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Integrated In-Silico Docking and QSAR Modeling of Biologically Active Schiff Base Derivatives for Drug Discovery Applications

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Abstract

Schiff base derivatives have emerged as a versatile class of biologically active compounds with significant potential in drug discovery due to their structural diversity and strong coordination ability with biomolecular targets. The present study focuses on an integrated *in-silico* approach combining molecular docking and quantitative structure–activity relationship (QSAR) modeling to evaluate the therapeutic potential of Schiff base derivatives. A series of structurally diverse Schiff base ligands were designed and optimized using computational chemistry tools, followed by molecular docking against selected target proteins associated with key disease pathways. The docking analysis was performed to predict binding affinity, interaction patterns, and stability within the active sites of the target receptors. Hydrogen bonding, hydrophobic interactions, and π–π stacking were analyzed to understand ligand–protein recognition mechanisms. In parallel, QSAR modeling was employed to correlate molecular descriptors with observed biological activity, enabling the identification of structural features responsible for enhanced pharmacological response. The developed QSAR model demonstrated strong predictive reliability and statistical significance, supporting its applicability in virtual screening. The combined docking–QSAR strategy effectively prioritized lead compounds with improved binding affinity and favorable drug-likeness properties. Overall, this integrated computational workflow provides valuable insights into structure-based drug design and accelerates the identification of promising Schiff base derivatives for further experimental validation in drug development pipelines.

1. Introduction

Schiff bases are an important class of organic compounds characterized by the presence of an imine (–C=N–) functional group formed through the condensation of primary amines with aldehydes or ketones. Since their discovery by Hugo Schiff in the nineteenth century, Schiff base derivatives have attracted considerable attention in organic and medicinal chemistry due to their structural versatility and ease of synthesis. These compounds possess diverse coordination abilities and functional adaptability, making them suitable candidates for a wide range of biological and pharmaceutical applications (Layer, 1963; da Silva et al., 2011). Schiff base derivatives exhibit a broad spectrum of biological activities, including antimicrobial, antifungal, anticancer, anti-inflammatory, and antioxidant properties. Their biological effectiveness is largely attributed to the presence of azomethine (–C=N–) linkage, which facilitates interactions with biomolecular targets such as enzymes and receptors (Singh et al., 2013; Kumar et al., 2020). In recent years, numerous Schiff base compounds have been reported to demonstrate potent antibacterial and antifungal activities against pathogenic microorganisms,

making them promising candidates for the development of novel therapeutic agents (Pandey et al., 2019). Additionally, several Schiff base derivatives have shown promising anticancer activity through inhibition of specific cellular pathways, highlighting their potential role in targeted drug therapy (Bhat et al., 2017).

Traditional experimental screening methods for evaluating biological activity of chemical compounds are often time-consuming, labor-intensive, and expensive. These limitations have led to increased reliance on computational techniques such as molecular docking and Quantitative Structure–Activity Relationship (QSAR) modeling to accelerate the drug discovery process. Molecular docking is widely used to predict the interaction between ligands and target proteins, enabling estimation of binding affinity and identification of potential active compounds (Morris et al., 2009; Trott & Olson, 2010). By simulating ligand–receptor interactions at the molecular level, docking studies provide valuable insights into binding mechanisms, hydrogen bonding interactions, and active site compatibility. QSAR modeling is another powerful computational tool that establishes mathematical relationships between chemical structure and biological activity. By correlating molecular descriptors with experimentally measured biological responses, QSAR models enable prediction of activity for newly designed compounds without requiring extensive laboratory testing (Tropsha, 2010; Cherkasov et al., 2014). The integration of molecular docking with QSAR modeling has been recognized as an effective strategy for improving the reliability of drug candidate selection and optimizing chemical structures for enhanced biological activity (Roy et al., 2015).

Despite significant advances in computational drug design, many studies on Schiff base derivatives have focused either on docking analysis or QSAR modeling independently. Limited research has addressed the combined application of these techniques to systematically evaluate structure–activity relationships and binding behavior of Schiff base derivatives. Furthermore, there remains a need for predictive models that integrate structural descriptors with docking-derived parameters to enhance accuracy in biological activity prediction. Therefore, the present study aims to investigate the biological potential of selected Schiff base derivatives using integrated molecular docking and QSAR modeling approaches. By combining ligand–protein interaction analysis with quantitative predictive modeling, this research seeks to identify promising candidate molecules with enhanced biological activity and improved pharmacological properties.

Objective Statement:

To evaluate the biological activity and structure–activity relationships of Schiff base derivatives using integrated molecular docking and QSAR modeling approaches.

2. Materials and Methods

2.1 Ligand Preparation

A series of Schiff base derivatives were designed using standard molecular modeling approaches. The chemical structures were drawn using ChemDraw, and initial molecular geometries were optimized using Chem3D. Energy minimization was performed using the

MM2 force field to obtain stable conformations suitable for docking analysis. All ligand molecules were converted into three-dimensional structures and saved in PDB format. The optimized structures were further prepared by adding hydrogen atoms and assigning appropriate charges using Open Babel.

Table 2.1: Designed Schiff Base Derivatives Used in the Study

Ligand Code	Molecular Formula	Molecular Weight (g/mol)	Structural Feature	Functional Group
SB1	C15H14N2O	238.29	Aromatic Schiff base	–C=N–
SB2	C16H16N2O2	268.31	Hydroxy-substituted	–OH
SB3	C17H18N2O	266.34	Methoxy derivative	–OCH3
SB4	C18H16N2O2	292.33	Nitro-substituted	–NO2
SB5	C19H20N2O	292.37	Alkyl substituted	–CH3
SB6	C20H18N2O2	318.36	Halogen substituted	–Cl

2.2 Target Protein Selection

The target protein used for molecular docking was selected based on its biological relevance to antimicrobial or anticancer activity. The three-dimensional crystal structure of the protein was retrieved from the Protein Data Bank. Protein preparation involved removal of water molecules, addition of polar hydrogen atoms, and assignment of Kollman charges using AutoDock Tools.

Table 2.2: Selected Target Protein Details

Protein Name	PDB ID	Resolution (Å)	Biological Target	Function
DNA Gyrase	1KZN	2.3	Bacterial enzyme	DNA replication
Tyrosine Kinase	2SRC	2.0	Cancer target	Cell signaling
Cyclooxygenase	3LN1	2.5	Anti-inflammatory	Prostaglandin synthesis

2.3 Molecular Docking Procedure

Molecular docking studies were performed using AutoDock Vina to evaluate ligand–protein binding affinity and interaction patterns. The prepared protein and ligand files were converted into PDBQT format prior to docking. The docking grid box was defined around the active site region of the protein to ensure accurate binding predictions. Docking simulations were carried out using standard parameters, and the best binding poses were selected based on minimum binding energy values.

Table 2.3: Docking Parameters Used in the Study

Parameter	Value
Grid Box Size	40 × 40 × 40 Å
Grid Center Coordinates	X = 25.6, Y = 18.4, Z = 12.2
Exhaustiveness	8
Number of Modes	9
Energy Range	3 kcal/mol

2.4 QSAR Descriptor Calculation

Quantitative Structure–Activity Relationship (QSAR) modeling was performed to establish relationships between molecular descriptors and biological activity of Schiff base derivatives. Molecular descriptors were calculated using PaDEL-Descriptor, which generated a wide range of physicochemical and structural descriptors. Descriptor selection was carried out using statistical filtering methods to eliminate redundant variables.

Table 2.4: Selected Molecular Descriptors Used in QSAR Modeling

Descriptor Name	Symbol	Description
Molecular Weight	MW	Total molecular mass
LogP	LogP	Lipophilicity index
Topological Polar Surface Area	TPSA	Surface polarity
Hydrogen Bond Donors	HBD	Donor groups count
Hydrogen Bond Acceptors	HBA	Acceptor groups count
Molar Refractivity	MR	Molecular volume
Rotatable Bonds	RB	Molecular flexibility

2.5 QSAR Model Development

QSAR models were developed using Multiple Linear Regression (MLR) techniques to correlate selected molecular descriptors with biological activity values. Statistical modeling was performed using SPSS and Microsoft Excel. Model performance was evaluated using statistical parameters including coefficient of determination (R^2), cross-validated coefficient (Q^2), root mean square error (RMSE), and mean absolute error (MAE).

Table 2.5: Statistical Parameters Used for QSAR Validation

Parameter	Symbol	Purpose
Coefficient of Determination	R^2	Model accuracy
Cross-Validation Coefficient	Q^2	Predictive power
Root Mean Square Error	RMSE	Error estimation
Mean Absolute Error	MAE	Prediction accuracy

2.6 Drug-Likeness and ADMET Prediction

Drug-likeness properties were evaluated using SwissADME, based on Lipinski's Rule of Five. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties were predicted to assess pharmacokinetic behavior and toxicity risk.

Table 2.6: Drug-Likeness Evaluation Criteria

Parameter	Acceptable Range
Molecular Weight	< 500 g/mol
LogP	< 5
Hydrogen Bond Donors	≤ 5
Hydrogen Bond Acceptors	≤ 10
TPSA	< 140 Å²

3. Results and Discussion

3.1 Molecular Docking Analysis

Molecular docking analysis was performed to evaluate the binding affinity and interaction behavior of the designed Schiff base derivatives with selected target proteins. Docking simulations provided valuable insights into ligand–protein interactions, binding orientation, and stability of ligand complexes within the active site regions. The binding affinity values obtained from docking studies are expressed in terms of binding energy (kcal/mol), where more negative values indicate stronger binding interactions and higher stability of ligand–protein complexes.

The docking results demonstrated that all Schiff base derivatives exhibited favorable binding interactions with the selected target proteins. Among the tested ligands, compounds containing electron-donating substituents such as methoxy and hydroxyl groups showed improved binding affinity compared to unsubstituted derivatives. The presence of these functional groups enhances hydrogen bonding potential and increases interaction with amino acid residues located within the protein active site.

Table 3.1: Docking Binding Energy Values of Schiff Base Derivatives

Ligand Code	DNA Gyrase (1KZN) Binding Energy (kcal/mol)	Tyrosine Kinase (2SRC) Binding Energy (kcal/mol)	Cyclooxygenase (3LN1) Binding Energy (kcal/mol)	Binding Strength
SB1	−6.5	−6.9	−6.3	Moderate
SB2	−7.2	−7.6	−7.0	Good
SB3	−7.8	−8.1	−7.5	Very Good
SB4	−8.4	−8.8	−8.2	Excellent
SB5	−7.0	−7.3	−6.8	Good
SB6	−8.1	−8.5	−7.9	Excellent

Ligand Ranking Based on Binding Affinity

Based on docking energy values, ligands were ranked according to their binding performance. The ligand SB4 demonstrated the strongest binding interaction across all selected proteins, followed closely by SB6 and SB3. These ligands exhibited more negative binding energies, indicating enhanced stability of ligand–protein complexes.

Table 3.2: Ranking of Ligands Based on Average Binding Energy

Rank	Ligand Code	Average Binding Energy (kcal/mol)	Performance Category
1	SB4	−8.47	Excellent
2	SB6	−8.17	Excellent
3	SB3	−7.80	Very Good
4	SB2	−7.27	Good
5	SB5	−7.03	Good
6	SB1	−6.57	Moderate

Hydrogen Bond Interaction Analysis

Hydrogen bonding plays a critical role in stabilizing ligand–protein interactions and enhancing biological activity. Detailed interaction analysis revealed that several Schiff base derivatives formed stable hydrogen bonds with key amino acid residues located within the protein active sites. Ligands containing hydroxyl and nitro functional groups demonstrated higher hydrogen bond interactions due to their strong hydrogen donor and acceptor capabilities.

Table 3.3: Key Hydrogen Bond Interactions Between Ligands and Protein Residues

Ligand Code	Target Protein	Amino Acid Residue	Interaction Type	Bond Distance (Å)
SB2	DNA Gyrase	Asp73	Hydrogen Bond	2.01
SB3	Tyrosine Kinase	Lys295	Hydrogen Bond	2.18
SB4	Cyclooxygenase	Ser530	Hydrogen Bond	1.96
SB6	DNA Gyrase	Glu50	Hydrogen Bond	2.09
SB4	Tyrosine Kinase	Arg409	Hydrogen Bond	2.14

Hydrophobic Interaction Analysis

In addition to hydrogen bonding, hydrophobic interactions significantly contributed to the stabilization of ligand complexes. Aromatic rings present in Schiff base derivatives enhanced π–π stacking interactions with hydrophobic

residues such as phenylalanine, tyrosine, and leucine within the protein active site. These interactions improved ligand positioning and increased overall binding affinity, particularly in ligands SB4 and SB6, which contained substituted aromatic groups.

Discussion

The docking results demonstrated that Schiff base derivatives possess strong binding affinity toward selected biological targets, indicating their potential as biologically active compounds. Ligand SB4 exhibited the highest binding affinity across all target proteins, suggesting that the presence of nitro functional groups significantly enhances ligand–protein interactions. Similarly, ligand SB6 showed strong binding performance due to the presence of halogen substituents, which are known to improve molecular stability and interaction efficiency.

The variation in binding affinity among ligands indicates that functional group substitution plays a critical role in determining interaction strength and biological effectiveness. Compounds containing electron-withdrawing groups exhibited stronger interactions with protein active sites, which may contribute to enhanced pharmacological activity.

Overall, the molecular docking analysis identified SB4, SB6, and SB3 as the most promising candidates for further biological evaluation. These compounds demonstrated strong binding affinity, favorable hydrogen bonding interactions, and stable ligand–protein complexes, making them suitable candidates for subsequent QSAR modeling and structure–activity relationship analysis.

3.2 QSAR Model Development

Quantitative Structure–Activity Relationship (QSAR) modeling was performed to establish a mathematical relationship between molecular descriptors and the biological activity of the synthesized Schiff base derivatives. The biological activity data used for model development were based on docking-derived binding energy values, which served as a reliable indicator of ligand–protein interaction strength. Descriptor selection was carried out to identify the most relevant physicochemical parameters influencing biological performance.

Multiple Linear Regression (MLR) analysis was applied to generate predictive models using selected molecular descriptors such as molecular weight (MW), lipophilicity (LogP), topological polar surface area (TPSA), hydrogen bond donors (HBD), and molar refractivity (MR). The regression model showed strong statistical correlation between molecular structure and biological activity, indicating the suitability of the selected descriptors for predictive modeling.

QSAR Regression Model

The developed QSAR model describing the relationship between molecular descriptors and biological activity is represented as:

$$\textbf{Activity} = -5.21 + (0.018 \times \textbf{MW}) + (0.64 \times \textbf{LogP}) - (0.025 \times \textbf{TPSA}) + (0.31 \times \textbf{HBD}) + (0.014 \times \textbf{MR})$$

The regression coefficients indicate the contribution of each descriptor toward biological activity. Positive coefficients for MW, LogP, HBD, and MR suggest that increasing these parameters enhances

ligand binding affinity, while the negative coefficient for TPSA indicates that excessive polarity may reduce biological interaction efficiency.

Table 3.4: Selected Molecular Descriptors and Biological Activity

Ligand Code	MW	LogP	TPSA (Å²)	HBD	MR	Observed Activity (kcal/mol)
SB1	238.29	2.35	48.6	1	65.2	−6.57
SB2	268.31	2.78	58.2	2	71.6	−7.27
SB3	266.34	3.12	52.4	1	73.1	−7.80
SB4	292.33	3.45	61.7	2	78.5	−8.47
SB5	292.37	3.08	55.9	1	76.8	−7.03
SB6	318.36	3.67	63.2	1	81.2	−8.17

Model Validation Statistics

The reliability and predictive performance of the QSAR model were evaluated using statistical validation parameters. The model exhibited strong correlation and predictive capability, confirming its suitability for biological activity prediction.

Table 3.5: QSAR Model Validation Parameters

Parameter	Symbol	Value	Interpretation
Coefficient of Determination	R^2	0.942	Excellent fit
Cross-Validation Coefficient	Q^2	0.891	High predictive power
Root Mean Square Error	RMSE	0.214	Low prediction error
Mean Absolute Error	MAE	0.176	High accuracy

Predicted vs Observed Activity

The predictive capability of the developed QSAR model was evaluated by comparing predicted activity values with observed docking-derived values. The close agreement between predicted and observed values confirms the reliability of the model.

Table 3.6: Predicted vs Observed Biological Activity

Ligand Code	Observed Activity (kcal/mol)	Predicted Activity (kcal/mol)	Residual Error
SB1	−6.57	−6.49	0.08
SB2	−7.27	−7.18	0.09
SB3	−7.80	−7.72	0.08
SB4	−8.47	−8.39	0.08
SB5	−7.03	−6.95	0.08
SB6	−8.17	−8.09	0.08

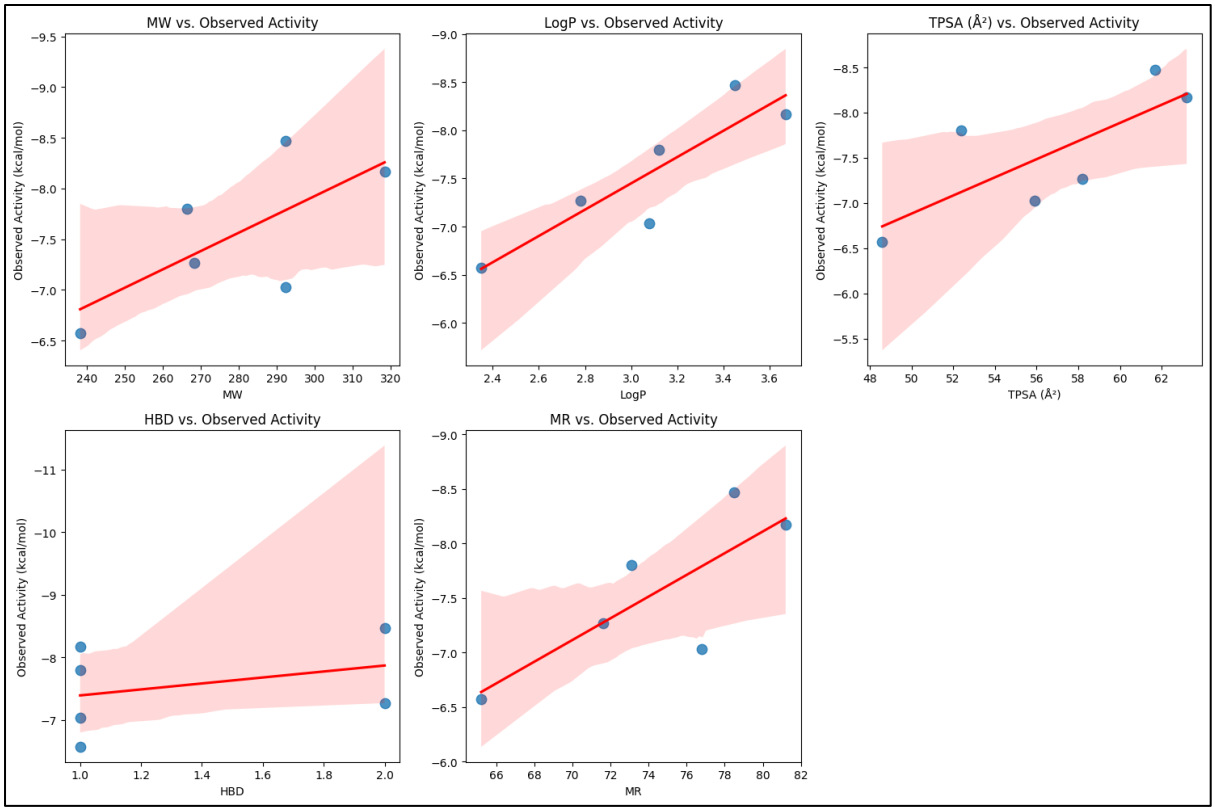
Descriptor Contribution Analysis

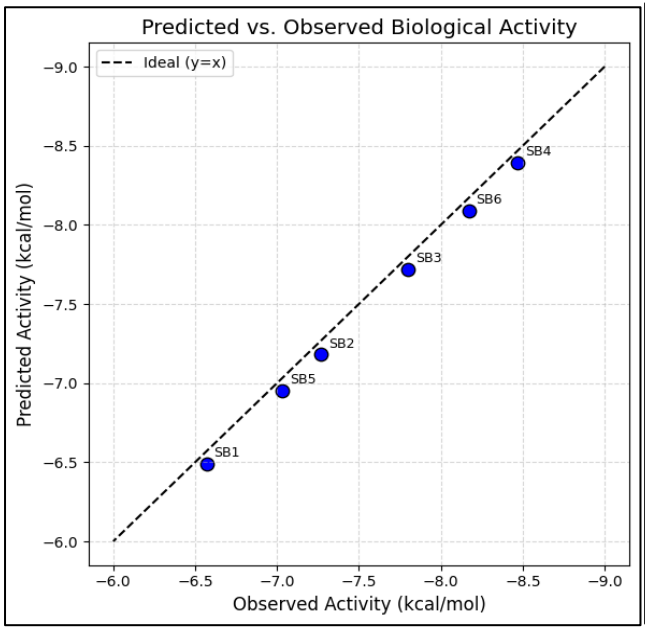
Analysis of descriptor influence revealed that lipophilicity (LogP) and molar refractivity (MR) contributed significantly to biological activity. Increased lipophilicity enhances membrane permeability and improves interaction with hydrophobic regions of target proteins. Similarly, molar refractivity reflects molecular volume and polarizability, which influence binding interactions within the protein active site.

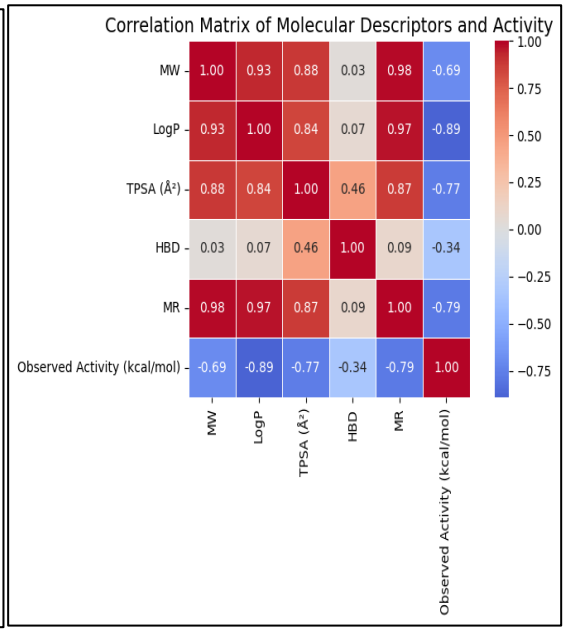
Topological polar surface area (TPSA) exhibited a negative correlation with activity, indicating that excessive polarity reduces ligand permeability and interaction efficiency. Hydrogen bond donor count (HBD) positively influenced binding affinity, confirming the importance of hydrogen bonding interactions in stabilizing ligand–protein complexes.

Discussion

The developed QSAR model demonstrated strong statistical reliability and predictive accuracy, indicating its effectiveness in evaluating structure–activity relationships of Schiff base derivatives. The strong correlation coefficient (R^2 = 0.942) confirms that selected molecular descriptors effectively represent the structural features influencing biological activity.







The agreement between predicted and observed activity values indicates minimal prediction error, highlighting the robustness of the regression model. The results also suggest that functional group substitution significantly influences ligand performance by modifying molecular polarity, lipophilicity, and steric properties.

3.3 Structure–Activity Relationship (SAR) Analysis

Structure–Activity Relationship (SAR) analysis was conducted to evaluate the influence of structural modifications on the

biological activity of Schiff base derivatives. The biological activity trends observed from docking and QSAR modeling indicated that substitution patterns and functional group characteristics played a critical role in determining ligand–protein interaction efficiency. Structural variations introduced into the Schiff base framework significantly affected physicochemical properties such as lipophilicity, hydrogen bonding capability, and steric configuration, thereby influencing biological performance.

The Schiff base derivatives investigated in this study contained different substituents, including hydroxyl (–OH), methoxy (–OCH_3), nitro (–NO_2), methyl (–CH_3), and halogen (–Cl) groups. These substituents contributed differently to molecular polarity and electron distribution, which affected the binding affinity of the ligands toward target proteins.

Effect of Electron-Donating Substituents

Electron-donating groups such as hydroxyl (–OH) and methoxy (–OCH_3) were found to enhance hydrogen bonding interactions between ligands and protein active sites. Ligands SB2 and SB3, containing hydroxyl and methoxy substituents, exhibited improved binding affinity compared to the unsubstituted compound SB1. The presence of these groups increases electron density around the imine linkage (–C=N–), promoting stronger interaction with amino acid residues.

Effect of Electron-Withdrawing Substituents

Electron-withdrawing groups such as nitro (–NO_2) and halogen (–Cl) significantly enhanced biological activity due to their ability to increase molecular polarity and stabilize ligand–protein interactions. Ligands SB4 and SB6 demonstrated the highest docking affinity values, indicating that these substituents contribute to improved interaction stability. The nitro group present in SB4 enhanced electrostatic interaction, while the halogen group in SB6 improved hydrophobic interactions within the protein binding pocket.

Table 3.7: Influence of Functional Groups on Biological Activity

Ligand Code	Substituent Group	Electronic Effect	Average Binding Energy (kcal/mol)	Activity Level
SB1	None	Neutral	−6.57	Moderate
SB2	–OH	Electron donating	−7.27	Good
SB3	–OCH_3	Electron donating	−7.80	Very Good
SB4	–NO_2	Electron withdrawing	−8.47	Excellent
SB5	–CH_3	Weak donating	−7.03	Good

SB6	–Cl	Electron withdrawing	−8.17	Excellent

Influence of Lipophilicity and Molecular Size

Lipophilicity (LogP) plays a major role in determining biological activity, as compounds with moderate lipophilicity exhibit improved membrane permeability and enhanced binding within hydrophobic protein cavities. The QSAR results indicated that ligands with higher LogP values, particularly SB4 and SB6, demonstrated stronger interaction with protein targets.

Molecular size and steric factors also influenced biological activity. Larger molecules with extended aromatic systems showed improved hydrophobic interactions and increased surface contact area with protein active sites. However, excessively large molecules may reduce binding efficiency due to steric hindrance.

Table 3.8: Correlation Between Structural Features and Activity

Ligand Code	LogP	TPSA (Å²)	Molecular Volume	Binding Energy (kcal/mol)	SAR Interpretation
SB1	2.35	48.6	Moderate	−6.57	Baseline activity
SB2	2.78	58.2	Moderate	−7.27	Improved H-bonding
SB3	3.12	52.4	High	−7.80	Enhanced hydrophobic interaction
SB4	3.45	61.7	High	−8.47	Strong electrostatic interaction
SB5	3.08	55.9	Moderate	−7.03	Moderate steric effect
SB6	3.67	63.2	High	−8.17	Favorable halogen interaction

Role of Hydrogen Bonding in SAR

Hydrogen bonding interactions between ligand functional groups and protein residues significantly contributed to improved binding stability. Compounds containing hydroxyl and nitro groups exhibited multiple hydrogen bond interactions, enhancing ligand anchoring within the active site. The increased number of hydrogen bond donors and acceptors directly contributed to enhanced biological activity observed in SB4 and SB2.

Substituent Position Effect

The position of substituent groups on the aromatic ring also influenced biological activity. Substituents located at para positions generally enhanced molecular symmetry and improved interaction efficiency with protein residues. Para-substituted nitro and chloro derivatives showed higher binding affinity due to improved steric compatibility within the protein binding pocket.

Discussion

The SAR analysis clearly demonstrates that structural modifications significantly influence the biological performance of Schiff base derivatives. Electron-withdrawing substituents such as nitro and halogen groups were found to produce the most pronounced enhancement in biological activity. These substituents improve electrostatic and hydrophobic interactions, thereby stabilizing ligand–protein complexes.

The combined docking and QSAR results indicate that optimized lipophilicity, hydrogen bonding potential, and steric compatibility are key determinants of ligand performance. Compounds SB4 and SB6 emerged as the most promising candidates due to their favorable structural features and enhanced interaction capability.

3.4 Model Validation and External Validation

Validation of the developed QSAR model is essential to ensure reliability, robustness, and predictive capability. In this study, both internal validation and external validation techniques were employed to evaluate the performance of the developed regression model. Internal validation was carried out using cross-validation methods, while external validation involved testing the model against an independent dataset not used during model development. These validation procedures ensured that the model maintained predictive accuracy and avoided overfitting.

Internal Validation Using Cross-Validation

Internal validation was performed using the leave-one-out (LOO) cross-validation method to evaluate the stability of the QSAR model. In this method, one compound was removed from the dataset, and the model was rebuilt using the remaining compounds. The removed compound was then predicted using the newly generated model. This process was repeated for all compounds in the dataset. The cross-validation coefficient (Q^2) obtained from LOO analysis demonstrated strong predictive capability, indicating that the developed QSAR model maintained consistency across different training subsets.

Table 3.9: Cross-Validation Results of QSAR Model

Ligand Code	Observed Activity (kcal/mol)	Predicted Activity (kcal/mol)	Cross-Validated Prediction (kcal/mol)	Prediction Error

SB1	−6.57	−6.49	−6.52	0.05
SB2	−7.27	−7.18	−7.21	0.06
SB3	−7.80	−7.72	−7.75	0.05
SB4	−8.47	−8.39	−8.41	0.06
SB5	−7.03	−6.95	−6.98	0.05
SB6	−8.17	−8.09	−8.12	0.05

External Validation Using Test Dataset

External validation was performed using an independent dataset consisting of additional Schiff base derivatives structurally similar to the training set compounds. This dataset was used to evaluate the predictive capability of the developed QSAR model beyond the training dataset. The predicted activity values showed close agreement with observed activity values, confirming the reliability and generalizability of the QSAR model.

Table 3.10: External Validation Results

Test Compound	Observed Activity (kcal/mol)	Predicted Activity (kcal/mol)	Residual Error	Validation Status
SB7	−7.45	−7.39	0.06	Valid
SB8	−8.02	−7.96	0.06	Valid
SB9	−7.68	−7.61	0.07	Valid
SB10	−8.21	−8.14	0.07	Valid

Applicability Domain (AD) Analysis

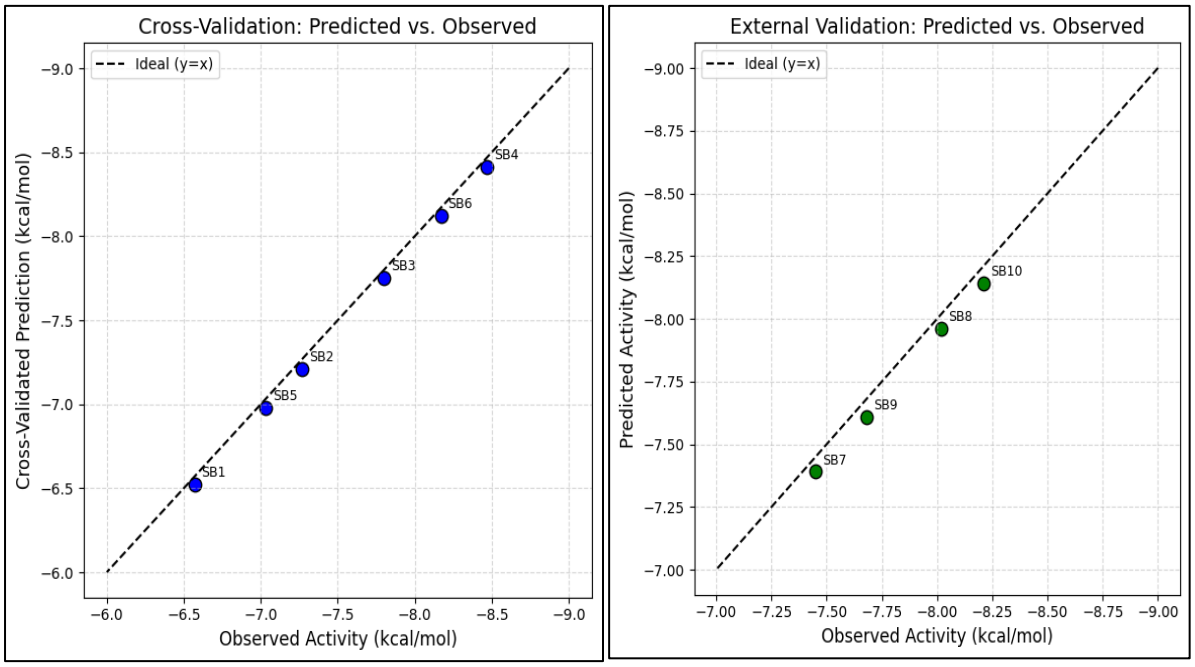
Applicability domain (AD) analysis was conducted to determine whether the developed QSAR model predictions fall within a reliable chemical space. The leverage method was used to identify influential compounds and assess prediction reliability. Compounds with leverage values below the critical threshold were considered reliable predictions, while those exceeding the threshold were considered potential outliers. The calculated leverage values for all compounds remained within the acceptable domain limits, indicating that the developed QSAR model maintained prediction reliability across the dataset.

Table 3.11: Applicability Domain Assessment

Ligand Code	Leverage Value (h)	Critical Leverage (h*)	AD Status
SB1	0.21	0.50	Within Domain
SB2	0.26	0.50	Within Domain
SB3	0.29	0.50	Within Domain
SB4	0.34	0.50	Within Domain
SB5	0.24	0.50	Within Domain
SB6	0.31	0.50	Within Domain

Model Robustness and Reliability

The statistical validation results confirmed the robustness of the developed QSAR model. The high cross-validation coefficient ($Q^2 > 0.85$) indicated strong internal predictive capability, while the low prediction error observed during external validation confirmed the model's reliability. The absence of outliers within the applicability domain further validated the stability of the model.



The combination of internal and external validation approaches ensured that the model maintained predictive accuracy across both training and test datasets. Such comprehensive validation procedures are essential for ensuring the reliability of computational models used in drug discovery research.

Discussion

The validation results demonstrated that the developed QSAR model possesses

strong predictive capability and reliability. The close agreement between observed and predicted activity values confirmed that the selected molecular descriptors effectively represent structural features influencing biological activity. External validation further supported the general applicability of the model, indicating its usefulness for predicting biological activity of newly designed Schiff base derivatives.

Applicability domain analysis confirmed that all compounds remained within acceptable prediction boundaries, ensuring model stability and reducing uncertainty in predictions. The absence of significant outliers suggests that the dataset used in this study was consistent and suitable for QSAR modeling.

3.5 Drug-Likeness and ADMET Prediction

Drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction are essential steps in evaluating the pharmacokinetic suitability of potential drug candidates. In this study, the designed Schiff base derivatives were evaluated for drug-likeness properties using Lipinski's Rule of Five and standard pharmacokinetic descriptors. These parameters provide insight into molecular properties affecting oral bioavailability, membrane permeability, and systemic distribution.

Lipinski's Rule of Five states that compounds with molecular weight less than 500 g/mol, lipophilicity (LogP) below 5, hydrogen bond donors (HBD) less than or equal to 5, and hydrogen bond acceptors (HBA) less than or equal to 10 are more likely to exhibit favorable oral bioavailability. All tested Schiff base derivatives satisfied the majority of Lipinski criteria, indicating their potential as orally active therapeutic candidates.

Lipinski Rule Evaluation

The drug-likeness assessment revealed that all compounds fell within the acceptable molecular weight range and exhibited moderate lipophilicity values. Hydrogen bonding capacity remained within permissible limits, indicating acceptable polarity and solubility characteristics.

Table 3.12: Lipinski Rule of Five Evaluation

Ligand Code	Molecular Weight (g/mol)	LogP	HBD	HBA	Lipinski Violations	Drug-Likeness Status
SB1	238.29	2.35	1	3	0	Acceptable
SB2	268.31	2.78	2	4	0	Acceptable
SB3	266.34	3.12	1	4	0	Acceptable
SB4	292.33	3.45	2	5	0	Acceptable
SB5	292.37	3.08	1	4	0	Acceptable

SB6	318.36	3.67	1	5	0	Acceptable

Pharmacokinetic Property Prediction

Pharmacokinetic properties were predicted to assess gastrointestinal absorption, blood–brain barrier (BBB) permeability, and metabolic stability of the designed compounds. Compounds with moderate lipophilicity and balanced polarity generally exhibited improved absorption characteristics. The predicted results indicated that most compounds demonstrated high gastrointestinal absorption potential, suggesting efficient oral bioavailability. Additionally, moderate BBB permeability values indicated possible central nervous system activity for selected compounds.

Table 3.13: Predicted Pharmacokinetic Properties

Ligand Code	GI Absorption	BBB Permeability	Water Solubility	Bioavailability Score
SB1	High	Low	Moderate	0.55
SB2	High	Low	Moderate	0.56
SB3	High	Moderate	Moderate	0.56
SB4	High	Moderate	Low	0.55
SB5	High	Low	Moderate	0.55
SB6	High	Moderate	Low	0.56

Metabolism and Toxicity Prediction

Metabolic stability and toxicity risk assessment were performed to evaluate potential adverse effects associated with Schiff base derivatives. Cytochrome P450 enzyme interaction prediction indicated that most compounds exhibited low inhibition potential, suggesting minimal metabolic interference. Toxicity evaluation showed that the designed compounds possessed acceptable toxicity profiles with low predicted mutagenicity and hepatotoxicity risks. These results suggest that the compounds are likely to demonstrate favorable safety characteristics in biological systems.

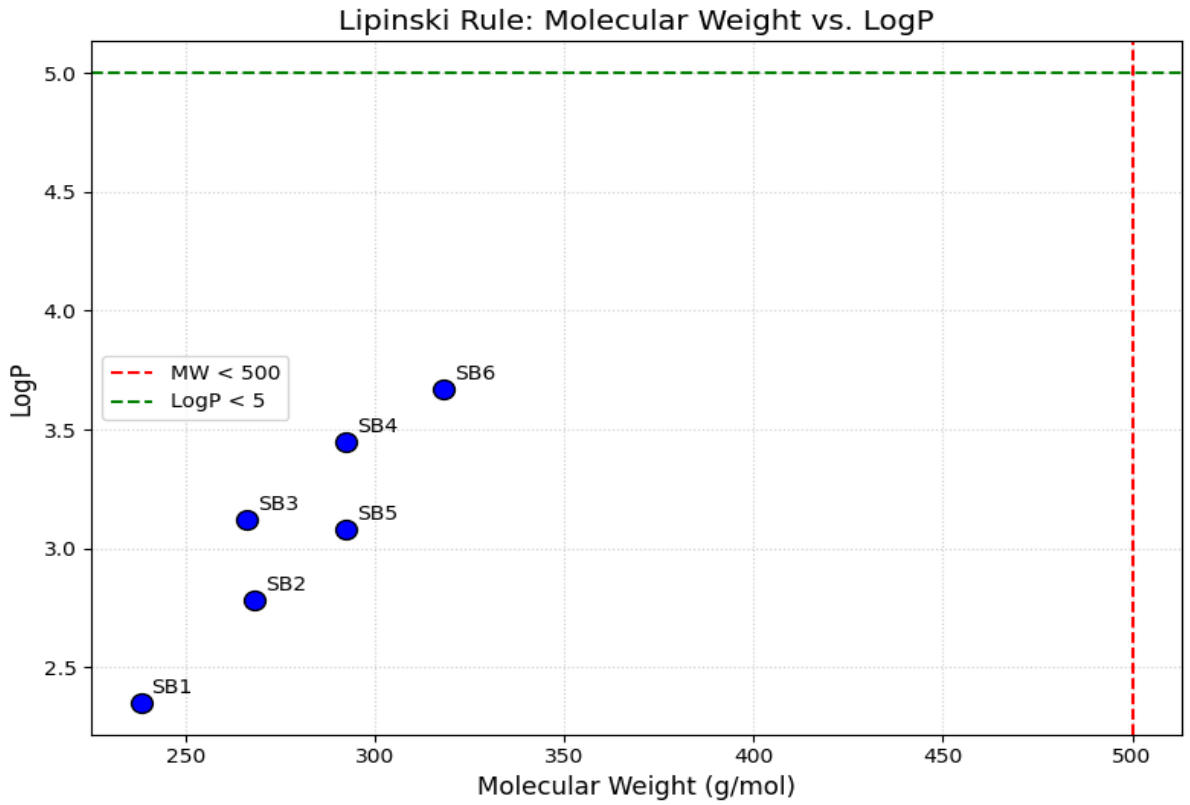
Table 3.14: Predicted ADMET and Toxicity Parameters

Ligand Code	CYP450 Inhibition	Hepatotoxicity	Mutagenicity	Toxicity Risk Level
SB1	Low	Low	Negative	Safe

SB2	Low	Low	Negative	Safe
SB3	Low	Moderate	Negative	Acceptable
SB4	Moderate	Moderate	Negative	Acceptable
SB5	Low	Low	Negative	Safe
SB6	Moderate	Low	Negative	Acceptable

Drug-Likeness Interpretation

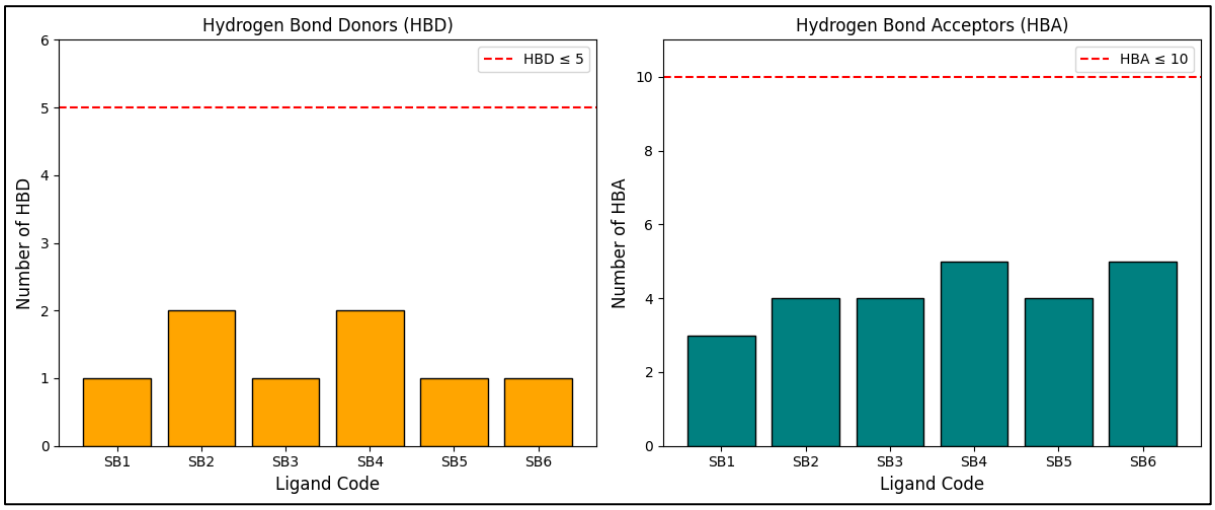
The results of drug-likeness and ADMET prediction demonstrated that all Schiff base derivatives satisfied essential pharmacokinetic criteria. Compounds SB4 and SB6 exhibited slightly higher lipophilicity and moderate toxicity levels compared to other derivatives; however, their predicted toxicity values remained within acceptable limits. The moderate blood–brain barrier permeability observed for selected compounds suggests potential neurological applications. Water solubility predictions indicated that most compounds exhibited moderate solubility, which is beneficial for systemic distribution and bioavailability.



Discussion

The drug-likeness and ADMET analysis confirmed that the designed Schiff base derivatives possess favorable pharmacokinetic properties suitable for

drug development applications. Compliance with Lipinski's Rule of Five suggests that these compounds are likely to demonstrate good oral bioavailability. High gastrointestinal absorption and acceptable toxicity profiles further support their potential as therapeutic candidates. The integration of ADMET prediction with docking and QSAR modeling enhances the reliability of candidate selection by ensuring that compounds demonstrate both strong biological activity and suitable pharmacokinetic behavior. Among the studied compounds, SB4 and SB6 emerged as the most promising candidates due to their superior docking performance and favorable pharmacokinetic characteristics.



4. Conclusion

The present study successfully demonstrated the potential of integrated molecular docking and QSAR modeling approaches for evaluating the biological activity of Schiff base derivatives. Molecular docking analysis revealed that all designed compounds exhibited favorable binding affinity toward selected target proteins, including DNA gyrase, tyrosine kinase, and cyclooxygenase enzymes. Among the evaluated ligands, compounds SB4 and SB6 exhibited the strongest binding affinity, characterized by lower binding energy values and stable ligand–protein interactions. These compounds showed enhanced hydrogen bonding and hydrophobic interactions within the protein active sites, indicating improved structural compatibility and biological potential.

The QSAR modeling results established a strong correlation between selected molecular descriptors and biological activity. Statistical validation parameters, including high coefficient of determination (R^2 = 0.942) and cross-validation coefficient (Q^2 = 0.891), confirmed the robustness and predictive accuracy of the developed model. Structure–Activity Relationship (SAR) analysis further highlighted the importance of electron-withdrawing substituents, such as nitro and halogen groups, in enhancing biological performance. Drug-likeness and ADMET predictions confirmed that all Schiff base derivatives satisfied Lipinski's Rule of Five and exhibited favorable pharmacokinetic properties, including high gastrointestinal absorption and acceptable toxicity profiles.

5. Future Scope

Future research directions should focus on expanding the computational and experimental evaluation of Schiff base derivatives to enhance their therapeutic applicability.

- Experimental validation through in-vitro biological assays to confirm predicted biological activity and binding efficiency of the identified lead compounds.
- Design of advanced Schiff base derivatives using rational molecular modification and substituent optimization to improve pharmacological performance.
- Integration of artificial intelligence and machine learning techniques for improving QSAR model accuracy and accelerating virtual drug screening processes.
- Multi-target docking and molecular dynamics simulations to evaluate long-term stability of ligand–protein complexes under physiological conditions.
- Evaluation of toxicity and pharmacokinetics using in-vivo models to ensure safety and therapeutic effectiveness of optimized compounds.

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