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Analysis of Binding Properties of Phosphoinositide 3-kinase Through *In silico* Molecular Docking

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Abstract

Phosphoinositide 3-kinases (PI3-kinases) are increasingly considered to have a key role in intracellular signal transduction in health and disease. Particularly the enzymes plays vital role in wide range of cancer such as breast, ovarian, myeloid leukemia, prostate, Small Cell Lung cancer (SCLC) etc., Compounds such as Wortmannin, LYS2002 are the inhibitors of PI3-kinases but these compounds shown adverse side effects . Hence five natural flavanoids having inhibitory effects on PI3-kinase namely Andrographolide, Kaempferol, Luteolin, Quercetin and Gingerol were taken for *in silico* prediction of binding affinities of the protein PI3- kinase. Our reports can be used to develop new inhibitors with better binding affinities towards the protein PI3-kinase protein. For the binding analysis the catalytic subunit of the protein PI-3 Kinase p110α was taken for the study as it considered being a potential target in cancer treatment.

Keywords: PI-3 Kinase Inhibitors; *In silico* Binding affinities; Molecular Docking

Introduction

Phosphoinositide 3-kinases (PI 3-kinases or PI3Ks) are a family of related enzymes that are capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). PTEN/PI3K/AKT constitutes an important pathway regulating the signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation and cell growth (Carnero A et al., 2008). Genomic mutations, alterations of the PI3K-AKT regulatory network, underlie such diseases as cancer, glucose intolerance (diabetes mellitus), schizophrenia, and/or autoimmune diseases (Noguchi et al., 2008). In particular the PI 3-kinases generate and convey signals that have an important role in cancer (Stein *2001)*. PI3-kinases are ubiquitously expressed, are activated by a high proportion of cell surface receptors, especially those linked to Tyrosine kinases, and influence a bewildering variety of cellular functions and events. The majority of the research on PI 3-kinases has focused on the Class I PI 3-kinases. Class I PI 3-kinases are composed of a catalytic subunit known as p110. Many literature studies has proven that PI 3-Kinases to be the most significant contributor to activation of cancer in human such as ovarian cancer (Bellacosa et al., 1995; Yuan et al., 2000; Shayesteh et al., 1999), breast cancers (Nakatani et al., 1999), myeloid leukaemia (Vanhaesebroeck et al., 1999), glioblastoma, prostatic, endometrial and endometroid ovarian cancer [Ali et al., 1999).Apart from these frequent and early involvement of the PI3-kinase pathway was observed in lung cancer specifically small cell lung cancer (SCLC)(Pierre *et al*., 2004; Moore et al., 1998). A number of compounds such as wotmannin (Powis et al., 1994), demethoxyviridin (Woscholski et al., 1994), LY294002 (a morpholino derivative of the broad-spectrum kinase inhibitor quercetin (Vlaho et al., 1994) that inhibit PI3-kinases have been identified. It is important to emphasize that wortmannin and, particularly, LY294002 display little selectivity within the PI3-kinase family. Both compounds lose specificity at high concentrations

and showed less potent for this group of enzymes. More over Inhibitors of PI 3-Kinase have unacceptable toxicity if administered continuously in protein trafficking and in DNA repair and cell cycle checkpoint control is likely to be undesirable. The potential toxicity of PI 3-kinase inhibitors can probably best be limited by compounds extracted from natural source. Flavonoids provide a large number of interesting natural compounds that are consumed daily and exhibit more or less potent and selective effects on some signaling enzymes as well as on the growth and proliferation of certain malignant cells in vitro (Laurence et al., 1999). *In silico* molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure-based drug design (Brooijmans et al., 2003).Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the receptor. Hence here we have taken In*silico* molecular docking to analyze the binding properties of the enzyme PI 3-kinase with the flavanoids.

Flavanoids Taken for Binding Analysis with PI3 Kinase

Natural flavanoids such as Andrographolide from *Andrographis paniculata,* Gingerol from *Zingiber officinale* , Kaempferol from tea, broccoli, *Delphinium*, Witch-hazel, grapefruit etc. , Luteolin from *Chromolaena odorata* and Quercetin from *Allium cepa* were taken. All these compounds were shown to exhibit anticarcinogenic, anti diabetic and antimicrobial effects and their references were shown in Table I. For all the four compounds namely andrographolide, kaempferol, luteolin and quercetin except gingerol literature proof has been available to shown inhibitory effects towards PI 3-kinase except for Glycerol Hence the compounds were taken for the study of binding affinities towards the protein PI 3-kinase, despite their lack of strict specificity, the study provided valuable bases for the prediction of natural compounds could be specific inhibitors of PI 3-kinase. Through this we could predict that these compounds exhibit diverse effects by inhibiting PI 3-kinases

Materials and Methods

Bioinformatics online databases such as pubmed, PDB and Pubchem, were used. PubMed database developed by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM) is designed to provide access to citations from biomedical journals. From PubMed we have collected literatures on PI 3-kinases,and flavanoids.

Understanding the interactions between proteins and ligands is crucial for the pharmaceutical and functional food industries. The experimental structures of these protein/ligand complexes are usually obtained, under highly expert control, by time-consuming techniques such as X-ray crystallography or NMR. These techniques are therefore not suitable for routinely screening the possible interaction between one receptor and thousands of ligands. To overcome this limitation, computational algorithms (i.e. docking algorithms)

S.No	Compound	Biological Effects
1	Andrographolide	PI3 kinase (Yu BC et al., 2003). Anti Diabetic(Tsai HR et al., 2004) Antioxidant(Lin FL et al., 2009) Anticancer(Rajagopal S et al., 2003) Antimicrobial(Chang RS et al., 1991)
2	Kaempferol	PI3 Kinase (Labbé D et al., 2009) Antimicrobial(Tereschuk ML et al.,2004) Anticancer(Jeong JC et al., 2009) Antidiabetic(Fang XK et al., 2008) Antioxidant(Verma AR et al., 2009)
3	Luteolin	PI3 Kinase (Zhong Yao Cai, 2006) Antimicrobial, Anticancer, Antidiabetic, Antioxidant (López-Lázaro M, 2009)
4	Gingerol	Antimicrobial(Park M et al., 2008) Anticancer(Lee SH et al., 2008) Antidiabetes(Sekiya K et al., 2004) Antioxidant(Masuda Y et al., 2004)
5	Quercetin	PI3 Kinase (Labbé D et al., 2009) Antimicrobial(Tereschuk ML et al.,2004) Antidiabetic(Fang XK et al., 2008) Antioxidant(Verma AR et al., 2009 Anticancer (Kim EJ et al., 2008)

Table I: Inhibitors taken for the study and their Multiple Biological Effects.

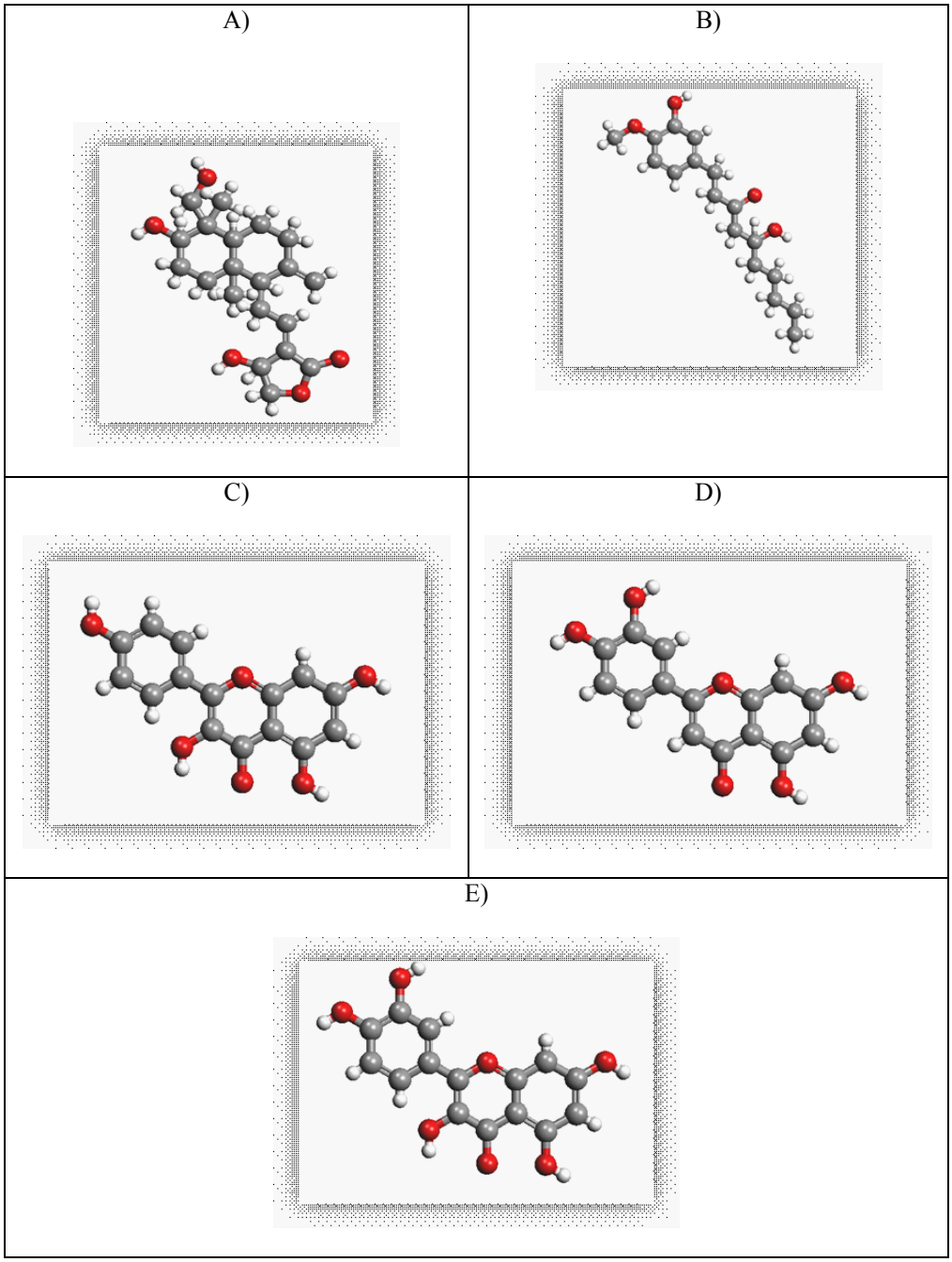

Figure 3: Molecular structures of the Flavanoids
Two dimensional structures of A)Andrographolide, B) Gingerol, C) Kaempferol, D) Luteolin E) Quercetin (retrieved from NCBI-Pubchem Compound Database).

have been developed that uses the individual structures of the receptor and ligand to predict the structure of their complex.

Docking

A number of powerful software programs, e.g. AutoDock, HEX, GOLD, FlexX, DOCK, Glide, Surflex, LigandFit, have been developed over the past several decades to carry out docking calculations, and good success in both binding mode and binding affinity prediction has often been achieved in selected test cases. We used a new shape-based method, LigandFit, for accurately docking ligands into protein active sites. The method employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions. A shape comparison filter is combined with a Monte Carlo conformational search for generating ligand poses consistent with the active site shape. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein-ligand interaction energies. The method appears quite promising, reproducing the X-ray structure ligand pose within an RMSD of 2A. A high-throughput screening study applied to the thymidine

kinase receptor is also presented in which LigandFit, when combined with LigScore, an internally developed scoring function, yields very good hit rates for a ligand pool seeded with known actives (Venkatachalam et al., 2003). Thus docking analysis of Gingerol, kaempferol, luteolin, andrographolide and Quercetin with PI3 Kinase was carried out by Ligand Fit of Discovery studio (Version 1.7, Accelry's Software Inc.). The software allows us to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores the ways in which these five molecules and the enzyme PI3 Kinase fit together and dock to each other well, like pieces of a three-dimensional jigsaw puzzle. The collection of Gingerol, kaempferol, luteolin, andrographolide and Quercetin and PI3 Kinase complexes was identified via docking and their relative stabilities were evaluated using their binding affinities.

Docking Protocol

Ligand Preparation

The three dimensional structures of anticancer compounds like Gingerol, kaempferol, luteolin, andrographolide and Quercetin were downloaded in .sdf format from Pubchem database. Hydrogen Bonds were added and the energy was minimized using CHARMm force field. Molecular weight, log *P* and number of Hydrogen-bond donors and acceptors for the active principles were noted (shown in Table III). All the five molecules were satisfied Lipinski's drug properties and their two dimensional structures were shown in Figure 3.

Protein Selection

Sequences of Phosphoinositide 3-kinases catalytic subunit alpha isoform were retrieved from swissprot for various species in FASTA Format for multiple sequence alignment and for phylogenetic analysis using ClustalW. Phylogenetic analysis revealed that *Mus musculus* and Bovine were closely related to Human (Shown in Fig 1), but the three dimensional structures were available only for Human and *Sus scrofa*. Hence their structures were retrieved and compared for further analysis.

There are several PDB structures available for the same protein and they are listed in table II along with their resolution and length. The PDB structure which was chosen for our study has a good resolution of 2.00 when compared to other structures. To predict the binding mechanism accurately, PDB structure (PDB ID: 1E7U) of *Sus scrofa* PI3 Kinase was chosen for the interaction analysis which is of 961 aminoacids. The PDB structure was also compared using the DALI server to find the structural alignment using the RMSD value as shown in Fig 2. As the RMSD score for the three dimensional structures of human PI3 kinase and Sus scrofawere below 2.00A^0, the structure from *Sus scrofa* could be taken for further analysis.

Protein Preparation

The ligands and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMm force field.

Docking Studies

The active site of the protein was first identified and it is defined as the binding site resulted in a cavity size of 3475 point units. There is evidence that wortmannin alkylates a lysine residue at the putative ATP binding site of p110α (Wymann et al.,1996). LY294002, in contrast, is a pure competitive inhibitor of ATP. The X-ray structure of wortmannin, LY294002 and several broad-spectrum kinase inhibitors, including quercetin in complex with p110, confirms the mechanism of inhibition and offers a basis for designing more specific compounds (Walker et al.,2000). Thus Binding sites were defined based on the ligands already present in the PDB file (i.e. ATP binding site region) which were followed

S.No	Molecules	Molecular weight (<=500)g/mol	XLog P (<=5)	H-Donor	H-acceptor
1	Andrographolide	350.4492 [g/mol]	2.9	3	5
2	Gingerol	294.38594 [g/mol]	3	2	4
3	Kaempferol	286.2363 [g/mol]	1.9	4	6
4	Luteolin	286.2363 [g/mol]	0.7	4	6
5	Quercetin	302.2357 [g/mol]	1.1	5	7

Table III: Lipinski properties of the five flavanoids (Values obtained from Pubchem)

For each molecule, many orientations and conformations are sampled; based on these configurations, each molecule is scored for complementarity to the receptor and ranked relative to the other members of the database.

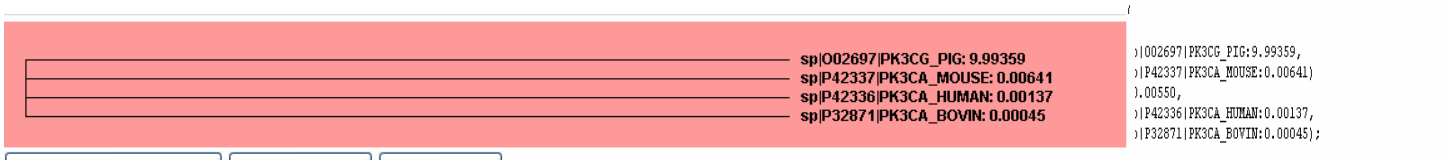

Figure 1: Phylogentic analysis of Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform PI3 kinase sequences.

S.No	PDB ID	Resolution	Length	Species
1	1E90	2.70	961	*Sus scrofa*
2	3CST	3.20	966	Homo sapiens
3	1E8Z	2.40	966	Homo sapiens
4	1E7V	2.40	961	*Sus scrofa*
5	1E8W	2.50	961	*Sus scrofa*
6	2CHX	2.50	966	Homo sapiens
7	3CSF	2.80	966	Homo sapiens
8	3DBS	2.80	960	Homo sapiens
9	3ENE	2.40	959	Homo sapiens
10	2CHZ	2.60	966	Homo sapiens
11	2CHW	2.60	966	Homo sapiens
12	2A5U	2.70	966	Homo sapiens
13	1E8X	2.20	961	*Sus scrofa*
14	3DPD	2.85	966	Homo sapiens
15	2V4L	2.50	966	Homo sapiens
16	2A4Z	2.90	966	Homo sapiens
17	1HE8	3.00	965	Homo sapiens
18	1E7U	2.00	961	*Sus scrofa*

Table II: Summary of three dimensional structures available for PI3-kinase in ProteinDataBank.

by site sphere definition. Here site 1 was chosen as the binding site and the site sphere size was set to(434.375 A° ^ 3, Partition level 1).The determination of the ligand binding affinity was calculated using LigScore and PLP1,JAIN and Dock score were used to estimate the ligand-binding energies. Apart from these, other input parameters for docking were set as default options.

Results and Discussion

Molecular Docking continues to holds great promise in the field of Computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions (Irwin et al., 2002). Number of reports citing successful application of CADD in developing specific drugs in different therapeutic areas is expanding rapidly. A very interesting example which can also serve as a proof of principle of the *in silico* approach involves a type I TGF β receptor kinase inhibitor. The same molecule (HTS-466284/LY-364947), a 27 nM inhibitor, was discovered independently using virtual screening by Biogen IDEC (Singh et al., 2003) and traditional enzyme and cell-based high-throughput screening (Sawyer JS et al., 2003). Another *in silico* modeling drug development program led to clinical trials of a novel, potent, and selective anti-anxiety, anti-depression 5-HT_{1A} agonist in less than 2 years from the start and requiring less than 6 months of lead optimization and synthesis of only 31 compounds (Becker et al., 2006). It is estimated that docking programs currently dock 70 – 80% of ligands correctly (Congreve et al., 2005).

Validation of Docking Results

To ensure that the ligand orientation obtained from the docking studies were likely to represent valid and reasonable binding modes of the inhibitors, the LigandFit program docking parameters had to be first validated for the crystal structure's Active site (PDBid 1E7U). Protein Utilities and Health protocol of Discovery's studio was used to find out the active sites in the structure and it was found that the active site contains amino acids such as ASP950 TYR867 MET804 GLU880 LYS808 VAL882 SER806 ILE831 ILE879 ASP964 LYS833 TRP812. Results of docking showed that LigandFit determined the optimal orientation of the docked inhibitor, exactly to these active sites.

The low RMS deviation of between the docked and crystal ligand coordinates indicate very good alignment of the experimental and calculated positions especially considering the resolution of the crystal structure (2.00Å) shown in table IX.

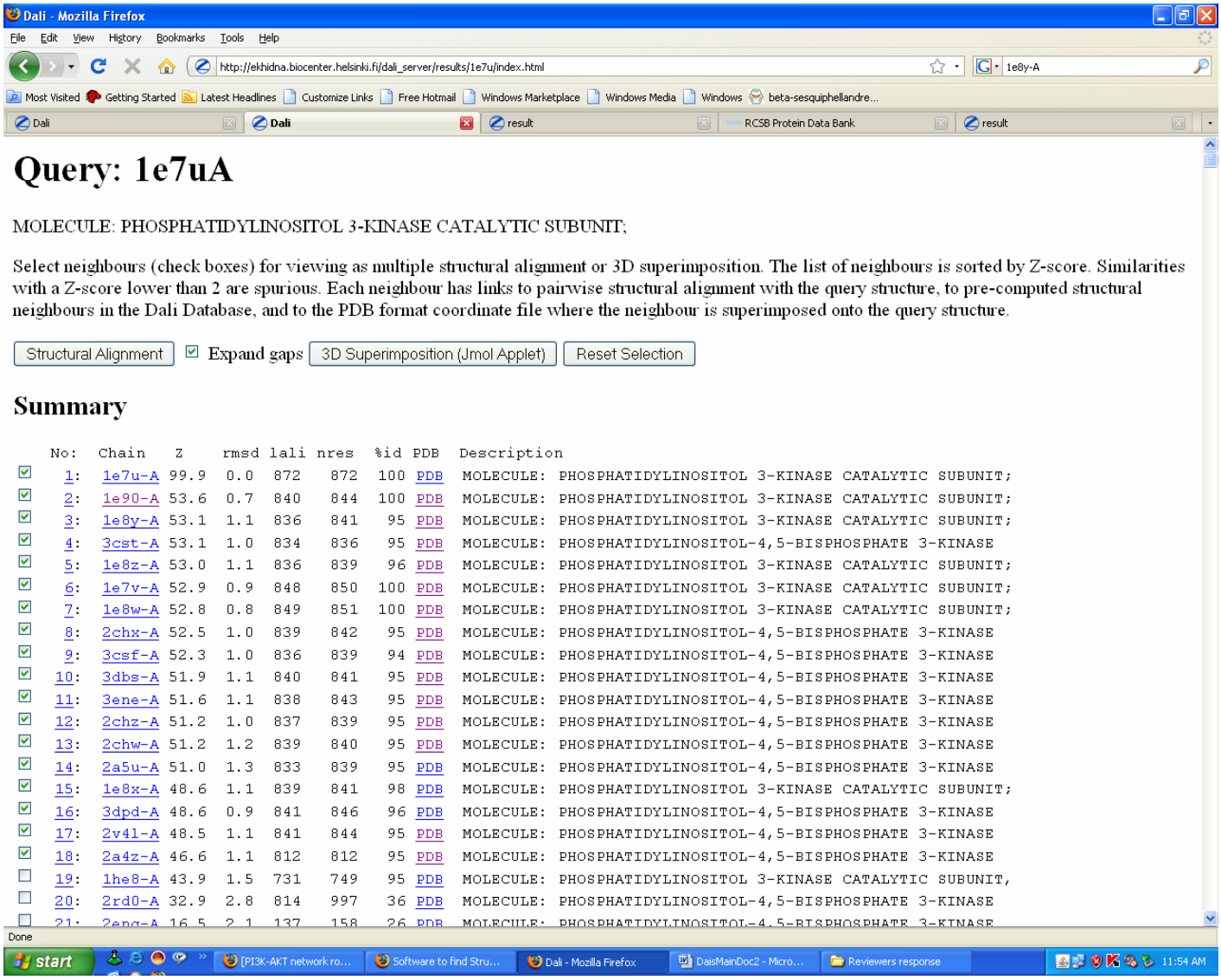

Figure 2: Structural alignment results using DALI.

Name	Ligscore1	Ligscore2	-PLP1	-PLP2	JAIN	-PMF	Dock Score
Andrographolide	4.58	4.33	42.84	47.06	2.4	111.9	62.735
Kaempferol	3.15	2.24	23.43	29.62	-0.89	66.36	65.058
Luteolin	4	3.7	30.73	37.11	1.23	56.66	69.14
Quercetin	3.76	3.81	35.11	42.99	0.74	57.5	71.407
Gingerol	3.64	4.07	41.25	44.27	-0.69	78.3	62.952

Table IX: Summary of docking information of the Top ranked poses of each flavanoids (values copied from the table browser window of Discovery studio2.1).

Here top ranked ligands were taken for binding affinity studies. The validation process consisted of two parts: (i) Hydrogen bond details of the top-ranked docked pose and (ii) prediction of Binding energy between the docked ligand and the enzyme using various score calculated using Discovery studio (DJD, 2 LigScore2, 3 LigScore1, 3 PLP, 45 PMF, 46 and JAIN47 scores were taken for the analysis.

Hydrogen Bond Details

A close view of the binding interactions of PI3 kinase with the flavanoids Andrographolide, Kaempferol, Quercetin, Luteolin and Gingerol were shown in Fig. 4. Ligand is coloured in Yellow (in balland-stick drawing) where as amino acids involved in Hydrogen bonding where shown in blue colour.

As shown in Fig. 4A, there are five hydrogen bonds (shown as green dotted lines) formed between the compound Andrographolide and PI3 Kinase. Those residues involved in forming hydrogen bonds with the enzyme were: (2 hydrogen bonds) Lys 802, Lys 807, Asp-950, Asp-964. Quercetin forms two hydrogen bonds (shown as green dot-

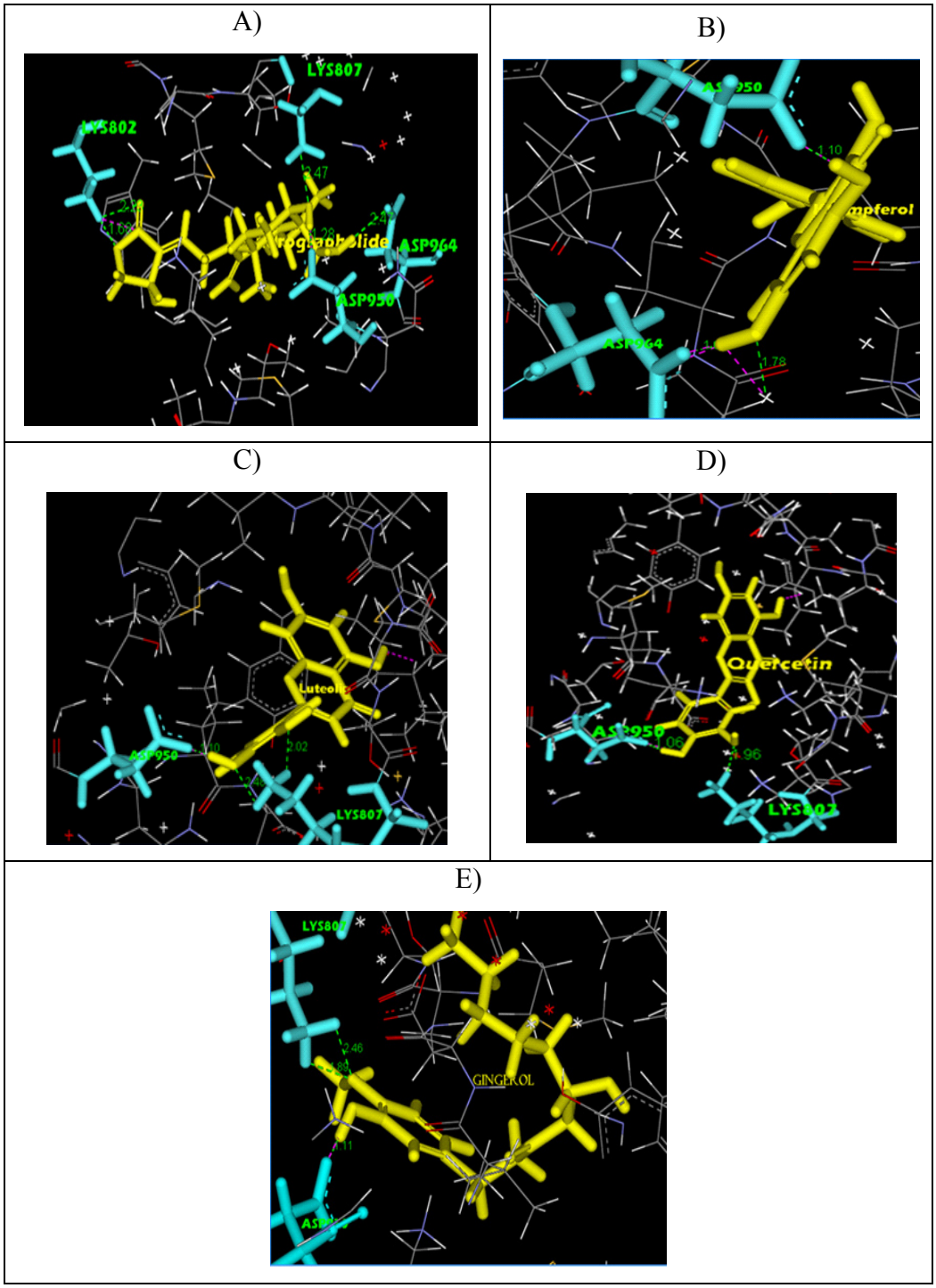

Figure 4: Summary of Docked Pose of the five anticancer compounds

Docking models of (A) Andrographolide (B) Kaempferol (C) Luteolin and (D) Quercetin and (E) Gingerol with PI3- kinase. The green dot lines denoted the hydrogen bonds. All the amino acid residues which involved in molecular interactions were shown in Blue color and the Ligands were shown in yellow color.

ted lines in Fig.4B) and the residues involved in forming the hydrogen bonds from the enzyme were: Lys-807 and ASP-950. Kaempferol forms two hydrogen bonds (shown as green dotted lines in Fig.4C) and here the residues involved in forming the hydrogen bonds from the enzyme were: Asp-950 and Asp 964.Table 4 showed the detailed information of the hydrogen bonds. Luteolin and Gingerol forms three hydrogen bonds (shown as green dotted lines in Fig.4D and Fig 4E) and the residues involved in forming the hydrogen bonds from the enzyme were: Lys-807 and Asp-950. The detailed atoms in forming the hydrogen bonds are given in Table IV, V,VI,VII and VIII for each flavanoids separately, which may provide useful information for in-depth understanding binding mechanism of the compound to the active site of the protein.

Docking Score and RMSD Values

As a result of docking there were 10 different conformations were generated for andrographolide, Quercetin, Kaempferol, luteolin and for gingerol. But only for top ranked docked complex the scores were copied from the table browser view of Discovery studio for binding affinity analysis. Table IX shown the different score values of top ranked ligands. The score values include Ligscore1&2 (Protein-Ligand Affinity Energy)(Krammer et al., 2005), PLP1, PLP2 (Steric and H-bonding intermolecular function, Higher PLP scores indicate stronger receptor-ligand binding (larger pK_i values)) (Gehlhaar et al., 1995,1999), JAIN(sum of five interaction terms namely Lipophilic interactions,Polar attractive interactions ,Polar repulsive interactions ,Solvation of the protein and ligand ,An entropy term for the ligand)(Jain 1996), PMF(developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex, A higher score indicates a stronger receptor-ligand binding affinity) (Muegge 2006; Muegge et al., 1999) and Dockscore(Candidate ligand poses are evaluated and prioritized according to the DockScore function) . The determination of the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein. The two scoring methodologies namely LigScore and PLP1 were used to estimate the ligand-binding energies. Larger score value indicates better ligand-binding affinity.

Conclusion

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the enzyme PI3 Kinase and the drugs to explore the binding mechanism of flavanoids to the PI3 kinase enzyme. They are Andrographolide, Gingerol, Kaempferol, luteolin and Quercetin. When the enzyme docked to the five anticancer compounds the scores obtained were shown in Table.:

Andrographolide (Dock score = 62.735), Quercetin (Dock score= 71.407), Kaempferol (Dock score= 65.058), Luteolin (Dock score= 69.14) and gingerol (Dock score=62. 952). Based on all the Dock score values it was predicted that

Andrographolide:

Amino acid	Atom in amino acid	Position	Atom in Ligand	Hydrogen Bond length(A^0)
LYS	HZ1	802	O5	2.228000
LYS	HZ1	802	O3	1.675000
LYS	HZ3	807	O1	2.474000
ASP	OD2	964	H51	2.474000
ASP	OD2	950	H47	1.278000

Table IV: Hydrogen Bond interactions between the enzyme PI3 Kinase and the ligand Andrographolide (Results were analysed using Hbond Monitor of Discovery studio.2.1). Second and third column represents the atoms of amino acid and ligand involved in hydrogen bond formation. Position represents the position of aminoacid in the enzyme.

Kaempferol

Amino acid	Atom in amino acid	Position	Atom in Ligand	Hydrogen Bond length(A^0)
ASP	OD2	950	H29	1.099000
ASP	OD2	964	H30	1.042000

Table V: Hydrogen Bond interactions between the enzyme PI3 Kinase and the ligand Kaempferol (Results were analysed using Hbond Monitor of Discovery studio.2.1). Second and third column represents the atoms of amino acid and ligand involved in hydrogen bond formation. Position represents the position of aminoacid in the enzyme.

Luteolin

Amino acid	Atom in amino acid	Position	Atom in Ligand	Hydrogen Bond length(A^0)
LYS	HZ2	807	O6	2.484000
LYS	HZ3	807	O5	2.02000
ASP	OD2	950	H31	1.098000

Table VI: Hydrogen Bond interactions between the enzyme PI3 Kinase and the ligand Luteolin (Results were analysed using Hbond Monitor of Discovery studio.2.1). Second and third column represents the atoms of amino acid and ligand involved in hydrogen bond formation. Position represents the position of aminoacid in the enzyme.

Quercetin

Amino acid	Atom in amino acid	Position	Atom in Ligand	Hydrogen Bond length(A^0)
LYS	HZ3	807	O6	1.958000
ASP	OD2	950	H32	1.056000

Table VII: Hydrogen Bond interactions between the enzyme PI3 Kinase and the ligand Andrographolide (Results were analysed using Hbond Monitor of Discovery studio.2.1). Second and third column represents the atoms of amino acid and ligand involved in hydrogen bond formation. Position represents the position of aminoacid in the enzyme.

Gingerol

Amino acid	Atom in amino acid	Position	Atom in Ligand	Hydrogen Bond length(A^0)
LYS	O3	807	HZ3	2.459000
LYS	O3	807	HZ1	1.888000
ASP	OD2	950	H44	1.108000

Table VIII: Hydrogen Bond interactions between the enzyme PI3 Kinase and the ligand Andrographolide (Results were analysed using Hbond Monitor of Discovery studio.2.1).
Second and third column represents the atoms of amino acid and ligand involved in hydrogen bond formation. Position represents the position of aminoacid in the enzyme.

the ligands Quercetin and Luteolin were have similar and good binding affinities towards the protein. It was also predicted that the compound gingerol showed good binding affinities towards the protein when compared to others. For all the four compounds like kaempferol, Quercetin, luteolin and Andrographolide literature proofs were available to indicate that they inhibit PI3-Kinase but for gingerol there is no such a proof is available. Here through i*n silico* approach it was predicted that the compound gingerol also shown to inhibit PI3-Kinase as it had good Ligscore and PLP1 when compared to Quercetin and Luteolin. Hydrogen bond formation also makes important contributions to the interactions between ligand and the enzyme. Here a maximum of four hydrogen bonds were formed between the protein and the ligand Andrographolide followed by three hydrogen bonds were formed between the enzyme and the ligand Gingerol and luteolin. Thus the concept of protein-Ligand interaction helps in analyzing the binding properties of the protein PI3-Kinase with its inhibitors. The study report also concluded that the residues Lys 802, Lys-807, Asp-950, Asp 964 plays an important role in binding mechanism. Hence drugs such as Luteolin and Gingerol which were shown similar binding mechanism and good docking score to quercetin could be the lead one to target the PI3 Kinase. Our results provide insight into the structural requirement for the activity of the inhibitor and the most favorable binding mode of the top ranking compounds will be useful in designing new derivatives of Luteolin and gingerol as PI3 kinase inhibitors similar to the quercetin derivative of LYS2002.

Future Perspectives

Understanding the interactions between proteins and ligands is crucial for the pharmaceutical and functional food industries. The experimental structures of these protein/ligand complexes are usually obtained, under highly expert control, by time-consuming techniques such as X-ray crystallography or NMR. Molecular modeling and molecular docking methods still have a long way to run before producing completely reliable results. This could be achieved by NMR screening remains a multifaceted and unique technique that is sensitive to both structure and dynamics and that can monitor the binding of low molecular weight ligands to biological macromolecules in the early stages of drug discovery due to its ability to detect even very weak binders.

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