Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):352-357

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Molecular docking analysis of anticancerous interactions of salinomycin

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ABSTRACT

To study the mode of action of salinomycin for its anticancer activity, by analysing salinomycin interactions with DNA, potassium channel and Wnt signalling proteins. PatchDock was used to study the Protein-Ligand interactions initially and detailed analysis were performed with AutoDock 4.2.1. DNA-Ligand interaction were also performed with AutoDock. The docking results were interpreted using PyMol and LigPlot+ softwares. Among the studied DNA types, salinomycin showed highest affinity to classic intercalation sites with a free binding energy value of -7.33 Kcal/Mol. Salinomycin also demonstrated good affinity towards potassium channel protein with binding energy of -6.84Kcal/Mol in AutoDock and ACE value of -435.0Kcal/Mol in PatchDock. Finally, among the studied Wnt/β-catenin signalling proteins, salinomycin showed highest affinity towards Protein Kinase A (PKA) with binding energy of -7.94Kcal/Mol with an inhibition constant of 1.53µM and towards Casein Kinase 1 gamma (CK1γ) with binding energy of -7.81Kcal/Mol with an inhibition constant of 5.45µM. This molecular docking analysis study concludes that, the mode of action of salinomycin for its ability to reduce the level of β-catenin and LRP-6 could be due to synergistic inhibition of PKA and CK1γ. Additionally, if salinomycin is to cause DNA damage directly, it would do so at classical intercalation sites. As an ionophore, salinomycin demonstrated good affinity towards human potassium channel. However, the most significant report of this study is that, anticancer activity of salinomycin by blocking the Wnt/β-catenin signal could potentially be achieved by inhibiting the activity of phosphorylating proteins PKA and CK1γ.

Keywords: AutoDock, DNA-Ligand docking, LigPlus, PatchDock, Protein-Ligand docking, PyMOL, Salinomycin, Wnt/β-catenin signalling.

INTRODUCTION

Salinomycin, a well known monocarboxylic polyether antibiotic, is a well known anticoccidant used in poultry feeds, which has been identified to be a potential anti-cancer drug [1, 2]. It is believed to be a novel compound since it is capable of killing apoptosis resistant cancer cells and cancer stem cells [1]. This monocarboxylic polyether antibiotic with a molecular formula of C42H70O11 was first obtained from *Streptomyces albus* [3, 4]. Since this group of molecules are ionophoric in nature with major affinity to potassium ions, it is believed that, salinomycin interacts with potassium channels and promotes the potassium ion efflux from mitochondria and cytoplasm [4 - 6].
At present, cancer stem cells are one of the major challenges in cancer treatment; these cancer stem cells are capable of self-renovation and tumour initiation; cancer stem cells also have well defined anti-apoptotic proteins and up-regulated drug-efflux pumps [7 - 11]. Report by Gupta et.al, 2009 proved that, salinomycin is capable of destroying cancer stem cells, thus marking this poultry feed antibiotic as a novel anticancer agent [1]. Review on salinomycin done by Shuang Zhou et.al, 2013, summarized the anticancer reports on salinomycin ever since its first report [11]. In this review, the reviewers summarized three possible mechanism of action of salinomycin for its anticancer activity, based on previous in-vitro reports. These three mechanisms include, i) DNA damage; ii) Potassium ion efflux and iii) Wnt/β-catenin signal inhibition [11].

Wnt signalling pathway is one of the evolutionarily conserved pathway and is important for cell fate determination, polarity, motility and stem cell renewal; it has two major types i) β-catenin dependant and ii) β-catenin independent; Fz receptor is a major receptor and LRP-6 is an important co-receptor in the Wnt signal complex; disturbance due to mutation in Wnt/β-catenin pathway would lead to cancer [12 - 14]. Extensive study by Desheng Lu, et.al, 2011, reported that, upon salinomycin treatment to cancer cells, the phosphorylation of LRP-6 and β-catenin were prevented and thus, the cancer cells underwent apoptosis due to the break in the chain of Wnt/β-catenin signalling pathway [15]. Christof Niehrs and Jinlong Shen 2010, reported the list of kinase proteins involved in phosphorylation of LRP-6, i.e., GSK3, CK1γ, GRK5/6 and PKA [16]. Shin-ichiro Hino, et.al, 2005, reported that, Protein Kinase-A (PKA) is an important phosphorylation protein for β-catenin [17]. Based on these reports, this in-silico molecular docking study to analyse these interactions was performed, to identify the mode of action of salinomycin as anticancer agent.

EXPERIMENTAL SECTION

Salinomycin structure was downloaded from PubChem, with the accession ID: 72370.

PDB structures of proteins were downloaded from RCSB website, with the following ID: 4HGL (CK1γ); 4ACG (GSK3β); 4TND (GRK5); 3NYN (GRK6); 3WB5 (PKA); and 4BW5 (Potassium Channel).

DNA structures were also downloaded from RCSB website, with the following ID: 101D (Minor Groove); 1BWG (Major Groove); 1AU5 (Covalent Cross-linking); 367D (Threading Intercalation); and 1Z3F (Classic Intercalation). These five DNA structures were chosen based on the previous study reported by Yocheved Gilad et.al, 2014 [18]. AutoDock-4.2 was used for DNA-Ligand docking [18, 19].

AutoDock 4.2 was also used for Protein-Ligand docking [20]. PatchDock was additionally used for Protein-Ligand docking analysis [21, 22]. PyMOL and LigPlot+ were used to analyse the docking solutions and confirmation obtained from AutoDock and PatchDock.

RESULTS

DNA-Ligand Docking

AutoDock 4.2.1 was used to dock the ligand molecules with DNA receptors. Table 1 summarises the results obtained from AutoDock for DNA-Ligand interaction. Ethidium bromide was used as positive control, which demonstrated high affinity to DNA compared to salinomycin. Salinomycin demonstrated overall low affinity to DNA. Among the studied DNA structures, salinomycin demonstrated significant interaction with classical intercalation site with a free binding energy of -7.33Kcal/Mol, while ethidium bromide demonstrated a significant binding energy of -9.09Kcal/Mol. Figure 1 shows the binding/interaction of salinomycin with classical intercalation site. Salinomycin showed less than 5.91Kcal/Mol binding energy for the remaining four DNA structures. Overall affinity of salinomycin towards DNA was low compared to the studied positive control.

Table 1: AutoDock results of salinomycin and ethidium bromide with 5 DNA structures

DNA Type	PDB ID	Ethidium Bromide	Salinomycin
Classic Intercalation	1Z3F	-9.09	-7.33
Minor Groove	101D	-8.49	-5.91
Covalent Crosslink	1AU5	-6.37	-5.89
Threaded Intercalation	367D	-9.27	-5.08
Major Groove	1BWG	-6.33	-3.52

Human potassium channel docking

Salinomycin demonstrated significant affinity towards Human potassium channel protein. In AutoDock, the docking significance was -6.84Kcal/Mol of binding energy and inhibition constant of 9.61μM, forming one hydrogen bond with Ala317 (2.39Å). In PatchDock analysis, the combination gave an ACE value of -435Kcal/Mol. Both analyses signify that, the ligand molecule has good affinity towards Human Potassium Channel protein (Table 2). Figure 2 shows positioning of salinomycin on the human potassium channel protein.

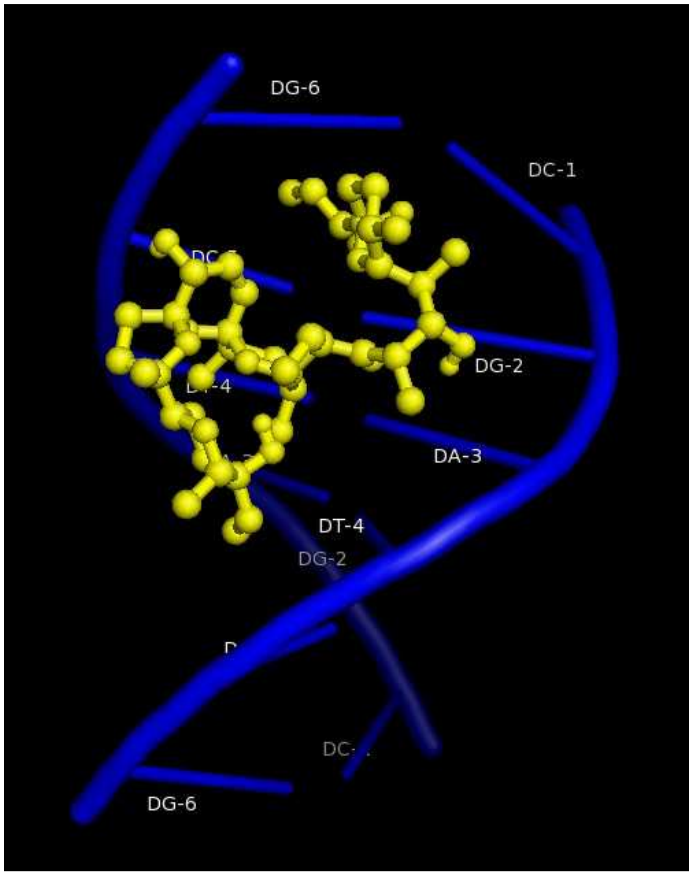

Figure 1: Positioning of salinomycin at DNA classic intercalation site (PyMOL view)

Table 2: Protein-Ligand interaction of salinomycin and potassium channel

AutoDock	PatchDock
Binding Energy	ACE Value
-6.84	-435.0
Inhibition Constant	PatchDock Score
9.61µM	7060

Wnt/β-catenin pathway proteins

AutoDock and PatchDock analysis for interaction of salinomycin with five chosen proteins were tabulated in Table 3 and Table 4. According to AutoDock results, Protein Kinase-A (PKA) and Casein Kinase 1 gamma (CK1γ) showed highest significance. Salinomycin demonstrated highest affinity to PKA with binding energy of -7.94Kcal/Mol and inhibition constant of 1.53µM; second highest affinity to CK1γ with binding energy of -7.81Kcal/Mol and inhibition constant of 5.45µM. Salinomycin formed 3 hydrogen bonds with PKA, at Gln-274 (2.86Å; 3.14Å) and Ser-252 (2.96Å). Salinomycin formed 3 hydrogen bonds with CK1γ, atAsn-53 (3.06Å), Lys-77 (2.73Å) and Lys-110 (3.24Å). Figure 3 shows the LigPlot+ analysis of the AutoDock results for salinomycin with [a] PKA and [b] CK1γ. The polar interactions are strong and the inhibition constant is less than 10µM, signifying the inhibition ability of the ligand molecule.

According to PatchDock analysis, salinomycin had highest affinity to CK1γ with an ACE value of -556.66Kcal/Mol and second highest affinity to PKA with an ACE value of -479.89Kcal/Mol.

Table 3: AutoDock results of salinomycin interaction with chosen protein kinases

Protein Name	PDB ID	Binding Energy	Inhibition Constant	No.of. H-Bond
Protein Kinase A	4WB5	-7.94	1.53µM	3
Casein Kinase 1 gamma	4HGL	-7.81	5.45µM	3
G-protein coupled Receptor Kinase-6	3NYN	-6.3	24.04µM	3
Glycogen Synthase Kinase-3 beta	4ACG	-5.7	66.36µM	4
G-protein coupled Receptor Kinase-5	4TND	-5.13	174.09µM	3

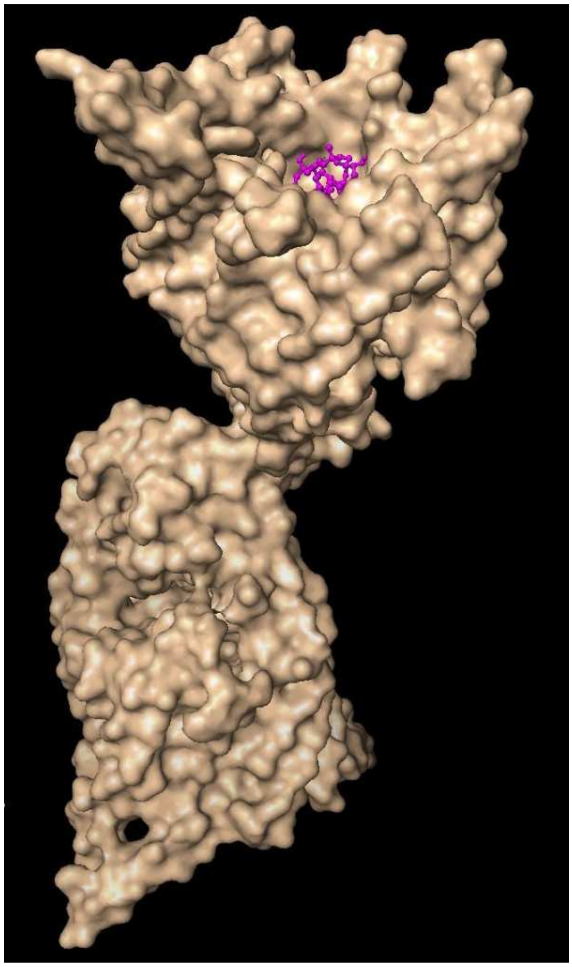

Figure 2: Positioning of salinomycin in human potassium channel (PyMOL view)

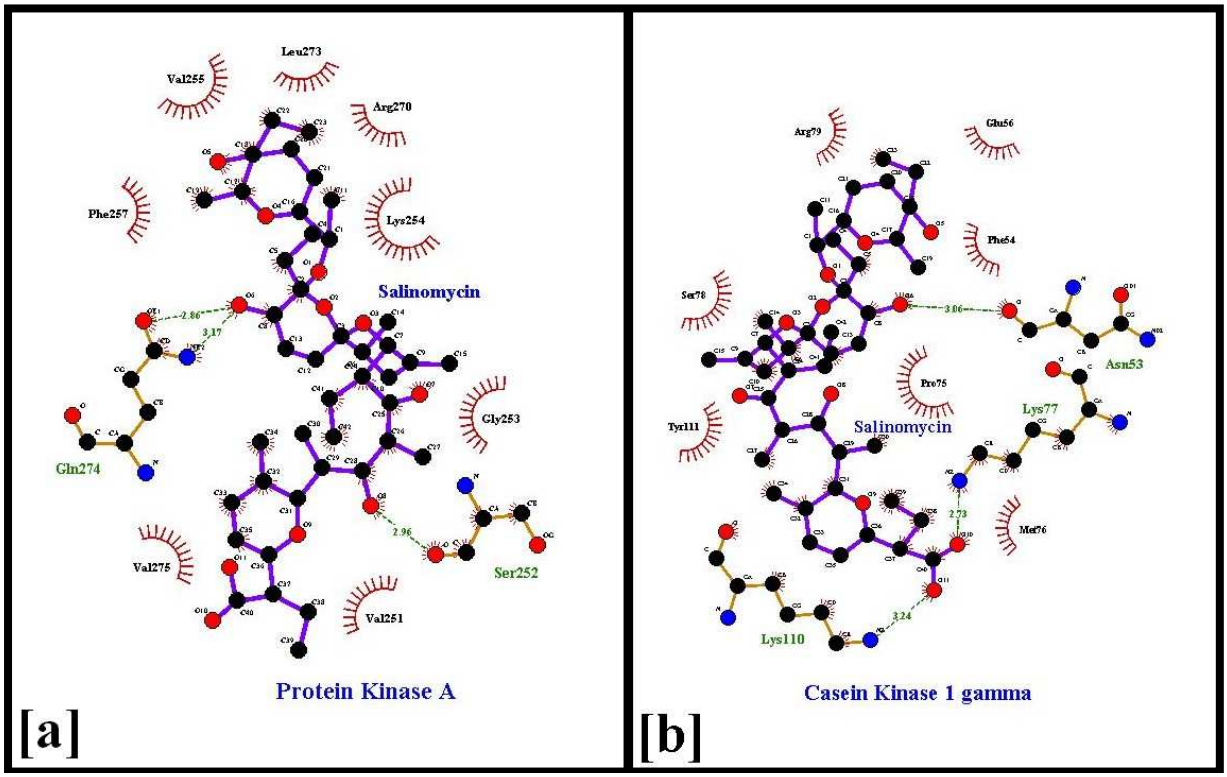

Figure 3: Interactions of salinomycin with [a] PKA and [b] CK1γ (LigPlot+ view)

Table 4: PatchDock analysis of salinomycin interaction with chosen protein kinases

Protein Name	PDB ID	PatchDock Score	ACE Value
Casein Kinase 1 gamma	4HGL	6234	-556.66
Protein Kinase A	4WB5	5830	-479.89
Glycogen Synthase Kinase-3 beta	4ACG	7380	-286.4
G-protein coupled Receptor Kinase-6	3NYN	7740	-231.12
G-protein coupled Receptor Kinase-5	4TND	6616	-218.39

DISCUSSION

Salinomycin is an extensively studied antibiotic compound which is recently finding its way into the clinical market as an anticancer drug. Recent review on salinomycin by Shuang Zhou, et.al, 2013 summarized the recent reports and studies on salinomycin as an anticancer drug. This report also summarized the possible mechanism of action of salinomycin on cancer cells, i.e., i) DNA damage, ii) potassium ion efflux and iii) Wnt signal inhibition. Here in this in-silico analysis, we studied these three possible mechanisms of salinomycin.

Salinomycin has been reported for its ability to increase DNA damage by several reports [23]. Hence, to study the mode of interaction with DNA, salinomycin was docked with five different DNA structures as previously reported by Yocheved Gilad and Hanoch Senderowitz, 2013 [18]. This report concludes that, AutoDock can be used to qualitatively predict the mode of interaction of ligands with DNA. It is also reported, DNA-ligand docking performed in AutoDock can be considered qualitatively significant [18, 19]. Ethidium bromide was used as a positive control since it is one of well known DNA intercalaters and has high affinity towards nucleic acids. Upon comparison of salinomycin and ethidium bromide interactions with the 5 chosen DNA fragments, salinomycin demonstrated low affinity towards DNA (as shown in Table 1). This difference could be due to the huge molecular weight difference between the two ligands. However, among the five studied DNA receptor molecules, salinomycin showed highest affinity to classical intercalation site, where a preformed intercalation gap is available. Interaction with remaining four structures showed no major significance. Hence, this DNA-ligand docking analysis, suggests that, if salinomycin is to cause DNA damage directly by interacting with DNA, it would happen so, preferably at the preformed intercalation sites.

Salinomycin belongs to a group of monocarboxylic polyester antibiotic also known as ionophores, which are known for its ability to interact with potassium channels and increase the efflux of potassium ions from mitochondria and cytoplasm [4 - 6]. Hence, salinomycin was docked with human potassium channel, using AutoDock and PatchDock (as shown in Table 2). As expected, salinomycin demonstrated significant interaction with potassium channel, signifying its affinity towards this protein. Salinomycin displayed -6.84Kcal/Mol free binding energy and 9.61µM inhibition constant in AutoDock and displayed -435.0Kcal/Mol Atomic Contact Energy (ACE) value in PatchDock.
Salinomycin has been studied in-vitro for its ability to interfere with Wnt/β-Catenin signalling [15]. Wnt signalling is a key pathway for cell cycle and cell survivability of cancer cells. Desheng Lu et.al., 2011, demonstrated that, salinomycin induces apoptosis by decreasing LRP-6 and β-Catenin levels in cancer cells. Further, they have also confirmed that, the phosphorylation of LRP-6 and β-Catenin was prevented, thus their degradation was promoted. Christof Niehrs and Jinlong Shen 2010, reported that LRP-6 is phosphorylated by GSK3, PKA, GRK5/6 and CK1γ. Shin-ichiro Hino, et.al., 2005, reported that, β-catenin is phosphorylated by PKA. Hence, we studied the interactions between salinomycin and phosphorylating protein kinases i.e., GSK3, PKA, GRK5/6 and CK1γ, using AutoDock and PatchDock (as shown in Table 3 and Table 4). Among the studied proteins, salinomycin demonstrated significant interaction with PKA and CK1γ. In AutoDock-4.2 analysis, salinomycin showed -7.94Kcal/Mol binding energy and 1.53µM inhibition constant for PKA; while, -7.81Kcal/Mol binding energy with 5.45µM inhibition constant for CK1γ. In PatchDock analysis, salinomycin demonstrated higher affinity to CK1γ than to PKA, i.e., ACE value of -556.66Kcal/Mol for CK1γ and ACE value of -479.89Kcal/Mol for PKA was observed. In either case, CK1γ is a major phosphorylating protein kinase for LRP-6 and PKA is a phosphorylating protein kinase for both LRP-6 and β-catenin. Inhibiting both CK1γ and PKA synergistically, would prevent phosphorylation of LRP-6 and β-catenin. Thus this analysis suggests that, salinomycin prevents the phosphorylation of LRP-6 and β-catenin by inhibiting PKA and CK1γ.

CONCLUSION

To conclude this study, this analysis suggests that, salinomycin reduces the levels of LRP-6 and β-catenin proteins in cancer cells, by inhibiting their phosphorylating proteins PKA and CK1γ. This could be the major mode of action of salinomycin for its anticancer activity. However, salinomycin is also known for its ability to increase DNA damage, thus, the DNA-ligand docking analysis suggests that, if salinomycin is to cause DNA damage by direct interaction with DNA, it would do so at the classical intercalation sites. As a known ionophore, salinomycin

demonstrated good affinity towards human potassium channel. But the interaction with DNA and potassium channel lack in-vitro evidences, hence, the salinomycin interaction with potassium channel and DNA could not be considered as significant results. Hence, the key finding in this in-silico analysis is that, supporting the data published by Desheng Lu, et al 2011, salinomycin ability to reduce the levels of β-catenin and LRP-6 proteins in cancer cells could be due to inhibition of PKA and CK1γ.

Acknowledgement
The authors thank VIT University for providing lab facility to carry out this research work.

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