Bioactive Evaluation of the Protox Inhibitory Potential of Quercetin

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Abstract: **A common flavonoid found in common foods like fruits and vegetables, quercetin is well-known for its health benefits, which include lowering edema, combating oxidation, and even preventing cancer. However, we have to overlook the potential drawbacks—its toxicity, particularly when it comes to modified forms utilized in treatments. In this study, we examined the oral toxicity of quercetin and three related chemicals using computer-based predictions through ProTox-3.0. Lethal dosages (LD50), toxicity levels, organ impacts, and their interactions with bodily pathways were among the parameters we studied. With an LD50 of 159 mg/kg (class 3), quercetin may be moderately hazardous, according to our data; however, the variations appear to be safer at 5000 mg/kg (class 5). They are all at risk for lung and kidney problems, as well as certain receptor interactions.**

This gives us a better idea of their safety, though real-lab tests are still needed. For initial checks, computer techniques such as these are quick, humane, and animal-free.

***Index Term*- Quercetin, Protox, LD-50, Toxicity**

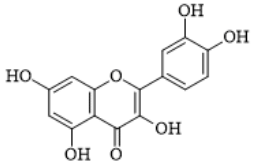
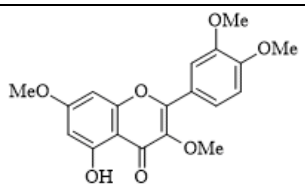
1. INTRODUCTION

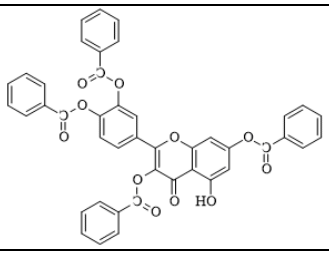
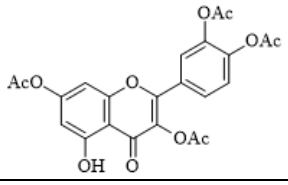
Scientifically known as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, quercetin is found in many foods, including tea, berries, onions, and apples [1]. Because of its potential to fight cancer cells, reduce inflammation, and serve as an antioxidant, it is often used in research [2]. However, excessive dosages could reverse the effects and make it a pro-oxidant that damages cells [3]. Comparing them is important since their safety may alter if we modify quercetin to create derivatives, perhaps to enhance the body's absorption of it or address particular problems [4].

Animals are frequently used in traditional toxicity assessments, which is time-consuming, expensive, and packed with ethical issues. In order predict dangers, in silico techniques such as ProTox-3.0 employ sophisticated algorithms that compare molecules and learn from data [5]. The GHS method is used to classify oral toxicity; LD50 indicates the class, which ranges from extremely toxic (I, ≤5 mg/kg) to essentially innocuous (VI, >5000 mg/kg) [5]. Here, we're using ProTox-3.0 to examine the expected toxicities of quercetin and three of its derivatives in order to aid in the development of safer treatments.

2. MATERIALS AND METHODS

The following four compounds were analysed for toxicity evaluation.

Compound	Structure	Molecular Weight	SMILES
Quercetin		302.24	Oc1cc(O)c2c(=O)c(O)c(c3cc(O)c(O)cc3)oc2c1
Methyl Quercetin		358.34	c12c(cc(cc1O)OC)oc(c(c2=O)OC)c1ccc(c(c1)OC)OC

Benzoyl Quercetin		662.73	c12c(cc(cc1O)OCc1ccccc1)oc(c(c2=O)OCc1ccccc1)c1ccc(c(c1)OCc1ccccc1)OCc1ccccc1
Acyl Quercetin		470.38	c1(c(=O)c2c(cc(cc2oc1c1ccc(c(c1)OC(=O)C)OC(=O)C)OC(=O)C)O)OC(=O)C

These structures were input into ProTox-3.0 based on their SMILES notations and physicochemical properties.

2.1 In Silico Toxicity Prediction

Toxicity predictions were performed using ProTox-3.0, a web-based tool that employs 61 machine learning models trained on extensive datasets for endpoints like acute toxicity, organ toxicity, and metabolic interactions. Inputs included SMILES strings, and outputs encompassed predicted LD50, toxicity class, average similarity to training data, prediction accuracy, and probabilities for active/inactive status across categories. Predictions with probabilities >0.5 were considered active. Data were exported in CSV format for analysis.

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity

The predicted LD50 and toxicity classes are summarized in Table 1(Figure 1-4). Quercetin (Compound 1) exhibited the highest toxicity with an LD50 of 159 mg/kg (Class 3), while all derivatives showed LD50 values of 5000 mg/kg (Class 5), indicating lower acute toxicity. Prediction accuracies ranged from 70.97% to 100%, with average similarities to training compounds between 83.38% and 100%.

Compound	Predicted LD50 (mg/kg)	Toxicity Class	Average Similarity (%)	Prediction Accuracy (%)
1	159	3	100	100
2	5000	5	99.19	72.9
3	5000	5	83.38	70.97
4	5000	5	85.13	70.97

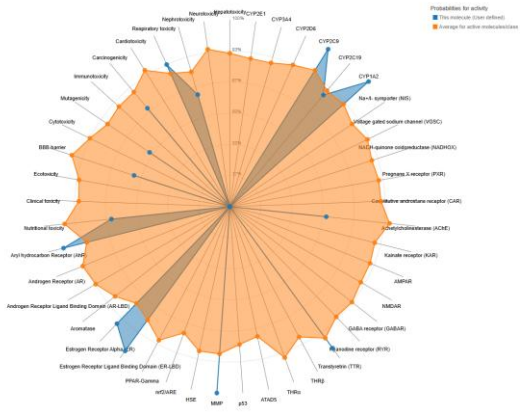


Figure-1 Compound-1

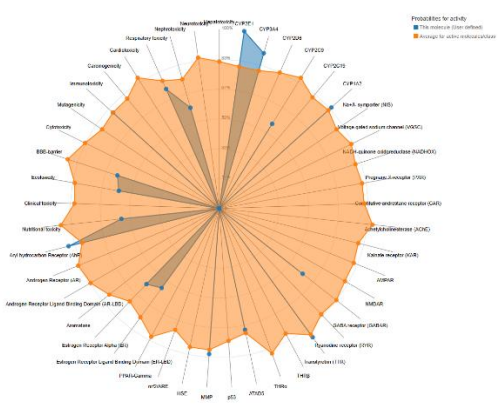


Figure-2 Compound-2

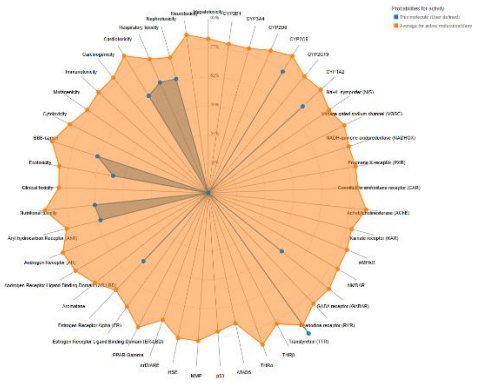


Figure-3 Compound-3

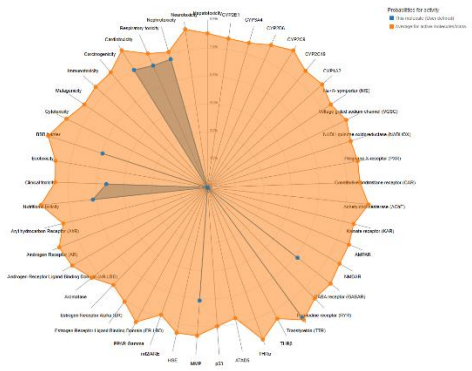


Figure-4 Compound-4

3.2 Organ Toxicity and Endpoints

Organ toxicities and general endpoints are detailed in Table 2. All compounds were predicted inactive for hepatotoxicity and neurotoxicity but active for nephrotoxicity and respiratory toxicity. Cardiotoxicity was inactive in Compounds 1 and 2 but active in 3 and 4. For endpoints, variations included active carcinogenicity and mutagenicity in Compound 1, and active ecotoxicity in Compounds 2 and 3.

Classification	Target	1	2	3	4
Organ Toxicity	Hepatotoxicity	Inactive (0.69)	Inactive (0.69)	Inactive (0.8)	Inactive (0.69)
	Neurotoxicity	Inactive (0.89)	Inactive (0.79)	Inactive (0.88)	Inactive (0.83)
	Nephrotoxicity	Active (0.62)	Active (0.58)	Active (0.63)	Active (0.71)
	Respiratory Toxicity	Active (0.83)	Active (0.73)	Active (0.64)	Active (0.71)
	Cardiotoxicity	Inactive (0.99)	Inactive (0.64)	Active (0.60)	Active (0.73)
Toxicity Endpoints	Carcinogenicity	Active (0.68)	Inactive (0.57)	Inactive (0.57)	Inactive (0.57)
	Immunotoxicity	Inactive (0.87)	Active (0.79)	Inactive (0.68)	Inactive (0.54)
	Mutagenicity	Active (0.51)	Inactive (0.70)	Inactive (0.68)	Inactive (0.63)
	Cytotoxicity	Inactive (0.99)	Inactive (0.99)	Inactive (0.88)	Inactive (0.85)
	BBB Permeability	Active (0.53)	Active (0.59)	Active (0.63)	Active (0.58)
	Ecotoxicity	Inactive (0.53)	Active (0.57)	Active (0.52)	Inactive (0.53)
	Clinical Toxicity	Inactive (0.53)	Inactive (0.53)	Inactive (0.59)	Active (0.53)
	Nutritional Toxicity	Active (0.63)	Active (0.55)	Active (0.61)	Active (0.61)

(Probabilities in parentheses; Active if >0.5)

3.3 Nuclear Receptor Signalling and Stress Response Pathways

All compounds showed activity in Aryl Hydrocarbon Receptor (AhR) and Estrogen Receptor (ER) pathways, with variations in others (e.g., Mitochondrial Membrane Potential active in most). Detailed probabilities are available in the supplementary data.

3.4 Molecular Initiating Events and Metabolism

Common active events included Transthyretin (TTR) binding and GABA receptor interaction. Metabolic predictions highlighted CYP1A2 activity in Compounds 1 and 2.

3.5 Toxicity Targets

Potential bindings were predicted for targets like ESR1, ESR2, and NR1I2 across all compounds, suggesting endocrine disruption potential.

4. DISCUSSION

The results align with known quercetin toxicity, where moderate acute toxicity (Class 3) is reported

due to its pro-oxidant behavior at high concentrations. Derivatives exhibited reduced acute toxicity, possibly due to structural modifications increasing molecular weight and lipophilicity, which may alter absorption and distribution[6]. Active nephrotoxicity and respiratory toxicity predictions warrant caution, as flavonoids can induce oxidative stress in these organs[7]. The activation of ER and AhR pathways supports quercetin's role as a phytoestrogen, potentially beneficial for anticancer applications but risky for endocrine-related toxicities.

Compared to literature, quercetin's LD50 predictions are consistent with experimental values around 160-200 mg/kg in rodents, validating ProTox-3.0's accuracy. Derivatives show promise with lower toxicity, but active cardiotoxicity in larger molecules (Compounds 3 and 4) suggests size-dependent effects[8]. Limitations include the reliance on computational models, which may not capture all in vivo interactions; thus, these predictions should guide, not replace, empirical studies.

5. CONCLUSION

This in silico evaluation reveals that while quercetin poses moderate oral toxicity risks, its derivatives appear safer with higher LD50 values. Shared concerns in nephrotoxicity, respiratory effects, and endocrine pathways highlight areas for further research. ProTox-3.0 proves valuable for early-stage screening, promoting ethical and efficient toxicity assessment in flavonoid-based drug development.

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