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Insilico Molecular Docking of STAT3 Protein With Bioactive Compounds from Costus Igneus against Hepatocellular Carcinoma

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KEYWORDS

Costus igneus, diosgenin, STAT3, hepatocellular carcinoma, anti-cancer

A B S T R A C T

Costus igneus commonly known as insulin plant belongs to costaceae family has been proven to possess anticancer activity. Phytochemical investigations reveal the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids and steroids. Hepatocellular carcinoma (HCC), also called malignant hepatoma, is the most common type of liver cancer. Most cases of HCC are as a result of either a viral hepatitis infections (hepatitis B or C) metabolic toxins (alcohol or aflatoxin) conditions like hemochromatosis and alpha 1-antitrypsin deficiency. Several human cancers, including hepatocellular carcinoma shows activation of STAT3. Diosgenin, a natural steroidal saponin isolated from *Costus igneus* rhizome is a novel blocker of the STAT3 activation pathway, with a potential role in the treatment of HCC. The activation of c-src,JAK,JAK2 implicated in STAT3 activation were also suppressed by saponin. The present study focuses on identifying diosgenin in *Costus igneus* rhizome having anticancer potential to effectively inhibit the action of STAT3.Diosgenin were docked against target proteins STAT3, JAK, IL-6 using AutoDock 4.2. Molecular docking analysis for STAT3 with diosgenin is more interactive and binding strongly at the active site. Therefore diosgenin may act as a potent inhibitor of STAT3 and could be used to treat HCC.

Introduction

Costus igneus commonly known as insulin plant in India belongs to the family *costaceae*. *It is* a newly introduced plant in India from South and Central America. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers. In southern India, it usually grows as an ornamental plant and its leaves are used as a dietary supplement in the treatment of

diabetes mellitus. It has been proven to possess various pharmacological activities like hypolipidemic, antidiuretic, antioxidant, anti-microbial and anti-cancer. Further, various phytochemical investigations reveal the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins,saponins, flavonoids, steroid, and appreciable amounts of trace elements. Bioactive compounds quercetin and diosgenin, a steroidal sapogenin, were isolated from *costusigneus* rhizome by high performance thin layer charmatography (HPTLC) (Kalailingam, 2011). Activation of STAT3 has been shown in several human cancers and transformed cell lines including hepatocellular carcinoma (HCC).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. The cells of origin are believed to be the hepatic stem cells, although this remains the subject of investigation. Tumors progress with local expansion, intrahepatic spread and distant metastases.

HCC is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected. The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC (Alison, 2005).

STAT3was initially discovered in the context of the specificity of the interferon (IFN) signaling (Aggarwal *et al*., 2009; Aggarwal, 2006; Darnell, 1994; Aggarwal, 2009(a); Aggarwal, 2009(b)) STAT3 was at first described in interleukin-6 (IL-6) stimulated hepatocytes as a DNA-binding factor capable of selectively interacting with an enhancer element in the promoter region of acute-phase genes(Akira,1994). Later, it was found that STAT3 can be activated by the entire IL-6 family of cytokines and growth factors such as epidermal growth factor (EGF) (Yu *et al.*, 2009; Turkson, 2000, haftchenary, 2011). Subsequently, the potential oncogenic role of STAT3 was established by the expression of constitutively activated STAT3 in various tumorcell lines including breast, colon, gastric, lung, head neck, skin and prostate (Yoshimura *et al*., 2009; Sansone, 2007,Yin 2006, Levy 2002). The STAT family comprises seven members namely, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6.The entire STAT family can be divided into two groups. The first group consists of STAT2, STAT4 and STAT6.The second group includes STAT1, STAT3 and STAT5, which are activated in different tissues through various ligands and are involved in IFN-γ signaling, development of mammary glands, and embryogenesis.

Constitutively activated IL-6/STAT3 signaling has been detected in a wide variety of human cancers including liver cancer and is considered an important factor for cancer initiation, development, and progression (Aggarwal *et al*., 2009; Karin, 2009; Yu, 2007; Calvisi, 2006). Most HCC appears in cirrhotic livers after years of chronic liver inflammation caused by hepatitis viral infection, alcoholic and non-alcoholic steatohepatitis (Serag *et al*., 2007; Llovet, 2008). Various factors can active hepatic STAT3 signaling such as inflammatory cytokines, growth factors, hormones, and hepatitis viral proteins (Asahina, 2010) .Several cytokines (such as IL-6, IL-6 family cytokines andIL-22) that activate STAT3 in hepatocytes have been shown to promote

HCC cell growth in vivo (Gao *et al*., 2005; Radaeva, 2004). Recently, Park *et al*. reported that localized production of IL-22 in the liver promotes hepatocytes survival and proliferation, thereby accelerating the HCC development after DEN challenge (Naugler, 2007). Moreover, emerging evidence suggests that the cytokines downstream of STAT3play an important role in the development of liver cancer park (Park *et al*., 2011; Kusaba *et al*., 2007)

In the present report, it was investigated whether diosgenin, a steroidal saponin isolated from costus igneus can modulate the STAT3 signaling pathway. It was found that diosgenin inhibited both constitutive and inducible activation of STAT3 with no effect on STAT5. The activation of c-Src, JAK1 and JAK2 implicated in STAT3 activation, were also suppressed by this saponin. Pervanadate reversed the diosgenin-induced down regulation of STAT3, suggesting the involvement of a protein tyrosine phosphatase. Indeed, it was found that diosgenin can induce the expression of Src homology 2 phosphatase 2 (SH-PTP2) that correlated with down regulation of constitutive STAT3 activation. Overall, these results suggest that diosgenin is a novel blocker of the STAT3 activation pathway, with a potential role in the treatment of HCC and other cancers (Fernandez, 2010).

Materials and Methods

Uniprot

The mission of UniProt is produced by the UniProt Consortium, a collaboration between the European (PIR). Uniprot is to provide the scientific community with a comprehensive, high quality, freely accessible resource of protein sequence and function information. The uniprot knowledgebase (uniprotKB**)** is the center access point for extensive curated protein information, include function, classification, and cross reference. The UniProt Knowledgebase, the centre piece of the UniProt Consortium's activities, is an expertly and richly curated protein database, consisting of two sections called UniProtKB/ Swiss-Prot and UniProtKB/ TrEMBL http://www.uniprot. org). (Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB) and the Protein Information Resource

PDB

The RCSB PDB is an information portal for researchers and students interested in structural biology. At its center is the PDB archive – the sole international repository for 3-dimensional structure data of biological macromolecules. The RCSB PDB integrates a variety of production-level data and software resources, and shares research results and software. The RCSB PDB is dedicated to fostering new scientific advances by providing accurate, consistent, and well-annotated 3-D structure data that is delivered in a timely and efficient way to a wide audience. Allthe3D crystal structural information about the target proteins was obtained from the Protein Data Bank (PDB).The PDB is a crystallographic database for the 3D structural data of large biological molecules, such as protein and nucleic acids. The PDB is a key resource in area of structural biology, such as structural genomics.
http//www.rcbs.org/pdb/home/home.do

PubChem

Pubchem is designed to provide information on biological activities of small molecules, generally those with molecular weight less than 500 Daltons. PubChem's integration with NCBI's Entrez information retrieval

system provides sub/structure, similarity structure, bioactivity data as well as links to biological property information in PubMed and NCBI's Protein 3D Structure Resource http://pubchem.ncbi.nlm.nih.gov/

ACD/ChemSketch

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, and polymers. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P*. Chemsketch is a molecular modeling program used to create and modify images of chemical structures. Also, there is a software that allows molecules and molecular models displayed in two and three dimensions, to understand the structure of chemical bonds and the nature of the functional groups

Open Babel

Open Babel is free software, a chemical expert system mainly used for converting formats. Due to the strong relationship to informatics this program belongs more to the category cheminformatics than to molecular. It is available for Windows, Unix, and Mac OS. It can be used to produce structures of organic molecules, names of organic molecules as well as Lewis structures, 3D structures, space filling models or ball and stick models, among other things. Open Babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials. Biochemistry or related areas.

Auto Dock

Auto dock is molecular modeling simulation software. It is especially for protein-ligand docking. Auto dock 4 is available under the GNU General Public License. Auto dock is available under the Apache license. Auto dock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Auto Dock 4.2 now has a free-energy scoring function that is based on a linear regression analysis, the AMBER force field, and an even larger set of diverse protein-ligand complexes with known inhibition constants than we used in Auto Dock 3.0. The best model was cross-validated with a separate set of HIV-1 protease complexes, and confirmed that the standard error is around 2.5kcal/mol. This is enough to discriminate between leads with milli and micro and nanomolar inhibition constants http://autodock.scripps.edu/

PyMOL

PyMOL is an open-source, user-sponsored, molecular visualization system created by Warren Lyford DeLano and commercialized initially by DeLano Scientific LLC, which was a private Software Company dedicated to creating useful tools that become universally accessible to scientific and educational communities. It is currently commercialized by Schrödinger, Inc. PyMOL can produce high-quality 3D images of small moleculesand biological macromolecules, such as proteins.PyMOL has been written mostly in the Python language (www.python.org), while the time-critical parts of the system have been coded in C. This way, Python programs interact

most easily with the PyMOL GUI. (www.rubor.de).

Result and Discussion

Sequence retrieval

The sequence of human (homo sapiens) STAT3, IL6 and JAK areretrieved from UNIPROT database, its accession number is P40763,P05231 and O60674 respectively.

Structure retrieval

The 3d structures of these proteins namely STAT3, IL6 and JAK wereretrieved from PDB structure database and PDB ids are shown Table 2.

Table.1 Drug Targets

S.NO	Drug Targets	Cancer
1	STAT3	Gastric cancer, lung cancer, breast cancer, prostate cancer, liver cancer
2	Il-6	Breast cancer, oral cancer, pancreatic cancer
3	JAK	Liver cancer.

Table.2 Drug Targets and PDB id

S.No	Protein	Pdb ID	Chain
1	STAT3	5AX3	A
2	IL-6	4ZS7	A
3	JAK	5C9Z	A

Table.3 Lipinski's properties of the compounds of diosgenin:

Ligand molecule	Molecular weight	xlogp3 value(<=5)	Hydrogen bond donar	Hydrogen bond acceptor
Diosgenin	414.62058 g/mol	5.7	1	3

Table.4 Shows the interaction between the diosgenin and STAT3

Stat3		Diosgenin	Distance (Å)	Binging Score (kcal/mol)
Residue	Atom	Atom		
GLU-316	N	O	2.9	-7.67
THY-130	OH	H	2.0	

Table.5 Shows the interaction between the diosgenin and IL6

IL6		Diosgenin	Distance (Å)	Binding Score (kcal/mol)
RESIDUE	ATOM	ATOM		
SER-58	O	H	2.4	-7.23

Table.6 Shows the interaction between the diosgenin and JAK

Jak		Diosgenin	Distance (Å)	Binding Score (kcal/mol)
Residue	Atom	Atom		
ARG-256	NH2	O	3.1	-7.2

Table.7 Overall docking results between PROTIENandLIGANDS

Ligands and proteins	Key Residues	Docking energy (Kcal/Mol)	No. of Hydrogen Bonds
Diosgenin and STAT3	GLU-316, THY-130	-7.67	2
Diosgenin and IL-6	SER-58	-7.23	1
Diosgenin and JAK	ARG-256	-7.2	1

Fig.1

Fig.2

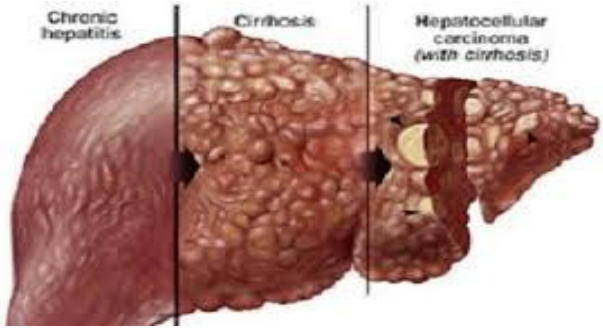


Fig.3 3D Structure of Drug Targets Visualization Using Rasmol

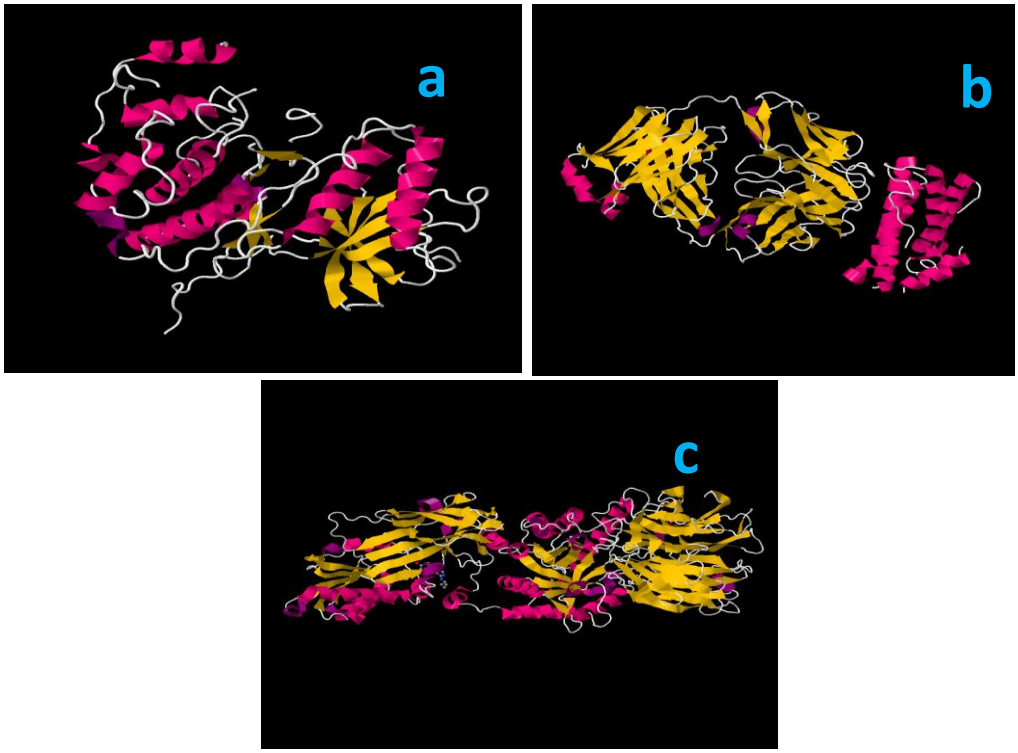
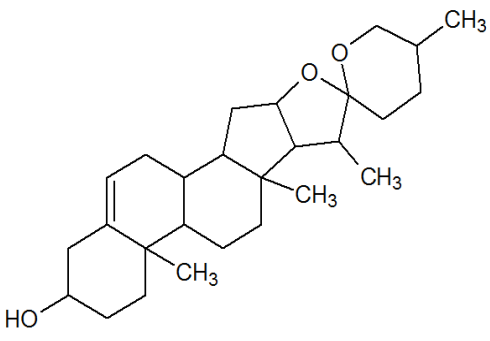
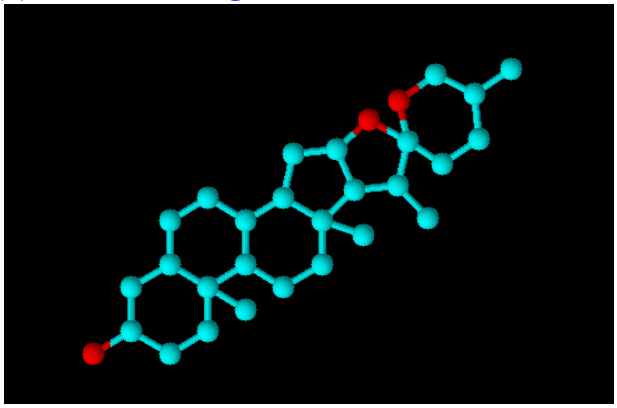


Fig.4 Structure of diosgenin (a) 2D (b) 3D drawn using ACD Chemsketch



(a)



(b)

Fig.5 STAT3 AND DIOSGENIN: (a) Docking score (b) finalconformation (c) visualization of hydrogen bonds between STAT3 and diosgenin using pymol

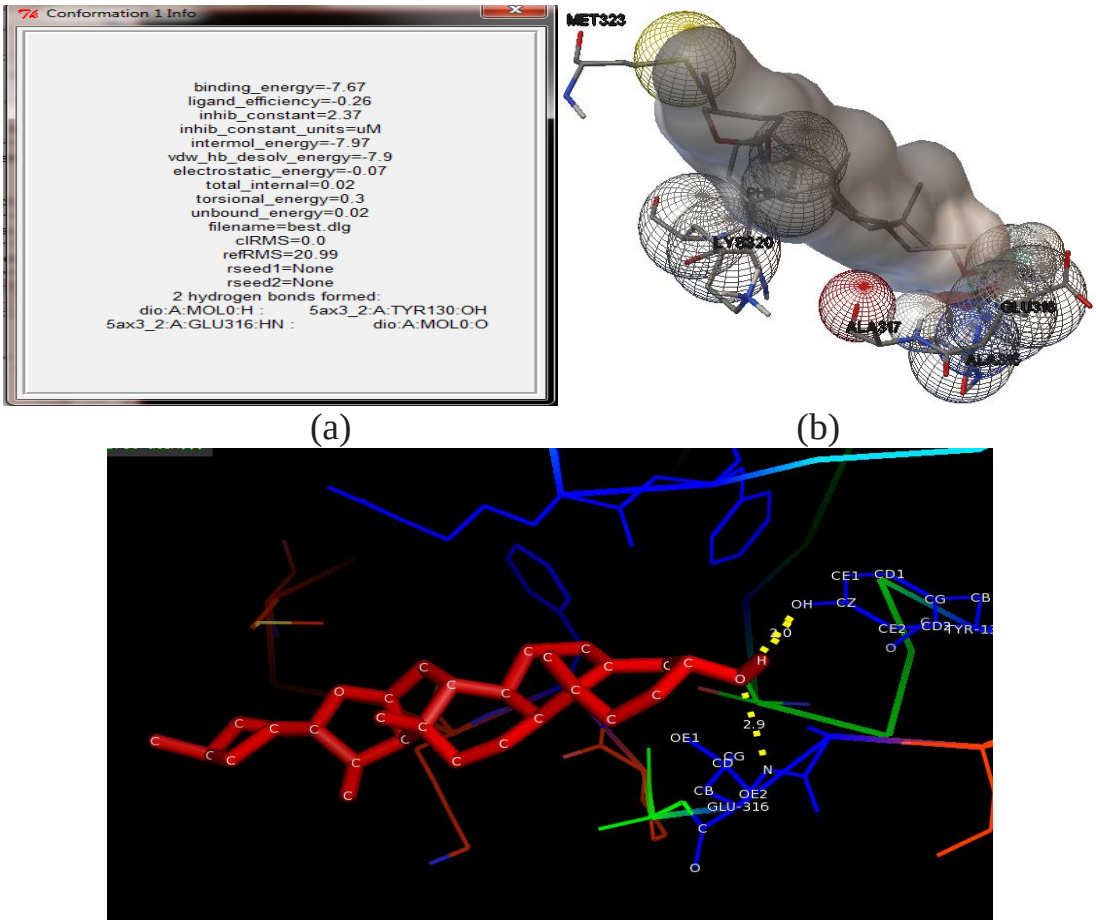
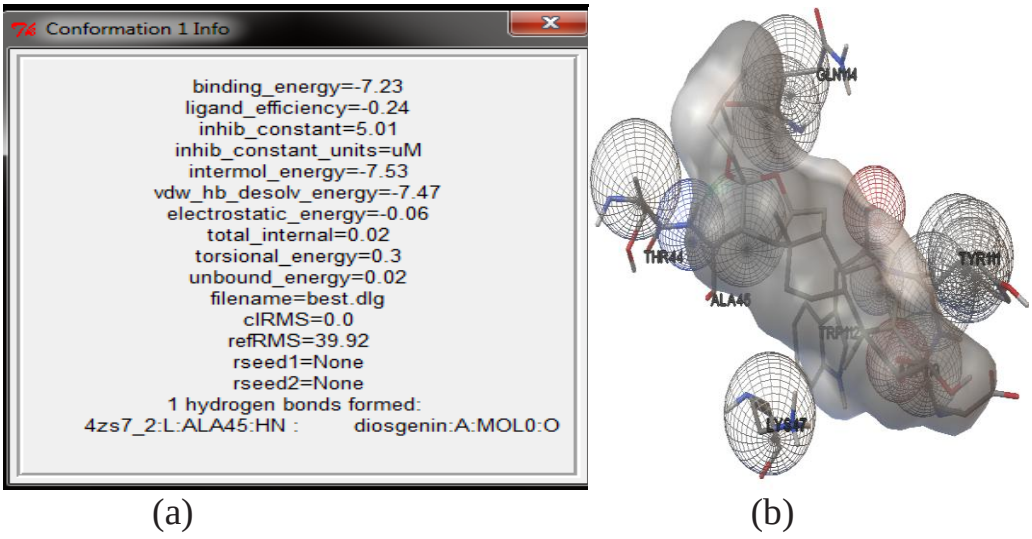
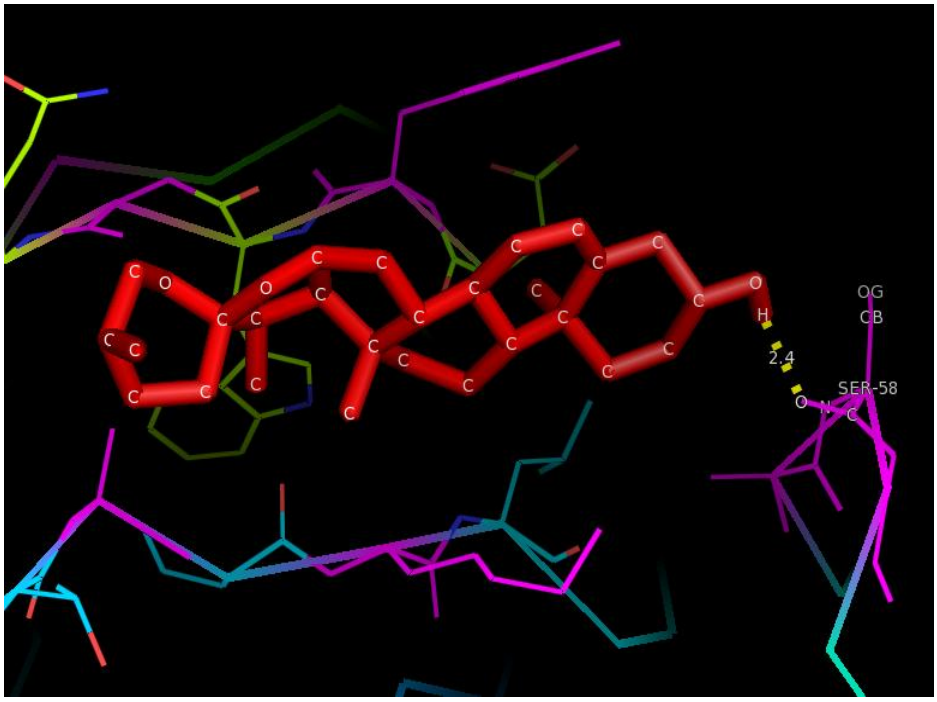


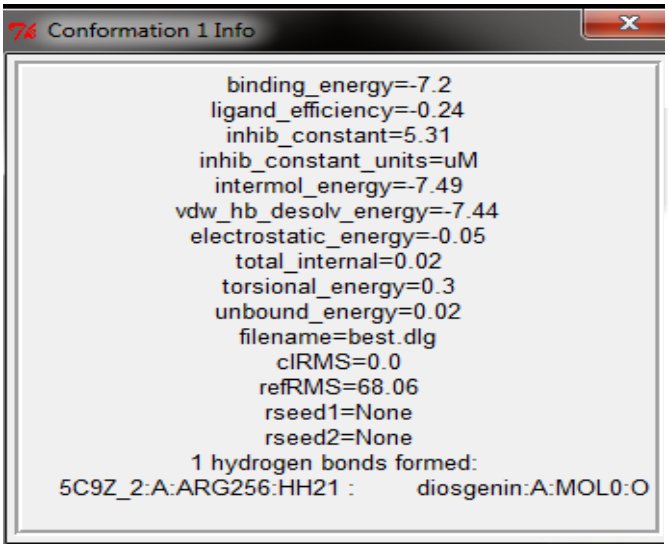
Fig.6 IL6 AND SIOSGENIN: (a) Docking score (b) finalconformation (c) visualization of hydrogen bonds between IL-6 and diosgenin using pymol



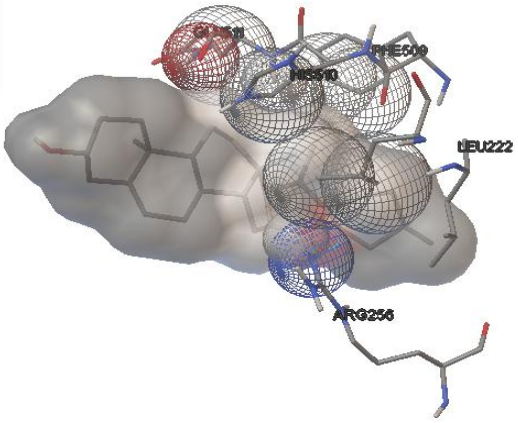


(c)

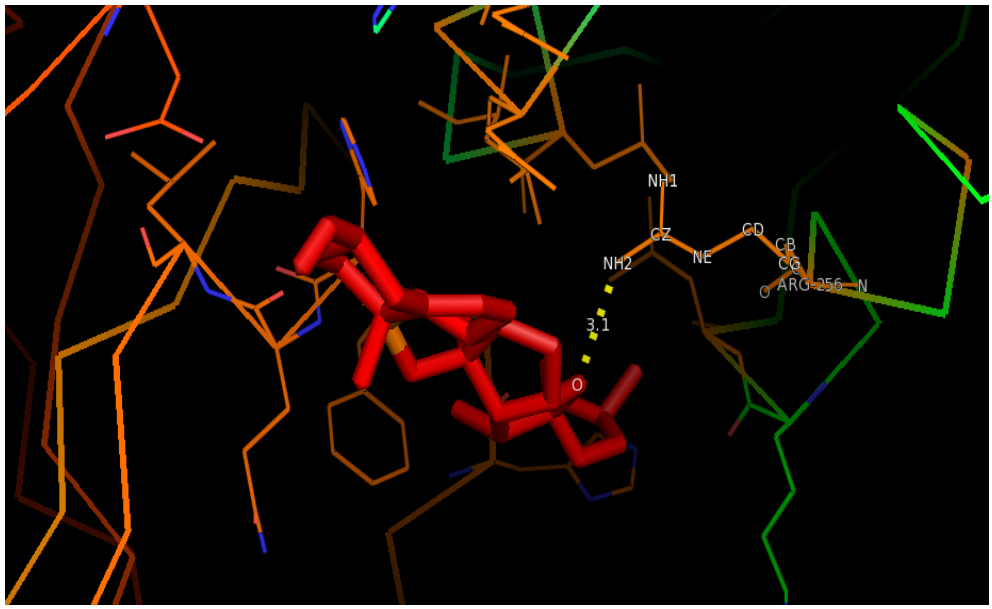
Fig.7 JAK AND DIOSGENIN: (a) Docking score (b) finalconformation (c) visualization of hydrogen bonds between JAK and diosgenin using pymol



(a)



(b)



(c)

Figure1: Structure of (a) STAT3, (b)IL-6,(c) JAK (The above color indicates pink-helix, yellow-beta sheet, & white-turns).

Ligand identification

The 2d structure of the diosgenin is drawn using ACD Chemsketch and saved in MDL MOL format and converted to PDB (3D) using open babel converter.

Docking of diosgenin with STAT3,IL6 andJAK

The diosgenin from *Costus igneus* is docked with drug targets such as STAT3.IL6, JAK.The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. Auto Dock (Good sell *et al*., 1996; Jones, 1997) requires pre-calculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 126,126 and 126 Å (x,y and z) to include all the amino acid residues that present in rigid macromolecules. AutoGrid4.2 program, supplied with AutoDock4.2 was used to produce grid maps. The Lamarckian Genetic Algorithm (LGA) (Morris, 1998) was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The diosgenin and STAT3 binding energy are shown in figures 4a, 5a and 6a, final conformations are shown in figures 4b,5b and 6b. Hydrogen bond between the donor and acceptor atoms is shown in table 4, 5 and 6.

The diosgenin from *costus igneus*is docked against STAT3. The docked structures were analyzed and the interactions were seen. Diosgenin interacts with STAT3, IL-6, and JAK forming hydrogen bonds and has good binding affinity with the docking score of -7.67,-7.23 and -7.3 kcal/mol, respectively.

These sites could be the best possible binding sites to inhibit the STAT3, IL-6 and JAK proteins. Hence, diosgenin from costus igneus posses anticancer activity and used for the treatment of liver cancer.

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