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Investigating SAR Insights into Royleanones for P-gp Modulation †

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Abstract: Multidrug resistance (MDR) presents a significant challenge in modern pharmacotherapy, greatly diminishing the effectiveness of chemotherapeutic agents. A primary mechanism contributing to MDR is the overexpression of P-glycoprotein (P-gp), also known as MDR1, encoded by the ABCB1 gene, which hampers the success of cancer treatments. Plants from the *Plectranthus* genus (Lamiaceae) have been traditionally acknowledged for their diverse therapeutic applications. The principal diterpene from *Plectranthus grandidentatus* Gürke, 7α-acetoxy-6β-hydroxyroyleanone (Roy), has demonstrated anti-cancer properties against various cancer cell lines. Previously synthesized ester derivatives of Roy have shown improved binding affinity to P-gp. This study employs previously acquired in vitro data on the P-gp activity of Roy derivatives to develop a ligand-based pharmacophore model, highlighting critical features necessary for P-gp modulation. Utilizing this data, we predict the potential of five novel ester derivatives of Roy to modulate P-gp in vitro against resistant NCI-H460 cells. In silico structure-activity relationship (SAR) studies were conducted on 17 previously synthesized royleanone derivatives. A binary classification model was constructed, distinguishing inactive from active compounds, generating 11,016 Molecular Interaction Field (MIF) descriptors from structures optimized at the DFT theory level. After variable reduction and selection, 12 descriptors were chosen, resulting in a model with two latent variables (LV), using only 34.14% of the encoded information for calibration (LV1: 26.82%; LV2: 7.32%). The activity prediction of new derivatives suggested that four have a high likelihood of activity, which will be validated in future *in vitro* biological assays.

Keywords: SAR; royleanones; P-gp; multi-drug resistance; *Plectranthus*

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1. Introduction

Multidrug resistance (MDR) remains a major challenge in pharmacotherapy, significantly reducing the effectiveness of many cancer treatments. MDR is linked to around 90% of cancer-related deaths [1,2]. A key factor in MDR is the overexpression of P-glycoprotein (P-gp), also known as MDR1, which is encoded by the ABCB1 gene. P-gp is an efflux pump from the ATP-binding cassette (ABC) family and expels various antineoplastic agents, such as anthracyclines, vinca alkaloids, and taxanes, from cancer cells, lowering drug concentrations and contributing to treatment resistance. This overexpression is also seen with targeted therapies like imatinib [3]. While early P-gp inhibitors like verapamil (VER) improved drug retention in MDR cells, their severe side effects highlighted the need for better options [4]. Newer inhibitors like tariquidar (Tqd) have shown promise but come with significant toxicity risks [5]. Both VER and Tqd function by increasing intracellular drug concentrations through competition at the P-gp binding site, but better-tolerated modulators are urgently needed.

Natural products have gained attention as potential sources of bioactive compounds for MDR therapy. The Lamiaceae family, which includes plants like mint and rosemary, contains various promising compounds, particularly in the genus *Plectranthus*. These plants, widely used in traditional medicine, have around 350 species rich in diterpenes, such as royleanones, known for their anticancer effects [6–8]. One notable compound, 7α-acetoxy-6β-hydroxyroyleanone (Roy, Figure 1), isolated from *Plectranthus grandidentatus*, has shown anticancer activity against various cell lines [6,8–10]. Though Roy itself is not a P-gp substrate, previous work has shown that modifying its structure can improve its binding affinity for P-gp. Substitution at positions C-6 and C-12 of Roy's structure, particularly with aromatic groups, may enhance its biological activity [6,8]. For example, a chloro-benzoyloxy derivative of Roy showed strong MDR-reversal activity in a 2020 study, similar to the known P-gp inhibitor Dex-VER [7].

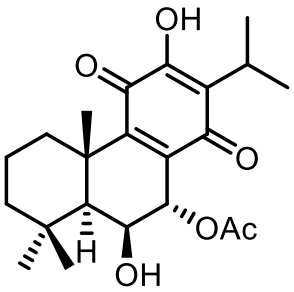

Figure 1. Natural compound 7α-acetoxy-6β-hydroxyroyleanone (Roy), extracted from *Plectranthus grandidentatus* Gürke**.**

To further improve the efficacy of Roy derivatives, Structure-Activity Relationship (SAR) studies help map the relationship between chemical structure and biological function. These studies allow researchers to design better compounds by identifying essential features for P-gp inhibition. Using in vitro data from earlier studies, this research aims to showcase critical features necessary for P-gp modulation in resistant cancer cells, with the goal of advancing MDR cancer therapies.

2. Methods

Seventeen royleanone derivatives, previously synthesized and evaluated by our research team [6,8,11] (Figure 2) for their potential to inhibit P-gp in combating multidrug-resistant tumors, were utilized for SAR studies in silico. To create a classification model, the compounds were assigned as a binary dependent variable, where inactive compounds were labeled as 1 and active compounds as 2.

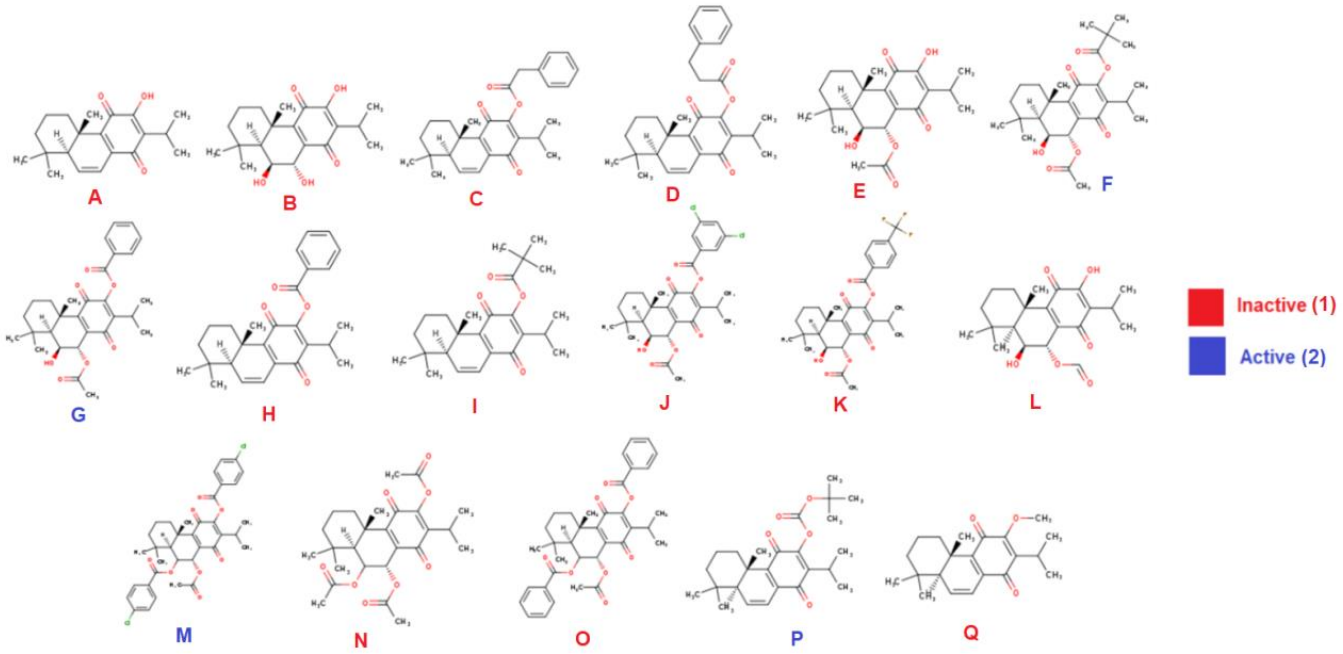

Figure 2. Seventeen derivatives of the natural compound Roy used for the in silico study.

Three-dimensional structures were created in HyperChem 7 software and optimized using density functional theory (DFT) in Gaussian 09, utilizing the B3LYP functional with the 6-311g++(d,p) basis set. Electrostatic potential (ESP) partial charges were computed via the CHELPG method, and structures were aligned based on their common diterpene nucleus.

Molecular Interaction Fields (MIF) descriptors were derived from the ESP charges and optimized geometries using Coulomb and Lennard-Jones potentials through the LQTA-QSAR approach [12]. A virtual grid measuring 18 × 13 × 13 Å with 1 Å cubes was used, and MIF descriptors were calculated in the LQTAGrid module with a NH4+-type probe [12]. Variables with a standard deviation below 0.1, correlation with the class vector below 0.3, and inter-descriptor correlations above 0.9 were excluded, keeping the one with the highest correlation with activity. Variable selection used a genetic algorithm (max variables: 20; population size: 500; migration rate: 0.2; crossover rate: 0.5; mutation rate: 0.2) [13]. Consistent auto-scaling of descriptors was applied according to QSAR pre-processing guidelines [14].

Partial Least Squares (PLS) regression was employed to develop the final model [15]. Model quality was evaluated using R2, RMSEC, F-test, Q2LOO, and RMSECV. The model's ability to differentiate active and inactive compounds was analyzed via scatterplots in QSAR Modeling [16] and Pirouette 4 software.

3. Results & Discussion

A total of 11,016 MIF descriptors were generated using DFT-optimized structures. After variable reduction, 12 descriptors were selected, resulting in a model with two latent variables (LV1: 26.82%, LV2: 7.32%), utilizing 34.14% of the descriptor information. LV-based models allow for internal validation using QSAR statistics, as shown in the regression Equation (1):

$$\text{Class} = 0.5052 - 0.0012*(D1) + 0.0029*(D2) - 0.0016*(D3) + 0.0012*(D4) + 0.0109*(D5) - 0.0341*(D6) + 0044*(D7) + 0.0049*(D8) + 0.0055*(D9) + 0.0065*(D10) - 0.2095*(D11) + 0.0939*(D12) \quad (1)$$

The model accounts for 73.5% ($R^2 = 0.735$) and predicts 62.4% ($Q^2LOO = 0.624$) of the variance, indicating strong discrimination.

In the case of inactive compounds, most aromatic substituents at position R1 are located in the upper section of the model, where the two descriptors associated with reduced biological activity (D1 and D5) are situated. For active compounds, only one derivative appears in this area. While this might seem contradictory, D1 and D5 are among the least significant descriptors in the model, with D1 being the least significant overall. Descriptor D3, which is also in this region, ranks as the second most important descriptor. Thus, it can be suggested that this region correlates with a lack of activity. Although modifications involving this region should not be entirely excluded in the synthesis of future derivatives (as indicated by descriptor D3), avoiding interactions in this area may enhance the chances of producing active derivatives. The third descriptor that negatively impacts activity (D12) is located in the lower region near the acetyl groups connected to carbon 7. This structural characteristic is present in only one active compound, suggesting that occupying this position may hinder the activity. The other five key descriptors (D6, D7, D10, D11, and D12) are arranged around the central axis of the rolyeanone nucleus. Among them, the Lennard-Jones descriptor D6 is the most significant, positioned near aromatic substituents found only in two derivatives, one active and one inactive. The difference in compound M is the presence of a p-chloro group on the aromatic ring, which enhances liposolubility and lowers the substituent's electronic density. The P-gp binding site is notably hydrophobic, with many residues featuring aromatic groups [17]. This hydrophobicity explains the higher prevalence of Lennard-Jones descriptors (9) compared to Coulomb descriptors (3). The chlorine atom's impact on the electronic density of the aromatic substituent may influence various π interactions with the binding site. Based on these descriptors, new derivatives are currently being syntheised and evaluated, with preliminary results pointing to 4 derivatives with a high likelihood of activity (data not shown).

4. Conclusions

Using a collection of previously synthesized and tested derivatives, a structure-activity relationship (SAR) model was developed based on MIF descriptors, which effectively categorized the dataset into active and inactive compounds. The interpretation of the model enhanced its credibility, as the chosen descriptors were linked to the structural features of both the derivatives and the binding site of P-gp modulators. Ultimately, these descriptors have predicted the activity of the newly synthesised derivatives which are currently being validated through in vitro assays.

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