

Molecular docking, dynamics, and ADME studies of new isatin derivatives as histone deacetylase (HDAC) inhibitors

Hanan Adil Salih^{*1}  , Rafid M. Hashim²  ,
Basma M. Abd Razik¹  , Ghazi Al Jabal^{**3}  

¹Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq.

²Department of Pharmaceutical Chemistry, College of Pharmacy, Uruk University, Baghdad, Iraq.

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biomedical Sciences, MAHSA University, Bandar Saujana Putra, 42610, Jen Jarom, Selangor, Malaysia.

*Corresponding Author¹ email: hanan.adil96@uomustansiriyah.edu.iq

ORCID: <https://orcid.org/0009-0004-6264-7130>

**Corresponding Author² email: Ghaziahmad@mahsa.edu.my

ORCID: <https://orcid.org/0000-0002-9031-0816>

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Abstract:

Histone deacetylase-2 (HDAC-2) is an important therapeutic target in cancer treatment. Isatin derivatives have attracted considerable interest in medicinal chemistry because of their diverse biological activities. The present study aimed to investigate a series of newly designed isatin derivatives as HDAC-2 inhibitors using computational approaches. Molecular docking was performed against HDAC-2 protein (3MAX) to evaluate the binding affinity and interactions of the proposed compounds. ADME analysis was performed to assess their pharmacokinetic and drug-likeness properties. The most promising derivative was subjected to molecular dynamics (MD) simulation for 200 ns to evaluate the stability of the protein–ligand complex and characterize key molecular interactions throughout the simulation period. Among the investigated compounds, Compound 2 exhibited the most favorable binding affinity toward HDAC-2 with a docking score of -9.866 kcal/mol, slightly exceeding that of the reference inhibitor SAHA -9.707 kcal/mol. The compound demonstrates stable interactions with binding site residues and maintained interaction with zinc ion. MD simulations demonstrated stable protein-ligand interactions, minimum structural fluctuations, and preservation of the overall protein secondary structure throughout the simulation. These findings suggest that Compound 2 could be a promising isatin-based scaffold for the development of new HDAC-2 inhibitors.

Keywords: Isatin, histone deacetylase, isocyanate, molecular docking, molecular dynamics.

1. Introduction

Cancer is a widespread disease globally that is most frequently caused by tobacco

smoking, alcohol consumption and genetic disorders such as development or differentiation in cells, known as tumor



oncogenes or suppressor genes^(1,2). During early embryonic development, the genome undergoes extensive epigenetic reprogramming through mechanisms such as DNA methylation, histone modifications, and interactions with non-coding RNAs⁽³⁾. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes dynamically modulate the acetylation of histone proteins⁽⁴⁾. Inhibition of HDAC enzymes constitutes a promising approach for cancer therapy⁽⁵⁾. The FDA sanctioned the use of vorinostat, panobinostat, belinostat, and romidepsin as HDAC inhibitors for cancer treatment figure-1(a), either in combination with other antineoplastic agents or as monotherapies. Three HDAC inhibitors now employed in clinical practice utilize the hydroxamate moiety as the zinc-binding group (ZBG). However, hydroxamate has been reported to exhibit poor pharmacokinetics and considerable toxicity⁽⁶⁾.

Isatin (1H-indole-2,3-dione) is a heterocyclic molecule that Erdmann and Laurent identified as an oxidation product of indigo by the use of nitric and chromic acids figure-1(b)^(7,8). Isatin is recognized for its function as a precursor in the production of diverse chemicals utilized in the formulation of specific medications aimed at particular tissues and cells⁽⁹⁾. Isatin, or 2,3-dioxindoline, is an indole derivative featuring keto groups at the 2 and 3 positions of the ring. The isatin ring system has a pyrrole ring fused to a benzene ring⁽¹⁰⁾. This compound is found in various plants, such as *Isatis tinctoria*, *Couroupita guianensis*, and *Calanthe discolor*⁽¹¹⁾. Isatin can be used to manufacture a variety of heterocyclic compounds. Particularly, positions 2 and 3, mainly the keto groups, can participate in condensation and addition processes at the C=O bond. Isatin derivatives can participate in Mannich and Michael processes, as well as N-alkylation and N-acylation through the amide functional group⁽¹²⁻¹⁴⁾.

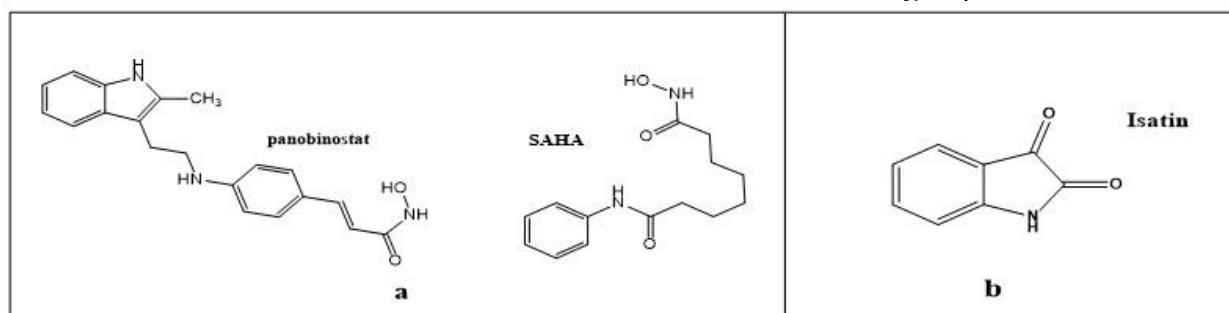


Figure 1: (a) structure of some FDA HDAC inhibitor (b) structure of isatin

Schiff bases of Isatin scaffolds show enormous applications in the biological field like anti-bacterial, anti-fungal and anti-cancer activities⁽⁷⁾. Numerous halogenated compounds, particularly fluorine-containing aromatic compounds, have garnered significant attention due to their biological activity⁽¹⁵⁾. Semaxanib and indirubin, two isatin-derived chemicals, are currently utilized in clinical settings or under clinical investigation to address various illnesses^(16,17). It was discovered that isatins-

hydrazine derivatives were effective against carcinosarcoma^(18,19). Isothiocyanate and isocyanate have been reported as an anticancer agent for several carcinomas, from cervical cancer to hepatoma^(20,21).

2. Materials and methods

2.1 Molecular Docking

The five isatin derivatives were initially drawn in ChemDraw as shown in figure2, followed by the conversion of their

two-dimensional sketches into three-dimensional conformational models. The initial geometry optimization followed the same multistep protocol commonly applied in ligand-based computational studies. Molecular mechanics optimization was first performed to generate reasonable starting geometries, followed by semi-empirical refinement to obtain low-energy conformers suitable for docking analysis. All ligand preparation, optimization, and docking procedures were carried out using the Schrodinger Maestro software suite⁽²²⁾. The crystal structure of the target protein (PDB ID: 3MAX; resolution 2.05 Å) was retrieved from the Protein Data Bank. Protein preparation was performed using the Protein Preparation Wizard, which included assignment of bond orders, addition

of missing hydrogen atoms, optimization of protonation states, removal of non-essential crystallographic water molecules, and restrained minimization of the protein structure. Ligand preparation was conducted using LigPrep to generate the most probable ionization states, assign proper stereochemistry, and minimize the structures using the OPLS force field⁽²³⁾. A receptor grid was generated around the active site of the 3MAX, the grid box was centered to the active site with dimensions 20 x 20 x 20 Å and molecular docking was performed using Glide. During docking, the receptor was kept rigid while full ligand flexibility was allowed. The best docking poses were selected based on Glide Score values and visual inspection of key interactions within the binding pocket⁽²⁴⁾.

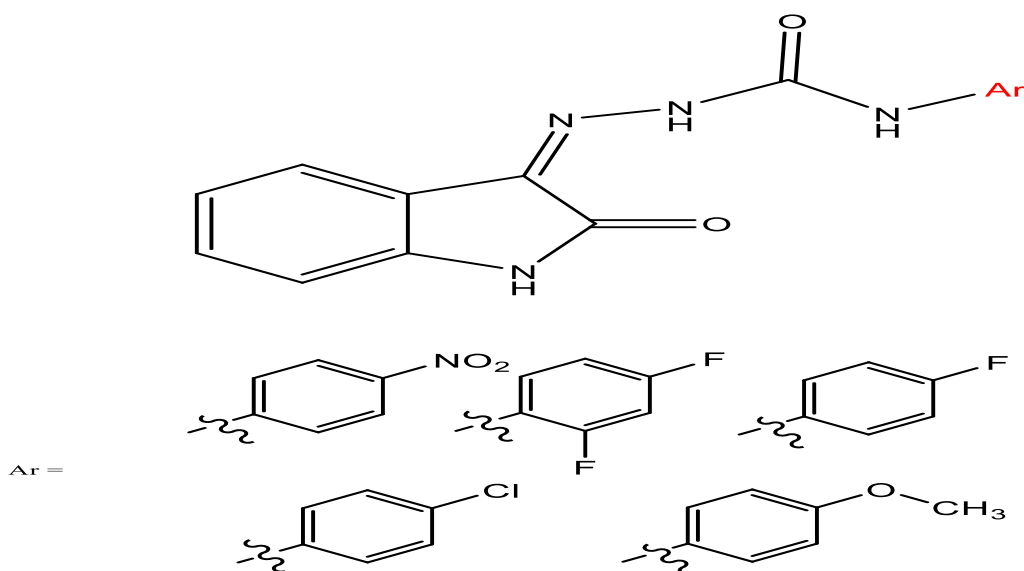


Figure 2: structure of tested derivatives

2.2. ADME study

In silico ADME profiling was made for all isatin derivatives using the SwissADME online platform. The structures of the derivatives were converted into SMILES format and uploaded to the server for the

prediction of pharmacokinetic, physicochemical, and drug-likeness properties. The molecular weight, hydrogen bond acceptors and donors, topological polar surface area (TPSA), lipophilicity (Log P), and the quantity of rotatable bonds were

calculated. Pharmacokinetic characteristics, including blood–brain barrier permeability, gastrointestinal absorption, and P-glycoprotein substrate prediction, were assessed. Drug-likeness was evaluated according to the Lipinski rule of five ⁽²⁵⁾.

2.3 Molecular Dynamic

Molecular dynamics simulations were performed using Schrodinger LLC's Desmond software program⁽²⁶⁾. The NPT ensemble was employed across the simulation, maintaining a temperature of 300 K, 200 ns, 48,429 atoms, 14,180 water molecules, 364 residues and a pressure of 1 bar. The studied compound 2 experienced a relaxation time of 1 ps throughout the 200 ns simulations. Trajectory analysis was performed using RMSD, RMSF, secondary structure assessment, protein–ligand interaction profiling and physicochemical property evaluation to evaluate the stability and binding behavior of the compound 2 ligand complex, which were subsequently preserved for later research ⁽²⁷⁾

3. Results

3.1 HDAC-2 inhibition and ADMET study results:

Analysis of how **Compound 1** binds to HDAC-2 exhibited an affinity score equal to -3.779 kcal/mol. **Compound 1** the ligand exhibited a stable binding mode within the HDAC-2 active site, forming hydrogen bond with **Asp104**, and showing potential coordination with the catalytic ion **Zn379**. Additionally, hydrophobic interactions with residues such as **Phe210**, **Leu98**, and **Met35**, along with π stacking involving aromatic rings (notably with **Phe155**), contributed to

the overall stabilization and enhanced binding affinity of the ligand within the pocket. Meanwhile, **Compound 2** had affinity score of -9.866 kcal/mol. demonstrated a stable binding mode within the protein active site, exhibiting potential coordination with the zinc ion **Zn379**. Hydrogen bond interactions were observed with **Gly143** and **Gly154**, which played a crucial role in stabilizing the ligand within the binding tunnel. Additionally, strong hydrophobic interactions with residues such as **Phe155**, **Phe210**, **Leu144**, and **Met35**, and the aromatic ring interaction. **Compound 3** record affinity score of -4.803 kcal/mol. The ligand exhibited stability in the active site, showing potential coordination with the catalytic **Zn ion Zn379**. also hydrogen bond was observed with **Gly154** and **Gly143**, and strong hydrophobic interactions with **Phe155**, **Phe210**, **Leu144**, **Met35**, and **Leu276**, were involved with aromatic residues. -9.538kcal/mol. was recorded for **Compound 4** displayed a stable binding conformation within the binding site, with potential coordination toward **Zn379**. A hydrogen bond interaction was observed with **Gly154**. Moreover, strong hydrophobic interactions with residues such as **Phe155**, **Phe210**, **Leu144**, **Met35**, and **Leu276**, along with aromatic rings. Lastly **compound 5** affinities score equal to -2.369 kcal/mol. The ligand adopted a stable binding pose within the protein, also interaction with **Zn379** was existed. **Gly154** formed a hydrogen bond with **compound 5** which stabilize it, strong hydrophobic interactions between aromatic rings and **Phe155**, **Phe210**, **Leu144**, **Met35**, and **Tyr308** were recorded. **Figure2 (1-5)** illustrates all these interactions.



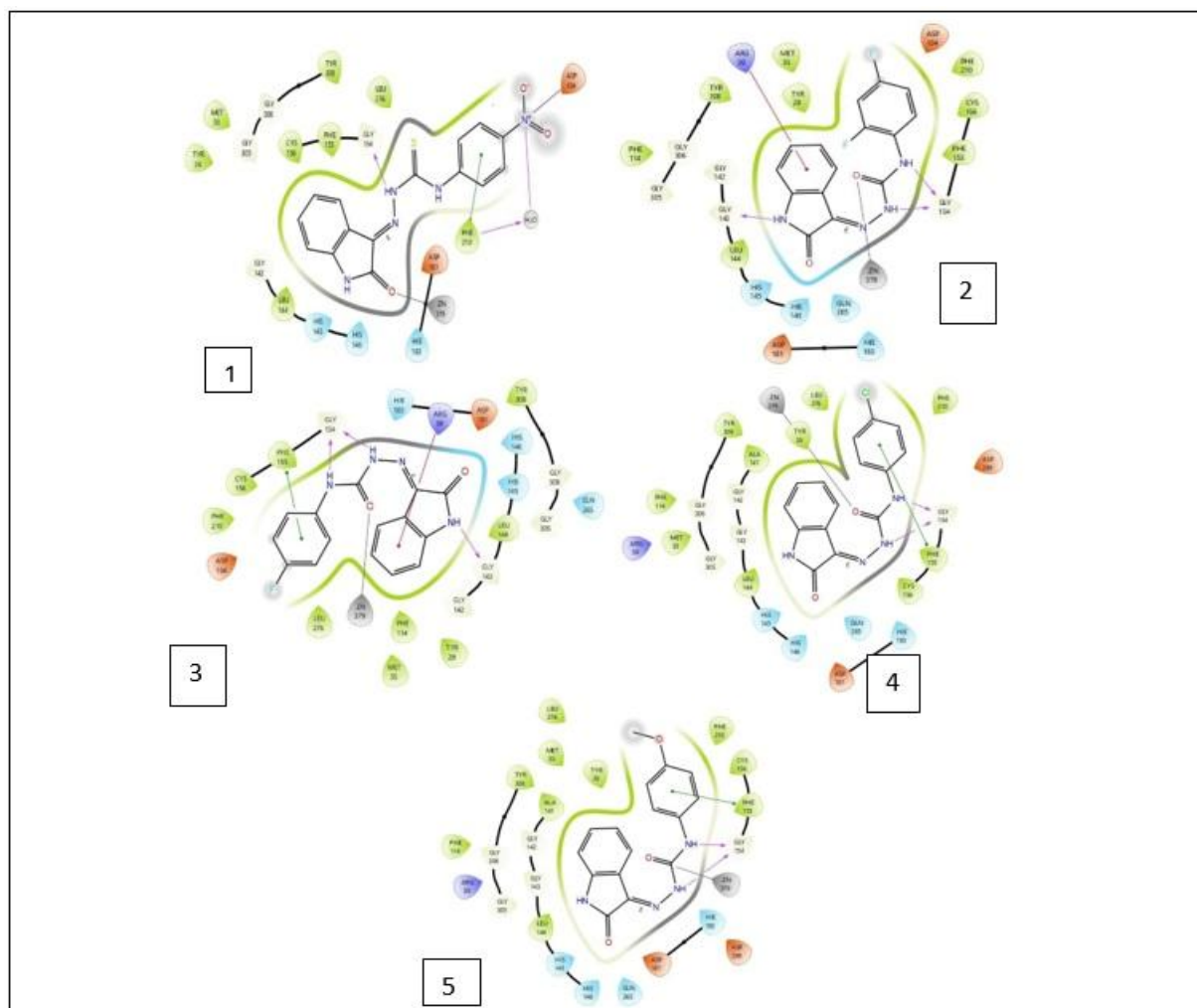


Figure 3: 2D of (Compound 1- 5) against HDAC-2.

Table 1: results of docking

Target protein	Tested compound	Docking (Affinity) score
HDAC 2 3MAX	Compound 1	-3.779
	Compound 2	-9.866
	Compound 3	-4.803
	Compound 4	-9.538
	Compound 5	-2.369
	SAHA	-9.707

For the ADME results, all compounds exhibit appropriate physicochemical and pharmacokinetic properties including gastrointestinal absorption, lipophilicity and water solubility also compounds show

moderate to high drug likeness properties, molecular weights and H-bonds donor and acceptor are shown in table 2 and table 3 for each compound.

Table 2: Pharmacokinetic and ADME parameters for all compounds

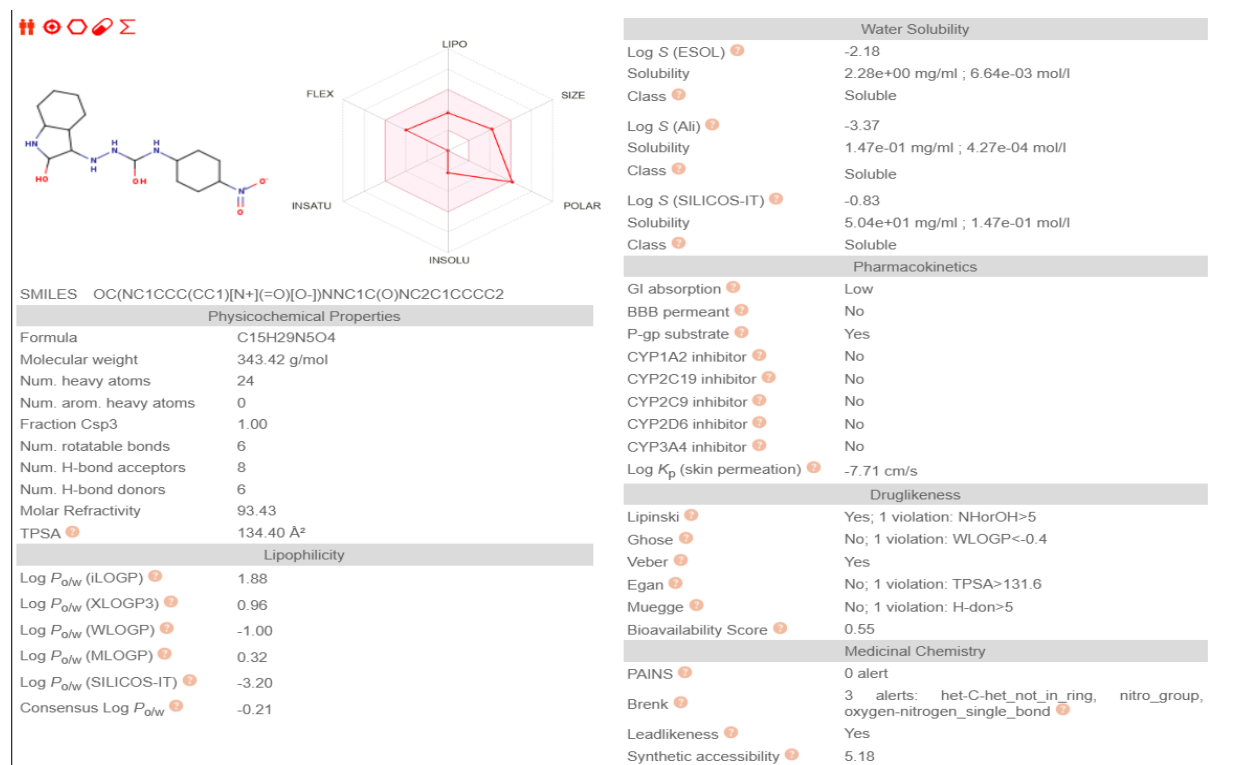
Molecule	M.W	H.B.A	H.B.D	M.R(m ³ /mol)	TPSA(°A ²)	G.I absorption
compound 1	343.4	8	6	93.43	134.4	Low
compound 2	316.3	5	3	82.36	82.59	High
compound 3	298.3	4	3	82.4	82.59	High
compound 4	314.7	3	3	87.45	82.59	High
compound 5	310.3	4	3	88.94	91.82	High
SAHA	264.32	3	3	73.33	78.43	High

M.W: molecular weight, H.B.A: hydrogen bond Acceptor, H.B.D: hydrogen bond donor, M.R: molar refractivity, TPSA: total polar surface area, G.I: gastrointestinal.

Table 3: ADME screening for proposed compounds.

Molecule	B.B.B permeant	P-gp substrate	Lipinski violations	Bioavailability Score
compound 1	No	Yes	1	0.55
compound 2	No	No	0	0.55
compound 3	No	No	0	0.55
compound 4	No	No	0	0.55
compound 5	No	No	0	0.55
SAHA	No	No	0	0.55

B.B: blood brain barrier, P-gp: P glycoprotein.

**Figure 4: ADME study for Compound 1**

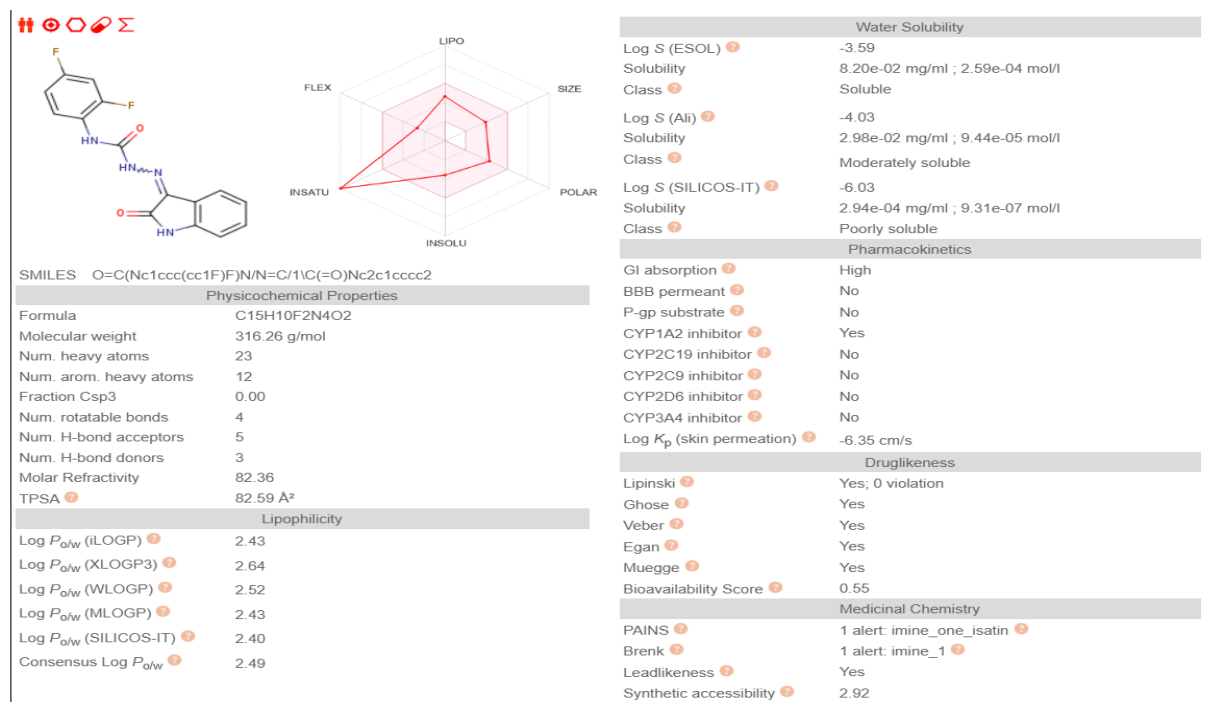


Figure 5: ADME study for Compound 2

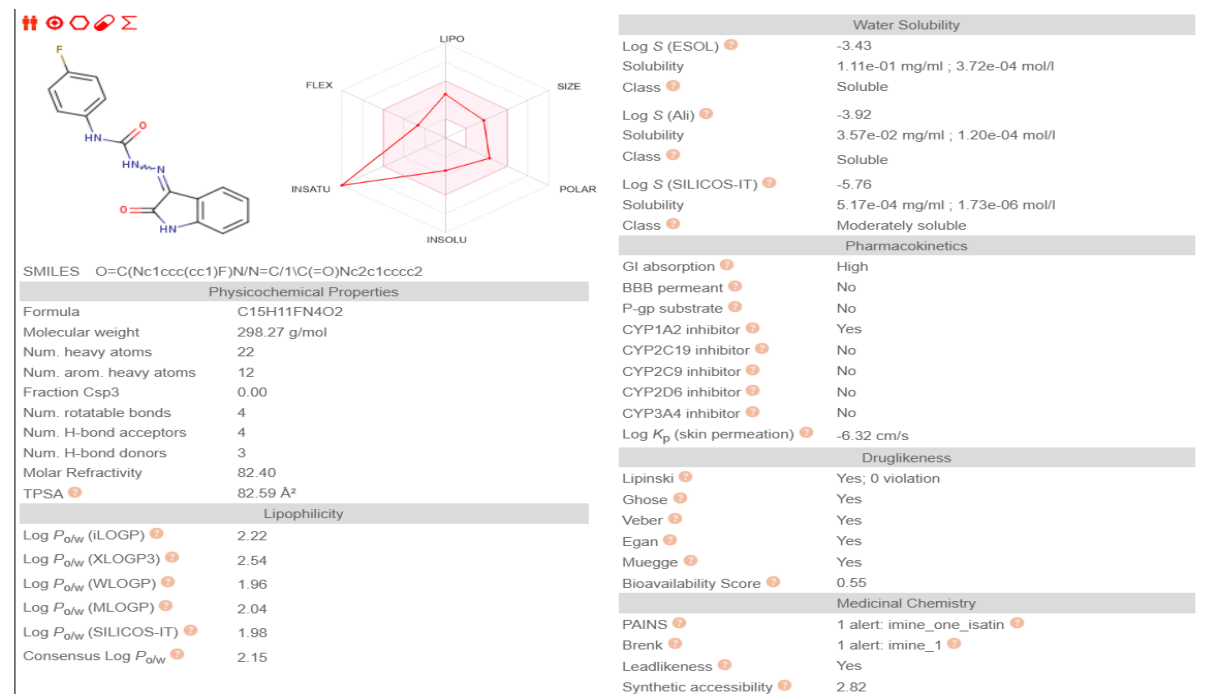


Figure 6: ADME study for Compound 3

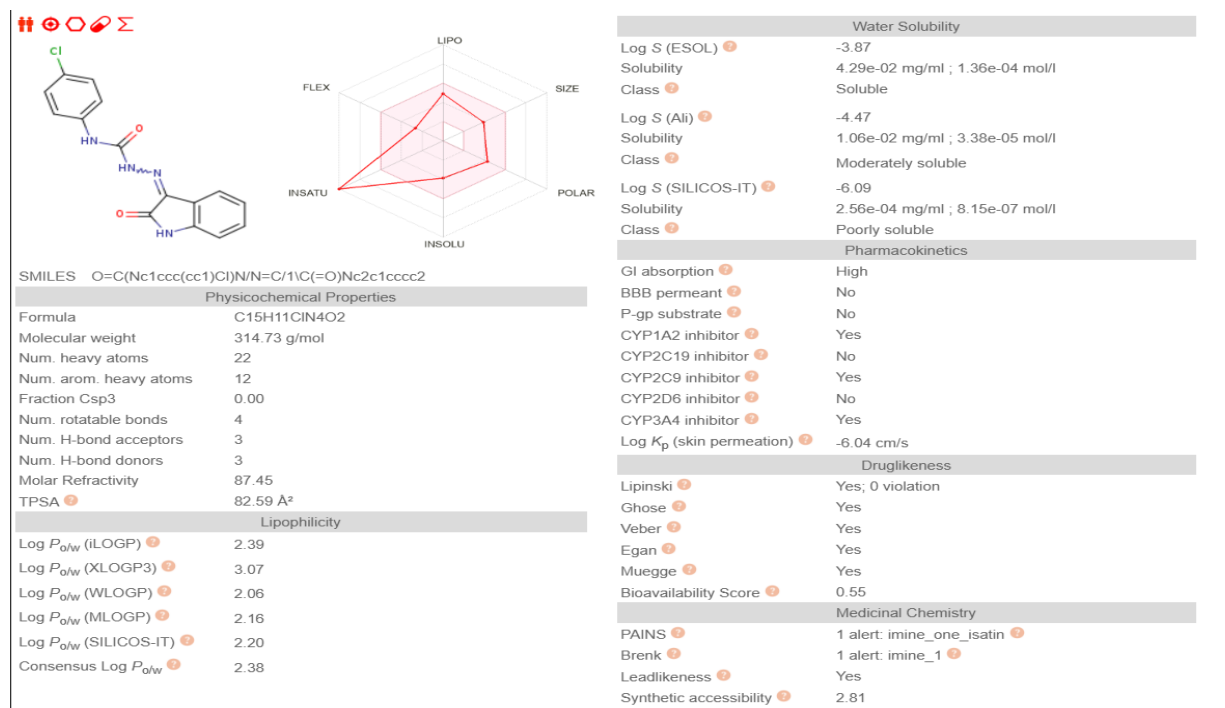


Figure 7: ADME study for Compound 4

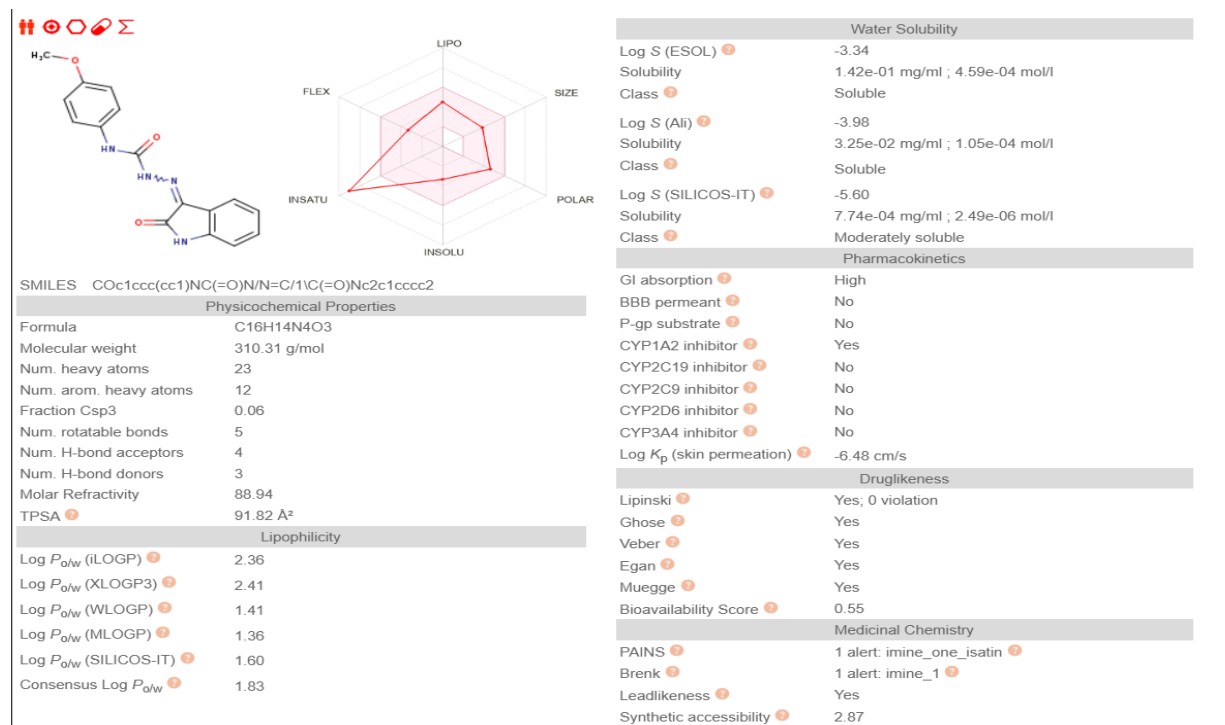


Figure 8: ADME study for Compound 5

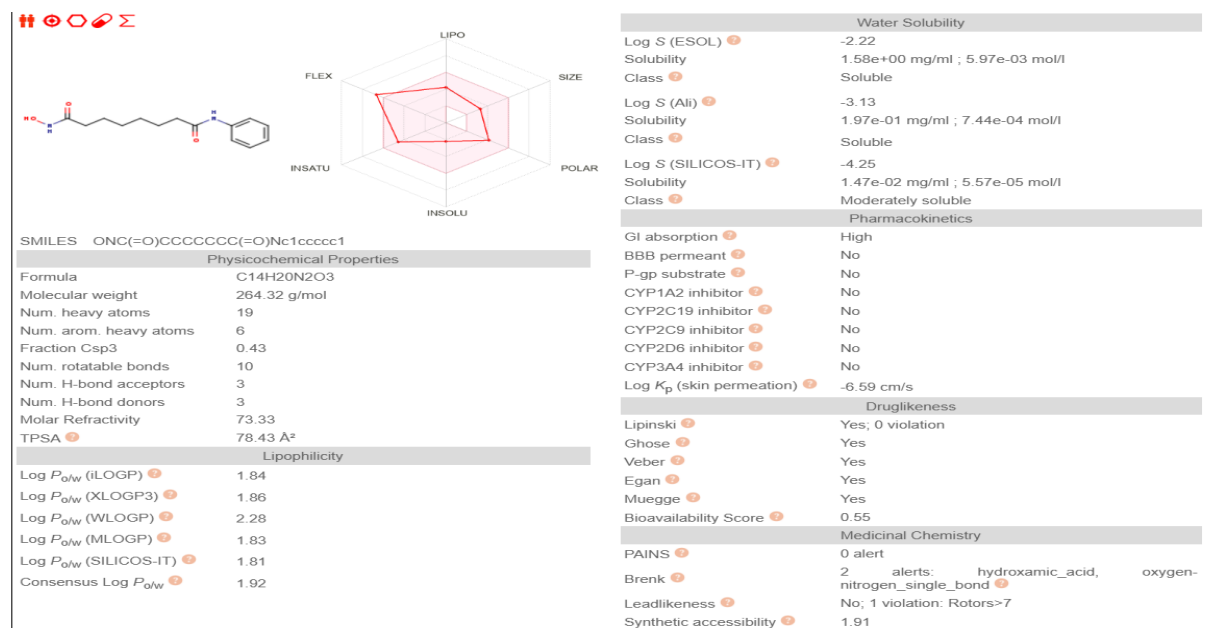


Figure 9: ADME study for SAHA

3.2 Molecular dynamic study results:

The molecular stability of the best docked compound was evaluated, hydrogen bonds donar and acceptor, hydrophobic interactions, zinc coordination and a thorough analysis and evaluation of the interaction between investigated compounds with HDAC-2 binding pocket was also conducted. Investigation using 200 ns MD simulations. We reported and examined the ligand and protein complexes obtained root mean square deviations (RMSDs) with respect to the initial locations inside the active site.

3.2.1 protein-ligand RMSD and RMSF Analysis:

As shown in figure 10 the MD analysis revealed that the protein backbone remained in a stable conformation throughout the simulation, with C α RMSD values stabilizing around ~1.2–1.5 Å after the equilibration phase. The ligand RMSD fluctuated moderately within ~1.0–2.0 Å, meaning that the ligand remained stable within the binding pocket, showing no significant displacement from the active site. A minor fluctuation was

observed during the early simulation phase (~20–50 ns), suggesting minor conformational adjustments before equilibrium was reached. The RMSF profile shows that the protein-ligand complex remains stable over the simulation, with small fluctuations (<1.0 Å) showing that the protein structure is not very flexible. It is important to note that the residues that involved in ligand binding showed very little change, which means that the ligand–protein complex is stable. The results indicate that the protein-ligand complex remained stable, with only localized flexibility that did not significantly affect ligand binding within the active site of protein.

3.2.2 protein secondary structure Analysis:

Secondary structure analysis indicate that HDAC-2 protein remained its structural integrity throughout simulation period. The α -helix content was 33.34%, β -strand content was 10.05%, the total secondary structure elements represented 43.40% of total protein structure, leading overall stability during the simulation period

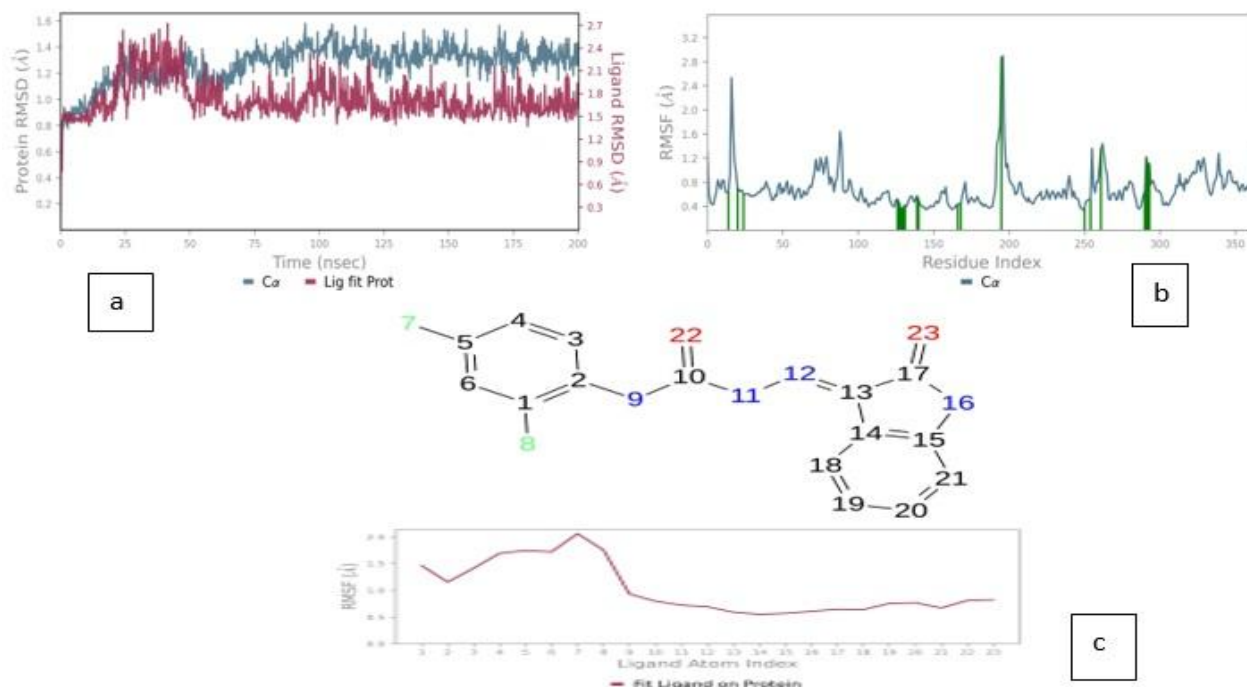


Figure 10: RMSD and RMSF of Compound 2 against HDAC-2.

3.2.3 Analysis of protein-ligand histogram:

Analyses of Compound 2 indicated a consistent interaction inside the HDAC-2 active region during the simulated period. The interaction analysis indicated that the ligand exhibits hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges. Hydrogen bond interactions were noted with **His146**, **Gly154**, and **Gln265**, demonstrates their essential function for ligand stability in the binding pocket. An ionic interaction between **Asp269** and **Asp181** was observed, showing how important negatively charged residues are in the ligand binding pocket. The ligand also had moderate hydrophobic interactions with residues like **Leu144**, **Phe155**, and **Phe210** which stabilized

the aromatic rings in the hydrophobic pocket. Interactions with water bridges also make the ligand more stable in the binding site, especially **Gly306**, **Gln265**, and **Arg39**. This suggests that interactions with the solvent are very important for keeping the ligand–protein complex stable. Coordination with the catalytic **Zn** ion was detected, demonstrating the ligand's ability to engage with the metal center of the HDAC, which is needed for inhibitory action.

Overall, compound2 and HDAC-2 had a strong, stable and different multiple interactions pattern which contributes to the proposed activity.

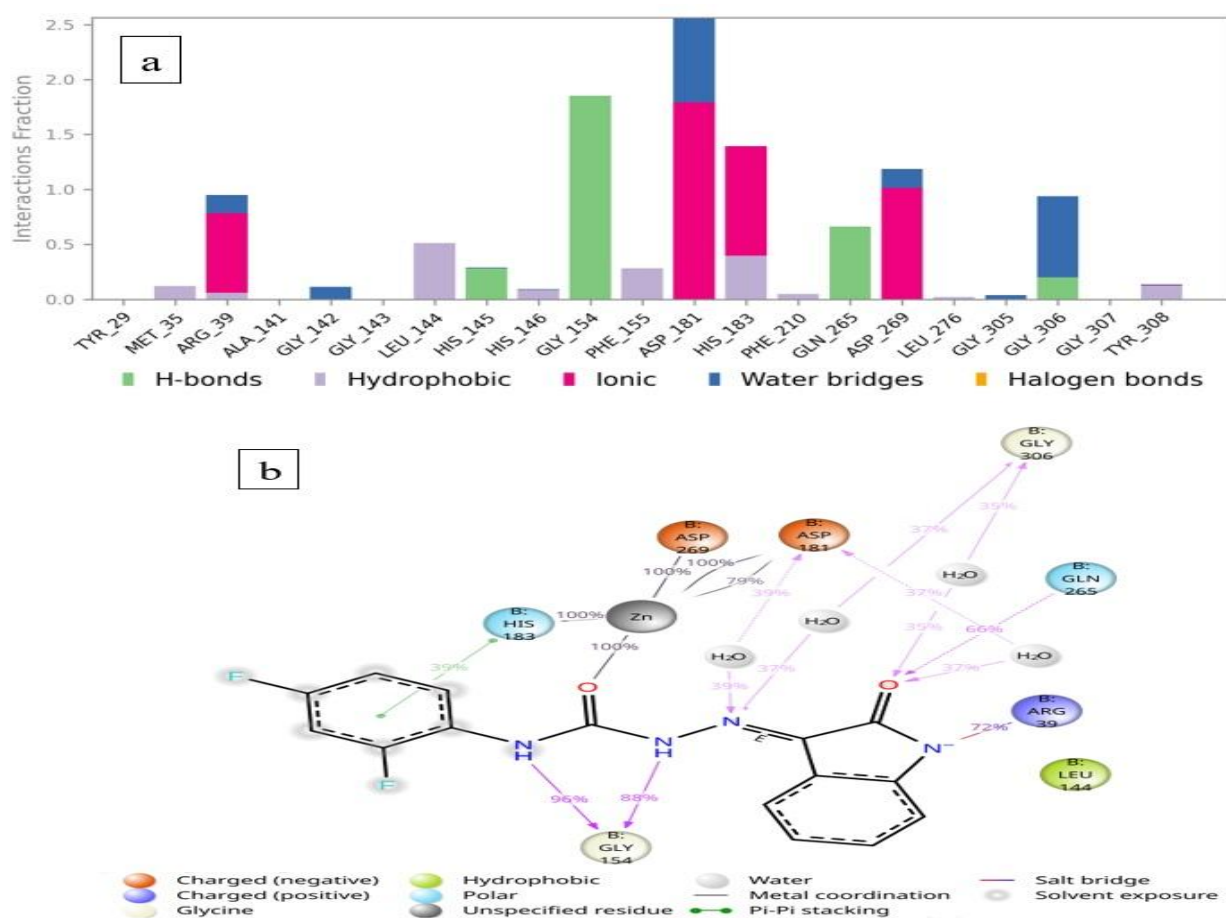


Figure 11: Protein ligand interaction.

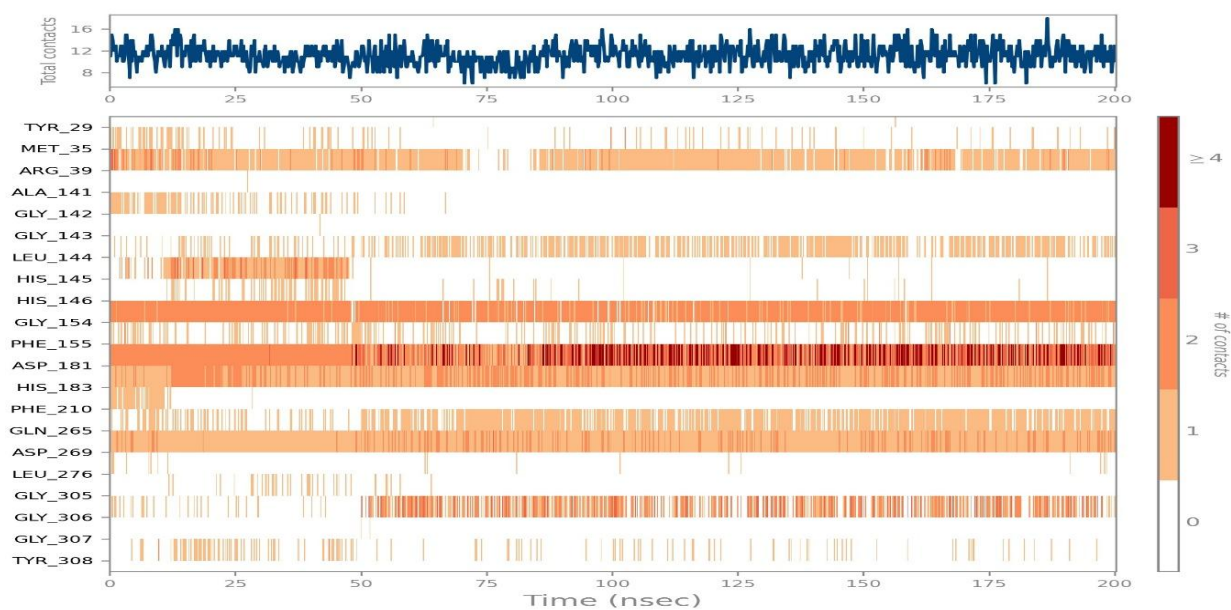


Figure 12: Heat map of protein ligand interaction during 200 ns simulation period.

4. Discussion

The interactions compound 2 and **HDAC-2** were evaluated to assess the potential activity. The simulation focused on the affinity scores of the binding and the multiple interactions which occurred during the testing and includes hydrogen bonds, hydrophobic interactions, and Zn ionic interactions all act to stabilize the ligand in the active site. The compound studied showed considerable results of binding affinities for **HDAC-2**; (-9.866 kcal/mol) which is slightly higher than reference SAHA (-9.707 kcal/mol). The interaction was characterized by a combination of hydrophobic π -interactions, hydrogen bonds, and metal ionic interactions, particularly with **Gly143, Gly154, Phe155 and Zn379** for **HDAC-2 compared with SAHA which interact with Asp104, His146 and Tyr308**. This binding suggests that metal coordination is important for the stability of the protein-ligand complex, with hydrophobic interactions, isatin provide additional rigid hydrophobic interactions gives an important role in the binding specificity unlike reference which contain flexible aliphatic linker. The reference drugs, **SAHA**, provided valuable benchmarks for evaluating the potential activity of this compound as HDAC inhibitor. Considering the drug likeness properties, number of hydrogen bonds acceptor and donor and all other ADME results the best result from the above work is the interaction of **Compound 2** with **HDAC-2**, which showed the high binding affinity score of **-9.866 kcal/mol**. although these results are preliminary, they provide a rationale reason for synthesis and purification of the derivative and further biological evaluation.

5. Conclusion

Compound 2 showed the most promising result due to its favorable ADME properties, acceptable lipophilicity and aqueous solubility, high protein binding affinity,

extensive bonding network, and competitive performance against reference drug **SAHA**. The combination of hydrophobic π -interactions, hydrogen bonds, and metal ion coordination suggests that **Compound 2** may represent a promising **HDAC-2** inhibitor, making it an excellent candidate for further experimental validation and potential therapeutic development.

6. Acknowledgment

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