Ligand based virtual screening to design novel potent inhibitors for human NGAL involved in cancers

Guide

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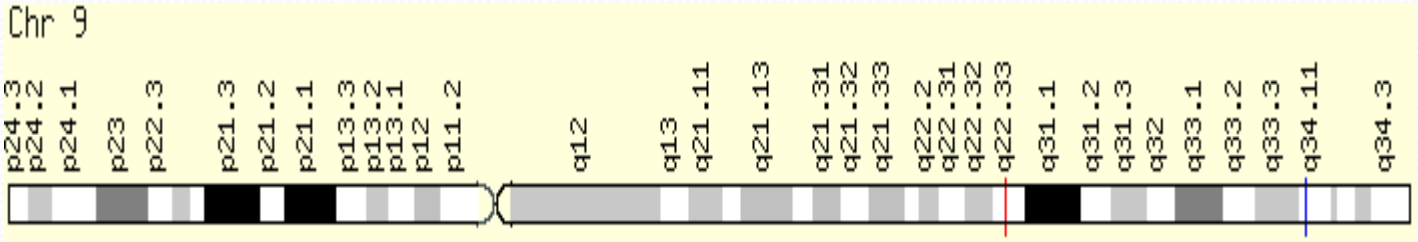
CANCERS

- Cancer is a class of diseases characterized by uncontrolled cell growth in which cells undergo alterations of their normal functions that progressively lead to the genesis of a tumor.
- There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected.
- Neutrophil-gelatinase associated lipocalin(NGAL) was generally found in activated human neutrophils but it is also expressed in human tissues kidney, prostate and epithelia of the respiratory and alimentary tracts.

GENE Mapping



NGAL is located on 9th chromosome with a band pattern of 9q34.11

Chromosome :9

Location:9q34.11

NGAL as cancer target

- Neutrophil gelatinase–associated lipocalin (NGAL) a 25 kDa glycoprotein covalently bound with matrix metalloproteinase-9(MMP-9) in human neutrophils.
- NGAL delivers iron from extracellular milieu(medium) into the cells because it has been described that iron could play an important role in cancer and apoptosis.

Functions of NGAL

- NGAL is involved in multiple processes such as apoptosis, innate immunity and renal development.
- NGAL delivers innate immunity by sequestrating iron leading to limit bacterial growth.

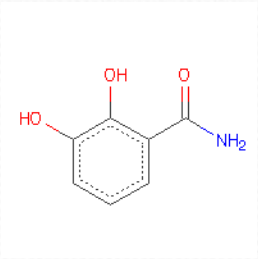
Diseases associated with human NGAL

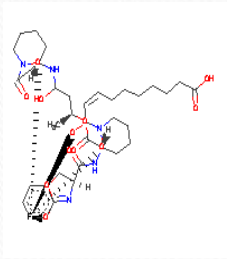
- Over expression of human NGAL leads to variety of cancers namely breast, lung and pancreas.

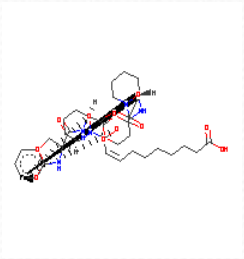
- NGAL has been proposed as an early biomarker in pancreatic cancer which leads to adhesion, invasion and angiogenesis.

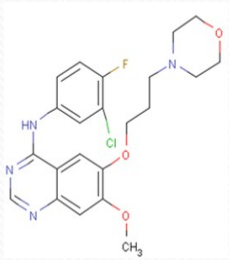
- Reduction of NGAL-induced cellular invasion is due to inhibition of focal adhesion kinase (FAK) phosphorylation.
- Angiogenesis reduction is mainly due to inhibition of vascular endothelial growth factor (VEGF) secreted by these cells.
- Therefore controlling NGAL over expression would control PaCa angiogenesis and metastasis, so NGAL is considered as a biomarker for PaCa progression and manipulation.

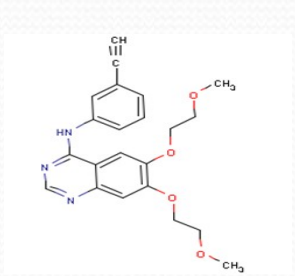
Inhibitors for human NGAL











2,3 DIHYDROXYBENZAMIDE CARBOXYMYCOBACTIN S CARBOXYMYCOBACTIN T GEFITINIB IRESSA ERLOTINIB TARCEVA

Holmes *et al*., 2005

(Siderocalin also binds carboxymycobactin, potentially defending against mycobacterial infections through iron sequestration)

Xiaoyan cui *et al*., 2009

(NGAL-dependent Apoptosis Resistance in NSCLC)

OBJECTIVES

- Annotation of structural and functional aspects of human NGAL.
- Prediction of binding site residues.
- Lead identification and optimization through computational docking and high throughput virtual screening.

MATERIALS AND METHODS

RCSB PDB PROTEIN DATA BANK

INHIBITORS FROM PDB, LITERATURE

Ligand databases

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

ACTIVE SITE RESIDUES

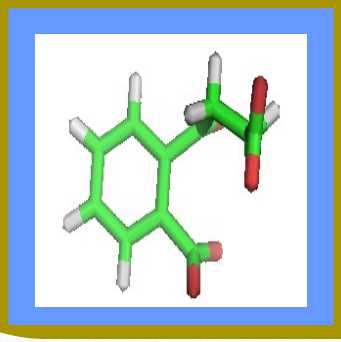
In-house library

Docking

SCHRÖDINGER (Maestro v9.2)

Virtual screening

Lead '1'

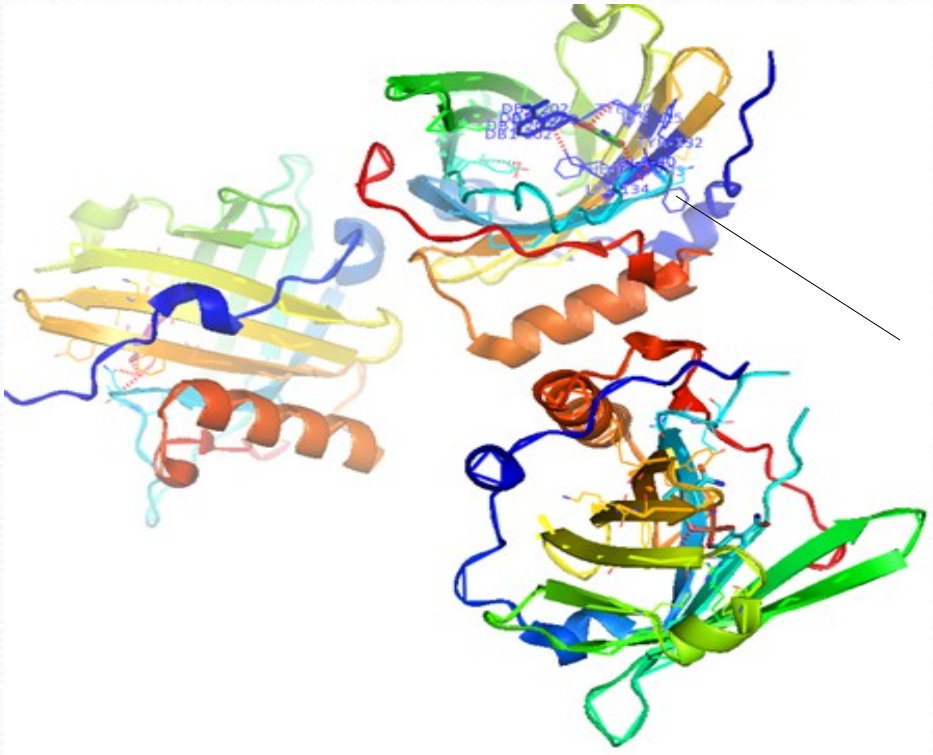


Scoring

Docked complex

RESULTS AND DISCUSSION

Co-crystal structure of human NGAL

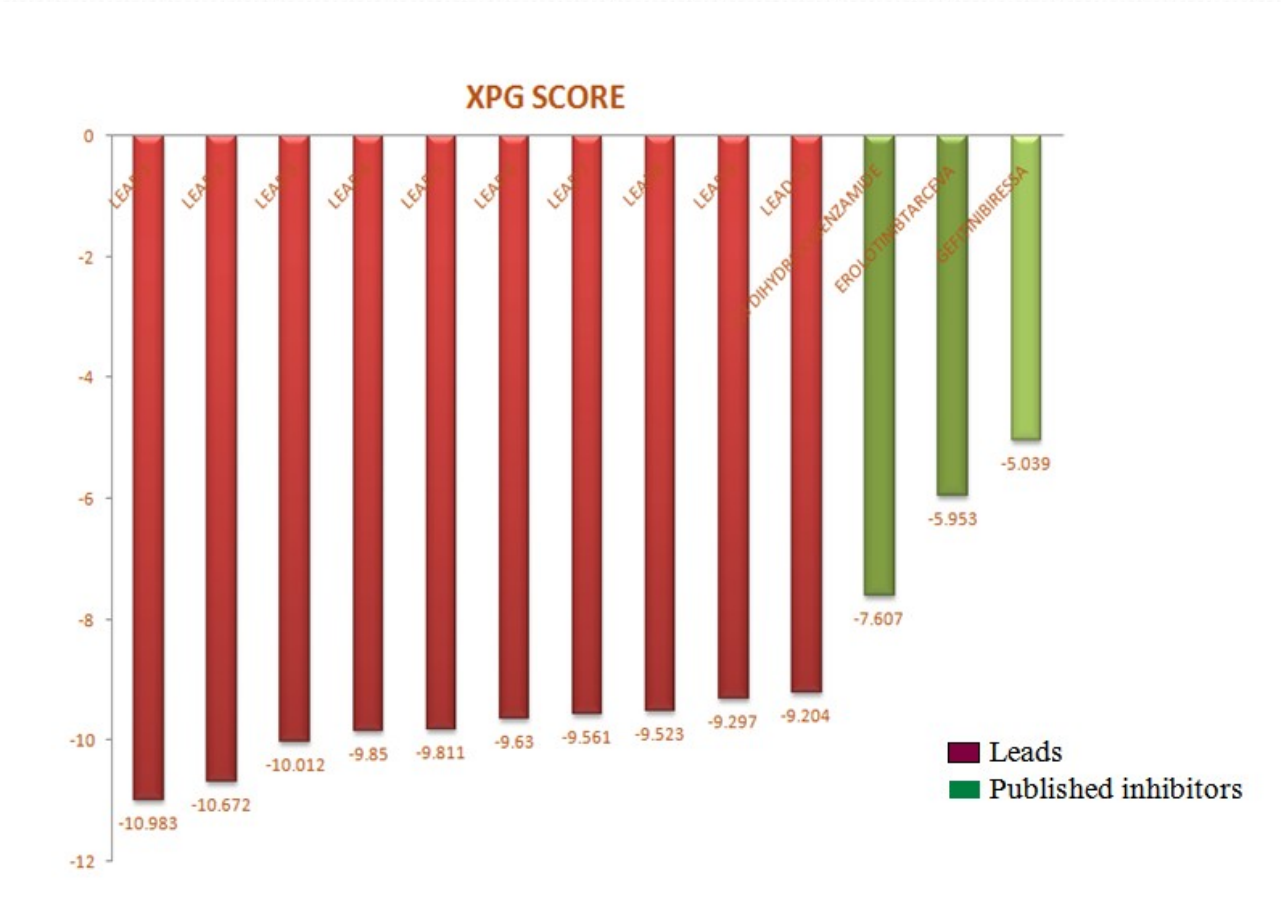


2,3 dihydroxy benzamide

1X71

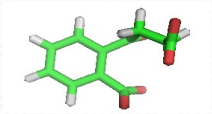
Ligand binding site residues are Ala 39, Ala 40, Ile 41, Tyr 52, Ser 68, Leu 70, Arg 81, Tyr 106, Phe 123, Lys 125, Tyr 132 and Lys 134

Docking scores for existing inhibitors and proposed leads of human NGAL



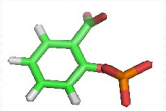
Structure of ten proposed leads

Lead ‘1’



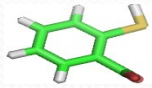
Mol. Weight : 222.2 D
XP Gscore : -10.672 K cal/mol

Lead ‘2’



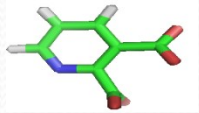
Mol. Weight : 218.1 D
XP Gscore : -10.983 K cal/mol

Lead ‘3’



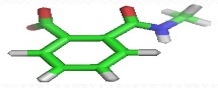
Mol. Weight : 154.9 D
XP Gscore : -10.012 K cal/mol

Lead ‘4’



Mol. Weight : 167.12 D
XP Gscore : -9.850 K cal/mol

Lead ‘5’



Mol. Weight : 178.18 D
XP Gscore : -9.811 K cal/mol

Lead ‘6’

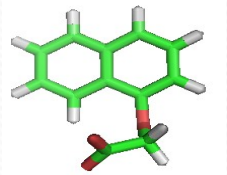
Mol. Weight : 151.16 D
XP Gscore : -9.630 K cal/mol

Lead ‘7’

Mol. Weight : 166.13 D
XP Gscore : -9.361 K cal/mol

Lead ‘8’

Mol. Weight : 202.21 D
XP Gscore : -9.523 K cal/mol

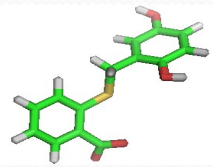


Lead ‘9’

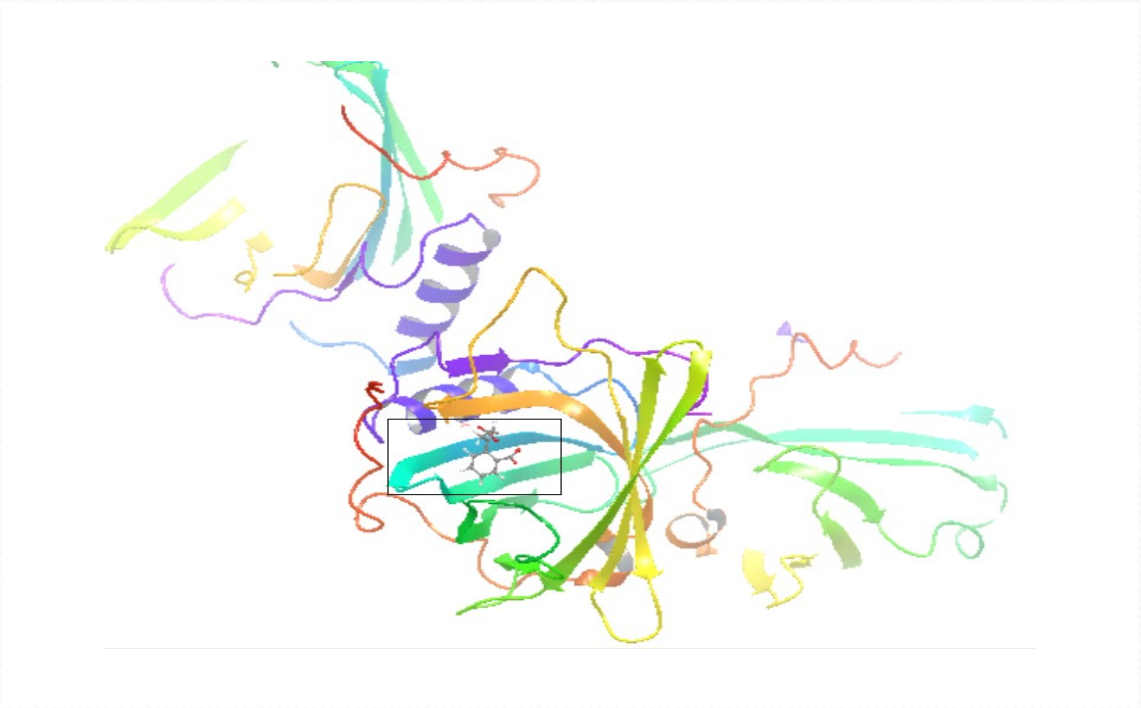
Mol. Weight : 258.23 D
XP Gscore : -9.297 K cal/mol

Lead ‘10’

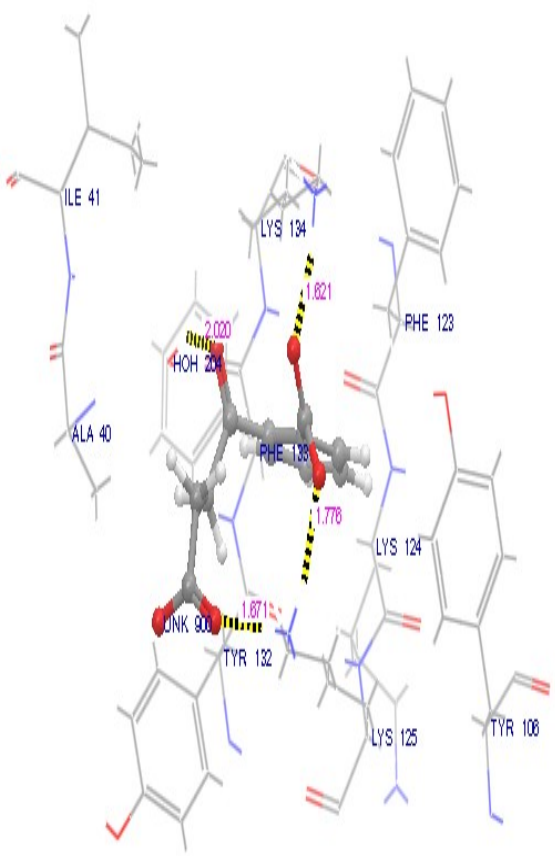
Mol. Weight : 276.31 D
XP Gscore : -9.204 K cal/mol



Docking complex of lead '1' with human NGAL



Hydrogen bond network between lead '1' and human NGAL



The lead **'1'** molecule was identified as potential target for the human NGAL. Lead **'1'** molecule binds to target protein and forms 2 hydrogen bonds with LYS 124 and LYS 134. These residues coincide with the published ligand for human NGAL protien. Schrodinger software suite gives docking score for the lead **'1'** molecule -10.98 kcal/mol.

Hydrogen bond residues

Lys -124

Lys -134

CONCLUSION

- The present study on human NGAL is through *in silico* approach to find out the more potent inhibitor to block its functional activity.
- Comparative analysis for the published inhibitors and Lead 1 had revealed that the binding mode of the docking complexes corroborating well. Lead 1 interaction was stronger with less XP Gscore, more number of hydrogen bonds and good van der Waals interactions.
- In view of the analysis lead 1 would be considered for designing potential inhibitor for cancer therapy would open up new avenues for designing of NGAL inhibitors if synthesized and tested in animal models.

ACKNOWLEDGEMENT

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