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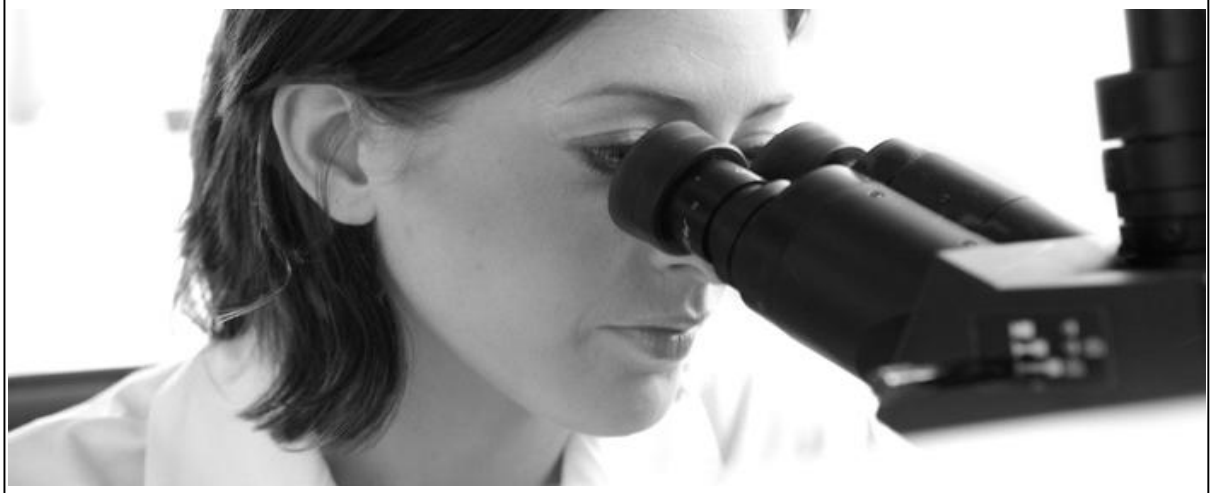

Somenath Bhattacharya

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RESEARCH ARTICLE

QSAR MODELLING & VALIDATION OF PHARMACOPHORES WITH REFERENCE TO THEIR ANTI-CANCER ACTIVITY AGAINST JAK2 RECEPTOR USING IN SILICO DRUG DESIGNING TOOLS

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ABSTRACT

Cancer is abnormal cells division without control & is able to invade other tissues. Cancer cells can spread to other parts of the body through the blood or lymph systems. Major cancer therapy (basically lung colorectal, breast & prostate cancer) approaches that directly target receptor (JAK2). Cucurbitacin derivatives are class of biochemical compounds (highly oxidized tetracyclic triterpenoids) that some plants - members of the family cucurbitaceae, that includes the common pumpkins & gourds. In the last few years, cucurbitacin derivatives had been shown to inhibit proliferation & induced apoptosis utilizing a long array of *in vitro* & *in vivo* cancer cell models. The three-dimensional structure of a potential drug (ligand) on its possible target site is superimposed by docking. Docking programs operate by placing the ligand in the target area & then attempting to orientate the ligand so that its binding groups line up with the complementary groups of the target. Here, molecular docking, receptor-ligand interactions, binding energy calculations, pharmacophores modelling, pharmacophores-based screening, scaffolds designing, various molecular descriptors calculations & Quantitative structure activity relationship (QSAR) predictions were employed in a screening strategy to identify inhibitors for JAK2 receptor.

INTRODUCTION

Cancer is one of the most prevalent disease in many countries worldwide. Cancer can be generally described as an uncontrolled growth and spread of abnormal cells in the body. Cells are basic units of life. All organisms are composed of one or more cells. Normally, cells divide to produce more cells only when the body needs them [(1)]. Sometimes cells keep dividing & thus creating more cells even when they are not needed. When this happens, a mass of tissue forms, this mass of extra tissue is called a tumor. Tumors are found in all kinds of tissue & can be benign or malignant. Cancer is not a single disease. It is a group of more than 200 different diseases [(1)].The exact cause of the disease remains enigmatic, but inhibition of the defence mechanism responsible for the elimination of disturbed cells is generally accepted as a background of carcinogenesis. The multidrug-resistance (MDR) [(2)] of tumor cells to chemotherapeutic agents is a major problem in the clinical treatment of cancer. MDR is defined as the ability of malignant cells exposed to chemotherapeutics to develop resistance to a broad range of drugs due to the members of the ATP (adenosine triphosphate) binding cassette proteins. The search for novel anti-cancer agents currently targets chemical entities that selectively induce apoptosis or reverse MDR [(2)]. Approximately 60% of all drugs now undergoing clinical trials for the multiplicity of cancers are either natural products or compounds derived from natural sources [(2)]. Cancer increases continuously due to increased life span & growth of population. The international agency for research on different types of cancers (IARC) produced 'GLOBOCAN' in 2008 which provided the most accurate assessment of the global cancer burden & showed that a majority of the 12.7 million new cases of cancer (both sexes) & the 7.6 milllion cancer deaths (both sexes) worldwide occurred in developing countries [(3)].

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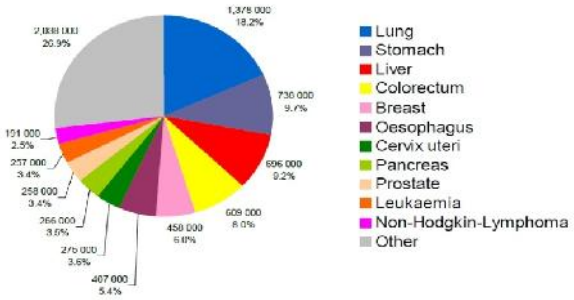

Figure 1 Global burden of cancer deaths worldwide occurred in developing countries per year [3]

Cucurbitacins are the class of highly oxidized tetracyclic triterpenoids. Cucurbitacins are chemically classified as steroids, formally derived from cucurbitane, a triterpene hydrocarbon-specifically, from the unsaturated variant cucurbita-5-ene [4]. Natural & semisynthetic cucurbitacins show promising anti-cancer activities ranging from anti-proliferation. Cucurbitacins were extracted from the cucurbitaceae family including the common pumpkins, citrus & gourds such as Trichosanthes cucumerina (snake gourd), Trichosanthes kirilowii (chinese cucumber) etc.

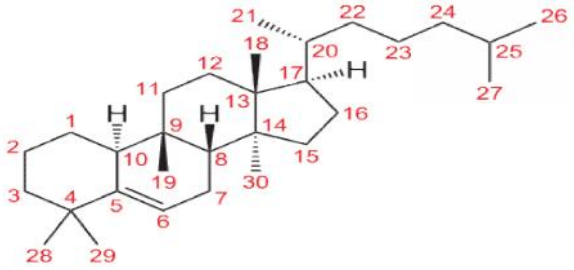

Figure 2 General structure of cucurbitacin skeleton with numbering [4]

Selection of Target: Cucurbitacin derivatives are hypothesized to be selective inhibitors of the JAK-STAT pathway [5].

JAK2: Janus kinase 2 (commonly called JAK2) is a non-receptor tyrosine kinase. It is a member of the Janus kinase family & has been implicated in signaling by members of the type II cytokine receptor family (Example: Interferon receptors) [5].

Mechanism of JAK-STAT pathway & selection of cytokine (interferon, interleukin) as an activator: The JAK-STAT pathway [5] [Figure 3] is involved in responses to many cytokines. Dimerisation of these receptors occurs when the cytokine binds & this attracts a cytosolic tyrosine kinase unit (JAK2) to associate with phosphorylate, the receptor dimer. Among the targets for phosphorylation by JAK2 is a family of transcription factors (STATS).

These are SH2-domain proteins that bind to the phosphotyrosine groups on the receptor JAK2 complex & are themselves phosphorylated [5]. Thus activated STAT (signal transducer & activator of transcription) migrates to the nucleus & activates gene expression. Gene transcription creates some oncogenes to develop cancers ((specially lung colorectal, breast & prostate cancer).

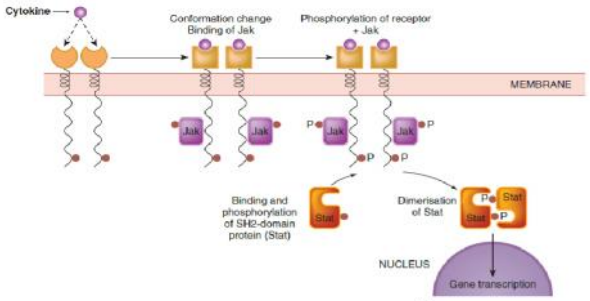

Figure 3 JAK-STAT Pathway [5]

Bioinformatics Tools

Soft wares

Auto dock tools**,** Accelrys Discovery studio 3.5 visualizer**,** UCSF Chimera 1.10**,** Ligand Scout 3.12**,** Rasmol**,** Chem utra draw 12 + serial**,** Chem sketch**,** Marvin sketch**,** Chem T**,** Accelrys draw 4.2**,** Padel-descriptor**,** NCSS 10**,** Analysing It.

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. Pharmagist: (http://bioinfo3d.cs.tau.ac.il/PharmaGist/)
6. ZINC pharmer: (zincpharmer.csb.pitt.edu/)
7. ALOGPS: (www.vcclab.org/lab/alogps/)
8. Clustal omega: (www.ebi.ac.uk/tools/msa/clustalw2/)
9. E-dragon: (www.vcclab.org/lab/e-dragon/)
10. Molfeat: (jing.cz3.nus.edu.sg/cgi-bin/molfeat2012/m old el. cgi/)
11. Model:(jing.cz3.nus.edu.sg/cgi-bin/model2012/molfe at.cgi/)

METHODS

1. From previously published articles, selection of diseases (specially lung, colorectal, breast & prostate cancer) based on current global data on percentage of death records in every year according to 'GLOBOCAN' [3] in 2008 produced by the international agency for research on all cancers in every year in all developing countries & selection of various target (protein or receptor) due to which these diseases were occurred.
2. From previously published articles, various standard compounds (activators or inhibitors) were selected for target (JAK2). The 3D (three-dimensional) structures of these compounds were downloaded from pubchem web server.
3. From previously published articles, various test compounds (different cucurbitacin derivatives) were selected based on their activation against the target [JAK2]. The 3D structures (three-dimensional) of selected targets were downloaded from the official web server of protein data bank.
4. Conformations of downloaded ligands (standard & test compounds) & conformations of downloaded proteins

were generated by using discovery studio 2.5 visualizer software.

5. Docking sites of each receptor were identified by using ligplot from which the amino acid residues & coordinates were obtained for crystal complex protein by using PDBsum web server.
6. Docking of standard compounds was done to the active site of JAK2 (3FUP) receptor.
7. Docking of test compounds was done to check the binding of different test compounds (cucurbitacin derivatives) to the activator attachment site of JAK2 (3FUP) receptor.
8. Best docked standard molecules with different sets were selected for generation of pharmacophores according to lowest maximum binding energy suitable for creating many hydrogen bonds for binding of various ligands to the active site of JAK2 (3FUP) receptor.
9. The features of the prepared pharmacophores of standard molecules with analyses of features cost were prepared by using pharmagist web server.
10. Test compounds (different cucurbitacin derivatives) were fitted with pharmacophores of various standard compounds for JAK2 (3FUP) receptor by using ligandscout 3.12.
11. According to highest cost difference of pharmacophores of various standard compounds, highest correlation coefficient of pharmacophore of various standard compounds, best fitted highest score of maximum test compounds (different cucurbitacin derivatives) with pharmacophores of various standard compounds, pharmacophores of various standard compounds were selected for JAK2 (3FUP) receptor.
12. Scaffold molecules were also generated based on mainly pharmacophoric features of various standard compounds for JAK2 (3FUP) receptor by using chem ultra draw software.
13. Scaffold molecules were also docked to the active site of JAK2 (3FUP) receptor.
14. Various substitutions with scaffold structure were also added by using chem ultra draw software to check the docking of substituted scaffold molecules to the active site of JAK2 (3FUP) receptor.
15. Different molecular descriptors of best docked substituted scaffold molecules to the active site of JAK2 (3FUP) receptor were also calculated by using padel-descriptors software & the multinomial graphs were plotted with correlation coefficients (R^2), standard error (SE) & equation by using analysing it software for QSAR (quantitative structure-activity relationship) analysis of JAK2 (3FUP) receptor.
16. Best docked substituted scaffold molecule (with lowest maximum binding energy for JAK2 receptor) was selected for prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity properties), carcinogenicity, mutagenicity & components for fulfilling Lipinski's [(7)] rule.

RESULTS AND DISCUSSION

Structure of downloaded JAK2 (3FUP) receptor (protein) from official website of protein data bank

Receptor (protein) code of JAK2: 3FUP
Classification: Transferase
Structure weight: 69810.15 kDa
Source organism: *Homo sapiens*
Type: Crystal complex structure

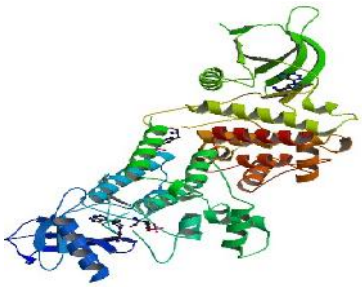

Figure 4 Downloaded structure of JAK2 (3FUP) receptor (ribbon structure) from website of protein data bank

Structure of active site of prepared JAK2 (3FUP) receptor (protein)

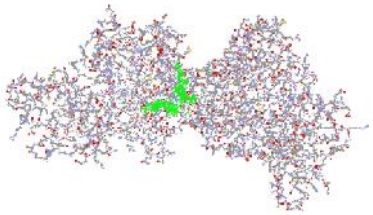

Figure 5 Structure of active site of prepared receptor JAK2 (3FUP) [In this figure, the green portion represents active site for JAK2 (3FUP) receptor]

Coordinates (X, Y & Z) & volume or size of the active site of JAK2 (3FUP) receptor (protein) for binding of each ligand

Table 1 Coordinate & volume or size of active site of JAK2 (3FUP) receptor (protein) [The volume or size of active site of JAK2 (3FUP) receptor must be greater than the volume of the each ligand]

Name of the receptor (protein)	Code	X	Y	Z	Volume or size of the active site (Å)
JAK2	3FUP	-53.325	34.539	17.395	712.125

Docking of various standard compounds to the active site of JAK2 (3FUP) receptor (Figure 6)

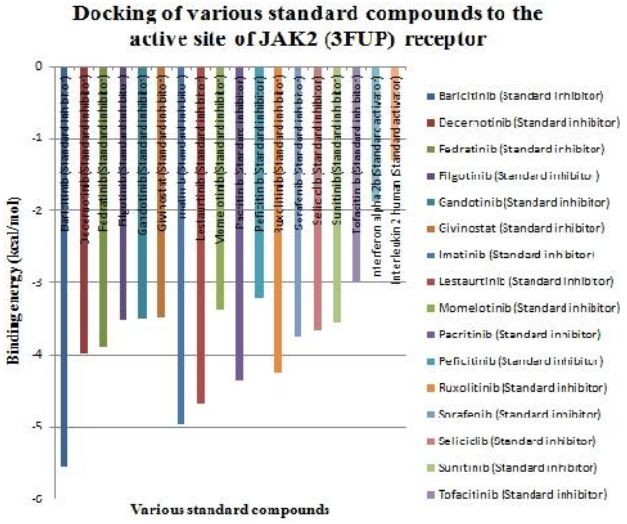

Figure 6 Graphical representation for docking of various standard compounds to the active site of JAK2 (3FUP) receptor [Baricitinib has lowest maximum binding energy for stable binding to the active site of JAK2 [3FUP] receptor]

Table 2 Docking of various standard compounds to the active site of JAK2 (3FUP) receptor [In this table, the deep black colour row represents lowest binding energy of ligands (for both standard compounds & test compounds) for JAK2 (3FUP) receptor]

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	**Baricitinib (Standard inhibitor)**	**-5.561**	304.6
2.	Decernotinib (Standard inhibitor)	-3.978	337.2
3.	Fedratinib (Standard inhibitor)	-3.877	351.3
4.	Filgotinib (Standard inhibitor)	-3.514	301.4
5.	Gandotinib (Standard inhibitor)	-3.501	321.3
6.	Givinostat (Standard inhibitor)	-3.485	420.5
7.	Imatinib (Standard inhibitor)	-4.964	436.2
8.	Lestaurtinib (Standard inhibitor)	-4.679	356.3
9.	Momelotinib (Standard inhibitor)	-3.364	426.0
10.	Pacritinib (Standard inhibitor)	-4.356	412.6
11.	Peficitinib (Standard inhibitor)	-3.210	389.6
12.	Ruxolitinib (Standard inhibitor)	-4.258	271.7
13.	Sorafenib (Standard inhibitor)	-3.741	348.9
14.	Seliciclib (Standard inhibitor)	-3.654	318.3
15.	Sunitinib (Standard inhibitor)	-3.547	345.9
16.	Tofacitinib (Standard inhibitor)	-2.998	276.7
17.	Interferon alpha 2b (Standard activator)	-1.874	320.0
18.	Interleukin 2 human (Standard activator)	-1.296	414.2

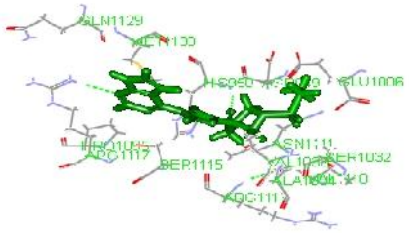

Figure 7 Docking of standard compound (Baricitinib has lowest maximum binding energy for stable binding to the active site of JAK2 [3FUP] receptor) to the active site of JAK2 (3FUP) receptor

Docking of various test compounds (cucurbitacin derivatives) to the active site of JAK2 (3FUP) receptor (Figure 8)

Table 3 Docking of various test (cucurbitacin) compounds to the active site of JAK2 (3FUP) receptor [In this table, the deep black colour row represents lowest binding energy of ligands (for both standard compounds & test compounds) for JAK2 (3FUP) receptor]

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Cucurbitacin A (Test inhibitor)	-4.565	428.0
2.	Cucurbitacin B (Test inhibitor)	-3.645	399.8
3.	Cucurbitacin C (Test inhibitor)	-3.847	407.4
4.	Cucurbitacin D (Test inhibitor)	-3.794	427.1
5.	Cucurbitacin E (Test inhibitor)	-2.986	411.5
6.	Cucurbitacin F (Test inhibitor)	-3.075	473.5
7.	Cucurbitacin H (Test inhibitor)	-4.317	416.3
8.	Cucurbitacin I (Test inhibitor)	-4.254	411.4
9.	Cucurbitacin J (Test inhibitor)	-3.917	378.3
10.	Cucurbitacin K (Test inhibitor)	-3.569	387.8
11.	**Cucurbitacin L (Test inhibitor)**	**-5.854**	473.2
12.	Cucurbitacin O (Test inhibitor)	-3.078	402.6
13.	Cucurbitacin P (Test inhibitor)	-3.966	456.2
14.	Cucurbitacin Q (Test inhibitor)	-3.014	383.2
15.	Cucurbitacin R (Test inhibitor)	-3.441	394.5
16.	Cucurbitacin S (Test inhibitor)	-3.412	385.0
17.	Dihydro Cucurbitacin B (Test inhibitor)	-2.845	403.6

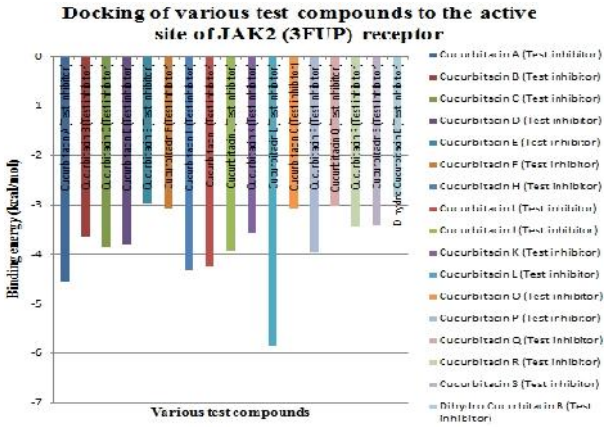

Figure 8 Graphical representation for docking of various test compounds to the active site of JAK2 (3FUP) receptor [Cucurbitacin L has lowest maximum binding energy for stable binding to the active site of JAK2 [3FUP] receptor]

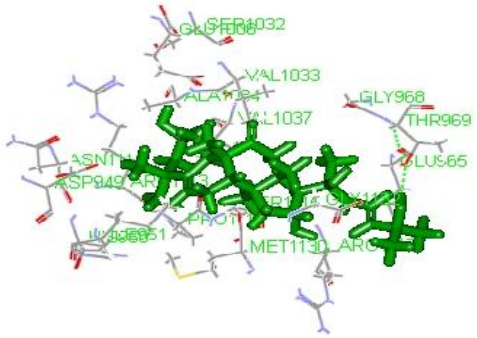

Figure 9 Docking of test compound (Cucurbitacin L has lowest maximum binding energy for stable binding to the active site of JAK2 [3FUP] receptor) to the active site of JAK2 (3FUP) receptor

Preparation of pharmacophores of various standard compounds for JAK2 (3FUP) receptor prepared by using pharmagist web server

Set: Decernotinib, Fedratinib, Sorafenib, Seliciclib & Sunitinib (Divide these compounds according to the range of binding energy)

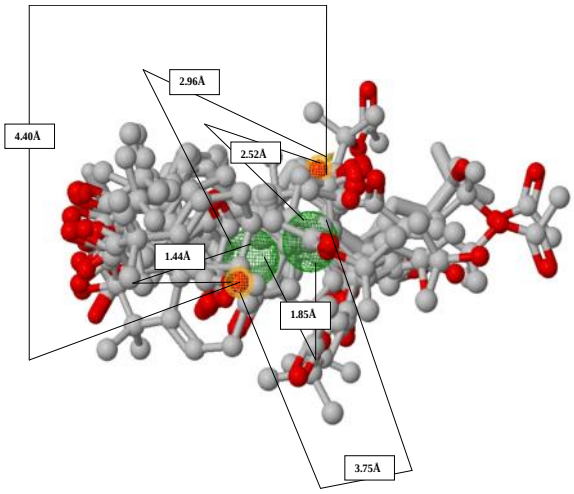

Figure 10 Pharmacophores with distances between various features (having highest score: 66.514 & maximum fitted scores with various test or cucurbitacin compounds) of various standard compounds for JAK2 (3FUP) receptor prepared by using pharmagist web server

Coordinates (X, Y & Z) & radius of pharmacophores of various standard compounds prepared by using pharmagist web server for JAK2 (3FUP) receptor

Table 4 Coordinates (X, Y & Z) & radius of pharmacophores of various standard compounds prepared by using pharmagist web server for JAK2 (3FUP) receptor

Sl. No.	Name of the pharmacophoric feature	Colour of the pharmacophoric feature	X	Y	Z	Radius
1	Hydrogen bond acceptor	Orange	-0.32	-2.00	5.21	0.50
2	Hydrogen bond acceptor	Orange	-1.36	4.41	-3.69	0.50
3	Hydrophobic	Green	0.50	-3.14	-2.50	1.10
4	Hydrophobic	Green	-3.34	1.00	-1.25	1.10

Cost analysis of this pharmacophoric model of various standard compounds generated for JAK2 (3FUP) receptor

Table 5

Null cost	Total cost (error cost+weigh cost+configure cost)	Fixed cost	Correlation coefficient	Error cost	Weigh cost	Configure cost	Cost difference (total cost-nullcost)
13.0254	66.3254	38.5214	0.944122	24.2547	6.33214	30.58684	53.3

Cost analysis of pharmacophores of various standard compounds for JAK2 (3FUP) receptor [According to the highest cost difference pharmacophores of various standard compounds, highest correlation coefficient pharmacophores of standard compounds, highest pharmacophores fit score (in bits) of maximum cucurbitacins derivatives (If the fit score of maximum cucurbitacins derivatives are in between 40-60 bits, there is an 80-95% chance that cucurbitacins derivatives fit with the pharmacophores of various standard compounds & if the fit score of maximum test compounds (different cucurbitacin derivatives) are more than 60 bits, there is greater than 95% chance that test compounds (different cucurbitacin derivatives) fit with the pharmacophores of various standard compounds, the pharmacophores of various standard compounds were selected for preparation of scaffold molecule for selected receptor]

represents the highest fit score of different cucurbitacin derivatives)

Preparation of scaffold molecule with different substitution sites for JAK2 (3FUP) receptor

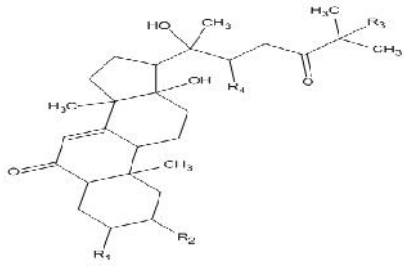

Figure 11 Preparation of scaffold molecule with diffferent substitutions sites for JAK2 (3FUP) receptor

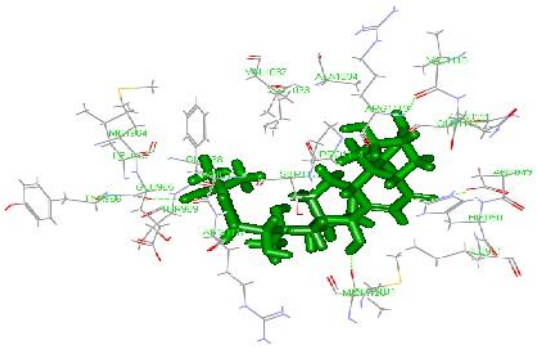

Figure 12 Docking of best docked substituted scaffold molecules (Figure 11: R_1 = OH, R_2 = OH, R_3 = OH & R_4 = $OCOCH_3$ has lowest maximum binding energy for proper binding to the active site of JAK2 [3FUP] receptor) to the active site of JAK2 (3FUP) receptor

Pharmacophores fit (matching) score of test compounds (different cucurbitacin derivatives) with pharmacophores of standard compounds by using ligandscout 3.12 software for JAK2 (3FUP) receptor

Sl. No.	Name of test compound	Pharmacophores fit score (Must be greater than 55)
1	**Cucurbitacin C**	**61.7500**
2	Cucurbitacin B	61.4800
3	Cucurbitacin E	61.4400
4	Cucurbitacin J	61.2000
Sl. No.	**Name of test compound**	**Pharmacophores fit score (Must be greater than 55)**
5	Cucurbitacin L	61.1200
6	Cucurbitacin A	60.1900
7	Cucurbitacin Q	60.0000
8	Cucurbitacin K	59.1500
9	Cucurbitacin P	59.1200
10	Cucurbitacin O	58.8900
11	Cucurbitacin S	58.4700
12	Cucurbitacin R	58.2300
13	Cucurbitacin D	58.1400
14	Cucurbitacin I	58.0000
15	Cucurbitacin F	57.1478
16	Cucurbitacin H	57.1000
17	Dihydro Cucurbitacin B	56.9600

Table 6 Pharmacophores fit score of test compounds (different cucurbitacin derivatives) with pharmacophores of various standard compounds for JAK2 (3FUP) receptor by using ligandscout 3.12 (In this above table, deep black colour row

Docking of different substituted scaffold molecules to the active site of JAK2 (3FUP) receptor

Table 7 Calculation of different molecular descriptors of different substituted scaffold molecules for JAK2 (3FUP) receptor [In this table, the deep black colour row represents lowest binding energy of ligands for JAK2 receptors]

Sl. No.	R_1	R_2	R_3	R_4	Binding energy (kcal/mol)	Molecular weight (gms) Must be 800 gms or 500 daltons	Volume (Å)
1	OH	OH	OH	OH	-5.011	494.62	407.4
2	OH	OH	OH	OCH_3	-5.114	508.64	325.6
3	**OH**	**OH**	**OH**	**$OCOCH_3$**	**-6.210**	**536.65**	**357.0**
4	OH	OH	OH	CH_3	-4.154	492.64	366.2
5	OH	OH	OH	COOH	-3.336	522.63	378.2
6	OH	OH	OH	H	-3.114	478.62	371.4
7	OH	OH	OH	NH_2	-3.025	493.63	359.3
8	OH	OH	OH	$C_6H_6O_2$	-2.685	586.71	415.2
9	OH	OH	OH	OC_2H_5	-2.147	522.67	420.3
10	OH	OH	OH	Cl	-3.014	513.06	400.1
11	OH	OH	OH	Br	-3.658	557.51	419.3
12	OH	OH	OH	I	-3.225	604.51	444.4
13	OH	OH	OH	F	-3.147	496.61	382.3
14	OH	OH	OH	NO_2	-3.347	523.62	400.2
15	OH	OCH_3	OH	OH	-5.510	508.64	321.6
16	OH	$OCOCH_3$	OH	OH	-5.010	536.65	380.0
17	OH	CH_3	OH	OH	-4.574	492.64	378.2
18	OH	COOH	OH	OH	-3.478	522.63	370.3
19	OH	H	OH	OH	-3.014	478.62	396.3
20	OH	NH_2	OH	OH	-3.205	493.63	351.3
21	OH	$C_6H_6O_2$	OH	OH	-2.557	586.71	410.3
22	OH	OC_2H_5	OH	OH	-3.168	522.67	420.3
23	OH	Cl	OH	OH	-3.617	513.06	412.3
24	OH	Br	OH	OH	-3.644	557.51	418.3
25	OH	I	OH	OH	-3.368	604.51	443.6
26	OH	F	OH	OH	-3.074	496.61	388.3
27	OH	NO_2	OH	OH	-3.149	523.62	401.3
28	OCH_3	OH	OH	OH	-5.368	508.64	321.6
29	$OCOCH_3$	OH	OH	OH	-5.974	536.65	368.5
30	CH_3	OH	OH	OH	-4.017	492.64	316.2
31	COOH	OH	OH	OH	-3.324	522.63	355.4
32	H	OH	OH	OH	-3.650	478.62	378.6
33	NH_2	OH	OH	OH	-3.621	493.63	374.5
34	$C_6H_6O_2$	OH	OH	OH	-2.671	586.71	410.3
Sl. No.	**R_1**	**R_2**	**R_3**	**R_4**	**Binding energy (kcal/mol)**	**Molecular weight (gms) Must be 800 gms or 500 daltons**	**Volume (Å)**
35	OC_2H_5	OH	OH	OH	-2.357	522.67	436.2
36	Cl	OH	OH	OH	-3.380	513.06	394.7
37	Br	OH	OH	OH	-3.887	557.51	422.3
38	I	OH	OH	OH	-3.489	604.51	415.3
39	F	OH	OH	OH	-3.174	496.61	371.4
40	NO_2	OH	OH	OH	-3.104	523.62	402.4
41	OH	OH	OCH_3	OH	-5.541	508.64	315.8
42	OH	OH	$OCOCH_3$	OH	-5.814	536.65	366.0
43	OH	OH	CH_3	OH	-4.770	492.64	389.0
44	OH	OH	COOH	OH	-3.745	522.63	380.1
45	OH	OH	H	OH	-3.789	478.62	386.4
46	OH	OH	NH_2	OH	-3.632	493.63	368.4
47	OH	OH	$C_6H_6O_2$	OH	-2.588	586.71	445.1
48	OH	OH	OC_2H_5	OH	-3.128	522.67	404.5
49	OH	OH	Cl	OH	-3.478	513.06	397.6
50	OH	OH	Br	OH	-3.648	557.51	427.5
51	OH	OH	I	OH	-3.581	604.51	430.7
52	OH	OH	F	OH	-3.630	496.61	398.9
53	OH	OH	NO_2	OH	-3.027	523.62	400.5
54	CH_3	CH_3	CH_3	OH	-5.063	488.70	403.8
55	CH_3	CH_3	CH_3	OCH_3	-5.022	502.73	328.9
56	CH_3	CH_3	CH_3	$OCOCH_3$	-6.045	530.74	342.3
57	CH_3	CH_3	CH_3	CH_3	-4.620	486.73	390.5
58	CH_3	CH_3	CH_3	COOH	-3.356	516.71	318.8
59	CH_3	CH_3	CH_3	H	-3.368	472.70	350.3
60	CH_3	CH_3	CH_3	NH_2	-3.145	487.71	377.4
61	CH_3	CH_3	CH_3	$C_6H_6O_2$	-2.026	580.79	372.3
62	CH_3	CH_3	CH_3	OC_2H_5	-3.168	516.75	384.5
63	CH_3	CH_3	CH_3	Cl	-3.255	507.14	360.5
64	CH_3	CH_3	CH_3	Br	-3.346	551.60	440.2
65	CH_3	CH_3	CH_3	I	-3.229	598.60	400.1
66	CH_3	CH_3	CH_3	F	-3.186	490.69	396.9
67	CH_3	CH_3	CH_3	NO_2	-3.001	517.70	408.5
68	CH_3	OH	CH_3	CH_3	-4.963	488.70	410.2

Sl. No.	R_1	R_2	R_3	R_4	Binding energy (kcal/mol)	Molecular weight (gms) Must be 800 gms or 500 daltons	Volume (Å)
69	CH_3	OCH_3	CH_3	CH_3	-4.935	502.73	342.3
70	CH_3	$OCOCH_3$	CH_3	CH_3	-5.368	530.74	350.4
71	CH_3	COOH	CH_3	CH_3	-4.562	516.71	310.3
72	CH_3	H	CH_3	CH_3	-4.014	472.70	347.5
73	CH_3	NH_2	CH_3	CH_3	-3.650	487.71	368.3
74	CH_3	$C_6H_6O_2$	CH_3	CH_3	-2.120	580.79	371.5
75	CH_3	OC_2H_5	CH_3	CH_3	-3.000	516.75	380.3
76	CH_3	Cl	CH_3	CH_3	-3.063	507.14	387.2
77	CH_3	Br	CH_3	CH_3	-3.125	551.60	414.2
78	CH_3	I	CH_3	CH_3	-3.235	598.60	400.1
79	CH_3	F	CH_3	CH_3	-3.152	490.69	396.9
80	CH_3	NO_2	CH_3	CH_3	-3.998	517.70	407.3
81	OH	CH_3	CH_3	CH_3	-4.952	488.70	405.3
82	OCH_3	CH_3	CH_3	CH_3	-4.856	502.73	350.3
83	$OCOCH_3$	CH_3	CH_3	CH_3	-4.841	530.74	364.5
84	COOH	CH_3	CH_3	CH_3	-3.952	516.71	313.6
85	H	CH_3	CH_3	CH_3	-3.632	472.70	340.5
86	NH_2	CH_3	CH_3	CH_3	-3.586	487.71	372.3
87	$C_6H_6O_2$	CH_3	CH_3	CH_3	-2.441	580.79	365.3
88	OC_2H_5	CH_3	CH_3	CH_3	-3.126	516.75	361.2
89	Cl	CH_3	CH_3	CH_3	-3.268	507.14	374.3
90	Br	CH_3	CH_3	CH_3	-3.065	551.60	405.6
91	I	CH_3	CH_3	CH_3	-3.428	598.60	398.6
92	F	CH_3	CH_3	CH_3	-5.025	490.69	385.6
93	NO_2	CH_3	CH_3	CH_3	-3.271	517.70	408.9
94	CH_3	CH_3	OH	CH_3	-4.820	488.70	406.3
95	CH_3	CH_3	OCH_3	CH_3	-4.210	502.73	355.3
96	CH_3	CH_3	$OCOCH_3$	CH_3	-3.632	530.74	361.3
97	CH_3	CH_3	COOH	CH_3	-3.456	516.71	323.3
98	CH_3	CH_3	H	CH_3	-3.996	472.70	337.8
99	CH_3	CH_3	NH_2	CH_3	-3.323	487.71	360.2
100	CH_3	CH_3	$C_6H_6O_2$	CH_3	-2.358	580.79	378.3
101	CH_3	CH_3	OC_2H_5	CH_3	-3.329	516.75	396.3
102	CH_3	CH_3	Cl	CH_3	-3.415	507.14	374.2
103	CH_3	CH_3	Br	CH_3	-3.420	551.60	390.3
104	CH_3	CH_3	I	CH_3	-3.262	598.60	392.2
105	CH_3	CH_3	F	CH_3	-3.133	490.69	386.3
106	CH_3	CH_3	NO_2	CH_3	-0.845	517.70	407.1

QSAR studies (Hansch analysis): multinomial graph plotted of activity binding energy vs various percent of values of molecular descriptors of best docked substituted scaffold molecules (Figure 11 & Table 7) for JAK2 (3FUP) receptor:

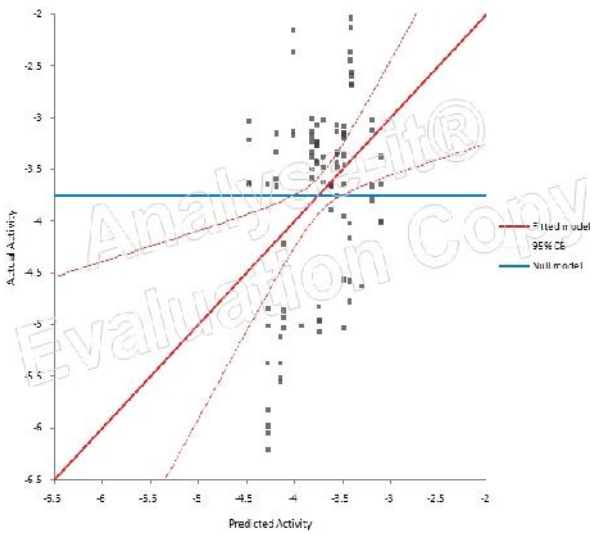

Figure 13 Multinomial graph of Hansch analysis for JAK2 (3FUP) receptor

From this graph, R^2 (correlation coefficient) = 0.614, n (total number of compounds) = 98, SE (standard error) = 0.182, F (Fischer test) = 31.66 & p (power test) = 0.0001

Equation: Activity = 40.15 + 2.851 LogP (partition coefficient) + 2.921 Hammett constant (electronic parameter) + 0.02465 Molar refractivity (steric parameter) Here, CB means confidence band.

For QSAR studies of natural or herbal compounds, the R^2 (correlation coefficient) value greater than 0.50 or r values (correlation coefficient) greater than 0.60 were usually regarded as representing an acceptable degree of accuracy provided that they were obtained using a reasonable number of results with a suitable standard deviation. This shown that 80% of the data are now satisfactorily accounted for by the chosen parameters Ideally for QSAR (quantitative structure-activity relationship) studies, SE (standard error) value was as low as possible.

Reasons for selection of Hansch analysis than any other analytical method for QSAR (quantitative structure-activity relationship) studies: From previously published articles, the Hansch analysis [(10)] has many advantages rather than any other analytical method such as, Free Wilson analysis –

1. Easy in rationalizing results & explaining why a substituent at a particular position is good or bad for activity.
2. Effect of all substituents may be additive.
3. Simple equation was generated to make more meaningful equation.

4. Ii was very quick & fast process rather than any other analytical method.

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

Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked substituted scaffold molecule for JAK2 (3FUP) receptor (substituted scaffold molecule - Figure 11: R_1 = OH, R_2 = OH, R_3 = OH & R_4 = $OCOCH_3$)

Table 8 Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked substituted scaffold molecule for JAK2 (3FUP) receptor

Name of compound	BBB (Blood brain barrier) level	Absorption level	Solubility level	Hepatotoxicity level	LogP (Must be less than 5)
Scaffold (JAK2)	Undefined	Good	Good	Non-toxic	3.252

Prediction of FDA (Food & drug administration) male & female rat carcinogenicity of best docked substituted scaffold molecule for JAK2 (3FUP) receptor (substituted scaffold molecule - Figure 11: R_1 = OH, R_2 = OH, R_3 = OH & R_4 = $OCOCH_3$)

Table 9 Prediction of male & female rat carcinogenicity of best docked substituted scaffold molecule for JAK2 (3FUP) receptor by FDA (Food & drug administration)

Name of compound	Prediction of carcinogenicity for male rat	Prediction of carcinogenicity for female rat	Prediction of Mutagenicity
Scaffold (JAK2)	Non-carcinogen	Non-carcinogen	Non-mutagen

CONCLUSION

From the above discussed topics it is clear that cancers (specially lung, colorectal, breast & prostate cancer) in now-a-days most severe diseases for whole world cause the highest extent of mortality according to the global data of 'GLOBOCAN' in 2008 produced by the international agency for research (IARC) on different types of cancers. These types of cancers are potentially fatal disease caused mainly by environmental factors that mutate genes encoding critical cell-regulatory receptors or proteins.

binding energy (-6.210) for stable binding of this molecule to the active site of JAK2 receptor & was highly active against JAK2 receptor than various standard drugs.

In QSAR (quantitative structure-activity relationship) studies, for Hansch analysis (Figure 13) of substituted scaffold molecules (Figure 11 & Table 7) for JAK2 receptor including R^2 (correlation coefficient) = 0.614 & SE (standard error) = 0.182, the data was acceptably accounted for by the chosen parameters. Substituted scaffold molecule for JAK2 receptor (Figure 11: R_1 = OH, R_2 = OH, R_3 = OH & R_4 = $OCOCH_3$) was non-toxic (Table 8), non-carcinogenic (Table 9) for both male & female rat, non-mutagenic (Table 9) after prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties & was fulfilled all components of Lipinski's rule (Table 10).

Table 10 Prediction of all components for fulfilling Lipinski's rule of best docked substituted scaffold molecule for JAK2 (3FUP) receptor

Name of compound	Molecular weight [gms] (not greater than 500 daltons or 800 gms)	Partition coefficient [LogP] (not greater than 5)	Polar surface area [Å^2] (not greater than 190 Å^2)	Number of total rotatable bonds (not greater than 10)	Number of total atoms (range between 20-70)	Molar refractivity (range between 40-130)	Number of total hydrogen bond donors (not greater than 5)	Number of total hydrogen bond acceptors (not greater than 10)
Scaffold (JAK2)	536.65	2.785	130.193	6	64	123.6328	4	9

Prediction of all components for fulfilling Lipinski's rule of best docked substituted scaffold molecule for JAK2 (3FUP) receptor (substituted scaffold molecule – Figure 11: R_1 = OH, R_2 = OH, R_3 = OH & R_4 = $OCOCH_3$) [Table 10]

Components for fulfilling the Lipinski's rule: Lipinski's rule [12] states that, in general, an orally active drug has no more than one violation of the following components:

1. Hydrogen-bond donor (the total number of nitrogen-hydrogen & oxygen-hydrogen bonds) in a molecule is not more than 5.
2. Hydrogen-bond acceptor (all nitrogen or oxygen atoms) in a molecule is not more than 10.
3. Molecular weight (MW) of a molecule is less than 500 daltons or 800 gms.
4. Octanol-water partition coefficient (LogP) of a molecule is not greater than 5.
5. Polar surface area (PSA) of a molecule is not greater than 190 Å^2.
6. The range of molar refractivity (MR) of a molecule is in between 40 to 130.
7. The range of total number of atoms in a molecule is in between 20-70.
8. The range of total number of rotatable bonds in a molecule is not greater than 10.

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