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RESEARCH ARTICLE

In Silico Target Prediction of 6-Gingerol and Similar Compounds as Potential Anticancer Agents

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SUMMARY

Ginger (Zingiber officinale Roscoe) has been widely recognized for its culinary and medicinal applications; however, its potential anticancer activity remains understudied. This research aimed to identify novel lead compounds exhibiting high bioavailability and low toxicity, akin to 6-gingerol, through an in silico screening approach using ChemMine Tools depending on the PubChem fingerprints. The screened compounds were further analyzed using target fishing servers to identify putative kinase targets. Molecular docking studies were conducted on selected kinases including BRAF, JAK1/2, ERK1(MAPK3), and p38γ. Computational analysis was performed to evaluate the pharmacokinetics, drug-likeness, and toxicity profiles of the compounds, shedding light on their safety and bioavailability. Notably, the following compounds exhibited promising anticancer potential: 6-gingerol, vanylglycol, vanillylmandelic acid, L-(+)-vanilmandelic acid, dehydrozingerone, 2-methoxyestrone, methylvanillate, dihydroconiferyl aldehyde, pratensein, acetovanillone, acetosyringone, licochalcone B, isoferulic acid, curcumin PE, and coniferaldehyde. These findings suggest that these compounds warrant further investigation as potential candidates for anticancer therapies and should be considered for future lead optimization studies.

Key Words: *6-gingerol, anticancer, chemical similarity, computational toxicity, molecular docking, pharmacokinetics, target fishing*

Potansiyel Antikanser Ajanlar Olarak 6-Gingerol ve Benzer Bileşiklerin In Silico Hedef Tahmini

ÖZ

Zencefil (Zingiber officinale Roscoe), mutfak ve tıbbi uygulamalarıyla yaygın olarak tanınmaktadır; ancak potansiyel antikanser aktivitesi yeterince araştırılmamıştır. Bu araştırma, PubChem parmak izlerine bağlı olarak ChemMine araçlarını kullanan bir in silico tarama yaklaşımı aracılığıyla 6-gingerol'e benzer şekilde yüksek biyoyararlanım ve düşük toksisite sergileyen yeni öncü bileşikleri tanımlamayı amaçlamıştır. Taranan bileşikler, varsayılan kinaz hedeflerini belirlemek için hedef balıkçılık sunucuları kullanılarak daha fazla analiz edildi. BRAF, JAK1/2, ERK1(MAPK3) ve p38γ dahil olmak üzere seçilen kinazlar üzerinde moleküler yerleştirme çalışmaları yapılmıştır. Bileşiklerin farmakokinetiğini, ilaca benzerliğini ve toksisite profillerini değerlendirmek, güvenliklerine ve biyoyararlanımlarına ışık tutmak için hesaplamalı analizler yapılmıştır. Özellikle, aşağıdaki bileşikler umut verici antikanser potansiyeli sergilemiştir: 6-gingerol, vanilglikol, vanilmandelik asit, L-(+)-vanilmandelik asit, dehidrozingeron, 2-metoksiestron, metilvanillat, dihidrokoniferil aldehit, pratensein, asetovanillon, asetosiringon, likokalkon B, izoferulik asit, kurkumin PE ve koniferaldehit. Bu bulgular, bu bileşiklerin anti-kanser tedaviler için potansiyel adaylar olarak daha fazla araştırılması gerektiğini ve gelecekteki öncü optimizasyon çalışmaları için dikkate alınması gerektiğini göstermektedir.

Anahtar Kelimeler: *6-gingerol, antikanser, kimyasal benzerlik, hesaplamalı toksisite, moleküler yerleştirme, farmakokinetik, hedef tahmini*

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INTRODUCTION

Cancer, a leading cause of death worldwide, poses significant challenges to effective treatment. Chemotherapy remains a widely utilized therapeutic approach for all stages of cancer (Rayan et al., 2017). However, it is associated with high doses, severe side effects due to toxicity, activation of pro-survival pathways with prolonged exposure, and the emergence of treatment resistance (Rastogi et al., 2015; Rayan et al., 2017). To overcome these drawbacks, researchers have turned their attention to natural resources. Various alkaloids, flavonoids, terpenoids, polysaccharides, saponins, and other natural substances are known to have anticancer activities by modulating immune function, inducing autophagy, or inhibiting cell proliferation (Rayan et al., 2017). Ginger (*Zingiber officinale Roscoe*), a member of the Zingiberaceae family and genus *Zingiber*, has a long history of use as a culinary spice and herbal medicine. Rich in bioactive compounds, ginger contains phenolic compounds such as gingerols, shogaols, and paradols, with 6-gingerol, 8-gingerol, 10-gingerol, and terpene compounds being the major polyphenols found in fresh ginger. Recent studies have revealed a range of biological effects exhibited by these bioactive compounds, including antioxidant, anti-inflammatory, antimicrobial, and intriguingly, anticancer properties. Numerous studies have demonstrated the anticancer effects of 6-gingerol in various human cancers, including leukemia, breast, colon, pancreatic, prostate, and liver (Wang et al.,2014; Zhang et al., 2017; Mao et al., 2019). In particular, the hydroxyl group-containing aliphatic chain moiety a in 6-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) (Figure 1) has been linked to its biological activities (Wang et al.,2014).

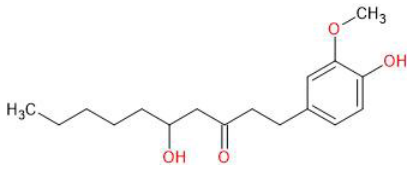


Figure 1. Structure of 6-gingerol

Kinases are cellular intermediaries that facilitate the phosphorylation process by transferring the gamma-phosphate group from an ATP molecule to the serine (Ser), threonine (Thr), or tyrosine (Tyr) residues in target molecules. They play a pivotal role in regulating cellular signaling pathways by controlling a wide range of cellular functions such as transcription, metabolism, nervous system activity, immune response, cell division, and migration (Modi et al., 2019; Schwarz et al., 2019; Wang et al., 2021). Dysregulation and mutations of protein kinases have been associated with a range of human diseases including cancer, diabetes, autoimmune disorders, cardiovascular diseases, inflammatory conditions, and neurological disorders. As a result, protein kinases have emerged as key therapeutic targets, particularly in cancer treatment (Roskoski, 2016).

In silico target fishing is widely used in drug discovery to discover the most probable biological targets of a given molecule (Galati et al., 2021). This approach allows us to anticipate the biological activity of a given molecule in question or its mechanism of action. Additionally, target fishing can be useful for predicting side effects, revealing multiple effects of a drug, and uncovering new applications for existing drugs (Cereto-Massagué et al., 2015). A commonly used similarity approach is the target fishing strategy, which suggests that molecules with similar structural patterns may exhibit comparable bioactivities and, thus, interact with similar targets (Galati et al., 2021).

In light of existing research, this study aims to identify novel lead compounds that are similar to 6-gingerol and exhibit potent anticancer activity, while also demonstrating high bioavailability and a low toxicity profile. For this purpose, an *in silico* screening was performed using ChemMine Tools based on PubChem fingerprints, to search for compounds with structural similarity to 6-gingerol. To investigate the anticancer potential of 6-gingerol and the identified similar compounds, they were screened using *in silico* target fishing servers, focusing on their putative

kinase targets. Selected kinase targets were then used in docking studies to assess potential interactions with the compound dataset. Furthermore, computational assessments were performed to determine the pharmacokinetics, drug-likeness, and toxicity profiles of these compounds, with the goal of highlighting the safety and bioavailability of both 6-gingerol and its structurally similar compounds. The results of this computational study include the discovery of putative kinase targets of 6-gingerol-like molecules and potential lead compounds for further development.

MATERIAL AND METHOD

Compounds Studied

The primary focus of this study was on 6-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone), a known active anti-tumor compound. In addition to 6-gingerol, compounds with chemical structures similar to 6-gingerol were identified for further investigation. The selection of structurally similar compounds was based on a predetermined cut-off value. For the similarity search, Tanimoto score calculations were performed using PubChem fingerprints in ChemMine Tools (https://chemminetools.ucr.edu/eisearch/query/) (Backman et al., 2011).

In silico Target Fishing

The biological activities of 6-gingerol were predicted using the PASSOnline, Swiss Target Prediction, MolTarPred, and SEA web servers (Keiser et al., 2007; Filimonov et al., 2014; Peón et al., 2017; Daina et al., 2019). These servers utilize fingerprint similarity to known bioactive compounds to generate a list of potential biological targets for the molecules under investigation. The first tool used in this study was PASSOnline, which can be accessed at (http://www.way2drug.com/PASSOnline/predict.php). The SMILES format of 6-gingerol was submitted to the PASSOnline server, and the results were subsequently evaluated. This tool provides estimates with an average accuracy of 95% for approximately 4,000 different types of biological activity. Compound activity prediction is based on structure-activity relationship analysis of a training set of over 300,000 compounds (Filimonov et al., 2014). The PASSOnline output provides a list of expected activity types, along with estimated probabilities expressed as probable activity (Pa) and probable inactivity (Pi). Pa and Pi values range from 0 to 1. When with Pa> Pi were considered potential for a given compound. When Pa is greater than 0.7, there is a greater likelihood that the compound has novel pharmacological activity while being structurally similar to an existing drug. Pa values between 0.5 and 0.7 indicated a lower probability of experimental pharmacological activity. When Pa is less than 0.5, the likelihood of finding the compound's activity experimentally is low, suggesting its potential as a parent compound to study biological activity (Filimonov et al., 2014).

The second server used in this study was Swiss Target Prediction (http://www.swisstargetprediction.ch/). This server, which has been operational since 2014, employs ligand-based target prediction to identify the most likely protein targets for a given bioactive small molecule. Predictions were performed by assessing the 2D and 3D similarity between the input query and compounds with known *in vitro* activity data in the ChEMBL library (ChEMBL 23 is the version used). The 2D similarity measure used the Tanimoto index on FP2 (2D) with a threshold of 0.65. For 3D similarity, multiple conformations of the molecule were generated, and Manhattan distances were calculated for each conformation. Target scores were calculated using logistic regression with the 2D and 3D similarity scores of the most similar ligands. The server provided a list of up to 100 potential protein targets for the 6-gingerol compound, with target scores ranging from 0 to 1. The same approach was applied to its structurally similar compounds (Daina et al., 2019).

The third method used in this screening was the MolTarPred web server (http://moltarpred.marseille.inserm.fr/). It was used to predict protein

targets utilizing ChEMBL22 data. When 6-gingerol was submitted, the server determined the Tanimoto similarity between the Morgan fingerprint of the query molecule and each of the 607,659 ChEMBL compounds that will be tested. The predicted targets were obtained from the top 10 most similar molecules to the query. To claim that a drug candidate is among the 607,659 ChEMBL and has single-protein targets annotated with activity equal to or less than 10 micromolar, the confidence score needed to be between 7.8 and 9. However, if the query contained more than one protein target with activity less than 7.8 micromolar, further prospective validation of these targets was required (Peón et al., 2017).

The Similarity Ensemble Approach (SEA) server was the fourth tool used to select the putative biological target for the query (https://sea.bkslab.org/). This server employs a 2D ligand-based similarity ensemble approach to predict the biological targets of a compound based on its resemblance to ligands found in a reference database, such as ChEMBL. The comparison of the input ligands (6-gingerol and related compounds) was performed against all ligands within the target sets, using the Tanimoto similarity of ECFP4 fingerprints. The Tanimoto similarities were summed, and z-scores were calculated for each ligand. This tool has been successfully applied to identify new targets for old drugs and natural products, and to predict side effects and potential anatomical therapeutic indications (ATCs) of approved drugs (Keiser et al., 2006; Wang et al., 2016; Irwin et al., 2018). In this study, the query (6-gingerol) and its similar molecules were screened using these *in silico* approaches, with the corresponding SMILES serving as the query input. The resulting list of predicted targets was downloaded and further analyzed manually, with a particular focus on kinase targets.

Molecular Docking

To prepare the compounds for molecular docking, ionization and protonation were performed based on physiological pH. The compounds were subjected to conformational search in the Molecular Operating Environment (MOE) v2019.01 (MOE Release V2019.01; ULC) software from Chemical Computing Group ULC, Montreal, QC, Canada was employed for conformational search using the Stochastic Conformational Search module. The global minimum energy of each molecule was minimized using the MMFF94x force field. The structure of the proteins [V-Raf Murine Sarcoma Viral Oncogene Homolog B1 (BRAF) active and inactive conformation PDB ID: 3OG7, 6N0Q, Janus Tyrosine Kinase 1 (JAK1) active conformation PDB ID: 3EYG, Janus Tyrosine Kinase 2 (JAK2) active and inactive conformation PDB ID: 2B7A, 3UGC, Extracellular Signal-Regulated Kinase 1 (ERK1) PBD ID: 2ZOQ, human p38 mitogen-activated protein kinase p38γ (MAPK) active and inactive conformation PDB ID:1CM8, PDB ID:6UNA] were downloaded from Protein Data Bank (https://www.rcsb.org/) (Berman et al., 2000). Unnecessary chains, ions, water molecules, and solvents were removed, and hydrogen atoms and partial charges were added to the protein structures. Missing loops and side chains were also fixed. Each conformation of the compounds was docked into the structures using MOE with London dG rescoring and triangle matcher placement settings (MOE Release V2019.01; ULC).

The Pharmacokinetic Properties and Drug-likeness

The *in silico* evaluation of pharmacokinetic properties and drug-likeness, including absorption, distribution, metabolism, and excretion (ADME) properties, was conducted for 6-gingerol and similar molecules. The SwissADME web server was used to assess these properties (http://www.swissadme.ch/) (Daina et al., 2017). Additionally, the excretion properties of the compounds were predicted by using the pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/prediction) (Pires et al., 2015). The user-friendly web interface allows the input of structures in SMILES format and after submission, the results

were presented in an output table. Drug-likeness is a crucial concept in the screening of drug candidates in the early stages of drug discovery, with a focus on achieving oral bioavailability. Lipinski's Rule of Five (Ro5) is a widely used criterion for evaluating the drug-like properties of molecules. According to this rule, a molecule may exhibit poor absorption or permeation if it violates more than two of the following criteria, it: molecular weight (MW) < 500, number of hydrogen bond donors (HBD) < 5 (counting the sum of all NH and OH groups), octanol/water partition coefficient Log P < 5, number of hydrogen bond acceptors (HBA) < 10 (counting all N and O atoms) (Bickerton et al., 2012; Leeson et al., 2015; Daina et al., 2017). In addition, there were two other descriptors were identified by Veber *et al.* (Veber et al., 2002): Number of Rotatable Bonds (NRB) <10 and Polar Surface Area (PSA) <140 $Å^2$. Furthermore, the ability of the investigated compounds to cross the blood-brain barrier (BBB) was also evaluated as a distribution parameter (Daina et al., 2017).

Cytochrome P450 (CYP450) enzymes, particularly the major isoforms CYP1A2, 2C9, 2C19, 2D6, and 3A4, play a significant role in the metabolic biotransformation and elimination of drugs. Inhibition of these isoenzymes can lead to toxic or adverse effects due to drug-drug interactions (Daina et al., 2017). Drug clearance is measured by the proportionality constant (CLtot) and expressed as a log(mL/min/kg). It is related to bioavailability and is important in determining dosing rates to achieve steady-state concentrations. The Organic Cation Transporter 2 (OCT2) was also determined to determine whether the compounds were substrates for it or not. It plays an essential role in the clearance of drugs and endogenous substances, and was evaluated by using the pkCSM server (Pires et al., 2015).

Toxicity Assessment

Toxicity prediction is a critical aspect of the drug development process, as many drugs fail to reach the market due to ineffectiveness or adverse effects. Computational approaches have been widely used to assess the potential cytotoxicity and adverse effects of chemical structures on both normal and tumor cells, as well as general organ toxicity. These approaches help to identify structures that can improve efficacy and therapeutic success (Capuzzi et al., 2016). In this study, the drug cytotoxicity on tumor and non-tumor cells was computationally screened using PASS CLC Pred (http://www.way2drug.com/cell-line/index.php) (Lagunin et al., 2018). Adverse effects commonly associated with anticancer drugs, such as hepatotoxicity and cardiotoxicity, were evaluated. Hepatotoxicity and hERG I-II inhibitory activities of the compounds were predicted by using pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) (Pires et al., 2015). Hepatotoxicity prediction was also performed using the ProTox-II program (https://tox-new.charite.de/protox_II/index.php?site=compound_input) (Banerjee et al., 2018).

Furthermore, the investigated compounds were evaluated for mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity. The ProTox-II program was used to predict their effects on stress response pathways, specifically mitochondrial membrane potential (MMP) and phosphoprotein tumor suppressor (p53) (Banerjee et al., 2018). The SMILES structures of the compounds were used to predict the aforementioned toxicity endpoints.

RESULTS AND DISCUSSION

Similarity Search

A similarity search was performed using the Chemmine server to identify chemical structures similar to the query compound, 6-gingerol. The similarity cut-off value was set at 0.80, resulting in 49 compounds, including 6-gingerol (Table S1). All of these similar compounds are originated from natural sources and exhibit diverse biological activities. Here are some examples of the activity of the investigated compounds.

Calycosin (N-G-12) is a bioactive phytoestrogenic isoflavone that is isolated from *Trifolium pratense*

(red clover), which is able to suppress carcinogenesis and progression by modulating estrogen receptor (ER)β expression. Furthermore, the antiproliferative effects of calycosin were found to be mediated through ER ß-dependent regulation of the The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathways in human osteosarcoma cell line (MG-63) (Tian et al., 2020). While Curcumin (N-G-5), Demethoxycurcumin (N-G-24), and Tetrahydrocurcumin (N-G-41) are bioactive non-flavonoid polyphenolic compounds extracted from the rhizome of the plant *Curcuma longa* and found in the spice turmeric. Many studies have been conducted to assess the effects of curcumin metabolites, analogs, or derivatives. Curcumin or its metabolite tetrahydrocurcumin has been shown to promote cell death in chemotherapy-resistant (Ara-C) human AML cells (HL-60) using two different methods. Indeed, curcumin mainly induced apoptotic cell death mainly via the regulation of poly(ADP-ribose) polymerase (PARP), caspase-9, and caspase-3, whereas tetrahydrocurcumin primarily promoted autophagy mainly via the regulation of LC3 and p62. Tetrahydrocurcumin has also been used in human NSCLC A549 cells. The therapy suppressed cell growth and induced autophagic flux by suppressing the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway and increasing Beclin 1 gene expression (Benvenuto et al., 2020). Additionally, pratensein (N-G-39) is a member of the flavonoid family that is found in many plants such as *Trifolium pratense.* The anticancer activity of pratensein was recently demonstrated in breast cancer cells. The result of the study showed that pratensein can induce caspase-3 and Bcl-2-associated X protein (Bax) and increase the expression level of p53. It also inhibits B-cell lymphoma 2 (Bcl-2) activation in a time-dependent manner (Behbahani et al., 2020). In addition, vanillyl alcohol (N-G-18) is one of the phenolic compounds that can be found in a variety of plants that can be produced enzymatically from the reduction of vanillin. A previous study has shown the anti-angiogenic activity of vanillyl alcohol by using the chick chorioallantoic membrane (CAM) assay. The results showed that vanillyl alcohol has a significant anti-angiogenic effect due to the decrease in membrane transport, possibly due to its methoxy group at the 3-position (Jung et al., 2008). Eugenol (N-G-3) is the active component of the essential oil isolated from clove (*Syzygium aromaticum*) and has antimutagenic, antigenotoxic, and anti-inflammatory properties. It also has cytotoxic activity and can induce cell death in various cancer cells (Carvalho et al., 2015). Methyleugenol (N-G-6), on the other hand, is a substituted allylbenzene that can be found in many foods and essential oils. It has been shown to induce cytotoxicity in rat and mouse hepatocytes and leukemia. Methyleugenol has also been reported to enhance the inhibition of cancer cell growth and induce apoptosis when it was combined with cis-platin (Carvalho et al., 2015; Yi et al., 2015).

Licochalcone B (Lico B) (N-G-38), which shares a similar structure to 6-gingerol, is a recently synthesized molecule belonging to the chalcone group extracted from licorice root, also known as *Glycyrrhiza* root. Chalcone groups serve as precursors to flavonoids. Numerous *in vitro* and *in vivo* studies have demonstrated the therapeutic effects of Lico B, particularly its anti-inflammatory, anticancer, antioxidant, and hepatoprotective properties. In a 2016 *in vitro* study, Lico B was found to significantly inhibit cell proliferation in oral squamous cell carcinoma (OSCC) cells, depending on the duration and concentration of treatment. Treatment with Lico B up-regulated pro-apoptotic proteins such as Bax and down-regulated anti-apoptotic proteins such as BH3 domain-only death agonist protein and B-cell lymphoma-extra large, while promoting myeloid leukemia cell differentiation protein Mcl-1 (Bax). Lico B induced apoptosis through the release of cytochrome C and loss of mitochondrial membrane potential. In addition, these studies provided evidence for the activation of multiple caspases and the cleavage of the PARP protein following Lico B therapy. Based on these findings, Lico B is considered a promising natural drug for the treatment of human oral cancer

through its induction of apoptosis (Maria Pia et al., 2019).

The Putative Anticancer Target of the Molecules

To identify the most promising target for 6-gingerol and its related compounds, a comprehensive screening process was conducted. First, the anticancer activities of 6-gingerol were evaluated using four different web servers. Subsequently, the potential targets of small molecules sharing a 6-gingerol scaffold were identified by screening the compounds through various web servers, namely PASS Online, Swiss Target Prediction, MolTarPred, and SEA. Each server employs different algorithms to predict potential targets. The results from each server are presented in Tables S3, S4, S5, and S6. Based on the methods used and the results of these computational analyses, the most notable anticancer activities were identified. The corresponding results are presented in Table S2. Given the data collected, our focus for the subsequent docking studies was primarily centered around tyrosine kinases, including B-RAF, JAK1/2, ERK1 (MAPK3), and p38γ. These kinases have been strongly implicated in a variety of diseases, particularly cancer, which is the main focus of our study.

Evaluation of Docking on the Selected Targets with the Studied Molecules

To identify the most promising targets for 6-gingerol and related compounds, a comprehensive screening process was conducted. The anticancer activities of 6-gingerol were first evaluated using various web servers, and the potential targets of similar compounds were identified through screening using various algorithms. The focus was primarily on tyrosine kinases, including B-RAF, JAK1/2, ERK1, and p38γ, which are strongly associated with cancer.

The majority of approved protein kinase inhibitors work by interacting with the ATP-binding pocket and acting as competitive enzyme antagonists. In particular, the interaction with the hinge region is critical for ATP binding and substrate phosphorylation. Successful kinase inhibitors have been developed based on this strategy (Roskoski, 2016; Wang et al., 2021). Most BRAF inhibitors also interact with the ATP-binding and hinge regions, known as type I inhibition (Roskoski, 2016; Wang et al., 2017; Umar et al., 2020). Docking studies on the BRAF protein with the X-ray data of 3OG7 showed that the co-crystallized ligand (PLX4032) formed two hydrogen bonds with Cys532 and Gln530, in the hinge region. Vanylglycol (N-G-42) and N-G-1 were identified as potential type I inhibitors of BRAF kinase by forming two hydrogen bonds with the specific hinge region residues with docking scores: -5.341 and -7.585, respectively (Figure 2).

Selective BRAF inhibitors that target the RAF-MEK-ERK pathway without inducing paradoxical activation are desired. Interaction with the DFG loop enhances inhibitor potency, known as type II inhibition (Ramurthy et al., 2020). Docking studies on inactive BRAF revealed that the conformation of the co-crystallized ligand [N-(4-methyl-3-(1-methyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)phenyl)-3-(trifluoromethyl)benzamide] formed hydrogen bonds with specific residues Phe595, Asp594, and αC-Glu501. L-(+)-vanilmandelic acid (N-G-44) showed potential as a selective type II inhibitor of BRAF with a docking score of - 5.5003 (Figure 2).

In general, most of the studied compounds studied with the active conformation of the BRAF protein by forming hydrogen bonds with hinge residues. The side chain hydroxyl group of certain compounds (N-G-12, 17, 26, 31, 38, 39, 40, 42, and 47) played an important role in interacting with the BRAF receptor (Figure S1).

Overall, the screening and docking studies identified potential targets and modes of inhibition for 6-gingerol and its related compounds, with a particular on kinase proteins.

(2a) (2b)

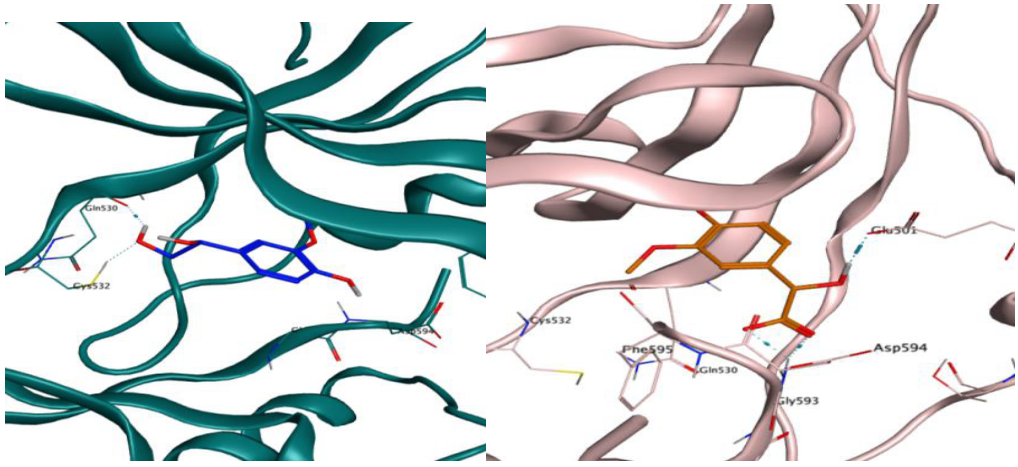


Figure 2. (a) Structure of BRAF^{V600E}: PDB ID: 3OG7 complex with: vanylglycol (N-G-42) and (b) Structure of BRAF: PDB ID: 6N0Q complex with: L- (+)-vanilmandelic acid (N-G-44)

JAK proteins are non-receptor tyrosine kinases that play critical roles in cytokine and growth factor signaling pathways. Dysregulation of the JAK-STAT signaling cascade may contribute to various immunological disorders and malignancies (Alicea-Velázquez et al., 2011; Sanachai et al., 2020). JAK1, JAK2, and JAK3 are frequently found in cancer cells, with JAK1 and JAK2 being particularly abundant, making them promising targets for anticancer agents (Lucet et al., 2006; Hornakova et al., 2011; Andraos et al., 2012; Sanachai et al., 2020). Most JAK inhibitors act as type I inhibitors, enhancing phosphorylation in the JAK activation loop while inhibiting kinase activity and phosphorylation of STAT proteins (Andraos et al., 2012).

Binding to JAK1 in its active state involves interactions with Glu957 and Leu959 residues in the hinge region (Williams et al., 2009; Lin et al., 2018). Docking studies on JAK1 demonstrated that the co-crystallized ligand (CP-690-550) formed hydrogen bonds with Glu957 and Leu959. When 6-gingerol and its similar molecules were docked into the active conformation of JAK1, paradol (N-G-30) formed a hydrogen bond with the carbonyl backbone of Leu959, indicating its potential as an inhibitor of JAK1 with a docking score of - 6.9492 (Figure 3).

Similarly, to achieve good binding to JAK2 in its active state, the inhibitor should interact with Glu930 and Leu932 residues in the hinge region. Docking studies on JAK2 revealed that the co-crystallized ligand (CMP6) formed hydrogen bonds with Glu930 and Leu932 (Lucet et al., 2006). In the case of dihydroconiferyl aldehyde (N-G-43), docking simulations showed that it formed two hydrogen bonds with the carbonyl backbone and the nitrogen backbone of Leu932, indicating its potential as a JAK2 inhibitor with a docking score of -5.478 (Figure 3).

Based on these findings, paradol (N-G-30) showed potential as a JAK1 inhibitor, while dihydroconiferyl aldehyde (N-G-43) showed potential as a JAK2 inhibitor. These results suggest that 6-gingerol and its related compounds have the potential to inhibit JAK proteins and may serve as promising agents in the development of anticancer treatments targeting the JAK-STAT signaling pathway.

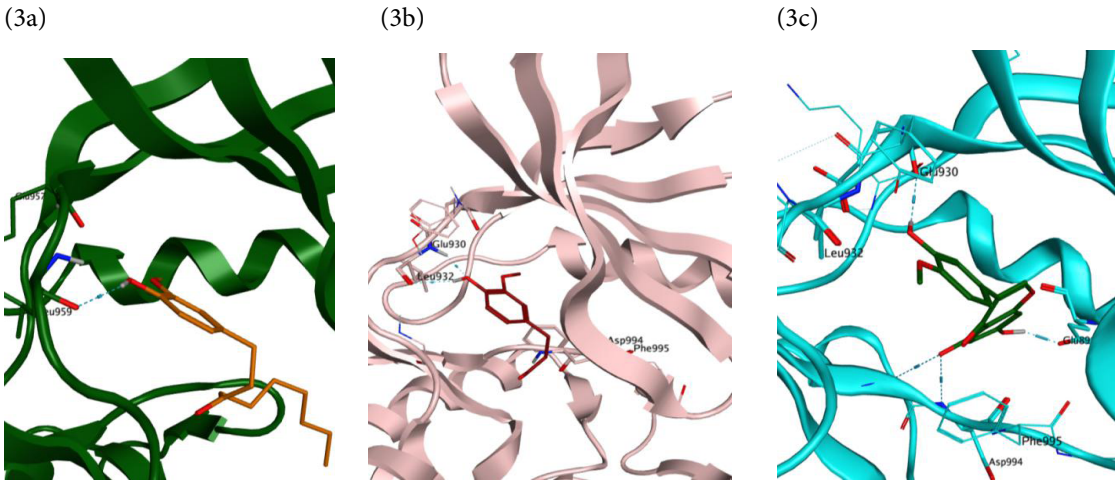


Figure 3. (a) Structure of JAK1: PDB ID-3EYG complex with paradol (N-G-30), (b) structure of JAK2: PDB ID-2B7A complex with dihydroconiferyl aldehyde (N-G-43), and (c) structure of JAK2: PDB ID: 3UGC complex with pratensein (N-G-39)

The development of a selective JAK2 inhibitor has been challenging due to the conserved ATP site of the JAK family. However, efforts are underway to discover selective JAK2 inhibitors to minimize undesired side effects (Lin et al., 2018). Inhibition of JAK2 activity can lead to several alterations in myeloid cell function. Binding to the inactive conformation of the JAK protein, known as DFG-out, reduces phosphorylation in the activation loop. Critical residues, including Leu932, αC-Glu898, and Asp994, play a pivotal role in achieving selectivity towards JAK2. Docking studies on JAK2 using X-ray data revealed that the co-crystallized ligand (NVP-BBT494) formed hydrogen bonds with Leu932, Asp994, and αC-Glu898 (Andraos et al., 2012; Lin et al., 2018). Pratensein (N-G-39) formed hydrogen bonds with Glu898, Glu930, and Asp994, indicating its potential as a selective JAK2 inhibitor with a docking score of -6.5816 (Figure 3).

For both JAK1 and JAK2, most of the compounds interacted with the active conformation of the proteins. The phenol group of the compounds formed hydrogen bonds with hinge region residues, predominantly Leu959 for JAK1 and Glu930 and Leu932 for JAK2. The phenolic hydroxyls of the compounds played an important role in the interaction with the receptors (Figure S2 and S3).

Mitogen-activated protein kinases (MAP kinases), such as ERK1 and ERK2, regulate cell proliferation, differentiation, and various physiological responses. Dysregulation of ERK signaling has been implicated in several cancers (Halder et al., 2019; Pegram et al., 2019). Most inhibitors targeting ERK1/2 exhibit a type I binding mode. Co-crystal structures of ERK1/2 5-iodotubericidin (5ID) have revealed a type I binding mode with hydrogen bonds formed with hinge residues (Met125 and Asp123) and Lys131 (Chaikuad et al., 2014). Docking studies on ERK1 showed that isoferulic acid (N-G-47) formed hydrogen bonds with Met125 and Lys131, indicating its potential as an ERK1 inhibitor with a docking score of -5.413 (Figure 4). The phenol group of 6-gingerol also formed a hydrogen bond with Met125. Several other compounds exhibited hydrogen bonding interactions with Met125 and Lys131 through their phenol and carbonyl groups or phenolic hydroxyl group and carboxylate group (Figure S4).

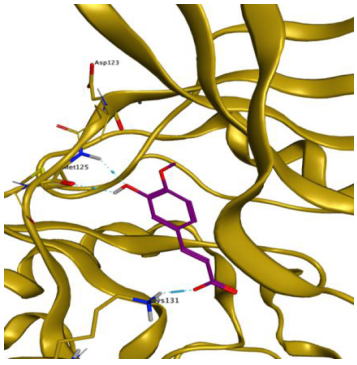


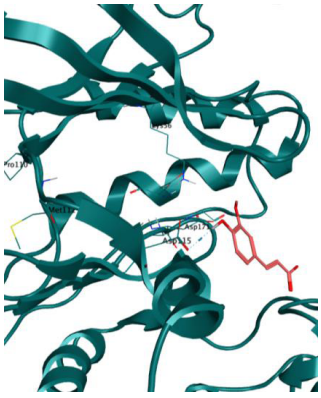
Figure 4. Structure of ERK1: PDB ID: 2ZOQ complex with isoferulic acid (N-G-47)

The p38 mitogen-activated protein kinase (MAPK) family consists of four isoforms: p38α, p38β, p38γ, and p38δ. These isoforms are activated by dual phosphorylation of tyrosine and threonine residues in a Thr-X-Tyr motif. The p38 MAPK pathway is involved in the regulation of the inflammatory response and may contribute to tumorigenesis. Elevated levels of p38γ have been observed in cutaneous T-cell lymphoma (CTCL) and various malignancies, making it a potential therapeutic target (Sahu et al., 2019; Zhang et al., 2021).

Docking studies were performed on both the active and inactive conformations of p38γ to understand how the compounds under study interact with the protein. X-ray data from 1CM8, using the non-hydrolyzable ATP-like inhibitor AMP-PNP, revealed two hydrogen bonds with the hinge residues (Met112 and Pro110) (Bellon et al., 1999). However, the compounds in this study did not interact with the hinge region. Instead, they showed interactions with residues located outside of the ATP pocket (Figure 5).

No co-crystallized ligands were available for the inactive conformation of p38, represented by the X-ray data of 6UNA. The binding pocket was identified based on the active conformation of p38, particularly involving αC-Glu74 and Asp171 of the DFG region (Bellon et al., 1999; Aoto et al., 2019). Docking studies on 6UNA showed that most of the compounds interacted with the hinge region or with Asp171 of the DFG region. For example, Curcumin PE (N-G-40) formed hydrogen bonds with the backbone of Met112, Phe111, Asp115, and αC-Glu74, indicating its potential as an inhibitor with a docking score of -5.979 (Figure 5). More information about the docking scores of the 6-gingerol-like series and the co-crystallized ligands is presented in (Table S7).

(5a)



(5b)

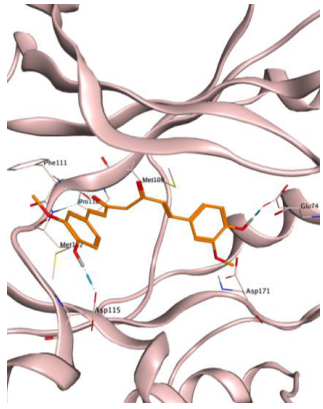


Figure 5. (a) Structure of p38γ: PDB ID: 1CM8 complex with Ferulic acid (N-G-2) and (b) structure of p38γ: PDB ID: 6UNA complex with curcumin PE (N-G-40)

Prediction of Drug-likeness and Pharmacokinetics Properties of the Molecules

To assess the drug-likeness and pharmacokinetic properties of the molecules, an analysis was conducted to ensure their potential as drugs. For this purpose, the SwissADME online tool, designed for easy submission and analysis of the results, was used to predict the drug-likeness and ADME properties. The results of drug-likeness and ADME properties are presented in Tables S8, S9, and S10, respectively. The evaluation showed that 6-gingerol and the similar molecules investigated in this study met Lipinski's and Veber's rules of five. This suggests that these molecules possess favorable oral bioavailability based on their physicochemical properties. These parameters are associated with desirable aqueous solubility and intestinal permeability, which are crucial initial factors in determining oral bioavailability. Furthermore, the bioavailability score (BA) was assessed, revealing that most of the molecules studied obtained a value of 0.55. This score indicates compliance with the Rule of Five and predicts a 55% bioavailability in rats, indicating a higher probability compared to the 10% threshold (Martin, 2005; Daina et al., 2017). Notably, N-G (2, 22, 46, 47, and 48) showed a BA value of 0.85. The synthetic accessibility was also evaluated using a scale ranging from 1 (easy to synthesize) to 10 (very difficult and complex to synthesize) (Daina et al., 2017). The synthetic accessibility of all the studied molecules falls within the range of 1-4, indicating that they are relatively easy to synthesize (Table S8).

The ADME properties of 6-gingerol and similar compounds are summarized in Tables S8 and S9. These compounds exhibit high gastrointestinal (GI) absorption, consistent with the Rule of Five (Ro5). P-glycoprotein (P-gp), a transmembrane efflux pump involved in drug transport, regulates the uptake and efflux of various drugs and toxins (Finch et al., 2014; Leeson et al., 2015). Our results showed that the molecules N-G-(16, 27, and 31) are the only substrates for P-gp among the molecules studied, while the others are not substrates for P-gp. Furthermore, the blood-brain barrier (BBB) controls cerebral homeostasis and provides the central nervous system (CNS) with a unique protection against the toxicity of many xenobiotics and pathogens (Weiss et al., 2009). Analysis of the molecules in this study indicates that the N-G (5, 9, 12, 14, 17, 22, 24, 28, 31, 38- 42, 44, and 46) lack the ability to cross the BBB, while the remaining compounds studied possess BBB permeability.

The results presented in Tables S8 and S9 are consistent with the BOILED-Egg (Brain Or IntestinaL EstimateD permeation predictive model) shown in Figure 6. The BOILED-Egg model predicts the behavior of small molecules based on their polarity and lipophilicity, using an egg visualization. Molecules located in the yolk of the egg are expected to pass through the BBB, while those in the white are expected to be absorbed by the human gastrointestinal tract (HIA) (Daina et al., 2016). Blue-white circles indicate molecules predicted to be effluxed from the CNS by P-glycoprotein (PGP+), while red-white circles represent molecules not expected to be effluxed by P-glycoprotein (PGP-). As depicted in Figure 6, most of the compounds, except those mentioned above, are located within the yolk of the egg, suggesting their permeability across the BBB. However, 6-gingerol and its similar compounds are predicted to be absorbed from the gastrointestinal tract.

Furthermore, our results indicate that most of the compounds do not inhibit CYP2C19, except for N-G-15, which exhibits inhibitory effects on CYP2C19. Among the compounds studied, N-G-(5, 12, 17, 24, 27, 32, 38- 41) specifically affects CYP3A4, while the remaining molecules do not inhibit this isoform. Only N-G-(1, 12, 15, 16, 17, 27, 30, 31, 32, 39, and 41) exhibit inhibition against CYP2D6, while the other compounds do not affect this isoform. Regarding CYP2C9, compounds N-G-(5, 24, 32, 38- 40) show inhibitory activity, while the remaining compounds do not impact this isoform. Moreover, the compounds

N-G-(1, 3, 6, 7, 11- 13, 15, 19, 23, 24, 29, 30, 33, 37-39, and 45) inhibit the CYP1A2 isoform, while the other compounds do not exhibit any effect on this isoform. Overall, the compounds N-G-(5, 12, 17, 24, 27, 32, 38- 40, and 41) show inhibitory activity against multiple CYP isoforms.

Regarding excretion, clearance was determined by calculating the sum of hepatic and renal clearance, which represents the rate at which a drug is eliminated from the body. A low total clearance value indicates a longer persistence of the drug in the body. In our study, most of the compounds exhibited low clearance rates. However, the compounds N-G-(1, 2, 4, 8- 10, 15, 16, 20, 30, 34, 35, 38, 46, 47, and 49) exhibited high clearance values (Table S10). The organic cation transporter 2 (OCT2), which is responsible for renal uptake, plays a critical role in the clearance of drugs and endogenous substances. When substances are OCT2 substrates, there is a possibility of adverse reactions when co-administered with OCT2 inhibitors (Pires et al., 2015). It is noteworthy that none of the compounds studied in this research were identified as OCT2 substrates. Therefore, it is unlikely that these compounds would interact or cause adverse effects when used with OCT2 inhibitors.

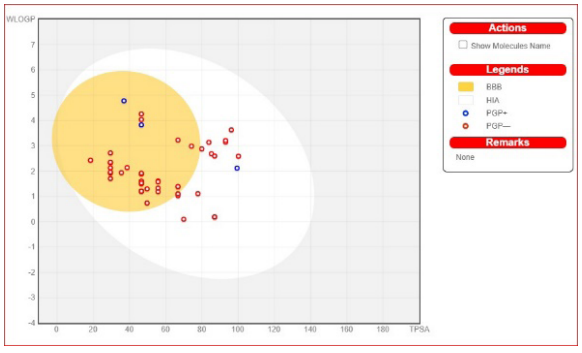


Figure 6. BOILED-Egg representation of the studied molecules

Prediction of the Toxicity Profiles of the Molecules

The toxicity profiles of the compounds were predicted using various computational tools, and the results are summarized in (Table S11, S12, and S13). Cytotoxicity predictions (Way2Drug) on tumor and non-tumor cell lines revealed that 21 compounds showed activity against various tumor cell lines from 8 different tissues. Among them, coniferaldehyde exhibited the highest predicted activity against 6 cell lines. Vanylglycol demonstrated the highest predicted activity (0.747) against PC-3 cells. However, the majority of the compounds did not exhibit significant effects on non-tumor cells when their probability was above 0.50, and only 6 compounds showed negative effects on non-tumor cells. This lack of activity was consistent with the predictions from ProTox-II (Table S12), which indicated that most of the compounds were expected to be safe with low probabilities of toxicity.

Hepatotoxicity predictions made by ProTox-II suggested that most of the compounds were unlikely to cause liver toxicity (Table S12). The compounds predicted to have positive effects had low probabilities, ranging from 50% to 52%. Furthermore, the toxicity effects of hepatotoxicity, hERG I inhibitor, and hERG II inhibitor were assessed using pkCSM (Table S13). Most of the compounds showed no significant effects on hepatotoxicity, hERG I inhibitor, and hERG II inhibitor.

Regarding the predictions of mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity by ProTox-II, not all compounds were expected to show mutagenic or cytotoxic effects. Only two compounds had low probabilities of a positive mutagenicity result, ranging from 53% to 67%. Immunotoxicity predictions revealed that 17 compounds, including the query compound, had a high probability of affecting the immune system (79% to 98%). For carcinogenicity, methyleugenol exhibited the highest potential to induce tumors, with a probability of 76%. Most of the compounds showed no toxicity to MMP and p53. However, seven compounds were predicted to have positive effects on MMP with probabilities ranging from 78% to 100%, and two compounds were predicted to have positive effects on p53 with

probabilities from 80% to 100%. Notably, curcumin showed a toxic effect on MMP and p53 with a high probability of 100%.

CONCLUSION

In this study, we used virtual screening to identify potential leads and molecular targets for natural chemical compounds, with a particular focus on 6-gingerol and its similar compounds. Through target prediction servers, we discovered anticancer targets, with a particular focus on kinase proteins due to their role in cellular signaling pathways (Roskoski, 2016; Wang et al., 2021).

Our findings showed that the compounds interacted with various kinases, including B-RAF, JAK1/2, ERK1, and p38γ. These interactions were determined by through molecular docking studies using MOE. The majority of the compounds acted as type I inhibitors, forming hydrogen bonds with specific residues in the hinge region of the active conformation of the target proteins.

The drug-like characteristics and ADMET properties of the compounds were also evaluated. They complied with Lipinski's and Veber's rule of five, indicating good oral bioavailability and absorption. However, some compounds inhibited multiple CYP isoforms, while most showed low clearance. None of the studied molecules were substrates for OTC2.

Toxicity assessments were conducted using computational approaches. Most compounds did not show cytotoxicity to normal cells, but several demonstrated toxicity to cancer cells. Coniferaldehyde, Vanylglycol, and L-(+)-vanilmandelic acid exhibited the highest potency on various cell lines. Each compound showed potent inhibitory activity against specific kinases. With respect to organ toxicity, the compounds did not show hepatotoxicity or hERG inhibitory effects on the liver and heart. None of the compounds exhibited mutagenicity or carcinogenicity, except for two with a low probability of positive mutagenicity. A few compounds had a high probability of affecting the immune system. Additionally, the majority of compounds were predicted to be inactive with respect to the MMP and p53 stress response pathways, indicating safe use for non-cancerous cells.

In conclusion, our computational approach identified the potential anticancer targets for 6-gingerol and its similar molecules including vanylglycol, vanillylmandelic acid, L-(+)-vanilmandelic acid, dehydrozingerone, 2-methoxyestrone, methylvanillate, dihydroconiferyl aldehyde, pratensein, acetovanillone, acetosyringone, licochalcone B, isoferulic acid, curcumin PE, and coniferaldehyde that could inhibit (B-RAF, JAK1/2, and ERK1 (MAPK3)) proteins primarily in their active state while they could modulate p38γ primarily in its inactive state. In addition, our computational analysis showed that the majority of the compounds have good drug-likeness and ADMET profiles without toxicity. In the future, *in vitro* enzymatic assays are planned to validate the computational results and optimize the lead compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS STATEMENT

Author contribution: Enise Ece Gurdal and Gulcin Tugcu contributed equally. Enise Ece Gurdal and Gulcin Tugcu designed the experiments. Nour Osama Al-Massri, Enise Ece Gurdal and Gulcin Tugcu performed computational studies. All authors contributed to the data analyses and writing of the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Alicea-Velázquez, N.L., & Boggon, T.J. (2011). The Use of Structural Biology in Janus Kinase Targeted Drug Discovery. *Current Drug Targets*, *12*(4), 546-555. doi: 10.2174/138945011794751528

Andraos, R., Qian, Z., Bonenfant, D., Rubert, J., Vangrevelinghe, E., Scheufler, C., Marque, F., Régnier, C. H., De Pover, A., Ryckelynck, H., Bhagwat, N., Koppikar, P., Goel, A., Wyder, L., Tavares, G., Baffert, F., Pissot-Soldermann, C., Manley, P. W., Gaul, C., Voshol, H., & Radimerski, T. (2012). Modulation of Activation-Loop Phosphorylation by JAK Inhibitors Is Binding Mode Dependent. *Cancer Discovery, 2*(6), 512-523. doi: 10.1158/2159-8290.CD-11-0324

Aoto, P.C., Stanfield, R.L., Wilson, I.A., Dyson, H.J., & Wright, P.E. (2019). A Dynamic Switch in Inactive p38γ Leads to an Excited State on the Pathway to an Active Kinase. *Biochemistry, 58* (51), 5160-5172. doi: 10.1021/acs.biochem.9b00932.

Backman, T.W., Cao, Y., & Girke, T. (2011). ChemMine tools: an online service for analyzing and clustering small molecules. *Nucleic Acids Research, 39* (Web Server issue), W486- W491. doi: 10.1093/nar/gkr320

Banerjee, P., Eckert, A.O., Schrey, A.K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research, 46* (W1), W257- W263. doi: 10.1093/nar/gky318

Behbahani, M., & Moghaddam, M. (2020). Study of Anticancer Activity of Pratensein and Pratensein Glycoside Isolated from Cuscuta kotchiana. *Journal of Molecular Biology Research, 10*(1), 73. doi: 10.5539/jmbr.v10n1p73

Bellon, S., Fitzgibbon, M.J., Fox, T., Hsiao, H.M., & Wilson, K.P. (1999). The structure of phosphorylated P38γ is monomeric and reveals a conserved activation-loop conformation, Structure. *Structure, 7*(9), 1057- 1065. doi: 10.1016/s0969 2126(99)80173-7.

Benvenuto, M., Albonici, L., Focaccetti, C., Ciuffa, S., Fazi, S., Cifaldi, L., Miele, M.T., De Maio, F., Tresoldi, I., Manzari, V., Modesti, A., Masuelli, L., & Bei, R. (2020). Polyphenol-Mediated Autophagy in Cancer: Evidence of In Vitro and In Vivo Studies. *International Journal of Molecular Sciences, 21*(18), 6635. doi: 10.3390/ijms21186635

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., & Bourne, P.E. (2000). The Protein Data Bank. Nucleic Acids Research, *28*(1), 235-242. doi: 10.1093/nar/28.1.235

Bickerton, G.R., Paolini, G.V., Besnard, J., Muresan, S., & Hopkins, A.L. (2012). Quantifying the chemical beauty of drugs. *Nature Chemistry, 4*(2), 90- 98. doi: 10.1038/nchem.1243

Capuzzi, S., Politi, R., Isayev, O., Farag, S., & Tropsha, A. (2016). QSAR Modeling of Tox21 Challenge Stress Response and Nuclear Receptor Signaling Toxicity Assays. *Frontiers in Environmental Science, 4*(3). doi: 10.3389/fenvs.2016.00003

Carvalho, A.A., Andrade, L.N., de Sousa, É.B., & de Sousa, D.P. (2015). Antitumor Phenylpropanoids Found in Essential Oils. *Biomed Research International, 2015*, 392674. doi: 10.1155/2015/392674.

Cereto-Massagué, A., Ojeda, M.J., Valls, C., Mulero, M., Pujadas, G., & Garcia-Vallve, S. (2015). Tools for in silico target fishing. *Methods, 71*, 98– 103. doi: 10.1016/j.ymeth.2014.09.006

Chaikuad, A., Tacconi, E.M.C., Zimmer, J., Liang, Y., Gray, N.S., Tarsounas, M., & Knapp, S. (2014). A unique inhibitor binding site in ERK1/2 is associated with slow binding kinetics. *Nature Chemical Biology, 10*, 853- 860. doi: 10.1038/nchembio.1629

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*, 42717. doi: 10.1038/srep42717

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Research, *47*(W1), W357– W364. doi: 10.1093/nar/gkz382

Daina, A., & Zoete, V. (2016). A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem, 11*(11), 1117- 1121. doi: 10.1002/cmdc.201600182

Filimonov, D.A., Lagunin, A.A., Gloriozova, T.A., Rudik, A.V., Druzhilovskii, D.S., Pogodin, P.V., & Poroikov, V.V. (2014). Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. Chemistry of Heterocyclic Compounds*, 50*(3), 444- 457. doi: 10.1007/s10593-014-1496-1

Finch, A., & Pillans, P. (2014). P-glycoprotein and its role in drug-drug interactions. Australian Prescriber, *37*(4), 137- 139. doi: 10.18773/austprescr.2014.050

Galati, S., Di Stefano, M., Martinelli, E., Poli, G., & Tuccinardi, T. (2021). Recent advances in in silico target fishing. *Molecules, 26* (17), 5124. doi: 10.3390/molecules26175124

Halder, A.K., Giri, A.K., & Cordeiro, M.N.D.S. (2019). Multi-Target Chemometric Modelling, Fragment Analysis and Virtual Screening with ERK Inhibitors as Potential Anticancer Agents. *Molecules, 24*, 3909. doi: 10.3390/molecules24213909

Hornakova, T., Springuel, L., Devreux, J., Dusa, A., Constantinescu, S.N., Knoops, L., & Renauld, J.C. (2011). Oncogenic JAK1 and JAK2-activating mutations resistant to ATP-competitive inhibitors. *Haematologica, 96*(6), 845- 853. doi: 10.3324/haematol.2010.036350

Irwin, J., Gaskins, G., Sterling, T., Mysinger, M., & Keiser, M. (2018). Predicted Biological Activity of Purchasable Chemical Space. *Journal of Chemical Information and Modeling, 58*, 148-164. doi: 10.1021/acs.jcim.7b00316

Jung, H., Song, Y., Lim, C., & Park, E. (2008). Anti-angiogenic, anti-inflammatory and anti-nociceptive activities of vanillyl alcohol. *Archives of Pharmacal Research, 31*(10), 1275-1279. doi: 10.1007/s12272-001-2106-1

Keiser, M.J., Roth, B. L., Armbruster, B. N., Ernsberger, P., Irwin, J.J., & Shoichet, B.K. (2007). Relating protein pharmacology by ligand Chemistry. *Nature Biotechnology, 25*(2), 197- 206. doi: 10.1038/nbt1284

Lagunin, A.A., Dubovskaja, V.I., Rudik, A.V., Pogodin, P.V., Druzhilovskiy, D.S., Gloriozova, T.A., Filimonov, D.A., Sastry, G.N., & Poroikov, V.V. (2018). CLC-Pred: a freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PLOS One, 13*(1). doi: 10.1371/journal.pone.0191838

Leeson, P.D., & Young, R.J. (2015). Molecular Property Design: Does Everyone Get It?. *ACS Medicinal Chemistry Letters, 6*(7), 722-725. doi: 10.1021/acsmedchemlett.5b00157

Lin, T.E., HuangFu, W.C., Chao, M.W., Sung, T.Y., Chang, C.D., Chen, Y.Y., Hsieh, J.H., Tu, H.J., Huang, H.L., Pan, S.L., & Hsu, K.C. (2018). A Novel Selective JAK2 Inhibitor Identified Using Pharmacological Interactions. *Frontiers in Pharmacology, 9*(1379). doi: 10.3389/fphar.2018.01379

Lucet, I.S., Fantino, E., Styles, M., Bamert, R., Patel, O., Broughton, S.E., Walter, M., Burns, C.J., Treutlein, H., Wilks, A.F., & Rossjohn, J. (2006). The structural basis of Janus kinase 2 inhibition by a potent and specific pan-Janus kinase inhibitor. *Blood, 107*, 176- 183. doi: 10.1182/blood-2005-06-2413

Mao, Q.Q., Xu, X.Y., Cao, S.Y., Gan, R.Y., Corke, H., Beta, T., & Li, H.B. (2019). Bioactive compounds and bioactivities of ginger (zingiber officinale roscoe). *Foods,8*, 1-21. doi: 10.3390/foods8060185

Maria Pia, G.D., Sara, F., Mario, F., & Lorenza, S. (2019). Biological Effects of Licochalcones. *Mini Reviews in Medicinal Chemistry, 19*(8), 647- 656. doi: 10.2174/1389557518666180601095420

Martin, Y.C. (2005). A bioavailability score. *Journal of Medicinal Chemistry, 48*(9), 3164- 3170. doi: 10.1021/jm0492002

Modi, V., & Dunbrack, R.L. (2019). Defining a new nomenclature for the structures of active and inactive kinases. *Proceedings of the National Academy of Sciences, 116*, 6818-6827. doi: 10.1073/pnas.1814279116

MOE. Molecular Operating Environment, Release $_{V}$2019.01; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2021

Pegram, L.M., Liddle, J.C., Xiao, Y., Hoh, M., Rudolph, J., Iverson, D.B., Vigers, G.P., Smith, D., Zhang, H., Wang, W., Moffat, J.G., & Ahn, N.G. (2019). Activation loop dynamics are controlled by conformation-selective inhibitors of ERK2. *Proceedings of the National Academy of Siences, 116*, 15463- 15468. doi: 10.1073/pnas.1906824116

Peón, A., Naulaerts, S., & Ballester, P. J. (2017). Predicting the Reliability of Drug-target Interaction Predictions with Maximum Coverage of Target Space. *Scientific Reports, 7*, 3820. doi: 10.1038/s41598-017-04264-w

Pires, D.E., Blundell, T.L., & Ascher, D.B. (2015). pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry, 58*(9), 4066-4072. doi: 10.1021/acs.jmedchem.5b00104

Ramurthy, S., Taft, B.R., Aversa, R.J., Barsanti, P.A., Burger, M.T., Lou, Y., Nishiguchi, G.A., Rico, A., Setti, L., Smith, A., Subramanian, S., Tamez, V., Tanner, H., Wan, L., Hu, C., Appleton, B.A., Mamo, M., Tandeske, L., Tellew, J.E., Huang, S., Yue, Q., Chaudhary, A., Tian, H., Iyer, R., Hassan, A.Q., Griner, L.A.M., La Bonte, L.R., Cooke, V.G., Abbema, A.V., Merritt, H., Gampa, K., Feng, F., Yuan, J., Mishina, Y., Wang, Y., Haling, J.R., Vaziri, S., Hekmat-Nejad, M., Polyakov, V., Zang , R., Sethuraman, V., Amiri, P., Singh, M., Sellers, W.R., Lees, E., Shao, W., Dillon, M.P., & Stuart, D.D. (2020). Design and Discovery of N-(3-(2-(2-Hydroxyethoxy)-6-morpholinopyridin-4-yl)-4-methylphenyl)-2(trifluoromethyl)isonicotinamide, a Selective, Efficacious, and Well-Tolerated RAF Inhibitor Targeting RAS Mutant Cancers: The Path to the Clinic. *Journal of Medicinal Chemistry, 63*, 2013- 2027. doi: 10.1021/acs.jmedchem.9b00161

Rastogi, N., Duggal, S., Singh, S.K., Porwal, K., Srivastava, V.K., Maurya, R., Bhatt, M.L.B., & Mishra, D.P. (2015). Proteasome inhibition mediates p53 reactivation and anticancer activity of 6-Gingerol in cervical cancer cells. *Oncotarget, 6*, 43310-43325. doi: 10.18632/oncotarget.6383

Rayan, A., Raiyn, J., & Falah, M. (2017). Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. *PLoS One, 12*, 1- 12. doi: 10.1371/journal.pone.0187925

Roskoski, R. (2016). Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. *Pharmacology Research, 103*, 26- 48. doi: 10.1016/j.phrs.2015.10.021

Sahu, V., Nigam, L., Agnihotri, V., Gupta, A., Shekhar, S., Subbarao, N., Bhaskar, S., & Dey, S. (2019). Diagnostic Significance of p38 Isoforms (p38α, p38β, p38γ, p38δ) in Head and Neck Squamous Cell Carcinoma: Comparative Serum Level Evaluation and Design of Novel Peptide Inhibitor Targeting the Same. *Cancer Research and Treatment, 51*(1), 313- 325. doi: 10.4143/crt.2018.105

Sanachai, K., Mahalapbutr, P., Choowongkomon, K., Poo-arporn, R., Wolschann, P., & Rungrotmongkol, T. (2020). Insights into the Binding Recognition and Susceptibility of Tofacitinib toward Janus Kinases. *Aerican Chemical Society Omega, 5*, 369-377. doi: 10.1021/acsomega.9b02800

Schwarz, D., Merget, B., Deane, C., & Fulle, S. (2019). Modeling conformational flexibility of kinases in inactive states. *Proteins: Structure, Function and Bioinformatics, 87*, 943-951. doi: 10.1002/prot.25756

Tian, W., Wang, Z.W., Yuan, B.M., & Bao, Y.G. (2020). Calycosin induces apoptosis in osteosarcoma cell line via ERβmediated PI3K/Akt signaling pathways. *Molecular Medicine Reports, 21*(6), 2349- 2356. doi: 10.3892/mmr.2020.11039

Umar, A.B., Uzairu, A., Shallangwa, G.A., & Uba, S. (2020). Docking-based strategy to design novel flavone-based arylamides as potent V600E-BRAF inhibitors with prediction of their drug-likeness and ADMET properties. *Bulletin of the National Research Center, 44*, 179. doi: 10.1186/s42269-020-00432-7

Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., & Kopple, K.D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry, 45*(12), 2615- 2623. doi: 10.1021/jm020017n

Wang, B., Wu, H., Hu, C., Wang, H., Liu, J., Wang, W., & Liu, Q. (2021). An overview of kinase downregulators and recent advances in discovery approaches. *Signal Transduction and Targeted Therapy, 6*, 1-19. doi: 10.1038/s41392-021-00826-7

Wang, G.M., Wang, X., Zhu, J.M., Guo, B.B., Yang, Z., Xu, Z.J., Li, B., Wang, H.Y., Meng, L.H., Zhu, W.L., & Ding, J. (2017). Docking-based structural splicing and reassembly strategy to develop novel deazapurine derivatives as potent B-Raf^{V600E} inhibitors. *Acta Pharmacologica Sinica, 38*(7), 1059- 1068. doi: 10.1038/aps.2016.173

Wang, S., Zhang, C., Yang, G., & Yang, Y. (2014). Biological properties of 6-Gingerol: A brief review. *Natural Product Communications, 9*(7), 1027-1030. doi: 10.1177/1934578x1400900736

Wang, Z., Liang, L., Yin, Z., & Lin, J. (2016). Improving chemical similarity ensemble approach in target Prediction. *Journal of Cheminformatics, 8*(1). doi: 10.1186/s13321-016-0130-x

Weiss, N., Miller, F., Cazaubon, S., & Couraud, P.O. (2009). The blood brain barrier in brain homeostasis and neurological diseases. *Biochimica et biophysica acta, 1788*(4), 842- 857. doi: 10.1016/j.bbamem.2008.10.022

Williams, N.K., Bamert, R.S., Patel, O., Wang, C., Walden, P.M., Wilks, A.F., Fantino, E., Rossjohn, J., & Lucet, I.S. (2009). Dissecting Specificity in the Janus Kinases: The Structures of JAK-Specific Inhibitors Complexed to the JAK1 and JAK2 Protein Tyrosine Kinase Domains. *Journal of Molecular Biology, 387*, 219- 232. doi: 10.1016/j.jmb.2009.01.041

Yi, J. L., Shi, S., Shen, Y. L., Wang, L., Chen, H. Y., Zhu, J., & Ding, Y. (2015). Myricetin and methyl eugenol combination enhances the anticancer activity, cell cycle arrest and apoptosis induction of cis-platin against HeLa cervical cancer cell lines. *International Journal of Clinical and Experimental Pathology, 8*(2), 1116– 1127.

Zhang, F., Zhang, J.G., Qu, J., Zhang, Q., Prasad, C., & Wei, Z.J. (2017). Assessment of anti-cancerous potential of 6-gingerol (Tongling White Ginger) and its synergy with drugs on human cervical adenocarcinoma cells. *Food and Chemical Toxicology, 109*, 910- 922. doi: 10.1016/j.fct.2017.02.038

Zhang, X.H., Chen, C.H., Li, H., Hsiang, J., Wu, X., Hu, W., Horne, D., Nam, S., Shively, J., & Rosen, S.T. (2021). Targeting the non-ATP-binding pocket of the MAP kinase p38γ mediates a novel mechanism of cytotoxicity in cutaneous T-cell lymphoma (CTCL). *FEBS Letters, 595*(20), 2570-2592. doi: 10.1002/1873-3468.1418