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ORIGINAL RESEARCH

Multitargeted molecular docking study of plant-derived natural products on phosphoinositide-3 kinase pathway components

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Abstract Phosphoinositide-3 kinase (PI3K) signaling pathway comprises of a cornucopia of protein molecules capable of regulating numerous cellular events, including cell survival, cell cycle regulation, angiogenesis, and apoptosis. Deregulation of PI3K downstream signaling is a phenomenon commonly seen in various types of cancer and also held responsible for poor prognosis and resistance to chemotherapy. Targeting PI3K signaling pathway has become a new and promising strategy in combating cancer. In the present study, PI3K signaling components PI3K, PDK1, Akt, and mTOR were chosen and 51 natural compounds along with 17 reference compounds were selected as ligand with the aid of PubMed published literature search. Ligands were docked to protein molecules by using Maestro 9.3 (Schrödinger Inc.). It was discovered in this study that compounds myricetin, quercetin, morin, luteolin, and emodin yielded excellent dock score with the proteins concluded with the help of docking free energy. The remarkable feature of these compounds are their various pharmacodynamics and pharmacokinetic characteristics, many of which are in accordance with the "Lipinski's Rule of five". The docking study carried out is an endeavor to portray the docking of these compounds with the proteins, to summarize the various Gscore, hydrogen bond, electrostatic bond, and to chart out various factors that are decisive for and also govern the protein–ligand interactions.

Keywords Cancer · Phosphoinositide-3 kinase · Natural product compounds · Maestro 9.3

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Introduction

Over the past few decades it became increasingly apparent that the most crucial strategy for drug discovery is targeted drug design (Drews, 2000). However, targeting single sites did not work in many of the multifactorial and complex diseases such as cancer and diabetes owing to the fact that many sites might be required to be targeted simultaneously. Moreover, the efficacy of single-target drugs for treating complex diseases might be compromised with different signaling pathways (Morphy, 2010). Multi-target therapeutics involves discovering a single agent that can act on two or more targets at the same time. It is presumed that this strategy is pharmacologically more significant and can be modeled because the pharmacokinetics and pharmacodynamics of a single agent targeted to multiple protein molecules are more predictable than simultaneous use of two or more single-targeted agents (Zhou *et al.*, 2013). PI3K signaling pathway proceeds through a cascade of protein molecules that regulate a number of cellular processes such as growth, proliferation, survival, and metabolism (LoPiccolo *et al.*, 2008; Sarker *et al.*, 2009). In human prostate cancer, the PI3K pathway is one of the most frequently activated signal transduction pathways apart from rat sarcoma (RAS) activated pathway and play a significant role in cellular growth and metabolism (Bartholomeusz and Gonzalez-Angulo, 2012; Morgensztern and McLeod, 2005). Receptor tyrosine kinases (RTK) family, including the RTK class I (human epidermal growth factor receptors) and RTK class II (insulin and insulin-like growth factor receptor), are also known to be regulated by the PI3K signaling pathway. Binding of hormones and growth factors to specific receptor molecules induce conformational changes in the latter, which in turn activates autophosphorylation and transphosphorylation of

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a cascade of tyrosine residues of small protein molecules. The gene Akt, that code for one of the PI3K downstream protein molecules of the same name, is flanked by two tumor suppressors genes viz., phosphatase and tensin homolog (PTEN) and Tuberous Sclerosis Complex ½ (TSC1/TSC2). It is the mutations in these tumor suppressor genes that are associated with various types of cancer initiation and progression (Hay, 2005). Thus, PI3K/Akt/mTOR pathway is an attractive target for the therapeutic intervention of a variety of cancers. PI3K, Akt, PDK1, and mTOR are activated by a number of cellular processes including expression of oncogenes, inactivation of tumor suppressor genes, tyrosine kinase receptors, and G-protein-coupled receptors. Akt is a central kinase intermediate in this PI3K signaling pathway that regulates apoptosis and cell proliferation. Phosphorylated Akt molecules exert cellular effects through a cascade of phosphorylation of downstream substrates such as mTOR that regulate the apoptosis and cell proliferation. Detailed PI3K/AKT/mTOR signaling pathway is illustrated in Fig. 1a.

At present, PI3K/AKT/mTOR pathway inhibitors that target individual components such as PI3K, PDK1, Akt, and mTOR are being developed and undergoing clinical evaluation for the treatment of lung, breast, and prostate cancer (Morgan *et al.*, 2009). However, due to a multitude of side effects and arguable efficacies there is a demand for newer compounds for the treatment of prostate cancer. Recent studies have revealed that many natural products isolated from plants inhibit PI3K pathway and exhibit potent anticancer activities (Chen *et al.*, 2005; Rao *et al.*, 2010; Zhang *et al.*, 2007). Due to a large number of physiologically active structures and known mechanisms, natural products have immense potential for prospective drug development, including the development of synthetic

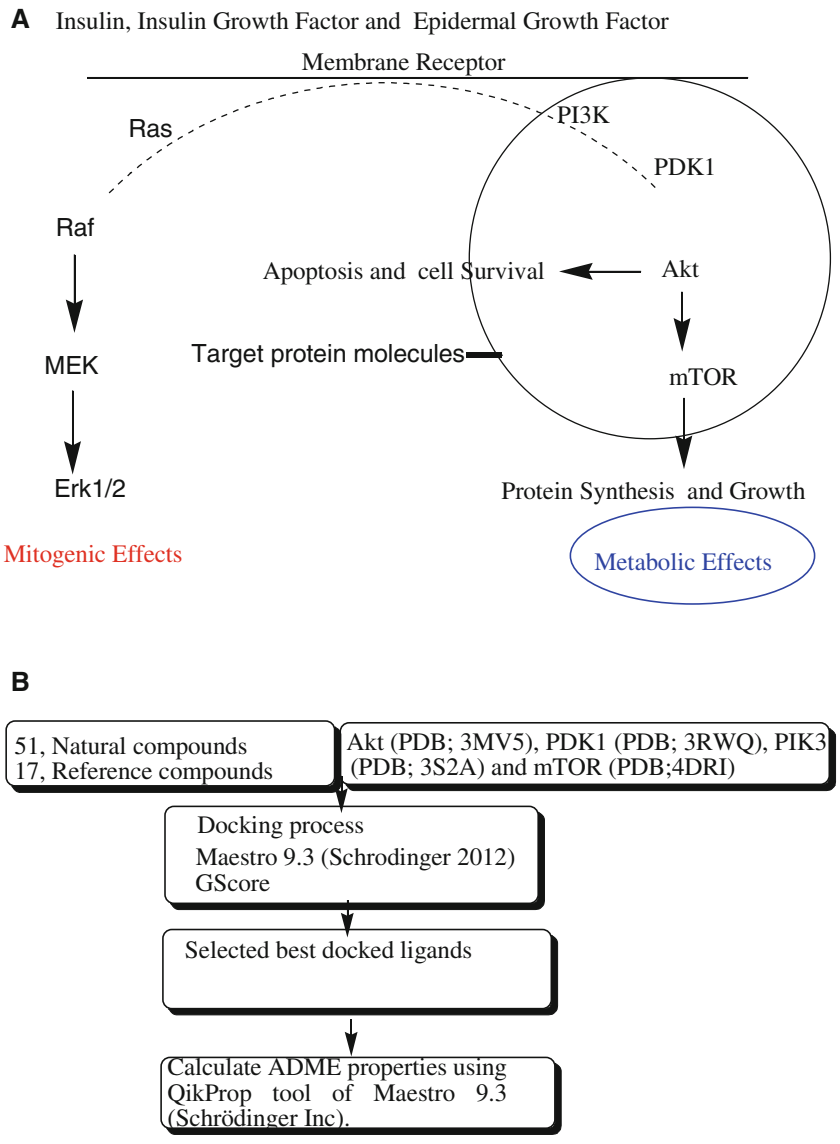

Fig. 1 **a** Activation of PI3K and RAS pathway by insulin, insulin-like growth factor, and epidermal growth factor receptors. **b** Schematic diagram of the molecular docking workflow

structural analogs. PI3K is located at a junction pivotal for numerous metabolic and tumor suppression signaling pathways. Deregulation of PI3K through inactivation of PTEN tumor suppression gene results in the activation of oncogene in addition to overexpression of small signaling protein molecules, which as a consequence disrupts the normal cascade of signaling pathways. High levels of PI3K activity are observed in variety of cancers including lung, prostate, and breast cancer, and also held responsible for poor prognosis and resistance to chemotherapy (Antonarakis *et al.*, 2010; Downward, 2008; Morgan *et al.*, 2009; Wong *et al.*, 2010). In this context, our aim of the present work is to find a natural product that can be developed as a suitable PI3K signaling pathway inhibitor by using molecular docking studies.

Methodology

Selection of ligand and protein molecules

Schematic diagram of the molecular docking workflow adopted in the present study is illustrated in Fig. 1b. Selected candidate ligand molecules were divided in two groups: (a) PI3K pathway inhibitor compounds were searched at http://www.ncbi.nlm.nih.gov/pccompound (Bolton *et al.*, 2008) using query molecules from published literature (Baldo *et al.*, 2008; Easton and Houghton, 2006; Faivre *et al.*, 2006; O'Reilly *et al.*, 2006; Watanabe *et al.*, 2011; Zhou *et al.*, 2010); and (b) natural compounds that had been reported in the published literature (Cho and Park, 2008; da Rocha *et al.*, 2001; Hillman, 2012; Huang *et al.*, 2012; Phosrithong and Ungwitayatorn, 2010; Roell and Baniahmad, 2011; Sarkar and Li, 2006; Sunil, 2012). Pharmacokinetics properties of selected compounds are detailed in Tables 1 and 2. The X-ray crystal structure of Akt, PDB; 3MV5 (Freeman-Cook *et al.*, 2010), PDK1, PDB; 3RWQ (Murphy *et al.*, 2011), PIK3, PDB;3S2A (Nishimura *et al.*, 2011), and mTOR, PDB;4DRI (März *et al.*, 2013) retrieved from the Protein Data Bank and proceeded to molecular docking analyses.

Molecular docking

Molecular docking studies using the selected ligand molecules were conducted using Maestro 9.3 molecular docking suite (Friesner *et al.*, 2004; Friesner *et al.*, 2006; Halgren *et al.*, 2004). The study included 51 plant-derived natural compounds and 17 reference compounds that have been previously reported to possess activity against PI3K pathway. Each of these compounds was docked into four different target protein molecules and the docking conformation possessing the lowest energy was selected. Input ligands molecules were prepared using respective wizard applications, where functions such as addition of hydrogen atoms, fixing of charges and orientation of groups, 2D to 3D conversion, corrected bond lengths and bond angles, low energy structure and correct chiralities, ionization

Table 1 List of PubChem molecules previously available PI3K inhibitors used as a control in this study

S.no.	Compounds (CID)	Mol. wt. (130–725 kDa)	Dipole	Volume (500–2000)	H-bond Donor (0–6)	H-bond Acceptor (2–20)	Q P log P oct (8.0–35.0)	Q P log Pw (4.0–45.0)	Q P log $P_{o/w}$ (−2.0 to 6.5)	Q P log S mol/L (acceptable range: −6.5 to 0.5).
1	2519	194.193	4.927	648.274	0	5	9.34	6.7	−0.068	−0.924
2	3071	246.311	4.243	835.901	2	0	12.853	6.476	4.22	−4.389
3	65064	458.378	6.825	1255.723	8	8.75	31.172	23.925	−0.243	−3.547
4	445154	228.247	2.312	786.572	3	2.25	13.599	9.347	2.012	−2.803
5	969516	368.385	2.877	1199.54	2	7	18.659	11.559	2.997	−4.35
6	5280961	270.241	4.527	807.798	2	3.75	14.142	9.887	1.678	−3.014
7	5284616	914.184	7.39	2694.357	1	19	39.759	17.803	6.647	−5.61
8	6442177	958.237	9.692	2838.193	1	20.7	42.103	19.028	6.727	−5.566
9	6918289	1030.3	9.678	3013.08	0	20.7	42.828	17.676	7.065	−5.467
10	9876378	966.222	13.88	2808.075	0	20.8	42.178	19.01	5.729	−4.669
11	9884685	348.36	2.419	1037.804	1	6.45	17.266	11.337	2.8	–4.323
12	11520894	990.219	8.214	2908.371	0	22.3	42.784	19.607	6.065	−4.589
13	11977753	469.545	5.802	1422.099	0	5.5	21.379	10.096	5.493	−8.144
14	16663243	900.2	9.008	2577.679	1	17	37.316	15.276	7.123	−4.452
15	24905147	308.342	1.892	951.373	4	4.75	19.076	13.566	1.885	−3.783
16	49836027	607.634	9.074	1736.677	0	9	28.846	16.145	5.761	−7.145
17	49867926	599.66	9.557	1642.108	3	11.75	30.808	19.279	3.901	−5.694

Table 2 List of PubChem molecules screened in this study

S.no.	Compounds (CID)	Mol. wt. (130–725 kDa)	Dipole	Volume (500–2000)	H-bond Donor (0–6)	H-bond Acceptor (2–20)	Q P log P oct (8.0–35.0)	Q P log Pw (4.0–45.0)	Q P log $P_{o/w}$ (−2.0 to 6.5)	Q P log S mol/L (acceptable range: −6.5 to 0.5).
1	932	272.257	3.889	840.858	2	4	14.728	10.219	1.658	−3.47
2	2543	310.435	1.711	1122.807	1	1.5	13.745	4.396	5.604	−6.637
3	3220	270.241	3.141	808.002	1	4.25	12.602	8.522	1.258	−3.069
4	5350	177.279	6.64	661.995	0	6.5	9.242	8.882	0.535	0.827
5	9064	290.272	4.628	872.256	5	5.45	19.89	15.596	0.481	−2.609
6	10494	456.707	3.894	1401.837	2	3.7	21.162	8.308	6.238	−7.067
7	16078	314.467	0.973	1147.852	1	1.5	13.759	3.962	5.694	−6.774
8	64945	456.707	3.856	1387.746	2	3.7	20.965	8.275	6.125	−6.917
9	68077	372.374	8.963	1087.198	0	6.25	15.729	7.732	3.241	−3.239
10	72276	290.272	2.942	872.747	5	5.45	19.683	15.562	0.494	−2.587
11	72277	306.271	4.452	892.591	6	6.2	21.772	17.64	−0.17	−2.356
12	72281	302.283	4.457	919.956	2	4.75	15.742	10.473	1.803	−3.782
13	72326	442.724	1.87	1393.324	2	3.4	20.187	7.483	5.92	−6.784
14	73641	488.706	4.498	1431.329	4	7.1	25.992	14.438	4.231	−5.37
15	73659	472.707	3.881	1414.592	3	5.4	23.674	11.514	5.168	−6.242
16	91469	242.274	2.982	798.666	2	2.25	13.082	8.215	2.761	−3.579
17	245005	645.745	7.859	1752.257	3	16.95	33.343	22.199	1.861	−2.42
18	259846	426.724	1.655	1378.31	1	1.7	17.885	4.492	7.043	−7.907
19	261265	430.626	3.05	1387.396	2	6.15	21.085	10.026	4.381	−6.176
20	312145	428.438	2.42	1155.175	0	11.2	19.064	13.078	0.24	−1.059
21	440917	136.236	0.314	621.544	0	0	5.044	−0.203	3.99	−4.003
22	441794	496.553	6.596	1340.516	5	12.35	28.675	20.626	0.968	−3.199
23	442793	294.39	3.872	1090.841	1	4.2	13.232	5.745	3.766	−4.418
24	446925	536.882	0.453	2234.739	0	0	21.324	−4.769	18.947	−21.076
25	457964	338.486	2.004	1041.085	4	5.85	19.149	12.301	2.001	−3.044
26	5270604	426.724	1.299	1373.759	1	1.7	18.106	4.738	7.02	−7.99
27	5280343	302.24	3.491	883.724	4	5.25	18.67	14.544	0.522	−3.1
28	5280373	284.268	4.292	860.146	1	3.75	13.02	8.02	2.522	−3.523
29	5280443	270.241	4.57	822.477	2	3.75	14.638	10.218	1.642	−3.377
30	5280445	286.24	4.704	843.559	3	4.5	16.591	12.301	0.96	−3.096
31	5280789	538.898	0.65	2258.201	0	0	21.816	−4.468	19.118	−21.32
32	5280863	286.24	3.653	840.186	3	4.5	16.394	12.278	1.059	−3.057
33	5280896	264.321	6.007	879.926	2	4.75	14.317	8.601	2.259	−2.599
34	5280899	568.881	1.318	2125.199	2	3.4	25.936	5.687	10.564	−12.471
35	5281605	270.241	7.17	818.653	2	3.75	15.089	10.185	1.757	−3.317
36	5281607	254.242	5.656	799.677	1	3	12.869	8.121	2.386	−3.647
37	5281612	300.267	5.118	901.465	2	4.5	15.652	10.473	1.79	−3.695
38	5281614	286.24	6.519	841.842	4	5.5	18.889	14.744	0.5	−2.796
39	5281616	270.241	4.525	817.401	2	3.75	14.551	10.181	1.794	−3.309
40	5281670	302.24	7.116	858.838	4	5.25	18.887	14.332	0.41	−2.753
41	5281672	318.239	4.894	882.348	5	6	20.432	16.443	−0.281	−2.564
42	5281707	268.225	4.88	774.132	2	4.5	14.593	10.923	1.319	−2.915
43	5281708	254.242	3.979	787.865	2	4	14.141	10.264	1.774	−2.957
44	5288382	560.643	10.69	1637.658	3.25	14.1	29.18	18.154	1.879	−2.874
45	6436722	542.93	0.633	2281.955	0	0	22.433	−4.282	18.967	−21.567
46	6441009	785.023	4.855	2215.801	9	21.15	48.736	33.891	1.639	−3.956
47	6857485	276.504	0.073	1033.576	0	0	10.507	−0.266	6.886	−8.574

Table 2 continued

S.no.	Compounds (CID)	Mol. wt. (130–725 kDa)	Dipole	Volume (500–2000)	H-bond Donor (0–6)	H-bond Acceptor (2–20)	Q P log P oct (8.0–35.0)	Q P log Pw (4.0–45.0)	Q P log $P_{o/w}$ (−2.0 to 6.5)	Q P log S mol/L (acceptable range: −6.5 to 0.5).
48	6917781	805.013	3.797	2224.026	5	14.05	40.191	21.528	6.333	−6.514
49	9548699	274.489	0.17	963.039	0	0	9.861	−0.523	8.186	−8.689
50	9548711	278.52	0.112	1081.565	0	0	10.365	−1.273	9.272	−9.918
51	9910986	401.846	7.334	1141.405	2	6.7	20.745	12.793	2.369	−3.673

Compounds; PubChem molecules screened in this study

Molecular weight (130–725 kDa)

Volume; Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution

Hydrogen bond donors (<5)

Hydrogen bond acceptors (<10)

Q P log Poct; was predicted partition coefficient of octanol/gas, (8.0–35.0)

Q P log Pw; was predicted partition coefficient of water/gas (4.0–45.0)

P log Pw; was Predicted octanol/water partition co-efficient log p (recommended range: −2.0 to 6.5)

Q Plog S; was Predicted aqueous solubility; S in mol/L (acceptable range: −6.5 to 0.5)

states, tautomers, stereochemistries, ring conformation, etc. was incorporated into ligands molecules followed by minimization and optimization in Optimized Potential for Liquid Simulations (OPLS_2005) force field (Jorgensen *et al.*, 1996; Jorgensen and Tirado-Rives, 1988; Shivakumar *et al.*, 2010). Finally, one conformation for each ligand was generated, and ready for docking. Consequently, input protein molecules were prepared using respective wizard applications, where changes such as addition of hydrogen atoms, assigning bond orders, create zero order bonds to metal, create disulfide bonds, fixing of the charges and orientation of groups were incorporated into the raw PDB structure. After the completion of ligand and protein preparations, a receptor-grid file was generated. For running the grid generation module, we have scaled Van der Waals radii of receptor atoms by 1.00 Å with a partial atomic charge of 0.25. The active site of the receptor provides an accurate scoring function with thermodynamic optimal energy and is calculated on a grid by various sets of fields. After the formation of receptor-grid file, flexible ligands with rigid receptor molecular docking were performed. The final energy evaluation is done on the basis of Gscore.

ADME properties studies

The majority of drug candidates don't succeed in clinical trials due to poor ADME properties. Thus, ADME properties of best docked compounds were predicted using QikProp application of Schrodinger 2012. Absorption, distribution, metabolism, excretion, and toxicity (ADME/T) properties of selected best docked ligands molecules were predicted. It predicts properties such as log BB, overall CNS activity, Caco-2 and MDCK cell permeability, and logKhsa for human serum albumin binding, etc. (Jorgensen and Duffy, 2002; Lu *et al.*, 2004).

Results and discussion

Table 3 summarizes results of the docking study based on binding conformations and energies. The lowest energy conformation, representing the best binding conformation of inhibitors to these receptor protein molecules, was identified by the molecular docking procedure. The complex formed by protein molecules with ligands yielded a plethora of information highlighting the conclusive role portrayed by numerous factors namely hydrogen bonds, salt bridges, metal interactions, lipophilic interactions, π-π, and π-cation interactions in the Protein–Ligand interaction profile. The data obtained from these Protein–Ligand docking molecule interactions, including their Gscore, hydrogen bond, electrostatic bond, and residues, are depicted in Table 3. In the present study, ten lead molecules showing high Gscore were chosen from each class of protein molecules under the study.

As evident in several reports, sulforaphane administration is also known to inhibit prostate cancer progression and pulmonary metastasis in TRAMP mice by reducing cell proliferation as a consequence of suppressing the Akt signaling pathway (Keum *et al.*, 2009; Singh *et al.*, 2009). Data underlined in Table 3 signifies that CID5350 (sulforaphane) was best docked against Akt yielded a Gscore value of −5.27 kcal/mol. The docking studies conducted put forth that sulforaphane had similar interactions against Akt as CID969516 (Curcumin), since sulforaphane not

Table 3 Lowest binding energy for the ligand-Akt (PDB; 3MV5), PDK1 (PDB; 3RWQ), PIK3 (PDB; 3S2A), and mTOR (PDB; 4DRI) interaction, along with scores for various interaction types, as detected by GLIDE

Protein	Compounds ID	GScore	Lipophilic E vdw	H-bond	Electro	Protein ligands interaction
Akt	5350	−5.27	−1.97	−0.63	−0.21	Ala 230
	969516	−5.24	−4.43	−1.97	−0.93	Arg C:4, Ala A:230 and Glu A:228
	442793	−4.01	−3.8	−2.17	−0.59	Asp A:292, Ala A:230 and Glu A:228
	5281672	−3.94	−1.72	−3.78	−1.08	Leu A:156, Lys A:158 and ThrC:5
	91469	−3.74	−3.13	−1.51	−0.72	Arg C:4, Ala A:230 and Glu A:228
	5280343	−3.67	−1.77	−3.33	−1.17	Asp A:292, Lys A:158 and ThrC:5
	3220	−3.36	−3.75	−1.12	−0.68	Glu A:228
	5280961	−3.34	−3.27	−1.21	−0.47	Arg C:4, Ala A:230 and Glu A:228
	5280445	−3.25	−1.8	−3.05	−0.99	Leu A:156, Lys A:158 and ThrC:5
	72281	−3.1	−3.22	−1.72	−0.72	Ala A:230 and Asp A:292
PDK1	5281672	−9.15	−3.69	−3.8	−1.21	Ser 160, Thr 222 and Glu 209
	969516	−9.12	−3.66	−3.19	−1.2	Ser 160, Ala 162 and Lys 111
	5281670	−8.96	−3.49	−4.02	−1.09	Ser 160, Thr 222, Ala 162 and Glu 209
	5280343	−8.55	−3.65	−3.32	−1.09	Ser 160, Thr 222 and Glu 209
	5280445	−8	−3.6	−2.84	−1.05	Ser 160, Thr 222 and Glu 209
	72281	−7.74	−3.35	−2.7	−0.8	Ser 160 and Thr 222
	5280373	−7.49	−3.14	−2.13	−0.81	Ser 160, Ala 162 and Thr 222
	5281612	−7.45	−3.61	−2.56	−0.84	Ser 160, Thr 222 and Glu 209
	3220	−7.39	−3.52	−2.1	−0.54	Ser 160 and Thr 222
	72277	−7.39	−2.89	−4.74	−1.36	Ser 160, Ala 162 and Lys 111
PIK3	5281672	−10.98	−3.13	−3.79	−1.96	Val 882, Lys 833, Asp 964
	65064	−10.82	−3.13	−5.66	−1.81	Asn 951, Val 882, Asp 964
	5280343	−9.89	−3.9	−3.06	−0.84	Glu 880, Val 882, Trp 812 and Tyr 867
	3220	−9.81	−3.41	−2.66	−0.67	Trp 812, Tyr 867, Val 882, Glu 880
	5281614	−9.69	−4.82	−2.4	−0.63	Tyr 867, Trp 812, Val 882 and Ala 885
	24905147	−9.22	−3.22	−1.84	−0.79	Glu 880 and Val 882
	9910986	−9.16	−4.38	−2.14	−0.7	Val 882, Trp 812 and Lys 890
	5280445	−9.13	−3.95	−2.94	−1.17	Val 882, Glu 880, Lys 890 and Asp 964
	5281670	−8.8	−3.96	−2.24	−0.51	Trp 812, Tyr 867, Val 882 and Lys 890
	5280961	−8.72	−4.31	−1.81	−0.86	Trp 812, Tyr 867 and Ala 885
mTOR	72276	−5.71	−1.2	−3.2	−1.03	Gln B:2099 and Arg A:73 and
	245005	−4.91	−1.65	−2.55	−1.02	Gln B:2099, Asp A:72 and Asn A:74
	65064	−4.74	−0.72	−3.27	−2	Asp A:68, Asp B:2102, Asp B:2096 and Lys B:2087
	5280343	−4.68	−1.42	−2.55	−0.82	Gln B:2099, Asp B:2102 and Arg A:73
	457964	−4.67	−2.59	−1.33	−0.58	Asp B:2102 and Arg A:73
	441794	−4.61	−0.77	−2.91	−1.38	Asp B:2102, Arg A:2076, Glu B:2080 and Gln B:2102
	72277	−4.06	−1.63	−1.58	−0.8	Trp B:2084, Glu B:2080 and Asn A:74
	932	−3.83	−1.66	−1.38	−0.63	Asp B:2102 and Hid B:2106
	5280373	−3.73	−1.9	−1.31	−0.53	Asn A:74, Gln B:2102 and Hid B:2106
	3071	−3.72	−1.96	−1.22	−0.55	Gln B:2099,Hid B:2106 and Arg A:73

Ligand; PubChem IDs of the lead molecules

GScore; Glide extra precision scores (kcal/mol)

Lipophilic E Vdw; Chemscore lipophilic pair term and fraction of the total protein–ligand vdw energy

HBond; Hydrogen-bonding term

Electro; Electrostatic rewards

Protein ligands interaction; π–π stacking, π–cat interaction and hydrogen bond between the ligands and protein

only showed better docking interaction with Akt in comparison to the known PI3K inhibitor but also showed binding conformation similar to Curcumin (Fig. 2). Thus, it could be considered as an Akt inhibitor.

CID5281672 (Myricetin), CID5280343 (Quercetin), CID5281670 (Morin), and CID5280445 (Luteolin) are naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables. These natural compounds have ability to oxidize the C-ring of the basic benzo-γ-pyrone structure to different extend and differentiates them from each other. Docking studies have revealed that Myricetin, Quercetin, Morin, and Luteolin docked better or with similar binding conformation with PDK1 and PI3K than any other PI3K-specific inhibitors, as listed in Table 1. Myricetin, Quercetin, and Morin differ from each another in the position of addition or substitution of hydroxyl groups on the phenyl moiety. Interaction profile of these natural compounds revealed that both chromone as well as phenyl moiety are indispensable for protein–ligand interactions (Fig. 3). There are two types of hydrogen bonds involved in protein–ligand interactions in both chromone as well as phenyl moiety–hydrogen bond with back bone and hydrogen bond with side chain. Structure of the PDK1 complexed with Myricetin, Quercetin, Morin, and Luteolin revealed that weak interactions, such as hydrogen bonding and hydrophobic interactions, are key players in stabilizing energetically-favored protein–ligand interaction (Fig. 4). Protein–Ligand interactions highlighted that amino acid Leu88, Leu159, Ala109, Val143, Tyr161, Ala162, Ala162, and Leu212 are involved in hydrophobic interaction; Ser160, Glu209, and Thr222 are involved in, π–π stacking and hydrogen bond interactions. Furthermore, the structure of the PI3K complexed with Myricetin, Quercetin, Morin, and Luteolin outlined the importance of amino acids Pro810, Trp812, Tyr867, Ile 881, Val882, Ala885, Ile879, Met 953, and Phe961, in hydrophobic interactions and hydrogen bonding with Val882,-Lys833, Asp964 residues (Fig. 5).

A number of reports confirm that Myricetin plays a crucial role in the inhibition of PC-3 cell apoptosis in a dose-dependent manner. Although a noticeable synergistic effect on inhibition of cell proliferation was observed when Myricetin was used in combination with Myricitrin, Myricetin alone was also equally adept in inhibiting the cell proliferation of HL-60 (leukemia), HepG2 (hepatoma), and T24 cells in a dose- and time-dependent manner (Morales and Haza, 2012; Sun *et al.*, 2012; Zhang *et al.*, 2013). Moreover, Myricetin was also found to inhibit H_2O_2-induced apoptosis in Chinese hamster lung fibroblast (V79-4) cells. CID5280343 (Quercetin) has been shown better binding with PDK1 and PI3K with Gscore of −8.55 and −9.89 kcal/mol, respectively, and even better than previously available PI3K-specific inhibitors represented in Table 1. In-vitro and in vivo experiments supported that Quercetin could inhibit proliferation and induce apoptosis of human PC-3 cells (Zhu *et al.*, 2011). Quercetin is observed to arrest the cell cycle at the G0/G1 phase and induce apoptosis of PC-3 by endoplasmic reticulum stress and mitochondrial apoptosis signaling pathway (Liu *et al.*, 2012). Foremost Quercetin has been found to be capable of reducing cell viability and also induce apoptosis in prostate cancer cells by down regulating the AKT, mTOR, and P70S6K expressions (Pratheeshkumar *et al.*, 2012). It was also noted that combination of Quercetin and EGCG synergistically inhibited cell proliferation, induced apoptosis in PC-3 cells, and arrested cell cycle (Wang *et al.*, 2012). Morin is observed to induce apoptosis in HL-60 and hepatocellular cells by activation of caspase-3

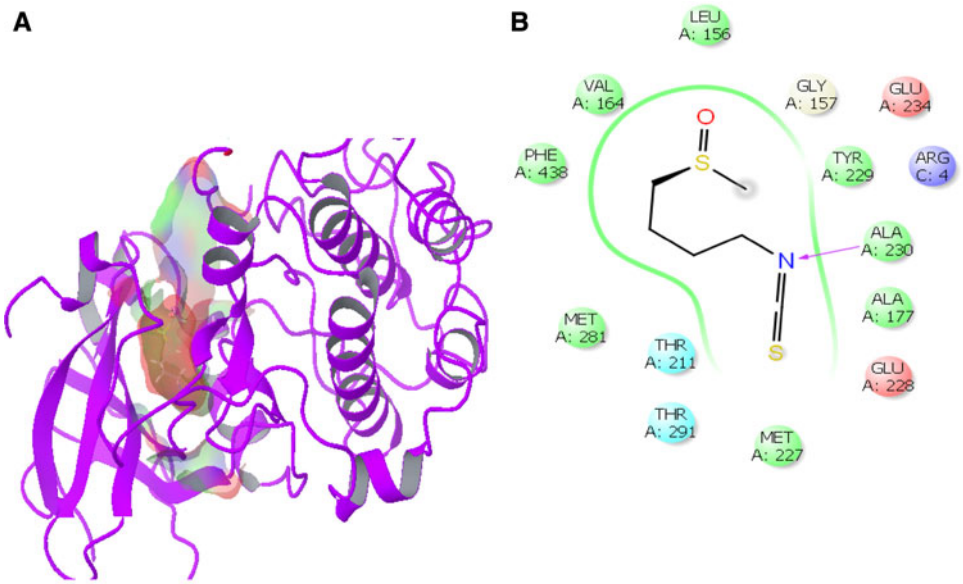

Fig. 2 **a** Ribbon presentation of Akt protein molecule with CID5350. **b** Protein ligands interaction profile of Akt with CID5350

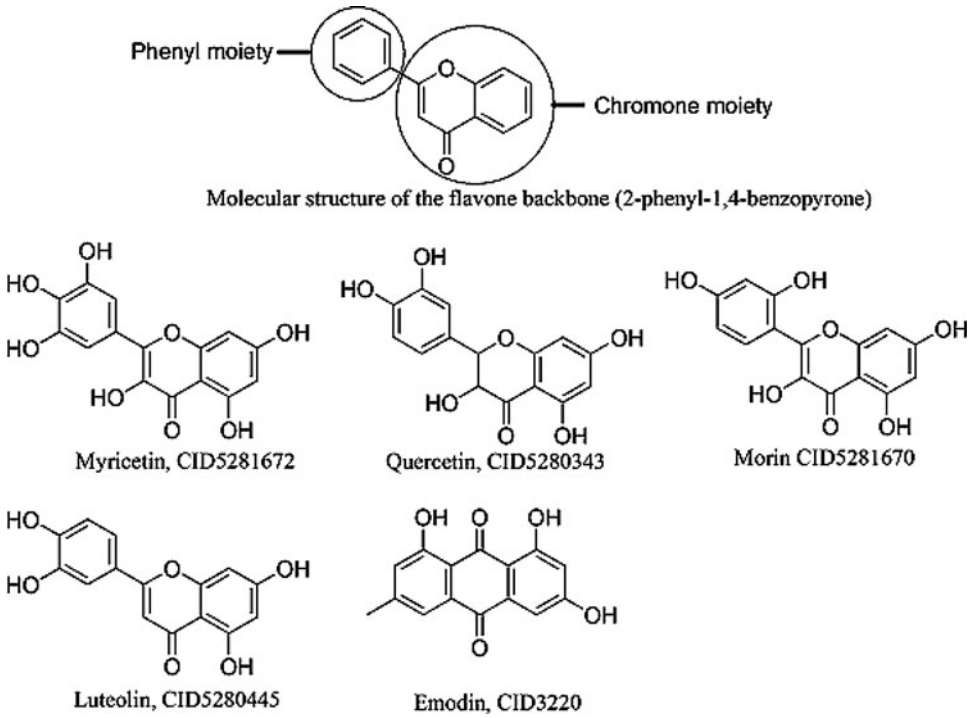

Fig. 3 Chemical structures of the lead molecules

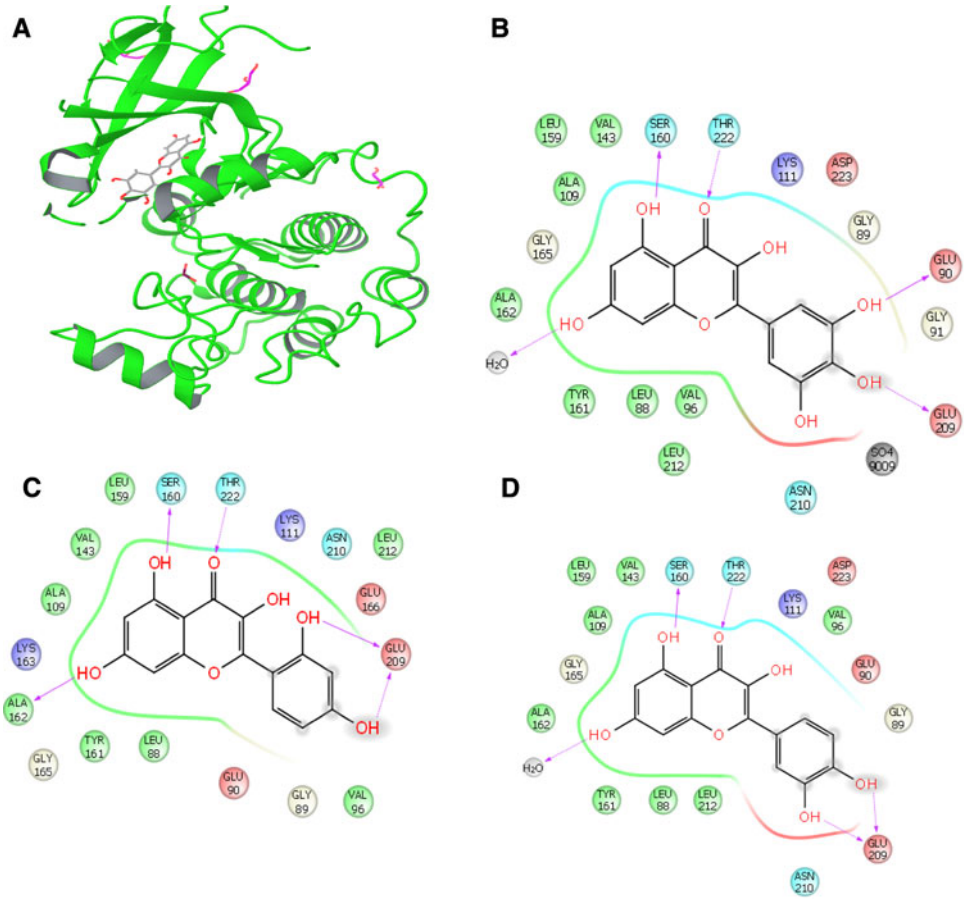

Fig. 4 **a** The ribbon presentation of PDK1 protein molecule with CID5281672. **b** Protein ligands interaction profile of PDK1 with CID5281672. **c** Protein ligands interaction profile of PDK1 with CID5281670. **d** Protein ligands interaction profile of PDK1 with CID5280343

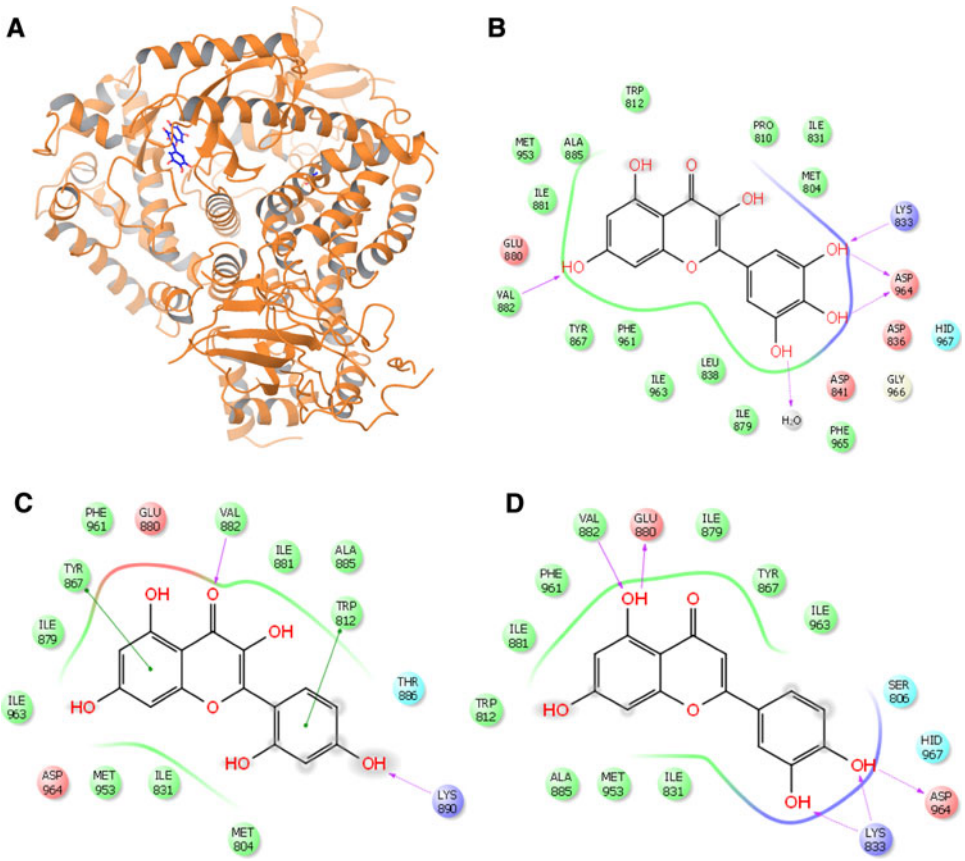

Fig. 5 **a** Ribbon presentation of PIK3 protein molecule with CID5281672. **b** Protein ligands interaction profile of PIK3 with CID5281672. **c** Protein ligands interaction profile of PI3K with CID5280343. **d** Protein ligands interaction profile of PDK1 with CID5280445

cysteine-aspartic acid protease (KUO *et al.*, 2007; Sivaramakrishnan and Devaraj, 2010). Luteolin is one of the most common naturally occurring flavonoids present in edible plants. Luteolin could sensitize cancer cells to inhibit proliferation and induce apoptosis through suppressing cell survival pathways such as PI3K, nuclear factor kappa β (Kim

Table 4 Evaluation of drug-like properties of the lead molecules by Qikprop (Schrodinger 9.3)

S.no.	Compounds (CID)	Q P log $P_{o/w}$ (−2.0 to 6.5)	Q P log HERG (acceptable range: above −5.0)	QPP Caco (nm/sec) <25-poor >500-great	Q P log BB (−3 to 1.2)	QPP MDCK (nm/sec) <25-poor >500- great	Q Plog Kp (−8.0 to −0.1)	Q P log Khsa (Acceptable range: −1.5 to 1.5).	Percent Human Oral Absorption >80 %-High <25 %-Poor
1	5350	0.535	−3.934	116.229	−0.016	6998.824	−1.907	−1.454	67.042
2	5281672	−0.281	−4.869	7.525	−2.827	2.507	−6.323	−0.491	28.028
3	5281670	0.41	−4.923	23.268	−2.244	8.492	−5.333	−0.35	53.806
4	5280343	0.522	−5.356	21.055	−2.418	7.623	−5.353	−0.316	53.688
5	5280445	0.96	−5.051	42.097	−1.946	16.119	−4.851	0.19	61.636
6	3220	1.258	−4.357	79.111	−1.541	31.877	−4.71	−0.101	68.288

Ligand; PubChem IDs of the lead molecules

Q P log $P_{o/w}$ (−2.0 to 6.5) Predicted octanol/water partition co-efficient log p; (range: −0.20 to 6.5)

Predicted IC50 values for blockage of HERG K^+ channels; (acceptable range: above −5.0)

QPP Caco-Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells is a model for the gut blood barrier; (nm/sec) <25-poor >500-great

Q P log BB-Predicted brain/blood partition coefficient

QPP MDCK-Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood–brain barrier; (nm/sec) <25-poor >500-great

Q P log KP-Predicted skin permeability; Q P log K_{hsa}- Prediction of binding to human serum albumin; (acceptable range: −1.5 to 1.5)

Percentage of human oral absorption; (<25 % is poor and >80 % is high)

et al., 2012; Lin *et al.*, 2008; Lopez-Lazaro, 2009). Another natural compound that showed good binding interaction with PDK1 and PI3K is CID3220 (Emodin), a naturally occurring anthraquinone purgative resin present in the roots and barks of numerous plants, molds, and lichens. In our docking studies, Emodin have shown better binding conformation with PDK1 and PI3K (−7.39 and −9.81 kcal/mol, respectively) than any other PI3K-specific inhibitors (listed in Table 1). Myricetin, Quercetin, Morin, and Luteolin have shown potential health benefits owing to the anticancer activity of these phytochemicals; their properties being attributed to the phenolic hydroxyl groups as well as chromone moiety attached to the flavonoid structure.

ADME studies

Pharmacokinetic and pharmacodynamic properties of natural compounds were assessed through the Qikprop application of Maestro 9.3. Myricetin, Quercetin, Morin, Luteolin, and Emodin were found to be promising based on their docking free energy score as well as from percent bioavailability point of view except Myricetin for which percent bioavailability was found to be 28.02. Most interesting aspect of Myricetin, Quercetin, Morin, Luteolin, and Emodin are their excellent Q P log $P_{o/w}$, Q P log HERG K^+ channels, Q P log BB, Q P log KP, Q P log K_{hsa} values which satisfy the Lipinski's Rule of Five (Table 4). However, these compounds do not have very good QPP Caco and QPP MDCK values and also didn't fulfill the "Lipinski's Rule of five". Therefore, structural modifications and optimization to predict structures that have better QPP Caco and QPP MDCK activity are required for further designing multitargeted PDK1 and PI3K inhibitor.

Conclusion

In this study state-of-the-art molecular docking simulation techniques were used preliminarily to investigate the potential multi-target inhibition of the PI3K/AKT/mTOR signaling pathways. Analysis of best docking poses of compounds indicate that there are at least five compounds, viz. Myricetin, Quercetin, Morin, Luteolin, and Emodin, which have significantly better energy scores than previously reported PI3K pathway inhibitors. These compounds have the best docking free energy score against PDK1 and PI3K but the ADME parameters of these compounds were found to be unsatisfactory for developing into therapeutics. Structure modification is a prerequisite for increasing pertinent scores like QPP Caco, QPP MDCK, and percent human oral absorption. Nevertheless, compounds identified in the present investigation have good potential for the prospective development of novel structural analogs which can be used as multi-targeted agents for anticancer drug development. Protein–ligand interaction profile highlighted that lipophilic interactions are the main force at the active site of protein molecules, while hydrogen bonding and π–π stacking interactions also contributes to some extent. Results of these studies can be useful to understand protein–ligand interactions that are required to enhance the cell growth inhibitory activity as well as ADME properties. Further experimental studies are required for the experimental validation of our findings.

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