

Original Research Article

In silico profiling, molecular docking, and RMSD prediction of novel chalcone-schiff base hybrid derivatives as HDAC2 inhibitors

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Abstract

Purpose: To investigate the pharmacokinetic properties and binding affinities of a series of chalcone-Schiff base hybrid derivatives.

Methods: The pharmacokinetic properties and binding affinities of a series of chalcone-Schiff base hybrid derivatives (compounds I - XI) for HDAC2 enzyme were evaluated using computational techniques (SwissADME wizard and Schrödinger software).

Results: All investigated compounds were predicted to exhibit high gastrointestinal absorption and to comply with drug-likeness parameters, such as Lipinski's rule. The compounds (I - XI) displayed comparable binding interactions with the reference ligand (SAHA), particularly Zn²⁺ coordination. Compounds I, II, III, V, VII (no substitution, ortho OH, para OH, para NO₂, and para-Cl substitutions) showed higher binding affinities in kcal/mol with the active site of HDAC2 (-10.330, -10.852, -10.508, -10.282, and -9.811) compared with SAHA (-9.609).

Conclusion: Compounds I - XI showed favorable pharmacokinetic properties, characterized by high predicted gastrointestinal absorption and compliance with Lipinski's drug-likeness criteria, suggesting potential suitability as active drug candidates.

Keywords: Chalcone, HDAC2 inhibitors, *In silico* profiling, Molecular Docking, Schiff Base

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INTRODUCTION

Reversible acetylation and deacetylation of histone proteins are post-translational pathways controlling epigenetic regulation processes such as cell differentiation, genomic stability preservation, and gene expression. This is achieved by restructuring chromatin and regulating access of transcriptional machinery to

DNA [1]. This process consists of two opposing steps. The forward step involves the acetylation of ε-amino group of lysine residues catalyzed by histone acetyltransferase enzymes (HATs), leading to chromatin relaxation and facilitated gene expression. The backward step involves ε-amino group deacetylation of lysine residues catalyzed by histone deacetylase (HDAC)

enzymes, leading to chromatin compaction and gene silencing [2].

Several diseases such as cancer, neurodegenerative disorders and numerous inflammatory conditions have been associated with HDAC protein activity aberration and deregulation. As a result, HDAC proteins have become therapeutic targets in drug discovery and development [3]. To date, there are 18 discovered isoforms of HDAC enzymes. Among the classical HDAC isoforms, histone deacetylase 2 (HDAC2) is regarded as one of the most biologically significant and clinically relevant. It belongs to class I HDAC family and is predominantly nuclear, where it forms complexes with transcriptional repressors to regulate cell growth, apoptosis, and DNA repair pathways. It is also a zinc-dependent metalloprotein in which a zinc ion plays a critical role in catalytic activity [4]. Overexpression or hyperactivation of HDAC2 has been implicated in the development and progression of several malignancies, including colorectal, breast, gastric, prostate, and lung cancers, where it is frequently associated with poor clinical outcomes, increased tumour aggressiveness, and treatment resistance. Furthermore, HDAC2 contributes to neuronal dysfunction in disorders such as Alzheimer's disease by suppressing genes involved in synaptic plasticity and memory formation. Therefore, inhibition of HDAC2 is considered a compelling therapeutic approach to restore regular gene expression and reestablish normal cellular function [5].

Several HDAC inhibitors have been approved by FDA, such as vorinostat (also known as SAHA), panobinostat, and belinostat. However, numerous significant drawbacks were reported with limited clinical utility, such as undesired side effects resulting from off-target toxicity and lack of isoform selectivity, including cardiotoxicity, thrombocytopenia, gastrointestinal (GI) disturbances, resistance, and poor pharmacokinetic characteristics leading to administration of high therapeutic doses. Also, development of selective HDAC isoform inhibitors was challenging due to structural similarity within the catalytic zinc-binding pocket among HDAC isoforms [6]. As a result, the need for designing and developing new HDAC inhibitors with novel molecular scaffolds has become necessary to achieve improved selectivity, reduced toxicity, and enhanced drug-likeness profiles, especially for HDAC2 [7].

Chalcones are $\alpha\beta$ -unsaturated ketones with various biological effects such as anticancer, antimicrobial, anti-inflammatory, antidiabetic, and

antioxidant activities. They have numerous structural characteristics such as the ability to take part in several intermolecular interactions, including hydrogen bonding and π - π stacking, contributing to their pharmacological diversity [8]. Schiff bases, characterized by the presence of azomethine ($-\text{C}=\text{N}-$) linkage, have attracted considerable attention due to their broad spectrum of biological activities, including anticancer, antimicrobial, and antiviral properties. The pharmacological potential of azomethine is primarily associated with its ability to coordinate metal ions, modulate enzyme active sites, and interact with multiple biological targets through diverse non-covalent interactions [9,10]. As a result, chalcones and Schiff bases have attracted significant attention as privileged pharmacophores in medicinal chemistry. Therefore, designing a new chalcone-Schiff base molecular hybrid has been considered a promising approach to produce multitarget compounds with enhanced binding affinity and optimized pharmacokinetic characteristics, with potentially synergistic pharmacological activities. Formation of a single molecular pharmacophore by integrating chalcone and Schiff base moieties has been recently investigated in anticancer research, including developing several derivatives with the ability to disrupt tubulin polymerization, modulate epigenetic pathways, induce apoptosis, and generate reactive oxygen species [11].

Chalcone-Schiff base hybrids represent a valuable chemical framework for designing next-generation HDAC inhibitors due to their structural flexibility and capacity to engage in crucial interactions within enzyme active sites. However, the potential of these hybrids as selective HDAC2 inhibitors remains underexplored, and a systematic computational investigation may provide essential insights before proceeding to experimental synthesis and biological evaluation. The process of identification and optimization of novel therapeutic candidates has significantly accelerated with recent advances in computational chemistry techniques, especially *in silico* drug design. Currently, researchers are able to predict molecular interactions, biological activities, pharmacokinetic properties and evaluate drug-likeness characteristics with high proficiency and minimal experimental cost [12].

Molecular docking (MD) is the most popular computational method for predicting how a small molecule will orient and bind to a protein's active site. It provides insight into the strength and nature of ligand-protein interactions, including but not limited to hydrogen bonding, hydrophobic contacts, π - π stacking, and coordination to

metal ions [13]. Regarding HDAC2 enzyme, ligand coordination with a zinc ion is crucial for inhibitory activity, which is identified by MD [14]. Root mean square deviation (RMSD) is a metric used in MD to measure the average distance between the atoms of a docked ligand's pose and a reference pose, typically the experimentally determined native pose. A low RMSD value (often $< 2 \text{ \AA}$) indicates that the docked pose is similar to the reference pose, suggesting a good docking prediction. These computational tools, combined with *in silico* pharmacokinetic profiling such as ADME (absorption, distribution, metabolism, and excretion) prediction, provide a comprehensive understanding of the therapeutic potential of compounds before laboratory experimentation [15]. This study investigated a series of novel chalcone-Schiff base derivatives (compounds I - XI) which were designed and evaluated as potential HDAC2 inhibitors using an integrated *in silico* workflow. Molecular docking studies and RMSD calculations were conducted to predict binding affinity, key active-site interactions, zinc-ion coordination patterns, and to assess the stability of promising complexes. Additionally, ADME predictions were used to evaluate drug-likeness and identify molecules with favorable pharmacokinetic characteristics. These approaches identified promising chalcone-Schiff base candidates with strong inhibitory potential against HDAC2, laying the groundwork for future synthesis and pharmacological evaluation, such as *in vitro* and *in vivo* anticancer screening.

EXPERIMENTAL

In silico profiling study

This study was conducted to evaluate the predicted pharmacokinetic characteristics of the investigated compounds using SwissADME website (<https://www.swissadme.ch>). Marvin JS tool was utilized to sketch the chemical structure of the designed compounds (I - XI), which were converted to SMILES. The generated SMILES were submitted to the SwissADME server for analysis.

Molecular docking study and RMSD calculation

Protein Data Bank (PDB) website (<https://www.rcsb.org>) was used to retrieve the crystallographic structure of HDAC2 enzyme (PDB ID: 4LXZ). The protein file was uploaded to the MD software (Schrodinger Suite, 2025-1). The protein preparation module was activated to prepare the protein for docking by minimizing its 3D energy and removing unnecessary chains and residues. The substructure panel was chosen and workspace entry was loaded. All the ligands of chain A were deleted except for the reference ligand, SAHA, and the zinc residue. All the remaining options in this panel, along with the preparation workflow and diagnostics panels, were maintained in default settings. Receptor grid generation module was utilized to specify the space in which the ligand would be docked within the active site of HDAC2 protein. The SAHA 3D structure was selected and specified as custom site center. Chemical structures of compounds I - XI along with the chemical structure of the reference ligand (SAHA) were sketched using 2D sketcher panel and saved in the workspace. LigPrep module was used to prepare the 3D conformation of investigated ligands for docking. 'Add metal binding states' was activated and all remaining options were kept in the default state. Molecular docking was conducted using the Glide module. Extra precision (XP) mode was selected and all remaining options were maintained in the default state. Finally, docking scores (in kcal/mol) and docking visualization images were recorded. The RMSD was calculated utilizing superposition module. The redocked reference ligand, SAHA, was pinned and the Superimpose Structures panel was selected and RMSD scores for compounds I - XI were recorded [16].

RESULTS

In silico profiling study

This study was conducted using SwissADME wizard to evaluate the pharmacokinetic properties of compounds I - XI (Figures 1 to 11).

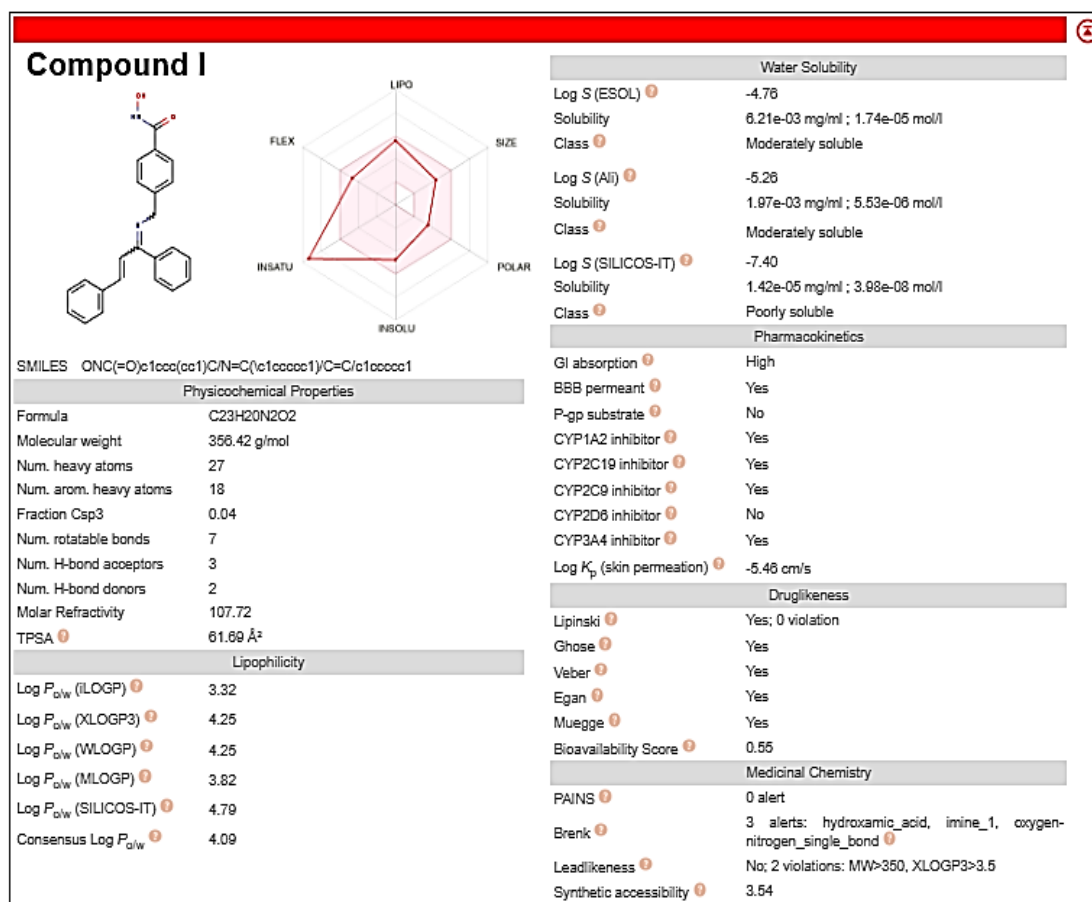


Figure 1: Predicted pharmacokinetic and drug-likeness characteristics of compound I



Figure 2: Predicted pharmacokinetic and drug-likeness characteristics of compound II

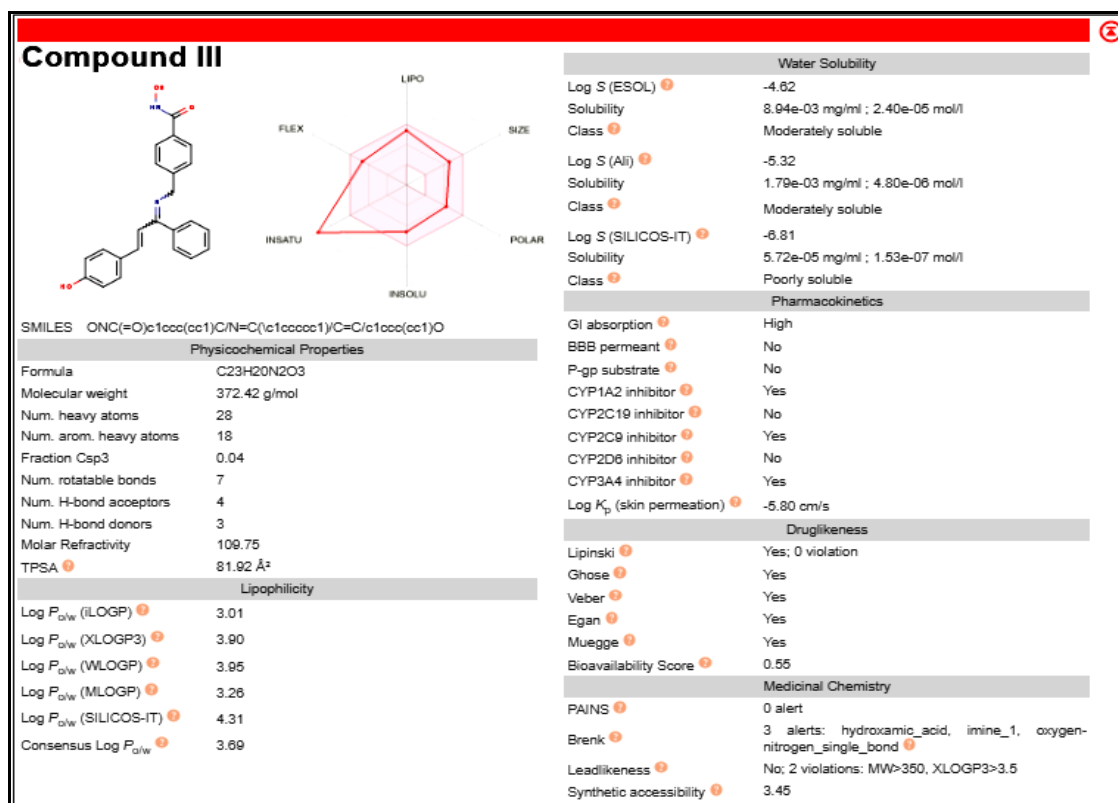


Figure 3: Predicted pharmacokinetic and drug-likeness characteristics of compound III

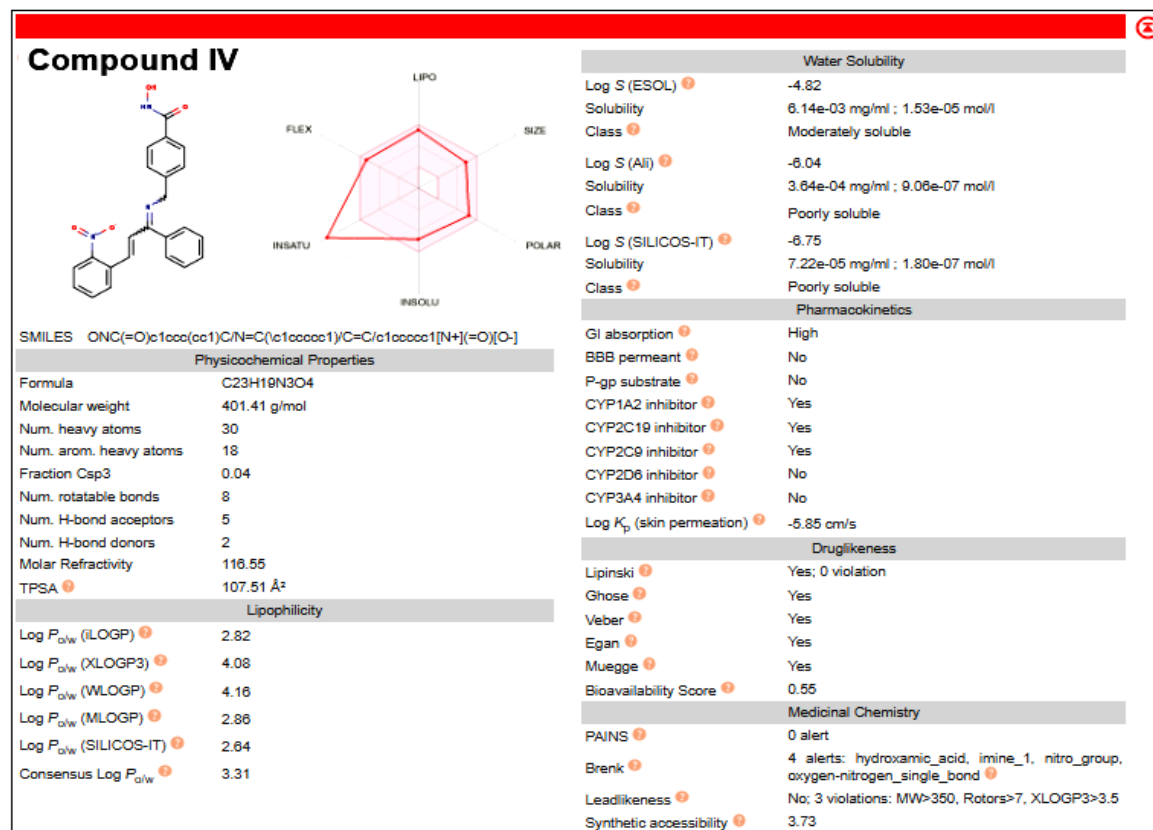


Figure 4: Predicted pharmacokinetic and drug-likeness characteristics of compound IV

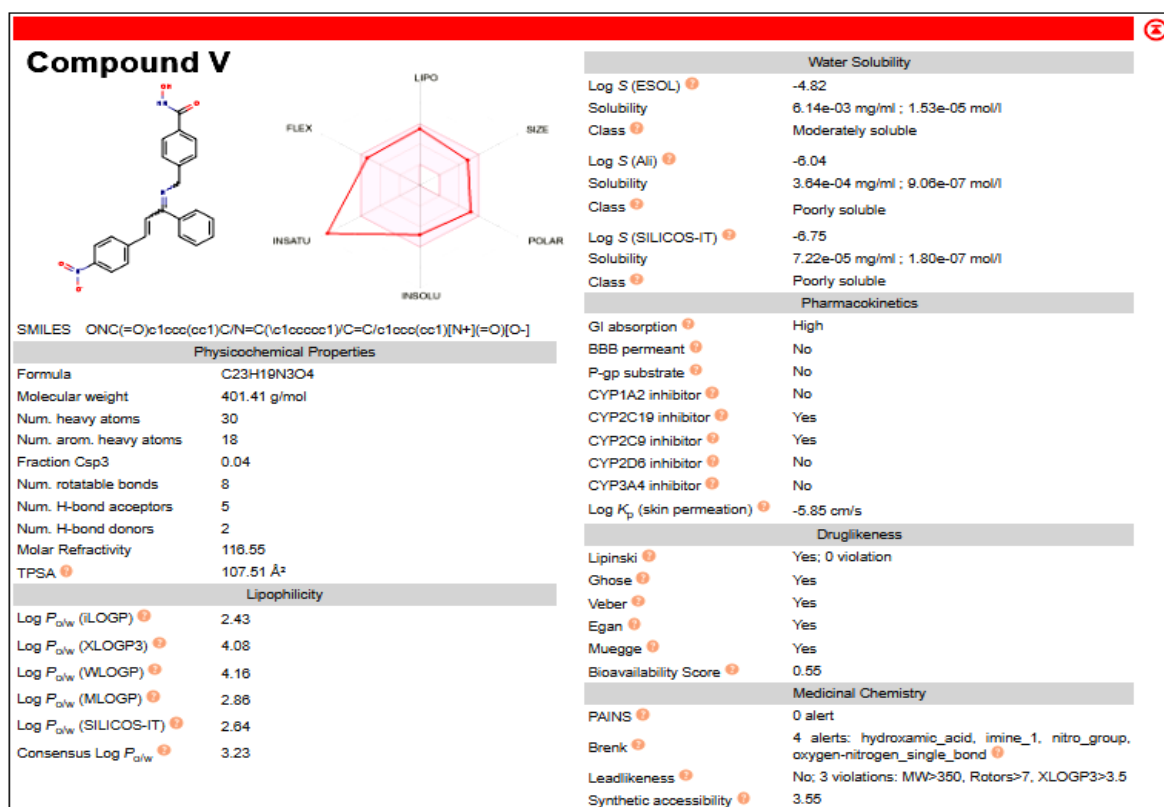


Figure 5: Predicted pharmacokinetic and drug-likeness characteristics of compound V

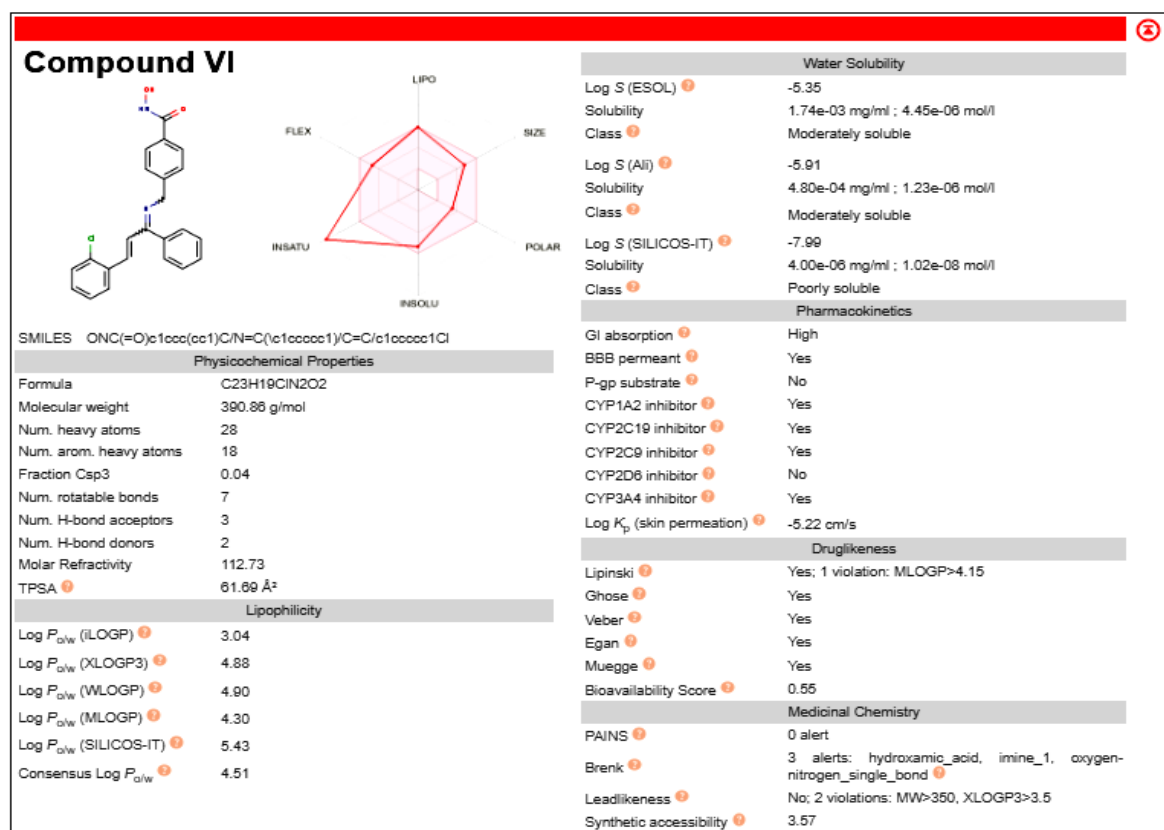


Figure 6: Predicted pharmacokinetic and drug-likeness characteristics of compound VI

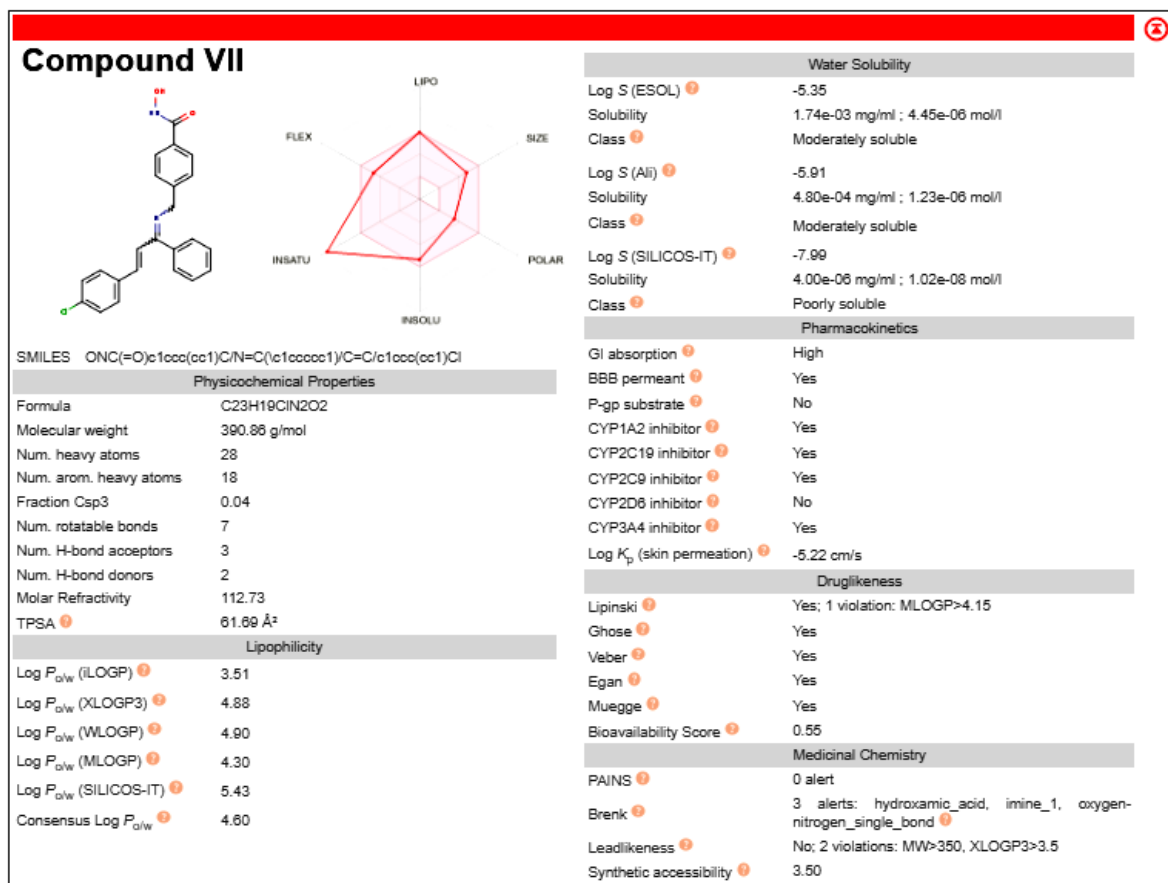


Figure 7: Predicted pharmacokinetic and drug-likeness characteristics of compound VII

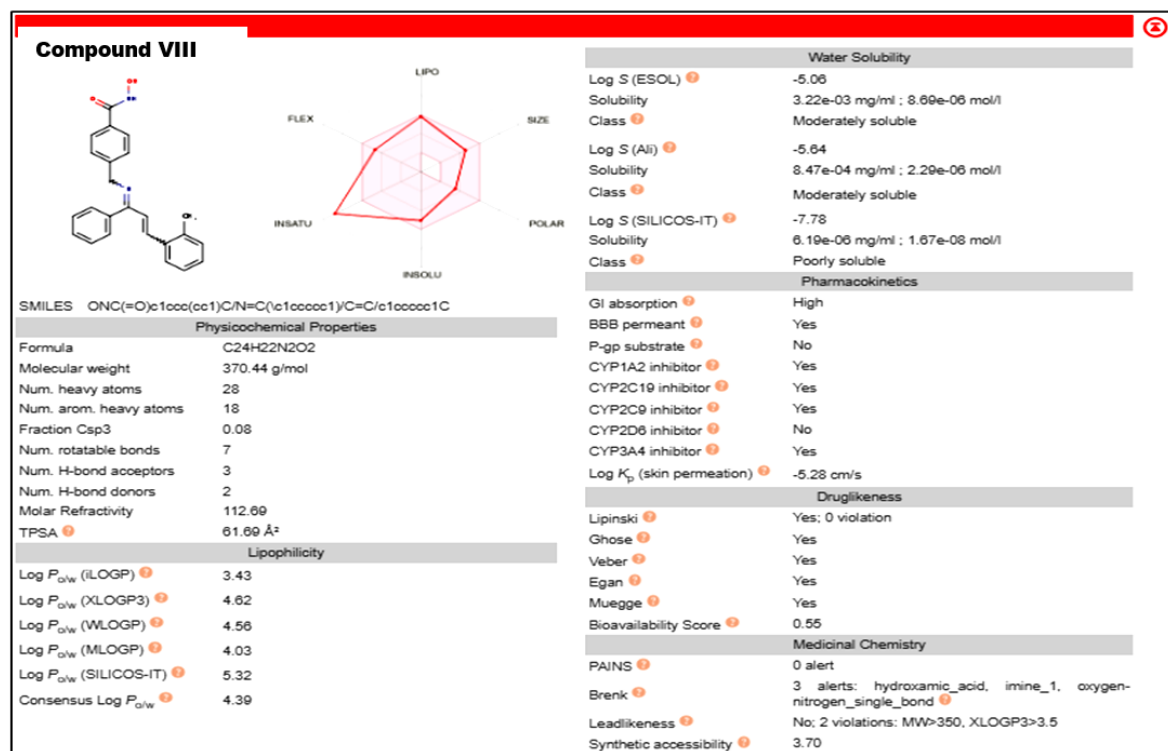


Figure 8: Predicted pharmacokinetic and drug-likeness characteristics of compound VIII

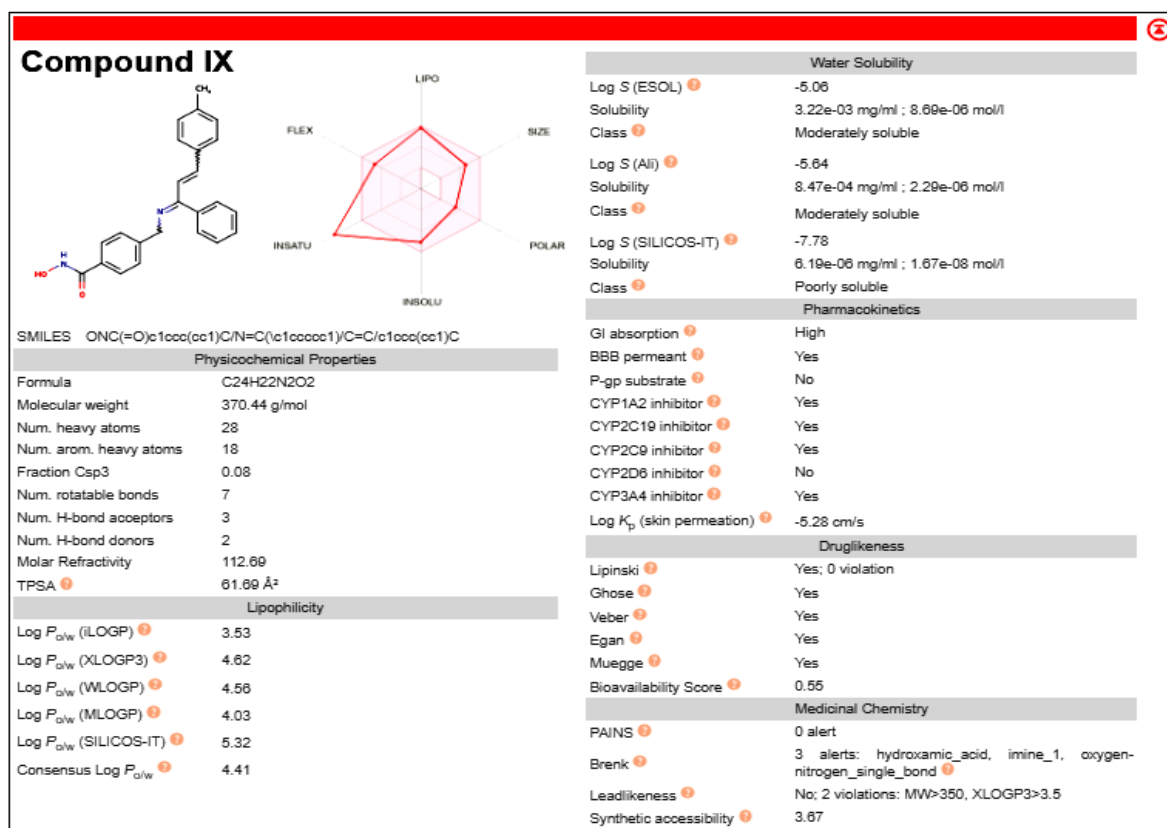


Figure 9: Predicted pharmacokinetic and drug-likeness characteristics of compound IX

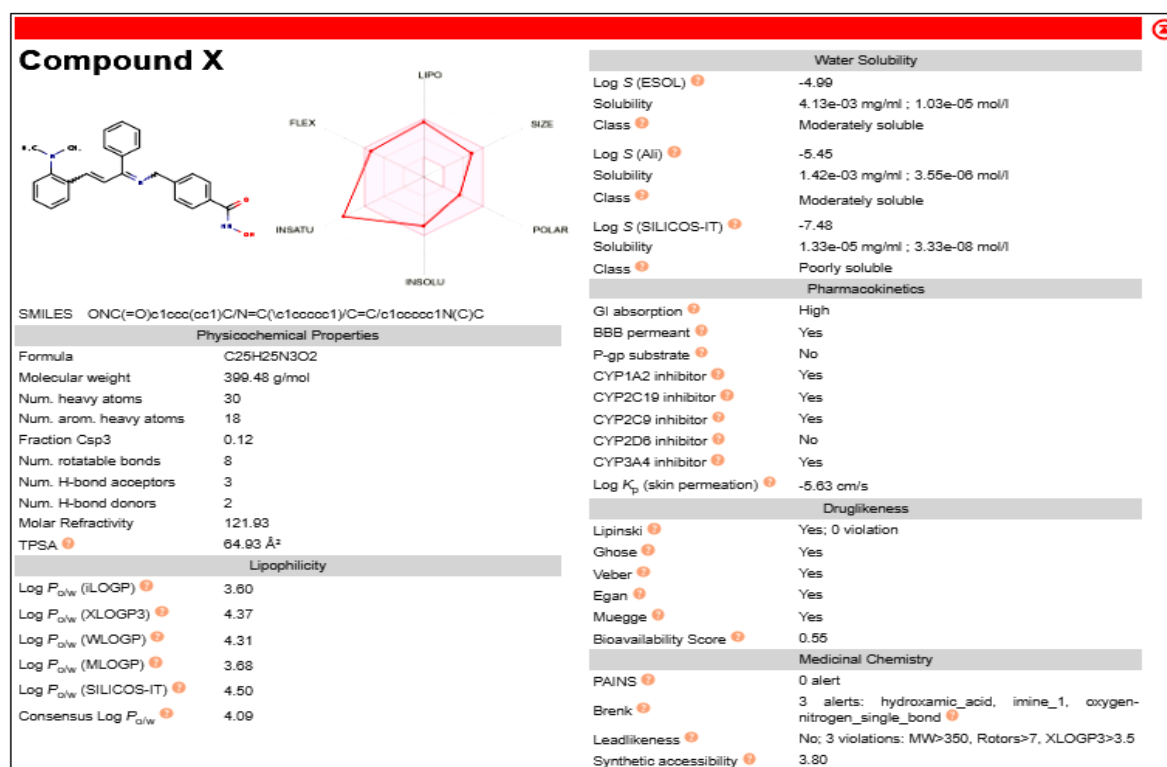


Figure 10: Predicted pharmacokinetic and drug-likeness characteristics of compound X



Figure 11: Predicted pharmacokinetic and drug-likeness characteristics of compound XI

Molecular docking and RMSD analysis

This was achieved using Schrodinger software (2025-1, Glide module) to calculate binding affinity and visualize interaction poses of compounds (I - XI) with amino acid residues in the catalytic pocket of HDAC2 enzyme (PDB ID: 4LXZ). The XP mode (extra precision) was used instead of SP (standard) mode, which increased the credibility of docking scores (Table 1).

Synthetic route

A synthetic route has been proposed for the investigated compounds (Figure 13, Table 2).

DISCUSSION

The study showed that all the investigated compounds (I - XI) have high predicted GI absorption. Also, they were predicted to permeate the blood-brain barrier (BBB), except for compounds II-V. This may be due to polar aromatic substituents (ortho OH, para OH, ortho NO₂, and para NO₂ for compounds II, III, IV, and V). The aforementioned moieties reduced the lipophilicity of compounds II-V, which is a critical requirement for passive diffusion through BBB [17]. This may suggest potential applicability in targeting brain tumors. Furthermore, the predicted results showed that all the investigated compounds were not P-gp substrates, which may lead to lower incidence of multidrug-resistant cancer cells and brain efflux [18,19].

Table 1: Docking scores, binding affinities of compounds I - XI and SAHA with amino acid residues in the catalytic pocket of HDAC2 enzyme and RMSD results

Compound symbol	Docking scores (Kcal/mol)	Amino acid residues in the catalytic pocket of HDAC2	RMSD
I	-10.330	Zn401, Tyr308, Phe155, His146, His145	0.0228
II	-10.852	Zn401, Tyr308, Phe210, Phe155, His145, Asp104	0.0199
III	-10.508	Zn401, Tyr308, Phe155, His146, His145, Glu103	0.0179
IV	-9.141	Zn401, Phe155, Gly154	1.3500
V	-10.282	Zn401, Tyr308, Phe155, His146, His145, Asp104	1.6300
VI	-8.746	Zn401, Phe210, His183, Asp104	0.0130
VII	-9.811	Zn401, His183, Phe155, Asp104	0.0087
VIII	-9.154	Zn401, Tyr308, Phe210, Phe155, Gly154	0.0069
IX	-9.141	Zn401, Phe155, His145	0.0037
X	-9.108	Zn401, Tyr308, Phe210, Phe155, His145	2.0411
XI	-9.040	Zn401, Tyr308, Phe155, Gly154, His145	2.0185
SAHA (reference ligand)	-9.609	Zn401, Tyr308, His145, Asp104	

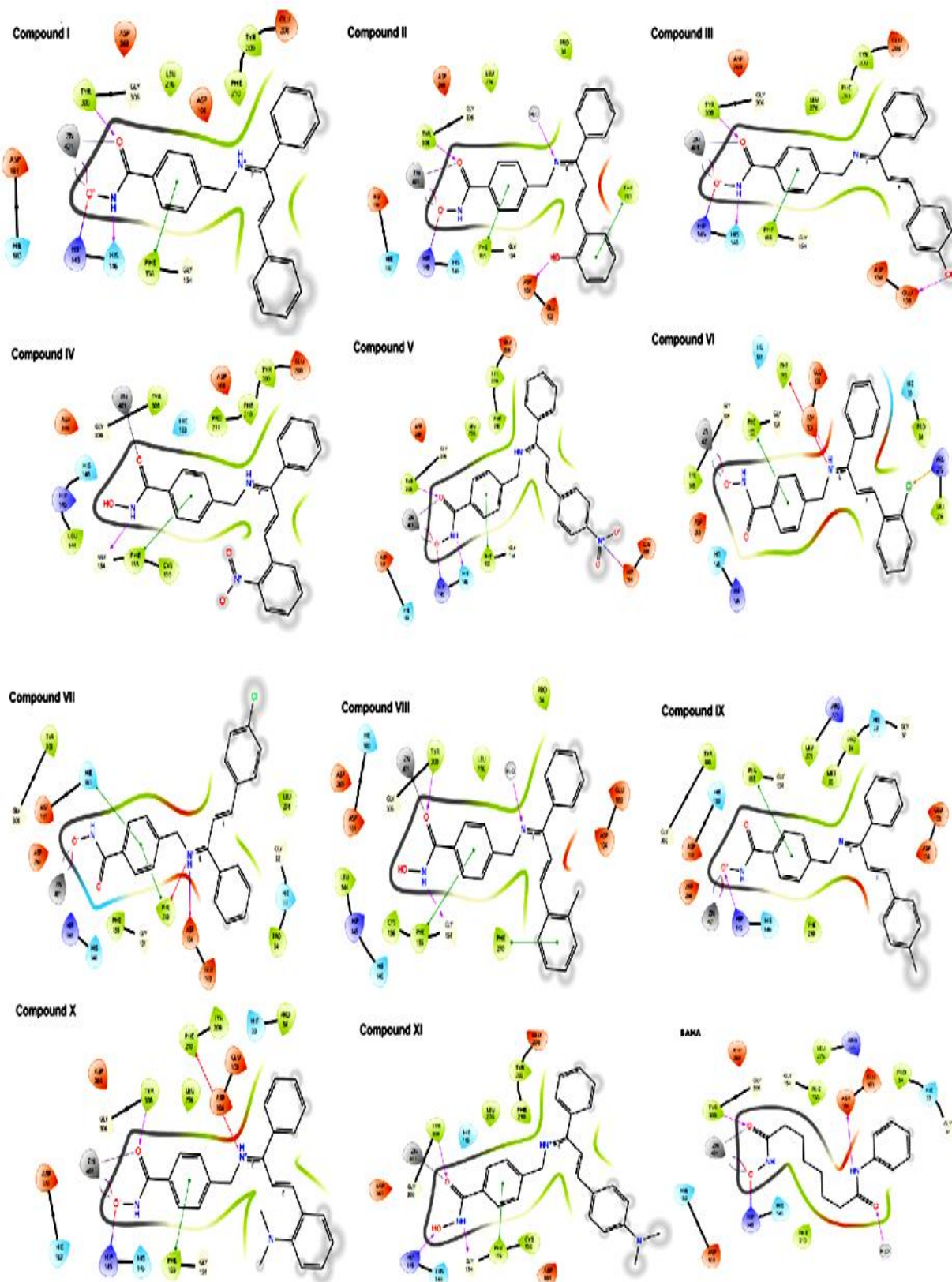
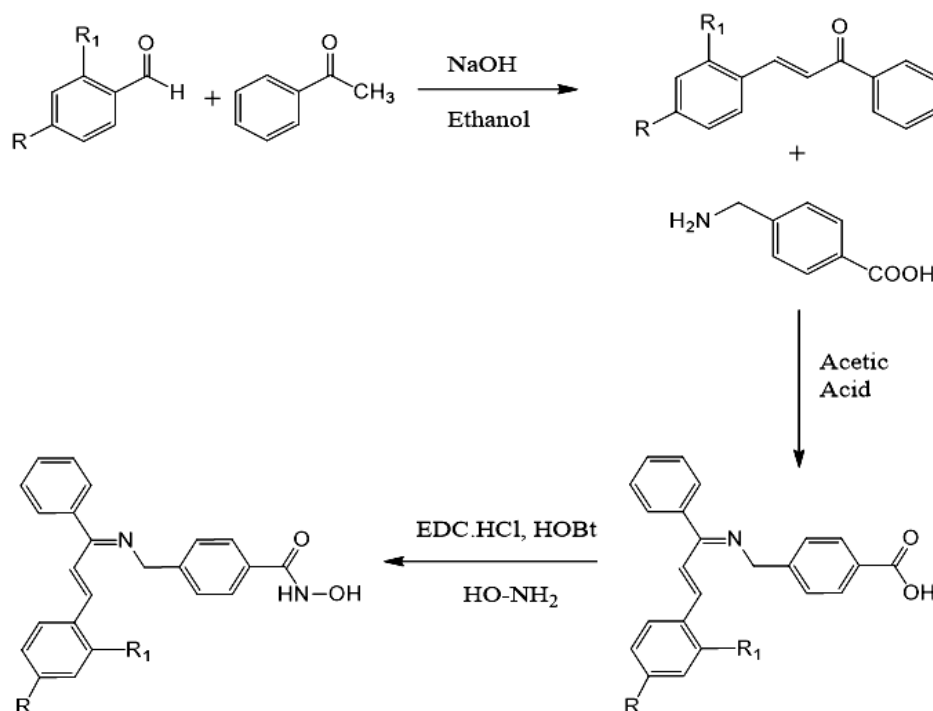


Figure 12: Visualization of compounds I - XI and SAHA binding with the amino acid residues in the active site of HDAC2 enzyme

**Table 2:** List of constituents per compound

Compound	R ₁	R
I	H	H
II	OH	H
III	H	OH
IV	NO ₂	H
V	H	NO ₂
VI	Cl	H
VII	H	Cl
VIII	CH ₃	H
IX	H	CH ₃
X	N(CH ₃) ₂	H
XI	H	N(CH ₃) ₂

Figure 13: Suggested scheme for synthesizing compounds I – XI. **Key:** EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide), HCl (hydrochloric acid), HOBT (1-Hydroxybenzotriazole)

With respect to drug-likeness, all the investigated compounds displayed complete compliance with no rule violations. On the other hand, violations of medicinal chemistry criteria are due to the effect of hydroxamate and imine moieties, molecular weight, XLOGP3, and number of rotors.

However, these violations may not necessarily preclude oral bioavailability because various orally active FDA-approved medications showed comparable measurements, such as vorinostat and cefixime. Finally, the synthetic accessibility was predicted to be 3.45 - 3.8, which is considered an acceptable range for the synthesis of chemical compounds [20]. These findings indicated that compounds I - XI may have potential oral therapeutic effects. All the investigated compounds (I - XI) displayed comparable binding interactions with the

reference ligand (SAHA), particularly Zn²⁺ coordination, which is critical for inhibitory activity of HDAC2. Compounds I, II, III, V, VII (no substitution, ortho OH, para OH, para NO₂, and para-Cl substitutions) showed more favorable docking scores in kcal/mol with the active site of HDAC2 (-10.330, -10.852, -10.508, -10.282, and -9.811) compared to SAHA (-9.609). These findings suggested that the moieties played a crucial role in keeping the orientation of the compounds at the right position for binding with the active site, or participated in the binding (capping process) with surrounding amino acid residues; ortho OH with Asp104, para OH with Glu103 and para NO₂ with Asp104 for compounds II, III, and V, respectively, which strengthened binding interaction and improved docking scores.

It is important to mention that the hydroxyl group is electron-donating, while the nitro group is electron-withdrawing, which may have significantly influenced electronic distribution of the aromatic ring and consequently affected π - π stacking interactions with aromatic amino acid residues such as Phe155. The results showed that the investigated compounds had RMSD values less than 2 Å (except compounds X and XI, with RMSD values slightly > 2 Å). These compounds were predicted to have comparable docking poses with the reference ligand (SAHA). The docking results further suggested that compounds I - XI may show significant *in vitro* and *in vivo* HDAC2 inhibitory activity. The high RMSD scores for compounds X and XI may be due to the effect of the positions of aromatic rings (perpendicular orientation in compound X and steric effect of the N, N-dimethylamino group in compound XI), which affected alignment of the compound in the protein active site. Furthermore, hybridizing chalcone with Schiff base may produce multi-target synergistic effect and further optimize future *in vitro* and *in vivo* results. Current results were promising and encouraging for future synthesis of compounds I-IX and conducting *in vitro* and *in vivo* pharmacological evaluation.

CONCLUSION

Compounds I - XI showed favourable pharmacokinetic properties, characterized by high predicted gastrointestinal absorption and compliance with Lipinski's drug-likeness criteria, favourable docking interactions with HDAC2, particularly compounds II, III, V, and VII, suggesting potential suitability as active drug candidates. Further studies should explore the proposed synthetic pathways and conduct *in vitro* and *in vivo* pharmacological evaluation.

DECLARATIONS

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Ethical approval

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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