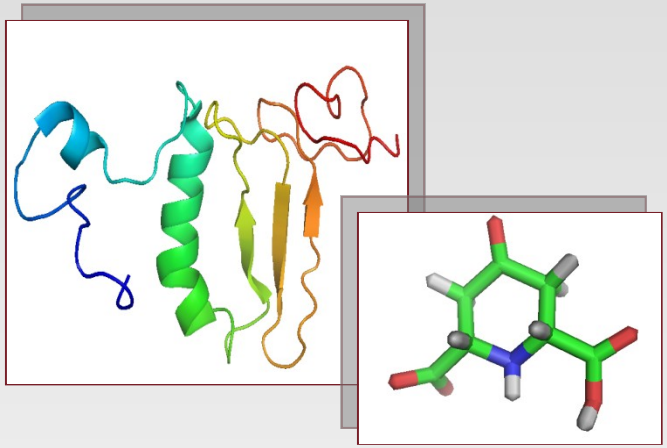
Docking-based virtual screening for the exploration of potential antagonists for human IGFBP6



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Insulin-like growth factor binding protein-6

IGF-AXIS

The insulin-like growth factors (IGFs) are polypeptides with high sequence similarity to insulin. But whilst insulin has predominantly metabolic effects, the IGFs are far more potent growth factors and anabolic agents.

They are present ubiquitously in the circulation and in the extracellular space.

Ligands

- IGF-I
- IGF-II

Receptors

- IGF-I
- IGF-II/mannose 6-phosphate
- Insulin, including insulin receptor isoform A

IGF binding proteins

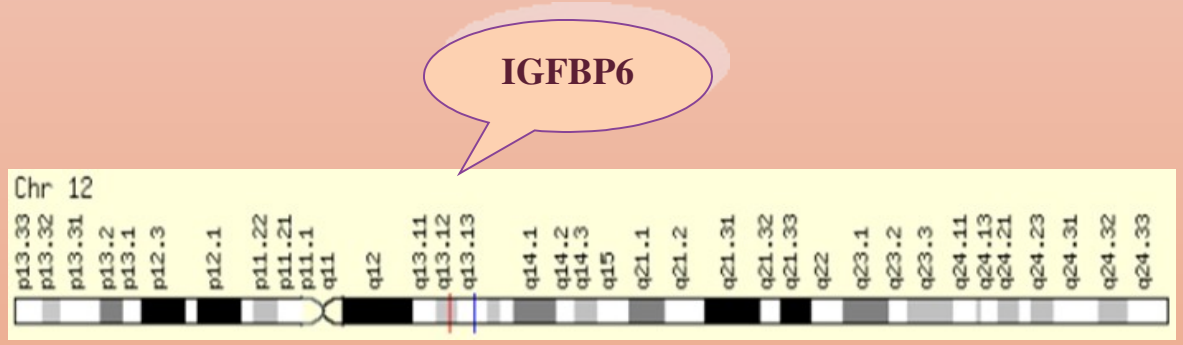
- IGFBP-1
- IGFBP-2
- IGFBP-3
- IGFBP-4
- IGFBP-5
- IGFBP-6

Function of IGF system:

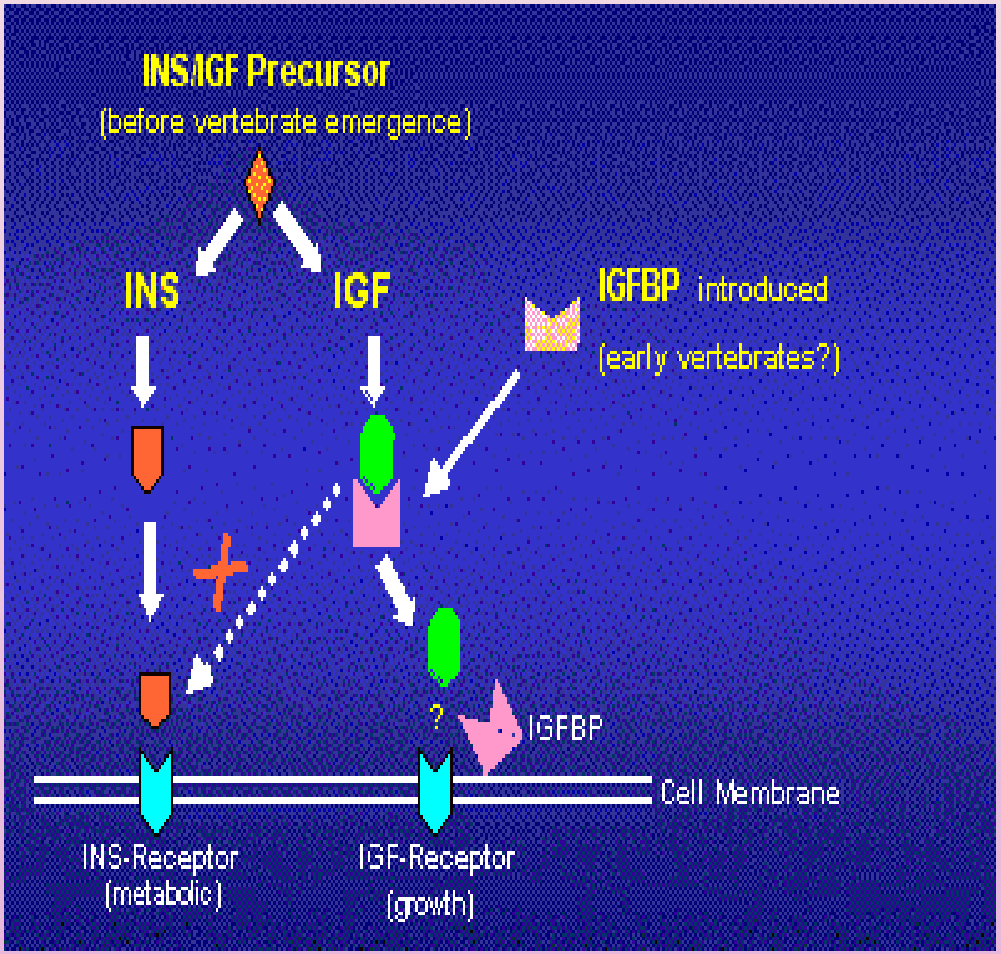
- Cell growth, metabolism and survival
- Aging
- Promotion of cell proliferation
- Inhibition of cell death (apoptosis).

Insulin-like growth factor-binding protein-6

- IGFBP6, a member of IGF-axis shows preferential affinity for insulin-like growth factor (IGF) II.
- Length: 240 amino acids
- Mol.wt: 25322.4D
- **Domain regions** : (i) Thyroglobulin type-1
  (ii) IGFBP
- **Function**: IGFBP-6 act as a crucial regulator in IGF system plays an important role in *osteogenesis* and *bone function*. It regulates (either stimulate or inhibit) the growth promoting effects on cell culture and alter the interaction of IGFs with their cell surface receptors.
- **Gene location** : 12q13.



Regulatory Roles of IGFBPs at the Cellular Level

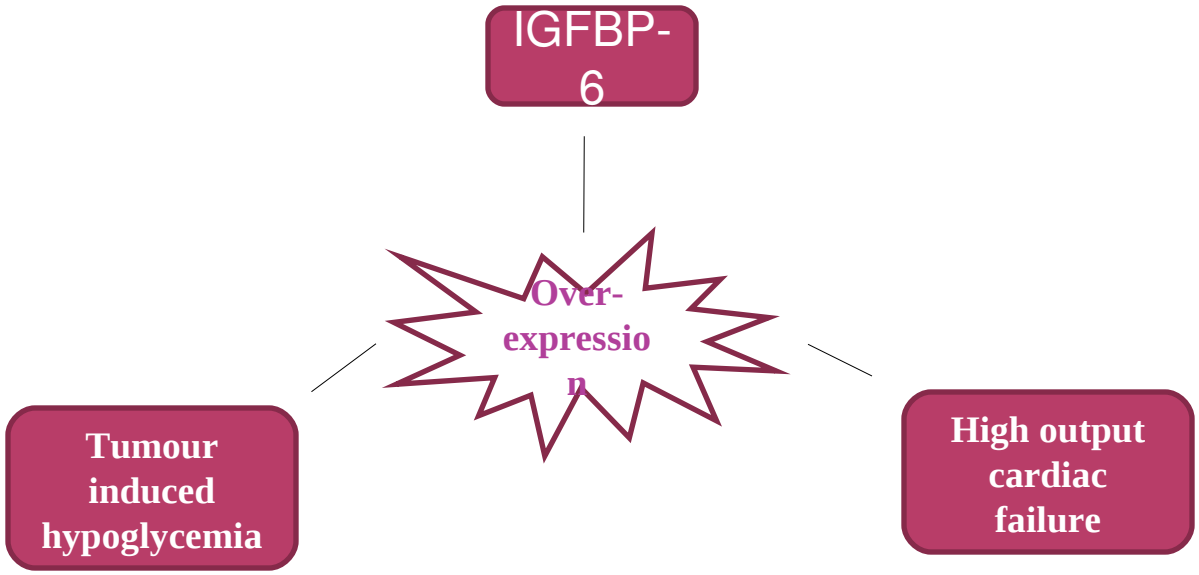


- IGFBP possess as high or higher IGF-binding affinities than that of the cellular IGF receptor, meaning that they are centrally positioned to influence IGF receptor binding and therefore the activation of cell growth.
- They also play a key role in regulating the availability of IGF to its own cellular receptor.

IGFBP-6 involvement in pathological conditions

•IGFs are important for both the regulation of normal physiology, as well as a number of pathological states, including cancer (IGF-1 stimulates growth of both prostate and breast cancer cells) and diabetes.

•IGFBP-6 elevated levels are expressed in tumour-induced hypoglycemia (Hoekman K *et al*., 1999, Clin Endocrinol) and high output cardiac failure(Sasaki *et al*., 2007, Int J Hematol).



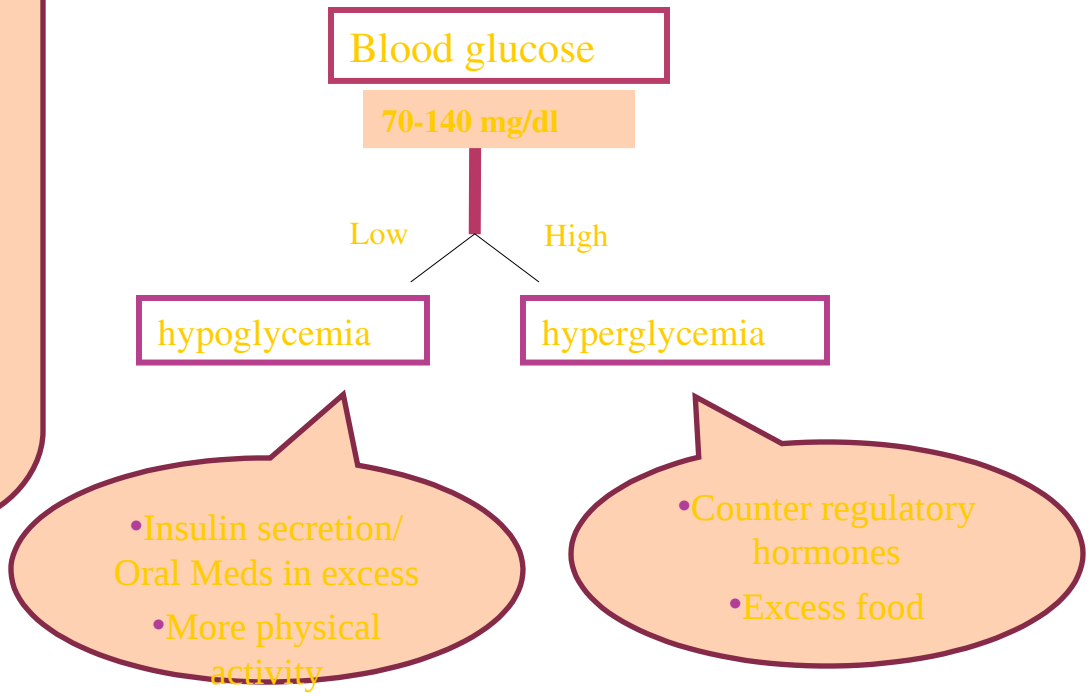
HYPOGLYCEMIA

- Blood sugar levels outside the normal range (70-140 mg/dl) may be an indicator of a medical condition. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia.

Effects can range from mild dysphoria to more serious issues such as seizures, unconsciousness, and (rarely) permanent brain damage or death.

Symptoms

- Anxiety
- Blurred vision
- dizziness
- Depression
- Fatigue
- Headache
- Irritability
- mood swings
- Confusion
- Mental disturbances



INHIBITORS

- IGFBP-6 over expression causes severe hypoglycemia and some cardiovascular diseases. Hence inhibitors are suggested to regulate the elevated levels of protein.
- The current IGFBP-6 inhibitors available in clinical practice are of less efficacy, leaving room for further improvement.

Acitretin
Adapalene
Alitretinoin
Bexarotene
Cycloheximide
Dexamethasone
Diethylstilbestrol
Etretinate
Forskolin
Isotretinoin
Retinal
Retinoic acid
Retinol
Tamoxifen
Tazarotene

From literature

(Rohan *et al.*, Endocrinology,1993)
(Zhou *et al.*, Endocrinology,1996)
(Hayford *et al.*, Growth horm IGF Res.,1998)
(Koike *et al.*, British journal of cancer, 2005)
(Martin *et al.*, Endocrinology,1995)
(Yan *et al.*, Growth horm IGF Res., 2001)
(Zhou *et al.*, Biochimica et Biophysica acta, 2001)
(Tanos *et al.*, Anticancer res.,2003)

Aim

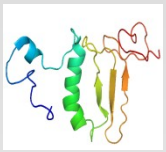
Docking-based virtual screening for the exploration of potential antagonists for human IGFBP6

Objectives

- Retrieval of IGFBP-6 structure.
- Prediction of ligand binding sites.
- Lead identification and optimization through computational docking and high throughput virtual screening.

Materials and methods

Retrieval of structure ⟷ RCSB PDB PROTEIN DATA BANK



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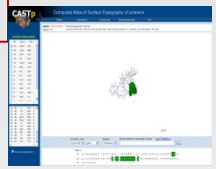
Identification of ligand binding sites ⟷ CASTp



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Lead identification and optimization ⟷ Ligand.info

Harvard ChemBank - 2,344 records
E-MSD ChemPDB - 4,009 records
KEGG Ligand - 10,005 records
Anti-HIV NCI - 42,689 records
Druglikeness NCI - 192,323 records
Unannotated NCI - 15,237 records
AKos GmbH - 544,391 records
Asinex Ltd. - 348,276 records

↓

Docking studies ⟷ SCHRÖDINGER

RESULTS AND DISCUSSION

STRUCTURE OF IGFBP-6

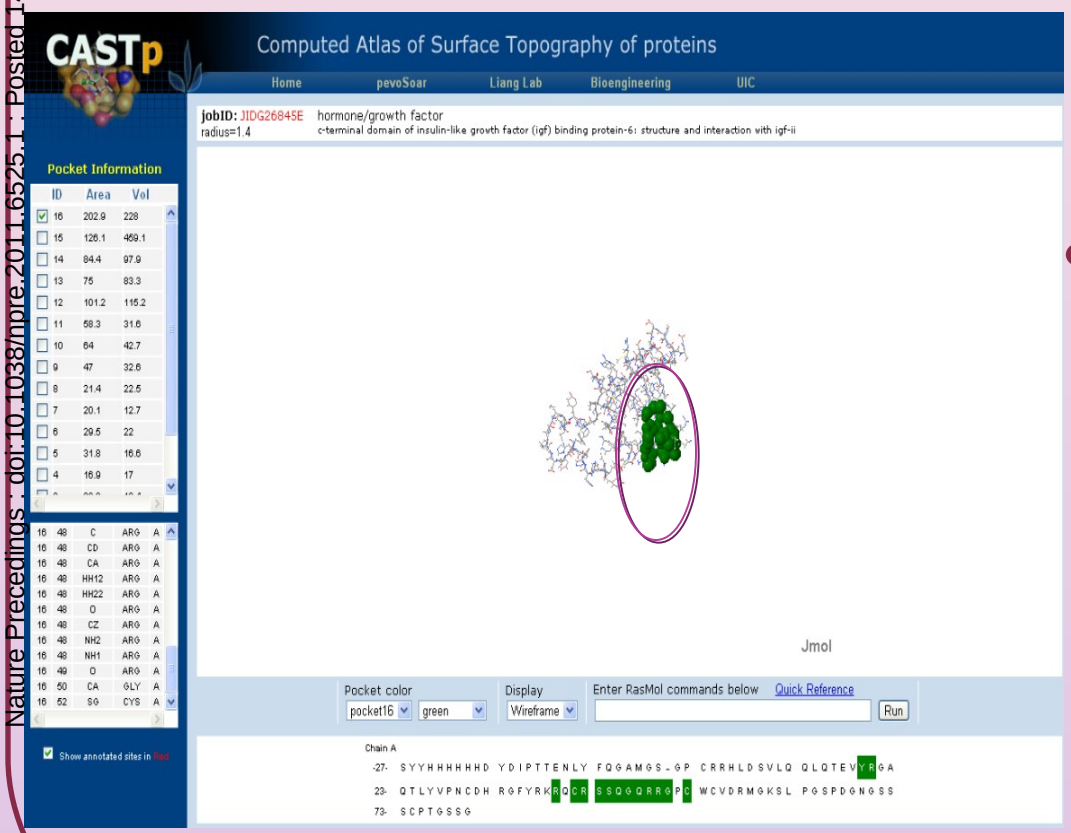
- The structure of IGFBP-6 was retrieved from PDB (Id=1RMJ).
- The 3D structure of IGFBP-6 was determined through nuclear magnetic resonance (NMR).
- The protein sequence consists of 18% helix (2 helices; 20 residues) and 14% beta sheet (5 strands; 16 residues).

RCSB PDB PROTEIN DATA BANK

1RMJ

Identification of ligand binding sites

- The ligand binding sites of IGFBP-6 were predicted through CASTp analysis. The amino acid residues constituting the functionality of protein are Tyr 19, Arg 20, Arg 39, Cys 41, Arg 42, Ser 43, Ser 44, Gln 45, Gly 46, Gln 47, Arg 48, Arg 49, Gly 50, and Cys 52.



Lead identification and optimization

Fifteen published IGFBP6 inhibitors obtained through literature subjected to structural analogue search (Ligandinfo)

50 analogues from each database

- Harvard ChemBank - 2,344 records
- E-MSD ChemPDB - 4,009 records
- KEGG Ligand - 10,005 records
- Anti-HIV NCI - 42,689 records
- Druglikeness NCI - 192,323 records
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Ligprep-5759 compounds

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Post-ligprep-11473 compounds

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Lipinski and reactive filters-7520 compounds

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Three levels of docking procedures (HTVS,SP,XP)-138 leads

138 leads

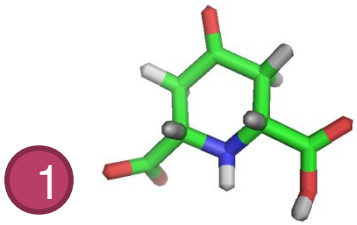
Contd…

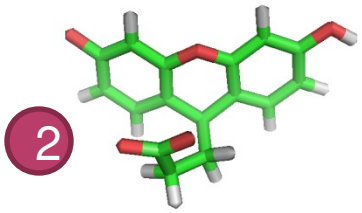
138 leads

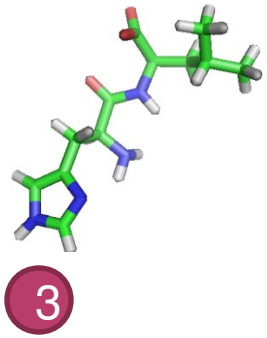
ADME properties

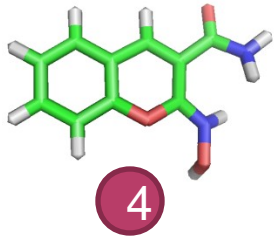
XP G scores

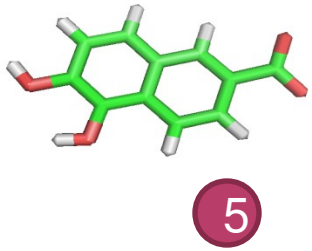
6 leads











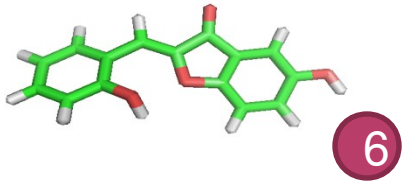
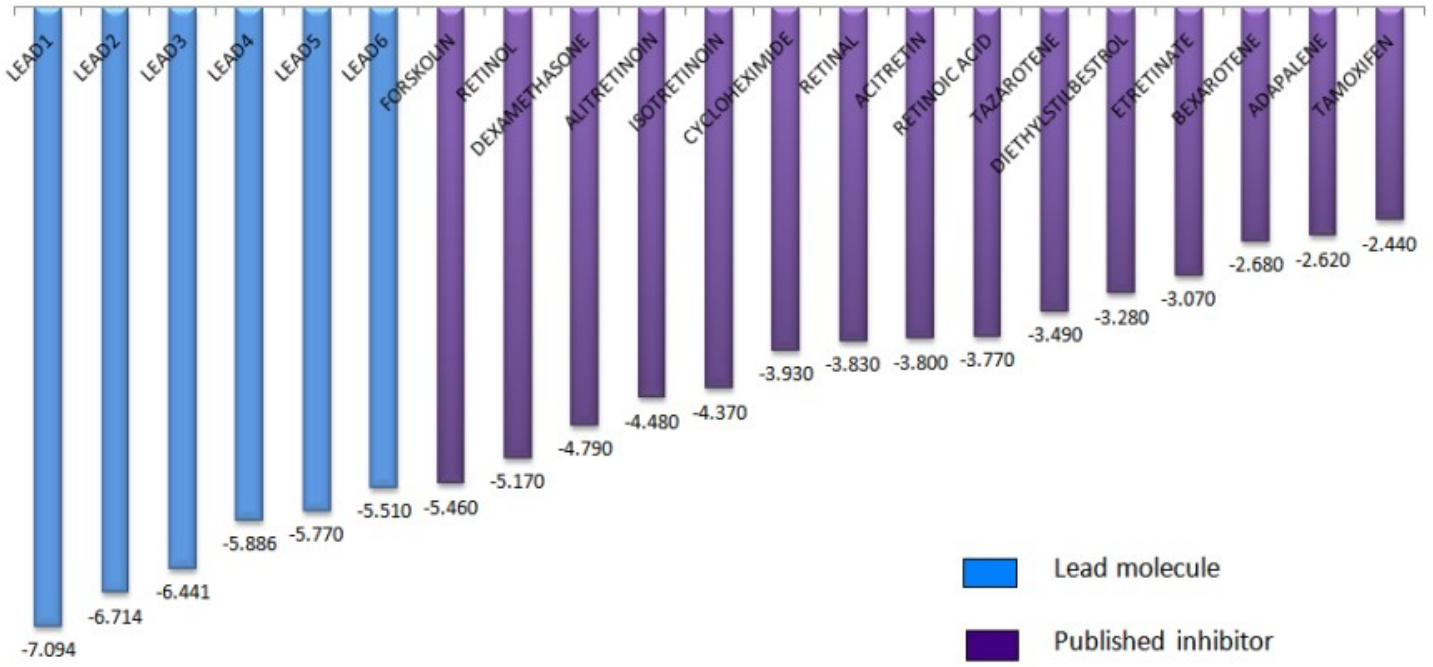


CHART OF LEADS

S.no.	Name of chemical compound	Mol.formula	Mol.wt (Daltons)	XP Gscore (kcal/mol)
1	Chelidamic acid	$C_7H_9NO_5$	187.15	-7.094
2	3,6-dihydroxy xanthene-9-propionic acid	$C_{16}H_{14}O_5$	286.28	-6.714
3	Benzotriazole tri-ethanolamine	$C_{12}H_{20}N_4O_3$	268.32	-6.441
4	6-methoxy 3-nitro quinoline	$C_{10}H_8N_2O_3$	204.19	-5.886
5	5,6-dihydroxy-2-naphthoic acid	$C_{11}H_8O_4$	204.18	-5.770
6	9,10-anthracenedione-1,8 dihydroxy 3-methylate	$C_{15}H_{10}O_4$	254.24	-5.510

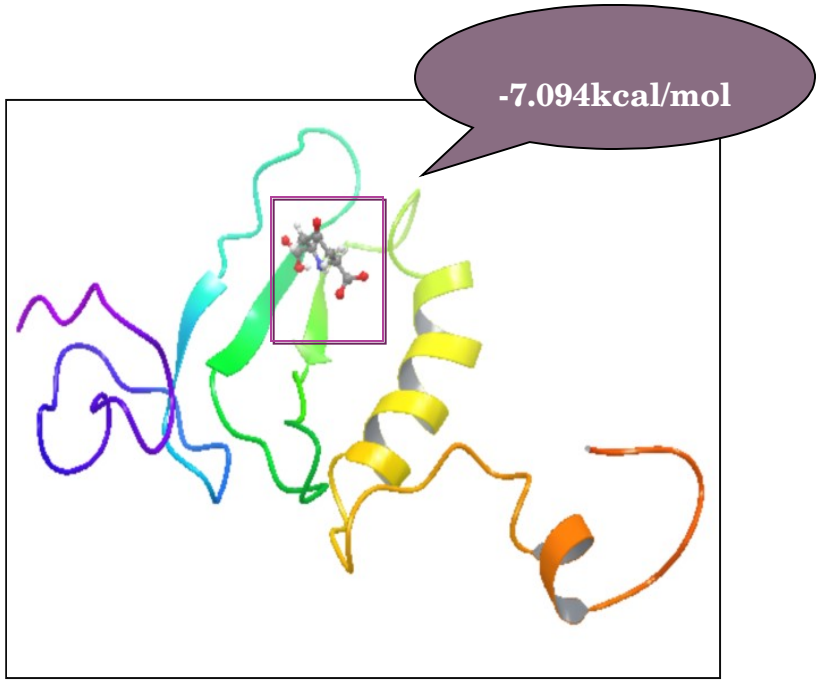
Comparision of existing inhibitors and proposed leads

- The six proposed leads are having better docking scores compared to fifteen existing inhibitors.

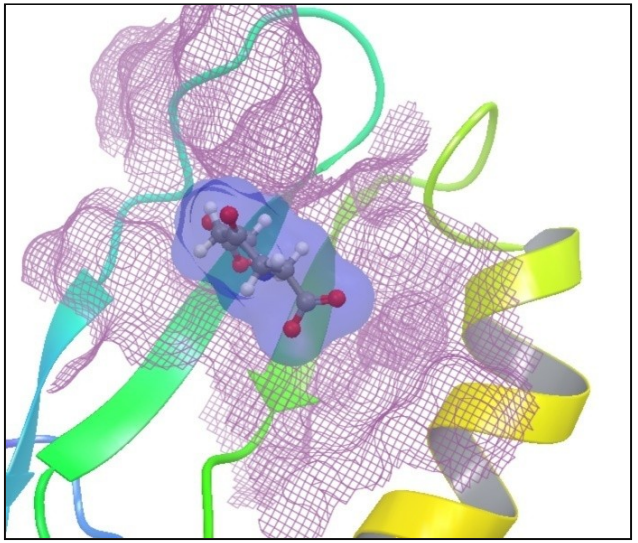


DOCKING INTERACTIONS

- Lead 1, *Chelidamic acid* was docked with IGFBP-6 with good binding affinity and XP Gscore of -7.094 k cal/mol.
- Mol.wt 187.15 kcal/mol.



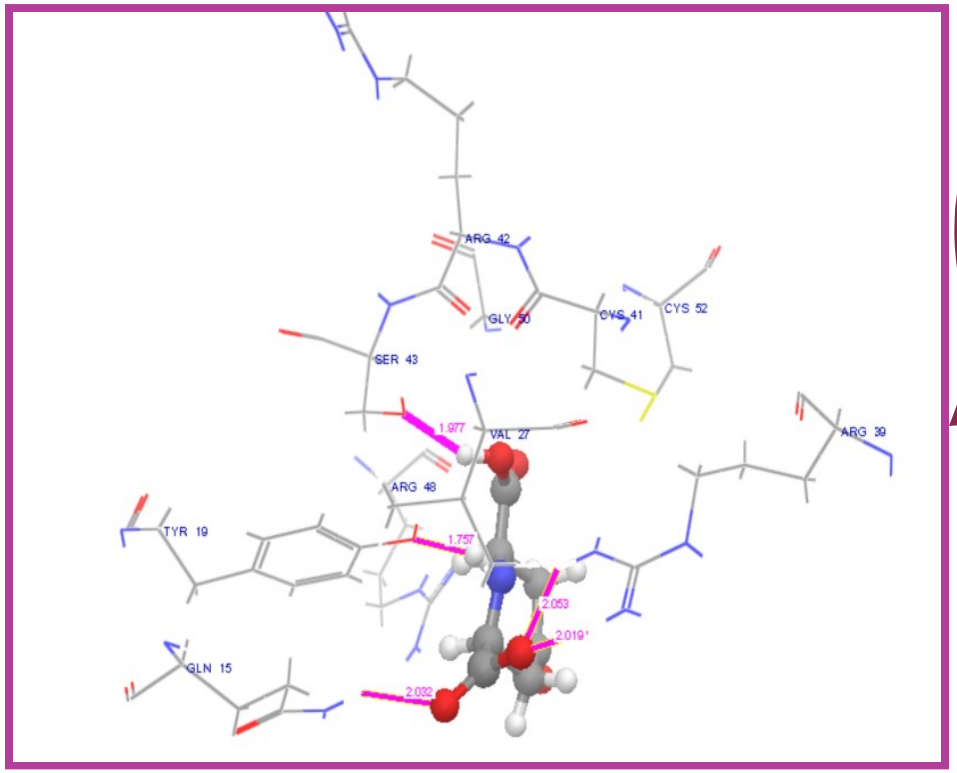
Docking complex



van der Waal interactions

Hydrogen bond interactions between Lead1 and IGFBP6:

•The residues Arg-39, Gln-15, Tyr-19 and Ser-43 are participated in hydrogen bond interactions. Of these, Arg-39 formed 2H-bonds, Gln-15 formed 1H- bond, Tyr-19 formed 1H-bond and Ser-43 formed 1 H-bond with the lead 1.These are present in ligand binding sites responsible for the functionality of IGFBP6.



Arg39- 2Hbonds (2.019,2.053)
Gln15- 1Hbond (2.032)
Tyr19- 1Hbond (1.757)
Ser43- 1Hbond (1.977)

CONCLUSION

- IGFBP-6 is the protein belonging to IGF family cause diseases like hypoglycemia, cancers and also some cardiovascular diseases during its elevated levels. Hence it is taken as a drug target.
- The present study was carried out to find more potent inhibitors to block its activity in diseased conditions.
- Docking results showed that six leads are having better XP Gscores compared to current IGFBP-6 inhibitors in clinical practice possessing good pharmacological & ADME properties and hence proposed as potent antagonists for IGFBP6.
- The lead 1 is 'Chelidamic acid', a novel chemical compound having good binding affinity with IGFBP-6 and its docking complex revealed an XP Gscore of -7.094 k cal/mol.
- Thus, it is hoped that the six newly identified leads if synthesized and tested in animal models would hold promise for drug discovery against IGFBP-6 .

ACKNOWLEDGEMENT

- I express my deep sense of gratitude to the honorable Dr. A. Umamaheswari, Coordinator of BIF & Head of the Department, Bioinformatics, SVIMS, Tirupati for her support, guidance and valuable suggestions.
- I am highly thankful to DBT, ministry of science and technology, Govt. of India for providing all the necessary facilities to carryout project.

Thank you