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INTERACTIONS OF SHIKONIN A POTENT ANTITUMOR DRUG WITH ITS KNOWN PROTEIN TARGETS

Shruthi Vijayakumar, Adhithya Ragunathan, Lokesh Ravi*

School of Biosciences and Technology, VIT University, Vellore-632014

ABSTRACT: In this in silico study, we have validated the interaction of Shikonin with various targets using AutoDock 4. Shikonin, a naphthoquinone was found to be a promising anti-cancer drug. It was found to be a poor inducer of drug resistance and so it is potentially regarded as a powerful alternative in the case of cancer cells which gain resistance to conventional anti-cancer drugs. Various researchers have proved that Shikonin targets multiple proteins namely DNA Topoisomerase 1, Polo Like Kinase 1, phosphorylating proteins in ERK pathway, Proteasomes, anti-apoptotic proteins and PKM2. The poor drug resistance induction nature of Shikonin was believed to be due to its ability to target these multiple proteins. This in-silico study serves as a further validation for Shikonin's mode of action. The most significant interaction of Shikonin was found to be against PKM2 with a free binding energy of -8.63 Kcal/Mol and Ki value of 473.6 nM with 6 hydrogen bonds.

Keywords: Shikonin, PKM2, AutoDock, PyMOL, BCL-2.

***Corresponding Author: Dr. Lokesh Ravi** Ph. D.

School of Biosciences and Technology, VIT University, Vellore-632014

*Email Address: lokesh.ravi@vit.ac.in

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1. INTRODUCTION

Cancer has been one of the leading causes of death in the past decade. In 2012 the number of new cancer cases has increased to 14.1 million globally [1]. This has led to a commensurate increase in the demand for drugs. One of the major obstacles interfering with the efficacy of cancer chemotherapy is cancer drug resistance. The cancer cells adapt to chemotherapeutic agents. This results in the stimulation from the drugs being attenuated by the cancer cells [2]. The common reason for procuring resistance to a broad range of anticancer drugs is due to the expression of energy-dependent transporters that detect and eject anticancer drugs from cells [3]. Cellular factors could also be a cause of drug resistance. This includes activation of DNA repair, activation of detoxification system, blocked apoptosis [2]. To circumvent this problem drugs which are toxic to cancer cells but incompetent to resistance is desired. Shikonin, a naphthoquinone extracted from the roots of *Lithospermum erythrorhizon* which is traditionally a Chinese herb is a potent antioxidant with anti-inflammatory, antiviral and cancer-preventing properties. Shikonin exhibits significant cytotoxic activity against multiple cancer cell types in vitro and in vivo, and a number of studies have previously established a potential role for shikonin as a candidate therapeutic agent in the treatment of cancer.[4]. Shikonin and its analogues exhibit anticancer activity by inhibiting a variety of proteins. Those proteins are Topoisomerase I, polo-like kinase1 (PLK1), phosphorylating proteins in the Ras-Raf-MEK-ERK pathway, 20S proteasome, 26S proteasome, Bcl-2, Bcl-xl, PKM2 [5-9]. Topoisomerase enzymes alter the topological states of DNA which is required for critical cellular processes like DNA transcription and replication [10]. Polo-like kinases (Plks) play key roles during multiple stages of mitosis in addition to less well understood roles during G1/S and in response to DNA damage [11]. The Ras-Raf-ERK kinase (MEK)-extracellular-signal-regulated kinase (ERK) cascade couples signals from cell surface receptors to transcription factors. This regulates gene expression. Depending upon the stimulus and cell type, this pathway transmits signals, which results in the prevention or induction of apoptosis or cell cycle progression [12]. The proteasome is essential in the ATP-dependent proteolytic pathway and is responsible for the degradation of most cellular proteins. The 20S (700-kDa) proteasome contains multiple peptidase activities that mainly function through a proteolytic mechanism involving a threonine active site. The 26S (2000-kDa) complex degrades ubiquitinated proteins. [13]. Bcl-2 and bcl-xl proteins are

the regulators of apoptosis. They also have critical roles involved in autophagy, calcium handling and mitochondrial dynamics [14, 15]. Pyruvate kinase catalyzes the last step within glycolysis, the dephosphorylation of phosphoenolpyruvate to pyruvate, and is responsible for net ATP production within the glycolytic sequence. In contrast to mitochondrial respiration, energy regeneration by pyruvate kinase is independent from oxygen supply and allows survival of the organs under hypoxic conditions often found in solid tumors [16].

2. MATERIALS AND METHODS

3D structures of the target proteins were downloaded from RCSB PDB website (http://www.rcsb.org/pdb). DNA Topoisomerase 1 (1TL8), Polo like Kinase 1 (4O56), 20S Proteasome (5A0Q), 26S Proteasome (5A5B), K-RAS (4OBE), RAF-1 (3OMV), MEK-1 (3VVH), MEK-2 (1S91), BCL-2 (2XA0), BCLXL (4TUH) and PKM2 (3SRH).The ligand molecule Shikonin (PubChem ID: 479503) was downloaded from the Pubchem website (http://pubchem.ncbi.nlm.nih.gov). Meta pocket analysis was done to identify binding sites in the target proteins [17]. AutoDock 4.2.1 was used to perform the protein-ligand docking analysis [18]. The protein molecule was edited prior to docking, by removing all non-aminoacid residues in the structure file. In AutoDock the protein was introduced, and editing was done by removing water molecules, adding polar hydrogen atoms and merging non polar hydrogen atoms. Kolman charges were added and the editing was saved. Then ligand molecule was introduced. Grid box was set to cover the binding site for each of the target proteins. Docking was performed by adopting Lamarckian genetic algorithm. PyMOL [19] and LigPlus [20] software were used to analyze the docking results obtained from AutoDock.

3. RESULTS AND DISCUSSION

Interactions with PKM2

Shikonin demonstrated highest affinity towards PKM2 (Pyruvate Kinase M-2) with a free binding energy of -8.63Kcal/mol, Ki value of 473.6nM and formed 6 hydrogen bonds. The hydrogen bonds were formed with Leu-352 (1.9Å), Lys-310 (2.1Å), Tyr-389 (1.8Å), Ala-387 (2.0Å; 3.4Å), and His-28 (2.2Å). Among all the other docking interactions, shikonin displayed highest significance towards PKM2. Interaction between shikonin and PKM2 is given in Figure.1.

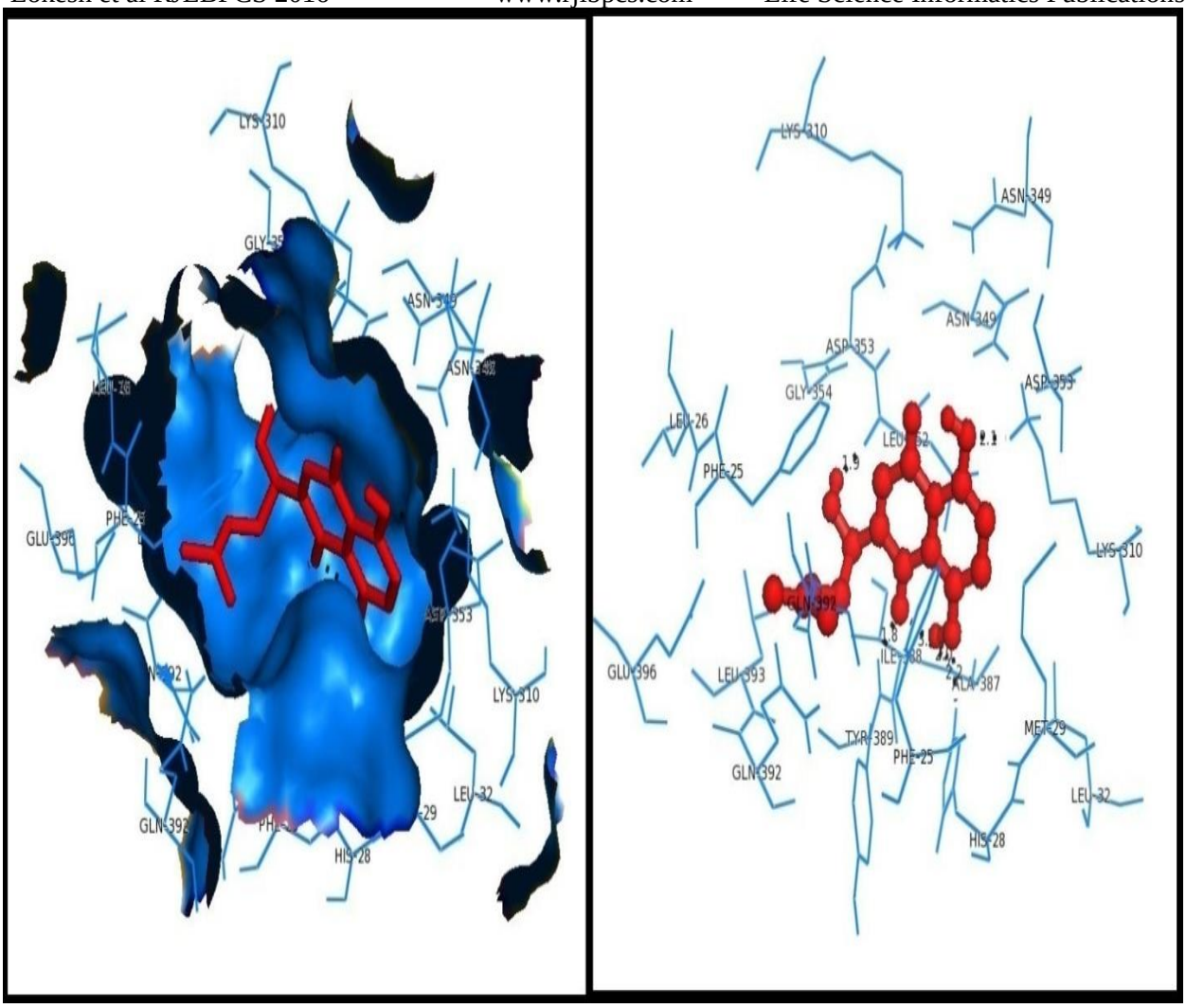

Figure.1: Interactions between shikonin and PKM2

Interaction with Proteins in ERK Phosphorylation

Shikonin is reported to inhibit the phosphorylation of ERK protein. Among the four major ERK phosphorylating proteins, shikonin showed highest significance towards MEK1 protein with -7.44 Kcal/mol free binding energy and Ki value of 187.26nM with 7 hydrogen bonds. The 7 hydrogen bonds were found to be with Val-211 (1.9Å), Phe-209 (2.3Å, 2.6Å), Asp-208 (2.0Å, 2.3Å, 2.6Å) and Lys-97 (1.8Å). Shikonin also displayed significant affinity towards KRAS (-7.78Kcal/mol) and MEK2 (6.99Kcal/mol) proteins, as the Ki value were below 10µM (2µM and 7.52µM respectively). The least significance was observed with RAF1 protein which displayed a free

binding energy of -6.76 Kcal/mol and a Ki value of 11.12μM while forming 3 hydrogen bonds. The docking result for all the four proteins is tabulated in Table.1.

Target proteins	Binding energy (Kcal/Mol)	Inhibition Constant	No. of hydrogen bonds
MEK1	-7.44	3.5 μM	4
KRas	-7.21	5.19 μM	5
MEK2	-6.99	7.52 μM	4
Raf1	-6.76	11.12 μM	3

Table.1: Interactions of shikonin with ERK phosphorylating proteins

Interaction With Proteins That Are Directly Inhibited By Shikonin

Shikonin is reported to inhibit some proteins directly. Among the four known targets of shikonin, it showed highest significance towards DNA Topoisomerase 1 protein with -7.25 Kcal/mol free binding energy and Ki value of 4.87 μM with 8 hydrogen bonds. Shikonin also displayed significant affinity towards Polo Like Kinase 1 (-7.12 Kcal/mol), 26S Proteasomes (-7 Kcal/mol) proteins and 20S Proteasomes (-6.96 Kcal/mol) as the Ki value were below 10μM (6 μM, 7.36 μM and 7.89 μM respectively). The docking results of these proteins are tabulated in Table.2.

Target proteins	Binding energy (Kcal/Mol)	Inhibition Constant	No. of hydrogen bonds
DNA Topo isomerase 1	-7.25	4.87 μM	8
Polo like Kinase 1	-7.12	6 μM	5
26S Proteasomes	-7	7.36 μM	5
20S Proteasomes	-6.96	7.89 μM	8

Table.2: Interactions of shikonin with known inhibitors

Interaction With Anti Apoptotic Proteins

Shikonin showed highest binding affinity against BCL2 with a binding energy of -8.4 Kcal/Mol and a Ki value of 697.06 nM by forming 6 hydrogen bonds. The 6 hydrogen bonds were found to be Arg-127 (1.6Å), Phe-124 (2.9Å), Gly-128 (2.2Å), Pro-123 (1.9Å), Ala-126(2.9Å), Arg-127 (1.6Å). It is the second best interaction among the studied proteins in this work. It also displayed significant interaction against BCL-XL with a free binding energy of -7.48 Kcal/Mol and Ki

value of 3.31 μM forming 1 hydrogen bond. This suggests that, BCL2 protein could be the second most likely target for shikonin next to PKM2. A result of the docking interactions of shikonin with anti-apoptotic proteins is given in Table.3.

Target proteins	Binding energy (Kcal/Mol)	Inhibition Constant	No. of hydrogen bonds
Bcl2	-8.4	697.06 nM	6
Bclxl	-7.48	3.31 μM	1

Table.3: Interactions of shikonin with anti-apoptotic proteins

4. CONCLUSION

Shikonin is known for its excellent anti-cancer activity. J Chen et al., in 2011 demonstrated that, shikonin inhibits PKM2, a protein responsible for energy generation through glycolysis. Inhibition of PKM2 eventually leads to energy depletion, thus preventing further cell proliferation [9]. C Baily in 2000 found that DNA topoisomerase 1 is inhibited by Shikonin [5]. Thus shikonin is also proven to inhibit DNA replication. Y Masuda et al., in 2003 displayed that Shikonin inhibits Polo Like Kinase 1 [6]. S Kim et al., in 2001 found that Shikonin inhibits the phosphorylation of ERK by inhibiting Ras, Raf, MEK proteins [7]. H Yang et al., in 2009 displayed that Shikonin inhibits both 20S and 26S proteasomes [8]. From the obtained results, it is clear that, shikonin had second most significant interaction with BCL2 protein, one of the most preferred drug targets for anti-cancer activity. Among the many proteins that are targeted by shikonin, BCL2 could be one the most preferred or highly significant drug target, next to PKM2. Thus, by inhibiting and regulating multiple proteins, shikonin exerts its anticancer activity. One of the prominent features of Shikonin is that it is a poor inducer of cancer drug resistance. This was reported by W Hao et al., in 2013 that the poor cancer drug resistance induction of Shikonin is due to its ability to target multiple proteins. In this study, some of the proven protein targets for shikonin have been analyzed for the interaction site. This also provides guidelines that; shikonin could also act as an anti-apoptotic protein inhibitor. This in-silico analysis gives further understanding of how shikonin interacts with the cancer drug target proteins.

REFERNCES

1. UK research centre 2015
2. Hao, W.; Jiansheng, X.; Qiangrong, P.; Beibei, W.; Danqing, H.; Xun, H.; Anticancer Agent Shikonin Is an Incompetent Inducer of Cancer Drug Resistance. PLoS One, v.8, n.1, p.1-7, 2013.
3. Gottesman, M.M.; Mechanisms of cancer drug resistance. Annu Rev Med, v.53, p.615-627, 2002.
4. Hao, Z.; Qian, J.; Yang, J.; Shikonin induces apoptosis and inhibits migration of ovarian carcinoma cells by inhibiting the phosphorylation of Src and FAK. OncolLett, v.9, n.2, p.629-633, 2015.
5. Bailly C (2000) Topoisomerase I poisons and suppressors as anticancer drugs. Curr Med Chem 7: 39–58.
6. Masuda Y, Nishida A, Hori K, Hirabayashi T, Kajimoto S, et al. (2003) Betahydroxyisovalerylshikonin induces apoptosis in human leukemia cells by inhibiting the activity of a polo-like kinase 1 (PLK1). Oncogene 22: 1012–1023.
7. Kim SH, Kang IC, Yoon TJ, Park YM, Kang KS, et al. (2001) Antitumor activities of a newly synthesized shikonin derivative, 2-hyim-DMNQ-S-33. Cancer Lett 172: 171–175.
8. Yang H, Zhou P, Huang H, Chen D, Ma N, et al. (2009) Shikonin exerts antitumor activity via proteasome inhibition and cell death induction in vitro and in vivo. Int J Cancer 124: 2450–2459.
9. Chen J, Xie J, Jiang Z, Wang B, Wang Y, et al. (2011) Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2. Oncogene.
10. LI, K.W.; Randall, K.J.; Sidney, M.H.; Inhibition of topoisomerase I function by nitidine and fagaronine. Chem. Res. Toxicol, v.6, n.6, p.813–818, 1993.
11. Lowery, D.M.; Lim, D.; Yaffe, M.B.; Structure and function of Polo-like kinases. Oncogene, v.24, n.2, p.248-259, 2005.
12. Chang, F.; Steelman, L.S.; Lee, J.T.; Shelton, J.G.; Navolanic, P.M.; Blalock, W.L.; Franklin, R.A.; Mccubrey, J.A.; Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention.

Leukemia, v.17, n.7, p.1263-1293, 2003.

13. Coux, O.; Tanaka, K.; Goldberg, A.L.; Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem, v.65, p.801-847, 1996.
14. HARDWICK, J.M.; SOANE, L.; Multiple functions of BCL-2 family proteins. Cold Spring HarbPerspectBiol, v.5, n.2, p.1-22, 2013.
15. Yelena, M.J.; Courtney, G.S.; Anuja, C.; Ningli, C.; Erinn, L.S.; Linda, Z.P.; David, A.; Michael, K.; Elizabeth, Y.; Bcl-xL/Bcl-2 coordinately regulates apoptosis, cell cycle arrest and cell cycle entry. EMBO J, v.22, n.20, p.5459–5470, 2003.
16. Peter, Vaupela.; Louis, Harrison.; Tumor Hypoxia: Causative Factors, Compensatory Mechanisms, and Cellular Response. Oncologist, v.9 p.4-9, 2004.
17. Zhang, Z.; LI, Y.; Lin, B.; Schroeder, M.; Huang, B.; Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction. Bioinformatics, v.27, n.15, p.2083-2088, 2011.
18. Nidhi, S.; Sudandiradoss, C.; Jayanthi, A.; Screening of Furanone in Cucurbitamelo and Evaluation of its Bioactive Potential Using In Silico Studies. Interdiscip Sci, p.1-8, 2016.
19. Baugh, E.H.; Lyskov, S.; Weitzner, B.D.; Gray, J.J.; Real-time PyMOL visualization for Rosetta and PyRosetta. PLoS One, v.6, n.8, p.1-5, 2011
20. Sahu, C.; Sen, K.; Pakhira, S.; Mondal, B.; Das, A.K.; Binding affinity of substituted ureido-benzenesulfonamide ligands to the carbonic anhydrase receptor: a theoretical study of enzyme inhibition. J Comput Chem.v.34, n.22, p.1907-1916, 2013.