AN IN SILICO APPROACH TOWARDS BREAST CANCER THERAPY USING HSP90 AS TARGET:

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Bachelor of Technology

In

Biomedical Engineering

By

Ranjit Kumar Biraji

(109BM0688)

Under the Guidance of

Dr. Subhankar Paul

Department of Biotechnology & Medical Engineering

National Institute of Technology

Rourkela-769008, Odisha, India

2013

Dr. Subhankar Paul
Associate Professor
Department of Biotechnology & Medical Engineering
National Institute of Technology, Rourkela, Odisha, India

Certificate

This is to certify that the thesis entitled "**An in silico approach towards breast cancer therapy using Hsp90 as a target."** by **Ranjit Kumar Biraji (109bm0688)** submitted to the National Institute of Technology, Rourkela for the Degree of Bachelor of Technology is a record of bonafide research work, carried out by his in the Department of Biotechnology and Medical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

Prof. Subhankar Paul

Associate Professor

Department of Biotechnology and Medical Engineering

NIT Rourkela, 2013

Acknowledgement

If words are reflected as symbols of appreciation and token of acknowledgement, then words play the role of thanks to exhibit the deeply embedded feeling of gratitude. I am greatly indebted to, who either through guidance, discussion or providing facilities for the thesis work, have served as a beacon light or crowned my efforts with success. With an overwhelming sense of pride and genuine obligation I express my deep sense of heartfelt gratitude and regards to my guide **Dr. Subhankar Paul**, Department of Biotechnology and Medical Engineering for giving me an opportunity to do the project work in an independent arena.

I consider it a privilege to express my gratefulness to **Mr. Sailendra Mahanta**, **Mr. Deependra K. Ban,** and **Mr. Uday K. Gupta** for their constant support and advice in my project work. It is an immense pleasure to thank my friends **Prasanna, Ritiritao** and all others for their persistent encouragement and day –to-day support. I sincerely thank all my faculty members for their blessings. Finally I am grateful to my parents **Mrs. Anar devi Biraji**, **Mr. Jagat Biraji** and family members for their endurance, love, wishes and support, which helped me in completion of my work. Above all, I thank **Almighty** who showered his blessings upon us.

(Ranjit Kumar Biraji)

CONTENTS

Abstract

Breast cancer is a common malignancy and a lifetime risk among females worldwide. It has been observed that Hsp90 is over expressed in many breast cancer cells. Hsp90 is a highly conserved molecular chaperone and its functions that involve stress response, homeostatic control and assembly of wide range of other chaperones in various biological processes. Hsp90 inhibition has been reported to be a therapeutic approach in breast cancer.

In the present investigation, Hsp90 was docked with co-chaperones, anti-cancer drugs and Hsp90 C-terminal domain was docked with its inhibitors analogues of novobiocin with the help of Hex 6.3 docking software. The results showed that Hsp90 had high affinity to bind with Trap1. In order to inhibit Hsp90 a wide range of ligands (anti-cancer drug) were selected and docked by Hex 6.3 keeping ATP as control. Geldanamycin having binding energy -315.36 kcal/mol showed highest inhibition among all. In this work, in order to inhibit Hsp90 C-terminal domain a wide range of ligands were selected and docked by Hex 6.3. Coumermycin A1 having binding energy of -395.09 kcal/mol showed highest among all. Due to poor solubility and cytotoxicity results, Novobiocin was modified into fifteen analogues using ChemBioDraw Ultra 13.0.

Therefore Analogue 4 was found to be the best Hsp90 C-terminal domain inhibitor by Hex 6.3 docking software having binding energy 355.05 kcal/mol.

List of Tables:

List of Figures

CHAPTER 1: INTRODUCTION

1.1 Introduction

Normally cell growth takes place in a control and co-ordinate manner. But sometimes due to external factors cells grow and become uncontrollable. And this uncontrolled growth leads to formation of tumor. The tumor may be a benign one or a malignant one. Benign ones do not form cancer while malignant ones not only form cancer but also metastasize to other parts of the body that lead to death. All this happen because of disturbances in cell cycle and regulatory pathways. The reason may be genetic mutation, functional aberration in cell cycle, effect of environmental factor and age factor. There are many different types of cancer affecting different parts of body, most of which are termed according to the place of origin.

1.1.1 Breast cancer

Cancer is the main cause of death worldwide, approximately 7.6 million deaths (around 13% of all deaths) in 2008, with an expected 13.1 million deaths in 2030. And breast cancer covers 22.9% of all cancers in women. In 2008, breast cancer caused 13.7% of cancer deaths in women all over the world. There is a lifetime risk of developing breast cancer. The occurrence of breast cancer in India is on the escalation and is quickly becoming the number one cancer in females. The importance of the situation is apparent after working through current data from Indian Council of Medical Research (ICMR). It is testified that one in 22 women in India is expected to suffer from breast cancer during her lifespan**.**

Hanahan et al, 2000, stated that genetic disorder allows a cell to procure six characteristics that are specific to most cancers. Those are

(i) Self-capability in cell growth signalling

(ii) Non-responsive to anti-growth signalling

(iii) Ability to evade apoptosis

(iv) Persistent angiogenesis

(v) Tissue invasion and metastasis

(vi) Indefinite replicative potential

The genetic instability of cancer cells allow them to escape the molecular targeting of signalling pathway, which makes them insensitive to targeted therapeutics. Thus continuous attack on multiple points or nodes of a cancer cell's network of overlapping signalling pathways should be affected than the inhibition of one or a few individual signalling nodes.

The maximum onco-proteins and their web are entangled with hsp90 molecular chaperone.

1.1.2 Heat shock protein 90 (Hsp90)

Heat shock protein 90 (Hsp90), a molecular chaperone that plays important role in folding, stabilizing, activating and maintaining the conformational integrity of its client proteins through its ATPase activity. Cancer cells use the Hsp90 chaperone to protect the mutated onco-proteins from misfolding and proteasomal degradation. It is found that Hsp90 ATPase activity is up regulated nearly 100 fold in cancer cells. And the reason may be the up regulation of its function

Hsp90 is a member of superfamily (includes DNA gyrase, Histidine kinase and DNA mismatch pair) that contains an ATP binding pocket which is different from ATP binding cleft of protein kinases. The evolutionary conserved chaperone structure consists of three domains. These domains are composed of nearly 732 amino acids. It has two isomers α and β, mainly present in cytosol. The N –terminal domain is an amino terminal domain that contains a fold known as bergerat fold which is having the ATP and drug binding site. The middle domain is having a co-chaperone interacting motifs that provide docking sites for client proteins and co-chaperones which play a part in forming the active ATPase. The C-terminal domain is the carboxyl terminaldomain that is having a dimerization motif, which is a second drug binding region and site of interaction of other co-chaperones. Dimerization of Hsp90 monomer via C-terminus is crucial for chaperoning function.

1.1.3 Bioinformatics and Computational Biology

Now-a-days the enhancement of Bioinformatics and Cheminformatics is paving the way for easy drug designing. Computational biology and Bioinformatics have the potential to speed up drug discovery processes, reducing the costs of the processes and changing the way the drugs are designed. Rational drug design facilitates and speeds up the drug designing processes that involves various method of identifying novel compounds. One advanced method is the docking of the drug molecule or ligand or inhibitor with the target. The site where the drug binds is known to the site of action, which is responsible for the pharmaceutical effect is the target. *Docking* is the method by which two molecules bind to each other in 3D space. In addition, regression based or knowledge based scoring functions can be useful to compute the free enthalpy of ligand binding. There are various tools, software and servers meant for docking calculations. They may be rigid, flexible, and semi flexible docking. There are different databases store macromolecular 3D structure and ligand structure, which are extracted from NMR co-ordinates used for docking and simulations. Thus computational biology or In Silico approach is developing day by day with refinement. It is becoming a promising field and with the help of this the time and cost of biological work related to drug discovery, molecular interaction is reducing.

1.2 Objective

- To investigate the binding affinity of the interaction between Hsp90 and other chaperones.
- To identify the amino acid residues located at the binding interface.
- To design novel Novobiocin analogues as Hsp90 C-terminal domain-inhibitors.
- To analyse the effect of Hsp90 C-terminal domain inhibition of various novel Novobiocin analogues on the binding affinity between Hsp90 C-terminal domain and its inhibitors.

CHAPTER 2: LITERATURE REVIEW

2. Literature review

2.1 Heat shock proteins

When a cell experiences environmental stress, either its cycle stops, or slows down its original functions, such as transportation, DNA, RNA and protein synthesis. However, a set of proteins, called stress proteins, which are mainly expressed under these adverse conditions of stress response in rise in the outside temperature, called heat shock proteins (Hsp).They are also called molecular chaperones.

In 1962 Feruccio Ritossa discovered the heat shock response, who observed an amplification of special sections of Drosophila melanogaster chromosomes (heat shock puffs) when heat treatment was given to the flies.

Most of the heat shock proteins are molecular chaperones

Hartl et al, 1996 defined chaperone as "proteins that bind to and stabilize an otherwise unstable form of other protein and, by exact binding and release, enable its correct fate *in vivo*: be it folding, oligomer assembly, transportation to a certain subcellular compartment, or removal by degradation".

Multhoff et al, 1996 discovered the expression of heat shock proteins on cancer cell surface.

2.2 Heat Shock Protein 90KDa (Hsp90)

Hsp90 is a cytosolic protein, nearly present 1-2% in the cytosol. Its concentration varies depending on cells. (Nollen et al, 2002 and Ghaemmaghami et al, 2003). It is over expressed in cancer cells and estimated to be 2.8% in colon cancer cells. (Whitesell et al, 2005 and Pick et al, 2007).

2.3 The structure of Hsp90

The eukaryotic structure of Hsp90 having 40% similarity with its prokaryotic form (Bardwell et al, 1987) and is a dimeric phosphoprotein (Spence et al, 1989).Hsp90 contains two chaperone-sites, one on its N-terminal domain, and other one on the C-terminal domain. There are also other binding sites for calmodulin, peptidyl prolyl isomerases and other co-chaperones. Hsp90 forms dimers. ATP binds to its N-terminal domain, and changes its conformation. (Minami et al, 1994, Prodromou et al, 1997 and Stebbins et al, 1997).

Hsp90 contains an ATP binding pocket in Its N-terminal domain, where Geldanamycin binds. (Stebbins et al, 1997 and Obermann et al, 1998) and yeast Hsp82 possess an adenine nucleotide binding site (Prodromou et al, 1997, revised view in Prodromou et al, 2000). Comparing the structures of N-terminal domain of human Hsp90 without ligand and with ATP / AMPPCP was given by Li et al, 2012. Human Hsp90 alpha consists of approximately 732 amino acids, three domains (N-terminal-terminal, middle and C-terminal) and a charge linker. (Huai et al, 2005, Harris et al, 2004 and Dutta et al, 2000).Co-chaperones and client proteins association regulate the ATPase activity of Hsp90 (Whitesell L. et al, 2005).

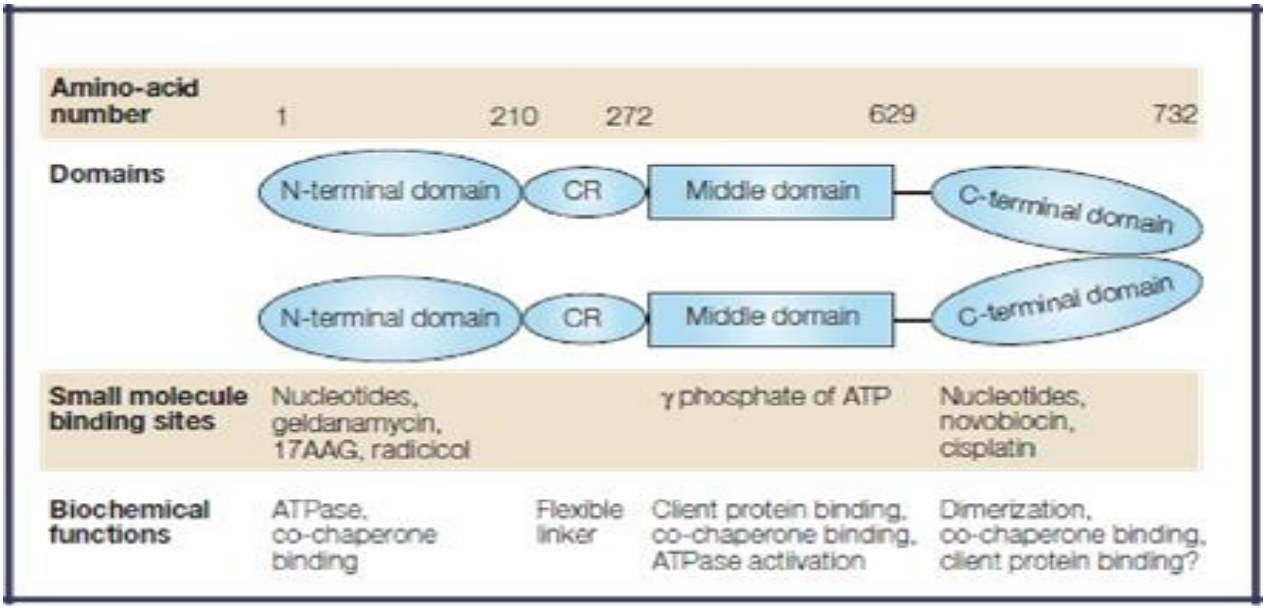


Figure 1: Structure of Hsp90 (Adapted from Ref: *Whitesell* et *al, 2005)*

2.4 The Functions of Hsp90 in various biological processes.

Hsp90 can efficiently bind to the target proteins and is crucial for their folding, maturation and maintaining in the folding competent. (Hartl et al, 1996, Buchner et al, 1999, and Csermely *et al*, 1998). Members of Hsp90 bind to various peptides in vivo and in vitro. (Menoret et el, 1999) Hsp90 involvement in signalling processes, poses threat to cellular function. (Blum et al, 2000). Extracellular Hsp90 interacted with the receptor CD91 (Basu et al, 2001 and Cheng et al, 2008). Role in disassembly of transcriptional complexes: Hsp90 and p23 are recruited to chromatin-bound glucocorticoid receptor; promoter-bound p23.Hsp90 inhibits GR, TR, NFκB, an AP-1 (Freeman et al, 2002).

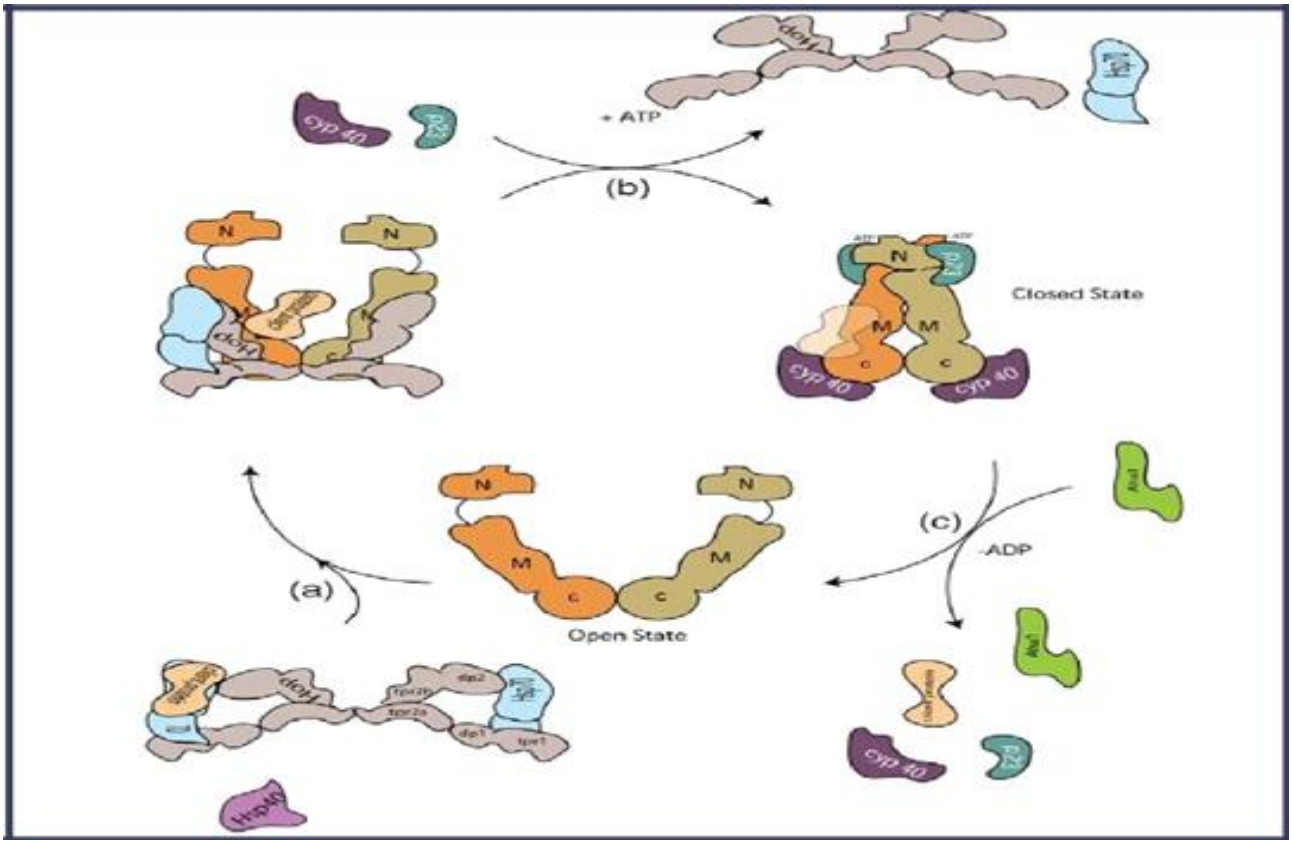


Figure 2: The Model of the conformational cycle of Hsp90 (Adapted from Ref: *Grossmann* et *al, 2008)*

2.5 <u>Overexpression of Hsp90 in Breast cancer cells.</u>

Hsp90 interacts with a number of proteins in breast carcinogenesis. Hsp90 equivalents include estrogen receptors, p53 protein, hypoxia-induced transcription factor HIF-1alpha, protein kinase Akt, Raf-1 MAP kinase and a number of receptor tyrosine kinases, such as erbB2 (Beliakoff J et al, 2004).Elevation of Hsp90 expression seems to be a trait of breast cancer and may be one of the coping mechanisms that cancer cells exhibit under stress (Conroy et al, 1996 and Yano et al, 1996). It is a promising target for breast cancer therapy (Whitesell et al, 2005) High Hsp90 level is detected in invasive breast carcinomas (Zagouri et al, 2008) and is associated with reduced survival in breast cancer (Pick et al, 2007).

2.6 Hsp 90 Inhibition

There are various natural and synthetic molecules that have been declared as promising in cancer therapy via disrupting the complex of ATP-HSP90-client proteins by targeting hsp90 N-terminal ATP binding pocket. Geldanamycin was the first inhibitor that led the way for other inhibitors to represent Hsp90 a therapeutic target for cancer therapy. (Schulte et al, 1997) Hsp90 binding drugs: deoxyspergualin (Nadeau et al, 1994) and Geldanamycin and relatives (Whitesell et al, 1994).

Geldanamycin A and Herbimycin A induce degradation of Raf, receptor tyrosine kinases (Schulte et al, 1997), CFTR (Loo et al, 1998) through proteasome, and transfected nNOS (Bender et al, 1999). Cancer cells with mutant p53 are more sensitive to Hsp90 inhibitors than cells with wild type p53.Oxime derivative of Radicicol with better pharmacology (Soga et al, 1999). Coumarin antibiotics such as Novobiocin bind Hsp90 and reduce levels of Hsp90 clients (Marcu et al, 2000). Geldanamycin A derivative WX514 .17AAG ,17DMAG expresses much improve results.(Clarke et al 2000 and Xu et al, 2001) Geldanamycin stimulate association of CHIP with client (erbB2) and facilitates degradation (Xu et al, 2002) and targets tumor cells because of a 100-fold higher affinity of their Hsp90 complexes (Kamal et al, 2003). A review on Hsp90 inhibition to elucidate a new strategy for protein kinases has been done. (Sreedhar et al, 2004).

A new class of Hsp90 inhibitors by structure based drug designing has been discovered. (Paul et al, 2005). Other novel Hsp90 inhibitors discovered the natural triterpenoids celastrol and gedunin, by chemical genomics (Hieronymus et al, 2006). Celastrol interrupts Hsp90 interaction with Cdc37 and function without hindering ATP binding (Zhang et al, 2008 and Zhang et al, 2009).The involvement of computational biology and the docking studies on anticancer drugs

has been much helped (Alex et al, 2009).A review on all Hsp90 inhibitors up to date given by HUIFANG HAO 2010, Detailed thermodynamic analysis of drug binding to human and yeast Hsp90 (Zubrienė et al, 2010). GA/17AAG target VDAC resulting in membrane depolarization of mitochondria and increased intracellular Ca2+ (Xie et al, 2011).3D structure elucidation and macromolecular interactions on Hsp90 help in establish drug designing (Madej et al, 2011).

Chapter 3:
MATERIALS AND METHODS

3. Materials and Methods

3.1 Tools and Bioinformatics softwares used

3.1.1 Pubchem (pubchem.ncbi.nlm.nih.gov)

3.1.2 NCBI

3.1.3 Swissprot

3.1.4 PHYRE 2 server (**P**rotein **H**omology/analog**Y** **R**ecognition **E**ngine)

3.1.5 UCSF Chimera 1.7

3.1.6 PDB(pdb.org)

3.1.7 Blast

3.1.8 Open babel GUI software

3.1.9 Rasmol

3.1.10 Hex 6.3

3.1.11 ChemBioDraw Ultra 13.0

3.2 Protocol followed

3.2.1 Interaction between Hsp90 and other chaperones

Retrieval of amino acid sequences of Hsp90 protein from NCBI

↓

3D structure of Hsp90 Protein was modelled and obtained by PHYRE server.

↓

3-D structure of Hsp70 and Hsp40 is retrieved from PDB (Protein Data bank).

↓

Energy minimization of all 3D structure of proteins had done by Chimera.

↓

Docking of individual protein and protein complex respectively had done by Hex 6.3.

3.2.2 Inhibition of Hsp90

Retrieval of 3D structure Hsp90 C-terminal domain was from PDB (3Q6M).

A collection of small ligand molecule was from Pubchem database.

OpenBabel GUI converted all the ligand structures from .sd file format to .pdb format.

Energy minimization of ligands was by UCSF Chimera 1.7.

Docking of hsp90 C-terminal domain with all ligands was done by Hex 6.3.

Analysis of the binding energy obtained from docking.

3.3 Methodology

3.3.1 Retrieval of amino acid sequences of Hsp90 protein from NCBI

NCBI stands for National Centre for Biotechnological Information. It is established as a division of National Library of Medicines at National Institutes of Health. The NCBI responsible for creating automated systems of knowledge about molecular biology, biochemistry, and genetics, providing the use of such databases and software by the research and medical community; collect biotechnology information both nationally and internationally; and execution research on advanced methods of computer-based information processing for examining the structure and function of biologically important molecules. The URL for this database is http://www.ncbi.nlm.nih.gov.

i. The above mentioned URL was browsed.

ii. In search option, protein was mentioned and hsp90 human was typed.

iii. There were many results of the search but the accession number AAI21063 was selected and the sequences were retrieved in fasta format.

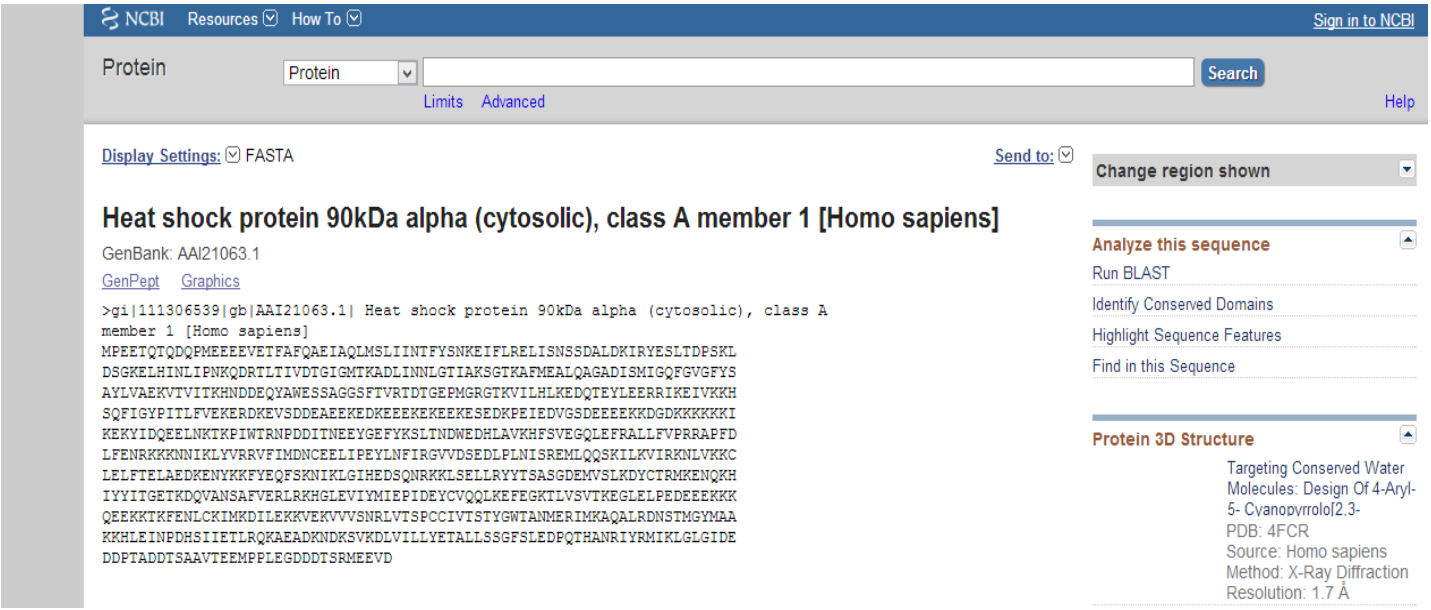


Figure 3: Retrieval of amino acids from NCBI

3.3.2 Retrieval of 3D structure of Hsp90 Protein modelled by PHYRE server.

PHYRE is an automatic fold recognition server for calculating the structure and function of the protein sequence that got submitted in the server. It is used for academic users only. It uses the principle and technique of Homology Modelling and relies on Hidden Markov Models.

i. The Fasta format of amino acid sequences was pasted and Phyre 2 search was clicked.

ii. After 5 h the results were sent to the user given email address.

iii. The modelled structure was retrieved from the link sent by the server.

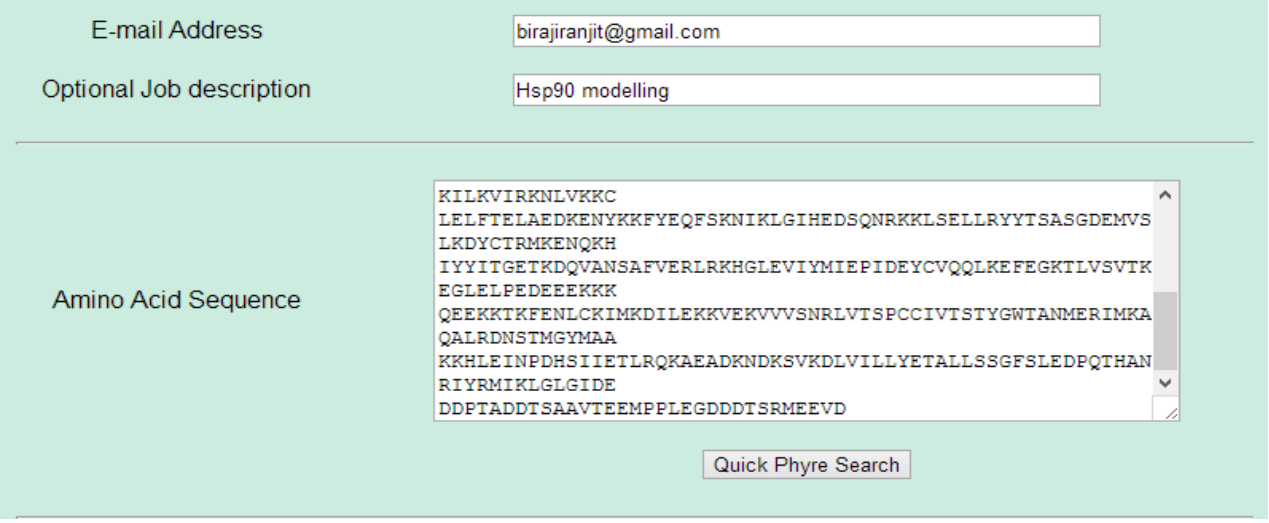


Figure 4: Submission of amino acids in PHYRE server

3.3.3 Retrieval of 3-D protein structure of Hsp70, Hsp40 from PDB (Protein Data bank).

The PDB (Protein Data Bank) is the universal store of Structural data of Biological macromolecules, founded in Brookhaven National Laboratories (BNL) in 1971.It provides Structural information of the macromolecules assessed by X-ray crystallographic, NMR Methods. This is very much important as the understanding of shape will lead to the way how it functions. As biological macromolecule like protein is having a structure to function relationship. Hence an accurate knowledge of structure is needed to know the varying functions. This server is free of use.one can easily download the structure in pdb file format or fasta format.

i. The URL www.pdb.org was browsed.

ii. In the search option Individual protein name was typed.

iii. After selecting the definite PDB ID, the structure was downloaded and saved in .pdb format.

3.3.4 Energy minimization of all 3D structure of proteins by UCSF Chimera 1.7.

UCSF CHIMERA 1.7 is an extensible programme for visualization and analysis of molecular structure and related data including density maps, supramolecular associations, sequence alignments, docking results, routes and conformational ensembles. One of the best features is the structural editing job. It can minimize the energy of molecules providing them high stability.

i. Chimera window was opened.
ii. From the option file, the 3D structure of protein was retrieved.
iii. The total residues were selected.
iv. From the tool option, by the structure editing option, minimized structure option was clicked.
v. The minimized structure was saved in .pdb format.

3.3.5 Collection of small ligand molecules from Pubchem.

Pubchem is a database of chemical structures of small organic molecules and contain information of their biological activity, origin and related literatures. It is executed and updated by NCBI and is freely available. Millions of compound structures and data seta can be freely downloaded in .sd format or chemical (CID) format

i. Pubchem page was retrieved by browsing Pubchem.ncbi.nlm.nih.gov.
ii. In search bar individual inhibitors name was typed and entered.
iii. All the available ligands of therapeutic target database retrieved in .sd file format.
iv. The ligands retrieved were Taxol, Geldanamycin A1, Radicicol, Herbimycin, Vinblastine, Vincristine, Novobiocin, Cisplatin, Epigallocatechin-3-Gallate (EGCG), Itraconazole (ITZ-1), KU-135, KU-32, ATP.

3.3.6 Conversion of all the structures from .sd file format to pdb by OpenBabel GUI Software 2.3.1

OpenBabel 2.3.1 is a chemical toolkit designed to interpret the various language of chemical data. It allows searching, converting, and analyzing chemical data. It supports Cheminformatics, molecular modeling, and bioinformatics. It covert chemical data from one file format to another.

i OpenBabel 2.3.1 window was opened.

ii The input format was selected as .sd and the output format as .pdb.

iii From the input option .sd file was browsed.

iv Click on the convert option to convert .sd file format to .pdb file format.

v Then the selected files were generated in the .pdb file format and save it for future use.

.

3.3.7 Modification of ligand molecules by ChemBioDraw Ultra 13.0.

ChemBioDraw Ultra 13.0 is a tool for drawing chemical structures, adding or deleting functional group or atoms, queries and reactions. Assigning stereochemistry, charge, valence, radicals and isotopes to each atom can be done and moreover single, double, triple bonds and aromatic forms can also be created.

ChemBioDraw Ultra 13.0 provides a wide range of Cheminformatics tools supporting molecule manipulation and processing, SMILES and SD file conversion,

normalization of molecules, creation of tautomers, molecule disintegration, calculation of several molecular properties needed in QSAR, molecular modelling and drug design.

i. ChemBioDraw window was opened.

ii. The ligand .pdb format was retrieved.

iii. Addition, deletion of functional group changes were made keeping in mind to increase solubility.

iv. The new molecules were saved in .pdb format.

3.3.8 Docking by Hex 6.3

Hex 6.3 is an Interactive Molecular graphics program developed by Dave Ritchie for estimating docking calculations and displaying docking modes of pairs of protein and ligand molecules. HEX is used as the docking tool which calculates intermolecular "energies" by adding up all intermolecular interactions (e.g. van der Waals, electrostatic) that occur between a ligand and protein target.

i. Hex manual window was opened.

ii. From the file, both receptor and ligand separately were opened from the path location defined.

iii. By the option control, docking was selected and activated.

iv. Lastly the binding energy (ΔE) produced by docking action was saved carefully.

v. The docking complex was saved from the file option in the .pdb format for future analysis.

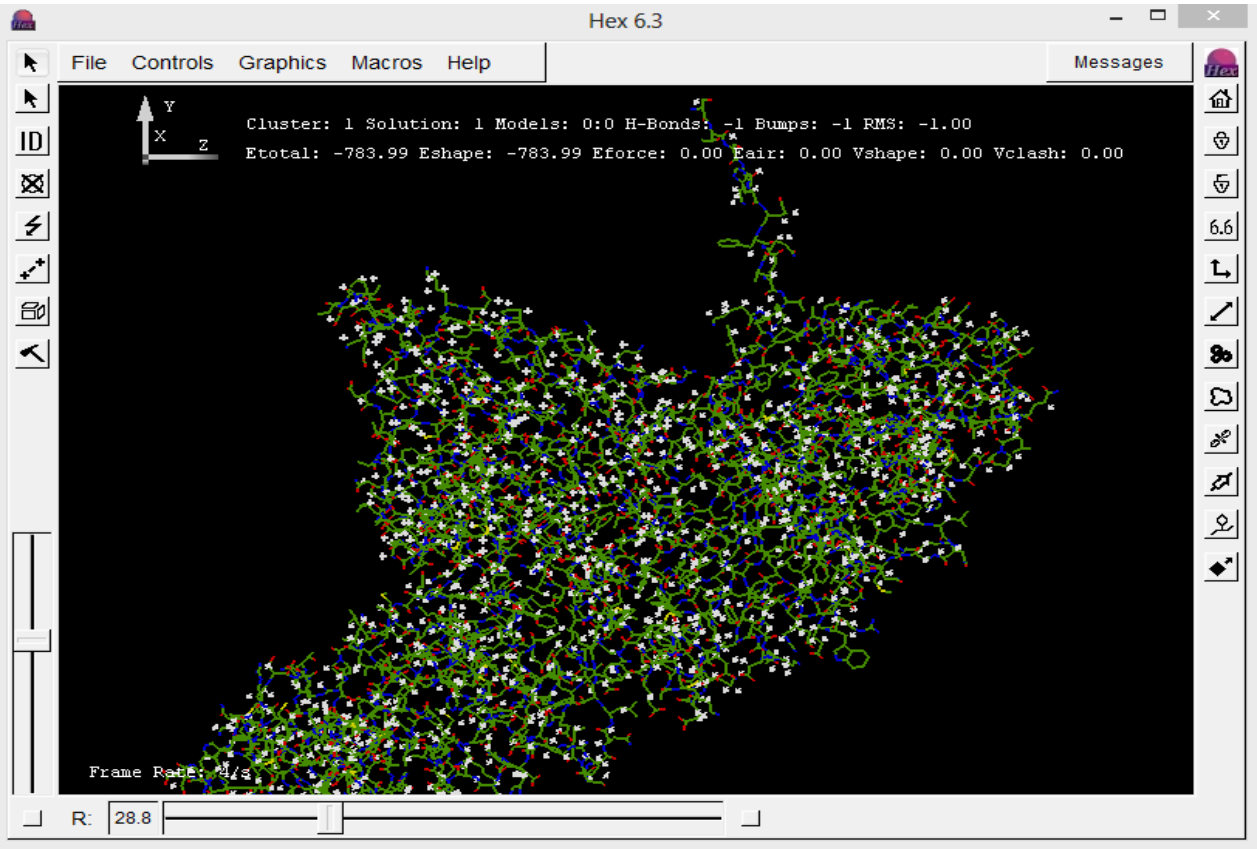


Figure 5: Docking of Hsp90 with Trap1 by Hex 6.3.

The fifteen analogues of Novobiocin that are created by ChemBioDraw are presented below:

On 7th position of Oxygen:

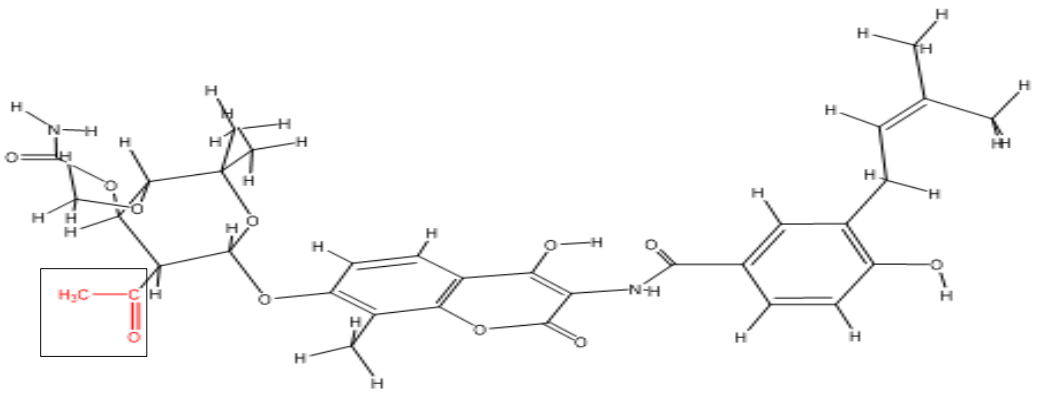


Figure 6.a: Analogue-1

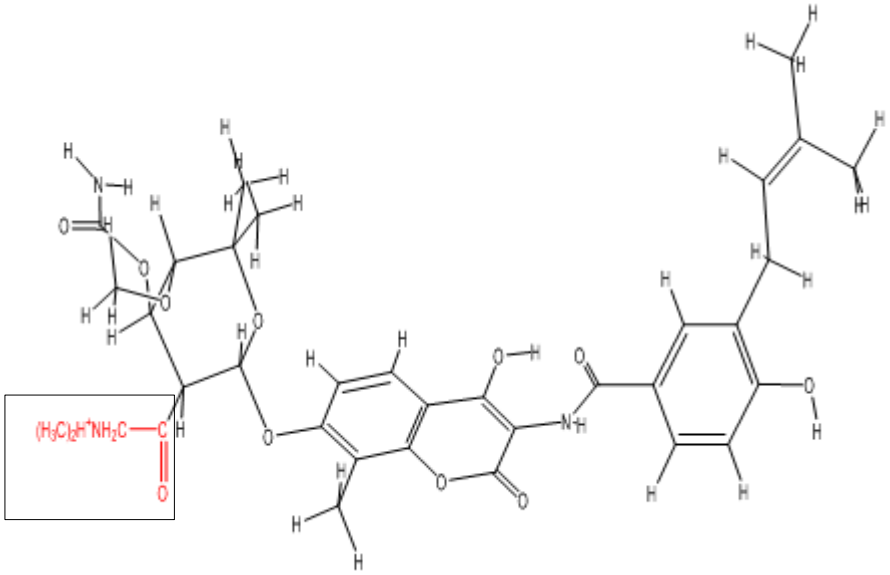


Figure 6.b: Analogue-2

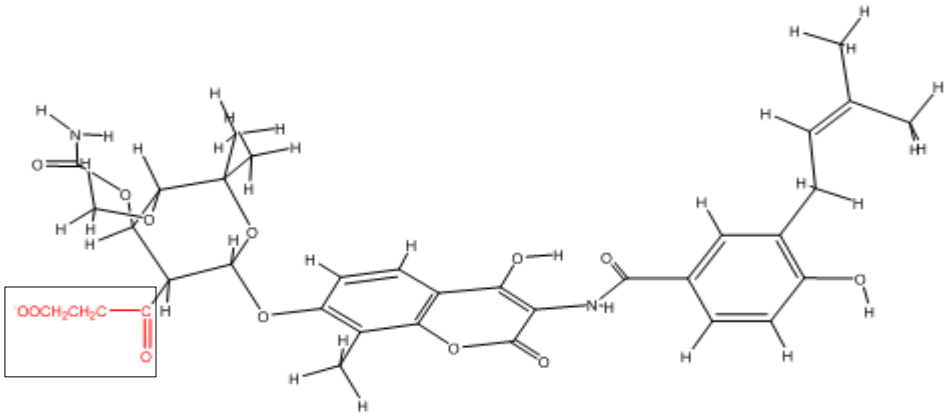


Figure 6.c: Analogue-3

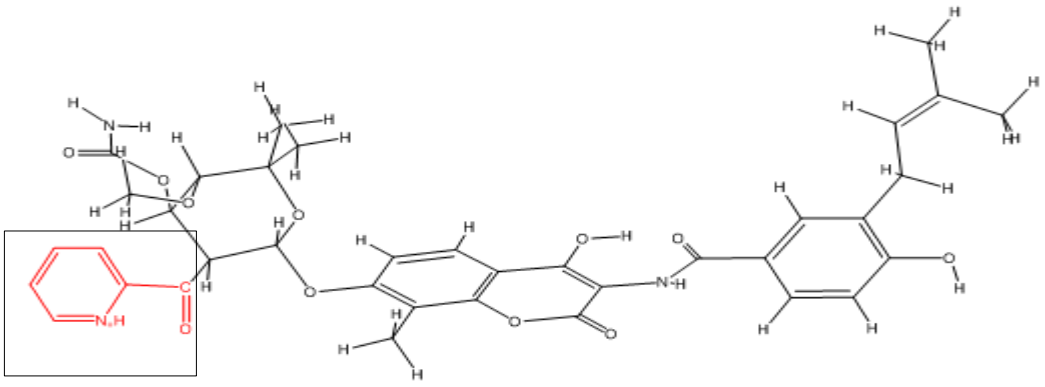


Figure 6.d: Analogue-4

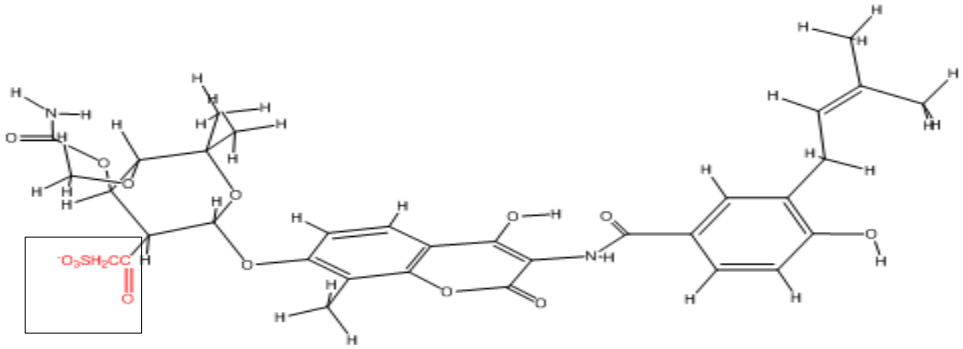


Figure 6.e: Analogue-5

On 8th position of Oxygen:

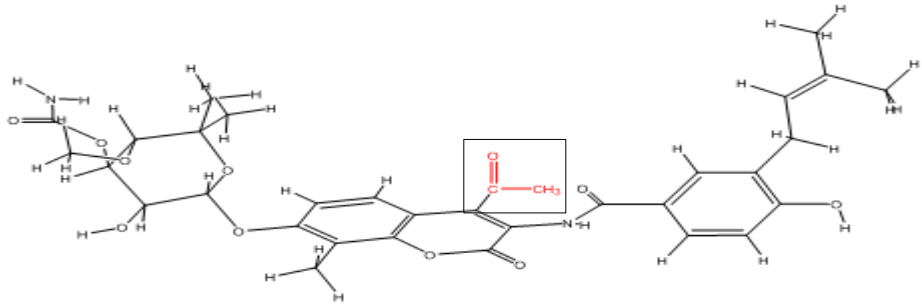


Figure 6.f: Analogue-6

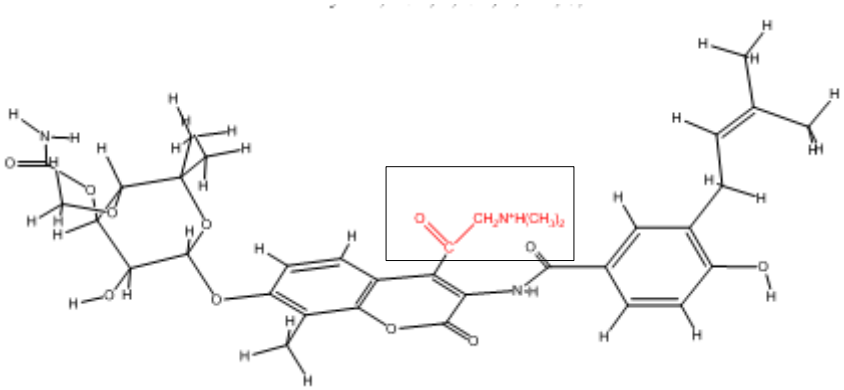


Figure 6.g: Analogue-7

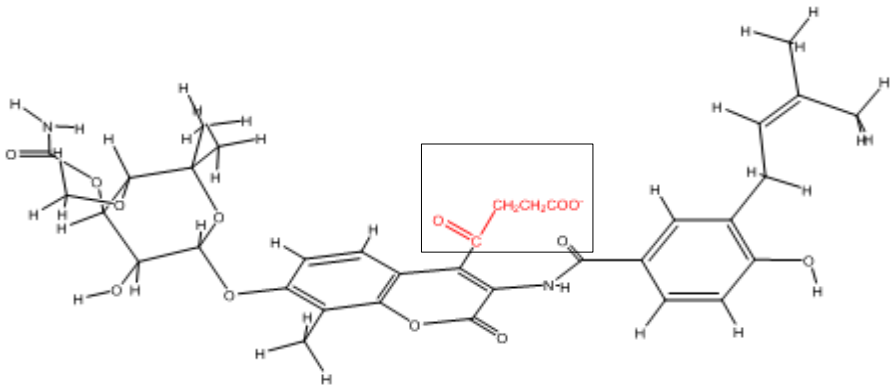
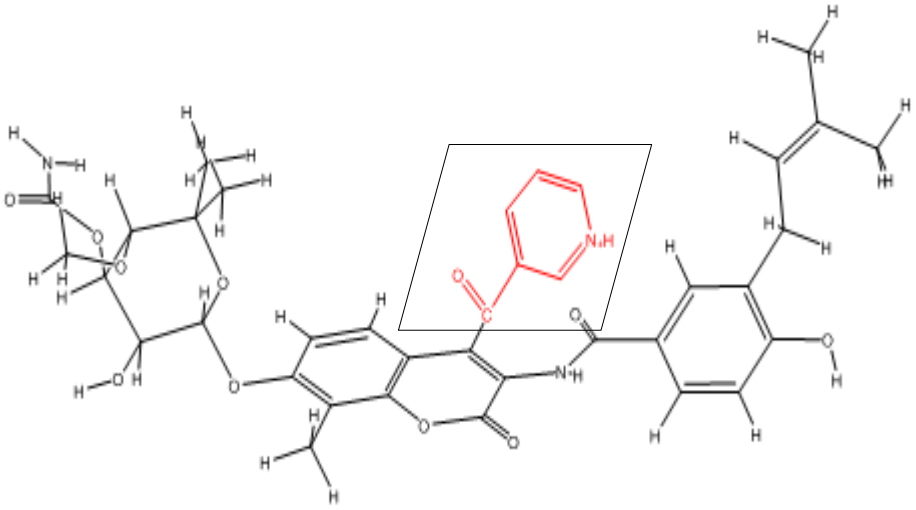


Figure 6.h: Analogue-8



S

Figure 6.i: Analogue-9

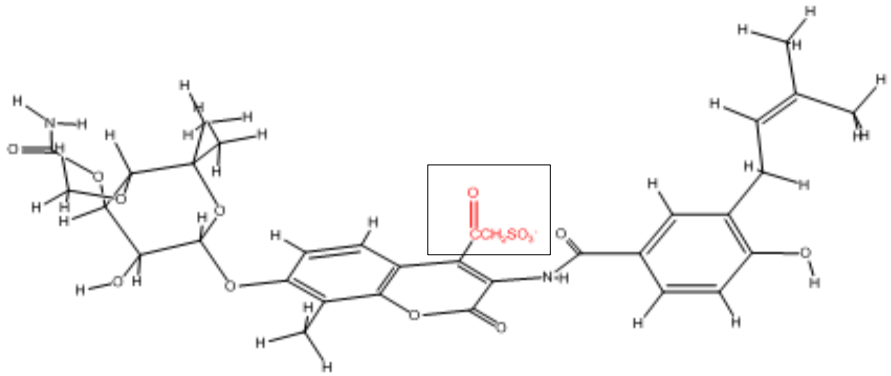


Figure 6.j: Analogue-10

On 11th position of Oxygen:

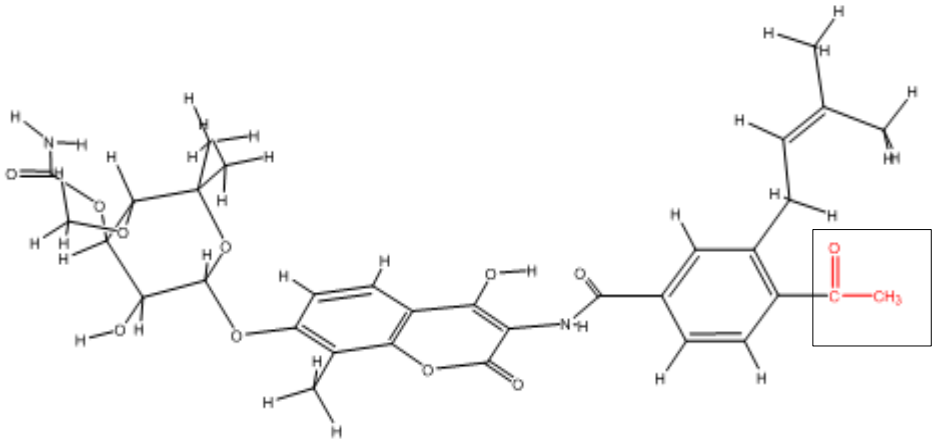


Figure 6.k: Analogue-11

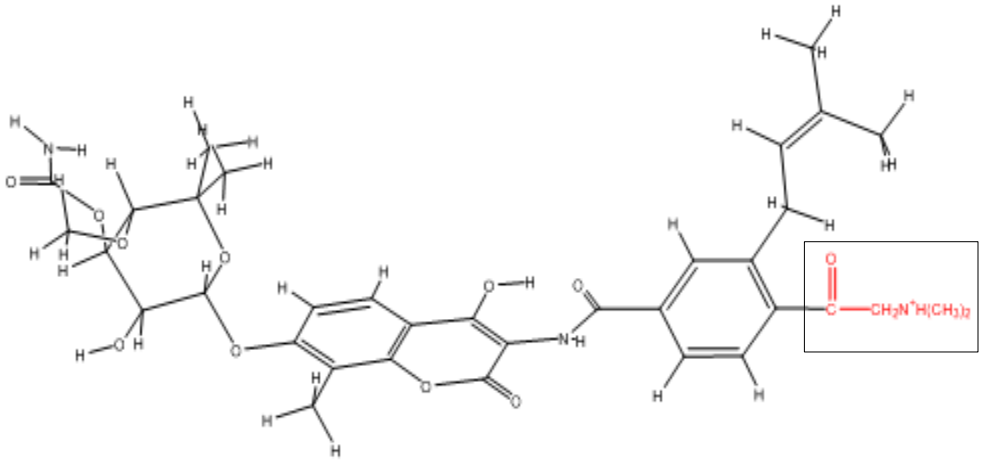


Figure 6.l: Analogue-12

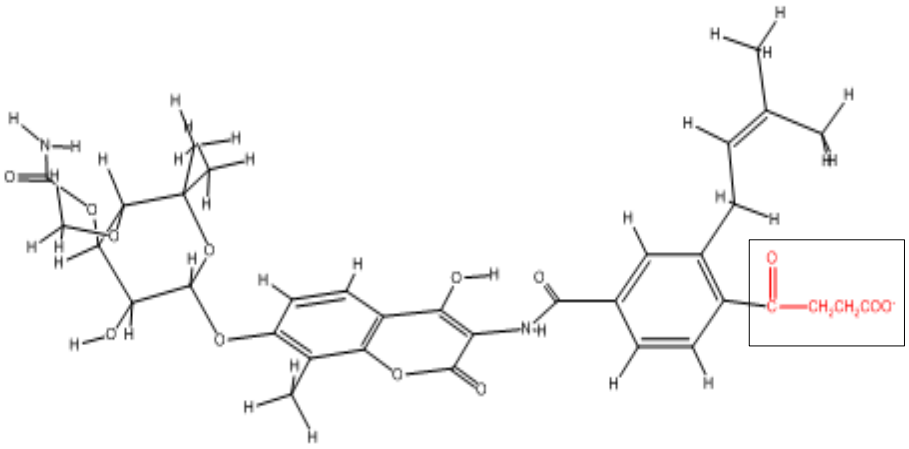


Figure 6.m: Analogue-13

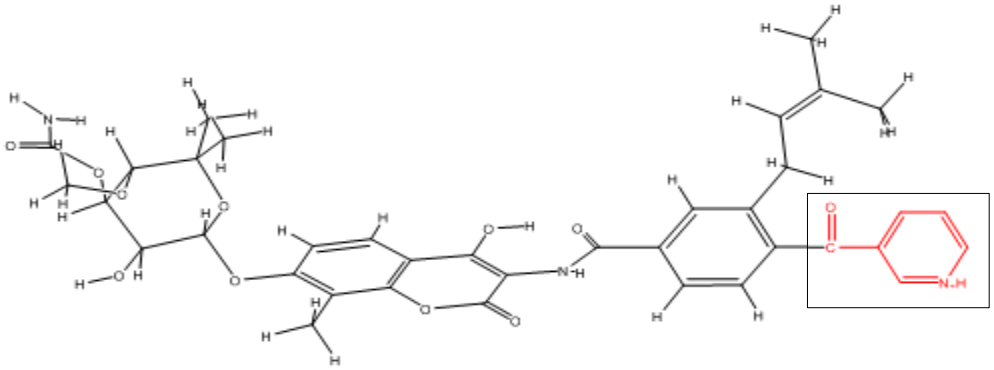


Figure 6.n: Analogue-14

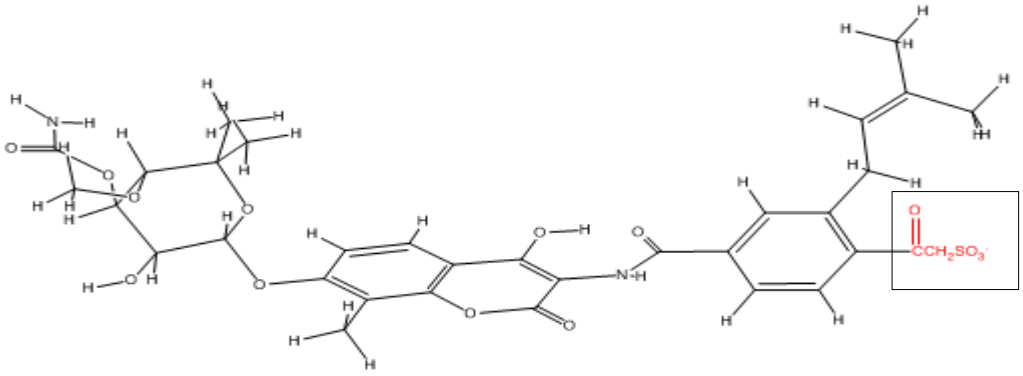


Figure 6.o: Analogue-15

Figure 6. (a, b, c……o) Represent all the fifteen Analogues of Novobiocin designed by ChemBioDraw Ultra 13.0.

Chapter 4: Results And Discussion

Results and Discussion

4.1 Interaction between Hsp90 and other chaperones.

The Hsp90 structure was modelled, aligned, and compiled in one full length 3D structure. This structure contains the entire three domains: N-terminal, middle and C-terminal domain. The spiral looks in the structures are α- helices and the arrow ones are β-sheets.

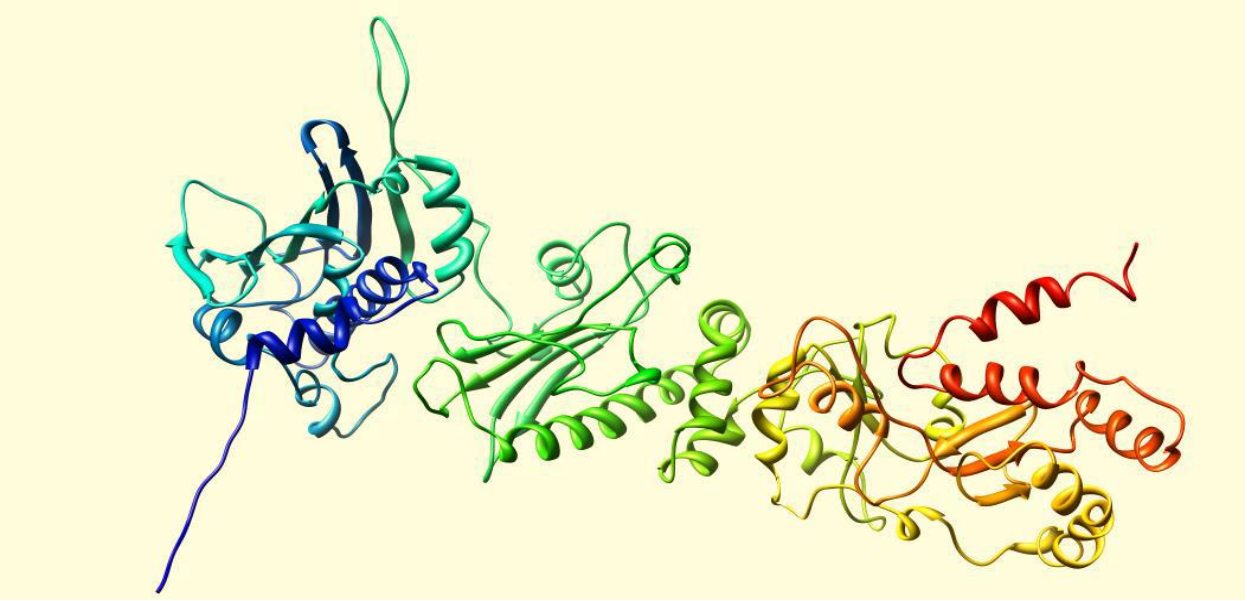


Figure 7: Modelled 3D structure of Hsp90 α by PHYRE 2 server

4.1.1 Hex 6.3 Docking Results (protein - protein)

The whole docking procedure has been done sequentially. First the Hsp90 docked with Hsp70, Hsp90-Hsp40, Hsp70-Hsp40, Hsp90+Hsp70 docked with Hsp40 separately. Then Hsp90 docked with its co-chaperones (p23, Hop, p50, Aha1, Grp94.Trap1). First the Hsp90 N-terminal domain was used, and then followed the middle domain and the whole hsp90 full length. The docking results are summarized in Table .The findings of the results are solely based on the docking energy value and the interaction at the binding sites. The more negative the value, the more stable the complex is and more binding affinity. According to the energy funnel theory less energy depicts highly stable conformation. Hence more energy would be needed to break the complex that means high dissociation energy.

Protein	Co-chaperones	Energy (Kcal/mol)
Hsp90	Hsp70	-652.49
Hsp90	Hsp40	-621.42
Hsp70	Hsp40	-633.23
Hsp90+Hsp70	Hsp40	-667.01
Hsp90	P23	-584.26
	Hop	-508.47
	P50	-552.95
	Aha1	-579.53
	Grp94	-350.55
	Trap1	**-783.99 ***

Table1: Hsp90 docking calculation by Hex 6.3 software.

The interpretation of the results of Table 1 is summarized below.

The docking energy of Hsp90 with Trap1 is -783.99 kcal/mol. This infers the high binding affinity of Hsp90 towards the Trap1 than other co-chaperones. The Hsp90 with Trap1 complex is more stable.

4.2 Inhibition of Hsp90

All the selected ligands were docked with Hsp90 and the results are shown in Table 2.

Chaperone	Ligand Molecules	E-Value (Kcal/mol)
Hsp90	Taxol	-312.57
	Herbimycin	-297.55
	Radicicol	-231.40
	Geldanamycin	**-315.25 ***
	Vinblastine	-291.16
	Vincristine	-294.45

Table 2: The docking energy calculations targeting Hsp90 by Hex 6

The docking of ligands was carefully observed and their interaction and orientations were also monitored. Table 2, Result showed that Geldanamycin having highest binding affinity (-315.36 Kcal/mol), second to that Taxol also scored high (-312.57 Kcal/mol) and third one the Herbimycin (-297.55 Kcal/mol) scored well (-311.70 Kcal/mol). Hence Geldanamycin inhibit the most.

4.3 Inhibition of Hsp90 C-terminal domain

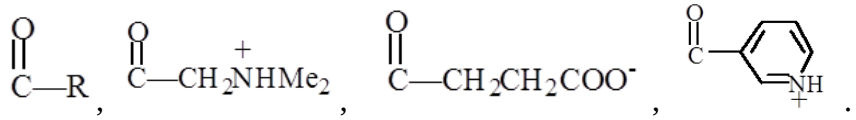
All the selected Hsp90 C-terminal domain ware docked with Hsp90 C-terminal domain and the results are shown in Table 3.

Chaperone	Ligand Molecule	E-value (Kcal/mol)
Hsp90 C-terminal	ATP	-262.78
	A4	-204.62
	Cisplatin	-88.99
	Clorobiocin	-334.54
	Coumermycin A1	**-395.09** *
	EGCG	-334.54
	ITZ-1	-334.54
	KU-32	-1
	KU-135	-215.00
	Novobiocin	-317.57
	Taxol	-310.66

Table 3: The docking energy calculations targeting Hsp90 C-terminal domain by Hex 6.3

The docking of ligands was carefully observed and their interaction and orientations were also monitored. Table 3, Result showed that Coumermycin A1 having highest binding affinity (-395.05 Kcal/mol), second to that ITZ-1 also scored high (-344.75 Kcal/mol) and third one the Clorobiocin and EGCG scored good (-334.54 Kcal/mol).All the ligands except Cisplatin, KU-32 and KU-135 showed high binding affinity than the ATP molecule,which was kept as control to row down the analysis. Hence these ligands having high affinity than ATP can be used as Hsp90 C-terminal domain inhibitors. As the ligands are more competent than ATP molecule, that's why when they will enter the cytosol, they may replace ATP and would bind the catalytic site of Hsp90 which will further interrupt its chaperoning function.

Now, the result showed Novobiocin is a potent inhibitor that got matched with the literature studies. According to the research works on Novobiocin stated that it couldn't enter into the clinical trials because of its poor solubility and toxicity. The reason may be the solubility. As we know Animal cells are more aqueous in nature, hence water soluble substances easily soluble in cytosol and water insoluble form precipitates. And any extra thing in the cytosol leads to cell toxicity. This is why all the research works are going on to modify Novobiocin structure to increase its solubility. This thought inspired us to work on the modification of Novobiocin structure. With the help of ChemBioDraw Ultra 13.0, fifteen analogues were made keeping in mind that it will increase solubility. Hence the functional groups that enhance solubility are –



And the functional groups enhance electronegativity that will help in hydrogen bonding. Hence at different position the changes were made randomly with the functional group and somewhat hit and trial method.

The docking calculations were made by Hex 6.3 and tabulated in Table 4.The results showed the binding affinities of the Novobiocin Analogues. According to Hex docking Analogue 4 scored high i.e. -355.05 Kcal/mol, it mean it has high binding affinity than the others. Other analogues scored high referring to the control ATP molecule. The analogues showed good binding affinity than ATP molecule except Analogue 5 and 10.

Chaperone	**Novobiocin Analogues**	**E-Value**
Hsp90 C-terminal	Analogue 1	-319.46
	Analogue 2	-348.02
	Analogue 3	-321.05
	Analogue 4	**-355.05 ***
	Analogue 5	-170.69
	Analogue 6	-329.68
	Analogue 7	-339.35
	Analogue 8	-341.17
	Analogue9	-339.70
	Analogue 10	-170.69
	Analogue 11	-332.24
	Analogue 12	-324.79
	Analogue 13	-338.33
	Analogue 14	-335.94
	Analogue 15	-325.43
	ATP	-265.78

Table 4: The docking energy calculations of Novobiocin Analogues by Hex 6.3

4.4 Effect of inhibition on binding affinity of Hsp90 C-terminal domain and Novobiocin.

Previously the findings showed good affinity between Hsp90 C-terminal Domain and Coumermycin A4 and Novobiocin and its Analogues which worked in a chaperoning complex. Hence Again the Inhibition studies have undergone and some suitable candidate are found along with the Literature‘s best ones. Hsp90 C-terminal domain docked with specific inhibitors to analyse the consequences. All these studies were based on docking energy calculation by hex only.

Chaperone	Ligands	E-Value (Kcal/mol)
Hsp90 C-terminal	Coumermycin A1	-395.09
	Novobiocin	-317.57
	Analogue 4	-355.05
	Analogue 2	-348.02
	Analogue 8	-341.17

Table 5: Docking calculation between Hsp90 C-terminal domain and Coumermycin A4 and Novobiocin and its Analogues

The Table 5 summarized all the docking calculations between Hsp90 C-terminal domain with Coumermycin A4 which inhibit C-terminal most among all the ligands and Novobiocin and its analogues. After studying the above table it is clear that the Analogues of Novobiocin are good inhibitors than the Novobiocin. The energy value changed to less negative. As previously stated the more negative e-value the more the stable the complex would. When the Hsp90 C-terminal domain docked with the different inhibitors, there must be some energy changes to each other, which mean the docking result of Hsp90 C-terminal domain with different inhibitors are different.

Chapter 5:
Conclusion
And
Future Perspectives

5.1.Conclusions

Interest in Hsp90 inhibitors for the treatment of cancer and neurodegenerative diseases has grown exponentially since identification of Geldanamycin as the first Hsp90 inhibitor in 1994. Over the last 25 years, several classes of Hsp90 inhibitors have been identified, each with unique mechanisms of inhibition and each displaying somewhat differing biological effects. Several of these inhibitors have been clinically evaluated for a variety of different cancers, unfortunately, the results of these trials has somewhat been disappointing. The results from these trials emphasize the need to thoroughly understand the biology of the target, to better understand how different classes of inhibitors affect the molecular chaperone, and to determine how cellular environments affect drug efficacy.

Several strategies exist that may help circumvent the known pitfalls resulting from Hsp90 inhibition. First, inhibitors of the C-terminal domain do not cause induction of the heat shock response, but maintain anti-proliferative activity. Compounds in this class should not suffer from dosing and scheduling issues observed with N-terminal inhibitors. Second, the development of isoform-selective inhibitors may result in fewer negative effects upon Hsp90 inhibition. All known Hsp90 inhibitors exhibit *pan*-inhibition, i.e. they target all four human isoforms simultaneously. An isoform-selective inhibitor will not only help delineate the roll of each isoform, but may prove to be clinically more viable. Lastly, inhibitors that work by alternate mechanisms, such as co-chaperone disruptors, provide a mechanism to disrupt only a subset of Hsp90 client proteins, and may also result in less undesired effects. In summary, Hsp90 is still an attractive therapeutic target, but new strategies for inhibition are necessary to overcome the clinical liabilities observed for prototypical N-terminal inhibitors.

5.2 Future perspectives

All the above findings are established with the help of promising, highly developed and reliable tools and software of computational biology. In this modern era of Insilco, every work is first tested by Virtual screening or Insilico designing, then only it goes for invitro and invivo analysis. The protocol follows like Insilico designing, Insitu designing, Invitro analysis and final invivo analysis

The crucial biological functions performed by Hsps90 and the dependency of cancer cells on the marked functions of Hsp90 make itself as an attractive target for anti-cancer chemotherapeutics. Among the trademarks of cancer, up-regulation of growth signals and apoptotic interruption are the most vital. As maximum growth signals rely on Hsp90 for their functional stability, Hsp90 become an ideal molecule to interfere in complicated web of oncogenic pathways. Therefore, drugs targeting Hsp90 are more advantageous than the oncogene pathway inhibitors.

References

- Bardwell J and Craig EA, Eukaryotic mr 83,000 heat shock protein has a homologue in Escherichia coli, 1987, Proceedings of the National Academy of Sciences, 84(15):5177.
- Beliakoff J and Whitesell L., Hsp90: An emerging target for breast cancer therapy, 2004, Anticancer Drugs, 15(7):651.
- Brough P. A., Barril X., Beswick M., Dymock B. W., Drysdale M. J. and Wright, L., (2005), 3-(5-Chloro-2, 4- dihydroxyphenyl)-pyrazole-4-carboxamides as inhibitors of the Hsp90 molecular chaperone, Bioorg. Med. Chem. Lett., 15, 5197–5201.
- Buchner J., Hsp90 & co.–a holding for folding, 1999, Trends Biochem Sci, 24(4):136-141.
- Clarke P.A., Hostein I., Banerji U., Stefano F.D., Maloney A., Walton M., Judson I. and Workman P., Gene expression profiling of human colon cancer cells following inhibition of signal transduction by 17-allylamino-17-demethoxygeldanamycin, 2000, an inhibitor of the hsp90 molecular chaperone, 19, 4125–4133.
- Conroy S.E. and Latchman D.S., Do heat shock proteins have a role in breast cancer? 1996, Br. J. Cancer, 74, 717–721.
- Csermely P., Schnaider T., Soti, C., Prohaszka Z.and Nardai G, the 90-kDa molecular chaperone family structure, function and clinical applications. A comprehensive review, 1988, *Pharmacol. There*, *79*, 129-168.
- Dutta R., Inouye M. and GHKL, An emergent ATPase/kinase superfamily, 2000, *Trends Biochem. Sci*, *25*, 24-28.
- Loo M. A., Jensen T. J., Cui L., Hou Y.-x., Chang X., And Riordan J. R., Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome, 1998, *EMBO J.*, *17*, 6879-6887.
- Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A and Dephoure N, Global analysis of protein expression in yeast,2003, Nature, 425(6959):737-741.
- Hanahan D and Weinberg RA., The hallmarks of cancer, 2000, Cell, 100(1):57-70.
- Harris S. F., Shiau, A. K. and Agard D. A., The crystal structure of the carboxy-terminal dimerization domain of htpG, the Escherichia coli Hsp90, reveals a potential substrate binding site2004, *Structure*, *12*, 1087-1097.
- Hartl FU, Molecular chaperones in cellular protein folding, 1996, chaperone, 9:47-50.

- Hieronymus, Lamb, Ross, KNPeng, XP Clement and Rodina A, Gene expression signature-based chemical genomic prediction identifies a novel class of Hsp90 pathway modulators, 2006, Cancer Cell, 10:321–30.
- Huai Q., Wang H., Liu Y., Kim, H.-Y., Toft D. and Ke H, Structures of the N-terminal and middle domains of E. coli Hsp90 and conformational changes upon ADP binding,2005, *Structure, 13*, 579-590.
- Kamal A., Thao L., Sensintaffar J., Zhang L., Boehm M. F., Fritz L. C. and Burrows F. J., A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors,2003, Nature, 425, 407- 410.
- Lai B.T., Chin N.W., Stanek A.E., Keh W. and Lanks K.W., Quantitation and intracellular localization of the 85K heat shock protein by using monoclonal and polyclonal antibodies, 1984, Mol. Cell. Biol., 4, 2802–2810.
- Loo M. A., Jensen T. J., Cui L., Hou Y.-x., Chang X.-B. And Riordan J. R., Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome, 1998, EMBO J., 17, 6879-6887.
- Marcu M. G., Chadli A., Bouhouche, I., Catelli M. G. and Neckers L., The heat shock protein90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone, 2000, J. Biol. Chem., 37181-37186.
- Maruya M., Sameshima M., Nemoto T. and Yahara I., 1999, J. Mol. Biol. 285, 903–907.
- Minami Y, Kimura Y, Kawasaki H, Suzuki K and Yahara I. The carboxyl-terminal region of mammalian HSP90 is required for its dimerization and function in vivo, 1994, Mol Cell Biol., 14(2):1459-64.
- Multhoff G. and Hightower LE., Cell surface expression of heat shock proteins and the immune response, 1996, Cell Stress Chaperones. 1(3):167.
- Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents, 2002, *Trends Mol. Med., 8*, S55-S61.
- Nollen EAA and Morimoto RI, Chaperoning signaling pathways: Molecular chaperones as stress-sensing heat shock' proteins, 2002, J Cell Sci., 115(14):2809-16.
- Obermann WMJ, Sondermann H, Russo AA, Pavletich NP and Hartl FU. In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis, 1998, J Cell Biol., 143(4):901-10.

- Pick E, Kluger Y, Giltnane JM, Moeder C, Camp RL and Rimm DL, High HSP90 expression is associated with decreased survival in breast cancer,2007, Cancer Res.,67(7):2932.
- Prodromou C, Panaretou B, Chohan S, Siligardi G, O'Brien R and Ladbury JE, The ATPase cycle of Hsp90 drives a molecular'clamp'via transient dimerization of the N-terminal domains,2000, EMBO J., 19(16):4383-92.
- Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW and Pearl LH., Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone, 1997, Cell., 90(1):65-7
- Schulte T.W., Destabilization of Raf-1 by geldanamycin leads to disruption of the Raf-1–MEK–mitogen-activated protein kinase signalling pathway, 1996, Mol. Cell. Biol., 16, 5839–5845.
- Soga S., Kozawa T., Narumi H., Akinaga S., Irie K., Matsumoto K., Sharma S. V., Nakano H., Mizukami T., and Hara, M., 1998, J. Biol. Chem. 273, 822–828.
- Sreedhar AS and Csermely P., Heat shock proteins in the regulation of apoptosis: New strategies in tumor therapy: A comprehensive review, 2004, Pharmacol Ther., 101(3):227-57.
- Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU and Pavletich NP, Crystal structure of an Hsp90-geldanamycin complex: Targeting of a protein chaperone by an antitumor agent, 1997, Cell, 89(2):239-50.
- T.W. Schulte, W.G. And L.M. Neckers, Geldanamycin-induced destabilization of Raf-1 involves the proteasome, 1997, Biochem. Biophys. Res. Commun, 239,655–659.
- W.B. Pratt and D.O. Toft, Steroid receptor interactions with heat shock protein and immunophilin chaperones, 1997, Endocr. Rev. 18, 306–360.
- Whitesell L and Lindquist SL. HSP90 and the chaperoning of cancer, 2005, Nature Reviews Cancer, 5(10):761-72.
- Xie Q., Wondergem R., Shen Y., Cavey G., Ke J., Thompson R., Bradley R., Daugherty-Holtrop J., Xu Y. and Chen, E., Benzoquinone ansamycin 17AAG binds to mitochondrial voltage-dependent anion channel and inhibits cell invasion, 2011, Proc. Natl. Acad. Sci, 108, 4105–4110.
- Xu W., Mimnaugh E., Rosser M.F., Nicchitta C., Marcu M., Yarden Y. and Neckers L., Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and

is mediated by the chaperone protein Hsp90, 2001, J. Biol. Chem, 276, 3702–3708.

- Xu W, Mimnaugh EG, Kim JS, Trepel JB and Neekers LM, Cell Stress Chaperones,2000, 7:91–96.
- Yano M., Naito Z., Tanaka S. and Asano G., Expression and roles of heat shock proteins in human breast cancer, 1996, *Jpn. J. Cancer Res*, *87*, 908–915.
- Zagouri F., Nonni A., Sergentanis T.N., Papadimitriou C.A., Michalopoulos N.V., Lazaris A.C., Patsouris E. and Zografos G.C, Heat shock protein90 in lobular neoplasia of the breast, 2008, BMC Cancer, 8, 312.
- Zhang M, Boter M, Li K, Kadota Y, Panaretou B, Prodromou C, Shirasu K and Pearl LH, cell stress chaperone, 2008, Embo J., 27:2789–2798.