Hasni Arsad[12*], Zaharaddeen Ayuba Sullubawa[2], Zaleha Md Toha[2]

[1]Advanced Management of Liver Malignancies (Liver Enrich) Research Program, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Penang, Malaysia

[2]Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Penang, Malaysia

*Corresponding author
Hasni Arsad
hasniarsad@usm.my

Exploring the Liver Cancer Therapeutic Potential of *Clinacanthus nutans* Phytochemical through *In Silico* Approaches

Abstract – Hepatocellular carcinoma (HCC) is a major cause of cancer-related mortality worldwide and is frequently associated with the overexpression of Glypican-3 (GPC3), Vascular Endothelial Growth Factor Receptor (VEGFR) and Platelet-Derived Growth Factor Receptor (PDGFR). These proteins play key roles in tumour proliferation and angiogenesis, making them critical therapeutic targets. Clinacanthus nutans (CN), a traditional Southeast Asian medicinal herb, exhibits anticancer properties; however, the specific phytochemical constituents responsible for activity against liver cancer targets remain underexplored. This study evaluated four phytochemicals identified in the dichloromethane (DCM) fraction of CN leaf extract using molecular docking and pharmacokinetic predictions. Among the evaluated compounds, N-(4-methoxyphenyl)-2-hydroxyimino-acetamide demonstrated the strongest binding affinities, with docking scores of −6.4 kcal/mol (PDGFR), −5.5 kcal/mol (VEGFR) and −5.0 kcal/mol (GPC3), and exhibited favourable ADMET features such as predicted intestinal absorption (70.44%) and moderate blood–brain barrier permeability (log BB = −0.238). These results suggest this compound may serve as a promising multi-target inhibitor for liver cancer therapy. Further biological validation is required.

Keywords: Clinacanthus nutans, hepatocellular carcinoma, molecular docking, Glypican-3, VEGFR, PDGFR

1 INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 90% of all primary liver cancers and remains a major contributor to cancer mortality worldwide, particularly in Asian countries where viral hepatitis and metabolic liver disease are highly prevalent. Despite the availability of chemotherapeutic and targeted biological agents such as sorafenib, regorafenib, and lenvatinib, significant challenges persist including low response rates, systemic toxicity, and rapid tumour adaptation leading to treatment resistance. This highlights an urgent need to identify new therapeutic molecules capable of targeting critical oncogenic pathways in HCC progression (1).

Several molecular targets have been implicated in promoting tumour proliferation, vascularisation, and immune evasion in HCC. Glypican-3 (GPC3) plays a key role in regulating Wnt/β-catenin signalling and is commonly overexpressed in HCC but not in healthy liver tissue, making it a promising therapeutic biomarker (2). Meanwhile, VEGFR and PDGFR are major mediators of angiogenesis, facilitating tumour blood vessel formation necessary for tumour growth, metabolic support, and metastasis. Drugs that simultaneously modulate these signalling pathways may provide increased efficacy and reduce tumour escape mechanisms (3).

Clinacanthus nutans (CN), a medicinal herb widely used in Malaysia, Thailand, and Indonesia, has gained attention due to its reported anti-inflammatory, antiviral, and anticancer properties. However, while multiple compounds have been isolated from CN leaf extracts, the specific phytochemicals responsible for anti-HCC activity are not fully elucidated. Computational molecular docking and ADMET profiling can support the prioritisation of phytochemicals for preclinical evaluation by predicting binding behaviour and pharmacokinetic suitability.

This study evaluates four phytochemicals identified in the dichloromethane (DCM) fraction of CN leaves for their multi-target binding affinity against GPC3, VEGFR, and PDGFR, and characterises their pharmacokinetic properties using in silico prediction models.

2 MATERIALS & METHODS

2.1 *In silico* docking analysis

Phytochemical constituents of CN were identified from the dichloromethane (DCM) leaf extract using GC-MS. Four major compounds were selected: N-(4-methoxyphenyl)-2-hydroxyimino-acetamide, 9,12-octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl)ethyl ester, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z), and pentadecanoic acid, 14-methyl-, methyl ester. Protein structures for GPC3, VEGFR and PDGFR were retrieved from the RCSB PDB and prepared using AutoDockTools. Ligands were geometry-optimised and converted to PDBQT format. Molecular docking was performed using AutoDock Vina. Pharmacokinetic profiles including drug-likeness, GI absorption and BBB permeability were predicted using SwissADME and pkCSM. 2D ligand–protein interaction maps were generated using LigPlot, while the 3D structural complexes were visualised and rendered using PyMOL (4, 5, 6)

2.0 Result and discussion

The docking results clearly demonstrated that N-(4-methoxyphenyl)-2-hydroxyimino-acetamide displayed the strongest binding affinity across all three molecular targets—GPC3, VEGFR, and PDGFR—compared to the other three phytochemicals (7, 8, 9). As shown in Table 1, the binding energy scores for this compound were −5.0 kcal/mol for GPC3, −5.5 kcal/mol for VEGFR, and −6.4 kcal/mol for PDGFR, indicating a more stable ligand–receptor interaction.

In contrast, the remaining compounds demonstrated moderate docking scores ranging from −3.3 to −5.4 kcal/mol, underscoring their comparatively weaker affinity for these targets. The ability of a single compound to strongly interact with multiple pathways is particularly meaningful in hepatocellular carcinoma (HCC), where tumour progression is driven by overlapping proliferative and angiogenic signaling networks. Thus, N-(4-methoxyphenyl)-2-hydroxyimino-acetamide may play a more central role in mediating the anticancer properties of Clinacanthus nutans, while the other constituents may contribute to supportive or synergistic biological effects.

In addition to binding score comparison, the binding interactions observed for N-(4-methoxyphenyl)-2-hydroxyimino-acetamide provide further insight into its potential mechanistic role. In the PDGFR active site, this compound formed stable hydrogen bonds with residues Arg558 and Glu556, along with hydrophobic interactions involving Leu839, Phe604, and Gly602. These interactions contribute to anchoring the ligand within the receptor pocket, explaining its favourable binding energy. Notably, PDGFR has been strongly associated with tumour angiogenesis, extracellular matrix remodelling, and stromal activation in HCC. Therefore, inhibition of this receptor may suppress tumour vascular support and limit metastatic expansion. Meanwhile, the compound's affinity for VEGFR suggests potential to impede new blood vessel formation, further enhancing its antiangiogenic potential (10, 11).

The pharmacokinetic predictions further reinforce the suitability of this compound as a drug development candidate. The molecular weight of 194.19 g/mol and moderate lipophilicity (logP 1.09) fall well within optimized ranges for oral bioavailability. The predicted intestinal absorption rate of 70.44% suggests that the compound is likely to be efficiently absorbed following oral delivery, supporting potential clinical practicality. Moreover, its moderate predicted blood–brain barrier permeability (log BB −0.238) implies that it is distributed primarily in peripheral tissues, which is desirable for anticancer agents to avoid central nervous system toxicity.

The remaining fatty acid ester derivatives, although showing weaker docking affinity, should not be overlooked because such compounds have been reported to influence oxidative stress modulation, inflammatory signaling attenuation, and membrane structure integrity, all of which are biologically relevant to the tumour microenvironment. Their roles may therefore complement the direct receptor inhibition demonstrated by N-(4-methoxyphenyl)-2-hydroxyimino-acetamide. This reflects a common characteristic of botanical extracts, where therapeutic effects arise from synergistic multi-component interactions rather than from a single active molecule alone (12, 13).

However, while the in silico findings provide a strong theoretical basis, experimental validation remains essential. The next phase should include cytotoxicity screening using hepatoma cell lines such as HepG2 and Huh7, followed by cell signalling and kinase phosphorylation assays to confirm pathway inhibition. Additionally, in vivo tumour xenograft models will be critical in evaluating pharmacodynamic behaviour, systemic

tolerance, and therapeutic efficacy. Overall, this study highlights the potential of Clinacanthus nutans as a source of novel multi-target agents for HCC treatment and identifies N-(4-methoxyphenyl)-2-hydroxyimino-acetamide as a promising lead molecule deserving further translational investigation.

3.0 CONCLUSION

This study provides computational evidence that the dichloromethane (DCM) leaf extract of Clinacanthus nutans contains phytochemicals with potential inhibitory activity against key molecular targets implicated in hepatocellular carcinoma. Among the four evaluated compounds, N-(4-methoxyphenyl)-2-hydroxyimino-acetamide demonstrated consistently stronger binding affinities towards GPC3 (−5.0 kcal/mol), VEGFR (−5.5 kcal/mol), and PDGFR (−6.4 kcal/mol) compared to the fatty acid ester derivatives, which exhibited moderate binding profiles. The favourable predicted pharmacokinetic features of N-(4-methoxyphenyl)-2-hydroxyimino-acetamide, including good intestinal absorption and acceptable molecular properties, further support its potential as a viable lead compound for therapeutic development.

The ability of this compound to interact with multiple oncogenic targets suggests a multi-target inhibitory mechanism, which is advantageous in overcoming pathway redundancy and resistance commonly observed in HCC. These findings highlight N-(4-methoxyphenyl)-2-hydroxyimino-acetamide as a promising candidate for further in vitro cytotoxicity testing, kinase inhibition assays, and in vivo validation to substantiate its pharmacological relevance and therapeutic potential.

ACKNOWLEDGEMENT

This study was supported by the Advanced Management of Liver Malignancies (Liver Enrich) Research Program, Advanced Medical and Dental Institute, Universiti Sains Malaysia.

REFERENCES

[1] Liu J, Park K, Shen Z, et al. Immunotherapy, targeted therapy, and their cross talks in hepatocellular carcinoma. Front Immunol. 2023;14:1285370. doi:10.3389/fimmu.2023.1285370 [frontiersin.org]

[2] Luan C-H. Ginsenosides as Multi-target Agents against Hepatocellular Carcinoma: Mechanisms and Therapeutic Prospects. Nat Cell Sci. 2025;3(3):e00016. doi:10.61474/ncs.2025.00016 [cellnatsci.com]

[3] Tong J, Tan Y, Ouyang W, et al. Targeting immune checkpoints in hepatocellular carcinoma therapy: toward combination strategies with curative potential. Exp Hematol Oncol. 2025;14:65. doi:10.1186/s40164-025-00636-5 [ehoonline....entral.com]

[4] Poyatos-Racionero E, et al. Validation of an AI-powered computational chemistry workflow for streamlined drug discovery. Mol Cancer Ther. 2025;24(10_Suppl):C008. doi:10.1158/1535-7163.TARG-25-C008 [aacrjournals.org]

[5] Frontiers Research Topic. Molecular Docking and Dynamics in Computational Cancer Drug Discovery. Front Oncol. 2026 (accepted). Link [frontiersin.org]

[6] Garg P, et al. Artificial Intelligence–Driven Computational Approaches in the Development of Anticancer Drugs. Cancers. 2024;16(22):3884. doi:10.3390/cancers16223884 [mdpi.com]

[7] Jahagirdar S, et al. Isolation and characterization of a potent anticancer fraction from Clinacanthus nutans: an integrated in vitro and in silico study. In Silico Pharmacol. 2025;13:182. doi:10.1007/s40203-025-00471-w [link.springer.com]

[8] Sun Q, et al. Multiplatform metabolomics reveals anticancer metabolites in Clinacanthus nutans against nasopharyngeal carcinoma. Ind Crops Prod. 2025;236:121905. doi:10.1016/j.indcrop.2025.121905 [colab.ws]

[9] Toha ZM, et al. Exploring Clinacanthus nutans leaf extracts on antiproliferative effects against MCF-7 cells. Future J Pharm Sci. 2022;8:49. doi:10.1186/s43094-022-00437-1 [fjps.sprin...eropen.com]

[10] Chaudhari PJ, et al. Recent updates on potential of VEGFR-2 small-molecule inhibitors as anticancer agents. RSC Adv. 2024;14:33384–33417. doi:10.1039/D4RA05244G [pubs.rsc.org]

[11] Zhao B, et al. Targeted inhibition of PDGFRA with avapritinib enhances lenvatinib efficacy in hepatocellular carcinoma. J Exp Clin Cancer Res. 2025;44:139. doi:10.1186/s13046-025-03386-8 [jeccr.biom...entral.com]

[12] Mikkili I, et al. Synergistic strategies for cancer treatment: leveraging natural products and molecular targets. Beni-Suef Univ J Basic Appl Sci. 2024;13:96. doi:10.1186/s43088-024-00556-z [bjbas.spri...eropen.com]

[13] Kumari P, et al. Synergistic effects of natural compound combinations in breast cancer therapy. Adv Sci Technol Innov. 2025;127–140. doi:10.1007/978-3-031-80189-1_10 [link.springer.com]

Figure 1. Binding interaction of N-(4-methoxyphenyl)-2-hydroxyimino-acetamide with PDGFR. The ligand is accommodated within the PDGFR active site and stabilised through multiple hydrogen bonds (green dashed lines) and van der Waals interactions (light green shadowed residues), indicating a strong and favourable binding conformation. Additional π–cation (orange) and π–alkyl (purple) interactions contribute to ligand anchoring within the hydrophobic pocket. These interactions support the potential inhibitory effect of the ligand on PDGFR-mediated signalling in hepatocellular carcinoma

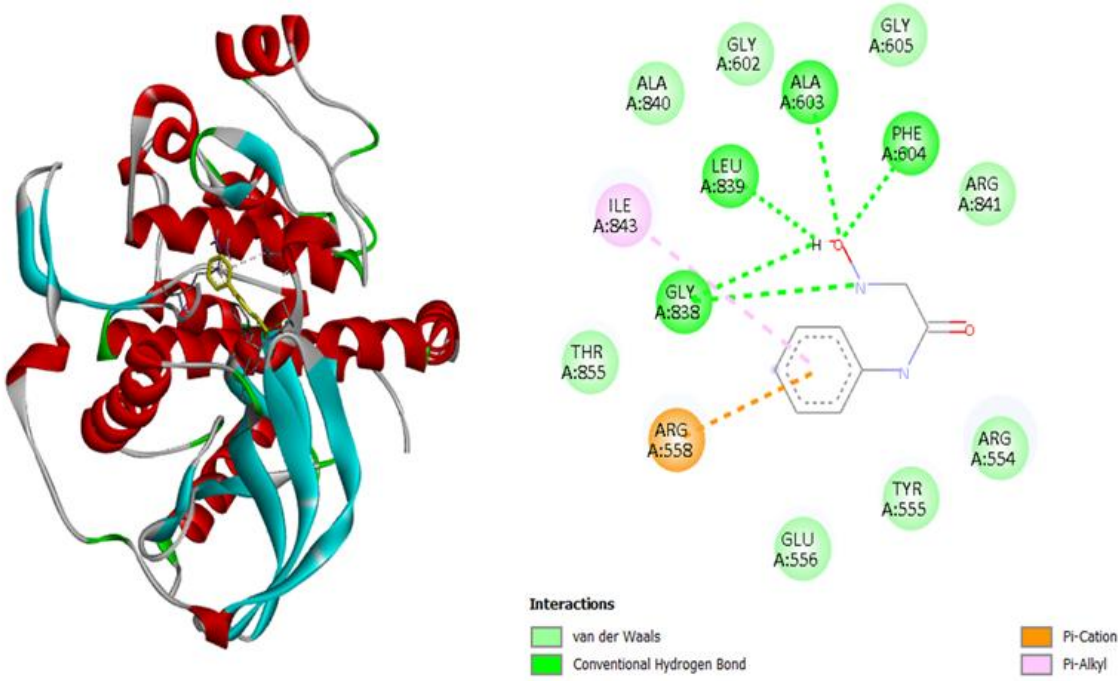

Table 1. Docking Scores of Clinacanthus nutans Phytochemicals Against GPC3, VEGFR, and PDGFR

No.	Receptor–Ligand Complex	Docking Score (kcal/mol)
1	GPC3 – N-(4-methoxyphenyl)-2-hydroxyimino-acetamide	−5.0
2	GPC3 – 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	−3.4
3	GPC3 – 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)	−3.5
4	GPC3 – Pentadecanoic acid, 14-methyl-, methyl ester	−3.4
5	VEGFR – N-(4-methoxyphenyl)-2-hydroxyimino-acetamide	−5.5
6	VEGFR – 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	−3.3
7	VEGFR – 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z)	−4.3
8	VEGFR – Pentadecanoic acid, 14-methyl-, methyl ester	−3.8
9	PDGFR – N-(4-methoxyphenyl)-2-hydroxyimino-acetamide	−6.4
10	PDGFR – 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	−4.0
11	PDGFR – 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z)	−5.4
12	PDGFR – Pentadecanoic acid, 14-methyl-, methyl ester	−5.3