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HARRINGTONINE FROM CEPHALOTAXUS HARRINGTONIA: MOLECULAR DOCKING STUDIES

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ABSTRACT

Objective: It is known that harringtonine, a natural compound of the cephalotaxine alkaloid class, has anticancer effects. The major goal of this work is to identify the molecular mechanism underlying Harringtonine's anticancer effect. Three different receptors with significant anticancer effects were used in this computational study: Procaspase7 (Pro7), Protein Kinase B (PKB), and Vascular Endothelial Growth Factor Receptor-2 (VEGFR2). **Materials and Methods:** Autodock and Pyrx The molecular docking element of the computational chemistry technique was completed using Vina, and the outcomes were displayed in the 2D interfaces of the discovery studio program. The sorts of chemical bonds that were generated between the target receptor and the ligand as well as the scores for the affinity for binding process were assessed during docking evaluation. **Results:** The docking scores for PKB were -6.7 kcal/mol, VEGFR2 was -7.4 kcal/mol, and procaspase 7 was -7.4 kcal/mol. **Conclusion:** The harringtonine binding affinity score revealed a dominant inhibitory action of the procaspase 7 and PKB receptors in comparison to its native ligand-receptor.

KEYWORDS: Protein kinase B, VEGFR2, Harringtonine, *Cephalotaxus harringtonia*, binding affinity, docking simulation, and procaspase 7.

INTRODUCTION

The evergreen tree *Cephalotaxus harringtonia*., native to southern and northeastern China, has a compound called harringtonine (HT), a cephalotaxine alkaloid with anticancer properties.[1, 2] Among the murine leukemias that it is effective against are the L1210, P388, L615, L7212, and the 6MP-resistant line of L615. Cephalotaxine's structure has been established, as have those of related alkaloids.[3-5] HT and homoharringtonine make up the majority of these alkaloids, and research on murine experimental tumor systems has demonstrated that they are active. In HeLa cells, HT prevents chain start during protein synthesis, which subsequently prevents DNA synthesis.[6]

In clinical trials for acute myeloblastic, acute monoblastic, and erythroleukemic leukemia in China, hastingtine showed signs of therapeutic benefit. Tachycardia was a significant barrier to the usage of harringtonine.[7-8]

Hamburger and Salmon's recently created human tumor stem cell assay offers a potentially helpful method for assessing the effectiveness of novel drugs against human malignancies.[9-11] Recently, multiple institutes have revealed that there is a link between the action of conventional cytotoxic drugs in this system and their therapeutic efficacy against the specific human malignancy.[12,13] In addition, the assay can be used to compare new anticancer medications and structural analogs to the original chemical. In earlier research employing this assay, we discovered that harringtonine demonstrated anticancer action in melanoma, mesothelioma, sarcoma, and adenocarcinomas of unknown origin.[14,15]

Reverse docking is a well-liked technique for simulating atomic-level interactions between a tiny molecule (ligand) and a known macromolecule. Candidate compounds can be evaluated using molecular docking and other bioinformatic methods prior to performing in vitro cell culture-based assays or chemical changes, which can speed up the overall drug development process. Information regarding the activity of the molecule against the targeted receptor can be obtained from the binding affinities of the target drug and the receptor as well as the type of bond formed.[16]

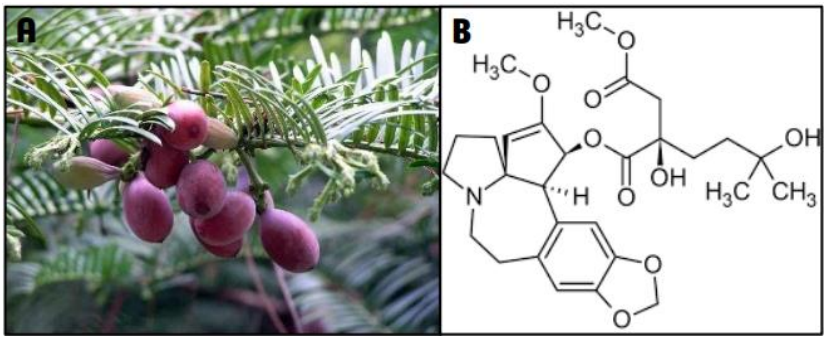

Figure 1: (a) *Cephalotaxus harringtonia;* (b) Harringtonine Structure.

In this article, procaspase 7 (PDB ID: 1K88) and receptor kinase for vascular endothelial growth factor R2 (VEGFR2; PDB ID: 1VR2) are used as "control" proteins. Protein kinase B (PKB; PDB ID: 1GZN), the molecular target of Harringtonine, was chosen as the protein of interest. PKB gets involved in the metabolism of cellular proteins and phosphorylation, two processes involved in cell division, growth, and death. The development of prostatic intraepithelial neoplasia or cancers (such T-cell lymphoma) can be monitored by measuring PKB accumulation.[17] The angiogenesis cycle is regulated by vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs). In addition to being in charge of the pathological angiogenesis that leads to tumor development, VEGFR2 also regulates vascular permeability, cell expression, and antiapoptotic effects.[18] Procaspase 7 is a polypeptide chain with 303 amino acid residues. Caspases are created by first activating and then deactivating the amino acid sequence Ile-Gln-Ala-Asp-2-Ser-Gly. Consequently, 105 short-chain residues and 175 big-chain residues were generated. Procaspase 7 is an enzyme that kills cells.[19]

MATERIALS AND METHODS

Software and Tools

AutoDock Vina 1.1.2, PyRx, and the Visualizer from Discovery Studio.

Preparing the ligands.

Scientific details about the ligands for Harringtonine are presented in table 1.

Table 1: Ligands used in the research.

No	Ligand	Molecular Formula	References
1	Harringtonine	$C_{28}H_{37}NO_9$	[2]
2	RPRTSSF	$C_{39}H_{66}N_{14}O_9$	[12]
3	Cilengitide	$C_{15}H_{27}N_7O_8$	[13]
4	RGDS	$C_{36}H_{59}N_{13}O_{11}$	[14]

Target receptor and ligand preparation

A protein data bank (http://www.rcsb.org) provides the target receptor proteins procaspase 7, receptor kinase R2, growth factor endothelial factor R2, and protein kinase B (PDB ID: 1GZN) available.

On http://pubchem.ncbi.nlm.nih.gov, we can obtain the 3D ligand structure. After that, a PDBQT file format conversion was performed on the ligand file created by energy minimization.

Investigation of Docking Parameters

Using AutoDock Vina 1.1.2, the molecules of hastingtonine and their organic ligands were docked. The ligand movement area can continue to be adaptive and move to the best spot for interactions with the target receptor because of the one-unit grid box spacing. The docking data were evaluated using ratings for binding affinity and the type of interaction made.

RESULTS AND DISCUSSION

Introduction of the Target Protein and Ligand

First-stage energy minimization was finished using Harringtonine and the natural ligands from each receptor, and the file format was modified to PDBQT. The physicochemical properties of ligands and their three-dimensional (3D) structure are shown in Table 2 and Figure 2.

Table 2: The ligand's physiochemical characteristics.

No	Ligand	Molecular Weight (g/mol)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	XLogP3-AA	Minimize Energy
1	Harringtonine	531.6	2	10	0.5	1305.65
2	RPRTSSF	875.0	14	14	-7.7	1277.93
3	Cilengitide	588.65	7	8	-1.0	1216.28
4	RGDS	433.42	9	10	-7.3	390.38

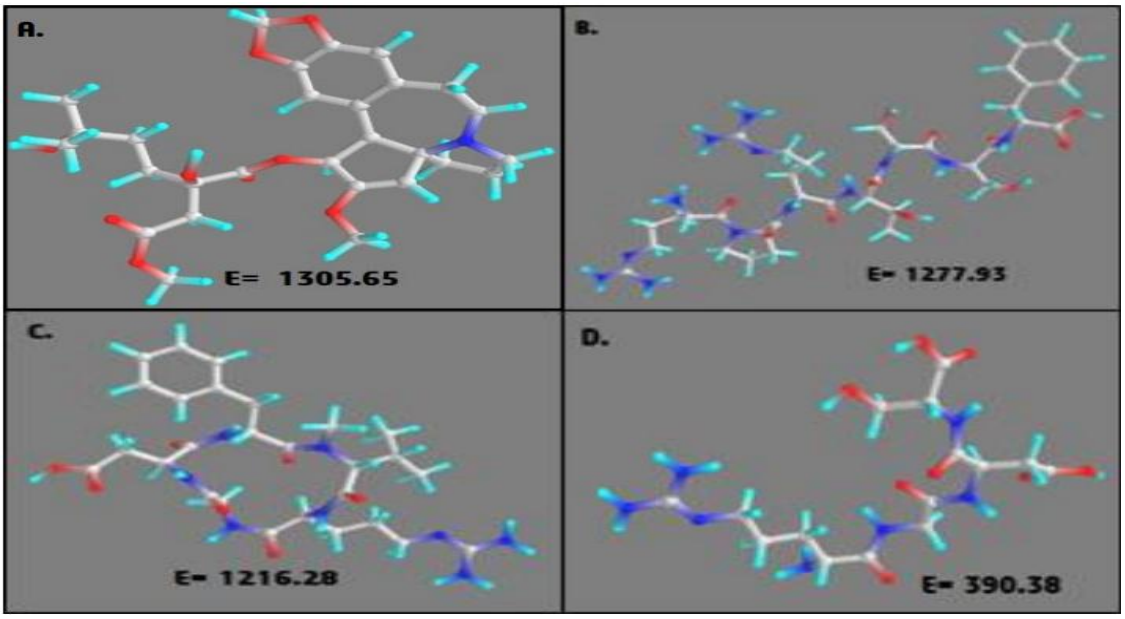

Figure 2. 3D ligand structure and energy minimized results.

(A) Harringtonine, (B). RPRTSSF, (C) Cilengitide, (D) RPRTSSF.

After the receptor protein was converted to PDBQT format, docking simulations were performed on each natural ligand. The binding affinity values for each receptor were then compared to the targets of each receptor and the active component, Harringtonine. Figure 3 depicts the target receptor protein's three-dimensional structure.

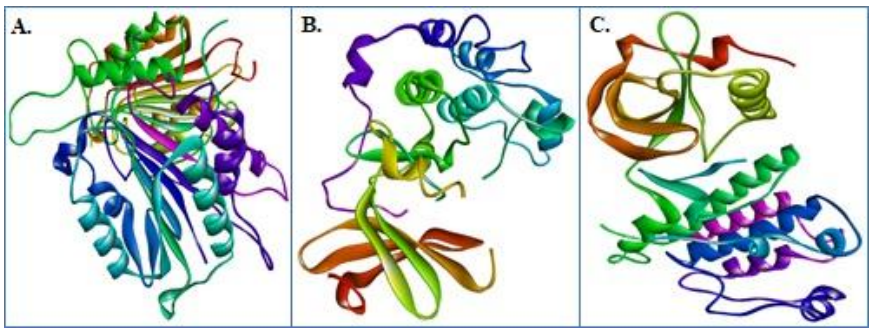

Figure 3: 3D structure of receptor, (A) Procaspase 7, (B) Protein Kinase B, (C) VEGFR2.

Docking analysis of ligand-receptor interactions

Harringtonine's docking to the PKB target receptor

The RPRTSSF ligand and the PKB receptor sites on Arg 274 Phe 163, Thr 162, Val 198, Lys 181, and Gly 295 of Harringtonine interact with each other, according to the outcomes of the two-dimensional molecular docking simulation.

According to the results of two-dimensional molecular docking simulations, the receptor targets Harringtonine's Arg 274 Phe 163, Thr 162, Val 198, Lys 181, and Gly 295 PKB engage with RPRTSSF ligands at the same amino acid. Harringtonine forms 2 hydrogen bonds with PKB receptors on Asp 293, and Lys 181.

In comparison to the RPRSSF ligand, the harringtonine ligand bound at the PKB receptor with a value that was -6.7 kcal/mol greater than RPRTSSF -6.6 kcal/mol.

Harringtonine's docking to the VEGFR2 target receptor

The results of a docking research showed that the harringtonine and cilengitide ligands interacted with the VEGFR2 receptor at its amino acid receptors Arg 1066, Leu 840, Val 848, Ala 866, Asp 1046, and Asp 923 in an equivalent manner. As opposed to the cilengitide ligand, which made three hydrogen bonds at the receptor, harringtonine only created one hydrogen bond at Asn 923.

Harringtonine's binding affinities were weaker than those of cilengitide, coming in at only -7.2 kcal/mol than cilengitide -8.2 kcal/mol.

Harringtonine's docking to the Procaspase 7 receptor

The procaspase 7 receptors on the procaspase 7 receptor are identical for the RGDS and Harringtonine ligands, as predicted by docking the two ligands to the receptor on Thr 90, Arg 87, Arg 187, His 144, and Ser 239 of the receptor. The RGDS ligand and the receptor established six hydrogen bonds, which were composed of the amino acids Ser 231, Arg 187, Asp 93, Ser 239, Arg 233, and Arg 87. Three hydrogen bonds are formed on Arg 187, Arg 233, and Asn 88 by the Harringtonine ligand and receptor.

When compared to the RGDS ligand, which exhibited binding affinities of -6.9 kcal/mol and -7.4 kcal/mol, respectively, the docking results indicated that the Harringtonine ligand had a higher binding affinity.

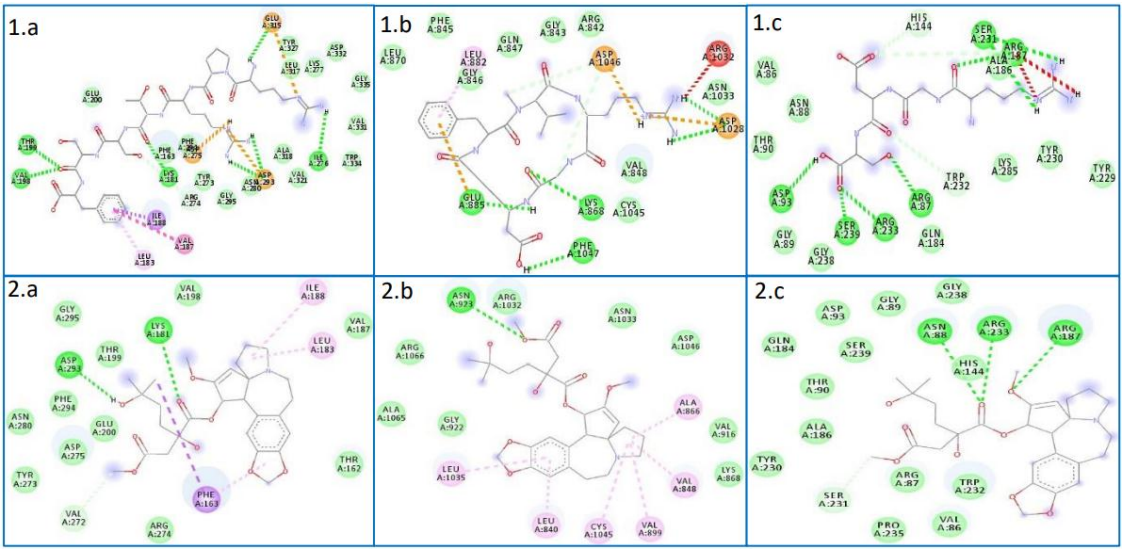

Figure 4: Interaction of ligands and target receptors: (1.a) RPRTSSF interaction with PKB; (1.b) Cilengitide interaction with VEGFR2; (1.c) RGDS interaction with Procaspase7; (2.a) Harringtonine with PKB; (2.b) Harringtonine with VEGFR2; (2.c) Harringtonine with Procaspase 7.

Table 4: Comparative binding affisnity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Vascular Endothelial Growth Factor R2	Cilengitide Harringtonine	-8.2 -7.2
2	Procaspase 7	RGDS Harringtonine	-6.9 -7.4
3	Protein Kinase B	RPRTSSF Harringtonine	-6.6 -6.7

CONCLUSIONS

According to data from docking simulations, Harringtonine molecules showed higher binding affinity values than the native ligands of procaspase 7receptor and protein kinase b. This demonstrates that the inhibitory mechanism of Harringtonine, an anticancer drug, involves the receptor.

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