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# In-Silico Evaluation of FDA-Approved Drugs as PDE5 Inhibitors for Treatment of Hepatocellular Cancer

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**Abstract:** Hepatocellular carcinoma (HCC) is a predominant cause of cancer-associated mortality, characterized by restricted treatment alternatives and unfavorable prognosis. Phosphodiesterase type 5 (PDE5) plays an important role in cancer development; thus, repurposing FDA-approved PDE5 inhibitors for HCC therapy, focusing on essential pathways like apoptosis and angiogenesis, would provide safe and effective treatment alternatives. This work seeks to assess the potential of FDA-approved drugs from the DrugBank library as PDE5 inhibitors against HCC-related targets by in-silico methods, thus identifying a safe and effective PDE5 inhibitor for HCC therapy. Molecular docking was conducted to evaluate the binding affinities of PDE5 inhibitors. Furthermore, ADMET profiling was performed utilizing web servers such as SwissADME, pkCSM, and ProTox to identify candidates possessing ideal drug-like characteristics. Molecular dynamics simulation was then utilized to confirm the stability and interactions of the highest-performing compounds. The best docked stable drug candidate was then docked with critical oncogenic proteins (PDE5, VEGFR-2, caspase-9, and caspase-7). Metabolite 4-hydroxy Nebivolol (DBMET02738) and Nabumetone ( ) had high docking scores of -12.781 and -12.573 kcal/mol with PDE5 and VEGFR-2 or Caspase-9, with excellent ADMET profiles and stability in the molecular dynamics simulation. Their binding affinities surpassed well-established drugs for HCC, such as Sorafenib or Regorafenib. Nebivolol metabolite (DBMET02738) and Nabumetone metabolite (DBMET02532) exhibit potential as repurposed PDE5 inhibitors for hepatocellular carcinoma treatment, according to their robust protein-binding affinities, advantageous pharmacokinetics, and minimal toxicity profiles.

**Keywords:** *in-silico* molecular docking; MD simulation; HCC; phosphodiesterase 5; cGMP; VEGFR-2; caspase-9.

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## 1. Introduction

Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer [1], represents a significant global health burden due to its high incidence and mortality rates [2,3]. Primarily originating within a background of chronic liver disease, HCC is often associated with underlying conditions such as hepatitis B (HBV) or hepatitis C (HCV) infections, excessive alcohol consumption, and non-alcoholic fatty liver disease (NAFLD) [4,5]. Globally, it is the fifth most common cancer in men and the seventh in women, ranking as the third leading cause of cancer-related deaths. The distribution of HCC varies geographically, reflecting the prevalence of risk factors such as HBV in Asia and Sub-Saharan Africa and rising

NAFLD-associated HCC in Western countries [6]. Unfortunately, late-stage diagnosis and limited therapeutic efficacy contribute to the poor prognosis of advanced HCC, underscoring the urgent need for innovative therapeutic strategies [7].

To facilitate the discovery and preclinical assessment of new HCC treatments, the use of animal models has proven essential. Diethylnitrosamine (DEN)-induced HCC, in particular, is widely used for its ability to emulate human liver disease and malignancy progression [8,9]. DEN acts as a potent hepatocarcinogen by inducing oxidative stress and DNA damage [10], leading to chronic inflammation, liver fibrosis, and subsequent tumor formation [11,12]. This model has enabled the identification and preliminary testing of potential therapeutics, including conventional chemotherapy agents, targeted therapies [13,14], and, recently, phosphodiesterase type 5 (PDE5) inhibitors [15]. Among these, Sorafenib has become the standard therapy for HCC [16]. Yet, treatment outcomes remain modest, driving further research into other promising drug classes and potential repurposing options [17].

PDE5 inhibitors are primarily recognized for their applications in managing erectile dysfunction and pulmonary arterial hypertension due to their ability to relax smooth muscle via modulation of cyclic guanosine monophosphate (cGMP) levels [18]. Interestingly, recent studies have unveiled a broader therapeutic potential for PDE5 inhibitors, suggesting their anticancer effects may extend across several mechanisms [19]. These inhibitors modulate cGMP signaling pathways, influencing cellular processes such as proliferation, apoptosis, angiogenesis, and immune modulation [20]. For instance, elevated PDE5 expression observed in HCC suggests that inhibiting PDE5 may restore cGMP signaling to suppress tumor cell growth [21]. Additionally, PDE5 inhibitors have shown potential in enhancing apoptotic signaling, reducing immunosuppressive cell populations, impeding angiogenesis, and improving chemotherapeutic delivery in solid tumors [22].

Given the complex landscape of HCC treatment, repurposing FDA-approved PDE5 inhibitors presents an attractive avenue for drug discovery [23]. These compounds, with their established safety profiles, allow for accelerated preclinical and clinical evaluation, expediting the potential delivery of novel anticancer therapies [24]. In-silico methodologies such as molecular docking, ADMET prediction [23], and molecular dynamics simulations offer an efficient and cost-effective means to screen and evaluate the binding affinities, stability, and pharmacokinetics of these inhibitors [25] against HCC-relevant targets, particularly VEGFR-2 and caspase proteins implicated in tumor progression and cell death [26]. By harnessing the FDA DrugBank library, this study aims to identify unknown PDE5 inhibitors with significant anticancer potential for HCC treatment.

This article presents a comprehensive in-silico evaluation of unknown PDE5 inhibitors against key molecular targets involved in DEN-induced HCC [27], emphasizing their mechanistic viability and regulatory implications for anticancer therapy. Through this approach, we address a critical gap in the therapeutic arsenal against HCC, aiming to provide a strategic foundation for clinical translation of these repurposed compounds.

## 2. Materials and Methods

### *2.1. Data acquisition.*

#### 2.1.1. FDA drugbank library.

The drug molecules were sourced from the FDA-approved section of the Drugbank database (https://www.drugbank.ca/), which provides a rich repository of information on various approved drugs. The database was queried to extract a list of FDA-approved compounds, focusing specifically on those with detailed molecular structures, pharmacological properties, and established clinical applications. The dataset included information on the active ingredients, drug categories, and molecular interactions, facilitating a comprehensive analysis for potential repurposing in the treatment of hepatocellular carcinoma (HCC).

### *2.2. Selection of PDE5 inhibitors.*

To identify suitable PDE5 inhibitors for evaluation as anticancer agents, specific criteria were applied to filter compounds from the DrugBank library. Firstly, Target Mechanism was prioritized, selecting only compounds categorized as phosphodiesterase type 5 inhibitors to ensure specificity in targeting the PDE5 enzyme, which plays a critical role in modulating cGMP levels within cells. Secondly, Indications and Approved Use were considered, focusing on FDA-approved inhibitors used to treat conditions such as erectile dysfunction or pulmonary hypertension. This approach leverages the known safety profile of these drugs, facilitating their potential repurposing for cancer therapy. Lastly, Chemical Structure and Properties were taken into account, with emphasis on molecular characteristics such as solubility and stability, to ensure the selected compounds align with the desired pharmacological profile for anticancer efficacy. This systematic selection process enhances the relevance and safety of PDE5 inhibitors in their novel application against hepatocellular carcinoma (HCC) [28].

This systematic approach ensured that only relevant PDE5 inhibitors with potential applications in HCC treatment were included for further evaluation.

### *2.3. Molecular docking studies.*

The molecular docking studies aimed to evaluate the binding affinity of selected PDE5 inhibitors with key protein targets involved in DEN-induced hepatocellular carcinoma (HCC). Protein Selection was carefully conducted based on the biological significance of each protein in tumorigenesis and therapeutic potential. The primary target, cGMP-Specific Phosphodiesterase 5 (PDE5), was selected due to its role in regulating intracellular cGMP levels (PDB ID 6L6E), which influence apoptosis and proliferation in cancer cells. Additionally, caspases and VEGF receptors (PDB ID 1NW9 and 3VHE), known to be implicated in HCC progression, were included based on literature emphasizing their role in cancer-related signaling pathways. Structures for these proteins were sourced from the Protein Data Bank (PDB) to ensure accuracy in the docking simulations (https://www.rcsb.org/) [29,30]. Molecular Docking Software utilized included AutoDock Vina, for its efficiency in high-throughput virtual screening and reliable binding affinity predictions, and the Schrödinger Suite (Glide) (Schrodinger Release: Maestro 13.9, Schrodinger, LLC, New York, NY), which offers advanced capabilities in evaluating molecular interactions with high precision, allowing

for detailed scoring and visualization of docking poses. Docking Parameters included Binding Affinity, measured in kcal/mol, where more negative values indicated stronger predicted interactions between PDE5 inhibitors and target proteins. Various Interaction Types were analyzed, such as Hydrophobic Interactions from non-polar side chains, Hydrogen Bonds that stabilize ligand-receptor complexes, and Salt Bridges contributing to binding affinity. These parameters provided insights into the potential efficacy of the selected PDE5 inhibitors as anticancer agents against HCC-associated proteins [31].

*2.4. Pharmacokinetic and ADMET profiling.*

To assess the pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of selected PDE5 inhibitors, SwissADME, pkCSM, and ProTox tools were utilized. SwissADME (http://www.swissadme.ch/index.php# #) was employed to predict key pharmacokinetic properties such as drug-likeness, solubility, permeability, and potential metabolic pathways, ensuring that the compounds exhibit favorable profiles for drug development. pkCSM (https://biosig.lab.uq.edu.au/deeppk/) provided further insights into the toxicity profiles, evaluating parameters like hepatotoxicity, cardiotoxicity, and interactions with key metabolic enzymes [32,33]. ProTox 3.0 (https://tox.charite.de/protox3/), a virtual lab for the prediction of compound toxicities, is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the number of animal experiments [34]. Results Criteria focused on two main benchmarks: Oral Bioavailability with a minimum threshold of 30% to support effective oral administration, and Toxicity Profiles with an emphasis on low predicted toxicity, particularly low hepatotoxicity, to ensure safety for potential therapeutic application in HCC. Compounds meeting these criteria were identified as promising candidates for further investigation in anticancer research.

*2.5. Molecular dynamics (MD) simulations.*

Molecular dynamics (MD) simulations were conducted to assess the stability and behavior of the protein-ligand complexes obtained from the docking studies. Schrodinger Desmond MD simulation package (https://www.schrodinger.com/platform/products/desmond/) was used on the Ubuntu Linux OS. The protein-ligand complexes were solvated in a water box, and ions were added to neutralize the system. The simulations were run for [specific duration, e.g., 100 ns]. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and potential energy were tracked to evaluate the stability of the complexes over time [25].

The results of the MD simulations were analyzed to determine the dynamic stability and conformational changes of the protein-ligand complexes, providing insights into the potential efficacy of the PDE inhibitors.

*2.6. Criteria for selecting potential anticancer agents.*

Selection criteria included high binding affinity to target proteins, favorable ADMET properties (e.g., oral bioavailability, low toxicity), and stability in molecular dynamics simulations.

Comparing the identified compounds with known PDE5 inhibitors, such as sildenafil, and known anticancer drugs such as regorafenib, is a smart strategy. This stage validates the

potential efficacy of the novel inhibitors by confirming their similarity in mechanisms of action with known medicines [35,36]. The discovered compounds are compared with established PDE5 inhibitors, such as sildenafil, to assess their potential [37]. Traditional PDE5 inhibitors primarily target erectile dysfunction but have shown anticancer potential by affecting pathways such as cGMP, VEGFR-2, and caspase 7-9, which play roles in cancer cell apoptosis and inhibition of proliferation. Sorafenib and other HCC drugs also inhibit specific kinase pathways but may not directly interact with PDE5 [38].

This compliance assessment aimed to facilitate a smoother transition from in-silico findings to preclinical and clinical evaluations, ensuring that potential PDE5 inhibitors are not only promising in theory but also viable for therapeutic application in hepatocellular carcinoma.

## 3. Results and Discussion

### *3.1. Molecular docking outcomes.*

#### 3.1.1. Top docking hits.

The molecular docking studies yielded several promising PDE5 inhibitors with significant binding affinities for the selected cGMP (PDB ID 6L6E) target proteins. Initially, we acquired the structure of PDE5 protein cGMP (PDBID: 6L6E) from the Protein Data Bank and visualized it using the Maestro module of the Schrödinger suite (Figure 1A). The protein preparation wizard then processed the acquired protein, clipping all chains (Figure 1B).

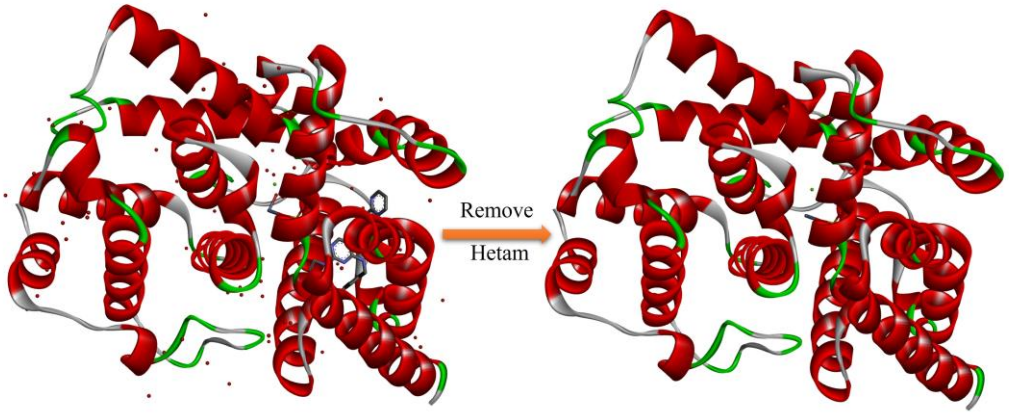


**Figure 1.** Human PDE5 Protein **(A)** Structure of cGMP protein (PDBID: 6L6E) optimized from Protein Data Bank; **(B)** Processed cGMP structure with removing water and hetatm.

The top-scoring hits are summarized in Table 1, which lists their respective docking scores (kcal/mol) and types of interactions observed.

**Table 1.** Docking score of the best 5 FDA-approved DrugBank compounds via Xglide.

| S. No | DrugBank Library compounds | cGMP-based PDE5 inhibitors | |
|---|---|---|---|
| | | Docking score | XP GScore |
| 1 | Nebivolol (4-hydroxy Nebivolol-DBMET02738) | -12.781 | -12.807 |
| 2 | Suprofen (Suprofen glucuronide-DBMET00164) | -12.765 | -12.765 |
| 3 | Nabumetone (reduced O-desmethyl-3-hydroxy-nabumetone glucuronide-DBMET02532) | -12.573 | -12.573 |
| 4 | Fenofibrate (Reduced Fenofibric Acid- DBMET02799) | -12.501 | -12.501 |
| 5 | Ketoprofen (Ketoprofen glucuronide- DBMET00589) | -12.125 | -12.125 |

DBMET02738, DBMET00164, DBMET02532, DBMET02799, and DBMET00589 are the top five FDA-approved DrugBank library compounds. Their docking scores ranged from -12.78 to -12.125 (Table 1).

3.1.2. Binding mode analysis.

The docking analysis of FDA-approved compounds from the DrugBank library against cGMP-based PDE5 identified five promising candidates with strong binding affinities, as measured by docking scores (in kcal/mol) using XGlide. Among them, Nebivolol (4-hydroxy Nebivolol - DBMET02738) demonstrated the highest binding affinity, with a docking score of -12.781 kcal/mol, likely stabilized by hydrophobic contacts and hydrogen bonds, particularly from its hydroxyl group. Suprofen (Suprofen glucuronide - DBMET00164) closely followed, with a docking score of -12.765 kcal/mol, and likely anchors within the PDE5 binding site through its glucuronide group, which facilitates hydrogen bonding and polar interactions. Nabumetone (reduced O-desmethyl-3-hydroxy-nabumetone glucuronide - DBMET02532) showed a docking score of -12.573 kcal/mol, with the presence of hydroxyl and glucuronide groups contributing to hydrogen bonding and a flexible structure that enhances binding adaptability. Fenofibrate (Reduced Fenofibric Acid - DBMET02799) scored -12.501 kcal/mol, leveraging hydrophobic interactions due to its lipid-lowering structure to form a stable inhibitor complex. Lastly, Ketoprofen (Ketoprofen glucuronide - DBMET00589) presented the lowest binding affinity among the top five, with a score of -12.125 kcal/mol, yet still displayed substantial stability through hydrogen bonding facilitated by its glucuronide conjugate. These results suggest that structural features such as hydroxyl and glucuronide groups, along with hydrophobic regions, play a critical role in stabilizing PDE5 interactions, making these compounds valuable candidates for further anticancer research.

The analysis of bond lengths and specific residues involved in these interactions provided insights into the mechanisms by which these PDE5 inhibitors may exert their effects, warranting further investigation.

We employed the SiteMap module of the Schrodinger suite to ascertain the active site of the preprocessed proteins. We utilized SiteMap (Figure 2 and Table 2) to find one possible active site for screening.

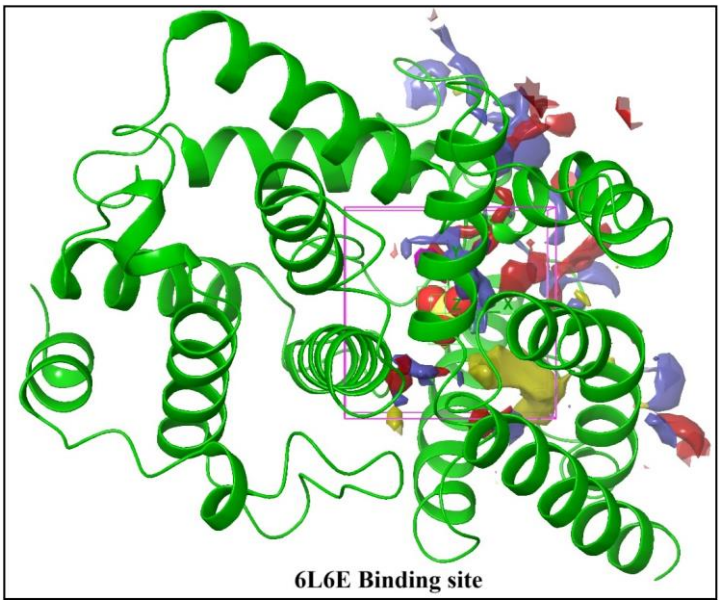


**Figure 2.** Binding sites acquired via SiteMap

**Table 2.** Binding sites parameters via Schrodinger SiteMap.

| Binding sites parameter | 6L6E |
|---|---|
| **PDB resolution** | 1.92 |
| **SiteScore** | 0.945 |
| **size** | 610 |
| **Dscore** | 1.044 |
| **volume** | 291.000 |
| **phobic** | 0.352 |
| **philic** | 0.560 |
| **balance** | 0.628 |
| **don/acc** | 0.901 |

Figure 3 illustrates the surface binding sites of the cGMP (6L6E) protein with the unknown DrugBank PDE5 inhibitors DBMET02532 and DBMET02799, visualized using Schrodinger's Maestro software. The image highlights the interaction between the inhibitor and the protein, showing the specific amino acid residues involved in the binding process. Key residues for DBMET02532, such as TYR 612, VAL 660, and PHE 786, and DBMET02799, such as ASN 661, VAL 660, and PHE 786, are labeled, indicating their roles in the binding site. The 3D representation of the protein includes various colored helices and loops, with the binding site rendered in dark colors to emphasize the interaction. The inset displays the chemical structure of DBMET02532 and DBMET02799, providing a clear view of the inhibitors' molecular features.

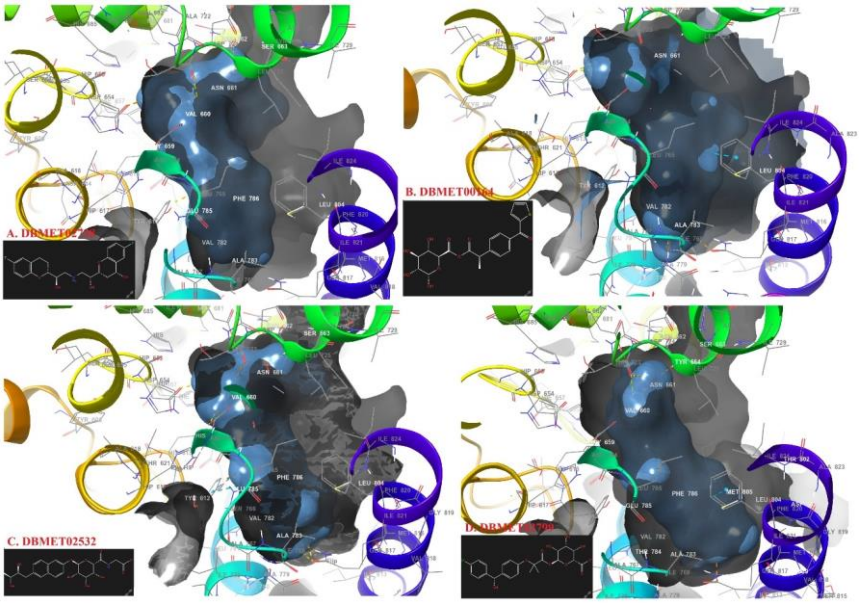


**Figure 3.** Surface binding sites of cGMP (6L6E) protein – unknown DrugBank PDE5 inhibitors acquired via Schrodinger surface (Binding site).

3.1.3. 3D and 2D cGMP based unknown drugbank PDE5 inhibitors structure analysis.

Furthermore, docking studies showed that covalent energy and hydrogen bonds are very important for the best binding affinity of the cGMP-based PDE5 inhibitors as potential anticancer agents targeting DEN-induced hepatocellular carcinoma (HCC). The two- and three-dimensional docking poses showed that ligand binding with PDE5 cGMP is significantly influenced (Figures 4 and 5).

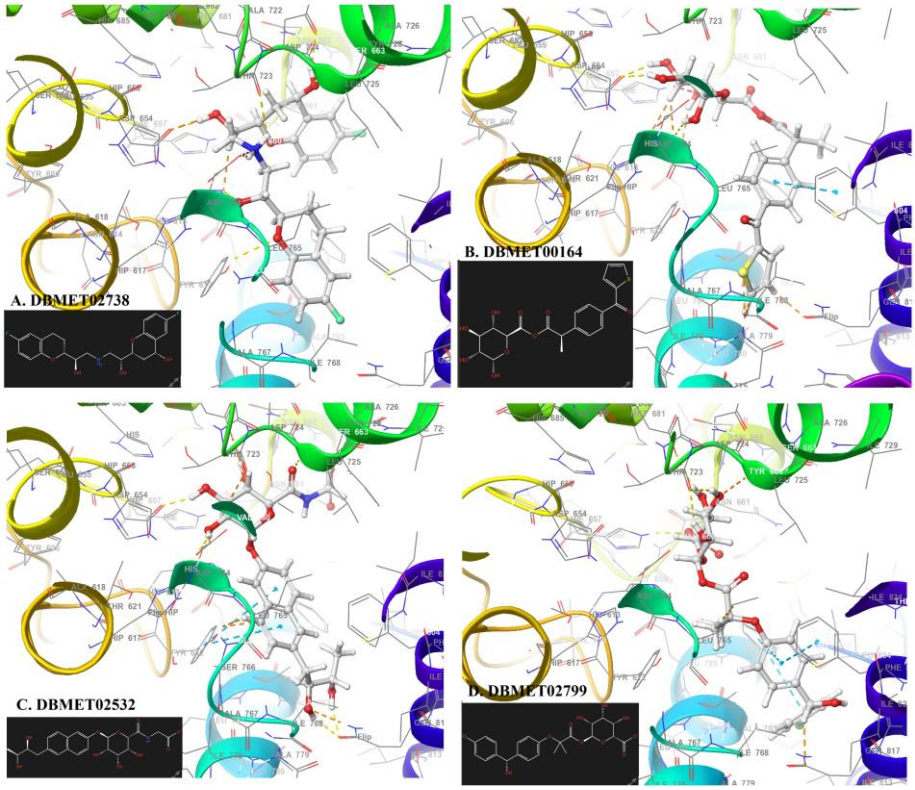


**Figure 4.** 3D best docked cGMP (6L6E) protein – unknown DrugBank PDE5 inhibitors interaction.

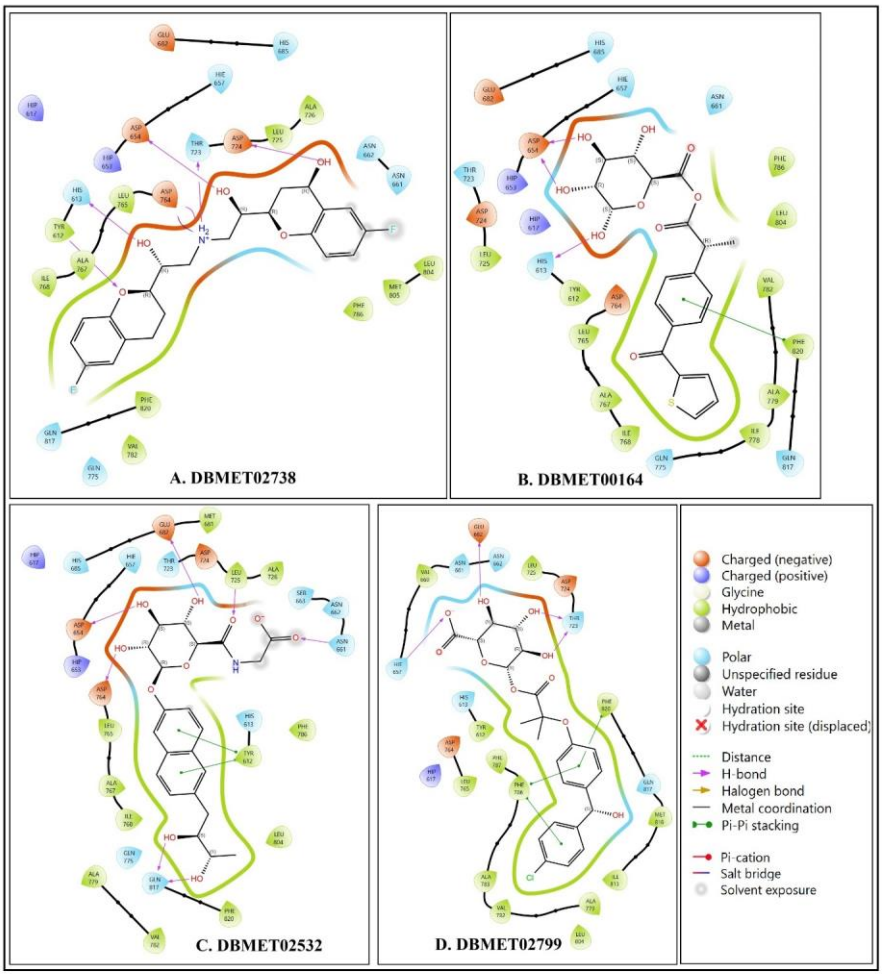


**Figure 5.** 2D best docked cGMP (6L6E) protein – unknown DrugBank PDE5 inhibitors interaction using the Maestro tool in Schrödinger.

*3.2. ADMET and pharmacokinetic profiling results.*

3.2.1. Drug-like properties.

Table 3 presents the drug-like properties of the five best-selected unknown PDE5 inhibitors from the DrugBank library as potential anticancer agents against DEN-induced hepatocellular carcinoma (HCC), assessed using pkCSM and SwissADME tools. Each compound's molecular weight and physicochemical properties, such as LogP (lipophilicity), number of rotatable bonds, hydrogen bond acceptors, and donors, are analyzed to determine their suitability for oral bioavailability and drug-likeness. Compounds DBMET02738, DBMET00164, DBMET02532, and DBMET00589 exceed the typical molecular weight for lead-like compounds, indicating they may require optimization for improved lead-likeness. DBMET02799, however, aligns with the "lead-like" criteria, showing favorable properties with a LogP of 3.14 and fewer acceptors and donors, suggesting better membrane permeability and oral bioavailability. In terms of bioavailability scores, DBMET02799 also stands out with a score of 0.85, indicating it may be more readily absorbed than the others, which generally score lower (0.11–0.55). Additionally, synthetic accessibility scores suggest that DBMET02799 is more feasible to synthesize (2.69) than the other compounds (ranging from 4.48 to 5.04), supporting its potential as a viable lead compound. Overall, these drug-like properties suggest that while all five compounds show some potential, DBMET02532 and DBMET02799 are particularly promising for further development as an anticancer agent for HCC due to their favorable bioavailability, lead-likeness, and synthetic accessibility.

**Table 3.** Drug-like properties of the best-selected unknown DrugBank PDE5 inhibitors as potential anticancer agents targeting DEN-induced hepatocellular carcinoma (HCC) using pkCSM and SwissADME.

| Descriptor | DBMET02738 | DBMET00164 | DBMET02532 | DBMET02799 | DBMET00589 |
|---|---|---|---|---|---|
| Molecular Weight | 421.43 | 436.43 | 408.4 | 320.77 | 430.4 |
| LogP | 2.85 | 0.91 | -0.22 | 3.14 | 1.06 |
| Rotatable Bonds | 6 | 7 | 6 | 5 | 7 |
| Acceptors | 8 | 9 | 9 | 4 | 9 |
| Donors | 4 | 4 | 6 | 2 | 4 |
| Surface Area | 91.18 | 178.83 | 156.91 | 66.76 | 150.59 |
| Leadlikeness | No | No | No | Yes | No |
| Synthetic accessibility | 4.48 | 4.9 | 4.83 | 2.69 | 5.04 |
| Bioavailability score | 0.55 | 0.55 | 0.11 | 0.85 | 0.11 |

Table 4 summarizes the pharmacokinetic properties of the top-selected unknown PDE5 inhibitors from the DrugBank library, evaluated as potential anticancer agents for DEN-induced hepatocellular carcinoma (HCC) using Deep-PK and SwissADME. Two compounds, DBMET02738 and DBMET02799, demonstrate high gastrointestinal (GI) absorption, suggesting they may be more bioavailable when administered orally. Notably, DBMET02799 also shows blood-brain barrier (BBB) permeability, implying potential central nervous system (CNS) activity, which could be beneficial or a limitation depending on the therapeutic context. P-glycoprotein (P-gp) substrate status varies, with DBMET02738, DBMET00164, and DBMET00589 identified as P-gp substrates, indicating a likelihood of efflux from cells, which could impact intracellular drug concentration and efficacy. In terms of cytochrome P450 enzyme interactions, DBMET02738 and DBMET02799 inhibit CYP2D6, with DBMET02738 also inhibiting CYP3A4, raising the potential for drug-drug interactions that could complicate co-administration with other therapies. Skin permeation values (Log Kp) indicate that DBMET02799 has the highest skin permeability (-5.86), which could influence transdermal

absorption if relevant. Additionally, total clearance data suggest DBMET02799 has notably lower clearance (-26.296), indicating a prolonged half-life and sustained activity in the system. None of the compounds serve as renal OCT2 substrates, which minimizes the likelihood of renal transport-related issues. Overall, DBMET02532 and DBMET02799 appear to have the most favorable pharmacokinetic profile, with high GI absorption, BBB permeability, moderate P-gp interaction, and low clearance, positioning them as strong candidates for further investigation in HCC treatment.

**Table 4.** ADME-related drug-like Pharmacokinetic Properties of the best-selected unknown DrugBank PDE5 inhibitors as potential anticancer agents targeting DEN-induced hepatocellular carcinoma (HCC) using Deep-PK and SwissADME.

| Pharmacokinetics | DBMET02738 | DBMET00164 | DBMET02532 | DBMET02799 | DBMET00589 |
|---|---|---|---|---|---|
| GI _Absorption | High | Low | Low | High | Low |
| BBB _ Permeability | No | No | No | Yes | No |
| P-gp _substrate | Yes | Yes | No | No | Yes |
| CYP1A2 _inhibitor | No | No | No | No | No |
| CYP2C19 _inhibitor | No | No | No | Yes | No |
| CYP2C9 _inhibitor | No | No | No | No | No |
| CYP2D6 _inhibitor | Yes | No | No | Yes | No |
| CYP3A4 _inhibitor | Yes | No | No | No | No |
| Log Kp_ (skin permeation) | -6.79 | -8.05 | -8.33 | -5.86 | -7.69 |
| Total Clearance | 0.849 | 0.134 | 0.772 | -26.296 | 2.653 |
| Renal OCT2 _substrate | No | No | No | No | No |

3.2.2. Toxicity profiles.

Table 5 presents the oral toxicity profiles of the top-selected PDE5 inhibitors from the DrugBank library, predicted using ProTox 3.0. The LD50 values indicate a wide range of lethal doses, with DBMET00164 having the lowest $LD_{50}$ (71 mg/kg, indicating high toxicity and Toxicity Class 1), while DBMET02532 has the highest $LD_{50}$ (3750 mg/kg, indicating lower toxicity and Toxicity Class 5). Most compounds fall under Toxicity Classes 4 or 5, suggesting moderate to low acute toxicity, except for DBMET00164, which is considerably more toxic. Interestingly, all compounds are non-hepatotoxic and neurotoxic, suggesting a potentially safe profile concerning liver and brain health. However, all compounds exhibit nephrotoxicity and respiratory toxicity, which could limit their therapeutic use or require monitoring during clinical trials. Cardiotoxicity is observed in all except DBMET02532 and DBMET02799, while DBMET02799 also shows mild carcinogenic potential, a unique finding among the compounds. Immunotoxicity is notable only in DBMET02738, which may affect the immune response. Although none of the compounds demonstrate mutagenicity or cytotoxicity, which is favorable for cancer treatments, the active BBB barrier crossing ability of most compounds indicates potential CNS effects, warranting further evaluation. Lastly, ecotoxicity is observed in DBMET02799, potentially impacting environmental safety. Overall, DBMET02532 and DBMET02799 present relatively safer toxicity profiles, albeit with considerations for nephrotoxicity, respiratory toxicity, and specific side effects such as carcinogenicity in DBMET02799. These findings provide a balanced perspective on the safety and potential risks of these PDE5 inhibitors as anticancer candidates for HCC.

**Table 5.** Oral toxicity prediction of the best-selected PDE5 inhibitors as potential anticancer agents targeting DEN-induced hepatocellular carcinoma (HCC) using ProTox 3.0.

| Organ Target | DBMET02738 | DBMET00164 | DBMET02532 | DBMET02799 | DBMET00589 |
|---|---|---|---|---|---|
| LD50 | 2000mg/kg | 71mg/kg | 3750mg/kg | 2400mg/kg | 3220mg/kg |
| Toxicity Class | 4 | 1 | 5 | 5 | 5 |
| Hepatotoxicity | Inactive | Inactive | Inactive | Inactive | Inactive |

| Organ Target | DBMET02738 | DBMET00164 | DBMET02532 | DBMET02799 | DBMET00589 |
|---|---|---|---|---|---|
| Neurotoxicity | Inactive | Inactive | Inactive | Inactive | Inactive |
| Nephrotoxicity | Active | Active | Active | Active | Active |
| Respiratory toxicity | Active | Active | Active | Active | Active |
| Cardiotoxicity | Active | Active | Inactive | Inactive | Active |
| Carcinogenicity | Inactive | Inactive | Inactive | Active | Inactive |
| Immunotoxicity | Active | Inactive | Inactive | Inactive | Inactive |
| Mutagenicity | Inactive | Inactive | Inactive | Inactive | Inactive |
| Cytotoxicity | Inactive | Inactive | Inactive | Inactive | Inactive |
| BBB-barrier | Active | Active | Inactive | Active | Active |
| Ecotoxicity | Inactive | Inactive | Inactive | Active | Inactive |
| Clinical toxicity | Active | Active | Active | Inactive | Active |
| Nutritional toxicity | Inactive | Inactive | Active | Inactive | Inactive |

*3.3. Molecular dynamics simulation results.*

Molecular dynamics simulations indicated that the selected PDE5 inhibitors maintained structural integrity in complex with their target proteins over the simulation duration. The Root-Mean-Square Deviation (RMSD) of the complexes remained below 2.0 Å, indicating stable conformations. Binding energy analysis revealed that DBMET02532 exhibited a binding energy of -91.062 kcal/mol. The Molecular Dynamics Simulation results for the ligand DBMET02532 reveal detailed insights into its interactions and stability within the protein binding site over a 100 ns simulation at 310 K.

3.3.1. Protein and ligand RMSD.

Figure 6A illustrates the RMSD (Root Mean Square Deviation) trajectories for both the protein backbone (Cα) and the ligand (fitted on the protein) over the simulation period, which measures their stability within the binding complex of DBMET02532.

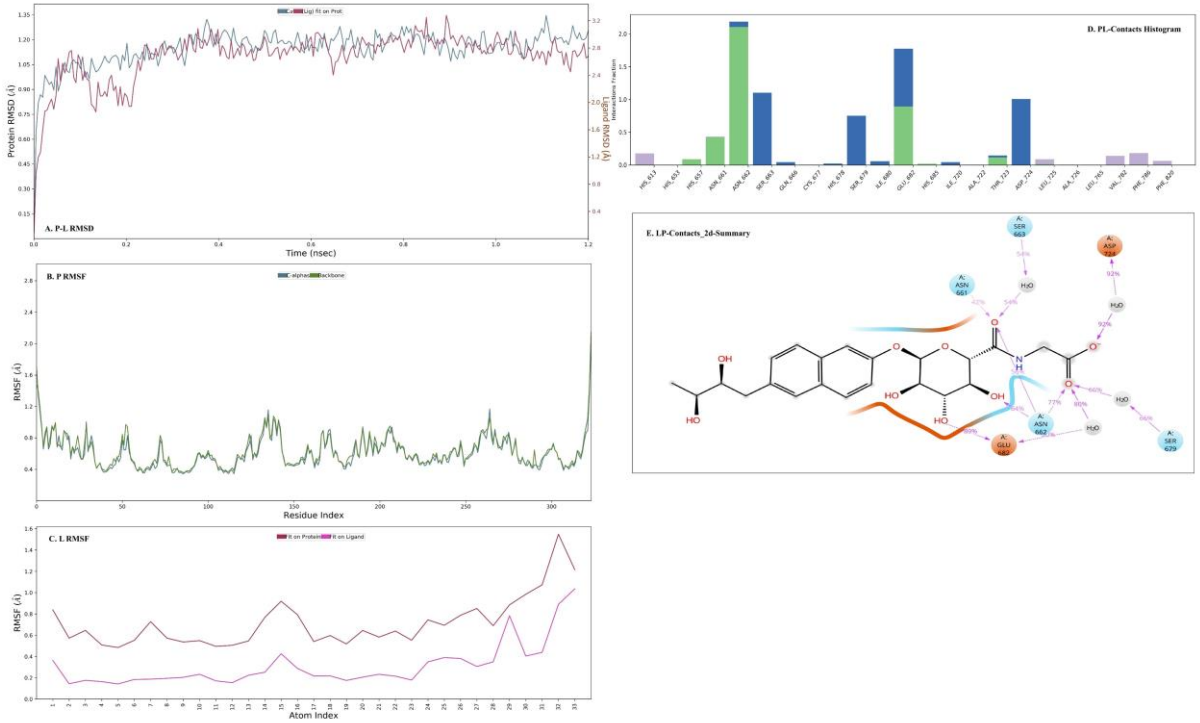


**Figure 6. (A)** P-L RMSD; **(B)** Protein RMSF; **(C)** Ligand RMSF; **(D)** protein-ligand contact histogram; **(E)** protein-ligand contacts of the top docked ADMET favorable DrugBank compound of DBMET02532.

The Protein RMSD of the protein backbone (blue line) gradually increases during the initial phase of the simulation, reaching a relatively stable range around 0.8-1.2 Å after approximately 0.2 ns. This indicates that the protein structure undergoes some initial

conformational adjustments but stabilizes soon after, showing no major deviations over the remaining simulation time. This stability suggests that the protein maintains its structural integrity and does not undergo significant conformational changes.

The Ligand RMSD of the ligand (pink line) exhibits a similar pattern, with initial fluctuations that settle around a stable range of approximately 2.6-3.2 Å. The ligand RMSD being higher than the protein RMSD is expected, as ligands often experience more flexibility within the binding pocket. The steady RMSD values after the initial phase imply that the ligand has found a stable orientation within the binding site and maintains consistent interactions with the protein.

Overall, the RMSD trajectories for both the protein and ligand indicate that the DBMET02532-protein complex is stable, with no significant structural drift. The stability of both components suggests that DBMET02532 remains well-bound and potentially effective in maintaining interactions within the target site over time. This stable binding is promising for its efficacy as a potential anticancer agent.

### 3.3.2. Protein and ligand RMSF.

The second plot, Figure 6B, presents RMSF for the protein alone, with the backbone and C-alpha atoms specifically highlighted (green and blue lines, respectively). The similarity in RMSF values between the backbone and C-alpha atoms suggests that the flexibility pattern observed is consistent across the core structural elements of the protein. The peaks in the RMSF values often align with flexible loops or terminal residues, while lower RMSF regions likely represent stable core areas within secondary structure elements.

The ligand's RMSF plot Figure 6C independently highlights fluctuations across its atomic indices. It provides information on which parts of the ligand interact more rigidly with the protein versus regions that may rotate or bend during simulation. Higher fluctuations in certain ligand atoms could indicate regions that are less tightly bound to the protein or may indicate flexible linkers within the ligand itself.

Comparing the fluctuations of both protein and ligand can reveal the interaction sites and suggest which residues or atoms may be critical for binding stability.

This RMSF analysis helps identify which parts of the protein and ligand are more dynamic, aiding in understanding the interaction's nature and stability, which could be relevant for the ligand's efficacy as a potential therapeutic agent.

### 3.3.3. Protein-ligand interactions.

The interaction analysis indicated persistent hydrogen bonds, hydrophobic contacts, ionic interactions, and water bridges between DBMET02532 and the protein, with hydrogen bonds being particularly prominent. These contacts were maintained for significant portions of the simulation, underscoring stable interactions.

The PL-Contacts Histogram Figure 6D displays the interaction fractions between the protein and ligand at various residue positions. The x-axis lists different amino acid residues, such as HIS_613 and ASN_661, while the y-axis represents the interaction fraction. The bars are color-coded, indicating different types of interactions or contributions to the interaction fraction. ASN_662 shows the highest interaction fraction, dominated by the green-colored interaction. GLU_682 and ASP_724 also exhibit high interaction fractions, mainly from blue-

colored interactions. Residues like HIS_613, HIS_657, and VAL_782 have lower interaction fractions, as indicated by shorter bars.

The "LP-Contacts_2d-Summary" Figure 6E illustrates the 2D interactions between the protein and ligand, offering a comprehensive view of the binding sites. Key amino acid residues like ASN 661, SER 663, and ASP 724 are shown interacting with the ligand, with percentages indicating the interaction strength. The ligand's functional groups, including OH groups, are highlighted, demonstrating their role in these interactions. Water molecules are also depicted, mediating some interactions and stabilizing the complex. This detailed visualization aids in understanding the binding mechanisms and can inform drug design efforts, emphasizing the critical interaction points for optimizing ligand efficacy.

This visual representation is crucial for understanding the binding sites and interaction strengths between the protein and the ligand. Such analysis is invaluable in drug design, offering insights into which residues are most involved in the interaction, thus informing the development of more effective therapeutics.

3.3.4. Torsion profile.

Figure 7 of the ligand torsion profile provides a detailed visualization of the rotational flexibility and preferred conformations of the ligand. It highlights the distribution of torsion angles, revealing the energy landscape associated with these conformations. Peaks in the plot indicate stable conformations, while troughs represent less stable or unfavorable states. By examining this profile, one can identify the most stable conformations that the ligand is likely to adopt, as well as regions of steric hindrance that limit rotation. This information is crucial for understanding the ligand's behavior in biological systems and optimizing its design for better binding affinity and specificity in drug development.

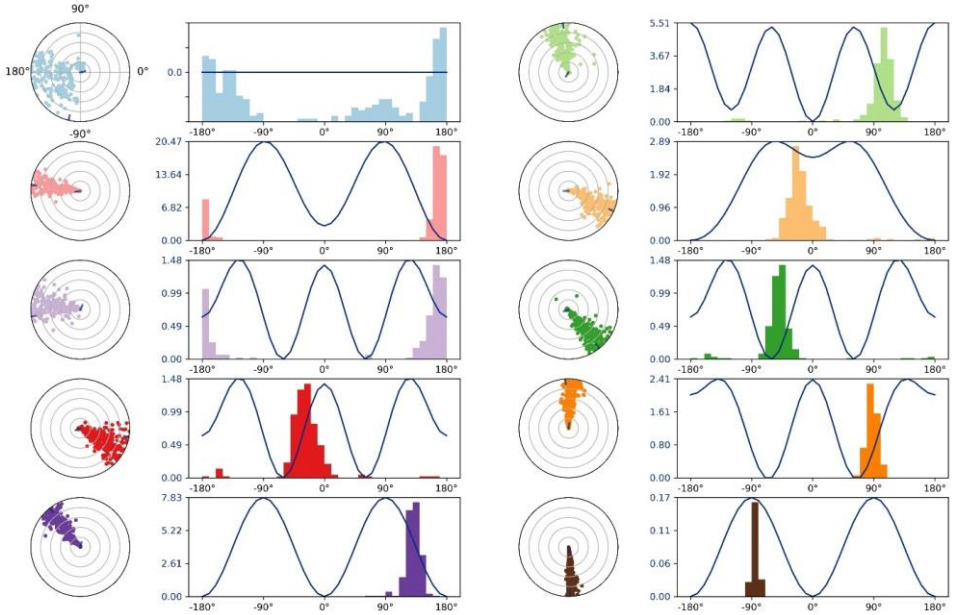


**Figure 7.** Ligand torsion profile of the top docked ADMET favorable DrugBank compound of DBMET02532.

3.3.5. Secondary structure analysis and ligand properties.

Protein compactness is quantified using rGyr. The text indicates the mean distance of protein atoms from the center of mass. Lower rGyr values suggest a more compact structure, whereas higher values denote a more extended structure. MolSA refers to the solvent-

accessible surface area of a molecule. It elucidates interactions between proteins and their environments. SASA quantifies the solvent-accessible surface area of proteins. SASA is employed to investigate protein-ligand interactions. Polar atoms, specifically oxygen, nitrogen, and hydrogen, are located within the Polar Surface Area (PSA). PSA forecasts the transmembrane passage of molecules.

Plot Figures 8 illustrate the variation of parameters over time or across different conditions. Trends over time illustrate the changes in each parameter throughout the specified duration. A decrease in rGyr may indicate increased protein compactness. Identify correlations among variables. A decrease in SASA coupled with an increase in MolSA may suggest protein folding and diminished solvent exposure. Examine the alterations in these parameters resulting from ligand binding. Ligand binding can reduce solvent-accessible surface area (SASA) and radius of gyration (rGyr) as the protein undergoes conformational folding around the ligand. Analyzing the variations in these metrics can elucidate the structural dynamics of the protein and its interactions with ligands.

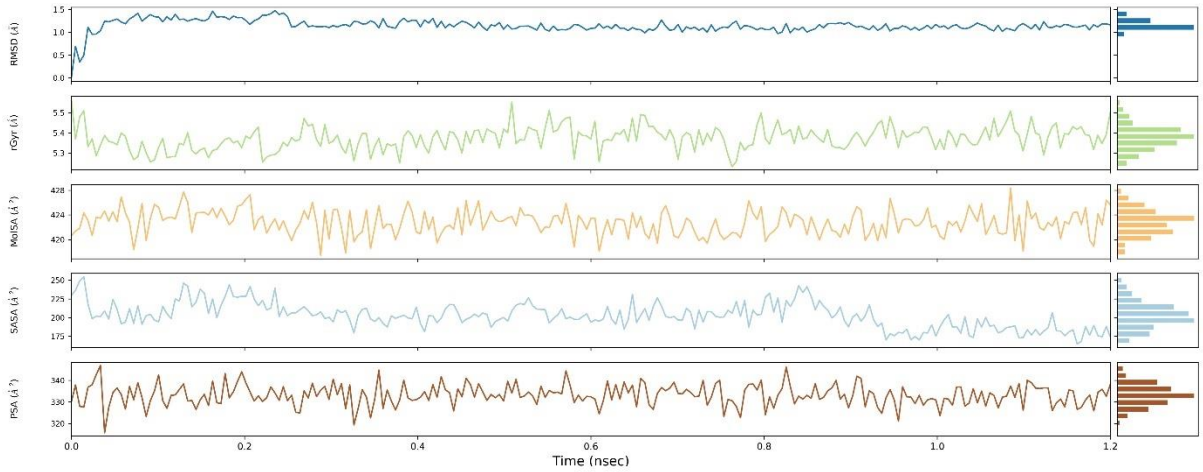


**Figure 8.** rGyr and SASA P-L properties of the top docked ADMET favorable DrugBank compound of DBMET02532.

These findings collectively suggest that DBMET02532 demonstrates a strong and stable binding interaction with the protein, marked by minimal conformational changes and persistent protein-ligand contacts. This stability may contribute positively to the ligand's efficacy in its intended application.

*3.4. Comparison with existing anticancer agents.*

The molecular docking study presented in Tables 6 and 7 provides a comparative analysis of known FDA-approved anticancer agents and DrugBank library compounds against key proteins involved in hepatocellular carcinoma (HCC) treatment. The three anticancer proteins used for docking include VEGFR-2 (PDBID: 3VHE), Caspase-9 (PDBID: 1NW9), and Caspase-7 (PDBID: 1F1J). The binding affinities are represented by docking scores in kcal/mol, where more negative values indicate stronger binding.

**Table 6.** Comparison of the binding affinity of the best docked known PDE5 inhibitors versus known anticancer agents against HCC.

| S. No | Known PDE5 Inhibitors | Binding affinity with cGMP (6L6E) | Known anticancer agents | Binding affinity with VEGFR-2 (3VHE) |
|---|---|---|---|---|
| 1 | Tadalafil | -10.5 | Regorafenib (11167602) | -10.068 |
| 2 | Vardenafil | -9.9 | Sorafenib (216239) | -9.277 |

| S. No | Known PDE5 Inhibitors | Binding affinity with cGMP (6L6E) | Known anticancer agents | Binding affinity with VEGFR-2 (3VHE) |
|---|---|---|---|---|
| 3 | Sildenafil | -9.8 | Lenvatinib (9823820) | -5.703 |
| 4 | Avanafil | -9.3 | Cabozantinib (25102847) | -4.388 |

**Table 7.** Comparison of Binding Affinity of best docked known FDA-approved anticancer agents versus unknown PDE5 inhibitors (DrugBank library compounds).

| Ligand | Docking Score (kcal/mol) | | | |
|---|---|---|---|---|
| | cGMP (6L6E) | VEGFR-2 (3VHE) | CASPASE-9 (1NW9) | CASPASE-7 (1F1J) |
| FDA-approved known anticancer agents | | | | |
| Sorafenib (216239) | -6.389 | -9.277 | -5.021 | -5.126 |
| Lenvatinib (9823820) | -5.096 | -5.703 | -4.751 | -5.485 |
| Regorafenib (11167602) | -6.255 | -10.068 | -6.371 | -8.331 |
| Cabozantinib (25102847) | -7.899 | -4.388 | -6.743 | -6.952 |
| DrugBank library compounds | | | | |
| Nebivolol (4-hydroxy Nebivolol-DBMET02738) | -12.781 | -9.139 | -7.756 | -13.162 |
| Suprofen (Suprofen glucuronide-DBMET00164) | -12.765 | -9.697 | -8.715 | -11.645 |
| Nabumetone (reduced O-desmethyl-3-hydroxy-nabumetone glucuronide-DBMET02532) | -12.573 | -8.101 | -11.35 | -11.196 |
| Fenofibrate (Reduced Fenofibric Acid-DBMET02799) | -12.501 | -8.055 | -8.147 | -10.688 |

Figure 9 compares the binding affinities of two different types of drugs: Standard Phosphodiesterase 5 (PDE5) inhibitors (Tadalafil) with the cGMP protein (6L6E) and Standard anticancer drugs with the VEGFR-2 protein (3VHE). The binding affinity is quantified in terms of free energy (ΔG), with more negative values indicating stronger binding.

Tadalafil and cGMP binding affinity is -10.5 kcal/mol, suggesting a strong interaction between Tadalafil and the cGMP protein. Anticancer drug (Regorafenib) and VEGFR-2: The binding affinity is -10.068 kcal/mol, indicating a slightly weaker but still substantial interaction between the anticancer drug and the VEGFR-2 protein.

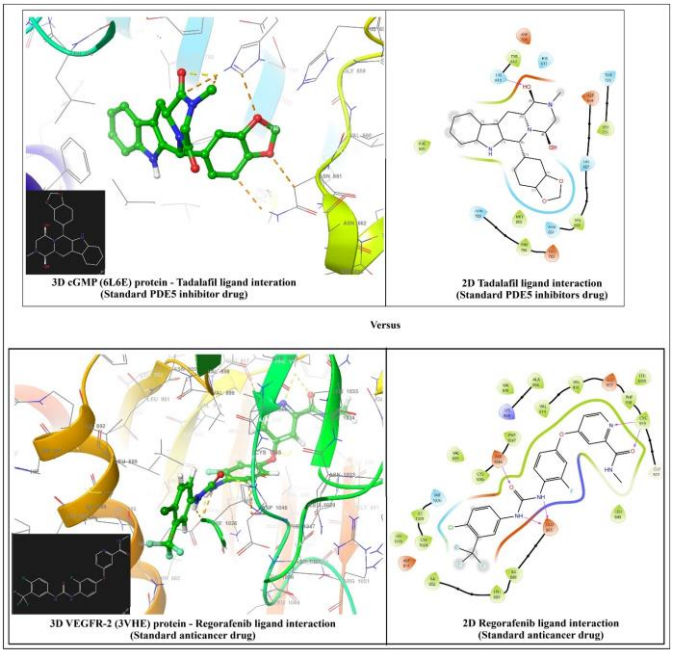


**Figure 9.** Comparison of Std PDE5 inhibitors (Tadalafil) with cGMP (6L6E) protein (-10.5 kcal/mol) vs Std anticancer drug with VEGFR-2 (3VHE) protein binding affinity (-10.068 kcal/mol).

This comparison highlights the effectiveness of both drug types in binding to their respective target proteins, which is crucial for their therapeutic functions. Tadalafil, a PDE5 inhibitor, is commonly used to treat erectile dysfunction by enhancing the effects of nitric

oxide, leading to increased blood flow [39]. On the other hand, anticancer drugs (Regorafenib) targeting VEGFR-2 aim to inhibit angiogenesis, thereby preventing tumor growth [40].

Figure 10 provides a comprehensive comparison of the molecular docking results of the high-affinity PDE5 inhibitor DBMET02532 with two different targets: cGMP and Caspase-9. In the 3D and 2D representations, DBMET02532's interaction with cGMP shows a stronger binding affinity of -12.573 kcal/mol, indicating a robust and stable interaction. This suggests that DBMET02532 is highly effective as a PDE5 inhibitor. In contrast, when docked as an anticancer agent with Caspase-9, the binding affinity is slightly lower at -11.35 kcal/mol, but still significant, indicating its potential efficacy in inhibiting Caspase-9 activity. The visual comparisons highlight the different binding modes and interactions of DBMET02532 with its targets, providing valuable insights into its multifunctional therapeutic potential.

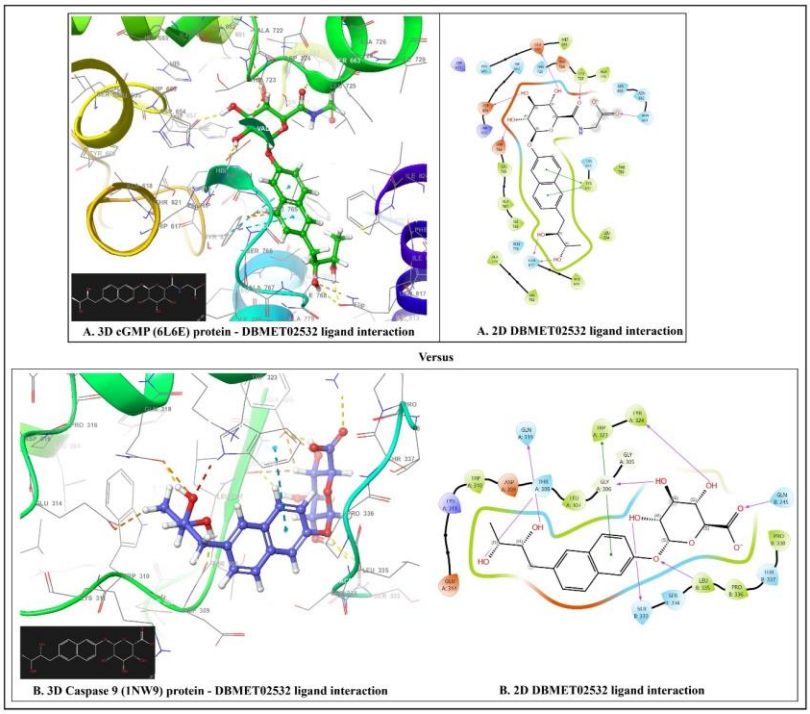


**Figure 10.** 3D and 2D comparison of molecular docking of affinity recently docked PDE5 inhibitor **(A)** DBMET02532 as a PDE5 inhibitor with cGMP (-12.573 kcal/mol); **(B)** DBMET02532 as an anticancer agent with Caspase-9 (-11.35 kcal/mol).

This study explored unknown PDE5 inhibitors from the DrugBank library to evaluate their potential anticancer properties against DEN-induced hepatocellular carcinoma (HCC) through in silico methodologies. The findings identified several compounds exhibiting significant binding affinity for the target proteins involved in HCC, particularly DBMET02532 and DBMET02799, which consistently showed favorable docking scores, pharmacokinetics, and toxicity profiles. These findings underscore that hydrophobic and hydrogen-bond interactions play a crucial role in stabilizing PDE5 interactions with cancer-related proteins, thereby supporting their further exploration as potential therapeutic agents.

The binding stability observed in molecular dynamics simulations, especially for DBMET02532, demonstrated promising interaction stability over the simulation period, with RMSD values stabilizing below 2.0 Å, indicating potential efficacy in sustaining interactions at the target site. Additionally, ADMET profiling revealed that DBMET02799 had high gastrointestinal absorption, blood-brain barrier permeability, and low clearance rates, suggesting prolonged systemic activity. However, moderate nephrotoxicity and respiratory

toxicity identified for DBMET02532 and DBMET02799 highlight potential challenges that might need addressing in future optimizations.

Compared to known anticancer agents (Sorafenib, Lenvatinib, and Regorafenib), the top-performing unknown PDE5 inhibitors displayed enhanced binding affinities, with docking scores as low as -12.781 kcal/mol, suggesting greater interaction stability with HCC-relevant targets. These results align with previous research indicating that PDE5 inhibitors might interfere with cancer cell proliferation by modulating cyclic GMP (cGMP) signaling pathways, a known regulator of cellular proliferation and apoptosis in HCC.

## 4. Conclusions

This in silico study demonstrated that the unknown PDE5 inhibitors DBMET02532 and DBMET02799 possess strong binding affinity, favorable pharmacokinetic properties, and relatively low toxicity profiles, marking them as promising candidates for HCC treatment. Molecular docking, dynamics simulations, and pharmacokinetic assessments collectively indicate that these compounds hold potential as effective anticancer agents targeting PDE5, a crucial mediator in HCC progression. Future studies involving in vitro and in vivo validations will be essential to confirm these compounds' anticancer efficacy, safety, and potential for clinical translation, ultimately contributing to the development of novel treatments for hepatocellular carcinoma.

## Author Contributions

Conceptualization, A.K. and D.S.R.; methodology, A.K.; software, A.K.; validation, A.K. and D.S.R.; formal analysis, A.K.; investigation, A.K.; resources, D.S.R.; data curation, A.K.; writing—original draft preparation, A.K.; writing—review and editing, D.S.R.; visualization, A.K.; supervision, D.S.R. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

The data of this study will be available from the corresponding author upon reasonable request.

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No.

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## Conflicts of Interest

No potential conflict of interest was reported by the author(s).

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