTox 3.0 to obtain an overview of the toxicity profiles and estimated median lethal dose (LD<sub>50</sub>).

The overall results were used to evaluate the potential of pelargonidin derivatives as EGFR inhibitor candidates based on predicted activity, binding characteristics, drug-likeness, and ADMET profiles.

## RESULTS

### QSAR Modelling

QSAR analysis was performed using 19 compounds in the training set and molecular descriptors comprising HOMO, LUMO, dipole moment, molecular volume, and molecular mass. Three regression models were obtained using different combinations of descriptors, as presented in Table 1.

**Table 1.** QSAR regression equations

| Model | Equation                                                                                                                                                                 |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1     | $\text{Binding affinity} = -39.072 - 38.705 (\text{HOMO}) - 35.823 (\text{LUMO}) - 0.105 (\text{Dipole Moment}) - 0.001 (\text{Volume}) + 0.020 (\text{Molecular Mass})$ |
| 2     | $\text{Binding affinity} = -39.281 - 38.952 (\text{HOMO}) - 36.238 (\text{LUMO}) - 0.107 (\text{Dipole Moment}) + 0.018 (\text{Molecular Mass})$                         |
| 3     | $\text{Binding affinity} = -38.304 - 38.212 (\text{HOMO}) - 35.651 (\text{LUMO}) + 0.015 (\text{Molecular Mass})$                                                        |

Model 3 was selected as the best model because it exhibited the highest F-calculated/F-table ratio of 3.863 while incorporating three molecular descriptors. This model yielded an R value of 0.847, an R<sup>2</sup> value of approximately 0.717, an adjusted R<sup>2</sup> value of 0.661, and a q<sup>2</sup> value of 0.6763. The PRESS value was 3.203204, with an MAE of 0.3351 kcal/mol and an RMSE of 0.4106 kcal/mol. The relationship between the actual and predicted binding affinity values is presented in Figure 1.

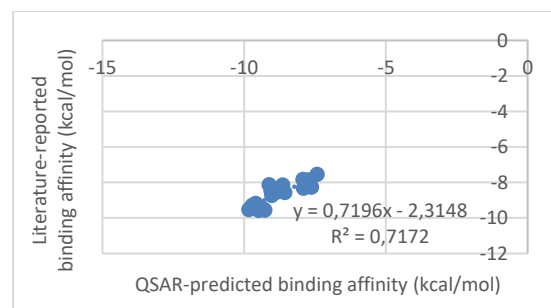


**Figure 1.** Correlation between literature-reported and QSAR-predicted binding affinity values for the modelling dataset (n = 19).

The correlation plot yielded a linear regression equation of  $y = 0.7196x - 2.3148$ , with an R<sup>2</sup> value of 0.7172.

### Prediction of Binding Affinity of Pelargonidin Derivatives

The selected QSAR model was used to predict the binding affinity of ten pelargonidin derivatives based on their HOMO, LUMO, and molecular mass values. The prediction results are presented in Table 2.

**Table 2.** Molecular descriptors and predicted binding affinity values of pelargonidin derivatives

| Compound                        | HOMO     | LUMO     | MW (g/mol) | Binding affinity |
|---------------------------------|----------|----------|------------|------------------|
| 3,5 Dimethyl Pelargonidin       | -0.43992 | -0.20921 | 329.29     | -9.09588         |
| 3-Acetoxy Pelargonidin          | -0.452   | -0.21753 | 329.29     | -8.33766         |
| 3-Chloro Pelargonidin           | -0.43946 | -0.20787 | 299.3      | -9.61108         |
| 3-Fluoro Pelargonidin           | -0.44217 | -0.21008 | 289.23     | -9.57964         |
| 3-Methoxy-5-Methyl Pelargonidin | -0.43101 | -0.21242 | 315.3      | -9.74206         |
| 5-Acetoxy Pelargonidin          | -0.44434 | -0.20916 | 329.28     | -9.58892         |
| 5-Fluoro Pelargonidin           | -0.44429 | -0.20915 | 289.23     | -9.53193         |
| 3-Methyl Pelargonidin           | -0.45176 | -0.21691 | 289.24     | -8.96969         |
| 5-Methoxy Pelargonidin          | -0.44954 | -0.21557 | 301.28     | -8.92169         |
| 5-Methyl Pelargonidin           | -0.43362 | -0.2097  | 285.27     | -9.67315         |

### Molecular Docking Validation and Results

Validation was performed by redocking the native ligand into the EGFR receptor with PDB ID 5UG8, chain A. The grid box dimensions were set to  $40 \times 40 \times 40$ , with centre coordinates of  $X = -13.156$ ,  $Y = 15.093$ , and  $Z = -25.719$ .

**Table 3.** Molecular docking validation results

| Parameter                 | Result |
|---------------------------|--------|
| Best conformation         | 8      |
| Binding energy (kcal/mol) | -8.60  |
| Inhibition constant (nM)  | 493.72 |
| RMSD (Å)                  | 1.150  |

The RMSD value of 1.150 Å was below the commonly applied threshold of 2 Å for evaluating redocking poses. This result supports the use of the selected docking parameters for subsequent analyses of the test compounds.

The docking results for pelargonidin, its derivatives, the native ligand, and osimertinib as the reference compound are presented in Table 4.

**Table 4.** Molecular docking results of the test compounds against the EGFR receptor

| Compound                        | Binding energy (kcal/mol) | Ki (nM) |
|---------------------------------|---------------------------|---------|
| Native ligand                   | -8,60                     | 493,72  |
| Osimertinib                     | -9,17                     | 190,43  |
| Pelargonidin                    | -9,41                     | 127,07  |
| 3-Methoxy-5-Methyl Pelargonidin | -9,73                     | 74,17   |
| 5-Methyl Pelargonidin           | -9,63                     | 86,77   |
| 5-Acetoxy Pelargonidin          | -9,50                     | 109,17  |
| 5-Fluoro Pelargonidin           | -9,45                     | 118,52  |
| 3-Fluoro Pelargonidin           | -9,45                     | 118,52  |

Pelargonidin and all selected derivatives evaluated through molecular docking exhibited more negative predicted binding energy values than the native ligand. Among the derivatives evaluated, 3-Methoxy-5-Methyl Pelargonidin exhibited the most negative binding energy, at -9.73 kcal/mol, with a predicted Ki of 74.17 nM.

### Molecular Interaction Analysis

Interactions between the ligands and EGFR were examined using BIOVIA Discovery Studio 2025. Molecular interactions were characterized by examining several types of contacts,

including classical hydrogen bonding, carbon–hydrogen bonding,  $\pi$ -mediated hydrophobic interactions, van der Waals forces, and energetically unfavourable contacts. The amino acid residues involved

in these contacts were reviewed to compare the predicted binding patterns of pelargonidin derivatives with those of the native ligand and osimertinib.

**Table 5.** Interactions between the ligands and EGFR amino acid residues

| Compound                        | Interaction type           | Amino acid residues                                                            |
|---------------------------------|----------------------------|--------------------------------------------------------------------------------|
| Native ligand                   | Conventional hydrogen bond | MET793                                                                         |
|                                 | Carbon–hydrogen bond       | GLN791                                                                         |
|                                 | Pi-sigma                   | VAL726, LEU718, LEU844                                                         |
|                                 | Pi-alkyl                   | ALA743, MET790, LYS745, CYS775, CYS797, MET793                                 |
|                                 | van der Waals              | THR854, PHE856, GLY719, LEU792, GLY796, PRO794, ASP800                         |
| Osimertinib                     | Conventional hydrogen bond | CYS797                                                                         |
|                                 | Carbon–hydrogen bond       | TYR801                                                                         |
|                                 | Pi-sigma                   | VAL726, LEU718                                                                 |
|                                 | Pi-alkyl                   | ALA743, LEU844, MET790, MET793, GLN791, CYS775                                 |
|                                 | van der Waals              | GLY719, PHE795, GLY796, PRO794, ASP800, THR854, PHE856, LYS745, LEU792, LYS728 |
| Pelargonidin                    | Conventional hydrogen bond | GLN791, LYS745, MET793, THR854, ASP855, LEU718                                 |
|                                 | Pi-alkyl                   | ALA743, VAL726, MET790, CYS775                                                 |
|                                 | Pi-sigma                   | LEU844                                                                         |
|                                 | van der Waals              | LEU792, GLY796, PHE856, GLY719                                                 |
| 3-Methoxy-5-Methyl Pelargonidin | Conventional hydrogen bond | MET793, GLN791, LEU718, THR854, LYS745                                         |
|                                 | Carbon–hydrogen bond       | GLN791                                                                         |
|                                 | Pi-sigma                   | LEU718, GLY796                                                                 |
|                                 | Pi-alkyl                   | ALA743, VAL726, LEU844, MET790                                                 |
|                                 | van der Waals              | PHE795, PRO794, LEU792, CYS775, ASP855, PHE856, GLY719                         |
|                                 | Unfavourable interaction   | GLY796                                                                         |
| 5-Methyl Pelargonidin           | Conventional hydrogen bond | ASP855, LYS745, THR854, GLN791, MET793, LEU718                                 |
|                                 | Alkyl                      | MET790                                                                         |
|                                 | Pi-alkyl                   | ALA743, VAL726, LEU844, CYS775, LEU718                                         |
|                                 | van der Waals              | PHE856, GLY719, LEU792, GLY796                                                 |
| 5-Acetoxy Pelargonidin          | Conventional hydrogen bond | GLN791, MET793, LYS745, THR854, ASP855                                         |
|                                 | Pi-alkyl                   | MET790, CYS775, LEU844, VAL726, ALA743, LEU718                                 |
|                                 | van der Waals              | LEU792, GLY719, GLY796, PHE856                                                 |

| Compound              | Interaction type           | Amino acid residues                            |
|-----------------------|----------------------------|------------------------------------------------|
| 5-Fluoro Pelargonidin | Conventional hydrogen bond | GLN791, MET793, LYS745, THR854, ASP855, LEU718 |
|                       | Pi-sigma                   | LEU718                                         |
|                       | Pi-alkyl                   | MET790, CYS775, ALA743, VAL726, LEU844         |
|                       | van der Waals              | LEU792, GLY796, GLY719                         |
|                       | Halogen interaction        | GLY796 (fluorine)                              |
| 3-Fluoro Pelargonidin | Conventional hydrogen bond | GLN791, MET793, LYS745, THR854, ASP855, LEU718 |
|                       | Carbon-hydrogen bond       | GLY796                                         |
|                       | Pi-sigma                   | LEU718                                         |
|                       | Pi-alkyl                   | MET790, CYS775, ALA743, VAL726, LEU844         |
|                       | Halogen interaction        | GLY796 (fluorine)                              |

### Evaluation of Lipinski's Rule of Five

Compound suitability for drug development was assessed by applying Lipinski's Rule of Five in conjunction with computational prediction methods to characterize their drug-likeness profiles. The assessment considered molecular weight, LogP, HBD, and HBA. The results are presented in Table 6.

**Table 6.** Evaluation of Lipinski's Rule of Five

| Compound                        | MW (g/mol) | Log P | HBD | HBA | Compliance |
|---------------------------------|------------|-------|-----|-----|------------|
| Osimertinib                     | 499,61     | 4,5   | 2   | 8   | ✓          |
| Pelargonidin                    | 271,24     | 3,2   | 4   | 4   | ✓          |
| 3-Methoxy-5-Methyl Pelargonidin | 315,3      | 3,5   | 4   | 5   | ✓          |
| 5-Methyl Pelargonidin           | 285,27     | 3,5   | 4   | 4   | ✓          |
| 5-Acetoxy Pelargonidin          | 329,28     | 3,1   | 4   | 6   | ✓          |

| Compound              | MW (g/mol) | Log P | HBD | HBA | Compliance |
|-----------------------|------------|-------|-----|-----|------------|
| 5-Fluoro Pelargonidin | 289,23     | 3,3   | 4   | 4   | ✓          |
| 3-Fluoro Pelargonidin | 289,23     | 3,3   | 4   | 4   | ✓          |

All evaluated compounds met the four physicochemical requirements of Lipinski's Rule of Five, exhibiting molecular weights of  $\leq 500$  g/mol, LogP values no greater than 5, a maximum of five hydrogen bond donors (HBD), and up to ten hydrogen bond acceptors (HBA).

### Pharmacokinetic and Toxicity Predictions

Based on in silico analyses using pkCSM and ProTox 3.0, the absorption, distribution, metabolism, excretion, and toxicity profiles of pelargonidin, its modified derivatives, and osimertinib as the reference EGFR-TKI were evaluated.

**Table 7.** Absorption profiles of the test compounds

| Compound                           | Caco-2<br>(10 <sup>-6</sup><br>cm/s) | HIA<br>(%) | LogKp  |
|------------------------------------|--------------------------------------|------------|--------|
| Osimertinib                        | 1,138                                | 93,42      | -2,735 |
| Pelargonidin                       | -0,089                               | 87,29      | -2,735 |
| 3-Methoxy-5-Methyl<br>Pelargonidin | 0,842                                | 82,10      | -2,747 |
| 5-Methyl<br>Pelargonidin           | 0,799                                | 85,82      | -2,743 |
| 5-Acetoxy<br>Pelargonidin          | 0,731                                | 75,92      | -2,736 |
| 5-Fluoro<br>Pelargonidin           | 0,743                                | 85,62      | -2,735 |
| 3-Fluoro<br>Pelargonidin           | 0,716                                | 83,54      | -2,741 |

**Table 8.** Distribution profiles of the test compounds

| Compound                           | VDss (log<br>L/kg) | logBB  |
|------------------------------------|--------------------|--------|
| Osimertinib                        | 1,081              | -1,129 |
| Pelargonidin                       | 0,649              | -1,061 |
| 3-Methoxy-5-Methyl<br>Pelargonidin | -0,068             | -1,361 |
| 5-Methyl<br>Pelargonidin           | -0,049             | -1,174 |
| 5-Acetoxy<br>Pelargonidin          | -0,046             | -1,442 |
| 5-Fluoro<br>Pelargonidin           | 0,499              | -1,389 |
| 3-Fluoro<br>Pelargonidin           | -0,116             | -1,350 |

**Table 9.** Metabolic profiles based on predicted CYP enzyme inhibition

| Compound                           | CYP<br>1A2 | CYP<br>2C19 | CYP<br>2C9 | CYP<br>2D6 | CYP<br>3A4 |
|------------------------------------|------------|-------------|------------|------------|------------|
| Osimertinib                        | No         | Yes         | Yes        | No         | Yes        |
| Pelargonidin                       | Yes        | Yes         | No         | No         | No         |
| 3-Methoxy-5-Methyl<br>Pelargonidin | Yes        | No          | No         | No         | Yes        |

| Compound                  | CYP<br>1A2 | CYP<br>2C19 | CYP<br>2C9 | CYP<br>2D6 | CYP<br>3A4 |
|---------------------------|------------|-------------|------------|------------|------------|
| Pelargonidin              |            |             |            |            |            |
| 5-Methyl<br>Pelargonidin  | Yes        | Yes         | Yes        | No         | Yes        |
| 5-Acetoxy<br>Pelargonidin | Yes        | No          | No         | No         | Yes        |
| 5-Fluoro<br>Pelargonidin  | Yes        | Yes         | Yes        | No         | No         |
| 3-Fluoro<br>Pelargonidin  | Yes        | Yes         | Yes        | No         | Yes        |

Note: "Yes" indicates predicted enzyme inhibition according to pkCSM.

**Table 10.** Excretion profiles of the test compounds

| Compounds                          | Total Clearance<br>(log mL/min/kg) | Substrate<br>OCT2 |
|------------------------------------|------------------------------------|-------------------|
| Osimertinib                        | 0,632                              | Ya                |
| Pelargonidin                       | 0,576                              | Tidak             |
| 3-Methoxy-5-Methyl<br>Pelargonidin | 0,811                              | Tidak             |
| 5-Methyl<br>Pelargonidin           | 0,766                              | Tidak             |
| 5-Acetoxy<br>Pelargonidin          | 0,911                              | Tidak             |
| 5-Fluoro<br>Pelargonidin           | 0,686                              | Tidak             |
| 3-Fluoro<br>Pelargonidin           | 0,679                              | Tidak             |

**Table 11.** Predicted toxicity profiles of the test compounds

| Compound                        | Toxicity class (1–6) | LD50 (mg/kg) | Hepatotoxicity | Carcinogenicity | Mutagenicity | Cytotoxicity |
|---------------------------------|----------------------|--------------|----------------|-----------------|--------------|--------------|
| Osimertinib                     | 3                    | 100          | No             | No              | No           | No           |
| Pelargonidin                    | 5                    | 3919         | No             | No              | No           | No           |
| 3-Methoxy-5-Methyl Pelargonidin | 5                    | 5000         | No             | No              | No           | No           |
| 5-Methyl Pelargonidin           | 5                    | 3919         | No             | No              | No           | No           |
| 5-Acetoxy Pelargonidin          | 5                    | 5000         | No             | No              | No           | No           |
| 5-Fluoro Pelargonidin           | 5                    | 3919         | No             | No              | No           | No           |
| 3-Fluoro Pelargonidin           | 5                    | 3919         | No             | No              | No           | No           |

Pelargonidin and all its derivatives were predicted to belong to toxicity class 5, with LD<sub>50</sub> values ranging from 3,919 to 5,000 mg/kg. In contrast, osimertinib was predicted to belong to toxicity class 3, with an LD<sub>50</sub> of 100 mg/kg. Pelargonidin and all its derivatives were also predicted to be negative for hepatotoxicity, carcinogenicity, Ames mutagenicity, and cytotoxicity.

## DISCUSSION

### QSAR Modelling

Model 3 was selected as the best QSAR model because it had the highest F-calculated/F-table ratio of 3.863 and included three descriptors: HOMO, LUMO, and molecular mass. The smaller number of descriptors resulted in a simpler model than Models 1 and 2 (Anwar et al., 2024).

The R value of 0.847 and R<sup>2</sup> value of 0.661 indicated a relatively strong relationship between the selected descriptors and the modelled binding affinity (BA). The R<sup>2</sup> value indicated that 66.1% of the variation in BA within the modelling dataset could be explained by the three descriptors, whereas the remaining variation was associated with other factors not included in the model. The use of molecular descriptors to

establish mathematical relationships between chemical structure and biological activity is a fundamental principle of QSAR analysis (Dwi Setiani et al., 2021).

The negative coefficients for HOMO and LUMO indicated that increases in these parameters tended to produce more negative predicted binding affinity values, whereas the positive coefficient for molecular mass indicated a tendency towards less negative binding affinity values. HOMO represents a molecule's electron-donating tendency, whereas LUMO represents its electron-accepting tendency. Changes in these parameters resulting from structural modifications may affect electron distribution and molecular interactions with biological targets (Raval & Ganatra, 2022).

Model validation yielded a PRESS value of 3.203204 and a q<sup>2</sup> value of 0.6763, indicating reasonably good predictive performance. Further evaluation using MAE and RMSE produced values of 0.3351 and 0.4106 kcal/mol, respectively. The RMSE value, which was only slightly higher than the MAE value, indicated relatively small prediction errors and suggested that extreme outliers did not substantially

influence the model (Maattallaoui et al., 2025; Vasilev & Atanasova, 2025).

Based on the correlation plot in Figure 1, the regression equation was  $y = 0.7196x - 2.3148$ , with an  $R^2$  value of 0.7172. This result demonstrated that the model accounted for 71.72% of the variability observed in the experimentally determined binding affinity. The distribution of points suggested a good linear relationship, supporting the consistency of the model's predictions (Anwar et al., 2024).

### Prediction of Binding Affinity of Pelargonidin Derivatives

Differences in binding affinity values indicated that structural modifications produced changes in molecular characteristics and predicted binding affinity. The combination of methoxy and methyl groups in 3-Methoxy-5-Methyl Pelargonidin yielded the most negative binding affinity value, suggesting that electronic characteristics and molecular size contributed to the predicted binding affinity. This finding was also consistent with the QSAR model, which identified HOMO, LUMO, and molecular mass as descriptors contributing to variations in binding affinity.

### Molecular Docking Validation and Results

Molecular docking validation yielded an RMSD value of 1.150 Å. This value was below the commonly used threshold of 2 Å for assessing the similarity between a redocked pose and the ligand position in the crystal structure. These findings support the suitability of the docking protocol for further analysis (Raval & Ganatra, 2022).

The molecular docking results showed that pelargonidin and all tested

derivatives exhibited more negative binding energy values than the native ligand. The native ligand demonstrated a binding free energy ( $\Delta G$ ) of  $-8.60$  kcal/mol and an inhibition constant ( $K_i$ ) of 493.72 nM. Osimertinib, serving as the reference EGFR tyrosine kinase inhibitor (EGFR-TKI), showed corresponding values of  $-9.17$  kcal/mol and 190.43 nM. Meanwhile, the unmodified pelargonidin compound exhibited a  $\Delta G$  of  $-9.41$  kcal/mol, with a predicted  $K_i$  of 127.07 nM. More negative  $\Delta G$  values and lower  $K_i$  values indicate a tendency towards higher binding affinity for the receptor (Raval & Ganatra, 2022).

The five pelargonidin derivatives with the highest predicted binding affinities exhibited  $\Delta G$  values ranging from  $-9.45$  to  $-9.73$  kcal/mol. 3-Methoxy-5-Methyl Pelargonidin produced the most favourable results, with a  $\Delta G$  of  $-9.73$  kcal/mol and a  $K_i$  of 74.17 nM, followed by 5-Methyl Pelargonidin ( $-9.63$  kcal/mol; 86.77 nM), 5-Acetoxy Pelargonidin ( $-9.50$  kcal/mol; 109.17 nM), and 5-Fluoro Pelargonidin and 3-Fluoro Pelargonidin, both of which exhibited a  $\Delta G$  of  $-9.45$  kcal/mol and a  $K_i$  of 118.52 nM. These results indicated that the five derivatives had higher predicted binding affinities than pelargonidin, osimertinib, and the native ligand based on the docking parameters used. However, these docking results represent computational predictions, and further experimental studies are required to confirm their actual EGFR inhibitory activities.

The increased predicted binding affinity of 3-Methoxy-5-Methyl Pelargonidin was hypothesised to be associated with the combination of methoxy and methyl groups. The methoxy

group may act as a hydrogen bond acceptor, whereas the methyl group may enhance hydrophobic interactions within the binding pocket. The combination of these substituents may influence the orientation and stability of the compound's interactions with EGFR. Methoxy group modifications in flavonoids have also been reported to potentially enhance binding affinity through changes in structural complementarity and hydrophobic interactions (Loveday & Echeverría, 2021). Acetoxy groups have also been reported to potentially increase compound lipophilicity (Summerfield & Pattison, 2026). Meanwhile, the effect of methyl groups in enhancing hydrophobic contacts and optimising ligand orientation within the binding pocket is known as the magic methyl effect (Taldaev et al., 2022).

### **Compound Interactions with the EGFR Binding Pocket**

Visualisation of the molecular docking results showed that pelargonidin and its derivatives with the highest predicted binding affinities interacted with several residues in the EGFR ATP-binding pocket, particularly GLN791, LYS745, MET793, THR854, ASP855, LEU718, ALA743, VAL726, MET790, CYS775, and LEU844. MET793 is an important residue in the EGFR inhibitor pharmacophore and serves as one of the principal anchoring points for tyrosine kinase inhibitor binding. The parent compound, pelargonidin, formed conventional hydrogen bonds with GLN791, LYS745, MET793, THR854, ASP855, and LEU718, as well as hydrophobic interactions with ALA743, VAL726, MET790, CYS775, and LEU844. This interaction pattern indicated that pelargonidin could occupy the EGFR binding pocket through various

interactions that may contribute to complex stability (Gad et al., 2023).

3-Methoxy-5-Methyl Pelargonidin formed conventional hydrogen bonds with MET793, GLN791, LEU718, THR854, and LYS745, as well as a carbon-hydrogen bond with GLN791. Hydrophobic interactions involving pi-sigma and pi-alkyl interactions included LEU718, GLY796, ALA743, VAL726, LEU844, and MET790, accompanied by van der Waals interactions with several residues surrounding the binding pocket. This compound retained several key residues that also interacted with pelargonidin, particularly GLN791, MET793, LYS745, LEU718, MET790, ALA743, VAL726, and LEU844. This extensive interaction pattern was consistent with its most negative  $\Delta G$  value ( $-9.73$  kcal/mol) and lowest  $K_i$  value ( $74.17$  nM). Although one unfavourable interaction with GLY796 was identified, the other favourable interactions were associated with the highest predicted binding affinity among the tested derivatives.

5-Methyl Pelargonidin retained interactions with GLN791, LYS745, MET793, THR854, and LEU718 and formed hydrophobic interactions with MET790, ALA743, VAL726, LEU844, CYS775, and LEU718. The addition of a methyl group did not eliminate the principal interactions observed for pelargonidin but maintained hydrophobic contacts within the binding pocket. This finding was consistent with the improvement in predicted binding affinity from pelargonidin ( $\Delta G$   $-9.41$  kcal/mol;  $K_i$   $127.07$  nM) to 5-Methyl Pelargonidin ( $\Delta G$   $-9.63$  kcal/mol;  $K_i$   $86.77$  nM).

Among the fluorinated compounds, 5-Fluoro Pelargonidin and 3-Fluoro

Pelargonidin retained interactions with key residues, including GLN791, MET793, LYS745, THR854, ASP855, LEU718, MET790, CYS775, ALA743, VAL726, and LEU844. Both compounds also exhibited halogen interactions with GLY796. They produced identical  $\Delta G$  values of  $-9.45$  kcal/mol and  $K_i$  values of  $118.52$  nM. Thus, changing the fluorine substitution position at the positions examined did not produce a difference in docking scores, although differences in local interaction patterns were observed.

#### Prediction of Lipinski's Rule of Five

Based on the Lipinski's Rule of Five predictions, all analysed compounds, including pelargonidin, its modified derivatives, and osimertinib as the reference drug, met all Lipinski criteria. These criteria included molecular weight (MW) below  $500$  Da, LogP below  $5$ , no more than five hydrogen bond donors (HBD), and no more than ten hydrogen bond acceptors (HBA). As the compound with the highest predicted binding affinity, 3-Methoxy-5-Methyl Pelargonidin had an MW of  $315.301$  g/mol, a LogP of  $3.5203$ , an HBD count of  $4$ , and an HBA count of  $5$ . These values remained within the specified limits, indicating drug-like characteristics.

The physicochemical criteria established by Lipinski's Rule of Five serve as an initial screening tool to assess whether a compound exhibits drug-like properties and the potential to achieve adequate bioavailability following oral administration. Compliance with these criteria indicated that structural modifications to the pelargonidin derivatives did not cause the principal parameters to exceed the specified limits. Consequently, all tested compounds remained within the physicochemical

range associated with drug-likeness (Lipinski et al., 1997).

#### Pharmacokinetic and Toxicity Predictions

For the absorption parameters, the increased predicted Caco-2 permeability of the modified compounds compared with pelargonidin indicated changes in their predicted permeability across an intestinal epithelial model, although their values remained lower than that of osimertinib. Conversely, Human Intestinal Absorption (HIA) values decreased following structural modification. This difference indicated that increased Caco-2 permeability was not necessarily accompanied by increased intestinal absorption. Meanwhile, the negative Skin Permeability ( $\log K_p$ ) values indicated low predicted skin permeability. These parameters should be evaluated together to provide an overview of the compounds predicted absorption characteristics (Pires et al., 2015).

Compared with pelargonidin, the modified derivatives exhibited lower predicted steady-state volumes of distribution ( $VD_{ss}$ ), suggesting differences in their anticipated distribution profiles within the body. However,  $VD_{ss}$  does not directly indicate a compound's ability to reach target tissues, as distribution is also influenced by physicochemical properties and interactions with biological components. Furthermore, the modified derivatives showed more negative predicted  $\log BB$  values than pelargonidin, suggesting a reduced likelihood of crossing the blood-brain barrier compared with the parent compound. These two parameters provide preliminary information about compound distribution but are insufficient to determine distribution superiority without

considering pharmacological characteristics and the location of the drug target (Pires et al., 2015).

For the metabolism parameters, variations in predicted inhibition of cytochrome P450 (CYP) isoenzymes indicated that structural modifications to pelargonidin could affect its interactions with metabolic enzymes. All pelargonidin-based compounds were predicted to inhibit CYP1A2 and not inhibit CYP2D6, whereas their predicted inhibition of CYP2C19, CYP2C9, and CYP3A4 varied. 3-Methoxy-5-Methyl Pelargonidin and 5-Acetoxy Pelargonidin exhibited more limited inhibition profiles, whereas 5-Methyl Pelargonidin and 3-Fluoro Pelargonidin were predicted to inhibit a greater number of CYP isoenzymes. These differences indicated variations in the potential for metabolic interactions among the compounds, which should be considered during drug candidate development (Pires et al., 2015).

For the excretion parameters, the increased predicted Total Clearance values of the modified compounds compared with pelargonidin indicated higher predicted clearance. This change could affect systemic compound exposure; however, higher clearance does not necessarily indicate a better pharmacokinetic profile because absorption and distribution must also be considered. Furthermore, all modified compounds were predicted not to be substrates of Organic Cation Transporter 2 (OCT2), in contrast to osimertinib, which was predicted to be an OCT2 substrate. These findings indicated differences in the predicted involvement of OCT2 in compound disposition (Pires et al., 2015).

Toxicity prediction classified pelargonidin and its derivatives as class 5,

with estimated LD<sub>50</sub> values between 3,919 and 5,000 mg/kg. In contrast, osimertinib fell into class 3, with an LD<sub>50</sub> of 100 mg/kg. Within the prediction model, higher LD<sub>50</sub> values corresponded to lower predicted acute toxicity. Furthermore, all pelargonidin-based compounds were predicted to exhibit negative results for hepatotoxicity, carcinogenicity, Ames mutagenicity, and cytotoxicity. The higher predicted LD<sub>50</sub> values of 3-Methoxy-5-Methyl Pelargonidin and 5-Acetoxy Pelargonidin compared with the parent compound indicated differences in predicted acute toxicity following structural modification (Pires et al., 2015).

## CONCLUSION

This in silico investigation evaluated the quantitative structure–activity relationship, predicted EGFR binding affinity, ligand–protein interactions, and drug-likeness characteristics of pelargonidin derivatives from barley (*Hordeum vulgare*). The selected QSAR model incorporated HOMO energy, LUMO energy, and molecular mass, with an R<sup>2</sup> value of 0.661 and a q<sup>2</sup> value of 0.6763. Molecular docking identified 3-Methoxy-5-Methyl Pelargonidin as the derivative with the most favourable predicted binding energy (–9.73 kcal/mol) and a predicted inhibition constant of 74.17 nM. The compound also met the assessed criteria of Lipinski’s Rule of Five and showed predicted pharmacokinetic and toxicity characteristics that support further evaluation. These findings provide a computational basis for prioritising 3-Methoxy-5-Methyl Pelargonidin for subsequent investigation. However, experimental validation, including in vitro and in vivo studies, is required to determine

its actual EGFR inhibitory activity, pharmacological effectiveness, and safety.

### CONFLICT OF INTEREST

The authors declare that they have no competing interests related to this research.

### AUTHORS' DECLARATION

The authors affirm the originality of this article and accept responsibility for any claims or liabilities arising from its contents.

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