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IN SILICO DOCKING STUDIES OF PHYTOCOMPOUNDS ON PI3K/NF-KB MEDIATED SIGNALLING PATHWAY

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ABSTRACT

Cancer cell invasion and metastasis are multistep processes influenced by the over expression of cell-secreted proteolytic enzymes such as Matrix metalloproteinases(MMPs).Phosphatidylinositide 3-kinase/Nuclear Factor of kappaB signaling pathways have been known to be involved in regulating MMP-9 expression. Synergistic targeting of these pathways using NF-κB and PI3K inhibitors may have great potential for cancer treatment. This paper focuses on identifying phytocompounds having anticancer potential to effectively inhibit PI3K and NF-κB. Thirty five phytocompounds with anticancer properties were utilized for the study. After screening out using Lipinski rule of five and ADMET, five compounds namely allixin, capsaicin, eugenol, niazimicin and piperine were docked with PI3K and NF-κB proteins. Niazimicin exhibited interaction for PI3K and NF-κB with residues CYS 633, ASP 632, GLN 392 and LYS 145 respectively. Niazimicin, a phytocompound of *Moringa Oleifera* which is an underexploited vegetable crop with medicinal properties showed maximum interaction with the targets.

KEYWORDS: **Cancer Metastasis MMP-9 Phosphatidylinositide 3-kinase Nuclear factor of kappaB Niazimicin *Moringa oleifera***

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INTRODUCTION

Cancer is a potentially fatal disease characterized by uncontrolled cell growth. Most of the emphasis in cancer research had been on the pathogenesis of primary tumors. But now, the major concern while diagnosing is whether the cancer has metastasized. Metastatic disease is usually a signal of immediate patient death. Understanding the complex pathways involved in cancer cell invasion and metastasis is a prerequisite step in identifying molecular targets for cancer therapy. Metastasis involves the progression of tumor in situ to an invasive tumor. It consists of a cascade of linked, sequential steps by which a subset or individual cancer cells disseminate from a primary location to distant secondary organs or tissues. Invasion involves tumor cell adherence to cells and to the extracellular matrix (ECM), proteolytic degradation of the surrounding tissue and acquisition of tumor cell motility [1]. The secretion of extracellular proteases plays an important role in cancer cell metastasis [2]. Of these proteases, the matrix metalloproteinases (MMPs), a group of zinc-dependent ECM-degrading enzymes play a pivotal role in tumor invasion, migration, host immune escape, extravasation, angiogenesis, and tumor growth. MMPs especially MMP-9 have increased expression for various types of tumor progression [3].

The elevation in MMP-9 production was mediated by the enhanced activity of Phosphatidylinositide 3-kinases (PI3K) [4]. PI3K is unique for its involvement in the developmental stages of tumor. The PI3K family constitutes a large family of lipid and serine/threonine kinases divided into different classes. They are composed of regulatory and catalytic subunit. The oncogenic transformation has been reported to be induced by the over expression of different isoforms of the various subunits such as the catalytic subunit, p110. This is contributed by the mutation in the genes encoding these subunits [5,6,7]. PI3K regulates motility of invasive cancer cells by the activation of transcription factor, nuclear factor of kappaB (NF-κB) [8,9,10]. NF-κB is a sequence-specific transcription factor involved in inflammatory and innate immune responses. Although this factor is expressed in an inactive state in most cells, cancer cells express an activated form of NF-κB.

PI3K regulated downstream cellular events include inactivation of several proapoptotic factors like BAD, procaspase-9, Forkhead transcription factors etc and the activation of transcription factors that upregulate antiapoptotic genes, including NF-κB [11,12,13,14]. MMP-9 has a NF-κB binding site in its promoter region which is activated through the PI3-k pathway [15,16]. The expression of tumor MMP-9 is therefore in an NF-κB dependent manner and is correlated with PI3K pathway [17]. Inhibition of the PI3K pathway components could synergize with, or overcome resistance to the existing cancer therapies[18]. Inhibitors could either target all isoforms of a particular protein or specifically a particular isoform. A drug targeting an upstream pathway component is unlikely to respond to downstream-activated mutation. Therefore inhibitors targeting both upstream and downstream components could be a breakthrough in cancer therapy. Plant kingdom represents an excellent reservoir of organic compounds, many of which have been used for medicinal purposes [19,20]. Phytocompounds could serve as lead for developing novel agents having good efficacy in various diseases [21,22]. Exploration of these phytochemical constituents and their pharmacological screening will thus provide basis for developing new life saving anticancer drugs.

METHODS

Protein selection and preparation of protein data bank file

Crystal structures of PI3K (1E8Y) with resolution 2.00A° and NF-κB/p50 (1NFK) with resolution 2.3A° were downloaded from protein data bank (PDB) (www.rcsb.org). The protein structures were subjected to preparation prior to minimization. Crystallographic water molecules and ligands were removed from the protein. Chemistry of the proteins were corrected by adding hydrogen. Energy minimization was performed

using CHARMm force field to relax the confirmation and to remove steric overlap.

Ligand preparation

About 35 phytocompounds with anticancer properties were selected for the study. The three dimensional structure of these phytocompounds was downloaded in .sdf format from Pubchem database (www.ncbi.nlm.nih.gov/pubchem). Lipinski's properties like molecular weight, log P and number of Hydrogen-bond donors and Hydrogen-bond acceptors for the active phytocompounds were calculated and ligands were screened on the basis of these properties. The ADMET properties for the phytocompounds which satisfies the Lipinski properties were calculated by using the toxicity prediction protocol of Discovery studio (ADMET). The ADMET properties include aqueous solubility, blood-brain penetration level, cytochrome 450 (CYP450), hepatotoxicity, human intestinal absorption and plasma protein binding level.

Docking

The binding sites of both proteins were predicted using receptor-ligand interaction protocol. For each binding site, the ligands were docked to get the poses of interaction. Ligand fit protocol was used for performing docking. The ligand binding affinity was calculated using LigScore, PLP1 and PLP2. JAIN and Dock score were used to estimate the ligand-binding energies. All the other input parameters were set as default options.

Toxicity prediction

Ligands with cLogP lesser than 5, logS greater than -4, molecular weight lesser than 450, positive value for drug likeness and maximum drug score, possess qualities of less toxic traded drugs. Toxicity risk assessment and drug properties are displayed with green indicating low risk, yellow indicating medium risk and red indicating high risk.

RESULTS AND DISCUSSION

Binding site prediction

The active sites were predicted for the energy minimized structures of 1E8Y and 1NFK with receptor – ligand protocol of discoverystudio. 37 and 17 sites were predicted for 1E8Y and 1NFK respectively.

Ligand selection

Molecular weight, logP, number of hydrogen bond donors and acceptors for the active phytocompounds according to Lipinski rule of five [23,24] are tabulated in Table-1.

Table 1
Lipinski properties of the active phytocompounds

SL. No.	COMPOUND NAME	PLANT	COMPOUND ID	MOLECULAR WEIGHT [G/MOL]	XLOGP3-AA	H-BOND DONOR	H-BOND ACCEPTOR
1	ALLIXIN C12H18O4	ALLIUM SATIVUM	86374	226.26892	2.8	1	4
2	APIGENIN C15H10O5	RICINUS COMMUNIS	5280443	270.2369	1.7	3	5
3	ANTHOCYANIN C15H11O+	BRASSICA OLERACEA	145858	207.24724	-	0	0
4	CAPSAICIN C18H27NO3	CAPSICUM ANNUUM	1548943	305.41188	3.6	2	3
5	β-CAROTENE C40H56	IPOMOEA BATATAS	5280489	536.87264	13.5	0	0
6	CATECHIN C15H14O6	ACACIA CATECHU	9064	290.26806	0.4	5	6
7	CIRSILINEOL C18H16O7	OCIMUM SANCTUM	162464	344.31544	2.9	2	7
8	COROSSOLONE C35H62O6	ANNONA MURICATA	11093061	578.86318	9.4	2	6
9	CROCIN C44H64O24	CROCUS SATIVUS	5281233	976.96456	2.5	14	24
10	β-CRYPTOXANTHIN C40H56O	CARICA PAPAYA	5281235	552.87204	12.3	1	1
11	CUCURBITACIN B C32H46O8	CUCUMIS SATIVUS	5281316	558.70284	2.6	3	8
12	CYANIDIN C15H11ClO6	SYZYGIUM CUMINI	128861	287.244	-0.75	5	6
13	ELLAGIC ACID C14H6O8	TERMINALIA ARJUNA	5281855	302.19264	1.1	4	8
14	EMBELIN C17H26O4	EMBELIA RIBES	3218	294.38594	5.4	2	4

15	EMBLICANIN A C34H22O22	EMBLICA OFFICINALIS	9810915	782.52528	1.6	12	22
16	EMODIN C15H10O5	ALOE BARBADENSIS	3220	270.2369	2.7	3	5
17	EUGENOL C10H12O2	SYZYGIUM AROMATICUM	3314	164.20108	2	1	2
18	FLAVOPIRIDOL C21H20CINO5	DYSOXYLUM BINECTARIFERUM	5287969	401.8402	3.3	3	6
19	GALLIC ACID C7H6O5	PUNICA GRANATUM	370	170.11954	0.7	4	5
20	GIRINIMBINE C18H17NO	MURRAYA KOENIGII	96943	263.33368	4.7	1	1
21	GLUCORAPHENIN C12H20NO10S3-	RAPHANUS SATIVUS	9548613	434.4829	-2.1	4	13
22	INDIRUBIN C16H10N2O2	WRIGHTIA TINCTORIA	5359405	262.2628	2.3	2	3
23	LUPEOL C30H50O	AEGLE MARMELOS	259846	426.7174	9.9	1	1
24	MANGIFERIN C19H18O11	MANGIFERA INDICA	535838	422.33962	0.4	8	11
25	MORIN C15H10O7	MACLURA POMIFERA	5281670	302.2357	1.5	5	7
26	NIAZIMICIN C16H23NO6S	MORINGA OLEIFERA	5471459	357.42192	0.7	4	7
27	NIMBOLIDE C27H30O7	AZADIRACHTA INDICA	100017	466.5229	2.2	0	7
28	OLEANOLIC ACID C30H48O3	SYZYGIUM AROMATICUM	10494	456.70032	7.5	2	3
29	ORIENTIN C21H20O11	OCIMUM SANCTUM	5281675	448.3769	-0.2	8	11
30	PIPERINE C17H19NO3	PIPER NIGRUM	638024	285.33766	3.5	0	3
31	PEDUNCULAGIN C34H24O22	EMBLICA OFFICINALIS	442688	784.54116	0.9	13	22
32	PEONIDIN-3-GLUCOSIDE C22H23O11+	BAUHINIA VARIEGATA	443654	463.41142	-	7	10
33	PLUMBAGIN C11H8O3	PLUMBAGO ZEYLANICA	10205	188.17942	2.3	1	3
34	WEDELOLACTONE C16H10O7	ECLIPTA ALBA	5281813	314.2464	2.4	3	7
35	WITHAFERIN C28H38O6	WITHANIASOMNIFERA	265237	470.59772	3.8	2	6

Twenty one ligands out of thirtyfive satisfied Lipinski's rule of five. The compounds for docking with PI3K and NF-κB were chosen based on their toxicity. Table-2 shows the ADMET (Absorbtion, Distribution, Excretion, Metabolism, Toxicology) properties of the compounds which already satisfied the Lipinski properties. Many compounds even after getting approval failed to reach market due to its toxicity [25]. After screening the 21 phytocompounds, five compounds namely allixin, eugenol, capsaicin, piperine and niazimicin were selected for docking studies.

Table 2
ADMET properties of active phytocompounds

SL.No.	COMPOUND	ABSORPTION LEVEL	BBB LEVEL	SOLUBILITY LEVEL	HEPATOTOXICITY	CYP2D6	PPB LEVEL	ADMET ALOGP98	ADMET PSD_2D
1	ALLIXIN	0	2	3	0	0	2	1.872	55.976
2	APIGENIN	0	3	3	1	1	2	2.41	88.679
3	ANTHOCYANIN	0	0	2	1	0	2	4.158	12.554
4	CAPSAICIN	0	1	3	0	1	0	3.91	59.856
5	CATECHIN	0	4	3	1	1	0	2.021	113.07
6	CIRSILINEOL	0	3	3	1	0	2	2.603	94.652
7	CYANIDIN	0	4	3	1	1	1	2.947	116.631
8	ELLAGIC ACID	1	4	3	1	0	1	1.584	135.723
9	EMODIN	0	3	3	1	1	2	2.568	97.048
10	EUGENOL	0	1	3	0	0	2	2.579	29.745
11	FLAVOPIRIDOL	0	3	2	1	1	1	3.108	92.029
12	GALLIC ACID	0	3	4	1	0	1	0.733	100.562
13	GIRNIMBINE	0	0	1	1	1	2	4.596	23.985
14	INDIRUBIN	0	3	3	1	1	2	1.876	60.222
15	MORIN	1	4	3	1	0	2	1.63	130.308
16	NIAZIMICIN	0	3	3	0	0	0	1.564	102.046
17	NIMBOLIDE	0	3	2	1	0	0	2.758	91.247
18	PIPERINE	0	1	3	0	0	0	2.864	38.513
19	PLUMBAGIN	0	2	3	1	0	2	1.962	55.417
20	WEDELOLACTONE	0	4	2	1	1	2	2.802	110.161
21	WITHAFERIN	0	3	2	1	0	0	3.388	94.092

Docking of PI3K (1E8Y) and NF-κB (1NFK) with the five ligands

Docking of PI3K and NF-κB were performed with the selected ligands. The final docked conformations obtained for the different ligands were evaluated based on the docking score and the number of hydrogen bonds formed, as given in Table 3.

Table 3
Docking scores of the ligands & H-bonds formed with PI3K and NF-κB

SL.No.	COMPOUND	SITE		RESIDUE		NO: OF HYDROGEN BOND		DISTANCE		DOCK SCORE	
		PI3K	NF-κB	PI3K	NF-κB	PI3K	NF-κB	PI3K	NF-κB	PI3K	NF-κB
1	NIAZIMICIN	4	1	CYS633 ASP 632 GLN 392	LYS 145 (A) LYS145 (B)	4	3	1.08435 2.34921 2.3628 1.90658	1.70044 2.29293 1.85862	60.297	48.182
2	CAPSAICIN	4	1	ASP 632 CYS 633	LYS 145 HIS 141	2	2	2.16189 1.18959	2.35044 2.08842	56.406	41.275
3	PIPERINE	4	1	LEU 567	LYS 145 (A) LYS 145 (B)	1	2	2.48307	1.76552 1.54686	45.078	38.753
4	ALLIXIN	7	2	ASP 1047	LYS 92	1	3	1.17593	2.42464 1.88179 1.65508	41.448	39.128
5	EUGENOL	7	2	LYS 808 LYS 833 ASP 964	LYS 92	4	3	2.21853 2.04251 2.22903 1.10974	2.16012 2.1558 1.63428	37.572	45.093

Docking score is a measure of interaction of ligand to the active site of the target. Greater values of dock score and more number of H-bonds formed between the atoms in the active site of the targets and ligands indicate effective stable conformation of the bound target-ligand. The ligands showed interaction for site 4 and 7 of PI3K. Niazimicin showed the maximum dock score of 60.297 and hydrogen bond formation for the residues CYS 633, ASP 632 and GLN 392 in the site 4 of PI3K, as given in Figure 1. The ligands showed interaction for site 1 and 2 of NF-κB protein. Niazimicin once again showed the maximum dock score of 48.182 and hydrogen bond formation for the site1, LYS 145 residues in the A chain and B chain of NF-κB protein, as given in Figure 2.

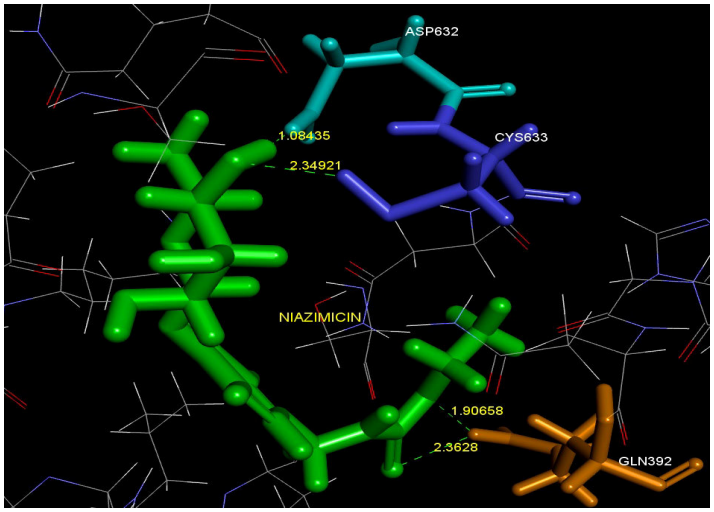

Figure 1
Docking of PI3K with Niazimicin

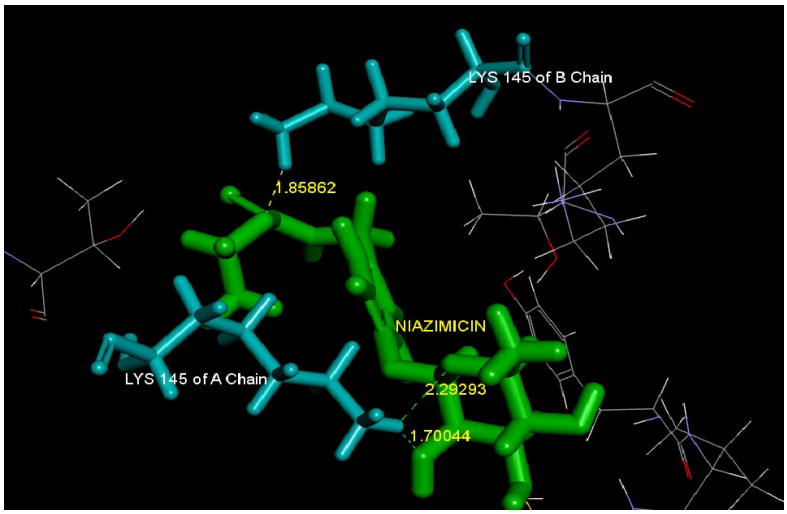

Figure 2
Docking of NF--κB with Niazimicin

Toxicity prediction

Toxicity analysis was performed for all the five ligands by OSIRIS property explorer. Ligands were drawn availing the drawing options of OSIRIS. The various molecular properties such as cLogP, solubility, molecular weight, drug likeness and drug score were calculated and displayed as given in Table 4.

Table 4
Toxicity analysis using OSIRIS property explorer

COMPOUND	MUTAGENIC	TUMORIGENIC	IRRITANT	REPRODUCTIVE EFFECT	CLOGP	LOGS	MOL.WT	DRUG LIKELINESS	DRUG SCORE
ALLIXIN	NO	NO	NO	NO	2.51	-2.94	226	-11.38	0.45
CAPSAICIN	NO	NO	YES	NO	4.2	-3.32	305	-9.65	0.3
EUGENOL	NO	NO	NO	YES	2.42	-2.05	164	-2.78	0.11
NIAZIMICIN	NO	NO	NO	NO	0.91	-2.57	357	-0.88	0.57
PIPERINE	NO	NO	NO	NO	3.63	-3.61	285	0.6	0.64

Toxicity risk assessment was interpreted as green indicating - low risk, yellow indicating - medium risk and red indicating - high risk. Of the five compounds, piperine and niazimicin showed favorable drug scores in the range of 0.64 and 0.57 respectively and therefore fall in the low to medium risk category. While comparing Table 3 and 4, it was observed that even though piperine exhibited a higher drug score, it's interaction with the target proteins were not satisfactory. However Niazimicin being the next compound with favorable drug score showed good inhibition for both PI3K and NF-κB, as given in Figure 3.

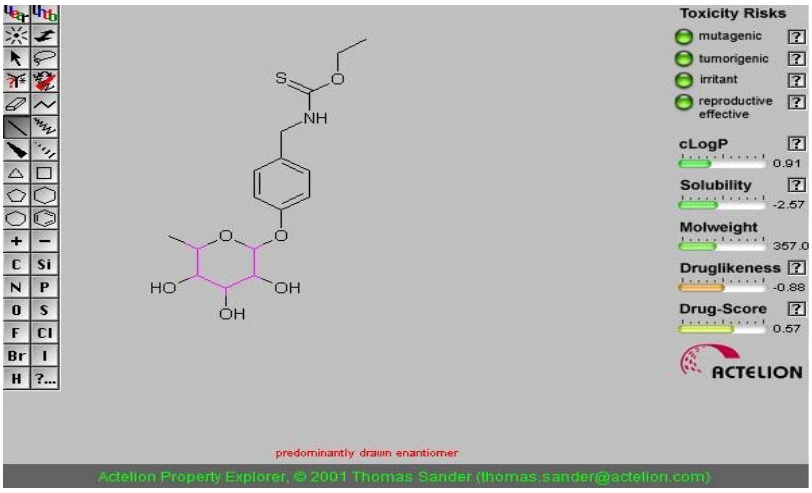

Figure 3
Toxicity analysis of Niazimicin using OSIRIS property explorer.

CONCLUSION

Among the five ligands, niazimicin which is an active component of *Moringa oleifera* showed maximum interaction for both PI3K and NF-κB with dock score values 60.297 and 48.182 respectively. Niazimicin satisfied all the qualities of less toxic drug except a negative value of -0.88 for drug likeness. However it gave a better drug score of 0.57. Also, the other factors such as mutagenicity, tumorigenicity, irritating effects and reproductive effects fall with in the desirable limits as indicated in the OSIRIS property explorer. It can therefore be categorized in the medium to low risk group. The therapeutic value of niazimicin can be effectively used to inhibit PI3K mediated NF-κB pathway. This study is an *in silico* approach to reiterate the application of these anticancer phytochemicals of *Moringa oleifera* as the potent and safe natural therapeutic agents in cancer drug design.

The medicinal properties of leaf, bark and flowers of the perennial vegetable crop, Moringa has been elucidated in various reports against several diseases such as diabetes, arthritis, AIDS, liver and kidney disorders etc. The perennial nature of the plant and its sustainability for humid tropical climate are highly advantageous for its large scale exploitation. It contains an enormous amount of nutritional content, loaded with calcium, iron, potassium, protein, vitamin A and C, and many more properties which help in promoting a healthy body to aid in cancer therapy. With increasing evidences supporting the molecular mechanism underlying the anticancer potential of Moringa coupled with considerations of quality, safety and efficacy could be a breakthrough in developing new low cost anticancer drugs and adjuvant to current therapy.

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