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IN – SILICO ANTI – CANCER DRUG DESIGNING FOR NF-kB AND ESTROGEN RECEPTOR INHIBITION

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ABSTRACT

Recent evidence suggests that activation of NF-kB contributes to the development of several types of human cancer. Our presented study used molecular dynamics to study the protein-ligand docking process. Anti- cancerous synthetic drugs, inhibiting NF-kB, present in the market (namely- 5-Aminosalicylic acid, Diethylmaleate, NF-kB Activation Inhibitor 3, Parthenolide, Rocaglamide, Rocaglamide AL, 2-(1, 8-napthyridin-2-yl)-Phenol) and natural drug Curcumin were docked in NF-kB active site (PDB entry- 1OOA) using BioMed CaChe. Curcumin showed higher activity and potency after molecular docking, so, was selected for further studies. Reported studies show that Estrogen receptors are over-expressed in around 70% of breast cancer cases, referred to as "ER-positive". Investigation to fit Curcumin and its analogues (present in PubChem compounds) in the binding pocket of Estrogen Receptor (PDB entry- 3ERT) was included in simulated docking experiments. In pharmacophore studies, active site residues for estrogen receptor are identified as MET343, LEU346, THR347, LEU349, ALA350, ASP351, GLU353, TRP383, LEU384, LEU387, MET388, LEU391, ARG394, PHE404, GLU419, GLY420, MET421, ILE424, LEU428, GLY521, HIS524 and LEU525. On energy minimization evaluation, results show that Curcumin, Curcumin2, Curcumin3, Turmeric Yellow,

Curcumin Bis-acetate provided approximately close values. The prediction of the suited molecule as cancer biomarker inhibitors can help us to build the structural analogues by using Three- Dimensional structural Activity Relationship (TSAR). Also studies illustrate Curcumin as GSK-3ß inhibitor involved in cancer pathway.

Keywords: Inhibition of NF-kB, Curcumin, Molecular Docking, Cancer, Estrogen Receptor, Breast Cancer, Curcumin Analogue

INTRODUCTION

Bioinformatics - Bioinformatics is conceptualizing biology in terms of molecules (in the sense of physical- chemistry) and then applying "informatics" techniques (derived from disciplines such as applied math, computer science and statistics) to understand and organize the information associated with these molecules, on a large scale **[1]**. Bioinformatics is associated with many medical applications like, in pharmacogenomics to personalize drugs for better bioavailability, to design new and better drugs, to develop better drug delivery system **[2]**. Bioinformatics is also essential for enhancing the discovery of new drugs **[3]**. By studying the interrelationships of protein expression and modification in health and disease, or drug treatment, proteomics can be applied to biomarker discovery and drug target validation **[4]**.

Cancer – An uncontrolled growth of normal cells known as Cancer, is of many types and can develop in almost any organ or tissue, such as lung, colon, breast, skin, bones, or nerve tissue. Cancer biomarkers can be used for prognosis: to predict the natural course of a tumor, indicating whether the outcome for the patient is likely to be good or poor (prognosis). They can also help doctors to decide which patients are likely to respond to a given drug (prediction) and at what dose it might be most effective (pharmacodynamics). Cancer biomarkers are present in tumor tissues or serum and encompass a wide variety of molecules, including DNA, mRNA, transcription factors, cell surface receptors, and secreted proteins **[5]**. These biomarkers are a parameter that can be used to measure the progress of disease or the effects of treatment.

NF-kB in Cancer – A constitutive NF-kB has been detected in most tumor cell types including esophageal cancer , laryngeal cancer, pharyngeal cancer, renal cancer, colon cancer, head and neck squamous carcinoma, lung cancer, bladder cancer, acute myelogenous leukemia, non-Hodgkin's lymphoma, B-cell lymphoma, adult T-cell leukemia, T-cell lymphoma, mantle cell lymphoma, multiple myeloma, acute lymphoblastic leukemia, cervical cancer, nasopharyngeal carcinoma, melanoma, thyroid cancer, liver cancer, breast cancer, ovarian cancer, and prostate cancer **[6,7]**.

Role of Estrogen Receptor- Breast cancer, the most common malignancy in women, was already known to be associated with the steroid hormone estrogen more than a century ago. The discovery of the estrogen receptor (ER) provided us not only with a powerful predictive and prognostic marker, but also an efficient target for the treatment of hormone-dependent breast cancer with antiestrogens **[8]**. The main function of the estrogen receptor is as a DNA-binding transcription factor that regulates gene expression. However, the estrogen receptor has additional functions independent of DNA binding **[9]**. Estrogen receptors are over-expressed in around 70% of breast cancer cases, referred to as "ER-positive". Two hypotheses have been proposed to explain why this causes tumorigenesis, and the available evidence suggests that both mechanisms contribute: First, binding of estrogen to the ER stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication, leading to mutations. Second, estrogen metabolism produces genotoxic waste. The result of both processes is disruption of cell cycle, apoptosis and DNA repair, and, therefore, tumor formation **[10]**.

Anti- Cancerous Drug: Curcumin – Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are natural phenols and are responsible for the yellow color of turmeric. Curcumin can exist in several tautomeric forms, including a 1,3-diketo form and two equivalent enol forms. The enol form is more energetically stable in the solid phase and in solution **[11]**. Its potential anticancer effects stem from its ability to induce apoptosis in cancer cells without cytotoxic effects on healthy cells. Curcumin can interfere with the activity of the transcription factor NF-κB, which has been

linked to a number of inflammatory diseases such as cancer **[12]**.

MATERIALS AND METHODS

3-D Structures Screening of Considered Biomarkers: Protein Data Bank (PDB) archive atomic coordinates structures of nearly all macromolecules. The PDB contains the coordinates of nearly 30,000 macromolecules structures (proteins, nucleic acids and carbohydrates as determined by X-ray and other diffraction – based techniques, NMR and other theoretical modeling) and is growing exponentially **[13]**. Nuclear Factor kappa B, NF-kB, transcriptional factor (PDB id -1OOA) and breast cancer specific biomarker, Estrogen Receptor (PDB id-3ERT), were considered. Resolution factor is 2.45 A^o and 1.90 A^o respectively with X- ray diffraction as the method of incorporation.

3-D Structure Screening of Drug Molecules: PubChem is free database of chemical structures of small organic molecules and information on their biological activities provided the structures of anti-cancerous synthetic drug and the natural drug Curcumin **(Table 1)**.

Identification of Active Site: The first step of molecular docking is the identification of active site of target protein. Binding of protein takes place at active site. Research data available for protein provided the information for the active site.

Protein – Ligand Docking: For molecular docking studies of NF-kB with their inhibitors available in market Biomed CaChe software was used. Fujitsu BioMed CaChe Software is used for optimizing leads by maximizing activity and improving the prediction of bioavailability. The novel compounds for testing and synthesis that are most likely to be successful are chosen. Protein – ligand docking process provided with the best fit ligand among them which was further used for the breast cancer specific biomarker (Estrogen Receptor). Curcumin showing high activity was selected and its analogues present in PubChem were also explored.

RESULTS AND DISCUSSIONS

Molecular Docking of NF-kB With Inhibitors: Drugs inhibiting the NF-kB activation during molecular docking gave the following results-

Table 1: Drugs Inhibiting NF-kB Available in the Market and Their Docking Score

NAME OF DRUG	DOCKING SCORE (Kcal/mol)
Curcumin	-172.96
Rocaglamide	-156.103
Diethylmalate	-127.752
Parthenolide	-122.074
NF-kB Activation Inhibitor 3	-115.726
Rocaglamide AL	-115.687
5-Aminosalicyclic acid	-92.049
2-(1,8-napthyridin-2-yl)-Phenol	-77.262

Molecular Docking Experiment

Docking of NF-kB Inhibitors can be seen below from **Figure 1-5.**

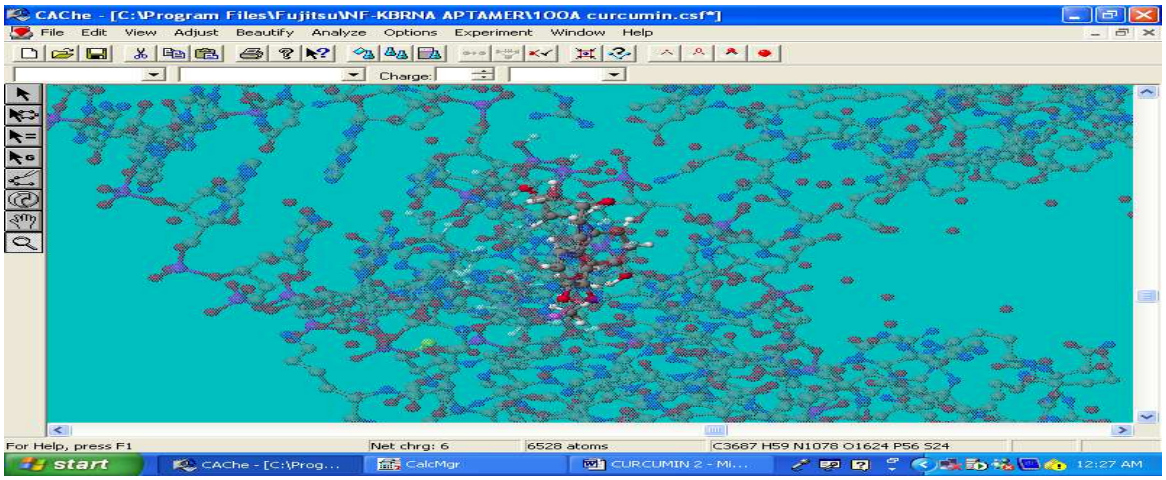

Figure 1: Docking of NF-kB with Curcumin

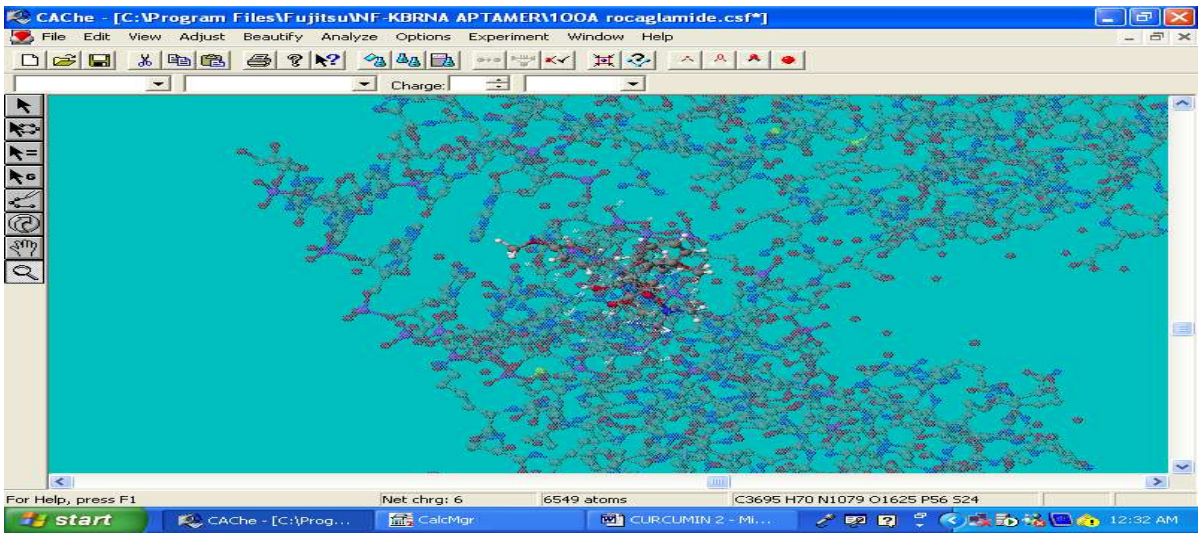

Figure 2: Docking of NF-kB with Rocaglamide

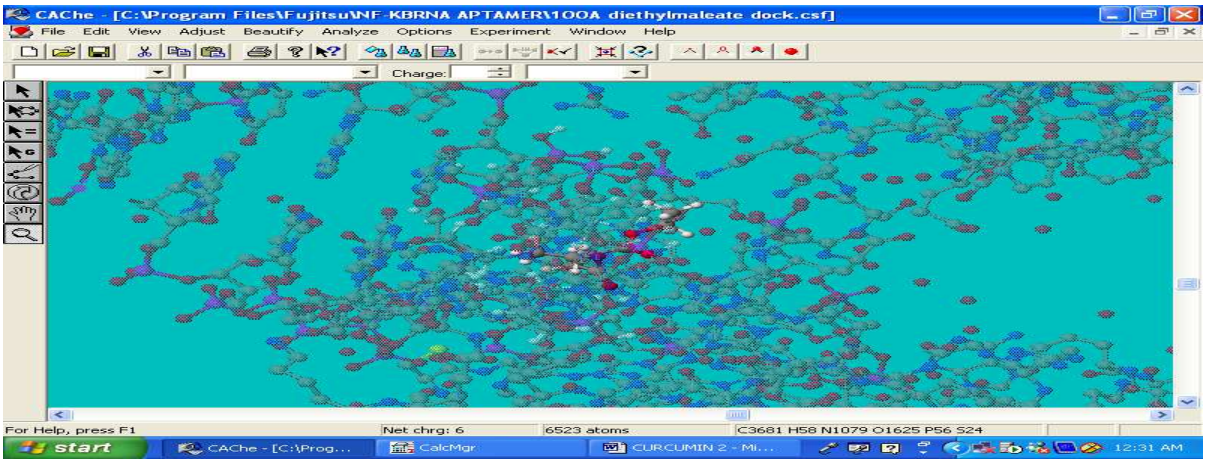

Figure 3: Docking of NF-kB with Diethylmalate

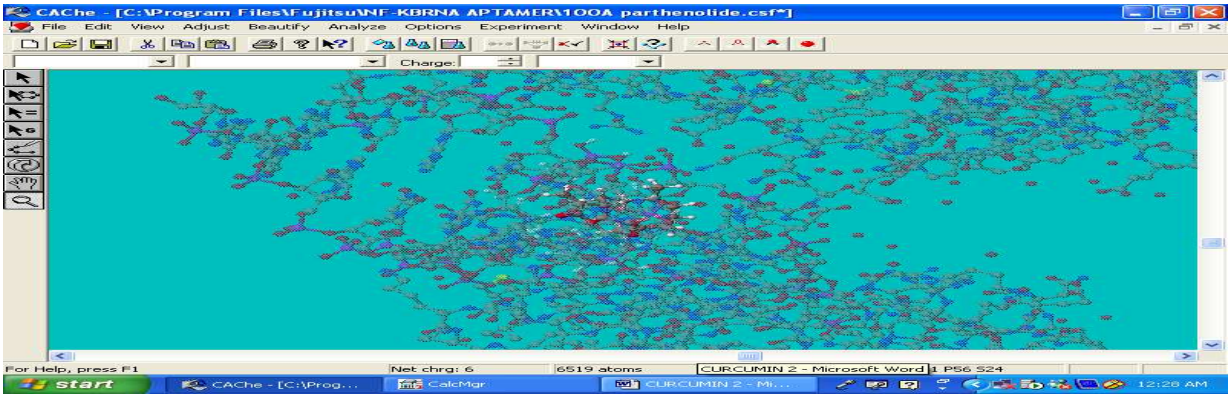

Figure 4: Docking of NF-kB with Parthenolide

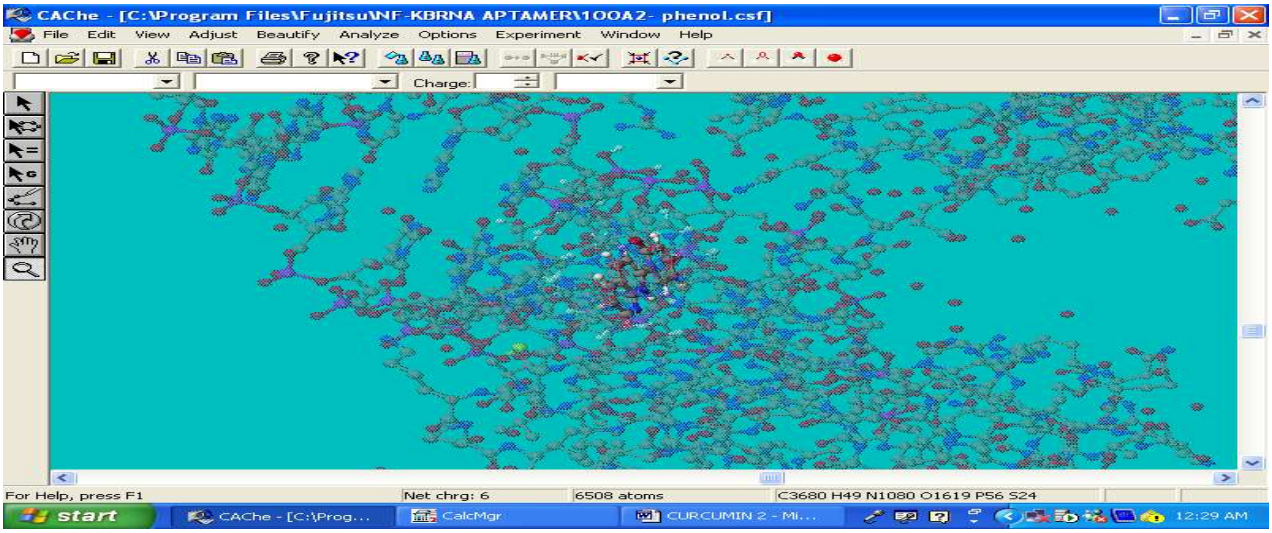

Figure 5: Docking of NF-kB with 2-(1,8-napthyridin-2-yl)-Phenol

Curcumin, Chosen as Best Ligand

According to the result table, curcumin showed the best minimization energy, so was selected for further studies.

Active Site Pharmacophore of Estrogen Receptor (Breast Cancer Specific Biomarker) In pharmacophore studies, by use of BioMed CaChe, active site residues for estrogen receptor were identified as MET343, LEU346, THR347, LEU349, ALA350, ASP351, GLU353, TRP383, LEU384, LEU387, MET388, LEU391, ARG394, PHE404, GLU419, GLY420, MET421, ILE424, LEU428, GLY521, HIS524 and LEU525 **(Figure 6)**.

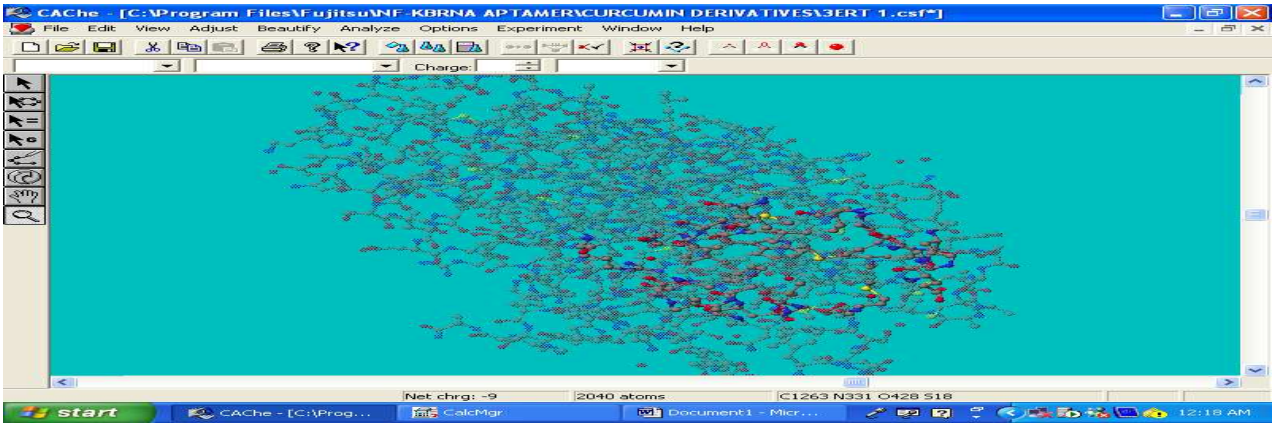

Figure 6: Active site of Breast Specific Biomarker, Estrogen Receptor

Docking With Curcumin Analogue PubChem database provided with the nineteen curcumin analogues. After docking these analogues with estrogen receptor best ligands were chosen.

Table 2: Docking scores obtained for Curcumin and its analogues with Estrogen Receptor

NAME	DOCKING SCORE (Kcal/mol)
Curcumin	**-431.797**
Demethoxycurcumin, Curcumin 2	**-373.795**
Bisdemethoxycurcumin, Curcumin 3	**-373.656**
Curcumin bis-acetate	**-431.587**
Curcuma longa L., NCIMech_000700	**-402.257**

Protein – Ligand Experiment Taking Estrogen Receptor as Biomarker

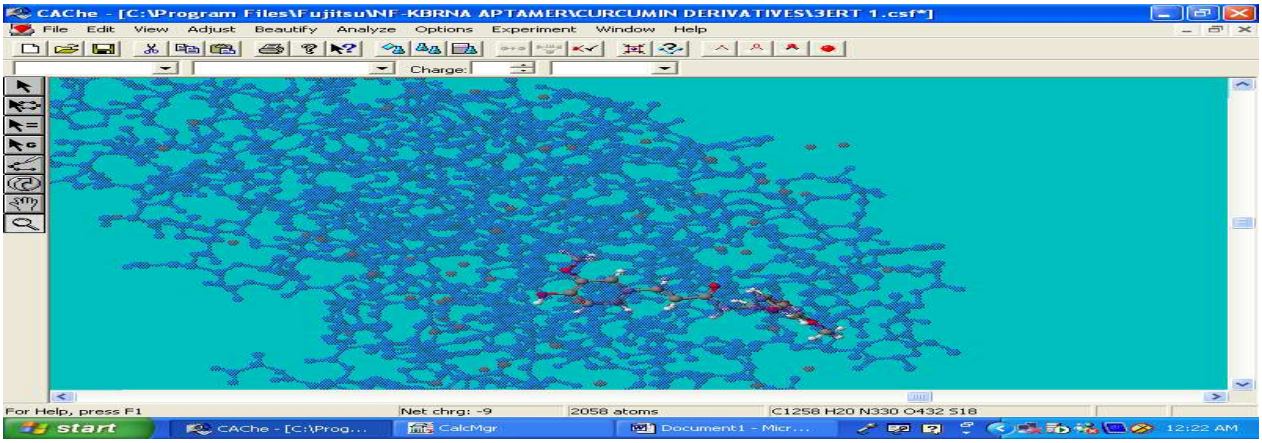

Figure 7: Docking of Estrogen Receptor with Curcumin

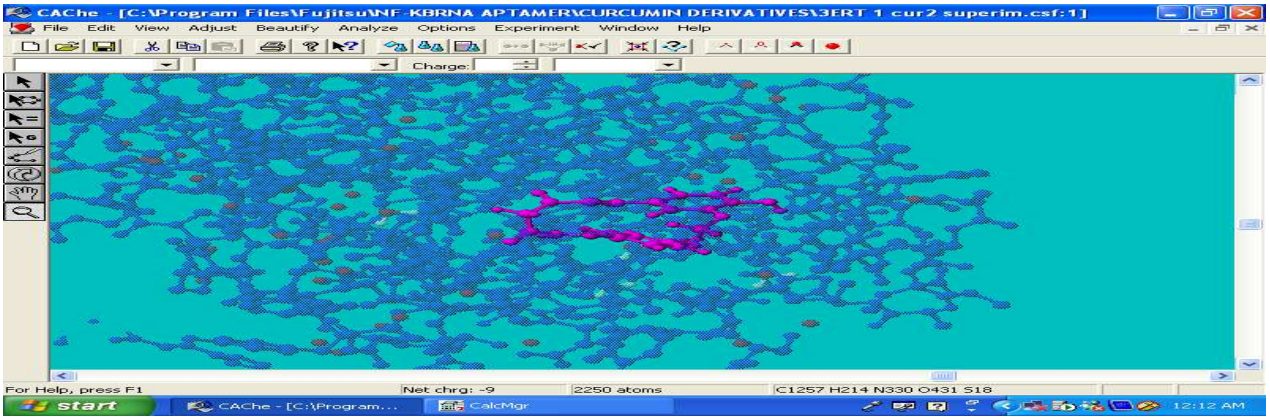

Figure 8: Docking of Estrogen Receptor With Curcumin 2

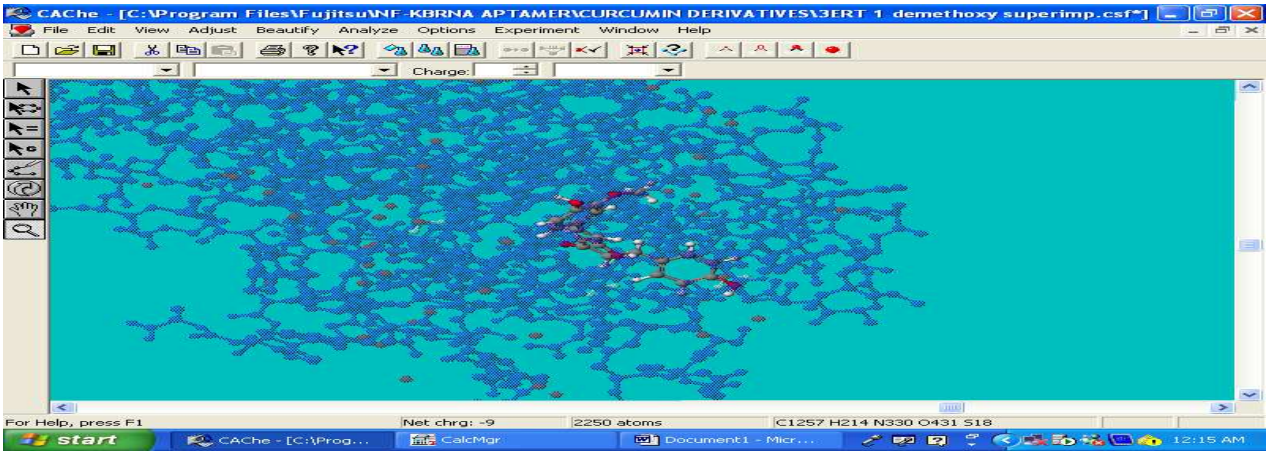

Figure 9: Docking of Estrogen Receptor With Demethoxycurcumin, Curcumin 3

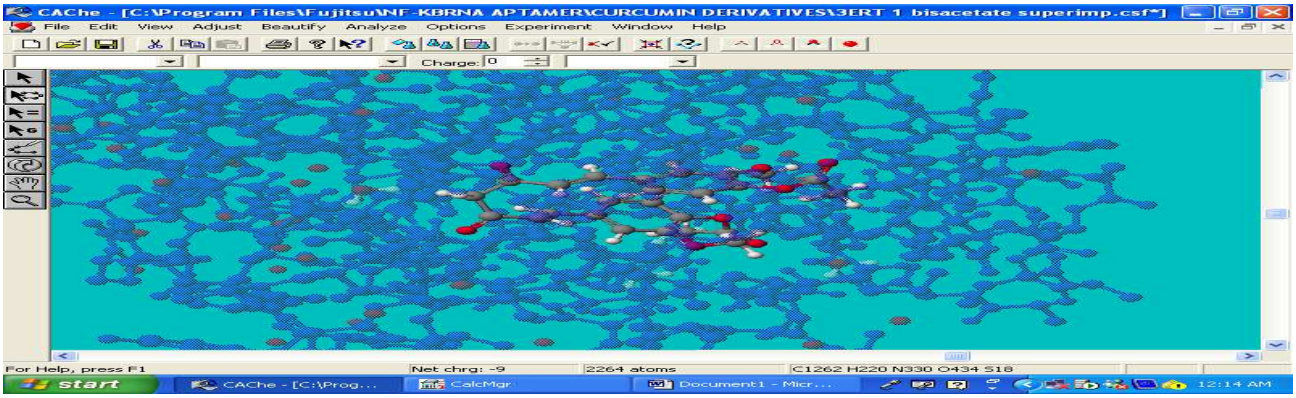

Figure 10: Docking of Estrogen Receptor With Curcumin Bis Acetate

Discussion

Activation of NF-kB blocks apoptosis and mediate tumor cell proliferation. Tumor cells frequently express constitutively activated form of NF-kB. Its activation induces resistance to chemotherapeutic agents. Several genes involved in tumor initiation, promotion and metastasis are regulated by NF-kB **[14]**. Several dietary agents like curcumin, resveratrol, lycopene etc. are natural chemopreventive agents that have been found to be potent inhibitors of NF-kB **[15]**. Activation of transcription factor NF-kB is suppressed by curcumin **[16]**. Curcumin inhibits the growth of multiple breast cancer cell lines, particularly those that result from exposure to environmental estrogens such as chemicals and pesticides. Also, curcumin,

estrogen, and estrogen mimickers gain entry into the cell through the aryl hydrocarbon receptor. Because curcumin competes for entry, it can crowd out damaging materials. According to researchers, curcumin blends well with other cancer inhibitors. For example, a curcumin-isoflavonoid combination suppressed the growth of estrogen receptor-positive cancer cells up to 95%.Curcumin Suppresses Metastasis in a Human Breast CancerXenograft Model:Association With Suppression of NF-κB, COX-2 and MMP-9 **[17]**. The results of present molecular modeling studies reveal that the NF-kB and Estrogen Receptor's interaction with best fit ligand, i.e. Curcumin, are similar to the results provided in the literature.

CONCLUSION

The investigation of protein – ligand docking experiment provided us with the best ligand to be chosen from the observed results. Nuclear factor kappa B (NF-kB) and breast cancer specific biomarker (Estrogen Receptor) were our target proteins. Potential inhibitors were collected from literature and their structures from PubChem. Molecular docking results were analyzed and Curcumin (IUPAC name - (1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6-heptadiene-3,5-dione), as showed the high activity was chosen for further analysis. Docking experiments were performed on the BioMed CaChe for NF-kB and Estrogen receptor and showed the ligand fits in the groove of proteins. Curcumin was found to be more potent among all inhibitors. Further studies can be carried to validate the results by constructing structural analogue using Three- Dimensional Structural Activity Relationship (TSAR).

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