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IN SILICO MOLECULAR DOCKING OF CUCURBITACIN DERIVATIVES FOR THEIR ANTI-PROLIFERATIVE ACTIVITY AGAINST TYK2 (TYROSINE KINASE 2) RECEPTOR

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RESEARCH ARTICLE

IN SILICO MOLECULAR DOCKING OF CUCURBITACIN DERIVATIVES FOR THEIR ANTI-PROLIFERATIVE ACTIVITY AGAINST TYK2 (TYROSINE KINASE 2) RECEPTOR

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ARTICLE INFO	ABSTRACT
	Drug discovery & development is an intense, lengthy & an interdisciplinary venture. *In silico* is an expression used to mean performed on computer or via computer simulation. *In silico* drug designing is a form of computer-based modeling whose technologies are applied in drug discovery processes. It has been of great importance to develop fast & accurate target identification and prediction method for the discovery of targeted drugs, construction of drug-target interaction as well as the analysis of small molecule. TYK2 (Tyrosine kinase 2) is critical receptor that creates gene transcription. However, mutational changes in these receptors leading to uncontrolled cellular proliferation or cell death. In humans, mutations in TYK2 is responsible for nearly 50% of lung cancers. In this paper in-silico docking were performed of natural cucurbitacin derivatives & various standard compounds that are thought to have potential to inhibit mutated TYK2 receptor. Out of 17 cucurbitacin derivatives & various standard compounds, Cucurbitacin O shows inhibition activity against TYK2 receptor. From this study, it is observed that Cucurbitacin O has promising inhibitory effect against lung cancer than other cucurbitacin derivatives & various standard compounds.

INTRODUCTION

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer & each is classified by the type of cell that is initially affected.

Cancer harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumors [1]. Tumors can grow with the digestive, nervous & circulatory systems & they can release hormones that alter body function. Tumors that stay in one spot & demonstrate limited growth are generally considered to be benign.

More dangerous, or malignant, tumors form when two things occur:

1. a cancerous cell manages to move throughout the body using the blood or lymphatic systems, destroying healthy tissue in a process called invasion.

2. that cell manages to divide & grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body & grows, invading & destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis & the result is a serious condition that is very difficult to treat. Lung cancers can start in the cells lining the bronchi & parts of the lung such as the bronchioles or alveoli. Lung cancers are thought to start as areas of pre-cancerous changes in the lung. The first changes in the genes (DNA) inside the lung cells may cause the cells to grow faster. These cells may look a bit abnormal if seen under a microscope, but at this point they do not form a mass or tumor. They cannot be seen on an x-ray & they do not cause symptoms.

There are 2 major types of lung cancer [1]:

- Small cell lung cancer (SCLC),

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- Non-small cell lung cancer (NSCLC).

SCLC often starts in the bronchi near the center of the chest, and it tends to spread widely through the body fairly early in the course of the disease. About 10% to 15% of all lung cancers are small cell lung cancer [1].

NSCLC starts in early versions of squamous cells, which are flat cells that line the inside of the airways in the lungs. About 85% to 90% of lung cancers are non-small cell lung cancer.
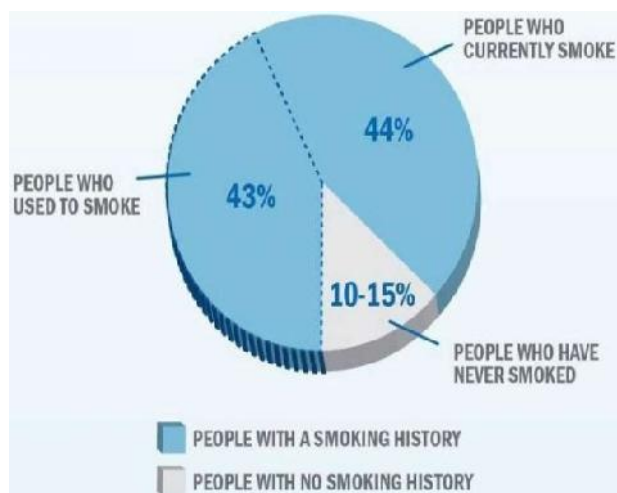

Figure 1 New cases of Lung cancer according to the American Cancer Society [1]

According to the American Cancer Society, Lung cancer is the most common cause of death in the world & accounts for nearly 1 or 2 of every 4 deaths related to cancer [1]. The World Health Organisation (WHO) estimates that worldwide, there were 1.59 million lung cancer-related deaths in 2012 [1].

Multi-drug resistance (MDR) has become the largest obstacle to the success of lung cancer chemotherapies [2]. So, the discovery of new drug is important for treatment of lung cancer. Cucurbitacin is a group of tetracyclic triterpenes derived from plants related cucurbitaceae family including the pumpkins, citrus & gourds such as *Trichosanthes kirilowii, Luffao perculata, Cayapo niatayuya, Citrullus colocynthis* etc [3]. At room temperature, cucurbitacin derivatives are generally crystalline substances & having poor water solubility [3].
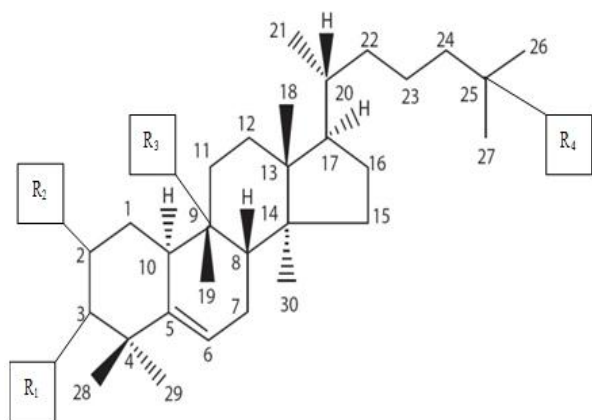

Figure 2 General structure of cucurbitacin skeleton [3]

Table 1 Different substitutions at different sites on chemical structures of different types of cucurbitacin derivatives [3].

Sl. No.	Name of compound	R_1	R_2	R_3	R_4
1	Cucurbitacin A	OH	OH	CH_3	$OCOCH_3$
2	Cucurbitacin B	OH	OH	H	$OCOCH_3$
3	Cucurbitacin C	OH	OCH_3	OH	$OCOCH_3$
4	Cucurbitacin D	OH	OH	H	OH
5	Cucurbitacin E	OH	CH_3	CH_3	$OCOCH_3$
6	Cucurbitacin F	OH	OCH_3	CH_3	OH
7	Cucurbitacin H	OCH_3	OCH_3	H	$OCOCH_3$
8	Cucurbitacin I	OH	OH	CH_3	OH
9	Cucurbitacin J	OF	CH_3	OH	OH
10	Cucurbitacin K	OCH_3	CH_3	OH	OH
11	Cucurbitacin L	$OCOCH_3$	OH	H	OH
12	Cucurbitacin O	OH	OCH_3	OH	CH_3
13	Cucurbitacin P	OH	OH	CH_3	OH
14	Cucurbitacin Q	OH	OH	CH_3	$OCOCH_3$
15	Cucurbitacin R	OCH_3	$OCOCH_3$	OH	OH
16	Cucurbitacin S	OH	$OCOCH_3$	H	OH
17	Dihydro Cucurbitacin B	OH	OH	H	OCH_3

Table 2 Different types & sources of cucurbitacin derivatives [3]

Sl. No.	Types of differerent cucurbitacins	Sources of differerent cucurbitacins
1	Cucurbitacin A	*Trichosanthes Cucumerina*
2	Cucurbitacin B	*Luffao perculata*
3	Cucurbitacin C	*Citrullus colocynthis*
4	Cucurbitacin D	*Trichosanthes kirilowii*
5	Cucurbitacin E	*Citrullus colocynthis*
6	Cucurbitacin F	*Trichosanthes kirilowii*
7	Cucurbitacin H	*Cayaponia tayuya*
8	Cucurbitacin I	*Cayapo niatayuya*
9	Cucurbitacin J	*Cayapo niatayuya*
10	Cucurbitacin K	*Trichosanthes kirilowii*
11	Cucurbitacin L	*Citrullus colocynthis*
12	Cucurbitacin O	*Trichosanthes kirilowii*
13	Cucurbitacin P	*Trichosanthes kirilowii*
14	Cucurbitacin Q	*Citrullus colocynthis*
15	Cucurbitacin R	*Cayaponia tayuya*
16	Cucurbitacin S	*Cayaponia tayuya*
17	Dihydro Cucurbitacin B	*Cayapo niatayuya*

Selection of Target: Cucurbitacin derivatives are hypothesized to be selective inhibitors of the RAS-RAF pathway.

TYK2: Non-receptor tyrosine-protein kinase TYK2 is an enzyme that in humans is encoded by the TYK2 gene. Cytokines play pivotal roles in immunity & inflammation by regulating the survival, proliferation, differentiation & function of immune cells, as well as cells from other organ systems. Cytokines including interleukins, interferons & hemopoietins activate the tyrosine kinases.

Mechanism of RAS-RAF pathway & selection of cytokine (interferon, interleukin) as an activator: The RAS-RAF pathway [4] [Figure 3] mediates the effect of many growth factors & mitogens. RAS which is a proto-oncogene product functions like a G-protein & conveys the signal (by GDP/ GTP exchange) from the SH2-domain protein, Grb2 (Growth factor receptor bound protein) which is phosphorylated by the RTK.

Activation of RAS in turn activates RAF which is the first of a sequence of three serine/threonine kinases, each of which phosphorylates & activates the next in line. The last of these mitogen activated protein (MAP) kinase phosphorylates one or more transcription factors that initiate gene expression, resulting in a variety of cellular responses including uncontrolled cell division or cancer.
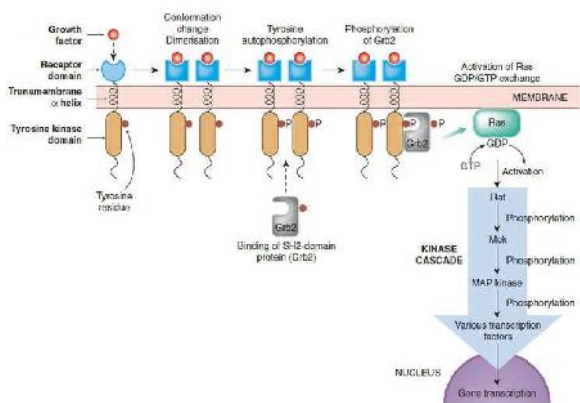

Figure 3 RAS-RAF Pathway [4]

Bioinformatics Tools

Soft wares: Auto dock tools**,** Accelrys Discovery studio 3.5 visualizer**,** UCSF Chimera 1.10**,** Rasmol**,** Padel-descriptor, Microsoft office excel 2007.

Web servers

1. Pubchem: (http://www.pubchem.ncbi.nim.nih.gov/)
2. Chemspider: (http://www.chemspider.com/)
3. RCSB protein data bank: (http://www.rcsb.org/)
4. PDBsum: (http://www.ebi.ac.uk/pdbsum/)
5. ALOGPS: (www.vcclab.org/lab/alogps/)
6. E-dragon: (www.vcclab.org/lab/e-dragon/)

METHODOLOGY

1. From previously published articles, disease (specially lung cancer) was selected according to 'WHO' [1] based on current global data on percentage of death records in 2012 on all cancers in all developing countries.
2. From previously published papers, selection of various target (protein or receptor) due to which these disease was occurred.
3. From previously published articles, various standard compounds (activators or inhibitors) were selected for target (TYK2). The 3D (three-dimensional) structures of these compounds were downloaded from pubchem web server.
4. From previously published papers, various test compounds (different cucurbitacin derivatives) were selected based on their activation against the target [TYK2]. The 3D structures (three-dimensional) of selected targets were downloaded from the official web server of protein data bank (PDB).
5. Conformations of downloaded ligands (standard & test compounds) & conformations of downloaded proteins were generated by using discovery studio 2.5 visualizer software.

6. Docking site of TYK2 was identified by using ligplot from which the amino acid residues & coordinates were obtained for crystal complex protein.
7. Docking of standard compounds was done to the active site of TYK2 (4OLI) receptor.
8. Docking of test compounds was done to check the binding of different test compounds (cucurbitacin derivatives) to the activator attachment site of TYK2 (4OLI) receptor.
9. Binding energies (kcal/mol) of various standard & test compounds were calculated by following formula, Binding energy (kcal/mol) = A+B+C-D, in where A = vanderwal energy + hydrogen bond energy + intermolecular interactions + desolvation energy + electrostatic energy, B = total internal energy, C = torsion energy & D = unbounded energy [5].
10. Best docked cucurbitacin derivatives (with lowest maximum binding energy against TYK2 receptor) was selected for prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity properties), carcinogenicity, mutagenicity & components for fulfilling Lipinski's [6] rule.

Lipinski's [6] rule: Good absorption are more likely when:

A. There are not greater than 5 H-bond donors.
B. The molecular weight is within 500 daltons or 800gms.
C. The LogP is within 5.
D. There are not greater than 10 H-bond acceptors.

RESULTS AND DISCUSSION

Structure of downloaded TYK2 (4OLI) receptor (protein) from official website of protein data bank (PDB)

Receptor (protein) code of TYK2: 4OLI
Classification: Transferase
Structural weight: 73936.42 kDa
Source organism: *Homo sapiens*
Type: Crystal complex structure

Figure 4 Downloaded structure of TYK2 (4OLI) receptor (ribbon structure) from website of protein data bank (PDB)

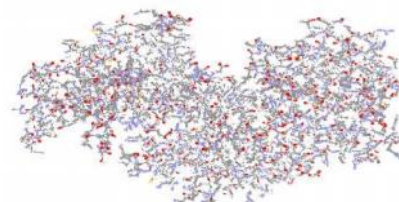***Structure of prepared TYK2 (4OLI) receptor (protein)***

Figure 5 Structure of prepared receptor TYK2 (4OLI)

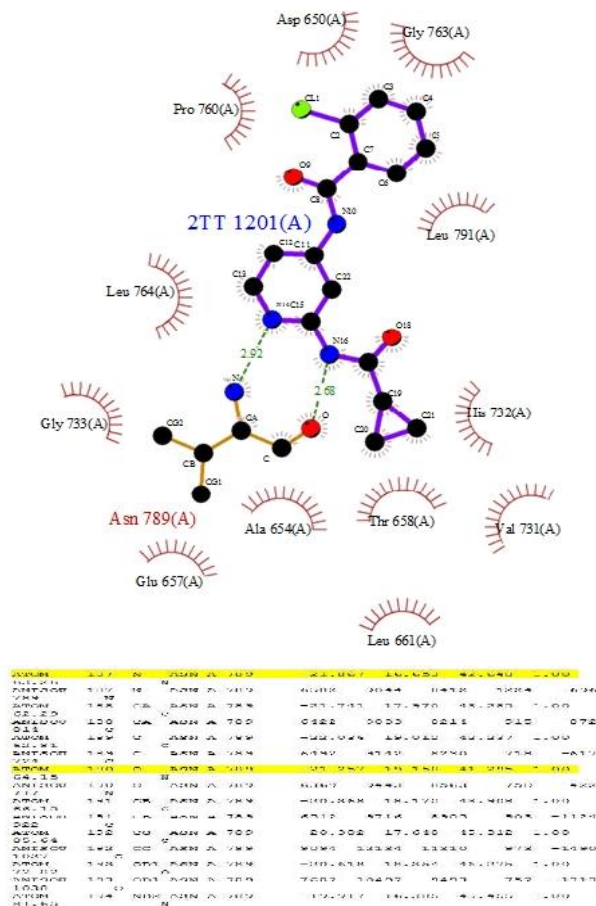*Selection of active site of TYK2 (4OLI) receptor (protein)*

Figure 6 Ligplot analysis of TYK2 (4OLI)

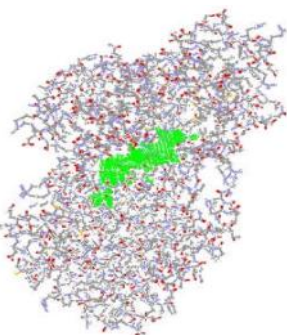*Structure of active site of prepared TYK2 (4OLI) receptor (protein)*

Figure 7 Structure of active site of prepared receptor TYK2 (4OLI) [In this figure, the green portion represents active site for TYK2 (4OLI) receptor]

Coordinates (X, Y & Z) & volume or size of the active site of TYK2 (4OLI) receptor (protein) for binding of each ligand

Table 3 Coordinate & volume or size of active site of TYK2 (4OLI) receptor (protein) [The volume or size of active site of TYK2 (4OLI) receptor must be greater than the volume of the each ligand or compound]

Name of the receptor (protein)	Code	X	Y	Z	Volume or size of the active site (Å)
TYK2	4OLI	-21.562	17.905	41.971	623.336

Docking of various standard compounds to the active site of TYK2 (4OLI) receptor **(Figure 8)**

Table 4 Docking of various standard compounds to the active site of TYK2 (4OLI) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for TYK2 (4OLI) receptor]

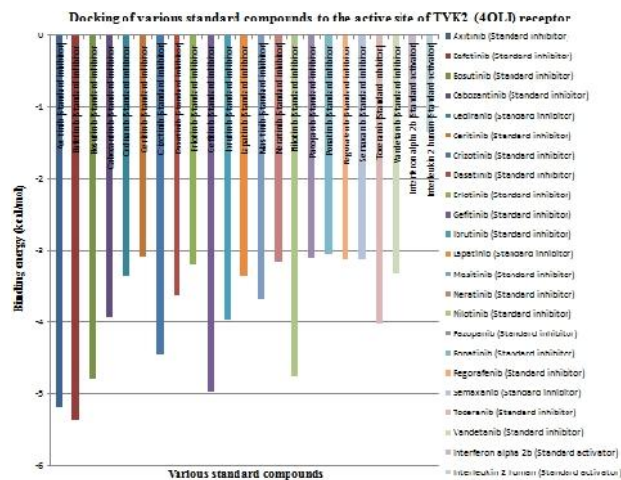| Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Axitinib (Standard inhibitor)	-5.179	303.6
2.	**Bafetinib (Standard inhibitor)**	**-5.367**	312.6
3.	Bosutinib (Standard inhibitor)	-4.781	347.6
4.	Cabozantinib (Standard inhibitor)	-3.920	389.6
5.	Cediranib (Standard inhibitor)	-3.360	355.6
6.	Ceritinib (Standard inhibitor)	-3.093	398.3
7.	Crizotinib (Standard inhibitor)	-4.437	365.4
8.	Dasatinib (Standard inhibitor)	-3.622	400.2
9.	Erlotinib (Standard inhibitor)	-3.203	398.3
10.	Gefitinib (Standard inhibitor)	-4.969	391.6
11.	Ibrutinib (Standard inhibitor)	-3.963	377.6
12.	Lapatinib (Standard inhibitor)	-3.358	401.3
13.	Masitinib (Standard inhibitor)	-3.678	331.1
14.	Neratinib (Standard inhibitor)	-3.159	327.5
15.	Nilotinib (Standard inhibitor)	-4.746	402.6
16.	Pazopanib (Standard inhibitor)	-3.107	387.3
17.	Ponatinib (Standard inhibitor)	-3.059	364.4
18.	Regorafenib (Standard inhibitor)	-3.121	381.2
19.	Semaxanib (Standard inhibitor)	-3.115	354.8
20.	Toceranib (Standard inhibitor)	-4.026	398.1
21.	Vandetanib (Standard inhibitor)	-3.312	371.2
22.	Interferon alpha 2b (Standard activator)	-1.158	320.0
23.	Interleukin 2 human (Standard activator)	-1.350	414.2

Figure 8 Graphical representation for docking of various standard compounds to the active site of TYK2 (4OLI) receptor [Bafetinib has lowest maximum binding energy for stable binding to the active site of TYK2 [4OLI] receptor]
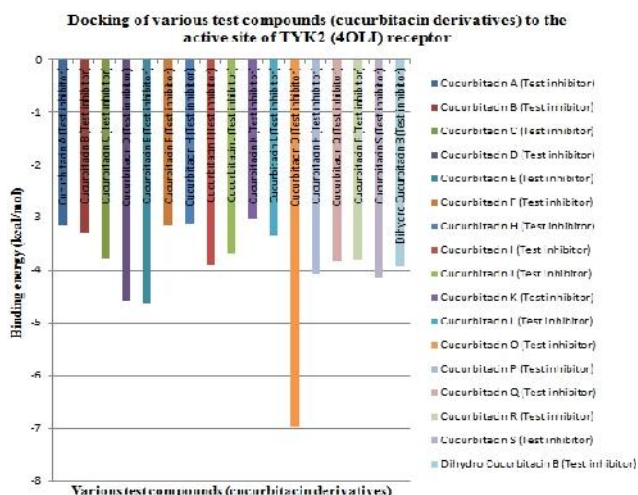

Figure 9 Docking of standard compound (Bafetinib has lowest maximum binding energy for stable binding to the active site of TYK2 [4OLI] receptor) to the active site of TYK2 (4OLI) receptor. [Total 3 Hydrogen-bonds: BAFETINIB: HG1 - A: GLY761: O, BAFETINIB: HG1 - A: ALA654: O, BAFETINIB: HG1 - A: ASN789: OG].
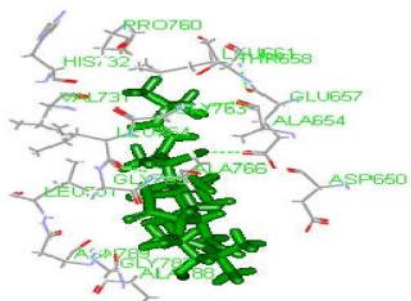
Docking of various test compounds (cucurbitacin derivatives) to the active site of TYK2 (4OLI) receptor (Figure 10)

Table 5 Docking of various test (cucurbitacin) compounds to the active site of TYK2 (4OLI) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for TYK2 (4OLI) receptor]

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Cucurbitacin A (Test inhibitor)	-3.142	428.0
2.	Cucurbitacin B (Test inhibitor)	-3.290	399.8
3.	Cucurbitacin C (Test inhibitor)	-3.778	407.4
4.	Cucurbitacin D (Test inhibitor)	-4.589	427.1
5.	Cucurbitacin E (Test inhibitor)	-4.615	411.5
6.	Cucurbitacin F (Test inhibitor)	-3.140	473.5
7.	Cucurbitacin H (Test inhibitor)	-3.112	416.3
8.	Cucurbitacin I (Test inhibitor)	-3.890	411.4
9.	Cucurbitacin J (Test inhibitor)	-3.682	378.3
10.	Cucurbitacin K (Test inhibitor)	-3.025	387.8
11.	Cucurbitacin L (Test inhibitor)	-3.339	473.2
12.	**Cucurbitacin O (Test inhibitor)**	**-6.976**	402.6
13.	Cucurbitacin P (Test inhibitor)	-4.061	456.2
14.	Cucurbitacin Q (Test inhibitor)	-3.816	383.2
15.	Cucurbitacin R (Test inhibitor)	-3.792	394.5
16.	Cucurbitacin S (Test inhibitor)	-4.144	385.0
17.	Dihydro Cucurbitacin B (Test inhibitor)	-3.903	403.6

Figure 10 Graphical representation for docking of various test compounds to the active site of TYK2 (4OLI) receptor [Cucurbitacin O has lowest maximum binding energy for stable binding to the active site of TYK2 [4OLI] receptor]

Figure 11 Docking of test compound (Cucurbitacin O has lowest maximum binding energy for stable binding to the active site of TYK2 [4OLI] receptor) to the active site of TYK2 (4OLI) receptor [Total 6 Hydrogen-bonds: CUCURBITACIN O: HG1 - A: GLY761: O, CUCURBITACIN O: HN1 - A: ALA654: O, CUCURBITACIN O: HN2 - A: LEU764: O, CUCURBITACIN O: HN1 - A: GLY787: O, CUCURBITACIN O: H61 - A: GLU763: OE1, CUCURBITACIN O: HN1 - A: ASN789: O]

Prediction of ADMET (Absorption, Distribution, Metabolism, Excretion & Toxicity) properties

Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Table 6 Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Name of compound	BBB (Blood brain barrier) level	Absorption level	Solubility level	Hepatotoxicity level	LogP (Must be less than 5)
Cucurbitacin O	Undefined	Good	Good	Non-toxic	2.032

Prediction of FDA (Food & drug administration) male & female rat carcinogenicity of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Table 7 Prediction of male & female rat carcinogenicity of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor by FDA (Food & drug administration)

Name of compound	Prediction of carcinogenicity for male rat	Prediction of carcinogenicity for female rat	Prediction of Mutagenicity
Cucurbitacin O	Non-carcinogen	Non-carcinogen	Non-mutagen

Prediction of NTP (National toxicology programme) male & female rat carcinogenicity of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Table 8 Prediction of male & female rat carcinogenicity of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor by NTP (National toxicology programme)

Name of compound	Prediction of carcinogenicity for male rat	Prediction of carcinogenicity for female rat	Prediction of Mutagenicity
Cucurbitacin O	Non-carcinogen	Non-carcinogen	Non-mutagen

Prediction of all components for fulfilling Lipinski's rule of best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Table 9 Prediction of all components for fulfilling Lipinski's rule of best docked best docked test molecule (Cucurbitacin O) against TYK2 (4OLI) receptor

Name of compound	Molecular weight [gms] (not greater than 500 daltons or 800 gms)	Partition coefficient [LogP] (not greater than 5)	Number of total hydrogen bond donors (not greater than 5)	Number of total hydrogen bond acceptors (not greater than 10)
Cucurbitacin O	550.63 gms	2.032	3	6

CONCLUSION

Cucurbitacin O (test compounds) had lowest maximum binding energy (-6.976) [Table 5] with total 6 hydrogen bonds than best docked standard compounds Bafetinib [Table 4] {binding energy (-5.367) with total 3 hydrogen bonds} for stable binding of Cucurbitacin O to the active site of TYK2 receptor & was highly active against TYK2 receptor than various standard compounds. Cucurbitacin O against TYK2 receptor was non-toxic (Table 6), non-carcinogenic (Table 7 & Table 8) for both male & female rat, non-mutagenic (Table 7 & Table 8) after

prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties & was fulfilled all components of Lipinski's rule (Table 9).

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