Section A-Research paper

COMPUTATIONAL DRUG DISCOVERY OF CERTAIN COMMERCIALLY AVAILABLE TERPENOIDS AS POTENTIAL LEAD MOLECULES FOR LIVER CANCER

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ABSTRACT

Background:

Liver cancer, the third most common cause of cancer-related deaths worldwide, is the fifth most common type of cancer. Resection patients typically have a low cure rate. Sorafenib is the only drug approved by the US Food and Drug Administration to treat advanced HCC, but it comes with substantial side effects, including a high risk of haemorrhaging. These restrictions necessitated the urgent need for innovative preventative and therapeutic approaches to managing this illness.

Objective: The goal of the current study was to use molecular docking studies to find the terpenoids that offered the best natural lead molecule for treating liver cancer. A few terpenoids are docked against the human P38 kinase for this function.

Methodology:

The initial molecular characteristics were computed using the Molinspiration program in accordance with Lipsink's rule of 5. Pharmacokinetic testing was carried out. The following compounds were chosen for this study: furanodiene, beta elemene, furanodienone, germacrone, curcumol, and andrographolide. All other selected terpenoids, with the exception of beta elemene, did not exhibit any violations, so they were picked for the AutoDock 4.2 docking investigations.

Results: According to the docking data, the chosen terpenoids showed excellent binding energies between -6.63 and -5.63 kcal/mol against the human P38 enzyme. The inhibition constant that was estimated to be in the range of 195.07 nM to 24.19 nM is consistent with the binding energies of the selected drugs. The terpenoids' intermolecular energies were lower than expected, ranging from -8.84 kcal/mol to -5.63 kcal/mol.

Conclusion: In comparison to Sorafenib, which was used as the reference, the screening revealed that Andrographolide had a binding energy of -6.63 Kcal/mol, which was somewhat greater. As a result, an *in silico* experiment revealed andrographolide as a candidate for further research in the treatment of hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC), the most common primary liver malignancy, affects adults. HCC is currently the leading cause of death in cirrhotic patients[1]. HCC is the third most typical cause of cancer-related mortality globally. The worst part of this disease, which most usually affects adult males, is that it is the fifth most common cancer in the world [2]. Each year, there are over 7.5 lakh new cases of HCC worldwide. Hepatocellular carcinoma has a very high death rate, hence an expeditious cure is required[3].

HCC is more prone to develop in those with liver disease, especially those with chronic hepatitis B and C[4]. Early signs are typically non existent, but later stages might result in symptoms like loss of weight, pain in the upper abdomen, or darkening of the skin (Jaundice). Treatment options include surgery, organ transplants, heating or freezing of cancer cells, and chemotherapy [5].

Many intracellular and cell surface kinases that cause angiogenesis, including as RAF (rapidly accelerated fibrosarcoma) kinases and VEGF receptors, are inhibited by the niacinamide and phenyl urea derivative sorafenib (Nexavar) [6]. It is the most significant standard treatment for advanced renal cell and hepatocellular carcinoma. It is also used to treat thyroid cancer that has developed a resistance to radioactive iodine therapy [7]. Sorafenib is one of the kinase inhibitors. It inhibits the protein kinase enzyme. Angiogenesis and growth signalling are the two conditions that sorafenib is intended to treat. In addition to inhibiting the RAF kinase enzyme, sorafenib also affects the VEGFR-2/PDGFR-beta signalling cascade. Sorafenib inhibits tumour angiogenesis by these mechanisms [8][9].

Drug designing is a vital strategy in the world of pharmaceutical chemistry for producing new compounds by chemically or molecularly modifying the lead moiety to create highly active molecules[10]. The assessment of the binding affinity of the complex and newly developed compounds in the receptor binding site is called docking of test compounds or newly developed components of structural-based drug design [11].

The goal of the current study was to use molecular docking studies to find the terpenoids that offered the best natural lead molecule for treating liver cancer. The following compounds were chosen for this study: Beta-elemene, Furanodiene, Furanodienone, Germacrone, Curcumol, and Andrographolide. Terpenoids are docked against the Human P38 kinase in order to discover the powerful lead molecule against Hepatocellular carcinoma as a prominent treatment. Sorafenib is the only medicine approved by the US Food and Drug Administration to treat advanced HCC. Unfortunately, there are some major side effects associated with this medicine, including a

substantial risk of bleeding [12,13]. Due to these drawbacks, new preventative and therapeutic approaches to this illness are required [14].

The current study was started to lessen the unfavourable effect with high pharmacological action with fewer side effects, and natural compounds from terpenoids have been picked for this study in order to combat these significant bad effects from the synthetic compounds as a medicine. In order to identify the potential lead chemical for treating liver cancer, insilico molecular docking was used in this investigation.

2. METHOD AND MATERIALS

Using software and databases:

2.1 The use of software

Molecular graphics laboratory (MGL) tools, AutoDock 4.2, Python 2.7, and Cygwin (a programme for data storage), respectively, were acquired from www.scripps.edu, www.python.com, and www.cygwin.com [18]. Chemsketch 2.5.5 and ACDLabs 2.5.5 were obtained from www.accelerys.com and www.acdlabs.com, respectively. Online smiling translation notation was done using cactus.nci.nih.gov/translate.

2.2 Preparation of the target enzyme

The crystal structure of the human P38 kinase (1IAN) protein database was downloaded from the research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (Fig. 1). As part of the AutoDock Tools' preparation of the target protein 1IAN, all hydrogen atoms were added to the macromolecule, a procedure necessary for the precise estimate of partial atomic charges [15]. Three-dimensional affinity grids of size 277 277 277 with 0.6 spacing were created for each of the following atom kinds: HD, C, A, N, OA, and SA in order to represent all potential atom types in a protein.

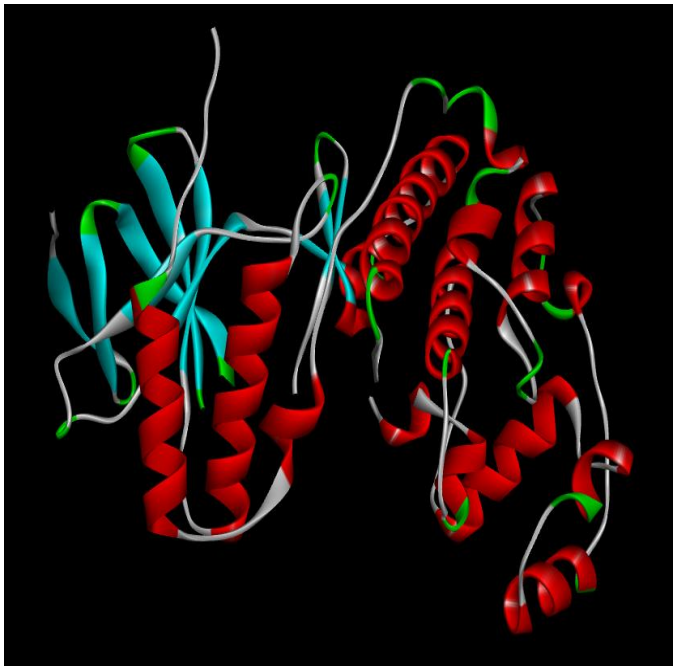


Fig. 1: Human P38 kinase RCSB protein data bank (1IAN)

2.3 Drug-like features of the ligands

The standard sorafenib and furanodiene, germacrone, furanodienone, curcumol, and andrographolide were among the ligands produced using ChemSketch and optimised using "Prepare Ligands" in AutoDock 4.2. (Fig.2). The enhanced human p38 kinase enzyme was docked into the optimised ligand molecules using "LigandFit" in AutoDock 4.2. [17].

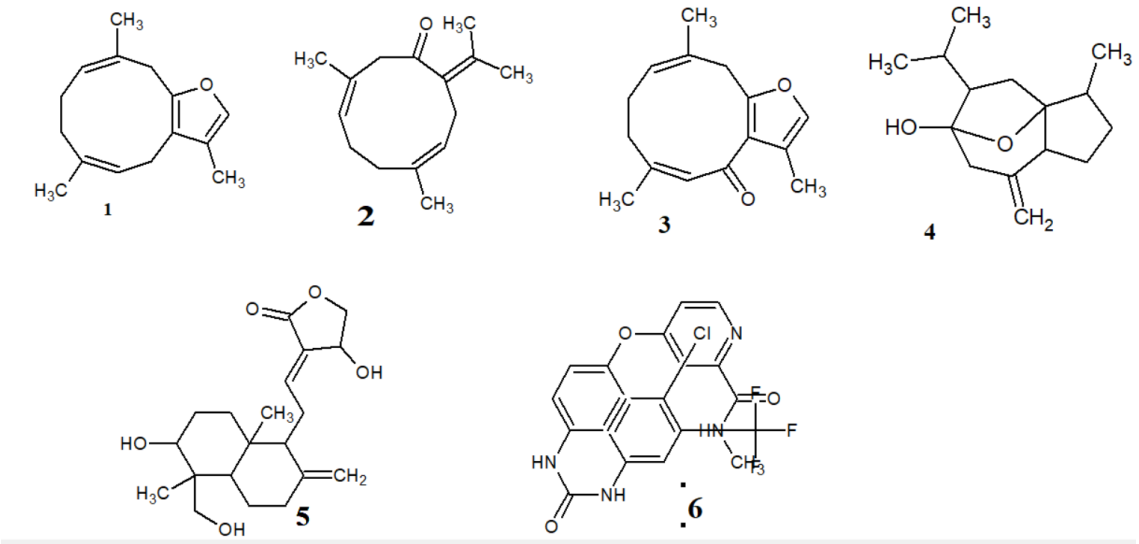


Fig. 2: The optimized ligand molecules (1 Furanodiene, 2 Germacrone, 3 Furanodienone, 4 Curcumin, 5 Andrographolide, and 6.Standard Sorafenib) were shown in **figure 2.**

2.4 Docking methodology

The largest database on the three-dimensional (3D) structures of enzyme targets and their multiplexes as determined by NMR spectroscopy, X-ray crystallography, and cryoelectron microscopy is the RCSB (Research Collaboratory for Structural Bioinformatics). [18]. The p38 kinase enzyme for humans was acquired from the Protein Bank of the Research Collaboratory for Structural Bioinformatics (RCSB) (PDB ID: 1IAN). Using the Accelrys Discovery Studio viewer, the PDB file of the target enzyme was created and the hetero atoms in the molecule were deleted (Fig. 1). An enhanced PDB format with the PDBQT file extension was used to coordinate files with atomic partial charges[19,20]. Chemical structures can be illustrated using a programme called ChemSketch. In figure 2, the terpenoids ligands, such as β-elemene, Furanodiene, Furanodienone, Germacrone, Curcumol, and sorafenib the standard, were created using ChemSketch and optimised using "Create Ligands" in AutoDock 4.2 for use in further docking studies. The improved terpenoids were docked into the updated target using AutoDock 4.2.

For ligand conformational searching, the Lamarckian genetic algorithm (LGA), a hybrid of a genetic algorithm and a local search method, was used[21]. By using this method, the docked molecule is first randomly confirmed into a population of people (genes). When each individual has been adjusted to acquire slightly different translations and rotations, the population of individuals is next exposed to the local search algorithm's energy minimizations [22].

The resulting energy is passed down to the following generation, and the cycle is repeated. The method is Lamarckian because each new generation of individuals is allowed to inherit the local search modifications of their parents. AutoDock 4.2 estimates Gasteiger charges rather than Kollman charges for each atom in the macromolecule, in contrast to prior versions of this program[23]. The PDB format was used to calculate the intended ligand and the enhanced protein for docking in AutoDock 4.2. While it is active, the.glg file performs AutoGrid calculations. AutoDock's execution is managed by the dlg file[24].

The binding site and its energy, conformational similarity, as well as other properties like the inhibition constant and intermolecular energy, may all be viewed when using the AutoDock Tools to assess the results of docking simulations. The projected free energy of the binding energy of the flavonoid molecule (kcal/mol) was used to calculate the inhibition constant (Ki) of each ligand [25,26].

Eventually, research was done on a single molecule in 10 different docked conformations. The binding energy, intermolecular energy, and inhibition constant of each of the selected terpenoids were assessed [27]. For each ligand, the top ten docking simulations against the target molecule were compiled. To generate diverse docked conformational poses, AutoDock 4.2 was ran many times [28]. Based on the docking characteristics obtained using AutoDock 4.2, the terpenoids were chosen for additional *in vitro* research.

3. RESULTS

The original Rule of 5 is the most needed promising profile development in novel drug discovery. The Rule of 5 takes on numeric values from 0 to 4 based on the potential difficulties of a compound on pharmacokinetic characteristics and whether the lead moieties are capable to reach further steps[29,30]. As such, Rule of 5 is an advantageous computational filter in drug candidate evaluation and the screening werewase in Medchem desig ner.In terms of ADMET Predictor models and descriptors, the Rule of 5 models administrates can be expressed as follow the following set of environments, excessive lipophilicity (MlogP) > 4.15, Molecular weight MWt) > 500, hydrogen bond donors (HBDH) > 5, hydrogen bond acceptors (M_NO) > 10 [31,32]

The compounds must be accepted if they only exhibit one violation; however, if they exhibit multiple violations, they must be excluded from the study. Similar to that in this study, beta elemene exhibits multiple violations, leading to its rejection and non-use in in silico studies. Using MedChem Designer, the terpenoids other than beta elemene's pharmacokinetic characteristics were studied (Table 1). All of the chosen terpenoids were evaluated and showed excellent pharmacokinetic characteristics, proving that they do not violate Rule of 5. The chosen terpenoids may be further screened for their in silico biological activity against the human P38 kinase enzyme based on the findings.

Table 1: Pharmacokinetic properties of the selected Terpenoids

Name	MWt	MlogP	S+logP	S+logD	T_PSA	HBDH	M_NO	Rule of 5 Number of Violations
Furanodiene	216.325	3.427	5.047	5.047	13.140	0.000	1.000	0
β-elemene	204.358	5.371	**5.361**	**5.361**	0.000	0.000	0.000	2
Furanodienone	230.309	2.728	3.766	3.766	30.210	0.000	2.000	0
Germacrone	218.341	3.427	3.896	3.896	17.070	0.000	1.000	0
Curcumol	236.537	3.427	3.194	3.194	29.460	1.000	2.000	0
Andrographolide	350.458	1.801	1.287	1.287	86.990	3.000	5.000	0
Sorafenib	464.834	3.196	5.073	5.063	92.350	3.000	7.000	0

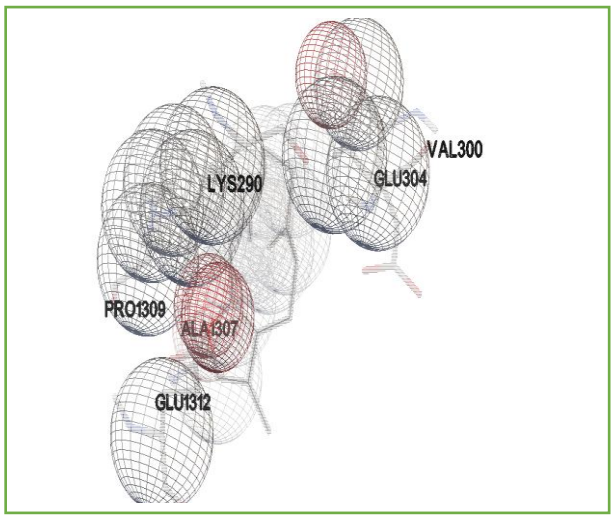
MWt - Molecular Weight; S+logP - "StarList" of ion-corrected experimental logP values; S+logD - octanol-water distribution coefficient; MlogP – Moriguchioctanol-water partition coefficient; T_PSA-Topological Polar Surface Area; HBDH - the count of HB donor protons; M_NO = total number of nitrogen and oxygen atoms (Ns and Os)

The in silico molecular docking of the terpenoids against the human p38 kinase enzyme was investigated in the current work. Using AutoDock 4.2, tests on molecular docking revealed that the binding energies of the chosen flavonoids ranged from -6.63 kcal/mol to -5.02 kcal/mol (table 2). All terpenoids demonstrated inhibitory binding energy to the human P38 kinase enzyme. The predicted binding energy of the popular drug sorafenib against the human p38 kinase enzyme was - 5.26 kcal/mol.

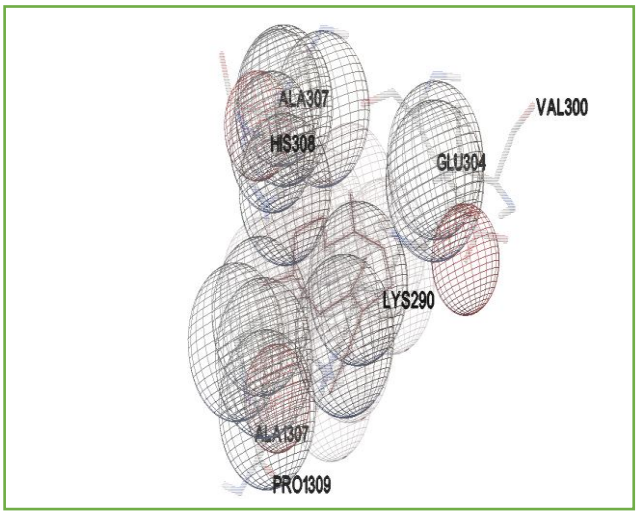
Table 2: Terpenoid's Binding energies based on their rank

Compounds		Compound binding energies (Kcal/Mol)								
Furanodiene	**-5.63**	**-5.62**	**-5.62**	**-5.62**	**-5.62**	**-5.62**	**-5.62**	**-5.61**	**-5.61**	**-5.61**
Furanodienone	**-5.98**	**-5.97**	**-5.97**	**-5.97**	**-5.97**	**-5.97**	**-5.96**	**-5.96**	**-5.95**	**-5.95**
Germacrone	**-6.30**	**-6.29**	**-6.29**	**-6.23**	**-6.21**	**-6.12**	**-6.09**	**-6.09**	**-6.07**	**-6.03**
Curcumol	**-5.72**	**-5.71**	**-5.70**	**-5.70**	**-5.68**	**-5.64**	**-5.62**	**-5.62**	**-5.59**	**-5.59**
Andrographolide	**-6.63**	**-6.62**	**-6.62**	**-6.60**	**-6.60**	**-6.59**	**-6.58**	**-6.58**	**-6.55**	**-6.54**
Sorafenib	**-5.26**	**-5.24**	**-5.24**	**-5.23**	**-5.19**	**-5.19**	**-5.17**	**-5.13**	**-5.11**	**-5.10**

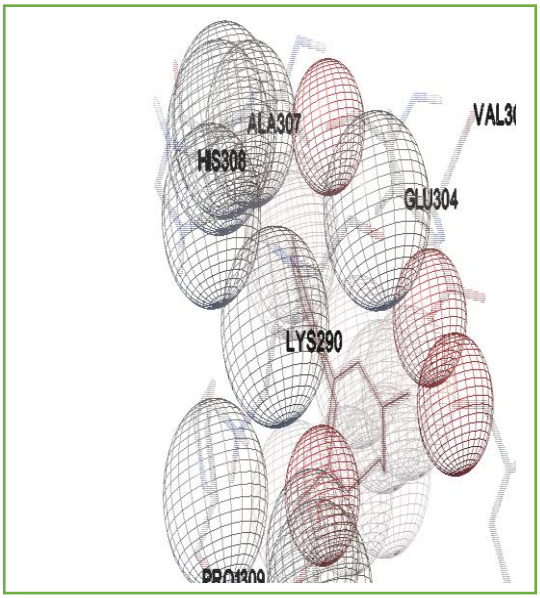
Figure 2: Terpenoids docked against the human p38 kinase enzyme



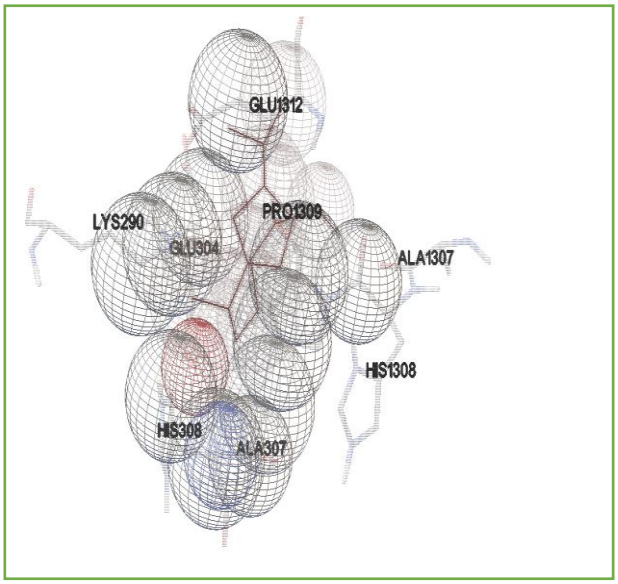
Furanodiene



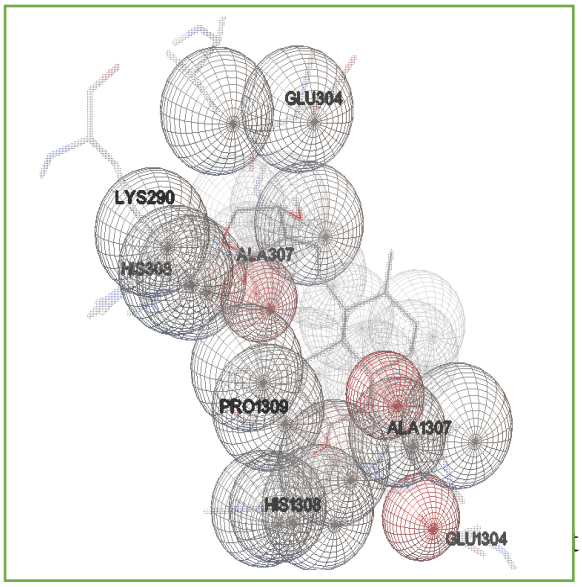
Germacrone



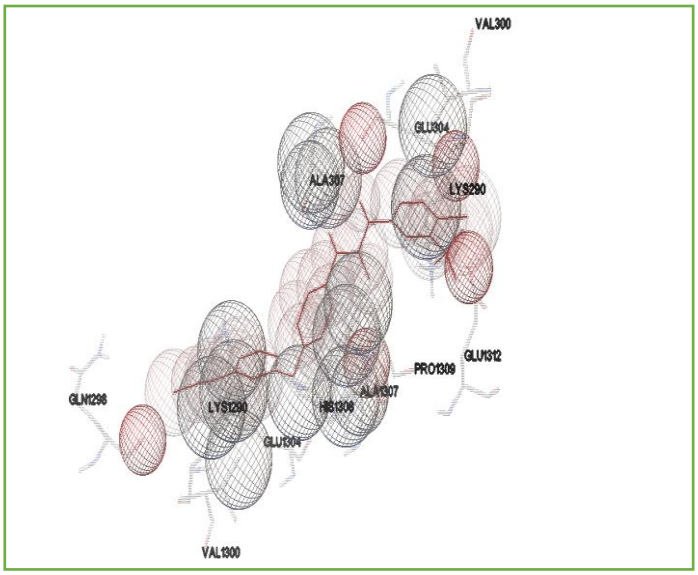
Furanodienone



Curcumol



Andrographolide



Sorafenib

In the aforementioned illustrations, the docking images were solely displayed with the amino acid residues' putative binding pockets facing the enzyme. The *in-silico* investigations were carried out with Autodock 4.2.

Table 3: Inhibition constant of the terpenoids based on their rank

Compounds		**Inhibition Constant of the compounds based on their rank (*nM, **mM)**								
Furanodiene	**75.14**	**77.28**	**78.31**	**85.12**	**89.34**	**99.26**	**101.11**	**105.21**	**109.23**	**112.20**
Furanodienone	**41.64**	**43.28**	**45.29**	**47.81**	**52.98**	**55.78**	**68.23**	**71.98**	**75.09**	**77.07**
Germacrone	**24.19**	**27.65**	**29.09**	**32.98**	**35.23**	**38.98**	**42.80**	**49.09**	**53.12**	**59.04**
Curcumol	**63.85**	**67.90**	**74.25**	**77.01**	**79.05**	**86.76**	**98.15**	**101.56**	**109.76**	**119.89**
Andrographolide*	**195.07**	**202.90**	**309.11**	**200.82**	**198.90**	**145.26**	**76.21**	**54.32**	**42.11**	**32.09**
Sorafenib	**6.81**	**28.98**	**39.87**	**54.21**	**76.90**	**89.01**	**107.34**	**109.76**	**154.21**	**196.23**

In addition, measurements of the inhibitory constant (Ki) and the intermolecular energy were made. According to Table 3, terpenoids showed inhibition constants ranging from 24.19 M to 195.07 M. All of the selected substances displayed reduced inhibition constants when compared to the standard. (6.81mM). Binding energy and the inhibition constant have a linear relationship. Thus, the kinase inhibitory effect of the chosen terpenoids was proven using molecular simulations.

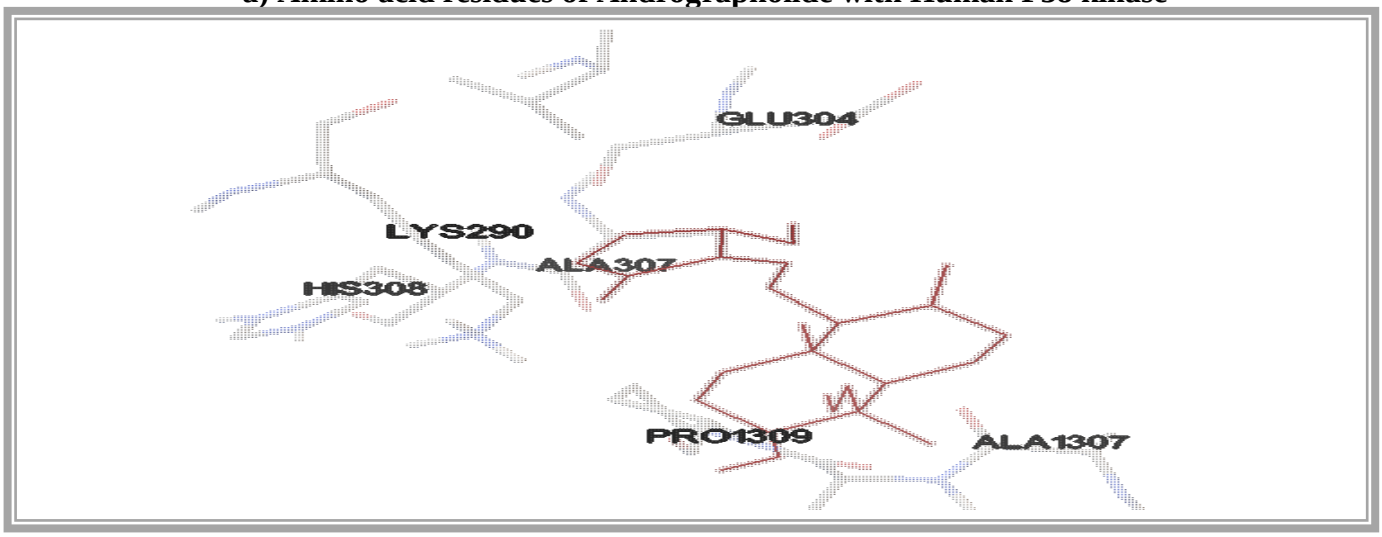
Table 4: Intermolecular energy of the Terpenoids based on their rank

Compounds		**Intermolecular energies of the compounds based on their rank (kcal/mol)**								
Furanodiene	**-5.63**	**-5.58**	**-5.51**	**-5.49**	**-5.45**	**-5.40**	**-5.38**	**-5.32**	**-5.29**	**-5.27**
Furanodienone	**-5.98**	**-5.96**	**-5.93**	**-5.90**	**-5.87**	**-5.65**	**-5.52**	**-5.40**	**-5.21**	**-5.07**
Germacrone	**-6.3**	**-6.0**	**-5.87**	**-5.67**	**-5.43**	**-5.20**	**-5.08**	**-4.97**	**-4.76**	**-4.62**
Curcumol	**-6.32**	**-6.02**	**-5.98**	**-5.87**	**-5.71**	**-5.60**	**-5.38**	**-5.10**	**-5.08**	**-4.92**
Andrographolide*	**-6.85**	**-6.67**	**-6.55**	**-6.40**	**-6.33**	**-6.21**	**-5.83**	**-5.57**	**-5.49**	**-5.20**
Sorafenib	**-8.84**	**-8.37**	**-8.76**	**-8.61**	**-8.03**	**-7.89**	**-7.71**	**-7.57**	**-7.43**	**-7.28**

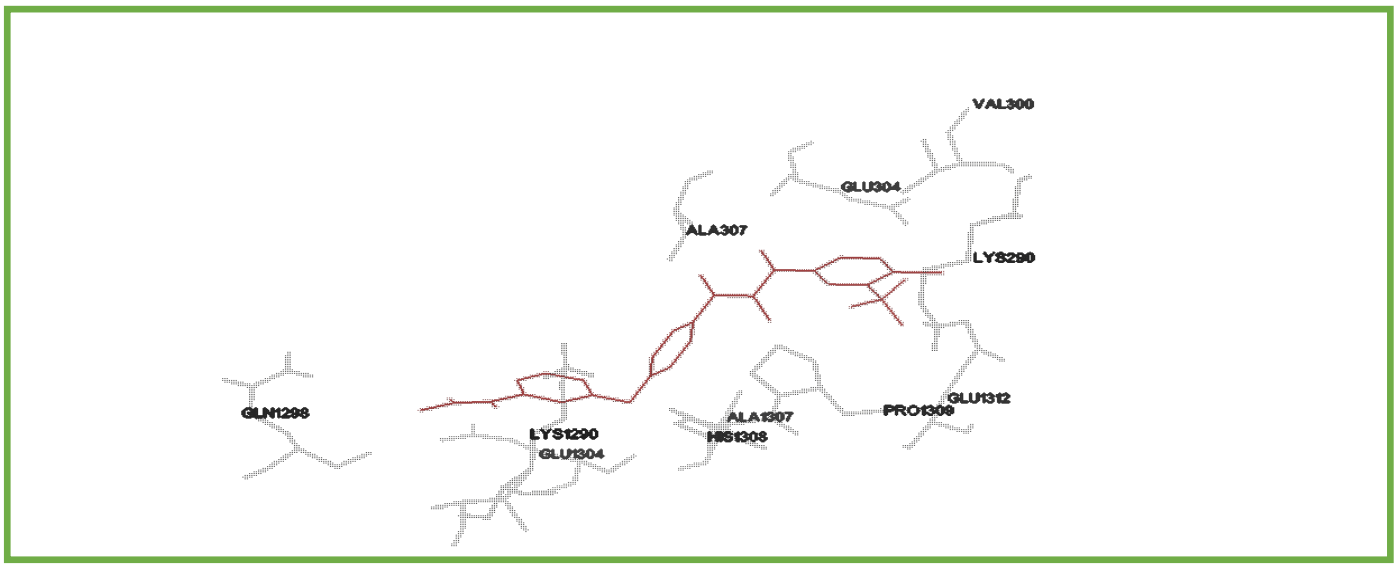
In contrast to the norm (-8.84 kcal/mol), the intermolecular energy of the compounds ranged from -8.84 kcal/mol to -5.63 kcal/mol, as indicated in table 4. Additionally, there is a direct relationship between the intermolecular energy and the binding energy. We saw a simultaneous drop in the binding energy and a decrease in the intermolecular energy of all the chosen terpenoids in the current experiment. Additionally, these discoveries increase the ability of all the selected terpenoids to inhibit human p38 kinase.

Fig. 3: Human p38 kinase enzyme conformational binding sites for andrographolide and conventional sorafenib

a) Amino acid residues of Andrographolide with Human P38 kinase



b) Amino acid residues of standard Sorafenib



The images in Figure 3 above clearly highlight the similarity in the binding sites of certain amino acids explained previously. This bolstered the kinase inhibitory potential.

Table 5: Docking orientations of selected Terpenoid and standard Sorafenib

Compound	Binding interaction with amino acid residue against Acetylcholinesterase enzyme
Andrographolide	**LYS 290**, **ALA 307**, HIS 308, **GLU 304**, **PRO 1309**, **ALA 1307**
Sorafenib (Standard)	GLN 1298, LYS 1290, GLU 1304, VAL 1300, **ALA 307**, **ALA 1307**, HIS 1308, **GLU 304**, **PRO 1309,** GLU 1312, VAL 300, **LYS 290**

In the current study, the terpenoids revealed the potential interaction against the enzyme Human P38 enzyme along with the binding site confirmations. Amino acid residues of the Andrographolide responsible for the Human p38 kinase inhibition was found to be, **LYS 290**, **ALA 307**, HIS 308, **GLU 304**, **PRO 1309**, and **ALA 1307**. Amino acid residues of standard sorafenib responsible for the kinase inhibition were found to be GLN 1298, LYS 1290, GLU 1304, VAL 1300, **ALA 307**, **ALA 1307**, HIS 1308, **GLU 304**, **PRO 1309,** GLU 1312, VAL 300, **LYS 290**

The amino acid residues such as **LYS 290**, **ALA 307, GLU 304**, **PRO 1309**, and **ALA 1307** are dynamically involved in the inhibition of Human p38 kinase in Andrographolide and compared with Sorafenib. This further clarifies that Andrographolide possesses similar binding orientation sites when compared with the standard Sorafenib.

4. Discussion

In the current work, the most promising natural lead molecule among the terpenoids for treating liver cancer was sought after using molecular docking studies. A few terpenoids are docked in the human P38 kinase for this purpose. Andrographolide was found in the screening to have a binding energy of -6.63 Kcal/mol, which, when compared to the binding energy, was higher than the standard drug Sorafenib's (-5.26 Kcal/mol). Andrographolide was thus found through *in silico* research as a lead for further consideration in the treatment of hepatocellular cancer [33],[34].

5. Conclusion

The current investigation shown that, among the chosen terpenoids, the potent lead molecule Andrographolide exhibited outstanding binding energy and inhibitory potential compared to that of the conventional sorafenib. For this p38 kinase inhibitory activity to be supported in the treatment of hepatocellular carcinoma, additional in vitro and in vivo models are required. By using molecular docking, terpenoids, in particular andrographolide, can be used to confirm the outcomes of the innovative approach used in the Insilco study.

Conflict of Interest

The author declares that there are no conflicts of interest

Acknowledgment

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References

1. Thoppil RJ, Bishayee A. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World J Hepatol. 2011 Sep 27;3(9):228-49.
2. Sacchettini JC, Poulter CD. Creating isoprenoid diversity. Science. 1997; 277:1788–1789.
3. Penuelas J, Munné-Bosch S. Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci. 2005;10:166–169.
4. Withers ST, Keasling JD. Biosynthesis and engineering of isoprenoid small molecules. *ApplMicrobiolBiotechnol.* 2007; 73: 980–990.
5. Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. Breast Cancer Res Treat. 2009; 115:223–239.
6. Wagner KH, Elmadfa I. Biological relevance of terpenoids. Overview focusing on mono-, di- and tetraterpenes. Ann NutrMetab. 2003;47:95–106.
7. Sultana N, Ata A. Oleanolic acid and related derivatives as medicinally important compounds. J Enzyme Inhib Med Chem. 2008;23:739–756.
8. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. Nat Prod Rep. 2009;26:72–89.
9. Cha,C. and Dematteo,R.P. (2005) Molecular mechanisms in hepatocellular carcinoma development. Best Pract. Res. Clin. Gastroenterol., 19, 25–37.
10. Goodsell DS, Morris GM, Olson AJ. Automated docking of flexible ligands: Applications of Autodock. J. Mol. Recog. 1996; 9:1-5.
11. Petit J, Meurice N, Kaiser C, Maggiora G. Softening the Rule of Five where to draw the line? Bioorg. Med. Chem. 2012; 20:53435351-5.
12. Okusaka T, Okada S, Ueno H, Ikeda M, Shimada K, Yamamoto J et al. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. Cancer 2002;95:1931–7.
13. Sasaki A, Kai S, Iwashita Y, Hirano S, Ohta M, Kitano S. Microsatellite distribution and indication for locoregional therapy in small hepatocellular carcinoma. Cancer 2005;103:299–306.
14. Sakurai T, Maeda S, Chang LF, Karin M. Loss of hepatic NF-kappa B activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. ProcNatlAcadSci U S A 2006;103:10544–51.

15. Owens J. Chris Lipinski discusses life and chemistry after the rule of five. Drug Discovery Today. 2003;8(1):12–6.
16. Remko M, Remková A, Broer R. A comparative study of molecular structure, PKA, lipophilicity, solubility, absorption and polar surface area of some antiplatelet drugs. International Journal of Molecular Sciences. 2016;17(3):388.
17. Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E.W., Jr. Computational methods in drug discovery. *Pharmacol. Rev.* **2014**, *66*, 334–395.
18. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001 Mar 1;46(1-3):3-26.
19. Lipinski C. A, Drug-like properties and the causes of poor solubility and poor permeability. J. Pharmacol. Toxicol. Methods, 2000,44, 235-249.
20. Shang W, Dai W, Yao C, Xu L, Tao X, Su H, et al. In vitro and in vivo evaluation of the main protease inhibitor FB2001 against SARS-COV-2. Antiviral Research. 2022;208:105450.
21. Rout S, Mahapatra RK. In silico screening of novel inhibitors of M17 leucine amino peptidase (lap) of Plasmodium vivax as therapeutic candidate. Biomedicine & Pharmacotherapy. 2016; 82: 192–201.
22. Tariq Q-un-N, Malik S, Khan A, Naseer MM, Khan SU, Ashraf A, et al. Xanthenone-based hydrazones as potent α-glucosidase inhibitors: Synthesis, solid state self-assembly and in Silico Studies. Bioorganic Chemistry. 2019; 84: 372–83.
23. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. Correction to "admetsar: A comprehensive source and free tool for assessment of Chemical Admet Properties." Journal of Chemical Information and Modeling. 2019;59(11):4959.
24. Anbuselvam M, Katherine CJ, Jeeva A, Ji H-F. Structure-based virtual screening, insilico docking, ADME prediction, and molecular dynamics studies for identification of potential NMT inhibitors against malaria. 2021;
25. Nakkala S, Modak C, Bathula R, Lanka G, Somadi G, Sreekanth S, et al. Identification of new anti-cancer agents against CENTERIN: Structure-based virtual screening, AutoDock and binding free energy studies. Journal of Molecular Structure. 2022;1270:133952.

26. El Aissouq A, Bouachrine M, Ouammou A, Khalil F. Homology modeling, virtual screening, molecular docking, molecular dynamic (MD) simulation, and ADMET approaches for identification of natural anti-parkinson agents targeting MAO-B protein. Neuroscience Letters. 2022;786:136803.
27. Semenets AP, Suleiman MM, Fedosov AI, Shtrygol SY, Havrylov IO, Mishchenko MV, et al. Synthesis, docking, and biological evaluation of novel 1-Benzyl-4-(4-(R)-5-sulfonylidene-4,5-dihydro-1h-1,2,4-triazol-3-yl)pyrrolidin-2-ones as potential nootropic agents. European Journal of Medicinal Chemistry. 2022;244:114823.
28. Suleiman M, Fedosov A, Shtrygol S, Havrylov I, Mishchenko M, Kovalenko S, et al. Novel betulindicarboxylic acid ester derivatives as potent antiviral agents: Design, synthesis, biological evaluation, structure-activity relationship and in-silico study. European Journal of Medicinal Chemistry. 2022; 244:114823.
29. Khanam M, Moin AT, Ahmed KA, Patil RB, Ripon Khalipha AB, Ahmed N, et al. Computational modeling of potential milciclib derivatives inhibitor-cdk2 binding through global docking and accelerated molecular dynamics simulations. Informatics in Medicine Unlocked. 2022; 33: 101069.
30. Kim E, Viatour P. Hepatocellular carcinoma: Old friends and new tricks. Experimental & Molecular Medicine. 2020;52(12):1898–907.
31. Raza A. Hepatocellular carcinoma review: Current treatment, and evidence-based medicine. World Journal of Gastroenterology. 2014;20(15):4115.
32. Bolondi L. Targeted therapies for hepatocellular carcinoma. Targeted Therapies for Hepatocellular Carcinoma. 2011;2–5.
33. Sung H, Ferlay J., Siegel R.L, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.CA Cancer Journal for Clinicians, 2021; 71(3),209-249.
34. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics.CA Cancer J Clin. 2022; 72: 7-33.