Section A-Research paper
ISSN 2063-5346

Molecular Docking Studies of Novel Andrographolide analogs using Phosphoinositide 3-kinase (PI3K) as a potent anti-cancer inhibitor

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DOI: 10.53555/ecb/2020.9.1.05

ABSTRACT:

Andrographis paniculata is an important herbal drug, it is used for cancer treatment and other various diseases. Andrographolide is the major chemical constituent of the extraction of Andrographis paniculata. It is a series of new andrographolide analogs that were designed based on structural activity relationship properties then minimized and docked against potential PI3K Inhibitor (PDB ID: 4XX5) using Schrödinger software (10.1). Docking studies of andrographolide analogs designed by substituting different chemical groups, The ligand 10(-8.885), 11(-8.808), 12(-8.415), 14(-8.133), 15(-8.079), showed high binding affinity against PI3K Inhibitor (PDB ID: 4XX5) Compared to the standard drug imatinib (-9.201). Binding affinity towards the active site of the protein. The target andrographolide analogs were evaluated for their *in vitro* anticancer activity against two cancer cell lines: non-small cell lung cancer A549 cell line and breast cancer MCF-7 cell line. Andrographolide analogs showed good potent anticancer activity compared with standard. These studies may pave a new way for better treatment for anticancer drugs.

KEYWORDS: Andrographolide, PI3K Inhibitor, Molecular docking, 4XX5, Cytotoxicity.

INTRODUCTION:

Andrographolide is a labdane diterpenoid that has been isolated from the leaves of *Andrographis Paniculata* (*Acanthaceae*) [1]. Andrographolide is a major phytoconstituent of the plant and has been recognized as a very important pharmacophore as a result of its key role as an inducer of apoptosis (Programmed cell death) against different types of cancers[2] in addition to different pharmacological effects (such as immunostimulatory, anti-viral, anti-inflammatory, antimalarial, anti-hyperglycemic[3], etc.

In recent years, Computer-aided Drug Design (CADD) has evolved as a big leap in the drug discovery process, with a rapidly increasing number of chemical and biological databases along with new and innovative software tools, all providing a much-improved basis for the design of ligands and inhibitors with the desired specificity. This discipline has long been divided into two types: (1) structure-based drug design and (2) ligand-based drug design.

Structure-based drug design uses the principle of complementarity between the receptor and the ligand and is applicable when the three-dimensional structure of the target is known, but the active site of the receptor is unknown, while ligand-based drug design is based on similarity, physicochemical parameters, and pharmacophore features. The latter is comparatively faster, and simpler, and is an extremely efficient method for virtual screening queries as compared to structure-based drug design, due to which it is more commonly employed by medicinal chemists. Moreover, structure-based drug design has some disadvantages, such as high complexity and computational cost. Molecular-based docking as a virtual screening strategy lacks accuracy and speed when processing large databases. It also has the risk of missing hits because of the inadequate representation of conformational space[4-6].

PI3K

Targeting signaling pathways that are deregulated in human cancer has been a promising approach in cancer therapy. The initial success of imatinib in chronic leukemia has validated this approach and has encouraged the development of additional therapies. To date, a large number of new drugs that target mutated proteins responsible for tumor growth have already been tested in clinical trials.

For example vemurafenib, a drug that targets the mutated version of B-Raf (v-RAF murine sarcoma viral oncogene homolog B1) has been demonstrated to prolong survival in metastatic melanoma. This represents a remarkable achievement, given the fact that advanced melanoma poorly responds to conventional treatments. Nevertheless, if targeted therapies prolong survival in advanced cancer patients, they do not cure cancer. Several drawbacks have been identified, the most important probably being the development of resistance by cancer cells and the tumor microenvironment[7-9].

The phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is frequently deregulated in cancer and accordingly represents the (PI3K)/Akt pathway as an important anticancer target. Indeed, the PI3K/AKT signaling pathway plays a major role in regulating cellular processes that are features of cancer such as cell proliferation, survival, or migration. Also, activating mutations of these enzymes are frequently observed in human cancer, leading to cancer growth. Thus, over the last years, drugs that target PI3K or AKT have been extensively developed and are being tested in clinical trials. However, similarly to what is observed for other targeted therapies, adaptive resistance limits the antitumor efficacy of these drugs. It is thus important to fully characterize the interactions between the components of the pathway to generate new concepts that can be exploited therapeutically [10-13].

MATERIALS AND METHODS:

Design and docking studies were performed for substituted-andrographolide analogues (17 ligands) with PI3K gamma protein inhibitor by the Schrodinger suite.

The ligands are prepared using the Ligprep module:

- The dataset of 17 substituted-andrographolide analogs was used for energy optimization and molecular docking studies. The respective structures were drawn by using ChemSketch (or ACDLABS 12.0).
- Maestro is used as a graphical user interface. With the help of the LigPrep module and following functions are performed:
- The drawn ligands were geometry optimized by using the optimized potentials for liquid simulations-2005 (OPLS-2005) force field with the steepest descent followed by truncated Newton Conjugate gradient protocol.

- LigPrep generates accurate, energy-minimized 3D molecular structures. LigPrep also applies sophisticated rules to correct Lewis structures and eliminate mistakes in ligands to reduce downstream computational errors.
- Maximum diversity: LigPrep optionally expands tautomeric and ionization states, ring conformations, and stereoisomers to produce broad chemical and structural diversity from a single input structure.
- The docking calculation generated a few poses for each ligand. The selection of the best pose was done on the interaction energy between the ligand and the protein as well as on the interactions the ligand shows with experimentally proven important residue.

Preparation of protein target structure

- The X-ray crystal structure of the protein was obtained from the Protein Data Bank (PDB) (www.rcsb.org). The PDB ID: 4xx5 (PI3K Gama protein inhibitor). The protein preparation wizard of the Schrodinger suite has been used to prepare protein. The prepared protein is loaded into a maestro environment and the active site is defined.
- All water molecules were removed, removing the solvent, heteroatoms, and pre-existing ligand molecules. The hydrogen atoms were added to the proteins and all-atom force field (OPLS-2005) charges and atom types were assigned. Preparation and refinement were done by running the Protein Prep job on the structure in a standard procedure. Minimizations were performed until the common root mean sq. deviation of non-hydrogen atoms reached 0.3 Å.
- Computing a ligand conformation and orientation relative to the site of the target protein. it's a graphical-automatic drug discovery system used for molecular docking, screening, and visualization of varied ligands.
- Receptor Grid: The grid center is defined for the active site and box sizes are set. The next step is to generate a glide grid. After the successful generation of the grids, prepared ligands are loaded into a maestro. Ligands are kept flexible, while the protein is rigid and docking started with extra precision mode (XP mode).
- The distribution of Log P shows the hydrophobic activity of the drug. The structures of the ligands were taken from ChemSketch and were docked by Schrodinger software. The optimized structures of the andrographolide analogs were consequently docked with the prepared proteins using Schrodinger.
- Finally, these analogs were evaluated for docking studies using the Glide application.

Computational methods with Glide Version 5.5

- All computational studies were carried out using Glide version 5.5, installed in a single machine running on an Intel Core i5 processor with 4GB RAM and 1 TB hard disk with Windows operating system.
- Glide docks flexible ligands into a rigid /flexible receptor structure by a rapid sampling of the conformational, orientational, and positional degrees of freedom of the ligand. There are three modes of running Glide which differ in how ligand degrees of freedom are sampled and in the scoring function employed. All three modes generate an exhaustive set of conformers for a ligand and employ a series of hierarchical filters to enable rapid evaluation of ligand degrees of freedom.
- The Glide Score scoring function is used to rank compounds docked by XP, SP, or HTVS Glide. XP Glide begins with XP Glide docking and then refines the predicted docking modes using an anchor-and-grow algorithm to more thoroughly sample ligand degrees of freedom and the Glide Score scoring function includes special recognition terms to identify and reward structural motifs important to the binding.

Anti-Cancer Screening

a) Cell line:

The human breast cancer cell line MCF-7 and the lung cancer cell line A549 were both cultivated in DMEM that was supplemented with 10% fetal bovine serum (FBS), 1 mM L-glutamine, 100 units/mL penicillin, and 100 g/mL streptomycin. Both cell lines were successfully cultured.

All media and supplements were purchased from Biological Industries (Hi-media). The cells were kept in a humidified atmosphere with 5% CO_2 at 37°C. All cultures were tested for mycoplasma contamination and were found to be negative.

b) Cytotoxicity assay

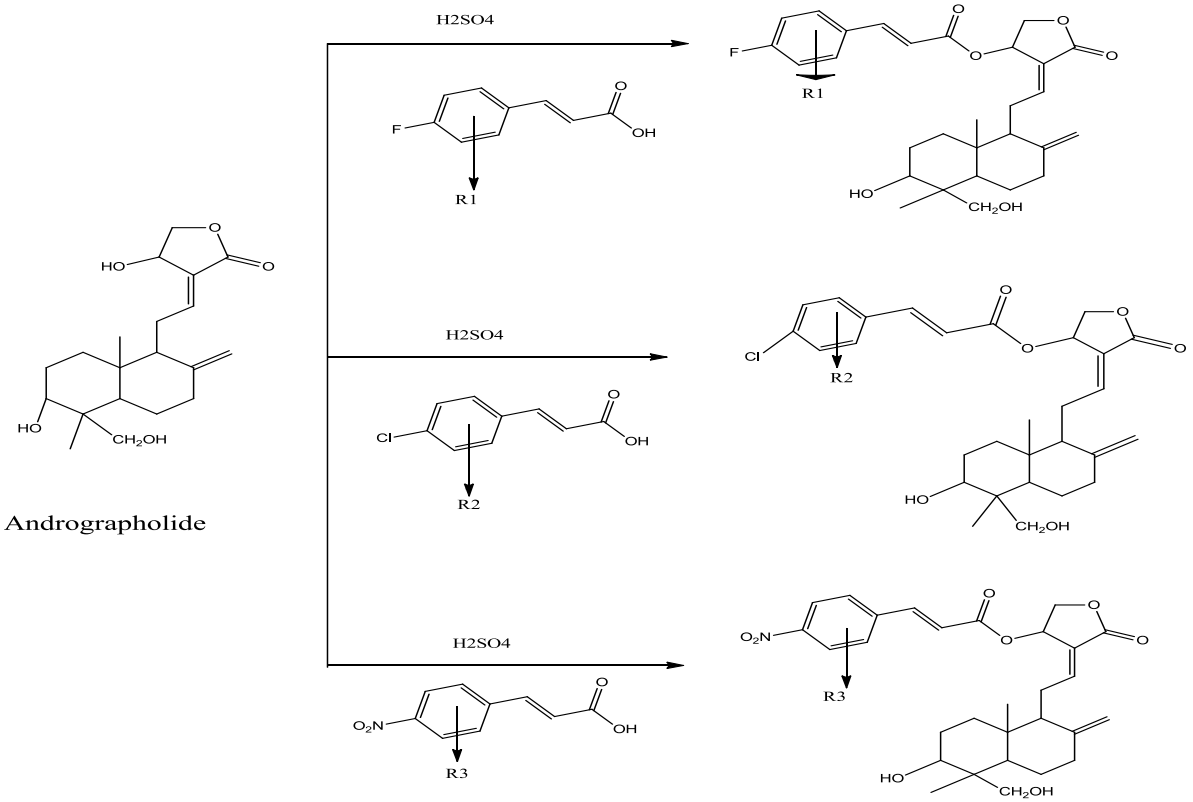
The MTT test is a colorimetric method for determining the amount of energy being used by a cell. It is predicated on the capability of cellular oxidoreductase enzymes that are reliant on nicotinamide adenine dinucleotide phosphate (NADPH) to reduce the tetrazolium dye MTT to its insoluble purple formazan.

The *in vitro* cytotoxic activity of each synthesized compounds **1-17** was evaluated against MCF-7 cell line (human breast cancer) cells using MTT colorimetric assay. Cancer cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 1 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin.

Trypsinization was used to harvest a cell line that was in the log phase of growth. The cells were then resuspended in full growth media to yield a total cell count of 5 x 104 cells/ml. Next, 195 l of the cell suspension was used to seed each well of the 96-well plates that had been prepared. The plates were kept in an incubator for a full 24 hours at 37 degrees Celsius and 5% carbon dioxide in a humidified air environment. Following an overnight incubation, 5 l of the media containing different concentrations of the substances andrographolide analogues (1-50 m) were tested against triple negative MCF-7 breast cancer cell lines (final concentrations of 1, 5, 10, and 20 g/ml). After another twenty-four hours, the plates were put back into the incubator. The maximum concentration of the chemicals that were used had a final concentration of 0.1% DMSO. In each plate, there were three control wells (cells that had not been exposed to test substances) as well as three blank wells (medium that contained 0.1% DMSO) to evaluate the viability of the cells. Following the completion of the treatment, the medium was withdrawn, and then 200 l of phenol red-free media that included MTT (1 mg/ml) was added to each well. This was followed by an incubation period of four hours. After incubation was complete, the culture media was discarded and 100 l of DMSO was added to each well. The absorbance of each well was then measured using a microplate reader set at 570 nm.

The concentration of any substance that, when compared to the control, resulted in a 50% inhibition of cell growth was referred to as the IC50. This value was derived using concentration-response curves using regression analysis.

Scheme: General Synthetic scheme of new Andrographolides



Andrographolide analogous

S.No	R_1	R_2	R_3
1	2-CH_3	2-CH_3	2-CH_3
2	2-C_6H_5	2-C_6H_5	2-C_6H_5
3	2-F	2-F	2-F
4	2-Cl	2-Cl	2-Cl
5	2-NO_2	2-NO_2	2-NO_2
6	3-CH_3	3-CH_3	3-CH_3
7	3-C_6H_5	3-C_6H_5	3-C_6H_5
8	2-OCH_3	2-OCH_3	2-OCH_3
9	2-AcO	2-AcO	2-AcO
10	3-OCH_3	3-OCH_3	3-OCH_3
11	3-AcO	3-AcO	3-AcO
12	2-OH	2-OH	2-OH

13	2-H	2-H	2-H
14	3-NH_2	3-NH_2	3-NH_2
15	3-OH	3-OH	3-OH
16	3-H	3-H	3-H
17	2-NH_2	2-NH_2	2-NH_2

Table-1: Andrographolide analogous

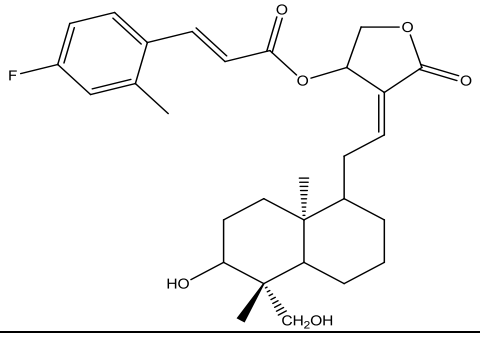
RESULT:

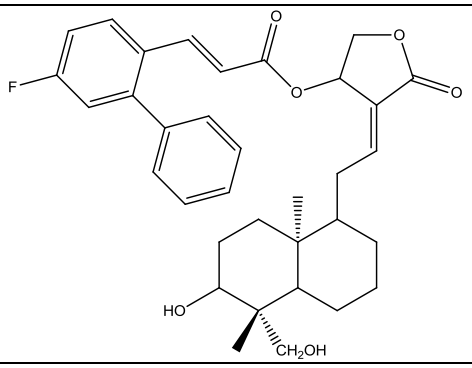
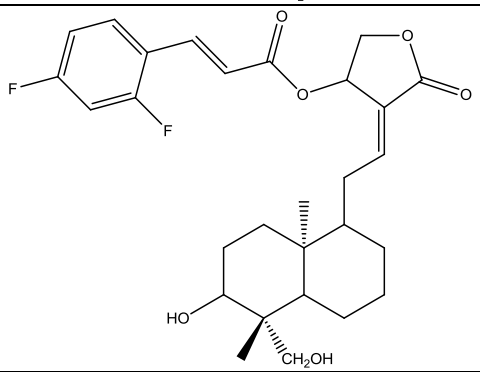
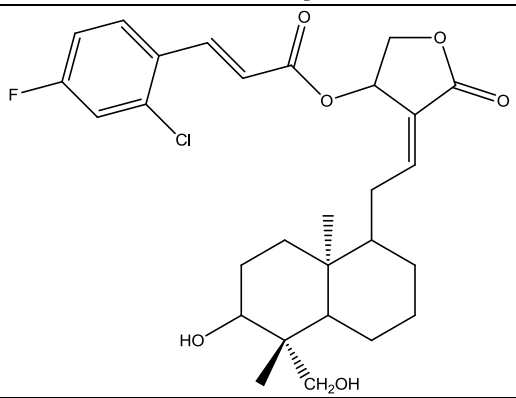
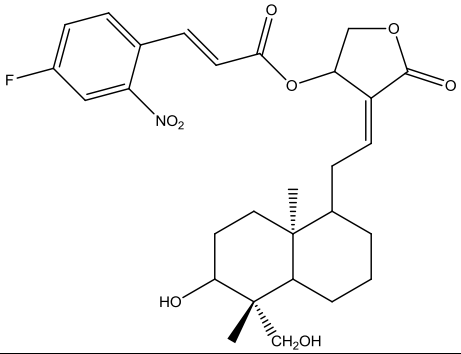
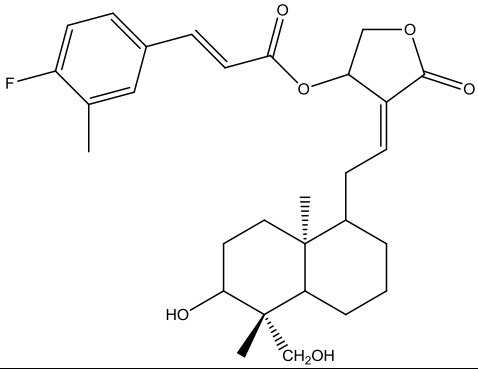
Molecular Docking

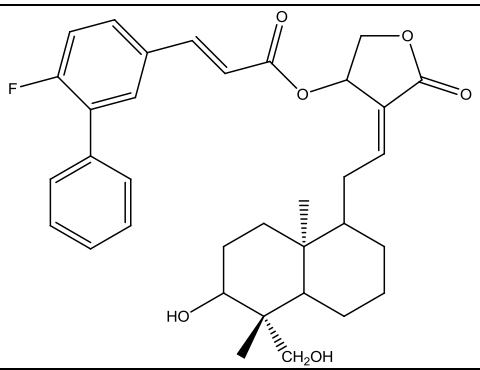
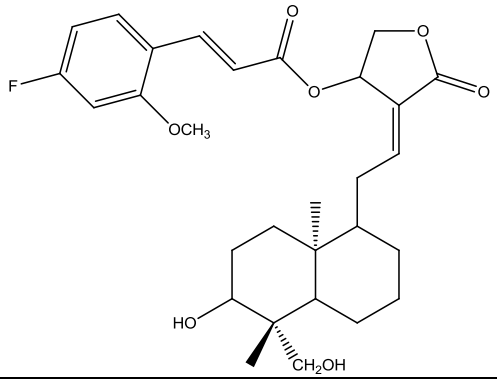
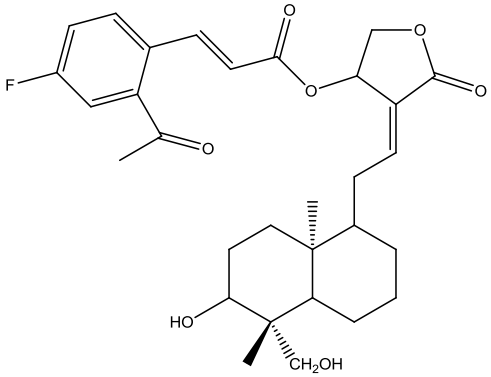
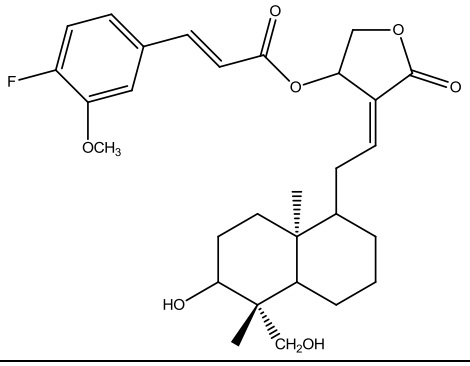
To have better insight into the interactions of PI3K inhibitors, docking studies were docked separately into the binding site of the receptor (PDB ID: 4XX5) using Glide (Grid-Based Ligand Docking With Energetics) software from Schrodinger. Receptor Vander Waals scaling for the non-polar atoms was set to 0.9 which makes the protein site "roomier" by moving back the surface of non-polar regions of the protein and ligand. This kind of change emulates to some extent the impact of breathing motion to the macromolecule site, it's a form of giving breathing to the receptor, and this approach softens the active site region of the receptor making it versatile.

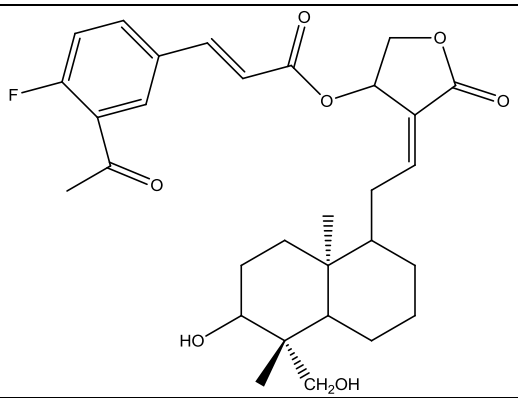
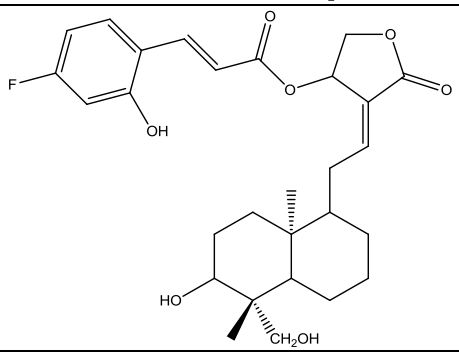
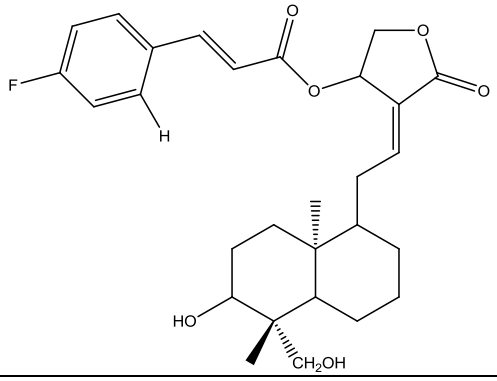
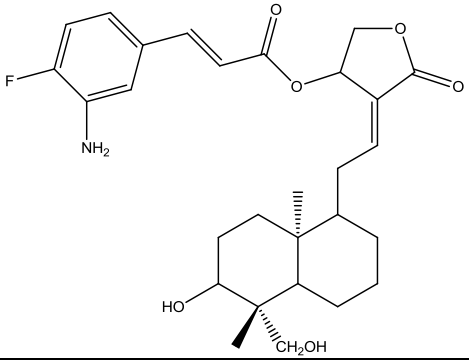
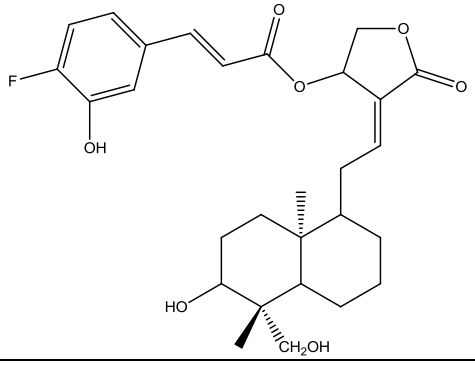
The prepared protein and the ligand were employed to build energy grids using the default value of protein atom scaling (1.0 Å) within a cubic box, centered on the centroid of the X-ray ligand pose. After Grid generation, the ligands were docked with the protein by using the Glide 5.5 module in extra precision mode XP-based minimization. The docked results were obtained from Glide.

Molecules selected from the plant sources were docked using Glide software and docked scores of those molecules were represented in Table-2, with their binding energy, number of hydrogen bonds, bond length, and interacting residues. Binding energies of the protein-ligand (drug) interactions are important to describe how fit the drug binds to the target macromolecule. After evaluating the number of geometries from the protein data bank (PDB ID: 4xx5) was selected for docking studies. However, to validate our docking procedure, we applied the same procedures to dock a well-known PI3 K inhibitor, (4xx5) into the binding pocket of this enzyme.

S.NO	Structure	Docking Score	XP H-Bond	Energy k.cal/mol
1		-5.717	-2.803	80.321

2		-5.384	-2.581	78.901
3		-6.340	-2.14	75.587
4		-7.675	-1.223	79.799
5		--5.012	-2.105	65.256
6		-7.934	-2.011	74.953

7		-7.811	-1.289	70.742
8		-7.788	-1.369	65.464
9		-7.703	-1.361	75.888
10		-8.885	-2.803	75.957

11		-7.995	-2.581	76.858
12		-8.415	-2.14	85.547
13		7.890	-1.223	80.933
14		-8.133	-2.105	82.615
15		-8.079	-2.011	74.709

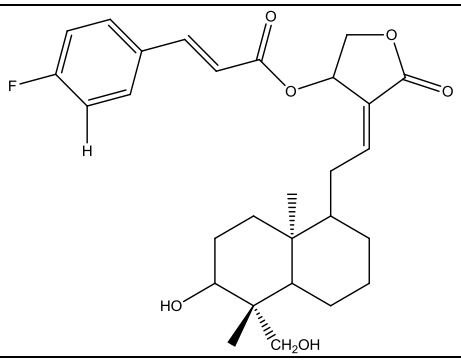
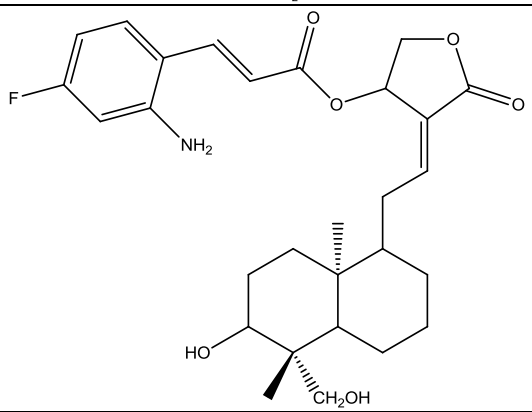
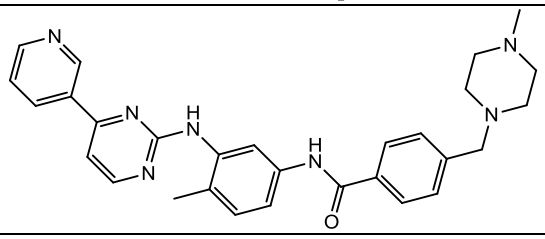
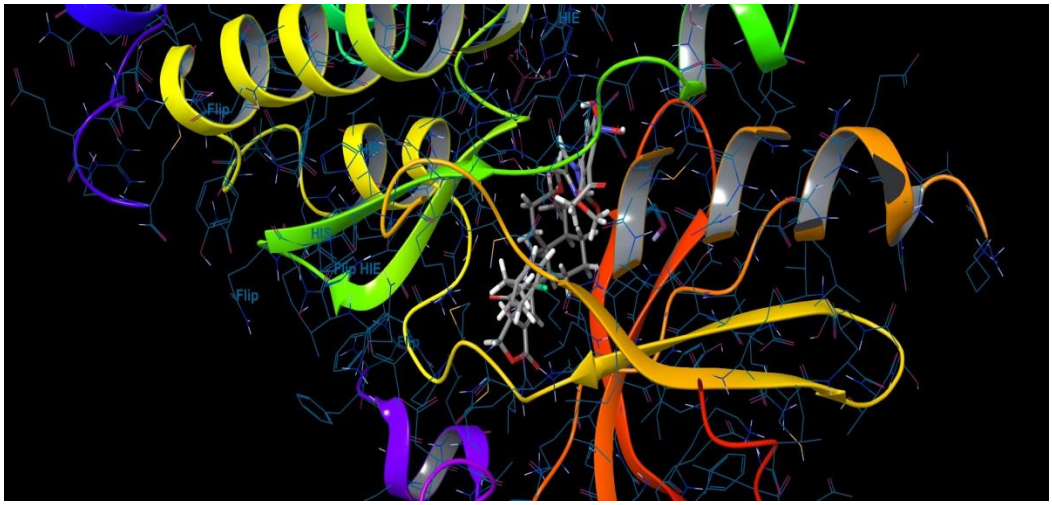
16		-7.988	-2.105	80.321
17		-6.266	-2.105	78.210
Standard		-9.201	-1.240	74.953

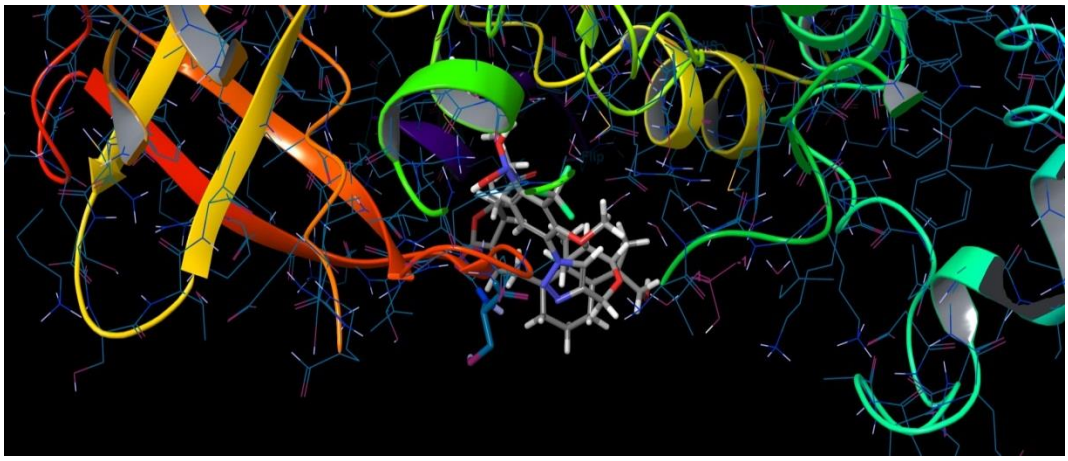
Table-2: Docked scores of Andrographolide analogous

Our docking simulation resulted in a very close model of the crystallographic structure, which supports our findings. Interestingly, ASP 318 seems to play a central role in the binding of 4xx5 within the PI3K binding site, as it forms hydrogen bonds. All ligands prepared by LigPrep were separately docked into the binding pockets of the PI3K inhibitor.

Docking simulations of all 17 compounds against protein resulted in a few best compounds that were evaluated based on the binding compatibility [docked energy (kcal/mol)] with the receptor. By analysing the docking score and the energy, the ligands, and the best compounds from docking are given in Figure-1.



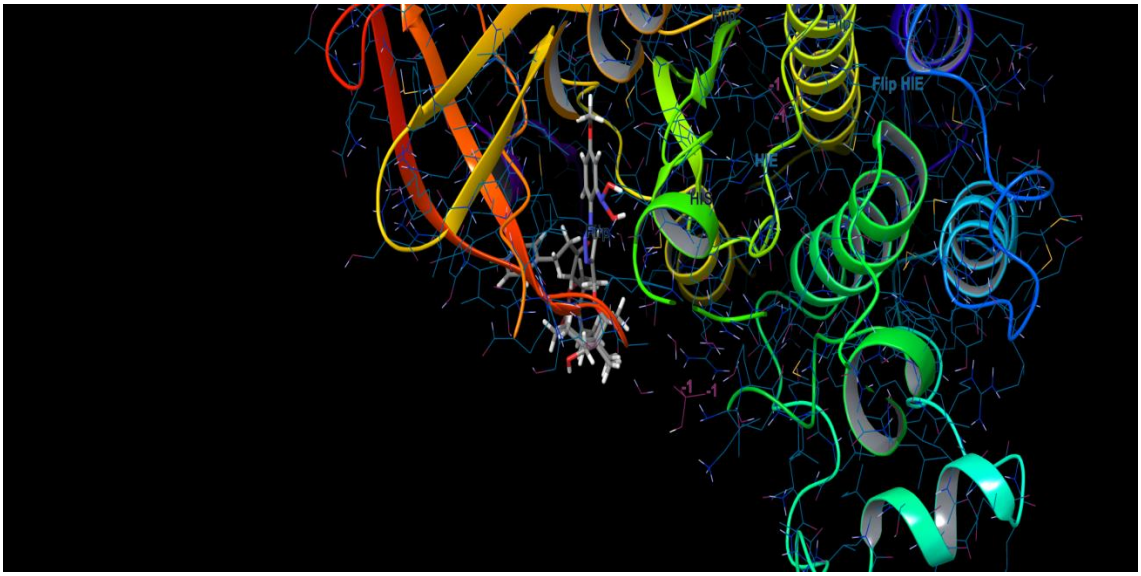
Ligand: 14



Ligand: 12

Ligand: 10

Ligand: 15



Ligand: 11

Figure-1: Molecular Docking 4XX5 Protein

Biological Activity

The American Type Culture Collection was the source for both the A549, which is a cell line derived from non-small cell lung cancer, and the MCF-7, which is a cell line obtained from human breast cancer. In order to keep the cells alive, Dulbecco's Modified Eagle's Medium (DMEM) was used. This medium was supplemented with 10% heat-inactivated fetal bovine serum, 10 g/mL of insulin, and 1% penicillin-streptomycin. in vitro antitumor activity of the newly synthesized andrographolide analogs according to the reported procedures, using doxorubicin as a standard treatment. Every experiment was carried out with the use of three replicates (three wells of the 96-well plate for each experimental condition), and the results of each and every one of the replicates were analyzed to see how the different experimental conditions impacted the outcomes. The values for the IC50 were found by combining an equation for a Boltzman sigmoidal concentration–response curve with the nonlinear regression fitting models that were offered by Graph Pad Prism version 5. This was done in order to get the values for the IC50.

Compound Code	**Ic50 in µM at 72 hrs**	
	A-549	**MCF-7**
1	46.32%±8.86	42.23%±1.4
2	39.28%±1.04	44.51%±1.38
3	46.17%±2.16	39.69%±0.07
4	42.39%±1.04	53.17%±2.16
5	61.75%±1.43	68.17%±1.27
6	48.28%±6.29	61.60%±2.08
7	43.47%±1.76	59.63%±4.22
8	59.13%±4.9	74.31%±7.82
9	50.93%±1.67	68.31%±1.27
10	28.03%±1.86	36.72%±0.35
11	50.75%±1.47	53.17%±1.27
12	32.28%±6.29	38.60%±2.13
13	40.16%±1.74	45.32%±4.4
14	38.63%±4.91	41.81%±7.82
15	40.93%±1.67	48.35%±1.27
16	53.17%±2.16	59.72%±0.33
17	61.75%±1.47	68.37%±1.20
Doxorubicin	21.48 ± 1.40	28.17%±2.84

Table-3: IC_{50} Values of andrographolide analogues

CONCLUSION:

The protein-ligand interaction plays a significant role in structural-based drug design. It has been demonstrated that the approach utilized in this study is successful in finding novel PI3K inhibitors. The ligand 10(-8.885), 11(-8.808), 12(-8.415), 14(-8.133), 15(-8.079), showed high binding affinity against PI3K Inhibitor (PDB ID: 4XX5) Compared to the standard drug imatinib (-9.201). They exactly fit into the active site region and the ligand formed more number H-bond interactions than the co-crystallized ligand. Therefore, this study states the importance of small molecules and their use to enhance protein-ligand interaction studies, *in silico.* From the docking results, we conclude that 10, 11, 12, 14, and 15 could be potential PI3K Inhibitors (PDB ID: 4XX5). The targeted andrographolide analogs were evaluated for their *in*

vitro anticancer activity against two cancer cell lines: non-small cell lung cancer A549 cell line and breast cancer MCF-7 cell line. Andrographolide analogs showed good potent anticancer activity compared with standard as a doxorubicin.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

ACKNOWLEDGMENTS:

The authors are heartily grateful to the Dean of Pharmacy, UCT, OU, and Schrodinger Software, USA. One of the authors is thankful to ICMR No.3/2/2/56/2018/Online Onco Fship/NCD-III for providing support.

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