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Computational Estimation of Compounds Isolated from *Asarum geophilum* as Potential Inhibitors of Janus Kinase 2 (JAK2) Protein

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ABSTRACT

Asarum geophilum is a narrowly distributed species in Vietnam with significant untapped bioactive potential. Numerous compounds, predominantly flavonoids have been isolated from *Asarum* species. This study aimed to screen and evaluate the inhibitory potential of flavonoid compounds (**1 – 18**) isolated from *Asarum geophilum* against the JAK2 protein using computational models. The interaction of the isolated compounds with JAK2 protein was investigated *in silico* via molecular docking, and molecular dynamics simulations. The molecular docking study successfully positioned eighteen isolated compounds from *Asarum geophilum* within the Janus kinase 2 (JAK2) protein active site. The isolated compounds demonstrated binding affinities ranging from -6.630 to -9.521 kcal/mol (mean: -8.209 kcal/mol). Nine top-performing ligands (compounds **7**, **8**, **9**, **11**, **12**, **13**, **14**, **16**, and **18**) with ΔG_{dock} < -8.0 kcal/mol were selected for further analysis. Conformational studies revealed these lead compounds formed stable interactions with key JAK2 amino acid residues. Notably, compound **14** exhibited exceptional binding characteristics (ΔG_{LIE} = -21.78 kcal/mol), with dissociation metrics significantly outperforming both other screened compounds and established JAK2 inhibitors. The ΔG_{LIE} values for all leads (-8.35 to -21.78 kcal/mol) substantially exceeded those of reference inhibitors, suggesting superior target engagement potential.

Keywords: *Asarum geophilum*, Janus kinase 2 protein, Inhibitors, Molecular docking.

Introduction

In modern pharmaceutical chemistry, computer-aided drug design (CADD) has emerged as a powerful tool for high-throughput compound screening to identify bioactive molecules capable of selectively inhibiting specific functional proteins. Evidence indicates that CADD significantly reduces the time and cost of de novo drug development.[1] Next-generation drug discovery and development leverage advanced genetic and biochemical screening platforms, including the utilization of alternative cell lines, regulatory mediators, and receptor-ligand interaction studies.[2] These assays elucidate the mechanism of action (MoA) of candidate compounds during early-stage drug development and enable precise identification of pharmacologically active constituents within complex extract libraries.[3] Virtual screening plays an indispensable role in modern drug discovery and development. This computational approach leverages advances in bioinformatics to virtually screen, characterize, and predict novel structures with potential high biological activity.[4] Virtual screening's key advantage is its ability to significantly reduce drug discovery costs and timelines. The method employs a multi-step filtering process, applying sequential screening criteria to systematically narrow down promising candidates.[5]

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Since virtual screening relies on computational simulations rather than physical experiments, it eliminates material costs and allows the evaluation of compounds that may not yet be synthesized. Depending on the study scope, virtual screening databases can encompass tens of millions of compounds, all of which can be analyzed in a single high-throughput screening campaign.[6]

In silico screening utilizes molecular docking simulations to predict receptor-ligand interactions, identifying ligands with optimal binding affinity based on the lowest calculated free energy (ΔG). These simulations rely on three-dimensional (3D) protein structures, typically derived from experimental data provided by structural biologists. The ligands, in turn, are well-defined chemical compounds with clearly documented structures and sources.[7,8]

A computational method, molecular docking enables the characterization of molecular mechanisms at protein binding sites (active sites) while elucidating fundamental biochemical processes.[9] The docking process comprises two essential steps: (1) predicting the ligand's binding configuration (including its spatial position and orientation, collectively referred to as the docking pose), and (2) evaluating the binding affinity. Consequently, molecular docking not only identifies biologically significant interactions but also quantifies binding strength through scoring functions, thereby enabling the classification of potential bioactive compounds based on their binding potency.[10,11] Using steered molecular dynamics (SMD) simulations, the Fast Pulling of Ligand (FPL) method has demonstrated effectiveness in evaluating relative binding affinities. This technique applies an external harmonic potential to mechanically extract the ligand from the protein's binding cavity. The resulting pulling work shows a strong correlation with experimental binding affinities, confirming the FPL method's efficacy for ranking potential inhibitors by their relative binding affinities across multiple target proteins.[12,13]

The Janus kinase (JAK) family has four distinct members (JAK1, JAK2, JAK3 and TYK2). Among them, JAK2 has become a significant therapeutic target due to its pivotal involvement in growth factors and cytokine receptor signaling. Aberrant JAK2 activity is associated with

various hematologic malignancies, such as lymphomas, leukemias, thrombocytosis, and myeloproliferative neoplasms.[14-19] Most JAK inhibitors achieve their effect by competitively binding to the ATP-binding pocket of JAK2's JH1 domain. Clinically approved inhibitors including ruxolitinib, tofacitinib, baricitinib, and oclacitinib demonstrate pan-JAK inhibition profiles.[20,21] However, their lack of isoform specificity often leads to off-target effects and associated adverse events. The development of next-generation, highly selective JAK inhibitors is expected to mitigate these side effects while improving therapeutic efficacy.[22]

The genus *Asarum* L. (Aristolochiaceae) encompasses more than 100 species distributed worldwide and holds significant importance in traditional herbal medicine. To date, phytochemical investigations have identified over 280 distinct compounds from *Asarum* species, with volatile oils, flavonoids, and lignans constituting the predominant bioactive constituents that serve as key chemotaxonomic markers. Contemporary research has revealed that *Asarum* extracts and their inherent bioactive compounds offer multi-faceted therapeutic benefits, including (1) analgesic and anti-inflammatory activities through COX-2 and cytokine modulation; (2) neuroprotective and cardioprotective effects mediated by antioxidant pathways; (3) antitussive and immunosuppressive actions via cough reflex suppression and immune cell regulation; and (4) antineoplastic and antimicrobial capacities against various cancer cell lines and pathogenic microorganisms. These findings demonstrate the genus's therapeutic potential for managing inflammatory conditions, neurological disorders, respiratory ailments, cardiovascular diseases, malignancies, and microbial infections. The diverse pharmacological profile of *Asarum underscores* its value as a source of potential drug candidates.[23-35]

Notably, *Asarum geophilum* is a narrowly distributed species in Vietnam with significant untapped potential, particularly in medicinal and conservation applications. From *Asarum geophilum* in Vietnam, 18 compounds were isolated including, chalcononaringenin 2′,4-di-O-*β*-D-glucopyranoside (**1**), asageoside (**2**), naringenin (**3**), naringenin 5-*O*-*β*-D-glucopyranoside (**4**), naringenin 7-*O*-*β*-D-glucopyranoside (**5**), naringenin 5,7-di-*O*-*β*-D-glucopyranoside (**6**), vitexin (**7**), isorhamnetin 3-*O*-*β*-D-glucopyranoside (**8**), isorhamnetin 3-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside] (**9**), cacticin (**10**), kaempferol (**11**), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**12**), luteoside (**13**), mauritianin (**14**), alangiflavoside (**15**), quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-galactopyranoside (**16**), quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-galactopyranosyl-7-*O*-*β*-D-glucopyranoside (**17**), and aureusidin 4,6-di-*O*-glucoside (**18**).[36] The isolated compounds were predominantly flavonoids, consistent with the characteristic chemical profile of *Asarum* species. These flavonoid compounds have demonstrated significant bioactivity in regulating cell proliferation, neural development, and particularly in hematologic malignancies, potentially through mechanisms involving the JAK2 protein. For the first time, this study aimed to screen and evaluate the inhibitory potential of compounds isolated from *Asarum geophilum* against the JAK2 protein, providing a foundation for further experimental research in drug development.

Materials and Methods

Molecular docking

The AutoDock Vina v1.2.3 measured the binding affinities of compounds from *Asarum geophilum* with JAK2 protein targets. The 3D crystal structure of JAK2 (PDB ID: 3IOK) was retrieved from the RCSB PDB database (http://www.rcsb.org/). The protein was desolvated and MGLTools was employed to add missing hydrogen atoms, assign Kollman charges, and save the file in .pdbqt format. The binding sites were identified and prepared for docking.[36] The geometric structures of *Asarum geophilum* compounds were sketched using MGLTools and Marvin JS, followed by energy minimization with the MMFF94s force field using OpenBabel's obminimize command.[37,38] For docking, the grid box was centered at *x* = 13.987, *y* = 11.713, *z* = 3.211 (Å), with dimensions of 24 × 24 × 24 Å³ and a grid spacing of 1 Å. The exhaustiveness parameter had a value of 400. The results were analyzed based on binding free energy (ΔG, kcal/mol), and the pose with the strongest affinity was selected. Discovery Studio Visualizer was used to visualize protein-ligand interactions.[37,38]

Molecular dynamics (MD) and FPL method

The GROMACS v2022 software was used to perform MD simulations. The JAK2 protein topology was prepared using the Amber99SB-ILDN force field.[39] Ligand parameterization was performed using the Amber force field with ACPYPE and AmberTools22.[40-42]

Geometric parameters and atomic charges were obtained from quantum mechanical calculations (DFT/B3LYP/6-31G(d,p)), with charges assigned using the restrained electrostatic potential method.[43] The protein-ligand complex was solvated in a triclinic periodic boundary box using the TIP3P water model and neutralized with counterions (Cl^- or Na^+). Positional restraints (1000 kJ·mol^{-1}·nm^{-2}) were applied to JAK2 Cα atoms. System relaxation was achieved through energy minimization followed by NVT and NPT equilibration using established protocols.[44] For FPL simulations, an external pulling force was applied along the Z-axis reaction coordinate to the ligand's center of mass in pre-equilibrated complexes, as described by Equation (1).[45,46]

$$F = k(\vartheta t - z) \quad (1)$$

Where, K denotes the cantilever's spring constant (k = 600 kJ·mol^{-1}·nm^{-2}), ϑ is the pulling speed (ϑ = 0.005 nm/ps), and represents the displacement of the compound's center of mass. For the FPL simulations, eight independent trajectories were performed for each protein-ligand complex to ensure adequate sampling. From there, the average pulling force (F_{max}) and external work (W) were computed. The free binding energy was predicted by the LIE method, as per equation (2) proposed by Mai *et al*. (2022).[45]

$$\Delta G_{bind} = 0.2\Delta E_{b-f}^{vdW} - 0.05\Delta E_{b-f}^{cou} + 2.9\Delta G_{SASA} + 8.5 \quad (2)$$

Where ΔE_{b-f}^{cou} is the variation in average electrostatic energy, and ΔE_{b-f}^{vdW} is the variation in van der Waals interaction energy.[47]

Results and Discussion

Molecular docking and refined molecular dynamics results

This method facilitates rapid screening of compound databases (including natural compounds from plants, marine organisms, etc.) to predict binding modes and affinities of target compounds. This computational strategy helps identify promising candidates for biological testing, thereby conserving time and resources in the drug development process.[48,49] In this study, data from 18 natural compounds isolated from *Asarum geophilum* were successfully docked within the active site of the JAK2 protein. The docking simulation results are presented in Table 1 and Figure 1. The complexes' binding affinities were calculated in the range of -6.630 to -9.521 kcal/mol, with an average value of -8.209 kcal/mol. According to the docking results, nine top-lead compounds (Table 1) with the best binding affinities to JAK2, having ΔG_{dock} values less than -8.024 kcal/mol (dock score of native inhibitor - 2-Aminopyrazolo[1,5-a]pyrimidines), namely **7**, **8**, **9**, **11**, **12**, **13**, **14**, **16**, and **18**, were selected for further investigation. Subsequently, potential conformational changes in the top-lead compounds were investigated to assess their interactions with JAK2 protein's binding region amino acid residues. Gohlke *et al*.[50] reported that ligand partial charges calculated via the PM6 method significantly enhance binding conformation and cluster population, leading to more accurate docking.[50] Figure 1 illustrates the specific interactions of the JAK2 protein with the nine potential compounds after docking and after molecular dynamics (MD) refinement. After transitioning from an implicit solvent environment (docking simulation) to an explicit solvent model (MD simulation), the structures of the protein-ligand complexes were partially refined. The results after MD simulating showed that in complex 7 - JAK2, hydrogen bonding with important amino acid residues Ser936, Lys857, and Asp994 was maintained.

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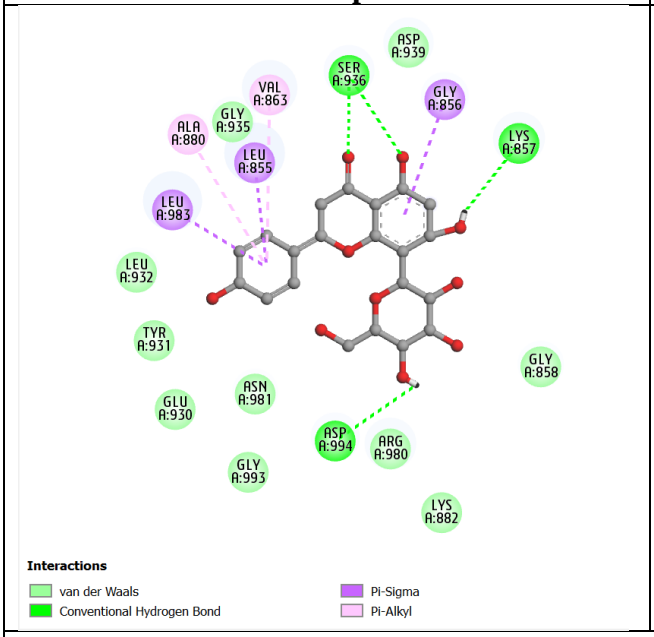
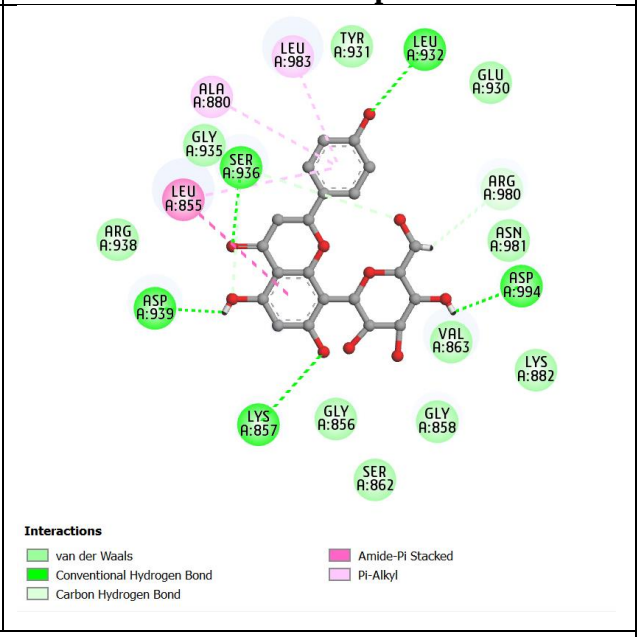
Docked pose	MD-refined pose
	
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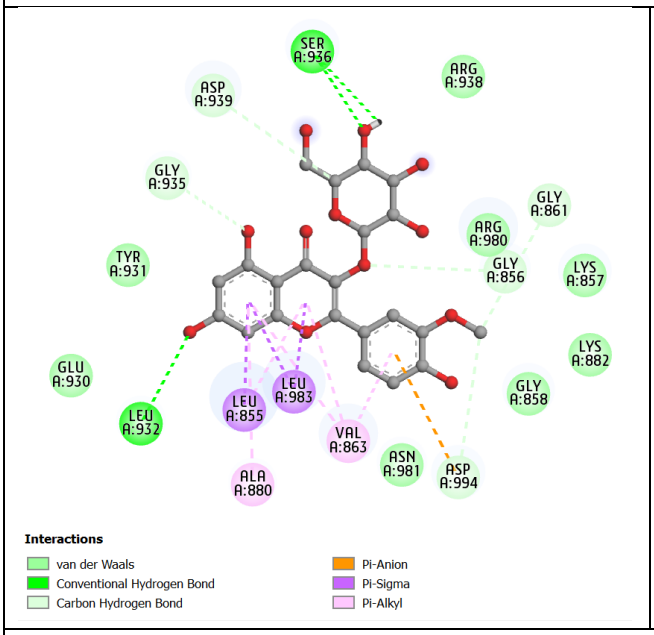	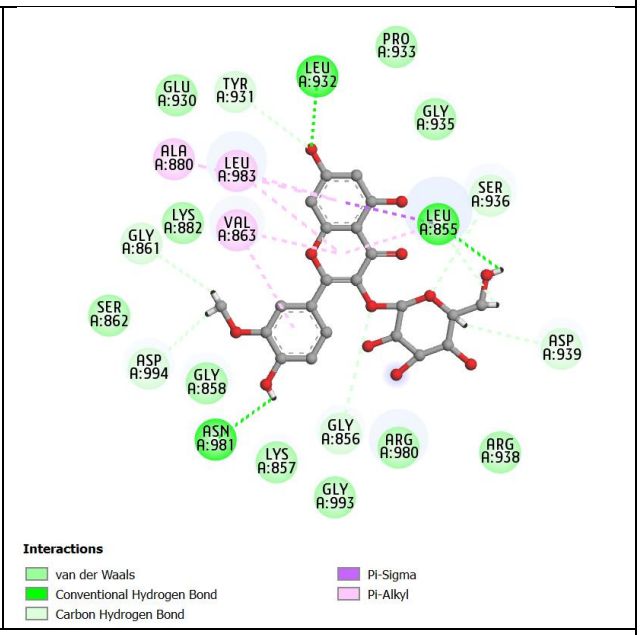
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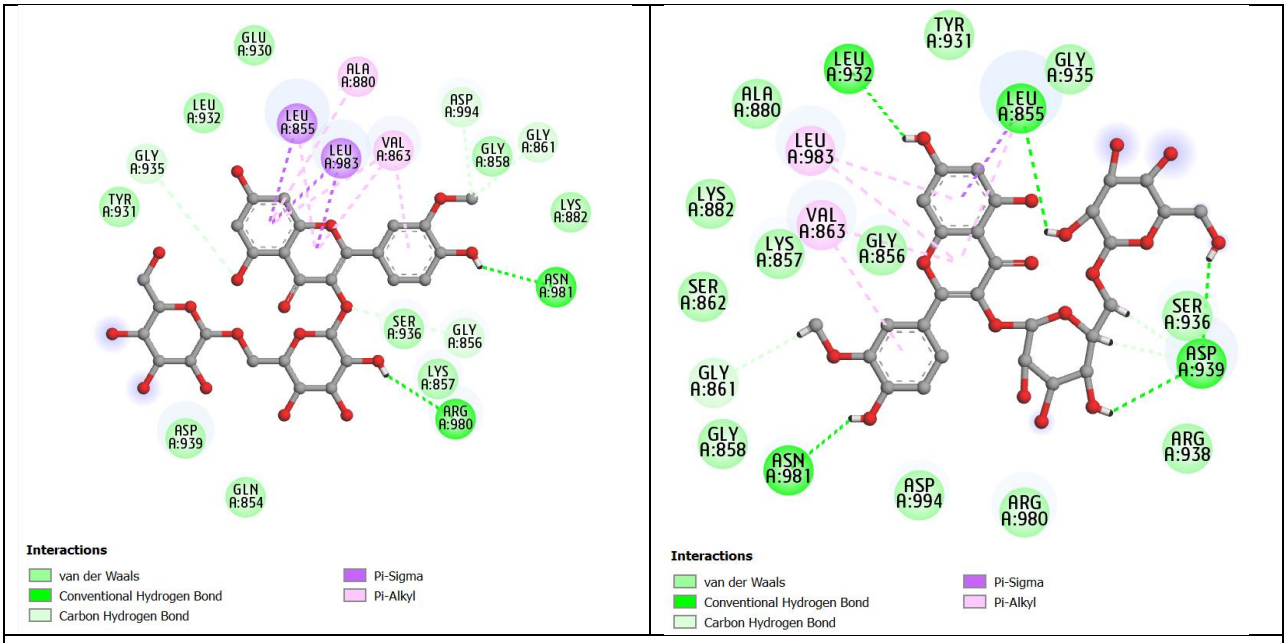

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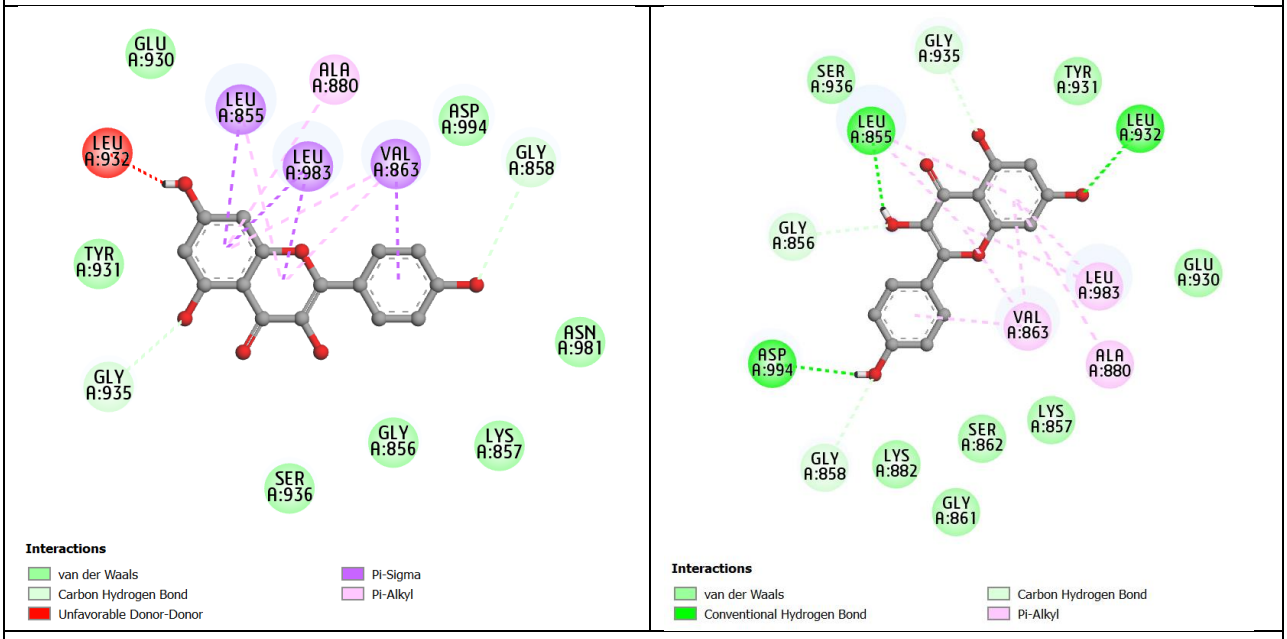

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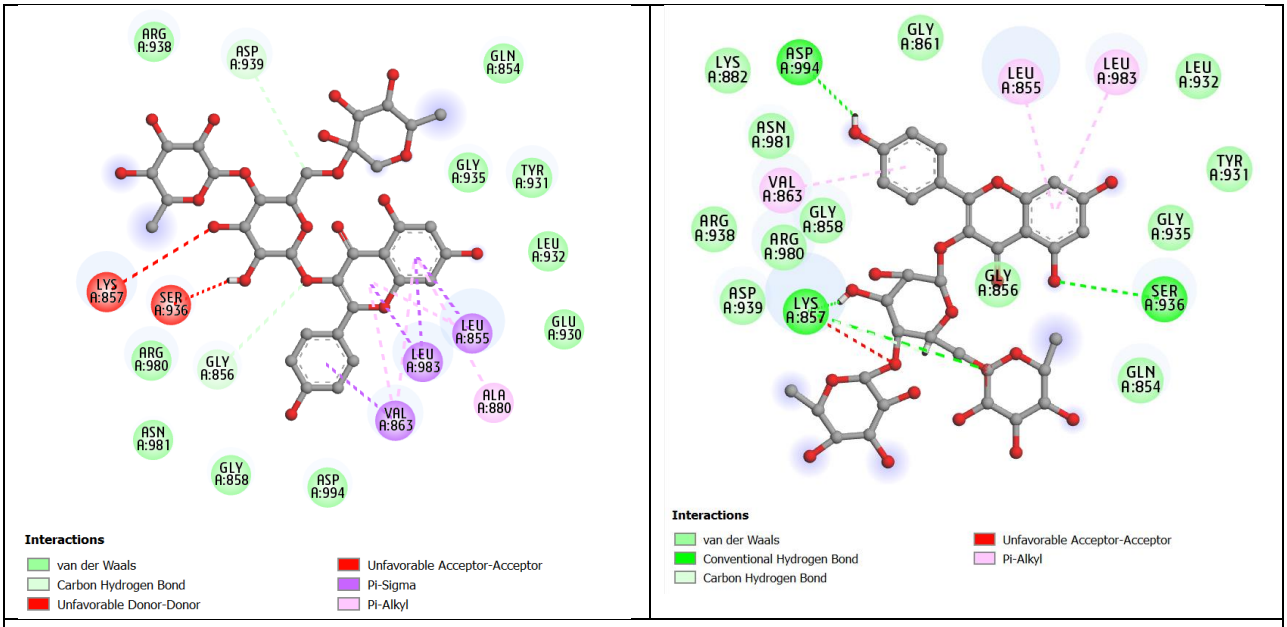

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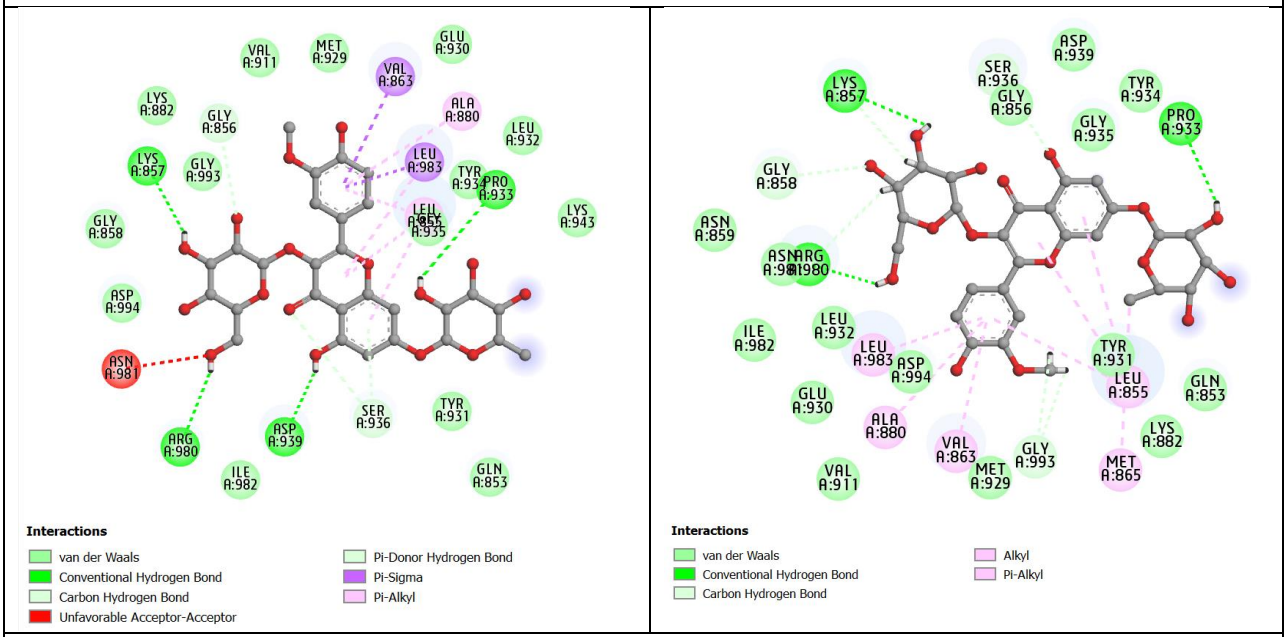

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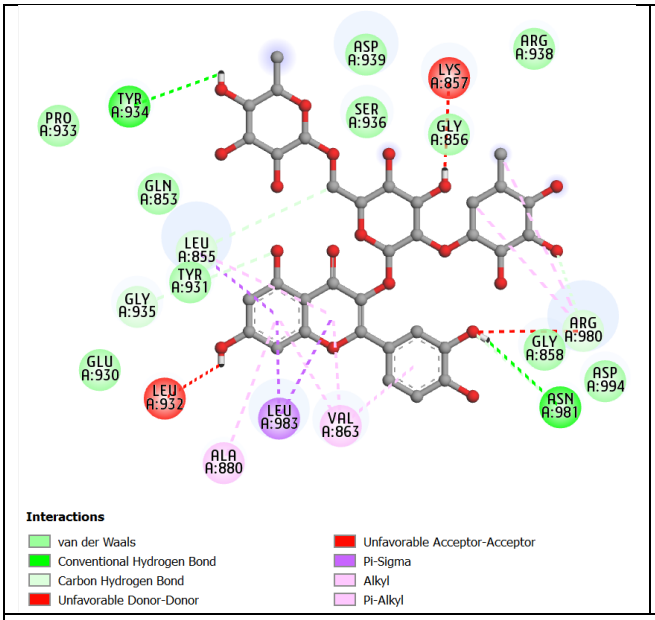

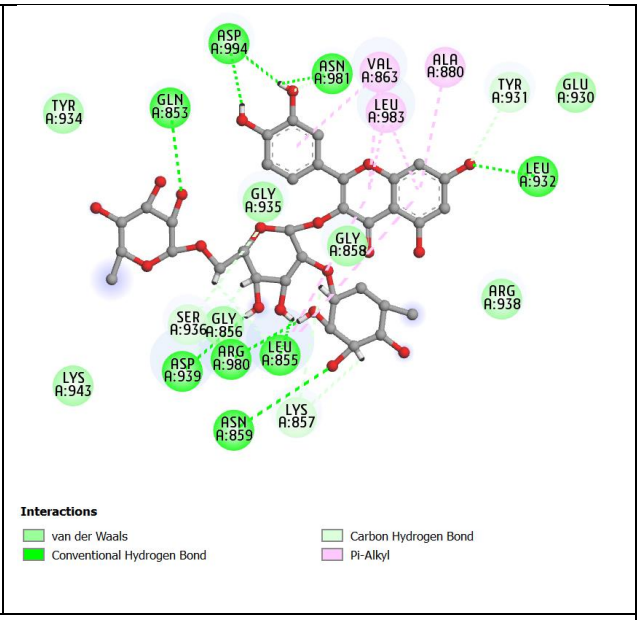

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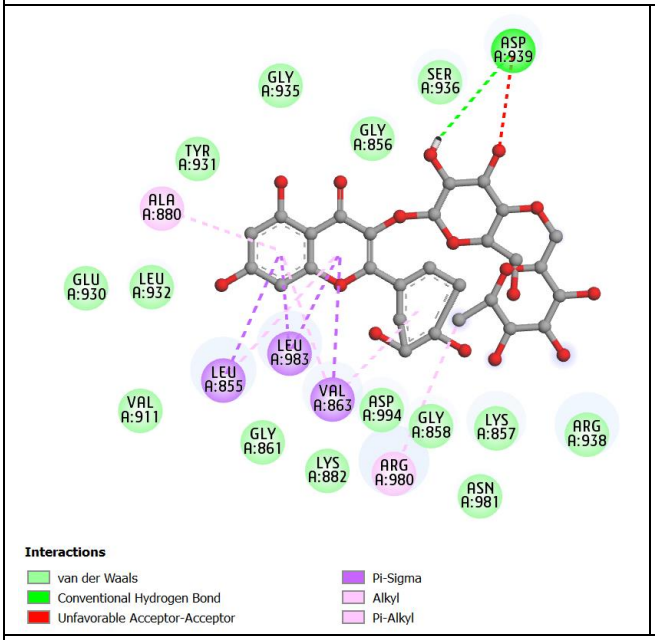

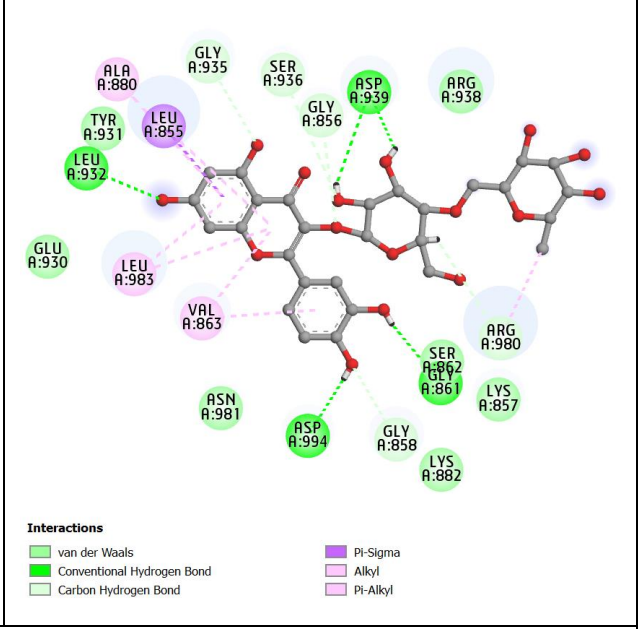

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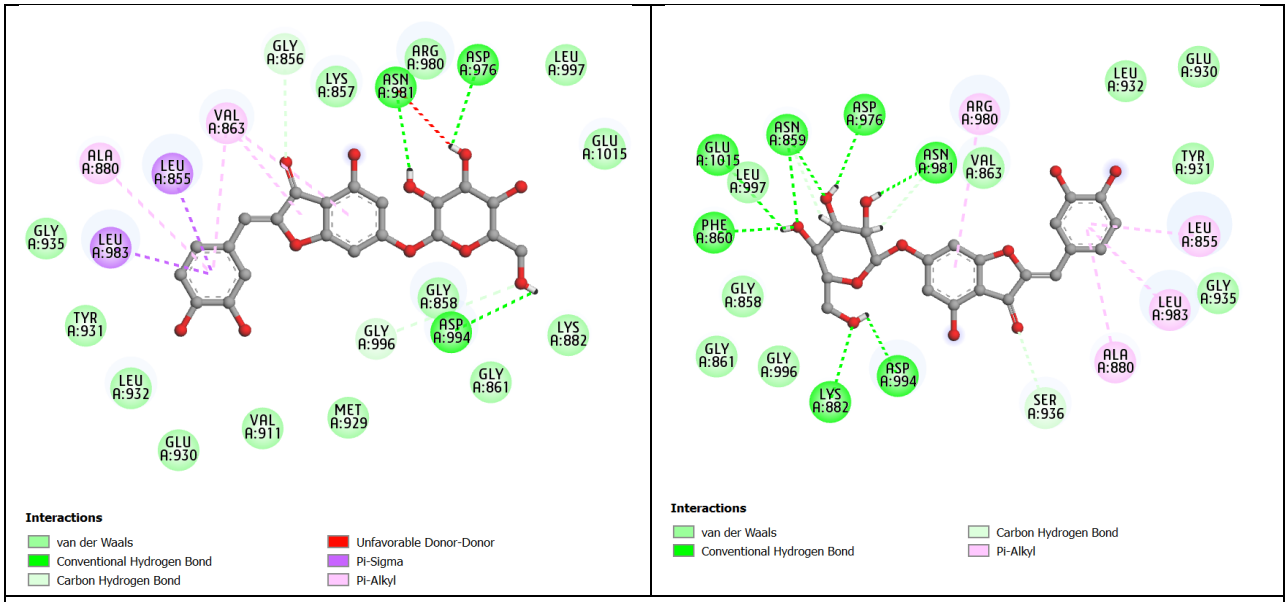

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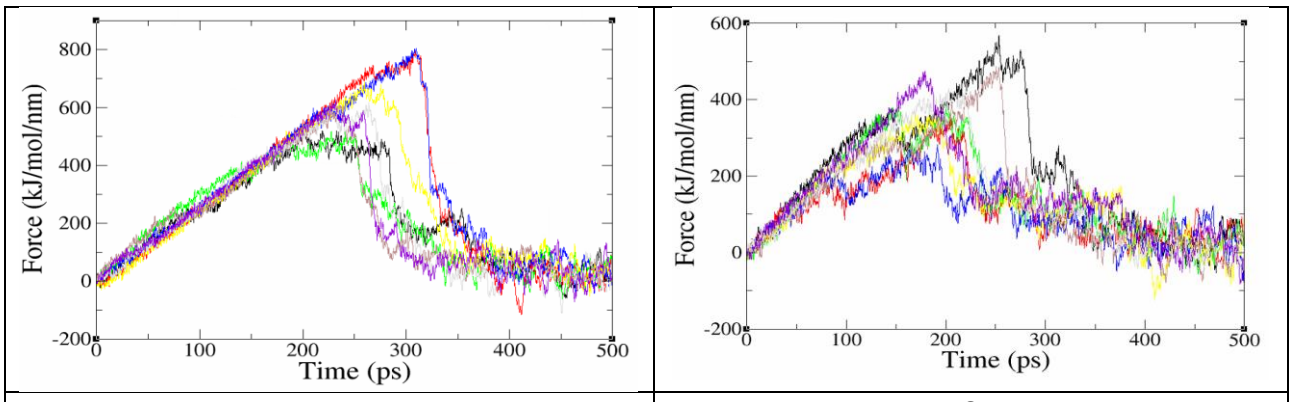
Figure 1: 2D visualization of the interaction of docked and refined-MD structures of compounds **7**, **8**, **9**, **11**, **12**, **13**, **14**, **16**, and **18** within the binding pocket of the JAK2 protein

Additionally, Asn981 formed a hydrogen bond with the molecule. Flavonoid **8** maintained hydrogen bonding interactions between the hydroxyl group at the C-7 position in the molecule and Leu932 throughout the MD simulation. Meanwhile, the hydroxyl group attached at the C-4' position in flavonoid **9** established a hydrogen bond with residue Asn981. Furthermore, compounds **8** and **9** also formed hydrogen bonds with other crucial amino acid residues, specifically Leu932 and Leu855. As for compounds **11** and **12**, no hydrogen bonds were found in the molecular docking simulation, but after undergoing MD simulation in a virtual physiological environment, hydrogen bonds were established. Specifically, compound **11** formed three hydrogen bonds with important amino acid residues Leu932, Leu855, and Asp994, while compound **12** in the binding pocket of JAK2 protein formed bonds with residues Asp994, Ser936, and Lys857. The JAK2-13 complex remained stable, maintaining three hydrogen bonds with essential amino acid residues, namely Lys857, Pro933, and Arg980, after running MD. Meanwhile, the 3'-OH of the C-ring in molecule **14** stabilized hydrogen bonding with residue Asn981. Compound **16** formed hydrogen bonds with amino acid residues Leu932, Asp939, Asp994, and Gly861, with Asp939 exhibiting stable interaction with this compound. Compound **18** established hydrogen bonds with residues Glu1015, Asn859, Asp976, Asn981, Phe860, Lys882, and Asp994 in the final trajectory of the MD simulation. As known, hydrogen bonds play a crucial role in inhibiting specific target activities. Therefore, these creation of bonds between important amino acid residues in the inhibitory region of JAK2 protein with the studied compounds, makes the compounds promising drug candidates.

Estimated binding affinity of top-lead compounds via FPL/LIE approach

In the previous report, the relative binding affinities of JAK2 inhibitors were calculated through the combination of FPL simulations and an optimized LIE model, demonstrating good efficiency with a correlation coefficient (R) of 0.82 between predicted and experimental values. Furthermore, the integration of these methods facilitated the rapid screening of the most promising JAK2 inhibitors from a diverse pool of existing compounds, offering potential candidates for further *in vitro* studies. The ΔG_{LIE} binding affinity values of the top-lead compounds are detailed in Table 2. It was found that the ΔG_{LIE} values range from -8.35 to -21.78 kcal/mol, with compound **14** exhibiting the strongest binding affinity to the JAK2 protein with ΔG_{LIE} = -21.78 kcal/mol. The average pulling works and forces of this compound also indicate its significant ability to dissociate the ligand from the active site of the JAK2 protein, surpassing other potential compounds examined (Table 1). Notably, when comparing the ΔG_{LIE} values of the nine top-lead compounds with known JAK2 inhibitors, the results show that these top-lead compounds exhibit significantly stronger binding capabilities (Figure 2). Therefore, these compounds may be the most promising candidates for *in vitro* biological experiments related to anti-inflammatory and anti-cancer properties.



7 **8**

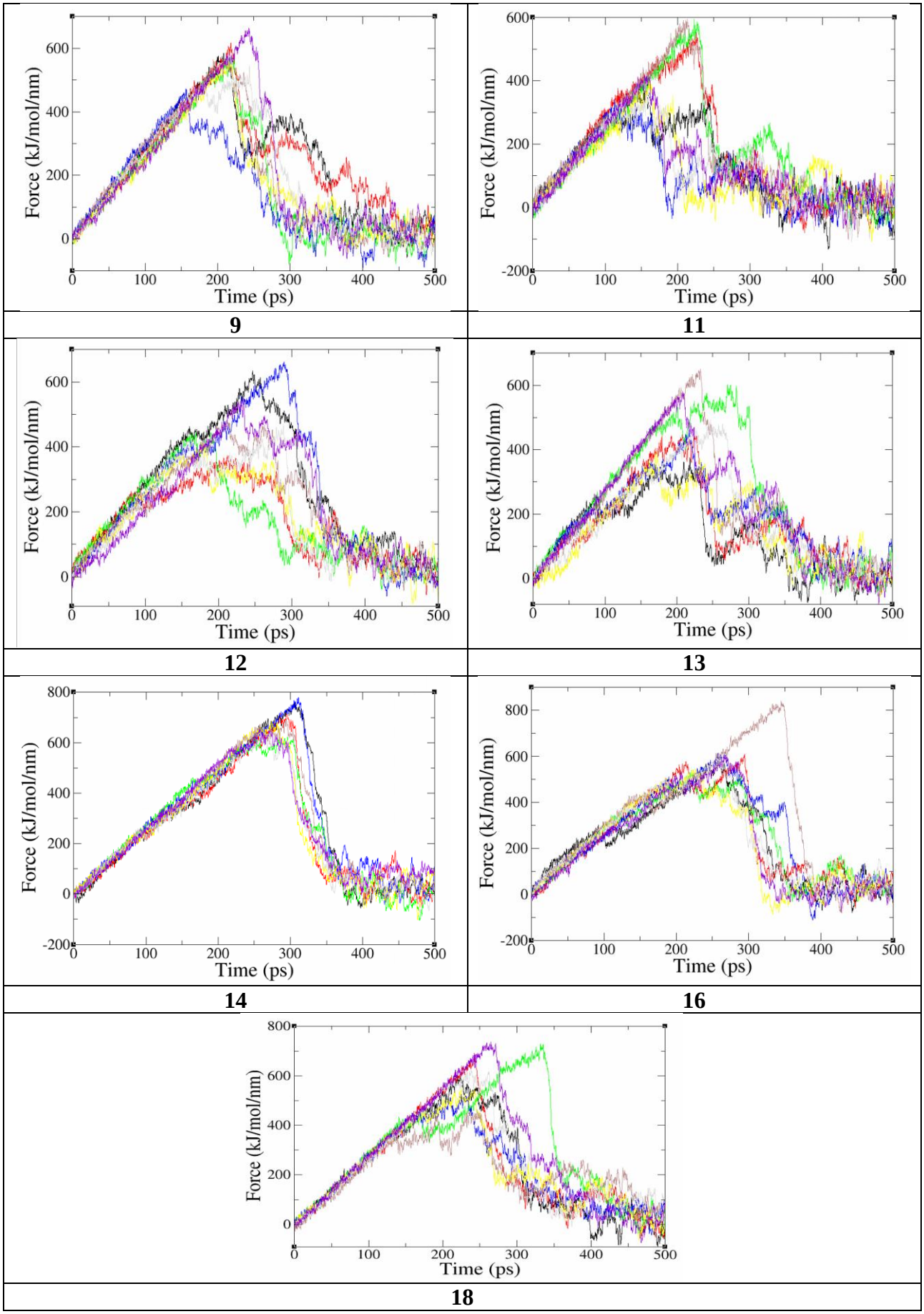


Figure 2: Force-time profiles of eight trajectories obtained from the FPL simulation of the top-lead compounds with JAK2 protein

Table 1: The estimated binding affinity, average maximum force, and average maximum work of top-lead compounds

Compound	Binding affinity (kcal/mol)	Average force (pN)	Average work (kcal/mol)
7	-9.352	955.760	124.171
8	-8.280	619.466	77.641
9	-8.487	847.101	104.399
11	-8.354	678.827	74.867
12	-8.831	744.653	110.668
13	-8.988	739.775	97.061
14	-8.787	1028.032	139.722
16	-8.927	915.758	130.491
18	-9.521	909.883	128.487

Table 2: Average interaction energy variances (kcal/mol) and LIE predictions for ligands

Compound	$\Delta E^{cou}_{b\text{-}f}$ (kcal/mol)	$\Delta E^{vdW}_{b\text{-}f}$ (kcal/mol)	ΔG_{SASA} (kcal/mol)	ΔG_{LIE} (kcal/mol)
7	13.44 ± 10.65	-19.79 ± 8.34	-6.15 ± 0.14	-13.96 ± 2.02
8	-6.93 ± 7.32	-19.14 ± 2.35	-6.51 ± 0.14	-13.86 ± 1.09
9	-4.89 ± 6.81	-25.52 ± 4.85	-7.22 ± 0.16	-17.31 ± 0.96
11	4.37 ± 6.92	-14.18 ± 1.78	-4.76 ± 0.11	-8.35 ± 0.87
12	3.38 ± 6.79	-26.54 ± 3.27	-6.80 ± 0.44	-16.70 ± 1.87
13	13.06 ± 8.40	-22.91 ± 5.32	-7.64 ± 0.24	-18.90 ± 1.81
14	-2.29 ± 8.87	-27.98 ± 2.20	-8.55 ± 0.21	-21.78 ± 1.25
16	12.67 ± 10.13	-25.70 ± 2.47	-7.57 ± 0.12	-19.23 ± 0.99
18	-1.77 ± 10.26	-22.26 ± 2.09	-7.22 ± 0.05	-16.81 ± 0.93

Conclusion

Finding from this study have revealed the significant potential of *Asarum geophilum*-derived compounds as novel JAK2 inhibitors. Compound **14** emerged as the most promising candidate, exhibiting superior binding compared to known JAK2 inhibitors. Its strong binding affinities and stable interactions with critical JAK2 active site residues, consistently maintained throughout MD simulations, suggest its potency as anti-inflammatory and anti-cancer therapies. These findings highlight three key research prospects. First, the top-performing compounds, particularly compound **14**, show superior binding compared to known JAK2 inhibitors, making them prime candidates for preclinical development targeting JAK2-related disorders. Second, the identified flavonoid scaffolds provide a valuable structural basis for rational drug design to optimize selectivity and potency. Third, this work validates *Asarum geophilum* as a rich source of bioactive compounds, significantly contributing to natural product-based drug discovery.

Future studies should prioritize structural modification of lead compounds, comprehensive *in vitro* and *in vivo* validation of JAK2 inhibitory activity, and ADMET profiling to advance promising candidates. This research not only expands the pool of JAK2-targeting agents but also underscores the untapped therapeutic potential of medicinal plant resources.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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