RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2401-2406| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ADMET PREDICTION AND MOLECULAR DOCKING SIMULATION OF PHYTOCONSTITUENTS IN *Boesenbergia rotunda* RHIZOME WITH THE EFFECTOR CASPASES TO UNDERSTAND THEIR PROTECTIVE EFFECTS

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ABSTRACT

Acute kidney injury is a condition when kidney function decreased abruptly, characterized by *e.g.* a decrease in the glomerular filtration rate. This condition is due to the activation of various signaling pathways, such as effector caspases, and thus can reverse kidney damage by interacting with these proteins, and eventually inhibiting the apoptotic process. This study aims to determine the molecular interactions of eight phytoconstituents in *B. rotunda* rhizome with the active binding pocket of caspase-3 and-7 and to predict the ADMET profile of these ligands. Results indicated that all ligands could occupy the binding pocket of both caspases, similar to those of caspase inhibitors, which means that these compounds could inhibit the apoptotic signaling pathway, thus protecting the cell. However, these ligands indicate a better docking score with caspase-7 than with caspase-3. Of all ligands, 4-hydroxypanduratin A and panduratin A showed the best affinity (-8.3 kcal/mol and -8.5 kcal/mol, respectively). ADMET prediction revealed that the excellent oral bioavailability belongs to 4-hydroxypanduratin A and pinostrobin (HIA >99%; Caco2 >70 μm/sec; BBB <1; PPB <50%).

Keywords: Apoptosis, *Boesenbergia rotunda*, Caspases, Panduratin A, Pinocembrin

RASĀYAN *J. Chem.*, Vol. 15, No.4, 2022

INTRODUCTION

Acute kidney injury (AKI) is a reversible condition when kidney function decreased abruptly, marked by an increase in certain bio-clinical parameters, *e.g.* kidney injury molecule 1 (KIM-1), and a reduced-glomerular filtration rate. This condition is caused by the activation of various signaling pathways such as NF-kappaB, pro-inflammatory cytokines, and caspases.[1] In apoptosis, caspases are classified into caspase initiators (caspase-2, -8, -9, -10) and effectors (caspase-3, -6, -7). Both are distinguished based on the pro-domain N-terminal of amino acid residues. Caspase initiators consist of more than 90 amino acid residues, while caspase effectors comprise about 20-30 residues in their pro-domain order. Certain Asp residues, particularly the second Asp in the D-E-V-D sequence that cleave, can activate the effector caspases.[2] It was reported that the substrate of caspase-3 constructs hydrogen bonds with residues 205-209, and hydrophobic contacts with phenylalanine and tryptophan.[3] In addition, the caspase substrate, interacts with caspase-3 catalytic cysteine and histidine diads, near the aspartate peptide bonds P1.[4] These catalytic diads are observed at the active sites of all caspases. Arg413 is a residue that unites the main chain and barrier chain of the peptide.[5] According to the cleavage-specificity profile of caspase-3 and caspase-7, these caspases were shown to be inherently redundant in terms of substrate cleavage during apoptosis.[6] Another study revealed that hydrogen bond interaction with residues 205-209 and hydrophobic contacts with Trp206 and Phe256, might be important in activating the executioner caspase-3.[7] Inhibition of caspase-3 occurs at residues Asp45, Ala46, Val48, Ile78, Trp79, and Arg81.[8] Moreover, inhibition of caspase-7 occurs at residues 198 and 206.[9] Currently, *in silico* method is acceptable and has a high contribution to the revelation of novel drug candidates with preferable pharmacological activity. This method has been successfully applied to predict the pharmacokinetic (ADMET) profile.[10] Many drug candidates fail at later stages of

http://doi.org/10.31788/RJC.2022.1547011

development. In more than half of the cases, drug failure may be due to the unacceptable nature of ADMET.[11] Molecular docking is also widely applied to study the binding interaction strength, mode, and drug interaction with amino acid residues of target receptor proteins.[12] Therefore, *in silico* methods are becoming increasingly popular as an initial screening for the discovery of compounds as drug candidates due to the relatively low cost required. *Boesenbergia rotunda* has been reported for its protective effect against thioacetamide intoxication in Sprague-Dawley rats as comparable to that of silymarin. Treatment of *B. rotunda* improved liver histopathology and levels of cytokines in rat serum.[13-14] Recently, it has also been reported that the panduratin A of *B. rotunda* has a renal protective effect *in vitro* and *in vivo*.[15] Considering this, our present study determines the potential of *B. rotunda* as a nephroprotective phytomedicine by exploring the molecular interaction of eight phytoconstituents in the rhizome of this plant with important amino acid residues in caspase-3 and caspase-7 and predict their ADMET profile.

EXPERIMENTAL

Hardware and Software

The hardware used was Asus-KHHGFQSN embedded with Microsoft windows, INTEL(R) Core (TM) i7-9750H processor, CPU 2.60 GHz, 2.59 GHz, and 8GB RAM. The software used was ChemDraw 19.0, AutoDock Vina, Pyrx, Pymol, and Discovery Studio Visualizer.

Molecular Docking

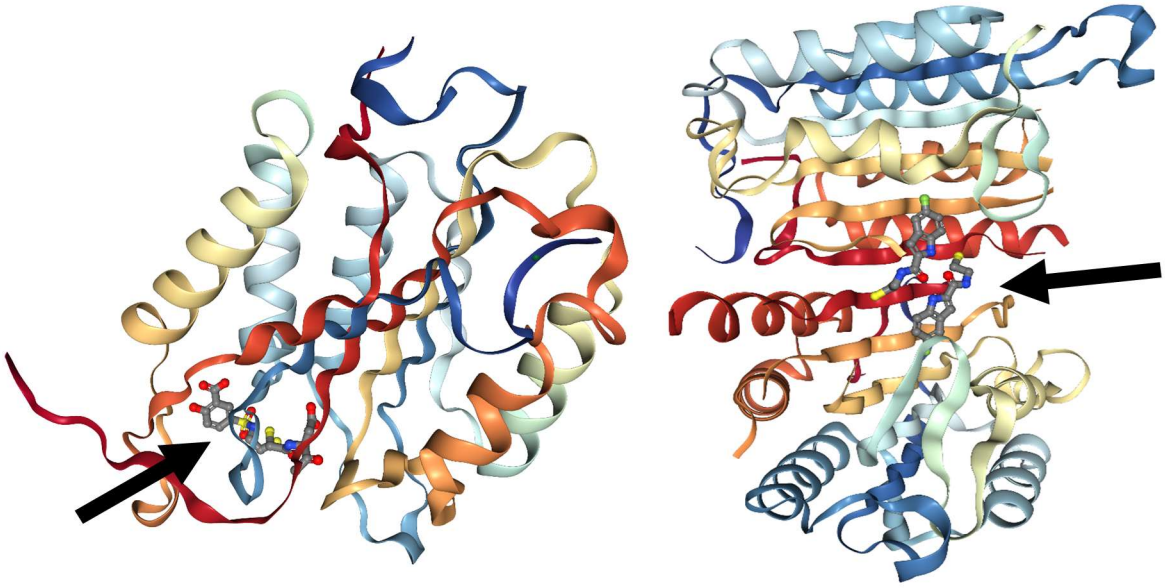
The 3D structure of the ligands was retrieved from the PubChem database: alpinetin (PubChem CID: 154279), cardamonin (PubChem CID: 641785), 4-hydroxypanduratin A (PubChem CID: 636530), naringenin 5-methyl ether (PubChem CID: 188424), panduratin A (PubChem CID: 6483648), pinocembrin (PubChem CID: 68071), pinostrobin (PubChem CID: 73201), and pinostrobin chalcone (PubChem CID: 5316793). The ligands were optimized using MMFF94 to obtain accurate geometric structures. The X-ray crystal structure of the macromolecules: caspase-3 (1NME; depicted in Fig.-1a) and caspase-7 (1SHL; depicted in Fig.-1b) were taken from https://www.rcsb.org/structure. Details of the macromolecules and the structure of the protein were made are presented in Table-1. The native ligand was separated and used as the standard.[17,18] AutoDock Vina was used for molecular simulation of docking of all ligands into binding pocket macromolecules. The scale factor for nonpolar atoms was changed to 0.8 for adjusting the flexibility of macromolecules, and all other parameters were set to default. The docking score was used to express the binding affinity of the macromolecular-ligand complex.

Prediction of ADMET

The AdmetSAR 2.0 application was used for predicting the pharmacokinetic profile of the ligands.[19,20] The structure of the compound is drawn or uploaded to a file in the format (*.mol) or by entering the Canonical SMILES format for analysis. The program will automatically calculate the value of cLogP, Human Intestinal Absorption (HIA), Blood Brain Barrier (BBB), and Plasma Protein Binding (PPB). Toxicity prediction using the Ames Test method was employed to test the mutagenesis of compounds and carcinogens as frameshift mutation. The program automatically displays a "positive" or "negative" result.

Table-1: Details of the Macromolecules

Description	Caspase-3	Caspase-7
PDB ID	1NME	1SHL
Resolution (Å)	1.60	3.00
Native ligand	3-(2-mercaptoacetylamino)-4-oxo-pentanoic acid	5-fluoro-1-indole-carboxylic acid-2-(mercapto-ethyl)-amide
Classification	Apoptosis/hydrolase	Hydrolase
Organism	*Homo sapiens*	*Homo sapiens*
Mutation	No.	Yes
Grid coordinates:		
Center x	27.8208	55.735
center_y	102.8152	18.9152
center_z	10.9511	9.5442



(a) (b)

Fig.-1: Structure of (a) Macromolecular-Ligand Complex for 1NME; (b) Macromolecular-Ligand Complex for 1SHL. Inhibitors in Both Caspases Are Depicted As Black Arrows

RESULTS AND DISCUSSION

Receptor Preparation and Validation

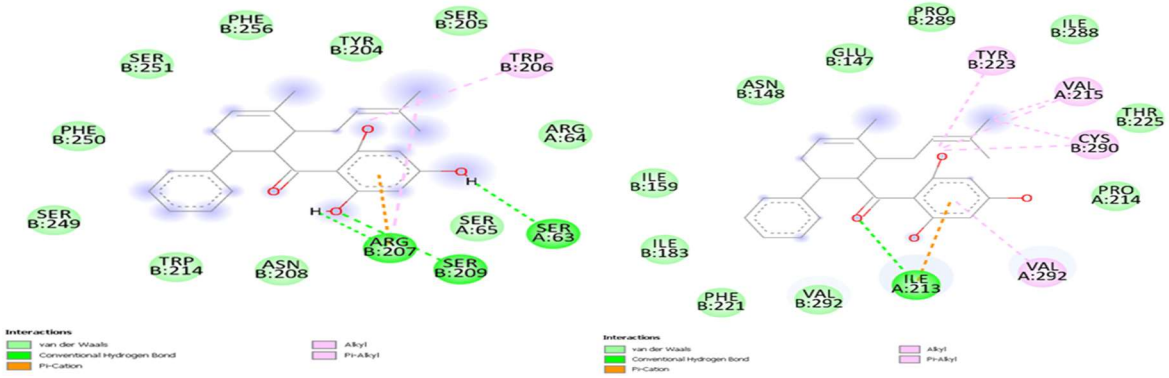
In this research, we studied the binding mode between eight phytoconstituents of *B. rotunda* (alpinetin, cardamonin, 4-hydroxypanduratin A, naringenin 5-methyl ether, panduratin A, pinocembrin, pinostrobin, pinostrobin chalcone), and the residues in the catalytic site of caspases to predict their nephroprotective activity. Validation of the docking method in terms of docking score and inhibition constant was displayed in Table-2.

Table-2: Comparison of the Docking Score of the Ligands with Caspase-3 and Caspase-7

Ligands	Caspase-3(1NME)		Caspase-7(1SHL)	
	Docking score (kcal/mol)	Ki	Docking score (kcal/mol)	Ki
Alpinetin	-7.4	0.997	-7.6	0.997
Cardamonin	-6.5	0.997	-7.7	0.997
4-hydroxypanduratin A	-7.2	0.997	-8.3	0.996
Naringenin 5-methyl ether	-7.0	0.997	-7.3	0.997
Panduratin A	-7.0	0.997	-8.5	0.996
Pinocembrin	-7.7	0.997	-7.4	0.997
Pinostrobin	-6.9	0.997	-7.6	0.997
Pinostrobin chalcone	-6.5	0.997	-7.6	0.997

Molecular docking results denote that all ligands, in general, indicate a better docking score with caspase-7 compared to caspase-3. Of all ligands, 4-hydroxypanduratin A and panduratin A showed the best affinity. Ligands that interact with specific amino acid residues at the catalytic center of caspases can competitively displace enzyme substrates and thus block subsequent apoptotic processes. Inhibition of this caspase may be useful in cases of renal failure given that renal cell death is one of the causes of AKI.

The molecular docking results showed that all tested compounds had better docking scores with caspase-7 than with caspase-3. Apoptosis occurs via cleavage of caspase-3 or caspase-7 in the extrinsic and intrinsic pathways. This activated caspase cleaves chromosomal DNA into internucleosome fragments, degrades cytoskeletal and nuclear proteins, and causes protein cross-linking. Morphological changes of apoptotic cells include cell degradation, changes and division of the nucleus, widening of the membrane, and decreased cell adhesion. In the final stage of apoptosis, there is the creation of apoptotic bodies, the release of ligands for phagocytic cells, and phagocytosis.[21]



(a) (b)

Fig.-2: Interaction of 4-Hydroxypanduratin A with (a) Caspase-3 and (b) Caspase-7. In Caspase-3, Hydrogen Bonds are Formed with Residues Ser63, Ser209, and Arg207, and Hydrophobic Interactions with Residues Arg207 and Trp206. In Caspase-7, Hydrogen Bonds are Formed with Residue Ile213, while Hydrophobic Interactions at Residues Ile213, Val215, Tyr223, Cys290, and Val292. Hydrogen Bonds are characterized by Green Dashed Lines. The Orange Dashed Lines Visualizes Pi-Cation Interaction. The Light Pink Dashed Lines Indicates Pi-Alkyl Interaction

In this study, several phytoconstituents, in particular, 4-hydroxypanduratin A and panduratin A (Fig.-2), interact with specific amino acid residues at the catalytic sites of caspases, where these ligands can competitively replace enzyme substrates so that further apoptosis can be inhibited. Inhibition of this caspase may be advantageous in cases of renal impairment, where cell death can be prevented. In another study, it was also reported that the two phytoconstituents were used as ligands for docking. The results showed that both of them are potent competitive inhibitors with the dengue virus protease type 2 NS2B/NS3 homology approach.[22] The *in silico* method has been developed and is widely used to develop and test pharmacological hypotheses. This method can be used to search for quantitatively active structural relationships, similarity searches, pharmacokinetics, homology models, molecular modeling, and other computational data analysis. These studies are also commonly used to discover and optimize new molecules that account for target affinity, absorption, distribution, metabolism, excretion, toxic properties, and physicochemical properties.[23] This *in silico* study focuses on caspase-3 and -7 to screen various *B. rotunda* plant components to explore their potential as renal protective drug candidates. These two caspases are known as apoptosis executive caspases, in contrast to caspase 9, which acts as initiating caspases.[9] Prior to performing the molecular docking study, a prediction of physicochemical (in terms of log P) and pharmacokinetic properties of the ligands was calculated to emphasize the critical parameters (bioavailability and toxicity). The results were presented in Table-3.

Table-3: Physicochemical and Pharmacokinetic Properties of Phytoconstituents in *B. rotunda* rhizome (in alphabetical order)

Ligands	cLogP	Absorption	Distribution		Toxicity
		HIA (%)	BBB	PPB(%)	Carcinogenicity/ mutagenicity
Alpinetin	3.1073	99.47	0.965	35.07	No
Cardamonin	3.0025	98.14	1.069	77.12	No
4-hydroxypanduratin A	5.7086	99.56	0.834	31.51	No
Naringenin 5-methyl ether	2.8129	99.47	0.955	45.07	No
Panduratin A	6.0116	99.58	0.906	82.19	No
Pinocembrin	2.8043	99.36	1.035	52.83	No
Pinostrobin	3.1073	99.36	1.029	35.11	No
Pinostrobin chalcone	3.0025	98.14	1.117	77.12	No

cLogP: calculated log P (acceptable range -2 to 6.5); HIA: human intestinal absorption (acceptable if >80%); PPB: plasma protein binding (lower value means higher fraction of free drug); BBB: blood-brain barrier (acceptable if <1)

Of all ligands, 4-hydroxypanduratin A and pinostrobin showed excellent oral bioavailability (HIA >99%; BBB <1; PPB <50%). Greater HIA denotes a better absorption from the intestinal tract upon oral administration, lower PPB means a higher the fraction of free drug that reaches the target of action.

Therefore, since 4-hydroxypanduratin A possesses both an excellent oral bioavailability (HIA >99%; BBB <1; PPB <50%) and a good affinity with caspase-7, the docking pose of this ligand with caspase-3 and caspase-7 is depicted in Fig.-2. Compound 4-hydroxypanduratin A, occupies the catalytic site of caspase-3 by building three hydrogen bonds with residues 63, 207, and 209. In addition, hydrophobic interactions with residues 206 and 207 were also detected. In caspase-7, hydrogen bonds are formed with residue Ile213, while hydrophobic interactions with residue Ile213, Val215, Tyr223, Cys290, and Val292. Pharmacokinetic profiling (ADMET) studies are very important in the process of searching for new drug candidates. In addition, a good drug candidate must not only be effective for its treatment target but must also have an appropriate ADMET profile at the treatment dose.[24] In this study, the ligands 4-hydroxypanduratin A and pinostrobin were reported to have an excellent pharmacokinetic profile with an HIA greater than 99%. HIA values indicate well-absorbed oral bioavailability greater than 70-100%. In addition, PPB values are less than 50%, i.e., more than 90% tightly bound and less than 90% loosely bound to less strong plasma protein binding compared to sites.[25] In recent decades, studies on the binding of plasma proteins that play an important role in controlling drug concentrations at therapeutic targets have become a major topic as a drug development strategy. The explanation of the "free drug theory" is used to explain that once equilibrium reaches a steady state in the absence of any energy-dependent processes, the amount of free drug in plasma equals the amount of free drug for pharmacologically targeted receptors in tissue plasma. In many cases, pharmacokinetic relationships have been described.[26] Candidate compounds without corresponding PPB values are discarded at the next drug screening stage. Therefore, calculating the value of PPB in candidate compounds is a very important first step, as well as prioritizing its development strategy. *B. rotunda* in many studies has shown various pharmacological activities.[27] Recently, its anti-inflammatory activity has been evaluated *in vivo*. The study reported that *B. rotunda* can inhibit Akt activation, thereby reducing the expression of NF-kappaB p65.[28] Inflammation is most common in cases of AKI. This results in renal ischemia-reperfusion. NF-kappaB is thought to act as an inflammatory mediator by being activated together with the induction of renal damage by ischemic-reperfusion.[29] In addition to the cell death signaling pathway via inhibition of caspases expression, inflammation regulated by the NF-kappaB signaling pathway is another promising target for the diagnosis and treatment strategy of kidney disease. Several molecules can strongly inhibit this signaling by targeting receptors such as IKK (IkappaB kinase), IkappaB, and transcription factors.

CONCLUSION

The molecular docking study of eight phytoconstituents contained in *B. rotunda* rhizome with the effector caspases (caspase-3 and-7) has been performed. This study proved that all tested ligands could interact with important residues in the catalytic site of both proteins, however, these ligands, in general, indicate a better docking score with caspase-7 compared to caspase-3. Of all ligands, 4-hydroxypanduratin A and panduratin A showed the best affinity. Additionally, the ADMET prediction also revealed that 4-hydroxypanduratin A and pinostrobin have excellent oral bioavailability. This study could add more insight to the discovery of a novel plant-based nephroprotective agent. However, further studies are still required to confirm its activity.

ACKNOWLEDGEMENT

The authors thank the Rector of Universitas Padjadjaran via the Directorate of Research and Community Engagement for funding the publication fee of this article. This research is in the framework of the first author's dissertation project.

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[RJC-7011/2022]