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Original article

Inhibition of leptin receptors through plant-derived ligands for liver fibrosis drug development

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Background: Liver fibrosis is a common health problem worldwide. Several drug-targets have been identified from the fibrosis pathway namely leptin, leptin receptors, adiponectin, and PPAR ã agonist. Suitable antifibrotic drugs are needed.

Objective: Present plant-derived ligands inhibiting the leptin receptor mediated pathway as antifibrotic drugs.

Methods: Bioinformatics was used to generate 3D-structure of leptin receptor to explore binding grooves of therapeutic targets. A molecular library with bioactive compounds from SE Asian medicinal plants was developed by using the bioactive compounds, docking with leptin receptor.

Results: The homology model of leptin receptor has showed similarities with crystal structures and folds reported previously for other cytokine family members. The Ramachandran plot shows that 89.1% of the residues are most favored region. Besides, 15 possible active sites were identified with active residues such as Ser180, Gln463, Pro477, IIe482, Leu519, Asn550, Pro564, Val600, Glu643, Lys665, Asp671, Ser675, Leu767, Pro803, and Glu834.

Conclusion: This study provides data on surface cavity, binding groove, and active sites of the leptin receptor, which will be useful for researchers to understand leptin receptor/complex. We hope that the strandline serve as a potential drug candidate for liver fibrosis in the near future.

***Keywords*:** Bioinformatics, drug target, hepatic fibrosis, leptin receptors, ligands

Hepatic fibrosis has been proved to be a life threatening complication worldwide. It is a result of chronic liver injuries that ultimately lead to cirrhosis of the liver. The stimulus that triggers hepatic fibrosis includes viruses, autoimmune diseases, metabolic disorders, and many more [1]. In normal condition, only 5 to 10% of the cells of the liver are composed of hepatic stellate cells (HSC) that are present in the subendothelial space between hepatocytes and sinusoidal endothelial cells. The hepatic stellate cells (HSC), previously known as lipocytes, Its cells, or perisinusoidal cells were recognized as the collagen-producing cells in the liver [2]. This cell type was first explained by von Kupffer in 1876. They are phenotypically activated during chronic liver diseases and causes fibrogenic properties [3]. Under stressed condition, induced by chronic or acute liver diseases, HSC undergo phenotypic changes resulting in the switching of cells from a quiescent vitamin A rich phenotype to myofibroblastic phenotype [1, 4]. De-novo fibrogenic properties are shown by activated HSCs, which will include secretion of proinflamatory cytokines and chemokines, proliferation of the cells and synthesis of matrix proteins, and inhibitors of matrix degrading protein in large excess. Those will ultimately lead to progressive scar formation in the liver [5-7].

To date, many approaches have been taken to inhibit or withdraw the injurious agent that causes fibrosis. Nonetheless, this approach is not that feasible and hence efforts are directed for developing liver-specific antifibrotic therapies. There is no specific antifibrotic treatment yet. Therefore, efforts are being made, in clinical trials, to evaluate small molecules that could be used for treating the deadly disease [8, 9]. Moreover, several experimental analyses have revealed that myofibroblastic activation of hepatic myofibroblasts derived from portal connective tissue, perivascular fibroblasts of portal and central veins and

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periductular fibroblasts activates the hepatic satellite cells for inducing fibrosis. Recent studies have focused on emerging antifibrotic therapeutic targets which are- PPAR γ agonist (peroxisome proliferator activated receptor gamma agonist), leptin, and leptin receptor, adiponectin, among others [7].

Leptin and leptin receptor can be potential drug target for this disease [7]. An obese gene is responsible for leptin synthesis and it derived from adipocytes cells whose work is to organize the food intake and energy equilibrium by using some kind of receptor (OB-R). Leptin serum levels are found to be increased in patients suffering from alcoholic cirrhosis of liver [10]. It has been reported that mice with leptin deficiency or having mutations in leptin receptor showed reduced level of liver fibrogenesis. This protein is imperceptible in the normal liver which is produced during fibrogenesis, and can be by activated hepatic myofibroblasts in vitro and in vivo especially which is obtained by thioacetamide [11, 12]. Therefore, any antagonists of leptin receptors can be promising drug candidate molecule hepatic fibrosis. Thus, leptin receptor blocking through antagonist has enormous potentials to cure hepatic fibrosis.

Ligand molecules may act as an antagonist of receptor also used as drug targets [13]. Several medicinal plants that have been used for a long time are the new source candidates of therapeutic molecules [14]. Therefore, the medicinal plants and their compounds such as flavonoid and terpenoid may act as ligands [15]. These plant-derived ligands have shown their potentials for the receptor inhibition and they may be less toxic as they have been used by people for generations [16]. Therefore, medicinal plants have enormous potential for mining the ligand molecules. In this study, we have developed drug candidate molecule for liver fibrogenesis through computer aided drug design. We have chosen the leptin receptor to develop the antagonist for hepatic fibrosis. For ligand molecule, we have selected some compounds from Chinese and Indian medicinal plants and prepared a molecule library from East Asian medicinal plants. We have performed docking analysis of small molecules with the leptin receptor. The binding energies from the docking

Materials and methods

3D structure of surface cavity and binding groove

We have collected information about leptin receptor and this functional protein sequences in FASTA format was collected from the National Center for Biotechnology information (NCBI) and used for further analysis [17]. The Accession number of the protein is P48357. We have performed the homology modeling to generate the PDB structure. PDB structure of the leptin receptor is not available in the PDB database. The sequence of human leptin receptor (LR), called the target sequence, was used for homology modeling and the process of homology modeling of leptin receptor has been followed by method of Poornima et al. with some modification [18]. The homology model of LR was generated using the SWISS MODEL server [19-21]. Finally, LR was evaluated from their Ramachandran diagrams using Swiss-PDB Viewer program [19]. The PyMOL(20), have been used for the generation of 3D as well as identification of binding grooves of therapeutic targets. It is an open-source, molecular visualization system that is used for structural biology. This software has been written by the python programming language. We have used ".pdb" files to generate the surface structure and the cavities of those proteins.

Active sites and preparation of compound library

The Q-Site Finder server was used to identify the potential possible active sites where ligand can bind. This program is available online [21]. This tool uses energy-based method for the prediction of protein-ligand binding sites. PubChem is a publicly available database of chemical molecules and their activities against biological assays [22, 23]. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). A small molecular library has generated seven compounds from East Asian medicinal plants especially from the medicinal Chinese and Indian medicinal plants. It retrieved the structures of the respective small molecules from pubchem.

Docking

Molecular docking has become an integral part of many modern structure-based drug discovery efforts. For docking, we have used the Hex software (version 5.0), which is a based on fourier transform (FFT)-based docking algorithm [24]. Minimum energy with docked molecule was calculated using ligand information.

Results

Surface cavity, binding groove, and molecular library

Surface cavity and binding grooves of leptin receptor has been provided in **Figure 1**. The protein is having several surface cavities. We have identified five major probable surface cavities. We have also constructed a molecular library from East Asian medicinal plants. Seven small molecules have been selected from three plants, which are artichoke (*Cynara scolymus)*, milk thistle *(Silybum marianum)*, and turmeric (*Curcuma longa*). The Pubchem ID of molecules has also been recorded in **Table 1**.

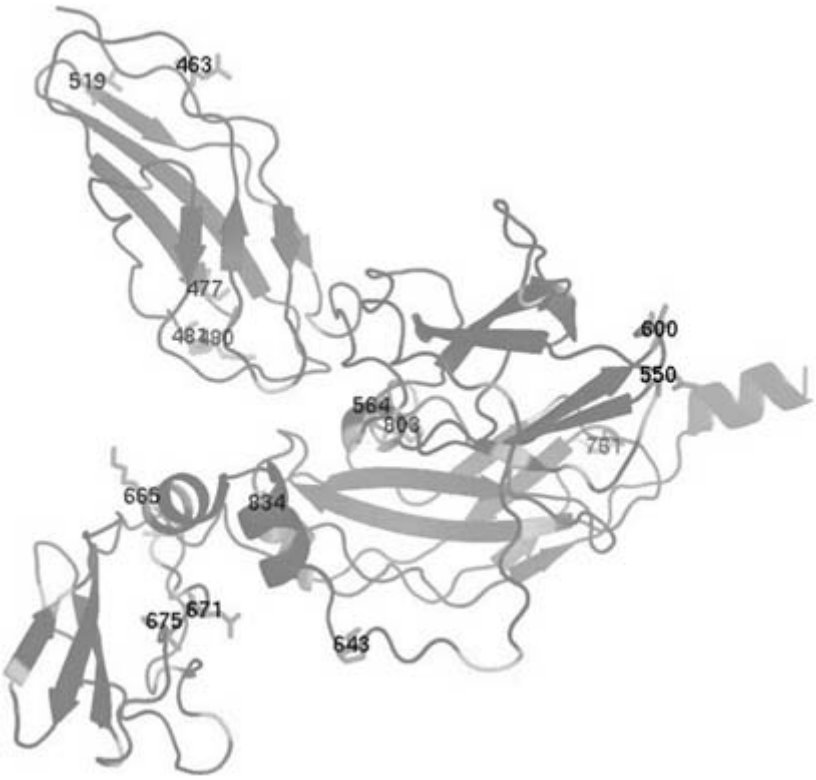

Figure 1. Residue plot of Ramachandran analysis using protein structure validation software (PSVS).

Table 1. Molecular library of different bioactive compounds and their plants which has been used as ligands in our study.

Plant Source	Bioactive compounds	Pubchem ID
Artichoke (*Cynara scolymus*)	Trimethylglycine (TMG)	CID 247
	Tetrandrine	CID 73078
	Cinarine	CID 6124212
Milk Thistle *(Silybum marianum)*	Silibinin	CID 31553
	Silydianin	CID 3033957
	Silychristin	CID 441764
Turmeric (*Curcuma longa*)	Curcumin	CID 969516

Possible active sites and docking

The active site has been generated through Q-Site finder and is shown in **Figure 2**. Ten possible active sites have been identified and the active sites with toggle surface have been depicted through the software. Docking results has been recoded as shown in **Table 2**. The graphical result of the different binding energies of different ligands during the binding leptin receptor has been shown in **Figure 3**. It shows the tetrandrine bind with the leptin receptor with lesser energy, which is -323.60 KJ/Mol. Conversely, the docking result has shown that leptin receptor also binds with the silybin, cinarine**,** tri-methylglycine, silychristin, silydianin, curcumin ligands with the different energy which are-273.59 KJ/Mol, -234.40 KJ/Mol, -126.95 KJ/Mol, -275.80 KJ/Mol, -265.50 KJ/Mol, -255.23 KJ/Mol respectively. While performing docking, we have drawn docking result while tetrandrine bind with the leptin receptor. We have taken a snapshot before and after docking (**Figure 4**).

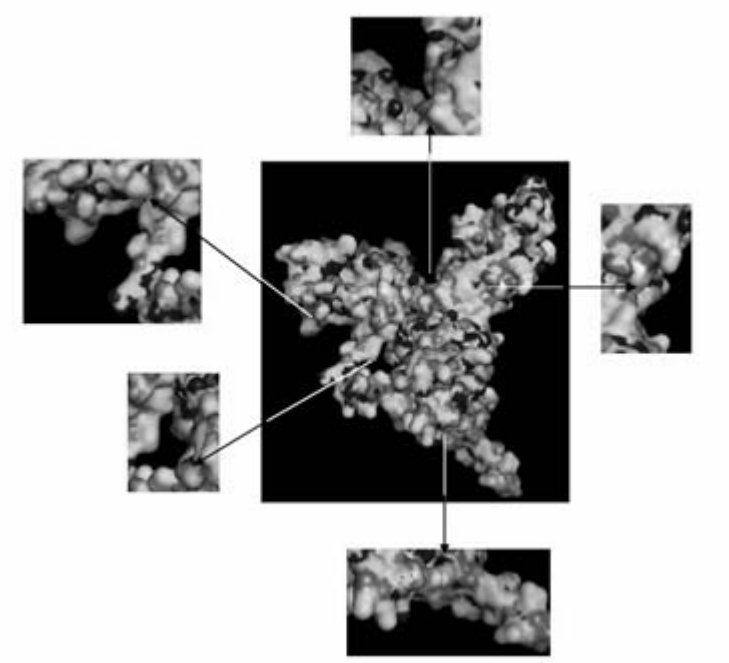

Figure 2. Surface cavity and binding grooves of the leptin receptor generated through PyMOL.

Table 2. Docking results with different parameters.

Parameters	Leptin receptor +Silybin	Leptin receptor +Cinarine	Leptin receptor +Tri-methylglycine	Leptin receptor +Silychristin	Leptin receptor +Tetrandrine	Leptin receptor +Silydianin	Leptin receptor +Curcumin
Cluster	1	1	1	1	1	1	1
Solutions	1	1	1	1	1	1	1
Models	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H-bonds	-1	-1	-1	-1	-1	-1	-1
Bumps	0	0	0	0	0	0	0
RMS	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00
E total (KJ/Mol)	-273.59	-234.40	-126.95	-275.80	-323.60	-265.50	-255.23
E shapes	-210.72	-202.10	-97.94	-214.96	-273.58	-230.88	-224.67
E force	-62.87	-32.31	-29.01	-60.83	-50.02	-34.62	-30.56
E air	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V shape	253.00	179.77	103.16	266.44	292.58	264.35	230.66
V clash	0.00	0.00	0.00	0.00	0.00	0.00	0.00

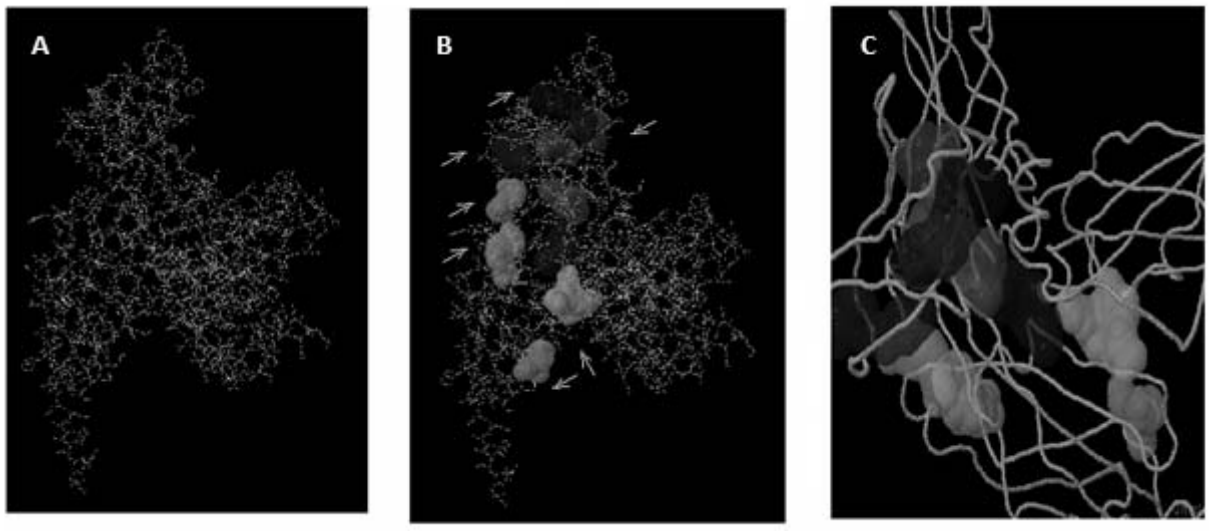

Figure 3. Binding sites identified by Q-site Finder program of leptine receptor. **A:** wireframe representation of the leptin receptor, **B:** wireframe representation of the leptin receptor with toggle ligands, **C:** leptin receptor representation through CHIME Interface with toggle ligands.

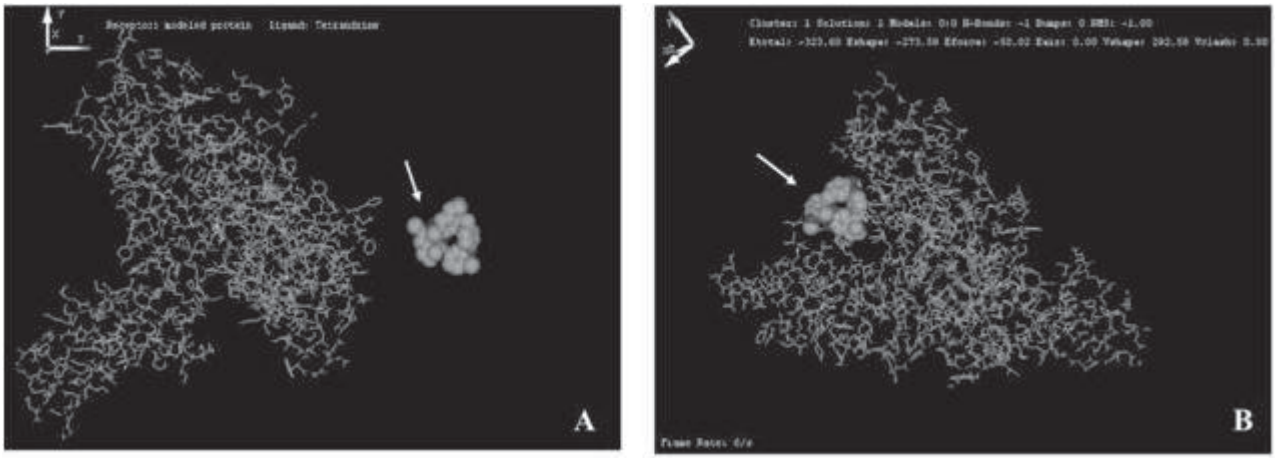

Figure 4. Docking result while performing docking with leptine receptor and tetrandrine as ligand. The snapshots of the tetrandrine ligand and the leptin receptor before docking (**A**) and after docking (**B**) which shows the binding site.

Discussion

Hepatic fibrosis affects several hundred million people worldwide. Presently, this disease is a major health hazard around the globe [25]. This disease is a conserved evolutionary response that causes tissue damage and provides chronic liver injury [26]. New strategies should be designed to reverse or block liver fibrosis. Understanding the molecular mechanisms of fibrosis and its progression provides several promising molecular targets for antifibrotic treatments [27-30]. To date, there are no regular treatments available for liver fibrosis. Several molecules have been studied to prevent fibrosis progression in animal model [31]. Most of the anti-fibrotic drugs are still in experimental stage [32]. In the present study, we have tried to develop antifibrotic agent computationally through blocking a target in the hepatic fibrosis pathway.

Target related approaches for the drug discovery is one of the important approaches for the drug discovery, directed to known molecular targets or fibrogenesis or fibrolysis pathways. Such target function can be blocked by small molecule inhibitors.

Small molecules can be developed by way of screening of existing compound libraries through bioinformatics. Bioinformatics is gaining importance in drug development and improvement, especially as *in silico* [33]. In this approach, some compounds with known activity can be optimized toward a target. Hence, we have generated compound libraries for the active agents from the medicinal herbs and have considered leptin receptor as drug target, which is part of the hepatic fibrosis pathway to block the receptor.

Our drug target, leptin receptor, is related to obesity (ob) gene and the gene is spliced to produce at least six isoforms (obRa–obRf, mainly obRa-obRd and obRf), which are identical in their extracellular and transmembrane domain [34]. This receptor contains two domains, first is extracellular and second is transmembrane. The first domain of this receptor consists of 816 amino acids. This extracellular contain two cytokine-like binding (Trp-Ser- X-Ser-Trp) motifs and a fibronectin Type III domain [35, 36]. From the crystal structure studies, the NMR studies have demonstrated that both leptin and leptin receptors take on a cytokine fold similar to the short-helix subfamily of cytokine folds, which create the leptin receptor as a typical member of the cytokine receptor family [37, 38]. After binding with the leptin, the leptin/ leptin receptor complex has been formed and the homology modeling of this complex has been provided by Hiroike et al [39]. Bogan *et al.* reported that this protein protein complex made up with of a small subset of binding energy and residues enriched with Trp, Tyr, Arg [40]. The major interface of leptin/receptor are Y441, R468 and R615 (leptin receptor) and R20 (leptin). On the other hand, minor interface were W583 and Y586 (leptin receptor). They have concluded that R20 (leptin) may be one of the constituent residues in the major interface. However, the structure of leptin receptor and the leptin/ leptin receptor complex has not yet been properly understood.

We have considered leptin receptor as drug target, which has been described by several researchers that leptin receptor can be a drug target for heptic fibrosis [7]. However PPAR γ, adiponectin can also be used as drug target. The lPPAR γ are largely found in adipocytes cells and are responsible for adipogenesis. They are a member of nuclear receptor super family of ligand dependent transcription factors. During HSC activation, expression of PPAR γ decreases to almost undetectable level and are expressed when exposed to PPAR γ agonists [41-44]. Finally, binding of PPAR γ with anti-diabetic thioazelinediones decreases progression of fibrosis [45]. However, it can be used as a drug target. Alternatively, adiponectin, product of adipocyte cells, can be a drug target for liver fibroblasts. Evidences support that during chronic liver diseases, adiponectin plays antifibrotic role [46]. However, leptin and its functional receptors (Ob-Rb) play a crucial role in hepatic fibrosis and this leptin mediated signaling pathway is one of the key pathways for the profibrogenic responses in the liver [47]. Therefore, we have chosen the leptin receptor as drug target.

Herbal medicines have become increasingly popular for several decades throughout the world [48]. Herbal drugs are very popular for liver diseases. Surveys from Europe and the United States have recorded that up to 65% of patients with liver disease take herbal preparations [49-52]. In this study, we have selected seven important bioactive compounds form three herbal medicines such asartichoke (*Cynara scolymus*), milk thistle *(Silybum marianum),* and turmeric (*Curcuma longa*). These have been used as liver medicine for decades with proven toxicity [53]. Hence, we have chosen seven important bioactive compounds from these plants. We noted from our docking result that the bioactive compound, tetrandrine binds with leptin receptor with lesser energy. This bioactive molecule is one of the most important bioactive compounds of artichoke and we have hypothesized for the leptin receptors inhibition through the tetrandrine.

We hope that our findings will be helpful to understand the structure of leptin receptor. The information about the surface cavity-binding groove and active site of leptin receptor will help future research. We are hopeful that strandline may be a good drug candidate for future.

All the authors declare that there is no conflict of interest in this work.

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