

Research Article



Flavonoid Derivatives as Matrix Metalloproteinase-1 and Dihydrofolate Reductase Inhibitors: An Integrated in Silico Approach with Molecular Dynamics Insights for Cosmeceutical Applications

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ABSTRACT

Flavonoids constitute an important class of polyphenolic compounds with considerable potential for cosmetic applications due to their diverse biological activities and favourable structural characteristics. Herein, some flavonoid derivatives comprising flavanone, flavonols and aurones, along with standards 8-methoxybutin and leptosidin, were evaluated for cosmeceutical applications that involve inhibiting capacity of matrix metalloproteinase-1 and dihydrofolate reductase using a computational approach. Physicochemical and ADMET studies revealed solubility, skin permeability, bioavailability and synthetic accessibility, while all derivatives satisfied drug-likeness criteria. No predicted concerns were observed for skin sensitization, eye corrosion, carcinogenicity, hERG inhibition and AMES mutagenicity. Molecular docking studies of flavonoid derivatives demonstrated favourable binding energy across the proteins - MMP-1 and DHFR, with superior affinities observed for several derivatives compared with the parent and reference compounds. Aurone derivative 4c (-9.12 kcal/mol) and flavanone derivative 5a (-9.63 kcal/mol) exhibited the highest binding affinities within their respective series. SAR analysis demonstrated that scaffold-dependent planarity, rigidity and π -conjugation strongly influenced ligand orientation and binding affinity, with hydroxyl groups facilitating hydrogen bonding and methoxy groups contributing hydrophobic and van der Waals contacts. Aurones showed preferential MMP-1 affinity, flavonols favoured DHFR binding, whereas the conformationally flexible flavanone scaffold provided balanced dual-target interactions. 100 ns molecular dynamics simulations of the top-ranked candidate-DHFR complex demonstrated its structural stability and favourable binding behaviour. These findings identify flavonoid derivatives as promising candidates for additional experimental validation to optimize their potential as anti-aging and anti-acne cosmetic agents.

Keywords: Flavonoids, Matrix metalloproteinase-1 inhibitors, Dihydrofolate reductase inhibitors, molecular dynamics simulation, Cosmeceuticals.

INTRODUCTION

Flavonoids have emerged as one of the most promising classes of cosmetic ingredients owing to their broad spectrum of pharmaceutical properties that effectively discourse multiple dermatological concerns. Flavonoids possess well documented cosmeceutical dermatological applications¹. Owing to their polyphenolic structure, flavonoids promote cure to dermatological issues through several mechanistic pathways. It includes suppression of tyrosinase activity, destruction of reactive oxygen and control of inflammatory routes¹. Skin aging is a complex process. It is due to oxidative stress, degradation of collagen, imbalance hormonal levels and prolonged ultraviolet irradiation, ultimately leading to wrinkles, skin laxity, dryness and reduced elasticity. Excellent antioxidant and anti-inflammatory characteristics of flavonoids enable them to effectively counteract these age-associated changes².

Flavonoids assist to maintain tegument integrity and prevent the aging process by scavenging free radicals, inactivating the enzymes responsible for collagen degradation and activating collagen synthesis³⁻⁵. Due to androgen hormonal imbalance, sebaceous gland gets distorted and the adolescents are predominantly get affected by an inflammatory disorder called as acne⁶. Elevated androgen levels stimulate excessive sebum production and promote keratinocyte hyperproliferation,

resulting in follicular blockage and sebum accumulation. This microenvironment favours the proliferation of *Propionibacterium acnes*, which subsequently triggers inflammatory responses leading to acne lesion formation⁷. *In silico* analysis provides an efficient, cost-effective, and reliable approach for evaluating molecular properties, biological activities, and safety profiles, thereby supporting the early-stage discovery and development of anti-acne and anti-aging agents⁸⁻¹⁰. Among the various subclasses of flavonoids, flavanones, flavonols and aurones possess considerable attention due to their varied biological activities and structural versatility. Therefore, these three flavonoid scaffolds were selected (Figure 1) in the present study to systematically investigate their multifunctional cosmetic potential using an integrated molecular docking and ADMET-based computational approach.

Molecular docking was performed against key protein targets matrix metalloproteinase-1 (MMP-1), Dihydrofolate reductase (DHFR). Collagenase enzyme, matrix metalloproteinase-1 is a zinc-based endopeptidase responsible for initiating the degradation of class I and III fibrillar collagens. These are the important components of structure of the extracellular matrix. Exposure to ultraviolet radiation, oxidative stress and chronic inflammation significantly influence mitogen-activated kinase protein thereby increasing the expression of MMP-1. It results in accelerated collagen degradation thereby causing the



formation of wrinkles and hence skin will lose its elasticity. Consequently, inhibition of MMP-1 has become an important strategy for the prevention of skin maturity and formulation of anti-aging cosmetic ingredients. The crystal structure of MMP-1 provides a reliable structural model for investigating enzyme–ligand interactions through molecular docking studies. DHFR is an important enzyme associated in folate metabolism during DNA synthesis and cellular proliferation. The dihydrofolate is reduced to tetrahydrofolate by the assistance of DHFR. Inhibition of microbial DHFR disrupts nucleotide biosynthesis and bacterial growth, it has been widely explored as an important target in the pursuit for antimicrobial agents.

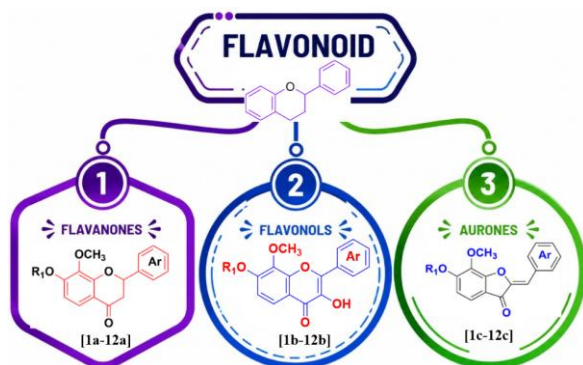


Figure 1: Flavonoids -flavanones, -flavonols and -aurones derivatives.

MATERIALS AND METHODS

The geometries of the flavonoid derivatives and reference compounds were optimized using Chem3D software with the MM2 force field. The 3D crystal structures of MMP-1 (1U4G) and DHFR (3FRE) were recovered from the Protein Data Bank. All co-crystallized ligands, cofactors and water molecules are removed from the protein. Auto Dock Tools¹¹ are used to perform docking operations. The docking grid was defined within a cubic grid box and the Lamarckian genetic algorithm is used to execute docking. Receptor grids were generated using the Auto dock grid module with grid dimensions of 100 Å × 100 Å × 100 Å and a grid spacing of 0.375 Å for MMP-1, while a grid size of 80 Å × 80 Å × 80 Å with the same grid spacing was employed for DHFR. Each ligand was docked in ten independent runs to identify the most favourable binding conformation. The resulting docking poses and ligand–protein relations were subsequently envisaged and analysed using UCSF Chimera¹² and BIOVIA Discovery Studio Visualizer¹³. In addition, the pharmacokinetic and toxicity profiles of the complexes were assessed by means of the SwissADME¹⁴ and DeepPKCSM network servers¹⁵. Molecular dynamics simulations were accomplished by via the online platform (<https://www.charmm-gui.org/>)^{16,17} to generate the required topology files for the top ranked complex based on the CHARMM36 force field. The top tiered complex is subjected to 100ns MD simulations with GROMACS 2020.6^{18,19}. Prior to simulation, the complex systems were protonated and solvated in a TIP3P water box with a diameter of 10m. The total charge of each system is

neutralized by introducing KCl ions. The systems initially underwent energy minimization for 1000ps. It is followed by equilibration under the NPT ensemble at 300 K and 1 bar pressure using a 2fs integration time step. Subsequently, a 100ns simulation was performed under the canonical NVT ensemble while preserving a constant number of particles, volume, and temperature. The resulting trajectories were analyzed in terms of root mean square deviation, root mean square fluctuation, solvent-accessible surface area, radius of gyration and the number of hydrogen bonds. These parameters were evaluated to assess the structural firmness, flexibility, compactness, solvent exposure and H-bonding behaviour of the top tiered complex throughout the simulation.

RESULTS AND DISCUSSION

ADMET and safety profile analysis

ADMET and safety profile parameters of the flavonoid derivatives are presented in Table 1 and 2. A comprehensive *in silico* ADME-toxicity assessment of flavonoid derivatives demonstrated favourable physicochemical, pharmacokinetic and safety characteristics, supporting their potential as multifunctional anti-aging and anti-acne agents. Across the entire series, the compounds exhibited good aqueous solubility, which is advantageous for topical cosmetic formulations and enhances their interaction with the skin surface. Skin permeability values (log Kp: -5.85 to -7.11 cm/s), together with TPSA values ranging from 63.22 to 109.36 Å², indicated a balanced polarity-lipophilicity profile. All derivatives exhibited 0.55 as a bioavailability score and low-to-moderate synthetic accessibility scores (3.00 to 3.63), suggesting favourable drug-likeness and practical synthetic feasibility. Importantly, all the derivatives fulfilled the Lipinski's rule of five. The pharmacokinetic and safety profiles of the 30 flavonoid derivatives, comprising flavanone (1a-12a), flavonol (1b-12b) and aurone (1c-12c) analogues, together with the reference compounds 8-methoxy butin and leptosidin, were evaluated to determine their suitability as prospective cosmetic ingredients for anti-aging and anti-acne applications. Since cosmetic actives are intended for repeated topical administration, favourable physicochemical properties, skin compatibility and a low toxicity profile are essential prerequisites prior to molecular interaction studies. All flavanone and flavonol derivatives were predicted to be soluble, whereas among the aurone derivatives only compound 30 exhibited moderate solubility. Overall, the high aqueous solubility observed for the majority of compounds is advantageous for cosmetic formulations, as it facilitates incorporation into aqueous or emulsion-based topical products and improves formulation flexibility. Skin permeability analysis revealed Log Kp values ranging from -6.36 to -6.79 cm/s for flavanones, -6.77 to -7.11 cm/s for flavonols, and -5.85 to -6.36 cm/s for aurones. These negative Log Kp values indicate controlled skin permeation, which is desirable for topical cosmetic agents because it promotes sufficient penetration into the epidermal layers while minimizing excessive systemic absorption. Among the investigated scaffolds, aurone

derivatives displayed relatively higher skin permeability than flavanone and flavonol analogues, suggesting that the aurone framework may possess improved skin penetration characteristics suitable for topical delivery. The total polar surface area (TPSA) of the investigated compounds ranged from 63.22 to 96.00 Å² for flavanones, 87.36 to 109.36 Å² for flavonols and 63.22 to 96.22 Å² for aurones. These TPSA values fall within ranges generally associated with balanced membrane permeability and adequate aqueous solubility. The comparatively higher TPSA of flavonol derivatives is attributable to their greater number of hydroxyl functionalities, which may enhance hydrogen-bonding capability and antioxidant potential while maintaining acceptable permeability characteristics. Drug-likeness assessment demonstrated excellent physicochemical characteristics across the entire dataset. All compounds satisfied drug likeness criteria without any violations, indicating supportive molecular weight, lipophilicity, H-

bonding capacity, molecular flexibility and polarity. Although these filters were originally developed for orally administered drugs, their compliance reflects a balanced molecular architecture and supports the overall developability of these flavonoid derivatives as bioactive cosmetic ingredients. All derivatives exhibited a bioavailability score of 0.55, representing a consistent balance of physicochemical parameters across the different flavonoid scaffolds. Furthermore, the synthetic accessibility scores ranged from 3.18 to 3.61 for flavanones, 3.22 to 3.63 for flavonols and 3.00 to 3.40 for aurones. These relatively low scores suggest that the designed derivatives are synthetically feasible and could be prepared without substantial synthetic complexity, an important consideration for large-scale production of cosmetic ingredients. All the compounds are reported in the literature and are supported by evidence²⁰.

Table 1: Physicochemical properties of Flavonoid derivatives.

C. No	A*	B*	C*	D*	E*	F*	G*	H*	I*	J*
1a	75.99	S	-6.51	0	0	0	0	0	0.55	3.18
2a	96.22	S	-6.79	0	0	0	0	0	0.55	3.26
4a	85.22	S	-6.71	0	0	0	0	0	0.55	3.39
5a	85.22	S	-6.64	0	0	0	0	0	0.55	3.36
6a	74.22	S	-6.57	0	0	0	0	0	0.55	3.50
7a	64.99	S	-6.36	0	0	0	0	0	0.55	3.29
8a	85.22	S	-6.64	0	0	0	0	0	0.55	3.37
10a	74.22	S	-6.57	0	0	0	0	0	0.55	3.50
11a	74.22	S	-6.57	0	0	0	0	0	0.55	3.47
12a	63.22	S	-6.42	0	0	0	0	0	0.55	3.61
1b	100.13	S	-6.91	0	0	0	0	0	0.55	3.22
4b	109.36	S	-7.11	0	0	0	0	0	0.55	3.40
6b	98.36	S	-6.97	0	0	0	0	0	0.55	3.50
7b	89.13	S	-6.77	0	0	0	0	0	0.55	3.35
8b	109.36	S	-7.11	0	0	0	0	0	0.55	3.42
9b	109.36	S	-7.11	0	0	0	0	0	0.55	3.47
10b	98.36	S	-6.97	0	0	0	0	0	0.55	3.52
11b	98.36	S	-6.97	0	0	0	0	0	0.55	3.52
12b	87.36	S	-6.82	0	0	0	0	0	0.55	3.63
1c	75.99	S	-6.00	0	0	0	0	0	0.55	3.00
3c	96.22	S	-6.36	0	0	0	0	0	0.55	3.16
4c	85.22	S	-6.21	0	0	0	0	0	0.55	3.19
5c	85.22	S	-6.21	0	0	0	0	0	0.55	3.19
6c	74.22	S	-6.06	0	0	0	0	0	0.55	3.30
7c	64.99	S	-5.85	0	0	0	0	0	0.55	3.10
8c	85.22	S	-6.21	0	0	0	0	0	0.55	3.19
9c	85.22	S	-6.21	0	0	0	0	0	0.55	3.26
10c	74.22	S	-6.06	0	0	0	0	0	0.55	3.29
11c	74.22	S	-6.06	0	0	0	0	0	0.55	3.29
12c	63.22	MS	-5.92	0	0	0	0	0	0.55	3.40
r1	96.22	S	-6.79	0	0	0	0	0	0.55	3.26
r2	96.22	S	-6.36	0	0	0	0	0	0.55	3.08

A*- TPSA; B*- Solubility; C*- log Kp (cm/s); D*- Lipinski; E*- Ghose; F*- Veber; G*- Egan; H*- Muegge; I*- Bioavailability Score; J*- Synthetic Accessibility; r1-8-methoxy butin; r2-leptosidin; S*-Soluble; MS*- Moderately Soluble.



Table 2: Safety parameters of Flavonoid derivatives.

C. No	A*	B*	C*	D*	E*	F*
1a	Safe	Safe	Safe	Safe	Safe	Safe
2a	Safe	Safe	Safe	Safe	Safe	Safe
4a	Safe	Safe	Safe	Safe	Safe	Safe
5a	Safe	Safe	Safe	Safe	Safe	Safe
6a	Safe	Safe	Safe	Safe	Safe	Safe
7a	Safe	Safe	Safe	Safe	Safe	Safe
8a	Safe	Safe	Safe	Safe	Safe	Safe
10a	Safe	Safe	Safe	Safe	Safe	Safe
11a	Safe	Safe	Safe	Safe	Safe	Safe
12a	Safe	Safe	Safe	Safe	Safe	Safe
1b	Safe	Safe	Safe	Safe	Safe	Safe
4b	Safe	Safe	Safe	Safe	Safe	Safe
6b	Safe	Safe	Safe	Safe	Safe	Safe
7b	Safe	Safe	Safe	Safe	Safe	Safe
8b	Safe	Safe	Safe	Safe	Safe	Safe
9b	Safe	Safe	Safe	Safe	Safe	Safe
10b	Safe	Safe	Safe	Safe	Safe	Safe
11b	Safe	Safe	Safe	Safe	Safe	Safe
12b	Safe	Safe	Safe	Safe	Safe	Safe
1c	Safe	Safe	Safe	Safe	Safe	Safe
3c	Safe	Safe	Safe	Safe	Safe	Safe
4c	Safe	Safe	Safe	Safe	Safe	Safe
5c	Safe	Safe	Safe	Safe	Safe	Safe
6c	Safe	Safe	Safe	Safe	Safe	Safe
7c	Safe	Safe	Safe	Safe	Safe	Safe
8c	Safe	Safe	Safe	Safe	Safe	Safe
9c	Safe	Safe	Safe	Safe	Safe	Safe
10c	Safe	Safe	Safe	Safe	Safe	Safe
11c	Safe	Safe	Safe	Safe	Safe	Safe
12c	Safe	Safe	Safe	Safe	Safe	Safe
r1	Safe	Safe	Safe	Safe	Safe	Safe
r2	Toxic	Safe	Safe	Safe	Safe	Safe

A*- Skin Sensitisation; B*- Eye Corrosion; C*- Biodegradation; D*- Carcinogenesis; E*- hERG; F*- Ames; r1-8-methoxy butin; r2-leptosidin.

Molecular docking analysis

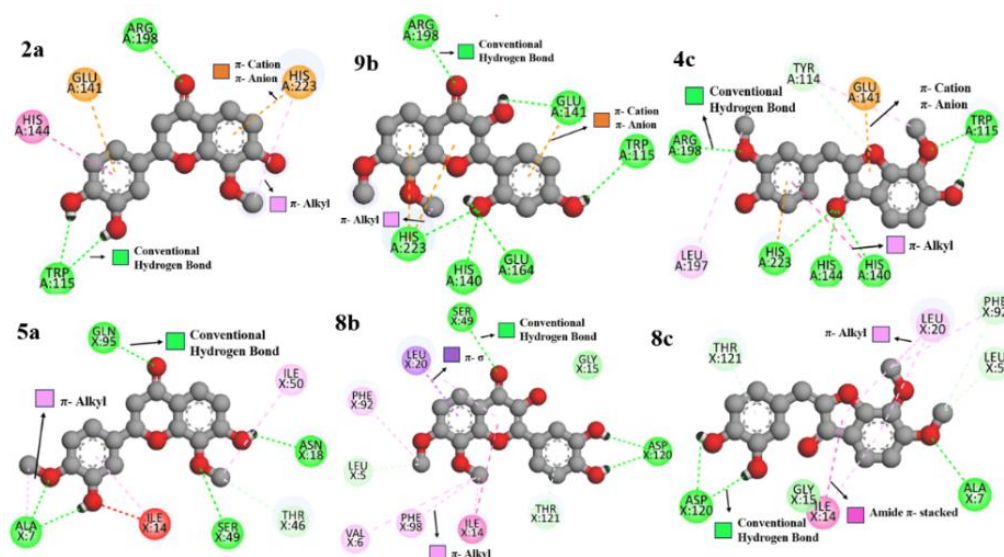


Figure 2: Interactions of potential candidates within the active site of proteins **2a**, **9b**, **4c** with MMP-1 and **5a**, **8b**, **8c** with DHRE.

Table 3: Binding energies of flavonoid derivatives.

a* **b*** **c***

S. No.	R ₁		C. No.	A*	B*	S. No.	R ₁		C. No.	A*	B*		
1.	H		1a	-7.28	-8.60	8.	CH ₃		8a	-8.65	-9.35		
			1b	-7.76	-9.27				8b	-8.14	-9.43		
			1c	-8.01	-8.42				8c	-8.02	-9.11		
2.	H		2a	-8.65	-9.60	9.	CH ₃		9b	-8.34	-8.92		
3.	H		3c	-7.84	-8.65				9c	-7.75	-8.59		
4.	H		4a	-7.5	-8.54	10.	CH ₃		10a	-7.58	-8.69		
			4b	-8.06	-9.23				10b	-7.32	-8.82		
			4c	-9.12	-8.63				10c	-8.76	-8.50		
5.	H		5a	-8.13	-9.63	11.	CH ₃		11a	-8.09	-9.53		
			5c	-8.30	-8.59				11b	-8.15	-9.39		
			6a	-7.39	-8.77				11c	-8.24	-8.48		
6.	H		6b	-7.92	-9.33	12.	CH ₃		12a	-7.25	-9.12		
			6c	-8.93	-8.48				12b	-7.49	-9.17		
			7a	-7.20	-8.26				12c	-7.88	-8.55		
7.	CH ₃		7b	-7.38	-8.77								
			7c	-7.66	-8.22								

a*- flavanone, b*-flavonol and c*-aurone derivatives. A*- B.E against MMP-1 in kcal/mol, B*- B.E against DHFR in kcal/mol.

Flavanone derivatives

The flavanone derivatives (**1a–12a**) exhibited binding energies ranging from -7.20 to -8.65 kcal/mol against MMP-1 and -8.26 to -9.63 kcal/mol against DHFR, demonstrating strong affinity toward both therapeutic targets (Table 3). Amid the investigated compounds, **2a** and **8a** displayed the highest binding affinity toward MMP-1, indicating that the presence of two hydroxyl substituents substantially enhances enzyme recognition. Hydroxyl groups increase the ability of the ligand **2a** to establish two hydrogen-bonding interactions with amino acid residues Trp115, Arg198 located within the catalytic pocket, thereby improving binding affinity. Further, π -cation/ π -anion contacts with Glu141 and His223, together with π -alkyl relations involving His144, stabilized the compound within the binding pocket (Figure 2). **8a** displayed conventional H-bonds with Trp115 and Arg198. Its π -anion relations with Glu141 and His223 added contribution to complex stabilization. Additionally, extensive *van der waals* interactions with Asp116, Tyr114, Asn112, Ala113, Phe129, Leu197, His140, Glu164, and Tyr155 reinforced its favourable binding within the MMP-1 active site. **5a** exhibited the chief binding affinity toward DHFR (-9.63 kcal/mol). It forms four conventional H-bonds with Gln95, Asn18, Ser49 and Ala7, together with a carbon hydrogen bond linking Thr46. In addition, alkyl and π -alkyl contacts with Ile50, Ile14, and Ala7 stabilized the complex

(Figure 2). **2a** and **11a** also demonstrated similarly favourable binding affinities toward DHFR. These findings suggest that both hydroxyl and methoxy substituents contribute positively to ligand binding by providing an optimal balance between H-bonding capability and hydrophobic interactions with the DHFR target. Hence molecular dynamics simulation was demonstrated to study the stability of the DHFR-**2a** complex.

Flavonol derivatives

The flavonol derivatives (**1b–12b**) demonstrated BE extending from -7.32 to -8.34 kcal/mol against MMP-1 and -8.77 to -9.43 kcal/mol against DHFR, indicating favourable interactions with both target proteins (Table 3). Amongst the evaluated derivatives, **9b** showed the strongest affinity to MMP-1 (-8.34 kcal/mol), followed closely by **11b** and **8b**. A docking score of **9b** is supported by an extensive hydrogen-bonding network involving Arg198, Glu141, Trp115, Glu164, His140 and His223. Besides conventional hydrogen bonds, electrostatic π -cation/ π -anion connections with Glu141 and His223, π -alkyl interaction with His223, contributed additional stabilization (Figure 2). Against DHFR, **8b** displayed a BE -9.43 kcal/mol and established a diverse interaction network. A conventional H-bonding interaction with Ser49 and Asp120, C-H bond and π -donor hydrogen bond interactions with Leu5 and Thr121,

contributed significantly to ligand stabilization. Furthermore, *van der waals* interactions involving Gly15, π - σ interactions with Leu20, amide- π stacked interactions with Ile14 and additional aromatic contacts involving Phe92, Phe98, Val6 and Leu20 reinforced the firmness of the ligand-protein complex (Figure 2). These outcomes indicate that the presence of hydroxyl substituents, either alone or in combination with methoxy groups, enhances binding within the DHFR active site by promoting hydrogen bonding, while maintaining favourable hydrophobic interactions.

Aurone derivatives

The aurone derivatives (**1c–12c**) displayed binding energies extending from -7.66 to -9.12 kcal/mol against MMP-1 and -8.22 to -9.11 kcal/mol against DHFR (Table 3). Among the evaluated compounds, **4c** demonstrated the strongest affinity toward MMP-1 with a binding energy of -9.12 kcal/mol. It is consistent with its extensive interaction profile. The ligand **4c** formed multiple conventional hydrogen bonds with Arg198, His140, His144, His223 and Trp115, along with a C-H bond involving Tyr114. Furthermore, π -anion interactions with Glu141 and His223, π - π stacking with His140 and alkyl/ π -alkyl interactions with Leu197 and Tyr114 significantly reinforced ligand stabilization within the active site (Figure 2). **6c** and **10c** also exhibited strong binding affinities toward MMP-1. It suggests that the combination of hydroxyl and methoxy substituents provide an optimal balance between hydrogen-bonding capability and hydrophobic interaction enabling efficient accommodation of the ligand within the MMP-1 active site. Such interactions are expected to enhance inhibition of collagen degradation, an important mechanism underlying anti-aging activity. **8c** demonstrated strong binding toward DHFR with a docking score of -9.11 kcal/mol. The ligand **8c** formed conventional hydrogen bonds with Asp120 and Ala7, while carbon hydrogen bond and π -donor hydrogen bond interactions with Thr121, Phe92 and Leu5 further strengthened the complex. In addition, *van der waals* interactions with Gly15, an amide- π stacked interaction with Ile14 and alkyl/ π -alkyl interactions involving Leu20 and Ile14 contributed to favourable hydrophobic stabilization within the binding pocket (Figure 2).

Structure activity relationship

Effect of flavonoid skeleton

The comparative evaluation of flavanone, flavonol, and aurone derivatives demonstrated that the flavonoid skeleton is the principal determinant of binding affinity and target selectivity. Differences in molecular planarity, rigidity, conjugation and spatial orientation of the three flavonoid cores significantly influenced ligand accommodation within the MMP-1 and DHFR binding pockets. Overall, aurone derivatives preferentially enhanced MMP-1 inhibition, whereas flavonol derivatives consistently exhibited superior DHFR affinity. Flavanone derivatives generally displayed balanced interactions with both targets, suggesting that scaffold geometry governs

molecular recognition more strongly than conserved aromatic substitution. This explains why compounds possessing identical substitution patterns - for example **1a,1b,1c**; **4a,4b,4c**; **5a,5c**; **6a,6b,6c**; **7a,7b,7c**; **8a,8b,8c**; **9b,9c**; **10a,10b,10c**; **11a,11b,11c**; **12a,12b,12c** - displayed different binding affinities.

Importance of hydroxyl groups

This is worth mentioning because almost every potent compound contains at least one OH group. Hydroxyl substitution positively influenced binding affinity by acting as an efficient hydrogen-bond donor and acceptor. Compounds containing one or two hydroxyl groups generally exhibited stronger docking scores than their corresponding dimethoxy analogues (*viz.*, **8a,b,c/12a,b,c**; **2a/6a**), indicating that hydrogen-bond formation contributes significantly to stabilization of the ligand-protein complexes (Figure 3).

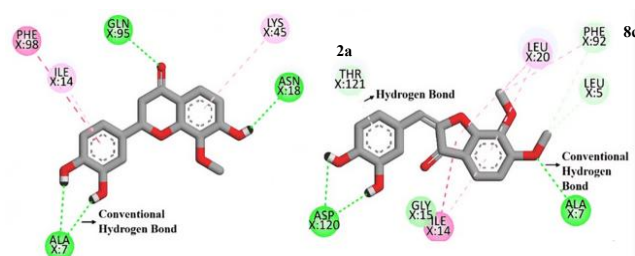


Figure 3: Interactions of **2a**, **8c** with in the active site of protein DHFR.

Importance of methoxy groups

Methoxy substitution primarily improved hydrophobic and *van der waals* interactions within the active-site pocket. When combined with hydroxyl groups, methoxy substituents improved the overall interaction profile by balancing hydrophobic contacts with hydrogen-bond formation. However, compounds containing only methoxy groups generally exhibited lower binding affinity than hydroxyl-containing analogues because of their reduced hydrogen-bonding capability.

Importance of the methyl group ($R_1 = CH_3$)

Introduction of a methyl substituent at R_1 produced only modest changes in docking affinity. The methyl group mainly contributed additional hydrophobic interactions and slight steric effects without substantially altering the overall binding mode, indicating that scaffold architecture exerted a greater influence than methyl substitution.

Complementarity between skeleton and substituents

Although hydroxyl and methoxy substituents contribute to protein recognition through hydrogen-bonding and hydrophobic interactions, their effect is highly dependent on the underlying flavonoid scaffold. The core structure determines the spatial orientation of these functional groups within the binding pocket, ultimately controlling interaction efficiency and target selectivity and it explains why identical substituents behave differently on different scaffolds.

Target selectivity

The results also indicate scaffold-dependent target selectivity, with aurone derivatives exhibiting greater preference for MMP-1, whereas flavonol derivatives consistently favoured DHFR inhibition. This suggests that structural differences among flavonoid cores influence complementary fitting within the distinct active-site architectures of the two proteins.

Molecular dynamics simulations studies

Molecular dynamics simulation was accomplished to study the stability of the DHFR-ligand **2a** complex. As shown in Figure 4, the SASA values of DHFR-**2a** complex endured stability throughout the simulation. It changes within a range of 86-91 nm², with an average value close to 89 nm². In the initial phase of the simulation (0-20ns), small fluctuations in SASA were observed as the protein-ligand complex underwent structural relaxation and adapted to the solvent environment. Following equilibration, it remained nearly constant with minor oscillations till the end of the simulation, indicating that the complex maintained a stable solvent-exposed surface without significant conformational expansion or collapse. No abrupt increases or decreases in SASA were detected, suggesting the absence of major structural rearrangements or unfolding events during the simulation.

The Rg values of DHFR-**2a** complex (Figure 4) continued stability throughout the simulation. It varies within a narrow range of 1.52-1.57 nm, with an average value of approximately 1.54 nm. In the initial stage of the simulation

(0-20 ns), minor variations in the Rg values were detected as the protein-ligand complex underwent conformational equilibration. Following this phase, the Rg profile remained nearly constant for the remainder of the simulation, exhibiting only small oscillations characteristic of the natural dynamic behaviour of proteins. A slight decline in Rg was noticed around 75ns, but the complex rapidly returned to its average value without any sustained deviation, indicating transient structural adjustments rather than irreversible conformational changes. The stable Rg profile indicates that DHFR maintained its compact tertiary structure throughout the simulation, and the binding of **2a** did not induce significant structural expansion or unfolding. It reflects favourable conformational stability during the simulation.

Hydrogen bond analysis of DHFR-**2a** complex (Figure 4) revealed that the total number of H-bonds remained same during the simulation. It fluctuates within a narrow range of 95-115 hydrogen bonds, with an average value of 105 hydrogen bonds. At the beginning of the simulation, the hydrogen-bond profile exhibited only minor fluctuations as the protein-ligand complex underwent equilibration. Following this initial adjustment, the number of hydrogen bonds persisted nearly constant throughout the 100ns simulation, with no abrupt loss or gain of hydrogen-bond interactions. The observed fluctuations are within the expected range of protein dynamics and reflect the continuous formation and disruption of transient hydrogen bonds with surrounding solvent molecules while maintaining the total structural integrity of the complex.

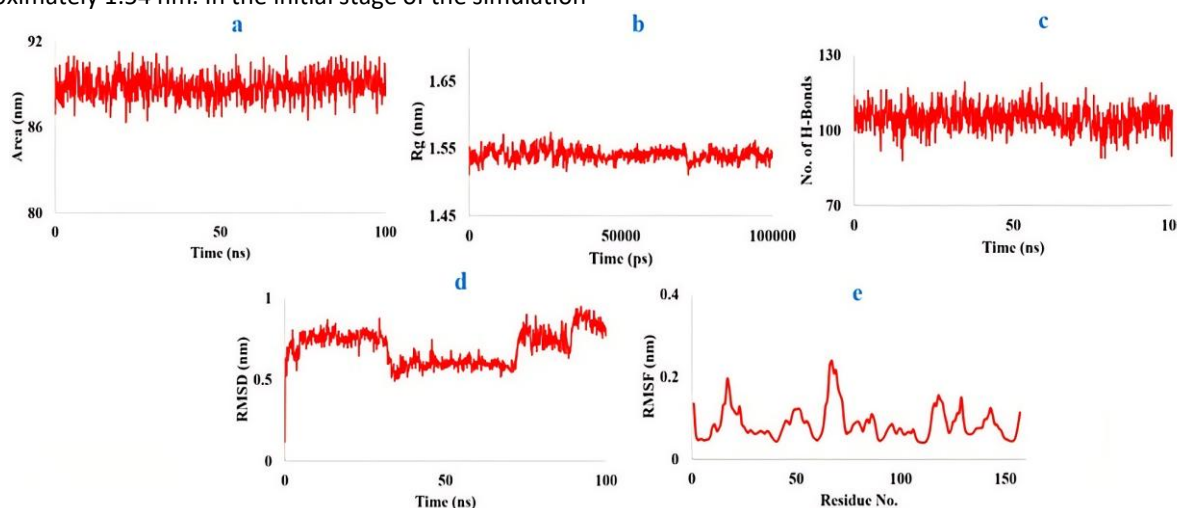


Figure 4: Molecular dynamics profile of the DHFR-**2a** complex revealing structural stability and interaction dynamics through a) SASA, b) Rg, c) hydrogen bond, d) RMSD and e) RMSF analyses.

The RMSD profile of DHFR-**2a** complex (Figure 4) exhibited an initial increase during the early stage of the simulation, followed by stabilization throughout the remaining simulation period, representing the formation of a stable complex. During the first 5ns, the RMSD increased rapidly from 0.15 nm to 0.70 nm, reflecting the initial structural relaxation of the protein and accommodation of the ligand within the active site. Between 5 and 30ns, the RMSD fluctuated around 0.75-0.82 nm, suggesting that the

complex reached an equilibrated conformation. A slight decrease in RMSD was observed between 30 and 70ns, where values remained relatively constant at approximately 0.55-0.65 nm, indicating enhanced structural stability and minimal conformational deviations. After 75ns, the RMSD increased moderately to 0.75-0.90 nm, followed by stabilization until the end of the simulation without any abrupt fluctuations or continuous drift. The observed RMSD fluctuations remained within an acceptable range (<1.0 nm),

demonstrating that the protein preserved its structural integrity throughout the simulation. The absence of large deviations indicates that the binding of **2a** did not make pronounced conformational changes or destabilization of DHFR. Instead, the ligand remained stably accommodated within the active site while allowing minor structural adjustments necessary for optimal protein-ligand interactions.

The root mean square fluctuation investigation of DHFR-**2a** complex (Figure 4) shows that the majority of amino acid residues displayed low RMSF values, ranging from approximately 0.04 to 0.10 nm, signifying that the protein maintained a stable conformation throughout the simulation. A few localized regions displayed relatively higher fluctuations, with prominent peaks observed around residues 15-20, 65-70 and 118-130, reaching a maximum RMSF of approximately 0.24 nm. These fluctuations are characteristic of flexible loop or solvent-exposed regions and are commonly observed during molecular dynamics simulations. Importantly, the fluctuations were confined to specific residues, while the remaining regions of the protein exhibited minimal atomic displacement, suggesting that the overall structural framework remained intact. The low RMSF values across most residues shows that binding of **2a** did not make substantial local structural perturbations in DHFR. Instead, the ligand maintained stable interactions with the active-site residues, restricting excessive flexibility within the binding pocket while allowing normal dynamic motion in peripheral loop regions. The absence of widespread high fluctuations further suggests that the protein retained its native conformation throughout the simulation.

The Rg, SASA, H-bond analyses, RMSD and RMSF results confirm that **2a** complex forms a stable and well-equilibrated complex with DHFR, substantiating its potential as a capable anti-acne candidate.

CONCLUSION

The present study provides an integrated *in silico* evaluation of flavonoid derivatives comprising flavanone, flavonol and aurone scaffolds for potential cosmetic applications. All derivatives exhibited favourable physicochemical, ADMET, drug-likeness and safety profiles, confirming their suitability as topical contenders. Molecular docking revealed favourable interactions with MMP-1 and DHFR, with binding stabilized by hydrogen-bonding, hydrophobic, π - π , π -alkyl and electrostatic interactions. All the derivatives demonstrated enhanced binding affinities relative to the corresponding parent and reference compounds. SAR analysis demonstrated that the flavonoid core predominantly governed binding affinity and target preference, while hydroxyl and methoxy substituents provided complementary polar and hydrophobic interactions. Aurones generally favoured MMP-1, flavonols showed stronger DHFR affinity and flavanones exhibited balanced dual-target inhibitor activity. Furthermore, 100ns molecular dynamics simulation of the top-ranked candidate-DHFR complex confirmed its structural stability and persistent protein-ligand association. Collectively, these

findings identify flavonoid derivatives as promising anti-aging and anti-acne cosmeceutical candidates, warranting further experimental validation through *in vitro* and *in vivo* studies.

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