In Silico Pharmacokinetic Profiling, ADMET Prediction, and Drug-Likeness Evaluation of Phenolic Constituents Identified in Honey: Implications for Asthma and Inflammatory Disorders

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Abstract

Introduction: Honey is a natural product of considerable medicinal interest owing to its diverse phenolic composition and reported therapeutic properties. The honey samples contain a variety of biologically active phenolic compounds; however, the drug-likeness and pharmacokinetic suitability of these constituents have not been systematically characterized.

Materials and Methods: In the present study, five phenolic compounds commonly identified honey samples, namely fumaric acid, p-hydroxybenzoic acid, p-coumaric acid, trans-2-hydroxycinnamic acid, and chrysin, were evaluated using the SwissADMET computational platform. Physicochemical properties, lipophilicity, aqueous solubility, pharmacokinetic parameters, drug-likeness compliance, and medicinal chemistry attributes were assessed for each compound. The BOILED-Egg model was employed to predict gastrointestinal absorption and blood-brain barrier permeability.

Results: All five compounds demonstrated high predicted gastrointestinal absorption and satisfied Lipinski's Rule of Five without violations. Among the investigated molecules, chrysin exhibited the highest lipophilicity (consensus Log P = 2.55) and molar refractivity, suggesting enhanced membrane permeability and protein-binding potential. p-Coumaric acid and trans-2-hydroxycinnamic acid displayed the most balanced physicochemical profiles with respect to oral bioavailability. None of the compounds were predicted to be P-glycoprotein substrates or cytochrome P450 inhibitors.

Conclusion: Given the established anti-inflammatory and immunomodulatory properties attributed to these compound classes in the literature, the present findings support their consideration as candidate molecules for further experimental investigation in the context of inflammatory disorders.

Keywords: ADMET; BOILED-Egg model; Drug-likeness; Honey; In silico; Pharmacokinetics.

1. Introduction

Honey is one of the oldest natural products used for medicinal purposes, with therapeutic applications spanning several millennia across diverse civilizations [1,2]. Beyond its nutritional value, honey has traditionally been employed in the management of wounds, gastrointestinal disorders, respiratory conditions, and infections [1]. Its antioxidant, antimicrobial, anti-inflammatory, and antiproliferative properties are primarily attributed to phytochemical constituents, particularly flavonoids and phenolic acids [2,3]. The composition of these bioactive compounds varies considerably with botanical and geographical origin, thereby influencing therapeutic potential [4,6]. Honey phenolics comprise two major groups: phenolic acids and flavonoids. Phenolic acids include hydroxybenzoic acid derivatives, such as gallic acid, p-hydroxybenzoic acid, and vanillic acid, and hydroxycinnamic acid derivatives, including caffeic, p-coumaric, ferulic, and cinnamic acids. Flavonoids include flavones (chrysin, apigenin), flavonols (quercetin, kaempferol, galangin), flavanones (pinocembrin, naringenin, hesperetin), and flavanonols (pinobanksin). Their relative abundance depends on nectar sources and is influenced by geographical, climatic, and seasonal factors [3-6]. These compounds can modulate oxidative stress, inflammatory signaling, and immune responses through multiple mechanisms [2,3].

Despite the growing literature on honey-derived phenolics, pharmacokinetic and drug-likeness evaluations have primarily focused on compounds identified in European, Mediterranean, Asian, and Australasian honey varieties [7]. Recent analyses of honey samples from southern Kazakhstan identified fumaric acid, p-hydroxybenzoic acid, p-coumaric acid, trans-2-hydroxycinnamic acid, and chrysin as commonly occurring phenolics across multiple sampling localities [7]. Plant-derived polyphenols have attracted increasing attention because of their pleiotropic pharmacological activities, favorable preclinical safety profiles, and long history of human consumption [1-5]. Their ability to interact with multiple inflammatory pathways may be advantageous in complex multifactorial diseases. Phenolic acids and flavonoids have been reported to suppress Th2 cytokine production, inhibit mast cell degranulation, reduce eosinophil recruitment, and attenuate mucus hypersecretion in experimental models of allergic airway inflammation and asthma [6,7].

Honey has traditionally been used for the symptomatic management of respiratory conditions, including cough, bronchitis, and asthma [7,8]. Its demulcent properties may alleviate bronchial irritation, while its phenolic constituents may modulate airway inflammation through antioxidant and anti-inflammatory mechanisms [7,8]. Several honey-derived phenolics suppress *NF-kB* and *MAPK* signaling, reduce pro-inflammatory cytokine production, and attenuate oxidative stress in preclinical models [9-11]. For example, chrysin attenuates IgE-mediated immune responses and airway hyperresponsiveness, whereas p-coumaric acid inhibits cyclooxygenase-2 (*COX-2*) and inducible nitric oxide synthase (*iNOS*) expression through *NF-kB* pathway suppression [9-11].

In silico approaches have become indispensable in contemporary drug discovery by enabling rapid assessment of absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties before experimental validation [12-15]. Computational platforms such as SwissADME facilitate prediction of physicochemical characteristics, drug-likeness, and pharmacokinetic behavior, supporting the identification of compounds with favorable pharmaceutical profiles [14,15]. Therefore, the present study aimed to evaluate the drug-likeness potential and pharmacokinetic profiles of five phenolic compounds commonly identified in honey in the context of inflammatory disorders.

2. Materials and Methods

2.1. Honey Samples and Compound Selection

Honey samples were collected from four distinct localities in southern Kazakhstan (Table 1). Based on analytical findings and literature data, five biologically active compounds consistently detected across the samples were selected for in silico evaluation: fumaric acid, p-hydroxybenzoic acid, p-coumaric acid, trans-2-hydroxycinnamic acid, and chrysin. The phenolic profile of Kazakhstani honey varies with nectar source, geographical origin, and climatic conditions. These compounds were selected because they were repeatedly detected in honey samples from different locations in southern Kazakhstan [4,5]. Here, "commonly identified" refers to their recurrence across independent samples rather than relative abundance. Other constituents were excluded because they were detected only in specific samples, occurred at trace levels, or lacked sufficient consistency for comparative in silico analysis. The selected compounds also represent major phenolic classes in honey, including hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids, providing a structurally diverse and representative subset for preliminary pharmacokinetic and drug-likeness screening.

Table 1. Geographical origins of honey samples collected from Kazakhstan

Sample Code	Location
B17	Kogam Village, Otrar
B18	Kelintobe Village, Zhanakorgan
B19	Shymkent
B20	Shieli

2.2. *In Silico* Pharmacokinetic and Drug-Likeness Assessment

The Simplified Molecular Input Line Entry System (SMILES) notations for each compound were retrieved from the PubChem database (National Center for Biotechnology Information, U.S. National Library of Medicine) [15-16] and are presented in Table 2.

Table 2. SMILES notations of selected bioactive compounds.

Compound	SMILES Notation
Fumaric acid	C(=C/C(=O)O)\C(=O)O
p-Hydroxybenzoic acid	C1=CC(=CC=C1C(=O)O)O
p-Coumaric acid	C1=CC(=CC=C1/C=C/C(=O)O)O
trans-2-Hydroxycinnamic acid	C1=CC=C(C(=C1)/C=C/C(=O)O)O
Chrysin	C1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O

The physicochemical, pharmacokinetic, and drug-likeness properties of the selected compounds were evaluated using the SwissADME web platform (http://www.swissadme.ch), a freely accessible computational tool developed by the Swiss Institute of Bioinformatics [15,16]. Toxicity parameters, including LD_{50} values and toxicity classes, were determined using the ProTox-3.0 online server [15,16]. Bioavailability radar plots were generated to provide a graphical overview of oral bioavailability-related properties across six dimensions: lipophilicity (LIPO), molecular size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX) [15-19]. The BOILED-Egg (Brain or IntestinaL EstimateD permeation) model was used to visualize predicted passive GI absorption and BBB permeation based on WLOGP and TPSA coordinates [16,17].

2.3 Molecular Docking Studies

The potential binding modes and intermolecular interactions of the bioactive compounds with the target protein were investigated using Maestro 14.5 (Schrödinger, 2025–3). The three-dimensional crystal structure was retrieved from the Protein Data Bank (PDB) under accession code 5KIR. Prior to docking, the protein was prepared using the Protein Preparation Wizard in Schrödinger Maestro 14.5, including addition of missing hydrogen atoms, assignment of partial charges, completion of missing side chains, and modeling of absent loop regions. Water molecules located more than 3 Å from the binding site were removed. The prepared structure was energy-minimized at pH 7.0 using the OPLS4e force field. Ligands were prepared with LigPrep by optimizing ionization states within pH 7.0 ± 2.0, verifying stereochemistry, and performing energy minimization using the OPLS3e force field. The receptor grid was generated around the protein active site, with standardized dimensions of 20 × 20 × 20 Å. For 5KIR, grid-center coordinates were defined according to the active site, and docking simulations were performed around these coordinates [18-20].

3. Results

3.1. Physicochemical Properties

The physicochemical properties of the five bioactive compounds are summarized in Table 3. Molecular weights ranged from 116.07 g/mol (fumaric acid) to 254.24 g/mol (chrysin), with all compounds below the Lipinski threshold of 500 g/mol [21]. Chrysin exhibited the highest number of aromatic heavy atoms (16 of 19), greatest molar refractivity (71.97), and most extensive ring system, indicating substantial potential for van der Waals and π–π stacking interactions with biological targets. TPSA values ranged from 57.53 Å² (p-hydroxybenzoic acid, p-coumaric acid, and trans-2-hydroxycinnamic acid) to 74.60 Å² (fumaric acid), remaining below the 140 Å² threshold associated with poor intestinal absorption [22]. All compounds had two hydrogen-bond donors and 3–4 acceptors, satisfying Lipinski criteria and indicating favorable intermolecular interaction potential [21]. Fsp3 was 0.00 for all compounds, reflecting their predominantly aromatic or unsaturated character and falling below the recommended 0.25 saturation threshold of the SwissADME bioavailability radar [14].

p-Coumaric acid and trans-2-hydroxycinnamic acid shared the molecular formula $C_9H_8O_3$ and molecular weight (164.16 g/mol) but differed in hydroxyl position. Although this isomerism does not alter the global physicochemical descriptors calculated by SwissADME, it may affect intramolecular hydrogen bonding, conformation, and biological activity [14,21-23]. Overall, chrysin and the hydroxycinnamic acid derivatives showed the most favorable profiles for biological target engagement.

These differences reflect the structural characteristics of the compounds. Fumaric acid, a non-aromatic dicarboxylic acid, has high polarity and hydrogen-bonding capacity but limited hydrophobic and π-stacking interactions. p-Hydroxybenzoic acid contains one aromatic ring with carboxylic acid and phenolic hydroxyl groups, resulting in moderate aromaticity and lipophilicity. The hydroxycinnamic acids, p-coumaric acid and trans-2-hydroxycinnamic acid, contain a propenoic acid side chain that increases conjugation and conformational rigidity. Chrysin is the most structurally complex compound, comprising three fused rings and two phenolic hydroxyl groups at C-5 and C-7 that may facilitate specific hydrogen-bonding interactions with molecular targets. These structural differences are reflected in their physicochemical profiles in molecular weight, molar refractivity (Table 3).

Table 3. Physicochemical properties of bioactive compounds.

Property	Fumaric Acid	PHBA	p-Coumaric Acid	t2HCA	Chrysin
Formula	$C_4H_4O_4$	$C_7H_6O_3$	$C_9H_8O_3$	$C_9H_8O_3$	$C_{15}H_{10}O_4$
MW (g/mol)	116.07	138.12	164.16	164.16	254.24
Heavy atoms	8	10	12	12	19
Aromatic heavy atoms	0	6	6	6	16
Fsp3	0.00	0.00	0.00	0.00	0.00
Rotatable bonds	2	1	2	2	1
H-bond acceptors	4	3	3	3	4
H-bond donors	2	2	2	2	2
Molar refractivity	24.41	35.42	45.13	45.13	71.97
TPSA (A2)	74.60	57.53	57.53	57.53	70.67

PHBA, p-hydroxybenzoic acid; t2HCA, trans-2-hydroxycinnamic acid; MW, molecular weight; Fsp3, fraction of sp3-hybridized carbon atoms; TPSA, topological polar surface area.

3.2. Lipophilicity Analysis

Lipophilicity is a key determinant of membrane permeability and oral bioavailability [22,23]. Consensus Log P values, calculated from five computational models, are presented in Table 4 and Figure 1. Chrysin showed the highest value (2.55), consistent with its extended aromatic system and relatively low polar surface area, suggesting favorable passive membrane permeation and hydrophobic protein interactions [21-23]. Trans-2-Hydroxycinnamic acid (1.40) and p-coumaric acid (1.26) fell within the optimal range for oral drug candidates (1.0–3.0), indicating balanced hydrophilic-lipophilic properties [23-25]. p-Hydroxybenzoic acid showed moderate lipophilicity (1.05), whereas fumaric acid had a negative Log P (-0.35), reflecting its highly hydrophilic character. Although all compounds fell within the broadly acceptable range (-0.7 to +5.0), fumaric acid may exhibit limited passive diffusion across lipid bilayers [15].

205 **Table 4.** Lipophilicity and Aqueous Solubility parameters of bioactive compounds.

Compound	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log P
Fumaric acid	0.32	-0.34	-0.29	-0.64	-0.81	-0.35
PHBA	0.85	1.58	1.09	0.99	0.74	1.05
p-Coumaric acid	0.95	1.46	1.38	1.28	1.22	1.26
t2HCA	1.09	2.03	1.38	1.28	1.22	1.40
Chrysin	2.27	3.52	2.87	1.08	3.02	2.55

Compound	Log S (ESOL)	Class	Log S (Ali)	Class	Log S (SILICOS-IT)	Class
Fumaric acid	-0.21	Very soluble	-0.76	Very soluble	1.34	Soluble
PHBA	-2.07	Soluble	-2.40	Soluble	-1.17	Soluble
p-Coumaric acid	-2.02	Soluble	-2.27	Soluble	-1.28	Soluble
t2HCA	-2.37	Soluble	-2.87	Soluble	-1.28	Soluble
Chrysin	-4.19	Mod. soluble	-4.69	Mod. soluble	-4.98	Mod. Soluble

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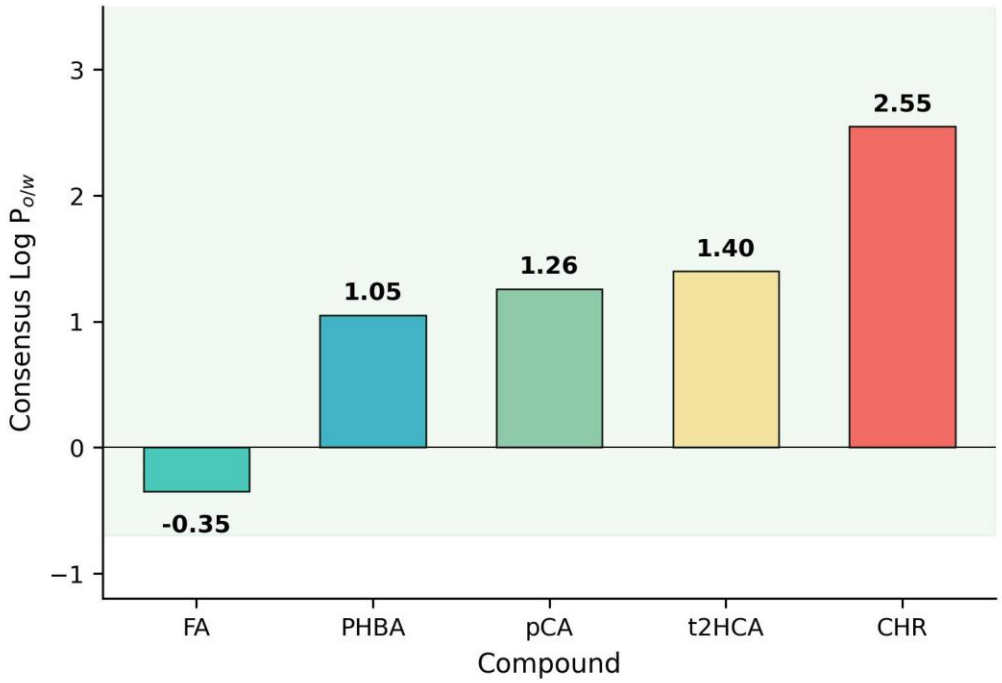

207 **Figure 1.** Consensus Log P values of the investigated bioactive compounds

208

3.3. Aqueous Solubility

The predicted aqueous solubility values obtained using ESOL, Ali, and SILICOS-IT are summarized in Table 4. Fumaric acid showed the highest predicted solubility, classified as "very soluble" by ESOL (log S = -0.21) and Ali (log S = -0.76), consistent with its low molecular weight and high polarity. p-Hydroxybenzoic acid, p-coumaric acid, and trans-2-hydroxycinnamic acid were classified as "soluble" by all three models, indicating favorable dissolution properties for oral administration [22-24].

Chrysin exhibited the lowest predicted solubility and was classified as "moderately soluble" (ESOL log S = -4.19; Ali log S = -4.69; SILICOS-IT log S = -4.98), likely reflecting its extended aromatic conjugation and higher lipophilicity. Thus, despite its favorable membrane-permeation and protein-binding characteristics, poor aqueous solubility may limit its oral bioavailability and require formulation strategies such as nanoencapsulation, cyclodextrin complexation, or solid dispersion [22-24]. The hydroxycinnamic acid derivatives showed a favorable balance between solubility and lipophilicity, suggesting potentially more predictable pharmacokinetic behavior (Table 4).

3.4. Pharmacokinetic Predictions

The predicted pharmacokinetic properties are presented in Table 5. All five compounds showed high predicted gastrointestinal (GI) absorption, consistent with their favorable Lipinski profiles and TPSA values [22-25]. Four compounds were predicted to cross the blood-brain barrier (BBB), whereas fumaric acid was not, consistent with its higher polarity (TPSA = 74.60 Å^2) and low lipophilicity in the BOILED-Egg model. None of the compounds were predicted to be P-glycoprotein (P-gp) substrates or inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. These predictions suggest a low likelihood of active efflux and CYP-mediated pharmacokinetic interactions, although experimental validation is required [25]. Chrysin showed the highest predicted skin permeation coefficient (log Kp = -5.35 cm/s), whereas fumaric acid showed the lowest (-7.25 cm/s), consistent with their lipophilicity profiles (Figure 2) [25].

241 **Table 5.** Predicted pharmacokinetic properties of bioactive compounds.

Parameter	Fumaric Acid	PHBA	p-Coumaric Acid	t2HCA	Chrysin
GI absorption	High	High	High	High	High
BBB permeant	No	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log Kp (cm/s)	-7.25	-6.02	-6.26	-5.86	-5.35

GI, gastrointestinal; BBB, blood-brain barrier; P-gp, P-glycoprotein; CYP, cytochrome P450; Log Kp, skin permeation coefficient; PHBA, p-hydroxybenzoic acid; t2HCA, trans-2-hydroxycinnamic acid.

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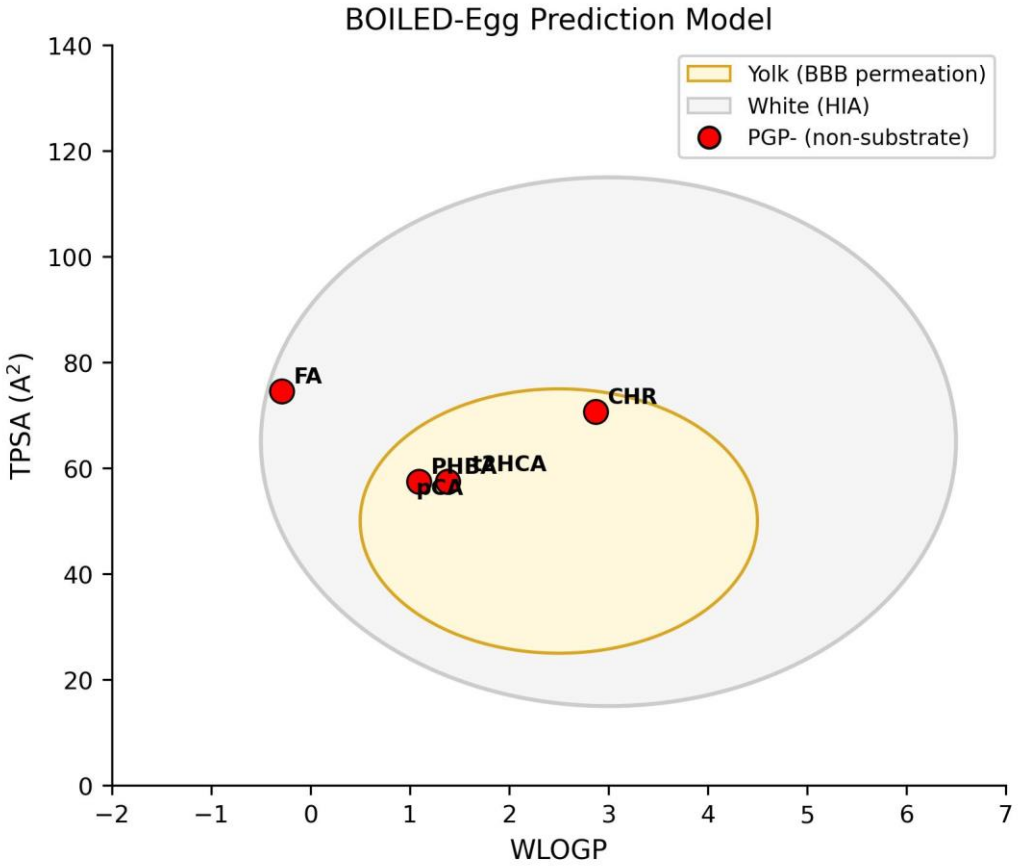


243 **Figure 2.** BOILED-Egg model predictions of compounds.

3.5. Drug-Likeness Assessment

Drug-likeness was evaluated using five complementary filters (Lipinski, Ghose, Veber, Egan, and Muegge), with results presented in Table 6. All five compounds satisfied the Lipinski, Veber, and Egan criteria without violations, supporting their basic suitability for oral drug development [22-25]. p-Coumaric acid and trans-2-hydroxycinnamic acid also passed the Ghose filter, whereas fumaric acid and p-hydroxybenzoic acid showed Ghose and Muegge violations, mainly due to their low molecular weights [23-25]. Chrysin was the only compound satisfying all five drug-likeness filters. However, its predicted bioavailability score (0.55) was lower than that of the other compounds (0.85), likely reflecting its lower aqueous solubility. This finding highlights the importance of the solubility–lipophilicity balance for oral bioavailability and the potential need for formulation optimization of chrysin [25].

Table 6. Drug-likeness evaluation and Medicinal chemistry attributes of bioactive compounds

Compound	Lipinski	Ghose	Veber	Egan	Muegge	BA Score
Fumaric acid	Yes; 0 viol.	No; 3 viol.	Yes	Yes	No; 2 viol.	0.85
PHBA	Yes; 0 viol.	No; 3 viol.	Yes	Yes	No; 1 viol.	0.85
p-Coumaric acid	Yes; 0 viol.	Yes	Yes	Yes	No; 1 viol.	0.85
t2HCA	Yes; 0 viol.	Yes	Yes	Yes	No; 1 viol.	0.85
Chrysin	Yes; 0 viol.	Yes	Yes	Yes	Yes	0.55

Compound	PAINS Alerts	Brenk Alerts	Leadlikeness	SA Score
Fumaric acid	0	1 michael_acceptor_1)	No; MW < 250	1.80
PHBA	0	0	No; MW < 250	1.00
p-Coumaric acid	0	1 (michael_acceptor_1)	No; MW < 250	1.61
t2HCA	0	1 (michael_acceptor_1)	No; MW < 250	1.85
Chrysin	0	0	No; XLOGP3 > 3.5	2.93

BA, bioavailability; viol., violation(s); PHBA, p-hydroxybenzoic acid; t2HCA, trans-2-hydroxycinnamic acid.

PAINS, pan-assay interference compounds; SA, synthetic accessibility; MW, molecular weight; PHBA, p-hydroxybenzoic acid; t2HCA, trans-2-hydroxycinnamic acid.

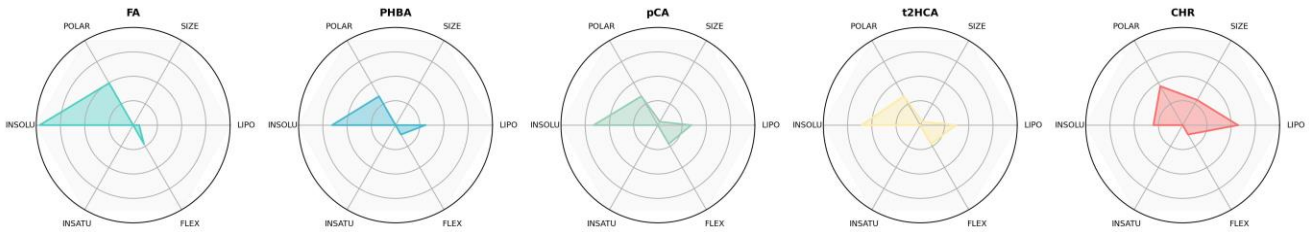


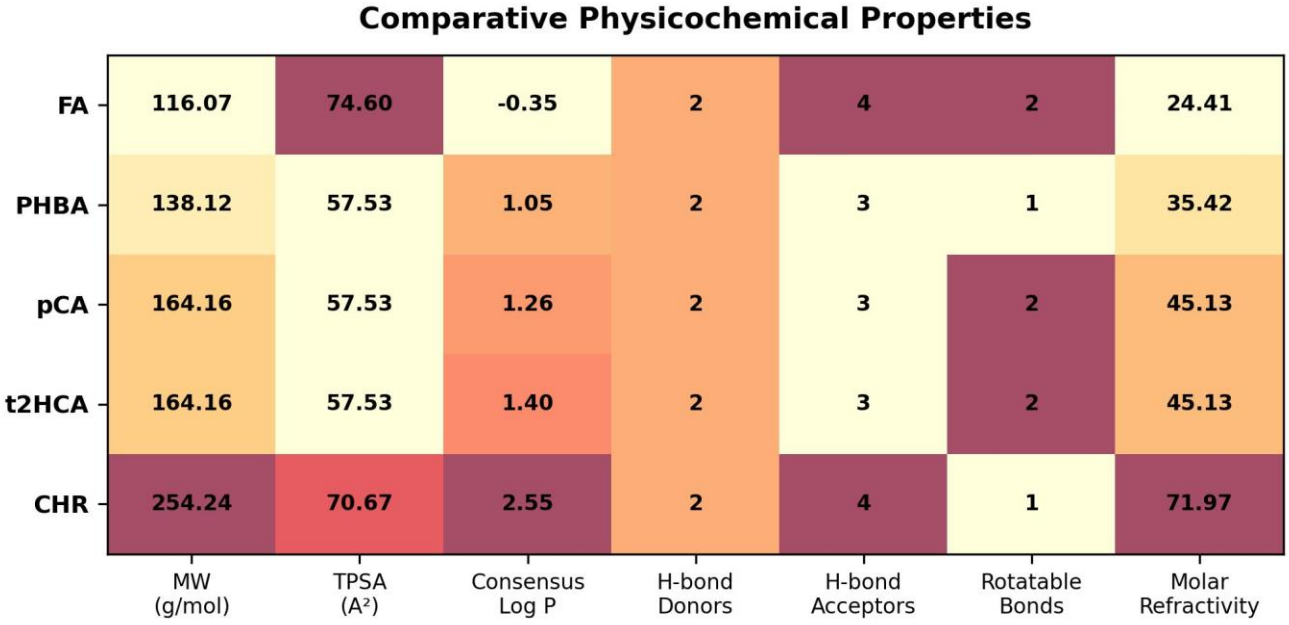
Figure 3. Bioavailability radar plots for bioactive compounds

3.6. Medicinal Chemistry Attributes

Medicinal chemistry assessment (Table 6) showed that none of the five compounds triggered PAINS alerts, suggesting a low likelihood of nonspecific reactivity [25]. Brenk michael_acceptor_1 alerts were identified for fumaric acid, p-coumaric acid, and trans-2-hydroxycinnamic acid due to their α,β-unsaturated carbonyl groups. Although this feature may indicate electrophilic reactivity, it is also common in approved drugs and natural products and does not preclude further development [25].

Synthetic accessibility scores ranged from 1.00 (p-hydroxybenzoic acid) to 2.93 (chrysin) on a 1–10 scale, indicating relatively favorable synthetic accessibility for all compounds. Combined with their natural occurrence in honey, these findings support their potential for further development [25].

Figure 4. Comparative physicochemical heatmap of the compounds



3.7. Integrated BOILED-Egg and Bioavailability Radar Interpretation

The BOILED-Egg model predicts gastrointestinal absorption and BBB permeation based on WLOGP and TPSA coordinates [23-25]. As shown in Figure 2, all five compounds were located in the white region, indicating high predicted human intestinal absorption, while four were also positioned in the yellow region (yolk), suggesting potential passive BBB permeation. Fumaric acid was outside the yolk, consistent with its high polarity and TPSA. All compounds were PGP−, indicating no predicted P-glycoprotein substrate activity.

Although BBB permeability may be relevant to neuroimmune interactions, its significance here should be interpreted cautiously because these findings are based solely on computational predictions. Accordingly, BBB permeability should be considered a general pharmacokinetic indicator rather than evidence of therapeutic central effects.

The bioavailability radar plots (Figure 3) showed that p-coumaric acid and trans-2-hydroxycinnamic acid had the most balanced profiles across the evaluated parameters. All compounds failed the saturation criterion (Fsp3 $\geq$ 0.25), reflecting their predominantly aromatic structures. Overall, integrated physicochemical, pharmacokinetic, and drug-likeness analyses identified p-coumaric acid and trans-2-hydroxycinnamic acid as the most favorable candidates, followed by p-hydroxybenzoic acid, chrysin, and fumaric acid.

3.8. Oral Toxicity Prediction and Molecular Docking Results

Toxicity was predicted using the ProTox-3.0 online server. Predicted LD_{50} values ranged from 1350 to 3919 mg/kg (Table 7), with chrysin showing the highest (3919 mg/kg) and fumaric acid the lowest value (1350 mg/kg). Toxicity classes were additionally predicted using the ProTox-II model, which ranks compounds from class 1 (most toxic) to class 6 (least toxic). Overall, the investigated honey-derived compounds showed relatively low predicted toxicity.

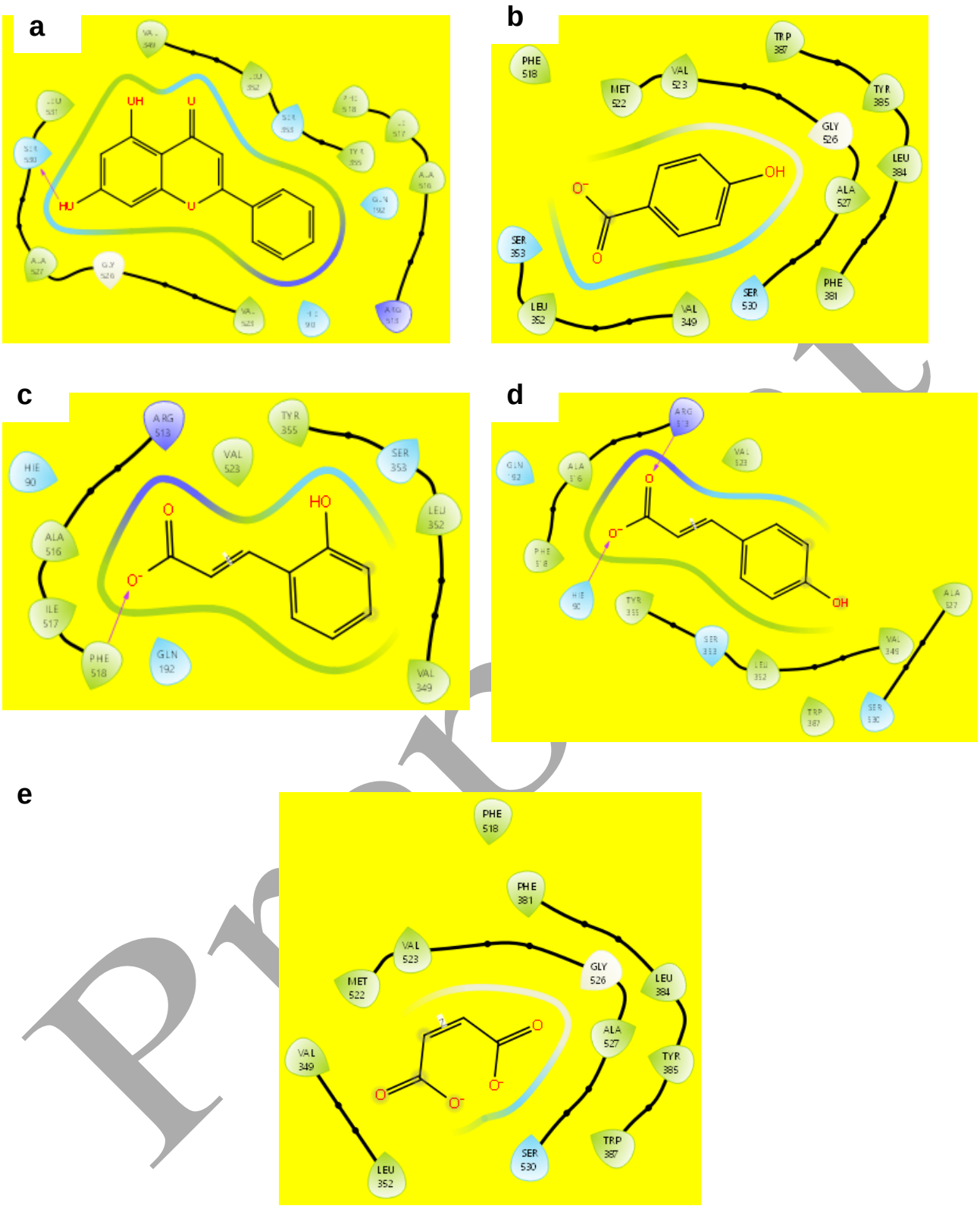
Table 7. Predicted Toxicity Parameters

Compound	LD50(mg/kg)	Toxicity Class
Fumaric acid	1350	4
p-Hydroxybenzoic acid	2200	5
p-Coumaric acid	2850	5
trans-2-Hydroxycinnamic acid	2850	5
Chrysin	3919	5

Compound	Docking Score
Chrysin	-8.412
p-Hydroxybenzoic acid	-6.522
trans-2-Hydroxycinnamic acid	-5.794
p-Coumaric acid	-5.633
Fumaric acid	-3.331

Molecular docking revealed marked differences in binding affinities among the compounds (Table 7). Chrysin showed the strongest predicted affinity (−8.412 kcal/mol), likely due to its extensive flavonoid scaffold and favorable hydrophobic complementarity. Among the phenolic acids, p-hydroxybenzoic acid (−6.522 kcal/mol) showed stronger binding than trans-2-hydroxycinnamic acid (−5.794 kcal/mol) and p-coumaric acid (−5.633 kcal/mol), whereas fumaric acid exhibited the weakest affinity (−3.331 kcal/mol), consistent with its smaller size and limited interaction capacity.

Chrysin formed hydrophobic interactions with Val523, Ala527, and Leu352, while p-hydroxybenzoic acid established polar contacts with Arg120 and Tyr355. Trans-2-Hydroxycinnamic acid and p-coumaric acid interacted with Arg120 through their acrylate side chains, with p-coumaric acid additionally interacting with Ser353. Fumaric acid interacted with Ser530 and Gly526 within a confined region of the active site (Figure 5). Overall, the docking results indicate that complementary hydrophobic and hydrogen-bonding interactions contribute to ligand stabilization within the target binding site.



318 **Figure 5. The docking result of bioactive molecules. a:** Chrysin **b:** p-Hydroxybenzoic acid **c:** trans-
319 2-Hydroxycinnamic acid **d:** p-Coumaric acid **e:** Fumaric acid

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321

4. Discussion

The pharmacokinetic and drug-likeness profiles established in the present study gain particular significance when considered alongside the reported biological activities of these compounds. Fumaric acid, through derivatives such as dimethyl fumarate (DMF), activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, enhancing antioxidant defenses while suppressing NF-kB-mediated pro-inflammatory cytokine production [6-7]. This dual mechanism is highly relevant to oxidative stress-related diseases, including inflammation and asthma. p-Coumaric acid suppresses COX-2 and iNOS expression via inhibition of NF-kB and MAPK signaling and reduces TNF-alpha and IL-6 production in inflammatory models [6-8]. Its balanced lipophilicity and high predicted GI absorption support the feasibility of these effects following oral administration. Similarly, trans-2-hydroxycinnamic acid modulates inflammatory pathways and exhibits antioxidant activity, with a pharmacokinetic profile comparable to that of p-coumaric acid [9-12]. Chrysin warrants particular attention due to its ability to suppress NF-kB, STAT3, and PI3K/Akt signaling, modulate mast cell activation, and attenuate IgE-mediated immune responses [6-12]. Experimental asthma models have shown reductions in airway hyperresponsiveness and eosinophilic infiltration [6-12]. Although its high molar refractivity and lipophilicity suggest strong receptor-binding potential, limited aqueous solubility remains a key pharmacokinetic challenge that may be overcome through nanocarrier systems or structural modifications. p-Hydroxybenzoic acid exhibits free radical scavenging activity through its phenolic hydroxyl group. Although related hydroxybenzoic acid derivatives reduce pro-inflammatory mediator expression [5-12], direct evidence for p-hydroxybenzoic acid remains limited. The coexistence of these five phenolics in honey suggests potential synergistic interactions. Multi-target modulation of the NF-kB, MAPK, and PI3K/Akt pathways may provide broader anti-inflammatory effects than single-target approaches [5-12]. Their diverse physicochemical properties, ranging from hydrophilic fumaric acid to lipophilic chrysin, indicate complementary biodistribution patterns that may contribute to the enhanced biological activity often observed in whole honey compared with isolated constituents. Overall, the ADMET and drug-likeness analyses indicate that p-coumaric acid, trans-2-hydroxycinnamic acid, and chrysin possess favorable pharmacokinetic characteristics for modulating inflammatory signaling. Their multi-target mechanisms, natural origin, and reported preclinical safety support further evaluation as complementary therapeutic candidates for inflammatory diseases. Several limitations should be acknowledged. SwissADMET predictions do not fully capture the complexity of in vivo pharmacokinetics, including first-pass metabolism, microbiome-mediated biotransformation, and tissue-specific accumulation. Likewise, the BOILED-Egg model predicts BBB permeability based solely on passive diffusion and excludes several active transport mechanisms. In addition, commonly used drug-likeness filters were developed primarily using synthetic compounds and may have limited applicability to low-molecular-weight natural phenolics. Future studies should validate these findings

through experimental ADME approaches, including Caco-2 permeability, microsomal stability, and plasma protein binding assays.

5. Conclusions

The present in silico investigation demonstrated that the five phenolic compounds commonly identified in honey samples from the southern regions of Kazakhstan possess favorable physicochemical, pharmacokinetic, and drug-likeness profiles that suggest their potential as candidates for further experimental investigations. It should be emphasized that the present findings are based exclusively on computational predictions and require experimental confirmation. Future studies incorporating molecular docking simulations, in vitro cell culture models, and in vivo animal models are warranted to validate the hypotheses generated in this study.

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Authors' Contribution:

Study concept and design: S.D.D and Z.S

Acquisition of data: E.S.G and T.D

Analysis and interpretation of data: E.S.G and S.D.D

Drafting of the manuscript: S.D.D, N.S, Z.S

Critical revision of the manuscript for important intellectual content: Z.S, A.O.

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Supplementary Materials

Not applicable.

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Institutional Review Board Statement

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Informed Consent Statement

Not applicable.

Data Availability Statement

All data generated or analyzed during this study are included in this published article. The SwissADME platform is freely accessible at http://www.swissadme.ch. Compound SMILES notations were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Conflicts of Interest

The authors declare no conflicts of interest.

Use of Artificial Intelligence Tools

The authors declare that an artificial intelligence (AI) tool was utilized during the preparation of this manuscript. Claude (Anthropic) was employed to assist with language editing and scientific writing refinement. All AI-generated content was critically reviewed, verified, and revised by the authors, who assume full responsibility for the accuracy, integrity, and originality of the final manuscript.

References

1. Samarghandian S, Farkhondeh T, Samini F. Honey and health: A review of recent clinical research. Pharmacogn Res. 2017;9(2):121-127. [DOI: 10.4103/0974-8490.204647]
2. Cianciosi D, Forbes-Hernandez TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, et al. Phenolic compounds in honey and their associated health benefits: A review. Molecules. 2018;23(9):2322. [DOI: 10.3390/molecules23092322]
3. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernandez TY, Mazzoni L, Giampieri F. The composition and biological activity of honey: A focus on Manuka honey. Foods. 2014;3(3):420-432. [DOI: 10.3390/foods3030420]
4. Kedzierska-Matysek M, Stryjecka M, Teter A, Skalecki P, Domaradzki P, Florek M. Relationships between the content of phenolic compounds and the antioxidant activity of Polish honey varieties as tool botanical discrimination. Molecules. 2021;26(6):1810. [DOI: 10.3390/molecules26061810]
5. Ongalbek D, Tokul-Olmez O, Sahin B, Kucukaydin S, Aydogmus-Ozturk F, Sicak Y, et al. Classification of buckwheat honey produced in Kazakhstan according to their biochemical

ingredients and bioactivities by chemometric approach. Food Chem. 2024;451:139409. [DOI: 10.1016/j.foodchem.2024.139409]

6. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. Lancet. 2018;391(10122):783-800. [DOI: 10.1016/S0140-6736(17)33311-1
7. Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. Nat Rev Immunol. 2018;18(7):454-466. [DOI: 10.1038/s41577-018-0007-5]
8. Pei K, Ou J, Huang J, Ou S. p-Coumaric acid and its conjugates: Dietary sources, pharmacokinetic properties biological activities. J Sci Food Agric. 2016;96(9):2952-2962. [DOI: 10.1002/jsfa.7578]
9. Neog MK, Joshua Pragasam S, Krishnan M, Rasool M. p-Coumaric acid, a dietary polyphenol ameliorates inflammation and curtails cartilage and bone erosion in the rheumatoid arthritis rat model. Biofactors. 2017;43(5):698-717. [DOI: 10.1002/biof.1381]
10. Taofiq O, González-Paramás AM, Barreiro MF, Ferreira ICFR. Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. Molecules. 2017;22(2):281. [DOI: 10.3390/molecules22020281]
11. Yao J, Jiang M, Zhang Y, Liu X, Du Q, Feng G. Chrysin alleviates allergic inflammation and airway remodeling in a murine model of chronic asthma. Int Immunopharmacol. 2016;32:24-31. [DOI: 10.1016/j.intimp.2015.12.031]
12. Sliwoski G, Kothiwale S, Meiler J, Lowe EW Jr. Computational methods in drug discovery. Pharmacol Rev. 2014;66(1):334-395. [DOI: 10.1124/pr.112.007336]
13. Ferreira LLG, Andricopulo AD. ADMET modeling approaches in drug discovery. Drug Discov Today. 2019;24(5):1157-1165. [DOI: 10.1016/j.drudis.2019.03.015]
14. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717. [DOI: 10.1038/srep42717]
15. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):W257-W263. [DOI: 10.1093/nar/gky318
16. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Res. 2023;51(D1):D1373-D1380. [DOI:10.1093/nar/gkac956]
17. Daina A, Zoete V. A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem. 2016;11(11):1117-1121. [DOI: 10.1002/cmdc.201600182]
18. **Schrödinger Release 2026-1**: LigPrep, Schrödinger, LLC, New York, NY, 2025.
19. **Schrödinger Release 2026-1**: Maestro, Schrödinger, LLC, New York, NY, 2025.

20. **Schrödinger Release 2026-1**: Protein Preparation Workflow; Epik, Schrödinger, LLC, New York, NY, 2024; Impact, Schrödinger, LLC, New York, NY; Prime, Schrödinger, LLC, New York, NY, 2025.

21. 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. [DOI: 10.1016/s0169-409x(00)00129-0]

22. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615-2623. [DOI: 10.1021/jm020017n]

23. Arnott JA, Planey SL. The influence of lipophilicity in drug discovery and design. Expert Opin Drug Discov. 2012;7(10):863-875. [DOI: 10.1517/17460441.2012.716032]

24. Delaney JS. ESOL: Estimating aqueous solubility directly from molecular structure. J Chem Inf Comput Sci. 2004;44(3):1000-1005. [DOI: 10.1021/ci034243x]

25. Mani R, Natesan V. Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism action. Phytochemistry. 2018;145:187-196. [DOI: 10.1016/j.phytochem.2017.11.008]