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Computational Design of Isatin–Sulfonamide Hybrids as Selective Carbonic Anhydrase XII Inhibitors with Anticancer Potential

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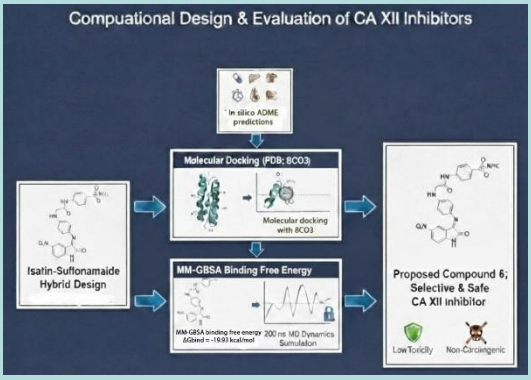
Abstract: Hypoxic solid tumors overexpress a transmembrane enzyme; Carbonic Anhydrase XII (CA XII) to regulate pH and promote tumor progression. Selective inhibition of CA XII is believed to be a promising anticancer approach. Many studies were designed to introduce a molecule with high selectivity to cancer associated CA isoforms, but they faced issues like selectivity, safety, and potency. This study aimed to design and computationally evaluate novel isatin-sulfonamide hybrids as effective and selective CA XII inhibitors with enhanced safety profile. Six hybrid compounds were designed and assessed using molecular docking against the CA XII active site (PDB:8CO3), utilizing the selective ligand V8O and non-selective acetazolamide (AZM) as references. Molecular docking identified compounds 5 and 6 as having highest affinity score (-7.069 kcal/mol) and (-6.614 kcal/mol) respectively. Further evaluation using MM-GBSA binding free energy estimation emphasized compound 6 as the most thermodynamically favorable candidate (ΔG_{bind} = -19.93 kcal/mol), principally determined by strong van der Waals as well as lipophilic interactions. The stability of the compound 6–CA XII complex was established over a 200 ns molecular dynamics simulation, which confirmed a stable binding position. In silico ADME predictions showed that most compounds, especially compound 6, have acceptable pharmacokinetic properties except oral bioavailability. In silico investigations indicate that all designed hybrids showed improved toxicity profile, rendering them of low acute toxicity (Class 5, LD_{50} 4000-5000 mg/kg) and predicted to lack carcinogenic potential, in contrast to the reference ligand V8O. In conclusion, these computational results present these derivatives, especially compound 6 as promising candidates with moderate suggested selectivity, and predicted to be safe CA XII-inhibitor for further experimental development as an anticancer agent.



Keywords: Carbonic Anhydrase XII; Isatin-Sulfonamide Hybrids; Molecular Docking; Molecular Dynamics; MM-GBSA

Introduction

One of the hallmarks of the solid tumor microenvironment is hypoxia. It is triggered by the rapid proliferation of tumor cells that is faster than the development of an adequate vascular supply (1). This oxygen-poor niche encourages tumor aggressiveness, invasion, and metastasis, as well as drug resistance. In addition, it induces adaptive molecular responses, such as the upregulation of hypoxia-inducible factors (HIFs) (2). One of the genes activated under hypoxic circumstances is Carbonic Anhydrase XII (CA XII), which encodes CA XII transmembrane enzyme. Consequently, it is overexpressed in several cancers, including breast, lung, and colorectal carcinomas (3). CA XII is a critical factor in sustaining pH homeostasis. It keeps the pH balanced through reversible hydration of carbon dioxide into bicarbonate and protons, catalyzed by carbonic anhydrase. This enhances tumor cell survival, invasion, and treatment resistance (4). Based on this observation, selective inhibition of CA XII has emerged as a promising strategy in combating hypoxic tumors with minimal or no effects on normal tissues that mainly express other CA isoforms (5). The efforts to identify isoform-selective CA inhibitors have increased in recent years. Sulfonamide-based compounds are considered interesting research candidates as a result of their strong zinc-binding capacity, which permits effective coordination with the catalytic zinc ion inside the CA active site (6). However, several primitive sulfonamides show non-selective inhibitory activity, resulting in off-target effects and toxicity (7). Hybridization can improve potency, selectivity, multitarget activity, and pharmacokinetic properties by

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combining pharmacophores within a single molecule (8). In CA inhibitor design, it allows the fusion of the zinc-binding sulfonamide group with structural elements that enhance isoform selectivity by exploiting differences in CA active sites (9). Isatin-based compounds have shown strong cytotoxicity and favorable selectivity toward cancer cells, and their incorporation into hybrids can provide additional anticancer effects beyond CA inhibition, producing dual-acting agents that target both tumor metabolism and proliferation pathways (10,11). Thus, combining sulfonamide and isatin scaffolds is a rational strategy for developing selective anticancer agents against CA IX and XII, where benzenesulfonamide binds the catalytic zinc ion and isatin interacts with the selectivity-determining regions of the enzyme active site (12,13). To overcome this problem, researchers explored the non-classical scaffolds, including isatin (1H-indole-2,3-dione), which has shown prominent anticancer and CA inhibitory potential (10). Recent studies revealed the CA inhibitory potential of isatin derivatives, especially upon functionalization with sulfonamide groups, thereby combining the zinc-binding pharmacophore with the multipurpose isatin moiety (14,15). This hybrid method aims to improve both selectivity and binding affinity toward cancer-bound CA isoforms such as CA XII. A famous example of a clinically advanced selective CA inhibitor is SLC-0111, which is a sulfonamide-based compound undergoing Phase I/II trials for hypoxic tumors (16). Its structure highlights the importance of both hydrophobic and polar interactions within the active site of CA XII. Based on this, it was hypothesized that intentional hybridization of the zinc-binding group of sulfonamides with the isatin scaffold may produce novel compounds with enhanced both selectivity as well as efficacy against CA XII. While SLC-0111 is a promising lead, it has limitations such as moderate potency and potential off-target effects. The "tail approach" applied to the tail of SLC-0111 molecule to increase binding affinity to the active site of transmembrane carbonic anhydrase, since they differ from CA II in their hydrophobic pocket. Molecular docking studies have identified higher binding affinity of some hybrids of SLC-0111 fitting inside the hydrophobic pocket of carbonic anhydrase. New compounds, like isatin-sulfonamide hybrids, are assessed to discover novel, more potent, and safer inhibitors (17). Recent studies on sulfonamide-isatin hybrids that showed potent CA XII inhibition include Secci and colleagues (2024) (9), Saied *et al.* work (18), Ibrahim et al. (19), Krymov and colleagues (2022) (20). In the present study, a number of isatin-sulfonamide derivatives were designed and evaluated computationally as selective CA XII inhibitors. Applying programs like molecular dynamics simulations, pharmacokinetic profiling, molecular docking, in addition to MM-GBSA free energy calculations, it was aimed to detect promising candidates for additional experimental evaluation as anticancer drugs. This computational study was carried out on six precisely designed hybrid compounds (Figure 1), which bear the essential zinc-binding sulfonamide group besides the structurally versatile isatin moiety. The aim of this molecular hybridization is to improve interactions with both hydrophobic and hydrophilic residues within the active site of carbonic anhydrase XII (CA XII). To predict the binding positions of these hybrids, molecular docking simulations were conducted utilizing the high-resolution crystal structure of CA XII (PDB ID: 8CO3). The obtained affinity values and orientations were compared against two reference ligands: V8O, a selective CA XII binder, and Acetazolamide (AZM), a non-selective pan-inhibitor. For additional evaluation of complex stability, molecular dynamics (MD) simulations were executed. In addition, binding free energies were calculated using the MM-GBSA tool to quantify and compare the predicted affinities of binding of the designed compounds.

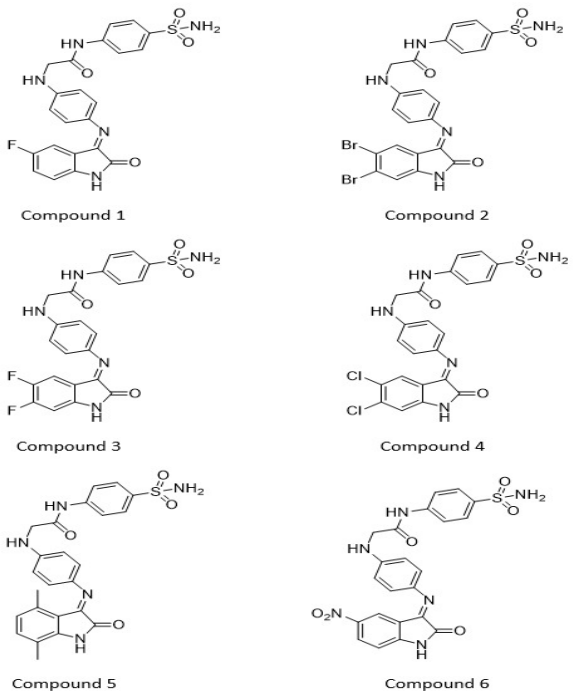


Figure 1: The chemical structures of the proposed compounds 1,2,3,4,5 and 6.

Computational method

Computational study was employed for six isatin-sulfonamide hybrids with the following IUPAC names:

Compound 1 (E)-2-((4-((5-fluoro-2-oxoindolin-3-ylidene)amino) phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Compound 2 (E)-2-((4-((5,6-dibromo-2-oxoindolin-3-ylidene) amino) phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Compound 3 (E)-2-((4-((5,6-difluoro-2-oxoindolin-3-ylidene) amino)phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Compound 4 (E)-2-((4-((5,6-dichloro-2-oxoindolin-3-ylidene) amino)phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Compound 5 (E)-2-((4-((4,7-dimethyl-2-oxoindolin-3-ylidene) amino)phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Compound 6 (E)-2-((4-((5-nitro-2-oxoindolin-3-ylidene) amino)phenyl)amino)-N-(4-sulfamoylphenyl)acetamide

Molecular Docking Study

The binding interactions and affinities of the six newly designed isatin-sulfonamide derivatives within the human carbonic anhydrase XII (hCA XII) active site were investigated by conducting molecular docking simulations. The crystallographic structure of hCA XII (PDB ID: 8CO3) was retrieved from the Protein Data Bank (8CO3 was selected for its high resolution (1.65 Å) and the presence of a co-crystallized selective inhibitor) and arranged for docking using Schrödinger's Protein Preparation Wizard (H-bond optimization, PROPKA at pH 7.4, and restrained minimization). Preparation steps involved the addition of hydrogen atoms, bond orders assignment, and removal of water molecules (only those structurally conserved within 5Å of the native ligand were retained) as well as the original ligands (21). Ligand preparation was performed using LigPrep with (Epik, the OPLS4e force field, and a pH 7.4 ± 2.0). Docking validation was performed by redocking of V8O yielding an RMSD=0.18Å which confirms the protocol accuracy, as in figure 5. The grid of docking was adjusted around the binding site of co-crystallized selective ligand (V8O), to define the

catalytic region. The Glide (XP) module in Schrödinger's suite was then used for flexible docking of the ligand conformations (up to 10 poses/ligand), enabling a comparative study of binding poses and interactions relative to both the selective reference ligand V8O and the non-selective inhibitor acetazolamide(22,23).

Molecular Mechanics, General Born Surface Area Binding Free Energy Calculations

The free energies of binding for the six designed isatin-sulfonamide derivatives, in addition to the selective ligand V8O and the non-selective ligand acetazolamide, against carbonic anhydrase XII (CA XII; PDB: 8CO3) were assessed using the MM-GBSA method, employed through the Prime module of the Schrödinger suite (24). The Optimized Potentials for Liquid Simulations 4-extended (OPLS4e) force field was applied to describe the potential energy of the system, while the VSGB 2.0 solvation model was used to account for solvent effects (25). The calculations were executed on the optimized ligand enzyme complexes to obtain the values of binding free energy (ΔG_{bind}), which offers a quantitative grading of the relative binding affinities and selectivity. In addition to the total ΔG_{bind}, energy terms like van der Waals contributions, lipophilic interactions, and hydrogen bonding energies were disintegrated to identify the main driving forces behind complex stabilization (26).

Molecular Dynamics Simulation (MDS) Study

The Desmond module within the Schrödinger software suite was used to perform molecular dynamics (MD) simulations to assess dynamic interactions, stability, and the conformational behavior of ligand–protein complexes(27).

After solvation of the enzyme–ligand complexes; CA XII bound to V8O (the maximum performing designed compound) and acetazolamide, in an orthorhombic box utilizing the TIP3P water model, and system neutralization via addition of sodium and chloride ions, the employed force field was OPLS4e under the NPT ensemble while keeping temperature and pressure at 300 K and 1.01325 bar, respectively, and a time step of 2 fs, and a cutoff distance of 9 Å (28).

A simulation run of 200 nanoseconds was executed for each complex, and trajectories were analyzed to evaluate key parameters such as root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and specific protein–ligand interactions. The diagram of simulation interaction provided insights into the persistence and nature of binding interactions over time, offering a dynamic perspective on complex stability and binding mode consistency within the CA XII active site.

ADME Prediction Study

To investigate the drug-like properties and pharmacokinetic profile of the six newly designed isatin-sulfonamide derivatives, an in silico ADME (Absorption, Distribution, Metabolism, and Excretion) assessment was applied utilizing the SwissADME platform (29). It is a widely used and freely accessible web tool developed by the Swiss Institute of Bioinformatics providing fast, and validated predictive models for key physicochemical and pharmacokinetic parameters, making it a standard choice in academic drug discovery. These parameters of the candidate compounds were estimated and compared with both the selective ligand V8O and the non-selective ligand acetazolamide. The predicted properties included molecular weight (MW), topological polar surface area (TPSA) number of hydrogen bond donors (HBD) and acceptors (HBA), and lipophilicity (LogP) (30). These parameters were assessed for the determination of compliance with updated bioavailability rules, as well as the oral absorption and blood-brain barrier penetration prediction (31). These computational estimates provide a preliminary validation of the compounds' pharmacokinetic behavior and drug-likeness.

Toxicity Study

In silico toxicity profiling was performed for the six designed isatin-sulfonamide derivatives, the selective ligand V8O, and the non-selective ligand acetazolamide to evaluate possible adverse effects and acute toxicity. Toxicity was estimated using the ProTox 3.0 web server (32), a freely available webserver that integrates molecular similarity and machine-learning models to predict 61 different toxicity endpoints, including acute toxicity (LD_{50}), organ toxicity (hepatotoxicity, neurotoxicity), and clinical toxicity (carcinogenicity, mutagenicity). Its predictive performance and accessibility have led to its widespread adoption in computational toxicology studies. It has an established track record in peer-reviewed literature for evaluating novel drug candidates at the discovery stage (33).

Results and Discussion

Molecular Docking Analysis for CA XII Inhibitors

Validation of docking was carried out, as shown in figure 5. Molecular docking simulations were conducted to evaluate the binding characteristics of the designed isatin-sulfonamide hybrids (compounds 1–6), acetazolamide (AZM), and the native ligand V8O against CA XII (PDB ID: 8CO3). The resulting docking scores spanned from –4.367 to -7.069 kcal/mol, as summarized in Table 1. These values indicate favorable binding affinities of all designed compounds toward the CA XII active site.

All compounds, as well as the references, formed hydrogen bonds with important residues in the catalytic pocket, principally Thr204 and Thr205. The participation of water mediated hydrogen bonds (e.g., with Ser136 and Pro206) further improves binding specificity, as solvation effects are identified to modify CA inhibitor recognition (26).

The direct coordination with the catalytic zinc ion (Zn^{2+}, labeled ZN301 in the table) represents a hallmark of effective sulfonamide based CA inhibitors. All designed compounds, in addition to both reference ligands, revealed stable coordination with ZN301, confirming the proper positioning of the sulfonamide zinc-binding group (ZBG) to chelate the metal ion. This interaction is important for the displacement of the water molecule bound to zinc and inhibition of the enzyme's hydration activity (6).

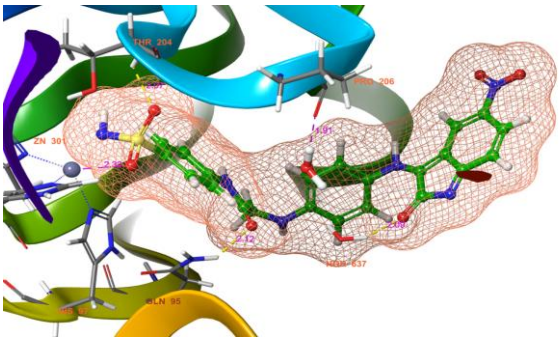
All compounds made numerous hydrophobic contacts with several residues, these hydrophobic interactions are critical for ligand stabilization and take part in the overall binding energy, as shown in recent MM-GBSA studies on CA inhibitors (24).

Compared to the reference inhibitors, compounds 5 and 6 exhibited supplementary hydrogen bonds and more extensive hydrophobic interaction networks.

Table (1): Type of interaction and docking scores for diverse isatin-sulfonamide derivatives within the active site of CA XII.

Compounds	Docking score Kcal/mol	Interaction			
		H-bonding with distance	Metal coordination	Hydrophobic	Pi-Pi stacking
1	-4.760	THR 204(2.04), SER136+H2O (1.96)	ZN 301	LEU 203, PRO 206, PRO 207, VAL 212, TRP 214, LEU 61, ALA 135, TYR 127, VAL 125, LEU 145, VAL 147	HIS 97
2	-4.367	THR 204(2.03), THR 205(2.16), SER136+H2O (2.47)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, TYR 7, TRP 5, ALA 135, ALA 138, TYR 127, VAL 125, LEU 145, VAL 147	HIS 97
3	-6.216	THR 204(2.09), PRO 206+H2O (1.91)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, TRP 5, TYR 7, ALA 135, ALA 138, VAL 125, TYR 127, LEU 145, VAL 147	HIS 97
4	-5.464	THR 204(2.05), THR 205(2.15), SER136 (2.61), GLN 95 (2.17)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, LEU 61, TRP 5, TYR 7, ALA 135, ALA 138, VAL 125, LEU 145, VAL 147	
5	-7.069	THR 204(2.01), THR 205(2.29), LYS 70+H2O (2.24),	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, ALA 135, TYR 20, VAL 125, TYR 127, LEU 145, VAL 147	
6	-6.614	THR 204(2.07), THR 205(2.20), GLN (2.12)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, ALA 135, TYR 7, TRP 5, VAL 125, TYR 127, LEU 145, VAL 147	
AZM	-5.782	THR 204(2.09), PRO 206+H2O (1.91)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, TYR 7, ALA 135, ALA 138, VAL 125, LEU 145, VAL 147	HIS 97
Native ligandV8O	-5.822	THR 204(2.07), THR 205(2.20), GLN (2.12)	ZN 301	LEU 203, PRO 206, PRO 207, CYS 208, VAL 212, TRP 214, TRP 5, TYR 7, ALA 135, ALA 138, VAL 125, TYR 127, LEU 145, VAL 147	HIS 97

A)



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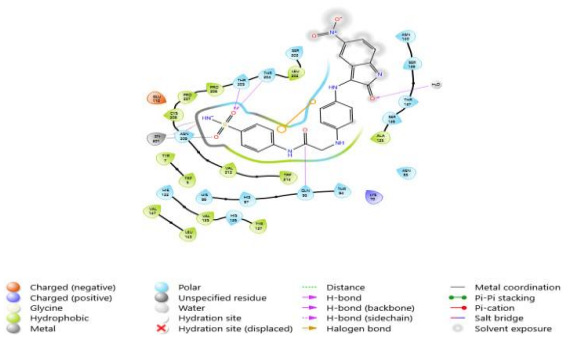
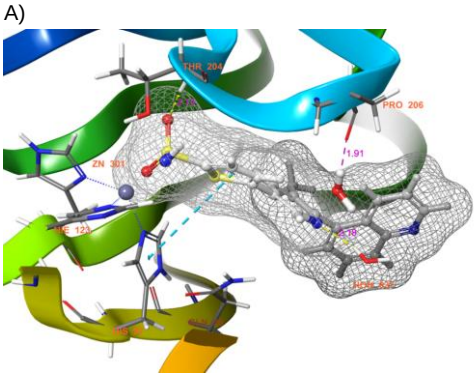


Figure 2: the interaction of compound 6 with the active binding site of CA XII in 3D (A) and 2D (B) structures.

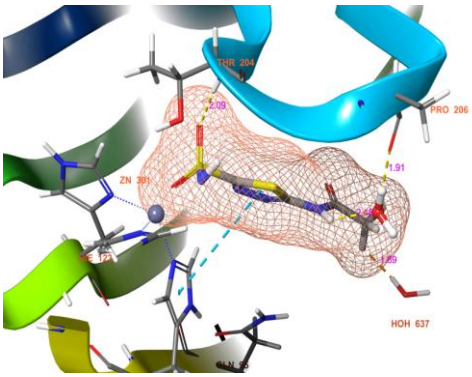
A)



B)

Figure 3: The interaction of native ligand (V8O) with the CA XII enzyme active site in 3D (A) and 2D (B) structures.

A)



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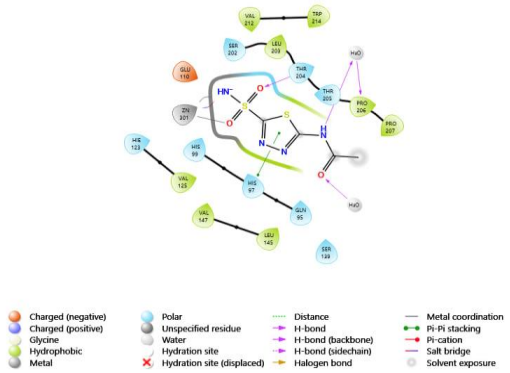


Figure 4: The interaction of AZM with CA XII enzyme active site in 3D (A) and 2D (B) structures.

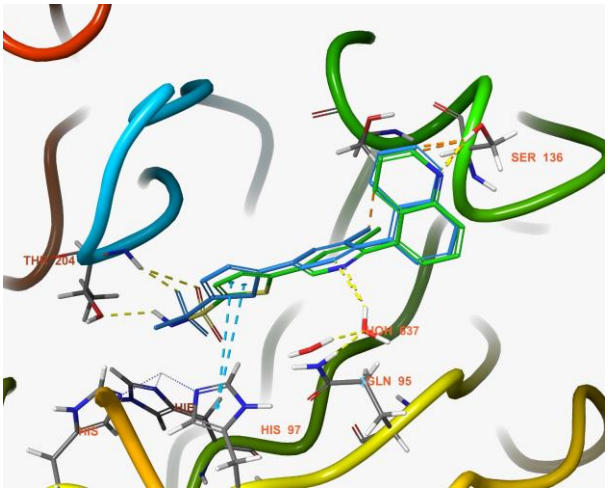


Figure 5: superimposition of redocked ligand over co-crystallized ligand

Molecular Mechanics/Generalized Born Surface Area Binding Free Energy Analysis

The free energies of binding for the derivatives (1–6), the non-selective inhibitor acetazolamide (AZM), and the selective native ligand V8O against carbonic anhydrase XII (CA XII; PDB ID: 8CO3) were assessed via the (MM-GBSA) method (Table 3). These values provide a numerical thermodynamic ranking of ligand affinity and identify the main interactions that stabilize the complexes (26).

The total ΔG_{bind} values extended from –6.12 to –19.93 kcal/mol for the designed derivatives. All six compounds showed more negative ΔG_{bind} than AZM (–9.27 kcal/mol), suggesting that the derivatives show stronger binding to the active site of CA XII. However, ΔG_{bind} of the selective native ligand V8O (–38.58 kcal/mol), is stronger (24).

Van der Waals contributions (–30.98 to –41.48 kcal/mol) and lipophilic interactions (–4.05 to –11.19 kcal/mol) were the primary drivers of binding, consistent with the hydrophobic contact networks observed in docking. Hydrogen bonding contributions were modest (–1.39 to –2.62 kcal/mol). The native ligand V8O also showed a strong lipophilic contribution, while AZM demonstrated a low lipophilic contribution, explaining its weaker hydrophobicity, and hence, a non-selective, lower affinity binding (34).

Notably, while compound 6 had a slightly lower docking score than compound 5 (–6.614 vs. -7.069 kcal/mol), it showed a more favorable MM-GBSA ΔG~bind~ (–19.93 vs. –17.96 kcal/mol). This difference is consistent with findings that MM-GBSA integrates solvation effects and dynamic sampling, providing a more thermodynamically rigorous ranking than docking scores (35). In comparison to the native ligand V8O, compound 6 is considered moderately active, but is still a promising scaffold.

Table (3): MM-GBSA values of different isatin-sulfonamide derivatives and the native ligand in CA XII enzyme (PDB ID:8CO3).

Compounds	ΔG_{bind} (Kcal/mol)	ΔG_{bind} van der Waals	ΔG_{bind} lipophilic	ΔG_{bind} H-bond
1	-16.44	-36.77	-8.71	-2.30
2	-15.69	-38.90	-11.19	-1.39
3	-14.35	-30.98	-5.92	-2.42
4	-6.12	-34.97	-4.05	-2.40
5	-17.96	-37.01	-7.63	-2.60
6	-19.93	-41.48	-10.09	-2.62
AZM	-9.27	-27.37	-2.58	-1.52
Native ligand V8O	-38.58	-40.58	-14.36	-1.97

Molecular dynamics

Molecular dynamics simulations of Carbonic Anhydrase XII (CA XII) in complex with compound 6 and the reference inhibitor V8O over 200 ns provide critical insights into the stability, flexibility, and interaction profiles of these inhibitors.

Root Mean Square Deviation (RMSD) analysis: In (figure 6 A) the Cα RMSD rapidly equilibrated within the first 15 ns and maintained a tightly controlled trajectory with a calculated mean value of 1.28 ± 0.14 Å, and the ligand in this complex underwent an initial conformational relaxation during the first 15 ns, subsequently settling into a rigid, low-fluctuation plateau for the final 75 ns of the simulation, yielding an overall trajectory mean of 3.75 ±0.42 Å.

While in (figure 6 B), Cα RMSD displayed exceptional backbone stability, centering at a mean RMSD of 1.32 ± 0.21 Å, and the ligand V8O in this complex remained structurally close to its original docked pose with a remarkably low mean RMSD of 1.25 ±0.48 Å.

In molecular dynamics, stability is defined more by equilibrium and low fluctuation (a flat plateau) than by just having a low absolute number. Hence, complex in (figure 6 A) represents a more stably bound system in its final state, while Complex in (figure 6 B) shows better pocket retention but higher structural volatility.

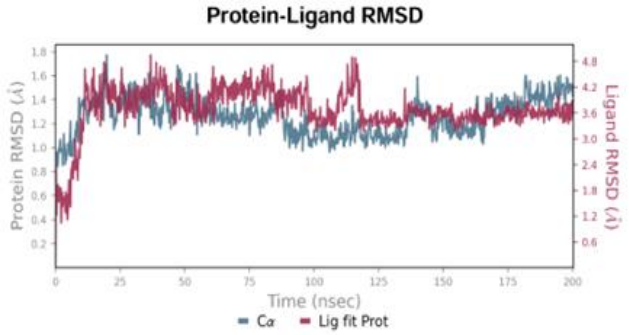
Root Mean Square Fluctuation (RMSF) plots (Figure 7) further dissect residue-wise flexibility. Both complexes show reduced fluctuations in the active site region (residues ~90–120 and 190–210), indicative of effective binding and stabilization of the catalytic pocket (7). However, compound 6 induces marginally higher flexibility in several loop regions adjacent to the active site, particularly around residues 130–150, which may reflect adaptive binding or less optimal steric complementarity compared to V8O.

The protein-ligand contact timelines (Figure 8) offer a detailed temporal map of non-covalent interactions sustained over the 200 ns simulation. Both inhibitors maintain essential polar interactions with key catalytic residues, including Thr199 and Glu117, which are crucial for zinc-binding group coordination—a hallmark of carbonic anhydrase inhibition (36).

Notably, V8O shows a higher occupancy of hydrophobic interactions involving residues such as Val121, Leu198, and Phe131 (Figure 9). This enhanced hydrophobic packing within the active site likely contributes to its superior stability, as observed in the RMSD analysis, and aligns with recent strategies to improve CA XII selectivity through van der Waals optimization (37).

Compound 6, while also engaging in hydrogen bonding and hydrophobic interactions, shows a more dynamic profile with intermittent water-bridged hydrogen bonds. These solvent-mediated interactions, although less persistent, may offer entropic advantages or facilitate induced-fit binding, which could be advantageous in certain pharmacological contexts (38).

A)



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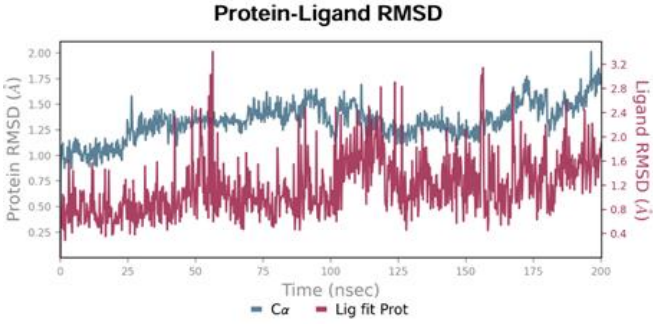

Figure 6: A) Compound 6 RMSD plot. B) V8O RMSD plot.

A)

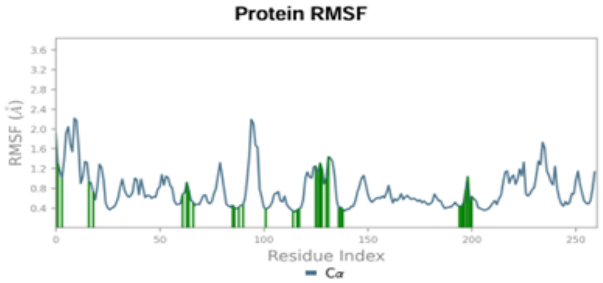

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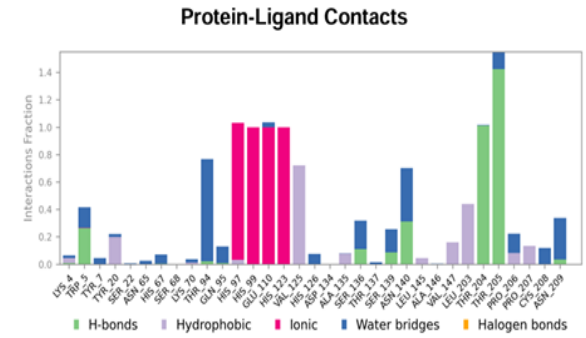
Figure 7: A) Compound 6 RMSF plot. B) V8O RMSF plot.

A)



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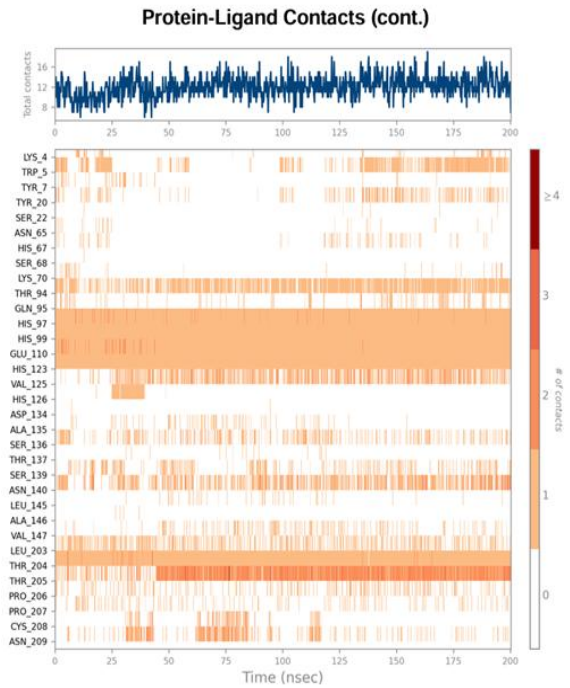
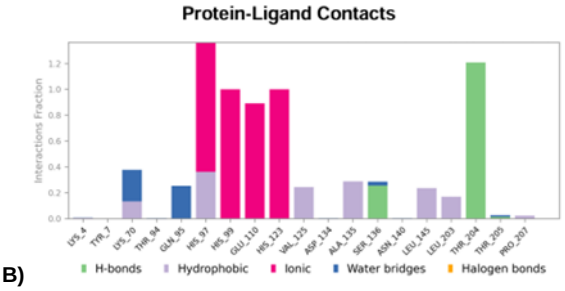


Figure 8: CA XII - contacts of compound 6. (A, B) explaining the binding interactions proportion through MDS in 200 ns.

A)



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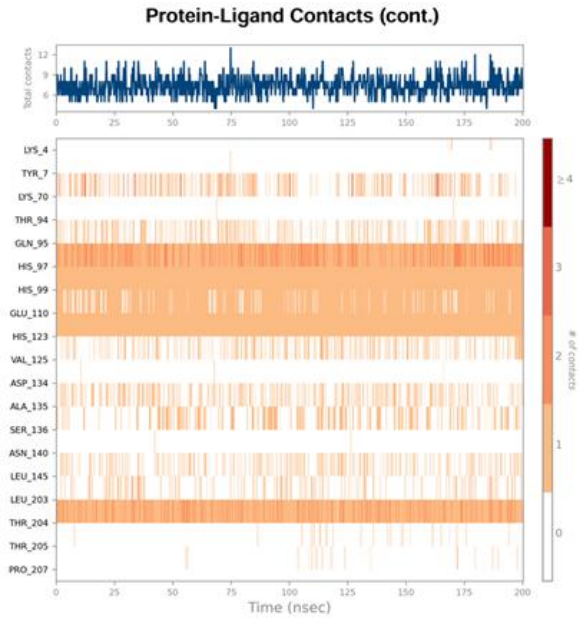

Figure 9: CA XII contacts of V8O (A, B) explaining the binding interactions proportion through MDS in 200 ns.

ADME Analysis and Pharmacokinetic Profiling

Molecular weight is the main factor of passive diffusion and oral absorption. According to Lipinski's rule of five, an ideal drug candidate should have an MW <500 Da to ensure reliable intestinal permeability(39). In this series, compounds 1, 3, 5, and 6 obey this limit, while compounds 2 and 4 exceed it.

LogP (partition coefficient) reflects the compound's hydrophobicity and influences solubility, membrane penetration, and distribution. For an oral drug it must be <5 (39). Most of the designed compounds showed LogP values within this limit. Compound 2 LogP (2.85), may reflect good membrane permeability but probably less aqueous solubility. Compound 6 has a low LogP (0.80), indicating higher hydrophilicity and enhanced solubility, but may reduce passive diffusion. Remarkably, AZM LogP (–0.7), reflects high hydrophilicity and poor blood-brain barrier penetration. At the same time, V8O has a LogP of 3.19, indicating high lipophilicity and enhanced tissue distribution, but also potential off-target accumulation.

All designed derivatives have 4 HBD, and HBA range from 6 to 8, also within the acceptable limit. Notably, V8O has only 1 HBD and 5 HBA, while AZM has 2 HBD and 6 HBA. The higher HBD/HBA ratio in the derivatives suggests a greater ability to engage in polar interactions with the CA XII active site, as indicated by strong hydrogen bond networks detected in the docking studies.

TPSA is a well-recognized indicator of passive membrane transport and blood brain barrier (BBB) penetration. For accepted intestinal absorption, a TPSA <140 Å² is generally required (40). Compounds 1–5 all have TPSA values (151.13 Å²), slightly over the ideal threshold, which may reflect moderate oral absorption with reduced BBB penetration. This is favorable for peripherally acting anticancer agents intended to reduce central nervous system adverse effects. Compound 6 shows a significantly higher TPSA (196.95 Å²), possibly due to additional polar groups, further limiting its passive diffusion.

The ADME profiles of the designed isatin-sulfonamide derivatives reveal a balanced pharmacokinetic profile. Most compounds follow the main Lipinski guidelines (MW <500, LogP 1–3, HBD ≤5, HBA ≤10). The values exhibited by these compounds make them moderately bioavailable orally, in addition to their limited CNS penetration, which is valuable for targeting peripheral tumors while minimizing neurotoxic adverse effects. Table 2 shows the most important pharmacokinetic properties of the proposed compounds, native ligand, and AZM.

Notably, the tested compounds are not substrates for P-glycoprotein (P-gp), suggesting they are not subject to active efflux, which may compensate for their low passive GI absorption.

Regarding metabolism, the compounds were found to inhibit CYP1A2, CYP2C9, and CYP3A4. While this indicates a potential for drug-drug interactions (DDI), such profiles are common in complex heterocycles like isatin-sulfonamides (41), and will be the focus of future structural optimization to improve metabolic selectivity.

Table (2): physicochemical properties of native ligand, AZM and isatin-sulfonamide derivatives.

Compound	Molecular weight(g/mol)	LogP	H-bond donors	H-Bond acceptors	TPSA	P-gp substrate
native ligand V8O	381.47	3.19	1	5	122.56	no
AZM	222.25	-0.7	2	6	151.66	no
1	467.47	1.94	4	7	151.13	no
2	607.27	2.85	4	6	151.13	no
3	485.46	2.20	4	8	151.13	no
4	518.37	2.72	4	6	151.13	no
5	477.54	2.28	4	6	151.13	no
6	494.48	0.80	4	8	196.95	no

Toxicity profile

In silico toxicity assessment of compounds 1-6 was performed using ProTox 3.0 and compared to V8O and acetazolamide (Table 4). All tested compounds, including the references, were predicted to be inactive for hepatotoxicity, neurotoxicity, and immunogenicity (42). Acetazolamide was predicted to be carcinogenic (active, 0.51), whereas all designed hybrids and V8O were predicted to be inactive for carcinogenicity (43).

For acute toxicity, according to the Globally Harmonized System (GHS) classification, compounds 1-6 fell into Class 5 (low toxicity), with predicted oral LD50 values ranging

from 4000 to 5000 mg/kg. In contrast, V8O was classified as Class 4, with a predicted LD50, of 400 mg/kg (44). The primary shared concern across the series was a predicted risk of

respiratory toxicity (probabilities of 0.67 0.80), a known class effect associated with the sulfonamide pharmacophore that warrants careful investigation in subsequent experimental studies and before clinical advancement in relevant in vitro (e.g., lung cell assays) and in vivo models. This would require specific mitigation strategies, such as formulation adjustments or targeted delivery, to minimize pulmonary exposure (45).

Table (4): toxicity profile of the native ligand, non-selective ligand, and isatin-sulfonamide derivatives.

Toxicity	Selective native ligand	Non-selective ligand	Compound1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Immunogenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Active 0.51	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Respiratory toxicity	Active 0.67	Active 0.72	Active 0.79	Active 0.76	Active 0.79	Active 0.80	Active 0.76	Active 0.70
Toxicity class	4	5	5	5	5	5	5	5
LD50	400mg/kg	4300mg/kg	5000mg/kg	4000mg/kg	5000mg/kg	5000mg/kg	5000mg/kg	5000mg/kg

Conclusion

This computational study recognizes novel isatin-sulfonamide hybrids, particularly compound 6, as a promising candidate with predicted selectivity as CA XII inhibitors for cancer treatment. These compounds reveal superior predicted binding affinity, complex stability in 200 ns MD simulations, and favorable pharmacokinetic characteristics when compared with standard inhibitors. Notably, they show a significantly enhanced predicted acute toxicity profile over the reference ligand V8O. Although the reference ligand V8O exhibited the highest MM-GBSA binding free energy, compounds 5 and 6 showed a more favorable balance of strong contributions from van der Waals as well as lipophilic bonds, in addition to improved predicted pharmacokinetic properties and a significantly enhanced predicted acute toxicity profile compared to V8O. These results suggest that these isatin-sulfonamide hybrids, especially compound 6 are strong lead candidates for further experimental development.

limitations of Study

limitations of computational study: although computational approaches are valuable for guiding research and optimizing experimental work, they cannot replace real experimental studies, since they often fail to predict the complexity of biological systems, protein flexibility, and long-term real-world behavior, as many simulations operate on reduced or idealized representations of the system.

Since no cross-isoform docking was carried out, definitive selectivity cannot be proven.

binding energy values (ΔG_{bind}) are relative estimations. Specifically, the neglect of solute entropy and the use of a continuum solvent model may lead to more favorable energy values than experimental results. However, since the same parameters were applied to both compound 6 and the reference (V8O), the relative ranking and the identification of the primary energy contributors (e.g., van der Waals vs. electrostatic) remain highly valid for lead optimization.

Future Perspectives

In silico results are considered theoretical predictions of compounds, as a result they require rigorous validation through real experimental studies to ensure accuracy. For example, in vitro testing, which includes testing the compounds against cancer cell lines, in addition to enzyme inhibition assays to verify their selective inhibition of CA XII.

Also, experimental safety assessments are required to confirm the improved safety profile and lower acute toxicity of new compounds.

Disclosure Statements

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.

Author's contribution

All authors contributed equally to this work.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article

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