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Original Research Article

Molecular Insights into the Anticancer Mechanism of Glycyrrhetinic Acid in Hepatocellular Carcinoma

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Abstract

The study aimed to predict the binding affinity and interaction patterns between DNMT1, DNMT2, DNMT3A, DNMT3B, TET-1, c-Myc, TET-2, NF-kB and methionine synthase in complex with Glycyrrhetinic acid (GA) using molecular docking simulations. In this study, A crystal structure of proteins (DNA methyltransferase 1, DNA methyltransferase 2, DNA methyltransferase 3A, DNA methyltransferase 3B, NF-kB, TET-1, c-Myc, TET-2 and methionine synthetase) was downloaded from the Protein Data Bank (PDB). the Auto Dock Vina and visualization by Discovery Studio and Chimera program were utilized for molecular docking study. The docking findings are examined to determine the docking pose based on binding affinity, hydrogen bonding, and other beneficial interactions (hydrophobic bond). In addition, it is used to visualise the proteins ligand interactions and analyze the binding pose of GA. Comparing the various binding energies and torsions of the test compound and the control revealed that the test GA had a perfect docking score, and it was predicted to possess comparable anti-tumour and anticancer activity.

Keywords: Molecular docking, HCC, Glycyrrhetinic acid (GA), DNA methyltransferases, Auto dock, epigenetics.

INTRODUCTION

Hepatocellular Carcinoma (HCC) is a very aggressive malignancy with few treatment options and a death rate that parallels its occurrence (Suresh *et al*., 2020). Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the fourth leading cause of cancer-related death globally (McGlynn *et al*., 2015).

The World Health Organization predicts that over a million people will pass away from liver cancer by 2030, based on yearly projections. The understanding of the molecular profiles, risk factors, and epidemiology of HCC has advanced significantly. Furthermore, logical methods for early detection, diagnosis, treatment, surveillance, and prevention have been established (Yang *et al*., 2019). In addition, early detection of HCC by surveillance is crucial for the treatment of high-risk patients.

Novel anticancer agents produced and identified from natural sources have received increased attention. These chemicals constitute an important alternative for the enhancement of existing mainstream cancer therapy (Zuo *et al*., 2022).

Glycyrrhetinic acid (GA), additionally known as enoxolone, is a triterpenoid derivative of beta-amyrin and the aglycone generated from the intestinal hydrolysis of glycyrrhizin, a pentacyclic triterpenoid found in licorice roots and rhizomes (Sharifi-Rad *et al*., 2021). GA naturally occurs as 18β-GA, generated from 18β-glycyrrhizin, and can be isomerized into the α-isoform in

alkaline circumstances (Pastorino *et al*., 2018). It inhibits cell proliferation, invasion, and metastasis, arrests the cell cycle, induces autophagy and apoptosis, and reduces immunosuppression, making it effective against HCC (Graebin, 2018). GA has been shown to effectively treat HCC through various mechanisms, including cell cycle arrest, autophagy, apoptosis (Tang *et al*., 2014), and reduced immunosuppression (Cai *et al*., 2016).

Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequences. Epigenetic gene patterns are critical in many biological processes, including embryonic development, genetic imprinting, and X-chromosome inactivation (Tsai and Baylin, 2011). Accordingly, these processes are essential for preserving cell identity and significantly impact development, stem cell renewal, genome integrity, and proliferation (Arechederra *et al*., 2020; Brien *et al*., 2016). Cancer is one human disease that is frequently associated with epigenetic dysregulation (Cheng *et al*., 2019).

Epigenetic events typically include chromatin remodelling, noncoding RNA impacts, DNA methylation, histone modification, and the readout of these alterations. Three roles—" writer," "reader," and "eraser"—can be applied to the components engaged in various modification patterns. The terms "writers" and "erasers" refer to enzymes that add or delete chemical groups from DNA or histones, respectively. "Readers" are proteins that can identify changed DNA or histones (Cheng *et al*., 2019).

Molecular docking is an essential tool in structural biology and computational chemistry. It forecasts the ligand's major binding mechanisms to a protein. This tool ranks and predicts the biological activities and mechanisms of action of several substances, including phytocompounds and synthetic chemicals (Morris and Lim-Wilby, 2008). Advancements in genetics and molecular biology have led to the identification of new targets for anticancer drugs. Structure-based drug design is a popular approach for finding molecules with specific anticancer properties (Kurian, 2024).

We used molecular docking simulations to estimate the binding affinity and interaction patterns of DNMT1, DNMT2, DNMT3A, DNMT3B, TET-1, c-Myc, TET-2, NF-kB, and methionine synthase in complex with Glycyrrhetinic acid (GA).

MATERIAL AND METHOD

Ligand preparation

The structure of Glycyrrhetinic acid (GA) was downloaded from PUBCHEM database in SDF format.

Protein Preparation

A crystal structure of proteins (DNA methyltransferase 1, DNA methyltransferase 2, DNA methyltransferase 3A, DNA methyltransferase 3B, NF-kB, TET-1, c-Myc, TET-2 and methionine synthetase) was downloaded from the Protein Data Bank (PDB) using the following IDs: 3AV6, 4FSX, 4UVT, 8EIH, 7W7L, 4LT5, 5I4Z, 7NE3 and 6WQ6 respectively.

Molecular Docking

Molecular docking analysis has been performed using the AutoDock Vina and visualization by Discovery Studio and Chimera program.

In silico pharmacokinetics evaluation of Glycyrrhetinic acid (GA)

Depending on pharmacokinetics, GA showed high gastric intestinal absorption, it does not affect blood- brain barrier (BBB) permeant, affects P-glycoprotein (P-gp) substrate, and has skin permeation of -5.27 cm/s. On the other hand, GA does not affect cytochrome P450 isomerase (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). On the other hand, the drug-likeness was performed using Lipinski, Ghose, Veber, Egan, Muegge and Bioavailability score. The Lipinski filter was the first to filter out any medication at the absorption or permeation level. An ideal drug has a molecular weight (MW) of less than 500 g/mol, Log of the octanol/water partition coefficient (log P) value of ≥ 4.15, and a maximum of two H-donor and four H-acceptor atoms (Lipinski *et al*., 2012). The Ghose filter defines the drug-likeness requirements as the following: The WLOGP ≥ 5.6, MW from 160 to 480, Molar Refractivity (MR) ≥ 130, and total atoms ≥70 (Ghose *et al*., 1999). The Veber (GSK) rule establishes drug-likeness limitations as rotatable bond count 1 and topological polar surface area (TPSA 75) (Veber *et al*., 2002). The bioavailability score was used to predict the possibility of a chemical having 0.85 oral bioavailability in rats (Martin, 2005). We conclude that GA does violation any existing drug-likeness rules including Lipinski, Ghose, Egan, and Muegge 1,3,1,1 respectively as shown in Figure 1.

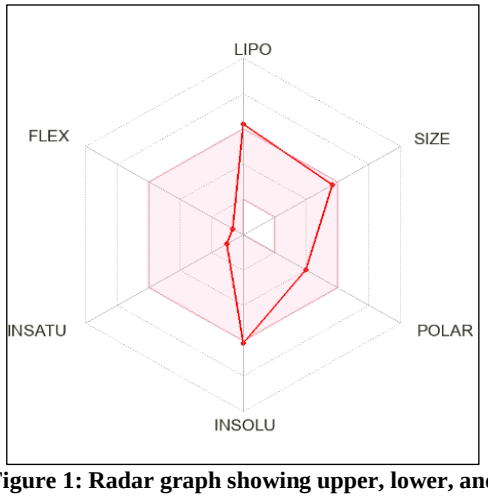


Figure 1: Radar graph showing upper, lower, and predicted values of various physicochemical and molecular properties of GA

RESULT

Docking of Glycyrrhetinic acid (GA) into DNA methyltransferase 1 (DNMT 1) Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of (DNMT1) (PDB ID: 3AV6). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligand (SAM) which was bonded with DNMT1 through a docking score –8.4 kcal/mol. The compound docked correctly into the active binding site of DNMT1(Table 1) with docking scores of -8.4 kcal/mol. GA bindings interact with a backbone of DNMT1 via hydrogen bonds (H-bonds) with ASP1419 and hydrophobic bonds with ILE1571(Table 2). This finding shows that therapy with GA decreased the hypermethylation activity in HCC-developed rats as revealed by the DNMT1 expression profile.

Docking of GA into DNA methyltransferase 2 (DNMT2) Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of (DNMT2) (PDB ID: 4FSX). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligand (SAH) which was bonded with DNMT2 through a docking score of -8.1 kcal/mol. The compound docked correctly into the active binding site of DNMT2 (Table 1) with docking scores of -8.1 kcal/mol. GA bindings interact with a backbone of DNMT2 via hydrogen bonds (H-bonds) with SER454 and a hydrophobic bond with TRP 476 (Table 2). This data indicates the hypermethylation activity in HCC-developed rats evidenced by DNMT2 expression profile, which was reduced upon treatment with GA.

Docking of GA into DNA methyltransferase 3A (DNMT 3A) Binding Site

We performed molecular docking studies using the catalytic binding pocket of DNMT3A (PDB ID: 4UVT) to comprehend the binding mode of GA. Herein, we report the binding pose of GA and compare it with the standard co-crystal ligand (SAH) which was bonded with DNMT3A through a docking score of -7.4 kcal/mol. The compound docked correctly into the active binding site of DNMT3A (Table 1) with docking scores of -7.4 kcal/mol. GA binding interacts with backbone of DNMT3A via hydrogen bonds (H-bonds) with Ser807, Ser10, Lys9, Arg8, Ala7, nr6, Gln5, Arg544, Gly543, Asp529, Cys524, Tyr536, Lys4, Cys541, Val546, Thr3, Asp531, Tyr533, Gln534, Met548, Arg2, Ala1, Glu578 and Ala575 (Table 2). This result demonstrates the hypermethylation activity in HCC-developed rats indicated by DNMT3A expression profile which has been reduced upon treatment with the GA.

Docking of GA into DNA methyltransferase 3B (DNMT 3B) Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of DNMT3B (PDB ID: 8EIH). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligand (SAH) which was bonded with DNMT3A through a docking score of -7.3 kcal/mol. The compound docked correctly into the active binding site of DNMT3B (Table 1) with docking scores of -7.3 kcal/mol. GA bindings interact with a backbone of DNMT3B via hydrogen bonds (H-bonds) with LYS517 and hydrophobic bonds with TRP522, LEU488 and PRO521 (Table 2). In HCC-developed rats, the results show a reduced DNMT3B expression profile following treatment with GA, these data demonstrate the hypermethylation activity in these rats.

Docking of GA into nuclear factor kabba (NF-kB) Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of NF-kB (PDB ID: 7W7L). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligan which was bonded with NF-kB through docking score -7.2 kcal/mol. The compound docked correctly into the active binding site of NF-kB (Table 1) with docking scores of -7.2 kcal/mol. GA bindings interact with a backbone of NF-Kb via hydrogen bonds (H-bonds) with PRO223, ARG311 and ARG49. (Table 2). GA binding interacts with a backbone of NF-kB via hydrophobic bonds with ARG313, HIS218 and ILE189 and carbon-hydrogen bonds with ALA225. These findings point to potential methylomic alterations in NF-kb in rats with HCC and imply that GA may have a regulatory function in their expression during HCC treatment.

Docking of GA into nuclear factor ten-eleven translocation 1 (TET-1) Binding Site

We performed molecular docking studies using the catalytic binding pocket of TE-1 (PDB ID: 4LT5) to comprehend the binding mode of GA. Herein, we report the binding pose of GA and compare it with the standard co-crystal ligan (OGA) which was bonded with TET-1 through a docking score of -7.4 kcal/mol. The compound docked correctly into the active binding site of TET-1 (Table 1) with docking scores of -7.4 kcal/mol. GA bindings interact with a backbone of TET-1 via hydrogen bonds (H-bonds) with THR79 and SER77. GA binding interacts with a backbone of TET-1 via a hydrophobic bond with PRO266, PHE78 and PRO113 (Table 2). This finding reveals that TET-1 protein expression increased significantly in rats treated with GA.

Docking of GA into c-Myc Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of DNMT3B (PDB ID: 5I4Z). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligand (GOL) which was bonded with c-Myc through a docking score of -7.6 kcal/mol. The compound docked correctly into the active binding site of c-Myc (Table 1) with docking scores of -7.6 kcal/mol. GA bindings interact with a backbone of c-

Myc via hydrogen bonds (H-bonds) with GLU36 and hydrophobic bonds with ALA54 and TYRB55 (Table 2).

Docking of GA into nuclear factor ten-eleven translocation 2 (TET-2) Binding Site

To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of TE-1 (PDB ID: 7NE3). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligan (MES) which was bonded with TET-2 through a docking score of -9.8 kcal/mol. The compound docked correctly into the active binding site of TET-2 (Table 1) with docking scores of -9.8 kcal/mol. GA bindings interact with a backbone of TET-2 via hydrogen bonds (H-bonds) with LEU1886 and GLU1413. GA binding interacts with a backbone of TET-2 via a hydrophobic bond with LEU1415, LEU1447, PHE1450, ARG1452 and PRO1885 (Table 2). According to this finding, rats given GA had a significant increase in TET-2 protein expression.

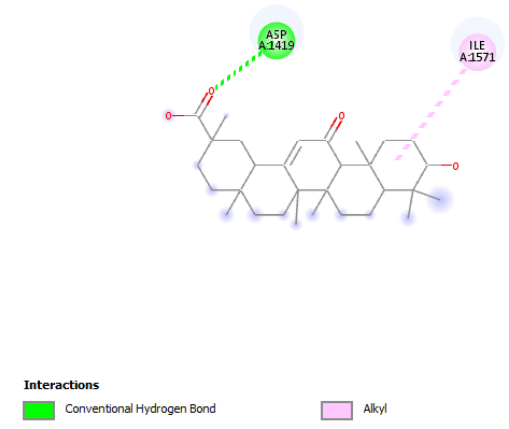
Docking of GA into nuclear factor Methionine synthetase Binding Site

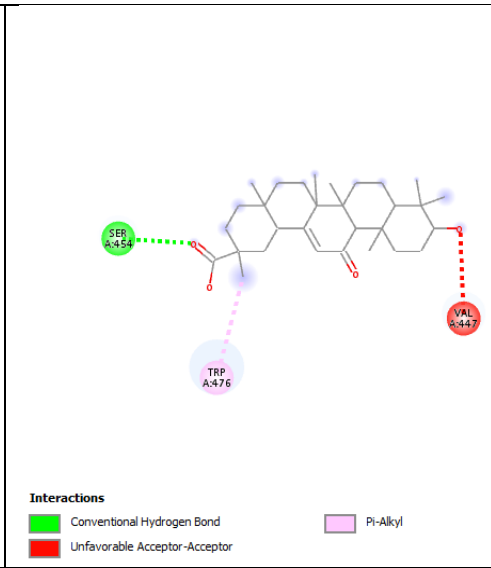
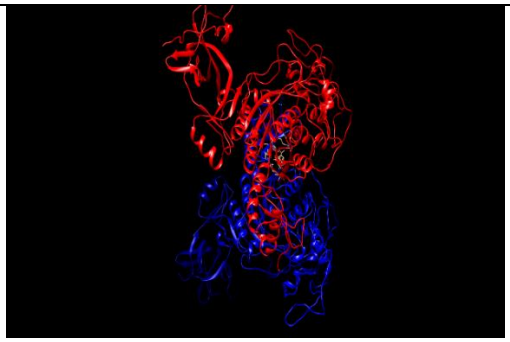
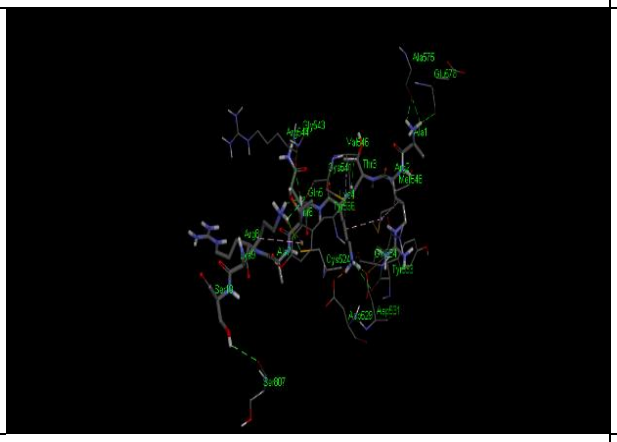
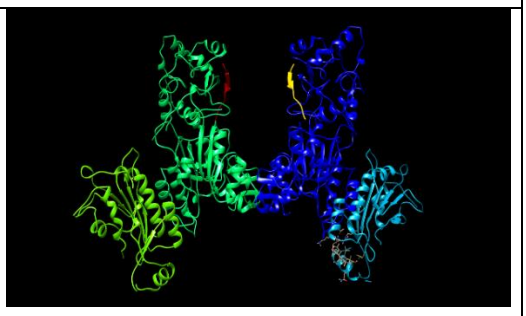
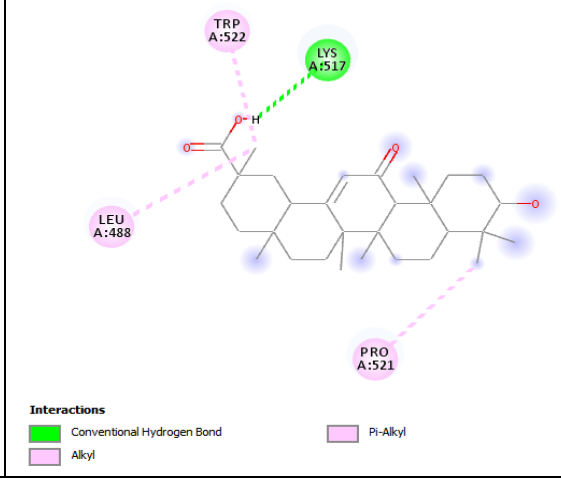
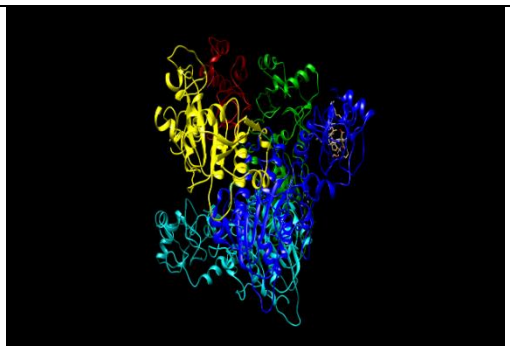
To comprehend the binding mode of GA we performed molecular docking studies using the catalytic binding pocket of Methionine synthetase (PDB ID: 6WQ6). Herein, we report the binding pose of GA and compare it with the standard co-crystal ligan (MET) which was bonded with Methionine synthetase through a docking score of -9.6 kcal/mol. The compound docked correctly into the active binding site of Methionine synthetase (Table 1) with docking scores of -9.6 kcal/mol. GA bindings interact with a backbone of Methionine synthetase via hydrogen bonds (H-bonds) with ASN15, GLY21 and ASN321. GA binding interacts with a backbone of Methionine synthetase via a hydrophobic bond with TYR13, TYR323 and HIS22 (Table 2).

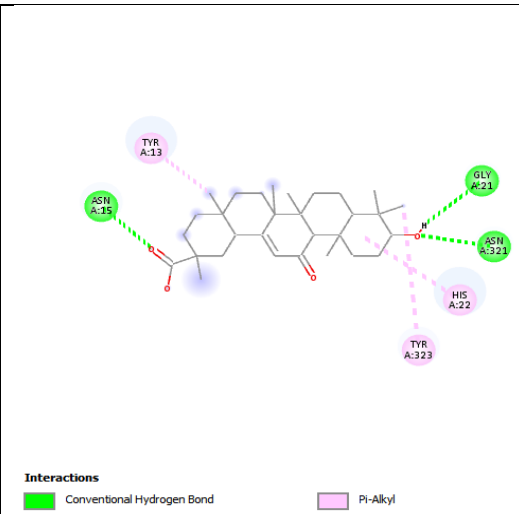
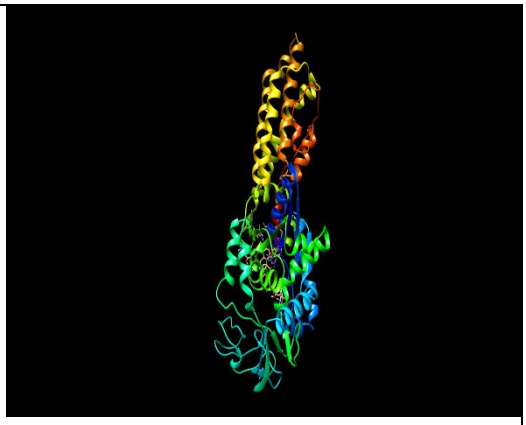
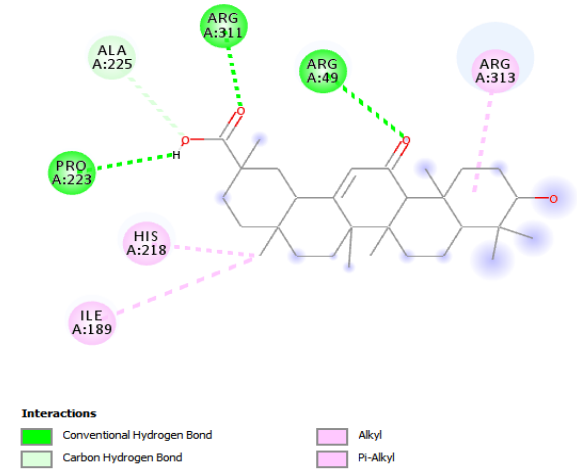
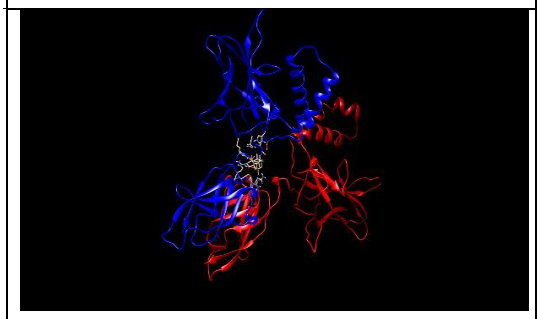
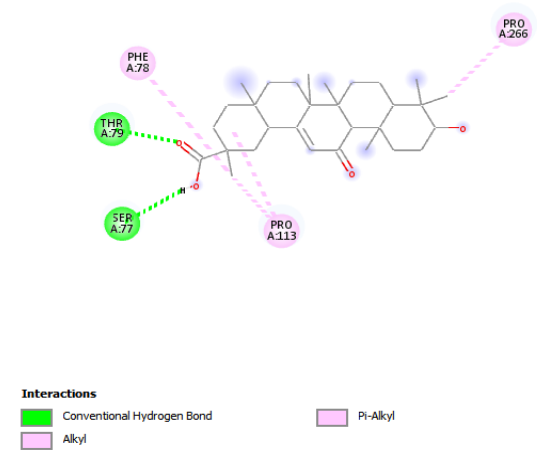
Table 1: Binding energies (kcal/mol) obtained after docking

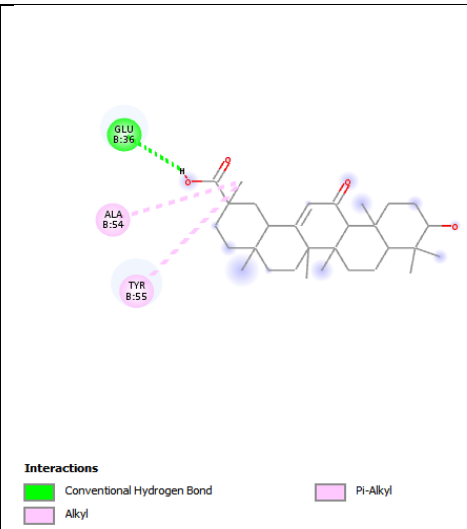
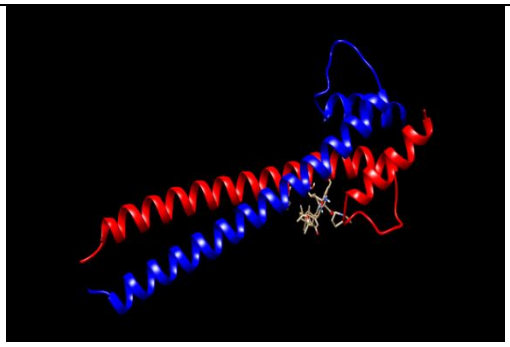
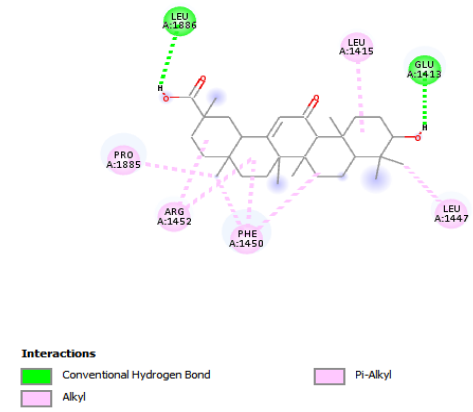
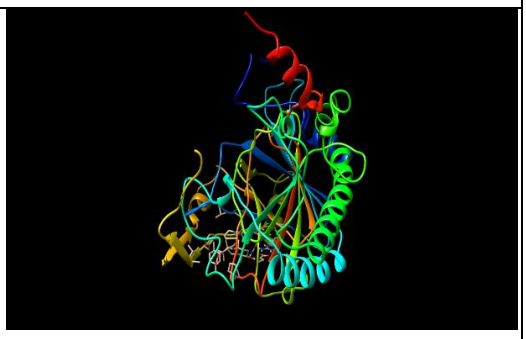
Protein	PDB-ID	Glid score	Co-crystalised ligands
DNA methyltransferase 1	3AV6	-8.4	SAM
DNA methyltransferase 2	4FSX	-8.1	SAH
DNA methyltransferase 3A	4UVT	-7.4	SAH
DNA methyltransferase 3B	8EIH	-7.3	SAH
NF-Kb	7W7L	-7.2	
TET-1	4LT5	-7.4	OGA
c-Myc	5I4Z	-7.6	GOL
TET-2	7NE3	-9.8	MES
methionine synthase	6WQ6	-9.6	MET

Table 2: 2D interaction diagrams between the docked ligands and their potential targets

Proteins	2D Interactions diagrams	3D interactions diagrams
DNMT-1		

DNMT2		
DNMT3A		
DNMT3B		

Methionine synthase		
NF-Kb		
TET-1		

c-Myc		
TET-2		

DISCUSSION

Molecular docking is essential in structural biology and computational chemistry. It forecasts the ligand's dominant binding mechanisms to a protein. This tool ranks and predicts biological activity and mechanisms of action for a vast number of substances (Kurian, 2024). The orientation of the ligands within the protein binding site will be revealed. Here The Protein Data Bank (PDB) provided the following IDs to download a crystal structure of proteins: 3AV6, 4FSX, 4UVT, 8EIH, 7W7L, 4LT5, 5I4Z, 7NE3, and 6WQ6, respectively. The proteins DNMT1, DNMT2, DNMT3A, DNMT3B, NF-kB, TET-1, c-Myc, TET-2, and methionine synthetase.

DNA methylation is a direct chemical alteration of DNA that mostly adds methyl groups to cytosine residues in the presence of a CpG dinucleotide (Robertson, 2005). This change is carried out by three highly conserved enzymes: DNA methyltransferase 1 (DNMT1), which maintains the methylation pattern of DNA, and DNMT3a and DNMT3b, which do de novo methylation (Edwards *et al*., 2017), which the GA is the primary licorice extract active ingredient, is the selection of ligand and interact with hydrogen bond with ASP1419 and with hydrophobic bond with ILE1571. utilized the binding pocket of DNMT1 (3AV6) that presents the binding pose of GA and contrasted with co-crystal ligand (SAM) with docking score -8.4 kcal/mol. Interestingly, GA into DNMT2 shows a binding pose with a co-crystal ligand (SAH) that has a docking score of -8.1 kcal/mol, which significant amino acid (SER454) via hydrogen bond. Moreover, DNMT3A gives a docking score of -7.4 kcal/mol which was reported with standard co-crystal ligand (SAH). It connected with GA via hydrogen bond w h amino acid (Ser807, Ser10, Lys9, Arg8, Ala7, nr6, Gln5, Arg544, Gly543, Asp529, Cys524, Tyr536, Lys4, Cys541, Val546, Thr3, Asp531, Tyr533, Gln534, Met548, Arg2, Ala1, Glu578 and Ala575) while DNMT3B note with standard co-crystal ligand (SAH) and demonstrate a docking score -7.3 kcal/mol. GA converse with it via hydrogen bond (LYS517). So, GA has a methylation inhibitor of HCC.

NF-κB is a transcription factor that regulates immunological response, inflammation, cell proliferation, survival, and development (Nakajima & Kitamura, 2013). GA into NF-kB binding interact with NF-kB suggesting the amino acid residues (PRO233, ARG311 and ARG49) that present the binding pose of

GA with NF-kB by docking score -7.2kcal/mol. Likewise, after retrieving NF-κB with UniProt ID P19838 from the UniProtKB Data Base, its energy was minimized. It was used to run molecular dynamic simulations using Gromacs 5.0.7 software. The GROMOS96 43A1 force field, which used the steepest descent approach and A significant role for amino acid residues (Lys52, Ser243, Asp274, Lys, 275) in the compound's anti-breast cancer activity was suggested by its binding interactions with the active site of NF-κB proteins (Mukund *et al*., 2019).

TET proteins are iron (II)- and α-ketoglutarate-dependent dioxygenases, consisting of 11 translocations. TET1, TET2, and TET3 catalyze further oxygenation events in DNA (5-methylcytosine) (Joshi *et al.*, 2022). Importantly, the molecular docking of GA into TET-1 gives a docking score of -7.4 kcal/mol and has a co-crystal ligand (OGA) which GA connected with GA via H-bond (THR79 and SER77) while TET-2 has a docking score of -7.6 kcal/mol, that compared with co-crystal ligand (MES) and suggest amino acid (LEU1415, LEU1447, PHE1450, ARG1452 and PRO1885.

Myc is an oncogene that plays an important role in oncogenic tumors such as breast, prostate, colon, and cervical cancer. It also causes lymphoma, myeloid leukemia, small-cell lung carcinomas, and neuroblastoma (Kalkat *et al*., 2017). So, docking into c-Myc indicates a docking score of -7.6 kcal/mol, when compared with co-crystal ligand (GOL). Through hydrogen bonds (H-bonds) with GLUB36, GA binding interacts with the c-Myc backbone.

Finally, methionine synthase demonstrates the binding pose of GA and comparison with the standard co-crystal ligand (MET) with a score of -9.6kcal/mol.

The inhibition of GA into proteins (DNMT1, DNMT2, DNMT3A, DNMT3B, TET-1, TET-2, NF-kB, c-Myc and methionine synthase) is highlighted. Therefore, GA may be a promising treatment option for HCC.

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