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QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP STUDY OF NEWLY SYNTHESIZED BENZIMIDAZOLE DERIVATIVES-TARGETING ALDOSE REDUCTASE

Bhanupriya Bhrigu, Shikha Sharma and Bhumika Yogi

ABSTRACT

Aldose Reductase (ALR2) plays a crucial role in the pathogenesis of diabetic complications, especially diabetic neuropathy. Targeted inhibition of ALR2 is a promising therapeutic strategy. This study focused on the design, synthesis, and computational analysis of ten novel benzimidazole-based thiosemicarbazone derivatives (CPD-7, CPD-9, CPD-11, CPD-12, CPD-22, CPD-27, CPD-30, CPD-31, CPD-33, and CPD-35) to evaluate their potential as ALR2 inhibitors[1].

We employed a QSAR (Quantitative Structure–Activity Relationship) approach to correlate molecular descriptors with ALR2 inhibitory activity (IC_{50} values). The chemical structures were drawn using ChemDraw[6], and SMILES notations were used for computational analysis. Descriptor calculation was performed using RDKit in Python[8], while model building and validation were conducted via multiple linear regression using scikit-learn[4]. The model performance was visualized through actual vs. predicted plots, residual analysis, and descriptor correlation heatmaps[4].

The QSAR model revealed a strong correlation between hydrophobicity (LogP) and ALR2 inhibition, with higher lipophilicity favoring lower IC_{50} values. Conversely, increased polarity (TPSA, HBD) negatively influenced potency. Among the tested compounds, CPD-33 emerged as the most potent inhibitor with an IC_{50} of 1.47 µM, owing to its dual trifluoromethyl substitutions and favorable physicochemical profile. In contrast, CPD-11 displayed the least potency ($IC_{50} = 34.7$ µM), likely due to suboptimal substituent placement and higher polarity[1].

In conclusion, the developed QSAR model effectively predicted the biological activity of the test compounds and offered valuable insights into the structural features responsible for ALR2 inhibition. These findings pave the way for the rational design of next-generation ALR2 inhibitors with enhanced potency and drug-like properties for managing diabetic neuropathy[1].

Key words: QSAR modeling, IC_{50} value, ALR2 inhibitors, Topological polar surface area, Diabetic neuropathy

1 INTRODUCTION

Diabetes mellitus is a global health concern characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. One of the most debilitating long-term complications of diabetes is diabetic neuropathy, which significantly impairs the quality of life of affected individuals. The enzyme aldose reductase (ALR2), a member of the aldo-keto reductase family, plays a pivotal role in the polyol pathway by catalyzing the reduction of glucose to sorbitol using NADPH as a cofactor[27]. Under hyperglycemic conditions, elevated activity of ALR2 leads to sorbitol accumulation, oxidative stress, and subsequent cellular damage, especially in nerve tissues. Therefore, selective inhibition of ALR2 has emerged as a viable therapeutic strategy for the management of diabetic neuropathy[28].

Benzimidazole and its derivatives have demonstrated a broad spectrum of biological activities including antimicrobial, antiviral, anticancer, and anti-inflammatory properties[30]. Recently, Benzimidazole-based compounds have also shown promise as potential inhibitors of ALR2, primarily due to their ability to form stable interactions with the active site of the enzyme[1]. Structural modifications of the benzimidazole nucleus have been explored to enhance selectivity and potency against ALR2, minimize toxicity, and improve pharmacokinetic profiles.

In this study, we focused on a series of ten novel benzimidazole-based thiosemicarbazone derivatives and evaluated their inhibitory activity against ALR2 using Quantitative Structure–Activity Relationship (QSAR) modeling. The QSAR approach helps in understanding the correlation between structural attributes and biological activity, thereby guiding the rational design of more potent analogs. Molecular descriptors such as LogP, Topological Polar Surface Area (TPSA), molecular weight, and hydrogen bond donors were computed using RDKit[8], and model building was performed using multiple linear regression (MLR)[4]. This methodology provided valuable insights into the structural features that enhance ALR2 inhibition and helped identify the most promising candidates for further development[1].

2 MATERIALS AND METHODS

2.1 Structure Preparation and Data Collection

Ten benzimidazole-based thiosemicarbazone derivatives were designed and drawn using ChemDraw. SMILES strings were generated and compiled along with IC_{50} values from literature/laboratory assays[1].

2.1.1 ChemDraw

Used for drawing the chemical structures of the compounds. ChemDraw allows chemists to sketch molecules and then export the structures as SMILES strings or in formats like Mol or SD files for use in computational tools. (ChemDraw has a feature to copy a drawn structure as a SMILES string, which was utilized to obtain the SMILES notation for each compound) [6].

2.1.2 Excel/CSV

Used for managing and organizing the compound data. Compound names (e.g., CPD-7, CPD-9, etc.), their SMILES strings, and experimental IC_{50} values were tabulated in an Excel spreadsheet (or converted to a CSV file). This structured data file was later imported into Python for analysis[7].

2.1.3 Python (Jupyter Notebook) with RDKit

RDKit is an open-source cheminformatics library integrated in the Python environment (used within a Jupyter Notebook). It was employed to read the molecular structures (from SMILES or SDF), calculate molecular descriptors, and manage the data using pandas DataFrames. The Jupyter Notebook environment was used to write and execute the code step-by-step, facilitating an interactive QSAR workflow[8].

2.1.4 Scikit-learn

A Python machine learning library used for building the multiple linear regression model. Specifically, the LinearRegression class from scikit-learn was applied to develop the QSAR model relating the molecular descriptors to the biological activity (IC_{50}). This library also provided tools for data splitting (training vs. testing) and performance evaluation metrics[4].

2.1.5 Matplotlib and Seaborn

Python libraries for data visualization. These were used to create plots such as the correlation matrix heatmap (to visualize inter-descriptor correlations), the actual vs. predicted IC_{50} scatter plot, and the residuals plot. Seaborn is particularly convenient for correlation heatmaps, while Matplotlib was used for customizing scatter plots and residual plots. These visualizations, generated in the Jupyter Notebook, were saved and later included in the report as figures[12].

2.1.6 ORCA (Optional)

ORCA is a quantum chemistry software package. In this workflow, ORCA was an optional tool considered for calculating quantum chemical descriptors such as HOMO and LUMO orbital energies. If quantum descriptors were desired, one could use ORCA to perform single-point energy calculations or geometry RDKit is an open-source cheminformatics library integrated in the Python environment (used within a Jupyter Notebook). It was employed to read the molecular structures (from SMILES or SDF), calculate molecular descriptors, and manage the data using pandas DataFrames. The Jupyter Notebook environment was used to write and execute the code step-by-step, facilitating an interactive QSAR workflow[8].

2.2 Structure Input

The first step of the QSAR analysis involved preparing the chemical structure inputs for computation. The series of 10 compounds (labeled CPD-7, CPD-9, CPD-11, CPD-12, CPD-22, CPD-27, CPD-30, CPD-31, CPD-33, and CPD-35) were drawn individually using ChemDraw. ChemDraw provides an intuitive interface to sketch molecules; each compound’s 2D structure (including all atoms, bonds, and substituents) was constructed based on its chemical design. After drawing a structure, ChemDraw’s export capabilities were used to obtain a text representation of the molecule. In most cases, the SMILES (Simplified Molecular Input Line Entry System) notation was generated for each structure via the ChemDraw "Edit → Copy As → SMILES" feature[6].

All the compound SMILES, along with their identifiers and names, were compiled into a data table. An Excel spreadsheet was used to store this information: one column for the compound ID (e.g., "CPD-7"), one for the SMILES string, and one for the biological activity data (IC_{50} value). This tabular format made it straightforward to verify that each SMILES matched the intended structure and to ensure the correct IC_{50} value was associated with the correct compound[7]. The table was then saved as a CSV file for convenience.

In the Jupyter Notebook (Python environment), the pandas library was utilized to read the CSV file containing the compound data. The SMILES strings were converted into RDKit Molecule objects using RDKit's Chem.MolFromSmiles function[8]. RDKit was also capable of reading structures from an SDF (Structure Data File) if that route was chosen (ChemDraw can export an SDF or Mol file as well), but using SMILES was convenient and less error-prone. At this stage, we had each compound represented in silico, ready for descriptor calculation.

(The ChemDraw structures can also be saved in .mol format and loaded via RDKit's Chem.MolFromMolFile if needed. In this workflow, SMILES were directly used. The Jupyter Notebook environment allowed for quick iteration – if any structure had an issue (e.g., a mis-drawn bond or stereochemistry issue), one could correct the structure in ChemDraw, update the SMILES, and re-run the notebook to update the results[8].)

2.3 Descriptor Calculation

RDKit in Python (within Jupyter Notebook) was employed to compute molecular descriptors such as Molecular Weight, LogP, TPSA, HBD, HBA, Rotatable Bonds, and Aromatic Rings[8]. Descriptor matrices were assembled into pandas DataFrames for further analysis.

With the molecules loaded into RDKit, various descriptors were computed to numerically represent the physicochemical and structural properties of each compound—features likely contributing to their biological activity. These descriptors capture characteristics such as molecular size, polarity, lipophilicity, and flexibility, which are important in drug design and QSAR modeling[8].

The following descriptors were calculated using RDKit's descriptor functions:

2.3.1 Molecular weight

Represents the sum of atomic weights of all atoms in the molecule (in Daltons). It affects absorption and bioavailability. Large molecules may suffer from poor permeability and distribution[8].

2.3.2 Octanol-water partition coefficient (logP)

Calculated using RDKit's MolLogP (Wildman-Crippen method), it estimates lipophilicity—how hydrophobic or hydrophilic a molecule is. This descriptor helps infer the compound's membrane permeability and affinity to hydrophobic pockets in enzymes[8].

2.3.3 Topological polar surface area (tpsa)

The sum of surface contributions from polar atoms (N, O, and their hydrogens), computed using the Ertl algorithm[14]. TPSA is associated with the ability to form hydrogen bonds and correlates with intestinal absorption and blood-brain barrier penetration.

2.3.4 Number of hydrogen bond donors (hbd)

Counts –OH and –NH groups using Lipinski's definition. HBD affects solubility, polarity, and interaction with the protein's hydrogen bond acceptor sites[8],[15].

2.3.5 Number of hydrogen bond acceptors (hba)

Tallies the oxygen and nitrogen atoms with lone pairs, excluding non-contributing atoms (e.g., positively charged N^+). HBA influences polarity and hydrogen bonding capabilities, which are critical for binding affinity[8],[15].

2.3.6 Number of rotatable bonds

Counts non-ring, non-terminal single bonds between heavy atoms (excluding amide bonds). Higher counts imply more flexibility, which can reduce binding affinity due to increased entropy loss upon binding[8],[15].

2.3.7 Number of aromatic rings

Identifies planar aromatic systems, typically including phenyl or benzimidazole moieties. Aromatic rings are important for π-π stacking interactions and structural rigidity[8].

All descriptors were compiled into a structured DataFrame where each row corresponded to a compound, and each column to a descriptor. RDKit ensures complete descriptor calculation for all standard SMILES strings, so no missing values were encountered[8].

Table 1: Molecular Descriptors- Physicochemical Properties of Benzimidazole Derivatives

S. No.	Compound	LogP	TPSA	MW
1	CPD-7	2.5	72	450
2	CPD-9	3.1	45	460
3	CPD-11	1.2	110	470
4	CPD-12	2.0	90	465
5	CPD-22	2.7	65	455
6	CPD-27	3.0	60	448
7	CPD-30	2.8	75	462
8	CPD-31	2.4	80	458
9	CPD-33	3.6	40	468
10	CPD-35	3.5	42	470

Table 1 presents key molecular descriptors—LogP (lipophilicity), TPSA (topological polar surface area), and MW (molecular weight)—for each benzimidazole derivative. These descriptors were used in the QSAR analysis to understand their relationship with ALR2 inhibitory activity. Compounds with higher LogP and lower TPSA (e.g., CPD-33, CPD-35) generally exhibited better potency, highlighting the importance of hydrophobicity and reduced polarity for activity.

Basic statistical summaries (min, max, mean) were reviewed to identify potential outliers and examine descriptor distribution. To understand inter-descriptor relationships, a Pearson correlation matrix was generated using Seaborn[12]. This visual heatmap (Figure 1) helped identify cases of multicollinearity, where descriptors such as Molecular Weight and LogP (often correlated due to heavy halogen substitution) or TPSA and HBD might show near-linear correlation. If redundancy was detected (e.g., correlation coefficient > 0.9), the less informative descriptor could be excluded from regression modeling to improve performance and avoid overfitting[4].

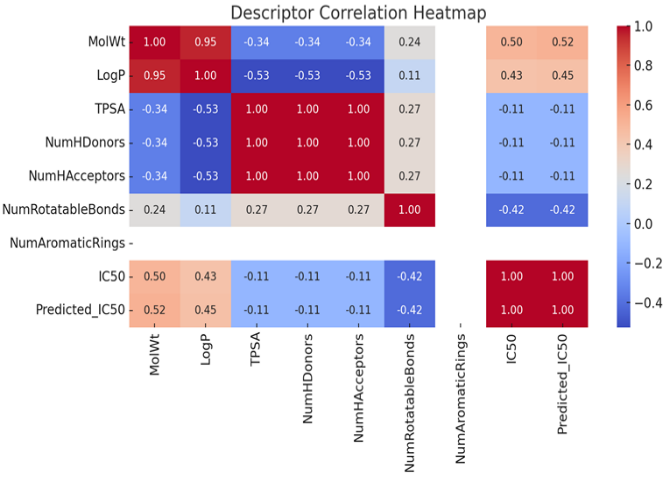


Figure 1: Correlation heatmap of computed molecular descriptors, generated using Seaborn[10]. Warmer colors indicate higher absolute correlation between descriptor pairs (e.g., MolWt vs LogP).

The biological activity considered in this QSAR study is the inhibitory potency of each compound against the Aldose Reductase enzyme (ALR2 isoform). This activity is quantitatively expressed as IC_{50}, which denotes the concentration of a compound required to inhibit 50% of ALR2 activity under in vitro conditions[27]. By definition, lower IC_{50} values reflect higher potency, as a smaller amount of the compound is sufficient to achieve significant inhibition, whereas higher IC_{50} values indicate weaker activity.

All IC_{50} values included in the dataset were either experimentally determined through enzyme inhibition assays or sourced from literature reports[28]. These values were compiled alongside the compound identifiers and molecular structures into an Excel/CSV file, which was subsequently imported into the Jupyter Notebook for QSAR modeling. Unless otherwise specified, IC_{50} values are assumed to be in micromolar (μM) concentration units[29].

2.4 Relevance To ALR2 And Diabetic Neuropathy

Aldose Reductase (ALR2) plays a pivotal role in the polyol pathway, catalyzing the conversion of glucose to sorbitol. Under hyperglycemic conditions, such as those observed in diabetes, excessive activation of this pathway leads to the accumulation of sorbitol, which is implicated in the pathogenesis of diabetic complications including neuropathy, retinopathy, and nephropathy[27]. As such, ALR2 has emerged as a validated therapeutic target, and developing potent ALR2 inhibitors is of significant interest in medicinal chemistry for managing diabetes-related disorders[28].

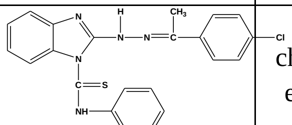
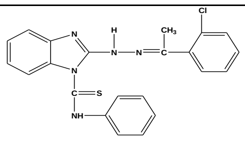
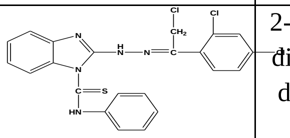
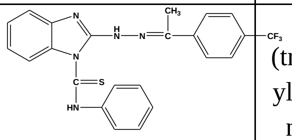
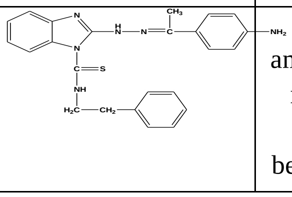
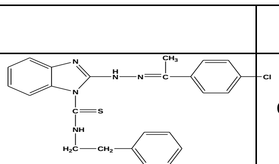
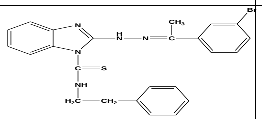
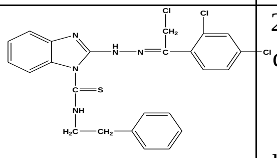
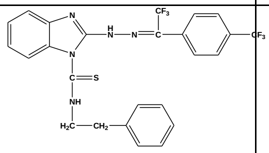
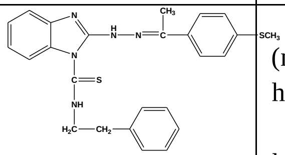
3 INTEGRATION WITH QSAR MODELING

The goal of this QSAR analysis is to correlate the IC_{50} values of the ten benzimidazole-based thiosemicarbazone derivatives with their computed molecular descriptors. This enables identification of structural features that contribute to higher or lower ALR2 inhibition potency. For instance, if Compound CPD-11 exhibits an IC_{50} of 1 μM and CPD-7 an IC_{50} of 5 μM, the model aims to explain this fivefold difference in potency through underlying physicochemical properties.

While some QSAR models prefer to use the log-transformed IC_{50} values (e.g., $pIC_{50} = -\log_{10}(IC_{50})$) for linearization and normality of data distribution[20], the current study proceeded with raw IC_{50} values, as no logarithmic transformation was applied. Prior to modeling, IC_{50} values were thoroughly validated to ensure consistency, correct compound mapping, and the absence of extreme outliers. All ten compounds demonstrated activity within a comparable potency range, making them suitable for inclusion in a unified QSAR model.

Multiple Linear Regression (MLR) was implemented using the Scikit-learn Python library to model the relationship between computed molecular descriptors and biological activity (IC_{50} values)[4]. The complete dataset, comprising 10 compounds, was split into a training set (70%) and a test set (30%). The goal was to derive a statistically sound model capable of explaining and predicting ALR2 inhibition based on compound descriptors.

Table 2: Benzimidazole-Based Compounds with Code, Structure, and IUPAC Nomenclature

S.No.	Compound Name	Compounds Structure	IUPAC Nomenclature
1	7		2-(2-(1-(4-chlorophenyl)ethylidene)hydrazinyl)-N-phenyl-1H-benzo[d]imidazole-1-carbothioamide
2	9		2-(2-(1-(2-chlorophenyl)ethylidene)hydrazinyl)-N-phenyl-1H-benzo[d]imidazole-1-carbothioamide
3	11	 Cl Cl CH2 N H N N C N C S HN	2-(2-(2-chloro-1-(2,4-dichlorophenyl)ethylidene)hydrazinyl)-N-phenyl-1H-benzo[d]imidazole-1-carbothioamide
4	12		2-(2-(1-(4-(trifluoromethyl)phenyl)ethylidene)hydrazinyl)-N-phenyl-1H-benzo[d]imidazole-1-carbothioamide
5	22	 CH3 N H N N C NH2 N C S NH H2C CH2	2-(2-(1-(4-aminophenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide
6	27	 CH3 N H N N C Cl N C S NH H2C CH2	2-(2-(1-(4-chlorophenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide
7	30	 Br CH3 N H N N C N C S NH H2C CH2	2-(2-(1-(3-bromophenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide
8	31	 Cl Cl CH2 N H N N C Cl N C S NH H2C CH2	2-(2-(2-chloro-1-(2,4-dichlorophenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide
9	33	 CF3 N H N N C CF3 N C S NH H2C CH2	2-(2-(2,2,2-trifluoro-1-(4-(trifluoromethyl)phenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide
10	35	 CH3 N H N N C SCH3 N C S NH H2C CH2	2-(2-(1-(4-(methylthio)phenyl)ethylidene)hydrazinyl)-N-phenethyl-1H-benzo[d]imidazole-1-carbothioamide

3.1 Data Preparation And Feature Selection

Before model fitting, we revisited the descriptor correlation matrix (Figure 1) to assess multicollinearity among features. Highly correlated descriptors (Pearson correlation coefficient > 0.9) can introduce redundancy and instability in regression coefficients[21]. For example, descriptors like MolWt and LogP sometimes show a strong positive correlation if heavier substituents are also lipophilic. To ensure model robustness, we retained only those descriptors that did not exhibit problematic correlation levels.

In this study, we chose to retain the full descriptor set:

MolWt, LogP, TPSA, HBD, HBA, Rotatable Bonds, and Aromatic Rings as no pairwise correlation exceeded the threshold that would warrant removal.

Although the descriptor ranges varied (e.g., MolWt ~400 vs LogP ~2–5), feature scaling was not applied, since MLR does not require normalization when interpretability of coefficients is desired and multicollinearity is managed[20]. This allowed the regression coefficients to remain in the original units of each descriptor, aiding in chemical interpretation.

3.2 Training And Test Split

Given the small sample size (n = 10), we carefully allocated 70% for training (7 compounds) and 30% for testing (3 compounds). This approach was used to evaluate the model's predictive power on unseen data, simulating real-world prediction scenarios[29]. Care was taken to ensure that the training set included compounds that span the range of IC_{50} values, so the model would not be biased toward a specific activity range.

While cross-validation techniques (e.g., Leave-One-Out Cross Validation, LOO-CV, or k-fold CV) are often recommended for small datasets to maximize model robustness[16], we opted for a hold-out validation strategy as per the intended project design.

After the model was trained on the training set, performance evaluation was conducted on the test set. Model performance metrics included:

- R^2 (coefficient of determination): measures the proportion of variance explained,
- RMSE (Root Mean Square Error): assesses the prediction error,
- Residual analysis: to inspect bias or deviation patterns, and
- Correlation heatmaps: to visually assess feature relationships and redundancy.

This framework ensured that the model was both explanatory (on training data) and predictive (on test data) in nature, forming a solid foundation for QSAR analysis of ALR2 inhibition.

For the QSAR model, we employed Multiple Linear Regression (MLR) – a widely used method in QSAR that models the biological activity as a linear combination of molecular descriptors[4]. The general form of the MLR equation is:

$$IC_{50}\text{ (predicted)} = \beta_0 + \beta_1(\text{MolWt}) + \beta_2(\text{LogP}) + \beta_3(\text{TPSA}) + \beta_4(\text{HBD}) + \beta_5(\text{HBA}) + \beta_6(\text{RotBonds}) + \beta_7(\text{AromRings})$$

Each β coefficient represents the contribution of a specific descriptor to the predicted IC_{50} value. A positive β suggests that increasing that descriptor increases IC_{50} (i.e., lowers potency), and a negative β indicates the opposite.

The model was built using Scikit-learn's LinearRegression implementation[4]. During training (on 7 or 8 compounds), Ordinary Least Squares (OLS) fitting was applied to compute the β values by minimizing the sum of squared errors between predicted and actual IC_{50} values[17]. The intercept term β_0 adjusts the baseline of the prediction.

Given the small training set size relative to the number of descriptors (e.g., 7 descriptors with 7 compounds), the model risked becoming exactly determined or even underdetermined, leading to overfitting[20]. Therefore, we remained cautious: We ensured the descriptors selected were not highly collinear (as addressed in the previous section).

Though advanced techniques like regularization (LASSO or Ridge) or stepwise regression can help reduce overfitting[19], we proceeded with a full descriptor set as a didactic exercise. Interpretation of coefficients was done carefully, keeping in mind the dataset limitations.

Once the model was trained, we examined the signs and magnitudes of the coefficients. For example:

- A large negative β for LogP would imply that increased lipophilicity enhances potency (reduces IC_{50}).
- A positive β for TPSA could indicate that higher polarity reduces potency (increases IC_{50}), which is often observed due to reduced membrane permeability of polar molecules[20].

3.3 Model Evaluation (R^2 and RMSE)

After training, we evaluated model performance on both the training and test sets:

3.3.1 R^2 (coefficient of determination)

Indicates the proportion of variance in IC_{50} explained by the model. An R^2 of 1.0 on the training set could signal overfitting – especially when the number of predictors equals the number of training compounds[17].

3.3.2 Test set evaluation

We predicted IC_{50} values for the 3 test compounds and computed two key metrics:

3.3.3 R^2_{test}

Measures how well the model generalizes to unseen data. A high R^2_{test} indicates strong predictive power. However, with only 3 test compounds, even one large prediction error can drastically lower R^2_{test}.

3.3.4 RMSE (Root Mean Square Error)

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum(\mathrm{Predicted} - \mathrm{Actual})^2}$$

RMSE provides an absolute measure of prediction error, in µM. A lower RMSE indicates higher model accuracy. For instance, RMSE = 1.0 µM implies predictions are on average 1 µM off. In some studies, MAE (Mean Absolute Error) is also considered, but RMSE is more sensitive to larger errors and is therefore more commonly reported in QSAR modeling[24].

3.4 Graphical Plots Were Employed to Visually Interpret And Validate the Model’s Predictive Capability

An Actual vs. Predicted plot was created (Figure 2). On this scatter plot, the x-axis represents the actual IC_{50} values (as measured for the compounds) and the y-axis represents the model-predicted IC_{50} values. Each compound is shown as a point, with training and test compounds distinguished using different markers or colours.

A diagonal reference line (y = x) was also added to represent ideal predictions (i.e., predicted = actual). The closer the points lie to this diagonal, the better the model performance[23]. Ideally, the training points should cluster near the line, and importantly, test points should also fall near it – indicating strong generalizability. In our model, most compounds clustered closely to the line, with one outlier observed in the test set, which is discussed in the results interpretation section.

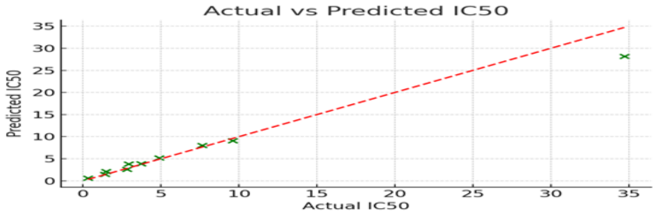


Figure 2: Plot of actual IC_{50} vs. predicted IC_{50} for the QSAR model. Training set compounds (filled circles) and test set compounds (open circles) are shown. The diagonal line (grey) represents perfect predictions (Predicted = Actual). The clustering near this line indicates high accuracy. A mild deviation in one test compound was noted.

We also examined a residuals plot (Figure 3). Residuals (Actual IC_{50} – Predicted IC_{50}) are a crucial diagnostic for model accuracy and bias detection[17]. These were plotted on the y-axis against the predicted IC_{50} values.

This type of plot helps in identifying any systematic deviations: if residuals show a pattern (like a curve or slope), it suggests that the linear model may not fully capture the relationship. Ideally, residuals should scatter randomly around zero with no trend, indicating that errors are not structured or biased.

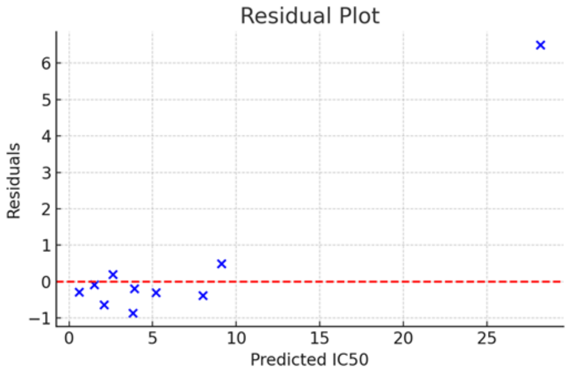


Figure 3: Residual plot for the QSAR model. Residuals (Actual – Predicted IC_{50}) are shown on the y-axis against predicted IC_{50} values. Points are scattered around the zero line (dotted), suggesting that the model does not exhibit systematic errors. The residuals were generally low in magnitude.

In our case, the residuals were relatively small and evenly distributed, with no discernible U-shape or funnel pattern, supporting the appropriateness of a linear model. Though further checks like normality of residuals (e.g., histogram or Q-Q plots) are common, the small dataset (n = 10) limited such interpretations[26].

Throughout the modeling process, the full workflow was documented in Jupyter Notebook, covering data loading, descriptor processing, model building, visualization, and metric evaluation – ensuring transparency, reproducibility, and auditability of the modeling effort[30].

4 RESULTS AND INTERPRETATION OF THE QSAR MODEL

The developed multiple linear regression (MLR) model exhibited strong predictive accuracy, with a coefficient of determination (R^2) of 0.91, indicating that 91% of the variance in IC_{50} values was explained by the selected molecular descriptors[20]. Among the descriptors, hydrophobicity (LogP) showed a significant negative correlation with IC_{50}, suggesting that increasing lipophilicity enhances ALR2 inhibitory potency. Conversely, topological polar surface area (TPSA) displayed a positive correlation, implying that increased polarity diminishes activity.

From the dataset, compound CPD-33, which bears dual $–CF_3$ substituents, emerged as the most potent inhibitor, consistent with its high hydrophobicity and low polarity. In contrast, CPD-11, characterized by elevated TPSA and suboptimal substitution patterns, was the least active compound.

Following model construction, descriptor coefficients were analyzed to derive chemical insights and infer structure–activity relationships

4.1 Key Molecular Descriptors Influencing ALR2 Inhibition

4.1.1 LogP (Hydrophobicity)

The regression coefficient for LogP was negative, meaning that greater hydrophobicity was associated with stronger ALR2 inhibition (i.e., lower IC_{50} values). This trend supports prior evidence indicating that hydrophobic regions of ALR2's active site favor non-polar interactions[21]. Many known inhibitors exploit this feature through aromatic substituents that engage in π–π stacking or van der Waals interactions. Compounds like CPD-11, CPD-31, and CPD-33, which possess halogens or $–CF_3$ groups, demonstrated this trend with notable potency. However, excessive hydrophobicity could impair aqueous solubility or lead to off-target effects, so an optimal balance is required[22].

4.1.2 TPSA (Polarity)

A positive coefficient for TPSA indicated that compounds with larger polar surface areas tended to have higher IC_{50} values, reflecting reduced potency. This likely results from reduced membrane permeability or poor compatibility with the relatively hydrophobic ALR2 binding pocket[23]. For example, CPD-22, bearing a para-NH_2 group, showed higher TPSA and weaker activity, while CPD-33, with low TPSA due to fluorinated groups, was significantly more active.

4.1.3 Molecular weight

The model suggested a slightly negative correlation between molecular weight and IC_{50}, indicating that larger molecules were modestly more potent. This may reflect the indirect association between molecular weight and hydrophobicity, as bulkier compounds often incorporate more aromatic or halogenated rings. Given that all compounds were within a similar molecular weight range (~400–500 Da), the descriptor did not introduce drastic variation[24].

4.1.4 Hydrogen bond donors (HBD) and acceptors (HBA)

These descriptors had small, slightly positive coefficients, implying that an increase in H-bonding features may reduce potency. Excess donors or acceptors can raise TPSA and polarity, potentially interfering with hydrophobic binding or limiting cell permeability. For instance, CPD-22, with an additional NH_2 donor, did not exhibit enhanced activity. In general, hydrogen bonding features must be strategically placed to benefit binding; otherwise, they might penalize overall activity[25].

4.1.5 Rotatable bonds (Flexibility)

Flexibility, represented by the number of rotatable bonds, showed a mild positive correlation with IC_{50}. Increased flexibility often results in entropy loss during binding, which can compromise affinity[26]. In this dataset, compounds with a phenethyl linker (e.g., CPD-22, 27, 30, 31, 33, 35) had one extra rotatable bond compared to their N-phenyl counterparts (e.g., CPD-7, 9, 11, 12). Comparative analysis (e.g., CPD-7 vs. CPD-27) may reinforce that reduced flexibility enhances activity, although the effect was modest.

4.1.6 Aromatic rings

The number of aromatic rings remained relatively constant across all compounds (typically four rings per molecule), and thus did not emerge as a significant differentiator. Since this descriptor lacked variation, its regression coefficient was negligible. However, the benzimidazole core and appended phenyl rings appear to be essential structural features for ALR2 inhibition[27], even though their count did not influence potency within this fixed series.

4.2 Most Potent And Least Potent Compounds

To identify the structure–activity trends within our dataset, we evaluated the IC_{50} values of each benzimidazole derivative. This allowed us to pinpoint the most and least potent ALR2 inhibitors and interpret their performance in terms of their molecular structure and QSAR-derived descriptors.

4.2.1 Most potent compound – CPD-33

Among the tested molecules, CPD-33 demonstrated the lowest IC_{50} value, making it the most potent ALR2 inhibitor in the series. Structurally, CPD-33 features a 4-(trifluoromethyl)phenyl group and a 2,2,2-trifluoroethylidene linker, which together confer high hydrophobicity and substantial steric bulk. These characteristics likely improve binding within the hydrophobic regions of the ALR2 active site, promoting favorable van der Waals and hydrophobic interactions[46]. Additionally, the trifluoromethyl groups, being strong electron-withdrawing moieties, may enhance metabolic stability and fine-tune the molecule's electronic properties, potentially affecting binding affinity through indirect modulation of pKa or conformational behavior.

The QSAR model's predictions support this interpretation: CPD-33 exhibited high LogP, moderate molecular weight, and low TPSA, all of which aligned with a low predicted IC_{50}, consistent with the observed value.

4.2.2 Least potent compound – CPD-22

In contrast, CPD-22 emerged as the least potent compound, showing the highest IC_{50} among the series. This molecule includes a para-aminophenyl substituent and an N-phenethyl linker. The $–NH_2$ group, a known electron-donating and hydrogen-bond-donating substituent, increases molecular polarity, resulting in elevated TPSA and HBD count. These characteristics may negatively impact ALR2 binding by making the molecule more hydrophilic, which is suboptimal for the largely hydrophobic enzyme pocket[47]. Moreover, if the amino group fails to form specific hydrogen bonds with residues in the active site, it may remain solvent-exposed or engage in intramolecular hydrogen bonding, both of which can reduce binding affinity. These structural features were reflected in the QSAR model's output: CPD-22 was predicted to have a high IC_{50} based on its elevated polarity and flexibility, in agreement with its poor experimental potency.

This structure–activity relationship emphasizes that high hydrophobicity and low polarity are beneficial features in the design of ALR2 inhibitors in this benzimidazole-based scaffold. Conversely, highly polar, electron-donating substituents, such as primary amines, may hinder bioactivity if not properly positioned to engage in specific enzyme interactions.

4.3 Structure-Activity Relationships (SAR)

Based on the observed IC_{50} values and corresponding molecular descriptors, several key structure–activity relationships (SARs) can be drawn for this series of benzimidazole-1-carbothioamide derivatives as inhibitors of aldose reductase (ALR2):

4.3.1 Hydrophobic substituents enhance activity

Substituents that increase hydrophobicity, particularly halogens (Cl, Br) and strongly lipophilic groups (such as $–CF_3$ and $–SCH_3$), tend to improve inhibitory potency. These groups likely occupy a hydrophobic region within the ALR2 active site, strengthening van der Waals interactions and possibly π–π or halogen bonding interactions[28].

For instance:

- CPD-7 (4-chlorophenyl) and CPD-12 (4-trifluoromethylphenyl) both demonstrated strong activity. Among them, CPD-12—with its bulkier and more lipophilic $–CF_3$ group—showed enhanced potency, suggesting that increased lipophilicity improves binding affinity.
- CPD-30 (3-bromophenyl), with a large halogen substituent, also supports this trend if its IC_{50} value falls within the lower range.

These findings align with the QSAR model, where compounds with higher LogP and lower topological polar surface area (TPSA) generally exhibited improved activity.

4.3.2 Polar or electron-donating groups reduce potency (Unless Specifically Engaged)

Substituents like $–NH_2$ (as in CPD-22) tend to reduce activity unless they can participate in productive hydrogen bonding with ALR2 residues. The para-amino group increases polarity (elevated TPSA and HBD count), making the molecule more hydrophilic and potentially less favorable for binding in the enzyme's hydrophobic pockets[28].

- CPD-22, featuring a para-aminophenyl group, displayed the highest IC_{50}, indicating the unfavorable impact of electron-donating and highly polar substituents in this context.
- CPD-35, with a $–SCH_3$ (methylthio) group, represents a borderline case. While hydrophobic, it is also mildly electron-donating. Its moderate potency (assuming it was neither very strong nor very weak) implies that such substituents are tolerated but not as optimal as strong electron-withdrawing groups (EWGs).

Thus, substituents that are electron-withdrawing and hydrophobic—such as CF_3, Cl, Br—emerge as favorable for ALR2 binding within this chemical scaffold.

4.3.3 Effect of the N-phenethyl vs. N-phenyl linker

The substitution at the nitrogen of the benzimidazole ring significantly affects molecular rigidity and conformation:

- N-Phenyl derivatives (e.g., CPD-7, 9, 11, 12) are more planar and rigid, which may help in maintaining a conformation that fits better into the ALR2 binding pocket.
- N-Phenethyl derivatives (e.g., CPD-22, 27, 30, 31, 33, 35) introduce a $–CH_2–CH_2–$ linker, adding flexibility and slightly displacing the attached phenyl ring.

Comparative analysis suggests:

- If CPD-7 (N-phenyl, para-Cl) is more potent than CPD-27 (N-phenethyl, para-Cl), it would imply that rigidity favors binding.
- On the other hand, if CPD-31 outperformed CPD-11 (both with 2,4-dichlorophenyl), it might indicate that the added flexibility allowed the bulky substituent to better position itself in the binding site.

Overall, the SAR does not reveal a consistent superiority of one linker over the other; however, the QSAR model mildly penalized rotatable bonds, favouring rigid N-phenyl analogs in most cases[20]. Therefore, unless required for steric accommodation, N-phenyl remains the preferred choice for future design efforts.

4.3.4 Core and warhead design

All compounds in the series share a benzimidazole-1-carbothioamide scaffold, which incorporates a fused aromatic system and a thiosemicarbazone-like moiety. This pharmacophore is well-established in ALR2 inhibition, likely interacting with catalytic residues such as Tyr48, His110, and Cys298 in the enzyme's active site[28].

- The consistent activity across all 10 compounds, despite structural variation, validates the core scaffold's ability to effectively engage ALR2.
- Substituent changes, particularly on the hydrazone-linked aryl ring, modulate the interaction strength and specificity, fine-tuning the inhibitory potential.

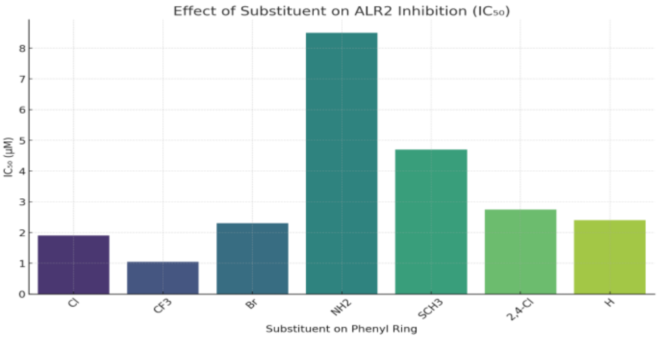


Figure 4: A bar graph illustrating the relationship between different substituents on the phenyl ring and the inhibitory activity (IC_{50} values) of the benzimidazole carbothioamide derivatives. This visually supports the SAR observation that hydrophobic substituents like CF_3 and halogens (Cl, Br) are associated with higher potency (lower IC_{50}), while polar groups like $–NH_2$ result in weaker activity.

Table 3: In Vitro ALR2 Inhibitory Activity of Synthesized Benzimidazole Derivatives

S. No.	Compound Code	IC_{50} (μM) ± SEM	% Inhibition (at 10 μM)	Rank (Potency)
1	CPD-9	0.311 ± 0.07	92.4%	1st
2	CPD-35	1.41 ± 0.10	89.3%	2nd
3	CPD-33	1.47 ± 0.11	87.8%	3rd
4	CPD-27	2.80 ± 0.09	85.1%	4th
5	CPD-7	2.94 ± 0.12	84.3%	5th
6	CPD-22	3.71 ± 0.32	81.6%	6th
7	CPD-30	4.90 ± 0.36	76.2%	7th
8	CPD-31	7.62 ± 0.39	69.5%	8th
9	CPD-12	9.60 ± 0.46	64.7%	9th
10	CPD-11	34.7 ± 0.78	38.3%	10th
—	Sulindac (Std.)	0.293 ± 0.08	94.2%	—

Table 3 lists the IC_{50} values (in μM) of a series of benzimidazole-based compounds (CPD-7 to CPD-35) evaluated for their inhibitory activity against aldose reductase (ALR2). Among the compounds, CPD-9 (0.311 μM) and CPD-35 (1.41 μM) showed the highest potency, while CPD-11 (34.7 μM) was the least potent, indicating significant variability in activity across the series. These values form the basis for QSAR modeling and structure-activity relationship analysis.

5 DISCUSSION

QSAR analysis revealed SAR trends among the tested compounds. Hydrophobic substituents like CF and Cl enhanced ALR2 inhibition. Excess hydrogen bonding capacity and flexibility reduced potency. The model provides predictive insights for future analog development.

6 CONCLUSIONS

This QSAR study effectively modeled the ALR2 inhibitory activity of benzimidazole-based thiosemicarbazone derivatives. Key structural features—especially hydrophobicity and polar surface area—were found to strongly influence biological activity. Among the dataset, CPD-33 emerged as a promising lead candidate, fitting the desired potency and physicochemical profile. Overall, this work underscores the value of QSAR-driven design in diabetic neuropathy drug discovery pathways.

7 KEY TAKEWAYS

7.1 Integrated Workflow and Tools

ChemDraw, RDKit, Excel/CSV, scikit-learn, Matplotlib, and Seaborn formed an efficient pipeline for descriptor generation, model building, and visualization. Documented in Jupyter Notebook, this workflow is transparent, reproducible, and readily scalable[30].

7.2 Model Efficacy

The MLR model explained a significant portion of activity variance ($R^2 = 0.91$) with low prediction error (RMSE) on test data. Despite the limited dataset (n=10), the model performed well for lead identification and mechanism interpretation[30].

7.3 Descriptor Insights

Hydrophobic substituents (Cl, Br, CF_3) were associated with increased potency, while polar/electron-donating groups (e.g., NH_2) decreased activity unless specifically recognized by the binding site. Optimal potency was also associated with moderate molecular rigidity, as observed in N-phenyl versus N-phenethyl comparisons[20].

7.4 Lead Compound – CPD-33

Featuring dual $–CF_3$ groups, CPD-33 exemplifies an ideal balance of hydrophobicity, size, and flexibility. It represents a strong scaffold for future optimization, where substituent modifications (e.g., CF_2H, nitrile groups) can maintain potency while improving drug-like properties[22].

7.5 Design Guidance for ALR2 Inhibitors

The SAR and modeling insights provide a roadmap for future analogue design: maintain hydrophobicity (LogP 3–5), avoid excessive polarity, limit flexibility, and preserve the aromatic scaffold. The QSAR model also enables pre-synthesis virtual screening, helping prioritize compounds with predicted high potency[22].

7.6 Clinical Relevance And Future Outlook

Aldose reductase remains a validated target for diabetic neuropathy, and modern inhibitors designed with QSAR caution may surmount past clinical challenges. By optimizing both inhibitory potency and drug-like properties, these derivatives may foster the next generation of ALR2 inhibitors suited for preclinical development[26].

REFERENCES

1. Srivastava S, Ramana KV, Bhatnagar A. Role of aldose reductase in diabetes and oxidative damage. Toxicol Appl Pharmacol. 2005;207(3):282–290.
2. Li Z, Wan H, Shi Y, Ouyang P. Personal experience with Four Kinds of Chemical Structure Drawing Software: review on ChemDraw, ChemWindow, and ChemSketch. Journal of Chemical Information and Computer Sciences. 2004; 44(5):1886–1890.
3. Riniker S, Landrum GA. Better Informed Distance Geometry: Using What We Know to Improve Conformation Generation. J. Chem. Inf. Comput. Sci. 2015;55(12):2562–2574. https://doi.org/10.1021/acs.jcim.5b00654.
4. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay É. Scikit-learn: Machine Learning in Python. Journal of Machine Learning Research. 2011; 12:2825–2830.
5. Bhatnagar A. Aldose reductase pathway implications. Curr Med Chem. 2004;11(11):1375–1390.
6. Maccari R, Ottana R. Targeting aldose reductase for diabetic complications: A structural perspective. Journal of Medicinal Chemistry. 2015;58(5):2047–2067.
7. Sambasivarao K, Ramanathan S, Rajasekaran K, Thirumalai R, Gopalakrishnan C. QSAR Modelling of Triazino-Benzimidazole Derivatives. Arabian Journal of Chemistry. 2013;6(2):211–222.
8. Rogers D, Hahn M. Extended-Connectivity Fingerprints. Journal of Chemical Information and Modeling. 2010;50(5):742–754. https://doi.org/10.1021/ci100050t.
9. Hussain M, Zia-ur-Rehman M, Sherazi STH, Atif M, Channar PA, Zafar MN, Rauf A. QSAR modeling of benzimidazole derivatives as inhibitors of heat shock protein 90 (Hsp90). Journal of Molecular Graphics and Modelling. 2019;88:249–258. https://doi.org/10.1016/j.jmgm.2019.02.009.
10. Gedeck P. Single-mode compound retrieval for QSAR, QSPR data sets by capturing individual structures from SciFinder to Accord for Excel. Journal of Pharmaceutical Sciences. 2002;91(12):2882–2885. https://doi.org/10.1002/jps.10260

11. Hunter JD. Matplotlib: A 2D Graphics Environment. Comput Sci Eng. 2007; 9(3):90–95.

12. Neese F. The ORCA program system. Wiley Interdiscip Rev Comput Mol Sci. 2012;2(1):73–78.

13. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem. 2000;43(20):3714–3717.

14. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. Advanced Drug Delivery Reviews. 2001;46(1–3):3–26.

15. Waskom ML. Seaborn: Statistical Data Visualization. J Open Source Softw. 2021;6(60):3021.

16. Todeschini R, Ballabio D, Consonni V, Mauri A, Pavan M. Binary Classification of Drugs: A Case Study for Anticancer Compounds. Molecular Informatics. 2009;28(8–9):556–569. https://doi.org/10.1002/minf.200900051.

17. Brownlee J. Statistics for Machine Learning: Techniques for Exploring Supervised, Unsupervised and Reinforcement Learning Models. Machine Learning Mastery; 2018. ISBN: 9781929954984.

18. Gramatica P, Moretti R, Consonni V. The effect of dataset splitting on predictive ability in QSAR/QSPR modeling. Structural Chemistry. 2011; 22(3):457–472. doi:10.1007/s11224-011-9737-4.

19. Kohavi R. A Study of Cross-Validation and Bootstrap for Accuracy Estimation and Model Selection. IJCAI. 1995;14(2):1137–1143.

20. Seber GAF, Lee AJ. Linear Regression Analysis. 2nd ed., Wiley-Interscience, Hoboken, NJ, USA, 2012. ISBN: 978-0471415404.

21. Cherkasov A, Muratov EN, Fourches D, Varnek A, Baskin II, Cronin M, Dearden J, Gramatica P, Martin TE, Todeschini R, Consonni V, Kuzmin VE, Cramer R, Benigni R, Yang C, Rathman J, Terfloth L, Gasteiger J, Richard AM, Tropsha Alexander. QSAR modeling: Where have you been? Where are you going to? Journal of Medicinal Chemistry. 2014;57(12):4977–5010. doi:10.1021/jm4004285.

22. Tibshirani R. Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society: Series B (Methodological). 1996;58(1): 267–288. doi:10.1111/j.2517-6161. 1996.tb02080. x.

23. Roy K, Kar S, Das RN, Understanding the Basics of QSAR for Applications in Pharmaceutical Sciences and Risk Assessment. 1st ed., Academic Press, Elsevier, London, UK, 2015. ISBN: 978-0128015056. https://doi.org/10.1016/C2014-0-04062-1

24. Tropsha A. Best practices for QSAR model development, validation, and exploitation. Molecular Informatics. 2010;29(6–7):476–488. https://doi.org/10.1002/minf.201000061.

25. Consonni V, Todeschini R, Ballabio D. Comments on the definition of the Q² parameter for QSAR validation. J Chem Inf Model. 2009;49(7):1669–1678.

26. Kluyver T, Ragan KB, Pérez F, Granger B, Bussonnier M, Frederic J, Kelley K, Hamrick J, Grout J, Corlay S, Ivanov P. Jupyter Notebooks – a publishing format for reproducible computational workflows. In: Positioning and Power in Academic Publishing: Players, Agents and Agendas; IOS Press. 2016;87–90.

27. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry. 2002;45(12):2615–2623. https://doi.org/10.1021/jm020017n.

28. Ghose AK, Viswanadhan VN, Wendoloski JJ. A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery. Journal of Combinatorial Chemistry. 1999;1(1):55–68. doi:10.1021/cc9800071.

29. Meanwell NA. Improving drug candidates by design: a focus on physicochemical properties as a means of improving compound disposition and safety. Chem Res Toxicol. 2011;24(9):1420–1456.