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Original Article

Colchicine as inhibitor of caspase-1 and GSTO-1 to regulate inflammation-induced pyroptosis in ischemic heart disease: A computational investigation

[Colchicina como inhibidor de la caspasa-1 y GSTO-1 para regular la piroptosis inducida por inflamación en la cardiopatía isquémica: Una investigación computacional]

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Abstract

Context: Inflammation-induced pyroptosis amplification contributes to the deterioration of ischemic heart diseases (IHD). Triggered by inflammasome activation, it provokes inflammatory reactions involving proteins such as glutathione transferase omega-1 (GSTO-1), NLRP-3 inflammasome, and caspase-1 (CASP-1), which might be prevented by colchicine. However, the mechanism remains to be explored.

Aims: To explore colchicine's potential to inhibit CASP-1, GSTO-1, and NLRP-3 to alleviate pyroptosis by mean computational methods.

Methods: Structures of colchicine, CASP-1, GSTO1-1, and NLRP3 were obtained from databases, followed by molecular docking and dynamics simulations to evaluate colchicine's inhibitory potential. NSC697923 and KT53 were used as reference compounds to validate the docking and molecular dynamics analyses.

Results: Molecular docking showed that colchicine binds to CASP-1 and GSTO-1 with lower binding energy than their native ligands, indicating strong inhibitory potential. Molecular dynamics simulations further confirmed that colchicine forms stable and robust interactions with CASP-1 and GSTO-1, supporting its effectiveness as an inhibitor. In contrast, colchicine's interaction with NLRP-3 was less stable, suggesting a lower inhibitory potential. Comparative analysis with NSC697923 and KT53, potent inhibitors of CASP-1 and GSTO-1, respectively, reinforced colchicine's stable conformation and sufficient hydrogen bonds despite its slightly higher binding affinity, underscoring its sustained interaction with CASP-1 and GSTO-1.

Conclusions: Colchicine showed a promising inhibitory potential agent against CASP-1 and GSTO-1, highlighting its potential for regulating inflammation in IHD. The stable binding of colchicine to CASP-1 and GSTO-1 indicated a potential inhibitory mechanism against inflammation-induced pyroptosis in IHD.

Keywords: caspase-1 inhibitor; colchicine; glutathione transferase omega-1 inhibitor; ischemic heart disease; pyroptosis.

Resumen

Contexto: La amplificación de la piroptosis inducida por la inflamación contribuye al deterioro de las cardiopatías isquémicas (CI). Desencadenada por la activación del inflamasoma, provoca reacciones inflamatorias que involucran proteínas como la glutatión transferasa omega-1 (GSTO-1), el inflamasoma NLRP-3 y la caspasa-1 (CASP-1), que podrían ser prevenidas por la colchicina. Sin embargo, el mecanismo aún está por explorar.

Objetivos: Explorar el potencial de la colchicina para inhibir CASP-1, GSTO-1 y NLRP-3 para aliviar la piroptosis a través de métodos computacionales.

Métodos: Las estructuras de la colchicina, CASP-1, GSTO1-1 y NLRP3 se obtuvieron de bases de datos, seguidas de simulaciones de dinámica y acoplamiento molecular para evaluar el potencial inhibidor de la colchicina. NSC697923 y KT53 se utilizaron como compuestos de referencia para validar los análisis de dinámica molecular y acoplamiento.

Resultados: El acoplamiento molecular mostró que la colchicina se une a CASP-1 y GSTO-1 con una energía de unión menor que sus ligandos nativos, lo que indica un fuerte potencial inhibidor. Las simulaciones de dinámica molecular confirmaron además que la colchicina forma interacciones estables y robustas con CASP-1 y GSTO-1, lo que respalda su eficacia como inhibidor. Por el contrario, la interacción de la colchicina con NLRP-3 fue menos estable, lo que sugiere un potencial inhibidor menor. El análisis comparativo con NSC697923 y KT53, potentes inhibidores de CASP-1 y GSTO-1, respectivamente, reforzó la conformación estable de la colchicina y los enlaces de hidrógeno suficientes a pesar de su afinidad de unión ligeramente superior, lo que subraya su interacción sostenida con CASP-1 y GSTO-1.

Conclusiones: La colchicina mostró un potencial inhibidor prometedor contra CASP-1 y GSTO-1, lo que destaca su potencial para regular la inflamación en la cardiopatía isquémica. La unión estable de la colchicina a CASP-1 y GSTO-1 indicó un posible mecanismo inhibidor contra la piroptosis inducida por inflamación en la cardiopatía isquémica.

Palabras Clave: cardiopatía isquémica; colchicina; inhibidor de la caspasa-1; inhibidor de la glutatión transferasa omega-1; piroptosis.

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Abbreviations: 2D: 2 Dimension; 3D: 3 Dimension; CASP-1: Caspase-1; GSTO-1: Glutathione Transferase Omega-1; IHD: Ischemic Heart Diseases; IL-18: Interleukin-18; IL-1β: Interleukin-1β; NL: Native Ligand; NLRP-3: Nucleotide-Binding Domain and Leucine-Rich Repeat Pyrin 3; PDB: Protein Data Bank; RMSD: Root-Mean-Square Deviation; RMSF: Root-Mean-Square Fluctuations; SMILES: Simplified Molecular Input Line Entry System.

INTRODUCTION

In recent years, molecular mechanisms underlying inflammatory responses in Ischemic Heart Disease (IHD) have become a focal point for novel therapeutic intervention researches (Vilela and Fontes-Carvalho, 2021). Especially, the involvement of inflammation following ischemic events has gained considerable interest in IHD treatment. Inflammasomes are cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses. Inflammasomes are not only activated by various cardiovascular risk factors and regulate the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18 (Liao et al., 2022), but are also activated after myocardial infarction (Li et al., 2014; Liao et al., 2022). The activated Nucleotide-Binding Domain and Leucine-Rich Repeat Pyrin 3 (NRLP-3) inflammasome contributes to unfavourable cardiac remodelling in the aftermath of acute myocardial infarction (Mezzaroma et al., 2021). The crucial role of NLRP-3 gives rise to the development of its inhibitor to improve the surveillance of patients with inflammation-induced cardiovascular diseases (Mezzaroma et al., 2021).

Glutathione transferase omega-1 (GSTO-1) has been identified as a novel regulator of the NLRP-3 inflammasome (Hughes et al., 2017). Its mechanism of action involves deglutathionylating NEK7, an element of the NLRP-3 inflammasome that has been newly recognized (Hughes et al., 2019). Moreover, the production of IL-1β and IL-18 is also induced by the GSTO-1 enzyme (Menon et al., 2014), further amplifying the inflammatory mechanisms in IHD. Thus, inhibiting GSTO1-1 represents a potential avenue to curtail the activation of these pro-inflammatory cytokines (Hughes et al., 2017; Menon et al., 2014; Xie et al., 2020).

Upon NLRP-3 activation, several pro-inflammatory cytokines will be produced. The downstream mechanism of NLRP-3-induced inflammation is highly associated with caspase-1 (CASP-1). CASP-1, a key endoprotease, is pivotal in the post-translational processing of pro-inflammatory cytokines, specifically IL-1β and IL-18 (Molla et al., 2020). Without CASP-1-dependent cleavage, the IL-1β and IL-18 are still inactivated and unfunctional stage (Syed et al., 2005). In addition, CASP-1 serves as a modulator in the inflammatory response to tissue injury, playing a dual role in amplifying the inflammatory cascade and promoting pyroptosis—a unique form of inflammatory programmed cell death (Franchi et al., 2009). The formation of inflammatory cell death is also dependent on the activity of CASP-1 (Fernandes-Alnemri et al., 2007). Given its central role in the maturation of these potent inflammatory mediators, inhibiting CASP-1 has emerged as a promising therapeutic strategy for developing anti-inflammatory agents to mitigate the inflammatory response in ischemic heart disease (Frantz et al., 2003; Liang et al., 2021).

Colchicine is well-known for its potent anti-inflammatory properties and has been used to treat various medical conditions (Schattner, 2022). Its diverse mechanisms of action suggest significant therapeutic potential in cardiac diseases (Zhang et al., 2022). Although colchicine has been shown to target NLRP-3 and CASP-1 in other disease settings (Amaral et al., 2023; Manukyan et al., 2013), its specific mechanism for inhibiting pyroptosis remains unclear in IHD. Pyroptosis is characterized by inflammasome activation, particularly under ischemic conditions, leading to the activation of caspase-1 and the cleavage of gasdermin D (Man and Kanneganti, 2015). This cleavage results in pore formation in the cell membrane, causing cell swelling, rupture, and release of pro-inflammatory contents (Man and Kanneganti, 2015).

This study aims to address the gap in understanding colchicine's role in IHD by investigating its potential to inhibit key molecular players in inflammasome-induced pyroptosis, specifically NLRP-3, GSTO-1, and CASP-1. Through a computational approach, the research seeks to elucidate the molecular interactions between colchicine and these pyroptosis-related proteins. By focusing on the pyroptosis pathway, this study aims to provide novel insights into colchicine's potential as a targeted and effective inhibitor of IHD-associated pyroptosis, despite some prior findings on its effects on NLRP-3 and CASP-1.

MATERIAL AND METHODS

Structure retrieval

The structure of colchicine was obtained from the PubChem database, referring to its isomeric SMILES code with compound identity (CID) 6167. On the other hand, the structure of CASP-1, GSTO-1, and NLRP-3 were retrieved from Protein Data Bank (PDB) with identity code 1RWX (Fahr et al., 2006), 6PNM (Xie et al., 2020), and 7ALV (Dekker et al., 2021), respectively. Native ligands (NL) from each crystallographic

Table 1. The grid box position setting is for specific docking protocol in this study.

Target Protein	X	Y	Z
Grid box dimension			
CASP-1	16.3321747686	16.6579012557	14.9358799491
GSTO-1	8.97244908938	11.9653983443	10.5992585186
NLRP-3	13.2102033301	14.5837998894	11.4017077595
Center point position			
CASP-1	36.3091479358	65.3314412068	7.03104689707
GSTO-1	16.8276109674	-4.53859432904	16.4124173114
NLRP-3	17.195385424	35.7051975751	125.402494697

structure were used as control molecules. The structure of NL was extracted from the PDB structure directly using PyMOL software.

Energy minimization and structural preparation

The geometry optimization step was performed to obtain optimum molecular docking results, as conducted in earlier work (Susilo et al., 2024). Colchicine and all noncovalent ligands were geometrically optimized using the tight-binding technique in XTB (Bannwarth et al., 2021). The structures were translated into .xyz format by OpenBabel software (O'Boyle et al., 2011) before beginning the geometry optimization process. The geometry optimization settings were set to the highest level possible to provide a comprehensive and accurate optimization process. The geometry-optimized structure was chosen as the ligand for future investigation.

Molecular docking, interaction analysis, and structural visualization

Molecular docking was performed using Vina 1.2.3 (Eberhardt et al., 2021; Trott and Olson, 2010) in the PyRx (Dallakyan and Olson, 2015) interface to predict interactions between Colchicine and each specified macromolecule. Colchicine and NL were set as flexible ligands, while proteins were treated as rigid macromolecules (Hermanto et al., 2019). The ligands were guided through grid box position to target the binding site of each target protein, as mentioned in Table 1. The docking simulations were run with an exhaustive value of 16 to ensure a thorough examination of potential binding configurations.

Molecular dynamics

YASARA (Krieger and Vriend, 2015) was selected to perform molecular dynamics analysis in an environment described in the previous studies (Hermanto et al., 2023). Every complex from molecular docking analysis was continued into molecular dynamics analysis with the following physiological conditions: pH 7.4; 0.997 g/mL water density, 1 bar pressure, 0.9% NaCl ions; and 310K temperature. The simulation was performed in a Cubic grid simulation box under an AMBER14 force field (Maier et al., 2015) for 150 ns. Free-binding energy analysis was also performed by employing molecular mechanics Poisson–Boltzmann surface (PBS) area equations in the YASARA binding energy macros (Konecny et al., 2012). This method approximates the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) calculation, excluding the entropy term derived from normal mode analysis, as is common in many MM/PBSA studies (Genheden and Ryde, 2015).

In silico data analysis

After docking, the ligand conformations with the best ranking and lowest binding affinity were selected for complex formation. The docking method was validated by redocking the native ligand to its receptor. Discovery Studio 2019 was employed to thoroughly analyze and visualize the interactions between amino acids and ligand atoms, comparing colchicine with native ligands in both two-dimensional (2D) and three-dimensional (3D) views. The 3D visualization of the entire complex was achieved using PyMOL.

Molecular dynamics analysis was conducted to assess structural flexibility and integrity by evaluating the root-mean-square deviation (RMSD) of atomic positions and root-mean-square fluctuations (RMSF) of residue positions (Syahraini et al., 2023). For free binding energy analysis, the final value was calculated using the following equation [1].

$$BindEnergy = E_{potRecept} + E_{solvRecept} + E_{potLigand} + E_{solvLigand} - E_{potComplex} - E_{solvComplex} \quad [1]$$

The RMSD of backbone atoms and RMSF were used to assess the stability of the entire complex, while the RMSD of ligand conformation and free

binding energy values were used to evaluate the stability of ligand structures and protein-ligand interactions, respectively (Hermanto et al., 2024).

Validation with reference compounds

To validate the simulation results, redocking was conducted to compare colchicine with reference compounds for each high-performing protein target, namely CASP-1 and GSTO-1. Based on previous studies, NSC697923 (Fig. 1A) was selected as the reference compound for CASP-1 (Cao et al., 2022), while KT53 (Fig. 1B) was chosen for GSTO-1 according to a 2011 study (Tsuboi et al., 2011). Both reference compounds underwent geometry optimization prior the docking process. Docking was carried out using Vina 1.2.3, targeting the colchicine binding sites on the crystallographic structures of CASP-1 and GSTO-1, as outlined in the earlier methodology. Molecular dynamics analysis was also performed alongside molecular docking, using similar simulation parameters and procedures as previously described. The resulting simulations were then compared to assess the binding stability of colchicine to CASP-1 and GSTO-1, respectively.

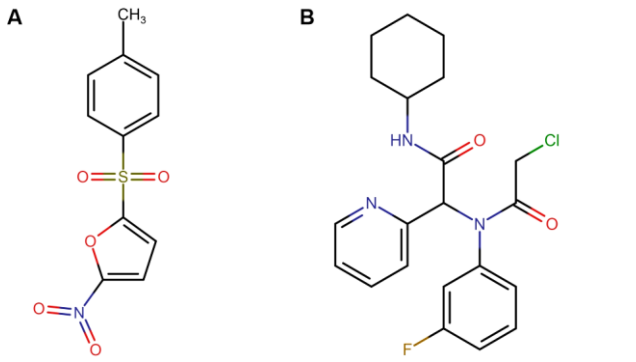

Figure 1. Two-dimensional visualization of the reference compounds used in this study. NSC697923 was a potent inhibitor of CASP-1 **(A)**, while KT53 is a selective inhibitor of GSTO-1 **(B)**.

RESULTS AND DISCUSSION

Colchicine is more likely to bind with CASP-1 and GSTO-1

Molecular docking revealed that colchicine was bound to CASP-1 and GSTO-1 at lower energy binding than their native ligand. Although colchicine interacted with NLRP-3 with low binding affinity, the energy remained higher than the native ligand of NLRP-3 (Table 2). Colchicine may perform better inhibitory activity with a lower energy binding than the native ligands of CASP-1 and GSTO-1. Lower binding affinity means colchicine formed strong interaction with the CASP-1 and GSTO-1 through the formation of solid interaction chemistry.

Ligand atoms-protein residues interaction was evaluated further to clarify colchicine's inhibitory potential against the protein targets. Interestingly, colchicine formed fewer interactions with CASP-1 compared to the native ligand, which is consistent with the observed binding affinity values. This event may occur due to the size of colchicine's chemical structure, which is smaller than that of the native ligand. Colchicine formed some hydrogen bonds accompanied by pi-pi bonds and several van der Waals interactions. However, more hydrogen bonds and pi-pi interaction were displayed by native ligand-CASP-1. In addition, colchicine has a binding site similar to native ligands. TRP340, ARG341, HIS342, VAL348, ARG352, PHE377, ASP381, ARG383, ALA384, and GLN385 were the residues involved in the formation of protein-ligand complexes either in colchicine and native ligands with CASP-1 (Fig. 2).

Colchicine-GSTO-1 showed a better chemistry interaction than the previous protein target. In general, the interactions between colchicine and GSTO-1 involve several key amino acid residues, including LEU56, TRP180, VAL127, PHE225, ILE131, PHE34, ARG183, and TYR229, similar to the interactions observed with the native ligand. Two hydrogen bonds at PRO124 and TYR229 and several hydrophobic bonds at CYS32, PRO33, and LEU56 were formed. Moreover, several van der Waals interactions also took place to strengthen the interaction between colchicine and GSTO-1. This interaction may be associated with the lower binding affinity colchicine-GSTO-1 than native ligand-GSTO-1. Again, a more complex chemical structure influences the residue interaction during protein-ligand complex formation (Fig. 3).

The molecular docking results reveal that both colchicine and the native ligand interact with the NLRP-3 protein at similar binding sites, forming various interactions with amino acid residues, including hydrogen bonds, hydrophobic interactions, and van der Waals forces. However, there are notable differences in the chemical interactions between the two complexes. While colchicine forms fewer pi-interactions than the native ligand, it creates a greater number of hydrogen bonds. Additionally, the conformational differences between colchicine and the native ligand within the binding site may influence their interactions with specific amino acid residues. Colchicine adopts a more extended conformation, allowing for additional contacts with the protein, whereas the native ligand has a more compact structure, potentially limiting its interactions (Fig. 4). These differences in chemical interactions and structural conformations may contribute to the distinct binding affinities and biological activities of colchicine and the native ligand with respect to NLRP-3.

Table 2. The binding affinity of colchicine compared to the native ligand of each target protein according to the calculation from molecular docking analysis.

Protein	Ligand	Binding affinity (kcal/mol)
CASP-1	Colchicine	-8.811
	Native Ligand	-6.425
GSTO-1	Colchicine	-7.107
	Native Ligand	-6.362
NLRP-3	Colchicine	-8.092
	Native Ligand	-9.202

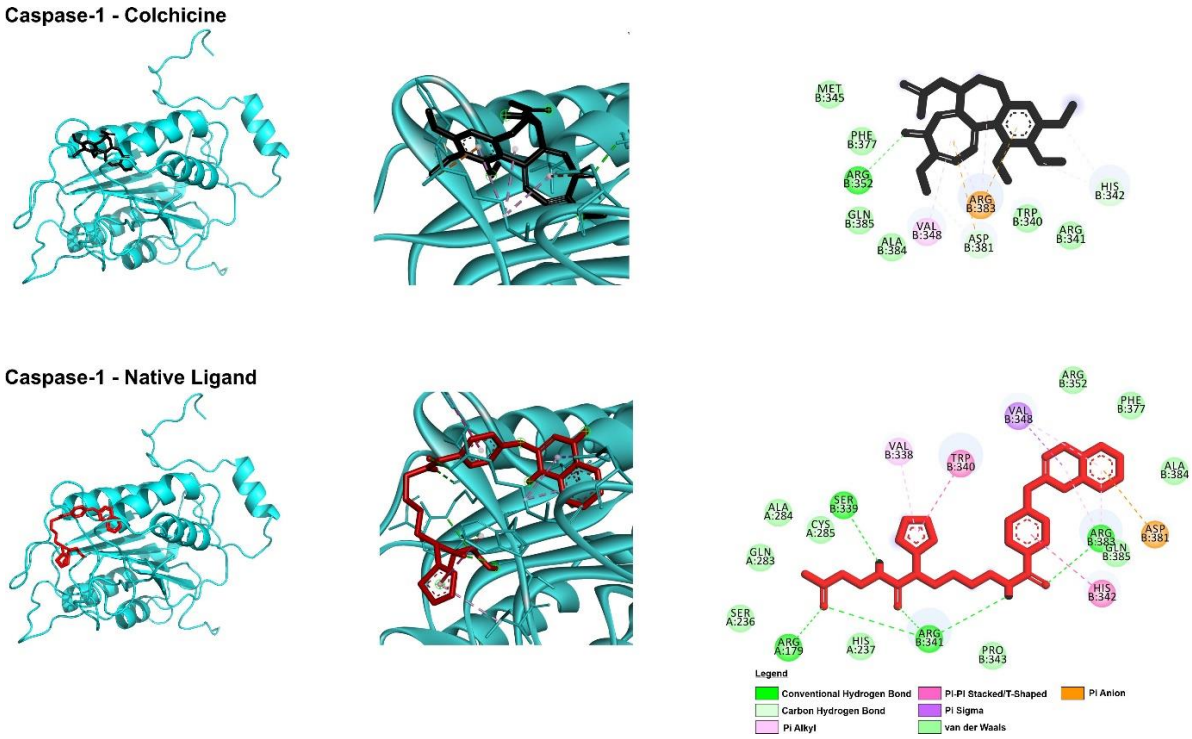

Figure 2. Three-dimensional visualization and two-dimensional schematic atom-residue interaction of caspase-1 complexed with colchicine or native ligand.

Protein was displayed in cyan ribbon, while the ligands were drawn in either black (colchicine) or red (native ligand) sticks.

It has been known that the compound's chemical structure is highly associated with the binding affinity from the molecular docking process. Vina employs the weighing method for each interaction chemistry and stereochemistry of the ligand to calculate the binding affinity of the protein-ligand complex (Trott and Olson, 2010). Ligands with more rotatable bonds with more steric interaction, particularly repulsive interaction, will produce less negative binding affinity (Du et al., 2016; Trott and Olson, 2010). Various robust interactions, such as hydrogen and hydrophobic bonds, contribute significantly during docking. Interestingly, the binding affinity positively correlates with ligands possessing a more repulsive and flexible structural conformation. This phenomenon is evident in the higher energy observed in the CASP-1-native ligand compared to the CASP-1-colchicine interaction. Here, the native ligand's structure influences the calculated energy required for binding with CASP-1. Conversely, structures resembling the native ligand, characterized by greater hydrophobic and hydrogen bonds, exhibit lower binding affinity, as exemplified in NLRP-3 complexes.

Molecular docking analysis has pinpointed CASP-1 and GSTO-1 as potentially promising targets for the pyroptosis-inhibiting activity of colchicine. Notably, colchicine demonstrates a lower binding energy with CASP-1 and GSTO-1 than the native ligands. This event suggests a higher likelihood of interaction between colchicine and both protein targets.

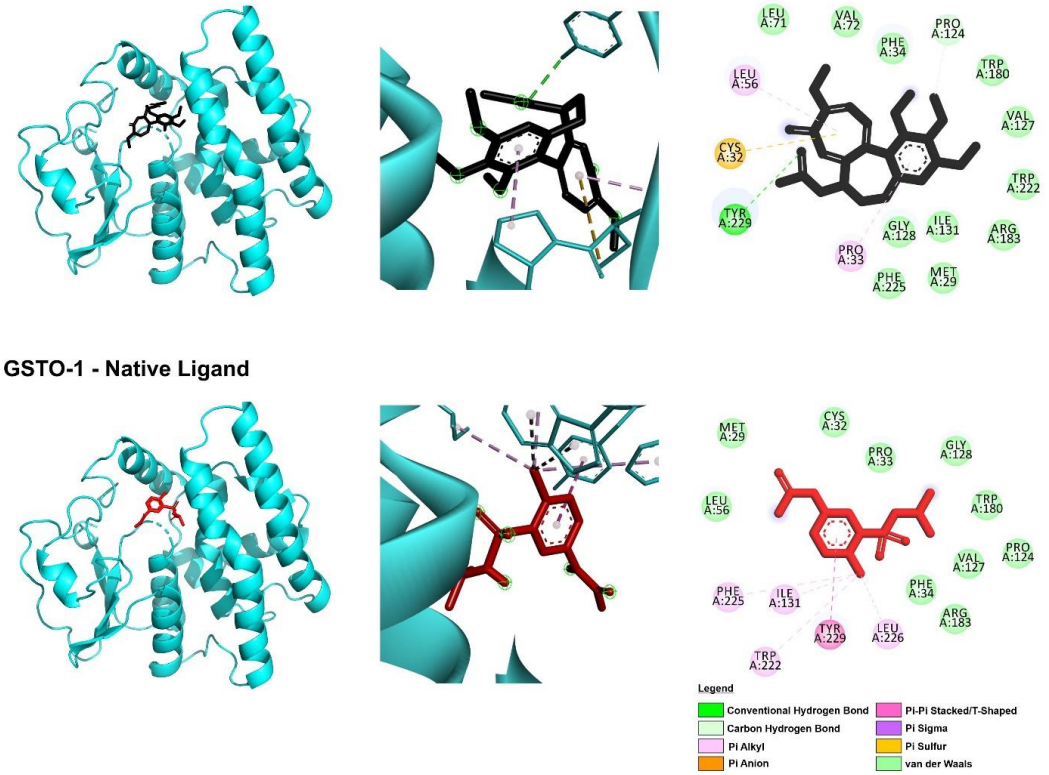

Figure 3. Three-dimensional visualization and two-dimensional schematic atom-residue interaction of GSTO-1 complexed with colchicine or native ligand.

Protein was displayed in cyan ribbon, while the ligands were drawn in either black (colchicine) or red (native ligand) sticks.

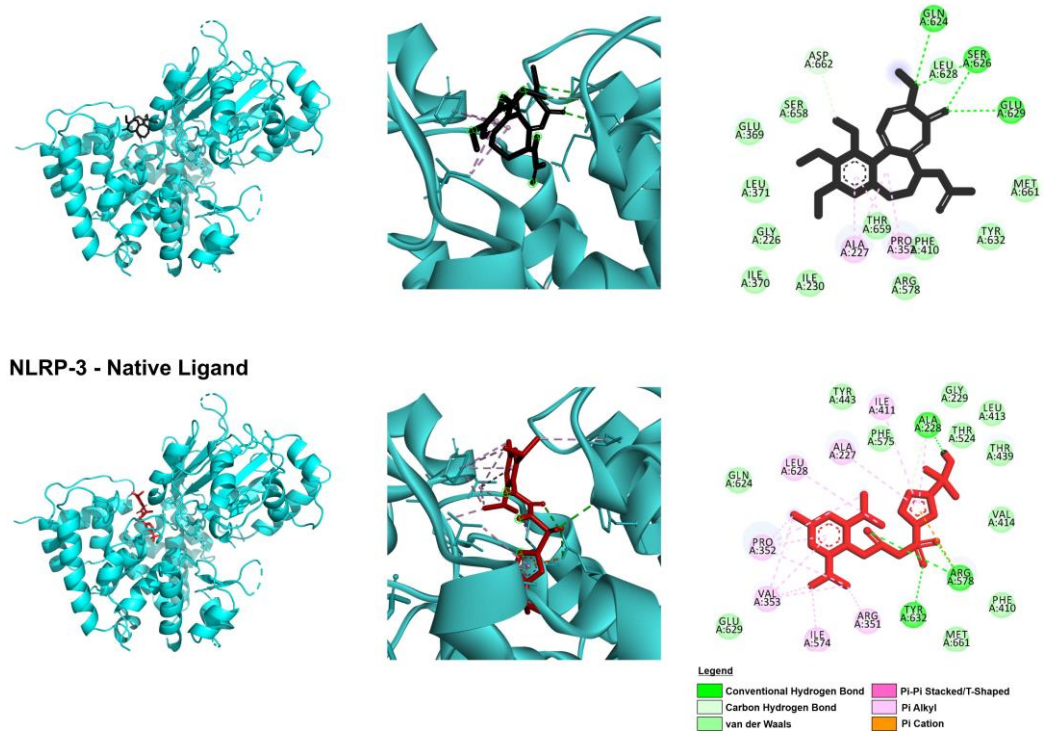

Figure 4. Three-dimensional visualization and two-dimensional schematic atom-residue interaction of NLRP-3 complexed with colchicine or native ligand.

Protein was displayed in cyan ribbon, while the ligands were drawn in either black (colchicine) or red (native ligand) sticks.

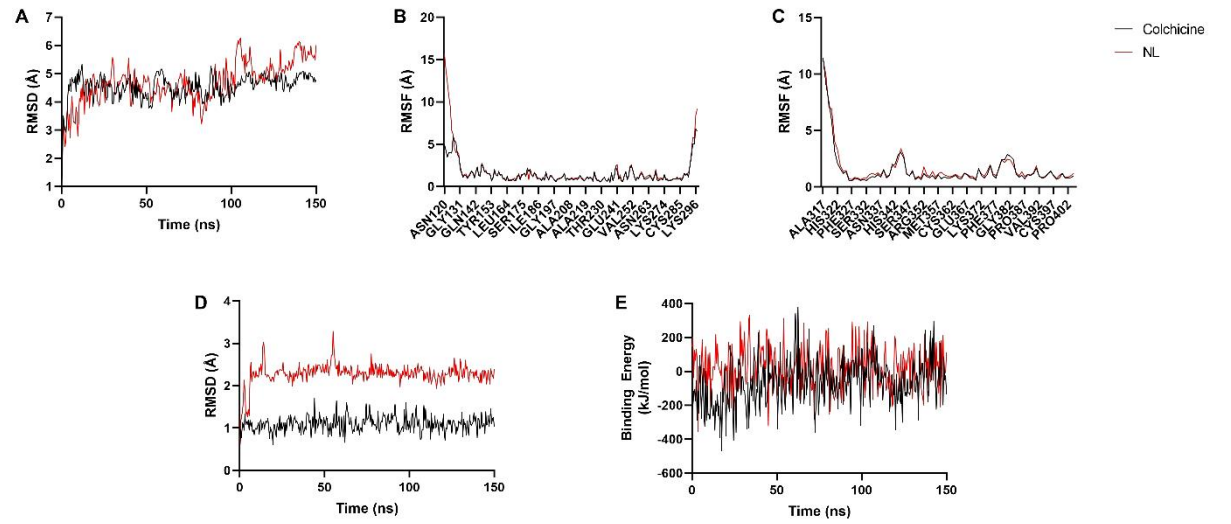

Figure 5. Structural dynamics of colchicine-CASP-1 and native ligand-CASP-1 complexes.

Several parameters were used to assess the stability of the protein, ligand, and complex according to the RMSD of backbone atoms **(A)**, RMSF of chain A **(B)**, RMSF of chain B **(C)**, RMSD of ligand conformation **(D)**, and binding-free energy calculations **(E)**. NL: Native Ligand.

Furthermore, the complex formation of CASP-1 and GSTO-1 with their native ligands shares several residues with the complex formed with colchicine. With its lower binding energy, colchicine emerges as a promising inhibitor, surpassing the potential of the native ligands in effectively modulating the targeted protein interactions.

Colchicine inhibits CASP-1 and GSTO-1 in a stable manner

Comprehensive molecular dynamics simulations (MDS) were systematically conducted for each complex to elucidate the inhibitory efficacy of colchicine against the designated protein targets. Analysis of the MDS data reveals a superior interaction profile of colchicine with CASP-1 compared to the native ligand of CASP-1. Notably, the interaction between colchicine and CASP-1 did not impact structural stability and integrity, as evidenced by the consistently low RMSD values of backbone atoms (Fig. 5A). This structural stability is further substantiated by the RMSF data, which delineates the absence of structural alterations in colchicine binding with CASP-1, as well as with the native ligand, at a per-residue level across both chains, A and B (Fig. 5B-C). Besides, the structural robustness of colchicine is underscored by its outperformance in stability compared to the native ligand, as evidenced by the lower RMSD of ligand conformation values for colchicine relative to the native ligand (Fig. 5D). However, it is noteworthy that a parallel pattern in the binding-free energy of colchicine-CASP-1 and native ligand-CASP-1 complexes is observed (Fig. 5E). These nuanced findings collectively contribute to a comprehensive understanding of colchicine's potential as a highly stable and effective inhibitor against CASP-1, laying the groundwork for further investigations into its therapeutic applications.

Consistent with the findings in the investigation of colchicine's interaction with CASP-1, parallel outcomes were observed in the simulation of colchicine and its native ligand complexed with GSTO-1. The complexes' structural integrity and robustness were scrutinized, revealing minimal alterations as elucidated by the RMSD values of backbone atoms (Fig. 6A) and the RMSF data for each residue of GSTO-1 (Fig. 6B). Colchicine demonstrated superior conformational stability compared to its native ligand counterpart, as evident in the conformational RMSD analysis (Fig. 6C). This heightened stability in the conformation of colchicine during its interaction with GSTO-1 is proposed to exert a significant influence on the binding-free energy of the colchicine-GSTO-1 complex, as indicated by the discernibly lower energy value in comparison to the complex formed by the native ligand and GSTO-1 (Fig. 6D). These interconnected findings across both CASP-1 and GSTO-1 complexes collectively underscore the robust and stable nature of colchicine's interactions, providing compelling evidence for its potential as a highly effective inhibitor against key protein targets implicated in pyroptosis and inflammatory processes.

Examining NLRP-3 complexes through structural dynamics simulations provided insights that contrast the interactions observed in the CASP-1 and GSTO-1 complexes. In this context, colchicine exhibited a less robust interaction and compromised integrity compared to the native ligand of NLRP-3. The binding of colchicine to NLRP-3 induced an elevation in the

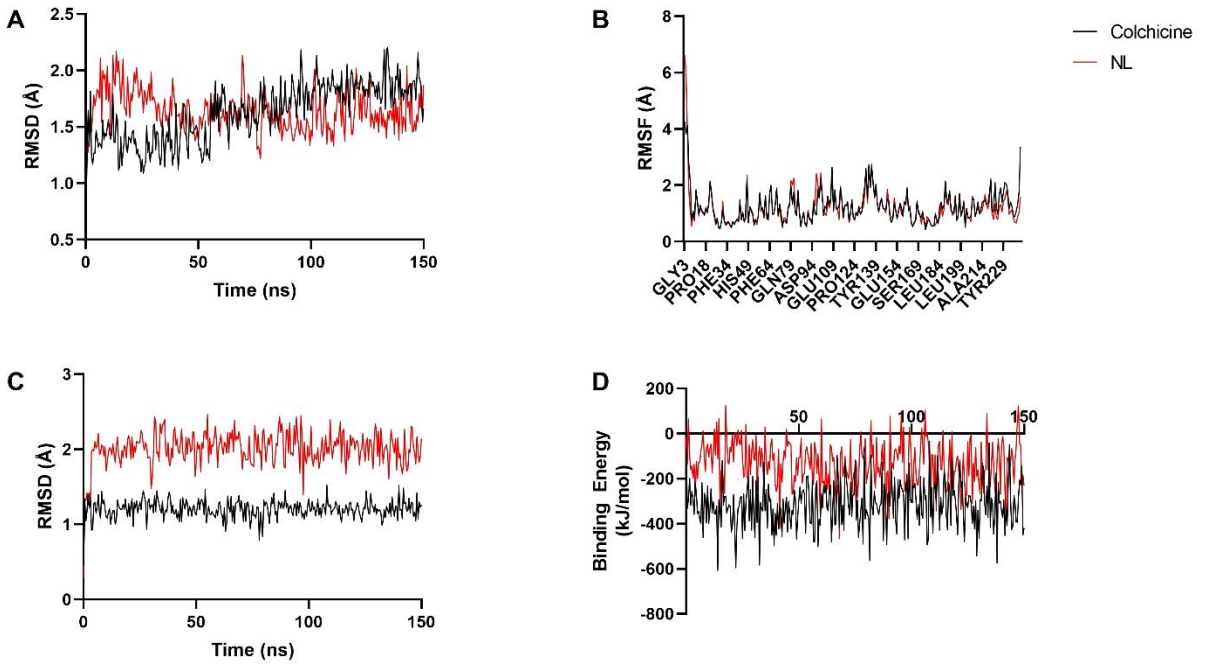

Figure 6. The stability of the GSTO-1 complex with colchicine and its native ligand was evaluated through various parameters.

The parameters include the RMSD of backbone atoms **(A)**, RMSF of individual residues **(B)**, RMSD of ligand conformation **(C)**, and calculations of binding-free energy **(D)**. NL: Native Ligand.

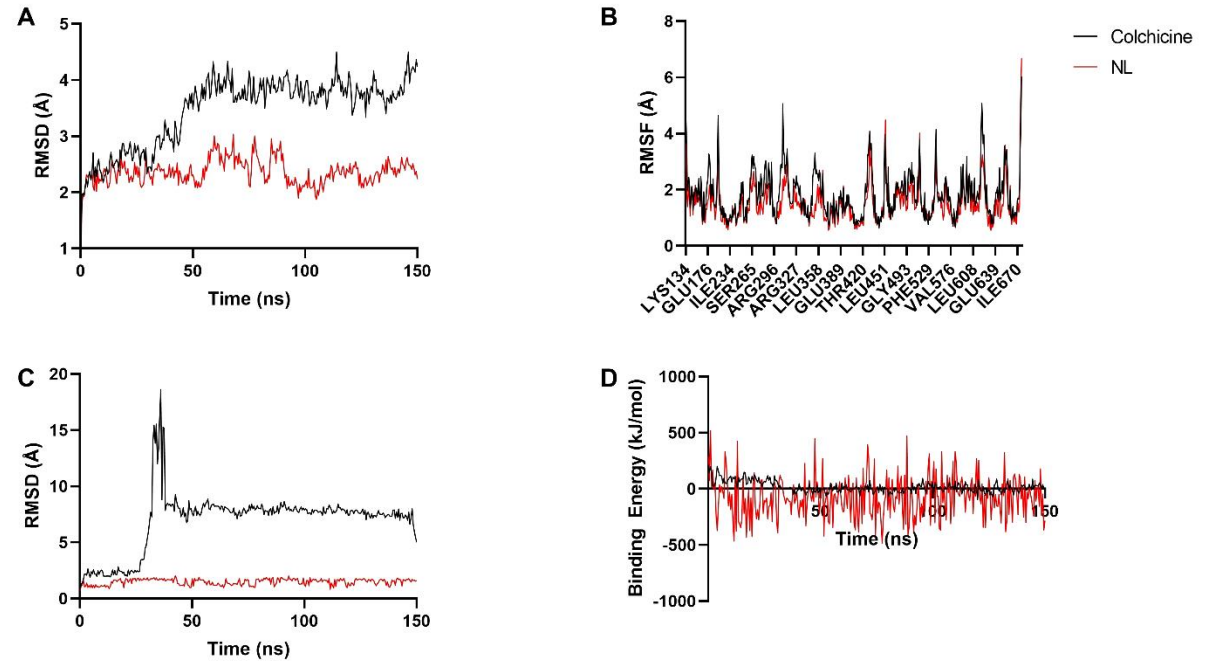

Figure 7. The structural dynamics of the NLRP-3 complex were analyzed using colchicine and the native ligand as ligands.

Various parameters, including the RMSD of backbone atoms **(A)**, RMSF of individual residues **(B)**, RMSD of ligand conformation **(C)**, and binding-free energy calculations **(D)**, were employed to evaluate the stability of the protein, ligand, and complex. NL: Native Ligand.

RMSD values of backbone atoms, indicating a significant impact on the structural stability of the complex (Fig. 7A). The RMSF data further elucidated this influence on structural integrity, revealing a more pronounced fluctuation in residues within the colchicine-NLRP-3 complex than its native ligand counterpart (Fig. 7B). Moreover, colchicine demonstrated inferior structural stability during its binding with NLRP-3, as evidenced by the conformational RMSD analysis (Fig. 7C). Consequently, this compromised structural sta-

bility contributed to elevated binding-free energy of the colchicine-NLRP-3 complex relative to the complex formed by the native ligand and NLRP-3 (Fig. 7D). These contrasting observations across CASP-1, GSTO-1, and NLRP-3 complexes underscore the nuanced nature of colchicine's interactions with distinct protein targets, necessitating a comprehensive understanding of its behavior in diverse biological contexts.

The exhaustive MDS has provided a nuanced understanding of the intricate interactions between colchicine and pivotal protein targets central to pyroptosis, namely CASP-1, GSTO-1, and NLRP-3. Notably, within the CASP-1 and GSTO-1 complexes, colchicine exhibited a superior binding affinity and conformational stability compared to their respective native ligands. These observations, supported by minimal RMSD values and heightened structural robustness (Rohman et al., 2023), set colchicine as a promising inhibitor with the potential to effectively modulate inflammation-induced pyroptosis through precise targeting of CASP-1 and GSTO-1. However, a discernibly different dynamic unfolded within the NLRP-3 complex, where colchicine manifested compromised structural integrity and stability relative to its native ligand. Elevated RMSD values and increased residue fluctuation in the colchicine-NLRP-3 complex underscore a less favorable interaction (Rohman et al., 2023), emphasizing the need for judicious consideration of colchicine's applicability across distinct protein targets. These findings underscore the intricate and context-dependent nature of colchicine's interactions, providing a compelling rationale for tailored therapeutic strategies contingent upon the underlying molecular dynamics of specific inflammatory pathways.

Validation of colchicine interaction to CASP-1 and GSTO-1 with reference compounds

Previous data indicated that colchicine acts as a stronger inhibitor of CASP-1 and GSTO-1 than NLRP-3. Redocking with reference compounds assessed colchicine's inhibitory mechanism, revealing only minor differences in binding affinity compared to the CASP-1 inhibitor NSC697923 (Fig. 8A-B). MDS showed that colchicine exhibited enhanced inhibitory potential, with increased stability reflected in RMSD of backbone atoms (Fig. 8C), RMSF per residue (Fig. 8D-E), ligand conformation (Fig. 8F), hydrogen bond count (Fig. 8G), and binding energy estimates (Fig. 8H). The colchicine-CASP-1 complex was more stable and formed more hydrogen bonds than the NSC697923-CASP-1 complex, contributing to binding energy stability despite slight fluctuations.

A comparative analysis of colchicine with KT53, an inhibitor of GSTO-1, yielded similar results. Docking analysis showed minor differences in binding affinity between colchicine-GSTO-1 and KT53-GSTO-1 complexes (Fig. 9A-B). MDS indicated that colchicine maintains a stable complex with GSTO-1, as evidenced by a stable RMSD trajectory of backbone atoms (Fig. 9C) and consistent RMSF values with minimal differences compared to the KT53-GSTO-1 complex (Fig. 9D). In contrast, KT53 showed higher fluctuations in ligand RMSD (Fig. 9E), suggesting potential instability. Colchicine formed more stable hydrogen bonds with GSTO-1 (Fig. 9F) and exhibited lower binding energy (Fig. 9G), indicating a stronger interaction to exhibit stronger inhibitory activity.

This study reveals a novel mechanistic insight into colchicine's potential as a treatment for IHD, particularly due to its anti-inflammatory and anti-pyroptotic effects through inhibition of CASP-1 and GSTO-1. CASP-1 plays a crucial role in activating pro-inflammatory cytokines, driving inflammation and inflammasome-mediated cell death, including pyroptosis (Franchi et al., 2009; Syed et al., 2005). Inhibiting CASP-1 reduces inflammation by blocking cytokine activation, inflammasome activity, and programmed cell death, as supported by prior research (Caruso et al., 2022; Dhani et al., 2021; Liang et al., 2021; McKenzie et al., 2018). Clinical studies also indicate colchicine's CASP-1 inhibition reduces NLRP-3 inflammasome activation, alleviating inflammation (Robertson et al., 2016). Consequently, colchicine's ability to inhibit pyroptosis in IHD cardiomyocytes may be linked to its CASP-1 inhibitory action.

Furthermore, this study reveals, for the first time, a mechanism by which colchicine inhibits GSTO-1 to prevent pyroptosis. Colchicine's impact is noted upstream in inflammasome activation, specifically by restraining GSTO-1. Given GSTO-1's pivotal role in NLRP-3 inflammasome formation (Hughes et al., 2019), inhibiting GSTO-1 mitigates inflammation (Laliberte et al., 2003; Xie et al., 2020). Despite the absence of direct evidence linking GSTO-1 and IHD, inhibiting GSTO-1 emerges as a promising strategy for averting pyroptosis by hindering inflammation. Therefore, colchicine's newfound inhibition of GSTO-1 in preventing pyroptosis signifies a groundbreaking contribution to our understanding of its cardioprotective activity, representing colchicine's inaugural report of such GSTO-1 inhibition.

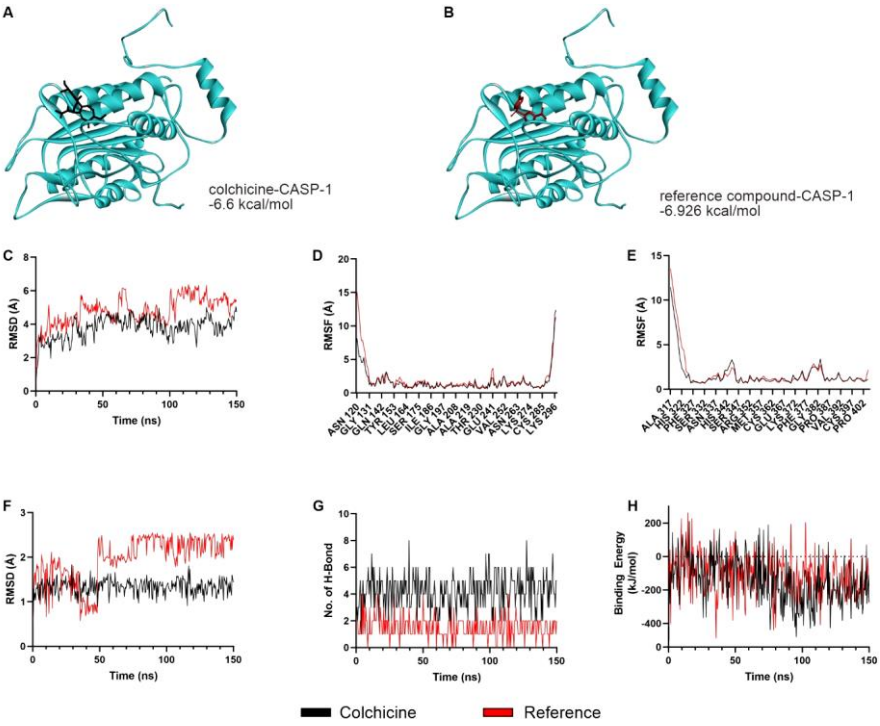

Figure 8. Molecular interaction of CASP-1 with colchicine and reference compound.

This figure depicts the molecular dynamics simulation of CASP-1 in complex with colchicine **(A)** and a reference compound **(B)**. Binding affinities are indicated. Protein was visualized in cyan ribbon, while the ligands were visualized in black or red sticks for colchicine and reference compound, respectively. The stability of the protein-ligand complexes is assessed by RMSD of backbone atoms **(C)**, RMSF of individual residues in chains A **(D)** and B **(E)**, ligand RMSD **(F)**, hydrogen bond formation **(G)**, and binding energy variation **(H)**. The black and red lines represent colchicine and reference compound, respectively.

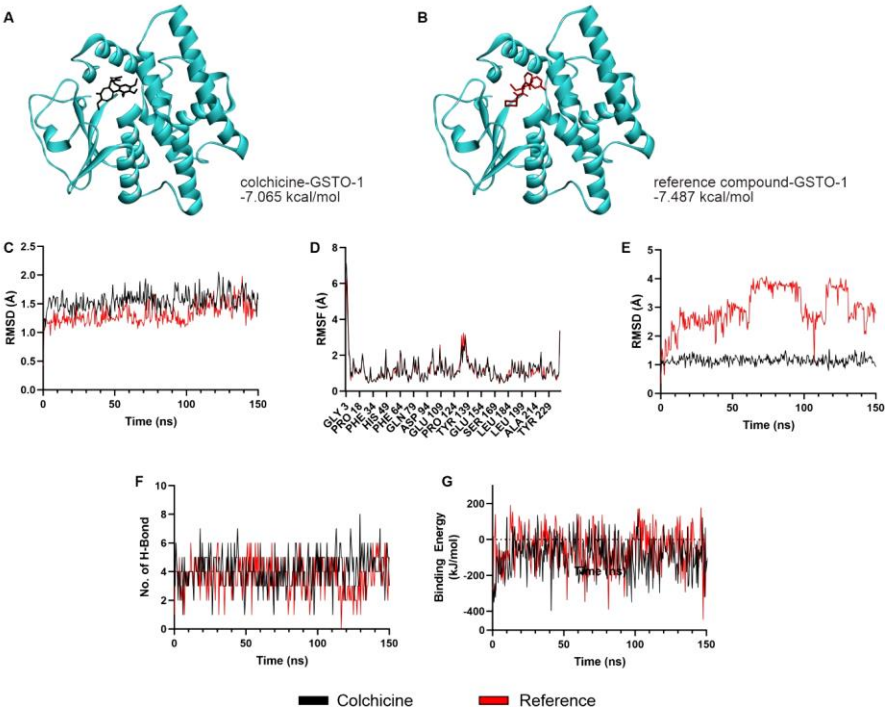

Figure 9. Molecular interaction of GSTO-1 with colchicine and reference compound.

This figure illustrates the molecular dynamics simulation of GSTO-1 bound to colchicine **(A)** and a reference compound **(B)**, with binding affinities noted. The protein is shown in cyan ribbon, while colchicine and the reference compound are displayed as black and red sticks, respectively. Complex stability is evaluated through backbone RMSD **(C)**, per-residue RMSF **(D)**, ligand conformation RMSD **(E)**, hydrogen bond formation **(F)**, and binding energy variation **(G)**. Black and red lines denote colchicine and the reference compound, respectively.

CONCLUSION

Colchicine demonstrates a preferential binding affinity for CASP-1 and GSTO-1 over NLRP-3, indicating selectivity in its interaction. Its stable association with CASP-1 and GSTO-1, confirmed through comparative analysis with reference inhibitors, suggests a durable and potent interaction. This stable binding implies an inhibitory mechanism that may reduce inflammation-induced pyroptosis. Colchicine likely achieves its anti-inflammatory effects by specifically targeting and inhibiting CASP-1 and GSTO-1, thus preventing pyroptosis and moderating inflammation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Satrijo B	RohmanMS	Asmoro AA	Susanti H	Lyrawati D	Muhammad RF
Concepts or ideas	x	x				
Design	x					
Definition of intellectual content		x				
Literature search	x					
Experimental studies	x					
Data acquisition	x					
Data analysis	x	x				
Statistical analysis						
Manuscript preparation	x	x				x
Manuscript editing		x	x	x	x	x
Manuscript review	x	x	x	x	x	x

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