

Analysis on in-Silico Identification of Novel Activator of Pyruvate Kinase M2

Pankaj Sharma^{1*}, Manvender Singh¹ and Sangeeta Sharma²

¹Department of Biotechnology, UIET, Maharshi Dayanand University Rohtak, Haryana, India

²School of Life Sciences, IIMT University, Meerut, India.

ABSTRACT

Pyruvate kinase M2 isoform (PKM2) in a less active state (dimer form) regulates the rate-limiting step of glycolysis that switches the glucose metabolism to aerobic glycolysis in tumor cells and thus promotes cell proliferation. Allosteric regulated PKM2 switches low to high activity state and prevent growth of cancer. Activators of PKM2 promote tetramer formation and suppress tumorigenesis. We present a structure based virtual screening of a diverse chemical compound collection (Diverse-lib) to identify novel activators of Pyruvate kinase M2 (PDB ID: 4G1N) from *Homo sapiens*. In order to rank potential small molecule hits, two separate docking algorithms were used to produce a consensus score. Four compounds out of 99,288 leads having lowest binding affinity even lower than control NZT compound were identified as activators of PKM2 using MTiOpenScreen and MTiAutoDock servers. Further, these best predicted compounds were subjected for physicochemical, pharmacokinetic and toxicological investigation using preADMET tool and cross verified by SwissADME tool. Compound PubChem SID 17517397 was satisfied all the ADME/Tox parameters out of four activators. In the AutoDock Vina and AutoDock programmes, the binding energy of compound SID 17517397 was -10 kcal / mol and -11.11 kcal/mol with PKM2. Compound SID 17517397 had human intestinal absorption, Caco2 cell permeability, Plasma Protein Binding and Blood-Brain Barrier penetration values as 94.18%, 23.86, 89.50%, 3.30, respectively, which indicates that it is in the range of well absorbed and active compound range. Compound had negative carcinogenicity value in mouse and rat. Therefore, it is concluded that compound could be promising novel activator for PKM2 as drug target but it must be verified by experimental studies.

KEY WORDS: ADMET/TOX, CANCER, MTIAUTODOCK, MTIOPENSREEN, PYRUVATE KINASE M2

INTRODUCTION

In cancer cells, the metabolism differs significantly from that of healthy cells (Kim and Dang, 2006; Vander

Heiden et al., 2009; Zhao et al., 2013). In normal cell rely on glycolysis to produce energy but in tumor cells it switches the glucose metabolism to aerobic glycolysis and this mechanism is called Warburg effect (Warburg, 1956; DeBerardinis et al., 2008). Tumor's glycolysis interventions are a novel approach for targeted anti-cancer therapies (Chen et al., 2007; Gatenby and Gillies, 2007; Porporato et al., 2011). In cancer cell metabolism, the regulation of Pyruvate kinase M2 isoform (PKM2) plays a key role. The last rate-limiting enzyme in the glycolytic pathway is pyruvate kinase (PK), which catalyses the transfer of a phosphate group from phosphoenolpyruvate to ADP to obtain pyruvate and ATP. There are four distinct subtypes of Pyruvate kinase. PKL isoforms exist predominantly

Article Information: *Corresponding Author: pank_lko456@rediffmail.com

Received 12/12/2020 Accepted after revision 23/03/2021

Published: 31st March 2021 Pp- 236-242

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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/14.1/34>

in the liver, kidney and red blood cells, whereas PKR is mostly present in red blood cells (Gupta and Bamezai, 2010; Israelsen et al., 2013, Wong et al., 2013; Yang and Lu, 2013; Otto et al., 2016).

In myocardium, skeletal muscle and brain tissue, PKM1 is distributed, and in tissues such as the brain and liver, PKM2 is distributed (Israelsen et al., 2013). For cancer metabolism and tumour development, PKM2 is essential, yet tetramer and dimer of PKM2 consist of the same monomer (Ashizaw et al., 1991; Yang and Lu, 2015). There are substantially different biological effects between the tetramer and dimer form (Muñoz-Colmenero et al., 2015). In the sense of glucose metabolism, the tetramer primarily plays the role of pyruvate kinase and controls glycolysis and dimer PKM2 as a switch for energy metabolism and material synthesis (Dombrauckas et al., 2005). In the dimer state, PKM2 can enter the nucleus to regulate gene expression, epithelial-mesenchymal transition (EMT), invasion and metastasis and cell proliferation. Zhang et al. (2019) first used the switching effect of PKM2 in glucose metabolism to expand and enrich the Warburg effect (Zhang et al., 2019).

Endogenous and exogenous activators allosterically regulate the change between PKM2 dimers and tetramers. PKM2 dimers are tetramerized using activators that allow PKM2 to behave like PKM1 induces reversal of the Warburg effect in cancer cells. Phosphorylation or acetylation of native tetrameric PKM2 in cancer cells causes a transition to a dimeric/monomeric form, which translocate into the nucleus and causes oncogene transcription. However, it is unclear how these post-translational modifications (PTMs) cause PKM2 to lose its oligomeric state. Nandi et al. (2020) performed crystallographic and biophysical studies of PKM2 mutants containing residues that mimic phosphorylation and acetylation Nandi et al. (2020).

They discovered that PTMs cause a significant structural reorganization of the binding site for fructose 1, 6-bisphosphate (FBP), an allosteric activator, affecting the interaction with FBP and causing oligomerization disruption (Nandi et al., 2020). In the current research, we have implemented a virtual screening approach to search out the novel activator for the treatment of cancer targeting the PKM2 protein. In-silico physicochemical, pharmacokinetic and toxicological properties of activators were analysed by preADMET and cross-checked with the SwissADME web tool (Lee et al., 2003; Daina et al., 2017).

MATERIAL AND METHODS

The 3D crystal structure of Pyruvate kinase isoform M2 in complex with an activator was retrieved from the Protein Data Bank with PDB ID: 4G1N (Kung et al., 2012). All the water molecules, Oxalate, Magnesium ions and N-(4-{[4-(pyrazin-2-yl) piperazin-1-yl] carbonyl} phenyl) quinoline-8-sulfonamide (NZT) were removed and polar hydrogen added to PKM2 protein for structure based virtual screening as well as molecular docking

processes. Small-molecule activator NZT bind PKM2 at the subunit interaction interface, a site that is distinct from fructose-1,6-bisphosphate (FBP) was used as activator binding site in molecular docking (Kung et al., 2012). The virtual screening was performed using the diverse chemical compound library (Diverse-lib) database. The compound library consisted of 99,288 diverse drug-like PubChem compounds from in house Diverse-lib database of RPBS Web portal. The compound library was filtered by using the criteria such as molecular weight <500 Dalton; hydrogen bond donor <5, hydrogen bond acceptor <10, octanol-water partition coefficient logP <5; Number of rotatable bonds <8, polar surface area <140 Å (Labbé et al., 2015).

Docking was carried out on the MTiOpenScreen server with AutoDock Vina and with AutoDock on MTiAutoDock server (Labbé et al., 2015). The rankings of AutoDock Vina and AutoDock were combined to construct a consensus list of compounds with both techniques that scored well. Validation and Optimization process of best predicted compounds had been processed by PreADMET tool and cross check by Swiss ADME tool, which are web-based application for predicting absorption, distribution; metabolism, elimination and toxicity (Lee et al., 2003; Daina et al., 2017). Using Python Molecular Viewer software, docking findings were visualized to display the 3D structure and position of activator binding to the protein (Sanner, 1999; Daina et al., 2017).

RESULTS AND DISCUSSION

Activator binding site analysis: Small molecule activator PubChem SID 17517397 bind PKM2 at the subunit interaction interface, a site different from that of the endogenous activator fructose-1, 6-bisphosphate (FBP). The activator binding sites of PKM2 comprises 8 residues on chain A such as TYR390, ASP354, GLN393, ILE389, PHE26, GLU397, LEU353, LEU394 and 7 residues on chain B as MET30, PHE26, TYR390, LYS311, LEU394, LEU353, ASP354. Binding of activator to PKM2 promoted a constitutively active enzyme state (tetramer form).

Structure based virtual screening: 1500 small molecules were screened after applying filter criteria in database. A gradient-based conformational search approach was employed by AutoDock Vina. The grid box parameters were set to values of 3.743 Å, -12.72, and 48.977 Å for the grid box center and 34 Å × 28 Å × 32 Å for the box dimensions. We use a total of 10 binding modes and 8 for exhaustiveness. The scoring of the docking poses produced and the ranking of the ligands were based on the empirical scoring function of Vina approximating the affinity of binding in kcal/mol. Top 100 compounds were selected based on their lowest binding energy and further docked with activator binding site of PKM2 using AutoDock. Docking results of both were shown in table 1.

Four compounds with PubMed SID 17517397, 26649876, 49737693, 4247715 were selected based on binding energy of compound with PKM2 protein having >-9.5kcal/mol in

AutoDock Vina and >-11 kcal/mol in AutoDock, which is even lower than control NZT compound (binding energy: -7.95 kcal/mol with PKM2 protein).

ADME/Tox properties of best predicted compounds: The properties of human intestinal absorption were critical for the production of drugs that purport to be orally administered (Zhao et al., 2001; Postigo et al., 2010). Human intestinal absorption (%HIA) values of four best predicted compounds with PubMed SID 17517397, 26649876, 49737693 and 4247715 were shown in table 2. These compounds have been identified in the category of well absorbed compounds (HIA: 70 ~ 100 %) (Yee, 1997). *In vitro* cell permeability Caco-2 is an important

test that measures drug intestinal absorption (Yazdanian et al., 1998). In the MDCK method, the cell permeability *in vitro* used canine kidney cells and has a shorter growth rate than the Caco-2 cells were used as a tool for the rapid analysis of permeability (Irvine et al., 1999). In the pharmaceutical industry, skin permeability is used to measure the toxicity of chemical products in the event of unintended skin touch (Singh and Singh, 1993). Moreover, the blood-brain barrier (BBB) was essential for drug pharmacology. PCaco-2, MDCK, Skin Permeability, PPB and BBB of four compounds were shown in table 2. Compound SID 26649876 had a BBB value greater than 2.0 and was graded in the central nervous system with high absorption (Ma et al., 2005).

Table 1. Docking results of Autodock Vina and Autodock programmes with physicochemical properties of compounds.

Sl. No	PubChem SID	BE (AutoDock Vina)	BE (AutoDock)	nRot	HBA	HBD	LogP	MW	TPSA
1	17517397	-10	-11.11	6	4	2	3.98	374.9043	58.2
2	26649876	-9.9	-11.34	6	6	1	3.29	374.4357	72.95
3	24838666	-9.6	-9.25	4	7	2	3.11	400.8587	99.94
4	49737693	-9.5	-11.28	8	7	1	3.65	413.8542	86.48
5	24339460	-9.5	-7.35	2	7	2	0.18	193.1628	96.51
6	4247715	-9.5	-11.71	6	7	1	3.77	398.4108	118.38
7	24798741	-9.4	-9.38	7	7	0	1.98	340.3764	85.26
8	4256388	-9.4	-9.34	1	4	0	3.1	218.2981	43.6
9	17477384	-9.4	-9.47	4	5	3	2.95	352.4069	97.64
10	26664864	-9.2	-8.57	1	5	0	1.63	204.1821	57.38
11	49736489	-9.2	-8.92	2	4	0	3.98	338.7876	46.34
12	17469113	-9.2	-8.24	3	4	1	2.75	260.1661	52.6
13	24779480	-9.2	-10.05	6	7	2	2.68	360.4108	153.7
14	17479262	-9.1	-9.95	5	6	0	3.88	295.2927	84.74
15	56317084	-9.1	-9.31	4	6	0	2.22	256.2136	78.36
16	26534815	-9.1	-9.85	2	5	0	3.39	317.386	103.68
17	7971621	-9.1	-8.93	3	7	1	1.52	331.3464	107.75
18	22413776	-9	-9.75	2	5	0	3.62	253.256	63.12
19	49716534	-8.9	-9.62	6	7	1	2.23	392.4477	113.63
20	49817894	-8.9	-8.26	6	6	1	2.41	356.2965	73.59
21	26537356	-8.9	-8.70	3	4	2	3.97	337.8028	57.78
22	17449251	-8.9	-10.08	6	7	1	3.88	369.3944	128.51
23	89852923	-8.9	-8.00	2	6	1	0.97	190.1588	87.39
24	862504	-8.9	-8.81	4	6	0	3.29	330.3401	73.93
25	849520	-8.9	-10.84	8	7	2	2.36	386.4216	106.02
26	4242876	-8.9	-8.57	2	3	1	3.49	275.3013	42.24
27	22402150	-8.8	-9.88	4	7	1	3.01	352.3672	121.87
28	26620089	-8.8	-11.71	6	6	1	3.18	414.5178	84.09
29	7967102	-8.8	-9.46	4	6	0	3.44	349.1363	78.36
30	57263884	-8.8	-10.18	10	7	2	2.99	372.415	97.64
31	24784913	-8.8	-9.11	6	6	0	3.24	376.4516	62.47
32	26650965	-8.8	-11.12	5	7	0	3.04	394.399	80.82
33	49674809	-8.8	-6.59	7	6	2	3.5	376.4052	95.15
34	24296145	-8.8	-8.14	2	7	1	3.1	323.3293	123.89
35	26725965	-8.8	-10.68	5	7	2	3.51	420.3899	102.68
36	17447282	-8.8	-8.45	2	4	1	3.59	305.7362	84.61

Table 1 Continue

37	24831749	-8.7	-9.88	6	5	0	3.53	335.3533	65.49
38	26649874	-8.7	-9.13	6	6	2	3.03	368.4263	76.66
39	7964748	-8.7	-10.28	5	7	1	3.03	375.442	111.78
40	17456133	-8.7	-8.24	4	6	3	2.55	329.3736	103.01
41	26647975	-8.7	-8.81	3	5	0	3.79	378.4641	43.21
42	26648535	-8.7	-7.27	5	5	1	3.47	353.4116	56.79
43	24809269	-8.7	-8.75	6	6	1	3.45	300.3092	84.15
44	17465009	-8.7	-6.39	1	4	2	3.69	329.3751	104.53
45	26533701	-8.7	-7.74	2	4	0	2.64	280.2021	48.03
46	26727266	-8.7	-9.89	4	6	0	3.29	327.3313	81.35
47	14730768	-8.7	-9.50	5	5	0	3.3	336.338	73.58
48	49737701	-8.7	-9.69	6	7	1	1.75	346.3777	82.82
49	24364860	-8.7	-6.77	3	4	2	2.98	296.3636	66.13
50	93577670	-8.7	-10.07	7	7	1	2.03	351.356	94.31
51	49645683	-8.6	-8.94	4	6	1	2.96	296.2775	88.28
52	17470642	-8.6	-9.50	5	5	2	3.37	361.3906	67.79
53	4246955	-8.6	-8.87	4	7	2	3.63	338.4069	84.73
54	17512468	-8.6	-7.58	1	5	4	3.06	284.3363	112.04
55	14744922	-8.6	-9.57	7	6	1	2.89	387.4494	97.92
56	26648015	-8.6	-7.70	7	7	2	2.02	390.4814	88
57	860027	-8.6	-7.02	2	4	0	3.48	362.4448	67.2
58	11534585	-8.6	-9.51	3	5	0	3.1	261.2813	67.39
59	851712	-8.6	-9.26	4	6	0	1.56	274.2984	103.14
60	16952534	-8.6	-8.96	4	5	1	3.96	297.3053	72.56
61	17402129	-8.5	-7.61	2	4	1	3.43	319.3324	58.11
62	24811377	-8.5	-11.15	7	7	0	3.64	350.3249	98.15
63	124949736	-8.5	-8.04	6	5	2	3.57	406.3982	67.43
64	24334833	-8.5	-9.20	3	5	1	3.97	292.2888	78.94
65	24293703	-8.5	-10.63	3	7	1	3.02	289.2667	129.19
66	24366734	-8.5	-10.40	4	4	1	3.98	327.7617	59.31
67	49640535	-8.5	-7.27	6	7	2	2.78	384.3793	97.28
68	57259063	-8.5	-7.65	5	6	1	2.76	327.3313	73.86
69	24807319	-8.5	-6.83	5	7	1	1.42	300.2662	86.75
70	24412722	-8.5	-5.59	7	7	1	2.08	434.5108	119.25
71	26729749	-8.5	-7.86	3	5	1	1.68	210.6204	63.5
72	144203702	-8.5	-5.71	9	5	2	3.85	414.3427	64.17
73	4247822	-8.5	-7.67	4	6	2	3.42	400.473	71.33
74	49718523	-8.5	-8.99	5	6	0	3.47	383.484	60.01
75	89855761	-8.5	-7.13	1	5	1	1.08	195.2184	68.01
76	3714881	-8.5	-10.69	6	6	0	3.84	365.4057	99.48
77	14720958	-8.5	-9.47	8	5	0	3.82	404.5014	57.69
78	17445644	-8.5	-9.34	5	5	1	3.9	336.2653	74.92
79	26652683	-8.4	-8.94	3	4	1	3.13	336.3612	93.48
80	24787424	-8.4	-9.58	4	7	1	1.19	234.2114	92.96
81	17452005	-8.4	-8.47	5	7	2	3.42	359.3813	96.02
82	4247583	-8.4	-9.82	6	7	2	1.22	388.4639	137.67
83	7977979	-8.4	-10.98	8	6	1	3.76	383.464	102.55
84	49733540	-8.4	-10.37	5	6	2	2.97	385.3905	108.86
85	24834152	-8.4	-8.08	6	6	3	2.43	387.4958	100.88
86	24348650	-8.4	-10.17	6	7	1	3.94	383.7852	97.04
87	24801340	-8.4	-7.24	2	4	0	2.74	277.2741	52.21
88	24365159	-8.4	-9.17	3	5	0	2.3	246.2619	67.41
89	847046	-8.4	-8.00	5	6	0	2.26	252.6571	69.9
90	104223077	-8.4	-9.72	8	6	2	1.78	354.3997	88.4
91	14722665	-8.4	-9.49	4	7	1	1.67	280.2566	122.81
92	17506536	-8.4	-9.37	4	6	0	2.82	292.3303	75.36

Table 1 Continue

93	24824418	-8.4	-10.57	1	6	2	1.69	293.2768	84.08
94	24320463	-8.4	-9.80	4	7	1	2.78	357.2846	98.39
95	24798468	-8.4	-10.25	6	6	2	2.65	338.4036	83.81
96	85269299	-8.4	-10.17	8	7	1	3	332.3511	101.22
97	26618413	-8.4	-8.21	4	7	2	1.03	383.4673	112.7
98	17515744	-8.4	-11.00	5	6	1	3.55	339.3684	116.05
99	24782536	-8.4	-10.26	3	7	2	1.94	272.2594	99.84
100	17453955	-8.3	-9.17	1	6	2	1.1	242.2334	83.54

BE: binding energy (kcal/mol), nRot:number of rotatable bonds, HBA: hydrogen bond donor Count, HBD: hydrogen bond acceptor Count, LogP: partition coefficient of a molecule between an aqueous and lipophilic phase, MW: molecular weight, TPSA:topological polar surface area.

Carcinogenicity is the toxicity in the body that causes cancer. Compounds SID 26649876 had positive carcinogenicity value in rat. Compounds SID 26649876, 49737693 and 4247715 had a BBB value less than 2.0 and was graded in the central nervous system with low absorption (Ma e al., 2005). Further, ADME properties of compounds PubChem SID 17517397, 26649876, 49737693 and 4247715 were analysed by SwissADME tool (Daina et

al., 2017). All the compounds were qualified five different rule-based filters such as Lipinski filter implemented rule-of-five, Ghose, Veber, Egan and Muegge rules shown in table 3 (Ghose et al., 1999; Egan et al., 2000; Muegge et al., 2001; Veber et al., 2002; Lipinski, 2004). The result provided in table 3 shows that all the compounds investigated have high gastrointestinal absorption and good skin permeation.

Table 2. ADMET properties of best predicted activators.

Pubchem SID	P _{Caco} -2 (nm/sec)	MDCK (nm/sec)	Skin Permeability	%HIA	%PPB	BBB	Carcinogenicity		
							Ames test	Mouse	Rat
17517397	23.86	0.11	-3.91	94.18	89.50	3.30	mutagen	-ve	-ve
26649876	31.32	0.13	-3.25	96.35	99.32	0.19	mutagen	-ve	+ve
49737693	26.33	1.55	-3.80	96.77	87.55	0.02	mutagen	-ve	-ve
4247715	19.85	0.84	-4.13	97.98	92.05	0.18	mutagen	-ve	-ve

PPB:Plasma Protein Binding, BBB: blood brain barrier.

Table 3. The pharmacokinetics and drug likeliness prediction of four best predicted compounds

Pubchem SID	GI	BBB	Log Kp (skin permeation Coefficient) (cm/s)	Bioavalia- bility Score	Lipinski	Ghose	Veber	Egan	Muegge
17517397	High	Yes	-5.76	0.55	Yes	Yes	Yes	Yes	Yes
26649876	High	Yes	-6.25	0.55	Yes	Yes	Yes	Yes	Yes
49737693	High	No	-6.23	0.55	Yes	Yes	Yes	Yes	Yes
4247715	High	No	-6.05	0.55	Yes	Yes	Yes	Yes	Yes

GI: Gastrointestinal absorption, BBB: Blood Brain Barrier penetration.

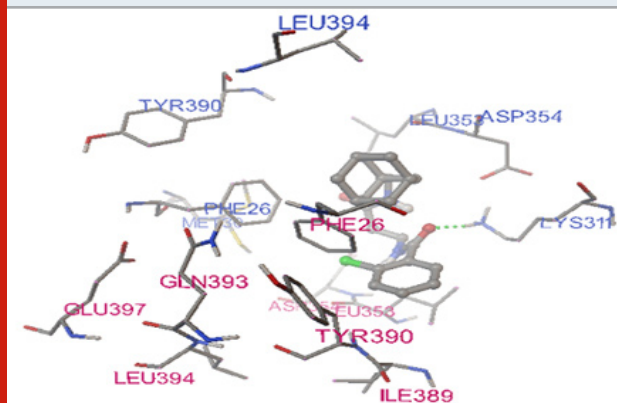
Compounds SID 49737693 and 4247715 indicate negative results in blood-brain barrier (BBB) permeation. The SwissADME estimates for passive human gastrointestinal absorption (GI) and permeation to the blood-brain barrier (BBB) consists of the BOILED-Egg model reading (Butina et al., 2002). Therefore, only one compound PubChem SID 17517397 qualifies all parameters of ADME /Tox on analysis of four best predicted compounds using PreADMET and SwissADME tools (Butina et al., 2002). Li et al. (2018) used structure-based virtual screening

to find successful activators targeting PKM2 in the ZINC database. ZINC08383544, a screened compound, promotes the development of PKM2 tetramers, effectively blocks PKM2 nuclear translocation, and inhibits tumour growth, suggesting that it may be a novel PKM2 activator (Li et al., 2018).

Visualization of protein-ligand interaction: Best docked complex was analyzed through Python Molecular Viewer

for their interaction study shown in figure 1. It is evident from this analysis that compound SID 17517397 was located at the subunit interaction interface of protein and was stabilized by hydrogen bonding.

Figure 1: Docking pose of compound SID 17517397 on PKM2 protein structure. One H-bond was formed between amino acid LYS311 of protein Chain B with compound. Chain A and B binding residues of PKM2 protein were colored with pink and royal blue, respectively.



CONCLUSION

The current research utilizes structure based virtual screening to identify human Pyruvate kinase M2 isoform (PKM2) protein activator that is required for cancer treatment. From thousands of chemical structures and a sequence of steps of rational refinement, including virtual screening, molecular docking and ADME/Tox studies, we identified compound PubChem SID 17517397 as novel activator of pyruvate kinase M2 protein as drug target for further experimental testing.

ACKNOWLEDGEMENTS

We would like to thank Professor Rahul Rishi, Director UIET, MDU, Rohtak, India and Dr. Sonia, HOD & Associate Professor, Department of Biotechnology, MDU, Rohtak, India for helping in preparation of the research paper.

Conflict of Interests: Authors declare no conflict of interests among themselves.

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