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IN SILICO INVESTIGATION OF THE MOLECULAR-LEVEL INTERACTIONS OF PHENOLIC COMPOUNDS IN PROPOLIS WITH THE MECHANOSENSITIVE PIEZO1 CHANNEL

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Abstract: The aim of this study is to evaluate the interactions between the A, B and C chains of the Piezo1 (6B3R) protein and selected natural phenolic and flavonoid compounds using molecular docking methods, and to comparatively examine chain-specific binding profiles. Molecular docking analyses were performed using Schrödinger Maestro software. The Piezo1 structure was obtained from the Protein Data Bank (PDB ID: 6B3R), and protein and ligand preparations were carried out under physiological pH conditions. Separate Glide grids were defined for chains A, B, and C; docking operations were performed in Glide SP and XP modes. Binding affinities were evaluated based on docking scores and Glide emodel values, and 2D and 3D interaction analyses were performed for the best poses. Additionally, the physicochemical and ADME properties of the selected ligands were calculated. Docking analyses revealed that ligand binding behaviour differed significantly between Piezo1 chains. In particular, epigallocatechin gallate (EGCG) and epicatechin gallate exhibited strong and stable binding profiles across all chains, with the highest binding affinity observed in the C chain. It was determined that the strong interactions were supported by multiple hydrogen bonds and aromatic interactions. This study reveals the chain-specific ligand binding properties of Piezo1 and demonstrates that natural polyphenolic compounds can form strong interactions with this mechanosensitive ion channel. The findings contribute to understanding the molecular basis of Piezo1-mediated mechanotransduction and provide a structural reference for future experimental studies, particularly in the context of cardiovascular mechanotransduction.

Keywords: Piezo1, 6B3R, Molecular docking, ADME properties, Phenolic compounds

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1. Introduction

Mechanical forces are one of the most fundamental physical stimuli to which cells are exposed, and the process by which these forces are converted into biochemical signals is defined as mechanotransduction. Mechanotransduction plays a critical role in regulating many fundamental biological processes, such as cellular proliferation, differentiation, migration, and survival. Mechanosensitive ion channels, which are involved in this process, are the primary molecular sensors that convert mechanical stress changes in the cell membrane into cellular responses via ion currents. Mechanosensitive ion channels have been shown to regulate processes such as proliferation, cell fate, and differentiation by responding to mechanical stimuli in various cell types (Otero-Sobrino et al., 2023). Furthermore, it has been reported that these channels also play important mechanistic roles in events associated with mechanotransduction, such as cell migration and directed migration (Canales Coutiño and Mayor, 2021).

Piezo1 is a cation channel capable of responding directly to mechanical stimuli due to its large trimeric structure and unique 'membrane dome' configuration. Piezo1's propeller-like trimeric architecture is characterised by locally bending the cell membrane into a dome shape, and this structure is adapted to regulate ion flow under mechanical stress (Guo and MacKinnon, 2017). Piezo1 activation facilitates the initiation of numerous signalling pathways by causing mechanical stress to result in calcium influx into the cell; it has been demonstrated that Ca^{2+} influx from Piezo1 activates downstream signalling pathways (Liu et al., 2025). The high-resolution structures of Piezo1 resolved by cryo-electron microscopy have revealed the structural basis for this channel's mechanical sensitivity and have made significant contributions to the investigation of mechanotransduction at the molecular level (Guo and MacKinnon, 2017). The structure of Piezo1, registered in the Protein Data Bank under code 6B3R, provides a reliable structural model for evaluating the functional architecture of the channel and the membrane dome

mechanism associated with mechanosensitivity. (RCSB Protein Data Bank, 2025).

Piezo1, although expressed in numerous tissues, plays a particularly prominent role in biological systems where mechanical stimuli are intense. In this context, it has been demonstrated that Piezo1 is activated in endothelial cells in response to fluid shear stress and directs physiological adaptation processes via cellular calcium signalling. Piezo1, activated by mechanical shear stress in endothelial cells, mediates Ca^{2+} influx and downstream signalling; this mechanism is critically important for vascular development and function. This property makes Piezo1 an important molecular component in the cardiovascular system for the perception of mechanical forces and the regulation of cellular responses. However, the role of Piezo1 was not directly addressed in the context of cardiovascular diseases in this study; rather, it was evaluated within the framework of the general biophysical and molecular principles of mechanotransduction (Hyman et al., 2017; Douguet et al., 2019).

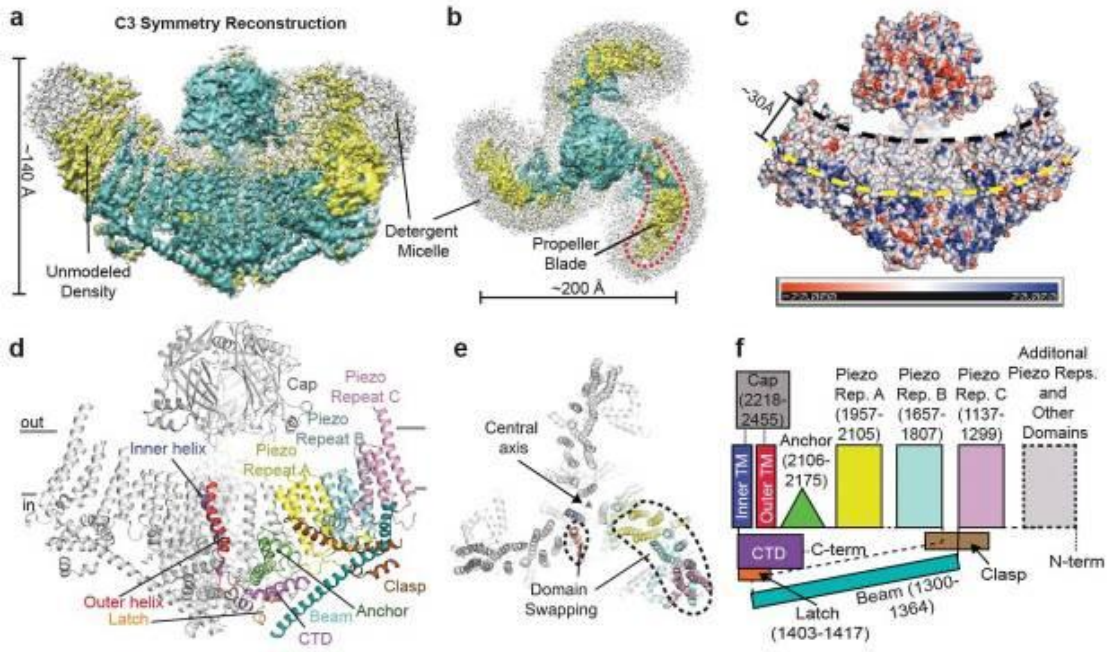

Figure 1. Architecture and domain arrangement of the Piezo1 core. Side (a) and top (b) views of the Piezo1 cryo-EM map refined with C3 symmetry imposed. Density corresponding to modeled regions is colored green. Less resolved density that could not be modeled is colored yellow. At lower thresholds (transparent gray map), scattered density likely originating from detergent micelle can be observed. c, surface electrostatics of the Piezo1 core model. Note the bent hydrophobic stripe representing the transmembrane region. Dotted lines approximate the extracellular (black) and cytosolic (yellow) membrane boundaries. d, e, side (d) and top (e) views of the Piezo1 core in cartoon representation, with each domain colored differently. In e, the cap domain is removed to highlight domain swapping between Piezo Repeat A and outer helix. f, schematic of the Piezo1 core domain arrangement. Dotted lines represent flexible regions that were not clearly resolved in the density maps (Saotome et al., 2018).

In recent years, growing interest in the possibility of modulating Piezo1 activity via small molecules has brought this protein to the fore as a potential pharmacological target. Studies on the modulation of Piezo1 have demonstrated that synthetic agonist Yoda1 and similar small molecules can directly influence Piezo1 activity; these molecules can modulate the channel's mechanical sensitivity by altering its conformation (Jiang et al., 2023). Natural phenolic and flavonoid compounds possess the capacity to form strong interactions with proteins due to their multiple hydroxyl groups, aromatic rings, and flexible structural properties. While the antioxidant and biological regulatory properties of these compounds are well established, their molecular interactions and binding behaviours with Piezo1 remain insufficiently elucidated. Comparative analysis of the ligand binding profiles of different Piezo1 chains is important for understanding the structural-functional organisation of this channel (Tang et al., 2022).

At this point, propolis stands out as a remarkable natural product. Propolis is a natural bee product with a rich and complex biochemical composition, formed by honeybees processing plant resins with beeswax and enzymes (Boulechfar et al., 2021). Although its chemical composition varies depending on geographical origin and botanical sources, it is essentially rich in phenolic acids (gallic acid, caffeic acid, ferulic acid) and flavonoids (quercetin, kaempferol, catechin, chrysin, pinocembrin) (Necip et al., 2023). These compounds are well characterised for their antioxidant, anti-inflammatory,

cytoprotective and cellular signalling regulatory properties. It has been demonstrated that propolis phenolics possess structural features that enable them to interact directly with ion channels, membrane proteins and mechanical signalling pathways, rather than being limited solely to free radical scavenging effects (Omer et al., 2024). In particular, multiple hydroxyl groups, aromatic rings and conformational flexibility enable these compounds to bind with high affinity to protein binding pockets. Recent molecular modelling and in silico studies have demonstrated that phenolic compounds derived from propolis can form specific and stable interactions with membrane proteins (Zhang et al., 2024; Lu et al., 2025).

However, the molecular-level interactions between the phenolic and flavonoid compounds in propolis and the mechanosensitive Piezo1 ion channel remain an area that has not yet been sufficiently elucidated in the literature. Considering the trimeric structure of Piezo1 and its chain-specific conformational differences, a comparative analysis of the binding profiles of ligands with the A, B, and C chains is important for a better understanding of the structural-functional organisation of the channel.

The aim of this study is to reveal the interaction potential of selected phenolic and flavonoid compounds commonly found in propolis with the A, B, and C chains of the Piezo1 (PDB: 6B3R) protein at the structural level using in silico molecular docking approaches. In this context, the comparative evaluation of chain-specific ligand binding properties and the identification of potential high-affinity binding regions are targeted. It is intended that the findings will contribute to a better understanding of the molecular basis of Piezo1-mediated mechanotransduction and provide a structural reference framework for future experimental and translational studies by revealing the potential of propolis-derived natural compounds in terms of Piezo1 modulation.

The aim of this study is to elucidate the interaction potential of the A, B, and C chains of the Piezo1 (6B3R) protein with selected phenolic and flavonoid compounds at the structural and molecular levels. In this context, the comparative evaluation of the chain-specific ligand binding properties of Piezo1 and the identification of potential high-affinity binding regions are targeted. It is anticipated that the findings will contribute to a better understanding of the molecular basis of Piezo1-mediated mechanotransduction and provide a structural reference framework for future experimental and translational studies targeting this channel.

2. Materials and Methods

2.1. Protein Preparation

Docking studies were conducted using the Schrödinger 2023-3 suite Maestro (https://www.schrodinger.com). In molecular docking studies, target protein structures were obtained from the Protein Data Bank (PDB) database as 6B3R (Structure of the mechanosensitive channel Piezo1 Method Electron Microscopy Resolution: 3.8 Å, Aggregation State: PARTICLE, Reconstruction Method: Single Particle, Starting Model: experimental) (Guo and MacKinnon, 2017). The Protein Preparation Wizard module in the Schrödinger Maestro interface was used to prepare protein structures for docking analysis. During this process, crystal water molecules and heteroatoms in the protein structures were examined; water molecules located outside the binding region were removed from the structure. Missing side chains and atoms were automatically completed, hydrogen atoms were added, and the protonation states of amino acids were adjusted according to physiological pH conditions (pH 7.4). The prepared protein structures were subjected to energy minimisation using the OPLS4 force field (Yıldırım et al., 2025a).

2.2. Ligand Preparation

The structures of ligands used in docking analyses were obtained from PubChem. Possible ionisation and tautomeric states of ligands were generated using the Epik tool within the physiological pH range (pH 7.0 ± 0.2). Ligand geometries were optimised while preserving stereochemical properties, and energy minimisation was performed under the OPLS4 force field (Çimentepe et al., 2025).

2.3. Grid Generation

For protein–ligand docking analyses, the binding site has been defined as three distinct chains, using ligands found in 6B3R structures as a reference. Grids for active binding sites were generated using the Receptor Grid Generation module, and grid sizes were optimised to allow ligand binding (Yıldırım et al., 2025b).

2.4. Molecular Docking Analysis

The prepared ligands were docked within the generated grid regions using the Glide module. Docking was performed in both Standard Precision (SP) and Extra Precision (XP) modes. Ligand conformational flexibility was allowed, while the protein structure was treated as rigid. The poses with the lowest binding free energy (GlideScore) were selected as the most probable ligand–protein binding conformations (Demirbağ et al., 2025).

2.5. Interaction Analysis and Visualisation

Docking results were analysed using the Maestro interface and Ligand Interaction Diagram tools. Hydrogen bonds, electrostatic interactions, hydrophobic interactions, π–π and π–cation interactions formed in ligand–protein complexes were evaluated in detail. The best docking poses were visualised for the purpose of comparative analysis of binding modes.

2.6. Physicochemical Properties and ADME Analysis

The ADME 3.0 Lab (https://adme.lab.imtm.cz) online platform was used in the study to evaluate the physicochemical properties and drug-likeness profiles of gallic acid and epigallocatechin gallate (EGCG) (Kaya et al., 2025a). The chemical structures of the ligands were uploaded to the system in SMILES format, and fundamental physicochemical parameters such as molecular weight, lipophilicity (LogP), total polar surface area (TPSA), hydrogen bond donor (HBD) and acceptor (HBA) counts, number of rotatable bonds, and molecular

volume were calculated (Kaya et al., 2025b).

Based on the data obtained, bioavailability radar charts were created to visually assess the suitability of the compounds in terms of absorption, distribution, metabolism, and excretion (ADME). The radar charts present a comparative overview of the compounds' calculated properties against the optimal lower and upper limits defined by ADME 3.0 Lab. This approach was used to comprehensively reveal the potential advantages and limitations of natural compounds in the drug development process. The results were evaluated to determine the compounds' positions in the drug-like chemical space and to identify potentially limiting parameters from a pharmacokinetic perspective.

3. Results

Three different Glide grids were defined corresponding to the A, B, and C chains for the 6B3R protein, and molecular docking studies were performed. When examining the docking score values, more negative values represent higher binding affinity (Necip, 2025). The glide emodel reflects the energetic stability of the binding pose; particularly negative emodel values indicate that the ligand is strongly and stably positioned in the active site (Yildirim et al., 2025b). Evaluating these two parameters together provides a more reliable interpretation than relying solely on the docking score.

Table 1. Docking score values (kcal/mol) of selected ligands with the 6B3R protein

	6B3R A chain		6B3R B chain		6B3R C chain	
	Docking score (kcal/mol)	Glide emodel	Docking score (kcal/mol)	Glide emodel	Docking score (kcal/mol)	Glide emodel
Caffeic Acid	-4.040	-32.258	-3.776	-30.738	-4.446	-41.569
Kaempferol	-4.096	-37.666	-4.691	-41.886	-4.754	-42.010
Quercetin	-5.151	-49.511	-4.172	-44.512	-4.250	-43.750
catechin	-4.681	-45.131	-4.362	-43.474	-4.334	-44.078
Epicatechin	-5.595	-47.051	-4.781	-40.773	-5.052	-42.017
Epicatechin gallate	-4.497	-54.766	-4.065	-53.416	-5.297	-63.737
Epigallocatechin gallate	-5.281	-66.379	-4.967	-60.545	-6.501	-73.776
Gallic acid	-5.048	-41.161	-5.568	-40.030	-5.216	-38.601
Naringenin	-5.101	-43.791	-5.101	-43.499	-4.375	-40.303

When examining the results for the 6B3R A chain, the lowest (best) docking scores were exhibited by the compounds epicatechin (-5.595 kcal/mol), EGCG (-5.281 kcal/mol) and quercetin (-5.151 kcal/mol). The presence of numerous hydroxyl groups and aromatic rings in these compounds indicates that they form strong hydrogen bonds, π–π interactions, and van der Waals interactions simultaneously with the active site on the A chain. In particular, the very low Glide emodel value of EGCG (-66.379) indicates that its binding to the A chain is not only strong but also highly energetically stable. Compounds with smaller or less functional groups, such as caffeic acid and kaempferol, have docking scores of -4.040 kcal/mol and -4.096 kcal/mol, respectively. We can conclude that steric fit and multiple interactions in the A chain are decisive for the binding strength.

The 6B3R B chain results are generally characterised by weaker docking scores compared to the A chain. However, gallic acid (-5.568 kcal/mol) and EGCG (-4.967 kcal/mol) are the compounds that stand out in the B chain. Despite its small molecular structure, gallic acid's better score in the B chain suggests that a narrower or more polar binding pocket may be present in this chain. However, the less negative Glide emodel values compared to the A chain suggest that the energetic stability of the ligand–protein complexes is lower. This indicates that the B chain offers a more limited or conformationally less favourable region for ligand binding.

The strongest interactions among the three chains appear to occur in the 6B3R C chain. In particular, EGCG (-6.501 kcal/mol; emodel: -73.776) and epicatechin gallate (-5.297 kcal/mol; emodel: -63.737) are the most favourable compounds in terms of both docking score and Glide emodel. Considering these values, we can say that the C chain has a binding pocket that is quite suitable for multifunctional and bulky phenolic compounds. Furthermore, the consistent and relatively strong binding profiles exhibited by flavonoids such as quercetin, catechin, and epicatechin on the C chain indicate that aromatic ring-to-ring π–π stacking and multiple hydrogen bonds play a more effective role in this chain.

When a comparative evaluation across chains is performed, it is noteworthy that the EGCG and epicatechin gallate compounds yield better results across all chains, but achieve maximum binding affinity and stability particularly in the C chain. This suggests that the C chain may play a more critical role in ligand binding in the functional activity of the 6B3R protein. The fact that compounds such as naringenin and caffeic acid show more limited variability between chains indicates that the binding of these ligands is based more on general polar interactions and cannot establish chain-specific strong interactions. The three distinct Glide grids defined

for the 6B3R protein provide significant insights into ligand binding behaviour, particularly indicating that the C chain represents the most favourable binding region for compounds containing polyphenolic and gallate groups. These findings indicate that chain-specific approaches and, in particular, flavonoid derivatives containing multiple hydroxyl groups should be prioritised as candidates in the design of 6B3R-targeted inhibitors. Molecular docking results clearly demonstrate that the ligand binding behaviours of the A, B, and C chains of the 6B3R protein differ significantly from one another. Therefore, the data are important in terms of both chain-specific binding tendencies and interaction strength dependent on ligand structure. The study demonstrates that selected phenolic and flavonoid compounds exhibit distinct binding tendencies across chains, indicating that they possess a highly suitable binding pocket (Yıldırım et al., 2025c). These results provide a strong foundation for advanced molecular dynamics simulations and experimental validation studies.

In the 2D molecular interaction between the EGCG ligand and the A chain of the 6BR3 protein, the ligand establishes multifaceted and strong interactions in its binding region. The numerous phenolic hydroxyl groups in the structure of EGCG form multiple hydrogen bonds, particularly with the negatively charged residues GLU 2545, GLU 2523, and GLU 2524, thereby providing the fundamental stabilisation of the complex. This dense hydrogen bond network contributes significantly to the stabilisation of EGCG in a suitable conformation within the binding pocket and to the increase in binding affinity. Furthermore, the observed π–cation interaction between the aromatic rings of EGCG and the positively charged side chain of the LYS 2179 residue indicates that the ligand–protein complex is further strengthened electrostatically. This interaction profile, involving hydrogen bonds, π–cation interactions, and hydrophobic contacts, demonstrates that EGCG exhibits high compatibility and potentially strong binding characteristics with the 6BR3 A chain.

The 2D interaction between the B chain of the 6BR3 protein and EGCG demonstrates that the ligand is stabilised in the binding site by both electrostatic and hydrophobic interactions. The phenolic hydroxyl groups of EGCG contribute to the fundamental binding stability of the complex by forming distinct hydrogen bonds, particularly with residues GLU 2524, GLN 1363, and ARG 1360. The π–cation interaction detected between the aromatic rings of LYS 2528 and EGCG stands out as an important factor increasing binding affinity. Hydrogen bonds, π–cation interaction, and hydrophobic contacts in the 6BR3 B chain of EGCG demonstrate a strong and stable binding through their synergistic effect.

The 2D interaction between the C-chain of the 6BR3 protein and EGCG also demonstrates that the ligand forms numerous complementary and strong interactions in the binding region. The phenolic hydroxyl groups of EGCG form multiple hydrogen bonds, particularly with the negatively charged residues ARG A 1360, TYR 1359, GLU 2523, and GLU 2524, thereby providing the main stabilisation of the complex. This hydrogen bond network significantly contributes to maintaining EGCG in a suitable conformation within the binding pocket. Furthermore, a distinct π–cation interaction was observed between the aromatic rings of EGCG and the positively charged side chain of the LYS 2179 residue, which contributes to the electrostatic strengthening of the ligand–protein complex. Polar/hydrogen bond-like interactions detected between the hydroxyl groups of EGCG and the LYS 2528 and ARG 1360 residues stand out as additional factors supporting binding affinity. The synergistic effect of hydrogen bonds, π–cation interactions, and hydrophobic contacts between EGCG and the 6BR3 C chain demonstrates stable and strong binding.

The 2D molecular interaction analysis between gallic acid and the A chain of the 6BR3 protein indicates that the ligand is stabilised primarily by hydrogen bonds and electrostatic/polar interactions in the binding region. The carboxylate group and phenolic hydroxyl groups of gallic acid form distinct hydrogen bonds, particularly with residues GLU 2524, ARG 1360, GLN 1363, and GLN 1356, ensuring strong attachment of the ligand to the active site. Additional hydrogen bonds formed between the phenolic –OH groups of the ligand and the GLN 1363 residue support the conformational fit in the binding pocket. The TYR 1359 residue, with its aromatic and partially hydrophobic character, contributes to the stability of the binding by contacting the phenyl ring of the ligand. However, due to the small and polar structure of gallic acid, hydrophobic interactions are limited, and binding occurs primarily through a hydrogen bond network and electrostatic complementarity. Overall, this interaction profile demonstrates that gallic acid exhibits specific and stable binding with the 6BR3 A chain.

The 2D molecular interaction analysis between gallic acid and the B and C chains of the 6BR3 protein indicates that the ligand is stabilised primarily by hydrogen bonds and electrostatic/polar interactions in the binding region. The carboxylate group and phenolic hydroxyl groups of gallic acid form distinct hydrogen bonds, particularly with residues THR 2538 and LYS 2528, ensuring strong binding of the ligand to the active site. Docking results reveal that the ligand exhibits high conformational fit with the target protein's active site through a multi-point binding model. The salt bridge formed, hydrogen bonds, and hydrophobic interactions occurring with aromatic amino acids, along with hydrophobic/π–π interactions occurring with aromatic amino acids, demonstrate that the ligand–protein complex possesses high stability and strong binding affinity (Amangeldinova et al., 2025). This interaction profile strongly supports the potential of ligands to be candidate inhibitors on the target protein.

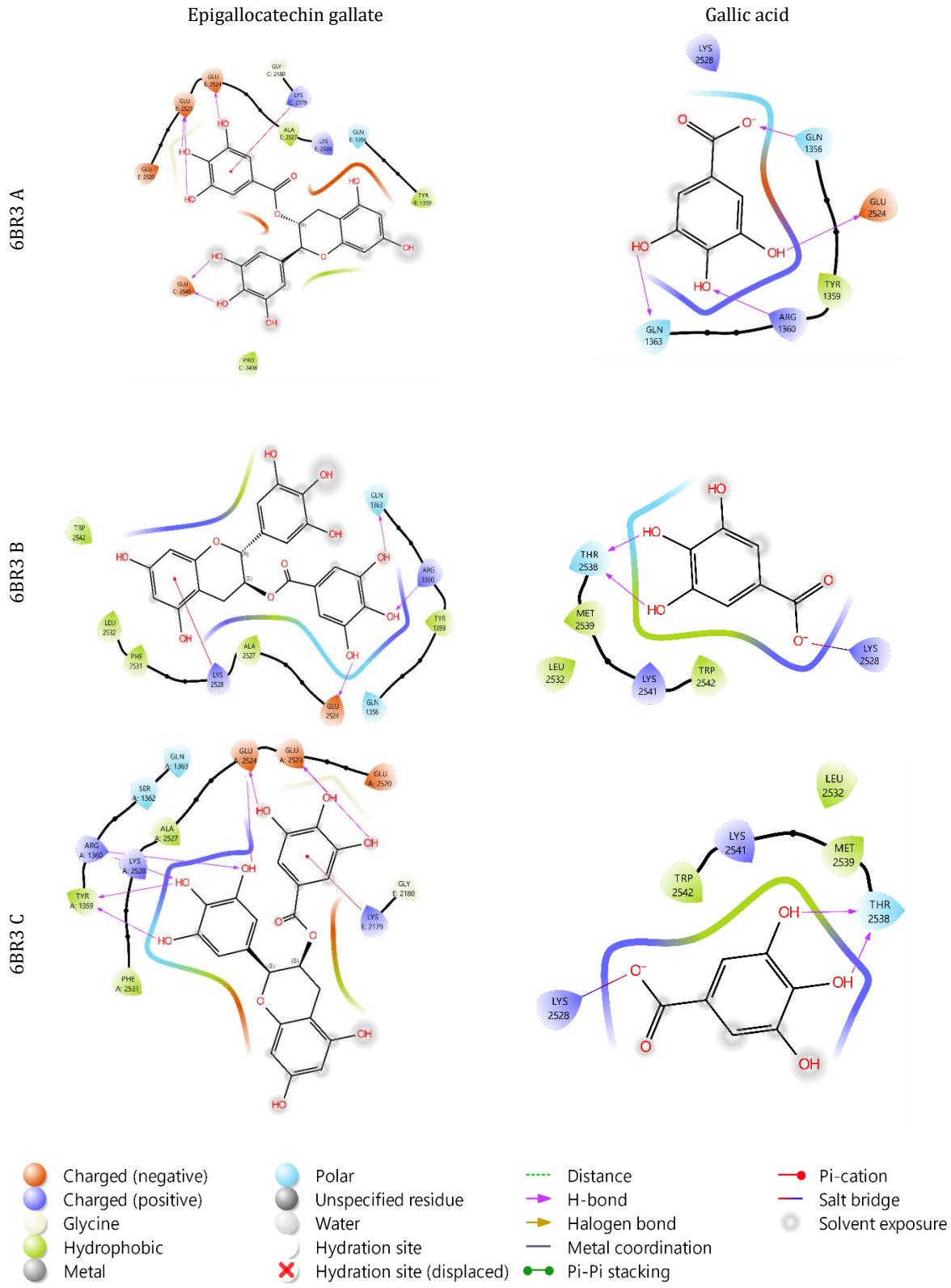

Figure 2. Two-dimensional (2D) interaction diagram of the ligand–protein complex.

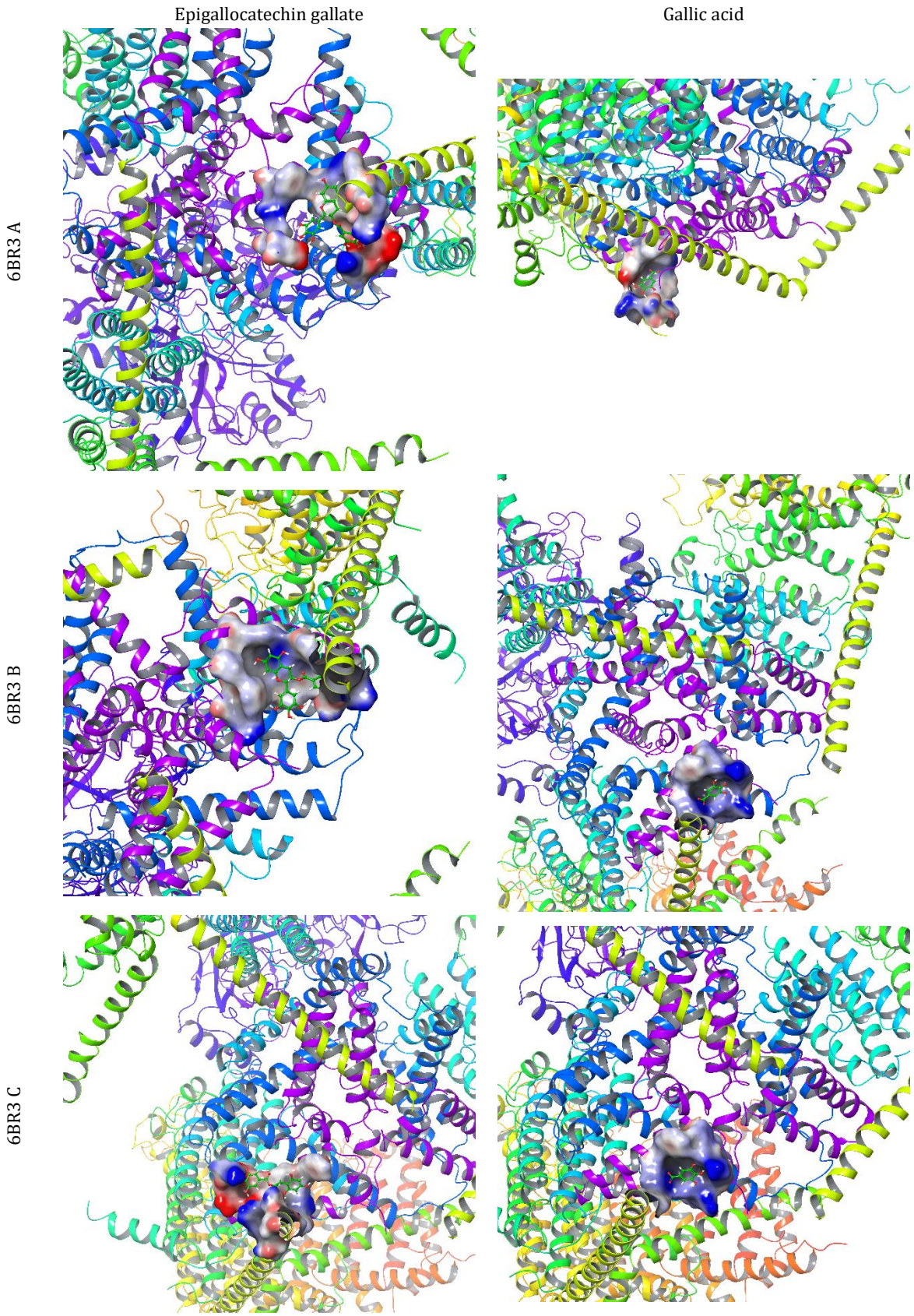

Figure 3. Two-dimensional (3D) interaction diagram of the ligand–protein complex.

Molecular docking analyses have demonstrated that all ligands bind to the active binding site of the target protein via multi-point interactions. However, it has been determined that binding profiles differ depending on the functional group diversity, molecular volume, and polar surface area of the ligands. These findings reveal that functional group richness and aromatic structures are decisive in strong and stable protein–ligand interactions, and that EGCG and gallic acid are the strongest potential inhibitor candidates.

The physicochemical and structural properties of Gallic Acid and EGCG compounds have been comparatively evaluated in terms of drug similarity and biological interaction potential (Table 2).

Table 1. Physicochemical property

	Gallic Acid	Epigallocatechin gallate	
Molecular Weight	170.02	458.08	Contain hydrogen atoms. Optimal:100~600
Volume	154.477	425.17	Van der Waals volume
Density	1.101	1.077	Density = MW / Volume
nHA	5.0	11.0	Number of hydrogen bond acceptors. Optimal:0~12
nHD	4.0	8.0	Number of hydrogen bond donors. Optimal:0~7
nRot	1.0	4.0	Number of rotatable bonds. Optimal:0~11
nRing	1.0	4.0	Number of rings. Optimal:0~6
MaxRing	6.0	10.0	Number of atoms in the biggest ring. Optimal:0~18
nHet	5.0	11.0	Number of heteroatoms. Optimal:1~15
fChar	0.0	0.0	Formal charge. Optimal:-4 ~4
nRig	7.0	24.0	Number of rigid bonds. Optimal:0~30
Flexibility	0.143	0.167	Flexibility = nRot /nRig
Stereo Centers	0.0	2.0	Stereo Centers. Optimal: £ 2
TPSA	97.99	197.37	Topological Polar Surface Area. Optimal:0~140
logS	-1.55	-3.483	The logarithm of aqueous solubility value.
logP	0.692	1.372	The logarithm of the n-octanol/water distribution coefficients at pH=7.4.
logD	0.615	1.Nis	The logarithm of the n-octanol/water distribution coefficient.
pka (Acid)	4.499	6.961	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
pka (Base)	2.582	2.754	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
Melting point	227.324	317.394	The predicted melting point of a compound is expressed in degrees Celsius (°C). Melting points below 25°C are classified as liquids, while melting points above 25°C are classified as solids.
Boiling point	326.02	453.046	The predicted melting point of a compound is expressed in degrees Celsius (°C). A normal boiling point below 25°C is categorized as a gas.

Gallic Acid has a molecular weight of 170.02 Da, and compared to EGCG (458.08 Da), it has a smaller and more compact structure. Both compounds fall within the 100–600 Da range recommended for drug-like molecules. However, EGCG's higher van der Waals volume (425.17 $Å^3$) and multi-ringed structure suggest that it may provide a larger contact surface area in protein binding sites. When examining hydrogen bonding properties, EGCG has significantly higher hydrogen bond acceptor (nHA=11) and donor (nHD=8) counts compared to Gallic Acid (nHA=5, nHD=4). This increases EGCG's potential to form multiple, strong hydrogen bonds with target proteins. However, the fact that the number of hydrogen bond donors in EGCG exceeds the recommended upper limit is considered a factor that may limit oral bioavailability. In terms of molecular flexibility, EGCG exhibits a more rotatable bond (nRot=4) and a higher flexibility value (0.167), while Gallic Acid shows a more

rigid structure (nRot=1; flexibility=0.143). This situation may increase EGCG's conformational adaptation ability to the binding pocket, while suggesting that Gallic Acid may have a more stable and predictable binding profile. The topological polar surface area (TPSA) for Gallic Acid is 97.99 $Å^2$, which falls within the recommended limits. In contrast, the TPSA value of EGCG is 197.37 $Å^2$, which is well above the optimal upper limit. This high polarity suggests that EGCG may have difficulty passing through cell membranes by passive diffusion and therefore may have limited oral absorption. When evaluating lipophilicity and solubility parameters, Gallic Acid is seen to have lower logP (0.692) and better aqueous solubility (logS = −1.55) values. EGCG, on the other hand, exhibits higher lipophilicity (logP = 1.372) and lower solubility (logS = −3.483). While these properties support EGCG's strong binding affinity to biological targets, they may pose disadvantages in terms of formulation and bioavailability. When examining acid–base properties, Gallic Acid's lower acidic pKa value (4.499) indicates a stronger acidic character compared to EGCG (6.961). This may lead to differences in ionisation degrees and interaction profiles with proteins under physiological pH conditions. In terms of thermal properties, EGCG's higher melting and boiling points suggest that the compound has a more stable and crystalline structure.

Gallic Acid exhibits a more advantageous profile in terms of drug similarity due to its suitable molecular size, balanced hydrogen bonding capacity, optimal TPSA value, and better aqueous solubility (Harwansh et al., 2024). EGCG, on the other hand, has the capacity to form strong protein–ligand interactions due to its high hydrogen bonding potential and large contact surface area. However, it exhibits limiting properties in terms of bioavailability due to its high polarity and low solubility (Saritha et al., 2024).

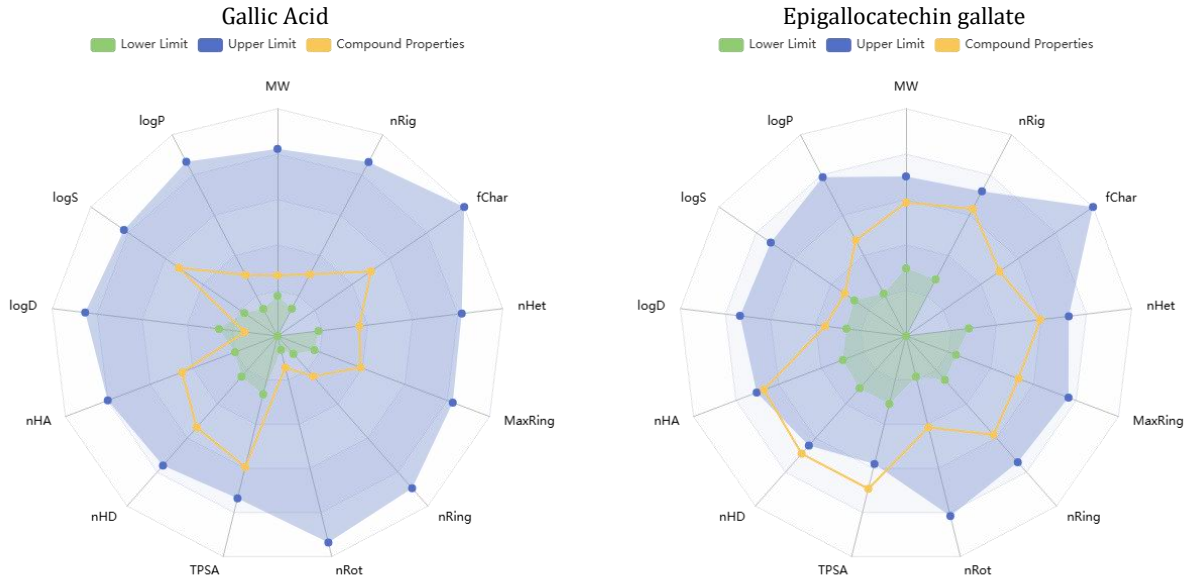

Figure 4. Visualisation of the physicochemical parameters related to ADME of gallic acid and epigallocatechin gallate using a radar chart.

Gallic acid and EGCG compounds are generally considered to fall within acceptable chemical limits in terms of drug-like properties. The graph shows that gallic acid has a profile close to the lower limits in terms of molecular weight and volume, which, thanks to its small molecular size, may facilitate its binding affinity to the active sites of target proteins. Conversely, the optimal range of hydrogen bond donor and acceptor counts indicates that gallic acid will form stable and specific interactions with proteins, particularly via hydrogen bonds (Daina et al., 2017). Another notable feature in the radar plot is that the compound has high polarity and low lipophilicity; this increases solubility in aqueous environments but is considered a factor that could limit passive membrane permeability. Furthermore, the low number of rotatable bonds indicates that gallic acid has a structurally rigid conformation, suggesting that it may form more stable complexes with the target protein through reduced entropic loss during binding.

Although EGCG falls within acceptable limits in terms of molecular weight and volume, it is seen to have a larger and more complex structure compared to gallic acid. The high number of hydrogen bond donors and acceptors supports EGCG's potential to form strong and multi-point hydrogen bonds with protein targets, due to its high content of hydroxyl groups. This property is an important advantage that could contribute to increased binding affinity in biological interactions such as ion channel modulation. Another parameter that stands out in the radar chart is EGCG's high polarity; while this increases its solubility in aqueous environments and compatibility with biological environments, it is also

considered a factor that could limit passive membrane permeability. Furthermore, the relatively high number of rotatable bonds indicates that the molecule has considerable conformational flexibility. While this offers an advantage in terms of adapting to the active sites of target proteins, it can also be considered a factor that may increase entropic losses during binding (Alam et al., 2024).

In general, radar chart data indicate that gallic acid and EGCG are natural compounds with strong biological interaction potential but with limitations from a pharmacokinetic perspective (Hadidi et al., 2024). Therefore, it is thought that it would be more rational to consider these compounds as lead molecules supported by structural optimisation or suitable carrier systems, and that this would be a rational approach for further drug development studies.

4. Discussion

In this study, the interactions between the A, B, and C chains of the Piezo1 protein (6B3R), one of the key regulators of mechanotransduction, and selected natural phenolic and flavonoid compounds were evaluated in detail using molecular docking approaches. The findings reveal that the ligand binding behaviour of Piezo1 exhibits distinct differences between chains and that compounds with a polyphenolic structure, in particular, can form strong and stable interactions with this channel. When docking scores and Glide emodel values were evaluated together, EGCG and epicatechin gallate compounds stood out in all chains, but the strongest binding affinity and energetic stability were obtained in the C chain. In particular, the markedly low docking score and emodel value exhibited by EGCG in the C chain suggests that this chain may possess a binding pocket more favourable for bulky and multifunctional ligands. This situation indicates that the different chains of Piezo1 may not be functionally equivalent in terms of ligand interactions and that chain-specific structural features play a decisive role in binding behaviour. Indeed, previous structural studies have revealed that Piezo1's trimeric propeller architecture and chains exhibit differences in terms of ligand access (Saotome et al., 2018). Furthermore, previous molecular docking studies have reported that EGCG and similar polyphenolic compounds can exhibit high binding affinity with various protein targets (Zhao et al., 2025).

Interaction analyses have revealed that ligands exhibiting strong binding form a multi-point interaction network with Piezo1. Compounds such as EGCG and gallic acid were observed to form hydrogen bonds via multiple hydroxyl groups, π–π and π–cation interactions via aromatic rings, and hydrophobic contacts in suitable regions. This interaction profile indicates that Piezo1–ligand complexes are advantageous not only in terms of binding affinity but also in terms of conformational and energetic stability. In particular, the dense hydrogen bond networks established with negatively charged glutamate residues support the high conformational fit of polyphenolic compounds into the Piezo1 binding pocket (Pan et al., 2022; Thien et al., 2024).

Interchain comparative analyses revealed that Chain A exhibited a selective binding profile in terms of volumetric fit and multiple interactions, whereas Chain B displayed a more limited and relatively weaker binding behaviour. In contrast, Chain C provided the most advantageous binding environment for gallate group-containing and multifunctional ligands. This suggests that the chains in the trimeric structure of Piezo1 may play different roles in terms of mechanical sensitivity and ligand interaction. For Piezo1, which is sensitive to conformational changes due to the nature of mechanotransduction, such chain-specific binding tendencies are a noteworthy finding in terms of functional modulation.

The fact that Piezo1 is an ion channel that responds to mechanical stimuli means that this protein plays a critical role, particularly in biological systems where mechanical stress is intense. It is known that Piezo1-mediated calcium influx in endothelial cells is activated in response to fluid shear stress and that Piezo1 regulates cell functions in response to shear stress (Guo and MacKinnon, 2017; Douguet et al., 2019; Zheng et al., 2022). In this context, it can be said that the strong ligand–Piezo1 interactions defined in our study provide structural clues for the molecular-level modulation of Piezo1-mediated mechanotransduction processes. However, it should be emphasised that this study does not aim to draw direct physiological or pathological conclusions; the data obtained provide a mechanistic and structural framework.

The evaluation of physicochemical and ADME parameters has revealed that ligands exhibiting strong binding exhibit different advantages and limitations in terms of biological applicability. Gallic acid exhibits a more advantageous profile in terms of drug similarity due to its suitable molecular weight, balanced polarity, and better aqueous solubility; whereas EGCG, despite forming strong protein–ligand interactions due to its high hydrogen bonding capacity and large contact surface area, exhibits limiting properties in terms of bioavailability due to its high topological polar surface area and low solubility. This situation demonstrates that strong binding affinity may not always correlate with optimal pharmacokinetic properties and that candidate compounds require multifaceted evaluation.

The fact that Piezo1 is an ion channel sensitive to mechanical stimuli places this protein in a functional position for detecting forces such as fluid shear stress and vascular wall tension that arise in the cardiovascular system (Douguet et al., 2019). It is known that Piezo1-mediated calcium influx in endothelial cells is associated with vascular tone regulation, nitric oxide bioavailability and modulation of inflammatory responses (Liu et al., 2025). The chain-specific ligand binding profiles described in this study suggest that different regions of

Piezo1 at the structural level may function as modules responding to mechanical signals. Although this study does not directly evaluate cardiovascular function, it can be said that the molecular findings obtained provide a structural basis for advanced experimental studies aimed at pharmacologically modulating Piezo1-mediated cardiovascular mechanotransduction.

4.1. Limitations of the Study

This study also has certain limitations. Molecular docking analyses are computational approaches that assume a rigid protein structure and reflect dynamic conformational changes to a limited extent. For a large and mechanically sensitive ion channel such as Piezo1, molecular dynamics simulations, free energy calculations, and experimental validation studies are required to better understand the dynamic effects of ligand binding. However, the chain-specific binding data presented in this study provide a strong starting point for such advanced analyses.

5. Conclusion

This study has demonstrated that the ligand binding behaviours of the A, B, and C chains of the Piezo1 (6B3R) protein differ significantly from one another. Molecular docking analyses indicate that natural compounds, particularly those with polyphenolic structures, can form strong and energetically stable interactions with Piezo1. The identified chain-specific binding profiles provide important molecular clues regarding the ligand-based modulation of Piezo1's structural regions that respond to mechanical stimuli. Considering the central role of Piezo1 in mechanotransduction processes in the cardiovascular system, these findings are thought to contribute to a better understanding of the molecular basis of Piezo1-mediated cardiovascular mechanotransduction and to provide a structural reference for future experimental and translational studies.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	B.A.	Ö.G.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

This study was conducted solely through the Scilio platform and did not involve direct intervention with humans or animals. Therefore, approval from an ethics committee was not required.

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