



Design, molecular docking, ADME, and Molecular Dynamics studies of new 6-amino coumarin derivatives as carbonic anhydrase inhibitors

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Abstract

New 6-amino coumarin derivatives were designed and computationally investigated as hCA IX-targeted compounds. Molecular docking, ADME prediction, and MD simulation were used to study their expected interactions and physicochemical and pharmacokinetic characteristics. ChemOffice was used to design the compounds' chemical structures, and GOLD was used to dock them with hCA IX (PDB ID: 4M2V). The compounds have ChemPLP fitness ratings ranging from 67.98 to 76.88, indicating various interaction patterns in the hCA IX active site, such as hydrogen bonding, short contacts, and interactions with the catalytic Zn^{2+} ion. Compounds 6c and 6e did not show predicted coordination with the catalytic Zn^{2+} ion as predicted. SwissADME analysis found no Lipinski criteria violations and gave preliminary ADME-related chemical characteristics. A 50-ns MD simulation of the selected the 6g–hCA IX complex demonstrated an initial conformational adjustment and a steady RMSD profile later in the trajectory. The ligand-binding area showed low variations, but surrounding regions showed more flexibility, according to RMSF analysis. Computational results provide preliminary insights into the compounds' binding and pharmacokinetic properties and assist their experimental research as possible hCA IX-targeted therapies.

Keywords: New 6-amino coumarin derivatives, hCA IX protein receptor, Molecular docking.

Introduction

Cancer is a major health problem characterized by uncontrolled proliferation and dissemination of malignant cells, which can impair normal tissue function and contribute to pain, organ failure, and cancer-related complications (S. Chen et al., 2023; Debela et al., 2021). Although surgery, radiotherapy, chemotherapy, targeted approaches, biological agents, and immune-mediated

therapies are available, the management of metastatic cancer remains challenging, emphasizing the need for continued investigation of molecular targets and new therapeutic strategies (Abbas & Rehman, 2018; Brown et al., 2023; Ghanghoria et al., 2018; Mokhtari et al., 2017; Reynolds et al., 2020).

Carbonic anhydrases (CAs) are metalloenzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton and contribute to acid–base regulation and other physiological processes (Aspatwar et al., 2022). The CA superfamily comprises eight evolutionary families: α , β , γ , δ , ζ , η , θ , and ι (Cainzos et al., 2021; Jensen et al., 2019). Mammalian α -CAs include cytosolic, mitochondrial, secreted, and membrane-associated isoforms with distinct tissue distributions and functions, and their dysregulated expression has been associated with several human diseases, including cancer (Abdoli et al., 2024; García-Llorca et al., 2024; Supuran, 2016).

Among the human isoforms, hCA IX and hCA XII have attracted particular interest in cancer research because of their association with tumor development and progression (Goud et al., 2021). hCA IX is involved in regulation of the tumor microenvironment, including pH regulation, and has been associated with cellular spreading and metastatic processes (Moi et al., 2025; Pastorekova & Gillies, 2019). Mammalian CAs possess a Zn^{2+} -dependent catalytic site coordinated by conserved histidine residues and a water/hydroxide molecule. The structural characteristics of this active site provide a basis for investigating small molecules that may interact with cancer-associated CA isoforms (Kim et al., 2020).

Coumarin (2H-1-benzopyran-2-one) is a heterocyclic scaffold found in several plant species and has been investigated for diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, anticoagulant, and anticancer effects (Bruna-Haupt et al., 2023; L. Chen et al., 2023; Toolabi et al., 2025). Nitrogen-containing heterocycles, particularly triazoles, are also important scaffolds in medicinal chemistry because of their structural diversity, synthetic accessibility, and reported biological activities. The 1,2,4-triazole ring can participate in hydrogen-bonding and other non-covalent interactions with biological targets, while substitution at different ring positions can influence the physicochemical and biological properties of its derivatives (Almasmoum et al., 2025; Goyal & Bhandari, 2024; Li et al., 2025).

Among 1,2,4-triazole derivatives, 1,2,4-triazole-3-thiol compounds are of particular interest because the thiol (–SH) group provides an additional site for molecular interaction and structural modification. Previous studies have reported biological activities for 1,2,4-triazole-3-thiol derivatives (Youssef et al., 2022). Based on these considerations, the present study focused on the design and computational investigation of new 6-amino coumarin derivatives incorporating a 1,2,4-triazole-3-thiol moiety as potential hCA IX-targeted compounds. Molecular docking was employed as a preliminary approach to investigate predicted binding modes within the hCA IX active site, while ADME prediction was used to assess selected physicochemical and pharmacokinetic properties. Molecular dynamics simulation was further performed to examine the stability and conformational behavior of a selected ligand–hCA IX complex. These computational approaches were intended to provide preliminary insights into the interaction and drug-likeness profiles of the designed compounds and to support further experimental investigation.

Computational Method

The structural design of the proposed 6-amino coumarin derivatives, guided by a comprehensive literature review, is illustrated in Figures 1 and 2. An *in silico* investigation was performed to assess the predicted interactions of the proposed compounds with human carbonic anhydrase IX

(hCA IX; PDB ID: 4M2V). The first molecular docking study in the hCA IX active site was performed by taking Brinzolamide (BRZ) as a reference compound to evaluate predicted binding poses and ligand–protein interactions, such as hydrogen bonds and other short-range contacts. This was followed by further evaluation of the ADME profiles for the designed compounds using SwissADME web server. Finally, molecular dynamics (MD) simulation was performed using the Desmond module of Schrödinger Suite 2025 to investigate the stability and conformational behavior of a selected ligand–hCA IX complex under simulated physiological conditions over a 50-ns trajectory.

2.1. Design of the Compounds

The chemical structures of the ligands (6a–g) were constructed using ChemOffice (ChemDraw 22.0.0). The resulting structures were then subjected to energy minimization using Chem3D prior to their preparation for the molecular docking procedure.

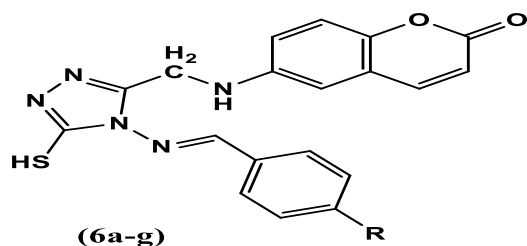
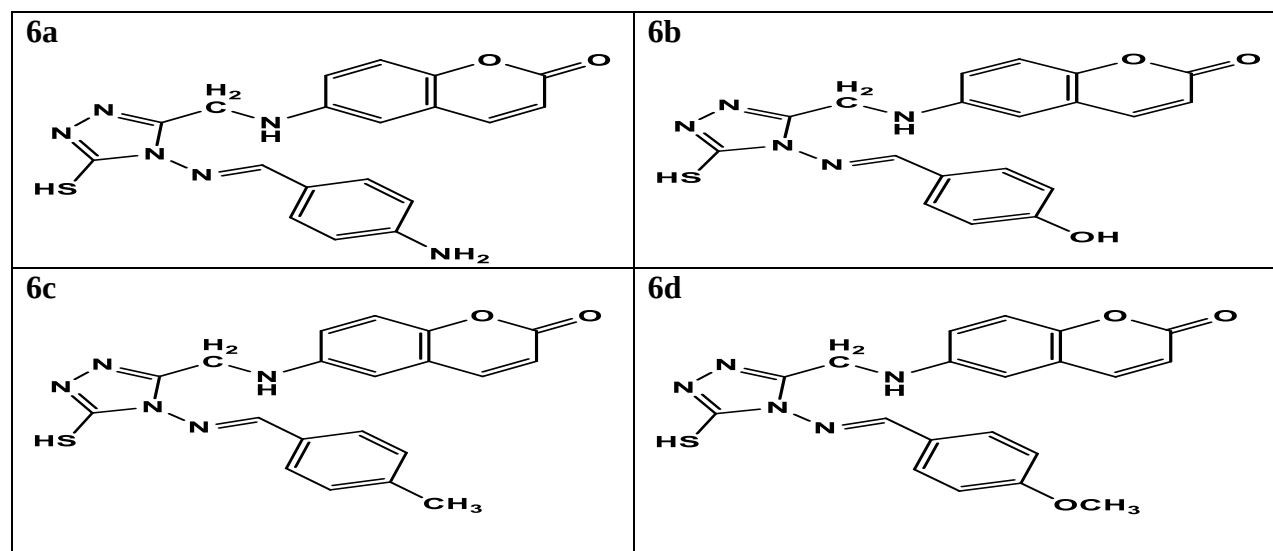


Figure (1): General structure of proposed Compounds



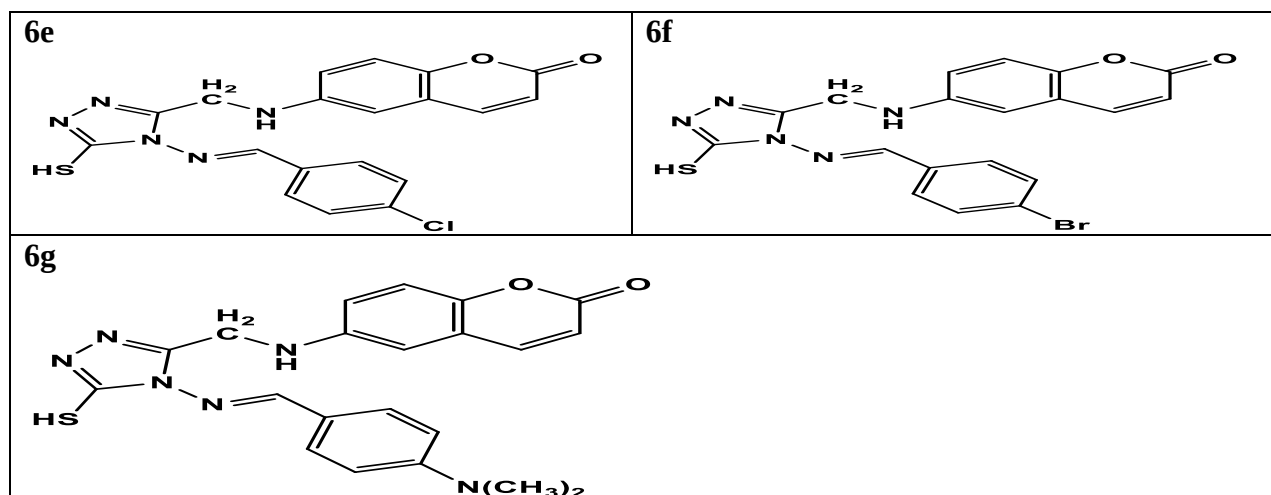


Figure (2): Structures of proposed Molecules

2.2. Molecular Docking

The crystal structure of human carbonic anhydrase IX (hCA IX) complexed with Brinzolamide was retrieved from the Protein Data Bank (PDB ID: 4M2V) and used as the receptor structure for molecular docking. Prior to docking, the protein structure was prepared by removing water molecules and adding hydrogen atoms to the protein residues to obtain appropriate protonation and ionization states. Molecular docking was performed using GOLD software (version 2024.3.0; Cambridge Crystallographic Data Centre). The binding site was defined to include all protein residues located within a 10 Å radius of the reference ligand in the crystal structure. The highest-ranked docking solution was retained according to the default configuration. As the fitness function for ranking the docking poses, ChemPLP was chosen.

2.3. Determination of ADME Properties

ADME properties of the designed compounds were predicted by the SwissADME web server. Other pharmacokinetic and drug-likeness properties, such as blood–brain barrier (BBB) permeation and gastrointestinal absorption were also analyzed. For the chemical structures production of all designed compounds, ChemDraw were used to prepare an initial structure and then its SMILES format was used for submission to SwissADME. The resulting predictions were employed for a first insight into pharmacokinetic and drug-likeness properties of the compounds and to eliminate compounds with potentially less favourable predicted profiles.

2.4. Molecular Dynamics Simulation

After the molecular docking analysis, a molecular dynamics (MD) simulation was run to learn more about the stability and dynamic behavior of a certain ligand–hCA IX complex. Compound 6g was selected for the MD simulation based on the molecular docking results and its predicted interactions within the hCA IX active site. The system was prepared using the Desmond module of Schrödinger Suite 2025 and solvated using the SPC water model in an orthorhombic simulation box with a 10 Å buffer. The OPLS4 force field was employed, and the system was neutralized with 0.15 M NaCl under near-neutral pH conditions. A 50-ns MD simulation was subsequently

conducted at 300 K to investigate the stability and conformational behavior of the 6g–hCA IX complex over time.

Results and Discussion

The study aimed to design new compounds with favorable predicted binding properties and acceptable ADME characteristics

3.1. Molecular Docking

Molecular docking was performed for the newly designed compounds (6a–6g) using GOLD Suite, with ChemPLP employed as the fitness function for evaluating the predicted docking poses. The docking analysis was used to investigate the ability of the compounds to fit within the hCA IX active site and to characterize their predicted interactions with the surrounding residues, including hydrogen bonding and hydrophobic contacts (Fu et al., 2018; Shivanika et al., 2020). The ChemPLP fitness scores of the docked compounds against hCA IX ranged from 67.98 to 76.88, as presented in Table 1 and Figure 3. Compounds 6c and 6e showed different interaction patterns within the active site and did not exhibit predicted coordination with the catalytic Zn²⁺ ion. These findings provide preliminary information on the predicted binding modes of the designed compounds within the hCA IX active site.

Table (1); Molecular docking results against hCA IX (PDB code: 4M2V) using GOLD software

Priority	Docking scores (HCA IX)	Interactions H- Bond	Short Contact Interaction
6a	72.3063	THR199 (2)	Zn, THR199, THR200 (6), VAL121 (2) and VAL143
6b	76.8894	THR200	Zn and LEU198
6c	70.0347	THR199 and GLN92	THR199 and GLN92
6d	67.9847	GLN92 and VAL121	Zn, GLN92, VAL121, VAL143 (2) and THR199 (2)
6e	70.5871	THR199 and GLN92	THR200, THR199, GLN92 and VAL121
6f	69.1919	GLN92(2)	Zn, GLN92(2), VAL143 (2) and THR199 (2)
6g	74.1122	ASN62, THR200 THR199	Zn, THR200 (A) (3), HIS94, THR199, VAL143(3) and VAL121
BRZ	69.0514	THR199 (2) and GLN92	Zn, GLN92, ASP32 and TRP5

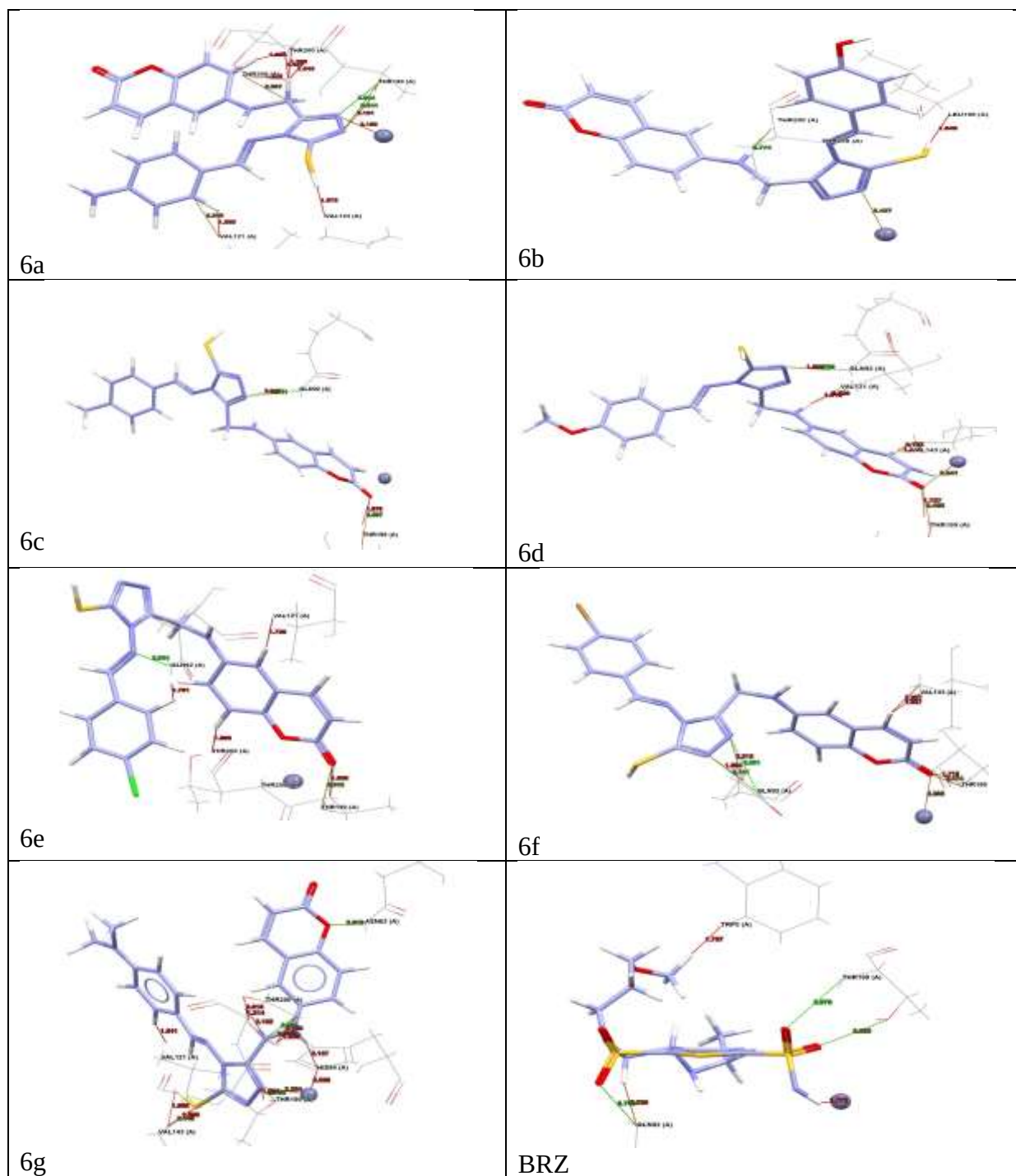


Figure (3); 3D interactions of the docked compounds with hCA IX using GOLD

3.2. ADME

ADME profiling provides preliminary information on the pharmacokinetic and drug-likeness characteristics of candidate compounds. In the present study, the SwissADME web server was used to predict the ADME-related properties of the designed compounds. The results of Lipinski's rule of five are summarized in Table 2. The evaluated compounds showed no violations of the assessed Lipinski criteria, including molecular weight (MW), hydrogen-bond donors (HBDs),

hydrogen-bond acceptors (HBAs), and rotatable bonds (X. Chen et al., 2020; Reddy et al., 2019)]. Additional parameters, including topological polar surface area (TPSA), molar refractivity (MR), bioavailability score, gastrointestinal absorption, and blood–brain barrier (BBB) permeability, were also evaluated. The predicted pharmacokinetic profiles of the designed compounds are presented in Table 3. These computational predictions provide preliminary information on the physicochemical, pharmacokinetic, and drug-likeness profiles of the designed compounds.

Table (2). Lipinski's rule of for all compounds and control compound

Compound	M.W	HBA	HBDs	Rotatable bonds
BRZ	383.51	8	2	7
6a	392.43	5	2	5
6b	393.42	6	2	5
6c	391.45	5	1	5
6d	407.45	6	1	6
6e	411.86	4	0	6
6f	456.32	5	1	5
6g	420.49	5	1	6
MWT: molecular weight, HBAs: hydrogen bond acceptors, HBDs: Hydrogen bond donors.				

Table (3). Pharmacokinetics properties for all proposed compounds and control compound

Name	MR	TPSA(Å ²)	GI absorption	BBB permeability	BS
BRZ	90.80	163.80	Low	No	0.55
6a	110.95	150.13	Low	No	0.55
6b	108.57	144.34	Low	No	0.55
6c	111.51	124.11	High	No	0.55
6d	113.03	133.34	High	No	0.55
6e	111.55	124.11	High	No	0.55
6f	114.24	124.11	High	No	0.55
6g	120.75	127.35	High	No	0.55

3.3. Molecular Dynamics

Molecular dynamics (MD) simulation of the 6g–hCA IX complex was performed to investigate its dynamic behavior and stability over the simulation period.

3.3.1. The RMSD

RMSD analysis of the 50-ns molecular dynamics trajectory was performed to assess the structural behavior of the complex consisting of hCA IX in complex with 6g. The RMSD of the protein backbone is 1.1 Å at the beginning and increased gradually to around 1.6 Å, during which a large

conformational change was recorded between 16 and 18 ns in both protein conformation as well as ligand. In comparison, the ligand RMSD increased to ~ 3.5 Å which showed that the ligand had reoriented in some ways in position about the binding site as well. After this transition, the RMSD profiles exhibited a mostly stable trend from roughly 20 ns until the end of each simulation. These observations indicate that the complex made a first conformational adjustment and then remained in an almost stable path during the later part of the simulation.

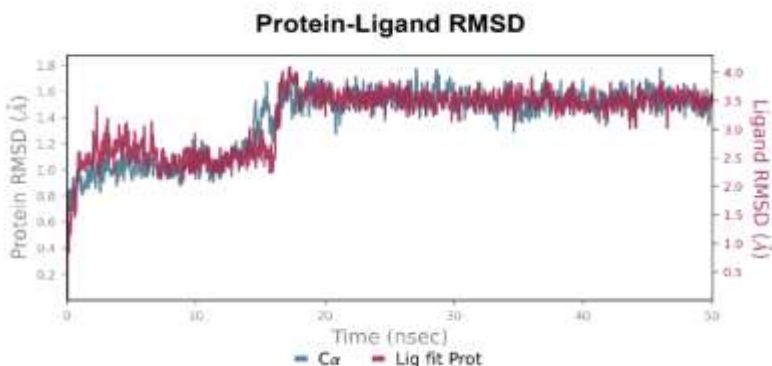


Figure (4); RMSD of the atoms of protein and the ligand over time (6g-CAIX complex).

3.3.2. RMSF

This residue-level flexibility of the 6g-hCA IX complex during 50 ns is deconvoluted further by RMSF analysis. The analysis revealed that larger fluctuations were primarily related to the unstructured terminal regions and surface loops, while the residues located in the binding site region appear to fluctuate less than about 0.8 Å. This observation means that residues enforced on the predicted ligand-binding region maintained stable conformations throughout simulation. The RMSF results, along with the form of the RMSD profile, imply that the 6g-hCA IX complex sustained a relatively constant interaction environment in this portion of the trajectory.

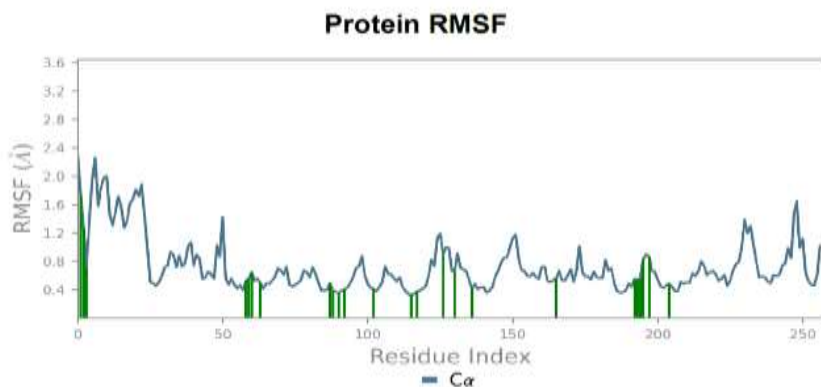


Figure (5); RMSF of protein (6g-CAIX complex).

3.3.3. Ligand RMSF

Ligand RMSF was assessed to study the flexibility of 6g ligand during a 50-ns molecular dynamics simulation. The profile produced from the RMSF plot indicated very small fluctuations in the middle of the ligand and relatively larger ones at either edge. This pattern suggests that the ligand was not uniformly flexible in its structure, and the terminal regions possessed higher

conformational mobility during the simulation. The ligand RMSF profile for the whole set of representative frames showed that the central part was more rigid, while flexibility came predominantly from atoms out of the core in peripheral regions.

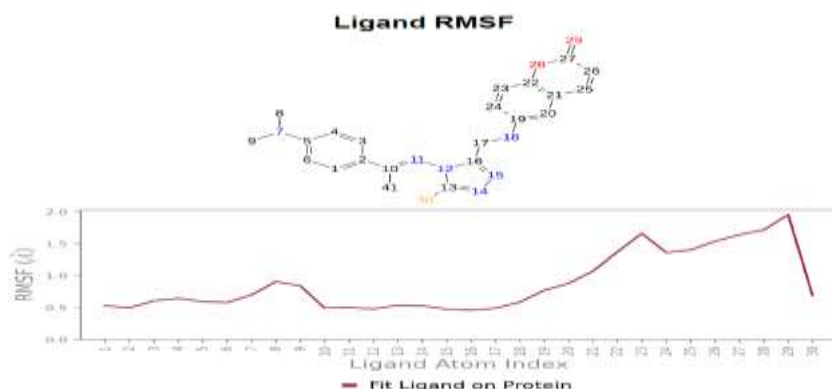


Figure (6); RMSF of Ligand (6g-CAIX complex).

4. Conclusions

The computational investigation provided preliminary insights into the predicted binding interactions, physicochemical properties, and dynamic behavior of the newly designed 6-amino coumarin derivatives against hCA IX. Molecular docking analysis showed that the compounds could be accommodated within the hCA IX active site, with ChemPLP fitness scores ranging from 67.98 to 76.88. The docking results also revealed different interaction patterns among the investigated compounds, including hydrogen bonding, short contacts, and interactions with the catalytic Zn^{2+} ion. Compounds 6c and 6e did not show predicted coordination with the catalytic Zn^{2+} ion, indicating differences in their predicted binding modes.

The SwissADME analysis provided preliminary information on the physicochemical and pharmacokinetic characteristics of the designed compounds, including their compliance with Lipinski's rule of five and selected ADME-related properties. Molecular dynamics simulation of the selected 6g-hCA IX complex showed an initial conformational adjustment between approximately 16 and 18 ns, followed by a relatively stable RMSD profile during the latter part of the 50-ns trajectory. RMSF analysis indicated comparatively limited fluctuations within the ligand-binding region, while greater flexibility was observed in some peripheral regions of the ligand.

Computational findings support the potential of the 6-amino coumarin derivatives investigated as candidates for further investigation against hCA IX. However, the present results are based on computational predictions and should not be considered direct evidence of hCA IX inhibitory activity. Further experimental studies are required to verify the predicted interactions and to determine the actual inhibitory potency, selectivity, pharmacokinetic properties, and biological activity of these compounds.

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