Advances and Applications in Mathematical Sciences
Volume 21, Issue 11, September 2022, Pages 6557-6570

IN SILICO SCREENING AND MOLECULAR DOCKING STUDIES OF POTENTIAL INHIBITORS OF $PP1\gamma_2$

RITIKA SAXENA, NABEEL AHMAD and SANJAY MISHRA

[1]School of Biotechnology
IFTM University
Moradabad (U.P.)-244102, India

[2]Professor
Dean and School of Allied Sciences
Dev Bhoomi Uttarakhand University
E-mail: nabeel.biotech@gmail.com

[3]Former Professor
School of Biotechnology IFTM University
Moradabad (U.P.)-244102, India
E-mail: sanjaymishra66@gmail.com

Abstract

Testis-specific $PP1\gamma_2$ is indispensable in the final stages of spermatogenesis. $PP1\gamma_2$ phosphatase activity has been found to be inversely associated to motility, with low activity in vigorously motile caudal spermatozoa and high activity in immotile caput spermatozoa. In recent study, $PP1\gamma_2$ was subjected to molecular docking study by screening of a ligand library of known natural phytochemical compounds which in turn may inhibit the catalytic activity of $PP1\gamma_2$ and ultimately resulting in high sperm motility. In our study, three compounds were identified as potential candidates which showed better binding energies. These phytochemicals were α-terpineol, Coumarin and 2-Phenylpropan-2-ol. Using these modern techniques, these molecules could be used to enhance sperm motility.

2020 Mathematics Subject Classification: 92-08.
Keywords: Spermatogenesis, $PP1\gamma_2$ Molecular Docking, Phytochemical Compounds, Sperm Motility.
*Corresponding author; E-mail: ritikashrivastava88@gmail.com
Received June 1, 2022; Accepted September 10, 2022

1. Introduction

Sperm maturation in the epididymis is critical for the development of male gamete function. In mammals, testicular spermatozoa are immotile and unable to fertilize ova. During the passage of spermatozoa through the epididymis, these functions are acquired, while concomitantly undergoing noticeable alterations in physico-chemical properties [1]. The epididymis, an androgen responsive tissue, is itself characterized by marked differences in secretary and adsorptive characteristics in its descending caput, corpus, and caudal segments [2-4]. The epididymis is in charge of maintaining luminal ion concentration, providing metabolic support for sperm viability, vesicular transportation for maturation, quality control, spermatozoa storage, and protection [5].

A key signaling protein, $PP1\gamma_2$ in spermatozoa was identified [6,7]. There have been few studies on protein phosphatases in spermatozoa until recently [8]. It has long been assumed that a protein phosphatase governs flagellar motility, based on the discovery that protein phosphatases in the reactivation media inhibit demembranated invertebrate spermatozoa from initiating movement [9-11].

In recent study, $PP1\gamma_2$ was subjected to molecular docking study and 50 natural phytochemicals library was prepared. Before docking, phytochemicals were subjected to the Lipinski rule of five (RO5). It is a rule of thumb for determining drug similarity or whether a chemical molecule with a specific pharmacological or biological activity has features that would make it a likely orally active medication in humans. The rule states that most "drug-like" molecules have Log $P \leq 5$, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5 [12]. Further, selected compounds were used to molecular docking. Molecular docking is a type of bioinformatic modelling technique in which two or more molecules combine to generate a stable adduct. It predicts the three-dimensional structure of any complex based on the binding characteristics of the ligand and target. Molecular docking provides a variety of potential adduct structures, which are scored and grouped together using the software's scoring mechanism [13]. LIGPLOT software was used to evaluate the results

of the molecular docking. LIGPLOT is a bioinformatics application that uses standard Protein Data Bank file input and docked molecules to build schematic 2-D representations of protein-ligand complexes.

2. Material and Methods

2.1 Construction of Phytochemical Library

To know the activity of natural phytochemicals against human target $PP1\gamma_2$ a library of 50 compounds was prepared from natural source through searching scientific literature.

2.2 Ligand Preparation

Each phytochemical's 3D structure was downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov) and converted to PDB files using Open Babel. The reference molecules which was co-crystallize with protein was employed as a reference. Protein Data Bank was used to obtain the structure of the reference molecule.

2.3 Protein Receptors Preparation

The 3D structure of protein $PP1\gamma_2$ from Homo sapiens was obtained from the Protein Data Bank (https://www.rcsb.org) (PDB ID 1it6). PyMOL software was used to remove all water molecules, ions, and ligands from the protein molecule. Thereafter, the MG Tools of AutoDock Vina software were used for adding the hydrogen atoms to the receptor molecule [14]. After that, the protein structure was saved in PDB format for further study.

2.4 Active Site Prediction

Before starting docking procedure, the x, y, and z coordinates of the reference molecule were retrieved using the "centerofmass" command line in PyMOL programme to determine the center of mass of the co-crystallized ligand. These coordinate sites are active site of protein. The coordinate sites of protein $PP1\gamma_2$ is (36.72, 16.55, 36.08).

2.5 Drug Likeness and ADMET Analysis

DruLi To open-source software was used to calculate drug-likeness in order to determine the cytotoxicity activity of compounds in humans. A

ligand's pharmacological importance is determined by its drug bioavailability or drug-likeness, which is based on physiochemical and structural features. As a result, DruLiTo software assessed all ligands for their druglike properties using Lipinski's five guidelines [15].

2.6 Molecular docking

Molecular docking was performed into the active site of $PP1\gamma_2$ domain using Autodock vina [14] under $PyRx$ that is a open source software (GUI version 0.8 of autodock). A grid box point of $PP1\gamma_2$ enzyme in x, y and z directions was built according to its coordinate site with a grid spacing of 25 A°. The ligand molecules remained flexible throughout the docking procedure, and the macromolecule was maintained rigid. Docking was used to generate a list of probable ligand orientations and conformations at the binding site. The best interactions better than that one of the positive controls were chosen with the lowest binding affinity pose or docking score. The Ligplot+ v.1.4.5 software was used to estimate molecule interactions between protein-ligand conformations, including hydrogen bonds and bond lengths.

2.7 Visualization

To find the interactions of amino acids between protein and ligand complex, the LigPlot+ v.1.4.5 programme was used to analyse 2D hydrogen and hydrophobic interactions of the protein-ligand complex structure. In each docking pose, LigPlot represented hydrophobic bonds, hydrogen bonds, and their bond lengths.

3. Results

3.1 Construction of Phytochemical Library

In view to less studied phytochemicals from plants as potential lead molecules against $PP1\gamma_2$ our study explored small molecules of plant origin to identify their binding affinity for the selected protein. 50 small molecules were collected from PubChem database (listed in table below along with their Pubchem CID), their property details and SDF file were obtained.

Table 1. List of compounds used in the study.

S. No.	Phytochemical	Pubchem CID
1.	Stigmasta-4,22-dien-3. beta.-ol	91744884
2.	Cholesterol	5997
3.	4-Ethoxy-6-piperidin-1-yl-[1,3,5]triazine-2-carboxylic acid amide	4417775
4.	9-octadecenamide	5353370
5.	Benzyl glucosinolate (glucotropaeolin)	6537197
6.	O-Ethyl-4-[(alpha-L-rhamnosyloxy)-benzyl]-carbamate	129712240
7.	Pterygospermin	72201063
8.	Moringyne	131751186
9.	Isooctanol	15450
10.	1,14-Tetradecanediol	88261
11.	Dibutyl phthalate	3026
12.	1-Hepten-4-ol	19040
13.	2,2,3,3,5,6,6-Heptamethyl- Heptane	23652
14.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	20393
15.	4,6,8- trimethyl-1-Nonene	41077
16.	2-ethyl-2-propyl-1-Hexanol	256822
17.	Squalene	638072
18.	2,4,6- cycloheptatrien-1-one,3,5-bis-trimethylsilyl-	610038
19.	cis-4-cyclopentene-1,3-diol	10148988
20.	3,8-Dimethyl-2,7-dioxaspiro [4.4] nonane-1,6-dione	18274
21.	2-Phenylpropan-2-ol3	12053
22.	Alpha citral	638011
23.	Tridecane	12388
24.	Heptadecane	12398
25.	4-hydroxy- Benzeneacetonitrile	26548
26.	Neophytadiene	10446
27.	6,10,14-trimethyl-2-Pentadecanone	10408

28.	2-Phenyltridecane	20636
29.	9-Hexadecyn-1-ol	145155
30.	Ethyl 9-hexadecenoate	5364759
31.	Stigmasterol	5280794
32.	cis -9- hexadecenal	5364643
33.	α-terpineol	17100
34.	benzyl isothiocyanate	2346
35.	Octadecenoic acid	172146
36.	2-Thiazolamine	2155
37.	Phenanthridone	1853
38.	Cinnoline	9208
39.	Coumarin	323
40.	Ribitol	6912
41.	Androstan	92877
42.	Ibogamine	100217
43.	Aristolocholic acid	2236
44.	dl-Mevalonic acid lactone	10428
45.	2-methyl-3-[4-t-butyl] phenyl- Propanoic acid	106694
46.	Stigmast-4-en-3-one	5484202
47.	Stigmast-4-en-3-one	5281365
48.	Cholesta-4,6-dien-3-one	3034666
49.	4',7-Dimethoxyisoflavone	136419
50.	Sinapyl alcohol	5280507

The SDF files of these 50 molecules were converted to PDB file using Open Babel, the screening of molecules was done using DruLito software. Similar study was conducted by Chandra et al., in which they utilized this software in their study to identify natural compounds against COVID-19 [16].

Table 2. Molecular Property determination of Ligands.

Sr. No.	Title	MW	logp	Alogp	HBA	HBD	TPSA	AMR	nRB	nAtom	N Acidic Group	RC	N Rigid B	nAromRing	nHB	SAlerts
1	91744884	426.39	11.188	1.896	1	1	20.23	129.2	5	81	0	4	29	0	2	1
2	5997	386.35	10.518	1.555	1	1	20.23	115.2	5	74	0	4	26	0	2	1
3	4417775	251.14	-0.047	-2.399	7	1	92.64	57.44	4	35	0	2	15	1	8	0
4	5353370	281.27	7.464	-2.738	2	1	43.09	69.14	15	55	0	0	4	0	3	2
5	6537197	409.05	-1.152	-0.694	8	5	199.8	93.91	7	45	1	2	20	1	13	4
6	1.3E+08	357.14	-0.927	-0.163	9	4	140.7	87.56	7	48	0	2	19	1	13	2
7	72201063	406.08	4.884	5.045	4	0	89.12	127.7	4	46	0	5	28	2	4	2
8	1.32E+08	312.12	0.46	-0.044	7	4	116.5	77.95	4	42	0	2	19	1	11	2
9	15450	130.14	3.066	-0.484	1	1	20.23	34.73	5	27	0	0	3	0	2	1
10	88261	230.22	4.776	-4.353	2	2	40.46	49.49	13	46	0	0	2	0	4	1
11	3026	278.15	3.646	-0.196	4	0	52.6	73.97	10	42	0	1	10	1	4	3
12	19040	114.1	1.872	-0.072	1	1	20.23	32.28	4	22	0	0	3	0	2	1
13	23652	198.23	8.173	4.481	0	0	0	65.17	4	44	0	0	9	0	0	0
14	20393	278.15	3.979	-0.119	4	1	63.6	74.53	9	42	1	1	11	1	5	2
15	41077	168.19	6.619	1.734	0	0	0	56.08	6	36	0	0	5	0	0	1
16	256822	172.18	4.388	-1.148	1	1	20.23	50.51	7	36	0	0	4	0	2	0
17	638072	410.39	11.482	8.552	0	0	0	144.8	15	80	0	0	14	0	0	2
18	610038	250.12	3.426	5.556	1	0	17.07	68.1	2	38	0	1	14	0	1	1
19	10148988	100.05	-0.254	-0.842	2	2	40.46	25.16	0	15	0	1	7	0	4	1
20	18274	184.07	1.057	-0.425	4	0	52.6	43.53	0	25	0	2	14	0	4	3
21	12053	136.09	1.229	1.342	1	1	20.23	46.07	1	22	0	1	9	1	2	0
22	638011	152.12	2.701	2.489	1	0	17.07	50.24	4	27	0	0	6	0	1	3
23	12388	184.22	7.737	-3.065	0	0	0	43.83	10	41	0	0	2	0	0	1
24	12398	240.28	10.013	-4.217	0	0	0	55.47	14	53	0	0	2	0	0	1
25	26548	133.05	0.228	0.714	2	1	44.02	42.09	1	17	0	1	9	1	3	0
26	10446	278.3	10.595	2.536	0	0	0	86.27	13	58	0	0	6	0	0	1
27	10408	268.28	8.221	1.035	1	0	17.07	77.65	12	55	0	0	6	0	1	0
28	20636	260.25	9.132	-0.944	0	0	0	74.83	11	51	0	1	8	1	0	1
29	145155	238.23	6.469	-2.562	1	1	20.23	60.59	11	47	0	0	5	0	2	2
30	5364759	282.26	7.798	-1.265	2	0	26.3	72.16	15	54	0	0	4	0	2	4
31	5280794	412.37	11.071	1.257	1	1	20.23	125.3	5	78	0	4	28	0	2	1
32	5364643	238.23	7.468	-1.719	1	0	17.07	62.17	13	47	0	0	3	0	1	3
33	17100	154.14	2.369	1.122	1	1	20.23	47.92	1	29	0	1	10	0	2	1
34	2346	149.03	2.198	1.26	1	0	44.45	49.69	2	17	0	1	8	1	1	2
35	172146	282.26	8.597	-3.316	2	1	37.3	65.66	15	54	1	0	4	0	3	2
36	2155	100.01	-0.223	-0.497	2	1	63.68	25.33	0	10	0	1	6	1	3	0
37	1853	195.07	1.422	1.138	2	1	29.1	65.02	0	24	0	3	17	2	3	0
38	9208	130.05	1.639	0.153	2	0	24.72	38.66	0	16	0	2	11	2	2	0
39	323	146.04	1.022	1.031	2	0	26.3	45.6	0	17	0	2	12	1	2	2
40	6912	152.07	-3.224	-2.429	5	5	101.2	32.44	4	22	0	0	5	0	10	0
41	92877	276.25	7.414	0.242	1	1	20.23	77.64	0	52	0	4	23	0	2	0
42	100217	280.19	2.802	-0.623	2	1	15.27	87.91	1	45	0	5	24	2	3	0

43	2236	341.05	2.02	0.575	5	1	108.1	89.34	3	36	1	4	25	3	6	3
44	10428	130.06	-0.036	-0.638	3	1	46.53	29.06	0	19	0	1	9	0	4	2
45	106694	220.15	3.698	2.945	2	1	37.3	68.97	4	36	1	1	12	1	3	0
46	5484202	412.37	11.588	1.956	1	0	17.07	124.4	6	78	0	4	27	0	1	0
47	5281365	290.26	6.168	4.804	1	1	20.23	99.13	10	55	0	0	10	0	2	1
48	3034666	382.32	10.393	2.633	1	0	17.07	117.5	5	70	0	4	26	0	1	0
49	136419	282.09	1.766	0.305	4	0	44.76	87.39	3	35	0	3	20	2	4	1
50	5280507	210.09	0.924	-0.333	4	2	58.92	62.22	4	29	0	1	11	1	6	0

Molecules failing Lipinski rule of 5 Molecules passing Lipinski rule of 5 .

DruLito software identified 33 molecules which had suitability for lead molecules owing to follow Lipinski rule of 5. The table below shows marking of molecules that fails Lipinski (highlighted with green-17 molecules) and that obeys it (highlighted with red-33 molecules).

Molecules that were screened for further docking

1. 4-Ethoxy-6-piperidin-1-yl-[1,3,5] triazine-2-carboxylic acid amide
2. Benzyl glucosinolate (glucotropaeolin)
3. O-Ethyl-4-[(alpha-L-rhamnosyloxy)-benzyl]-carbamate
4. Pterygospermin
5. Moringyne
6. Isooctanol
7. 1,14-Tetradecanediol
8. Dibutyl phthalate
9. 1-Hepten-4-ol
10. 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
11. 1-Hexanol, 2-ethyl-2-propyl-
12. 3,5-bis-trimethylsilyl-2,4,6- cycloheptatrien-1-one
13. cis-4-cyclopentene-1,3-diol
14. 3, 8-Dimethyl-2,7-dioxaspiro [4.4] nonane-1,6-dione
15. 2-Phenylpropan-2-ol
16. Alpha citral

17. 4-hydroxy-Benzeneacetonitrile

18. α-terpineol

19. benzyl isothiocyanate

20. 2-Thiazolamine

21. Phenanthridone

22. Cinnoline

23. Coumarin

24. Ribitol

25. Ibogamine

26. Aristolocholic acid

27. dl-Mevalonic acid lactone

28. 2-methyl-3-[4-t-butyl] phenyl-Propanoic acid

29. Stigmast-4-en-3-one

30. Stigmast-4-en-3-one

31. Cholesta-4,6-dien-3-one

32. 4',7-Dimethoxyisoflavone

33. Sinapyl alcohol

3.2 Target Protein

Prior to docking, all water and solvent molecules in the PDB file of $PP12$ were manually deleted because they were not determined to play a vital function in the protein. Polar hydrogen atoms were added and nonpolar hydrogen atoms were combined using Autodock Tools. The PDB to PDBQT format of the protein receptor was converted. The default settings for all other receptor preparation options were preserved.

3.3 Active Site Prediction

The coordinate site of active site of protein was calculated using "centreofmass" and VMD process as mentioned earlier. The coordinate site of the proteins was $PP1\gamma_2$ (36.72, 16.55, 36.08).

3.4 Pharmacophore Studies

Common pharmacophores for the ligands were analyzed by using PharmaGist and ZINCpharma web servers.

3.5 Molecular Docking Analysis

The docking data for the molecular target, *PP*12 revealed that three out of thirty-three candidate phytochemicals had a good binding affinity and better binding modes than the reference (CYU). For docking purposes, the 2D structures of screened phytochemicals were collected and saved.

The table below shows the list of ligands with best binding energy of the molecular target i.e. *PP*1γ2 Three compounds that were α-terpineol, Coumarin, 2-Phenylpropan-2-ol showed variable binding affinity with each target. For example, the binding affinity of α-terpineol was -7.1 kcal/ mol, Coumarin was -6.9 kcal/ mol and 2-Phenylpropan-2-ol was -6.7 kcal/ mol Thus, there have been fluctuations in binding energy in this range (−6.7 to −7.1 kcal/mol).

Table 3. Results Top 3 compounds (Following Lipinski's rule).

S. No	PubChem ID	Phytochemicals Name	Amino acid residues involved in protein-ligand interaction	Binding Energy
1.	-----	CYU (Reference)	Gln249, Glu252	-3.7
2.	l17100	α-terpineol	His125, Asp95, Asp92, His66, His248, Asp64	-7.1
3.	323	Coumarin	His66, Asp64, His248, Asp92, Asp95, His125	-6.9
4.	12053	2-Phenylpropan-2-ol	Asp95, His125, Tyr134, Asp92, Asp64, His66, His248, Arg221	-6.7

In case of *PP*1γ2 protein α-terpineol showed best results with -7.1 kcal/mol.

In comparison to reference molecules, all phytochemicals had slightly

lower binding energy. As a result, three phytochemicals are effective at inhibiting molecular targets.

To analyses Hydrogen bond interaction, hydrogen bond length and hydrophobic interactions with all receptors Ligplot analysis was carried out. Complexes of $PP1\gamma2$ with their best ligands were represented in the ligplots as shown below. The interacting amino acid residues are represented in the figure. The procedure was in consonance with the study conducted by Asthana et al., for carrying out the molecular docking study and analyzing the interactions of Pueraria tuberosa with vascular endothelial growth factor receptors [17].

3.6 2D Representation of Ligand and Receptor Interaction by Ligplot Program

The molecular targets references and the various screened phytochemicals integrated 2D interactions studied by hydrogen bond binding sites with the references and hydrophobic interactions with distinct residues as indicated in the figure 1. The green colour represents reference molecules, while the purple tint shows the phytochemicals that were screened. In diverse molecular targets, the common binding site was varied. The red sparking arcs depict residues creating hydrophobic interactions with phytochemicals, while the dotted green lines reflect hydrogen bonds with limitations. Protein residues in equivalent 3D locations are indicated by red circles and ellipses.

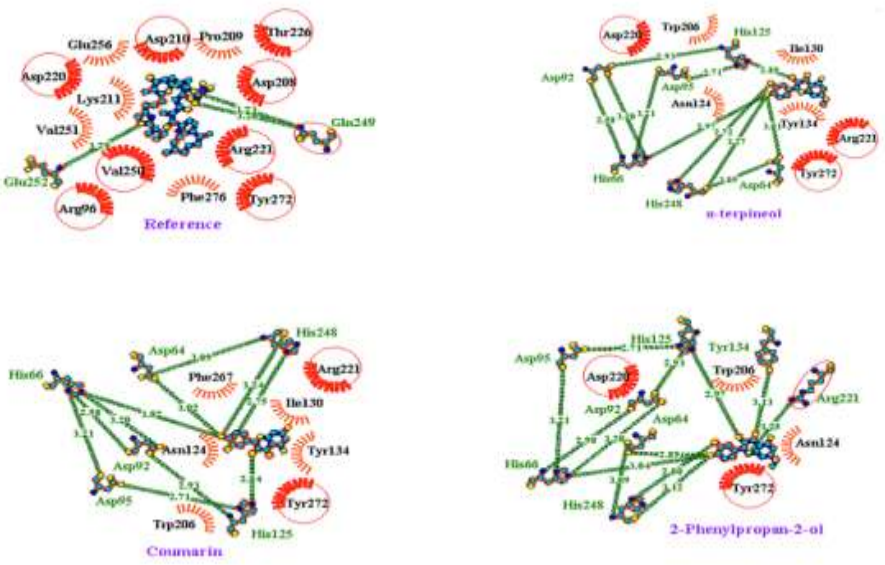

Figure 1. LigPlot result of *PP*1γ2 protein showing ligand interaction with residues in binding site.

4. Conclusion

The goal of this study was to find possible inhibitors of the *PP*1γ2 protein. Accordingly, a library of 50 molecules was docked with the protein for screening the potential inhibitors of the protein. From the analysis of molecular docking results and ligplot interaction, it was found that three compounds out of fifty are the potential candidates which showed better binding energies than the selected reference molecule. These phytochemicals were α-terpineol, Coumarin and 2-Phenylpropan-2-ol. The overall study suggested that these molecules could be used to inhibit the activity of *PP*1γ2, which is found to have an inverse relationship with sperm motility. Thus, by inhibiting the protein, the motility of sperm can be enhanced. This finding could play a key role for the researchers working in the area of fertilization.

Acknowledgement

The authors wish to thank everyone who has some way or the other helped in completion of this research work.

References

[1] E. Bianchi and G. J. Wright, Sperm meets egg: The genetics of mammalian fertilization, Annu. Rev. Genet. 50 (2016), 93-111.

[2] S. Breton, A. V. Nair and M. A. Battistone, Epithelial dynamics in the epididymis: role in the maturation, protection, and storage of spermatozoa, Andrology 7(5) (2019), 631-643.

[3] J. M. Bedford and D. D. Hoskins, The mammalian spermatozoon: morphology, biochemistry and physiology, Marshall's physiology of reproduction, Churchill Livingstone, Edinburgh, (1990).

[4] T. G. Cooper, G. M. Waites and E. Nieschlag, The epididymis and male fertility, A symposium report, Int. J. Androl. 9(2) (1986), 81-90.

[5] S. E. Ozkocer and E. Konac, The current perspective on genetic and epigenetic factors in sperm maturation in the epididymis, Andrologia 53(3) (2021), e13989.

[6] G. D. Smith, D. P. Wolf, K. C. Trautman, E. F. da Cruz e Silva, P. Greengard and S. Vijayaraghavan, Primate sperm contain protein phosphatase 1, a biochemical mediator of motility, Biol. Reprod. 54(3) (1996), 719-727.

[7] F. Y. Tang and D. D. Hoskins, Phosphoprotein phosphatase of bovine epididymal spermatozoa, Biochem. Biophys. Res. Commun. 62(2) (1975), 328-335.

[8] J. V. Silva, M. J. Freitas, J. Santiago, S. Jones, S. Guimarães, S. Vijayaraghavan, S. Publicover, G. Colombo, J. Howl and M. Fardilha, Disruption of protein phosphatase 1 complexes with the use of bioportides as a novel approach to target sperm motility. Fertil. Steril. 115(2) (2021), 348-362.

[9] E. Caroppo, Understanding sperm motility regulation: it's a long road ahead, Fertil. Steril. 115(2) (2021), 311-312.

[10] D. Takahashi, H. Murofushi, K. Ishiguro, J. Ikeda and H. Sakai, Phosphoprotein phosphatase inhibits flagellar movement of Triton models of sea urchin spermatozoa, Cell Struct. Funct. 10(4) (1985), 327-337.

[11] H. Murofushi, K. Ishiguro, D. Takahashi, J. Ikeda and H. Sakai, Regulation of sperm flagellar movement by protein phosphorylation and dephosphorylation, Cell Motil. Cytoskeleton 6(2) (1986), 83-88.

[12] C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46(1-3) (2001), 3-26.

[13] A. M. Dar and S. Mir, Molecular docking: approaches, types, applications and basic challenges. J. Anal. Bioanal. Tech. 8(2) (2017), 1-3.

[14] O. Trott and A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31(2) (2010), 455-461.

[15] P. Leeson, Drug discovery: Chemical beauty contest, Nature 481(7382) (2012), 455-456.

[16] T. Joshi, T. Joshi, P. Sharma, S. Mathpal, H. Pundir, V. Bhatt and S. Chandra, In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking, Eur. Rev. Med. Pharmacol. Sci. 24(8) (2020), 4529-4536.

[17] S. Asthana, T. Agarwal, S. Singothu, A. Samal, I. Banerjee, K. Pal, K. Pramanik and S. S. Ray, Molecular docking and interactions of pueraria tuberosa with vascular endothelial growth factor receptors, Indian J. Pharm. Sci. 77(4) (2015), 439-45.