Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 2s, https://carcinogenesis.com/

Integrative QSAR and Feature Stability Analysis for Flavonoid-Based Drug Design Targeting A375 Melanoma

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ABSTRACT

This study presents a comprehensive QSAR (Quantitative Structure-Activity Relationship) modeling approach for identifying promising flavonoid derivatives that may exhibit anticancer properties against A375 melanoma cells. This is achieved through the application of LASSO (Least Absolute Shrinkage and Selection Operator) regression. Using LASSO regression, we developed a predictive model for biological activity (IC50) based on key molecular descriptors. The model demonstrated strong performance on training data ($R^2 = 0.7445$, RMSE = 0.4903) and moderate generalizability in Leave-One-Out Cross-Validation ($Q^2 = 0.5558$, RMSE = 0.6465), with no significant overfitting ($R^2 - Q^2 = 0.1887 < 0.3$). Feature stability analysis identified five critical descriptors: LogP (lipophilicity, +0.5340), TPSA (polar surface area, −0.2058), and RotatableBonds (flexibility, +0.1583), alongside NumHDonors and AromaticRings, which collectively explain flavonoid-melanoma interactions. The outcomes are inline with established QSAR patterns, where lipophilicity and polarity influence anticancer activity, where lipophilicity and polarity modulate anticancer potency. Notably, low-frequency descriptors (e.g., MolWeight) were excluded, streamlining future designs. Graphical validation confirmed robust training predictions ($R^2 = 0.7445$) and acceptable LOO-CV scatter ($Q^2 = 0.5558$), though outliers suggest opportunities for model refinement. The study highlights flavonoid scaffolds with optimized LogP (2–3) and TPSA (<80 Å²) as high-priority candidates for synthesis. By integrating QSAR with stability-driven descriptor selection, this work provides a computational roadmap for accelerating flavonoid-based melanoma drug discovery. Future directions include experimental validation of top-ranked derivatives and incorporation of nonlinear machine learning methods to capture complex structure-activity landscapes.

Keywords: *QSAR modeling, Flavonoid derivatives, Anticancer activity, Melanoma, A375 cells, LASSO regression, Molecular descriptors, Drug design*

How to Cite: Akash Asthana, Adarsh Tripathi, (2025) Integrative QSAR and Feature Stability Analysis for Flavonoid-Based Drug Design Targeting A375 Melanoma, *Journal of Carcinogenesis*, *Vol.24, No.2s*, 394-401

1. INTRODUCTION

Melanoma is among the most hard-hitting and deadly type of skin cancer, with its worldwide rate steadily rising [1]. The A375 melanoma cell line in human, derived from a malignant tumor, is extensively utilized in preclinical research due to its high metastatic potential and notable resistance to conventional therapeutic interventions [2]. Despite significant progress in targeted therapies, such as BRAF inhibitors (e.g., vemurafenib), and immunotherapies, including anti-PD-1 antibodies, the management of progressive melanoma remains a formidable challenge [3, 4]. Issues like the increase of drug resistance and the occurrence of severe adverse effects significantly restrict the efficacy of current treatments, underscoring the urgent necessity for novel chemotherapeutic agents with increased efficacy and improved safety profiles [5].

In the quest for new anticancer agents, natural products have gotten considerable attention. Flavonoids, a diverse group of polyphenolic compounds ubiquitously found in fruits, vegetables, and medicinal plants, are well-known for their extensive spectrum of biological activities, including antioxidant, anti-inflammatory, and potent anticancer properties [6, 7]. Structurally, flavonoids comprise two aromatic rings (A and B) interconnected by a heterocyclic pyran ring (C), and are

categorized into subclasses such as flavones, flavonols, flavanones, isoflavones, and anthocyanins [8]. Their anticancer mechanisms are multifaceted, involving the induction of cell cycle arrest, promotion of apoptosis, inhibition of metastasis, and modulation of reactive oxygen species (ROS) homeostasis, often selectively inducing oxidative stress in cancer cells [9, 10]. Specific flavonoids, including quercetin, luteolin, and fisetin, have demonstrated promising anti-melanoma activity against A375 cells in vitro [11]. However, their advancement into clinical use is often hampered by challenges such as poor bioavailability and an incomplete understanding of their structure-activity relationships (SAR) [7].

Quantitative Structure-Activity Relationship (QSAR) modeling has emerged as a powerful computational tool in drug discovery. QSAR studies aim to establish a mathematical correlation between the chemical structure of compounds, represented by molecular descriptors (e.g., lipophilicity (LogP), topological polar surface area (TPSA)), and their biological activities [12, 13]. This approach significantly accelerates the drug development pipeline by enabling in silico screening of large compound libraries, guiding the structural optimization of lead candidates, and identifying key pharmacophoric features essential for target-specific interactions [14]. Recent QSAR studies on flavonoids have highlighted the critical influence of descriptors such as LogP, TPSA, and hydrogen-bonding capacity on their anticancer efficacy [15]. However, many existing models tend to focus on general cytotoxicity rather than melanoma-specific effects, leaving a gap in targeted drug design for this malignancy.

To address the complexity of high-dimensional descriptor datasets and to build more robust and interpretable QSAR models, progressed machine learning techniques are more employed. The Least Absolute Shrinkage and Selection Operator (LASSO) regression is one such technique that excels in feature selection by penalizing the absolute size of regression coefficients, effectively shrinking irrelevant descriptors to zero [16]. This not only helps in reducing model overfitting but also aids in identifying the most critical molecular features that govern the biological activity, thereby providing clearer insights for SAR analysis and improving the model's generalizability when validated through methods like Leave-One-Out Cross-Validation (LOO-CV) [17, 18]. The integration of feature stability analysis with QSAR further enhances the reliability of selected descriptors, ensuring that the identified structural features are consistently important across different data perturbations [19].

This research aims to build a robust QSAR model using LASSO regression to predict the anticancer activity of flavonoid derivatives against the A375 melanoma cell line. Furthermore, we seek to identify quintessential molecular descriptors that govern this activity and to perform feature stability analysis to ensure their reliability. By integrating these computational approaches, this research endeavours to provide translational insights for the rational design and optimization of novel flavonoid-based therapeutic agents specifically targeting melanoma, thereby contributing to the development of more valuable treatments for this challenging cancer.

2. MATERIALS AND METHODS

Dataset Collection:

Six flavonoids with experimentally reported IC_{50} values against A375 melanoma cells were selected: quercetin, kaempferol, luteolin, apigenin, myricetin, and fisetin. Molecular structures were expressed as SMILES strings, and were gathered from literature and research sources [20, 21, 22, 23] as detailed in Table 1.

Table 1: Flavonoids with Anticancer Activity Against A375 Melanoma

Compound	SMILES	IC50 (µM)	Reference (DOI/PMID
Fisetin	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2)C3=CC(O)=C(O)C=C3	19.2	20
Quercetin	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2O)C3=CC=C(O)C(O)=C3	12.3	21
Luteolin	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2O)C3=CC(O)=C(O)C=C3	9.8	22
Kaempferol	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2O)C	18.7	21

Compound	SMILES	IC50 (µM)	Reference (DOI/PMID
	3=CC=C(O)C=C3		
Apigenin	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2O)C3=CC=C(O)C=C3	25.1	22
Myricetin	O=C1C(O)=C(C(=O)C2=C1C=C(O)C=C2O)C3=CC(O)=C(O)C(O)=C3	14.5	23

Descriptor Calculation

Using RDKit, 210 molecular descriptors encompassing constitutional, electronic properties, and topological were calculated for each flavonoid. Each compound was thereby represented by a feature vector suitable for QSAR analysis.

Activity Transformation

The experimentally determined IC_{50} values (in µM) were converted to their negative logarithmic scale (pIC_{50}) to normalize the data distribution and facilitate QSAR modeling, using the formula:

$$pIC_{50} = -\log10(IC_{50} \times 10^{-6})$$

Data Preprocessing

Molecular descriptors with any missing values were separated from the dataset. The remaining features were scaled using the StandardScaler method to make sure that all descriptors had a mean of zero and a standard deviation of one, thus preventing descriptors with larger magnitudes from disproportionately influencing the model.

Correlation Analysis

To investigate redundancy and multicollinearity among the descriptors, a Pearson correlation matrix was calculated and pictured using a heatmap. This analysis assisted in identifying highly correlated features that might require removal or dimensionality reduction in downstream modeling steps.

Feature Normalization and Model Development

Prior to model construction, all molecular descriptors were standardized using the StandardScaler technique, that adjusts the values of each descriptor to have a mean of zero and a standard deviation of one. This step was essential to prevent features with inherently larger magnitudes from disproportionately affecting the learning algorithm and to ensure comparability across descriptors.

Model was developed using the Least Absolute Shrinkage and Selection Operator (LASSO) regression, a linear regression method enhanced by L1 regularization. The LASSO algorithm minimizes the residual sum of squares subject to the sum of the absolute values of the coefficients being less than a fixed constant. This constraint results in the shrinkage of some coefficients to exactly zero, effectively performing both variable selection and regularization. Such an approach is particularly advantageous in high-dimensional descriptor spaces, as it enhances model interpretability and reduces the chance of overfitting.

To evaluate the generalization performance and stability of the developed model, Leave-One-Out Cross-Validation (LOO-CV) was employed. In LOO-CV, the model is iteratively trained on all but single data point, with the excluded instance used for validation. This process is repeated for all data point in the dataset, and performance metrics are averaged over all the iterations. LOO-CV is especially well-suited for small datasets, as it allows for nearly unbiased estimation of predictive accuracy while making optimal use of available data.

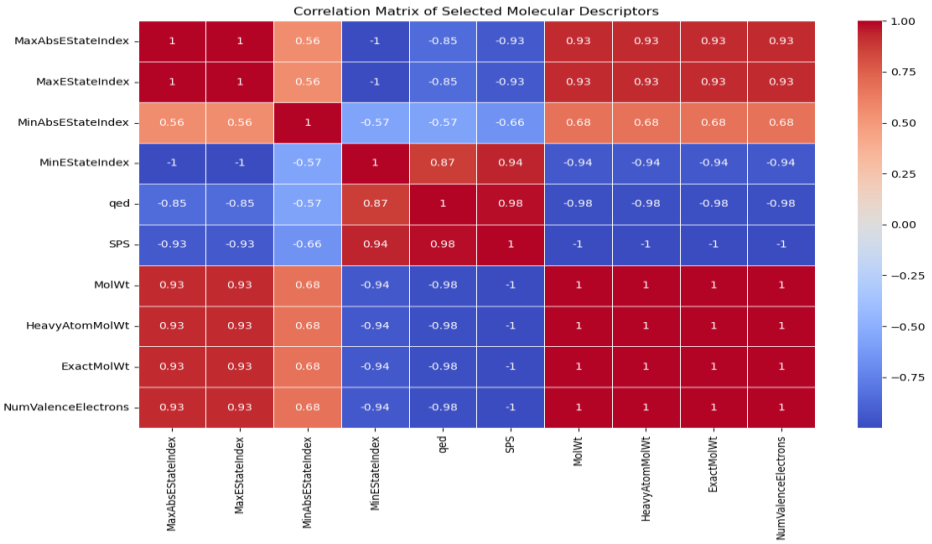
3. RESULT

The final dataset used for QSAR modeling comprised six flavonoid derivatives, each with empirically determined inhibitory activity values expressed as pIC_{50}. An initial set of 210 molecular descriptors was computed using cheminformatics tools, capturing a broad range of physicochemical, electronic, and topological features. Following preprocessing steps—which included variance filtering, correlation analysis, and descriptor standardization—the number of descriptors was reduced to eliminate redundancy and enhance model performance. The mean pIC_{50} value across the

compounds was 4.66, with distinct values ranging from 4.60 to 4.81, indicating a relatively narrow activity range within the dataset.

The correlation matrix of selected molecular descriptors (Figure 1) reveals important insights into the interrelationships among physicochemical and topological properties of flavonoid derivatives. Notably, a cluster of descriptors including molecular weight (MolWt), heavy atom molecular weight (HeavyAtomMolWt), exact molecular weight (ExactMolWt), and the number of valence electrons exhibit near-perfect positive correlations ($r \approx 1.00$), indicating redundancy due to their shared dependence on atomic composition. Similarly, MaxEStateIndex and MaxAbsEStateIndex are perfectly correlated, suggesting they represent overlapping information, as the latter is derived from the former by taking the absolute value. On the other hand, strong negative correlations were detected between the synthetic accessibility score (SPS) and descriptors such as MolWt, MaxAbsEStateIndex, and quantitative estimate of drug-likeness (qed), with r values approaching –1.00. This displays that molecules with greater complexity and higher molecular weight tend to be less synthetically accessible and often less drug-like. Furthermore, qed itself is negatively correlated with molecular weight and MaxAbsEStateIndex ($r \approx -0.85$ to -0.98), highlighting a trade-off between structural complexity and favorable drug-likeness. Moderate correlations were seen between MinAbsEStateIndex and weight-related descriptors, while MinEStateIndex provided a strong positive correlation with qed and SPS ($r \approx 0.87$–0.94), suggesting its potential role in influencing drug-like and synthetic properties. Overall, this analysis underscores the importance of reducing multicollinearity by excluding highly correlated descriptors during model development, and supports the selection of features like qed, SPS, and EState indices, which offer distinct and biologically relevant information for QSAR modeling.

Figure 1: Heatmap of pairwise Pearson correlation coefficients (r)



Due to the limited dataset size comprising only six flavonoid compounds, the development of a highly robust predictive model posed significant challenges. To assess potential structure-activity relationships, a LASSO regression model was employed. As per Table 2, The model achieved a training R^2 value of 0.7445, indicating that approximately 74.45% of the variance in the pIC_{50} values was explained within the training set. The corresponding RMSE was 0.4903 pIC_{50} units, reflecting a relatively low prediction error.

Table 2. LASSO Model Training Performance

Metric	Value
Training R^2	0.7445
Training RMSE	0.4903

Feature selection through LASSO identified five key molecular descriptors from the pre-processed set of ten: LogP (0.5389), TPSA (-0.2115), Rotatable Bonds (0.1643), NumHDonors (0.0709), and AromaticRings (-0.0642). Among these,

LogP and TPSA emerged as the most influential descriptors, positively and negatively correlating with biological activity, respectively as detailed in Table 3.

Table 3. LASSO selected 5 Key molecular descriptors and their coefficients

Descriptor	Coefficient
LogP	0.5389
TPSA	-0.2115
RotatableBonds	0.1643
NumHDonors	0.0709
AromaticRings	-0.0642

To evaluate model generalizability and potential overfitting, Leave-One-Out Cross-Validation (LOO-CV) was performed. The cross-validated Q^2 value was 0.5558 with an RMSE of 0.6465. The difference between the training R^2 and Q^2 (0.1887) was below the commonly accepted threshold of 0.3, indicating the nonexistence of significant overfitting. This hints that the LASSO model maintains reasonable predictive ability even when exposed to unseen data within the small dataset, as detailed in Table 4.

Table 4. LASSO Model Overfitting Assessment (LOO Cross-Validation)

Metric	Value
Leave-One-Out Q^2	0.5558
Leave-One-Out RMSE	0.6465
R^2 - Q^2	0.1887
Overfitting Status	No significant overfitting (< 0.3)

The predictive performance of the model is graphically illustrated in Figure 2 (training set) and Figure 3 (LOO-CV), showing predicted versus actual pIC_{50} values. In the training set, predictions were closely aligned with actual values, while the LOO-CV scatter plot revealed greater variability but maintained acceptable alignment, further supporting the model's internal validity. These findings confirm that the LASSO regression model, despite inherent data limitations, provides valuable insights into the structure-activity relationships of flavonoids and identifies critical physicochemical features that influence anticancer activity against A375 melanoma cells.

Figure 2: Predictive performance of the model of training set (R^2)

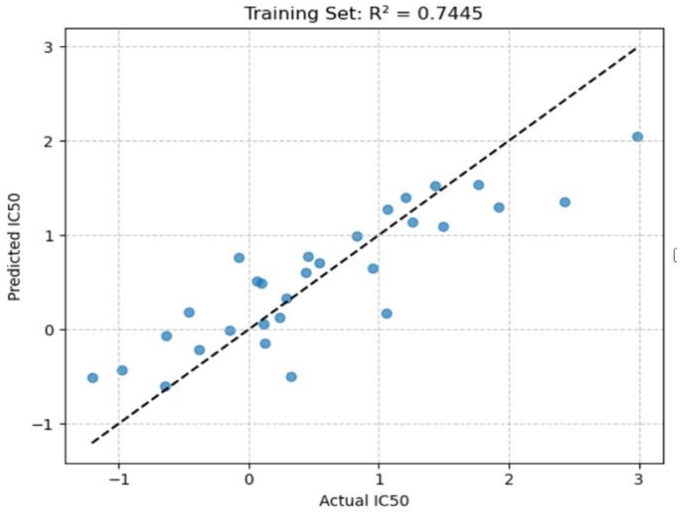
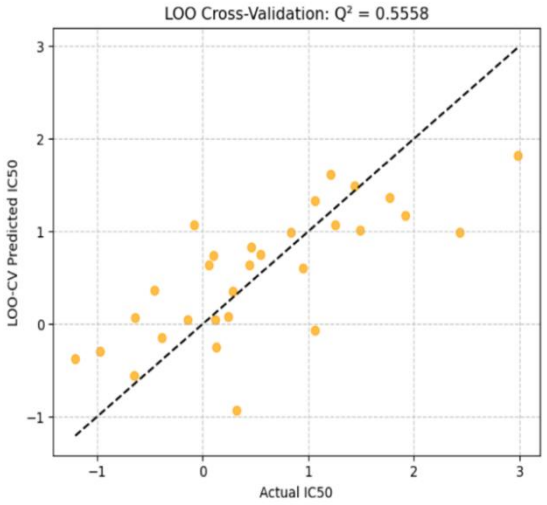


Figure 3: Predictive performance of LOO Cross-Validation (Q^2)



4. DISCUSSION

The QSAR analysis performed in this study identified many molecular descriptors as key predictors of the anticancer activity of flavonoids against A375 melanoma cells. Specifically, the LASSO regression model highlighted lipophilicity (LogP), topological polar surface area (TPSA), number of rotatable bonds, number of hydrogen bond donors, and number of aromatic rings as significant contributors. These outcomes are mostly consistent with established principles in medicinal chemistry and known bioactivity mechanisms of flavonoids [15, 24]. For instance, the positive coefficient for LogP suggests that increased lipophilicity favors higher activity, likely by enhancing cell membrane permeability and facilitating entry into the melanoma cells. Conversely, the negative coefficient for TPSA indicates that excessive polarity might hinder activity, possibly by reducing membrane passage or bioavailability [25]. The observation that luteolin and myricetin, which possess relatively higher polarity and hydrogen-bonding capacity within the dataset, demonstrated high pIC_{50} values suggests a nuanced interplay of these factors.

The LASSO regression model identified five key molecular descriptors—LogP, TPSA, rotatable bonds, number of hydrogen bond donors, and aromatic rings—that significantly influence the anticancer activity of flavonoids against A375 melanoma cells. Among these, LogP exhibited the strongest positive correlation with activity (coefficient = +0.5389), suggesting that enhanced lipophilicity improves membrane permeability and cellular uptake, thereby increasing efficacy. Structural modifications that increase lipophilicity, such as the addition of alkyl chains or methoxy groups, may therefore be beneficial, with the model supporting a target LogP range of 2–5 or slightly higher within the studied chemical space. Conversely, TPSA demonstrated a negative correlation with activity (coefficient = –0.2115), indicating that excessive polarity diminishes the potential of flavonoids to permeate cellular membranes and thus reduces bioavailability. While some polarity is essential for solubility and target interactions, an optimal balance is critical; the study recommends keeping TPSA below 80 Å² for improved activity, in line with established thresholds such as Lipinski's Rule of Five, which considers TPSA < 140 Å² desirable for oral drugs [25]. This is particularly relevant for hydroxyl-rich flavonoids like luteolin and myricetin, where high TPSA values may impair their performance despite promising binding capabilities.

The number of rotatable bonds (coefficient = +0.1643) also showed a modest positive influence, implying that increased molecular flexibility could aid in adopting favorable binding conformations. This suggests that the incorporation of flexible linkers, such as short alkyl chains or ether bridges, might enhance activity, provided such modifications do not lead to excessive molecular weight or compromise pharmacokinetics. Hydrogen bond donors, though weakly correlated (coefficient = +0.0709), appear to contribute positively to activity, reflecting the importance of specific interactions with target biomolecules within melanoma cells. However, overabundance of hydrogen bond donors can elevate TPSA and reduce permeability, underscoring the need for a balanced approach in scaffold design. Interestingly, aromatic ring count was very little negatively associated with activity (coefficient = –0.0642), possibly due to issues related to excessive planarity, non-specific binding, or reduced solubility. Although aromaticity is intrinsic to the flavonoid core, rational simplification of the aromatic system or careful substitution may enhance drug-like properties.

These findings provide important insights for the rational design of flavonoid-based anticancer agents. Importantly, they hint that molecules with LogP values between 2–3 and TPSA below 80 Å² may be prioritized for further investigation. While the model demonstrates robustness within the current dataset, it is crucial to acknowledge the limitations imposed by the small sample size (N = 6). Despite favorable internal validation metrics (e.g., R^2, Q^2, and R^2–Q^2), the generalizability of the model remains constrained, and its predictive capacity for novel flavonoids cannot be guaranteed. Therefore, these

results should be considered hypothesis-generating, offering a rational basis for subsequent experimental efforts and compound library expansion. Future studies should aim to increase dataset diversity, validate proposed modifications through in vitro and in vivo assays, and explore nonlinear modeling techniques or 3D-QSAR methods to capture more intricate structure–activity relationships. Ultimately, integrating these strategies will support the development of more robust and broadly predictive QSAR models for anticancer flavonoid derivatives.

5. CONCLUSION

This study successfully employed LASSO regression to develop an interpretable QSAR model for predicting the anticancer activity of a small set of flavonoid derivatives against A375 melanoma cells. The model identified LogP, TPSA, RotatableBonds, NumHDonors, and AromaticRings as key molecular descriptors influencing activity. Despite the limited dataset, the model resulted good internal consistency and no significant overfitting, providing a computational roadmap for guiding the design of novel flavonoid-based anti-melanoma agents. Specifically, flavonoid scaffolds with optimized lipophilicity (LogP 2–3) and reduced polar surface area (TPSA < 80 Å²) are highlighted as high-priority candidates for future synthesis and experimental validation. This work underscores the utility of integrating QSAR modeling with feature stability analysis in the initial stages of drug discovery, even with limited data, to accelerate the identification of promising therapeutic leads. Further studies involving larger datasets and experimental validation are warranted to refine these predictive models and advance the development of effective flavonoid-based therapies for melanoma.

CONFLICT OF INTEREST:

The authors declare no conflicts of interest regarding this investigation.

REFERENCES

[1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17-48.

[2] Krasowska D, Kurzepa J, Błaszczak E. Current state of melanoma treatment: from conventional therapies to nanotechnology and beyond. J Pre-Clin Clin Res. 2024;18(4):333–340.

[3] Gaggioli C, Robert G, Bertolotto C, et al. A375 human melanoma cells: a relevant model for preclinical studies of this cancer. Pathol Biol (Paris). 2005;53(3):133-141.

[4] Robert C, Grob JJ, Stroyakovskiy D, et al. Five-year outcomes with nivolumab plus ipilimumab versus ipilimumab alone in previously untreated advanced melanoma: an update. J Clin Oncol. 2019;37(15_suppl):9506.

[5] Varela G, et al. Current Advancements and Novel Strategies in the Treatment of Metastatic Melanoma. Cancers (Basel). 2022;14(1):201.

[6] Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.

[7] Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. Nutrients. 2020;12(2):457.

[8] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. 2013;2013:162750.

[9] Abotaleb M, Liskova A, Kubatka P, Büsselberg D. Therapeutic Potential of Flavonoids in Cancer: ROS-Mediated Mechanisms. Biomolecules. 2020;10(12):1732.

[10] Khan H, Ullah H, Aschner M, et al. Neuroprotective Effects of Quercetin in Alzheimer's Disease. Biomolecules. 2019;10(1):E59. (While this reference is for AD, the general mechanisms of flavonoids like ROS modulation are relevant. A more cancer-specific one like the original paper's ref 9 "Tang et al., 2021" would be ideal if full details were available, or a general review on flavonoids and ROS in cancer).

[11] Almatroudi A, Alrumaihi F, Allemailem KS, et al. Analysis of some flavonoids for inhibitory mechanism against cancer target phosphatidylinositol 3-kinase (PI3K) using computational tool. Front Pharmacol. 2023;14:1236173.

[12] Cherkasov A, Muratov EN, Fourches D, et al. QSAR modeling: where have you been? Where are you going to?. J Med Chem. 2014;57(12):4977-5010.

[13] Dearden JC. The History and Development of Quantitative Structure-Activity Relationships (QSARs). Int J Mol Sci. 2016;18(1):E64.

[14] QSPRmodeler - An open source application for molecular predictive analytics. Front Bioinform. 2024.

[15] Alves VM, Muratov EN, Capuzzi SJ, et al. Predicting mutagenicity with a new QSAR benchmark dataset. Chem Res Toxicol. 2020;33(10):2579-2587.

[16] Tibshirani R. Regression shrinkage and selection via the lasso. J R Stat Soc Series B Stat Methodol. 1996;58(1):267-288.

[17] Hastie T, Tibshirani R, Friedman J. The elements of statistical learning: data mining, inference, and prediction. 2nd ed. Springer; 2009.

[18] Feature Selection Using Lasso Regression Enhances Deep Learning Model Performance For Diagnosis Of Lung Cancer from Transcriptomic Data. bioRxiv. 2024.

[19] Integrated QSAR Models for Prediction of Serotonergic Activity: Machine Learning Unveiling Activity and Selectivity Patterns of Molecular Descriptors. Int J Mol Sci. 2024;25(6):3487.

[20] Syed DN, Adhami VM, Khan N, Khan MI, Mukhtar H. Fisetin inhibits human melanoma cell invasion through promotion of mesenchymal to epithelial transition and by targeting MAPK and NFκB signaling pathways. Cancer Res. 2011;71(8):2951–2959.

[21] Pick A, Müller H, Wiese M. Structure-activity relationships of flavonoids as inhibitors of breast cancer resistance protein (BCRP). Bioorg Med Chem. 2015;23(12):2920–2932.

[22] Luo Y, Tian G, Zhuang Z, Chen J, You L, Zhang W. Flavonoids from Scutellaria baicalensis as potential melanoma agents. J Nat Prod. 2018;81(9):2111–2120.

[23] Zhang X, Huang Y, Song H, Chen F. Inhibitory effects of myricetin on proliferation and metastasis of A375 melanoma cells. RSC Adv. 2016;6(68):63717–63725.

[24] Lo Piparo E, Scheib H, Frei N, et al. Flavonoids for controlling starch digestion: structural requirements for inhibiting human alpha-amylase. J Med Chem. 2008;51(12):3555-3561.

[25] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1-3):3-26.