ISSN: 0975-5772
Venkatesan Suba et al / Journal of Pharmaceutical Science and Technology Vol. 2 (11), 2010,356-360

The Novel Therapeutic Approaches of Breast Cancer Treatment by Inhibition of DNA Damage Signaling Pathways in erbb2 Gene

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Abstract
The term "breast cancer" refers to a malignant tumor caused by a genetic abnormality in the breast cancer. An alarming increase in breast cancer cases, demands a renewed effort to seek effective treatment. In this paper we report the noval therapeutic approaches of breast cancer treatment by inhibition of DNA damage signaling pathways in erbB2 gene. Specific protein causing the disease is identified as erbB2. Homology modeling of the protein is done by using SPDBV (Swiss Protein Data Bank viewer). Active site analysis is done through a site finder method. Standard available market drugs targeting the protein were identified and similar molecules were docked using Argus lab for these standard molecules. A data is created with all these molecules in VegaZZ. Virtual screening of these molecules is done by protein database docking method. The result obtained showed that HEXADECANOATE is interacting at lowest energy level with more amino acids in the potential active site. So, it is considered that ligand as the potential lead molecule to target the protein in treatment of breast cancer.

Key Words: *erbB2, docking, drug design, offline tool, SPDBV.*

Introduction

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display the traits of *uncontrolled growth* (growth and division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not. The term "breast cancer" refers to a malignant tumor that has developed from cells in the breast. Usually breast cancer either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Less commonly, breast cancer can begin in the stromal tissues, which include the fatty and fibrous connective tissues of the breast. Breast cancer is an uncontrolled growth of breast cells. To better understand breast cancer, it helps to understand how any cancer can develop.The erbB2 gene is also commonly referred to as Her-2/neu, especially by doctors and other clinicians. This gene is one member of a family of genes that provide instructions for producing growth factor receptors. Growth factors are proteins that stimulate cell growth and division. The official name of this gene is "v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)."The erbB receptor plays an important role in cancer progression[1]. Amplification of the erbB2 gene (location of erbB2 gene- fig-1) has been reported in breast cancer[2] resulting in over production of the erbB2 protein, which likely stimulates cells to grow and progression of cancerous tumor.

Computer assisted drug design[3, 4] approaches for treatment of cancer is promising factor, for drug research[5] because chemotherapy and surgery have not been fully effective against the high incident or low survival rate of the cancers[6]. Computational chemistry[7] programs allow one to generate molecular data including geometric, electronic properties, spectroscopic properties and bulk properties. QSAR[8,9] (Quantitative Structure Activity Relationship) represents an attempt to correlate structural or property descriptors of compounds with activities and its extinction is CoMFA[6] (Comparative Molecular Field Analysis) which is used to identify regions were increasing or decreasing a substituent constant would influence activity.

The Location of ERBB2 gene:

Cytogenetic Location: 17q11.2-q12
Molecular Location on chromosome 17: base pairs 35,097,918 to 35,138,440

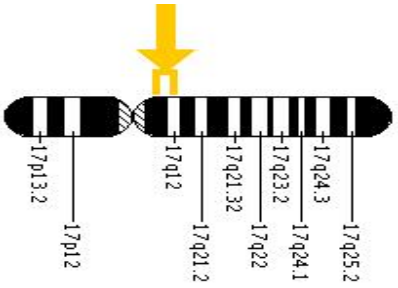

Figure 1

The ERBB2 gene is located on the long (q) arm of chromosome 17 between positions 11.2 and 12. More precisely, the erbB2 gene is located from base pair 35,097,918 to base pair 35,138,440 on chromosome 17.

Materials and Methods

NCBI:

National Center for Biotechnology Information maintained by National Institutes of Health (NIH) and National Library of Medicine (NLM). Established in 1988 as a national resource for molecular biology information. The information is retrived through Entrez server. Conducts basic and applied research in computational, mathematical, and theoretical problems in molecular biology and genetics, including genome analysis, sequence comparisons, sequence search methodologies, macromolecular structure, dynamics and interaction, and structure/function prediction. Establishes collaborative research projects in computational molecular biology with biologists, chemists, mathematicians, and computer scientists in NIH intramural laboratories, other government agencies, academia, and industry. Consults and advises governmental agencies and research laboratories in the application of computer-based analytical tools for studying molecular biology. Interacts with molecular biology groups to enhance wet-bench, laboratory-based research through the application of computational and theoretical approaches.

BIOEDIT:

BioEdit is a free program given by Brown lab (James W. Brown). BioEdit is a biological sequence editor that runs in Windows 95/98/NT/2000/XP and is intended to provide basic functions for protein and nucleic sequence editing, alignment, manipulation and analysis. BioEdit is not a powerful sequence analysis program, but offers many quick and easy functions for sequence editing, annotation and manipulation, as well as a few links to external sequence analysis programs. Sequence lengths and numbers are limited only by available system memory. Alignments >100 Mb have been edited on an average desktop with reasonable efficiency. The main goal of BioEdit is to provide a useful tool for biologists who do not want to have to know much about a program to utilize it. BioEdit is intuitive, menu-driven, and highly graphical and offers a graphical interface for users to run external analysis programs. The main functions are intended to be visible by simply playing with the menu options.

SWISS-PDB VEIWER:

Swiss-PdbViewer provides a user-friendly interface. This is under continuing development by Nicolas Guex & Manuel C. Peitsch of Geneva Glaxo Welcome Experimental Research. This allows analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain through the intuitive graphic and menu interface. This program allows the user to build models from scratch, simply by giving an amino-acid sequence. It submits the amino acid sequence to ExPASy to find homologous proteins, onto which you can subsequently align your sequence to build a preliminary three-dimensional model. It computes electrostatic potentials and carries out energy minimization to give a completely modeled protein and the user can all confirm whether all the residues are in the allowed regions of the Ramachandran plot. It also computes the molecular surfaces and cavities for the protein inorder to predict the active sites for it. It allows the user to

examine electron-density maps from crystallographic structures.

ARGUSLAB:

ArgusLab is a molecular modeling program that runs on Windows 98, NT, and 2000. ArgusLab consists of a user interface that supports OpenGL graphics display of molecule structures and runs quantum mechanical calculations using the Argus compute server.Generally helps the user in the study of docking of the ligand against the targets.

HYPERCHEM:

HyperChem is a sophisticated molecular modeling program that is known for its quality, flexibility, and ease of use. HyperChem unites 3-D visualization and animation with quantum chemical calculations, molecular mechanics and molecular dynamics. It puts more molecular modeling tools at fingertips than any other Windows program. It is equipped with several computational methods for calculating molecular geometry optimization, total energy, and bond angles and distances. It employs an intuitive point-and-click interface that allows for the easy assembly and calculation of molecule structures.

CAChe:

CAChe is a computer-aided molecular design (CAMD) modeling tool. CAChe enables to draw and model molecules and perform calculations on a molecule to discover molecular properties and energy values. CAChe displays experimental results in a variety of ways, such as, moving a molecule's atoms and bonds to produce an optimized or low-energy structure, showing electronic properties as surfaces superimposed on a molecule, producing three-dimensional energy graphs viewed alongside a series of low-energy conformations, showing experimental data as a range of values contained in the log file and output file automatically generated by each experiment. CAChe-UV-Visible Transitions: This indicates graphical representation that comprises of X-axis as the wave length and y-axis as the molar absorptivity. CAChe-IR-Visible Transitions: IR spectrum is a plot of wavenumber / wavelength (X-axis) vs percent transmittance / absorbance (Y-axis). With this design, without going to wet lab we can observe the maximum molar absorptivity (UV) & transmittance (IR) in our CAChe graphical preview.

RESULTS

Individual dock scores for standard available market drugs targeting the protein are in table-1. Dock scores of each amino acid with respective protein name and elapsed time for calculation are represented in table-2. QSAR properties for the final molecule are represented in table-3. From table-1, 2, 3 it is evident that HEXADECANOATE exhibits characteristic features. Hexadecanoate structure was represented in figure-2.

Hexadecanoate structure

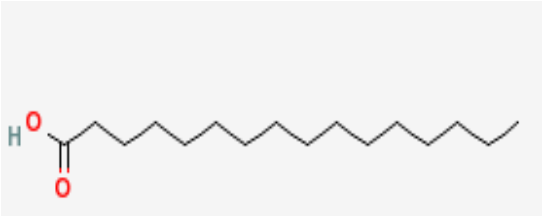

FIGURE-2

TABLE-1

Individual dock scores for standard available market drugs targeting the protein.

DRUGS	AMINO ACIDS	DOCK SCORES (kcal/mol)
BETAXALOL	Asp 30	**-7.19556**
CYCLOPHOSPHAMIDE	Phe 371	**-6.90071**
FLUROURACIL	Arg 434	**-5.96427**
GEMCITABINE	Ala 324	**-5.70766**
PALMITIC ACID	Leu 345	**-8.18794**

TABLE-2

Dock scores of each amino acid with respective protein name and elapsed time for calculation.

S.No	COMPOUND	AMINO ACIDS	DOCK SCORES (kcal/mol)	ELAPSED TIME FOR CALCULATION
1	HEXADECANOATE	Thr 23	-7.57758	1min.11 sec.
2	HEXADECANOATE	Val 25	-9.97404	1min.16 sec.
3	HEXADECANOATE	Cys 26	-9.35959	1min.
4	HEXADECANOATE	Thr 27	-8.61267	1min.5 sec.
5	HEXADECANOATE	Gly 28	-7.05893	59 sec.
6	HEXADECANOATE	Thr 29	-5.8243	45 sec
7	HEXADECANOATE	Asp 30	-8.64619	1min.6 sec.
8	HEXADECANOATE	Gln 57	-9.51531	1min.4 sec.
9	HEXADECANOATE	Gly 58	-7.60549	1min. 1 sec.
10	HEXADECANOATE	Asn 59	-9.66359	50 sec.
11	HEXADECANOATE	Gln 81	-8.07579	56 sec.
12	HEXADECANOATE	Thr 105	-6.78709	1 min. 8 sec.
13	HEXADECANOATE	Gln 106	-8.02335	1 min. 24 sec.
14	**HEXADECANOATE**	**Phe 291**	**-10.2459**	**56 sec.**
15	HEXADECANOATE	Pro 300	-9.54203	1 min. 30 sec.
16	HEXADECANOATE	Tyr 301	-8.46624	1 min. 6 sec.
17	HEXADECANOATE	Asn 302	-9.45694	58 sec.
18	HEXADECANOATE	Tyr 303	-9.57406	55 sce.
19	HEXADECANOATE	Leu 313	-8.87298	56 sec.
20	HEXADECANOATE	Val 314	-8.97683	1min. 9 sec.
21	HEXADECANOATE	Cys 315	-8.88593	1min. 5 sec.
22	**HEXADECANOATE**	**Gln 320**	**-10.2907**	**1min. 1 sec**
23	**HEXADECANOATE**	**Glu 321**	**-10.5065**	**52 sec.**

TABLE-3

QSAR Properties of final molecule.

S.No	PROPERTIES	VALUES
1	Net charge	0.00e
2	Surface area (approx)	881.27A^{02}
3	Surface area (grid)	538.41A^{02}
4	Volume	836.64 A^{03}
5	Hydration energy	-1.34Kcal/mol
6	Polarizability	20.45 A^{03}
7	Refractivity	66.83 A^{03}
8	Mass	224.17amu
9	Log-p	3.42

DISCUSSION

Gene information in Fasta format (of nucleotide and protein) are collected from online tool known as NCBI (National Centre for Biotechnology Information), which are submitted in offline tool named Bioedit for determining nucleotide and protein composition, primary structure prediction, secondary structure prediction and topology results are known from online tool known as ExPASY by the help of submitted data from Fasta format of protein. After prediction of the protein the homology modeling was carried out in SPDBV which is an offline tool.

In homology modeling the active site analysis was carried out in online tool known as Q-site finder in which the active sites of the protein are identified. Out of ten active sites the active site containing amino acids, are used for docking, in Argus lab which is an offline tool. By observing the dock scores, the lesser energy consumed molecule has been taken as the final molecule, for which the surfaces HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) & ESP mapped density surfaces are identified by using Argus lab. QSAR properties for the final molecule were identified by using Hyperchem tool.

CONCLUSION

Docking scores of similar molecules for selected drugs show that HEXADECANOATE exhibit characteristic dock score compare to other similar molecules. Dock score for Hexadecanoate ranges from -10.5065 to -5.8243. The dock score -10.5065 of Hexadecanoate is exhibited in case of GLU 321 amino acid with 52 seconds of elapsed time for calculation. -5.8243 dock score for Hexadecanoate is exhibited in case of THR29 with 45 sec as elapsed time for evaluation. Hexadecanoate exhibits characteristics dock score like -9.97404(in case of VAL25,elapsed time for calculation as 1min, 16 sec), -10.2459(incase of PHE 291, elapsed time for calculation as 56 seconds), and -10.2907 (in case of GLN320, elapsed time for calculation as 1minute,1second). This indicates Hexadecanoate is interacting at the lowest energy level with all the amino acids in the potential site. The values of QSAR properties like Net charges (0.00e), Hydration energy (-1.34 kcal/mol), Log P (3.42) also play a role in indicating the importance of Hexadecanoate, in treating the disease.

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