Research Article

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FMS-like tyrosine kinase 3 inhibitory potentials of some phytochemicals from anti-leukemic plants using computational chemical methodologies

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Abstract: Acute myeloid leukemia (AML) takes center stage as a highly prevalent and aggressive clonal disorder affecting hematological stem cells. FMS-like tyrosine kinase 3 (FLT3) mutations were prevalent in nearly 30% of the AML cases. However, efforts have led to the development of anti-mutant FLT3 drugs, such as midostaurin, gilteritinib, and quizartinib, to improve treatments. Currently, we are exploring the ability of compounds from anti-leukemic plants to be used in AML therapies, focusing on mutant FLT3 inhibition. Employing computational techniques such as drug-likeness assessment, molecular docking, pharmacokinetics properties profiling, molecular dynamics simulations (MDS), and free energy calculations, we identified 43 out of 57 compounds with oral drug potential. Notably, 7 out of 43 compounds, including flavopiridol, sanggenol Q, norwogonin, oblongixanthones A, oblongixanthones B, apigenin, and luteolin exhibited strong binding affinities ranging from −9.0 to −9.8 kcal/mol, surpassing the control drug gilteritinib (−6.3 kcal/mol). Notably, flavopiridol and norwogonin displayed highly favorable pharmacokinetics and low toxicity profiles. MDS confirmed the stability of their binding through parameters such as root mean square deviation, root mean square fluctuation, and radius of gyration (R_g) over 100 ns simulations. Flavopiridol and norwogonin emerge as promising candidates for the development of mutant FLT3 inhibitors. Therefore, experimental studies are warranted to validate their therapeutic potential.

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1 Introduction

Acute myeloid leukemia (AML) is a rapidly progressing hematologic malevolence that affects the bone marrow, blood cells, and other tissues [1]. It is typified by the abnormal growth, abnormal differentiation, proliferation, and poor differentiation of immature white blood cells that cause interference with the production of normal blood cells, leading to anemia, infections, and bleeding disorders [2].

The Cancer Society outfit (American Cancer Society, ACS) in the United States forecasted that approximately 60,650 new cases of leukemia alone would surface in 2022, with AML being the most disastrous subdivision and responsible for 48% of the leukemia-associated mortalities [3].

Despite significant advances in cancer therapy, the prognosis for AML remains deprived, with a 5-year survival frequency of less than 30% [4–6]. In older patients, the median survival outcome is between 5 and 10 months, including individuals with comorbidities whose body systems cannot endure penetrative chemotherapy [2]. One of the significant challenges in AML treatment is the development of drug resistance, which limits the effectiveness of conventional therapies such as chemotherapy and radiotherapy. Therefore, there is a need for novel and effective therapies that target specific molecular pathways involved in AML.

FMS-like tyrosine kinase 3 (FLT3) is a tyrosine kinase receptor that plays a critical role in normal hematopoiesis, the process by which blood cells are molded. It is overexpressed in up to 70% of the AML cases, and its activation has been linked to pathogenesis and progression of the disease. FLT3 mutations, particularly internal tandem duplications, and tyrosine kinase domain mutations, are frequent in AML and are connected with humble prognosis and drug resistance. Therefore, FLT3 has emerged as a viable therapeutic target in AML [7]. Several FLT3 inhibitors have been developed for AML treatment, including midostaurin, gilteritinib, and quizartinib. However, these drugs have shown limited efficacy and significant toxicities, and drug resistance remains a major challenge. Therefore, there is a necessity for the development of innovative and more effectual FLT3 inhibitors that can overcome drug resistance and reduce toxicity [8].

Plants have long been the go-to material in old-style medicine for the treatment of several diseases, cancer inclusive. Many associated plant compounds have been established to possess anti-cancer properties, and some have shown promise in AML treatment. For example, resveratrol, a polyphenol found in grapes and red wine, has been exposed to counter FLT3 activity and prompt apoptosis in AML cells [9]. Curcumin, a compound found in turmeric, has also been found to have anti-cancer properties and inhibits FLT3 signaling in AML cells [10].

Recently, there has been a growing interest in using natural plant products as a source of novel anti-cancer agents, particularly for treating AML. Natural products have several advantages over synthetic compounds, including their structural diversity, potential for multi-targeted activity, and low toxicity [11]. Furthermore, the use of natural products in cancer therapy has a long history of successful outcomes, including the discovery of taxanes, vinca alkaloids, and anthracyclines [12].

In recent years, computational drug discovery has arisen as an influential tool for the identification and optimization of unique drug candidates. Computer-based approaches that comprise molecular docking, virtual screening, and molecular dynamics simulations (MDS) [13–16] have been expanded to forecast the binding affinity and efficacy of potential FLT3 inhibitors. It is interesting to note that this technique can aid in the optimization of potential drugs, leading to their approvals being granted. Instances of some licensed drugs that were optimized using CADD are captopril, dorzolamide, oseltamivir, aliskiren, and nolatrexed [13,17,18].

The aim of this research is to investigate the potential of selected compounds from anti-leukemic plants as an anti-cancer therapy for AML, targeting the FLT3 protein using computer-enabled techniques such as drug-likeness, molecular docking, *in silico* pharmacokinetics profiling, MDS, and free energy calculations. The ultimate goal of the research is to identify novel and effective natural plant-derived compounds for treating AML that can overcome drug resistance and reduce toxicity.

2 Materials and methods

2.1 Ligand data sets

Fifty-seven bioactive compounds from 29 plants reported to possess anti-leukemic potential were chosen from the literature [19–22]. The control drug chosen for this study is gilteritinib, which is an approved medication in the treatment of degenerated/obstinate (R/R) AML with an FLT3 mutation. Table S1 illustrates the list of bioactive

compounds, source plants, their respective PubChem IDs, and canonical smiles.

2.2 Selection and preparation of the protein target

The three-dimensional (3D) structure of FLT3 co-crystallized with gilteritinib (PDB ID: 6JQR) at 2.20 Å resolution [23] was accessed through the Protein Database (PDB) (http://www.pdb.org/pdb). This structure was selected based on the fact that it was co-crystalized with a known approved drug for AML, low resolution, and recently submitted to the protein database. The structure of the target protein is shown in Figure 1. The preparation for docking and minimization of the protein was achieved through the dock prep module of UCSF-Chimera software. The proteins were liberated from all heteroatoms, such as co-crystallized ligands and water molecules. Additionally, charges and hydrogen atoms using gasteiger charge were added. Finally, minimization was achieved by employing the amber force field 94 fs (Amberff94fs) [24].

2.3 Screening for drug-like potentials

Fifty-seven bioactive compounds were sieved for druglikeness employing three rules, viz: Lipinski's [25], Veber's [26], and Egan's [27], and bioavailability score via the SwissAdme website [28]. Thus, compounds with no more than one violation of the three rules and a bioavailability score ≥0.55 are deemed fit for molecular docking against FLT3.

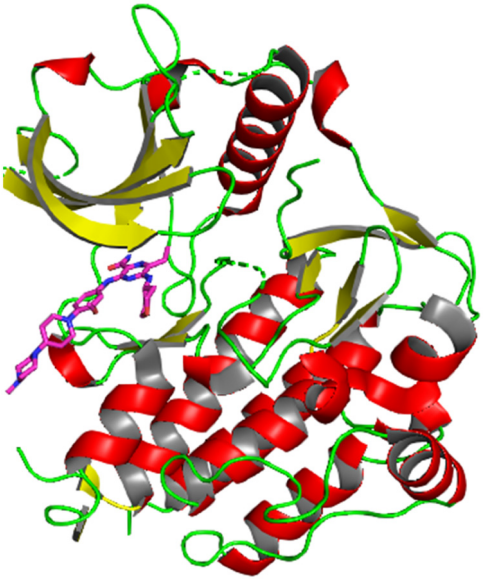

Figure 1: 3D structure of FLT3 bound to gilteritinib. FLT3 is presented in a cartoon with its α-helices (red), loops (green), and β-sheets (yellow). Gilteritinib is shown in purple, occupying the binding pocket.

2.4 Retrieval and preparation of ligands for molecular docking against FLT3

The 3D coordinates in the structure data file of gilteritinib (control drug) and 43 [42] compounds were retrieved from the chemical virtual store accommodated in the National Centre for Biotechnological Information called PubChem (PubChem (nih.gov)), which is a global leading collection of freely obtainable cheminformatics records. Likewise, the acquired compound structures were transformed into the best energetic and steady conformations choosing the Merck molecular force field (MMFF94) [29]; in addition, the optimization's system, conjugate gradient, by the use of the Open babel icon in Python Prescription suite (version 0.8).

2.5 Computer-assisted docking against FLT3

The docking steps were executed with the aid of AutoDock Vina, accommodated in open-source Python Prescription 0.8 [30] to secure probable binding geometries and binding energies (BEs) of compounds at the designated binding pocket of FLT3. The pocket was enclosed by adjusting the grid box with magnitudes (16.7026 × 16.8947 × 20.4031) Å, and the center was adjusted in line with the site of gilteritinib binding in the FLT3 binding cavity. The cavity comprises Leu818, Phe830, Ala642, Gly697, Tyr693, Leu616, Cys694, Glu692, and Asp829 [23]. After the docking simulation run, docking score (BE) of compounds below −9.0 and gilteritinib were submitted for molecular visualization procedures in order to explore their binding positions (3D) and molecular contact patterns (2D) via PyMOL© Molecular Graphics (version 2.4, 2016, Schrödinger LLC) [31] and Maestro 11.1's Ligand interaction option (Schrödinger 2017 ver.), respectively.

2.6 Prediction of *in silico* pharmacokinetics profiling

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) is imperative during the penultimate phase of drug discovery and design conduit to scrutinize the pharmacokinetics and pharmacodynamics of the projected compounds with the potential of becoming a drug. Here,

we employed ADMETSar server (http://lmmd.ecust.edu.cn/) to forecast the ADMET properties of the compounds with the best hits post-molecular docking analysis [32,33]. The web server was served with the simplified molecular-input line-entry system (SMILE) strings of the compounds from PubChem (https://pubchem.ncbi.nlm.nih.gov/compound/) through the search space and executed for ADMET features prediction.

2.7 Molecular dynamics simulations

The firmness of the protein–ligand (FLT3 with flavopiridol, norwogonin, and sanggenol Q) interactions was evaluated by performing MD calculations on the optimal docking postures. GROMACS 2020.2 software was deployed for the equilibration and production run stages of all MD simulations. The CHARMM36 forcefield was deployed to simulate the protein–ligand combination. The system was subsequently subjected to a constant number of particles, volume, and temperature and a constant number of particles, pressure, and temperature (NPT) ensembles to stabilize its temperature and pressure. It was simulated for a duration of 125 ps at a temperature of 300.15 K, with positional restrictions of 400 and 40 kJ/mol nm^2 for the backbone and side chains, respectively. The complex is ultimately exposed to a production simulation lasting 100 ns, conducted within an NPT ensemble at a temperature of 300.15 K and a pressure of 1 bar.

2.8 Trajectory analysis

The GROMACS program was employed to perform MD simulations. The root mean square deviation (RMSD) of atom positions was determined for both the ligands and FLT3 protein after fitting the FLT3 backbone using the gmx_rms subprogram. The RMSF was computed using the gmx_rmsf subprogram, based on the FLT3 C-alpha atoms. The R_g of all FLT3 atoms was calculated using the gmx_gyrate subprogram. The gmx_hbond subprogram was utilized to evaluate hydrogen bonds within the protein–ligand interface. The mass distance between FLT3 and the ligands was quantified during the simulation using the gmx_distance subprogram. Finally, the visual molecular dynamics (VMD) molecular graphics program was utilized for trajectory visualization and protein–ligand contact frequency analysis.

2.9 Molecular mechanics Poisson–Boltzmann surface area (MMPBSA) calculations

The systems selected for further research underwent MMPBSA calculations using g_mmpbsa, a computational tool within the GROMACS software package, which is employed to determine the binding affinity. In a broad context, the thermodynamic quantity known as the binding free energy, which characterizes the strength of interaction between the FLT3 and its ligand in a solvent, can be mathematically represented as

$$\Delta G_{\text{binding}} = \Delta G_{\text{complex}} - (\Delta G_{\text{protein}} + \Delta G_{\text{ligand}}).$$

$\Delta G_{\text{complex}}$ is the total free energy of the protein–ligand complex, and $\Delta G_{\text{protein}}$ is the total free energy of the isolated FLT3 and ligands in a solvent. By individually calculating the energy values for each residue and then adding them together, it was possible to determine the BE contribution of each residue. Given the limited compatibility of g_mmpbsa with specific GROMACS versions, it was necessary to recreate the binary run input file (.tpr) using GROMACS 5.1.4 to perform MMPBSA calculations with g_mmpbsa. The generation of the binary run input file required the utilization of three essential files: the molecular structure file (.gro), the topology file (.top), and the MD-parameter file (.mdp). These files were obtained via the MD process.

3 Results and discussions

AML is a form of leukemia that has high morbidity and mortality among adults and continues to be a major source of concern due to its poor prognosis and as a result of the limited efficacy of the regimens that are used in its management and treatment [5]. Consequently, the search for newer and alternative classes of anti-AML drugs remains unfaltering. To this end, this study aimed to identify potential inhibitors of FLT3 from plants with reported anti-leukemic activities.

In total, 57 bioactive compounds from 29 medicinal plants with anti-leukemic activities were identified and retrieved from the PubChem database. These compounds included alkaloids, ansamycins, flavonoids, terpenes, and phytosterols; following their retrieval, they were subjected to druglikeness evaluation aimed at identifying their fitness for use as oral drugs based on the rule of five (Ro5), Veber, and Egan. The Ro5 evaluates the fitness of small molecules to serve as drugs based on parameters, including the molecular weight <500 Da, number of hydrogen bond donors <10,

number of hydrogen acceptors <5, and octanol–water partition coefficient <5. The violation of more than one of the previously mentioned parameters may render small molecules unfit for use as oral drugs. Similarly, Veber and Egan's rules are considered a subset of the Ro5 due to overlapping parameters of the rules.

The screening identified 43 compounds out of the 57 compounds as having oral drug potential (Table S2). Following subjecting the compounds to the filtering criteria, the compounds with oral drug potentials were identified and subsequently subjected to a preparation pipeline aimed at making them fit for molecular docking. These included the addition of explicit hydrogens and energy minimization among others. The energy minimization of compounds is an essential step in the preparatory pipeline that compounds are subjected to prior molecular docking, which involves the optimization of the compound structures by adjusting bond lengths, angles, and torsion angles to reach a stable conformation [34,35]. This optimization enhances the accuracy of molecular docking predictions by providing more reliable starting structures for the docking simulations. It is worth noting that there exist several approaches to compound minimization, which include the molecular mechanics and the quantum mechanical approaches that utilize methods, including the semi-empirical method, density functional theory (DFT) method, and the *ab initio* methods. The molecular mechanics methods are commonly the go-to approach in the screening of compound libraries due to their balance between accuracy, computational power, and time. Similarly, the semi-empirical methods also provide a desirable balance between accuracy and required computational power, and they are commonly used in quantitative structure–activity relationship modeling and other drug discovery approach [36,37]. Compared to the DFT method, which requires high computing power, semi-empirical methods have been reported to produce DFT-level accuracy and exhibit excellent correlation with the experimental results [38,39]. It is worth noting that the molecular mechanics approach was used in this study, and they are widely used in molecular modeling for drug discovery due to their accuracy and time effectiveness [40,41]. Similarly, the structure of the FLT3 protein was also retrieved and prepared using a previously mentioned approach.

MDS of the compounds against the protein was performed to identify the hit compounds against the protein with gilteritinib as the control drug. Interestingly, the compounds exhibited high binding affinities for the protein, as revealed by their docking scores that ranged from −5.1 to −9.8 kcal/mol (Table S3). Notably, these compounds exhibited affinities that were much higher compared to that of gilteritinib, with compounds including sanggenol Q and flavopiridol exhibiting docking scores as high as −9.8 and −9.6 kcal/mol, respectively (Table 1).

To identify the potential drug candidates, the pharmacokinetic properties and toxicity profiles of the top seven hit compounds were studied (Table 2, Table S4). Notably, poor pharmacokinetics and toxicity profiles are the major reasons why most promising compounds fail in the later stage of clinical trials; hence, studying these properties has emerged as a viable means to circumvent these. Human intestinal absorption (HIA) is the process by which drugs that are orally administered are taken up from the gastrointestinal tract to the bloodstream prior to their distribution to target sites. All the selected hit compounds were predicted to be capable of being taken up into the bloodstream, and they also possessed a minimum bioavailability score of 55%, signifying their ability to be transported to the target site in high concentration upon absorption. Of all the hit compounds, only apigenin, luteolin, and oblongixanthones A were predicted to be Caco-2- permeable. While both the Caco-2 and HIA tests aim to give insights into the oral bioavailability of drugs, the HIA tests can be said to be the more accurate test due to their modalities that are based on humans as opposed to *in vitro* models. The P-glycoprotein serves as an efflux protein; it is known to

Table 1: Druglikeness and molecular docking outcomes of drug-like compounds from plants with anti-leukemic potentials against FLT3 protein

S/No.	Compounds	PubChem ID	MW	Lipinski #violations	Veber #violations	Egan #violations	Bioavailability Score	BE (kcal/mol)
1.	Flavopiridol	5287969	401.84	0	0	0	0.55	−9.6
2.	Sanggenol Q	11796489	422.47	0	0	0	0.55	−9.8
3	Norwogonin	5281674	270.24	0	0	0	0.55	−9.6
4.	Oblongixanthones A	25209069	326.3	0	0	0	0.55	−9.3
5.	Oblongixanthones B	25209201	478.58	0	0	1	0.55	−9.7
6.	Apigenin	5280443	270.24	0	0	0	0.55	−9.0
7.	Luteolin	5280445	286.24	0	0	0	0.55	−9.0
8.	Gilteritinib	49803313	552.71	2	3	0	0.17	−6.3

Table 2: ADMET profiling of hit plant compounds from different plants with anti-leukemic potential after molecular docking against FLT3 protein

ADMET profile	Apigenin	Flavopiridol	Luteolin	Norwoginin	Oblongixanthones A	Oblongixanthones B	Gilteritinib	Sanggenol Q
Ames mutagenesis	−	−	+	−	+	+	−	−
Toxicity (class)	III	III	II	II	III	III	III	III
Blood–brain barrier	−	+	−	−	−	−	+	+
Caco-2	+	−	+	−	+	−	−	−
Carcinogenicity	−	−	−	−	−	−	−	−
CYP1A2 inhibition	+	−	+	+	+	+	−	+
CYP2C19 inhibition	+	−	−	−	−	+	−	−
CYP2C9 inhibition	+	−	−	−	+	+	−	+
CYP2C9 substrate	−	−	−	−	−	−	−	−
CYP2D6 inhibition	−	−	−	−	−	−	−	−
CYP2D6 substrate	−	−	−	−	−	−	−	−
CYP3A4 inhibition	+	−	+	+	+	−	−	−
CYP3A4 substrate	−	+	−	−	+	+	+	+
CYP inhibitory promiscuity	+	−	+	+	+	−	−	+
Hepatotoxicity	+	−	−	−	+	+	+	+
hERG inhibition	−	+	−	−	−	+	+	+
HIA	+	+	+	+	+	+	+	+
HOB	−	−	−	−	−	−	+	−
Nephrotoxicity	−	−	−	−	−	−	−	−
Acute oral toxicity	1.484	2.658	1.998	2.037	2.215	2.557	2.361	1.688
P-gp inhibitor	−	−	−	−	−	+	+	+
P-gp substrate	−	+	−	−	−	−	+	+
PP binding	1.083	1.028	1.066	1.048	0.807	0.918	0.710	0.937
Subcellular localization	Mitochondria	Lysosomes	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Lysosomes	Mitochondria
UGT catalyzed	+	−	+	+	+	−	−	+
Water solubility	−2.777	−3.299	−2.999	−2.999	−3.189	−4.524	−3.200	−4.077

reduce the efficacy of drugs that serve as its substrates, while its inhibition also could lead to potential drug–drug interactions on co-administration of the inhibitor with some drugs. Among the selected hit compounds in this study, flavopiridol and sanggenol Q were found to be substrates of P-glycoprotein, which indicates the potential of the compounds to be effluxed out of the cell rapidly when administered as a drug. However, the effect of this phenomenon on their efficacy can be alleviated by the administration of a higher dosage of the drug, with consideration to potential dose-induced toxicity. Similarly, oblongixanthones B and sanggenol Q were found to be potential inhibitors of P-glycoprotein. The administration of both compounds as drugs could be limited in some clinical usage due to their potential to induce drug toxicity as a result of the inhibition of the transport of some toxic drug metabolites out of the cell by this protein.

The metabolism profiles of the hit compounds based on their potential to be metabolized in the phase I reaction mediated by cytochrome P450 (CYP450) enzymes were studied. The compounds were found to be metabolizable by at least one of the CYP450 isozymes involved in drug metabolism. However, some of the compounds were found to be inhibitors of some of the isozymes; hence, there must be selective administration of the compounds as drugs. Evaluation of the toxicity profiles of the hit compounds revealed oblongixanthones A and oblongixanthones B to be Ames-mutagenic; hence, they could be capable of causing potential alterations to nucleotide sequences and cause DNA damage. However, further studies revealed them non-carcinogenic; hence, the alteration caused did not give rise to molecular events that resulted in cancer development. Also, the other compounds were found to be non-carcinogenic. The potential of the compounds to serve as inhibitors of the human ether-a-go-go related gene (hERG), which codes for the potassium ion channel of the heart, was examined. Flavopiridol, oblongixanthones B, and sanggenol Q were found to be inhibitors of

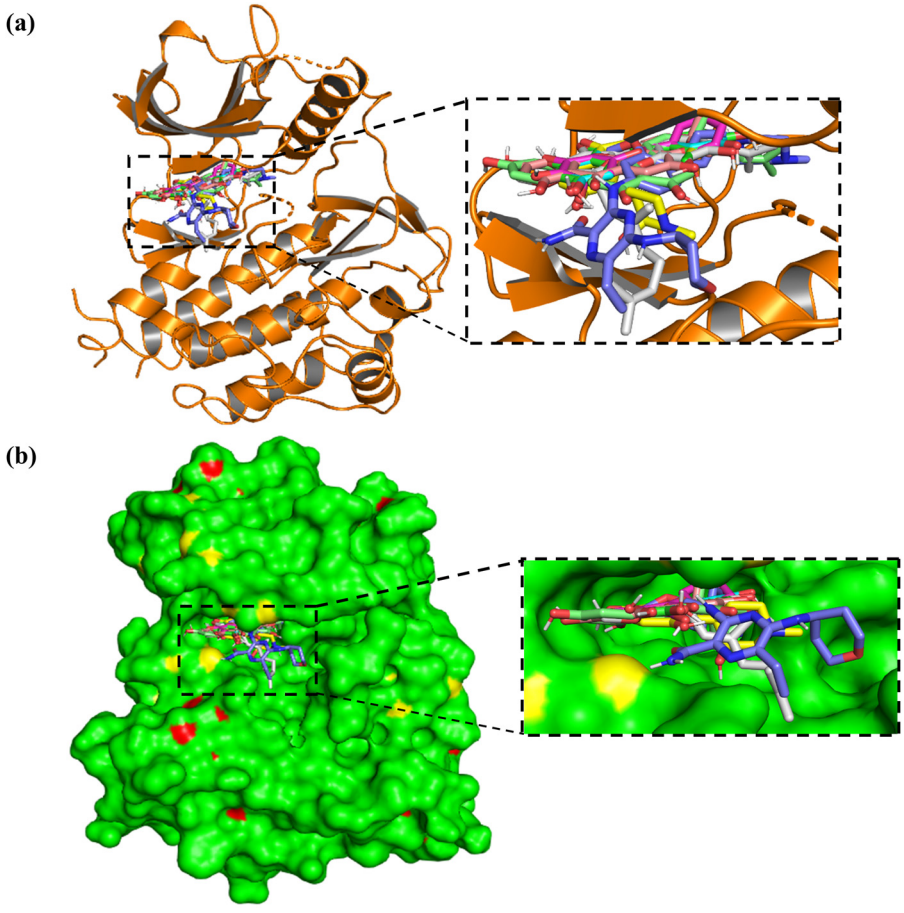

Figure 2: Molecular docking poses of hit compounds and gilteritinib against FLT3 in 3D rendition. (a) Cartoon and (b) molecular surface representations of binding poses of hit compounds (in sticks) in the binding pocket of FLT3. Apigenin (green), flavopiridol (yellow), gilteritinib (blue), luteolin (cyan), norwogonin (pink), obongixanthones A (pale pink), oblongixanthones B (gray), and sanggenol Q (light green) were observed to occupy similar portions of the binding pocket.

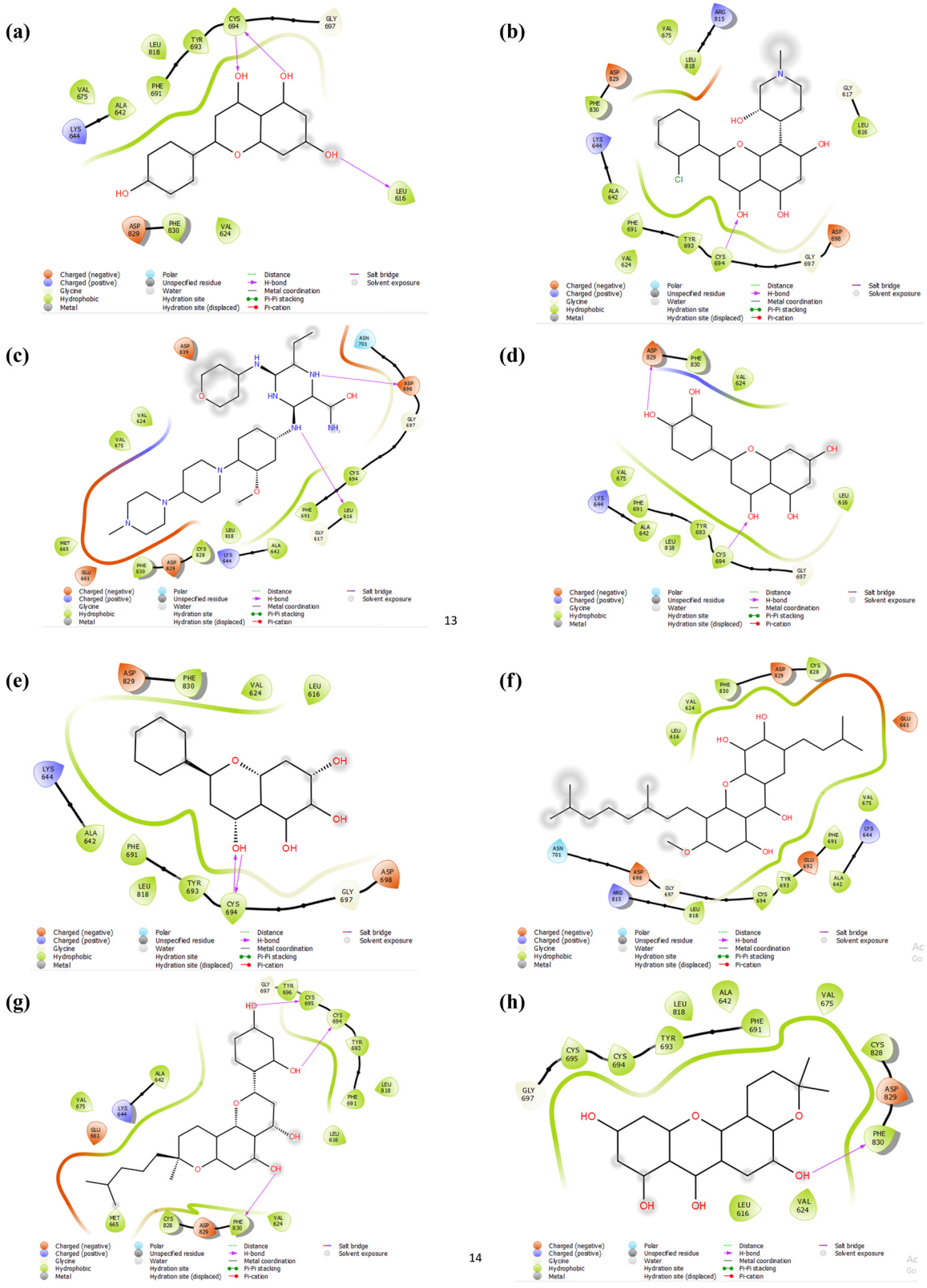

Figure 3: Molecular docking 2D interaction analyses of hit compounds and gilteritinib against FLT3. (a) Apigenin, (b) flavopiridol, (c) gilteritinib, (d) luteolin, (e) norwogonin, (f) oblongixanthones b, and (g) sanggenol q and (h) oblongixanthones A were observed to interact with amino acid residues found along the binding pocket of FLT3 via hydrogen bonding and hydrophobic interactions.

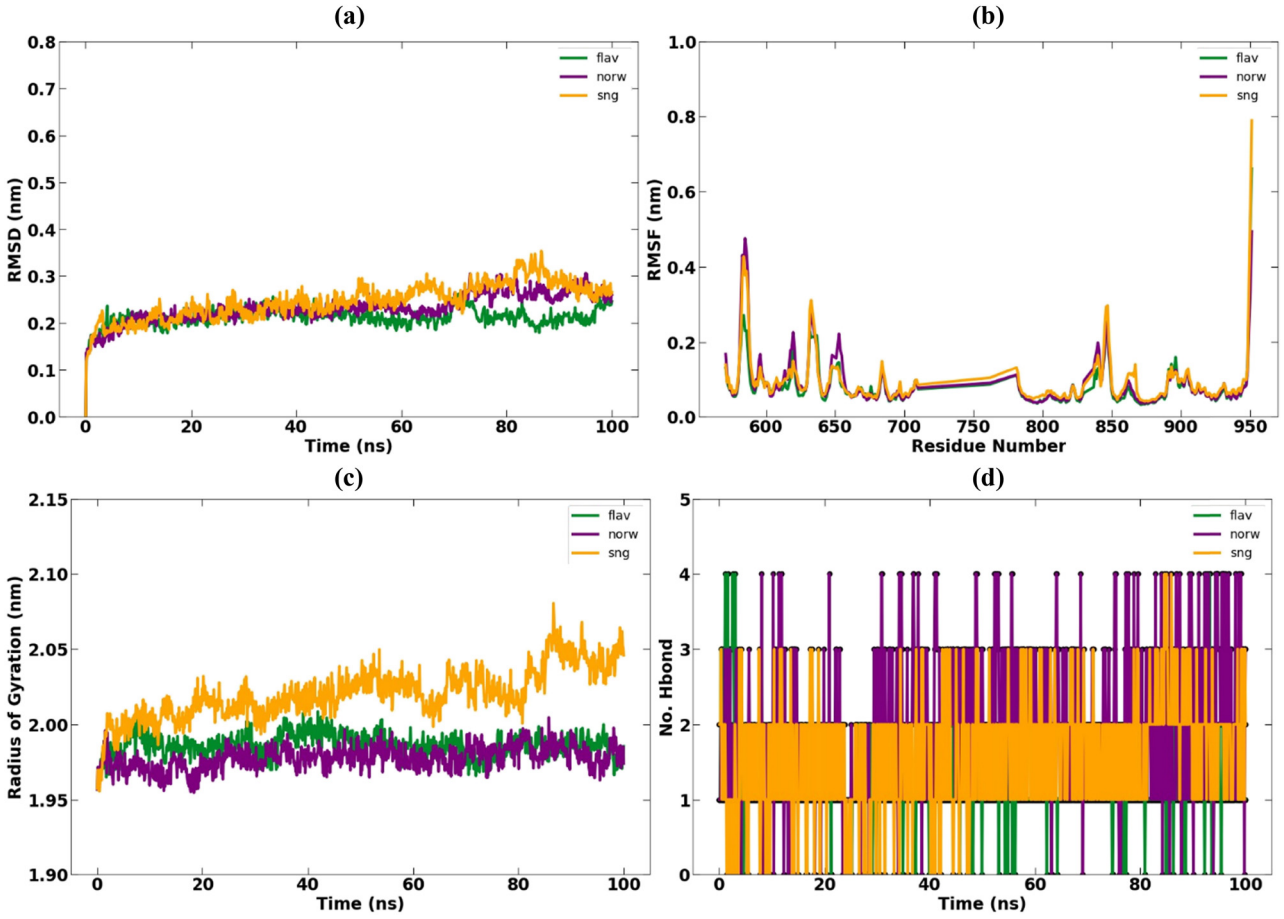

Figure 4: (a) RMSD, (b) RMSF, (c) radius of gyration, and (d) hydrogen bonding of the complexes during 100 ns MDS.

the hERG. This implies that the administration of the drug could lead to a condition referred to as cardiac arrhythmia. Apigenin, oblongixanthones A, oblongixanthones B, and sanggenol Q were found to be hepatotoxic; hence, their usage is dose-dependent (Figure 2).

After conducting molecular docking analysis, the subsequent molecular interaction assessments, facilitated through PyMOL and the ligand interaction module of Schrodinger Maestro 11.1, unveiled the robust binding of our investigated compounds within the active sites of the mutant FLT3 (Figure 3). Notably, all the identified hit compounds displayed similar interaction profiles to those of gilteritinib, the control drug. These interactions encompassed the formation of essential hydrogen bonds with Cys694, Leu616, and Gly697, as well as prominent hydrophobic interactions with Cys694, Tyr693, Phe691, Ala642, Leu818, Val675, Phe830, Val624, Leu616, and Ile62. Additionally, negative charge interactions were observed with Asp829, while positive interactions were noted with Lys644. An exception to this pattern was oblongixanthones B, which exhibited a distinctive polar interaction with Asn701. It is noteworthy that the binding pocket of the mutant FLT3 primarily comprises hydrophobic regions, reinforcing the compounds' affinity for the active site.

To assess the binding stability [42–45] of FLT3 protein–ligand complexes, including flavopiridol, norwogonin, and sanggenol Q, we conducted MDS at room temperature over a 100 ns duration. MDS were run for 100 ns at room temperature to evaluate the binding stability of FLT3–ligand complexes (flavopiridol, norwogonin, and sanggenol Q). After the simulation run, the trajectory data analysis showed that all ligands stayed bound to the ligand-binding groove inside the FLT3 pocket. The stability of each structure was evaluated by performing RMSD, RMSF, Rg, hydrogen bonding, average center of mass (COM) distance calculations between FLT3 and ligand, and MMPBSA calculations.

Figure 4a depicts the structure's complex RMSD during 100 ns simulations. After a simulation time of 20 ns, it was observed that all complex RMSD curves exhibited consistently low and steady values. The complex flavopiridol exhibits slight variations and fluctuations, followed by norwogonin and sanggenol Q. Figure 5b shows all complexes' RMSD and their corresponding curves. The RMSD curve of

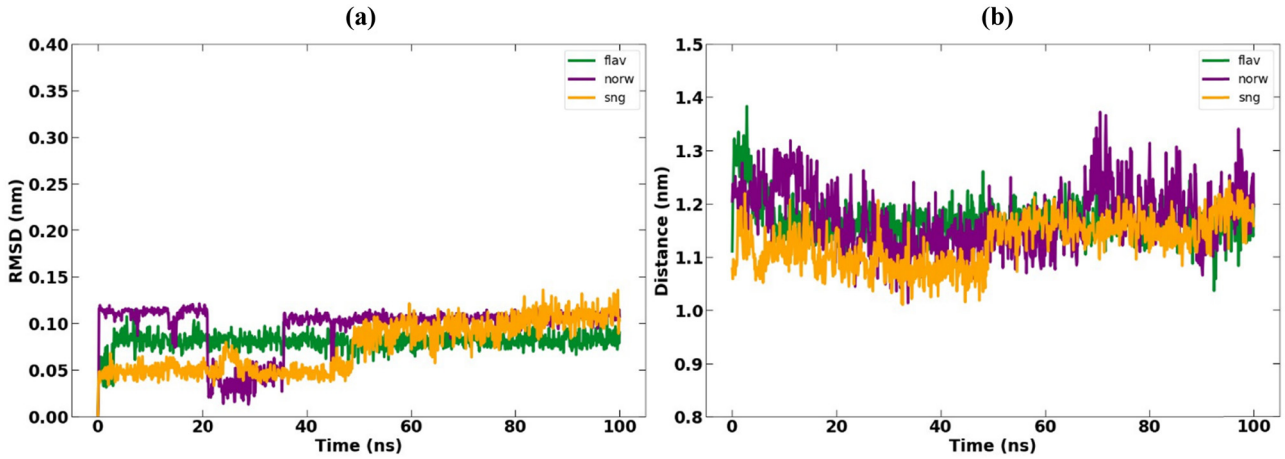

Figure 5: (a) Ligand RMSD and (b) the average distance between ligand and the protein of the complexes during 100 ns MDS.

the flavopiridol ligand exhibits minimal variations during the 100 ns simulation.

Additional experimental analyses, on the other hand, confirmed that the ligand norwogonin had the lowest binding affinity among the three ligands and the highest degree of variability in the ligand RMSD curve. The radius of gyration (Figure 4c) matches the results of the RMSD analysis for the complexes. All compounds show small changes (less than 0.5 Å) during the simulation. This suggests that the protein–ligand systems maintain a compact and stable conformation throughout the simulation. Although the ligand sanggenol Q remains stable within its binding site, R_g analysis indicates a gradual increase in contrast to flavopiridol and norwogonin. This suggests a protein conformation change, with the R_g value ranging from 19.5 to 20.1 Å. In order to determine the RMSF of the protein complex, we analyzed the positional deviations of the "C-alpha" atoms using GROMACS software. The compound usually experiences fluctuation intensities below 2.0 Å, except for specific residues corresponding to protein regions characterized by loops or turns (Figure 4b).

Figure 4d illustrates the cumulative count of hydrogen bonds established between the ligand and protein during a 100 ns simulation. Throughout the simulation, it was

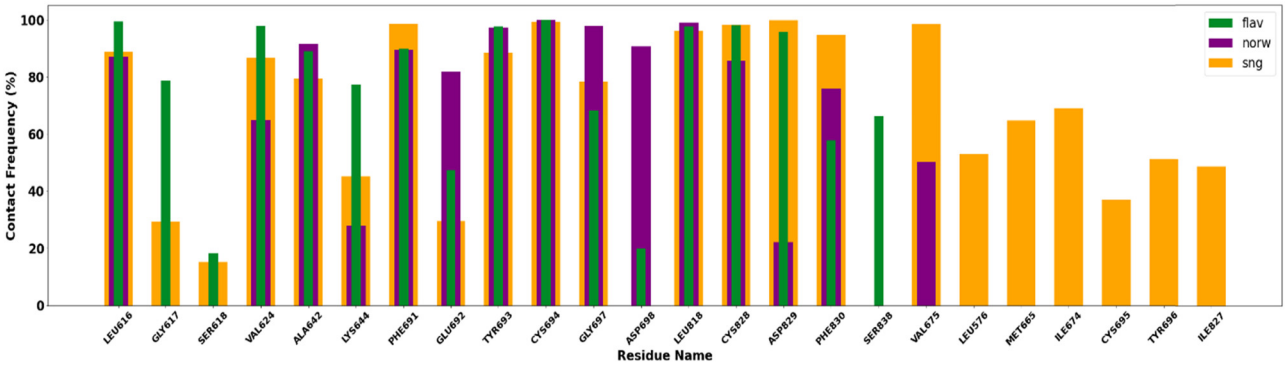

Figure 6: CF analysis of the complexes.

Table 3: Calculated binding free energies of the tested compounds (kJ/mol)

Complex	Δ*G*	van der Waals energy	Electrostatic energy	Polar solvation energy	SASA energy
Flavopiridol	−137.603 ± 13.791	−189.311 ± 6.884	−59.704 ± 13.073	131.023 ± 7.764	−19.610 ± 0.827
Norwogonin	−29.364 ± 17.416	−133.805 ± 7.506	−73.167 ± 30.426	191.326 ± 22.176	−13.718 ± 0.671
Sanggenol Q	−135.809 ± 25.042	−211.697 ± 13.615	−51.223 ± 22.348	149.476 ± 8.593	−22.364 ± 0.457

observed that all ligands consistently exhibited an average of one hydrogen bond.

The mean center-of-mass distance between the ligand and protein for a 100 ns simulation period is depicted in Figure 5b. The observed data indicate that the ligands remained at their respective binding sites, as evidenced by the minimum variability of the COM distance across all systems (less than 1.5 Å). Notably, high RMSD values indicate the inability of the ligand to maximize the interaction with the binding pocket of the protein being investigated; hence, the significant instability [46,47]. In order to conduct a more comprehensive assessment of the interaction between the protein and the ligands under investigation, a contact frequency (CF) analysis was carried out. This analysis utilized the contactFreq.tcl module in VMD, employing a cutoff distance of 4 Å. Among the three, ligand sanggenol Q has the most significant quantity and proportion of ligand interactions. The residues exhibiting the most significant carbon footprint percentages are depicted in Figure 6. The residues that exhibited the most significant CF throughout all simulations were Leu616, Val624, Ala642, Glu692, Tyr693, Cys694, Gly697, Leu818, Cys828, and Phe830.

The MMPBSA method was chosen to re-evaluate the complexes due to its effectiveness as a force field-based approach for calculating binding free energy. This approach is particularly advantageous compared to other methods, such as free energy perturbation or thermodynamic integration, as it offers a faster solution. The g-mmpbsa program was utilized to carry out the MM/PBSA calculation. The results of the binding free energies are detailed in Table 3.

4 Conclusions

This study investigated the potentials of 57 compounds sourced from 29 plants with reported anti-leukemic activities to serve as inhibitors of FLT3 using molecular modeling methods. Initially, the compound library was filtered based on three druglikeness rules: a process that led to the identification of 43 compounds with the potential to serve as oral drugs. Subsequent molecular docking simulation revealed flavopiridol, sanggenol Q, norwogonin, oblongixanthones A, oblongixanthones B, apigenin, and luteolin as the hit compounds based on their high affinities, as evident from their docking scores that ranged from −9.0 to −9.8 kcal/mol, while further screening revealed flavopiridol, sanggenol Q, and norwogonin as compounds with admirable ADMET properties. Also, MDS-based stability assessment of the compounds' interactions with the residues that constitute the binding pocket of the target revealed their stable interaction as evident from the RMSD values, which is less than >2.5 Å. In conclusion, the results of this study revealed flavopiridol, sanggenol Q, and norwogonin worthy of exploration in further computational and experimental studies aimed at developing therapeutic regimens aimed at combating AML via mutant FLT3 inhibition.

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