Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 9, 2015

Original Article

INSILICO ANALYSIS OF DIETARY AGENTS AS ANTICANCER INHIBITORS OF INSULIN LIKE GROWTH FACTOR 1 RECEPTOR (IGF1R)

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Received: 22 Jan 2015 Revised and Accepted: 15 Jul 2015

ABSTRACT

Objective: Insulin-like growth factor 1 receptor (IGF-1R) is over-expressed in a number of cancer cell lines and has been implicated to play a role in oncogenesis and suppression of apoptosis. Thus, the inhibition of IGF-1R activity leads to tumor regression and renders IGF-1R a plausible target for the development of anti-cancer drugs. Dietary agents are known to interfere with IGF signaling and offer a foundation for developing nontoxic agents that override any toxicity associated with synthetic IGF inhibitors. This study is designed to obtain structural motifs and active residues that preferentially interact with IGF1Rand to identify the phytochemicals from different plants and act as potential anticancer drug leads

Methods: Thus, six dietary agents known to interfere directly with circulating levels of IGF1R were undertaken for docking studies. A molecular docking simulation model of IGF1R with its bound ligand was established and validated to be used as a reference model for the study.

Results: The active site residuesGLU1080, MET 1082, GLU1081, GLU1027, GLU1145 and ARG1003 were found to play a significant role in binding mechanism. The ADME properties and drug likeliness of the ligands were rigorously analyzed under four criteria of known successful drug activity in the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, nuclear receptor ligand activity and enzyme inhibition. The dietary agents Apigenin and Luteolin demonstrated reliable interaction with IGF1R (-5.78Kcal/mol and-5.70 Kcal/mol respectively)and displayed good pharmacokinetics properties.

Conclusion: It is concluded that the explored dietary agents offer profound promise to be used natural inhibitors of IGF1R and thus may be useful for the preparation of different combinations and formulations for the management of tumors.

Keywords: IGF-1R Inhibitors, Dietary agents, Insilico modeling, Binding affinities, Molecular Docking.

INTRODUCTION

The insulin-like growth factor-1 receptor (IGF-1R) is a member of the receptor tyrosine kinase super family the ligand binding leads to receptor activation and phosphorylation of downstream substrates [1]. Signalling through IGFR-1 in normal cells leads to the activation of multiple intracellular pathways, mediated by the receptor-associated tyrosine kinase domain, PI-3 kinase, and by serine/threonine kinase (Akt), yielding growth and enhanced survival. In cancer cells, IGFR-1 plays an even more critical role because it contributes to the promotion of tumor growth by inhibition of the apoptosis, transformation, metastasis, and induction of angiogenesis through the vascular endothelial growth factor (VEGF) [2-4]. The IGF-1R is implicated in several cancers, including breast, prostate, and lung cancers [5].

A recent study reported thatIGF1R protein over expression may serve as an independent predictor of relapse and survival in laryngeal cancer [6]. As a drug target, the IGF system has a number of key features that lends itself to being appealing. The expressions of IGF-1R, the major signal trans ducing receptor of the pathway, appears to be necessary for malignant transformation in preclinical models [7]. Indeed, forced over expression of IGF-1R increases the timing and frequency of tumor development in animal models [8, 9]. Also, IGF-1 deficient mice have greatly reduced capacity to support tumor growth and metastasis [9, 10].

Thus targeting the IGF signalling pathway represents a promising strategy in the development of novel anti-cancer therapeutics. In general, many therapies like small molecule inhibition and targeted antibodies effectively block the IGF-1R and down regulate it's the expression [11]. Recently, Chowdhury and colleagues demonstrated the therapeutic potential of using a powerful IGF1R antagonist such as PQIP to inhibit colon cancer cell survival and trigger apoptosis [12]. Many studies emphasize that many adverse side effects like hyperglycemia and insulin resistance have been observed clinically with these IGF-1R targeted therapies [13]. In a competitive bid to explore new therapeutic agents, research has been focused to find out natural drugs that are cost effective, easily available and have lesser side effects.

Several phytochemicals. like flavonoids, carotenoids, polyphenols, flavonoids, iso flavonoids are natural chemo-preventive agents that have been found to be potent inhibitors of IGF1R pathway with anti-carcinogenic properties [16-29]. These compounds may block any one or more steps in the IGF1R signalling pathway. Therefore, in present study, we aimed to validate the above findings by using docking simulation studies and elucidate the feasible mechanistic aspects of above mentioned phytochemicals from different plants and obtain structural motifs that preferentially interact with IGF1R.

MATERIALS AND METHODS

Dietary agents taken for binding analysis with IGF-1R

Ligands of interest:-Apigenin (flavonoid),lycopene (carotenoid), curcumin(polyphenol), silibinin (flavonoid), genistein (isoflavonoid), and luteolin (flavonoid), that interfere directly with circulating levels of IGF-I and its receptor [16](table1) are searched on Pub Chem database (http://pubchem.ncbi.nlm.nih.gov) All these compounds were shown to exhibit anticarcinogenic, antidiabetic and antimicrobial effects.

Protein selection

Sequences of IGF1Recptor kinase were retrieved from Swiss Prot for various species in FASTA Format. Multiple sequence alignment was performed following phylogenetic analysis using Clustal W. Phylogenetic analysis revealed that *Mus musculus* and *Xenopus laevis* were closely related to Human (fig. 1), but the three dimensional structure was available only for Human.

Hence, their structures were retrieved and compared for further analysis. To predict the binding mechanism accurately, PDB structure (PDB ID: 2ZM3) of Homo *sapiens* IGF1R Kinase was chosen for the interaction analysis.

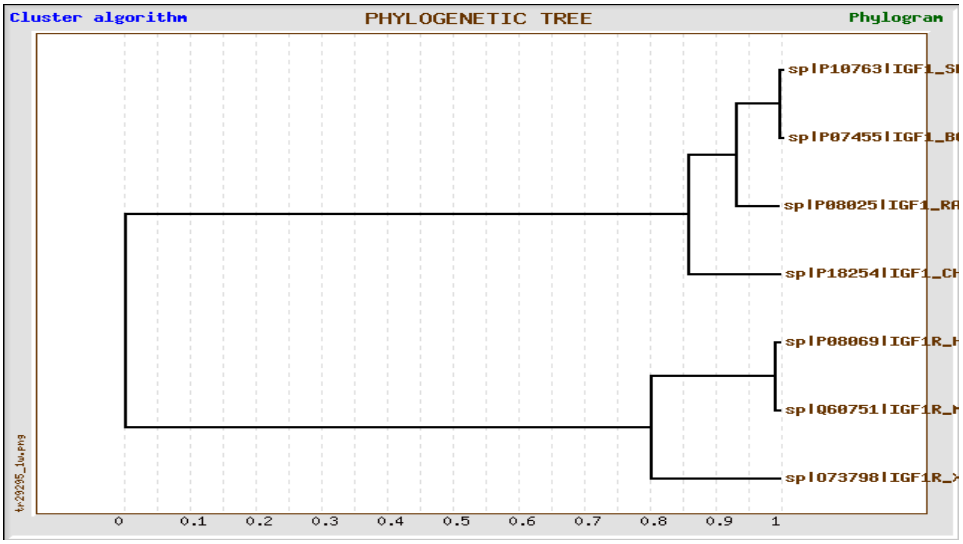

Fig. 1: Phylogentic analysis of insulin like growth factor 1 receptor-kinase sequences

Table 1: Chemo preventive inhibitors and their multiple biological effects

S. No.	Compound	Biological effect
1	Curcumin (polyphenol isolated from the rhizome of the plant *Curcuma longa)*	anti-inflammatory, anti-angiogenic, anti-oxidant, wound healing, anti-cancerous [17]
2	Genistein (isoflavonoid derived from soyabean *Glycine max)*	anti-cancer [18,19] anti-oxidant [20] Anti-angiogenic.
3	Apigenin (flavones derived from parsley, celery and chamomile tea)	anti-inflammatory effects, free radical scavenging properties, and anti-carcinogenic effects [21]
4	Lycopene,(carotenoid present in tomato)	Anti-cancer, Antioxidant [22], induction of cell-cell communication, growth control [23, 24].
5	Silibinin (flavonoid antioxidant foundin the milk thistle)	Anti-proliferative and apoptotic, Antioxidant and mitochondrial protective[25], Anti Photo-carcinogenic [26,27]
6	Luteolin(a bioactive flavonoid from Lonicera japonica)	anti-cancerous, anti-inflammatory, anti-allergic [28],anti-oxidant [29]

Computational tools

All computational studies were carried out using *Autodock 4.2* [30] installed in a single machine running on a 2.6 GHz Intel core2 duo processor with 1GB RAM and 300 GB hard disk with Windows XP as an operating system. The visualization tool, "Chimera" was obtained from portal http://www.cgl.ucsf.edu/chimera [31]. The "Autodock Tools", "Autogrid" and "Autodock-4.2" were downloaded from Scripps portal (http://autodock.scripps.edu). While the 3D protein model of IGF1 receptor complex *(*PDB 2ZM3) was downloaded from RSCB Protein Data Bank.

The Method involves thefollowing steps:

Ligand preparation

The three dimensional structures of six anticancer compounds Genistein, lycopene, curcumin, silibinin, apigenin and luteolin, were downloaded in. sdf format from PubChem database. Hydrogen Bonds were added and the energy was minimized using CHARMm force field and further, subjected to single step minimization using steepest descent method for 500 steps at RMS gradient of 0.01. Molecular weight, log *P* and number of Hydrogen-bond donors and acceptors for the active principles were noted (table 2). All the six molecules satisfied Lipinski's drug properties.

Protein preparation

The structure of human IGF1 receptor was retrieved from PDB (2ZM3). The substrate ligand-isoquinolinedione inhibitor (Bound ligand)) present in this protein was separated by Chimera. This was followed by removal of water molecules and correction of protein chemistry for missing hydrogen**.** The protein was subjected to two steps energy minimization to remove the bad steric clashes using steepest descent and conjugate gradient methods for 1000 steps at RMS gradient of 0.1 and 0.05 respectively. During the energy minimization process the backbone were fixing the backbone.

Table 2: Lipinski properties of the seven dietary nutrients (Values obtained from Pubchem)

S. No.	Molecules	Molecular weight<=500)g/mol	XLog P (<=5)	H-Donor	H-Acceptor
1	Curcumin	368.3799 [g/mol]	3.2	2	6
2	Genistein,	274.261547 [g/mol]	2.7	3	5
3	Apigenin	270.2369 [g/mol]	1.7	3	5
4	Lycopene	536.87264 [g/mol]	15.6	0	0
5	Silibinin	482.43618 [g/mol]	2.4	5	10
6	Luteolin	286.2363 [g/mol]	0.7	5	7

Designing of a molecular docking model of IGF1R and its bound ligand

The separated substrate ligand was also manually prepared for docking using software Autodock 4.2 tools by providing number of rotatable, non-rotatable and un-rotatable bonds to it. The necessary flexible residue present in the binding site of IGF1R was identified by individual docking of different amino acids involved in the binding of the bound ligand with the IGF1Receptor. Individual docking carried out in this manner revealed MET1028 residue to be responsible for best docking results. Thus, MET1028 was taken as flexible residue in the present case.

In order to study the binding site present in the receptor, an imaginary 3-dimansion grid box was formed covering a small portion of the receptor. The grid box was deliberately kept sufficiently large enough to encompass the entire binding pockets and nearby amino acids. In the present study, grid box size was adjusted to 60 × 56 × 58 with 0.375 Å spacing and was used for all docking runs. This grid box size was taken with respect to x center, y center and z center as 63.548, 55.601 and 15.016, respectively.

The software Autogrid was run to utilize grid parameters for building map files of receptor as well as ligand, required for the docking. Docking parameter file were prepared for each ligand using genetic algorithm (GA) population size 150, maximum number of energy evaluations (short) 250,000, maximum number of generations 27,000. In the present studies total ten GA cycles were performed with the rate of gene mutation 0.02.

Now, the prepared ligand (bound ligand) was docked in the active site of the prepared receptor protein by using software Autodock-4.2. All the molecular docking simulations were carried out using default docking parameters. This procedure was undertaken to optimize the molecular docking process, and the model thus prepared was validated firstly by calculating binding energy and secondly by observing the overlay and comparing the chemical resemblance, of the docked conformation with the crystallized structure of the ligand.

Further, the overlay of the docked and crystallized ligands were diagnosed by calculating the root mean square deviation (RMSD) between two sets of atomic coordinates. In the present case, the coordinates for crystallographic structure were taken as (x_c, y_c, z_c) and that for docked simulations as (x_d, y_d, z_d) and the values of RMSD were calculated by using following expression:

$$f(x) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_c - x_d)^2 + (y_c - y_d)^2 + (z_c - z_d)^2}$$

It is interesting to note that in the docking simulations, RMSD values below 1.5 Å are considered best results, when compared to crystallographic structures.

Docking simulations

The docking model of IGF1R with the bound ligand revealed the bindingsite in a cavity size of 234 Cubic Angstroms. Site 2 was chosen as the binding site with Min Coordinates: (-58, 16,-18) and Max Coordinates: (-44, 35,-1). The ligand binding affinity of IGF1R was calculated using AUTODOCK 4.2 and Dock score were used to estimate the ligand-binding energies. The parameters used for the docking process were:

Flexible Residue-MET1082

Grid Box= (50*46*40), Spacing= 0.375, X= 36.717, Y= 78.56 & Z= 56.137.

Finally, the docking simulations were conducted via a Lamarckian Genetic Algorithm. Ligand and MET1082 residue of the protein was considered to be flexible. The resulting complexes were clustered according to their root mean square deviation (rmsd) values and binding energies, were calculated using the Autodock scoring function. Apart from these, other input parameters for docking were set as default options.

Prediction of drug-likeliness of ligands

The drug-likeness for all compounds was accomplished for GPCR (G protein coupled receptor) ligand activity, ion channel modulator activity, kinase inhibition, nuclear receptor ligand activity and enzyme inhibition by Molinspiration [32].

RESULTS AND DISCUSSION

IGF-1R is currently being one of the most promising targets for modern cancer treatment. Recently, many natural inhibitors are emerging as potent multimodal cancer-preventing agent. Six dietary agents viz: apigenin, lycopene, curcumin, silibinin, genistein, and luteolin that are known to interfere directly with circulating levels of IGF-I and its receptor were selected for the study. In order to determine the active site residues present in the binding pocket of IGF1Rand screen effective IGF1R inhibitors, firstly, a molecular docking simulation model of IGF1R with its bound ligand was prepared and validated.

Validation of docking model of IGF1R with its natural bound ligand

The docking of IGF1R with bound ligand reported a binding energy of-9.88 k. Cal/mol and RMSD value o of 0.93 Å. (fig. 2). It is interesting to note that in the docking simulations, RMSD values below 1.5 Å are considered best results, when compared to crystallographic structures. The docked confirmation of the bound ligand was perfectly overlaid with the crystallized bioactive conformation of the bound ligand (fig. 3). The values of RMSD within prescribed limits further confirmed the presence of bound ligand in the IGF1Receptor. It was also found that the docked ligand had similar chemical interactions with the binding residues present in the IGF1Receptor, to those present in the crystal structure of the downloaded IGF1Receptor. Thus, the docking model of IGF1R and its bound ligand was validated and was used as a reference model for further study.

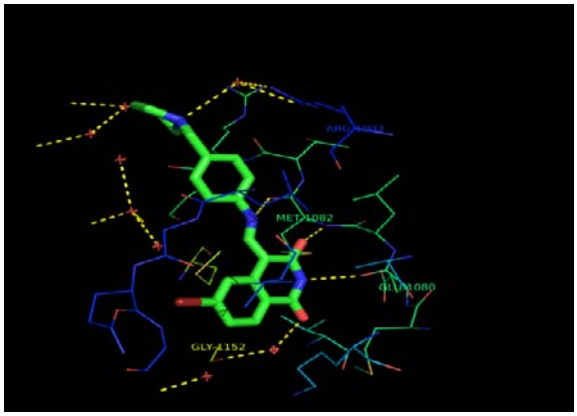

Fig. 2: Bound ligand docked to IGF1R

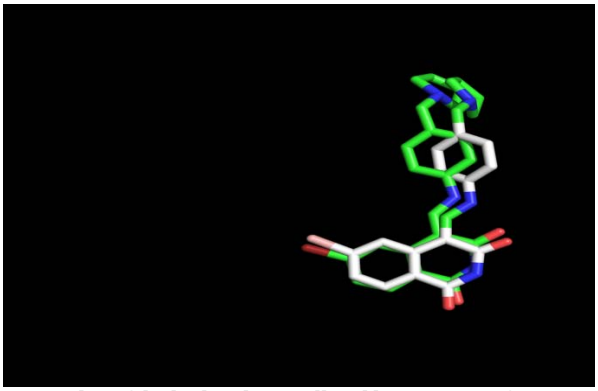

Fig. 3: Overlay of docked and crystallized bioactive conformation of bound ligand

Visulization of docked complex and analysis

The docked complex of protein and ligand was visualized by Ligplot [33]. Ligplot is a command line based program for automated plotting of protein–ligand interactions from the 3 -D structure coordinates file of protein–ligand complex and generates schematic diagrams of this interaction, showing interacting residues of protein and ligand, mediated by hydrogen bonds and hydrophobic interactions. H-bonds are indicated by dashed lines between the atoms involved, while an arc represents hydrophobic contacts with spokes radiating toward the ligand atoms they contact. The contacted atoms are shown with spokes radiating back (fig. 4, table 3).

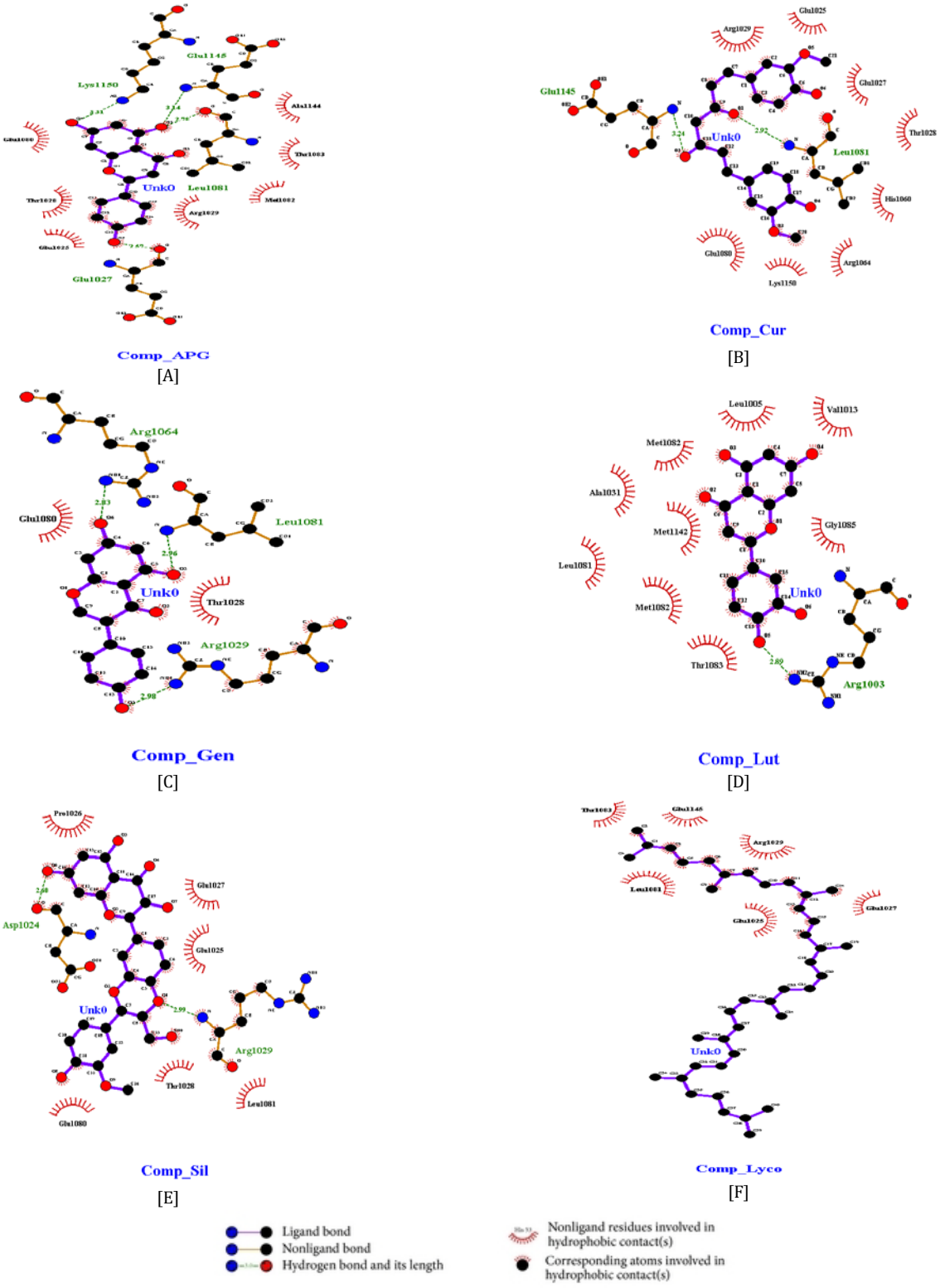

Fig. 4: Summary of docked pose of the six anticancer compounds (A. Apigenin B. Curcumin C. Genistein D. Luteolin E. Silibinin F. Lycopene). The green dot linesdenotes the hydrogen bonds and arcs show the residues involved in hydrophobic interactions

Table 3: Summary of docking information of the top ranked poses of each dietary agent

Name	Dock Score	Inhibition coefficient (KI)	Residues Involved in H-Bonding	Residues making Hydrophobiccontacts
Apigenin	-5.78Kcal/mol	58.26 µmol	Leu1081,Glu1027,Glu1145,Lys1150	Ala1144,Thr1083, Met1082,Arg1029,Glu1025, Thr1028,Glu1080
Curcumin	-3.54 Kcal/mol	2.54 mmoles	Leu1081,Glu1145	Glu1025, Glu1027, Thr1028,His1060, Arg1064,Lys1150, Glu1080, Arg1029
Genistein	-4.80 Kcal/mol	305.23 µmol	Arg1064, Leu1081, Arg1029	Glu1080,Thr1028
Luteolin	-5.7 Kcal/mol	66.43 µmol	Arg1003	Val1013,Gly1085, Thr1083,Met1082, Met1142,Met1082,Ala1031,Leu1081, Leu1005
Silibinin	-5-27 Kcal/mol	137.85 µmol	Arg1029,Asp1024	Glu1027,Glu1025,Thr1028,Leu1081,Glu1080
Lycopene	0.86 Kcal/mol	NA		Thr1083,Glu1154, Arg1029,Glu1027, Glu1025,Leu1081

The Ligplot study revealed that among the selected ligands (fig. 5), Apigenin had 7 hydrophobic and 4 hydrogen bond interactions, which is the highest in comparison with the number of interactions as compared to the rest of the molecules (table 3). Curcumin had 8 hydrophobic and 2 hydrogen bonds followed by Leutolin with 9 hydrophobic and 1 hydrogen bond. Silibinin had 5 hydrophobic and 2 hydrogen bonds.

Whereas, Curcumin formed two H-bonds and 8 hydrophobic interactions, it was noticed that Lycopene did not form any H-bond with the receptor and thus was shown to be a poor ligand of IGF1R.

From Table3 it is clear that the active site residues GLU1080, GLU1027, GLU1025, GLU1145, MET1028, ARG1003, ARG1064, ARG1029, LYS 1150, LEU 1081 and THR1028in the receptor cavityplay an important role in drug binding and interaction.

Drug-likeliness scores of Ligands

Table 4 shows that cLogP values and PSA values for Luteolin and Apigenin lie well within the optimum. The cLogP value of Luteolin and Apigenin 1.974 and 2.468 respectively contributes significantly to improved pharmacokinetics and pharmacodynamics of the ligands. The PSA value of Apigenin (90.895$A^{2)}$ suggests better intestinal suggesting that the administration of these agents may be beneficial to the patient and may have substantial affect. To support this contention, it can be noted that Luteolin and Apigenin have zero violations of the Rule of 5. Rule of 5 increases the probability that a potential chemotherapeutic will have favorable bioavailability. The well-known rule-of-five the Rule of 5 is a set of parameters devised to evaluate drug likeness better and aid the screening of potential drug "hits" identified through processes such as high throughput screening [34]. Applying this rule points out that most orally administered drugs have a molecular weight (MW) of 500 or less, a log P no higher than 5, five or fewer hydrogen bond donor sites, and 10 or fewer hydrogen bond acceptor sites (N and O atoms).

The structural features include surface area components and hydrogen-bonding potentials, and the properties include octanol/ water and water/gas log. The small deviation from average molecular mass, number of heavy atoms and molecular volume in these dietary agents suggests their adherence to preservation of atom type and functional group presence. In order to evaluate the expected bio-activity of drug structures it is constructive to apply screening criteria to populations of the potential drug candidates.

All the six dietary agents were analyzed in this manner (see Materials and Methods) under the criteria of GPCR ligand activity, ion channel modulation, kinase inhibition, nuclear receptor ligand activity and enzyme inhibition giving results presented in table 5. Also, the numerical values of the activities determined for Luteolin and Apigenin were favorable and superior to other ligands. Thus, it is clear that Luteolin and Apigenin have good solubility, stability and absorption and significant first passmetabolism. The drug likeliness score as calculated through Molinspiration reveals that they satisfy maximum parameters. (tables 4, 5).

Table 4: Calculated molecular properties by molinspiration

Compound	Optimum range	Apigenin	Luteolin	Curcumin	genstein	Silibilin	Lycopene
LogP	-5 to+5	2.463	1.974	2.303	2.268	1.465	9.977
TPSA	60 to 40	90.895	111.123	93.066	90.895	155.145	0
natoms		20	21	27	20	35	40
mw	150 to 500	270.25	286.239	368.385	274.208	482.441	536.888
nON	0 to 10	5	6	6	5	10	0
nOHNH	0 to 5	3	4	2	3	5	0
nviolation	0	0	0	0	0	0	2
nrotb		1	1	8	1	0	16
volume		224.049	232.067	332.182	224.049	400.862	601.871

Log P= water partition coefficient, TPSA=Molecular Polar Surface Area, MW= Molecular Volume, nrotb= no. of rotatable bonds, nviolation= violation of rule of five.

Table 5: Predicted bioavailability of the dietary agents

Compound	Apigenin	Luteolin	Curcumin	Genstein	Silibilin	Lycopene
GPCR ligand	-0.07	-0.02	-0.06	-0.22	-0.07	-0.07
Ion channel modulator	-0.09	-0.07	-0.2	-0.54	-0.05	-0.12
Kinase inhibitor	0.18	0.26	-0.26	-0.06	0.01	-0.06
Nuclear receptor ligand	0.34	0.39	0.12	0.23	0.16	0.29
Protease inhibitor	-0.25	-0.22	-0.14	-0.68	-0.02	-0.06
Enzyme inhibitor	0.26	0.28	-0.14	0.13	0.23	0.17

CONCLUSION

The proposed study predicts the binding affinities of the natural dietary agents to the Insulin like Growth factor 1 Receptor and the most effective binding site residues that play a significant role in the binding mechanism. GLU1080, GLU1027, GLU1025, GLU1145, MET1028, ARG1003, ARG1064, ARG1029, LYS 1150, LEU 1081 and THR1028residues are found to be significant binding site residues in the IGF1Receptor protein. Based on the results obtained, the dietary agents Luteolin and Apigenin are proposed as potential anticancer inhibitors of IGF1R that display reliable pharmacokinetics and pharmacodynamics features and minimum toxicity. Thus, these dietary agents can serve as efficient drug leads for designing anticancer inhibitors for the broad spectrum drug target IGF1R in various human malignancies.

ACKNOWLEDGMENT

Authors are highly grateful to department of Bioinformatics New Delhi and MPCST Bhopal for providing Biotechnology-Bioinformatics infrastructure facility to carry out this work.

CONFLICT OF INTERESTS

Declared None

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