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Osteoporozda Katepsin K'nın Terapötik Hedeflenmesi: Polifenolik İnhibitörlerin *In Silico* ve ADMET Analizi

Therapeutic Targeting of Cathepsin K in Osteoporosis: Analysis of Polyphenolic Inhibitors *In Silico* and ADMET

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ÖZET

Amaç: Osteoporozda artan kemik rezorpsiyonu, başlıca osteoklastların salgıladığı Katepsin K (CatK) enziminin kolajen yıkımındaki belirgin rolüyle ilişkilidir. Sentetik CatK inhibitörlerinde görülen güvenlik sorunları nedeniyle doğal bileşikler terapötik açıdan umut verici alternatifler sunmaktadır. Bu çalışmanın amacı, seçilmiş polifenollerin CatK enzimi ile etkileşimlerini *in silico* yöntemlerle değerlendirerek potansiyel inhibitör adaylarını belirlemektir.

Materyal ve Metot: CatK'nın kristal yapısı (PDB ID: 4X6H) hazırlanarak AutoDock Vina yazılımı ile moleküler docking analizleri gerçekleştirilmiştir. Ligandların bağlanma modları Discovery Studio Visualizer ile incelenmiş; hidrofobik ve hidrojen bağı gibi etkileşimler değerlendirilmiştir. Bileşiklerin farmakokinetik ve toksikolojik profilleri ADMETlab 3.0 aracılığıyla tahmin edilmiş, Lipinski kuralları, absorpsiyon, dağılım, metabolizma ve toksisite parametreleri analiz edilmiştir.

Bulgular: Docking analizleri, EGCG, Naringenin ve Genistein'in referans inhibitörden daha yüksek bağlanma affinitesi gösterdiğini ortaya koymuştur. Bu bileşiklerin CatK'nın katalitik çifti ve S1–S3 cepleriyle belirgin etkileşimler kurduğu saptanmıştır. ADMET sonuçları tüm bileşiklerin ilaç-benzeri özellikler taşıdığını, düşük toksisite riskine sahip olduğunu ve metabolik profillerinin farklılık gösterebildiğini göstermiştir.

Tartışma ve Sonuç: CatK'nın katalitik çifti ve seçicilik cepleriyle uyumlu etkileşimler, güçlü inhibitör adaylarının belirlenmesinde temel belirleyicidir. Çalışmada öne çıkan bileşiklerin bu bölgelerle doğrudan temas kurması, yüksek affinitelerini yapısal olarak açıklamaktadır. ADMET verileri genel bir uygunluk sunmakla birlikte, bazı bileşiklerde CYP enzimleriyle olası etkileşimler metabolik optimizasyon ihtiyacını göstermektedir. Elde edilen bulgular, bazı doğal polifenollerin CatK inhibisyonu açısından güçlü adaylar olduğunu ve yapısal etkileşimleri ile farmakokinetik profillerinin gelecekteki ilaç tasarımı çalışmalarına temel oluşturduğunu göstermektedir.

Anahtar Kelimeler: Osteoporoz, Katepsin K, Moleküler Kenetlenme, ADMET, Polifenoller

ABSTRACT

Objective: Increased bone resorption in osteoporosis is primarily associated with the significant role of the Cathepsin K (CatK) enzyme, secreted by osteoclasts, in collagen degradation. Due to safety concerns encountered with synthetic CatK inhibitors, natural compounds offer promising therapeutic alternatives. The aim of this study is to identify potential inhibitor candidates by evaluating the interactions of selected polyphenols with the CatK enzyme using *in silico* methods.

Materials and Methods: The crystal structure of CatK (PDB ID: 4X6H) was prepared, and molecular docking analyses were performed using AutoDock Vina software. The binding modes of the ligands were investigated using Discovery Studio Visualizer; interactions such as hydrophobic and hydrogen bonding were evaluated. The pharmacokinetic and toxicological profiles of the compounds were estimated using ADMETlab 3.0, and Lipinski's rules, absorption, distribution, metabolism, and toxicity parameters were analyzed.

Results: Docking analyses revealed that EGCG, Naringenin, and Genistein exhibited higher binding affinity than the reference inhibitor. These compounds were found to have significant interactions with CatK's catalytic binary and S1–S3 pockets. ADMET results showed that all compounds possessed drug-like properties, had a low risk of toxicity, and exhibited varying metabolic profiles.

Discussion and Conclusion: Consistent interactions with CatK's catalytic binary and selectivity pockets are key determinants in identifying strong inhibitor candidates. The direct contact of the prominent compounds in this study with these regions structurally explains their high affinity. While ADMET data offer general agreement, potential interactions with CYP enzymes in some compounds highlight the need for metabolic optimization. The findings demonstrate that some naturally occurring polyphenols are strong candidates for CatK inhibition, and their structural interactions and pharmacokinetic profiles form the basis for future drug design studies.

Keywords: Osteoporosis, Cathepsin K, Molecular Docking, ADMET, Polyphenols

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INTRODUCTION

Osteoporosis is a systemic skeletal disease that weakens the structure and strength of bone tissue, increasing fragility and thus raising the risk of fractures (1). Globally, one in three women and one in five men over the age of 50 will experience osteoporotic fractures during their lifetime (2). Although the disease is particularly common in postmenopausal women, its incidence is also increasing in geriatric men (3). Due to the significant hormonal changes (estrogen deficiency) that occur during menopause, bone mass loss begins approximately 15–20 years earlier in women compared to men (4). The risk of osteoporosis increases with factors such as advanced age, female gender, postmenopausal estrogen deficiency, previous fractures, diabetes, sarcopenia, chronic inflammatory diseases, hypogonadism, thyroid disorders, cancer treatments, long-term glucocorticoid use, smoking, alcohol, low BMI, and bariatric surgery (2, 5-7). Age and menopause constitute the most important risk factors for the disease. Osteoporosis is caused by these factors called primary osteoporosis. Other risk factors for the disease are the comorbidities of osteoporosis, and osteoporosis that occurs in this way is called secondary osteoporosis (7, 8).

The fundamental cellular mechanism of osteoporosis is the disruption of the balance between bone resorption and bone formation, with the process shifting towards bone destruction. Over time, this process leads to deterioration in the skeletal microstructure, a decrease in bone mass, and consequently an increased susceptibility and risk of fractures (9). In the postmenopausal period, estrogen deficiency accelerates osteoclastogenesis and bone resorption by increasing the production of receptor activator of nuclear factor κB ligand (RANKL) and decreasing osteoprotegerin (OPG). Furthermore, estrogen deficiency contributes to the pathophysiology of the disease through osteoimmune mechanisms, increasing RANKL and tumor necrosis factor (TNF)-α via T cell activation. Aging, on the other hand, reduces bone formation by promoting the differentiation of mesenchymal stem cells towards adipocytes instead of osteoblasts. All these processes lead to decreased bone density and quality, and an increased risk of fractures, as a result of increased resorption and decreased formation (10).

Bone resorption is carried out by osteoclasts, which are multinucleated cells of hematopoietic origin, and this process requires the coordination of a two-stage mechanism involving both the dissolution of the mineral component and the breakdown of the organic matrix. In the first stage, osteoclasts firmly adhere to the bone surface, forming an isolated, acidic microenvironment called a "resorption lacuna" (11). Osteoclasts lower the pH to approximately 4.5 by releasing protons (H^+) into this area via a vacuolar proton pump (V-ATPase). This acidic environment allows the dissolution of hydroxyapatite, the inorganic mineral component of

bone (3, 12). Following the demineralization process in an acidic environment, the second stage, displacement of the organic matrix, occurs. The dissolution of minerals exposes the bone organic matrix (approximately 90% Type I collagen). The breakdown of this organic matrix is primarily carried out by a cysteine protease enzyme called cathepsin K (CatK), which is activated in an acidic environment. This degradation process leads to increased bone resorption, which is the fundamental mechanism of osteoporosis (3, 11, 13).

Unlike other proteases, CatK stands out for its effectiveness in degrading collagen at the triple helix region (13). Therefore, specific inhibition of CatK is considered a viable therapeutic target for halting bone loss without affecting bone formation. However, to date, there are no approved drugs targeting CatK (14). Odanacatib, the leading candidate in this field, has reached Phase III clinical trials, demonstrating sustained increases in bone mineral density and a significant reduction in fracture risk (15, 16). However, the odanacatib Phase III study was stopped after a statistically significant increase in the risk of cerebrovascular events was observed (14). Other promising candidates, balicatib (Phase II), were discontinued due to skin reactions (morphea-like lesions) caused by its structure that led to accumulation in lysosomes, while ONO-5334 (Phase II) had its development terminated due to competition and marketing strategies (13-17). As a result of these shortcomings, there is currently no approved drug targeting CatK. Since CatK plays a central role in osteoclast activity, plant-derived phytochemicals offer potential as an alternative inhibitor for the treatment of osteoporosis. Unlike traditional synthetic inhibitors, phytochemical compounds such as dihydrotanshinone (DHT) have the advantage of targeting the allosteric sites of CatK, called exosides, thus preserving the enzyme's core catalytic activity towards other physiological substrates. This novel strategy aims to selectively block the enzyme's collagenase activity, thereby reducing the risk of "on-target, off-target" side effects (17).

Vitamin K subtypes and other naturally occurring naphthoquinone/phenanthrenquinone derivatives, such as beta-lapochone, have also shown CatK inhibition in the micromolar range(16). Phytochemicals such as AC-5-1, panduratin A, and cycloaltilisin 6 have been proposed as alternative CatK inhibitors due to their natural origin and lower risk of side effects (18). While the natural origin, structural diversity, and potentially favorable safety profiles of phytochemicals are attractive, the relatively low potential (high IC50) of most candidates compared to synthetic compounds and the lack of extensive in vivo studies present significant challenges. To overcome these limitations, future studies should focus on structural optimization through computational modeling (18).

Today, Computer-Aided Drug Design (CADD) methods are widely used in drug discovery processes to save time and cost. Molecular docking, one of the leading methods, is a powerful tool for predicting how small molecule ligands (polyphenols) bind to target proteins (CatK) and their binding affinity (19).

The aim of this study is to investigate the binding of several different polyphenols, known in the literature for their various biological activities, to CatK, a potential target in osteoporosis treatment, using *in silico* methods. Using molecular docking simulations, the interactions and binding energies of these compounds with the protein's active site were analyzed and compared with a reference inhibitor.

MATERIALS AND METHODS

Preparation of Protein and Ligand Structures

The three-dimensional structure of CatK was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org), and the 4X6H PDB-coded structure was used in this study. Protein preparation was performed using AutoDock Tools 1.5.7 software, and water molecules and all other cofactors/metabolites in the protein were removed. In addition, missing atoms were completed, polar hydrogen atoms were added, and Kollman coupled charges were assigned. The prepared protein structure was saved in PDBQT format for use in molecular docking analyses.

The chemical structures of the selected ligands and the reference inhibitor (RI) 4-Amino-N-(1-[(Cyanomethyl)carbamoyl] cyclohexyl)-3-Fluorobenzamide were obtained from the PubChem database in SDF format (Table 1). The three-dimensional conformations, format transformations, and appropriate protonation states of the ligands were determined using Open Babel GUI and AutoDock Tools 1.5.7 software. Subsequently, the geometry of the ligands was optimized using the UCSF Chimera 1.17.3 program, and they were saved in PDBQT format for docking studies and prepared for analysis.

Molecular Docking Protocol

Molecular docking simulations were performed using AutoDock Vina software to evaluate the possible binding modes and binding affinities of ligands with CatK. Computational accuracy and efficiency were ensured by setting the exhaustiveness parameter to 8. Ten binding positions were generated for each ligand and ranked based on their binding affinities (kcal/mol). The center coordinates were determined as x = 11.798, y = – 1.08, and z = – 11.75, and the spacing was set to 0.5 Å for the grid box created with dimensions 56 × 62 × 63(20). The binding positions obtained from the simulations were then analyzed using Discovery Studio Visualizer

software (21). The analyses included a detailed evaluation of hydrogen bonds, hydrophobic interactions, π–π stacking, π–cation interactions, and other complementary ligand-protein interaction types.

Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) Estimates

ADMETlab 3.0, an *in silico* prediction tool developed to characterize the pharmacokinetic and toxicological properties of a candidate molecule in drug discovery and development processes, was used in our study (22). SMILES (Simplified Molecular Input Line Entry System) codes, a text-based line encoding of compounds, were obtained from the PubChem database and used as the input format to the ADMETlab 3.0 web server in the analyses.

Drug similarity assessment was estimated based on a concept previously developed by Lipinski et al. (MW≤500; logP≤5; H-bond acceptor≤10; H-bond donor≤5)(23). Pharmacokinetic and toxicological profiles were predicted using ADMETlab 3.0. Through this platform, absorption parameters (HIA, CaCO-2 permeability), distribution characteristics (blood-brain barrier crossing (BBB), plasma protein binding rate), potential metabolic interactions (interaction with CYP450 isoforms), and excretion trends (LogS and LogP values) were evaluated. In toxicity analysis, AMES mutagenicity, hERG channel inhibition risk, and hepatotoxicity indicators were predicted. Thus, the drug similarity, pharmacokinetic behavior, and safety profile of the compounds were predicted holistically.

RESULTS

Results of Molecular Docking Analysis of CatK and Ligands

In the molecular docking study against CatK protein, binding affinities were obtained in kcal/mol (Table 1). The binding score of the reference inhibitor (RI) was determined as -6.9 kcal/mol. Among the compounds studied, epigallocatechin gallate showed the highest binding affinity with -7.7 kcal/mol. Naringenin followed with -7.1 kcal/mol and genistein with -7.0 kcal/mol. Davidigenin, phloretin, and sophoraflavanone G compounds received the same score as the reference inhibitor, -6.9 kcal/mol. Gingerol had the lowest binding affinity among the compounds studied, with a score of -6.1 kcal/mol.

Molecular docking analysis revealed hydrogen bonds, hydrophobic interactions, and other complement types in ligand-enzyme interactions (Table 2). The reference inhibitor formed conventional hydrogen bonds with ASN18, GLN21, and TRP184 residues in the active site; it also exhibited halogen (fluorine), π-donor hydrogen bond, and π–π stacking interactions with TRP184.

Table 1. Docking Scores and PubChem ID Information for Compounds

Compound	Ligand Short Name	PubChem ID	Docking Score (kcal/mol)
Reference Inhibitor (RI)	L1	CID: 91885514	-6.9
Epigallocatechin Gallate	L2	CID: 65064	-7.7
Naringenin	L3	CID: 439246	-7.1
Genistein	L4	CID: 5280961	-7.0
Davidigenin	L5	CID: 442342	-6.9
Phloretin	L6	CID: 4788	-6.9
Sophoraflavanone G	L7	CID: 72936	-6.9
Gingerol	L8	CID: 442793	-6.1

Epigallocatechin gallate formed conventional hydrogen bonds with GLY66, ALA137, and GLN19, while showing a π-sulfur interaction with CYS25. Naringenin formed conventional hydrogen bonds with TRP184 and GLN21, a carbon-hydrogen bond with GLN21, and a carbon-hydrogen bond type hydrophobic interaction with GLY20. Naringenin also exhibited a π–π stacking hydrophobic interaction with TRP184. Figure 1 shows the binding interactions of epigallocatechin gallate and naringenin, which had the lowest docking scores with the reference inhibitor (Figure 1).

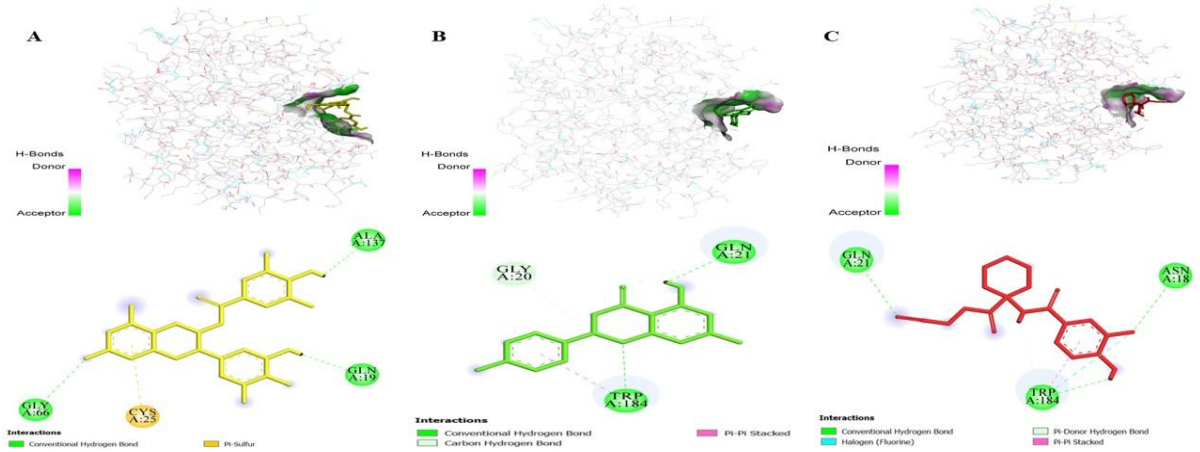

Figure 1. A: Interactions between epigallocatechin gallate and the amino acids of CatK; B: Interactions between naringenin and the amino acids of CatK; C: Interactions between the reference inhibitor and the amino acids of CatK

Genistein formed conventional hydrogen bonds with GLN21 and carbon-hydrogen bonds with TRP188; it also formed multiple π–π stacking hydrophobic interactions with TRP184. Davidigenin formed conventional hydrogen bonds with CYS25, HIS162, GLN19, and CYS22, and showed π–π stacking interactions with TRP184. Phloretin formed conventional hydrogen bonds with HIS162 and GLU59; exhibited carbon-hydrogen bonding with GLY65, π-anion

electrostatic interaction with ASP61, π-sulfur interaction with CYS25, and π-π stacking interactions with TYR67. Gingerol formed conventional hydrogen bonding and carbon-hydrogen bonding with GLN21; established π-π stacking and π-alkyl hydrophobic interactions with TRP184, while forming alkyl interactions with ALA137 and π-alkyl interactions with HIS162. Finally, sophoraflavanone G formed conventional hydrogen bonding with TRP184, exhibited π-sulfur interaction with CYS25, and π-π stacking and π-alkyl interactions with TRP184.

ADMET Results

Pharmacokinetic and toxicological properties of the compounds evaluated in the study were obtained by ADMET analyses (Table 3). It was observed that all compounds did not violate Lipinski's Five Rules, indicating that the molecules possess drug-like properties. When the absorption parameters were examined, the Human Intestinal Absorption (HIA) values were determined as 1 for the reference inhibitor and most compounds, revealing that these molecules have a high absorption potential. Caco-2 permeability values ranged from -5.572 to -5.204 log cm/s, with the lowest permeability recorded for Epigallocatechin gallate. The Blood-Brain Barrier (BBB) crossing probability, evaluated within the scope of distribution parameters, was 1 for all compounds except Epigallocatechin gallate, indicating that these molecules can cross into the central nervous system. Plasma protein binding rates ranged from 85.914% to 96.65%, with the highest binding observed by Sophoraflavanone G and the lowest by Epigallocatechin gallate. In terms of toxicity parameters, AMES mutagenicity, hERG channel inhibition, and hepatotoxicity probabilities were calculated as 0 for all studied compounds; these results indicate that the studied molecules do not carry genotoxic or cardiotoxic risks and show no signs of liver toxicity. Excretion-related water solubility (LogS) values ranged from -4.645 to -2.841; the lowest solubility was observed by Sophoraflavanone G, the highest by Epigallocatechin gallate. Lipophilicity (LogP) values ranged from 1.372 to 4.145, with Davidigenin showing the highest lipophilicity and Genistein showing the lowest. Metabolism results revealed that the compounds interacted with various CYP enzymes at different levels. CYP1A2 inhibition probabilities ranged from 0.003 to 0.548, with Naringenin and Epigallocatechin gallate exhibiting low inhibitory probabilities. In CYP2C19 inhibition, all compounds except Davidigenin (0.636) and Naringenin (0.038) showed low values.

Table 2. Details of the interaction between CatK and ligands.

Ligand	Category	Type	Residues (Distance A˚)
Reference Inhibitor (RI)	Hydrogen Bond; Halogen	Conventional Hydrogen Bond; Halogen (Fluorine)	ASN18 (3.22), GLN21 (3.11), TRP184 (2.37)
	Halogen	Halogen (Fluorine)	TRP184 (3.03)
	Hydrogen Bond	π-donor Hydrogen Bond	TRP184 (2.89)
	Hydrophobic	π-π Stacked	TRP184 (3.86)
Epigallocatechin Gallate	Hydrogen Bond	Conventional Hydrogen Bond	GLY66 (3.25), ALA137 (2.27), GLN19 (2.45)
	Other	π-sulfur	CYS25 (5.12)
Naringenin	Hydrogen Bond	Conventional Hydrogen Bond	TRP184 (3.10), GLN21 (2.09)
	Hydrogen Bond	Carbon Hydrogen Bond	GLN21 (3.42)
	Hydrophobic	Carbon Hydrogen Bond	GLY20 (3.48)
	Hydrophobic	π-π stacked	TRP184 (3.89)
Phloretin	Hydrogen Bond	Conventional Hydrogen Bond	HIS162 (3.14), HIS162 (3.19), GLU59 (2.39)
	Hydrogen Bond	Carbon Hydrogen Bond	GLY65 (3.39)
	Electrostatic	π-anion	ASP61 (4.33)
	Other	π-sulfur	CYS25 (5.71)
	Hydrophobic	π-π stacked	TYR67 (5.11)

Contd…

Table 2. Contd...

Gingerol	Hydrogen Bond	Conventional Hydrogen Bond	GLN21 (2.35)
	Hydrogen Bond	Carbon Hydrogen Bond	GLN21 (3.60)
	Hydrophobic	π-π stacked	TRP184 (4.20), TRP184 (4.80)
	Hydrophobic	Alkyl	ALA137 (3.86)
	Hydrophobic	π-alkyl	HIS162 (4.86), HIS162 (4.48), TRP184 (5.20), TRP184 (5.36), TRP184 (5.01)
Davidigenin	Hydrogen Bond	Conventional Hydrogen Bond	CYS25 (3.67), HIS162 (3.22), GLN19 (2.74), CYS22 (2.42)
	Hydrophobic	π-π stacked	TRP184 (3.91), TRP184 (4.48)
Genistein	Hydrogen Bond	Conventional Hydrogen Bond	GLN21 (1.93)
	Hydrogen Bond	Carbon Hydrogen Bond	TRP188 (3.40)
	Hydrophobic	π-π stacked	TRP184 (3.71), TRP184 (4.63), TRP184 (4.19), TRP184 (5.14)
Sophoraflavanone G	Hydrogen Bond	Conventional Hydrogen Bond	TRP184 (3.03)
	Other	π-sulfur	CYS25 (5.64)
	Hydrophobic	π-π stacked	TRP184 (4.02). TRP184 (3.98)
	Hydrophobic	π-alkyl	TRP184 (5.15)

Table 3. The results of the ADMET test with AdmetLab3.0 (I: Inhibitor, S: Substrate)

ADMET Parameter	L1	L2	L3	L4	L5	L6	L7	L8
Lipinski's rule	0	0	0	0	0	0	0	0
HIA	1	0	1	1	1	1	1	1
Caco-2 (Log cm/s)	-5.572	-6.448	-5.455	-5.352	-5.204	-5.253	-5.337	-5.275
BBB	1	0	1	1	1	1	1	1
PPB (%)	89.97	89.263	92.518	89.57	85.914	93.003	96.65	91.801
AMES	0	0	0	0	0	0	0	0
hERG	0	0	0	0	0	0	0	0
Hepatotoxicity	0	0	0	0	0	0	0	0
LogS	-3.463	-3.483	-4.021	-3.471	-2.841	-2.957	-4.645	-3.440
LogP	1.950	1.372	2.596	2.075	2.420	2.348	4.145	3.199
CYP1A2-I	0.548	0.007	0.027	0.003	0.016	0.007	0.081	0.004
CYP1A2-S	0.027	0.024	0.592	0.013	0.811	0.941	0.887	0.006
CYP2C19-I	0.222	0.015	0.038	0.004	0.004	0.009	0.636	0.003
CYP2C19-S	0.006	0.005	0.662	0.002	0.004	0.007	0.741	0.015
CYP2C9-I	0.767	0.003	0.613	0.007	0.016	0.008	0.767	0.004
CYP2C9-S	0.003	0.002	0.686	0.002	0.003	0.003	0.97	0.004
CYP2D6-I	0.957	0.004	0.17	0.985	0.007	0.012	0.965	0.003
CYP2D6-S	0.003	0.001	0.002	0.001	0.002	0.003	0.009	0.002
CYP3A4-I	0.895	0.005	0.844	0.014	0.011	0.012	0.865	0.008
CYP3A4-S	0.004	0.002	0.198	0.003	0.004	0.004	0.988	0.007

For CYP2C9, the reference inhibitor and Davidigenin showed the highest probability with a value of 0.767. In terms of CYP2D6, Gingerol (0.985) and Davidigenin (0.965) showed a significant inhibitory profile. In CYP3A4 inhibition, the reference inhibitor (0.895) and Naringenin (0.844) had high probabilities. When CYP substrate activity is examined, it is seen that Davidigenin, in particular, has a high probability of being a CYP3A4 substrate, with a value of 0.988.

DISCUSSION

The proteolytic activity of CatK is based on the characteristic catalytic pair formed by the CYS25 and HIS162 residues located in the enzyme's active site. These two critical amino acids form the functional core of the deep V-shaped active site cleft, which enables substrate binding and cleavage. Furthermore, the TYR67 and LEU208 residues, located near the active site, are key structural elements shaping the enzyme's pronounced substrate specificity towards collagen. These residues directly contribute to CatK's capacity to recognize, bind, and efficiently degrade collagen fibers(18). CatK inhibitors derive their binding strength and selectivity from three key subpockets (S1, S2, and S3) in the enzyme's active site. The S1 pocket contains the catalytic triad (CYS25, HIS162, ASN182) and prefers small or hydrophilic groups. The S2 pocket, formed by TYR67 and LEU209, plays a central role in the acquisition of selectivity; here, small or branched hydrophobic side chains are preferred. Finally, the S3 pocket is formed from the ASP61 residue (16).

Molecular docking analysis performed on CatK showed that a significant portion of the studied natural compounds exhibited higher binding affinity compared to the reference inhibitor. While the binding score of the reference inhibitor was -6.9 kcal/mol, Epigallocatechin gallate showed the highest binding affinity with -7.7 kcal/mol, significantly below this value. Naringenin (-7.1 kcal/mol) and Genistein (-7.0 kcal/mol) also stood out with binding scores exceeding the reference inhibitor. The fact that Davidigenin, Phloretin, and Sophoraflavanone G obtained the same binding score as the reference inhibitor suggests that the inhibitor potential of these compounds may be at least as high as the reference molecule. In contrast, Gingerol's value of -6.1 kcal/mol indicates a binding performance even lower than the reference inhibitor. When the binding modes were examined, it was observed that compounds achieving strong scores formed close interactions with CYS25 and HIS162, which constitute the catalytic core of CatK. In this study, the π-sulfur interaction of Epigallocatechin gallate with CYS25, the hydrogen bonding of Davidigenin with CYS25 and HIS162, and the hydrogen bonding of Phloretin with HIS162 indicate that these compounds strengthen their binding affinity by directly targeting the catalytic duo. Similarly, the frequent π-π stacking interactions observed with TRP184 appeared as a recurring motif in many strong ligands, as in the reference inhibitor; this suggests that aromatic surfaces play a critical complementary role in binding. Furthermore, the targeting of TYR67 and LEU209, key residues determining the selectivity of the S2 pocket, by certain ligands through interaction, particularly the π-π stacking interactions of Phloretin with TYR67, supports the CatK-specific binding profile of the compounds. Considering the hydrophobic–anionic character of the S3 pocket composed of ASP61, the π-anion interaction of Phloretin

with ASP61 exhibits a binding behavior consistent with the pocket architecture described in the literature. In this context, the interactions of the ligands with both the catalytic binary and the S1/S2/S3 pocket components explain the high binding affinities at the structural level. In contrast, Gingerol's more limited variety of interactions indicates that it does not establish sufficiently strong contact with either the catalytic binary or the selectivity pockets, supporting its weaker binding score. ADMET evaluation revealed that the compounds included in the study generally possess drug-like properties. The compliance of all compounds with Lipinski's rules indicates that these molecules possess suitable basic properties for pharmaceutical development. While high HIA values in absorption parameters are positive, the low Caco-2 permeability of Epigallocatechin gallate suggests that its bioavailability may be limited. The positive probability of BBB crossing in most compounds indicates that these molecules can be evaluated for CatK modulation studies related to the central nervous system. A wide range was observed in plasma protein binding rates, and high binding rates are an important parameter to consider in terms of systemic distribution. In the toxicity assessment, the low risk profile obtained for all molecules in terms of AMES, hERG, and hepatotoxicity creates a significant advantage in terms of reliability. In the metabolic interaction results, compounds exhibiting a high inhibitory probability on some CYP enzymes should be carefully evaluated in terms of potential drug-drug interactions. Findings such as the high probability of CYP3A4 substrate of davidigenin reveal that metabolic stability may vary on a molecular basis. Overall, compounds such as Epigallocatechin gallate, Naringenin, and Genistein stand out due to their strong interactions with catalytic binary and selectivity pockets, as well as their binding scores and reliable ADMET profiles. The fact that they exhibit binding motifs compatible with the structural features of CatK and critical residues determining substrate specificity, as described in the literature, supports the idea that these compounds are particularly strong inhibitor candidates. In this context, it can be said that the natural compounds evaluated in this study provide a valuable basis for structural rationality in the development of new derivatives for CatK inhibition.

The findings of this study, when compared with molecular interaction profiles of CatK inhibitors reported in the literature, confirm that interactions with the catalytic binary (CYS25–HIS162) and the S2 selectivity pocket (TYR67–LEU209) are key determinants of inhibitor efficacy. While previous studies have mostly evaluated synthetic inhibitors or a limited number of natural compounds, this research offers a unique contribution to the literature by comparatively considering structurally different natural molecules in the same context. The fact that epigallocatechin gallate, naringenin, and genistein show comparable or superior results

to the reference inhibitor in terms of both binding scores and interactions with critical amino acids associated with CatK specificity in the literature reveals that these compounds are strong candidates not only theoretically but also in terms of structural rationality. Furthermore, the detailed demonstration of S3 pocket-specific interactions and aromatic π-π stacking deepens the existing structural knowledge for CatK inhibitor design. The inclusion of ADMET analyses alongside docking results distinguishes this study from research focused solely on binding affinity, offering a more holistic assessment of the pharmaceutical applicability of candidate molecules. In these respects, the study provides an innovative framework that contributes to the rational design of CatK inhibitors, expands the existing literature, and clearly reveals the potential of natural compounds in this field.

CONCLUSION

This study comprehensively evaluated the inhibitory potential of selected natural compounds on the CatK enzyme in terms of both structural interactions and pharmacokinetic properties. Molecular docking results showed that Epigallocatechin gallate, Naringenin, and Genistein, in particular, exhibited strong binding motifs targeting selectivity pockets with their catalytic dual, resulting in higher affinity than the reference inhibitor. The compatibility of ligand-residue interactions with the known structural architecture of CatK supports the validity of these findings. ADMET analyses revealed that the compounds largely possessed drug-like profiles, with low toxicity risks and some metabolic differences requiring molecular-level optimization. Overall, the evaluated natural molecules offer strong candidates for CatK inhibition and provide an important structural basis for the rational design of novel derivatives. These results point to the therapeutic development potential of naturally derived CatK inhibitors.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Ethics Approval and Consent

This study does not require ethical approval.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors Contributions

All stages of this study were carried out by the authors.

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Data Availability Declaration

The datasets used and/or analyzed during the present study are available from the relevant author upon reasonable request.

Declaration of Generator AI and AI-Powered Technologies in the Writing Process

The authors used Grammarly and ChatGPT to improve the language and readability of the text. The authors reviewed and edited the content and are fully responsible for its accuracy. No data was generated or modified using AI tools.

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