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In Silico Study on 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one as a Candidate IL-1R and TNF-R Inhibitor

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Abstract

Inflammation is an important process that fights infections and allows the repair of tissues; however, chronic inflammation is implicated in many diseases, including autoimmune diseases and tumorigenesis. Central to this process are the Interleukin-1 receptor and Tumor Necrosis Factor receptor that mediate the action of the pro-inflammatory cytokines IL-1 and TNF-α, respectively. This work is aimed at the theoretical investigation of the possibility of 5-Hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one, a naturally occurring flavonoid with established anti-inflammatory properties, acting as an IL-1R and TNF-R inhibitor by in silico methods. In fact, molecular docking studies have indicated good binding affinities and identified crucial interactions with amino acid residues in the receptor binding sites. These observations strongly suggest that this compound is likely to inhibit IL-1R and TNF-R with high efficiency, leading to the modulation of the inflammation-related pathways. Further experimental validation is required to confirm these interactions and also to assess their therapeutic potential in inflammatory disorders.

Keywords 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one, IL-1R, TNF-R, Anti-Inflammatory agents

1.0 INTRODUCTION

Inflammation is a crucial biological response that helps protect us from threats like infections and injuries. It is mediated by several cells with the role of initiating inflammation, such as macrophages, neutrophils, and lymphocytes [1]. However, chronic inflammation can contribute to various health problems, including autoimmune diseases, cancer, and cardiovascular issues [2]. When it comes to inflammation a couple of key players stand out, the Interleukin-1 receptor (IL-1R) [3] and Tumor

Necrosis Factor receptor (TNF-R) [4]. IL-1R is involved in the signaling pathways of the pro-inflammatory cytokine IL-1, while TNF-R mediates the effects of TNF-α, another key pro-inflammatory cytokine. Dysregulation of these receptors is implicated in the pathogenesis of numerous inflammatory disorders, emphasizing the importance of effective inhibitors to modulate their activity [5].

The compound 5-hydroxy-2-(4-hydroxyphenyl)-6-7-dimethoxychromen-4-one, a naturally occurring flavonoid, has garnered attention due to its diverse pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities [6]. Flavonoids are known for their ability to interact with various biological targets, making them promising candidates for drug development. However, the specific molecular mechanism by which 5-hydroxy-2-(4-hydroxyphenyl)-6-7-dimethoxychromin-4-one exerts its effects on IL-1R and TNF-R remains to be elucidated [7].

In silico methods have transformed drug discovery and development. These computational approaches facilitate rapid screening and optimization of potential drug candidates, reducing time and costs compared to traditional experiments. Molecular docking predicts how small molecules bind to target proteins, while molecular dynamics simulations reveal dynamic changes in protein-ligand complexes over time. Molecular docking stands out as a pivotal technique that predicts how small molecules, such as 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one, bind to target proteins with remarkable precision and efficiency. By simulating the interaction between ligands and receptors at the atomic level, molecular docking provides insights into the specific binding affinity, orientation, and potential modes of interaction within the binding sites of IL-1R and TNF-R [8]. This study aimed to predict the binding affinity of 5-hydroxy-2-(4-hydroxyphenyl)-6-7-dimethoxychromen-4-one as an IL-1R and TNF-R inhibitor.

2.0 EXPERIMENTAL

2.1 Materials

This research uses an in silico approach using the webserver https://pubchem.ncbi. nlm.nih.gov/, https://ncbi.nlm.nih.gov, https://swissmodel.expasy.org, PyRx software, PyMol, and Biovia Discovery Studio 2021. This research material is the compound 5-hydroxy-2 -(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one with PubChem CID 188323 Cirsimaritin(Compound) and omeprazole compounds with PubChem CID 4594, IL-1R (GenBank: AAA16196.1) and TNF-R (NCBI Reference Sequence: NP_035741) proteins. 2). The IL-1R and TNF-R proteins underwent FASTA extraction which was then modeled using the webserver https://swissmodel.expasy.org.

The ligand and protein were entered into the PyRx software for molecular docking. The molecular docking results included binding affinity values, hydrogen bonds, hydrogen bond distances, and hydrophobic bonds. The ligand resulting from docking was saved in .pdb format, which was then docked with the protein target using PyMol software to form a protein-ligand complex. This complex was visualized using Biovia Discovery Studio 2021 software. The visualization showed the interaction between the ligand and amino acid residues.

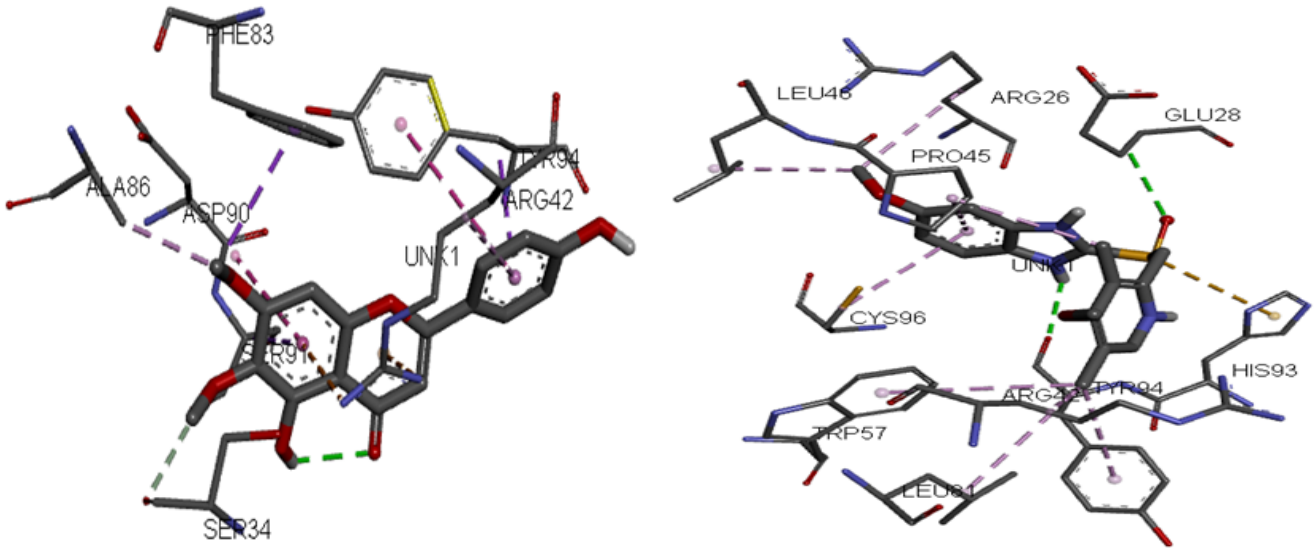
3.0 RESULTS AND DISCUSSION

3.1 Molecular Docking of Ligand and Protein Complexes (IL-1R)

Molecular docking data is shown in Table 1. The binding affinity value of IL-1R_188323 is more negative (-8.5 kcal/mol) compared to the binding affinity of IL-1R_4594 (-8.3 kcal/mol). Hydrogen bonds in the IL-1R_188323 protein-ligand complex show a hydrogen bond distance of more than 2.00 Å. Meanwhile, hydrogen bonds in the IL-1R_4594 protein-ligand complex show a hydrogen bond distance of more than 2.00 Å. In the protein ligand complex IL-1R_188323 and IL-1R_4594 there is a hydrophobic bond. The results of the molecular docking visualization are shown in Figure 1. In Figure 1 (a) the ligand 188323 binds to the amino acid residues ALA86, PHE83, TYR94, ARG42, SER34, SER91, ASP90, and UNK1. In Figure 1 (b) ligand 4594 binds to the amino acid residue LEU81; TRP57, ARG42, TYR94, HIS93, CYS96, PRO45, LEU45, ARG26, GLU28, and UNK1.

Table 1 Binding affinity of 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one with IL-1R.

Protein-Ligand Complex	Binding Affinity Value (kcal/mol))	RMSD (Å)	Hydrogen Bond Distance (Å)	Hydrogen Bonds	Hydrophobic Bonding
IL-1R_188323	-8.5	0.00	3.529 3.684	N:UNK1:C-A:LYS48:O A:ALA50:N-N:UNK1	A:ALA50:CB-N:UNK1 A:HIS95-N:UNK1 A:ALA50-N:UNK1:C N:UNK1:C-A:LEU43 N:UNK1:C-A:CYS44 N:UNK1-A:ALA50 N:UNK1-A:PRO52
IL-1R_4594	-8.3	0.00	2.649 2.088 2.398 3.519 3.879 4.107	N:UNK1:H - A:ASN130:OD1 N:UNK1:HN - A:CYS158:O N:UNK1:HN - N:UNK1:O N:UNK1:C - A:PRO148:O A:CYS158:N - N:UNK1 A:THR164:OG1 - N:UNK1	N:UNK1:C-A:VAL154 N:UNK1-A:CYS146 N:UNK1-A:CYS158 N:UNK1-A:CYS146 N:UNK1-A:CYS158



(a) (b)

Figure 1 Visualization of molecular docking of the ligand and protein complex (IL-1R).

In Figure 1(a), ligand 188323 showed significant binding interactions with several key amino acid residues in the IL-1R complex. The interactions were highly stable, as evidenced by the formation of hydrogen bonds and hydrophobic interactions with residues such as ALA86, PHE83, and TYR94. The involvement of ARG42 and SER34 also indicated that 188323 may have a strong affinity for the IL-1R binding site, which may lead to effective inhibition of receptor activity. The presence of UNK1 (unknown residue) in the interaction profile indicated the possibility of interactions with non-standard or unidentified residues, which may play an important role in ligand binding efficiency.

Similarly, in Figure 1(b), ligand 4594 shows strong binding interactions with several amino acid residues including LEU81, TRP57, and ARG42. Interactions with TYR94 and HIS93, particularly through hydrogen bonds, confirm the potential

efficacy of the ligand in stabilizing the IL-1R structure, thereby inhibiting its function. The presence of residues such as CYS96 and PRO45, along with an unknown UNK1 residue, suggests a complex interaction profile that may contribute to the inhibitory activity of the ligand.

3.2 Molecular Docking of Ligand and Protein Complexes (TNF-R)

Table 2 provides an overview of the molecular docking data. The TNF-R_188323 complex demonstrates a binding affinity of -6.1 kcal/mol, which is more negative than that of TNF-R_4594, also at -6.1 kcal/mol, indicating stronger interactions with the receptor. The hydrogen bonds formed in the TNF-R_188323 complex exhibit distances greater than 2.00 Å, similar to the hydrogen bonds observed in the TNF-R_4594 complex. Both TNF-R_188323 and TNF-R_4594 protein-ligand complexes also exhibit hydrophobic interactions. Figure 2 (a) illustrates that ligand 188323 interacts with amino acid residues ARG97, PRO52, ALA50, LEU43, CYS44, LYS48, HIS93, and UNK1, while Figure 2 (b) shows ligand 4594 binding with residues VAL154, THR164, PRO148, CYS146, CYS158, ASN130, and UNK1.

Table 2 Binding affinity 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one with TNF-R.

Protein-Ligand Complex	Binding Affinity Value (kcal/mol))	RMSD (Å)	Hydrogen Bond Distance (Å)	Hydrogen Bonds	Hydrophobic Bonding
TNF-R_188323	-6.1	0.00	2.504 2.842 3.467	N:UNK1:H - B:ARG42:NH1-N:UNK1 N:UNK1:O B:ARG42:NH2-N:UNK1 B:SER91:OG - N:UNK1:C-B:PHE83 N:UNK1:O B:SER91:CA-N:UNK1 N:UNK1:C - B:TYR94:CB-N:UNK1 B:SER34:O N:UNK1 - B:TYR94 B:ASP90:C,O;SER91:N-N:UNK1 N:UNK1:C-B:ALA86 N:UNK1-B:ARG42	
TNF-R_4594	-6.1	0.00	3.054 2.662	B:GLU28:N - N:UNK1:S-B:HIS93 N:UNK1:O N:UNK1:C-B:ARG42 N:UNK1:HN - N:UNK1:C-B:LEU81 B:TYR94:O N:UNK1:C-B:PRO45 N:UNK1:C-B:ARG26 N:UNK1:C-B:LEU46 B:TRP57-N:UNK1:C B:TYR94-N:UNK1:C N:UNK1-B:PRO45 N:UNK1-B:CYS96	

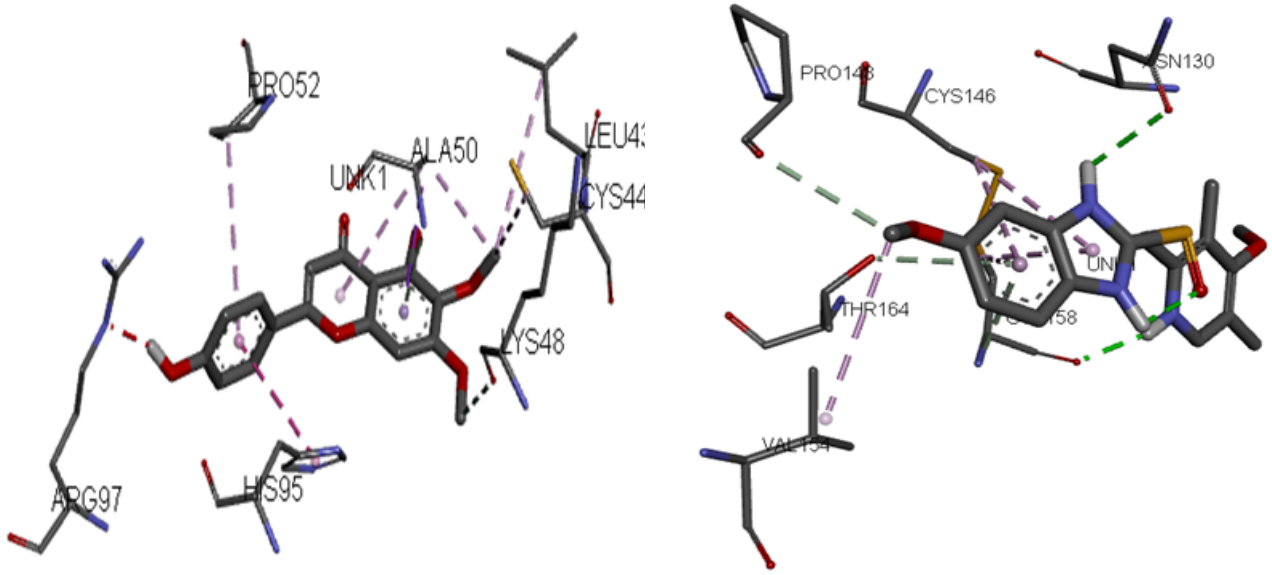


Figure 2 Visualization of molecular docking of the ligand and protein complex (TNF-R).

In Figure 2A, we see that ligand 188323 binds strongly, but this time it binds with a different set of amino acid residues in the IL-1R complex. Binding to ARG97, PRO52, and ALA50 suggests that 188323 may interact with different regions of the IL-1R compared to its interactions in Figure 1A. The involvement of residues such as LEU43 and HIS93, along with UNK1, suggests that 188323 may have multiple binding sites or modes of interaction within the IL-1R, thus increasing its potential as an inhibitor.

In Figure 2B, ligand 4594 exhibits interactions with a series of novel amino acid residues including VAL154, THR164, and PRO148. Consistent interactions with residues such as CYS146 and CYS158, along with the presence of ASN130, suggest that ligand 4594 may bind to different sites on the IL-1R, possibly providing different mechanisms of inhibition. The presence of UNK1 in this interaction profile again raises the possibility of novel or non-standard interactions that may be key to ligand efficacy.

3.3 Role of IL-1R and TNF-R to Promote Inflammation

Inflammation is a complex biological response that plays a crucial role in defending the body against infections and initiating tissue repair. This process involves a cascade of events that include the activation of immune cells, the release of signaling molecules, and the regulation of various pathways essential for maintaining homeostasis. However, when inflammation becomes chronic, it can lead to the development of a wide range of pathological conditions, including autoimmune diseases, cardiovascular disorders, and various forms of cancer [9]. Chronic inflammation is characterized by the persistent activation of immune responses, often driven by the continuous production of pro-inflammatory cytokines such as IL-1 and TNF-α. These cytokines exert their biological effects through binding to specific receptors, namely the IL-1R and TNF-R, which are central to the regulation of the inflammatory process [10].

The importance of IL-1R and TNF-R roles to promote inflammation lies in its function to activate inflammation after binding with its native ligand, which is IL-1 and TNF-α. Receptor-ligand interaction will dimerize the receptor via the TIR domain of the receptor, thus promoting recruitment of MyD88. MyD88 will act as an adaptor protein that will bind with IL-1R-associated kinases (IRAKs) and tumor necrosis factor receptor-associated factor 6 (TRAF6). It will create the MyD88-IRAKs-TRAF6 complex, thus activating further downstream signaling [11]. TNF-R will dimerize after binding with the ligand and recruit several proteins related to the activation of inflammation, such as TRADD which will bind cellular inhibitor of apoptosis (cIAP)1/2, TRAF 2/5, and RIP protein kinase. Both IL-1R and TNF-R dimerization will activate downstream signaling to promote the binding of TAB1 and TAB2 with TAK1, thus activating the IKK complex. The result will be activating NF-κB, a transcription factor that has a main role in activating the inflammation process [12].

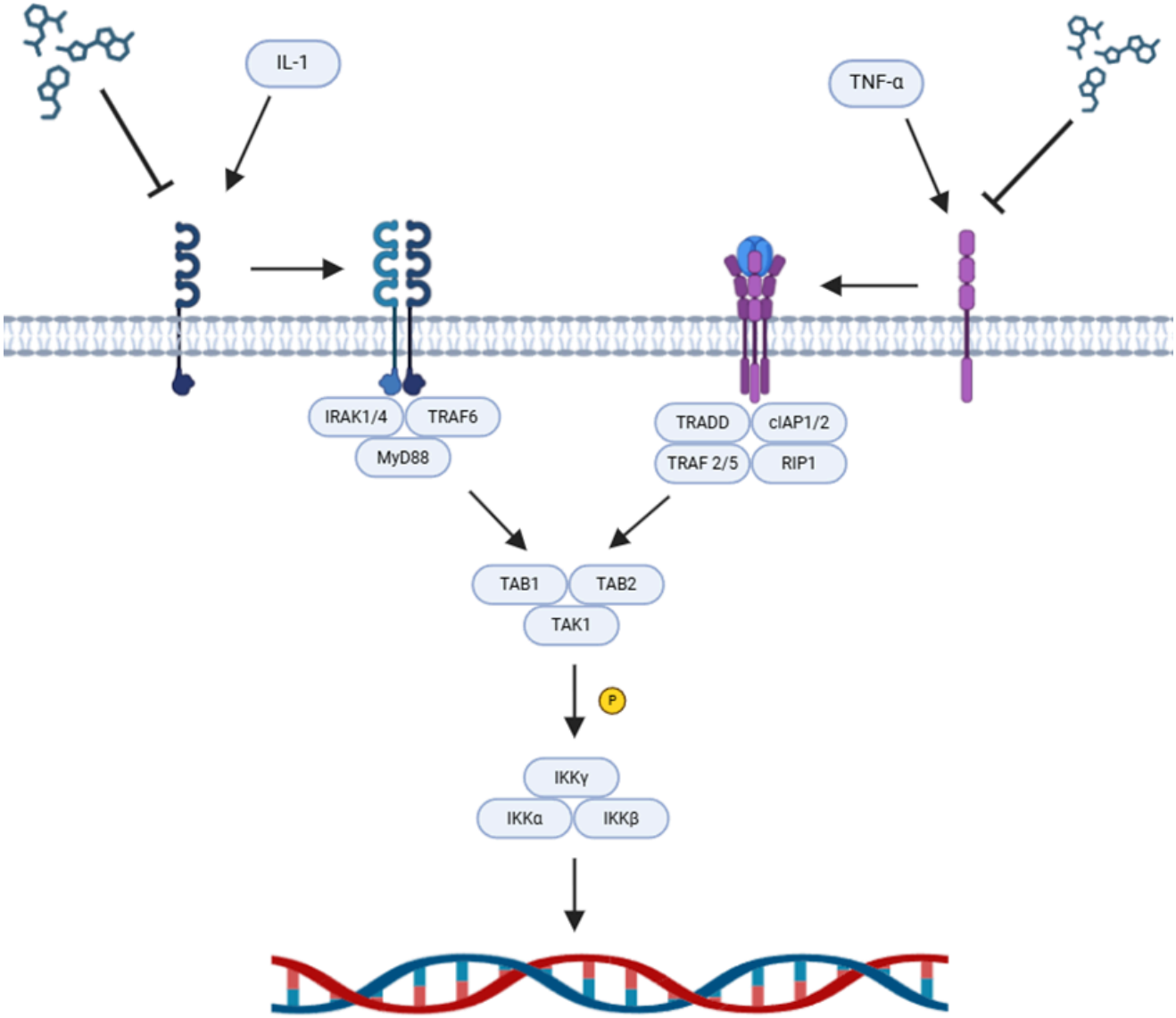


Figure 3. Proposed mechanism of 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one inhibiting IL-1R and TNF-R.

Given the pivotal role that IL-1R and TNF-R play in mediating inflammatory responses, they have become key therapeutic targets in the treatment of inflammation-related diseases. One promising class of compounds that have shown potential in modulating these receptors is flavonoids, a diverse group of polyphenolic compounds found in various fruits, vegetables, and medicinal plants. Flavonoids are known for their broad spectrum of biological activities, including antioxidant, anti-inflammatory, and immunomodulatory effects. Research has demonstrated that flavonoids can inhibit IL-1R activity through multiple mechanisms, including direct binding to the receptor, modulation of downstream signaling pathways, and reduction of cytokine [13]. These actions collectively contribute to the anti-inflammatory properties of flavonoids, making them attractive candidates for the development of therapies aimed at treating chronic inflammatory conditions.

Furthermore, flavonoids have been shown to inhibit TNF-α activity, thereby attenuating the inflammatory response in various disease models. For example, in rheumatoid arthritis, a condition characterized by chronic inflammation and joint destruction, flavonoids have been reported to reduce inflammation by inhibiting the binding of TNF-α to its receptor, thus blocking the activation of pro-inflammatory pathways [14]. This dual ability of flavonoids to target both IL-1R and TNF-R highlights their therapeutic potential in managing not only autoimmune diseases but also other inflammation-driven pathologies. Their natural origin and relatively low toxicity further underscore the importance of flavonoids as potential therapeutic agents in the ongoing battle against chronic inflammation and its associated diseases.

The compound under investigation, 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one, is a naturally occurring flavonoid that has garnered significant attention in recent years due to its diverse pharmacological activities, particularly its anti-inflammatory properties properties [15]. Flavonoids like this one are well-known for their ability to modulate various biological processes, making them promising candidates for therapeutic applications. However, despite the extensive research on its general pharmacological effects, the specific molecular mechanisms by which 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one interacts with key inflammatory receptors such as Interleukin-1 receptor (IL-1R) and Tumor Necrosis Factor receptor (TNF-R) remain largely unexplored [16]. Understanding these interactions is crucial, as IL-1R and TNF-R are central to mediating inflammatory responses, and their modulation could lead to significant therapeutic advancements in treating inflammation-driven diseases.

This study utilized in silico methods to explore the potential inhibitory effects of 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one on IL-1R and TNF-R. In silico techniques, particularly molecular docking, have become invaluable tools in modern drug discovery, allowing researchers to predict how small molecules interact with target proteins at the atomic level. Molecular docking was employed in this study to simulate the binding interactions between the flavonoid and the receptors, providing insights into the compound's binding affinity and potential modes of interaction [17]. The results from these simulations were compelling, revealing that 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one exhibited strong binding affinities towards both IL-1R and TNF-R, with binding energies calculated at -8.5 kcal/mol and -6.1 kcal/mol, respectively.

The visualizations from PyMol and Biovia Discovery Studio 2021 provided detailed insights into how 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one interacts with IL-1R and TNF-R at the molecular level. The compound forms strong hydrogen bonds and other interactions with key amino acids in these receptors, which may block the binding of pro-inflammatory cytokines like IL-1 and TNF-α. This suggests that the flavonoid could inhibit inflammation by interfering with these signaling pathways, highlighting its potential as a therapeutic agent for chronic inflammatory diseases. These findings also lay the groundwork for developing more potent inhibitors targeting IL-1R and TNF-R [9].

4.0 CONCLUSION

The in silico findings presented in this study strongly support the hypothesis that 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one could act as a potent inhibitor of IL-1R and TNF-R, key receptors involved in the inflammatory response. The molecular docking simulations reveal significant binding affinities and interactions between the compound and these receptors, suggesting a potential mechanism through which the flavonoid may modulate inflammatory pathways.The molecular docking results indicate that 5-Hydroxy-2-(4-hydroxyphenyl)-6,7dimethoxychromen-4-one may inhibit the IL-1R and TNF-R pathways by binding to key residues, potentially blocking the signaling involved in chronic inflammation. This suggests the compound could serve as an effective inhibitor, modulating inflammatory responses linked to autoimmune disorders and cancer.

These computational insights lay a solid groundwork for future research aimed at the rational design and development of novel anti-inflammatory agents that target IL-1R and TNF-R pathways. By advancing our understanding of these interactions, this study opens new avenues for the development of more effective therapies that could mitigate the effects of chronic inflammation and improve patient outcomes. While these results are promising, it is crucial to undertake further experimental validation, including in vitro assays and in vivo studies, to confirm the efficacy and safety of this compound in biological systems. Such studies will help to elucidate the precise mechanisms of action and assess the therapeutic potential of 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxychromen-4-one in treating various inflammatory disorders, including autoimmune diseases and cancer.

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