

# An enzoinformatics study targeting polo-like kinases-1 enzyme: Comparative assessment of anticancer potential of compounds isolated from leaves of *Ageratum houstonianum*

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## ABSTRACT

Natural products from plant sources, embracing inherently ample structural diversity than synthetic ones are the major sources of anticancer agents and will constantly play as protagonists for discovering new drugs. Polo-like kinases (PLKs) play a leading role in the ordered execution of mitotic events and 4 mammalian PLK family members have been identified. PLK1 is an attractive target for anticancer drugs in mammalian cells, among the four members of PLKs. The present study expresses the molecular interaction of compounds (1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester, squalene, 3,5-bis (1,1-dimethylethyl) phenol, Pentamethyl tetrahydro-5H-chromene, (1,4-Cyclohexylphenyl) ethanone and 6-Vinyl-7-methoxy-2,2-dimethylchromene) isolated from methanolic extract of leaves of *Ageratum houstonianum* with PLK1 enzyme. Docking between PLK1 and each of these compounds (separately) was performed using "Auto dock 4.2." (1,4-Cyclohexylphenyl) ethanone showed the maximum potential as a promising inhibitor of PLK1 enzyme with reference to  $\Delta G$  (-6.84 kcal/mol) and  $K_i$  (9.77  $\mu M$ ) values. This was sequentially followed by Pentamethyl tetrahydro-5H-chromene ( $\Delta G = -6.60$  kcal/mol;  $K_i = 14.58$   $\mu M$ ), squalene ( $\Delta G = -6.17$  kcal/mol;  $K_i = 30.12$   $\mu M$ ), 6-Vinyl-7-methoxy-2,2-dimethylchromene ( $\Delta G = -5.91$  kcal/mol;  $K_i = 46.68$   $\mu M$ ), 3,5-bis (1,1-dimethylethyl) phenol ( $\Delta G = -5.70$  kcal/mol;  $K_i = 66.68$   $\mu M$ ) and 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester ( $\Delta G = -5.58$  kcal/mol;  $K_i = 80.80$   $\mu M$ ). These results suggest that (1,4-Cyclohexylphenyl) ethanone might be a potent PLK1 inhibitor. Further, *in vitro* and *in vivo* rumination are warranted to validate the anticancer potential of (1,4-Cyclohexylphenyl) ethanone.

**Key words:** *Ageratum* sp., anticancer, docking, enzyme inhibition, natural compounds, polo like kinase

## INTRODUCTION

Natural compounds of higher plants origin can be lead compounds, conceding the design and rational aiming of new drugs with new therapeutic properties and novel mode of action.<sup>[1,2]</sup> A variety of these plant based natural compounds have been reported, which have significant anticancer activities; however, to reveal their modes of action is an animating challenge for medicinal chemists and pharmacologists. Furthermore, molecular docking,

which has shown an important and promising role in the drug discovery will help in digging out lead (active) compounds from these natural compounds.<sup>[3]</sup> Recently, scientific groups have focused on the tactics to initiatory explore the potential molecular targets and to confirm the inhibitory activity of these anticancer compounds.<sup>[4]</sup> The authors performed molecular docking using different enzymes and receptor proteins involved with cell cycle, cell growth, and DNA replication.<sup>[4]</sup>

Polo-like kinases (PLKs) pertaining to a family of serine-threonine kinases with a kinase domain at the N-terminus followed by one or two C terminal polo-box domains that are associated in substrate binding,<sup>[5]</sup> have a diversity of roles in cell cycle progression.<sup>[6]</sup> Among the

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four members of PLKs in mammalian cells, PLK1 is the best defined and it has also been discovered that PLK1 functions mainly in many aspects of mitotic progression, like controlling the entry into mitosis through the activation of the cdc 2/cyclinB complex,<sup>[7]</sup> centrosome maturation,<sup>[8]</sup> bipolar spindle formation,<sup>[8]</sup> sister chromatid separation,<sup>[9]</sup> anaphase promoting complex activation,<sup>[10]</sup> and affecting cytokinesis by phosphorylating NudC,<sup>[11]</sup> etc., Overexpression of PLK1 has been reported in many proliferating cancer cells, including colorectal cancer,<sup>[12]</sup> endometrial carcinomas,<sup>[13]</sup> breast cancer,<sup>[14]</sup> head and neck squamous cell carcinomas,<sup>[15]</sup> non-small cell lung cancer,<sup>[16]</sup> esophagus and stomach cancer,<sup>[17]</sup> pancreatic cancer,<sup>[18]</sup> ovarian cancer,<sup>[13]</sup> skin cancer,<sup>[19]</sup> and many others.<sup>[20]</sup> Interestingly, it was reported that due to PLK1 depletion normal cells can survive, but not the tumor cells.<sup>[21]</sup> Hence, PLK1 is a promising target in antitumor therapy. Increasing efforts have been made to identify small-molecule PLK1 inhibitors for preclinical development and clinical trials.

Scytonemin was the first published small molecular PLK1 inhibitor, a natural marine product isolated from cyanobacteria,<sup>[22,23]</sup> which is a micro molar non-specific Adenosine-triphosphate ATP competitor. In another study, Purpurogallin, which is the aglycon of several glycosides isolated from nutgalls and oak barks, has been reported as interception of the polo box domain of human polo like kinase-1.<sup>[24]</sup> The identification of natural compounds as a PLK1 inhibitor prompted us to further investigate the inhibitory activity of natural compounds isolated from plants. Plants belonging to *Asteraceae* species are a source of many biologically active compounds such as essential oils,<sup>[25-28]</sup> polyphenolic compounds,<sup>[29,30]</sup> flavonoids,<sup>[31-34]</sup> terpenoids,<sup>[32,35-38]</sup> phenolic acids,<sup>[31,34,39]</sup> alkaloids,<sup>[40]</sup> lignans,<sup>[31]</sup> saponins,<sup>[32,38,41]</sup> stilbenes, sterols,<sup>[34]</sup> polysaccharides,<sup>[35]</sup> and many others. Plants from *Asteraceae* family are commonly used in treatment of various diseases including cancer due to their bio-active properties.

We have previously identified six compounds 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester, squalene, 3,5-bis (1, 1-dimethylethyl) phenol, Pentamethyl tetrahydro-5H-chromene, (1, 4-Cyclohexylphenyl) ethanone and 6-Vinyl-7-methoxy-2, 2-dimethylchromene by Gas Chromatography-Mass Spectrometry GC-MS analysis in the (AB-2) active band isolated by preparative Thin Layer Chromatography TLC from the methanolic crude extract of leaves of *Ageratum houstonianum* (Family *Asteraceae*), which turned out to be a promising antioxidant. We further showed that the active band (AB-2) possessed significant antibacterial potential.<sup>[42]</sup> However, there is a lack of information about the mechanism of action and inhibitory effect of these biologically active natural compounds isolated from the species *A. houstonianum*

on PLK1 enzyme and possible molecular interactions between the two. Hence, the identification of the amino acid residues crucial to the interaction between PLK1 and these natural compounds is of due scientific interest. Such information is expected to aid in optimizing the safe and efficacious use of these natural compounds isolated from *A. houstonianum*. Furthermore, this study would be useful for scientists involved in drug design in their ongoing search for more potent and versatile PLK1-inhibitors. Currently, no crystal structure is available with the Protein Data Bank to aid in the characterization of the interaction between PLK1 and these compounds isolated from *A. houstonianum*. In the present study, the natural compounds identified by GC-MS analysis in the fraction (AB-2) were used as ligands for targeting PLK1 for the selection of most active inhibitor out of six compounds. This will suggest us the best PLK1 inhibitor, which might serve as a potential lead compound for further, analyzes and possible future pharmaceutical applications.

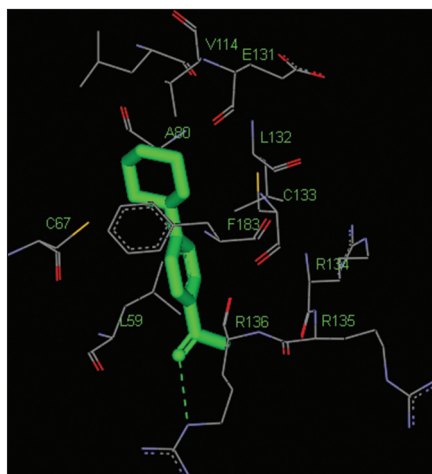
## MATERIALS AND METHODS

To determine how the structures of natural compounds 1,2-Benzenedicarboxylic acid bis (2ethylhexyl) ester, squalene, 3,5-bis (1,1-dimethylethyl) phenol, Pentamethyl tetrahydro-5H-chromene, (1,4-Cyclohexylphenyl) ethanone and 6-Vinyl-7-methoxy-2,2-dimethylchromene identified in the (AB-2) active band isolated from leaves of *A. houstonianum* contribute to their differential inhibitory activity against PLK1 kinase, virtual docking analysis with the kinase domain of PLK1 was performed. The crystal structure of PLK1 solved at 2.1 Å resolutions was retrieved from the Protein Databank (PDB ID code 2OWB). PDB structure of natural compounds 1, 2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester (Chemspider ID-21106505), squalene (Pubchem CID-638072), 3, 5-bis (1,1-dimethylethyl) phenol (Pubchem CID-70825), Pentamethyl tetrahydro-5H-chromene (Pubchem CID-605742), (1,4-Cyclohexylphenyl) ethanone (Pubchem CID-87715) and 6-Vinyl-7-methoxy-2,2 dimethylchromene (Pubchem CID-188454) were retrieved from Pubchem and Chemspider. Thereafter, each of these ligands (natural compound) was docked to the enzyme (PLK1) using “Auto dock 4.2” separately. For energy minimization of each of the ligand molecules, the MMFF94 force field was used. Gasteiger partial charges were added to the ligand atoms. We merged the non-polar hydrogen atoms and duly defined the rotatable bonds. Docking calculations were carried out on the protein model. With the aid of Auto dock tools, we added hydrogen atoms, Kollman united atom type charges, and solvation parameters. A grid of 40 Å × 40 Å × 40 Å with 0.375 Å spacing was designed via “Auto grid” aimed to target

the ATP-binding pocket of the catalytic site of PLK1. The values of x, y, and z co-ordinates used for targeting the “ATP-binding pocket” were 0.069, 23.58 and 66.741, respectively. We used the default set of parameters and dielectric functions included within the “Auto dock” program for the calculation of the van der Waals and the electrostatic terms. “Lamarckian genetic algorithm” and the “Solis and Wets local search method” were employed for docking simulations. We randomly set the initial position, torsions, and orientation of the ligand molecules. Hundred runs were used for each docking experiment. Each of the runs was terminated after 2,500,000 energy evaluations the population size being 150. Discovery Studio2.5 (Accelrys) was used for visualizations and figure-generation.

## RESULTS AND DISCUSSION

The natural compounds identified by GC-MS analysis in the (AB-2) active band isolated from methanolic crude extract of leaves of *A. boustonianum* exhibited differential inhibitory action against PLK1 kinase. The virtual docking results indicated that (1,4-Cyclohexylphenyl) ethanone exhibited strong binding to the kinase domain of PLK1, which could suggest it has stronger anti-PLK1 activity than the other compounds. The docking model showed that (1,4-Cyclohexylphenyl) ethanone was sandwiched into a “C” shaped cavity between the side chain of L59 from the N terminus and R136 from the C-terminus [Figure 1]. The kinase domain of PLK1 was found to interact with (1,4-Cyclohexylphenyl) ethanone through the amino acid residues R134, R135, R136, C133, F183, L132, A80, E131, V114, C67 and L59 [Table 1]. The free energy of binding and estimated inhibition constant (K<sub>i</sub>) for the “(1,4-Cyclohexylphenyl) ethanone-PLK1 kinase domain-interaction” were



**Figure 1:** Interaction of (1, 4- Cyclohexylphenyl) ethanone docked to the “catalytic site” or “kinase domain” of the human Polo Like Kinase 1 (PLK1). The ligand [(1, 4- Cyclohexylphenyl) ethanone] is shown in ‘stick’ representation

determined to be  $-6.84$  kcal/mol, and  $9.77$   $\mu\text{M}$ , respectively. Four carbon atoms of (1,4-Cyclohexylphenyl) ethanone, namely CD1, CB, CG1 and CG2 were predicted to be involved in hydrophobic interactions with amino acid residues L59, A80, V114, L132 and C133 of the enzyme. Total intermolecular energy of docking for (1,4-Cyclohexylphenyl) ethanone-PLK1 kinase domain-interaction was found to be  $-7.43$  kcal/mol. One of the oxygen atom O1 of (1, 4-Cyclohexylphenyl) ethanone was observed to make polar bonds with amino acid residue R136 of PLK1. At the same time, F183 (commonly a leucine or methionine in other kinases) was observed to further enhance the affinity through  $\pi$ - $\pi$  stacking with two carbon atoms of (1,4-Cyclohexylphenyl) ethanone (CD2 and CE2), which were present at the binding site. This was in contrast to “Beta carboline compounds-PLK1 interaction” where the affinity was further enhanced through  $\pi$ - $\pi$  stacking with benzene at the p-fluro benzyloxy group and seemed to play a significant role in docking.<sup>[43]</sup> In another study on PLK1 inhibitors, it has been found that F183 and compound (73) was interacting through an aromatic ring stacking, which had an important influence on the conformational equilibrium of the whole compound.<sup>[44]</sup> “Van der Waals,” “Hydrogen Bond,” and “Desolvation” energy components together contributed  $-7.36$  kcal/mol while the “Electrostatic” energy component was found to be  $-0.06$  kcal/mol. Total interacting surface area for (1,4-Cyclohexylphenyl) ethanone-PLK1 kinase domain-interaction was found to be  $595.73$   $\text{\AA}^2$  while hydrogen bonds and cation- $\pi$  interactions were absent.

**Table 1: Amino acid residues involved in natural compounds and PLK1’ interactions**

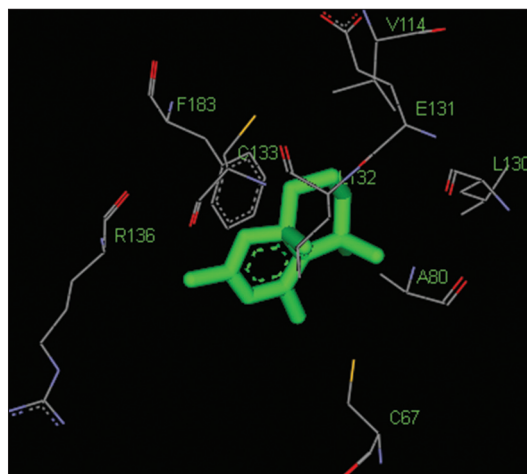
Ligands (natural compounds)	Interacting amino acid residues of catalytic domain of human-PLK1
(1,4-Cyclohexylphenyl) ethanone	R134, R135, R136, C133, F183, L132, A80, E131, V114, C67, L59
Pentamethyl tetrahydro-5H-chromene	C67, R136, F183, C133, L132, V114, E131, L130, A80
6-Vinyl-7-methoxy-2,2-dimethylchromene	R134, R136, L59, G60, C67, A80, F183, L132, C133, E131, V114, L130, E140
Squalene	R57, R134, E69, L132, L59, R136, S137, E140, G180, D194, A65, K82, K66, V114, L130, G60, K61, C133, G193, N181, F183, L131, A80, G62
3,5-bis(1,1-dimethylethyl) phenol	K82, L130, A80, C67, E131, L132, V114, L59, G60, R134, R136, F183, C133
1,2-Benzenedicarboxylic acid bis (2ethylhexyl) ester	K82, C67, E131, C133, A80, R134, R136, F183, G193, E69, R57, L132, L59, L130, G60, K61, D194, V114

PLK: Polo-like kinase

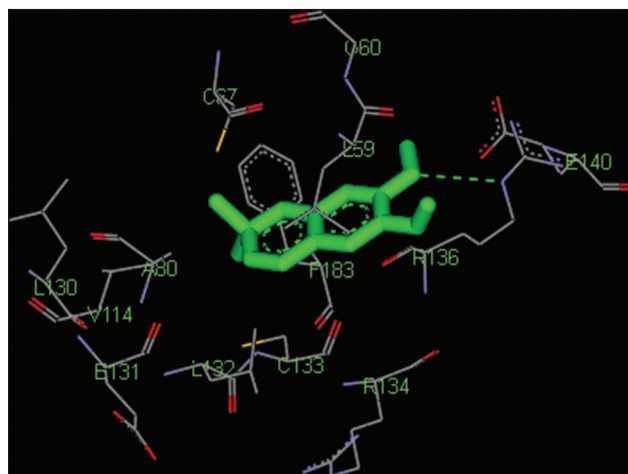
Chromenes are structurally simple compounds belonging to a large class of molecules known as benzopyrenes and chromen-4-one moiety is an integral part of many natural products. These compounds and related derivatives exhibit diverse biological activities, such as antitumor, leishmanicidal, and bacteriostatic that makes these compounds attractive for further exploration and screening as novel therapeutic agents.<sup>[45-47]</sup> In the present study, two chromenes, Pentamethyl tetrahydro-5H-chromene and 6-Vinyl-7-methoxy-2, 2 dimethylchromene were used as ligands to interact with catalytic kinase domain of human PLK1 enzyme. The catalytic domain of human PLK1 was determined to interact with Pentamethyl tetrahydro-5H-chromene through 9 amino acid residues, namely R136, F183, C133, L132, V114, E131, L130, A80 and C67 [Figure 2 and Table 1]. Accordingly, 6-Vinyl-7-methoxy-2, 2 dimethylchromene was found to interact with the catalytic domain of PLK1 with 13 amino acid residues namely, R134, R136, L59, G60, C67, A80, F183, L132, C133, E131, V114, L130 and E140 [Figure 3 and Table 1]. The free energy of binding and estimated  $K_i$  for the “Pentamethyl tetrahydro-5H-chromene-PLK1 kinase domain-interaction” were determined to be  $-6.60$  kcal/mol and  $14.58$   $\mu\text{M}$ , respectively. However, free energy of binding and estimated  $K_i$  for the “6-Vinyl-7-methoxy-2, 2 dimethylchromene-PLK1 kinase domain-interaction” were determined to be  $-5.91$  kcal/mol and  $46.68$   $\mu\text{M}$ , respectively. Elaboration of these interactions might aid in the design of PLK1 inhibitors focused on the backbone of Pentamethyl tetrahydro-5H-chromene. Four carbon atoms of Pentamethyl tetrahydro-5H-chromene, namely CB, CG1, CG2 and CD1 were predicted to be involved in hydrophobic interactions with six amino acid residues of the enzyme, namely C67, A80, V114, L130, L132 and C133. Total intermolecular energy of docking for Pentamethyl

tetrahydro-5H-chromene-PLK1 kinase domain-interaction was found to be  $-6.60$  kcal/mol. “Van der Waals,” “Hydrogen Bond,” and “Desolvation” energy components together contributed  $-6.63$  kcal/mol, the “Electrostatic” energy component being  $0.03$  kcal/mol. Total interacting surface area for Pentamethyl tetrahydro-5H-chromene-PLK1 kinase domain-interaction was  $580.768$   $\text{\AA}^2$ . No pi-pi or cation-pi and hydrogen bond interactions were observed.

In contrast to this, seven carbon atoms of 6-Vinyl-7-methoxy-2, 2 dimethylchromene, namely CB, CG, CG1, CG2, CD1, CE1 and CZ were predicted to be involved in hydrophobic interactions with seven amino acid residues of the enzyme, namely L59, C67, A80, V114, L130, C133 and F183. In addition to this  $\pi$ - $\pi$  interaction (CD2 and CE2) of 6-Vinyl-7-methoxy-2, 2 dimethylchromene with F183 was also observed while no cation-pi and hydrogen bond interactions were observed. However, total intermolecular energy of docking for 6-Vinyl-7-methoxy-2, 2 dimethylchromene-PLK1 kinase domain-interaction was found to be  $-6.45$  kcal/mol. “Van der Waals,” “Hydrogen Bond,” and “Desolvation” energy components together contributed  $-6.44$  kcal/mol, the “Electrostatic” energy component being  $-0.01$  kcal/mol. Total interacting surface area for 6-Vinyl-7-methoxy-2, 2 dimethylchromene-PLK1 kinase domain-interaction was  $600.396$   $\text{\AA}^2$ . Squalene is an intermediate in the cholesterol biosynthesis pathway, which is a triterpene.<sup>[48]</sup> It has been experimentally reported that squalene can effectively inhibit chemically induced colon, skin, and lung tumorigenesis in rodents.<sup>[49,50]</sup> During the past few years, squalene was also found to exhibit protective activities against several carcinogens.<sup>[51]</sup> In the present study, with the help of virtual docking we have studied the inhibitory activity of squalene against human PLK1 enzyme to predict the efficacy of squalene as a potential anticancer agent [Figure 4]. 24 amino acid residues, namely



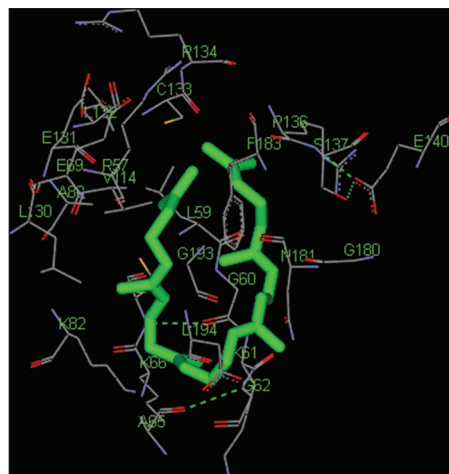
**Figure 2:** Interaction of Pentamethyl tetrahydro-5H-chromene docked to the “catalytic site” or “kinase domain” of the human polo like kinase 1. The ligand (Pentamethyl tetrahydro-5H-chromene) is shown in “stick” representation



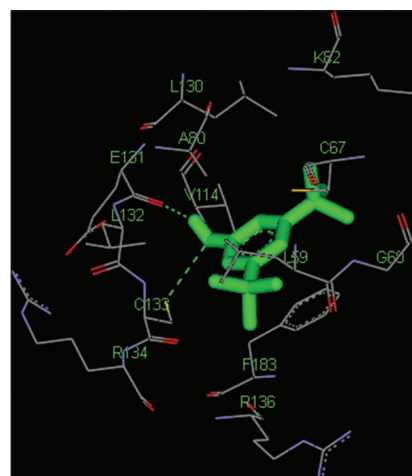
**Figure 3:** Interaction of 6-Vinyl-7-methoxy-2, 2-dimethylchromene docked to the “catalytic site” or “kinase domain” of the human polo like kinase 1. The ligand (6-Vinyl-7-methoxy-2, 2-dimethylchromene) is shown in “stick” representation

R57, R134, E69, L132, L59, R136, S137, E140, G180, D194, A65, K82, K66, V114, L130, G60, K61, C133, G193, N181, F183, L131, A80 and G62 of the catalytic domain of PLK1 were found to interact with squalene [Table 1]. Squalene displayed a free binding energy of  $-6.17$  kcal/mol and  $K_i$  of  $30.12$   $\mu\text{M}$  against catalytic domain of human PLK1 enzyme and total intermolecular energy of docking for squalene-PLK1 kinase domain-interaction was found to be  $-9.91$  kcal/mol. Four carbon atoms (CD1, CB, CG1 and CG2) of squalene were predicted to be involved in hydrophobic interaction with five amino acid residues (L59, A80, V114, L130, C133) of PLK1 enzyme catalytic domain. “Van der Waals,” “Hydrogen Bond,” and “Desolvation” energy components together contributed  $-9.91$  kcal/mol, the “Electrostatic” energy component being  $0.01$  kcal/mol. Total interacting surface area for squalene-PLK1 kinase domain-interaction was  $1041.859$   $\text{\AA}^2$ .

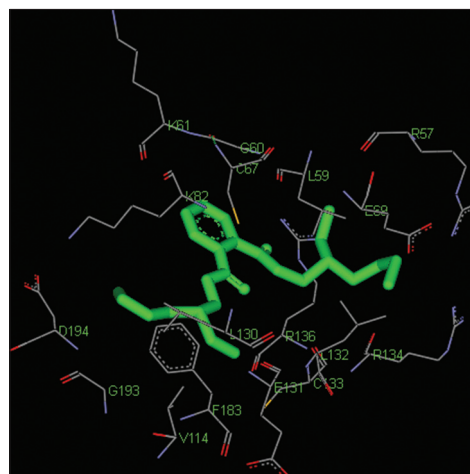
3,5-bis(1,1-dimethylethyl) phenol and 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester showed weaker interaction compared to the other compounds used in the study. Accordingly, the free energy of binding ( $\Delta G$ ) and  $K_i$ , were found to be  $-5.70$  kcal/mol and  $66.68$   $\mu\text{M}$  in case of 3,5-bis (1,1-dimethylethyl) phenol-PLK1 interaction [Figure 5] and  $-5.58$  kcal/mol and  $80.78$   $\mu\text{M}$  in case of 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester-PLK1 interaction [Figure 6], respectively. 13 amino acid residues of the catalytic domain of PLK1, namely K82, L130, A80, C67, E131, L132, V114, L59, G60, R134, R136, F183 and C133 were found to interact with 3,5-bis(1,1-dimethylethyl) phenol, while, eighteen amino acid residues (K82, C67, E131, C133, A80, R134, R136, F183, G193, E69, R57, L132, L59, L130, G60, K61, D194 and V114) were found to interact with 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester [Table 1]. Total intermolecular energy of docking for 3,5-bis (1,1-dimethylethyl) phenol-PLK1 interaction and 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester-PLK1 interaction were found to be  $-6.59$  kcal/mol and  $-8.95$  kcal/mol, respectively. Two carbon atoms of 3,5-bis (1,1-dimethylethyl) phenol, namely CB and CD1 were observed to make hydrophobic interaction with two amino acid residues L59 and L130; however, four carbon atoms of 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester, namely CD1, CB, CD2 and CE2 were found to be involved in hydrophobic interaction with four amino acid residues L59, C67, A80, and F183 of the catalytic domain of PLK1 enzyme. In case of 3,5-bis(1,1-dimethylethyl) phenol-PLK1 interaction “Van der Waals,” “Hydrogen Bond” and “Desolvation” energy components together contributed  $-6.51$  kcal/mol and the “Electrostatic” energy component being  $-0.08$  kcal/mol, while, in case of 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl)



**Figure 4:** Interaction of squalene docked to the “catalytic site” or “kinase domain” of the human polo like kinase 1. The ligand (squalene) is shown in “stick” representation



**Figure 5:** Interaction of 3,5-bis(1,1-dimethylethyl) phenol docked to the “catalytic site” or “kinase domain” of the human polo like kinase 1. The ligand (3,5-bis [1,1-dimethylethyl] phenol) is shown in “stick” representation



**Figure 6:** Interaction of 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester docked to the “catalytic site” or “kinase domain” of the human polo like kinase 1. The ligand (1,2-Benzenedicarboxylic acid bis [2 ethylhexyl] ester) is shown in “stick” representation

ester-PLK1 interaction “Van der Waals,” “Hydrogen Bond,” and “Desolvation” energy components together contributed  $-8.97$  kcal/mol and the “Electrostatic” energy component being  $0.02$  kcal/mol. Total interacting surface area for 3,5-bis(1,1-dimethylethyl) phenol-PLK1 interaction and 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester-PLK1 interaction were  $628.966 \text{ \AA}^2$  and  $1041.586 \text{ \AA}^2$ , respectively. A hydrogen bond interaction was observed between O1 of 3,5-bis (1,1-dimethylethyl) phenol and C133 of catalytic domain of PLK1 enzyme. During the study, no  $\pi$ - $\pi$  or cation- $\pi$  interaction involving either 3,5-bis (1,1-dimethylethyl) phenol or 1,2-Benzenedicarboxylic acid bis (2 ethylhexyl) ester with PLK1 was observed. Moreover, It was found that (1,4-Cyclohexylphenyl) ethanone-PLK1 kinase domain-interaction ( $K_i = 9.77 \mu\text{M}$  and  $\Delta G = -6.84$  kcal/mol) displayed a lower  $K_i$  and a higher (negative)  $\Delta G$  value compared to other compounds from the leaves of *A. boustonianum* involving the same binding sites of catalytic/kinase domain of PLK1 enzyme. Hence, the present study reveals that (1,4-Cyclohexylphenyl) ethanone is a stronger inhibitor of human PLK1 than the other compounds ([1,2-Benzenedicarboxylic acid bis [2 ethylhexyl] ester, squalene, 3,5-bis[1, 1-dimethylethyl] phenol, Pentamethyl tetrahydro-5H-chromene and 6-Vinyl-7-methoxy-2,2-dimethylchromene]) in terms of predicted  $K_i$  values and free energy of binding as well.

Further investigations are needed to establish the anticancer potential of (1, 4-Cyclohexylphenyl) ethanone. However, it can be safely stated that the present study on this natural compound reflects a hope for the development of novel agent of biomedical importance. Moreover, a similar study on a natural compound, Purpurogallin, which is the aglycon of several glycosides from nutgalls and oak-barks has been performed by Liao *et al.*<sup>[24]</sup> It was observed that Purpurogallin bound to the phospho-binding pocket of PLK-1 and induced fit analysis revealed that Purpurogallin filled the SpT pocket via  $\pi$ - $\pi$  stacking and hydrogen-bonding interactions, thereby providing a rationale for natural compound acting as a novel PLK-1 inhibitor.<sup>[24]</sup> It is noteworthy to mention that the natural compound ([1,4-Cyclohexylphenyl] ethanone) discussed herein is of due clinical significance. Different synthesized derivatives of ethanone have been already reported for their potential anticancer activities against various cell lines.<sup>[52-55]</sup> Hence, this study is expected to aid future design of more specific anticancer pharmacological compounds.

This study explores molecular interactions between human PLK1 and the natural compounds identified by GC-MS analysis in (AB-2) active band, which we isolated in another study, published previously. In the present study, we have provided a comparative account of the interactions of

different natural compounds found in active band AB-2 with PLK1 enzyme. Hydrophobic interactions play an important role in the correct positioning of these natural compounds within the catalytic site of PLK1 enzyme to permit docking. However, docking of (1, 4-Cyclohexylphenyl) ethanone to PLK1 is largely dominated by hydrophobic interactions followed by one  $\pi$ - $\pi$  interaction and one polar interaction. Such information may aid in the design of versatile PLK1-inhibitors. Further, *in vitro* and *in vivo* studies are warranted to explore the anticancer potential of (1,4-Cyclohexylphenyl) ethanone and to validate the findings presented herein. This study predicts that (1, 4-Cyclohexylphenyl) ethanone is a more efficient inhibitor of human PLK1 enzyme compared to other natural compounds used in the study with reference to  $K_i$  and  $\Delta G$  values.

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