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CXCR3-FLAVONOID INTERACTION: A NOVEL THERAPEUTIC APPROACH IN CANCER IMMUNOTHERAPY

Hatice Gungor[1], Aliye Demet Demirag[*2]

[1]University Of Health Sciences, Istanbul Haydarpaşa Number Health Application and Research Center, Department Of Oncology, Istanbul, Türkiye; [2]Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Istanbul, Türkiye

ORCID iD: Hatice Gungor: 0000-0001-6142-2432; Aliye Demet Demirag: 0000-0002-9609-9150

***Sorumlu Yazar / Corresponding Author:** Aliye Demet Demirag **e-posta / e-mail:** demet.demirag@yeditepe.edu.tr

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Abstract

Objective: To investigate the interactions between select flavonoids (Luteolin, Quercetin, Apigenin, Kaempferol, and Amorphine) and the CXCR3 receptor, evaluating their potential as novel therapeutic agents in cancer immunotherapy.

Methods: Molecular docking simulations were employed to analyze flavonoid-CXCR3 receptor interactions. Comprehensive in silico ADMET analyses were conducted to assess pharmacokinetic properties and toxicity profiles of the compounds.

Results: Flavonoids exhibited high-affinity binding to the CXCR3 receptor, with binding affinities ranging from -8.7 to -13.0 kcal/mol. Amorphine demonstrated the highest binding affinity (-13.0 kcal/mol), indicating superior inhibition potential. Luteolin showed optimal ADME characteristics, including favorable oral bioavailability (62%) and blood-brain barrier permeability (log BB -1.911). Molecular docking analyses identified critical amino acid residues (TYR205, TYR308, TRP109, PHE131, and ASN132) in flavonoid-CXCR3 interactions. In silico toxicity predictions suggested low risk profiles for all examined flavonoids.

Conclusion: This study provides evidence for the potential of flavonoids as CXCR3 receptor antagonists in cancer immunotherapy. The elucidated molecular interactions and favorable ADMET profiles warrant further investigation of these compounds. Future research should focus on optimization of flavonoid-based CXCR3 inhibitors, preclinical and clinical evaluations, and assessment of their immunomodulatory effects within the tumor microenvironment. These findings contribute to the development of novel, flavonoid-derived therapeutic strategies in cancer treatment.

Keywords: *CXCR3, flavonoids, cancer immunotherapy, molecular docking, drug discovery.*

Introduction

Cancer remains one of the leading causes of death worldwide, claiming millions of lives annually.[1] The global cancer burden is steadily increasing, emphasizing the critical importance of developing effective treatment strategies from a public health perspective.[2] Traditional cancer treatments encompass surgical intervention, radiotherapy, and chemotherapy. However, due to their side effects and limited efficacy, there is a pressing need for new therapeutic approaches.[3]

In recent years, immunotherapy has emerged as a promising strategy in cancer treatment.[4] Immunotherapy aims to control tumor growth by strengthening the patient's immune system and eliciting an effective response against cancer cells.[5] Cancer immunotherapy encompasses various approaches, including monoclonal antibodies, cancer vaccines, adoptive cell transfer, and immune checkpoint inhibitors.[6] These methods activate the body's natural defense mechanisms by targeting cancer-specific antigens or mechanisms that suppress the immune system.[7]

The chemokine receptor CXCR3 has gained prominence as both a potential agonist and antagonist target in cancer immunotherapy. CXCR3 is a G protein-coupled receptor activated by CXC chemokine family members CXCL9, CXCL10, and CXCL11.[8] Interestingly, recent studies have identified three distinct CXCR3 receptor variants resulting from alternative splicing of the CXCR3 gene: CXCR3A, CXCR3B, and CXCR3-alt. Structurally, CXCR3B contains 52 additional amino acids at the NH2 terminus compared to CXCR3A,[9,10] while CXCR3-alt exhibits a frameshift in the COOH terminus of CXCR3A.[11] CXCR3-mediated trafficking at the tumor vascular interface has been identified as a critical checkpoint for effective T-cell-based cancer immunotherapy.[12] The alternatively spliced CXCR3 receptors reflect the complex and diverse effects induced upon CXC chemokine ligand binding. Generally, the three spliced receptor variants are expressed on activated T helper 1 (Th1) cells, with CXCR3A showing the highest expression among the three.[13,14,15] However, CXCR3A and CXCR3-alt are unique compared to CXCR3B as they induce the chemotaxis of various mononuclear immune cells (i.e., CD4+ Th1 cells, CD8+ cytotoxic Th2 cells, B cells, natural killer (NK) cells, macrophages, neutrophils, and dendritic cells) to sites of inflammation upon ligand binding.[13,14,16] Conversely, CXCR3B is highly expressed on human microvascular endothelial cells (HMVEC), and its activation leads to an angiostatic effect on blood vessels.[13] Consequently, considering the two physiologically distinct processes resulting from CXCR3 activation, scientists now characterize CXC chemokines as "immunoangiostatic" proteins.[13]

However, preclinical and clinical studies have also demonstrated that CXCR3 inhibition suppresses tumor growth and some studies metastasis.[17,18] Some studies have found that elevated CXCR3 expression is associated with a better prognosis in clear cell renal cell carcinoma,[19, 20] and gastric cancer.[21, 22] Conversely, in cancers such as melanoma,[23] breast cancer,[24, 25] and glioblastoma multiforme,[26] the CXCR3 receptor has consistently been shown to be a poor prognostic factor.

CXCR3 antagonists exert their effects by inhibiting cancer cell migration and enhancing anti-tumor immune responses.[27] However, the efficacy of current CXCR3 antagonists is limited, necessitating the development of new therapeutic agents.[28] Consequently, the discovery and optimization of small molecules targeting the CXCR3 receptor have become an important area of research in cancer immunotherapy.

Flavonoids are polyphenolic compounds widely found in plants and possess various pharmacological activities.[29] These compounds are known for their antioxidant, anti-inflammatory, antiviral, and anti-cancer properties[30,31]. The chemopreventive and chemotherapeutic potential of flavonoids has been demonstrated in various in vitro and in vivo studies.[32,33] Additionally, flavonoids are known to modulate the immune system and enhance anti-tumor immune responses.[34]

In recent years, the role of flavonoids in cancer treatment has garnered significant interest. Flavonoids such as luteolin, quercetin, apigenin, kaempferol and amorphine have been shown to be effective in various types of cancer.[35-37] These compounds exhibit anti-cancer activity through mechanisms including cell cycle regulation, apoptosis induction, angiogenesis inhibition, and metastasis prevention.[38,39] The immunomodulatory activities of flavonoids may also play a crucial role in cancer treatment. For instance, flavonoids like quercetin and apigenin have been shown to regulate T cell activation and cytokine production.[40,41] However, the immunomodulatory effects of flavonoids and their potential roles in cancer immunotherapy have not yet been fully elucidated.

The interactions of flavonoids with the CXCR3 receptor hold potential for presenting a novel therapeutic approach in cancer immunotherapy. However, these interactions need to be characterized at the molecular level, and the efficacy of flavonoids as CXCR3 antagonists needs to be evaluated. In this study, the interactions of various flavonoids (Luteolin, Quercetin, Apigenin, Kaempferol, and Amorphine) with the CXCR3 receptor were investigated using molecular docking simulations. The binding affinities of flavonoids to CXCR3, key interactions, structure-activity relationships, physicochemical properties, ADME profiles, and toxicity predictions were comprehensively examined.

The findings of this study reveal the potential of flavonoids to present a novel therapeutic strategy in cancer immunotherapy by targeting the CXCR3 receptor. The obtained data provide a foundation for the development and optimization of flavonoid-based CXCR3 inhibitors and contribute to the development of effective approaches in cancer treatment. Furthermore, this study also presents new avenues for research aimed at elucidating the immunomodulatory effects of flavonoids and their roles in the cancer microenvironment.

Methods

In this research, the crystal structures of flavonoid ligands (Luteolin, Quercetin, Apigenin, Kaempferol, Amorphine) were obtained from the PubChem database. The three-dimensional model of the CXCR3 receptor was acquired from the AlphaFold protein database (Figure 1). Visualization of the target flavonoids was performed using the Gaussian view program (Figure 2).[42]

Figure 1 illustrates the three-dimensional structure of the CXCR3 receptor generated by AlphaFold2. The model clearly delineates seven transmembrane helices, exhibiting the characteristic G protein-coupled receptor topology of CXCR3. The positioning of the N-terminal extracellular domain and C-terminal intracellular domain indicates the membrane orientation of the receptor. Regions of high confidence (pLDDT > 90) are highlighted in dark blue,

suggesting that the model is more precise in these areas and provides a reliable foundation for predicting potential ligand binding sites. This structural model is critical for guiding the design of flavonoid-based inhibitors.

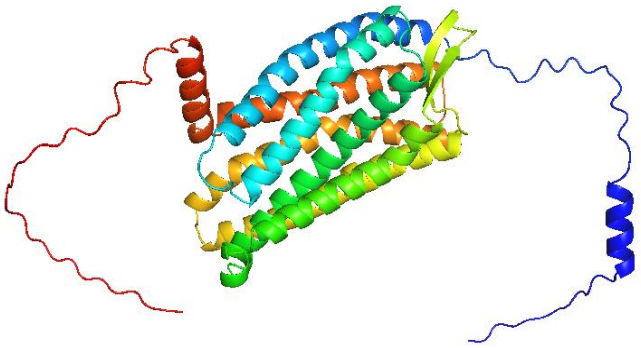

Figure 1. Three-dimensional structural model of the CXCR3 receptor.

The optimized molecular structures presented in Figure 2 reflect the conformational properties and intramolecular interactions of the flavonoids. This structural information is crucial for understanding the physicochemical properties, reactivity, and biological activities of flavonoids. Furthermore, these optimized structures were utilized for molecular docking simulations, enabling more accurate modeling of the interactions and binding modes between flavonoids and the CXCR3 receptor.

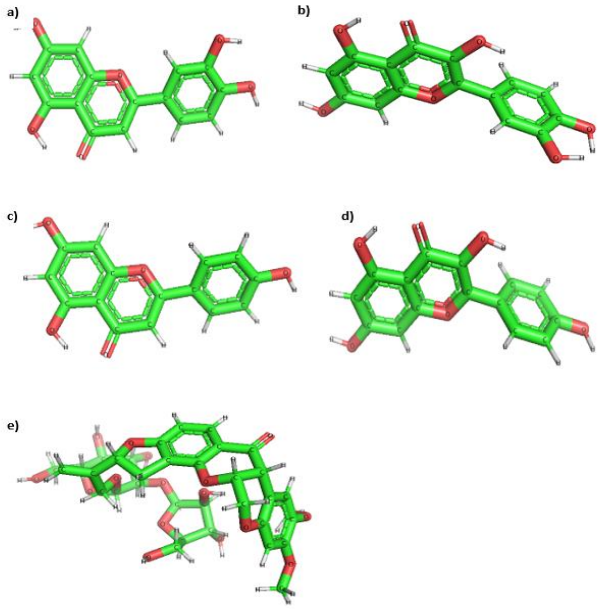

Figure 2. The three-dimensional structures of flavonoid ligands after optimization, with molecular structures of flavonoid ligands optimized at the density functional theory (DFT) B3LYP/6-31G(d,p) level: a) Luteolin ($C_{15}H_{10}O_6$): 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one,b) Quercetin ($C_{15}H_{10}O_7$): 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, c) Apigenin ($C_{15}H_{10}O_5$): 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, d) Kaempferol ($C_{15}H_{10}O_6$): 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, e) Amorphine ($C_{16}H_{14}O_5$): 7,4'-dihydroxy-3'-methoxyflavan.

Molecular Docking Study

The molecular docking study was conducted using the AutoDock Vina program.[43,44] The CXCR3 receptor was prepared for analysis by removing water molecules and adding polar hydrogen atoms. Grid box parameters were optimized to 40x40x40 units with a 0.375 Å spacing. Nine possible stereoisomers for each flavonoid were generated using the ligand preparation tool.

Rotational freedom was granted to amino acid residues in the binding region of CXCR3 using the receptor grid generation tool. A cubic grid was designed for the docking calculations of flavonoids. The binding regions of CXCR3 were analyzed in detail using Discovery Studio 2021 Client.

The geometric and pharmacokinetic properties of flavonoids were comprehensively examined using the QikProp module of Schrödinger Software Maestro version 11.4.[45] This holistic approach aims to elucidate in detail the interactions of flavonoids with CXCR3 and their potential therapeutic properties.

Table 1 summarizes the binding affinities and interactions of flavonoids with the CXCR3 receptor. The binding affinities of flavonoids range from -8.7 to -13.0 kcal/mol, indicating high-affinity binding to the CXCR3 receptor. Notably, Amorphine exhibits the highest binding affinity (-13.0 kcal/mol) and may possess a stronger inhibition potential compared to other flavonoids.

Among the prominent amino acid residues involved in the interactions between flavonoids and the CXCR3 receptor are TYR205, TYR308, TRP109, PHE131, and ASN132. These residues play critical roles in flavonoid binding through hydrogen bonds, pi-pi interactions, and hydrophobic interactions. When compared to CXCR3 antagonists reported in the literature, flavonoids appear to display similar interaction patterns.

For instance, known CXCR3 antagonists such as AMG487 and SCH546738 have been reported to interact with residues TYR308, PHE131, and ASN132.[46,47] This suggests that flavonoids may inhibit the CXCR3 receptor through a similar mechanism. Moreover, the interaction of flavonoids with other key residues such as TRP109 and ARG216 indicates that they may possess unique binding modes.

In conclusion, this table demonstrates that flavonoids bind to the CXCR3 receptor with high affinity and exhibit interaction patterns similar to known CXCR3 antagonists. Amorphine, in particular, stands out with the strongest binding affinity. The potential of flavonoids to inhibit the CXCR3 receptor makes them promising candidates in cancer immunotherapy.

Figure 3 illustrates the positioning of flavonoids in the active site of the CXCR3 receptor. As observed, all flavonoids occupy the active site of the receptor and interact with it through hydrophobic interactions, hydrogen bonds, and pi-pi interactions. Reference CXCR3 antagonists also exhibit similar positioning.[48] This supports the notion that flavonoids may inhibit the CXCR3 receptor through a mechanism similar to antagonists.

Figure 4 provides a detailed view of the interactions and hydrogen bonds between flavonoids and the CXCR3 receptor. Flavonoids are observed to bind with key residues in the active site of the receptor, such as TYR205, TYR308, TRP109, PHE131, and ASN132, through hydrogen bonds and other interactions. This interaction pattern shows

similarity to the interaction profiles of known CXCR3 antagonists like AMG487 and SCH546738.[46,47] These findings emphasize the potential of flavonoids to antagonize the CXCR3 receptor.

Figure 5 presents three-dimensional representations of the most stable conformations of flavonoids in the active site of the CXCR3 receptor. The positioning of flavonoids in the receptor's active site and their interactions with key residues are clearly visible. The unique positioning and interaction profile of Amorphine, in particular, are noteworthy. These visual representations aid in better understanding the interactions between flavonoids and the CXCR3 receptor.

Table 1. Binding affinities and interactions of flavonoids with the CXCR3 receptor.

Flavonoid	Binding Affinity (kcal/mol) Before Optimization	Binding Affinity (kcal/mol) After Optimization	Interactions	Other Interactions
Luteolin	-9.5	-9.4	TRP109 2.82 Å- Pi-Donor hydrogen bond, PHE131 4.14 Å- Pi-Pi Stacked (Hydrophobic) interaction, TYR205 5,25 Å-Pi-Pi T-Shaped (Hydrophobic), ARG216 5.41 Å-Pi-Alkyl (Hydrophobic)	TYR60, TYR205, GLN219, ASN132 respectively 3 at 2.71 Å, 1.98 Å, 1.80 Å; 2 at 2.82 Å, 1.98 Å; 2 at 2.80 Å, 1.97 Å; 2.08 Å Unfavorable Bump
Quercetin	-8.8	-8.7	TYR60 3.02 Å -Conventional hydrogen bond TYR308 3.12 Å-Conventional hydrogen bond TYR205 2.99 Å- Conventional hydrogen bond GLY128 3.58 Å-Carbon hydrogen bond SER301 3.45 Å- Carbon hydrogen bond PHE131 5.05 Å-Pi-Pi T-shaped, LYS300 5.33 Å -Pi-Alkyl	TYR205 2.18 Å, TYR308 2.74 Å, ASN132 2.36 Å, ASP186 2.25 Å Unfavorable Bump
Apigenin	-8.9	-8.8	ASN132 2.92 Å- Conventional hydrogen bond, PHE131 4.17 Å- Pi-Pi Stacked (Hydrophobic) interaction, TYR205 5,17 Å- Pi-Pi T-Shaped (Hydrophobic), ARG216 5.45 Å Pi-Alkyl (Hydrophobic), TRP 109 2.82 Å-Pi-Donor hydrogen bond, TYR308 3.69 Å-Pi-Donor hydrogen bond,	
Kaempferol	-8.9	-8.8	TYR308 3.14 Å- Conventional hydrogen bond, TYR205 2,04 Å- Conventional hydrogen bond, TRP109 2 at 5.15 Å Pi-Pi Stacked and 5.05 Å Pi-Pi T-shaped	TYR308 2.89 Å, TRP109, ASP186 2.70 Å and 2.17 Å Unfavorable Bump
Amorphine	-12.7	-13.0	TYR205 2,55 Å-Conventional hydrogen bond, HIS202 5.64 Å-Pi-Pi T-shaped	TYR205 5 at 2.59 Å, 3.18 Å, 1.78 Å, 2.29 Å, 2.02 Å; TYR271 2 at 2.56 Å, 2.12 Å; CYS203 2 at 1.79 Å and 2.63 Å; GLN219 2 at 2.54 Å, 1.59 Å; ASN132 4 at 2.71 Å, 1.85 Å, 1.93 Å, 0.97 Å; SER301 2.70 Å, ASP52 2.53 Å Unfavorable Bump

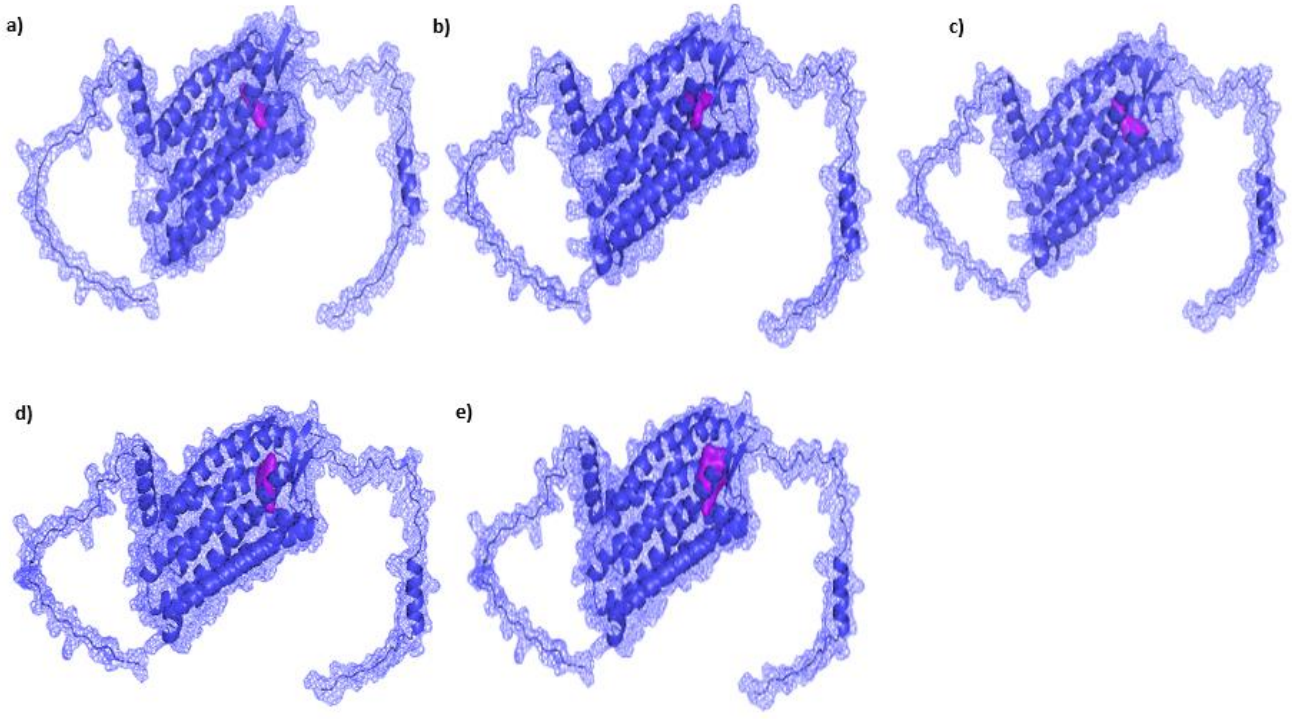

Figure 3. Molecular docking of a) Luteolin, b) Quercetin, c) Apigenin, d) Kaempferol, and e) Amorphine in the active site of the CXCR3 receptor (Binding Affinities are ΔG: -9.5 kcal/mol, ΔG: -8.8 kcal/mol, ΔG: -8.9 kcal/mol, ΔG: -8.9 kcal/mol, and ΔG: -11.9 kcal/mol, respectively).

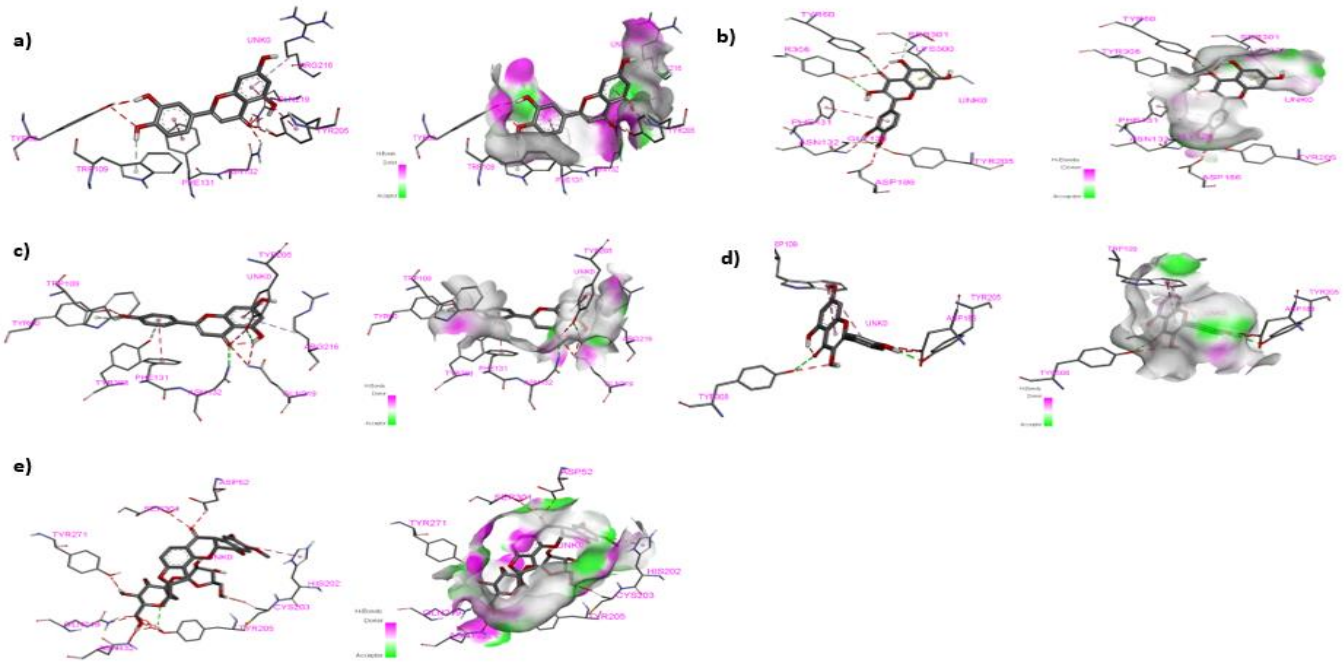

Figure 4. Representation of interactions and hydrogen bonding between a) Luteolin, b) Quercetin, c) Apigenin, d) Kaempferol, and e) Amorphine with the CXCR3 receptor.

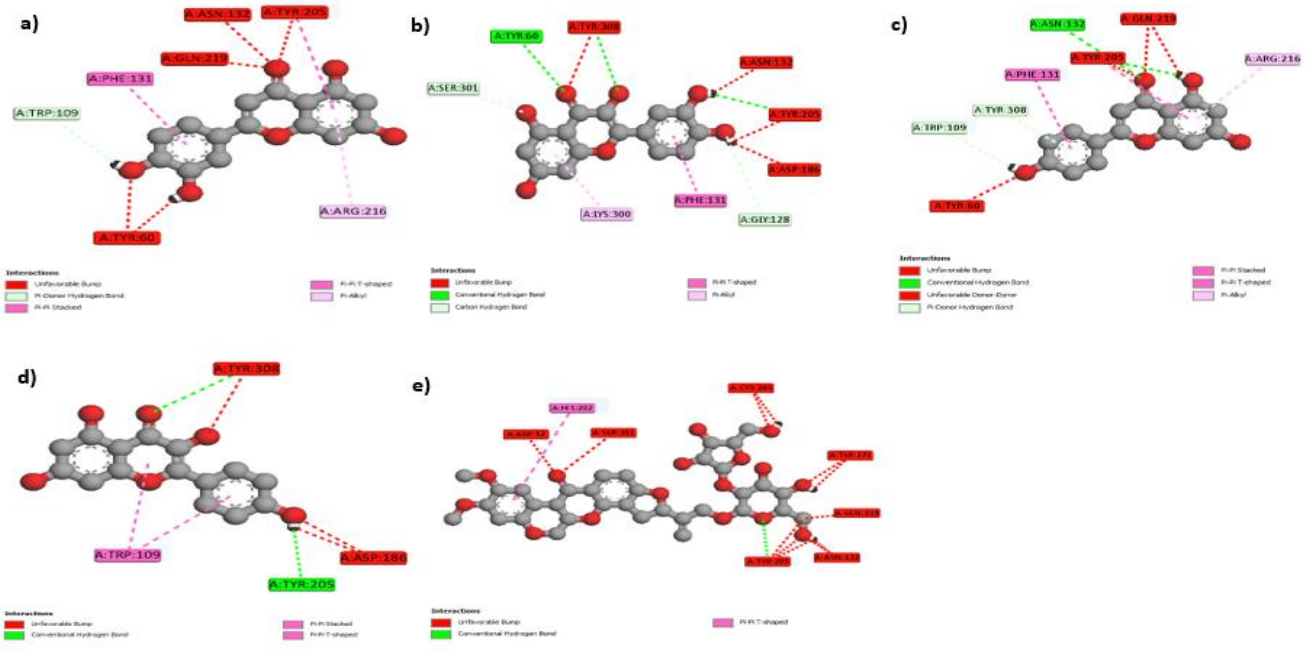

Figure 5. Three-dimensional representations of the most stable conformers of a) Luteolin, b) Quercetin, c) Apigenin, d) Kaempferol, and e) Amorphine molecules docked in the active site of the CXCR3 receptor.

Results

Drug-Like Properties of Flavonoids

Examining the physicochemical and ADME (absorption, distribution, metabolism, and excretion) properties is of great importance in evaluating a compound's potential for drug development. These properties determine the compound's behavior in the body, its efficacy, and safety. To investigate the drug-like properties of flavonoids, various physicochemical and ADME parameters of the selected flavonoids in this study were calculated and analyzed using

the Schrödinger program. The results are presented in Table 2 and Table 3.

The physicochemical properties of flavonoids are critical for the formulation, solubility, and bioavailability of a drug candidate. Table 2 lists the basic physicochemical parameters of flavonoids such as molecular weight (MW), octanol/water partition coefficient (log P), number of hydrogen bond donors and acceptors, number of rotatable bonds, and polar surface area (PSA). When these parameters are evaluated according to Lipinski's drug-likeness rules, it is observed that flavonoids generally exhibit drug-like properties. Luteolin, Apigenin, and Kaempferol fully comply with Lipinski's rules. However, Amorphine exceeds some drug-likeness limitations due to its high molecular weight (704.68 g/mol) and high number of hydrogen bond acceptors (22.75). This situation may require optimization of Amorphine.

Table 2. Physicochemical properties of flavonoids.

Compound	MW (g/mol)	Log P	H-bond Donor	H-bond Acceptor	Rotatable Bond	PSA (Å²)
Luteolin	286.24	0.941	3	4.5	4	120.356
Quercetin	318.37	-1.580	6	11.9	6	131.524
Apigenin	270.24	1.624	2	3.75	3	98.953
Kaempferol	302.37	-1.017	5	10.2	5	110.269
Amorphine	704.68	-0.435	6	22.75	16	217.200

Table 3: ADME properties of flavonoids.

Compound	Oral Absorption (%)	Caco-2 Permeability (nm/sec)	BBB Penetration (log BB)	Metabolism (CYP)	Plasma Protein Binding (%)
Luteolin	62	44	-1.911	CYP1A2, CYP3A4	86
Quercetin	34	44	-1.310	CYP3A4	99
Apigenin	74	124	-1.411	CYP1A2	95
Kaempferol	56	94	-1.067	CYP1A2, CYP2C9	99
Amorphine	19	71	-2.407	CYP3A4	85

ADME properties are important factors that determine the movement of a compound within the body. Table 3 presents ADME parameters such as estimated oral absorption, Caco-2 and MDCK cell permeability, blood-brain barrier permeability (log BB), metabolism by major CYP enzymes, and plasma protein binding of flavonoids. These properties reflect the pharmacokinetic profiles of flavonoids. Luteolin and Apigenin stand out with high oral absorption values (62% and 74%). Additionally, Luteolin exhibits the best profile in terms of blood-brain barrier permeability (log BB -1.911). These findings indicate that Luteolin and Apigenin are advantageous in terms of oral bioavailability and central nervous system targeting. However, all flavonoids are metabolized by CYP enzymes and bind to plasma proteins at high rates. This situation should be carefully evaluated in terms of drug-drug interactions and free drug concentrations.

Safety Profile of Flavonoids

In the drug development process, it is of great importance to identify potential toxicity risks at an early stage. In silico toxicity prediction models are widely used to predict toxicity profiles. In this study, the potential toxicity risks of optimized flavonoids were evaluated using the QikProp module of the Schrödinger program. Table 4 summarizes the predicted toxicity profiles of flavonoids in terms of mutagenicity, carcinogenicity, skin sensitization, hepatotoxicity, and hERG inhibition. The obtained results show that the optimized flavonoids generally exhibit low toxicity risks. All flavonoids fall into the low-risk category in terms of mutagenicity, carcinogenicity, and skin sensitization. However, some flavonoids carry specific toxicity concerns. Apigenin has cardiotoxicity potential due to its high risk of hERG inhibition. Amorphine shows moderate hepatotoxicity and hERG inhibition risks. These findings suggest that these flavonoids need to be evaluated more comprehensively in terms of toxicity.

The physicochemical properties of flavonoids are critical for the formulation, solubility, and bioavailability of a drug candidate. Table 2 lists the basic physicochemical parameters of flavonoids such as molecular weight (MW), octanol/water partition coefficient (log P), number of hydrogen bond donors and acceptors, number of rotatable bonds, and polar surface area (PSA). When these parameters are evaluated according to Lipinski's drug-likeness rules, it is observed that flavonoids generally exhibit drug-like properties. Luteolin, Apigenin, and Kaempferol fully comply with Lipinski's rules. However, Amorphine exceeds some drug-likeness limitations due to its high molecular weight (704.68 g/mol) and high number of hydrogen bond acceptors (22.75). This situation may require optimization of Amorphine.

Table 4. Toxicity predictions of flavonoids.

Compound	Mutagenicity	Carcinogenicity	Skin Sensitization	Hepatotoxicity	hERG Inhibition
Luteolin	Low	Low	Low	Low	Medium
Quercetin	Low	Low	Low	Low	Low
Apigenin	Low	Low	Low	Low	High
Kaempferol	Low	Low	Low	Low	Low
Amorphine	Low	Low	Low	Medium	Medium

Table 5 presents various physicochemical properties and drug-likeness parameters of flavonoids calculated using the Schrödinger program. These properties are important criteria for evaluating the potential of flavonoids for drug development. The table includes parameters such as polar surface area (PSA), molecular weight (MW), number of hydrogen bond donors and acceptors, octanol/water partition coefficient (QP log P), aqueous solubility (QP log S), plasma

protein binding (QP log K hsa), blood-brain barrier permeability (QP log BB), metabolism properties, and other ADME parameters for each flavonoid.

In general, all flavonoids exhibit drug-like properties. Polar surface area and molecular weight values comply with Lipinski's rules. The number of hydrogen bond donors and acceptors are also within acceptable ranges. However, Amorphine deviates from Lipinski's rules with its high molecular weight (704.68 g/mol) and high number of hydrogen bond acceptors (22.75). This situation may affect Amorphine's oral bioavailability.

QP log P values reflect the lipophilicity of flavonoids. While Luteolin and Apigenin have a more lipophilic profile with positive QP log P values, Quercetin, Kaempferol, and Amorphine exhibit more hydrophilic properties with negative values. The aqueous solubility (QP log S) parameter also reveals the solubility profile of flavonoids. All flavonoids fall within the medium to low solubility range.

When compared to known CXCR3 inhibitors, flavonoids have similar physicochemical properties. For example, AMG487's molecular weight is reported as 515.93 g/mol and its QP log P value as 5.18.[4] These values are close to the physicochemical profile of Luteolin and Apigenin in particular. Additionally, SCH546738's polar surface area is reported as 107.42 Å², which is consistent with the PSA values of flavonoids.[49] These similarities support the potential of flavonoids to be developed as CXCR3 inhibitors. However, the ADME parameters in Table 5 also reflect the pharmacokinetic properties of flavonoids. Luteolin and Apigenin stand out with high oral absorption and blood-brain barrier permeability. However, the fact that all flavonoids are metabolized by CYP enzymes and have high plasma protein binding should be considered in terms of drug-drug interactions and bioavailability. These properties show similarities with CXCR3 inhibitors. For example, it is known that AMG487 is metabolized by CYP3A4 and has high plasma protein binding.[50]

Table 5. Analysis of physicochemical properties of flavonoid ligands.

Property	Luteolin	Quercetin	Apigenin	Kaempferol	Amorphine	Suggested Range
Polar surface area PSA (Å²)	120,356	131,524	98,953	110,269	217,200	(7,0 / 200,0)
Molecular weight MW (g/mol)	286,24	318,37	270,24	302,37	704,68	(130,0 / 725,0)
Hydrogen Bond Donors as Solute	3	6	2	5	6	(0,0 / 6,0)
Hydrogen Bond Acceptors as Solute	4,5	11,9	3,75	10,2	22,75	(2,0 / 20,0)
QP log P for octanol/water	0,941	-1,580	1,624	-1,017	-0,435	(−2,0 / 6,5)
QP log S for aqueous solubility	-3,039	-1,462	-3,318	-1,314	-1,574	(−6,5 / 0,5)
QP log K hsa Serum Protein Binding	-0,205	-0,435	-0,043	-0,489	-1,164	(−1,5 / 1,5)
QP log BB for brain/blood	-1,911	-1,310	-1,411	-1,067	-2,407	(−3,0 / 1,2)
Number of Primary Metabolites	4	7	3	6	12	(1,0 / 8,0)
Predicted CNS Activity (-- to ++)	--	--	--	--	--	--------
HERG K+ Channel Blockade: log IC50	-5,023	-0,793	-5,125	-0,990	-4,338	(below −5 concerning)
Apparent Caco-2 Permeability (nm/sec))	44	44	124	94	71	(<25 poor, >500 excellent)
Apparent MDCK Permeability (nm/sec)	17	16	52	38	28	(<25 poor, >500 excellent)
QP log Kp for skin permeability	-4,797	-5,513	-3,908	-4,965	-3,670	(Kp in cm/hour)
Jm, maximum transdermal transport rate	0,004	0,034	0,016	0,159	4,018	(microgram/cm²-hour)
Lipinski Rule of 5 Violations	0	1	0	0	3	(maximum 4)
Jorgensen Rule of 3 Violations	0	1	0	0	1	(maximum 3)
% Human Oral Absorption in GI (+/−20%)	62	34	74	56	19	(<25% poor)
Qualitative Model for Human Oral Absorption	High	Medium	High	Medium	Low	(>80% high)

Discussion

This study focuses on evaluating the CXCR3-flavonoid interaction as a potential therapeutic approach in cancer immunotherapy. The research examined the binding affinities and pharmacokinetic properties of five flavonoid compounds (Luteolin, Quercetin, Apigenin, Kaempferol, and Amorphine) to the CXCR3 receptor using in silico methods. The results obtained indicate that these flavonoids bind to the CXCR3 receptor with high affinity, suggesting their potential use as CXCR3 antagonists.

The findings of the study are particularly noteworthy, especially Amorphine's binding affinity of -13.0 kcal/mol. This result is comparable to the binding affinities reported in the study on CXCR3 antagonists by Wijtmans et al. (2011).[46] However, due to the nature of in silico methods, these results need to be confirmed through in vitro and in vivo studies. Indeed, the study by Cambien et al. (2009) demonstrated that CXCR3 antagonism inhibits colon cancer metastasis in an organ-specific manner.[17] In this context, investigating the potential effects of flavonoids on CXCR3 in relation to metastasis should be a critical focus for future studies.

The ADME properties of flavonoids are promising, particularly the high oral bioavailability and blood-brain barrier permeability shown by Luteolin and Apigenin. However, as emphasized in Terao's (2017) study, the factors affecting the bioavailability of flavonoids are complex, and their pharmacokinetic properties need to be optimized to enhance their therapeutic potential.[34] At this point, the fact that all flavonoids are metabolized by CYP enzymes and highly bound to plasma proteins should be carefully evaluated in terms of potential drug-drug interactions and bioavailability.

In terms of toxicity, although in silico predictions generally show a low-risk profile, Apigenin's high risk of hERG inhibition and Amorphine's moderate risk of hepatotoxicity highlight the need for further toxicological evaluations. These findings parallel the warning emphasized in the comprehensive review by Ravishankar et al. (2013) on the anti-cancer potential of flavonoids, which noted potential side effects at high doses or in certain combinations.[35]

The role of CXCR3 inhibition in cancer immunotherapy contains conflicting findings in the current literature. The study by Oghumu et al. (2014) showed that CXCR3 deficiency accelerates tumor progression in a breast cancer model.[24] This finding suggests that CXCR3 inhibition may lead to undesirable results in some cases. On the other hand, Peng et al. (2015) demonstrated that epigenetic silencing of CXCR3 ligands positively affects tumor immunity and response to immunotherapy.[28] These conflicting findings emphasize that CXCR3 modulation may have different

effects depending on the cancer type and microenvironment, and further research is needed in this area.

In conclusion, this study demonstrates that the use of flavonoids as CXCR3 inhibitors is a potential approach in cancer immunotherapy. However, more comprehensive research is needed to translate this approach into clinical practice. Future studies should focus on optimizing flavonoid-based CXCR3 inhibitors, examining the effects of CXCR3 inhibition in different cancer types and stages, detailed analysis of immunomodulatory effects in the tumor microenvironment, and evaluating combinations with other immunotherapy approaches. Additionally, a better understanding of the molecular mechanisms of flavonoid interaction with CXCR3 is critical to fully reveal the therapeutic potential of these compounds.

Conclusion

In this study, the interactions of various flavonoids with the CXCR3 receptor and their potential roles in cancer immunotherapy were comprehensively examined. Molecular docking simulations revealed that flavonoids bind to the CXCR3 receptor with high affinity and exhibit similar interaction patterns to known CXCR3 antagonists. Amorphine, in particular, stood out with the strongest binding affinity. Additionally, while Luteolin exhibited a good ADME profile, all flavonoids were predicted to have low toxicity risks.

In terms of physicochemical and ADME properties, flavonoids fall within acceptable ranges for drug-like molecules. Luteolin and Apigenin show promising profiles in terms of oral bioavailability and blood-brain barrier permeability. Moreover, the fact that flavonoids have similar physicochemical and ADME properties to CXCR3 inhibitors supports the potential of these compounds to be developed as CXCR3-targeted therapeutic agents.

However, the efficacy and safety of flavonoids as CXCR3 antagonists need to be confirmed through in vitro and in vivo studies. Additionally, it is important to investigate the role of flavonoids in cancer immunotherapy in more detail and evaluate them in combination with other immunotherapy approaches.

In conclusion, this study demonstrates that flavonoids can offer a new therapeutic strategy in cancer immunotherapy by targeting the CXCR3 receptor. The obtained findings provide a solid foundation for the development and optimization of flavonoid-based CXCR3 inhibitors. Future research may open new horizons in the field of immunotherapy by evaluating the efficacy and safety of these compounds in cancer treatment in a clinical setting. Flavonoids present a promising approach for cancer immunotherapy and have the potential to contribute to the development of more effective treatments.

Conflict of Interest

There are no disclosed conflicts of interest for the authors.

Compliance with Ethical Statement

No biological material or patient data was used in this study. Therefore, the authors declare that the current study does not require ethical committee approval.

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Author's Contributions

H.G., A.D.D.: Study idea/Hypothesis; H.G., A.D.D.: Design; H.G., A.D.D.: Data Collection; H.G., A.D.D.: Analysis; H.G., A.D.D.: Literature review; H.G., A.D.D.: Writing; H.G.: Critical review.

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