Original Article

In-Silico Screening Of Phytochemicals For Anaplastic Lymphoma Kinase Positive Oncogenicity

Vivek Kumar[1]*, Dr. Pawan Jalwal[2] , Arti Soni[3] and Dr. Ramchander Khatri[4]

1. Research scholar, Department of Pharmaceutical Sciences, SBMNU, Rohtak, India
2. Faculty of Pharmacy, Department of Pharmaceutical Sciences, SBMNU, Rohtak, India
3. Faculty in Department of Pharmacy, Panipat Institute of Engineering & Technology, Samalkha, Panipat, Haryana (India)-132102.
4. Assistant Controller of Examination, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi (India)110017.

[1]vivekp2222@gmail.com,[2]pawan_jalwal@rediffmail.com,[3]artisoni26@gmail.com, [4]rckhatri@gmail.com

* Research Scholar in Department of Pharmaceutical Sciences,
Shri Baba Mastnath Institute of Pharmaceutical Sciences & Research,
Baba Mastnath University,
Rohtak-124021, Haryana, India
ORCID ID: 0000-0002-6514-8474
Email Id: vivekp2222@gmail.com,
DOI: 10.47750/pnr.2022.13.S10.170

Abstract

Anaplastic Lymphoma Kinase (ALK) receptor inhibitors have been in use to treat ALK positive oncogenic situations like Non Small Cell Lung Cancer (NSCLC) from last two decades but issues like Acquired Drug resistance (ADR) and lipophilicity associated poor penetrations always created a lacuna for these inhibitors to become a successful therapy. Despite the availability of three generations of FDA approved ALKIs at present we seek a need to identify few noval ALKIs among phytochemicals which are deemed to be less toxic and safer with better body penetrations. Current in-silico screening facilitated by Maestro 13.1 modeling interface by Schrodinger, LLC was conducted to screen a pool of about 1500 phytochemicals from 24 chemical classes such as Aloins, Limonoids, Curcumins, Benzimidazoles etc. The virtual protein 8ARJ, downloaded from Protein Data Bank was conditioned to generate receptor grid of ALK followed by creating pool of preconditioned phytochemical ligands having structures in view to contain certain cellular receptor based therapeutic activity along with structural similarities with binding cavity of receptor. Standard ALK inhibitors like Entrectinib, Alectinib etc were also evaluated as reference. This in silico study process includes an initial ADMET prescreening by QikPro wizard followed by docking an initial HTVS followed by standard precision and at last by extra precision mode. At last it can be concluded that; Curcumins phytochemicals must be researched & developed as a new generation of noval ALK inhibitors supported by fact that 10 out of top 18 phytochemicals belongs only to Curcumin family. Secondly phytochemicals identified as ALK inhibitors are Indirubin (Benzimidazole, Pubchem ID10177), Xanthoangelol (Limonoids, Pubchem ID 85134973) and Curcumin (Pubchem ID 442783), with their docking scores of -9.324, -9.254 and -9.025 respectively as compared to docking scores of standard ALK inhibitors like Entrectinib, Crizotinib and Brigatinib having docking scores of -9.361, -8.224 and -8.111 respectively.

Keywords: ALK Inhibitors, Lung Cancer, Docking, Curcumins, Alectinib.

1. INTRODUCTION

1.1 Anaplastic Lymphoma Kinase Receptor & Oncogenicity

ALK is basically a cluster of differentiation 246 (CD246) proteins which was discovered & identified in 1997 belonging to the family of Receptor Tyrosine Kinase (RTK) which acts as a receptor as well as an enzyme to facilitate catalytic reactions in biological bodies including Human beings. Structurally it consist a sequence of 1620 amino acids having a molecular weight of 180 kDa which can be differentiated into extracellular, intracellular and transmembrane domain, out to which the extracellular domain (ECD) is found unique and specific among all of 20 RTKs having 2 MAM (Meprin protein) and a LDL (Lipid) unit along with glycine rich area.[1][2][3][4] This ECD domain is an important part of this receptor to be researched as various fusion proteins can promote dimerization of two ALK receptor which converts this metabolic tool into oncogenic disturbances. [5] However this conversion or activation to oncogenicity may occurs by several other means such as mutations, cleavages at extracellular domains, phenomenon of dependence, glycosylation, presence of its isoform and abbrent forms as well. [6-13] this process of activation involves various intermediate proteins and transduction pathways as well which were studied and targeted simultaneously to make this therapy more versatile and effective. [14]

1.2 ALK Inhibitors & Approaches of Combination Therapy

The targeted approach to deal with this ALK positive oncogenicity requires use of specific Inhibitors of ALK which will block its oncogenic activation thereby controlling the tumor growth. Receptor based structural studies resulted in emergence of Crizotinib[15] which was regarded as first generation ALK inhibitor after its approval. Further optimizations and research resulted in development of subsequent generations like second generation (Ceritinib, Alectinib and Brigatinib) [16-18] and third generation (Lorlatinib) of ALKIs.[19] Further these ALKIs were researched with combination therapy approaches such as implementation of other anti-tumor therapeutic agents like anti-VEGF antibodies, EGFR inhibitors, immunotherapeutic agents and chemotherapeutic agents etc. which resulted in revolutionary success and improvement of overall prognosis in ALK associated oncogenic situations.

1.3 Need to Develop New ALK Inhibitors

This ALKIs therapy showed very effective results initially when used alone as well as in combination with conventional chemotherapeutic agents as well but in later stages of therapy problems like drug resistance which can either be acquired or present naturally in some patients along with lipophilicity associated penetration problems in CNS and tissues raised questions over the success of this therapy. Although equipped with three generation of fully approved ALK inhibitors along with some inhibitors in under clinical trial phase at present, there is a need to develop and identify new series of inhibitors as they belongs to primary part of the therapy along with above mentioned reasons like ADR and low lipophilicity associated penetration problems.[20][21][22] These drive to identify new ALKIs also seeks an opportunity to search noval ALK inhibitors among natural phytochemicals which are deemed to be less toxic and having better penetration profile in biological media.

1.4 Why In-Silico Identification

Now a day's recent technical advancements in field of computer aided drug designing enables us to conduct inexpensive, specific, fast and viable development of lead compound therefore this phytochemical identification project will be carried out on a suitable molecular modeling interface.[23][24] As these virtual screenings are precisely based on structural interactions between receptor and drug lead it also enable us to determine the precise chemical moiety along with its stereo specific features present in extracts from plant source or phytochemical. Generally these studies are carried out by downloading of the virtual protein from databases like Protein Data Bank or PDB which will be regarded as the target receptor under study along with downloading of large libraries of phytochemicals from online databases with a view to consider the therapeutic activity along with structural similarity with binding site of that receptor.

After downloading both of these entities are conditioned by software tools to make them suitable for docking studies. Before docking a fast preliminary screening based on ADMET properties is done to filter out non suitable phytochemicals from lengthy docking operations. Moreover these docking studies are carried out with different modes of precision to make the process fast and legible such as an initial & fast High Throughput Virtual Screening (HTVS) followed by Standard Precision (SP) mode and at last by Extra Precision (XP) mode. At last the results obtained can be interpreted based on their docking score comparison directly with docking scores of standard drug compounds obtained with similar protocol of in-silico methodology.[25]

2. MATERIALS & METHODOLOGY

In this in-silico screening following Hardware, Softwares and research methodologies will be adopted.

2.1 Hardware & Softwares

The hardware used will be Dell Inspiron 15-3567 desktop which is a 64-bit operating system having x64 based Intel(R) Core (TM) i3-6006U series with a speed of 2 Ghz having 4 GB RAM on which Microsoft Windows 10 Home will be used as an operating platform along with word processing software MS Office. The Maestro (13.1) Moleculer Modeling Interface by Schrodinger, INC will be used for all of the in silico operations and computational studies.

Protein Data Bank (PDB) will be used to download the concerned virtual protein receptor and phytochemicals which are to be screened will be downloaded from various online databases and specific websites such as https://coconut.naturalproducts.net/ etc.

2.2 Methodology

Selection of the ALK protein receptor;

Source of the protein to be considered will be Homo sapiens, with resolution near to 1.5 Å without any reported mutations and very missing residues which can be easily rectified by Protein preparation wizard of Maestro. Protein Data Base (PDB) from the site https://www.rcsb.org/ will be used to download the protein however it can be downloaded directly by file menu of Maestro. [26][27]

Retrieval of the Protein Receptor;

Protein Preparation Workflow (PPW) by Maestro 13.1 Molecular modeling Interface will be used to execute sub structural modifications like removal of molecules of solvent or water and other moieties followed by various pre processing such as assigning bond order, replacement of H atoms, creating zero order bonds to metal, creating disulfide bonds, kabat antibody annonations, filling missing loops etc. along with ionization of heteroatoms of the receptor protein at assumed biological pH 7±2 and by applying Epik function (Generation of Permeability of the Receptor followed by minimizing the protein structure by using the force field and convergence.[28]

Receptor Grid Generation;

Receptor Grid Generation (Glide) by Maestro 13.1 Molecular modeling Interface will be used to execute generation of grid specifying confinement of ligand (centroid of workspace or residue along with size of ligand selection). After selecting specific modifications the ligand will be selected manually in the Maestro workspace. The grid generated will be saved at convenient location and name which will be used in next steps of docking with prepared ligands. [29]

Collection of Phytochemicals;

Phytochemicals will be used in their mol format by use of online tools such as openbabel https://openbabel.org/ and by direct download of 2d mol format of phytochemicals from online database such as https://coconut.naturalproducts.net/. Extensive literature review on phytochemicals across many databases, research articles, journals etc. will be done in search of phytochemicals having cellular receptor based therapeutic activity along with structural similarity as well. [25]

Preparation of Ligand to be suitable for in-silico docking studies;

LigPrep by Maestro 13.1 Molecular modeling Interface which will change the orientation of phytochemical along with minimization of structure by force fields to make these phytochemical suitable to be used as ligand in docking studies.[31]

ADMET Studies;

QikPro wizard of Maestro 13.1 Molecular modeling will be used to interpret the properties such as Lipinski rule of 3 & rule of 5, Value of Qlog Kp, % Human Oral Absorption etc. After this ADME filtration majority of phytochemical will be excluded from the study as they are not suitable to be used against the receptor protein according to their pharmacokinetic profile. [30]

Docking Studies between Receptor & Ligands;

Ligand Docking (Glide) by Maestro 13.1 Molecular modeling Interface will be used for docking study by operating parameters such as use of Input charges, specifying number of atoms per ligand as well as number of rotating bonds etc. Furthermore different precision modes will be used in increasing order such as High Throughput Virtual Screening (HTVS), Standard Precision (SP) and at last by Extra Precision (XP). Various parameters w.r.t ligands can be adopted in this step such as Ligand sampling (flexible as well as rigid), allowing nitrogen inversions and ring conformations, allowing addition of Epik state penalties, rewards of intermolecular hydrogen bonds and enhancing planarity of conjugated pi groups etc. Finally the molecular docking will be carried out setting output parameters such as enabling pose viewing. RMSD calculations can be calculated for input ligand geometry. Computational facilities of the Maestro software will calculate their docking score which will be interpreted. [32]

Docking Studies between Receptor & Standard ALK Inhibitors;

Various Standard ALK inhibitors approved or to be approved will be downloaded in their 2d structure followed by the same procedure as applied to the phytochemical ligands discussed above, however the mode of docking used will be Extra precision (XP) only.

Interpretation of the Docking Results; Selection of the best phytochemical on the basis of docking score, oral absorption and lipid solubility will be done along with various probabilities depending on the results.

3. RESULTS AND DISCUSSIONS

3.1 Selection of the ALK Protein Receptor

First ALK protein to be considered was 7MZY from PDB, which is perfect to the resolution criteria i.e. 1.5 Å, exists without mutations and few non significant missing residues but due to absence of any binded ligand this protein was rejected. Another protein 4Z55 was considered and despite the presence of binded ligand and closeness to the resolution criteria this protein was rejected as it is reported to exhibit mutations. Further search among ALK proteins made us to study 5A9U which belongs to Homo sapiens along with binded ligand and acceptable resolution (1.6 Å) but due to the presence of various missing residues and mutation it has to be rejected.

At last we found a protein 8ARJ which was without any mutations, related to Homo sapiens and bound with a single carbonyl inhibitor (FIGURE 1). However the size of protein being 1.65 Å, which is a bit more than our desired criteria but can be managed easily similarly defects like few missing residue and water or solvent content can also be avoided because these defaults are easy to rectify by using protein prep workflow of Schrödinger molecular modeling interface.

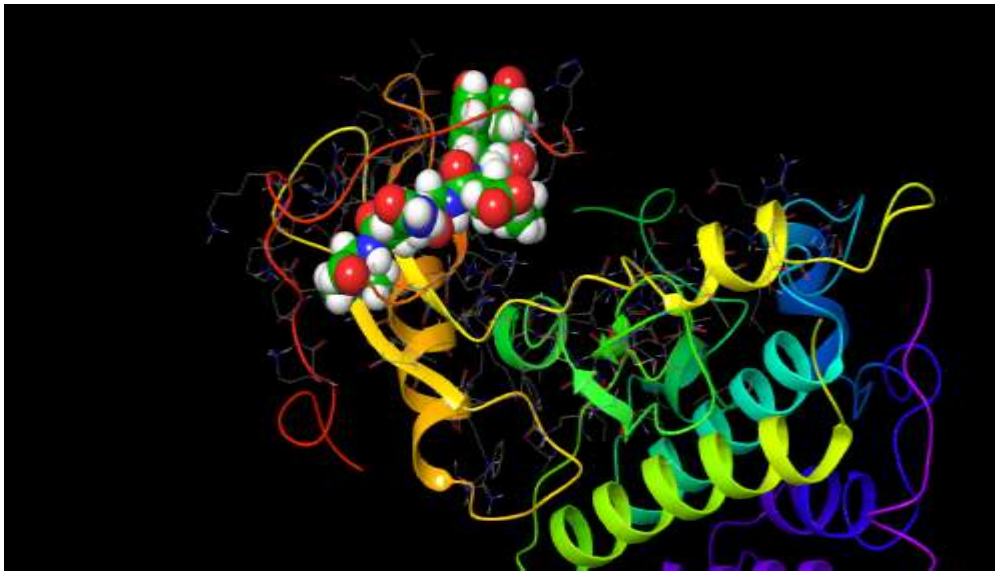


Figure 1: Selected 8ARJ showing its carbonyl inhibitor (CPK view)

3.2 Retrieval of Protein Receptor

Retrieved by Protein Preparation Workflow (PPW) .The ALK protein 8ARJ crystallized with a carbonyl inhibitor considered for the virtual screening studies was downloaded from protein data bank (PDB). Before downloading this protein receptor it is necessary to provide a working directory and create a project file with .prj extension with convenient name and location of our choice. The protein was selected and protein preparation workflow was executed, after opening of the PPW window the protein structure was reviewed and 63 molecules of solvent water was deleted followed by other necessary steps such as ionization of heteroatoms of the receptor protein at assumed biological pH 7±2 by Epik function of PPW which will help in generation of permeability of the receptor, hydrogen bonds are processed to avoid their interaction with OH groups of amino acids followed by conditioning of situations like missing hydrogen atoms, amino acid residues and adding the missing side chains. Finally the protein structure was minimised using the OPLS2005 force field having a convergence criteria of 0.45 Å.

3.3 Receptor Grid Generation

Facilitated by Receptor Grid Generation (Glide) tool of Maestro which will remove the bounded carbonyl ligand to generate the binding site of the receptor where the phytochemical will be docked to study the binding stability of the formed complex. The preconditioned receptor protein 8ARJ was selected and above grid generating tool was executed. After opening of 8ARJ receptor in above tool, the ligand was selected manually in the Maestro workspace, before execution command the location and name of generated grid in form of zipped folder was specified. The receptor grid generated here was used in final steps of docking with prepared ligands. (FIGURE 2)

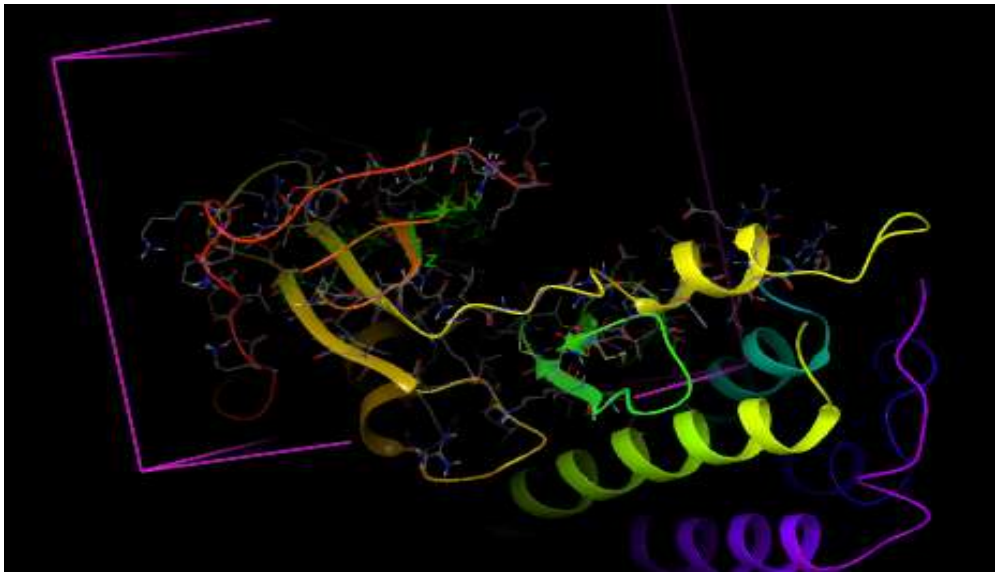


Figure 2: 8ARJ receptor protein after grid generation in Maestro workspace

3.4 Collection of Phytochemicals

Extensive literature review on phytochemicals across many databases, research articles, journals etc. was done in search of phytochemicals having cellular receptor based activity as they may have ability to inhibit ALK receptor as well along with a structural similarity approach between the phytochemical and binding site of receptor. However phytochemicals which were effective against lung cancer cases or believed to act through ALK receptors was preferred. About 1500 Phytochemicals from 24 chemical classes were downloaded in their 2D sdf format and then sketched or converted into their 2 D mol format by using online tools such as openbabel https://openbabel.org/ and by direct download of 2d mol format of phytochemicals from online database like https://coconut.naturalproducts.net/. The 2 D structures selected to be downloaded were considered as per their molecular weight, 2 D structural orientation and terminal features etc. After conversion into their mol format, these phytochemicals were imported into the file module of the modeling software from where they were processed for ADMET studies. (Table 1)

Table 1. Number of Phytochemicals downloaded from different chemical classes along with ADMET results

S.No	Class of Phytochemical	No. of structures downloaded	No. of Structure passed the ADME by QikPro
1.	ALOIN	03	00
2	ANTHOCYANIN	04	00
3	BENZIMIDAZOLE	55	15
4	BENZOATES	04	00
5	CAFFINE	16	00
6	CARBOXYLATE	13	00
7	CUCURBITACIN	198	55
8	CURCUMIN	18	15
9	CURCUMOL	05	05
10	CYMBINODIN	02	01

11	**EPHEMERANTHOQUINONE**	02	01
12	**HESPERDINES & COMPLEXES**	200	00
13	**LIMONOIDS**	147	34
14	**EPIGALLOCATECHIN**	01	00
15	**PODOPHYLLOTOXINS**	15	01
16	**PROCATECHUIC ACIDS**	06	05
17	**QUERCETIN**	132	00
18	**QUINONES**	69	14
19	**RESVERATROL**	16	09
20	**RUTIN & COMPLEXES**	200	01
21	**SALICIN & COMPLEXES**	150	00
22	**STYRENES**	17	13
23	**TAXOL**	05	00
24	**VITAMINE E & COMPLEXES**	200	00

3.5 ADMET Studies

Facilitated by QikPro wizard of Maestro where all of the phytochemicals were screened for ADMET studies. After selecting them from the project table and assigning proper name and location to the folder where filtered out phytochemicals were meant to be saved along with deselecting fast mode (to ensure better screening), a RUN command was executed for performing ADME studies. After execution the results were exported through generation of project file under sub menu WINDOW of Maestro interface. Results obtained were interpreted by selecting desired properties such as Lipinski rule of 3 & rule of 5, Value of Qlog Kp, % Human Oral Absorption etc from the property tree of project table. After this ADME filtration majority of phytochemical will be excluded and deleted from the project table as they are not suitable to be used against the receptor protein according to their pharmacokinetic profile. Out of 1500 phytochemicals pool only 169 phytochemicals were able to pass these ADMET studies. (Table 1)

3.6 Preparation of Ligand

Facilitated by Lig Prep wizard of Maestro in which phytochemicals passed in ADMET studies were meant to be processed further to convert them into ligand suitable for docking studies where the 2 dimensional structures of these phytochemicals was converted into 3 dimensional structures as they have to perform their docking with already prepared 3D protein receptor. The phytochemicals were processed to undergo many extensive processes such as restriction of number of atoms per ligand were restricted to 500 atoms per ligand, application of force fields OPLS2005 to minimize the structure of these phytochemicals to make them effective ligands, implanting ionization at body pH 7±2 by Epik tool of this wizard, a separate binding state for metallic ligands was not created, but desalting, without any tautomer generation was opted with maintenance of chirality. After implementation of all of the above process, a RUN command is executed and allowed to process till incorporation of prepared ligands in workspace window where the ligands were selected to append as a different group.

3.7 Docking Studies

Facilitated by Ligand docking module of Maestro (Glide) all of the ligands prepared above were selected at once along with incorporation of the generated grid created by receptor grid generation wizard in above proceedings. After opting both of the entities following options were implemented such as; application of input partial charge, imposing a restriction of 500 atoms per ligand, allowing 100 rotatable bonds per ligand, scaling of Van Der Waal radius to 0.8 along with 0.15 as partial cut off charge. Further in the setting sub menu ligand selection was kept flexible and addition

of Epik penalties to the docking score was applied. Docking studies was performed in three steps in their increasing order of sensitivity or precision such as HTVS (High Throughput Virtual Screening) which was performed on all of 169 ligands prepared in above proceeding. After incorporation of this HTVS results which was listed in a ranked order of docking score of all of the 169 phytochemicals out of which top 50 ligands were selected and docked again with Standard Precision mode followed by selection of top 20 out of these 50 ligands of standard precision result table which was docked again on third and last mode of precision i.e. Extra Precision mode (XP)

3.8 Docking Results (Test Ligands)

At the time of execution of docking process output parameters such as pose viewing including receptor along with limiting of number of poses and post docking minimizations were implemented. All of the computational facilities of the Maestro software were shown as values of docking score. A brief over look into result table clearly suggesting the importance of curcumin compounds as very potent series of leads effectively interacting with the ALK receptor proteins. However during interpretation of results properties such as oral absorption profile and lipophilicity property of ligand were also considered. The result table of top 18 phytochemicals of XP mode docking study (Table 2) which will be interpreted by comparison of docking score and absorption values of standard ALK Inhibitors docking study (XP) as reference.

Table 2: Docking values of Test Ligands along with their oral absorption and lipophilicity

TITLE	CHEMICAL CLASS	% ORAL ABS	QPlog Kp	R 3	R 5	DOCKING SCORE
1. CNP0177535	BENZIMIDAZOLE	96.799	-2.792	0	0	-9.324
2. CNP0235415	LIMONOID	90.513	-2.843	2	0	-9.254
3. CNP0270212	CURCUMIN	90.294	-2.693	1	0	-9.025
4. CNP0337918	CURCUMIN	82.927	-3.07	0	0	-8.910
5. CNP0207029	CURCUMIN	88.600	-2.852	0	0	-8.682
6. CNP0349190	BENZIMIDAZOLE	90.711	-2.569	0	0	-8.539
7. CNP0352332	CURCUMIN	80.461	-3.174	0	0	-8.413
8. CNP0138219	CURCUMIN	87.761	-2.798	0	0	-8.192
9. CNP0390161	CURCUMIN	81.163	-3.23	0	0	-8.011
10. CNP0242773	CURCUMIN	85.406	-3.595	0	0	-8.005
11. CNP0141102	CURCUMIN	85.398	-2.957	0	0	-7.819
12. CNP0262510	RESVERATROL	36.963	-4.721	1	1	-7.687
13. CNP0097810	BENZIMIDAZOLE	83.561	-3.067	0	0	-7.639
14. CNP0106311	CURCUMIN	81.802	-2.98	0	0	-7.630
15. CNP0323655	CYMBINODIN	86.642	-2.068	0	0	-7.408
16. CNP0104692	CURCUMIN	81.101	-2.924	0	0	-7.106
17. CNP0116963	ANTHOQUINONE	85.351	-2.822	0	0	-6.942
18. CNP0140175	CURCUMOL	69.945	-4.7	1	0	-6.686

3.9 Docking Results (Standard ALK Inhibitors)

For comparison of our docking studies results various approved or to be approved standard formulations were downloaded in their 2d format and converted into their mol format followed by importing them in Maestro workspace and subjected to ADME as well as Lig prep wizard and then followed by Ligand docking (glide) by extra precision method. The result obtained (Table 3) were regarded as reference against Ligand (test).

Table 3: Result table of standard ALK Inhibitors

Name	% Human Oral Absorption	QP log Kp	Rule Of Five	Rule Of Three	Docking score
ENTRECTINIB	77.852	-3.97	2	1	-9.361
CRIZOTINIB	91.963	-4.681	0	0	-8.224
BRIGATINIB	70.234	-5.854	1	0	-8.111
ALECTINIB	91.063	-5.08	0	1	-7.496
CERITINIB	62.966	-5.599	1	1	-7.25
LORLATINIB	83.084	-4.04	0	1	-3.133

3.10 Interpretation of Docking Results

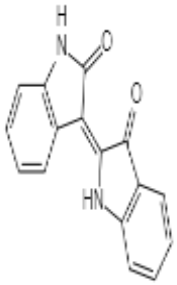
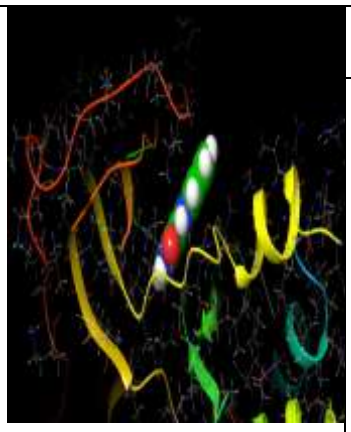
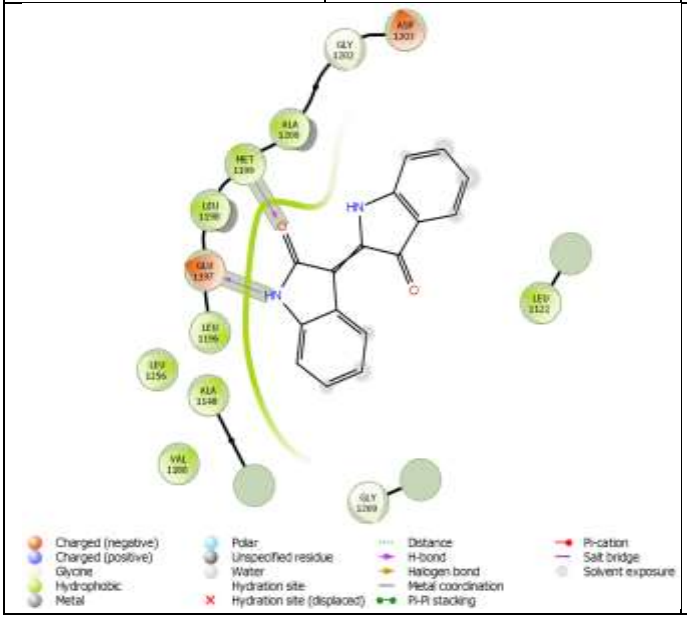
Results shown by our test ligands clearly interpret that as per their docking scores, all of the top eight test ligands can attain first three positions of docking result table of our standard ALK inhibitors along with the fact that almost all of the test ligands were giving a better rate of oral absorption as compared to standard drugs. Furthermore as per the literature studies almost all of the phytochemical are easy to procure and non toxic, which further enforces our conclusion that these phytochemicals can be considered as future lead compounds to develop new generation of ALK inhibitors which will be less toxic and will respond in ALK inhibition therapy where resistance may have been developed against older generations of Inhibitors. As a process of identification three phytochemicals which were identified as ALK inhibitors are Indirubin (Benzimidazole, CNP0177535), Xanthoangelol (Limonoids, CNP0235415) and at last a Curcumin (CNP0270212) with their docking scores of -9.324, -9.254 and -9.025 respectively. Key details about these three identified phytochemicals are briefed below with glimpse of docking studies in forms of outputs such as poses and ligand interaction 2d diagrams having details about the H-bonding acceptor and H-bond donors along with the spatial orientation justifying the charge distributions and steric properties of the complete molecule. These details mentioned w.r.t docking can also be used to processes like lead optimization and distribution of drug.

Indirubin

Among 1500 phytochemicals this phytochemical CNP0177535 (Coconut database Id) and PUBCHEM ID10177 belonging to chemical class of Benzimidazole also known as Indirubin with synonyms like Coroupitine B and Indigopurpurin having molecular weight of 262.263, C16H10N2O2 and IUPAC 1',2'-dihydro-1H,3H-[2,3'-biindolylidene]-2',3-dione. This phytochemicals showed a great value of oral absorption with a good lipophilic property value as well. (Table 4)

Table 4: Detailed result table of Indirubin along with ligand interaction 2D diagram

Indirubin	Docked Complex	Property	Values
		% Oral Abs	96.799
		Docking	-9.324
		QPlogKp	-2.792
		Total Atoms	30
		Heavy Atoms	20
		Bond Counts	23
		Number of C	16
		Minimal Rings	4

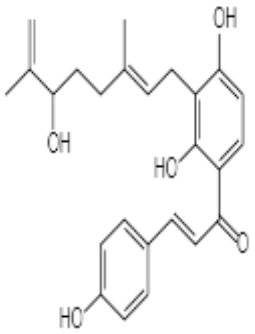
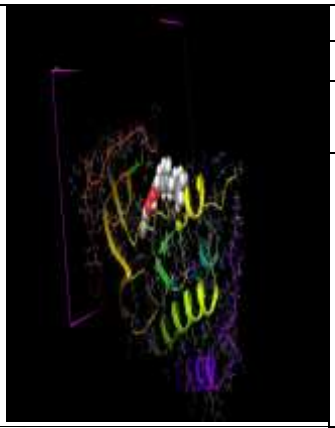
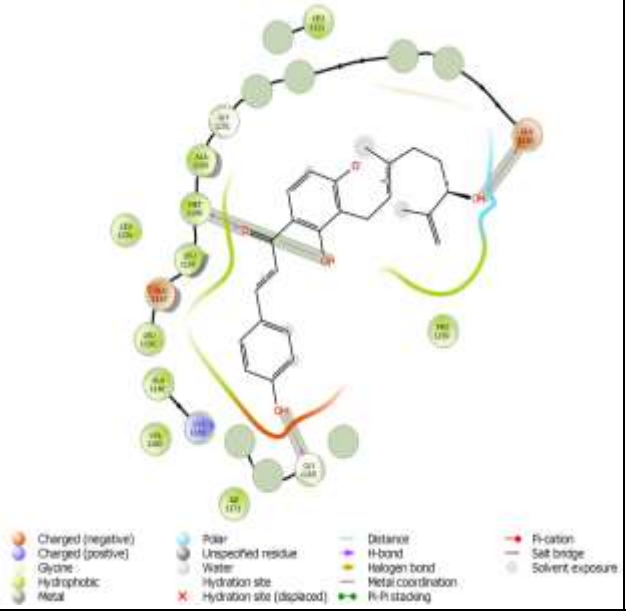
		NP-likeness Score	0.33
		Pubchem ID	10177
		2-D Ligand Interaction Diagram of Indirubin showing receptor forming two H-bonds along the center of ligand with MET 1199 & GLU-1197 along with hydrophobic push of LEU 1122 and covering the ligand with its symmetry.	

Xanthoangelol

This second ranked phytochemicals known as CNP0235415(Coconut database) PUBCHEM ID 85134973 related to chemical class of Limonoids known as Xanthoangelol B having molecular weight of 408.488, C25H28O5 and IUPAC Name: 1-[2,4-dihydroxy-3-(6-hydroxy-3,7-dimethylocta-2,7-dien-1-yl)phenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one. (Table 5)

Table 5. Detailed result table of Xanthoangelol along with ligand interaction 2D diagram

Xanthoangelol	Docked Complex	Property	Values
		%Oral Absorption	90.513
		Docking Score	-9.254
		QPlogKp	-2.843
		Total Atoms	58
		Heavy Atoms	30
		Bond Count	31

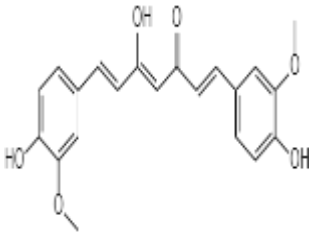
		Number Of C	25
		Minimal Rings	2
		NP-Likeness Score	1.48
		Pubchem Id	85134973
		2-D Ligand Interaction Diagram of Xanthoangelol B showing formation of H-bonds with MET 1199, GLU-1199 & GLY 1269 along with hydrophobic pushes of PRO. It is clearly evident that the H bonding covered the ligand by two terminals and one center part.	

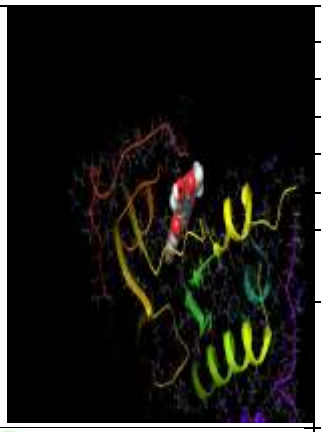
Curcumin

Followed by CNP0270212(Coconut database) PUBCHEM ID 442783 related to chemical class known as Curcumin having molecular weight of 368.380, C21H20O6 and IUPAC Name: 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one. One more interesting observation about this category is that as per docking result table, almost all of the associated curcumins showed a tremendous potential in their structures being an inhibitor of ALK receptor as there are 10 curcumins residing among top 18 phytochemicals out of 1500 which clearly indicates that by implementations of minor structural optimization these curcumins will be a part of these future ALK inhibition therapies for effective and safe treatment of ALK associated oncogenicity cases. Given below a detailed brief about the third and last phytochemical to be discussed in continuation of the above heading. (Table 6)

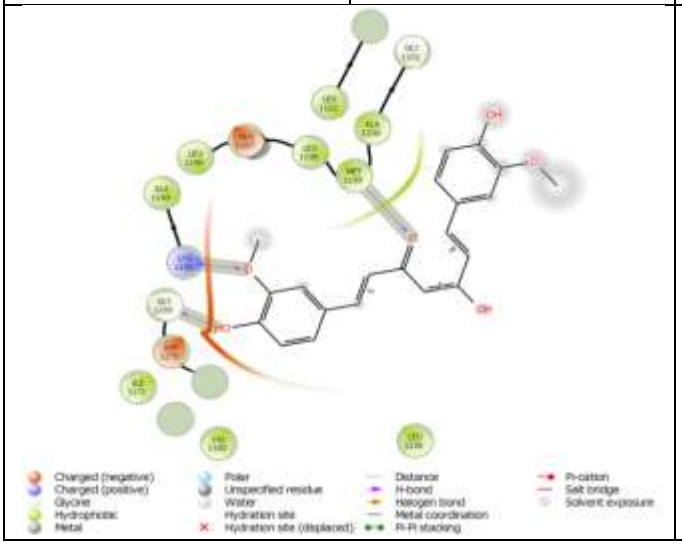
Table 6: Result table of Curcumin along with ligand interaction 2d diagram

CURCUMIN CNP0270212	DOCKED COMPLEX	PROPERTY	VALUES
		% ORAL ABS	90.294
		DOCKING SCORE	-9.025





QPlogKp	-2.693
Total atoms	47
Heavy atoms	27
Bond counts	28
Number of C	21
Minimal rings	2
NP-likeness score	0.66
Pubchem ID	442783



2-D Ligand Interaction Diagram of Curcumin showing formation of H-bonds with MET 1199, LYS-1150 & GLY 1269 along with symmetry coverage of ligand by receptor.

4. CONCLUSION

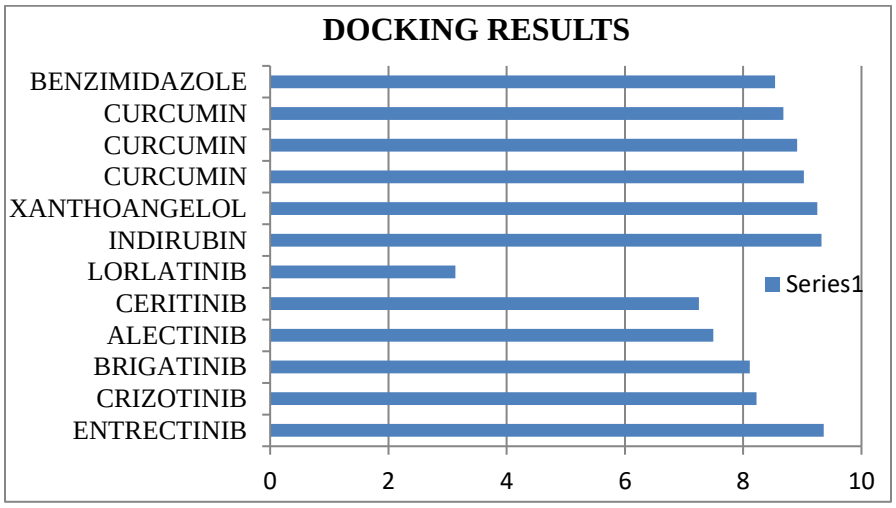


FIGURE 3. Graphical representations of Docking Scores

As per our docking study it can be concluded that despite being reported for anti tumor activity by cell cycle arrest mechanism or inhibitors of cyclin-dependent kinases (CDKs) and also reported as an antimicrobial as well, this phytochemical named as Indirubin (Pubchem ID 10177) can be obtained from its natural sources like Isatis tinctoria, Couroupita guianensis, and calanthe discolor, was found to be very active against ALK receptor as an inhibitor therefore it can be a lead to be developed as an ALK inhibitor, as a topper of docking studies and very high oral absorption rate and it was found capable to form two strong H-bonds through its center part and forced to bind to the receptor with hydrophobic push. Second candidate belongs to Limonoids named as Xanthoangelol B (Pubchem ID 85134973) showed a stronger bonding as they are capable of forming three H-bonds along both of the terminal and at center of its molecule as well. Although reported for blocking histidine kinase and can be obtained naturally from Angelica keiskei it showed very strong inhibitory effects on ALK receptor as well. At last despite being the 3rd candidate of our studies curcumins known for many reported medicinal properties obtained naturally from curcuma longa commonly known as turmeric having curcumin along with other closely associated compound were the most successful compounds for development of a series of ALK inhibitors as out of top 18 compound from docking studies about 10 was curcumins therefore these compound must be studied to in view to develop new generation of noval phytochemical ALK Inhibitors.

5. REFERENCES

[1] Iwahara T, Fujimoto J, Wen D, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene.1997; 14 (4), 439–449. doi 10.1038/sj.onc.1200849.

[2] Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 1994; 263 (5151), 1281–1284. Bibcode: 1994Sci...263.1281M. doi: 10.1126/science.8122112.

[3] Bilsland J.G., Wheeldon A., Mead A., et al. Behavioral and neurochemical alterations in mice deficient in anaplastic lymphoma kinase suggest therapeutic potential for psychiatric indications. Neuropsychopharmacol Off. Publ. Am. Coll. 2008**;** 33: 685–700. doi 10.1038/sj.npp.1301446.

[4] Bai RY, Ouyang T, Miething C, et al. Nucleophosmin-anaplastic lymphoma kinase associated with anaplastic large-cell lymphoma activates the phosphatidylinositol 3-kinase/Akt an apoptotic signaling pathway. Blood.2000; 96 (13), 4319-4327. doi: 10.1182/blood.v96.13.4319.h8004319_4319_4327.

[5] Murugan AK, Xing M. Anaplastic thyroid cancers harbor novel oncogenic mutations of the ALK gene. Cancer Res. 2011**;** 71:4403-11. http://dx.doi.org/ 10.1158/0008-5472.CAN-10-4041.

[6] Lin E, Li L, Guan Y, et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. Mol Cancer Res.2009; 7:1466-76. http://dx.doi.org/ 10.1158/1541-7786.MCR-08-0522.

[7] Cazes A., Louis-Brennetot C., Mazot P., et al. Characterization of rearrangements involving the ALK gene reveals a novel truncated form associated with tumor aggressiveness in neuroblastoma. Cancer Res. 2013; 73:195–204. doi: 10.1158/0008-5472.CAN-12-1242.

[8] Hallberg B., Palmer R.H. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology Nat. Rev. Cancer. 2013; 685–700. doi: 10.1038/nrc3580.

[9] Fujimoto M., Togashi Y., Matsuzaki I., et al. A case report of atypical Spitz tumor harboring a novel MLPH–ALK gene fusion with discordant ALK immunohistochemistry results. Hum. Pathol. 2018; 80:99–103. doi: 10.1016/j.humpath.2018.02.021.

[10] Inamura K., Kobayashi M., Nagano H., et al. A novel fusion of HNRNPA1-ALK in inflammatory myofibroblastic tumor of urinary bladder. Hum. Pathol. 2017; 69:96–100. doi: 10.1016/j.humpath.04.022.

[11] Amatu A., Somaschini A., Cerea G., et al. Novel CAD–ALK gene rearrangement is drugable by entrectinib in colorectal cancer. Br. J. Cancer. 2015; 113:1730–1734. doi: 10.1038/bjc.401.

[12] Aghajan Y., Levy M.L., Malicki D.M., et al. Novel PPP1CB–ALK fusion protein in a high-grade glioma of infancy. BMJ Case Rep. 2016; doi: 10.1136/bcr-2016-217189.

[13] Katayama R., Friboulet L., Koike S., et al. Two novel ALK mutations mediate acquired resistance to the next-generation ALK inhibitor alectinib. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2014; 20:5686–5696. doi: 10.1158/1078-0432.CCR-14-1511.

[14] Shaw A.T., Kim D.W., Mehra R., et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. N. Engl. J. Med. 2014; 370:1189–1197. doi: 10.1056/NEJMoa1311107.

[15] Bossi E, Aroldi A, Brioschi FA, et al. Phase two study of crizotinib in patients with anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma relapsed/refractory to chemotherapy. Am J Hematol. 2020 Dec; 95(12): E319-E321. doi: 10.1002/ajh.25967.Epub 2020 Sep 3.

[16] Farmer G. Non-Small-Cell Lung Cancer Standards of Care Challenged by a Cornucopia of New Drugs. Genetic Engineering & Biotechnology News, 30 (17), [October 2010].

[17] https://www.healio.com/news/hematology-oncology/20171106/fda-approves-alecensa-for-alkpositive-metastatic-nonsmall-cell-lung-cancer

[18] Soria JC, Tan DS, Chiari R, Wu YL, Paz-Ares L, Wolf J, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomized, open-label, phase 3 study. Lancet. 389 (10072).March2017; 917–929. doi: 10.1016/s0140-6736(17)30123-x.
[19] Nagasaka M, Ge Y, Sukari A, et al. A user's guide to lorlatinib. Critical Reviews in Oncology/Hematology. 2020; 151: 102969. doi10.1016/j.critrevonc.2020.102969.
[20] Vander Velde R, Yoon N, Marusyk V, et al. Resistance to targeted therapies as a multifactorial, gradual adaptation to inhibitor specific selective pressures. Nature Communications. 11 (1), 2020; 2393. Bibcode: 2020NatCo.11.2393V. doi: 10.1038/s41467-020-16212-w.
[21] "Novel Drug Approvals for 2017". FDA. [25 January 2021].
[22] Clinical trial number NCT03707938 for "Local Consolidative Therapy and Brigatinib in Treating Patients With Stage IV or Recurrent Non-small Cell Lung Cancer" at ClinicalTrials.gov, [October 2018].
[23] Miranker A, Karplus M. Functionality maps of binding sites: A multiple copy simultaneous search method. Proteins. 1994; 11(1): 29–34.
[24] Eisen MB, Wiley DC, Karplus M, et al. Hubbard RE. HOOK: A program for finding novel molecular architectures that satisfy the chemical and steric requirements of a macromolecule binding site. Proteins. 1994; 19(3): 199–221.
[25] Rajput PS, Kumari V, Mishra N. Medicinal plants contening anticancerous phytochemicals. Int J Complement Alt Med. 2018; 11(6): 414 -417. DOI: 10.15406/ijcam.2018.11.00437.
[26] Om Silakari, Pankaj Kumar Singh. Concepts and Experimental Protocols of Modelling and Informatics in Drug Design. Academic Press. 2021**;** Pages v-xii, https://doi.org/10.1016/B978-0-12-820546-4.00019-2.
[27] Gupta M, Sharma R, Kumar A. Docking techniques in pharmacology: How much promising? Comput Biol Chem. 2018**;** 76: 210-217. doi: 10.1016/j.compbiolchem.2018.06.005.
[28] https://www.schrodinger.com/training/maestro11/quick-start-guide/loading-and-preparing-a-protein-structure.
[29] https://www.schrodinger.com/training/videos/docking-receptor-grid-generation.
[30] Egan, William. Computational models for ADME. Annual Reports in Medicinal Chemistry. ANNU REP MED CHEM42. 2007; 449-467. 10.1016/S0065-7743(07)42029-2.
[31] https://www.schrodinger.com/kb/1609.
[32] https://www.schrodinger.com/science-articles/docking-and-scoring.