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MOLECULAR DOCKING STUDIES OF PHYTOCHEMICAL COMPOUND TARGETING AGAINST BREAST CANCER AND SYSTEMS BIOLOGY

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ABSTRACT

Breast cancer occurs when cells in breast tissue mutate and keep dividing. These abnormal cells form a tumor. A tumor is said to be malignant when these invade other parts of the breast. Up to 10% of all breast cancers are thought to be inherited, and most of the cases are due to defects in one or more genes, especially the BRCA1 or BRCA2 gene. The Breast Ovarian Cancer-1 BROVCA1 can be caused by mutation in BRCA1 gene on chromosome 17y. The genes confer cellular phenotypes consistent with a role in tumor formation. Different terms are applied in this area including computer aided drug designing, insilico drug design. Targeting Bcl2 family members become an important and attractive approach towards cancer therapy. In different malignancies the Bcl2 family proteins and Bcl2 like proteins are compound of anti-apoptotic machinery and are over expressed.

KEYWORDS

BRCA1, BRCA2 gene, BROVCA3, Mutation, Bcl2 and Insilico drug design.

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INTRODUCTION

Breast cancer occurs when cells in breast tissue mutate and keep dividing. These abnormal cells form a tumor. A tumor is said to be malignant when these invade other parts of the breast or metastasize to other areas of the body through the bloodstream or lymphatic system, a network of vessels and nodes within the body that plays a task in fighting infection. Up to 10% of all breast cancers are thought to be inherited, and most of the cases are due to defects in one or more genes, especially the BRCA1 or BRCA2 genes. Breast-ovarian cancer-1 BROVCA1 can be caused by mutation in the BRCA1 gene on chromosome 17q, BROVCA2 by

mutation in the BRCA2 gene on chromosome 13q12, BROVCA3 by mutation in the RAD51C gene on chromosome 17q22, and BROVCA4 by mutation in the RAD51D gene on chromosome 17q11. Furthermore, the PPM1D gene on 17q is commonly amplified in breast cancer and appears to lead to cell transformation by abrogating p53 tumor suppressor activity. Some genomic regions have been found to be amplified in breast cancer, including 8q24, 20q13, 11q12, and 8p12-p11[2].

The NCOA3and ZNF217 genes, located on 20q, undergo amplification in breast cancer; when over expressed, these genes confer cellular phenotypes consistent with a role in tumor formation[3]. In humans, six ant apoptotic members of the Bcl-2 family have been identified (Bcl-2, Bcl-xL, Bcl-B, Bcl-W, Bfl-1, and Mcl-1), in which Bcl-2 family proteins and Bcl-2 like proteins are a component of the anti-apoptotic machinery and are over expressed in different malignancies. Therefore, targeting Bcl-2 family members becomes an important and attractive approach towards cancer therapy. Use of computational techniques in drug discovery and development process is rapidly gaining in popularity, implementation and appreciation. Different terms are being applied to the current space, including computer-aided drug design (CADD), computational drug design, computer-aided molecular design (CAMD), computer-aided molecular modeling (CAMM), rational drug design, in silico drug design, computer-aided rational drug design.

Apoptotic regulators

Apoptosis is a regulated cellular suicide mechanism. The central regulator of the apoptosis is the Caspases. Apoptotic proteins can be categorized into two broad categories: those that modulate mitochondrial function and those that regulate the activation of caspases responsible for activation and execution of the apoptotic cascade. These proteins are involved in regulation of the intrinsic, mitochondrial apoptotic pathway. Protein BID is cleaved by the activation of pro-caspase-8 through the extrinsic pathway, and translocate to the mitochondrion to promote cytochrome C release. Apoptosis is additionally regulated by numerous signal transduction pathways, possibly through post-translational modifications in BCL-2 family proteins.

BID Protein

The anti-apoptotic protein Bcl-2, bind BID and inhibit BID's ability to activate Bax. As a result, the anti-apoptotic proteins Bcl-2 inhibit apoptosis by sequestering BID, leading to reduced Bax activation. The expression of BID is up regulated by the tumor suppressorp53, and BID has been shown to be involved in p53-mediated apoptosis.

Gene information

Full Name-BH3 interacting domain death agonist
Gene type-protein coding
Organism- Homo sapiens

Aim and Objective

The aim of the present study is by using a new anti-apoptotic BID subfamily targeting the Breast cancer using the Discover studio 2.0 software.

- To find a new target for Breast cancer.
- To find a new drugs for treating Breast cancer
- To perform molecular docking using Discovery studios 2.0

MATERIAL AND METHODS

Swiss Model- A fully automated protein homology-modeling server, accessible via the ExPASy web server, or from the program Deep View (Swiss Pdb-Viewer). The purpose of this server is to make protein modelling accessible to all life science researchers worldwide SWISS-MODEL is a fully automated protein structure.

Chem sketch- version 14.01

Chem Sketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers, and Markush structures. It conjointly includes options like calculation of molecular properties (e.g., mass, density, molar bending etc.), second and 3D structure cleanup and viewing, practicality for naming structures (fewer than fifty atoms and three rings), and prediction of log P. The package version of Chem Sketch doesn't embody all of the practicality.

Swiss PDB viewer- version4.1

Swiss-Pdb Viewer (aka Deep View) is an application that provides a user friendly interface

allowing to analyze several proteins at the same time. The proteins may be superimposed so as to deduce structural alignments and compare their active sites or the other relevant elements.

Discovery studios

Discovery Studio provides the most advanced modeling and simulation software solutions for life science researchers available today. From project conception to steer improvement, Discovery Studio includes a diverse collection of sophisticated software applications in a single, easy-to-use Linux- or Windows-based environment. Because Discovery Studio is built upon Pipeline Pilot platform, Accelrys scientific operating platform, any software that you need can be integrated into the research environment, whether it's from Accelrys, in-house developers, or other vendors to provide a truly customized solution.

Binding DB

It is a public, web-accessible database of measured binding affinities, focusing chiefly on the interactions of proteins considered to be candidate drug-targets with ligands that are small, drug-like molecules. Binding DB's web-interface provides a spread of browsing, query and data download tools. These embody browsing by the name of a macromolecule Target or by journal citation, query by chemical similarity and substructure, and downloads by target or query result.

Pub chem

Pub Chem may be an info of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology data (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). Pub Chem may be accessed at no cost through an online computer program. Millions of compound structures and descriptive datasets may be freely downloaded via FTP. Pub Chem contains substance descriptions and little molecules with fewer than one thousand atoms and one thousand bonds. More than eighty info vendors contribute to the growing Pub Chem info.

Uniprot

UniProt KB/Swiss-Prot may be a manually annotated, non-redundant protein sequence database. It combines data extracted from scientific literature and biocurator-evaluated process analysis. The aim of UniProt KB/Swiss-Prot is to produce all famed relevant data a few specific macromolecule. Annotation is often reviewed to stay up with current scientific findings. The manual associate degree notation of an entry involves elaborate analysis of the macromolecule sequence and of the scientific literature.

RESULTS AND DISSCUSION

Target selection

Target was selected based on the literature review. Anti-apoptotic protein BID was selected as the target.

New target identification by using comparative modeling

The protein sequence of BID was retrieved from UniProt and a new target was modeled using Swiss-model.

One model was generated and used as a target.

Structure Validation

The modeled protein was validated using swiss PDB viewer.

Ramachandran plot was generated and the proteins outside the allowed regions were dragged inside the allowed regions.

Ligand selection

Oxycodone is the common commercial drug used for treating breast cancer. A list of microbial ligands were taken based on the literature review.

Virtual screening of ligands

The ligands were screened against oxycodone using binding DB and the best ligand was choosed using the maximum smiliraity score.

Docking

One compound was selected based on the maximum similarity value and the compound was docked against the modeled protein.

Selected ligand (FDA approved Drug available commercially)- Oxycodone [Pub Chem ID: 5284603].

Protein or Receptor- BID [Uniprot ID: P10144].

Oxycodone was compared with the following phytochemical compounds and the maximum similarity value was found using Binding DB.

The selected compound among them is Catechin

(0.38) which had the maximum similarity value with oxycodone.

SYSTEMS BIOLOGY

Systems biology is the computational modeling of complex biological networks. It is a biology-based knowledge domain field of study that focuses on complicated interactions among biological systems.

Systems biology allows one to develop data repositories, and tools for simulation, analysis and visualization of system components such as biochemical networks. It also enables High Throughput molecular profiling and signal procession.

SBML (Systems Biology Markup Language)

A standard language for representing biochemical reaction networks in Systems Biology. It is a software-independent language for describing models common to research in many areas of computational biology, including cell signaling pathways, metabolic pathways, gene regulation, and others.

Networks are able to link with simulation and other analysis packages through Systems Biology Workbench (SBW). It is a collection of tools which includes programs for building, viewing and editing biochemical networks. Also, consists of tools for simulation, import and translation of models.

Cell Designer

Cell Designer is a Java -based program for constructing and editing of Biochemical networks. Networks are drawn based on the process diagram, with Systems Biology Graphical Notation system and #40; SBGN and #41; proposed by Kitano, and are stored using Systems Biology Markup Language (SBML). Recent version can import models in SBML and support display of biochemical networks.

Introduction to cell designer

Cell Designer may be a structured diagram editor for drawing gene-regulatory and organic chemistry networks. Networks are drawn supported the method diagram; with graphical notation system. The file stored will be an xml file representing the SBML model structure. Main Window consists of Menu, Toolbars, and the five Areas as shown below.

- Menu
- Toolbar
- Draw Area
- Tree Area
- Layer Area
- List Area
- Notes Area

PROTEIN KINASE PATHWAY

Protein kinases are enzymes that function by phosphorylating other proteins by chemically adding phosphate groups or codes. The codes instruct the cell to work, such as divide or grow. Phosphorylation is a common way of activating or inactivating enzymes and protein kinases are thus often found in signaling pathways.

KINASE PATHWAY

Kinase pathway is a chain of proteins in the cell that transfers a signal from a receptor present on the surface of the cell to the DNA in the nucleus of the cell. The signal starts when a signaling molecule binds to the receptor and ends when the DNA in the nucleus expresses a protein and produces some change in the cell. The pathway includes many proteins, including MAPK (mitogen-activated protein kinases) which communicate by adding phosphate groups to a neighboring protein, which acts as an "on" or "off" switch. When one of the proteins in the pathway is mutated, it can become stuck in the "on" or "off" position, which is a necessary step in the development of many cancers. The MAPKKK is typically activated by interactions with a small GTPase and/or phosphorylation by protein kinases downstream from cell surface receptors. Uncontrolled growth may be a necessary step for the event of all cancers. In many cancers, a defect in the MAP/ERK pathway leads to uncontrolled growth. Many compounds will inhibit steps within the MAP/ERK pathway, and therefore are potential drugs for treating cancer.

Kinase pathway generated using cell designer.

Gene switches

Some of the non-protein coding DNA regulates once and wherever genes are turned on and off and the way a lot of super molecule they turn out. The

regulative machinery works once proteins known as transcription factors bind to specific short sequences of polymer that flank the sequence, called transcription factor binding sites, and by doing so, switch genes on and off. Mutations to DNA sequences also play roles in turning genes on and off.

Lac operon pathway

The lac operon (lactose operon) is required for the transport and metabolism of lactose in E.coli. Although aldohexose is that the most well-liked carbon supply for many bacterium, the lac operon allows for the effective digestion of lactose when glucose is not available through the activity of beta-galactosidase. When disaccharide is needed as a sugar supply for the microorganism, the three genes of the lac operon can be expressed and their subsequent proteins translated: lacZ, lacY, and lac A.

If glucose is present, the lac operon uses a two-part control mechanism to ensure that the cell expends energy producing the enzymes encoded by the lac operon only when necessary. In the absence of disaccharide, the lac repressor, lacI, halts production of the enzymes encoded by the lac operon.

Gene expression

Gene expression is that the method by that sequencetic directions are accustomed synthesizes gene merchandise. These products are usually proteins, which go on to perform essential functions as enzymes, hormones and receptors. The process of gene expression includes the following steps: Transcription, processing, non-coding RNA maturation, RNA export, translation and protein folding.

Table No.1: Ligands that are similar to Oxycodone and their maximum similarity score obtained from binding DB when screened

S.No	Compound Name	Pubchem ID	Maximum similarity value
1	Catechin	9064	0.38
2	Terpenoid	3385	0.26
3	Gossypol	3503	0.22
4	Eremanthin	572	0.18
5	Coztunoloid	7559	0.17
6	Gallic Acid	370	0.14
7	Octylgallate	1253	0.2
8	Saponin	7422	0.2
9	Lupeol	846	0.1

Table No.2: Table showing the results of BID protein docked against oxycodone

S.No	Absolute Energy of Catechin	Relative Energy of Catechin	LibDock score	Total No. of poses	H Bonds
1	30.971	0.506	107.119	38	383

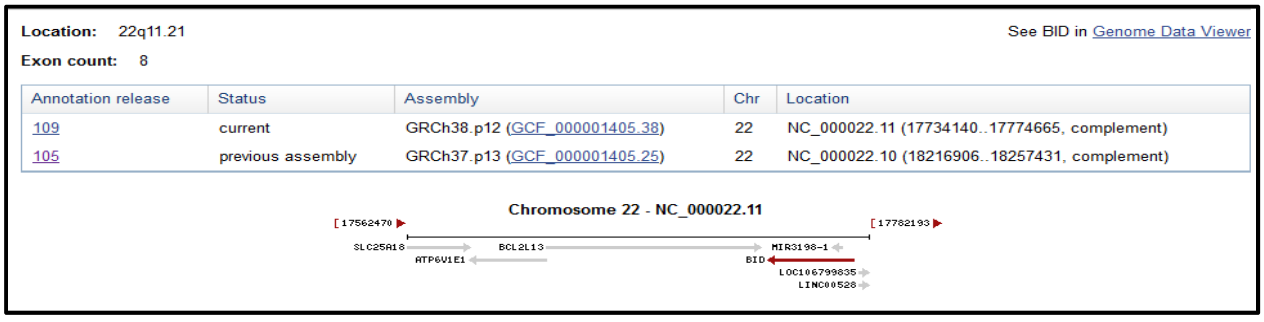

Figure No.1: location of the gene retrieved from NCBI

Figure No.2: protein modeling using Swiss- Model

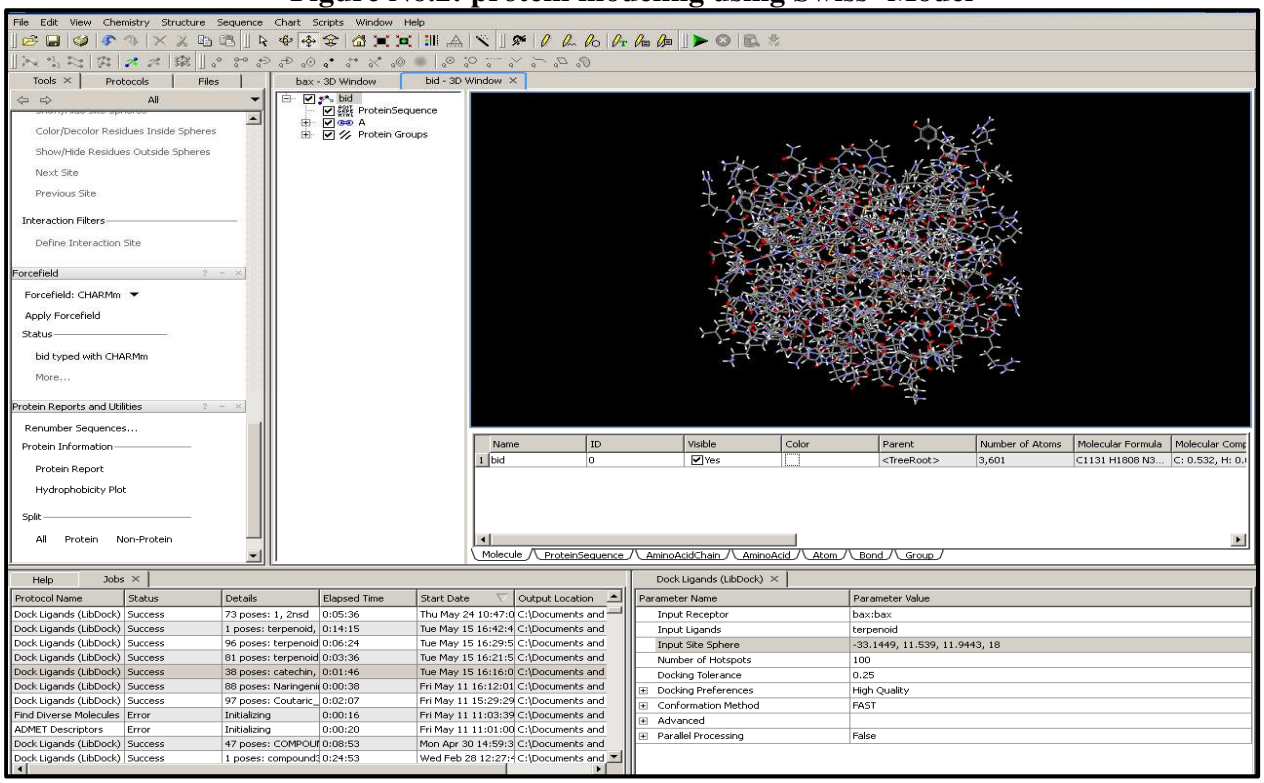

Figure No.3: The new model generated in Swiss model was then opened in Discovery studio using. pdb file extension, the hetero atoms and water molecules were removed

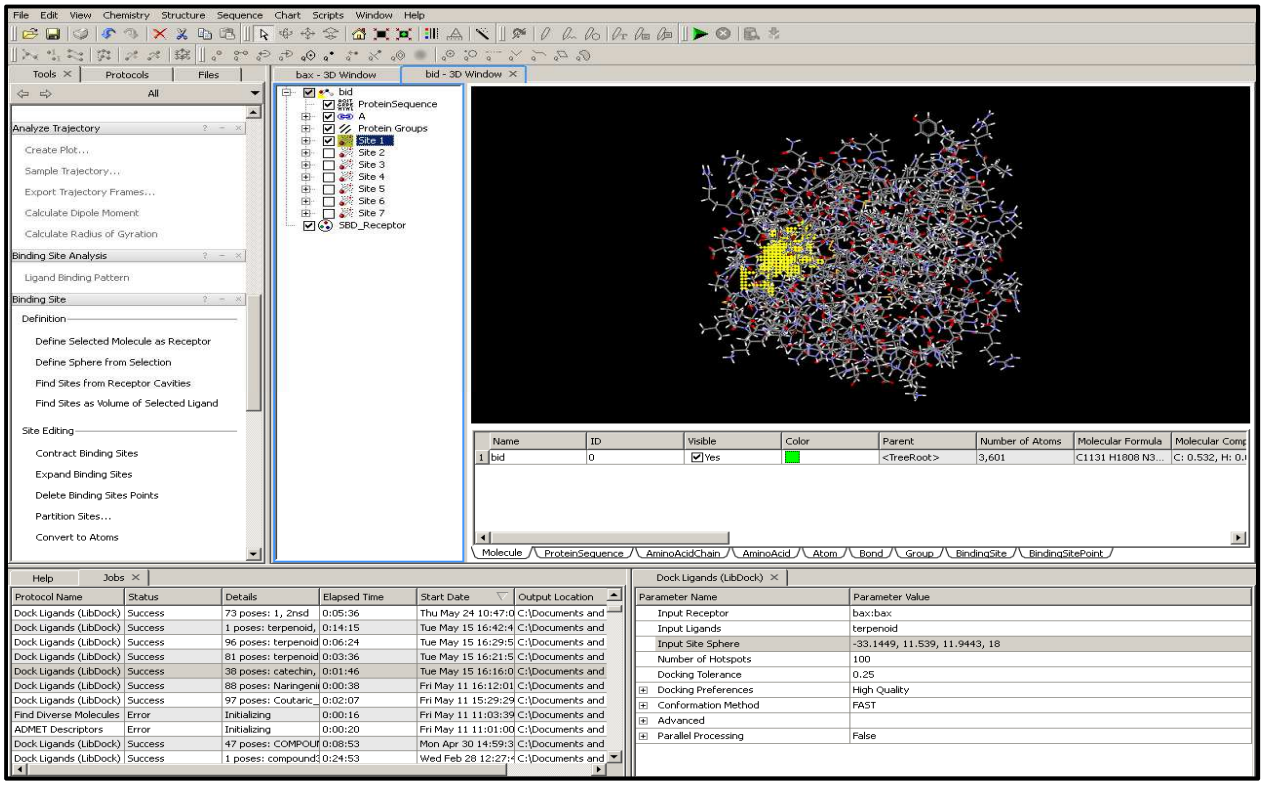

Figure No.4: The total number of active sites were viewed by defining the selected molecule as the receptor. The active site one was selected

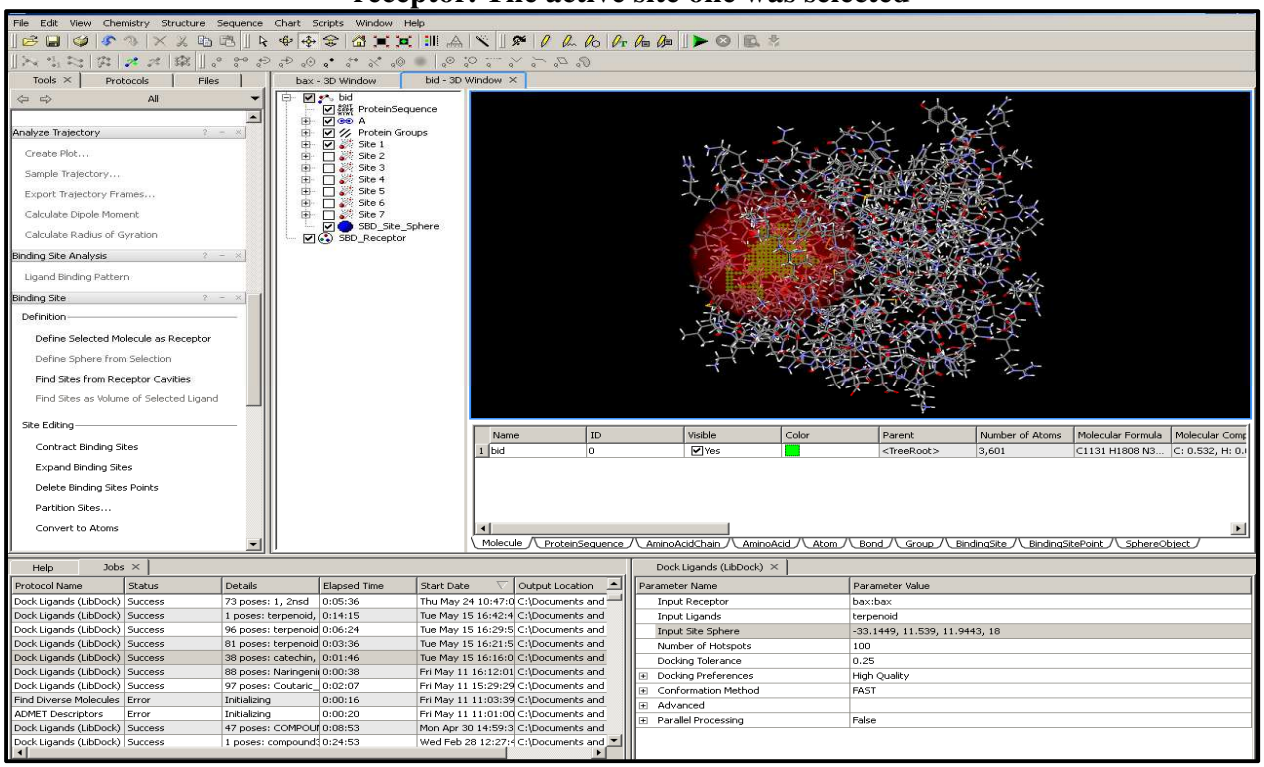

Figure No.5: The virtual analysis of biomolecule inside a human body can be studied by defining them inside a grid in molecular modeling. Discovery studio uses sphere that was defined in the selected active site

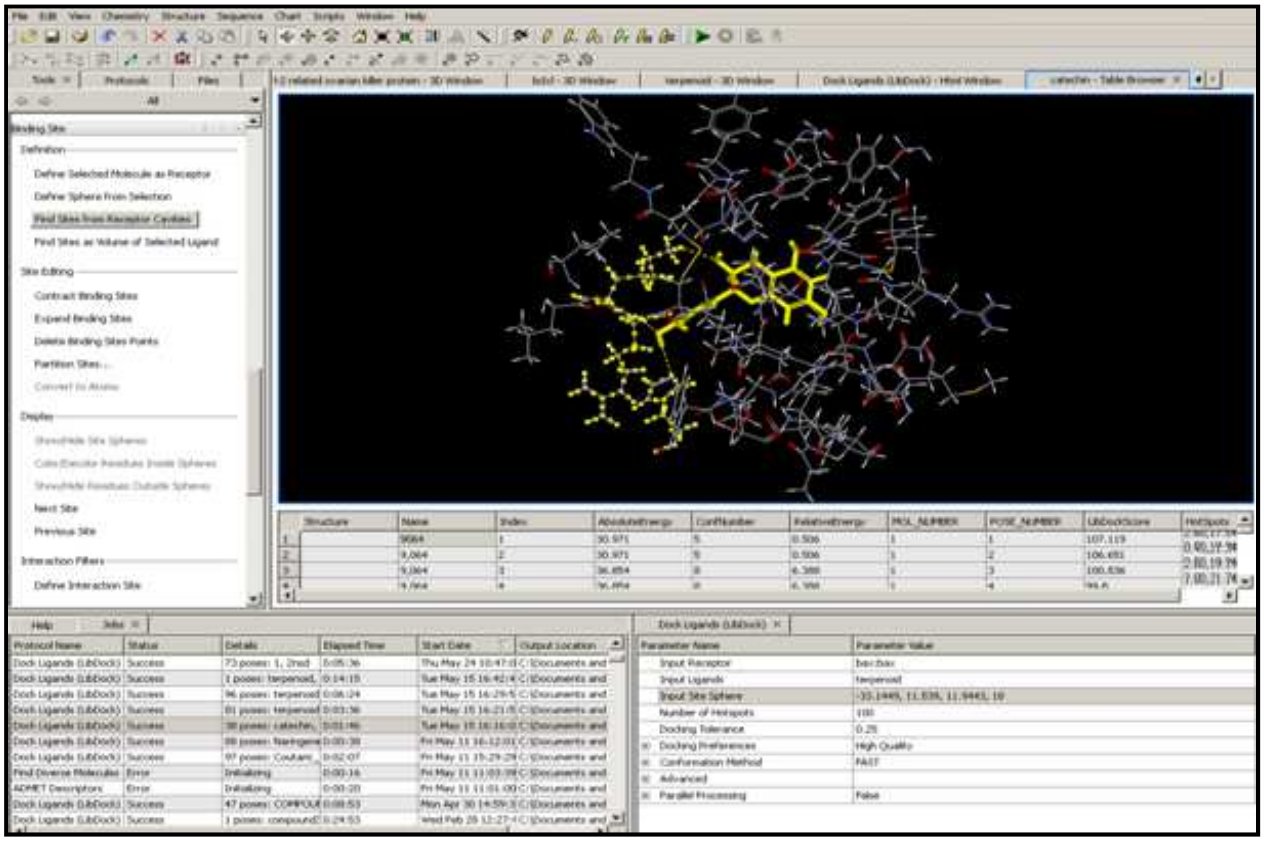

Figure No.6: The best ligand to xycodone was uploaded to the active site

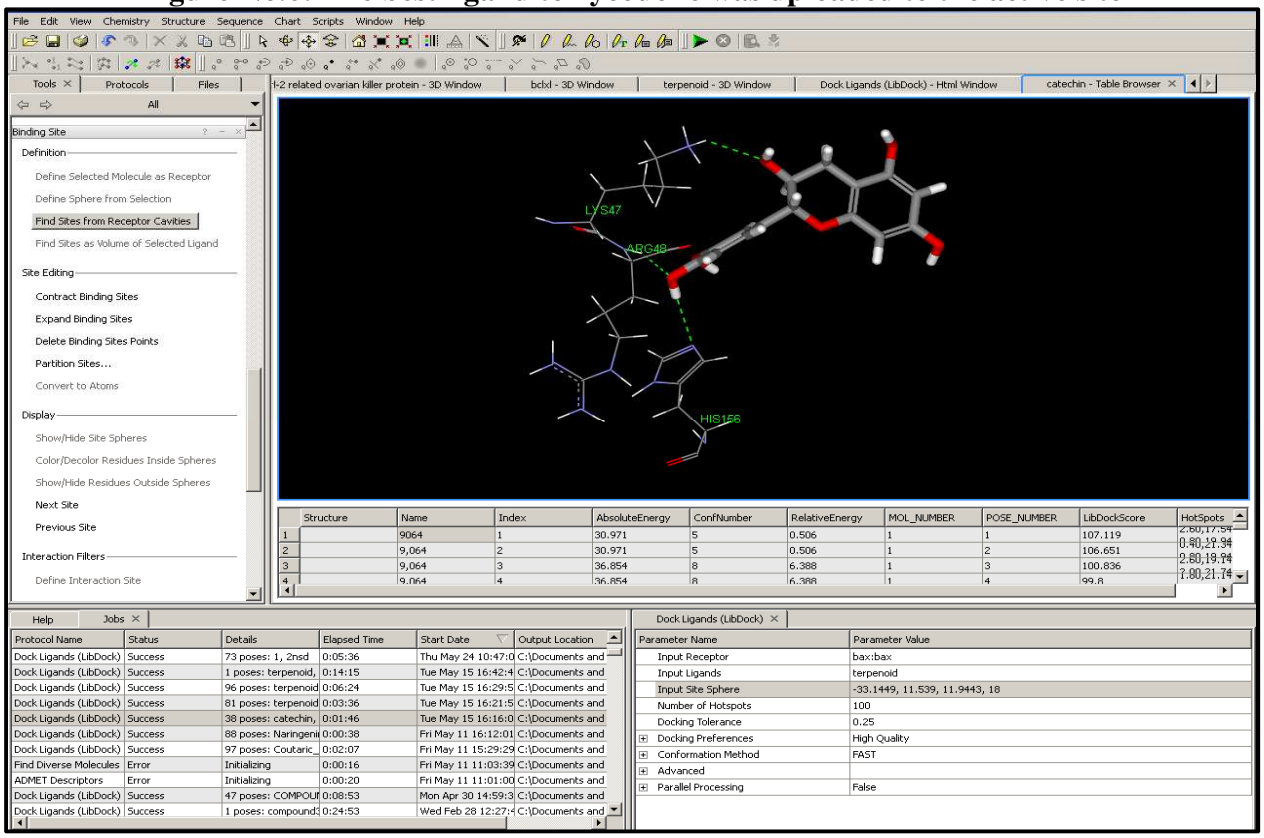

Figure No.7: Final results of docking

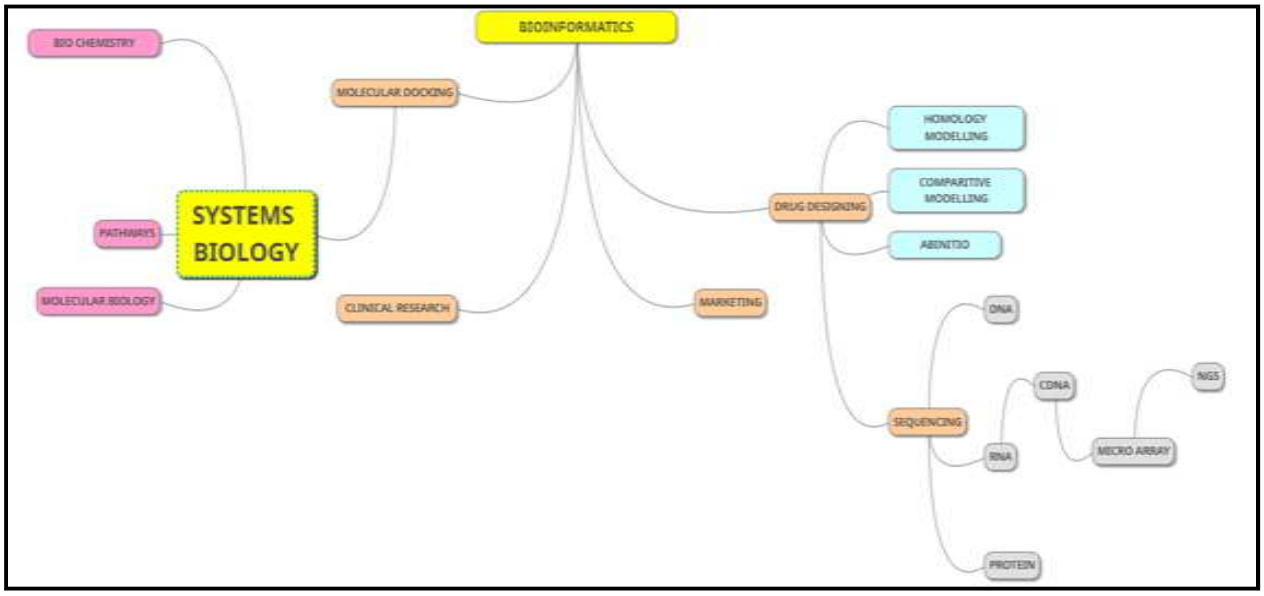

Figure No.8: Created using mindmup software

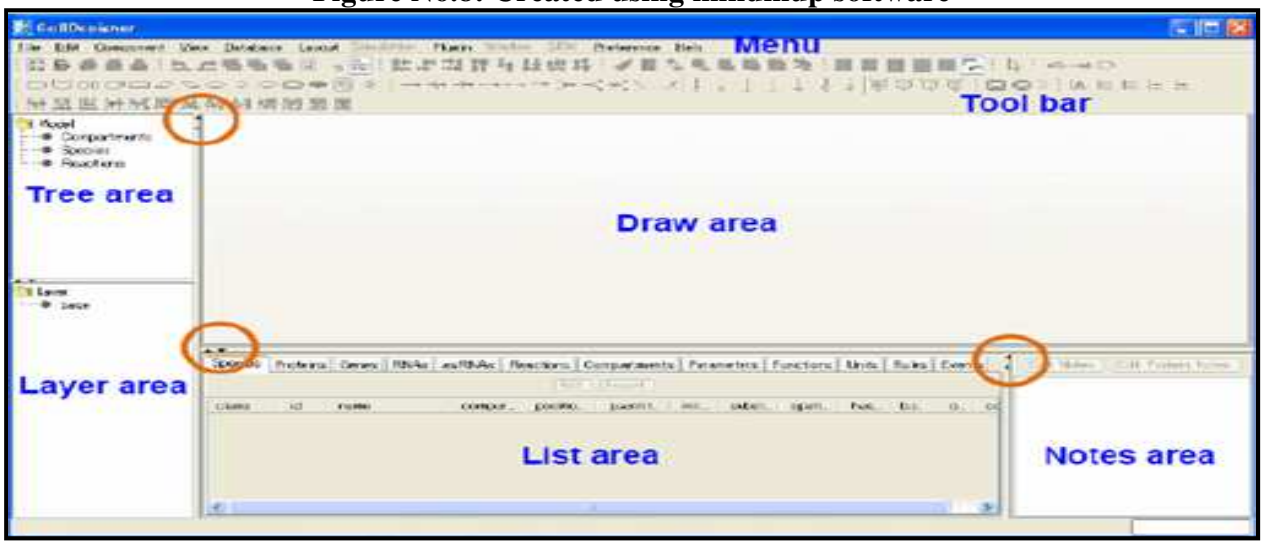

Figure No.9: Outline of cell designer

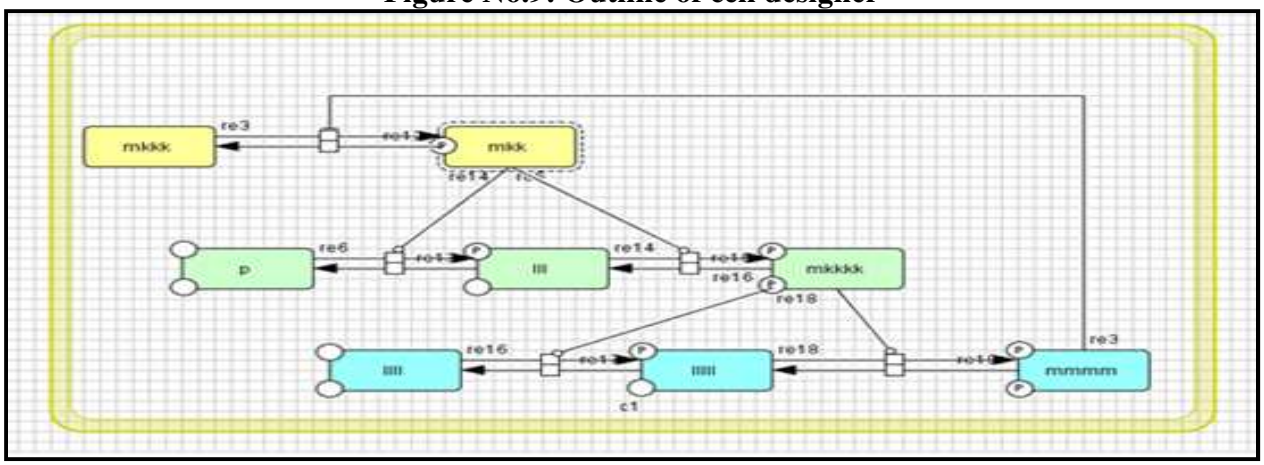

Figure No.10: Kinase pathway constructed using Cell Designer where the rectangles represent proteins, dotted lines represent the complex, circles represent phosphate groups

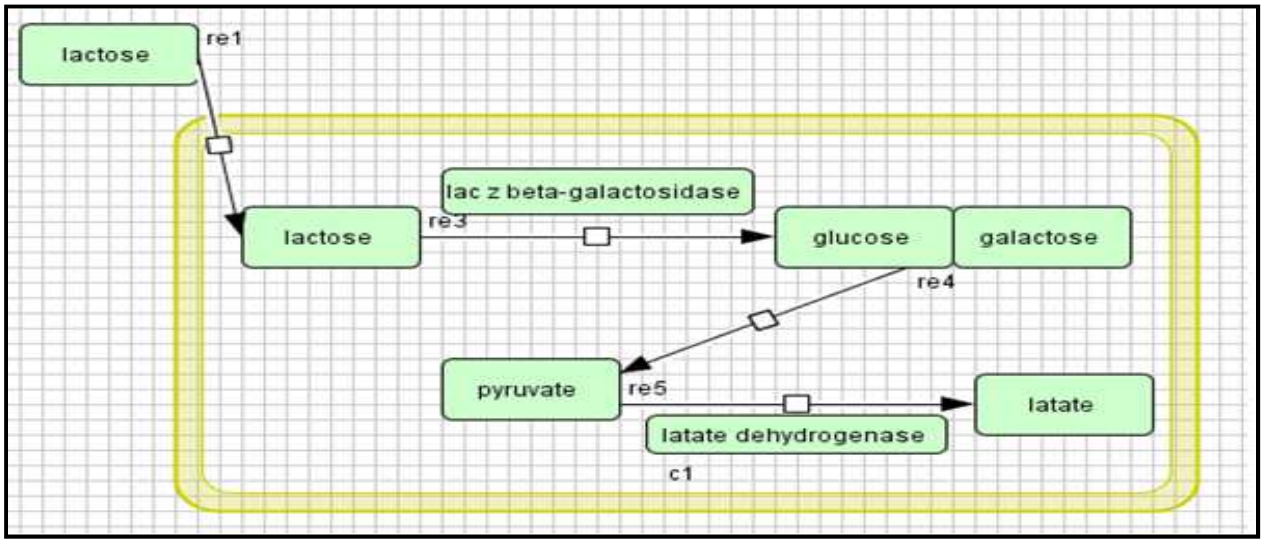

Figure No.11: Lac operon pathway

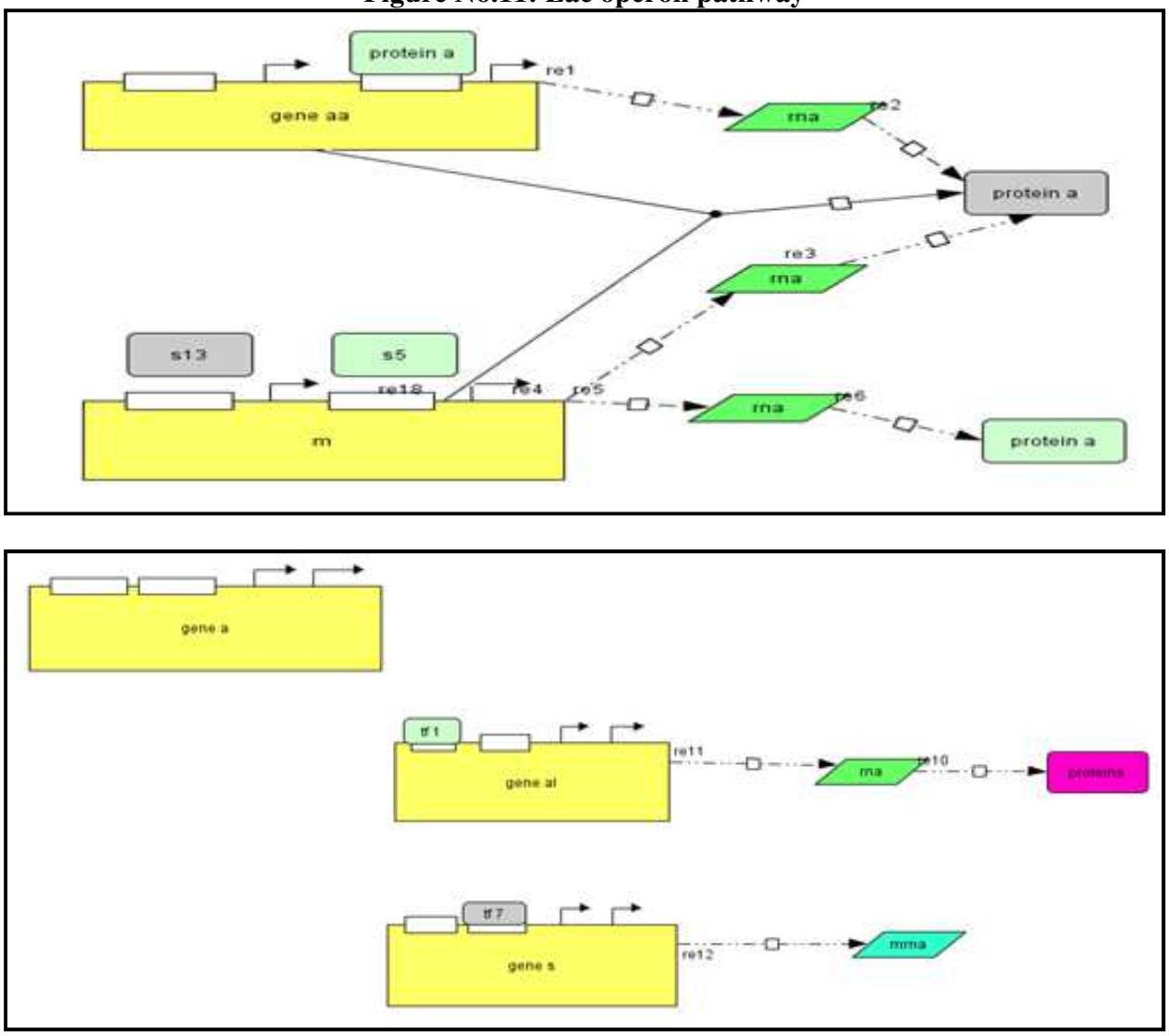

Figure No.12: Gene expression

THEPATHWAY OF PROTEIN KINASE WAS GENERATED USING CELL DESIGNER BASED ON KINETIC LAW

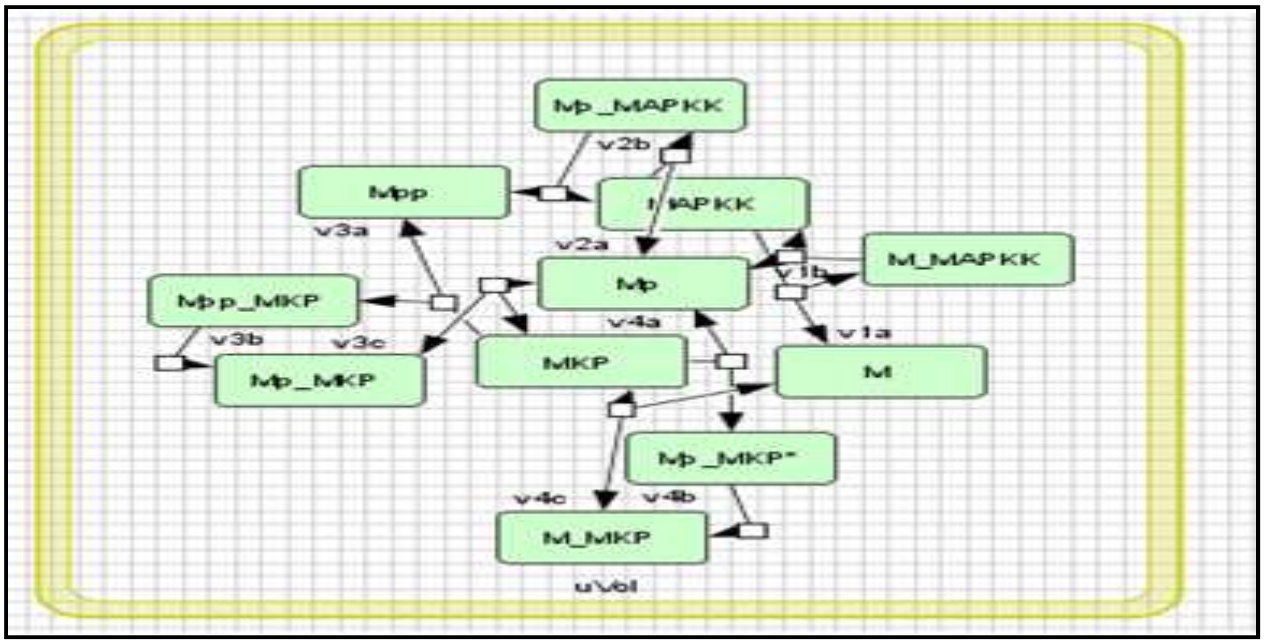

Figure No.13: Bio Med ID- 26

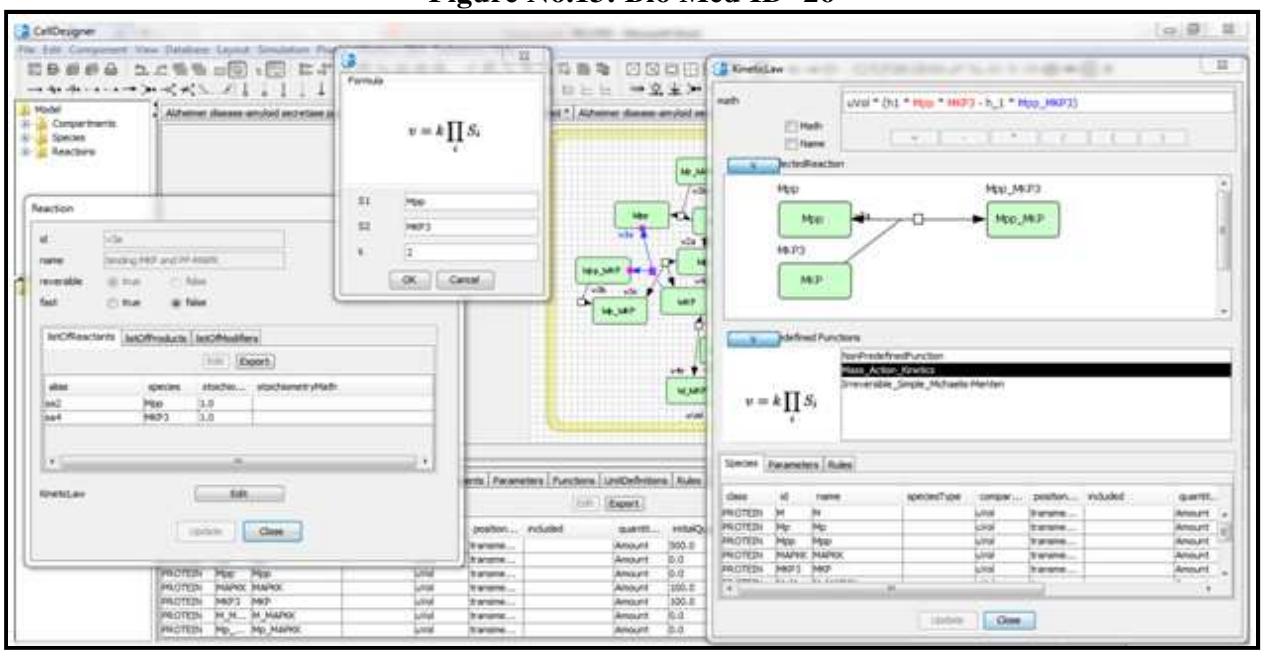

Figure No.14: Kinetic law of reactions was modified according to the instructions

Species | Proteins | Genes | RNAs | asRNAs | Reactions | Compartments | Parameters | Functions | UnitDefinitions | Rules | Events | SpeciesTypes | CompartmentTypes | InitialAssignments | Constraints

Edit | Export

class	id	name	speciesType	compar...	position...	included	quantit...	initialQuantity	sub...	hasO...	b.c.	c...
PROTEIN	M	M		uVol	transme...		Amount	500.0		false	false	false
PROTEIN	Mp	Mp		uVol	transme...		Amount	0.0		false	false	false
PROTEIN	Mpp	Mpp		uVol	transme...		Amount	0.0		false	false	false
PROTEIN	MAPKK	MAPKK		uVol	transme...		Amount	100.0		true	false	false
PROTEIN	MKP3	MKP		uVol	transme...		Amount	100.0		false	false	false
PROTEIN	M_M...	M_MAPKK		uVol	transme...		Amount	0.0		false	false	false
PROTEIN	Mp_...	Mp_MAPKK		uVol	transme...		Amount	0.0		false	false	false

Figure No.15: Initial values

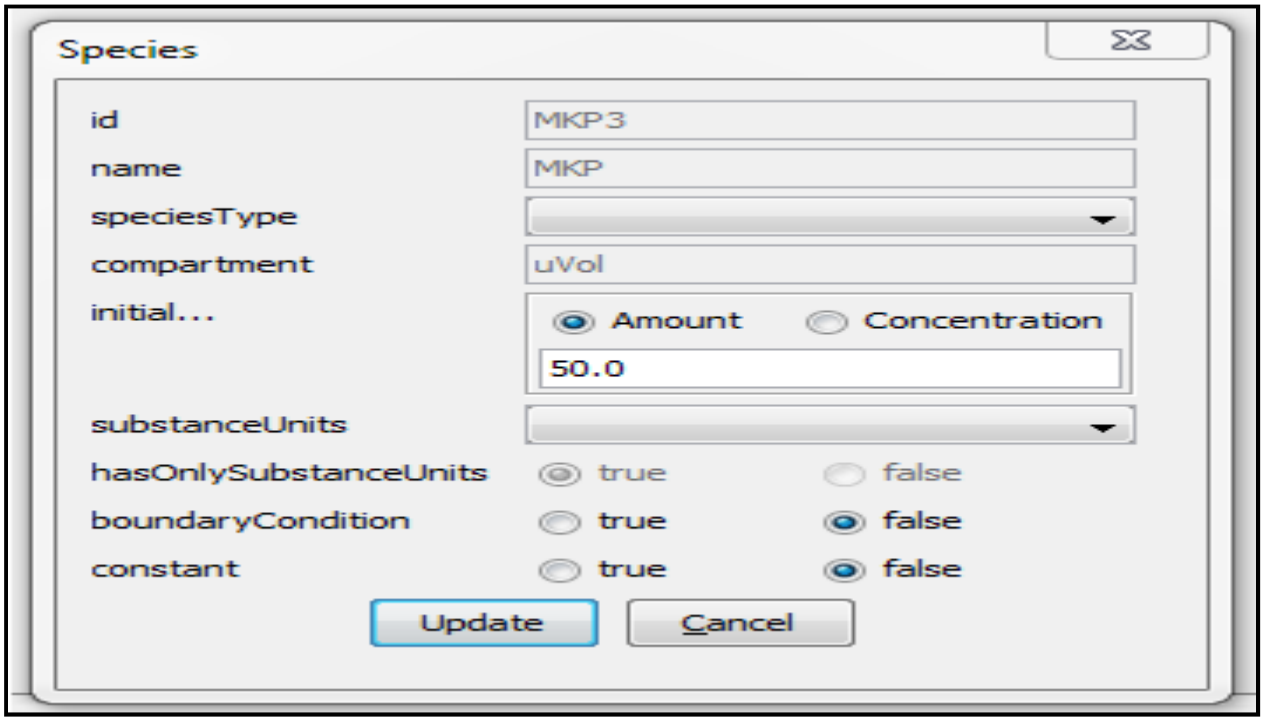

Figure No.16: Updating the initial values

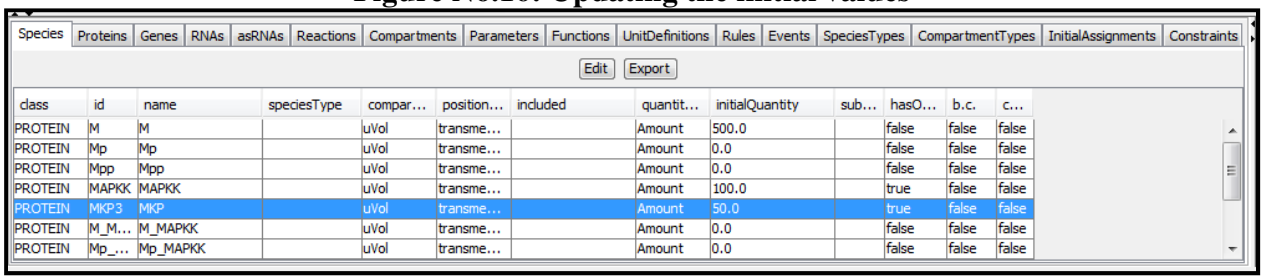

Figure No.17: Modified values

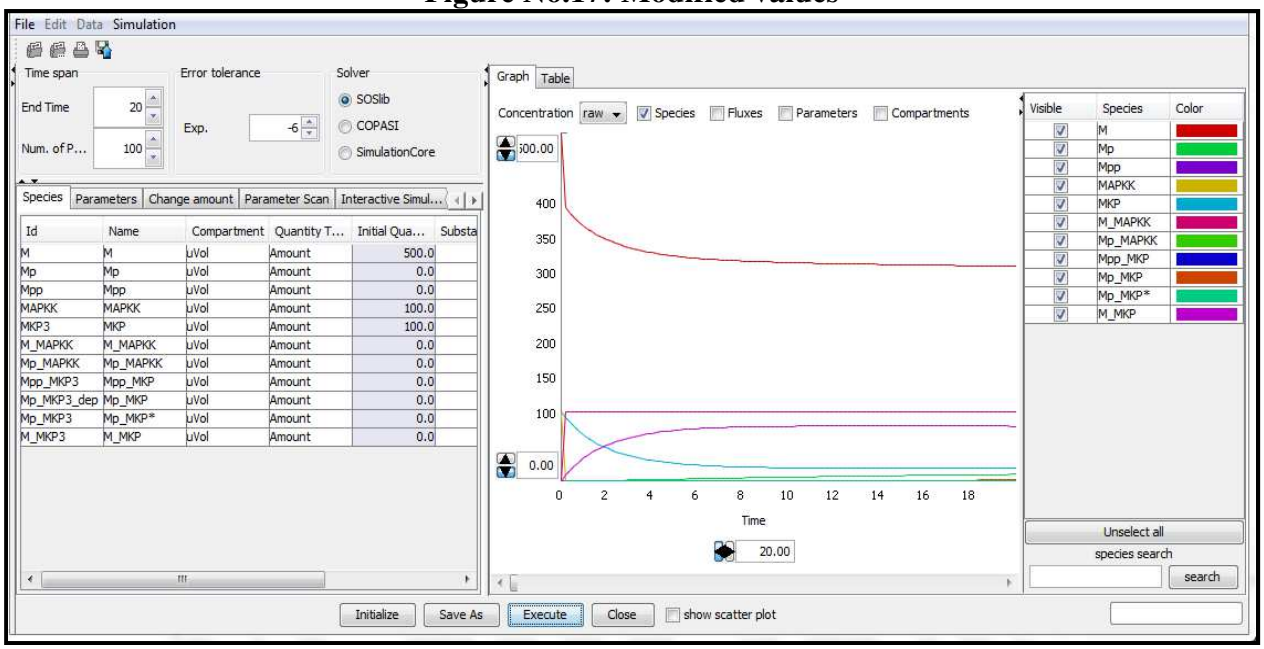

Figure No.18: Graph generated for the pathway- biomed id 26using cell Designer

CONCLUSION AND SUMMARY

The present study helps us to understand the interaction between the ligand Oxycodone and receptor BID protein and also explore their binding mode. Docking study was performed using Discovery Studios 2.1. The protein structure was modeled from Swiss-Model and used as a target for docking simulation shown in Figure No.3 -7. The compounds selected from the literature and screened against oxycodone using Binding DB are listed in Table No.1. Ligand were created and prepared for the docking procedure using Chem Sketch and SPDBV.

- Ligand Catechin and modelled BID protein as receptor were taken to explore their binding mode. Docking study was performed using Discovery Studios 2.1.
- It is found that the binding affinity of phytochemical compound oxycodone against the modelled protein is 30.972 Kcal/mol.
- This work evaluates the binding efficiency and the counteraction with the modelled protein.

The Protein-Ligand interaction plays a significant role in ligand based designing. In the present work we have taken the receptor that is modelled from Swiss-model and identified a bioactive phytochemical compound. When the receptor was docked with the ligand, the energy value obtained was (-30.971 kcal/mol) using Discovery Studios 2.1.From this we conclude that the phytochemical compound catechin obtained from literature review can be taken to further drug developmental studies.

In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of plant derived compounds. The results of our present study can be useful for the design and development of novel compounds having better inhibitory activity against breast cancer. This potential agent will be a promising candidate that can further be validated in wet lab studies for its proper function.

In future research work the ADMET (Absorption, Distribution, Metabolism, and Excretion, Toxicity) properties of these compounds can be tested in wet lab and research can proceed for clinical trials.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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