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Original Research Article

Network Pharmacology and Molecular Docking Study of 12-Methoxy-4-Methylvoachalotine (MMV) as an Anticancer Agent

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ABSTRACT

12-Methoxy-4-Methylvoachalotine (MMV) is a sarpagine indole alkaloid isolated from *Tabernaemontana macrocarpa* and has been implicated in the treatment of tumors and as an anti-snake venom. This study aims to explore MMV as an anticancer agent using *in silico* methods. The research integrates network pharmacology to identify key protein targets associated with MMV and molecular docking to evaluate the binding affinity and interaction profile of MMV with selected proteins implicated in anticancer mechanisms. The pharmacological network analysis identified ten key targets, including PTEN, HIF1A, CCND1, ESR1, AKT1, MTOR, MCL1, EGFR, and ERBB2, which are closely related to the mechanism of cell proliferation and apoptosis regulation. MMV molecular docking results against the five proteins (TP53, PTEN, ESR1, EGFR, and MCL1) showed lower binding affinity than the native ligands; however, the interaction patterns closely resemble those of the reference ligands. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions indicated low solubility but high gastrointestinal absorption potential. These findings provide important insights into the potential of MMV as an anticancer agent and emphasize the need for structural modifications to enhance its therapeutic efficacy.

Keywords: 12-methoxy-4-methylvoachalotine, Anticancer, Networking of pharmacology, Molecular docking, Prediction of absorption, Distribution, Metabolism, Excretion and Toxicity.

Introduction

Cancer remains a global health problem, with an estimated 19.98 million new cases and 9.74 million cancer-related deaths reported in 2022.[1] According to data from the Global Cancer Observatory (GLOBOCAN), in Indonesia, there were 408,661 new cases of cancer, underscoring the urgent need for improved prevention and treatment strategies.[2] The main challenge in cancer treatment is drug resistance, often associated with the extensive use of synthetic chemotherapeutic agents, which also exhibit severe side effects.[3] Therefore, research into new compounds that are safer and more effective is fundamental. 12-Methoxy-4-Methylvoachalotine (MMV), an indole alkaloid isolated from *Tabernaemontana macrocarpa*,[4] has been reported to exhibit biological activity, including the inhibition of snake venom phospholipase A2-like protein[5] and antifungal activity against *Trichophyton rubrum*.[6] Preliminary studies by Pereira *et al*.[7] suggest MMV's anticancer potential; however, its precise mechanism of action and interaction with target proteins remain unexplored.

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An *in silico* approach is often used as an initial step to guide and streamline experimental research by leveraging biological databases and software.[8] This technique provides an efficient preliminary method for evaluating bioactive compounds, leveraging computational models to predict pharmacological interactions.[8] Network pharmacology facilitates the identification of drug-target interactions within complex biological systems,[9] while molecular docking assesses ligand-receptor interactions at the atomic level, enabling the evaluation of potential binding affinities and structural compatibility.[10] In this study, the selected cancer-related MMV receptor targets will be analyzed using the molecular docking method to ensure that MMV can interact with the target receptor.

Materials and Methods

Materials

Computational analyses were conducted on an Acer Aspire A314-22 laptop using the following software tools: PLANTS, YASARA, MarvinSketch, CMD, Ligplus, Cytoscape 3.10.1 with the CytoHubba 0.1 plugin, and pkCSM. This study also utilized several online databases and web servers, including PubChem (https://pubchem.ncbi.nlm.nih.gov/), SwissTargetPrediction (http://swisstargetprediction.ch/), GeneCards (https://www.genecards.org/), and STRING (https://string-db.org/). Meanwhile, the materials used in this study include the SMILES structure, 2D and 3D structures of 12–Methoxy–4–Methylvoachalotine (MMV), and the 3D structures of the target proteins: 1A52, 1D5R, 5A7B, 6TFU, and 6NE5.

Networking of Pharmacology Analysis

The SMILES code for 12–Methoxy–4–Methylvoachalotine (MMV) was obtained from PubChem11 and entered into SwissTargetPrediction to identify its target protein.[12] All MVW target proteins with probability

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values >0 were processed into STRING[13] to determine the pharmacological network between proteins predicted to be able to interact with the MMV compound. The STRING data were analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. In addition, the data were also imported into Cytoscape,[14] and the most important proteins in this network were searched for using the CytoHubba plugin.[15]

Molecular Docking Analysis

Protein Selection

Protein structures were downloaded from the Protein Data Bank (http://rscb.org/) in .pdb format. The method used to determine the macromolecular structure is X-ray crystallography, which is suitable for large macromolecules and provides high-resolution data (RMSD < 3.5 Å) for the human organism (Homo sapiens).[16] Files were stored in the D:\VALIDATION folder. The selection of proteins was based on their relevance in signaling pathways that regulate cell growth, proliferation, and apoptosis, which are essential in cancer. The protein structures used have been determined experimentally, allowing analysis of the interaction between MMV compounds and target proteins.

Docking Validation Protocol

The first step after protein selection is to prepare the protein and the ref_ligand using YASARA. The target protein structure was downloaded from http://rscb.org/ in .pdb format and saved in the D:\VALIDATION folder. The protein file was then opened using YASARA via the File > Load > PDB file menu. Water molecules were removed, hydrogen atoms were added via the Edit > Add > Hydrogen to all menu, and the result was saved in YASARA Object (.yob) format. Next, the ligand present in the protein was removed, and the file was saved as protein.mol2. To prepare the ref_ligand, the .yob file was reopened, the protein was removed, and the result was saved as ref_ligand.mol2. The ligand was then opened in MarvinSketch for a clean 2D view, protonated at pH 7.4, and saved as ligand_2D.mrv. The ligand conformation was identified via the Tools > Conformation > Conformers menu, with 10 conformers, and then saved as ligand.mol2. The docking process was performed by opening cmd.exe and typing the command: PLANTS --mode bind ref_ligand.mol2 5 protein.mol2. The center coordinates and radius were recorded, then these values were inserted into the pc_4pyp.txt file and saved as pc_pdbid.txt. Next, the PLANTS --mode screen pc_pdbid.txt command is executed. After the process was complete, the lowest docking score was obtained by typing the command "more bestranking.csv". The RMSD calculation was performed by removing hydrogen atoms and comparing the docked ligand conformation with the reference ligand using YASARA. If the RMSD is below 2Å, the docking protocol is considered valid. The RMSD calculation results were saved as PDBID.sce in the D:\VALIDASI PDBID folder, concluding the validation process.

Molecular Docking of Ligand Test

The preparation stage of the test ligand begins by creating a folder named MMV_PDBID and copying important files, such as cmd.exe, PLANTS.exe, and the protein.mol2 into it. The molecular structure of MMV was drawn in MarvinSketch, protonated at pH 7.4, and saved as MMV_2D.mrv. After that, the conformation was searched using Tools > Conformation > Conformers, set to 10, and saved as MMV.mol2. For molecular docking, the cmd.exe file was opened. Plants –mode bind ref_ligand.mol2 5 protein.mol2 was input, and for screening, plants --mode screen pc_pdbid.txt was entered. After the process was complete, the cd results were viewed in the results folder, and the SCORE_RB_PEN column was used to sort the docking results in best-ranking.csv. For visualization, a PDB file from the YASARA docking results was generated by loading the protein.mol2, merging it with the ligand, and saving it as MMV_PDBID.pdb. It was opened in Discovery Studio to display the 2D diagram, and a screenshot was taken and saved as a JPEG. This step was repeated with the native ligand, saved as ref_ligand_PDBID.pdb, and Discovery Studio was finally closed after all visualizations were complete.

ADMET Prediction of Ligand Test

The Canonical SMILES of the MMV prepared in MarvinSketch were used for ADMET testing using pkCSM. pkCSM was accessed at https://biosig.lab.uq.edu.au/pkcsm/. The smiles were entered, and the necessary ADMET parameters —such as absorption, distribution, metabolism, excretion, and toxicity —were selected.

Results and Discussion

The analysis identified 98 proteins predicted to interact with MMV compounds targeting cancer, and then proceeded with pharmacological network analysis using STRING. This analysis was carried out to construct a network linking selected target proteins and the biological pathways associated with them (Figure 1).[17] STRING is a database containing more than 9 million proteins from various sources that can be used to predict protein interactions.[18] The results of the pharmacological network analysis of STRING were further analyzed using GO Biological Process and KEGG enrichment (Figures 2 and 3).

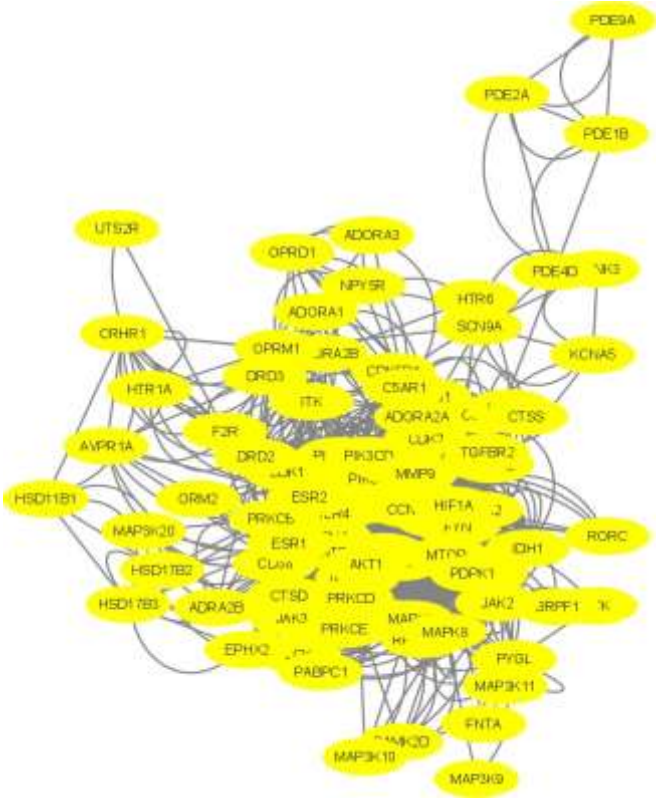

Figure 1: Pharmacological Network Model of 98 proteins targeted by the compound 12-Methoxy-4-Methylvoachalotine (MMV)

The results of this analysis are based on the False Discovery Rate (FDR), which is the expected proportion of false positives among the data points that are rejected. So the smaller the FDR value, the more accurate the analysis results are.[19] FDR is expressed in the form of -Log (p values); the greater the value, the lower the possibility of error. Pharmacological networks are becoming a viable method for accelerating the discovery of new drugs and clarifying how drugs act on different targets. Pharmacological networks describe diseases as disorders of complex biological networks and use computational methods to infer how drugs act from network topology.[20] In recent years, the emergence of pharmacological network methods has been able to define complex interactions between active compounds, targets, pathways, and related diseases, thus providing more scientific and efficient research ideas and methods for predicting active compounds in plants.[21] Several approaches can be used to analyze pharmacological

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networks, including GO and KEGG enrichment. GO enrichment is a tool for identifying biological mechanisms from a collection of genes or proteins derived from research data. There are three terms in GO: biological process (BP), molecular function (MF), and chemical component (CC).[22] KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment is a collection of manually curated biological signaling pathways that represent knowledge of molecular interaction and reaction networks.[23] From the results of GO enrichment (Figure 2) the pharmacological network of MMV compounds is predicted to be potentially involved in several biological process mechanisms such as pathways in cancer (Pancreatic cancer, Gastric cancer, Colorectal cancer, Lung cancer, etc.), biological processes related to virus therapy (Hepatitis B, Herpe virus, Epstein- Bar virus, etc.), also related to biological processes related to metabolic disorders (Endocrine resistance and Insulin resistance).

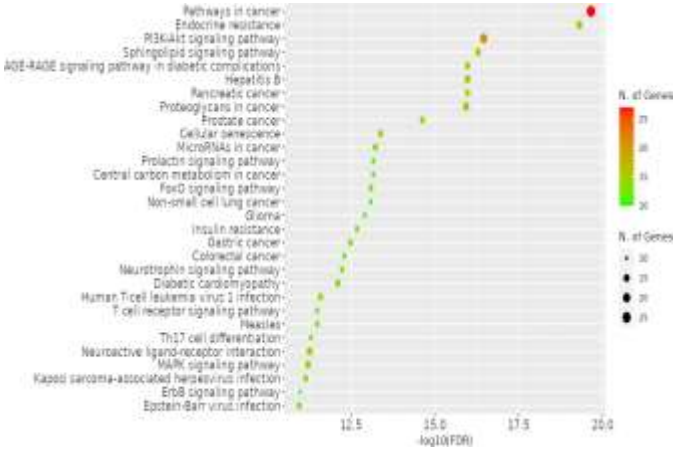


Figure 2: GO Biological Process and KEGG enrichment analysis. The X-axis shows the level of prediction accuracy, the Y-axis shows the type of Enrichment result, the color of the circle shows the greener the color, the more genes/proteins in the Enrichment type are involved, and the size of the circle shows the total number of genes/proteins in the Enrichment type.

The results of the KEGG enrichment analysis (Figure 3) in pancreatic cancer show that MMV compounds interact with proteins involved in apoptosis activity through the PI3K-Akt signaling pathway and the MAPK signaling pathway. Likewise, it is involved in proteins related to the cell cycle through the Jak-STAT, p53, and TGF-β signaling pathways. The PI3K-Akt and MAPK signaling pathways not only play important roles in regulating cell proliferation but are also involved in the mechanism of apoptosis.[24] The Jak-STAT signaling pathway is widely reported to be involved in promoting cell proliferation, angiogenesis, invasion, and chemotherapy resistance.[25] Activation of p53 is even more complex in its role in maintaining genomic balance, such as antiangiogenic mechanisms, triggering apoptosis, DNA repair, regulating metabolism, and cell cycle arrest.[26] Meanwhile, Jak-STAT is reported to be involved in the mechanisms of regulating cell proliferation and apoptosis.[27]

Furthermore, the analysis of essential proteins in the cancer network was conducted using the MCC (Maximal Clique Centrality) algorithm via the CytoHubba plugin in Cytoscape.[15] The results of this analysis are the top 10 proteins that have the most roles in the pharmacological network related to cancer (Figure 4). From the results of the analysis, it was found that the protein gene with the darkest intensity (TP53) (red) shows the most influential gene in this network. Furthermore, the brighter it is, the lower its influence (sequentially including the PTEN, HIF1A, CCND1, ESR1, AKT1, MTOR, MCL1, EGFR, and ERBB2 genes). The assessment of the most influential genes/proteins is based on their centrality in the sub-network. This centrality measures how vital a protein is in maintaining or influencing connections within the network.[15]

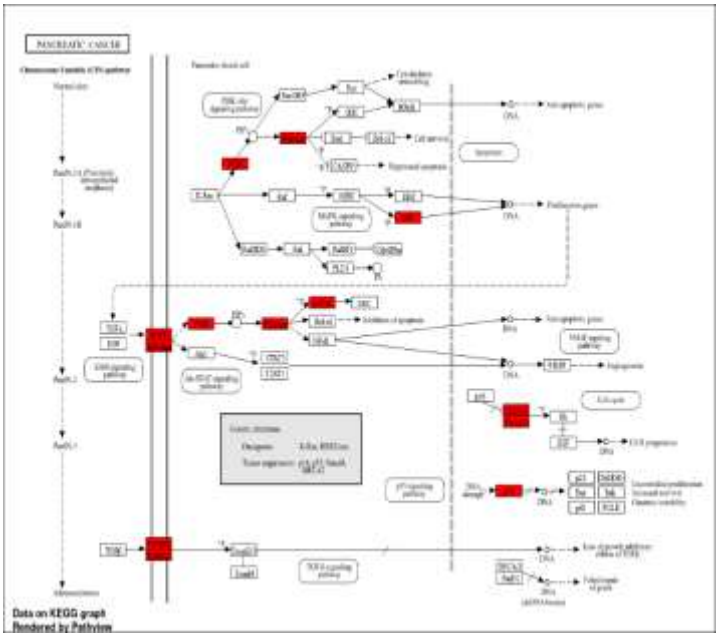
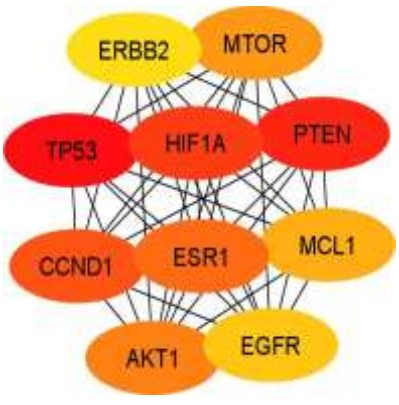


Figure 3: View of multiple MMV compound targets as anticancer in the "pancreatic cancer pathway"

TP53 is the most influential protein in this pharmacological network. P53 is known as "the guardian of the genome" because it controls the cell cycle, triggers apoptosis, and senescence.[28] Thus, this protein is essential for use and has been proven as a target for research on MMV compounds as an anticancer.

Several articles have used pharmacological networks to explain the mechanisms by which plant secondary metabolites act as anticancer agents. The *Physalis angulata* (L.) plant is predicted to suppress proliferation, inhibit metastasis, and trigger apoptosis in lung cancer, based on the Maximal Clique Centrality (MCC) CytoHubba analysis. A total of 10 most important genes (CDKN2A, KRAS, RBB2, PTEN, KRAS, NRAS, EGF, NFI, BRAF, and TP53) in the 3 anticancer mechanisms have also been used in the pharmacological network method in analyzing the role of secondary metabolites of the *F. sargassaceae* plant as anticancer in oral squamous cell carcinoma (OSCC).[29-30]



Rank	Node
1	TP53
2	PTEN
3	HIF1A
4	CCND1
5	ESR1
6	AKT1
7	MTOR
8	MCL1
9	EGFR
10	ERBB2

Figure 4: Top 10 Proteins predicted to interact with 12-Methoxy-4-Methylvoachalotine (MMV) compound related to cancer using the MCC method. The redder the resulting color, the important the protein is in this network.

Likewise, Okpako *et al.*[31] have reported the use of pharmacological networks to predict the potential of secondary metabolites of the *Aspilia pluriseta* plant as anticancer in prostate cancer. The results of this

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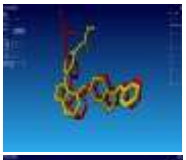
pharmacological network greatly assist subsequent analyses, such as molecular docking. The pharmacological network method facilitates the identification of potential protein targets. In contrast, the molecular docking method can describe the extent of interaction between the test compound and the selected target protein as its receptor.

Also, this study examined the effect of 12-methoxy-4-methylvoachalotine (MMV) from *Tabernaemontana catharinensis* on proteins involved in cancer mechanisms using the *in silico* method with PLANTS, involving 38 proteins from http://rscb.org/. Validation was carried out by redocking native ligands in YASARA, with the criteria of Root Mean Squared Deviation (RMSD) <2.Å to ensure prediction accuracy (Table 1). The process of removing water molecules was carried out to reduce irrelevant interactions, and adding hydrogen aimed to stabilize the ligand and increase binding affinity.[32]

Table 1: Validation results of the 5 most important proteins

PDB Code	Protein/Gene	Description	Native Ligand	RMSD score	Visualization of validation results
1A52	ESR1	Estrogen Receptor Alpha ligand-binding domain complexed to Estradiol.	EST	0.5319Å	
1D5R	PTEN	Crystal Structure of the PTEN Tumor Suppressor	TLA	1.0664Å	
6TFU	EGFR	Crystal Structure of EGFR T790M/V948R in Complex with Covalent Pyrrolopyrimidine 14d	N7K	1.9491Å	
5A7B	TP53	Structure of the p53 cancer Y220C bound to the stabilizing small molecule PhiKan5211	KMN	1.9991Å	
6NE5	MCL1	Discovery of Potent Myeloid Cell Leukemia-1 (Mcl-1) Inhibitors that Demonstrate in vivo Activity in Mouse Xenograft Models of Human Cancer	KJP	1.7096 A	

The molecular structure was made in 2D before conversion to 3D, with protonation at pH 7.4 to reflect physiological conditions. Validation of this native ligand is crucial because it can identify the optimal binding position, which is expected to yield a small RMSD, thereby helping predict the ligand's biological activity and increasing the compound's potential as a drug.[33] The molecular docking process of the MMV compound against the validated target protein begins with ligand preparation using MarvinSketch software version 5.2.5.1, where the MMV molecule is drawn in the application (Figure 5). Table 2 shows the docking results of MMV compounds with various receptors based on PDB codes, including docking score values for MMV and native ligands. The more negative the docking score, the more stable the ligand-receptor interaction.[34] If the docking score of the test compound is more negative than that of the native ligand, then the test compound shows better affinity. The greater stability of the interaction is positively correlated with increased potential biological activity.[35] Based on the docking results on the 5 receptors, the affinity of MMV is lower than that of the native ligand, but MMV still shows anticancer potential. In docking results for the 1A52 receptor, the native ligand has a score of -88.6142 kcal/mol, while MMV was only -76.2482 kcal/mol, indicating its low anticancer potential. Likewise, in the 1D5R receptor, the native ligand shows a score of -75.6527 kcal/mol, which is more stable than the MMV of -60.0084 kcal/mol. In the 6TFU receptor, the native ligand also shows a score of -111.678 kcal/mol, which is more stable than the MMV of -72.2072 kcal/mol. Table 3 presents a visualization of amino acid interactions between several target proteins and their native ligands, and 12-Methoxy-4-Methylvoachalotine (MMV). In this analysis, the similarity of amino acid interactions is essential for understanding the binding mechanism and the potential anticancer activity of MMV.

Table 2: Validation results of the 5 most important

Protein	PDB Code	Native Ligand	12-methoxy-4-Methylvoachalotine (MMV)
ESR1	1A52	-88.6142	-76.2482
PTEN	1D5R	-75.6527	-60.0084
EGFR	6TFU	-111.678	-72.2072
TP53	5A7B	-133.823	-85.8327
MCL1	6NE5	-138.068	-80.9839

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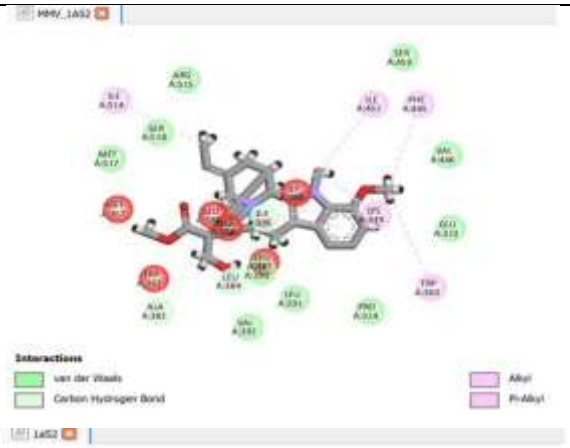
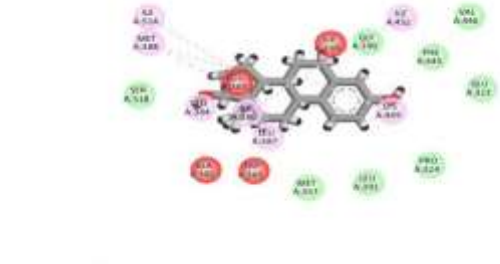
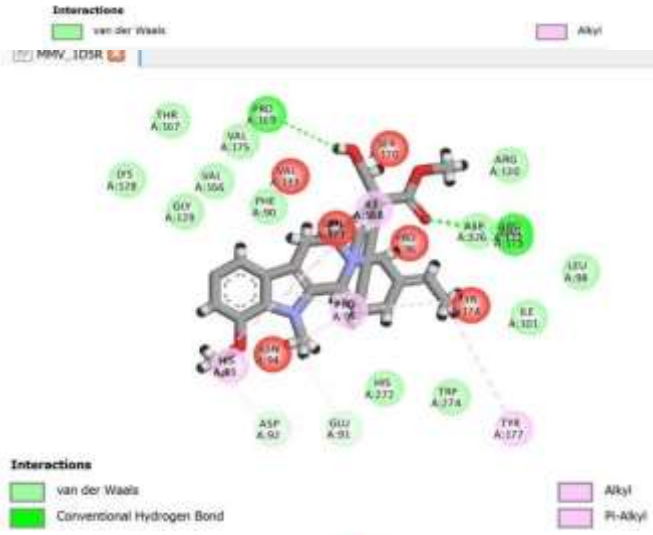
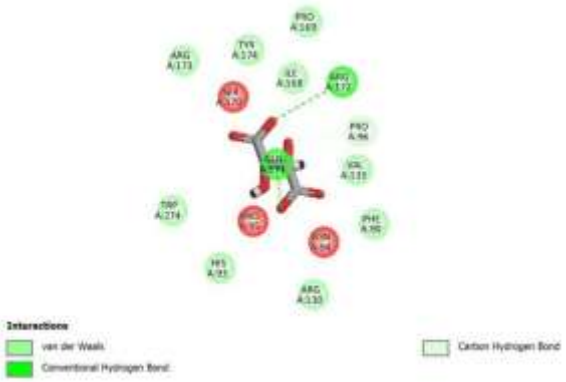
Table 3: Visualization of the interaction of the molecular docking result of MMV and native ligand receptors ESR1, PTEN, EGFR, TP53, and MGL1

No	Senyawa dan Reseptor	Interaction Visualization (2D)	Ligand Interactions with Amino Acids
1	MMV-ESR1 (1A52)		ARG515, SER450, **ILE452**, PHE445, MET517, **SER518**, **MET357**, **GLU 385**, **ILE386**, **ILE389**, **LYS449**, **VAL446**, **GLU323**, **TRP383**, **ALA382**, **LEU387**, **AGA390**, **LEU384**, **LEU391**, TRP360, VAL392, **PRO324**, **ILE514**
	Native ligand-ESR1 (1A52)		**ILE514**, MET388, **SER518**, **LEU384**, **GLU385**, **ILE386**, **LEU387**, **ALA382**, **TRP383**, **ILE389**, **GLY390**, **ILE452**, **VAL446**, **PHE445**, **GLU323**, **LYS449**, **MET357**, **LEU391**, **PRO324**
2	MMV-PTEN (1D5R)		THR167, **PRO169**, VAL175, LYS128, VAL166, **VAL133**, GLY129, PHE90, HIS93, **ASN94**, **SER170**, **ARG130**, **ILE168**, **PRO95**, **PRO96**, ASP326, **ARG172**, **ARG173**, **TYR174**, HIS272, **TRP274**, ASP92, GLU91, LEU98, ILE101, TYR177
	Native ligand-reseptor PTEN (1D5R)		**PRO169**, **ARG173** **SER170**, **TYR174**, **ILE168**, **ARG172**, **PRO96**, GLN171, **VAL133**, **TRP274**, **PRO95**, **HIS93**, **ASN94**, PHE90, ARG130

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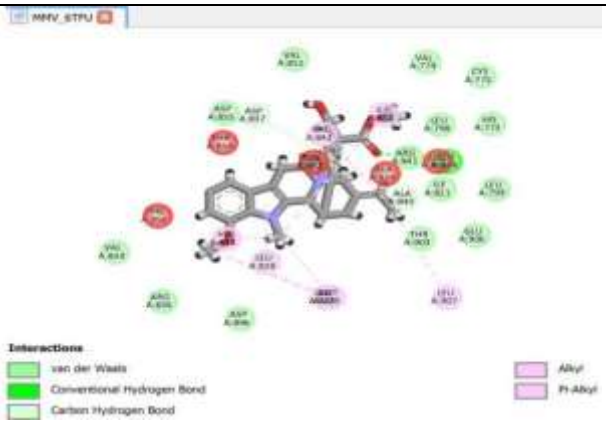
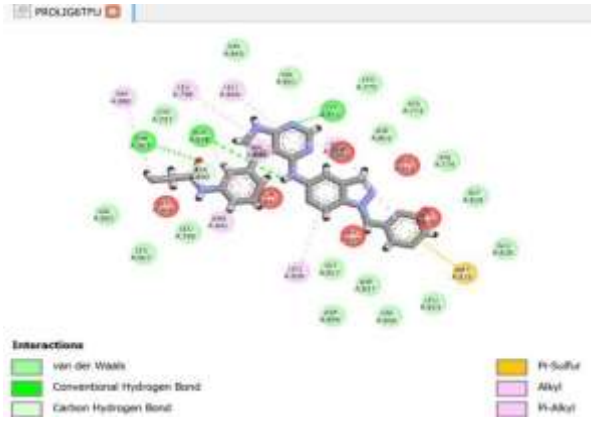
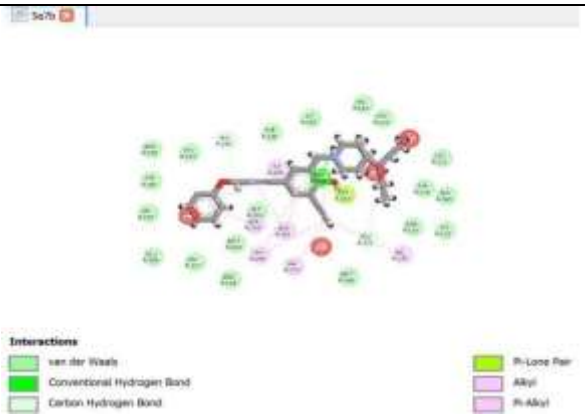
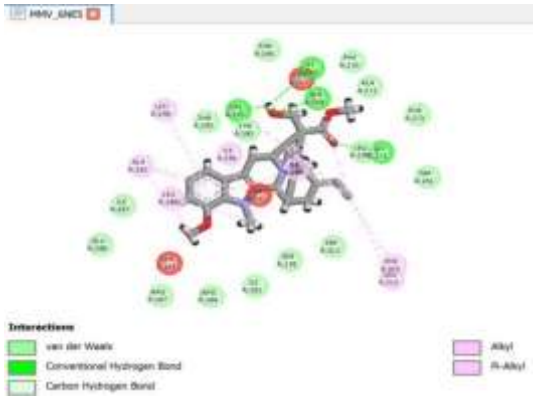
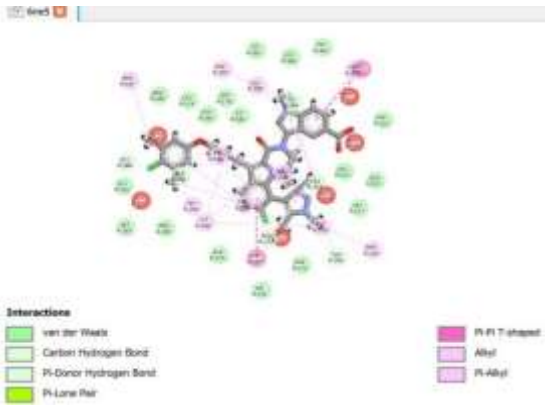
3	MMV-EGFR (6TFU)		**VAL834, PHE856, VAL851, VAL774,** CYS775, **ASP855, ASP837, ILE853, LEU798, THR854, VAL843, HIS773, ARG841, ASN842, ILE853, ALA839,** ILE821, **LEU799, ALA840, HIS835, LEU838, THR903,** GLU906, ARG836, **MET825, LEU907, ASP896, LEU833, LEU844, LYS852**
	Native ligand - EGFR (6TFU)		VAL845, **VAL851,** TRP880, **LEU798, LEU844,** CYS797, **THR903, ALA839, VAL843,** VAL902, GLU906, **ALA840, LEU907, LEU799, ARG841, ASN842,** CYS775, **LYS852, ASP855, HIS835, LEU838,** GLY857, **ASP837, HIS773, PHE856, VAL774, LEU833, ASP896, VAL834,** GLY824, **MET825,** GLU829, **THR854, ILE853**
4	MMV-TP53 (5A7B)		**HIS214,** LEU252 **SER269, MET160,** ALA150, ILE254, **VAL216, LEU133, LEU111,** ILE232, ALA161, **ILE255,** PHE113, **VAL197, ILE195, LEU194,** VAL173, **ILE251, THR253, TYR236,** **VAL272,** VAL143, **PHE270, TYR234, ARG196, TYR126,** CYS141 **SER215**

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

	Native ligand - TP53 (5A7B)		VAL143, **ILE255, LEU133, TYR234**, **ARG196,** HIS193, TYR205, **VAL197, HIS214, ILE195**, PHE113, **SER215**, ALA159, ALA161, **TYR236, VAL216, PHE270, THR253, LEU111, TYR126**, **SER269,** ASN131, LYS132, GLU204, VAL217, **MET160, LEU194, ILE251**, **VAL272**, GLU271, VAL274, ARG158, MET246
5	MMV-MCL1 (6NE5)		THR266, **PHE270**, **ALA272**, LEU290, **THR295, VAL265**, TYR185, **SER269, PHE273,** LEU298, **GLY271, ALA291, ILE294, ILE268, TRP261,** ILE287, **LEU186, GLU288,** SER183, **ARG187, ARG184, SER178, ILE181,** TRP312, **PHE315, LEU213, LEU267,** SER183
	Native ligand - MCL1 (6NE5)		LYS302, LEU306, TRP305, **ARG187,** PHE254, **ARG184,** LEU179, **THR295,** LEU298, **SER178, ILE181,** ILE264, **VAL265,** TRP212, **TRP261,** PHE319, **PHE315, GLU288**, GLU292, SER293, **ALA291,** LEU290, PRO289, **ILE294,** ALA275, **ALA272, GLY271, LEU267**, SER269, **LEU213,** GLN221, GLY217, **PHE270, THR266, PHE273,** PHE228, VAL274, **ILE268, LEU186**

Suppose the interaction between the test compound and the receptor's native ligand shows similarities; in that case, it is predicted that the test compound can interact with the same or a similar active site as the native ligand.[36] Thus, the test compound has the potential to mimic or replace the native ligand in its binding affinity as an anticancer. However, if the interaction similarity is not significant, it may indicate that the test compound does not effectively mimic the native ligand's biological activity.

In this study, the analysis showed that the amino acid interactions formed between MMV and target proteins were mainly similar to those involved in the binding of native ligands (amino acids marked in bold as in Table 3). This similarity suggests that MMV can interact with the same or similar active sites, potentially supporting anticancer activity. However, despite the similarity, the binding affinity of MMV was lower than that of the reference ligands. Other factors, such as the binding strength and conformation of the MMV molecule, may influence this. Therefore, to enhance MMV's anticancer potential, structural modifications that strengthen its interactions with target amino acids should be considered. This approach is expected to enhance MMV's anticancer mechanisms.

The results of the absorption prediction search showed that the MMV compound has a reasonably low water solubility of -2.95 (logmol/L). However, absorption in the human intestine reaches 99.63%. The Caco-

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2 permeability of 1.198 (log P_{app} in 10^6cm/s) indicates good permeability through intestinal epithelial cells, while the skin permeability of -2.907 (log Kp) indicates low efficiency in skin penetration. The distribution of the compound was measured by the volume of distribution (VDss) of 0.954 (log L/kg), indicating wide distribution in body tissues, and an unbound fraction (Fu) of 0.219, which increases the availability of pharmacologically active compounds. However, the permeability at the blood-brain barrier (BBB) of -0.186 (log BB) indicates difficulty in penetrating the barrier, thus limiting the effect on the central nervous system. Overall, this compound has high absorption potential in the digestive tract but limited penetration into the central nervous system. Metabolism testing results indicate that this compound is not a substrate for CYP2D6 or CYP1A2, but is a substrate for CYP3A4, which may contribute to drug interactions when given with other drugs metabolized by these enzymes. Inactivity as an inhibitor of CYP2C19, CYP2C9, and CYP2D6 suggests a low risk for interactions with other medications via these pathways. These results indicate that, despite concerns about drug interactions, the compound's metabolic profile is well managed.

The excretion test results showed a total clearance of 0.804 (log ml/min/kg), indicating moderate renal excretion, which can lead to accumulation in the body if not appropriately excreted. The positive status as an OCT2 substrate suggests that this compound is transported by an organic transporter that affects the rate and efficiency of excretion. These factors are essential for dose and potential toxicity. If the compound is not excreted efficiently, its concentration can increase and be dangerous, so understanding the excretion pathway helps design a safe dose. Toxicity tests showed that this compound did not have AMES toxicity, indicating safety against genetic mutations. However, the maximum tolerated dose of -0.313 (log mg/kg/day) indicates limitations at high doses. Acute oral toxicity (LD50) of 2.849 (mol/kg) and chronic toxicity (LOAEL) of 1.871 (log mg/kg bw/day) indicate potential toxic effects at high doses. Positive hepatotoxicity suggests an impact on liver function and warrants further evaluation. Risk and benefit assessment is vital before clinical therapy. More detailed pharmacokinetic data of the MMV compound are shown in Table 4 below.

Table 4: Pharmacokinetic and toxicity profile of the compound of 12-methoxy-4-methylvoachalotine

Model Name	Prediction Value	Unite
Absorption Properties		
Water solubility	-2.95	(log mol/L)
Caco-2 permeability	1.198	(log Papp in 10^6 cm/s)
Intestinal absorption (human)	99.633	(% absorp)
Skin Permeability	-2.907	(log Kp)
P-glycoprotein substrate	No	(Yes/No)
P-glycoprotein I inhibitor	No	(Yes/No)
P-glycoprotein II inhibitor	Yes	(Yes/No)
Distribution Properties		
VDss(Human)	0.954	(logL/kg)
Fraction unbound (Human)	0.219	(Fu)
BBB permeability	-0.186	(log BB)
CNS permeability	-2.97	(log PS)
Metabolism Properties		
CYP2D6 substrate	No	(Yes/No)
CYP3A4 substrate	Yes	(Yes/No)
CYP1A2 inhibitor	No	(Yes/No)
CYP2C19 inhibitor	No	(Yes/No)
CYP2C9 inhibitor	No	(Yes/No)
CYP2D6 inhibitor	No	(Yes/No)
CYP3A4 inhibitor	No	(Yes/No)
Excretion Properties		
Total Clearance	0.804	(log ml/min/kg)
Renal OCT2 substrate	Yes	(Yes/No)
Toxicity Properties		
AMES toxicity	No	(Yes/No)
Max. tolerated dose (human)	-0.313	(log mg/kg/day)
hERGI Inhibitor	No	(Yes/No)
hERGII Inhibitor	Yes	(Yes/No)
Oral Rat Acute Toxicity (LD50)	2.849	(mol/kg)
Oral Rat Chronic Toxicity	1.871	(log mg/kg_bw/day)

Conclusion

This study successfully demonstrated the potential of 12-Methoxy-4-Methylvoachalotine (MMV) as an anticancer agent through an in silico approach. The pharmacological network successfully identified the key targets of the MMV compound as an anticancer agent, including TP53, PTEN, ESR1, EGFR, and MCL1. The receptor targets are related to the mechanisms of cell growth and proliferation, regulation, and apoptosis. Molecular docking results showed that MMV interacted with various amino acids in the target protein's active site, acting as a native ligand. However, its affinity was comparable to that of the reference ligand. In addition, the physicochemical properties of MMV showed low solubility but good absorption, an essential aspect in drug development. Therefore, further research is needed to implement structural modifications to MMV to enhance anticancer activity and maximize its therapeutic potential.

Conflict of Interest

The authors declare no conflicts of interest.

Author's 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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