Volume-4, Issue-7, July-2015 • ISSN No 2277 - 8160

Research Paper Engineering

Structure Based Virtual Screening For Identification of Novel Survivn Inhibitor For Anticancer

*** Ganesan Vijayansiva** Department of Biotechnology, Madras University, Guindy Campus Chennai – 600025, Tamil Nadu -India. *Corresponding Author.

Ramarajan Sivasankaran Department of Biotechnology, Madras University, Guindy Campus Chennai – 600025, Tamil Nadu -India

ABSTRACT *Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors. Biologically, survivin has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis. Because of its upregulation in malignancy and its key role in apoptosis, proliferation and angiogenesis, survivin is currently attracting considerable attention as a new target for anti-cancer therapies. In these studies to identify potential inhibitor through virtual screening, molecular docking and ADME toxicity analysis revealed 2 hits compound with good docking score and included in ADME properties.*

KEYWORDS : ***Anticancer, Survivin*** **, Virtual screening, docking, ADMET.**

1. Introduction

Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, regulates two essential cellular processes, i.e., it inhibits apoptosis and promotes cell proliferation. Although expressed at high levels during fetal development, survivin is rarely expressed in normal healthy adult tissues. It is however, upregulated in the majority of cancers (1, 2). Because of this upregulation in malignancy, and its functional involvement in apoptosis as well as proliferation, survivin is currently attracting considerable interest both as a potential cancer biomarker3 and as a new target for cancer treatment.1 The aim of this article is to discuss approaches currently under investigation in targeting survivin for the treatment of cancer. As mentioned above, survivin is rarely expressed in normal differentiated tissues but is upregulated in the majority of malignancies. Early studies using SAGE analysis showed that survivin was the top fourth 'transcriptome' in a number of common human cancers. These early findings have been largely confirmed using immunohistochemistry and RT–PCR (3).The increased expression of survivin in cancer cells is thought to occur through upregulation of gene transcription, rather than stabilization of protein. Indeed, several tumor-associated signaling proteins, such as c-myc and STAT-39(4,5) have been shown to increase survivin expression while the tumor suppressors genes, p53,(6) APC(7) and PTEN(8) have been shown to decrease its expression.so survivin is an attractive drug target for cancer treatment. We have performed the virtual screening and molecular docking studies of the active 2 compounds for better understanding of the drug-receptor interaction.

2. MATERIAL METHODS

2.1 Protein Preparation

Energy minimisation of the resulting protein was carried out by using Maestro 9.0.111 protein preparation wizard (Schrodinger, LLC, 2008, New York, NY). The energy minimisation was carried out at the default cut off RMSD value of 0.30 Å using OPLS 2001 force field [9]. The possible conformation of the refined protein was obtained using procheck analysis visualized with the aid of Ramachandran plot [19, 20] by checking the dihedral Phi and Psi angles of amino acid residues.

2.2 Active Site

Identification and characterization of binding site is the key step in structure based drug design [10]. The active site region of the protein is identified by Castp [11]. This server analytically furnishes the area and the volume at the probable active site of each pocket to envisage the binding site.

2.4 Receptor Grid Generation

The scoring grid was generated using a box size of 30 ˚A ×30 ˚A ×30 ˚A and centered on the centroid within a box of dimension 27 ˚A ×16 ˚A ×46 ˚A that encloses the entire groove near the active site to fit the ligands[12]

2.5 Virtual Screening

Virtual screening has become a promising tool for identifying active lead/active compounds and has combined with the pipeline of drug discovery in most pharmaceutical companies [14]. Glide [15] module has been used for all the docking protocol. Among 72,000 small molecules of Zinc database that compounds have been used for screening and get less toxic compounds from the hits. The ligands were processed with the Lig Prep program to assign the suitable protonation states at physiological pH= 7.2±0.2. Conformer generation was carried out with the ConfGen torsional sampling and Ligand docking used OPLS_2005 force field. The van der Walls radii were scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties. There are three modes to screen the compound such as by HTVS, SP and XP in Glide modeule

2.6 Induce fit Docking

The protein structure of Survivin is applied with the induced-fit docking (IFD) method in the Schrodinger software suite [16]. All ligands were prepared using LigPrep and were optimized with the OPLS force field in the Macro Model module in Schrodinger[17]Ligands were docked to the rigid protein using the soften-potential docking in the Glide program with the vander Waals radii scaling of 0.8 for the proteins. The resulting top 20 poses of ligands were used to the protein plasticity using the Prime program in the Schrodinger suite. Residues having at least one atom within 5A˚ of any of the 20 ligand poses were subject to a conformational search and minimization while residues outside the zone were held fixed. In this way, the flexibility of proteins was taken into account [27].

2.7 ADME or pharmacokinetic predictions of best fit molecules

The ligands identified in IFD docked mode were subjected to predict the pharmacokinetic properties using Qikprop module of Schrodinger software suite[18]. Structures with unfavorable absorption, distribution, metabolism and elimination have been identified as the major cause of failure of candidate molecules in drug development. So there is an early prediction of ADME properties, with the objective of increasing the success rate of compounds reaching further stages of the development. Glide score, glide energy, visual inspection and ADME predictions were used as filtering in screening 2 hits for Survivin.

3. RESULT AND DISCUSSION

3. 1Virtual screening and docking studies

High-throughput screening is a computational technique to find potent small molecules against protein targets of Survivin. Various parameters such as Glide score, Glide energy and hydrogen bond interactions are used to assess which conformation or binding site orientation is best complement in the protein-binding site. Two main aspects were taken into account to assess the quality of docking methods: (i) Docking accuracy, which identifies the true binding mode of the ligand to the target protein, and (ii) Screening enrichment, which is a measurement of correlation between docking meth-

od and true binding ligands rather than random compound selection.

As explained in materials and methods we calculated our calculations in HTVS first, SP second and then XP mode. We filtered out 1,500 compounds from the HTVS process against the target GlgE protein. In the second stage compounds with Glide score > -6.00 were screened for Glide SP docking. In the next stage, only compounds with Glide SP score > -8.00 were screened for Glide XP mode docking. This third run identified 5 hits with best glide score.

3.2 Induce fit Docking Analysis

With the aim of investigating dynamic behavior of the active site during the binding of ligand, we performed IFD experiments on best ranked from virtual screening compound using Survivin based on glide scores. Glide score is an empirical scoring function that considers the energy contribution, the effects of the hydrophobicity as well as the hydrogen bonding and penalizes the steric clashes [29]. The best 2 compounds out of 1500 compounds screened and their corresponding chemical names are: **compound 1** (ZINC03830432): (6R,7R)-7-((R)-2-(4-Ethyl-2,3-dioxo-1-piperazinylcarboxamido)-2-(4-hydroxyphenyl)acetamido)-3-((1-methyl-1H-tetrazol-5-yl) thiomethyl)-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-en-2-carbonsaeure, **compound 2** (ZINC19594535): (6R,7R)-7-((R)-2-Amino-2-(p-hydroxyphenyl)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid.

3.3 Predicted ADME toxicity properties

QikProp Program predicted pharmacokinetic of the ligands. Predicted ADME properties values (Table 2) were analyzed with the recommended values such as Stars, QPlogHERG which are essential for drug design and The Lipinski's rule of 5, it is a rule of thumb to determine if a chemical compound with a certain biological activity[30]. All the Compounds that satisfy ADME properties are considered drug like. All the predicted ADME values are in the accepted ranges. Hence our in-silico analysis can conclude that these ligands can be act as survivin inhibitor.

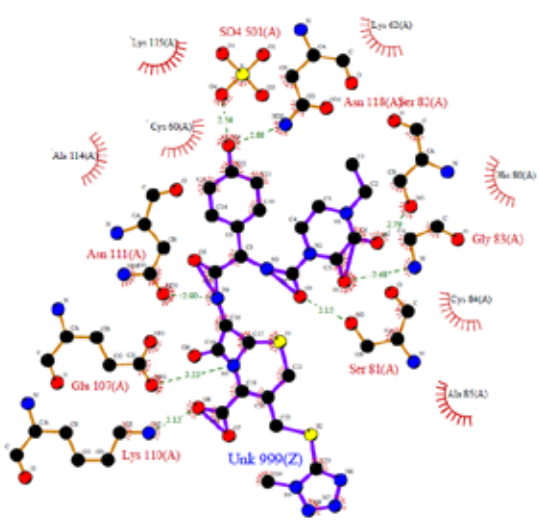
Table 1: .Binding interactions between survivin with hits compounds

S.no	Compounds name	Gide score	Glide energy
1	ZINC19594535	-11.821054	-66.173682
2	ZINC03830432	-7.721306	-64.082500
3	ZINC48056990	-7.36959	-43.316314
4	ZINC48056993	-7.13903	-48.266853
5	ZINC40163389	-7.98193	-43.156586
6	ZINC04349038	-7.05837	44.470794

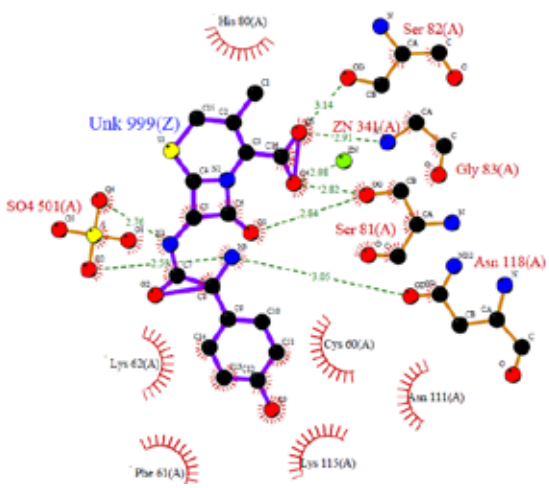
Table 2. ADME or pharmacokinetic predictions of the top five docked molecules

Compound name	H-bond donor	H-bond acceptor	PSA	Mol MW	#stars	QPlogBB	QPlog HERG	QPP Caco	QPP MDCK	QPpolrz
ZINC19594535	4	8	150	363	0	-1.72	-2.037	1.593	1.437	32.78
ZINC03830432	2	15	259	645	5	-4.76	-1.83	0.226	0.253	58.90
ZINC40163389	2	15	233	594	2	-3.002	-2.67	0.312	1.112	56.19
ZINC48056990	5	17	226	385	5	-3.044	-0.412	0.234	0.095	26.64
ZINC48056993	5	17	223	384	5	-3.455	-0.370	0.203	0.81	28.22
ZINC04349038	4	11	200	254	3	-2.405	-3.866	1.31	0.482	39.22

ZINC03830432- 6R,7R)-7-((R)-2-(4-Ethyl-2,3-dioxo-1-piperazinylcarboxamido)-2-(4-hydroxyphenyl)acetamido)-3-((1-methyl-1H-tetrazol-5-yl)thiomethyl)-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-en-2-carbonsaeure



ZINC19594535 - (6R,7R)-7-((R)-2-Amino-2-(p-hydroxyphenyl) acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid.



4. Conclusions

The present study was carried out to generate potential inhibitors for Survivin. A virtual screening of suitable drugs was performed, which identified **ZINC03830432** and **ZINC19594535** compounds proven to result in improved inhibition of cancer and active amino acid residues, which will be useful in designing other potent drugs and drug analogs. This study provides new insights into the identification of drugs in the in vitro laboratory. The novel molecular entities have the potential as leads which certainly aid in designing anti cancer mole-

cules in short span of time. As whole results throw light for future development of more potent and drug like inhibitors for Survivin.

REFERENCES

1. Altieri DC. Survivin, cancer networks and pathway-directed drug discovery.Nat Rev Cancer 2008;81:61–70. | 2. Li F. Survivin study: what is the next wave? J Cell Physiol 2003;1971:8–29. | 3. Duffy MJ, O'Donovan N, Brennan DJ, Gallagher WM, Ryan BM. Survivin: apromising tumor biomarker. Cancer Lett 2007;2491:49–60. | 4. Aoki Y, Feldman GM, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. Blood 2003;1014:1535–42. | 5. Sommer KW, Schamberger CJ, Schmidt GE, Sasgary S, Cerni C. Inhibitor of apoptosis protein (IAP) survivin is upregulated by oncogenic c–H–Ras. Oncogene 2003;2227:4266–80. | 6. Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. J Biol Chem 2002;2775:3247–57. | 7. Zhang T, Otevrel T, Gao Z, et al. Evidence that APC regulates surviving expression: a possible mechanism contributing to the stem cell origin of colon cancer. Cancer Res 2001;6124:8664–7. | 8. Koul D, Takada Y, Shen R, Aggarwal BB, Yung WK. PTEN enhances TNF-induced apoptosis through modulation of nuclear factor-kappaB signaling pathway in human glioma cells. Biochem Biophys Res Commun 2006;3502:463–71. | 8.Salam NK, Nuti R and Sherman WJ. Novel method for generating structure-based pharmacophores using energetic analysis. Chem. Inf. Model, 2009; 49: 2356–2368. | 9.Laskowski RA, Rullmannn JA, MacArthur MW, Kaptein R and Thornton J.M AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J. Biomol. NMR, 1996; 8: 477–486. | 10.Laskowsky RA, MacArthur MW, Moss DS, and Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures J. Appl. Crystallography,1993; 26: 283–291. | 11.Brindha V, Saravanan A and Manimekalai R. Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from Terminalia arjuna. Ind. J. Sci. Technol, 2009; 2: 22–26. | 12.Laurie AT and Jackson R. Q-SiteFinder: an energy-based method for the prediction of Protein–ligand binding sites .Bioinformatics 2005; 21: 1908–1916. | 13.Kawatkar S, Wang H, Czerminski R and McCarthy DJ. Virtual fragment screening: an exploration of various docking and scoring protocols for fragments using Glide. J. Comput. Aided Mol. Des, 2009; 23: 527–539. | 14.Louise-May S, Yang W, Nie X, Liu D, Deshpande MS, Phadke AS, Huang M and Agarwal A. Discovery of novel dialkyl substituted thiophene inhibitors of HCV by in silico screening of the NS5B RdRp Biol. Med. Chem. Lett, 2007; 17: 3905–3909. | 15.Friesner RA, Banks JL, Murphy R B, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shaw DE, Shelley M, Perry JK, Francis P and Shenkin PS. Glide. Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J. Med. Chem, 2004; 47: 1739-1749. | 16.Stahl M, Guba W and Kansy M . Integrating molecular design resources within modern drug discovery research: the Roche experience. Drug Discov Today, 2006; 11: 326–333. | 17.Schrodinger, LLC: Portland, OR, 2007, Web address: www.schrodinger.com. | 18.QikProp, version 3.4, Schrödinger, LLC, New York, NY, 2011