Indian Journal of Biochemistry & Biophysics
Vol. 60, September 2023, pp. 703-709
DOI: 10.56042/ijbb.v60i9.4056

Polyphenol MHQP as an allosteric inhibitor of Kinesin-5: Cease the molecular catwalk of "Drunken Sailor"

Manjari Shukla, Sushobhan Maji, Keshav Rajarshi & Sudipta Bhattacharyya*

Department of Bioscience & Bioengineering, Indian Institute of Technology, Jodhpur-342 030, Rajasthan, India

Received 30 May 2023; *revised 12 July 2023*

Human Kinesin-5 (KIF-11/Eg5), a major anticancer drug target, is a plus end-directed motor protein that is involved in spindle dynamics and principally involved in mitosis. In the present study, a computer-aided rational drug discovery approach has been applied to search for potential allosteric inhibitors against Eg5. Accordingly, virtual screening of naturally occurring secondary metabolites and their commercially available synthetic derivatives indicates 2-(9b- methyl-2,3,3a,4,5,9b-hexahydrofuro [3,2 c] quinolin-4-yl) phenol (MHQP), a hexahydrofuro [3,2-c] quinolone derivative as a potential therapeutic lead molecule against Eg5. The present study provides a structural glimpse of MHQP binding at the monastrol binding site of Eg5 with a vivid description of its plausible mode of Eg5 inhibition. Moreover, the *in silico* data also supports the superiority of MHQP over the well-characterized Eg5 inhibitor Arry-520 in terms of augmented binding affinity as well as to cope with Arry-520 resistant mutants of Eg5. Structure-guided mechanistic details of MHQP-induced inhibition of Eg5 and its predicted pharmacodynamics properties have been presented herein.

Keywords: Allosteric Inhibitor, Kinesin Eg5, MHQP, Molecular Dynamics Simulation

Kinesins belong to the class of motor proteins, and by using mechanochemical energy, they move along the microtubule. It is the widely explored protein family because of its various roles in different cellular functions like cellular division, meiosis, neuronal development, and transference of cellular cargo. Kinesins are crucial in the process of chromosome segregation and assist in the establishment of the bipolar spindle assembly. It also has several other roles in signal transduction[1-3]. Kinesin-5, sometimes referred to as Eg5, is a motor protein that assists in microtubule crosslinking as well as sliding[4]. Kinesin motor domains are the well-conserved regions among the various kinesins, and it incorporates two binding sites on each head; one binds the microtubule while the other incorporates nucleotides (ATP/ADP). The mitotic kinesin Eg5 synergy with the spindle during the mitosis helps in the stabilization of the bipolar spindle, and due to this feature, kinesin Eg5 is one of the explored drug targets for cancer[5,6]. The inhibition of Kinesin Eg5 does not interfere with the cytoskeletal process because its inhibition obstructs the cell division process and thus affects cell proliferation[7-9]. This intriguing feature validates Eg5 as an effective target for cancer cells. Kinesin inhibition can ruin the process of formation of bipolar spindle and eventually forms monopolar spindle[10,11]. Numerous drugs that can stop cancer cells from growing have been developed and tested in clinical settings, but the findings call for further study to be done in this area in order to develop an effective drug candidate that not only interacts with the target but also blocks the target's enzyme. Some inhibitors are reported to bind at the different sites of Eg5 kinesins, like on ATP-binding active sites (ex-thiazoles), but these inhibitors are less sought because they can interact with other ATP-binding proteins[12]. There are studies where off targeting has also been seen and for the solution of this problem many strategies has been developed like in one study mesoporous silica nanoparticles loaded with drug has been used to target the tumor cells specifically[13]. The inhibitors which can bind on the allosteric pocket of Eg5 are more desirable in terms of their high degree of target specificity[14-16]. The Eg5 has multiple prior reported allosteric binding sites like S1, S2, *etc*. (Suppl. Fig. 1), but their precise structural location is not very clear. The medication monastrol causes the production of monopolar spindles, and its binding site is an

*Correspondence:
E-mail: sudipta@iitj.ac.in
Suppl. data available at NOPR respiratory

allosteric site that has been described in great detail. The monastrol drug binding site is formed by hydrophobic loop5/ α2 helix /α3 helix pocket[14-16]. This site offers a peculiar feature *i.e*., lower toxicity and selectivity due to the presence of elongated loop5[17]. This site has some special features like the closed loop5 and tilted alpha helix4[18]. The binding of the drug in this site creates a transition in loop5 from "open" to "closed" confirmation. Because of this closed confirmation, the confirmational alteration of Eg5, which is required for the release of ADP, is inhibited even more[19]. To date, few other drugs are also reported to bind on monastrol drug binding sites like terpendol E, ispinesib, filanesib (Arry-520), and S-trityl-L cysteine (STLC)[20,21]. The computer-aided structural biological approach enables us to depict the possible mechanism of inhibition of these Eg5 inhibitors, their precise mode of interaction with the target protein as well as the drug-induced changes in the structural plasticity of the target protein. The process of drug binding entails complex structural transitions, which may not be easy to comprehend experimentally[22]. In this context, the molecular docking and molecular dynamics simulation study can be an efficient way to predict the atomic level spatial and temporal conformational perturbations upon the interaction between the receptor and ligand. In the kinesins-5 class, the loop5 region has an important role in ADP release as well as it communicates the structural changes in the neck linker and microtubule-binding region also[23]. Other important regions in the kinesin motor domains include the Switch I and Switch II regions, both of which are involved in the induction of nucleotide-driven conformational alterations[24]. Switch II region is also pivotal to forwarding the signal, which resulted from the structural change of the nucleotide-binding region to the relay helix, which is further located in the microtubule-binding site and then to the neck linker. In this way, the switch II region provides forward motility to Kinesins via nucleotide exchange-induced mechanical motion[25,26]. With all these postulates, it can be proposed that the small molecule inhibitors binding at the allosteric site surrounded by Loop5/ α2 helix /α3 helix can lead to the spindle collapse and eventually to mitotic arrest and ultimately arrest the growth of cancer cells. However, drug-resistant mutant variations of Eg5 kinesin are also reported. These mutants weaken and/or prevents the binding of the allosteric modulators to their respective binding sites. In this regard, resistance-resistant therapeutic leads are being sought globally, which can circumvent the oddities exerted by the drug-resistant Eg5 kinesin mutants. The present study focuses on screening some potent therapeutic leads of Kinesin Eg5, which can work as an allosteric inhibitor and can also be effective against drug-resistant Eg5 Kinesin variants. For this purpose, we performed *in silico* high throughput virtual screening of ligands with the library of bioactive plant secondary metabolites (commercially available as well as obtained from Indian medicinal plants). As we know that there are different plant secondary metabolites have been reported to be effective in treatment of different ailments[27,28] so we targeted the phytochemicals primarily. Several plant secondary metabolites have been reported to have the anti-cancer properties[29-31] to date but the mechanism of action is not precisely known. Phenols having three membered characteristic rings having in it is also known to have anti-cancer potential. Some of the polyphenols are reported for their anti-cancer effect are quercetin, curcumin, resveratrol with different mode of actions[32,33]. Further validation of the binding interactions and all atomistic molecular dynamic simulation studies presented herein divulges the plausible therapeutic action of the screened secondary metabolites against Eg5 kinesin and its drug-resistant mutated counterparts.

Materials and Methods

Virtual screening

The three-dimensional structure of Kinesin Eg5 (PDB Id:2PG2)[34] has been downloaded from the RCSB PDB database, and the full-length structure was modelled and energy minimized by Modeller[35] and Phenix suite[36], respectively. Against the selected drug target, we performed the virtual screening by following target-based rational drug designing approach. With an aim to find plausible anti-Eg5 bioactive secondary metabolites and their commercially available synthetic derivatives, we have set out the virtual screening with a total of 3200 small molecules [either obtained from Sigma Aldrich (www.sigmaaldrich.com) or retrieved from IMMPAT database[37]]. The multiple ligands docking strategy has been followed to do the receptor-guided virtual screening with PyRx software[38]. The top-scoring ligands obtained through the initial virtual screening were further verified through one-to-one molecular docking analysis using the blind docking protocol

(grid box size: X=68, Y=68, Z=68). From the top 11 compounds, the best small molecule [2-(9b-methyl-2,3,3a,4,5,9b-hexahydrofuro [3,2 c] quinolin-4-yl) phenol (abbreviated as MHQP)] ligand was selected on the basis of the best free energy binding score (ΔG). With this compound, further studies, including deep learning-based affinity predictions as well as all atomistic molecular dynamic simulation analysis, were carried out to predict the stability of the ligand-receptor (Eg5) complex under physiologically simulated conditions. Moreover, in order to check the effectivity of the screened ligand against the drug-resistant mutants of Eg5 Kinesin (D130A and L214A). The mutants have been modelled and subsequently docked with the identified ligand molecule using the same parameters which were used for the wild type Eg5 kinesin docking. The interaction profile of the best-selected ligand with its cognate protein target (Eg5 Kinesin) was analysed by using PyMOL (https://pymol.org/2/) and LigPlot (www.ebi.ac.uk/thornton-srv/software/LigPlus/)

ADMET & drug likeliness properties

The ADMET properties of the screened ligand molecule were checked with pkCSM[39] and Swiss ADME[40]. pkCSM checks the ADMET properties of small molecules. Checking the ADMET property is indispensable to consider a small molecule ligand as a plausible future therapeutic lead. Essentially, the ADMET properties of a molecule predict its drug likeliness through the analysis of its pharmacokinetics properties.

Molecular dynamics (MD) simulation analysis

The all-atomistic MD simulation analysis of the Eg5 Kinesin in its nucleotide (ADP) bound state was carried out in the presence and absence of the best-screened ligand (MHQP). The all atomistic simulation calculations were carried out for a 30ns time frame. For the MD simulation, NAMD 2.14[41] has been used, which uses the Chramm++ parallel programming. VMD (Visual Molecular Dynamics)[42] software was used for setup and trajectory analysis. Force field generation was done with Charmm Gui online Ligand Reader and Modeller tool[43]. The simulation was performed with an isothermal-isobaric ensemble (NPT) environment, which uses the Langevin dynamics. Solvation cubic box of 10 A° has been provided. The dcd frequency, xst frequency, and restart frequency were set to 5000, but the output energy was 500. System minimization was done for 1000 steps. The RMSF (Root Mean Square Fluctuation) plot was drawn with Origin software (http://www.originlab.com/), and structure analysis was done with PyMol.

Results and Discussion

The virtual screening of bioactive secondary metabolites and their commercially available synthetic derivatives against the antineoplastic drug target Kinesin Eg5 has yielded 11 lead compounds that show excellent Eg5 binding affinity in terms of free energy of binding (ΔG)(more than -8.0 kcal/mol). (Suppl. Table 1). These compounds were mainly having an affinity for allosteric drug binding sites of kinesin Eg5, like the classical monastrol binding site, as well as the newly reported sites S1 and S2. Among the selected compounds, 2-(9b- methyl-2,3,3a,4,5, 9b-hexahydrofuro [3,2 c] quinolin-4-yl) phenol (a hexahydrofuro[3,2- c]quinoline derivative abbreviated as MHQP)] was found to have the best ΔG of -10.6 kcal/moland occupies the well-characterized allosteric binding site of kinesin Eg5 *i.e*., the monastrol drug binding site. The binding site is located at the intersection of the α3 helix, α2 helix, and Loop 5 of the kinesin Eg5 motor domain. (Fig. 1A). The ligand MHQP is binding in the allosteric site, which provides it more target specificity and, as a result of that, may exert lesser side effects compared to the nucleotide-binding site targeting Eg5 inhibitors. Intriguingly, in a prior study, similar hexahydrofuro[3,2-c] quinolone derivatives have been found to inhibit the growth of MDAMB-231 breast cancer cells *in vitro*[44], although the molecular mode of therapeutic action of those derivatives remained obscured till date. Few other annotated structures of the MHQP are also reported for their anti-mitotic, anti-inflammatory properties via *in vitro* studies[45]. The mode of binding of MHQP resembles the submitted crystal structure of allosteric inhibitor Arry-520 (trade name: Filanesib, PDB Id: 6HKY), which is at its late stage of clinical trials for multiple myeloma. When we compared the binding energies of Arry-520 with MHQP using the same parameters and docking platform, we found that the binding affinity of MHQP was much better than the Arry-520 (ΔG of Arry-520 binding: -8.7 kcal/mol) with kinesin Eg5 and the docked ligand is also superimposing well with the bound Arry-520 (Suppl. Fig. 2A). It indicates that the proposed ligand is binding more efficiently at the same allosteric site compared to Arry-520. The 2D interaction plot of Arry-520 and MHQP with kinesin Eg5 shows that the

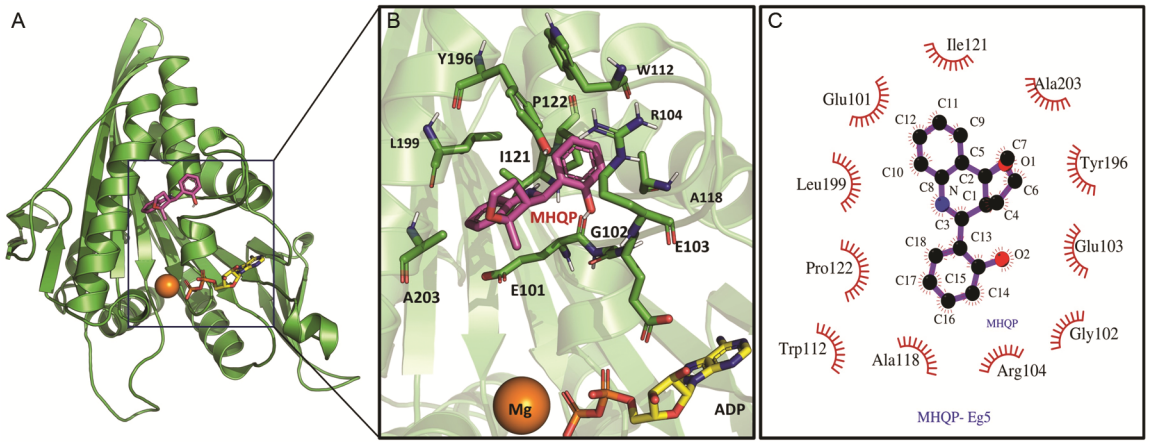

Fig. 1 — Molecular docking of MHQP with Kinesin Eg5 motor domain. (A) Binding site ofMHQP at the allosteric pocket of Eg5; (B) Three-dimensional interaction of MHQP with amino acid residues of monastrol binding pocket of Eg5; and (C) Two-dimensional interaction plot of Docked MHQP with Eg5 showing the hydrophobic interactions (represented by spiked red arcs) forming amino acids with the bound MHQP

amino acids which are having hydrophobic interaction with kinesin are common in both of the ligand's binding sites. (Except E200 & F224 in Arry-520) (Suppl. Fig. 2B & C). The deep learning-based Eg5 affinity prediction (pKD value) of MHQP was found to be 5.7. Drug likeliness of ligand MHQP has been checked with ADMET lab 2.0 and found that the ligand has been accepted according to the Lipinski rule of five (MW ≤500; logP £ 5; Hacc £ 10; Hdon £ 5), having no Lipinski violations. Intriguingly, in advanced-stage clinical trials of Arry-520, it has been observed that the drug is getting non-effective due to the emergence of two drug-resistant mutations in the monastrol-binding allosteric site (D130A and L214A). So, to check the effectiveness of MHQP against the drug-resistant Eg5 variants, further docking studies have been performed with these two mutated drug targets, Eg5_D130A and Eg5_L214A. The docking studies indicate that MHQP is showing significant binding affinities with both of these two mutants (with Eg5_D130A ΔG: -10.4 Kcal/mol and for Eg5_L214A ΔG: -9.8 kcal/mol) also. It stipulates that the MHQP may also be an effective inhibitor for the mutated Eg5 variants. In Eg5, the monastrol binding allosteric pocket has been formed by Loop5/α2/α3, and we found that the MHQP binding influences the opening and closure of the allosteric pocket, which is principally regulated by loop5. To investigate this mechanism, we measured the distance between two residues located on α3 helix (E200) and Loop5 (W112). One of the residues in loop5 that is crucial to the protein's activity is W112, which has been linked to the opening and closure of the Eg5 allosteric pocket. Interestingly, all atomistic MD simulations of Eg5/ADP complex in the presence and absence of the bound ligand, MHQP reflects a differential structural dynamic of the monastrol binding allosteric pocket. After the 30 ns simulation of the Eg5/ADP complex in the absence of MHQP, the distance between E200 and W112 was 8.2Å which represents the open conformation of this pocket; however, in the MHQP bound state of the Eg5/ADP complex; this distance got reduced to 7.3Å (Fig. 2A). Importantly, Loop 5 W112 residue, which maintained the so-called "closed conformation" of the monastrol allosteric pocket of Eg5, was forced into a downward position when the MHQP was bound to the Eg5/ADP complex (Fig. 2B). The RMSF (Root Mean Square Fluctuation) plot obtained from the MD simulation also shows that in the MHQP bound state, the amino acid region 100 to 115 (Loop-5) gets stabilized, which corroborates with lower fluctuation in RMSF value compared to the MHQP unbound state of the protein (Fig. 2C). It seems that the binding of MHQP causes the Eg5-L5/α2/α3 helix pocket to convert from an open conformation to a closed conformation. Importantly the flexibility of the loop5 region of Eg5 kinesin has been proposed to modulate the nucleotide exchange as well as the microtubule-binding properties[39]; hence the differential structural dynamics of the same loop in the context of MHQP binding may confer significant changes in the aforementioned properties of Eg5 kinesin. Again, the previous studies have shown that compared to the ATP-bound form of Eg5 Kinesin, the ADP-bound form is more structurally dynamic. To be more specific, the pliability of the Switch-I

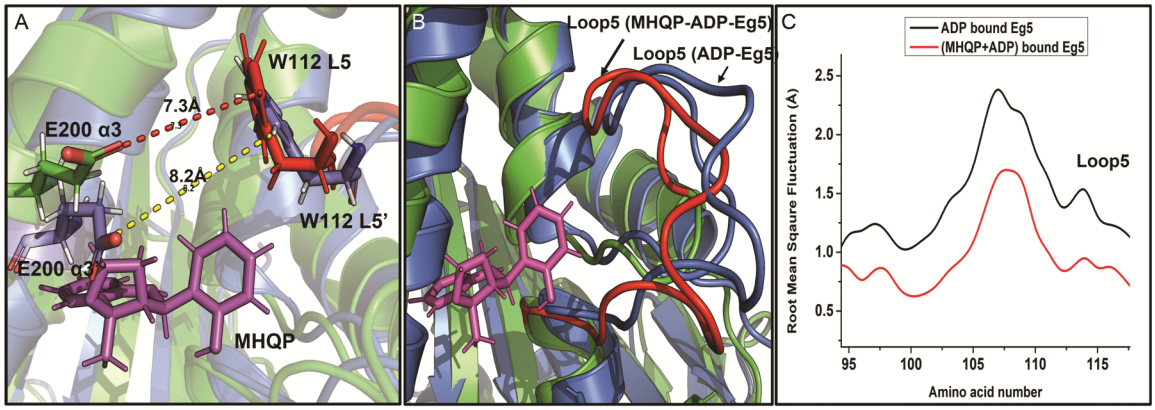

Fig. 2 — The subtle structural changes observed in MHQP free (blue-coloured structure) versus MHQP docked Eg5 (Green coloured structure). (A) The change in the distance betweenE200 (α3 helix) and W112 (Loop 5 abbreviated as L5) in MHQP docked state and in the absence of MHQP E200 (α3 helix) and W112 (Loop 5 abbreviated as L5); (B) The movement of Loop 5 in the presence of MHQP (red colour) and in the absence of MHQP (blue colour); and (C) The comparison in RMSF change in MHQP/ADP bound state (Red line) of Eg5 and in only ADP bound state of Eg5 (Black line)

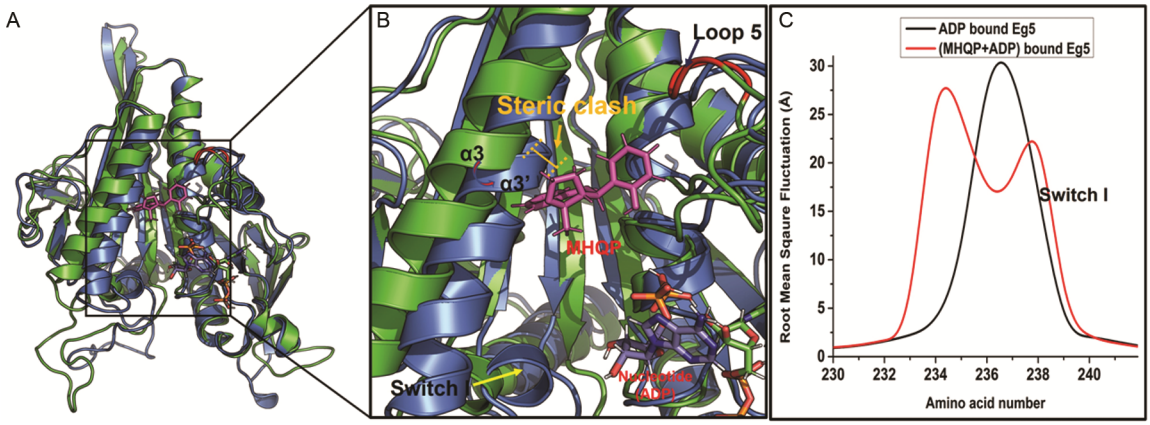

Fig. 3 — Structural change in alpha helix 3 of Kinesin Eg5 motor domain in the presence (green colour) and absence (blue colour) of MHQP (pink sticks). (A) The docked MHQP in the presence of ADP with Kinesin Eg5; (B) Zoomed panel showing the steric clash between MHQP bound and unbound states and thus the hindrance in the structural transition of ADP to ATP bound conformations of Eg5; and (C) The RMSF plot also supports the stabilization of the switch I region (230-240 amino acids) of Kinesin Eg5 in the presence of MHQP

region of Eg5 kinesin (amino acids: 217-240) is what allows for the release of ADP, which is then followed by the binding of incoming ATP (Fig. 3). With RMSF plot obtained from the MD simulation study of Eg5/ADP and Eg5/ADP/MHQP complexes, we observed that the Switch-I region got stabilized in the presence of MHQP (Fig. 3C). It specifies that the MHQP bound state halts the ADP bound conformation of the Eg5 motor domain by blocking the structural transition of the α3 helix (due to the steric clash of the bound ligand with the α3 helixin its ATP bound state), which is required to release the bound ADP molecule (Fig. 3B) to allow the subsequent binding of the next incoming ATP. Altogether, the *in silico* data presented herein unambiguously suggest MHQP binding to the monastrol site at the motor domain of Kinesin Eg5 may lead to the inhibition of this protein's movement by uncoupling the nucleotide exchange mediated structural dynamics, which ultimately leads to the binding and unbinding of the motor domain to the microtubule surface. The cease of Eg5 kinesin movement may further culminate into monospindle formation and, ultimately, apoptosis of the targeted cancer cells.

Conclusion

Herein, the *in silico* based rational drug discovery against the antineoplastic drug target Kinesin Eg5 using a library of bioactive secondary metabolites

and their commercially available synthetic derivatives led us to find a promising ligand MHQP (a derivative of hexahydrofuro[3,2-c] quinolone scaffold). The identified small molecule ligand is predicted to bind the classical monastrol binding site of Kinesin Eg5 with high affinity and thereby may plausibly uncouple the nucleotide mediated structural dynamics of the motor domain to further cease the molecular catwalk of this 'drunken sailor' (Eg5 Kinesin) on the ramp of microtubules. However, the high-resolution crystal structure(s) of Eg5/ADP/MHQP are needed to clarify these preliminary results to atomic details. Nonetheless, the present research may open up a new horizon of antineoplastic therapeutic research where naturally obtained hexahydrofuro[3,2-c] derivatives may serve as the plausible therapeutic leads against drug-resistant cancers.

Acknowledgement

The author is thankful to Indian Institute of Technology, Jodhpur for providing necessary facilities to conduct the experiments.

Conflict of interest

All authors declare no conflict of interest.

References

1 Mandelkow E & Mandelkow EM, Kinesin motors and disease. *Trends Cell Biol*, 12 (2002) 585.

2 Hirokawa N, Noda Y, Tanaka Y & Niwa S, Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol*, 10 (2009) 682.

3 Endow SA, Microtubule motors in spindle and chromosome motility. *Eur J Biochem*, 262 (1999) 12.

4 Garcia-Saez I & Skoufias DA, Eg5 targeting agents: From new anti-mitotic based inhibitor discovery to cancer therapy and resistance. *Biochem Pharmacol*, 184 (2021) 114364.

5 Ferenz NP, Gable A & Wadsworth P, Mitotic functions of kinesin-5. *Semin Cell Dev Biol*, 21 (2010) 255.

6 Waitzman JS & Rice SE, Mechanism and regulation of kinesin-5, an essential motor for the mitotic spindle. *Biol Cell*, 106 (2014) 1.

7 Kapoor TM, Mayer TU, Coughlin ML & Mitchison TJ, Probing Spindle Assembly Mechanisms with Monastrol, a Small Molecule Inhibitor of the Mitotic Kinesin, Eg5. *J Cell Biol*, 150 (2000) 975.

8 Wang Y, Wu X, Du M, Chen X, Ning X, Chen H, Wang S, Liu J, Liu Z, Li R, Fu G, Wang C, McNutt M, Zhou D & Yin Y, Eg5 inhibitor YL001 induces mitotic arrest and inhibits tumor proliferation. *Oncotarget*, 8 (2017) 42510.

9 Skoufias DA, DeBonis S, Saoudi Y, Lebeau L, Crevel I, Cross R, Wade RH, Hackney D & Kozielski F, S-trityl-L-cysteine is a reversible, tight binding inhibitor of the human kinesin Eg5 that specifically blocks mitotic progression. *J Biol Chem*, 281 (2006) 17559.

10 Henriques AC, Ribeiro D, Pedrosa J, Sarmento B, Silva PMA & Bousbaa H, Mitosis inhibitors in anticancer therapy: When blocking the exit becomes a solution. *Cancer Lett*, 440-441 (2019) 64-81.

11 Tillement V, Remy MH, Raynaud-Messina B, Mazzolini L, Haren L & Merdes A, Spindle assembly defects leading to the formation of a monopolar mitotic apparatus. *Biol Cell*, 101 (2009) 1.

12 Rickert KW, Schaber M, Torrent M, Neilson LA, Tasber ES, Garbaccio R, Coleman PJ, Harvey D, Zhang Y, Yang Y, Marshall G, Lee L, Walsh ES, Hamilton K & Buser CA, Discovery and biochemical characterization of selective ATP competitive inhibitors of the human mitotic kinesin KSP. *Arch Biochem Biophys*, 469 (2008) 220.

13 Hanif H, Nazir S, Mazhar K, Waseem M, Bano S & Rashid U, Targeted delivery of mesoporous silica nanoparticles loaded monastrol into cancer cells: an *in vitro* study. *Appl Nanosci*, 7 (2017) 549.

14 Jiang C, Chen Y, Wang X & You Q, Docking studies on kinesin spindle protein inhibitors: an important cooperative 'minor binding pocket' which increases the binding affinity significantly. *J Mol Model*, 13 (2007) 987.

15 Talapatra SK, Schüttelkopf AW & Kozielski F, The structure of the ternary Eg5-ADP-ispinesib complex. *Acta Crystallogr D Biol Crystallogr*, 68 (2012) 1311.

16 Yan Y, Sardana V, Xu B, Homnick C, Halczenko W, Buser CA, Schaber M, Hartman GD, Huber HE & Kuo LC, Inhibition of a Mitotic Motor Protein: Where, How, and Conformational Consequences. *J Mol Biol*, 335 (2004) 547.

17 Moores CA, Kinesin-5 mitotic motors: Is loop5 the on/off switch? *Cell Cycle*, 9 (2010) 1286.

18 Zhang W, Exploring the intermediate states of ADP-ATP exchange: a simulation study on Eg5. *J Phys Chem B*, 115 (2011) 784.

19 Cochran JC& Gilbert SP, ATPase mechanism of Eg5 in the absence of microtubules: insight into microtubule activation and allosteric inhibition by monastrol. *Biochemistry*, 44 (2005) 16633.

20 Nakazawa J, Yajima J, Usui T, Ueki M, Takatsuki A, Imoto M, Toyoshima YY & Osada H, A novel action of terpendole E on the motor activity of mitotic Kinesin Eg5. *Chem Biol*, 2003;10 (2) 131-7.

21 Chen GY, Kang YJ, Gayek AS, Youyen W, Tüzel E, Ohi R & Hancock WO, Eg5 Inhibitors have Contrasting Effects on Microtubule Stability and Spindle Integrity Depending on their Modes of Action. *Biophys J*, 112 (2017) 427a.

22 Orellana L, Large-Scale Conformational Changes and Protein Function: Breaking the *in silico* Barrier. *Front Mol Biosci*, 2019;6:117.

23 Goulet A, Behnke-Parks WM, Sindelar CV, Major J, Rosenfeld SS & Moores CA, The structural basis of force generation by the mitotic motor kinesin-5. *J Biol Chem*, 287 (2012) 44654.

24 Cochran JC, Gatial JE, III, Kapoor TM & Gilbert SP, Monastrol Inhibition of the Mitotic Kinesin Eg5. *J Biol Chem*, 280 (2005) 12658.

25 Kull FJ & Endow SA, Kinesin: switch I & II and the motor mechanism. *J Cell Sci*, 115 (2002) 15.

26 Sindelar CV& Downing KH, An atomic-level mechanism for activation of the kinesin molecular motors. *Proc Natl Acad Sci U S A*, 107 (2010) 4111.

27 Ganeshpurkar A, Chaturvedi A, Shrivastava A, Dubey N, Jain S, Saxena N, Gupta P & Mujariya R, *In silico* interaction of Berberine with some immunomodulatory targets: A docking analysis. *Indian J Biochem Biophys*, 59 (2022) 848.

28 Dwarampudi LP, Dhanabal SP, Farha S, Gade R, Shanmugam R & Raj KR, Phytochemical evaluation and anti-psoriatic activity of the ethanolic extract of the leaves of *Momordica charantia*. *Indian J Biochem Biophys*, 59 (2022) 751.

29 Shafique M & Sarma SP, Potential anticancer peptides design from the cysteine rich plant defensins: An *in silico* approach. *Indian J Biochem Biophys*, 59 (2022) 900.

30 Sathelly K, Kalagatur NK, Mangamuri UK, Puli COR & Poda S, Anticancer potential of *Solanum lycopersicum* L. extract in human lung epithelial cancer cells A549. *Indian J Biochem Biophys*, 60 (2022) 76.

31 Jenifer DR, Malathy B & Ariya S, *In vitro* and *in silico* studies on the biochemistry and anti-cancer activity of phytochemicals from *Plumbago zeylanica*. *Indian J Biochem Biophys,* 58 (2021) 272.

32 Cháirez-Ramírez MH, de la Cruz-López KG & García-Carrancá A, Polyphenols as Antitumor Agents Targeting Key Players in Cancer-Driving Signaling Pathways. *Front Pharmacol*, (2021) 12.

33 Bhosale PB, Ha SE, Vetrivel P, Kim HH, Kim SM & Kim GS, Functions of polyphenols and its anticancer properties in biomedical research: a narrative review. *Transl Cancer Res*, 9 (2020) 7619.

34 Pinkerton AB, Lee TT, Hoffman TZ, Wang Y, Kahraman M, Cook TG, Severance D, Gahman TC, Noble SA, Shiau AK & Davis RL, Synthesis and SAR of thiophene containing kinesin spindle protein (KSP) inhibitors. *Bioorg Med Chem Lett*, 17 (2007) 3562.

35 Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TA, Rempfer C, Bordoli L & Lepore R, SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*, 46 (2018) W296.

36 Zwart PH, Afonine PV, Grosse-Kunstleve RW, Hung LW, Ioerger TR, McCoy AJ, McKee E, Moriarty NW, Read RJ, Sacchettini JC & Sauter NK, Automated structure solution with the PHENIX suite. *Methods Mol Biol*, 426 (2008) 419.

37 Vivek-Ananth RP, Mohanraj K, Sahoo AK & Samal A, IMPPAT 2.0: An Enhanced and Expanded Phytochemical Atlas of Indian Medicinal Plants. *ACS Omega*, 8 (2023) 8827.

38 Dallakyan S & Olson AJ, Small-molecule library screening by docking with PyRx. *Methods Mol Biol*, 1263 (2015) 243

39 Pires DE, Blundell TL & Ascher DB, pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem*, 58 (2015) 4066.

40 Daina A, Michielin O & Zoete V, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*, 7 (2017) 42717.

41 Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L & Schulten K, Scalable molecular dynamics with NAMD. *J Comput Chem*, 26 (2005) 1781.

42 Humphrey W, Dalke A & Schulten K, VMD: Visual molecular dynamics. *J Mol Graph*, 14 (1996) 33.

43 Kim S, Lee J, Jo S, Brooks Iii CL, Lee HS & Im W, CHARMM-GUI ligand reader and modeler for CHARMM force field generation of small molecules. *J Comput Chem*, 38 (2017) 1879.

44 Chung PY, Tang JO, Cheng CH, Bian ZX, Wong WY, Lam KH & Chui CH, Synthesis of hexahydrofuro[3,2-c]quinoline, a martinelline type analogue and investigation of its biological activity. *Springerplus*, 5 (2016) 271.

45 Lin MW, Yang JS, Lu CC, Lin C, Kuo SC, Tsai FJ & Lee MR, 2-Phenyl-4-quinolone (YT-1) induces G2/M phase arrest and an intrinsic apoptotic mechanism in human leukemia cells. *Oncol Rep*, 39 (2018) 1331.