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Two Coumarin Compounds Isolated from Scadoxus Multiflorus as a Potential Therapeutic Candidates against Plasmodium Falciparum Enzymes through Insilco Approach

Akeem Ayodele Olaiya

olaiyaakeemayodele@gmail.com

Ahmadu Bello University

Yusuf Jimoh

Ahmadu Bello University

Tawakaltu Omolara Tijani

Ahmadu Bello University

Sakynah Abdullahi Musa

Ahmadu Bello University

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Abstract

The rising incidence of *Plasmodium falciparum* resistance to existing antimalarial drugs continues to undermine global malaria control efforts, particularly in developing countries where the disease remains a major cause of morbidity and mortality. Given the emergence of drug-resistant strains and the limited pipeline of new therapeutic leads, the search for novel antimalarial agents from natural sources is of critical importance. In this study, two coumarin derivatives isolated from *Scadoxus multiflorus* were investigated for their potential antimalarial activity using an in-silico approach. The molecular structures of the compounds were generated with ChemDraw and energy-minimized using Spartan14. Their pharmacokinetic properties and toxicity profiles were predicted using SwissADME and ProTox 3.0, respectively. Molecular docking was conducted with AutoDock Vina to evaluate binding interactions with key *P. falciparum* enzymes, including Falcipain-2, Falcipain-3, and Plasmepsin-2. Compound B12 (2-methyl-2H-chromen-7-ol) exhibited binding affinities between − 5.3 and − 5.6 kcal/mol, whereas compound C11 (7- methoxy-2H-chromen-2-one) demonstrated slightly stronger affinities ranging from − 5.9 to − 5.6 kcal/mol. Both compounds engaged in favorable hydrogen bonding, van der Waals interactions, and π–π stacking with active-site residues. Drug-likeness assessments confirmed compliance with Lipinski's rule of five, and toxicity predictions revealed acceptable safety margins (LD50: 500 mg/kg for B12 and 4300 mg/kg for C11). Collectively, these findings suggest that the tested coumarin derivatives possess desirable pharmacological properties and a multi-target binding profile, supporting their candidacy for further development as antimalarial agents. Experimental validation through in vitro and in vivo studies is warranted to confirm their therapeutic potential and advance them toward clinical applicability.

Introduction

Malaria remains one of the most pressing infectious diseases globally, with an estimated 249 million cases and over 600,000 deaths reported in 2022, the majority of which occurred in sub-Saharan Africa (WHO, 2023). The disease is caused by protozoan parasites of the genus *Plasmodium*, with *Plasmodium falciparum* being the most virulent and responsible for the most severe forms of the illness, while *Plasmodium vivax* contributes significantly to morbidity in Asia and Latin America (Erhirhe et al., 2021; Olaiya et al., 2025). Malaria transmission is influenced by a complex interplay of factors including vector species diversity, climatic conditions, and human behavioral patterns, with peak transmission typically observed during the rainy season in tropical and subtropical regions (Okello & Kang, 2019). Despite the introduction of vector control strategies and chemotherapeutic interventions, malaria continues to impose a considerable socioeconomic burden, disproportionately affecting children under five years and pregnant women (WHO, 2023).

Chemotherapy remains the cornerstone of malaria management, with artemisinin-based combination therapies (ACTs) serving as the recommended first line treatment for *P. falciparum* malaria since 2006 (Ouji et al., 2018). ACTs pair a fast-acting artemisinin derivative with a longer acting partner drug to reduce treatment failure and delay the emergence of resistance. However, the recent decline in ACT

efficacy in Southeast Asia, characterized by delayed parasite clearance and increasing treatment failures, highlights a concerning trend of artemisinin and partner-drug resistance (Blasco et al., 2017). Reports of molecular markers of resistance such as *kelch13* mutations further raise alarms that resistance could spread to Africa, as was the case with chloroquine and sulfadoxine pyrimethamine in previous decades (Coronado et al., 2021). This growing threat underlines the urgent need for novel chemotypes and innovative therapeutic strategies targeting unique biochemical pathways of *P. falciparum* to maintain progress toward malaria elimination.

Natural products have historically been a rich source of antimalarial agents, exemplified by the discovery of quinine from *Cinchona* bark and artemisinin from *Artemisia annua*. In this context, coumarins a structurally diverse class of benzopyran derivatives have emerged as promising scaffolds for drug discovery. They are widely distributed across higher plants, with umbelliferone being a well-characterized representative (Olaiya et al., 2024). Coumarins exhibit a broad range of pharmacological activities including antitumor, antimicrobial, anticoagulant, antioxidant, and anti-inflammatory effects, which have been attributed to their ability to modulate multiple biological targets (Bermejo et al., 2019). The chromone ring system present in coumarins is also a key structural motif in bioactive natural products such as α-tocopherol, a potent antioxidant known to prevent oxidative stress related pathologies including cancer and cardiovascular diseases (Bermejo et al., 2019).

Isocoumarins, polyketide-derived analogues of coumarins, further expand this pharmacological potential. Several members of this class, including monocerin and furobenzopyranone derivatives, have demonstrated activities against protozoan parasites, bacteria, and insects, making them attractive leads for anti-infective drug discovery (WHO, 2019; Coronado et al., 2021). Despite these promising findings, the antimalarial potential of coumarins from *Scadoxus multiflorus* has not been extensively studied.

The present study aims to bridge this knowledge gap by employing an in-silico approach to evaluate two coumarin derivatives isolated from *Scadoxus multiflorus*, designated compound B12 (2-methyl-2H-chromen-7-ol) and compound C11 (7-methoxy-2H-chromen-2-one), as potential inhibitors of key *P. falciparum* enzymes. Molecular docking, pharmacokinetic prediction (ADMET), and toxicity profiling were performed to assess their drug-likeness, safety, and interaction profiles with Falcipain-2, Falcipain-3, and Plasmepsin-2, critical enzymes involved in hemoglobin degradation and parasite survival. To the best of our knowledge, this is the first report to provide computational insights into these coumarin derivatives as multi-target therapeutic candidates for antimalarial drug development.

Material and method

2.1 Generation and Optimization of Molecular Structures

The two-dimensional (2D) chemical structures of the bioactive compounds B12 (2-methyl-2H-chromen-7-ol) and C11 (7-methoxy-2H-chromen-2-one), previously isolated from *Scadoxus multiflorus* (Olaiya et al., 2024), were initially generated using ChemDraw software to obtain accurate molecular

representations. Subsequently, the structures were converted to three-dimensional (3D) forms and geometrically optimized using Spartan14 software. Energy minimization was carried out to achieve the most stable conformations, thereby ensuring their suitability for downstream computational analyses.

2.2 Assessment of Bioavailability and Toxicity

The pharmacokinetic properties of the compounds, including absorption, distribution, metabolism, and excretion (ADME), as well as their drug-likeness and overall medicinal chemistry suitability, were predicted using the SwissADME web server (http://www.swissadme.ch/index.php) (Boudou et al., 2023). In addition, potential toxicological profiles were assessed using the ProTox 3.0 platform (https://comptox.charite.de/protox3/), which evaluates toxicity risks based on multiple molecular descriptors and predictive models.

2.3 Preparation of Ligands and Target Enzymes

For the molecular docking studies, the crystal structures of Falcipain-2 (PDB ID: 6JW9) (Chakraborty et al., 2021), Falcipain-3 (PDB ID: 3BWK) (Kerr et al., 2009), and Plasmepsin-2 (PDB ID: 1LF3) (Asojo et al., 2003), were obtained from the Protein Data Bank (PDB). Protein preparation was carried out in accordance with established protocols (Pettersen et al., 2004), which included the addition of hydrogen atoms, removal of crystallographic water molecules, and assignment of appropriate charges to optimize the structures for docking. The active sites of the enzymes were carefully defined to ensure precise ligand receptor interactions and enhance the reliability of the docking simulations.

2.4 Molecular Docking Studies

Molecular docking simulations were performed using AutoDock Vina, following validated protocols (Trott et al., 2010). This software was employed to predict the binding affinities and preferred binding conformations of the isolated compounds within the active sites of the selected target enzymes. Grid box parameters for Falcipain-2, Falcipain-3, and Plasmepsin-2, were carefully defined (Table 1) to ensure comprehensive coverage of the catalytic pockets and to facilitate accurate identification of the most favorable ligand enzyme interactions.

2.5 Analysis of Docking Results

Post-docking analyses were carried out using Biovia Discovery Studio to evaluate docking scores, binding conformations, and critical molecular interactions between the ligands and target enzymes. Key contacts, including hydrogen bonds, hydrophobic interactions, and π-related interactions, were examined to elucidate the structural determinants contributing to binding affinity. These analyses provided mechanistic insights into the potential inhibitory effects of the compounds on Falcipain-2, Falcipain-3, and Plasmepsin-2, thereby highlighting their relevance as prospective antimalarial agents.

Table 1
Grid-box parameters for the enzymes

Enzyme	Gridbox Size			Center		
	X	Y	Z	X	Y	Z
FP-2	**27**	**25**	**22**	**-8.889**	**15.368**	**-38.694**
FP-3	**18**	**17**	**14**	**5.96**	**-22.35**	**50.07**
Plm-2	**13**	**26**	**20**	**16.22**	**6.85**	**27.61**

Results and Discussion

3.1 Docking Parameters and Methodology

Molecular docking of B12 and C11 against FP-2, FP-3, and Plm-2 utilized tailored grid-box parameters (Table 1), with FP-2's 27 × 25 × 22 Å grid centered at (-8.889, 15.368, -38.694) and Plm-2 at 20. These settings, optimized via AutoDock Vina [Trott and Olson; 2010], to ensured precise binding predictions, underpinning the reliability of our *in-silico* inquiries.

3.2 Drug-Likeness of B12 and C11

The pharmacokinetic and physicochemical assessment of the isolated coumarin derivatives, B12 (2-methyl-2H-chromen-7-ol) and C11 (7-methoxy-2H-chromen-2-one), as presented in Table 3, showed strong compliance with Lipinski's rule of five, confirming their potential as orally bioavailable and drug-like molecules (Lipinski, 2001, 2004). Both compounds have relatively low molecular weights (162.19 and 176.17 g/mol, respectively), moderate lipophilicity (MLogP values of 1.62 and 1.34), and an acceptable number of hydrogen bond donors and acceptors. These parameters indicate good solubility and membrane permeability, two essential determinants for efficient gastrointestinal absorption. SwissADME predictions further classified both compounds as having high GI absorption, while toxicity evaluations (ProTox 3.0) revealed LD_{50} values of 500 mg/kg for B12 (toxicity class 4) and 4300 mg/kg for C11 (toxicity class 5), suggesting low acute toxicity and safe therapeutic potential. The lower toxicity of C11 may be associated with the methoxy moiety, which enhances metabolic stability and reduces oxidative vulnerability compared to the hydroxyl group in B12 (Boudou et al., 2023).

3.3 Binding Interactions and Affinities

The molecular docking results summarized in Table 3 and illustrated in Figs. 1–6 revealed that both compounds effectively interact with key Plasmodium falciparum enzymes (Falcipain-2, Falcipain-3, and Plasmepsin-2) responsible for hemoglobin degradation and parasite survival. In Falcipain-2 (PDB 6JW9), B12 and C11 demonstrated binding affinities of − 5.3 and − 5.9 kcal/mol, respectively, both slightly stronger than the co-crystallized inhibitor E64 (−5.1 kcal/mol). As observed in Figs. 1 and 4, both ligands

formed hydrogen-bond and π–π stacking interactions with essential residues such as Cys42, His174, and Asn173. These residues are part of the enzyme's catalytic dyad and active-site wall (Chakraborty et al., 2022; Jimoh et al., 2024). The chromen (benzopyran) ring present in both compounds provides a conjugated π-system that promotes van der Waals and π–π interactions with hydrophobic residues like Leu84, Ile85, and Ala175, stabilizing the ligand within the catalytic cavity. The hydroxyl group at position 7 in B12 can donate hydrogen bonds, enhancing binding affinity through polar interactions, while the methoxy group in C11 increases electron density on the aromatic ring, strengthening π–π stacking with aromatic residues such as Tyr78 and Phe236. These electronic effects explain the slightly improved affinity of C11 compared to B12.

In Falcipain-3 (PDB 3BWK), the docking analysis revealed moderate binding affinities of − 5.1 kcal/mol for B12 and − 5.2 kcal/mol for C11 compared with the reference inhibitor C1P (–7.1 kcal/mol). Both ligands occupied the enzyme's hydrophobic cleft and engaged in hydrogen bonding with residues Gly91 and Glu243, as well as π-interactions with Asn182, His183, and Tyr93, as depicted in Figs. 2 and 5. The coumarin nucleus again played a dominant role in these interactions by providing a planar aromatic surface that fits well within the protease's hydrophobic environment. The methoxy moiety in C11 acts as a mild electron donor, enhancing resonance delocalization across the chromen system and facilitating stronger π-stacking contacts. Meanwhile, the hydroxyl group of B12 may form intramolecular hydrogen bonding, slightly reducing its external hydrogen-bonding potential, which could account for its lower binding score.

For Plasmepsin-2 (PDB 1LF3), both compounds displayed binding energies of − 5.6 kcal/mol, compared to − 10.0 kcal/mol for the co-crystallized ligand EH5. Although the reference ligand binds more tightly due to its peptidic nature, both coumarin derivatives occupied the active pocket containing Asp34, Asp214, Tyr77, and Phe111, forming hydrogen bonds with the catalytic aspartates (Figs. 3 and 6). These residues are critical for the proteolytic activity of Plasmepsin-2 (Asojo et al., 2003). The planar coumarin ring promotes π–π stacking with Phe111 and Tyr77, while its conjugated oxygen atoms serve as hydrogen bond acceptors to stabilize the enzyme ligand complex. The electron-donating methoxy group of C11 enhances these interactions by increasing the aromatic ring's polarizability, whereas the hydroxyl group in B12 provides complementary hydrogen-bonding strength. Together, these features demonstrate that both functional groups (hydroxyl and methoxy) contribute uniquely to protein ligand stabilization through distinct electronic and spatial effects.

3.4 Comparative Insights and Novelty

Overall, the results in Tables 2 and 3 and Figs. 1–6 collectively indicate that the two coumarin derivatives possess desirable drug-like and binding properties. Their aromatic benzopyran core allows extensive van der Waals and π–π stacking within enzyme cavities, while the oxygen-containing substituents at position 7 fine-tune polarity and interaction specificity. Although their binding energies are moderately lower than those of the co-crystallized ligands, the similarities in binding orientation and contact residues underscore their potential as natural, non-peptidic scaffolds for further optimization. These interaction profiles agree with previous studies showing that substituted coumarins interact favorably with cysteine

protease residues via aromatic and hydrogen-bond contacts (Garba et al., 2024; Jimoh et al., 2024; Kerr et al., 2009), (Ouji et al., 2018; Olaiya et al., 2024; Olaiya et al., 2025).

Conclusion

The research work inquire the therapeutic promise of two coumarin compounds isolated from *Scadoxus multiflorus*, as a potential drug candidate for the management of malaria associated enzymes via *insilico* approach. The results showcase how these natural compounds can engage with distinct malaria targets, the combination of favorable pharmacokinetics, low toxicity, and effective binding interactions positions these compounds as promising candidates for developing next-generation antimalarial agents targeting multiple proteolytic enzymes in Plasmodium falciparum.

Declarations

Author Contributions

Olaiya Akeem Ayodele

Contribution: Conceptualization, Investigation, Methodology, Supervision, software

Resources, Software, Validation, Visualization, Writing-Original Draft, Writing - Review & Editing.

Jimoh Yusuf

Contribution: Methodology, software, Visualization, Writing – original draft, Writing – review & editing

Tijani Tawakaltu Omolara

Contribution: Conceptualization, Methodology, Formal Analysis, Project administration, Writing - review & editing

Abdullahi Sakynah Musa

Contribution: Investigation, Methodology, Formal analysis, Project administration, Resources, Software, supervision

Funding Declaration

Not Applicable

Clinical Trial Number

Not Applicable

Ethics, Consent to Publish Declaration

Not Applicable

Data Availability Statement

All the data used and/or analyzed during the current study are included in this work

Consent to Participate

Not Applicable

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Table 2 To 3

Table 2 To 3 are available in the Supplementary Files section.

Figures

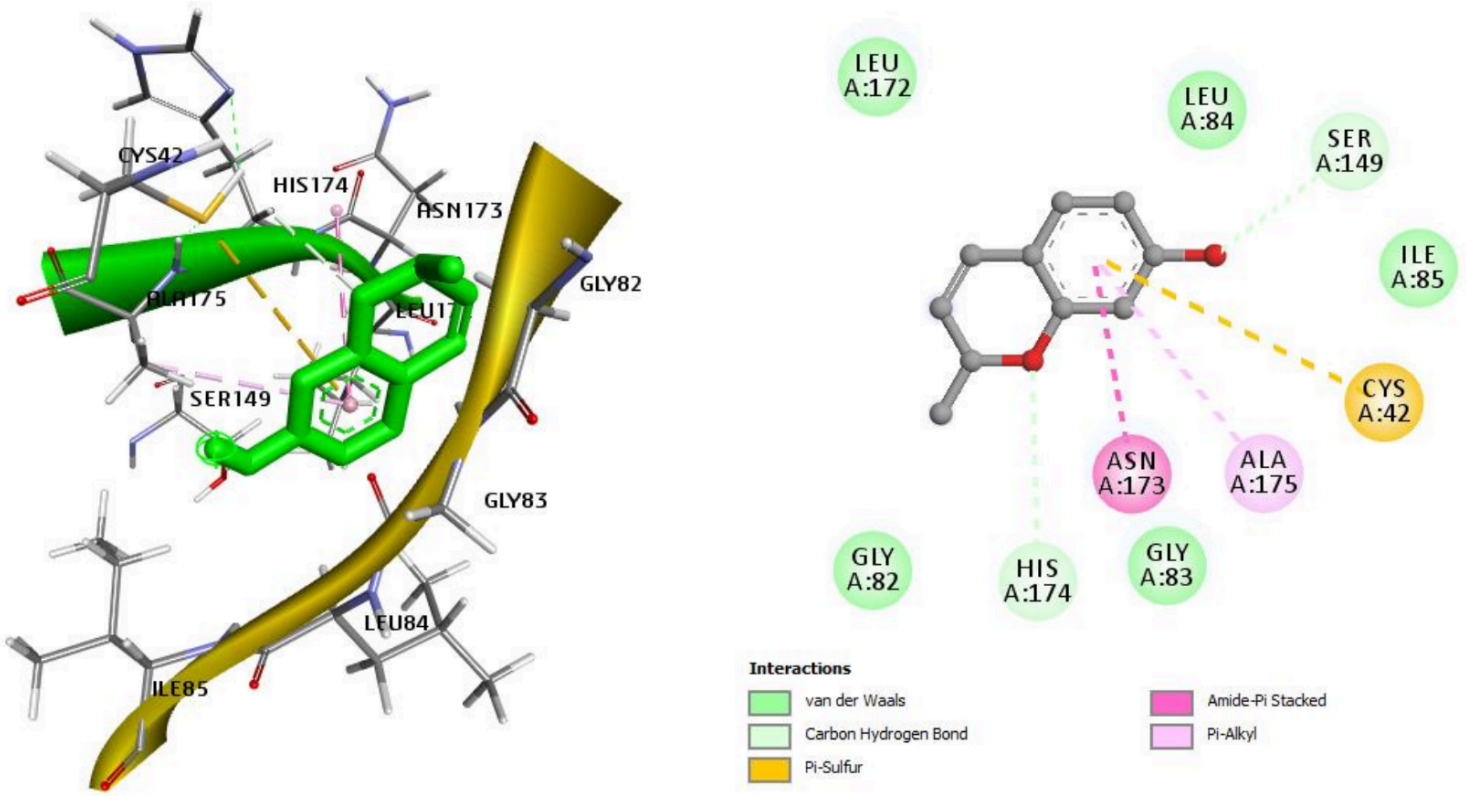

Figure 1

3D and 2D Binding pose interaction of B12 at the active site of falcipain-2

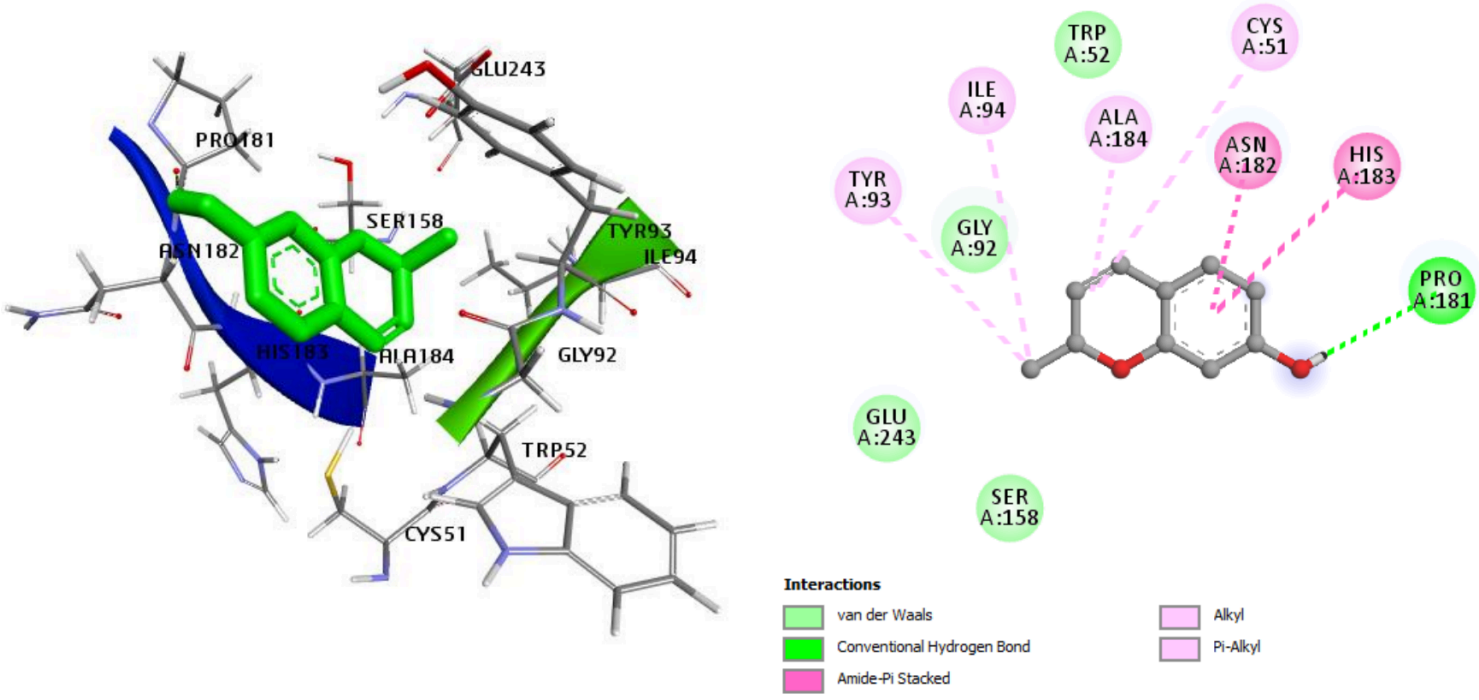

Figure 2

3D and 2D Binding pose interaction of B12 at the active site of falcipain-3

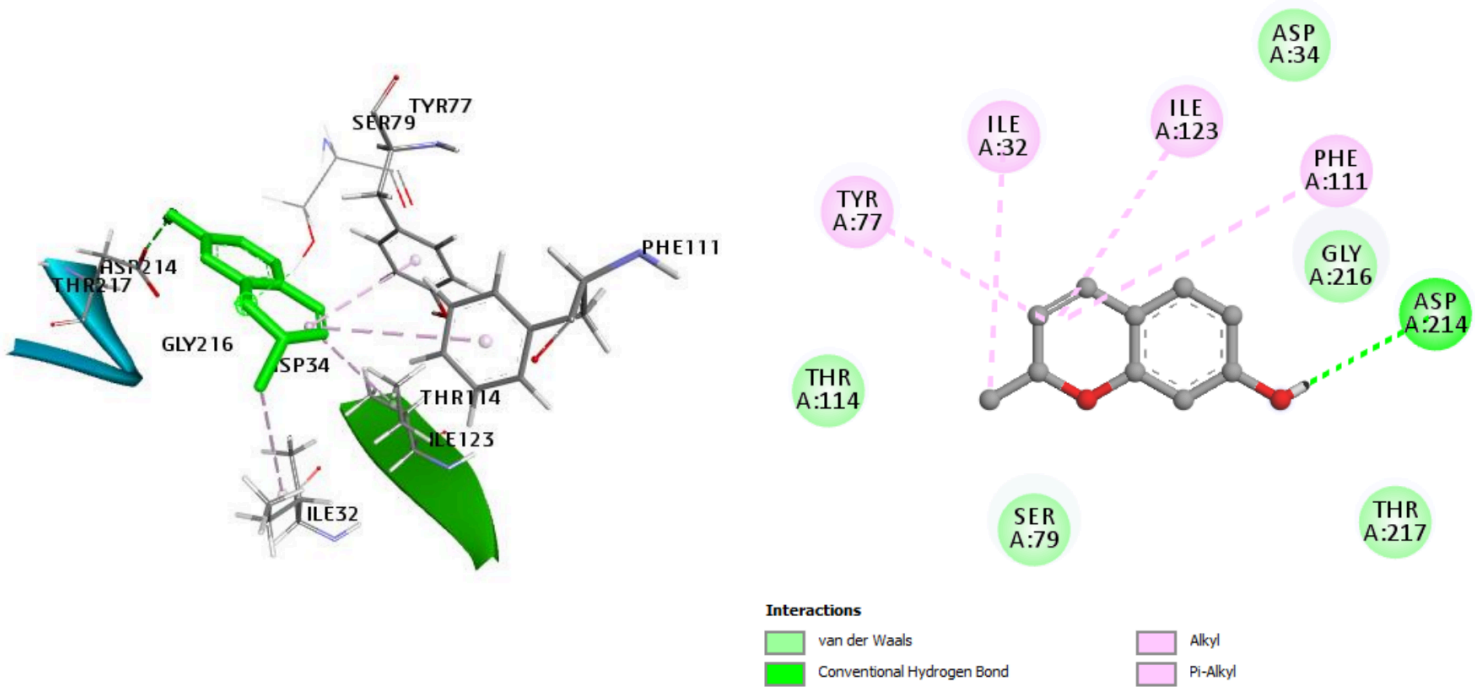

Figure 3

3D and 2D Binding pose interaction of B12 at the active site of plasmepsin-2

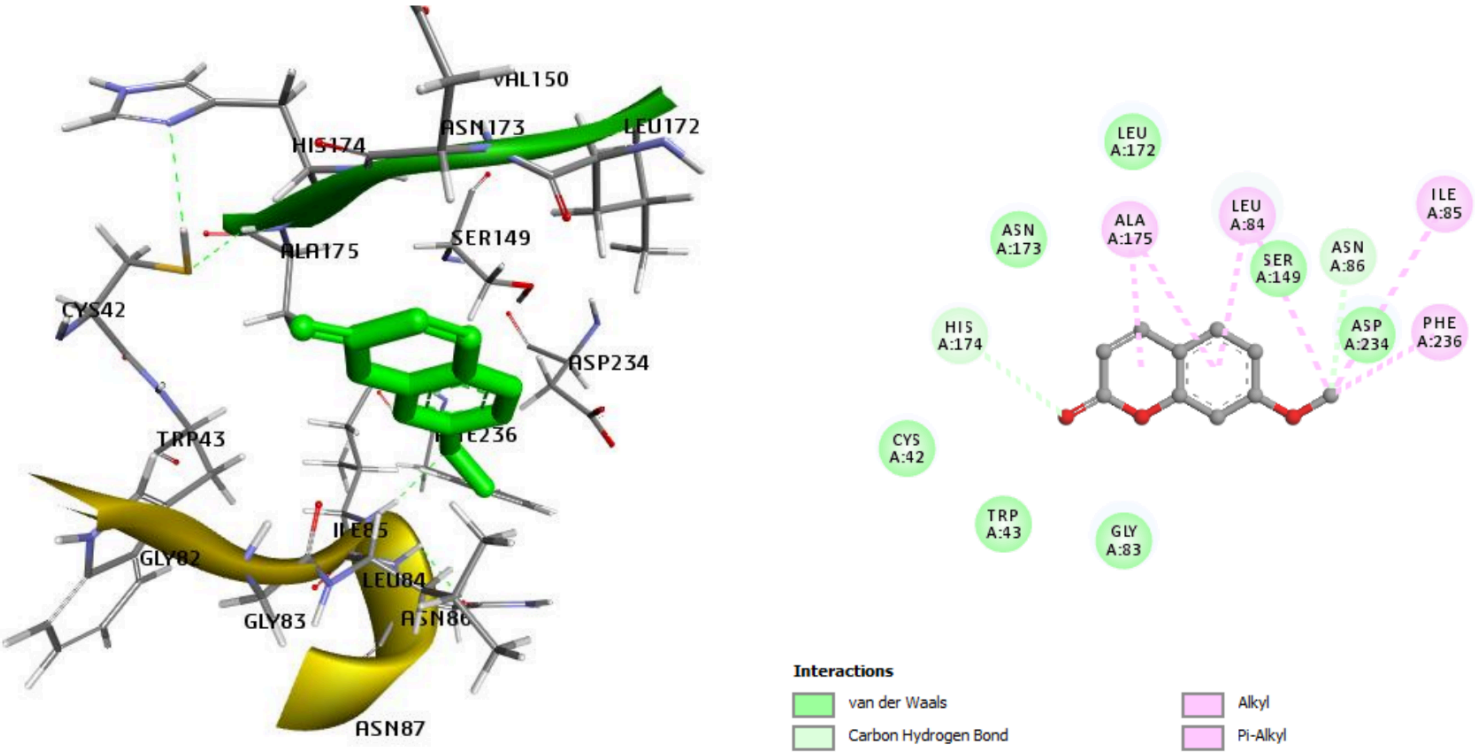

Figure 4

Figure 1: 3D and 2D Binding pose interaction of C11 at the active site of falcipain-2

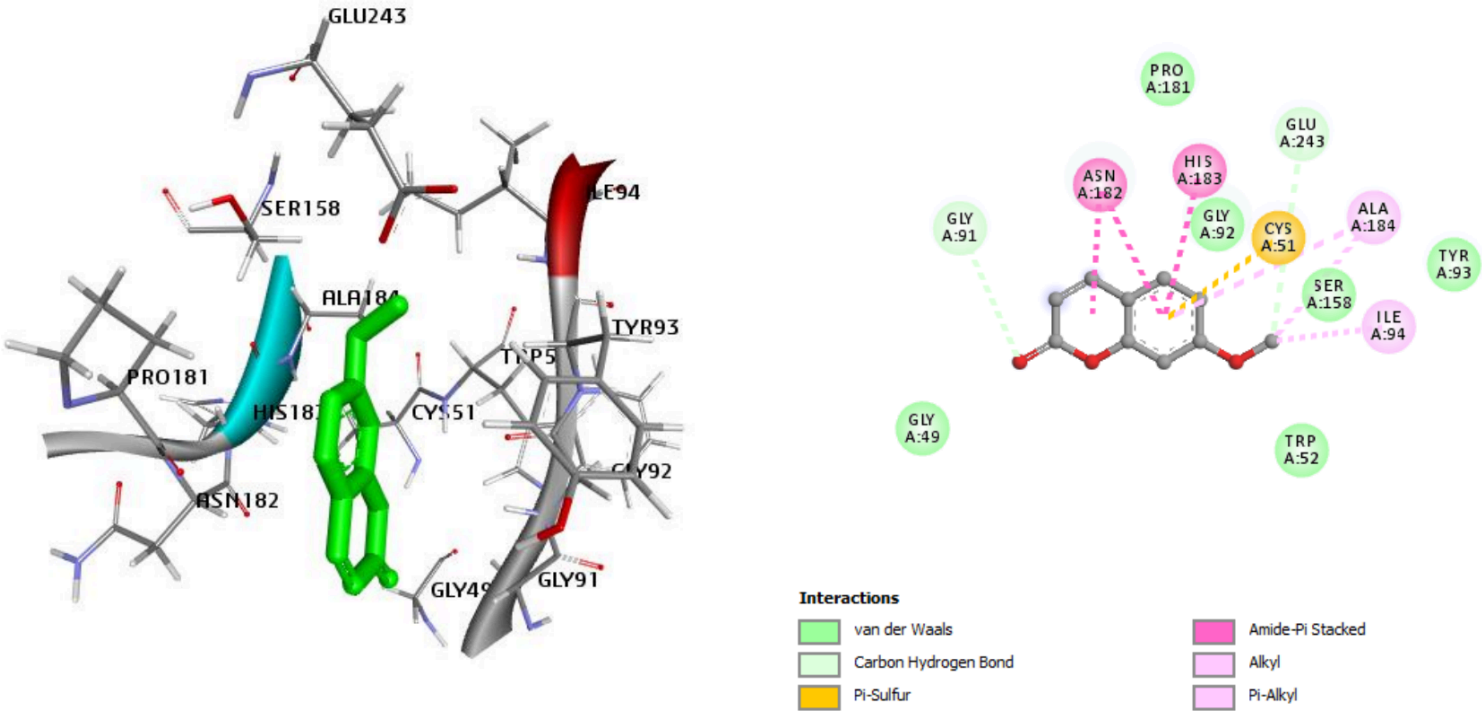

Figure 5

Figure 2: 3D and 2D Binding pose interaction of C11 at the active site of falcipain-3

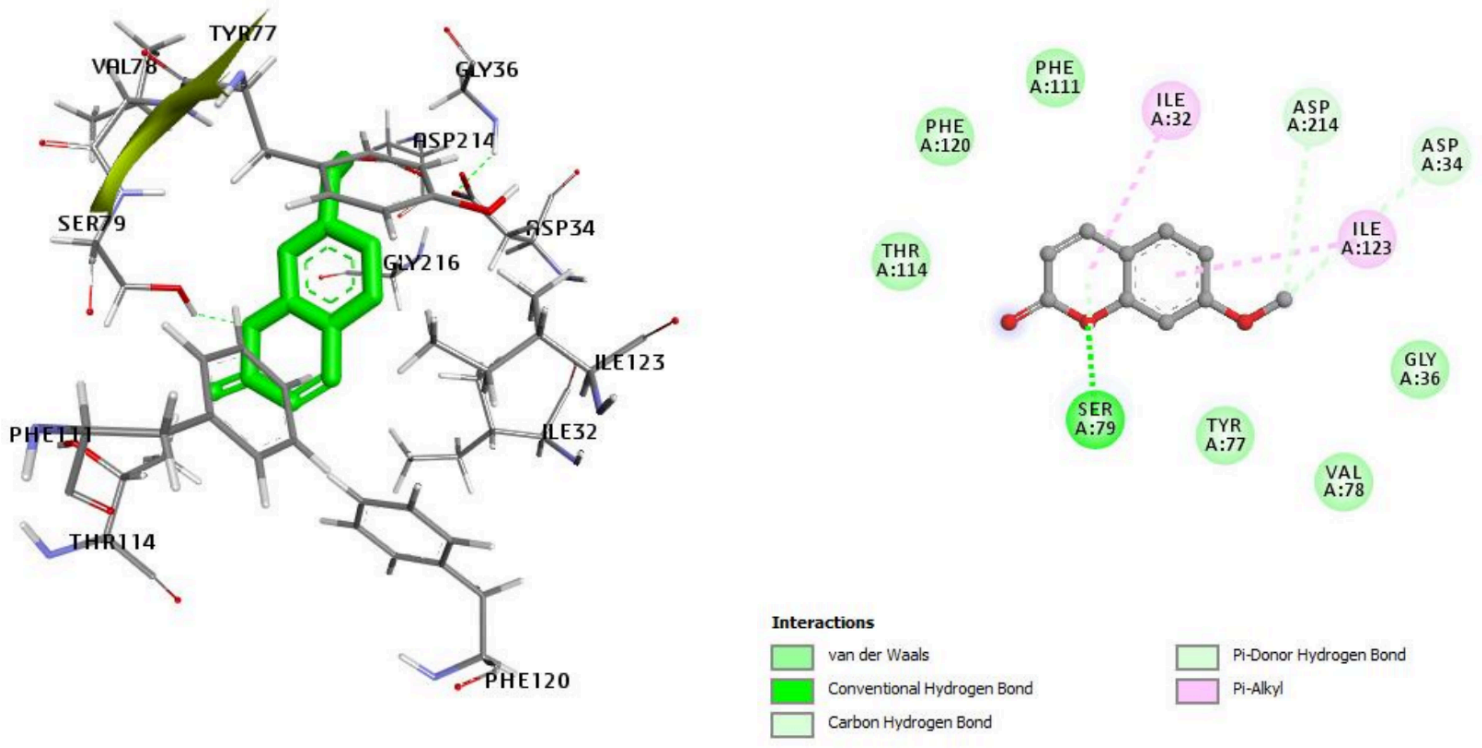

Figure 6

Fig 3: 3D and 2D Binding pose interaction of C11 at the active site of plasmepsin-2

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2and3.docx