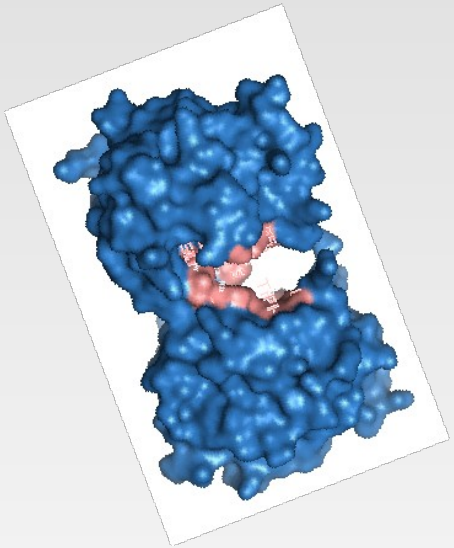
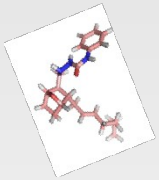
Identification of small molecule inhibitor of cyclophilin A using high throughput virtual screening and molecular docking studies





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SVIMS

- **Cyclophilin A** (CyPA) is a 20-kD chaperone protein with 165 a.a length.
- It is encoded by the gene PPIA (CyPA) and is located at the position 7p13 on the short arm of chromosome.
- EC 5.2.1.8-belongs to class Isomerase ; Subclass cis-trans isomerases and sub-sub class peptidylprolyl isomerase
- CyPA catalyzes the isomerization of peptide bonds from trans form to cis form at proline residues and facilitates protein folding.
- Cyclophilins are a family of highly conserved and ubiquitous proteins, termed immunophilins
- Thus, CyPA acts as acceleration factor in protein folding and assembly and the other roles include intracellular trafficking, signal transduction and transcription regulation.

Cyclophilin A

Over expression

Lee and Kim *Journal of Experimental & Clinical Cancer Research* 2010, **29**:97
http://www.jeccr.com/content/29/1/97

Journal of Experimental & Clinical Cancer Research

REVIEW Open Access

Current implications of cyclophilins in human cancers

Jinhwa Lee[1] and Sung Soo Kim*[2]

Abstract

Cyclophilins (Cyps), the intracellular receptor for immunosuppressant cyclosporine A (CsA), play important cellular roles through activities of peptidyl-prolyl cis-trans isomerase (PPIase) and chaperones. Cyps are structurally conserved and found in both prokaryotic and eukaryotic organisms, including humans which contain 16 Cyp isoforms. Although human Cyps were identified about 25 years ago, their physiological and pathological roles have only been the focus of attention recently because of their possible involvement in diseases and ailments such as HIV infection, hepatitis B and C viral infection, atherosclerosis, ER stress-related diseases and neurodegenerative diseases, *etc*. There are reports for upregulated Cyps in many human cancers and there are also strong correlations found between Cyps overexpression and malignant transformation. This review discusses the important and diverse roles of Cyps overexpression in human cancers. Understanding biological functions of Cyps will eventually lead to improved strategies for cancer treatment and prevention.

Introduction

Cyclophilins (Cyps) were initially identified as biological receptors for the immunosuppressive drug cyclosporine A (CsA) approximately 25 years ago. Later, they were shown to have peptidyl-prolyl cis-trans isomerase (PPIase) enzymatic activity which catalyzes cis-trans isomerization of peptide bonds preceding proline [1-6]. Cyps also possess chaperone activities. These two functions allow Cyps to be involved in proper folding of proteins in combination with other proteins. Although CsA is an effective inhibitor of Cyps, immunosuppressive activity of CsA is not the result of inhibition of the Cyps' activities. Rather, the Cyp-CsA complex accidentally inhibits calcineurin activity and thereby suppresses T-cell proliferation by interfering with downstream signal transduction [7].

Cyps are highly conserved from *E. coli* to humans throughout evolution. A total of 16 Cyp isoforms have been found in humans [8], but 7 major human Cyp isoforms, namely hCypA, hCypB, hCypC, hCypD, hCypE, hCyp40, and hCypNK [9], have been well characterized. They play diverse roles by localizing through unique domains for particular cellular compartments including the cytosol, endoplasmic reticulum (ER), mitochondria and nucleus. The clinical importance of Cyps has been implicated in diverse pathological conditions including HIV [10], hepatitis B and C viral infection, atherosclerosis [11,12], ER stress-related diseases such as diabetes, and neurodegenerative diseases. Cyps are also involved in normal cellular functions of muscle differentiation, detoxification of reactive oxygen species (ROS) [13], and immune response [14]. Their novel and unfamiliar nuclease activity similar to apoptotic endonucleases suggests a potential role in apoptotic DNA degradation. Overall roles of Cyps may encompass far more than already defined functions such as protein folding.

CypA overexpression in diverse types of cancer has been recently reported by many research groups. Subsequently, overexpression of other Cyps has also been repeatedly observed in various cancers. Although Cyps expression levels and patterns in many cancer types have been considerably well documented, the precise roles of Cyps in cancer are hardly defined. Here, we will discuss the implications of Cyps in cancer biology and particularly give emphasis on CypA that has been studied most extensively in diverse human cancers. Better understanding of Cyps' function in cancers may divulge their potential applications in cancer prevention, diagnosis, and treatment.

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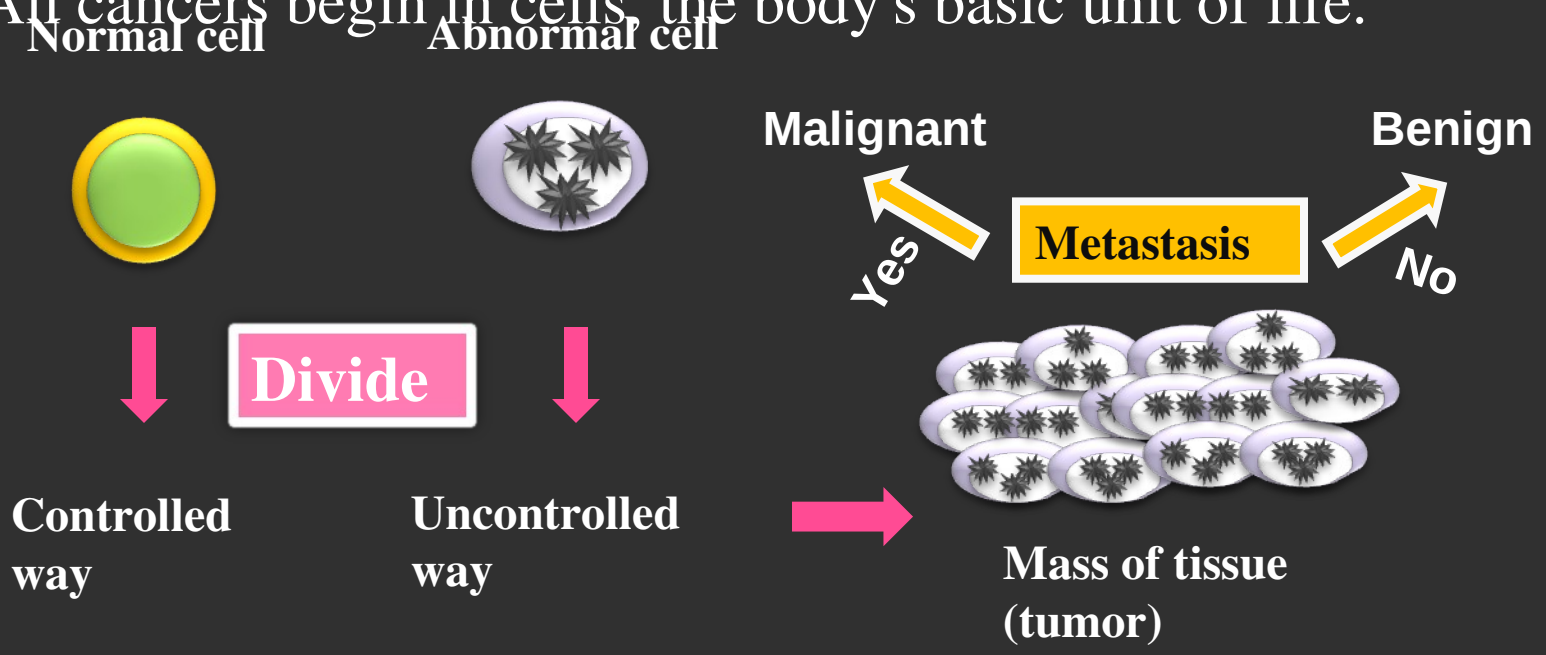
Table 1: Cyclophilin A in human cancers

Cancer type	Functions and implications of CypA in cancers	Contributers
Lung cancer	The first identification of CypA overexpression in lung cancer	**Campa et al.,** Cancer Res. (2003)
	Potential role of CypA in early neoplastic transformation and as a biomarker	**Howard et al.,** Lung Cancer (2004)
	Regulation of cancer growth, angiogenesisa and apoptosis through CypA knockdown and overexpression	**Howard et al.,** Cancer Res. (2005)
	Role of exogenous CypA in increased H446 cell growth through ERK1/2 pathway activation	**Yang et al.,** BBRC (2007)
Pancreatic cancer	Identification of CypA as a decreased factor by 5-aza-2-deoxycytidine	**Cecconi et al.,** Eletrophoresis (2003)
	Involvement of increased CypA in pancreatic carcinogenesis	**Shen et al.,** Cancer Res. (2004)
	Effect on the gene expression of several key molecules including NRPs, VEGF, and VEGFRs	**Li et al.,** Am J Surg (2005)
	Stimulation of cancer cell proliferation by increased CypA through CD 147 signaling	**Li et al.,** Cancer Res (2006)
	Association of increased CypA with tumor invasion, metastasis, and resistance to therapy	**Mikuriya et al.,** Int J Oncol (2007)
Hepatocellular carcinoma	Regulation of cancer cell proliferation and increase of hepatocarcinoma formation by interaction of increased CypA with calcineurin	**Corton et al.,** Cancer Let (1998)
	Identification as a useful HCC marker in tumor tissues	**Lim et al.,** BBRC (2002)
Breast cancer	Assessment of CypA down-regulation through proteomics in melphalan-resistant and -susceptible MCF-7 cell lines	**Hathout et al.,** J proteomic Res (2002)
	Role of CypA in cancer cell progression and regulation of JAK2	**Zheng et al.,** Cancer Res (2008)
Colorectal cancer	Identification of association of CypA with tumor development and tumor progression through protein profiling	**Melle et al.,** Int J Mol Med (2005)
	Role of CypA in COX-2-independent chemopreventive effect by celecoxib	**Lou et al.,** Cancer Epidemiol (2006)
	Upregualtion of CypA among5-fluorouracil (5-FU) response proteins for CRC chemotherapy	**Wong et al.,** Oncol Rep (2008)
Squamous cell carcinoma	Involvement in oncogenesis in SCC	**Chen et al.,** Proteomics (2004)
	Possible role as a malignant transformation-related protein in ESCC	**Qi et al.,** J Cell Biochem (2008)
Melanoma	High level expression in primary and metastatic melanoma	**Al-Ghoul et al.,** J Proteome Res (2008)
Prostate cancer	Preventing hypoxia- and cisplatin-induced apoptosis	**Choi et al.,** Cancer res (2007)
Glioblastoma multiforme	Increasing expression of CypA in human glioblastoma multiforme	**Han et al.,** Oncol Rep (2010)

Cancer

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues.

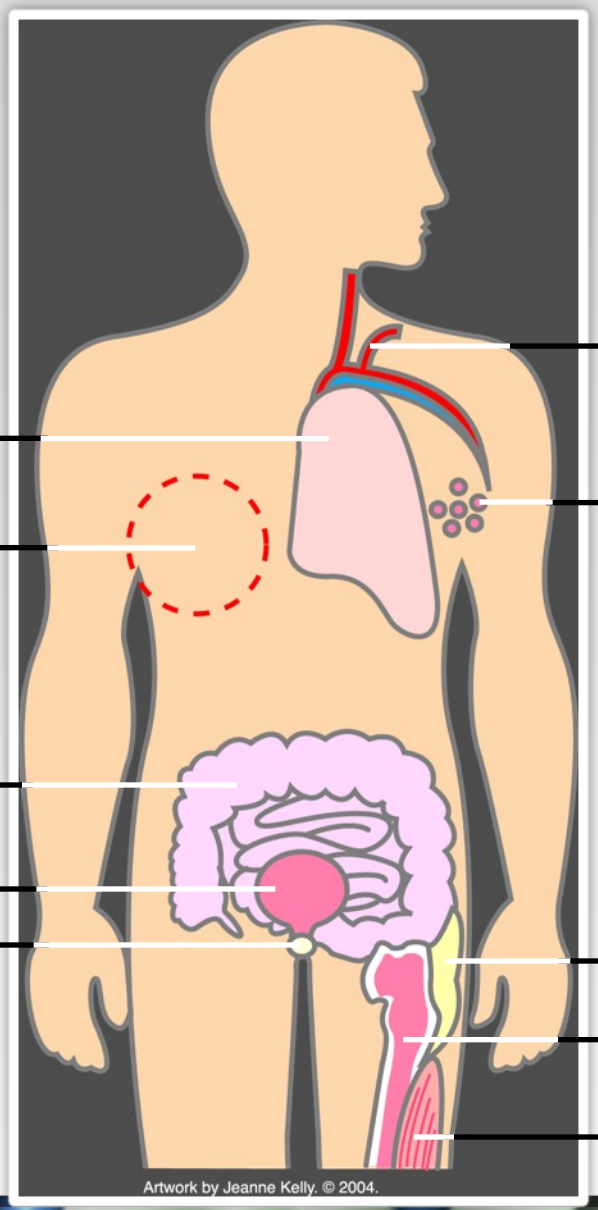
All cancers begin in cells, the body's basic unit of life.



Different Kinds of Cancer

Some common carcinomas:

- Lung
- Breast (women)
- Colon
- Bladder
- Prostate (men)



Leukemias:

- Bloodstream

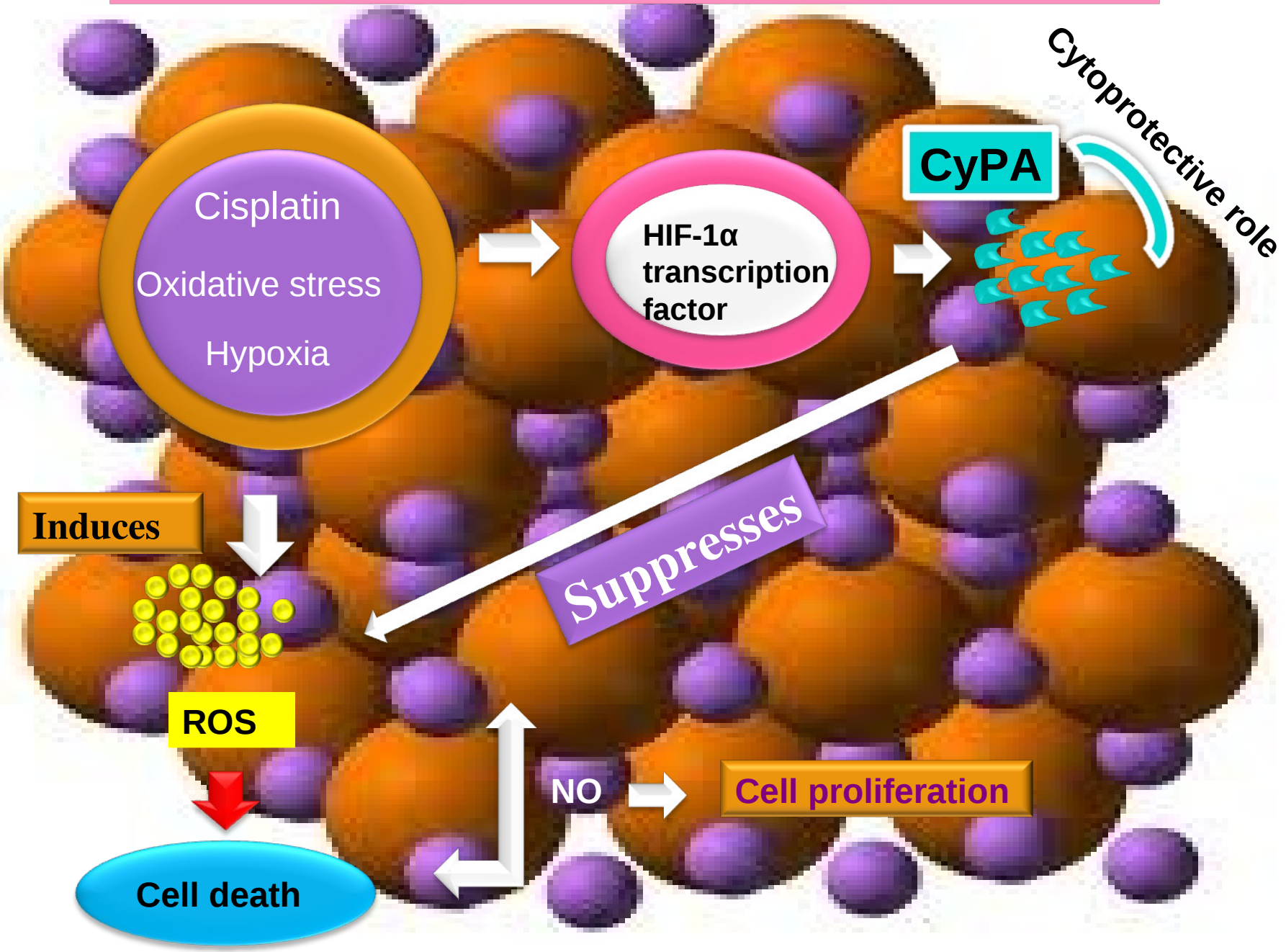
Lymphomas:

- Lymph nodes

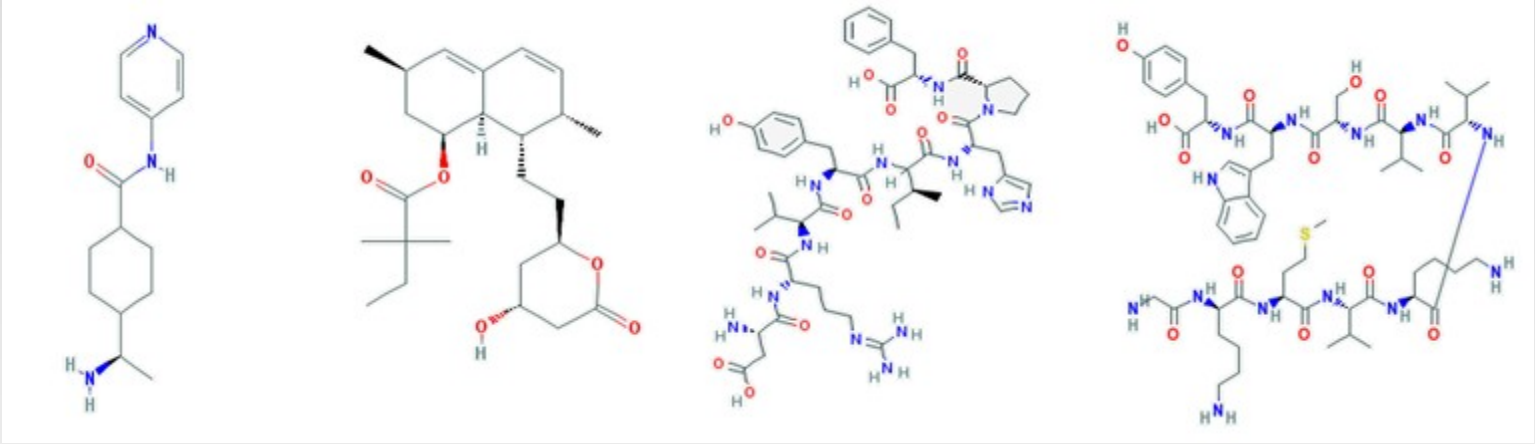
Some common sarcomas:

- Fat
- Bone
- Muscle

Mechanism of CyPA in cell proliferation of solid tumor



The existing inhibitors for cyclophilin A



Y 27632

Simvastatin

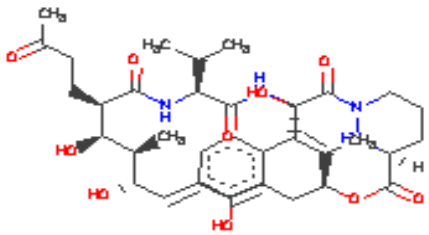
AT 1 a receptor blocker

ACE inbitor

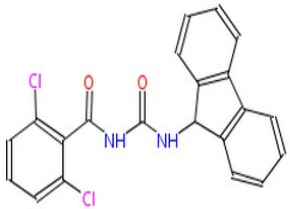
Satoh *et al.*, 2009

Habashi *et al.*, 2006

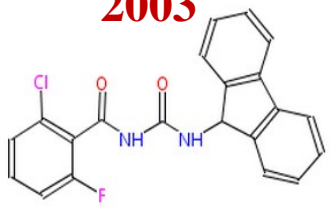
Ejiri *et al.*, 2003



Sanglifehrin A



3h



3i

Sedrani *et al.*, 2003

Shuaishuai *et al.*, 2009

Aim

Identification of small molecule inhibitor of cyclophilin A using high throughput virtual screening and molecular docking studies

Objectives

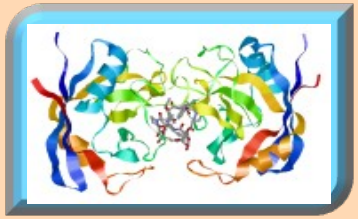
- *Phylogenetic analysis for the selected 17 proteins involved in cancer.*
- *Structural analysis for active site detection.*
- *Ligand based virtual screening for the existing inhibitors of human CyPA.*
- *Molecular docking studies using Schrödinger software suite 2010 (Maestro v9.1).*

WORK FLOW

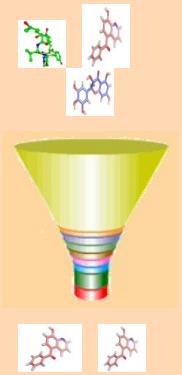
Retrieval of crystal structure PDB PROTEIN DATA BANK

Visualization & Active sites prediction

PyMOL Home Page

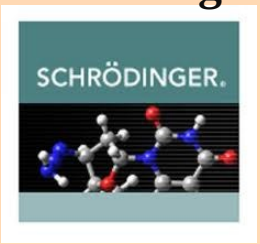


Structural analogue search



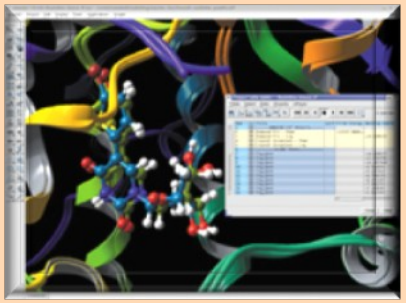
In-house library

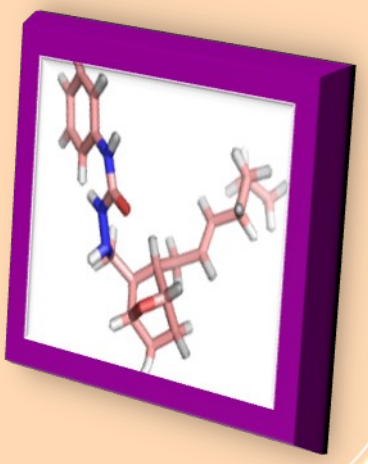
Docking studies



Maestro v9.1

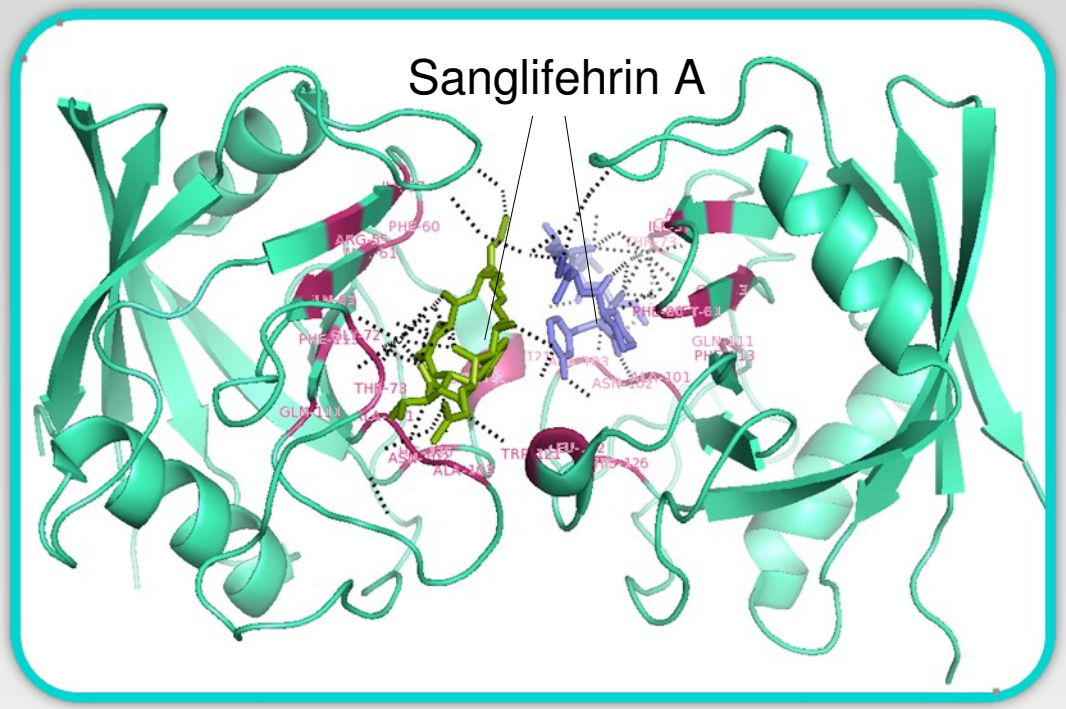
Docked complex





Results

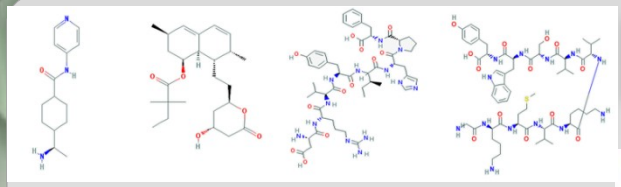
1NMK



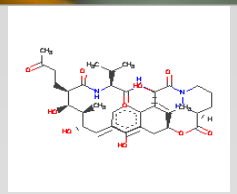
- X-Ray Diffraction.
- 2 similar chains-A,B.

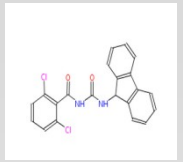
- DSSP program has predicted 13% helices (3 helices; 23 residues) and 33% beta sheets (13 strands; 56 residues).

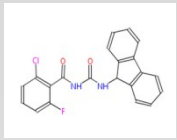
- The amino acid residues **Arg 55**, Ile 57, Phe 60, Met 61, **Gln 63**, **Gly 72, Thr 73, Ala 101**, **Asn 102,** Ala 103, Gln 111, Phe 113, Trp 121, Leu 122, **His 126** and water molecules at position1001, 1036, 1049, 1056, 1074, 1076, 1103, 1105 were found to be present in active site region of human CyPA protein around 4Å of the inhibitor sanglifehrin A.



7 existing inhibitors







50 analogues

2800 compounds

Ligand preparation

LigPrep – 2771 structures
Post LigPrep – 10,351 orientations
QikProp – 9929 (422 failed)
Lipinski filter - 4682

Protein preparation

Energy minimization – OPLS 2005
Grid generation – 20 x 20 x 20 Å

Glide

HTVS – 594
SP docking – 236
XP docking - 151

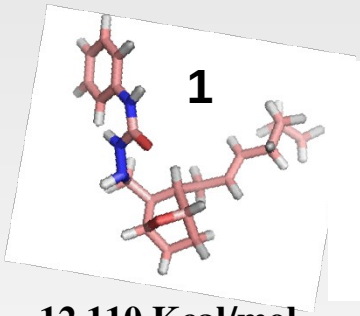
Out of 151

XP GScore

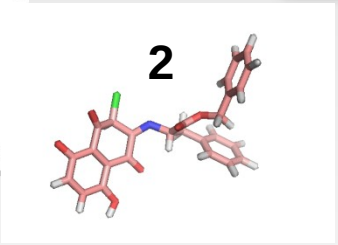
Better than existing inhibitors

30 compounds

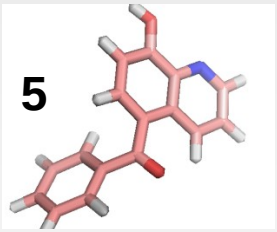
TOP 10 proposed leads



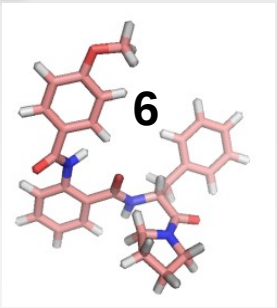
-12.110 Kcal/mol



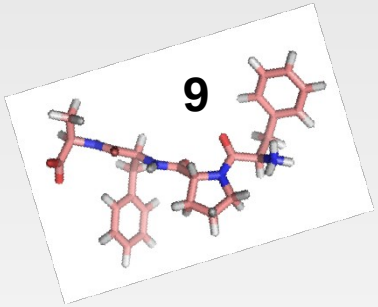
-11.635 Kcal/mol



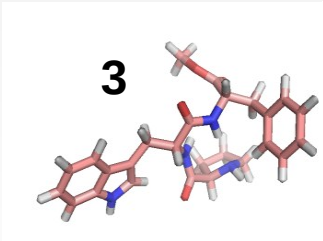
-10.897 Kcal/mol



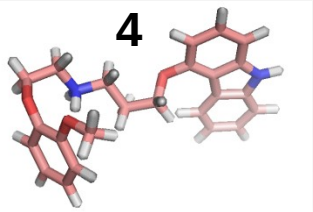
-10.837 Kcal/mol



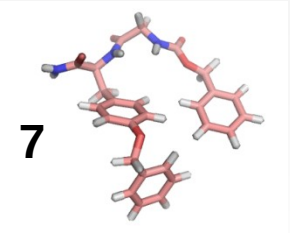
-10.166 Kcal/mol



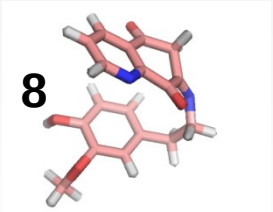
-11.101 Kcal/mol



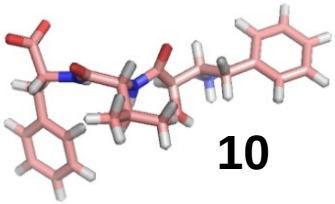
-11.054 Kcal/mol



-10.652 Kcal/mol



-10.448 Kcal/mol

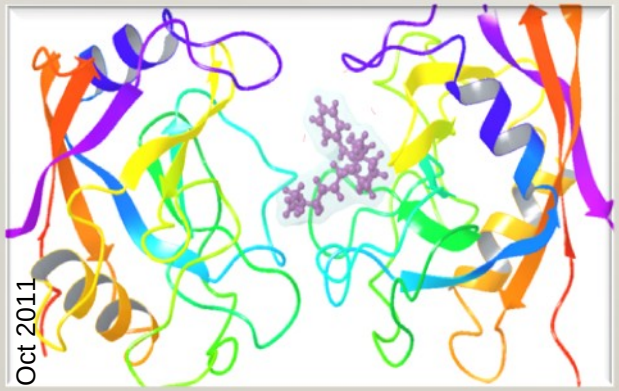


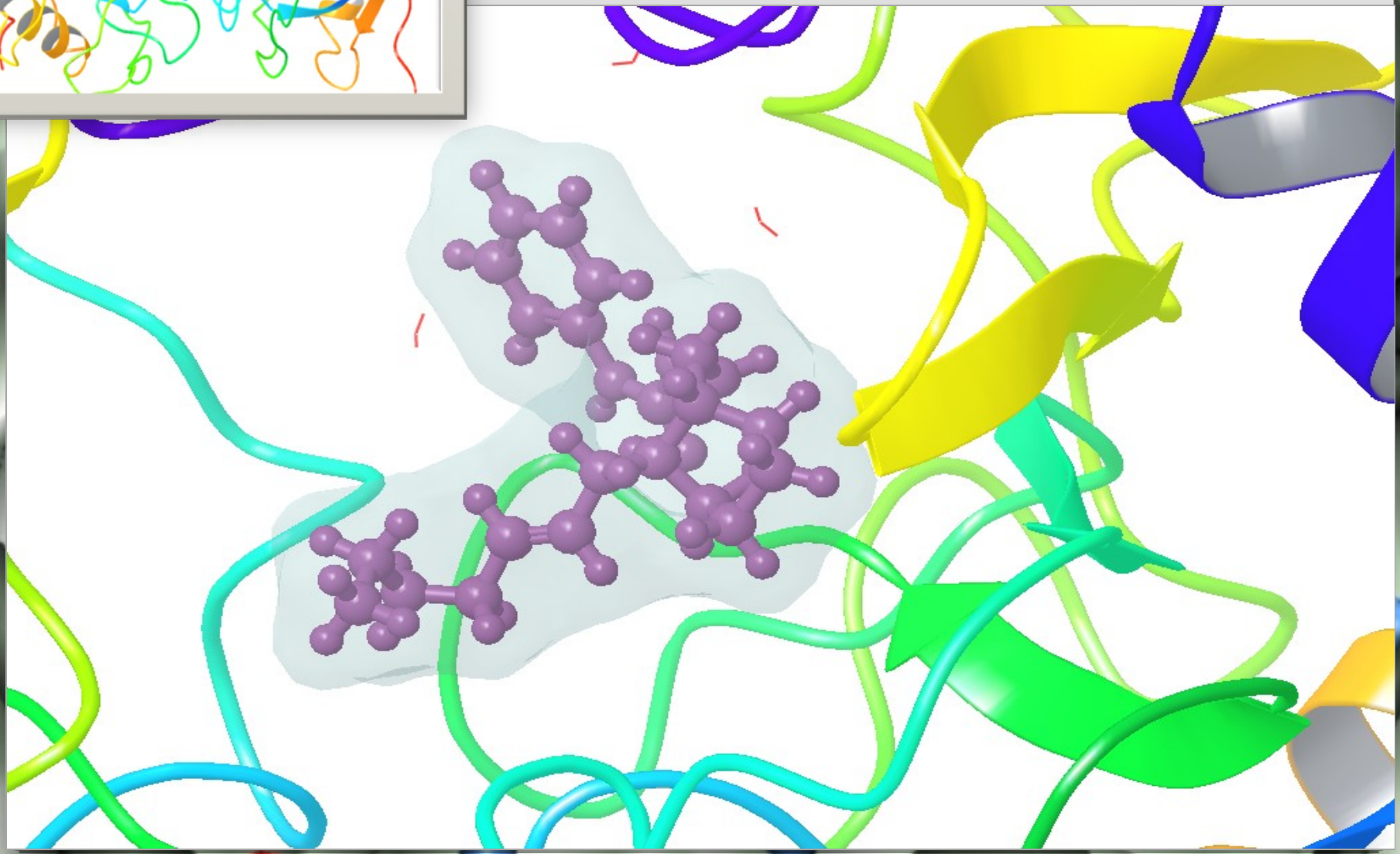
-10.152 Kcal/mol

The list of ten lead molecules

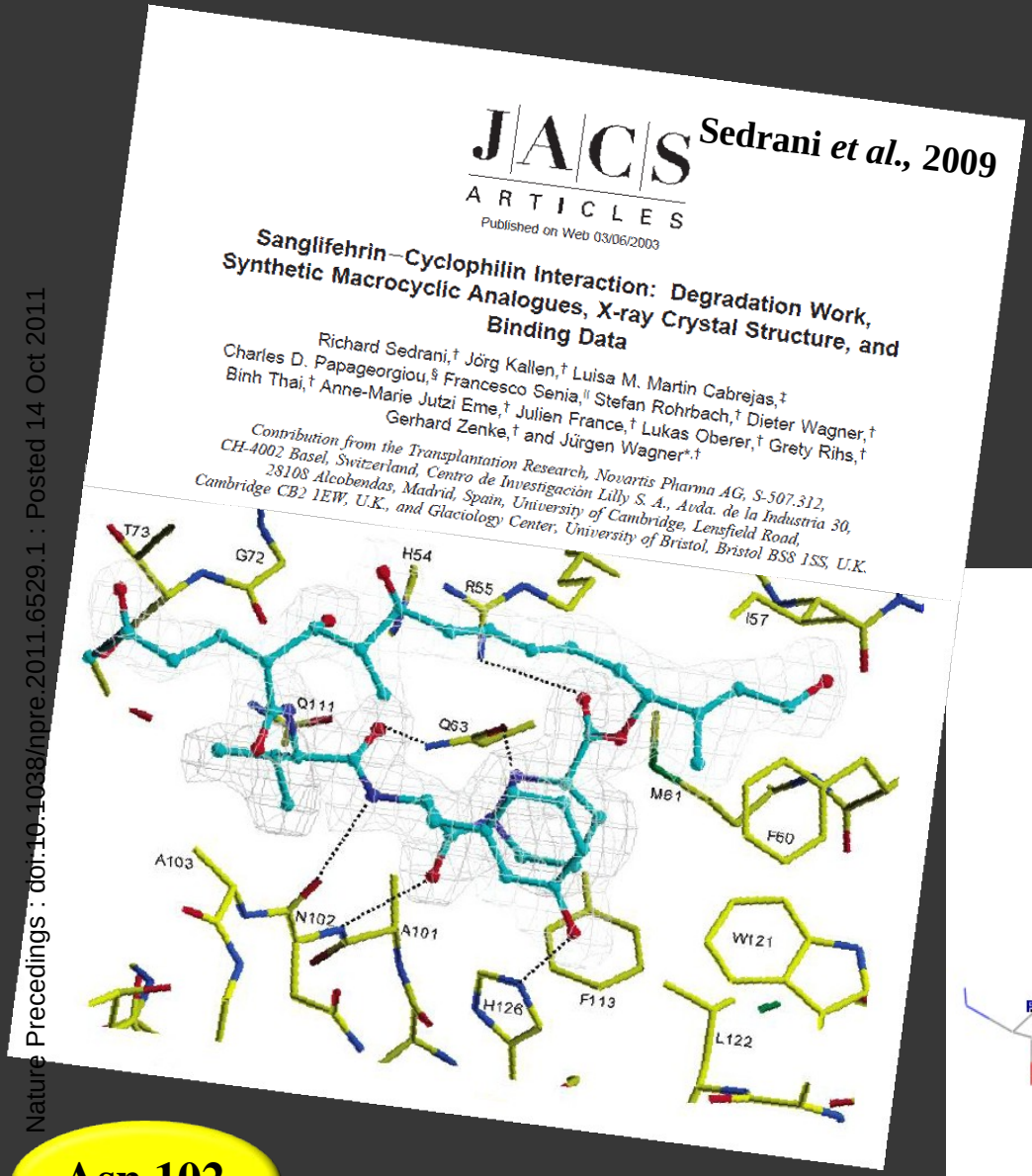
1. SQ-29548
2. Benzyl 2-((3-chloro-5, 8-dihydroxyl-1, 4 dioxo-1,4-dihydro-2-napthalenyl)amino-3-phenyl propanoate)
3. Methyl2-((3-(1H-indol-3-yl)-2-((2-pyrrolidinylcarbonyl)amino)propanoyl)amino)-3-phenyl propanoate
4. Carvedilol
5. (8-hydroxy-5-quinolinyl)(phenyl) methanone
6. C28H29N3O4
7. C2-(4-Benzyloxy-phenyl)-1-carbamoyl-ethylcarbamoyl]-methyl}-carbamic acid benzy
8. C18H16N2O4
9. N-(2-(((1-(2-amino-3-phenylpropanoyl)-2-pyrrolidinyl)carbonyl)amino)-3-phenyl propanoyl) alanine
10. C24H31N3O5

Docking complex of lead 1 with human cyclophilin A





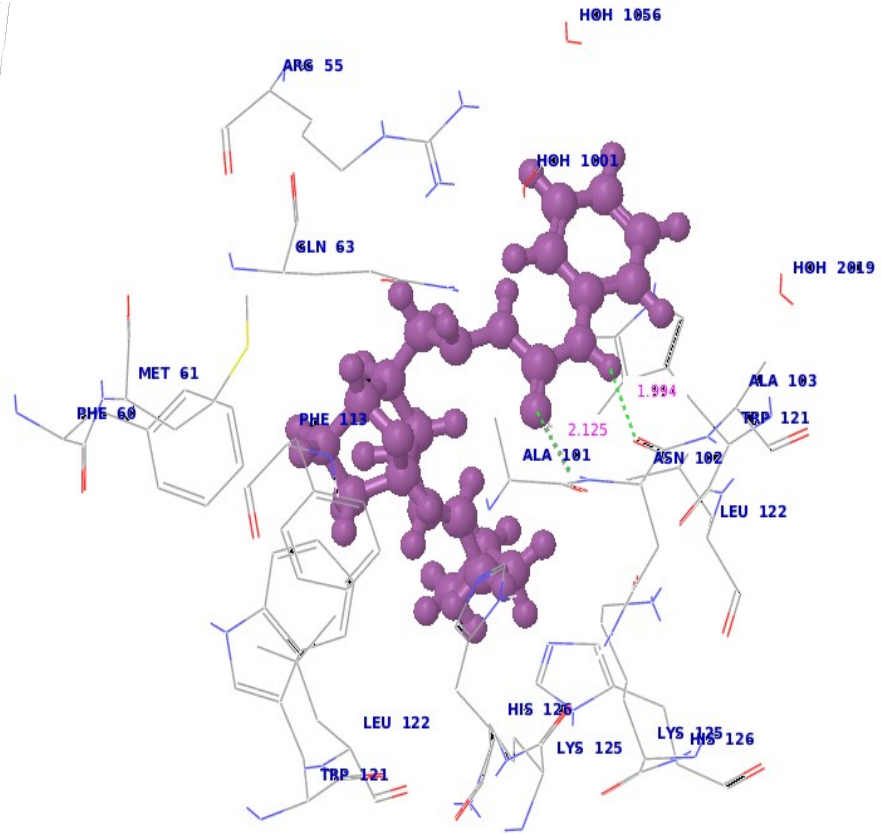
Comparative analysis



Asn 102

2 H-bonds – 2.125
1.994

Arg 55, Ile 57, Phe 60, Met 61, Gln 63, Gly 72, Thr 73, Ala 101, **Asn 102**, Ala 103, Gln 111, Phe 113, Trp 121, Leu 122, His 126



Treating high BP

Losartan
Antagonist
(AT 1a RB)

Common side effects

Head ache
dizziness
weakness
fatigue

Other

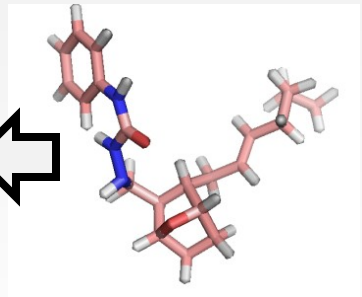
raised liver enzymes
cholestatic hepatitis
pancreatitis,
angio-edema

Poor

Pharmacokinetic & ADME Properties

Good

SQ-29548



Antagonist for TP receptor

CONCLUSION

- **CyPA over expression has been observed in diverse types of cancer (Lee and Kim, 2010) and also in some of the cardiovascular diseases like atherosclerosis, vascular stenosis, abdominal aortic aneurysm, cardiac hypertrophy etc., (Saoth *et al*, 2010).**

- **Identification of small molecule inhibitors through virtual screening along with molecular docking studies can act as starting point for new generation drug designing from previous studies.**

- **The docking result endeavors that 10 proposed leads were not violating ADME properties and have better XP GScore than the existing inhibitors.**

- **Lead-1 (SQ-29548) has been docked with human cyclophilin A with XPGScore -12.110 Kcal/mol.**

- **SQ-29548 already used as an antagonist for the human recombinant thromboxane receptor which supports the ten proposed leads as potential inhibitors.**

- **Thus, the novel leads identified could reduce the CyPA level which gains its cytoprotective role to combat against different cancers as well as cardiovascular associated diseases if synthesized and validated in animal model.**

Acknowledgement

I owe my deepest gratitude to the honorable Dr. A. Umamaheswari, Associate Professor and Head of the Department for her able guidance, valuable suggestions.

I am highly thankful to DBT, ministry of science and technology, Govt. of India for supporting this work under bioinformatics studentship fellowship program.

THANK You