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Predicting molecular mechanism of silymarin-potentiated diclofenac toxicity: Insight from *in silico* molecular docking

Cherdsak Boonyong [a], Suree Jianmongkol [b,*]

[a] Pharmacology and Toxicology Unit, Department of Medical Sciences, Faculty of Science, Rangsit University, Pathum Thani 12000, Thailand
[b] Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

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ABSTRACT

Silymarin was shown to enhance diclofenac toxicity by inducing the loss of mitochondrial membrane permeability (MMP) in Caco-2 cells, independent of endoplasmic reticulum stress. This study employed *in silico* molecular docking to further investigate the potential interaction between silymarin and specific mitochondrial proteins involved in the loss of mitochondria integrity, aiming to elucidate the underlying mechanism of potentiation. The target proteins for our docking analysis included mitochondrial complex I and III, voltage-dependent anion-selective channel (VDAC), and cyclophilin D (CypD). Our results indicated that diclofenac could bind to both mitochondrial complex I and III. In contrast, silymarin exhibited a strong interaction with mitochondrial complex I with the binding energy (ΔG) −7.74 kcal/mol and the inhibition constant (Ki) 2.12 μM, while not showing significant interaction with mitochondrial complex III. Additionally, silymarin had the potential to induce the opening of mitochondrial permeability transition pore by binding with VDAC in the outer mitochondrial membrane with ΔG −6.08 kcal/mol and Ki 34.94 μM. However, silymarin did not exhibit significant interaction with CypD in the inner mitochondrial membrane. Therefore, mitochondrial complex I and VDAC could be the potentiation targets of silymarin, resulting in the disruption of mitochondria integrity and enhancing the toxicity of diclofenac.

1. Introduction

Diclofenac is a non-selective inhibitor of cyclooxygenase (COX) commonly used for relieving pain and inflammation. Its notable adverse effect has been related to gastrointestinal damage which, in part, occurs from its inhibition against mitochondrial complex I activity and production of stress-induced apoptotic cell death [1–3]. Recently, we demonstrated that some natural antioxidants with ability to suppress endoplasmic reticulum (ER) stress such as epigallocatechin, quercetin and rutin could prevent diclofenac-induced apoptosis in Caco-2 cell, which is a widely accepted cell model for intestinal absorption, as well as in intestinal rat tissue [4,5]. Their "key" protective mechanisms involve inhibition of PERK-CHOP ER sensor activation, and maintaining mitochondrial permeability and function [4].

On the other hand, certain antioxidants such as phyllanthin and silymarin increased diclofenac toxicity in Caco-2 cells through suppression of survival responses and/ or increase of apoptosis [5]. For example, the potentiation effect of phyllanthin was caused by down-regulating the expression of p-Nrf-2/HO-1 proteins, activating PERK-CHOP proteins, disrupting mitochondrial membrane permeability (MMP), and inducing apoptosis [4]. Silymarin, a known hepatoprotective compound in milk thistle, enhanced apoptosis of diclofenac-treated Caco-2 cells possible through MMP disruption without generating ER stress [5]. It has been suggested that reduction of MMP could result from inhibition of mitochondrial complex I and III activities and opening of mitochondrial permeability transition pore (mPT pore) [6–10]. The mPT pore opening could occur due to the dissociation between a voltage-dependent anion channel (VDAC) in outer membrane and adenine nucleotide translocase (ANT) and cyclophilin D (CypD) in the mitochondrial matrix [11,12]. Thus, interference with these mitochondrial proteins could disrupt the integrity of mitochondria, leading to apoptosis induction. To date, the potentiation mechanism of silymarin targeting mitochondria integrity in relation to diclofenac toxicity has not been fully elucidated.

In this study, we applied *in silico* molecular docking to investigate potential interaction between silymarin and specific mitochondrial proteins responsible for the loss in MMP in order to gain better understanding of its sensitizing effect on diclofenac toxicity. The

* Corresponding author.
E-mail address: suree.j@pharm.chula.ac.th (S. Jianmongkol).

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protein–ligand recognition with the best binding poses of diclofenac and silymarin within sites of mitochondrial proteins particularly mitochondrial complexes I and III, VDAC, ANT, and CypD was analyzed. Our findings suggested that silymarin might exacerbate diclofenac-induced apoptosis by binding to mitochondrial complex I and VDAC. This binding interaction could lead to increased mitochondria dysfunction through the opening of mPT pore, which potentially contributes to the increased cytotoxicity of diclofenac. This *in silico* evidence provides a mechanistic insight into the specific targets of diclofenac toxicity associated with the loss of mitochondrial integrity.

2. Materials and methods

2.1. Ligand preparation

The chemical structures of diclofenac (PubChem CID: 3033) and silymarin (PubChem CID: 5213) were retrieved from NCBI the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/). The SDF format files of both compounds were converted to 3D chemical structure with PDB format files by using discovery studio 2021 Client (BIOVIA, San Diego, CA, USA). Two PDB files of compounds were converted to PDBQT file with AutoDoc Suite 4.2.6 (TSRI, La Jolla, CA, USA).

2.2. Protein preparation

RCSB Protein Data Bank (RCSB PDB) (https://www.rcsb.org/) was used to download the crystallographic structure of mitochondrial proteins of human respiratory complex I (PDB: 5XTD), human respiratory complex III (PDB: 5XTE), a VDAC (PDB: 6TIR) and CyPD (PDB: 4ZSC). The proteins were saved to PDB file. Each protein structure was removed the water molecules, added with the polar hydrogens and neutralized with Kollman united atom charges. The protein structure was saved again to PDB file and converted to PDBQT format files.

2.3. Docking simulation

Docking studies of diclofenac and silymarin interacting with mitochondrial proteins were performed using AutoDoc Suite 4.2.6 (TSRI, La Jolla, CA, USA) with PDBQT format files. The grid box measuring 60 × 60 × 60 Å with 0.375 Å spacing between points was generated to define docking site. Interactions between each ligand and mitochondrial proteins were run with Lamarckian genetic algorithm at 100 rounds, and the command was provided in Appendix A Supplementary data.

2.4. Analysis

After docking simulation was done, the highest ligand binding with mitochondrial proteins was observed according to binding energy (ΔG) and inhibition constant (Ki) (Appendix B. Supplementary data). The two-dimensional (2D) and three-dimensional (3D) intermolecular interactions were visualized using discovery studio 2021 Client.

3. Result and discussion

According to our previous study, the potential underlying mechanism by which silymarin potentiated diclofenac-induced apoptosis in Caco-2 cells involved the loss in MMP and mitochondrial integrity [5]. In our previous study, we demonstrated that when silymarin was present at a noncytotoxic concentration of 100 μM, the viability of diclofenac-treated Caco-2 cells significantly decreased from approximately 52–5 % after 72 h of exposure. Furthermore, the co-administration of silymarin and diclofenac led to a reduction in MMP from approximately 57–18 %, compared to diclofenac treatment alone [5]. In the present study, we demonstrated that both silymarin and diclofenac could inhibit mitochondrial complex I, but not mitochondrial complex III, based on *in silico* molecular docking predictions. In addition, silymarin was able to interact with VDAC proteins in outer mitochondrial membrane, but not with CypD in inner mitochondrial membrane. These findings further provided insight into the interaction between silymarin and target mPT pore architecture.

Mitochondria play key roles in activating apoptosis in mammalian cells [13]. Inhibition of mitochondrial respiratory complex I and III is a common cause that induces apoptotic cell death by disrupting the production of ATP and accumulation of reactive oxygen species (ROS), which subsequently results in mitochondria dysfunction [7,14,15]. Our docking results showed that both silymarin and diclofenac were able to bind with human mitochondrial complex I protein with binding affinity ΔG − 7.74 kcal/mol and −5.77 kcal/mol, respectively (Fig. 1 and Table 1). Due to the higher binding affinity, silymarin might be 27-fold more potent in inhibiting this enzyme than diclofenac, with the predicted inhibition constant (Ki) of 2.12 μM and 58.69 μM, respectively. Apparently, both silymarin and diclofenac could form bonds with various amino acids in the NADH dehydrogenase flavoprotein of the mitochondrial complex I protein [16]. As shown in Fig. 1 and Table 1, the residue GLY207 of the protein formed Pi-donor hydrogen bond with silymarin, while it formed an H-bond with diclofenac. In addition, both silymarin and diclofenac bonded to GLY121 through Pi-anion interaction. Unlike diclofenac, silymarin interacted with additional amino acid residues, including GLY90 ASP118, GLU121, ASP127, ASN244 through H-bond, GLU119 and GLU 121 through Pi-donor hydrogen bond, GLY120 through Unfavorable donor-donor bond. It was possible that the binding of diclofenac and silymarin to these amino acid residues has the potential to interfere with NADH dehydrogenase activity of mitochondrial complex I protein. It has been reported that decreased NADH dehydrogenase activity leads to the suppression of proton movement across the inner mitochondrial membrane, resulting in a decline in proton-driven ATP synthesis [10,13,17]. Consequently, this may contribute to the loss of MMP, followed by apoptotic cell death.

In addition to mitochondrial complex I, we also investigated the interaction between silymarin or diclofenac and mitochondrial complex III. It was reported that diclofenac was able to inhibit mitochondrial complex III [6]. As shown in Fig. 2 and Table 2, our docking results indicated that diclofenac exhibited a 2-fold higher binding affinity than silymarin, with respective values of ΔG at −8.78 kcal/mol and −4.62 kcal/mol. Furthermore, diclofenac demonstrated a significantly higher inhibitory potency compared to silymarin against this enzyme, with the predicted inhibition constant (Ki) values of 0.364 μM and 413.02 μM, respectively, representing a 1236-fold difference. Additionally, our findings indicated that silymarin exhibited a higher level of selectivity as an inhibitor against mitochondrial complex I compared to mitochondrial complex III. Both diclofenac and silymarin had the ability to form bonds with multiple amino acids found in the catalytic domain of cytochrome b-c1 complex within the mitochondrial complex III [16]. Specifically, they could form H-bond with MET383 and Pi-alkyl bond with ILE247. In addition, diclofenac could also bind to ALA385 with H-bond, PHE251 and LEU347 with Pi-alkyl bond, PHE344 with Pi-Pi stacked bond, TYP379 with Pi-cation bond and TYR379 with Unfavorable donor-donor bond. On the other hand, silymarin could bind to LEU246 and ALA385 with Alkyl and Pi-sigma bonds, PHE68, VAL70 and TYR379 with Pi-donor hydrogen bond and GLY384 with Unfavorable donor-donor bond. Inhibition of mitochondrial complex III can disrupt the proton gradient across the mitochondrial inner membrane, which results in the loss of MMP and the opening of mPT pore [18]. Consequently, the cellular production of ROS increases, while ATP synthesis decreases. These events may lead to apoptotic cell death [6,18]. Hence, our findings indicate that diclofenac induces apoptosis, partly by inhibiting mitochondrial complex III through its ability to bind with catalytic domain of cytochrome b-c1 complex. In addition, silymarin is able to enhance diclofenac toxicity by interfering with mitochondrial complex I.

The mPT pore is critical in maintaining the integrity and function of this organelle [11,12]. The mPT pore increases under cellular oxidative

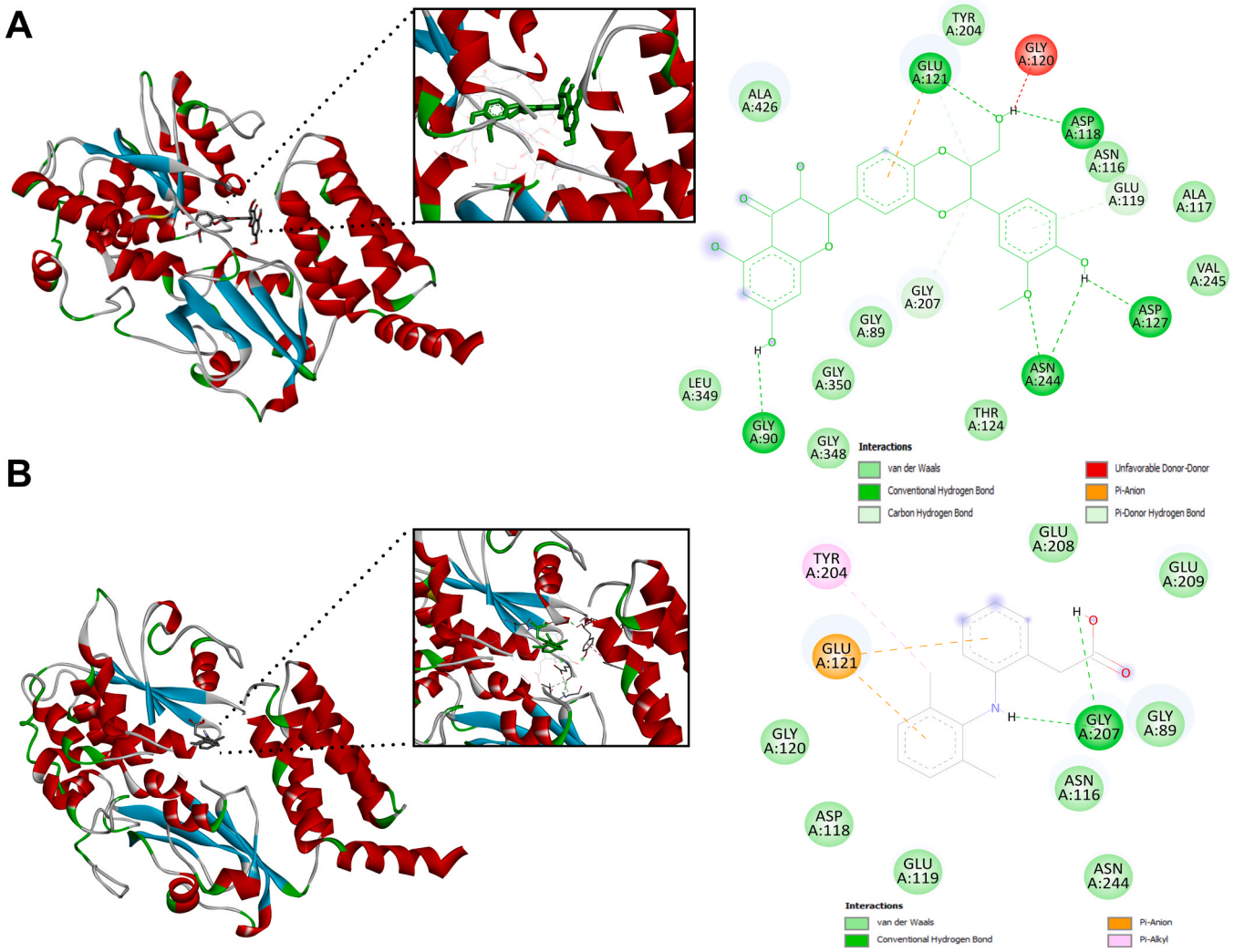

Fig. 1. Binding patterns and interaction of silymarin (A) or diclofenac (B) with mitochondrial complex I (PDB: 5XTD). The three-dimensional diagrams (left) showed the binding conformation of silymarin or diclofenac (green) with NADH dehydrogenase domain of mitochondrial complex I. The two-dimensional diagrams (right) displayed amino acid residues that may interact with the compounds.

Table 1
Docking results of silymarin and diclofenac with mitochondrial complex I.

Mitochondrial complex I	Compounds					
	Silymarin			Diclofenac		
Docking Parameters						
Binding affinity (kcal/mol)	-7.74			-5.77		
Ki (μM)	2.12			58.69		
Type of interactions	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**
H-bond	6	12.62	GLY90, ASP118, GLU121, ASP127, ASN244	2	3.90	GLY207
Pi-anion	1	3.93	GLU121	2	7.90	GLU121
Pi-alkyl				1	4.10	TYR204
Pi-donor hydrogen	3	9.49	GLU119, GLU121, GLY207			
Unfavorable donor-donor	1	1.28	GLY120			

stress and other certain pathological conditions such as skeletal muscle atrophy, diabetes and cardiovascular disease [12,19]. The opening of the mPT pore increases the permeability of the mitochondrial membrane, allowing the release of small molecules such as Ca^{2+} and cytochrome C into the cytoplasm. Consequently, this leads to a loss of MMP and ultimately triggers apoptosis [2]. The major components of mPT pore include VDAC, located on the outer mitochondrial membrane, as well as ANT and CypD, located on the inner mitochondrial membrane [11,12]. It has been demonstrated that both VDAC and CypD contribute to the sensitivity of mPT pore opening during cellular stresses [20]. According to our docking results, silymarin elicited a good binding affinity (ΔG −6.08 kcal/mol) and the predicted inhibition constant (Ki) of 34.94 μM, when binding to human VDAC (Fig. 3 and Table 3). In contrast, diclofenac did not show significant binding to VDAC protein. The interaction between silymarin and VDAC involves the formation of bonds with specific amino acid residues. These include H-bonds with

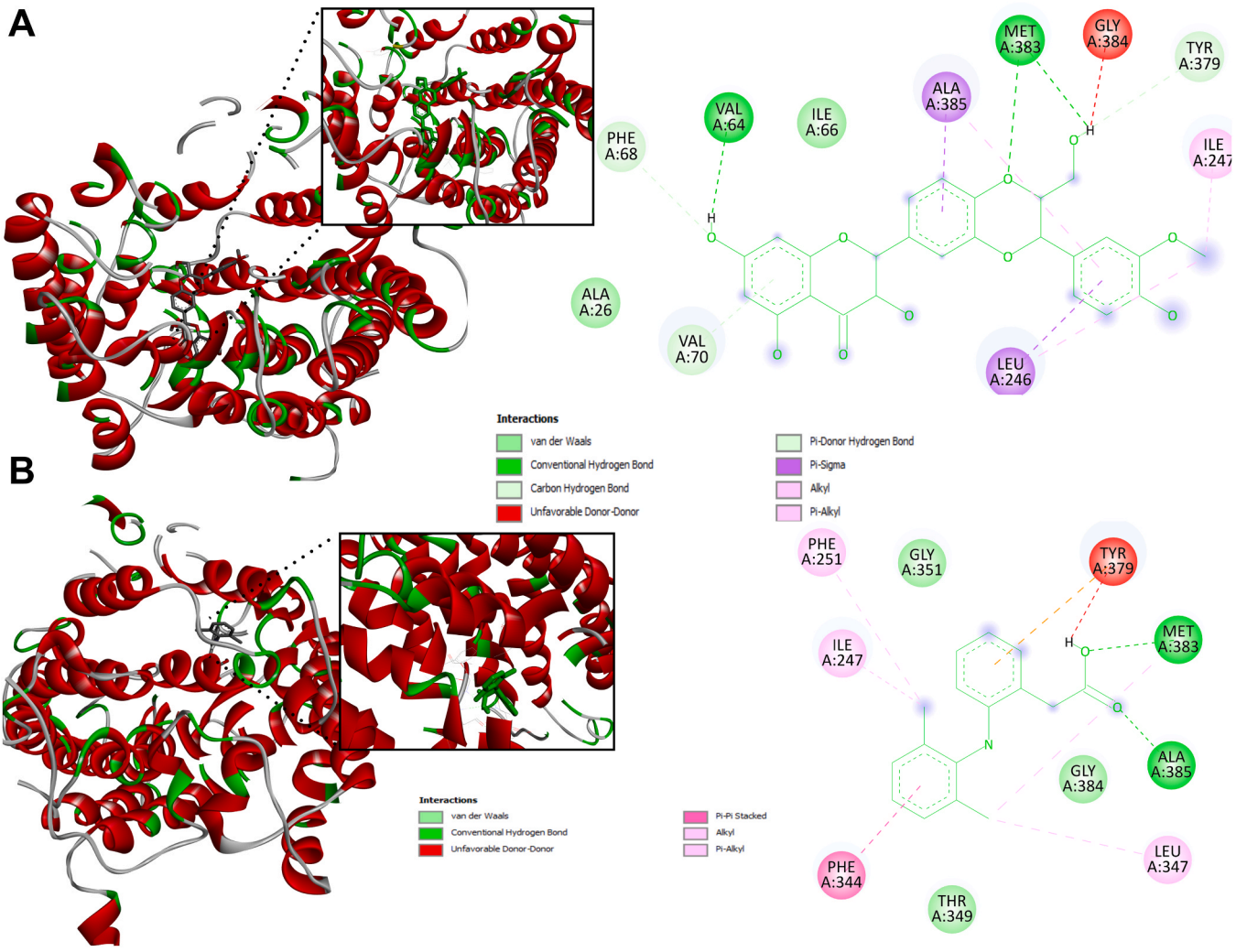

Fig. 2. Binding patterns and interaction of silymarin (A) or diclofenac (B) with mitochondrial complex III (PDB: 5XTE). The three-dimensional diagrams (left) showed the binding conformation of silymarin or diclofenac (green) with mitochondrial complex III at the catalytic domain of cytochrome b-c1 complex. The two-dimensional diagrams (right) displayed amino acid residues that may interact with the compounds.

Table 2
Docking results of silymarin and diclofenac with mitochondrial complex III.

Mitochondrial complex III	Compounds					
	Silymarin			Diclofenac		
Docking Parameters						
Binding affinity (kcal/mol)	-4.62			-8.78		
Ki (μM)	413.02			0.364		
Type of interactions	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**
H-bond	3	7.59	VAL64, MET383	2	7.81	MET383, ALA385
Pi-alkyl	1	3.71	ILE247	4	19.96	ILE247, PHE251, LEU347, MET383
Alkyl	2	10.66	LEU246, ALA385			
Pi-sigma	2	7.49	LEU246, ALA385			
Pi-donor hydrogen	3	9.98	PHE68, VAL70, TYR379			
Pi-Pi stacked				1	5.36	PHE344
Pi-cation				1	6.39	TYR379
Unfavorable donor-donor	1	2.06	GLY384	1	5.24	TYR379

LEU10, ASN156 and SER193, Pi-Alkyl bonds with ALA14, ARG139 and ALA141, Pi-sigma bonds with ARG15, Carbon hydrogen bonds with ASN183, and unfavorable donor-donor bonds with ASN156 (Fig. 3). These amino acids are located in the VDAC N-terminal helices of which this segment involves in the opening of mPT pore [21]. The binding of silymarin to these residues might induce the change of VDAC conformation, leading to subsequent opening of the mPT pore. It was reported that 2-methyl-5 (or 8)-hydroxy-furanonaphthoquinone (FNQ13) bound with VDAC and opened the mPT pore in HeLa cell line [22]. Consequently, the cytochrome C releases mediated cell apoptosis. Our results further demonstrated that neither silymarin nor diclofenac was able to interact with CypD protein (Fig. 4 and Table 4). Taken together, silymarin could target the mPT pore through interaction with VDAC, but not CypD proteins. The interaction between silymarin and the mPT pore

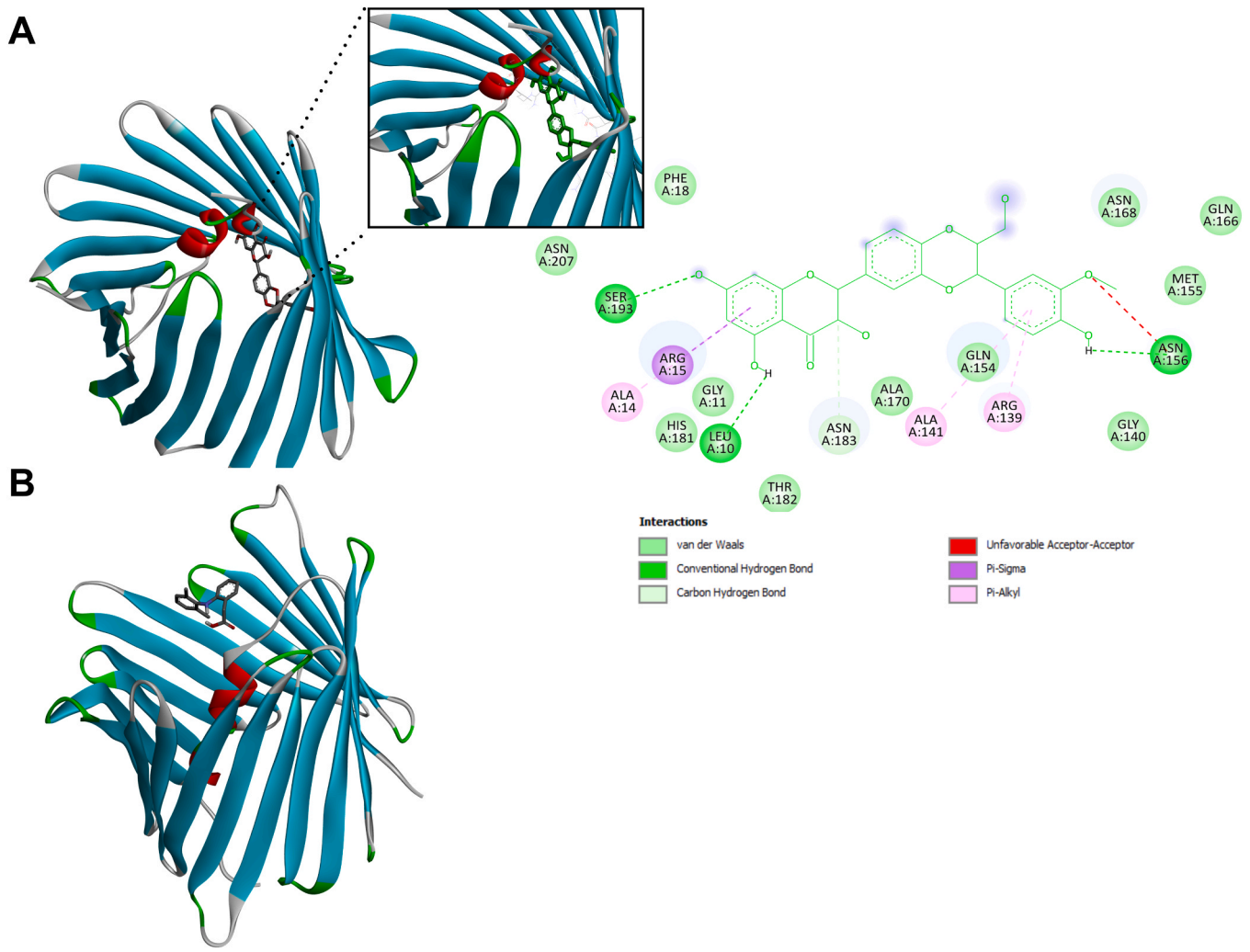

Fig. 3. Molecular docking of silymarin (A) or diclofenac (B) with voltage-dependent anion channel (VDAC) (PDB: 6TIR). The three-dimensional diagrams (left) showed the binding conformation of silymarin (green) with VDAC on the major binding site (red). No interaction between diclofenac and VDAC was observed. The two-dimensional diagrams (right) displayed amino acid residues that may interact with silymarin.

Table 3
Docking results of silymarin and diclofenac with voltage-dependent anion channel (VDAC).

Voltage-dependent anion channel (VDAC)	Compounds					
	Silymarin			Diclofenac		
Docking Parameters						
Binding affinity (kcal/mol)	-6.08			N.D.		
Ki (μM)	34.94			N.D.		
Type of interactions	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**	***n*-bonds**	**Average distance (Å)**	**Interacting amino acid**
H-bond	3	6.62	LEU10, ASN156, SER193			
Pi-alkyl	3	15.33	ALA14, ARG139, ALA141			
Pi-sigma	1	4.68	ARG15			
Carbon hydrogen	1	2.76	ASN183			
Unfavorable donor-donor	1	2.83	ASN156			

N.D.; Not detection

could lead to an increase in the loss of MPP and disrupt mitochondria integrity. These events could, in part, enhance diclofenac-mediated apoptosis. However, the concrete mechanisms underlying silymarin- and diclofenac-induced mitochondrial dysfunction still need experimental verification.

4. Conclusion

Silymarin and diclofenac have the potential to disrupt the integrity of mitochondria through direct interaction with mitochondrial complex I, III and VDAC proteins, as illustrated in Fig. 5. Diclofenac could disrupt electron transport chain at mitochondrial complex I and III, while silymarin exerted its disrupting effect at mitochondrial complex I. Moreover, by binding with the VDAC protein, silymarin could increase the opening of the mPT pore, which leads to the loss of mitochondrial integrity and enhance diclofenac -mediated apoptosis.

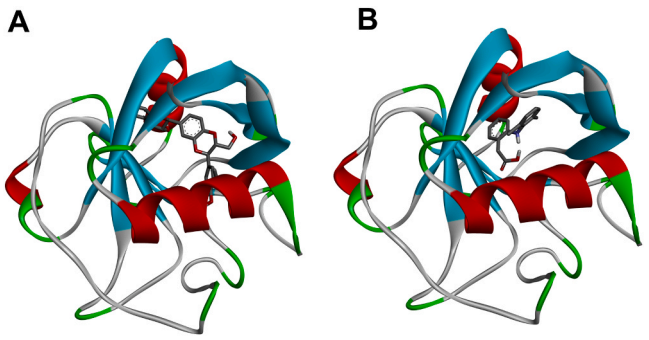

Fig. 4. Molecular docking of silymarin (A) or diclofenac (B) with cyclopilin D (CypD) (PDB: PDB: 4ZSC). The three-dimensional diagrams demonstrated no interaction between the compounds and CypD.

Table 4
Docking results of silymarin and diclofenac with cyclophilin D (CypD).

Cyclophilin D (CypD)	Compounds	
	Silymarin	Diclofenac
Docking Parameters		
Binding affinity (kcal/mol)	N.D.	N.D.
Ki (μM)	N.D.	N.D.

N.D.; Not detection
Figure legends

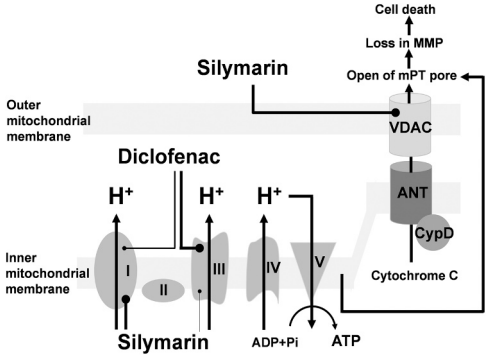

Fig. 5. Proposed interaction of silymarin with the specific mitochondrial proteins (i.e., Mitochondrial complex I and VDAC), contributing to its potentiation on diclofenac toxicity.

CRediT authorship contribution statement

Cherdsak Boonyong: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft preparation. **Suree Jianmongkol:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2023.10.001.

References

[1] M.V. Bielsa-Fernández, J.L. Tamayo-de la Cuesta, J. Lizárraga-López, J.M. Remes-Troche, R. Carmona-Sánchez, J.M. Aldana-Ledesma, J.M. Avendaño-Reyes, M. A. Ballesteros-Amozorrutia, M. De Ariño, L. de Giau-Triulzi, R. Flores-Rendón, H. Huerta-Guerrero, J.A. González-González, A. Hernández-Guerrero, E. Murcio-Pérez, J.O. Jáquez-Quintana, A. Meixueiro-Daza, J.R. Nogueira-de Rojas, H. Rodríguez-Hernández, R. Santoyo-Valenzuela, S.C. Solorzano-Olmos, L. F. Uscanga-Domínguez, F. Zamarripa-Dorsey, The Mexican consensus on the diagnosis, treatment, and prevention of NSAID-induced gastropathy and enteropathy, Rev. Gastroenterol. Mex. (Engl. Ed.) 85 (2020) 190–206.
[2] I. Bjarnason, C. Scarpignato, E. Holmgren, M. Olszewski, K.D. Rainsford, A. Lanas, Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs, Gastroenterology 154 (2018) 500–514.
[3] A.E. Chávez-Piña, R.R. Abril-Urías, D. Cerecedo, Mechanisms of damage involved in small intestinal tract caused by nonsteroidal anti-inflammatory drugs, Mex. J. Med. Res. ICSA 6 (2018) 1–5.
[4] C. Boonyong, N. Vardhanabhuti, S. Jianmongkol, Modulation of non-steroidal anti-inflammatory drug-induced, ER stress-mediated apoptosis in Caco-2 cells by different polyphenolic antioxidants: a mechanistic study, J. Pharm. Pharm. 72 (2020) 1574–1584.
[5] C. Boonyong, N. Vardhanabhuti, S. Jianmongkol, Natural polyphenols prevent indomethacin-induced and diclofenac-induced Caco-2 cell death by reducing endoplasmic reticulum stress regardless of their direct reactive oxygen species scavenging capacity, J. Pharm. Pharm. 72 (2020) 583–591.
[6] R. Ghosh, S.K. Goswami, L.F.B.B. Feitoza, B. Hammock, A.V. Gome, Diclofenac induces proteasome and mitochondrial dysfunction in murine cardiomyocytes and hearts, Int. J. Cardiol. 15 (223) (2016) 923–935.
[7] L. Lin, Y. Liu, S. Fu, C. Qu, H. Li, J. Ni, Inhibition of mitochondrial complex function-the hepatotoxicity mechanism of emodin based on quantitative proteomic analyses, Cells 8 (3) (2019) 263.
[8] S. Mazumder, R. De, S. Debsharma, S. Bindu, P. Maity, S. Sarkar, S.J. Saha, A. A. Siddiqui, C. Banerjee, S. Nag, D. Saha, S. Pramanik, K. Mitra, U. Bandyopadhyay, Indomethacin impairs mitochondrial dynamics by activating the PKCζ-p38-DRP1 pathway and inducing apoptosis in gastric cancer and normal mucosal cells, J. Biol. Chem. 294 (20) (2019) 8238–8258.
[9] N. Mnatsakanyan, H.A. Park, J. Wu, X. He, M.C. Llaguno, M. Latta, P. Miranda, B. Murtishi, M. Graham, J. Weber, R.J. Levy, E.V. Pavlov, E.A. Jonas, Mitochondrial ATP synthase c-subunit leak channel triggers cell death upon loss of its F_1 subcomplex, Cell Death Differ. 29 (9) (2022) 1874–1887.
[10] A.C. Roginski, Â.B. Zemniaçak, R.A. Marschner, S.M. Wajner, R.T. Ribeiro, M. Wajner, A.U. Amaral, Disruption of mitochondrial functions involving mitochondrial permeability transition pore opening caused by maleic acid in rat kidney, J. Bioenerg. Biomembr. 54 (4) (2022) 203–213.
[11] M. Bonora, C. Giorgi, P. Pinton, Molecular mechanisms and consequences of mitochondrial permeability transition, Nat. Rev. Mol. Cell Biol. 23 (4) (2023) 266–285.
[12] I.D. Boyenle, A.K. Oyedele, A.T. Ogunlana, A.F. Adeyemo, F.S. Oyelere, O. B. Akinola, T.I. Adelusi, L.O. Ehigie, A.F. Ehigie, Targeting the mitochondrial permeability transition pore for drug discovery: challenges and opportunities, Mitochondrion 63 (2022) 57–71.
[13] G. Vial, D. Detaille, B. Guigas, Role of Mitochondria in the mechanism(s) of action of metformin, Front Endocrinol. (Lausanne) 10 (2019) 294.
[14] H.A. Kalpage, J. Wan, P.T. Morse, M.P. Zurek, A.A. Turner, A. Khobeir, N. Yazdi, L. Hakim, J. Liu, A. Vaishnav, T.H. Sanderson, M.A. Recanati, L.I. Grossman, I. Lee, B.F.P. Edwards, M. Hüttemann, Cytochrome c phosphorylation: Control of mitochondrial electron transport chain flux and apoptosis, Int. J. Biochem. Cell. Biol. 121 (2020), 105704.
[15] A. Sreedhar, L. Aguilera-Aguirre, K.K. Singh, Mitochondria in skin health, aging, and disease, Cell Death Dis. 11 (6) (2020) 444.
[16] R. Guo, S. Zong, M. Wu, J. Gu, M. Yang, Architecture of human mitochondrial respiratory megacomplex I2III2IV2, Cell 170 (6) (2017) 1247–1257, e12.
[17] H. Wang, J. Luo, W. Tian, W. Yan, S. Ge, Y. Zhang, W. Sun, γ-Tocotrienol inhibits oxidative phosphorylation and triggers apoptosis by inhibiting mitochondrial complex I subunit NDUFB8 and complex II subunit SDHB, Toxicology 417 (2019) 42–53.
[18] W. Qi, C. Lu, H. Huang, W. Zhang, S. Song, B. Liu, (+)-Usnic acid induces ROS-dependent apoptosis via inhibition of mitochondria respiratory chain complexes and Nrf2 expression in lung squamous cell carcinoma, Int. J. Mol. Sci. 21 (3) (2020) 876.

[19] J.S. Bhatti, G.K. Bhatti, P.H. Reddy, Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies, Biochim. Biophys. Acta Mol. Basis Dis. 1863 (5) (2017) 1066–1077.
[20] Q. Hu, J. Wang, M. Irshad, S. Mao, H. Chen, Y. Song, X. Xu, X. Feng, Neuroprotective effects of the psychoactive compound Biatractylolide (BD) in Alzheimer's disease, Molecules 27 (23) (2022) 8294.
[21] R. Böhm, G.F. Amodeo, S. Murlidaran, S. Chavali, G. Wagner, M. Winterhalter, G. Brannigan, S. Hiller, The structural basis for low conductance in the membrane protein VDAC upon β-NADH Binding and voltage gating, Structure 28 (2) (2020) 206–214.
[22] E. Simamura, K. Hirai, H. Shimada, J. Koyama, Y. Niwa, S. Shimizu, Furanonaphthoquinones cause apoptosis of cancer cells by inducing the production of reactive oxygen species by the mitochondrial voltage-dependent anion channel, Cancer Biol. Ther. 5 (11) (2006) 1523–1529.