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Computational Simulation Studies of Various Flavonoid Subclasses on Treating Non-Small Cell Lung Carcinoma Associated with Cigarette Smoking

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Abstract

Lung cancer is one of the most prevailing cancers that cause death worldwide. Non-small cell lung cancer is getting tremendously increased day-by-day because of cigarette smoking. On exposure to the tobacco carcinogen- nicotine derived nitrosamine ketone (NNK) leads to the over expression of hepatocyte growth factor (HGF) and c-Met protein ,that triggers processes such as metastasis, angiogenesis, anti-apoptosis, enhanced cell growth and motility. Methotrexate treats NSCLC; with major side effects leucopenia and inflammation. So it was essential to propose a novel drug having more potential, least side effects and better therapeutic efficacy. The uptake of flavonoid foods can reduce the risk and further progression of lung cancer. Apart from antitumor activity, flavonoids also possess blood cell production and anti-inflammatory actions. Hence c-Met protein resides attractive binding sites for the invention of new drugs for NSCLC by the incorporation of computational simulation tools and softwares. The docking analysis was performed using Arguslab for the identification of best ligands by predicting the ligand conformation in the protein active sites and assessment of binding affinities. Among 28 ligands were docked with the protein (pdb id: 5EOB) all had shown higher docking scores than the standard drug methotrexate. Thus, we can conclude that flavonoids can become a promising lead in designing a new and improved drug target that are beneficial in NSCLC therapy. Further analysis can be conducted for the synthesis of these drug targets to determine its actions in both in vivo and in vitro studies.

Keywords: Non-Small Cell Lung Carcinoma (NSCLC), c-Met protein, flavonoids, Nicotine derived Nitrosamine Ketone (NNK), methotrexate, Arguslab 4.0.1version.

INTRODUCTION

Cancer is a condition of uncontrolled proliferation of cells. Non-small cell lung cancer (NSCLC) is a type epithelial lung cancer due to the formation of malignant cells in the tissues of the lungs. NSCLC accounts for 85% of all lung cancers and related deaths because of cigarette smoking habits. NSCLC is often serious or severe because it will not produce any symptoms until the disease is well developed. Histologically, NSCLC is categorized into adenocarcinoma, squamous cell carcinoma and large cell carcinoma [1]

Met pathway has prominent role in the carcinogenesis. C-met stimulation occurs via its natural ligand hepatocyte growth factor(HGF), over expression and amplification of c-met causes changes in the c-met receptor tyrosine kinase activity where the tyrosine phosphorylation sites will get more activated thereby alters various biological functions that causes enhanced cell motility,growth,migration and invasion,mitogenic,angiogenic,morphogenic,anti-apoptosis,metastasis in NSCLC patients[2].Cigarette smoking also has adverse impact on c-met expression,when exposed to tobacco carcinogen-NNK which contribute 89% in the cigarettes causes increased tumor multiplicity.In studies,it is observed that HGF over expression due to NNK exposure can cause high vascularization in lungs and induce lymphatic veessel growth too[3][4].

Methotrexate which treats NSCLC by preventing cells from utilizing the folate and prevent further DNA and RNA synthesis, slowing the proliferation of cancerous cells and causing their death.This action of methotrexate can also effect normal cells that leads to occurrence of significant side effects including inflammation and reduced blood cell counts[5].

The intake of flavonoids and incidence of lung cancer have an inverse relationship. Flavonoids of various classes posses numerous pharmacological activities antiinflammatory,antioxidant,antiulcer,antihepatotoxic,inhibition tumor growth and development,antiviral,antiallergic,enhanced blood cell production, provide protection against cardiovascular mortality and reduce all the activated biological functions in NSCLC. Flavonoids induce anticancer activity by causing cell cycle arrest or apoptosis of cancerous cells and are non carcinogenic,antimutagenic and produce less or no toxic effects to the normal cells.The phenolic nucleus in the flavonoids are responsible for the non-covalent interaction with protein[6]. Flavonoids are CYP450 1A2 and CYP450 3A4 enzyme inhibitor that are involved in the metabolism so it will provide prolonged duration of action in the body.It can inhibit tyrosine kinase activity and also inhibit the prostaglandin synthesis thereby promoting anti-inflammatory effect[7].Flavonoids provide antiangiogenesis activity by stopping the diffusion of oxygen and other nutrients to the rapidly growing tumor cells and leads to the cell death. Heamoglobin in the blood enhances the lipid peroxidation; the capuring of RBC membrane by peroxidants can led to hemolysis so flavonoids can prevent heamolysis too[8].

Various flavonoid subclasses and their food sources which they are present [9]:

Flavonoid subclasses	Compounds	Food sources
Flavon-3ol	Epigallocatechin,Epigallocatechin gallate,Theoflavin,Theorubin	Grapes, berries, apples
Flavonones	Eriodictyol, Hesperetin, Naringenin	Oranges,lemon
Isoflavons	Daidzein, Genistein, Glycitein, Biochanin A, Formononetin	Legumes,soyabeans
Anthocyanidins	Cyanidin, Delphinidin, Malvidin, Aurantinidin,Europinidin,Rosinidin,Pelargonidin, Peonidin, Petunidin	Red and purple grapes
Flavones	Apigenin, Luteolin, Baicalein, Chrysin	Parsley,celery
Flavonols	Isorhamnetin, Kaempferol, Myricetin, Quercetin	Onions,apples

So, c-met protein can become an attractive target for the cancer treatment by the involvement of computational simulation tools and softwares. The evaluation of drug likeness and ADME studies is performed. Docking approach is carried out to predict the ligand conformation as well as its orientation and position in the protein sites thereby assessing the binding affinities. By this way new and improved drug molecules having least side effects can be designed for the implementation of rational drug use through computer aided techniques[10][11].

MATERIALS AND METHODS

Preparation of Protein:

The three dimensional crystal structure of c-met protein with pdb id: 5EOB was obtained from protein data bank (PDB).The primary and secondary characterizations were calculated by the involvement of protparam and sopma[12].

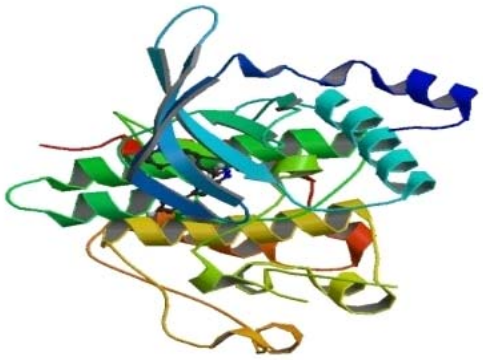

Fig 1.3D structure of pdb id: 5EOB.

Active Site prediction:

The active sites of selected target proteins were identified by using CASTp server (Computed Atlas of Surface Topography of proteins).The first pocket was chosen as the biologically most favorable active site for docking studies.

Identification of ligand :

Flavonoids have several subclasses were selected ligands. The 3D structure of the ligands was obtained from corina site, in association with chemsketch tool.

Evaluation of drug likeness:

Drug likenesses of these selected flavonoids were determined based on certain parameters mentioned in the Lipinski rule of 5 in association with molinspiration tool [13].

ADME and toxicity detection:

ADMET is defined as absorption, distribution, metabolism, excretion and toxicity. This reveals the pharmacokinetic profile of drug candidates for evaluating its pharmacodynamic activities.These properties were predicted using the admetSAR prediction tool[14].

Molecular docking:

Molecular docking is an analysis method to predict the binding orientation of small ligand molecule to their protein binding targets. This study was performed using Argus lab 4.0.1 version by involving software such as swiss pdbv.The interaction energy between protein and ligand were examined from their docking scores[15],[16].

RESULTS AND DISCUSSION

The suitable c-Met protein pdb id is selected for the docking analysis.The docking studies were carried out using Arguslab are given below:

Table 1. Protparam of c-met protein (pdb id: 5EOB)

Sl no:	Pdb id	No: of amino acid	Molecular weight	Theoretical pi	Extinction coefficient	Half life(hrs)	Instability index	Aliphatic index	Gravy
1	5HNI	312	35258.91	8.19	35785	1.4	37.71	95.19	-0.045
2	5HO6	312	35258.91	8.19	35785	1.4	37.71	95.19	-0.045
3	5EOB	319	35982.59	7.74	37275	30	39.57	92.82	-0.115
4	5T3Q	309	35163.70	8.19	38765	0.8	37.45	32.98	-0.133
5	4S14	259	29870.48	7.77	24910	30	43.68	92.63	-0.199
6	4S15	256	29810.51	5.58	27430	30	45.96	93.71	-0.138
7	4MXC	319	35258.91	8.19	37275	30	39.57	92.82	-0.115
8	4KNB	287	32505.84	8.68	37275	>20	37.41	93.66	-0.061
9	4GG5	319	35982.59	7.74	37275	30	39.57	92.82	-0.115
10	4GG7	319	35982.59	7.74	37275	30	39.57	92.82	-0.115

Table 2. Sopma of c-met protein (pdb id: 5EOB)

Sl no:	Pdb id	Alpha helix	pi helix	Beta turn	Random coil
1	5HNI	120	0	37	72
2	5HO6	120	0	37	72
3	5EOB	121	0	34	85
4	5T3Q	116	0	39	75
5	4S14	167	0	21	47
6	4S15	163	0	22	40
7	4MXC	121	0	34	85
8	4KNB	114	0	33	69
9	4GG5	121	0	34	85
10	4GG7	121	0	34	85

Table 3. Evaluation of druglikeness

Sl.no:	Ligands	miLogP	TPSA	natoms	Mol.Wt	nON	nONH	nviolations	nrotb	Volume
1	Aurantinidin	-0.52	112.31	21	287.25	6	5	0	1	234.81
2	Cyanidin	-0.75	112.31	21	287.25	6	5	0	1	234.81
3	Delphinidin	-1.04	136.52	22	303.25	7	6	1	1	242.83
4	Europinidin	-0.45	110.55	24	331.30	7	4	0	3	277.88
5	Pelargonidin	-0.26	92.08	20	271.25	5	4	0	1	226.79
6	Malvidin	-0.42	110.55	24	331.30	7	4	0	3	277.88
7	Peonidin	-0.44	101.32	22	301.27	6	4	0	2	252.34
8	Petunidin	-0.73	121.54	23	317.27	7	5	0	2	260.36
9	Rosinidin	0.10	90.32	23	315.30	6	3	0	3	269.87
10	Hespridin	-0.55	234.30	43	610.57	15	8	3	7	511.79
11	Eriodictoyl	1.63	107.22	21	288.25	6	4	0	1	238.28
12	Naringenin	2.12	86.99	20	272.26	5	3	0	1	230.26
13	Apigenin	2.46	90.89	20	270.24	5	3	0	1	224.05
14	Luteolin	1.97	111.12	21	286.24	6	4	0	1	232.07
15	Baicaelin	2.68	90.89	20	270.24	5	3	0	1	224.05
16	Chrysin	2.94	70.67	19	254.24	4	2	0	1	216.03
17	Kaempferol	2.17	111.12	21	286.24	6	4	0	1	232.07
18	Myrcietin	1.39	151.58	23	318.24	8	6	1	1	248.10
19	Quercetin	1.68	131.35	22	302.24	7	5	0	1	240.08
20	Isorhamnetin	1.99	120.36	23	316.26	7	4	0	2	257.61
21	Daidzein	2.56	70.67	19	254.24	4	2	0	1	216.03
22	Genistein	2.27	90.89	20	270.24	5	3	0	1	224.05
23	Glycitein	2.38	79.90	21	284.27	5	2	0	2	241.58
24	Biochanin a	2.80	79.90	21	284.27	5	2	0	2	241.58
25	Formononetin	3.10	59.67	20	268.27	4	1	0	2	233.56
26	Epigallocatechin	1.08	130.60	22	306.27	7	6	1	1	252.16
27	Epigallocatechin gallate	2.25	197.36	33	458.38	11	8	2	4	367.57
28	Theaflavin	1.66	217.59	41	564.50	12	9	3	2	459.20

By using protparama nd sopma tools the primary and secondarty characterization of c-Met protein were evaluated. From table.1 and table.2 on account of the parameters half life, instability index, gravy, aliphatic index from protparam and random coil from sopma the pdb id having higher values were selected pdb id:5EOB is choosen as the target for ligands to bind.

On considering the parameters of Lipinski rule of 5 that is millogp not greater than 5, molecular mass less than 500Da, NH and OH bonds not more than 5, nON bonds not more than 10and n violations 0.From table.3 it is clear that all the 28 ligands follow the rule,so posses good druglikeness.

From the table.4 it is clear that majority of the flavonoids having good intestinal absorption and CNS penetrability when compared with the standard drug methotrexate. Some ligands can inhibit CYP 1A2 and CYP 3A4 enzymes. They are non carcinogenic and have no AMES toxicity.Flavonoids posses comparatively least acute toxicity.The log S value is less when compared with the standard drug so the solubility will increase thereby enhances the absorption too.

From the table table.5 it is clear that among the docked 28 flavonoid ligands about 19 had shown better docking score than the standard drug methotrexate. The remaining ligands doesnot bind with the c-Met protein.So flavonoids were interacted well with the active sites of the c-Met protein.

Table 4. ADME and toxicity studies of the flavonoid subclasses using admetSAR tool.

Sl no:	ligands	Blood brain barrier	Human intestinal absorption	CYP Inhibitor		AMES toxicity	Carcinogenecity	LD 50 in rat(acute toxicity)	Log S
				CYP 3A4	CYP 1A2				
1	Aurantinidin	0.5202	0.5947	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.0306	2.6145
2	Cyanidin	0.5202	0.5947	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.0306	2.6145
3	Delphinidin	0.5202	0.5947	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.0306	2.6145
4	Europinidin	0.6875	0.6148	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.4101	2.6225
5	Pelargondin	0.7043	0.7824	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.2122	2.6732
6	Malvidin	0.5551	0.6456	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.4498	2.6111
7	Peonidin	0.5209	0.7047	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.3436	2.4291
8	Petunidin	0.7283	0.5078	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.1042	2.3665
9	Rosinidin	0.5370	0.7955	Non inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	-3.6663	2.7381
10	Hespridin	0.9466	0.6344	Non inhibitor	Non inhibitor	Non AMES toxic	Non carcinogenic	-2.6405	2.6228
11	Eriodictyol	0.5784	0.9223	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.3340	-3.45
12	Naringenin	0.6794	0.9670	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.5110	-3.1905
13	Apigenin	0.6364	0.9887	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	2.6983	-2.7765
14	Luteolin	0.5711	0.9650	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.0200	-2.9994
15	Baicaelin	0.5711	0.9650	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.0200	-2.9994
16	Chrysin	0.6364	0.9887	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	2.6983	-2.7765
17	Kaempferol	0.6286	0.9855	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.0825	-3.1423
18	Myrcietin	0.5711	0.9650	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.0200	-2.9994
19	Quercetin	0.5711	0.9650	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	3.0200	-2.9994
20	Isorhamnetin	0.6382	0.9783	inhibitor	inhibitor	Non AMES toxic	Non carcinogenic	2.7192	-3.2219
21	Daidzein	0.7448	0.9942	Non Inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	3.5363	-3.2055
22	Genistein	0.6785	0.6877	Inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	3.2988	-3.0925
23	Glycitein	0.5447	0.9898	Non inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	2.8186	-3.4244
24	Biochanin a	0.5674	0.9816	Inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	2.8328	-3.1911
25	Formononetin	0.7840	0.9949	Inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	2.9288	-3.4576
26	Epigallocatechin	0.5331	0.9654	Non inhibitor	Non inhibitor	Non AMES toxic	Non carcinogenic	1.8700	-3.1015
27	Epigallocatechin gallate	0.6047	0.8867	Non inhibitor	Non inhibitor	Non AMES toxic	Non carcinogenic	2.6643	-3.3141
28	Theaflavin	0.6153	0.9661	Inhibitor	Inhibitor	Non AMES toxic	Non carcinogenic	2.4019	-3.2123
29	Methotrexate (standard drug)	0.9467	0.8261	Non inhibitor	Non inhibitor	Non AMES toxic	Non carcinogenic	-3.0651	3.4955

Table 5. Docking scores obtained from Arguslab.

Sl no:	Ligands(flavonoid-subclasses)	Docking scores(kcal/mole)
1	Aurantinidin	-7.51268
2	Cyanidin	-7.2648
3	Delphinidin	-7.12549
4	Europinidin	-6.37104
5	Pelargondin	-7.34799
6	Malvidin	-7.00853
7	Peonidin	-7.49161
8	Petunidin	-6.34203
9	Rosinidin	-6.66783
10	Hespridin	-6.88237
11	Eriodictyol	-6.77009
12	Naringenin	No binding
13	Apigenin	No binding
14	Luteolin	-7.12322
15	Baicaelin	No binding
16	Chrysin	No binding
17	Kaempferol	-7.37186
18	Myrcietin	-6.86478
19	Quercetin	-7.26405
20	Isorhamnetin	-7.03017
21	Daidzein	No binding
22	Genistein	No binding
23	Glycitein	-6.9734
24	Biochanin a	No binding
25	Formononetin	No binding
26	Epigallocatechin	-6.35295
27	Epigallocatechin gallate	-7.40503
28	Theaflavin	No binding
29	Methotrexate(standard drug)	-5.96909

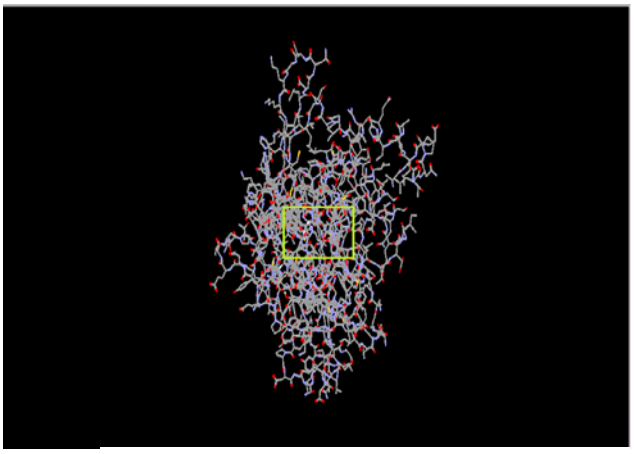

Fig 2.Docking of the c-Met protein pdb id: 5EOB with aurantinidin.

CONCLUSION

The aim of present study was to prove that flavonoids can be an appropriate drug molecule to treat NSCLC with least side effects and maximum antitumor activity.The lung cancer risk increases with the duration and intensity of cigarette smoking. From the docking approach using Arguslab we can conclude by considering the docking scores, that the flavonoids can be an appropriate key to treat NSCLC and can reduce the further progression in the body tha the standard drug methotrexate.Flavonoids can be used for the prevention side effects produced by the standard drug methotrexate that is inflammation,angiogenesis and reduced blood cell count.Hence it is applicable for the development of new and improved drug to treat NSCLC.These all were proved using computer aided tools and techniques. Further invitro amd invivo studies have to be performed to confirm the study.

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