

Computational Innovation of Novel Tetrazolium-Nicotinamide Derivatives as HPPD Inhibitors for Next- Generation Herbicides

TUKUR SAIDU

saidutukur@gmail.com

Ahmadu Bello University

Gideon Adamu Shallangwa

Ahmadu Bello University

Gbolahan Oladipupo Oduselu

West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), P.O Box LG 54, University of Ghana, Accra, Ghana

Adamu Uzairu

Ahmadu Bello University

Zakari Ya'u Ibrahim

Ahmadu Bello University

Muhammad Tukur Ibrahim

Ahmadu Bello University

Bitrus Dawi TARFA

Federal University of Agriculture, Mubi (FUAMB)

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AUTHORS:

- 1- Saidu Tukur (Corresponding author)
Department of chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria
Email: saidutukur@gmail.com
- 2- Professor Gideon Adamu Shallangwa
Department of chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria
Department of chemistry, Federal University of Agriculture, Mubi (FUAMB)
Email: gashallangwa@gmail.com
- 3- Dr. Gbolahan O. Oduselu
West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), P.O Box
LG 54, University of Ghana, Accra, Ghana
Email: gboduselu@gmail.com
- 4- Professor Adamu Uzairu
Department of chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria
Email: adamuuzairu@yahoo.com
- 5- Dr. Zakari Ya'u Ibrahim
Department of chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria
Email: zakariyyadibrahim@gmail.com
- 6- Dr. Muhammad Tukur Ibrahim
Department of chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria
Email: mtibrahim1988@gmail.com
- 7- Professor Bitrus Dawi TARFA,
Federal University of Agriculture, Mubi (FUAMB)
Email: btarfa@gmail.com

ABSTRACT:

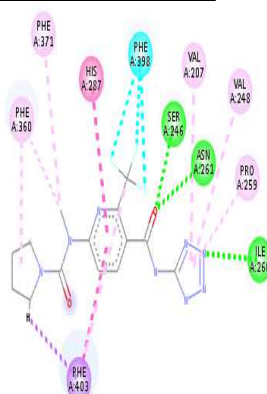
The escalating challenge posed by herbicide-resistant weed populations has intensified the search for new herbicidal agents with enhanced performance and distinct mechanisms of action. The reticence of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an essential enzyme in plant tyrosine degradation and carotenoid biosynthesis, remains an effective strategy for disrupting photosynthesis and causing plant lethality. In the contemporary work, a comprehensive computer coupling approach was employed to design and optimize tetrazolium nicotinamide-derived HPPD inhibitors using the 1TFZ crystal structure. This approach integrated 2D-QSAR analysis, molecular docking, structure-guided molecular modification, agrochemical-oriented ADMET evaluation, and molecular dynamics (MD) simulations. A reliable MLR-GFA QSAR model was established, demonstrating strong statistical robustness and acceptable predictive capability. Docking studies identified compounds 25-28 as potential HPPD inhibitors, with compound-26 displaying the most favorable binding affinity and a stable interaction pattern characterized by hydrogen bonding, halogen contacts, and aromatic interactions within the enzyme active site. Based on QSAR insights and binding-site interactions, focused modification at the -CF₃ substituent generated five new analogues (26a, 26b, 26c, 26d and 26e), four of which exhibited improved predicted herbicidal activity and enhanced binding behavior compared with the original scaffold. These modifications were attributed to improved electronic properties and better steric accommodation within the catalytic pocket. The evaluation of agrochemical-portrait and ADMET-related parameters indicated that, the premeditated compounds offer improved physicochemical profiles, environmental compatibility, and plant-specific safety compared to the commercial HPPD inhibitor mesotrione, with compound-26 emerging as the most promising candidate. In addition, a performance of 100 ns MD simulations verified the stability of the HPPD- compound-26 complex, as shown by consistent structural parameters, reduced flexibility, stable solvent exposure, and persistent hydrogen-bond formation. Following system equilibration, sustained hydrogen bonding highlighted the key stabilizing contributions of the tetrazolium and nicotinamide functional groups. In conclusion, compound-26 represents a strong structural template for next-generation HPPD-targeting herbicides, while -CF₃- position modification is confirmed as an operative approach for enlightening binding stability and predicted herbicidal efficacy. The study provides a solid computational framework to support future synthesis and experimental validation.

GRAPHICAL ABSTRACT

2D- QSAR

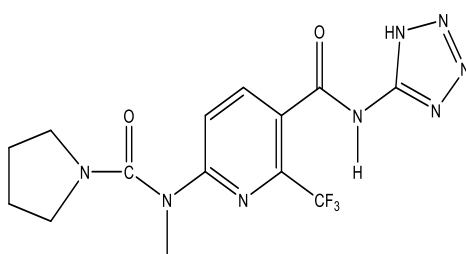
- ✓ LOF = 0.01824700
- ✓ $R^2 = 0.95761800$
- ✓ $R^2_{pred.} = 0.62928100$
- ✓ $Q^2_{cv} = 0.92339200$

Molecular docking



2D - receptor ligand -26 complex

Lead compound (ligand-26)



-CF₃ - Modification

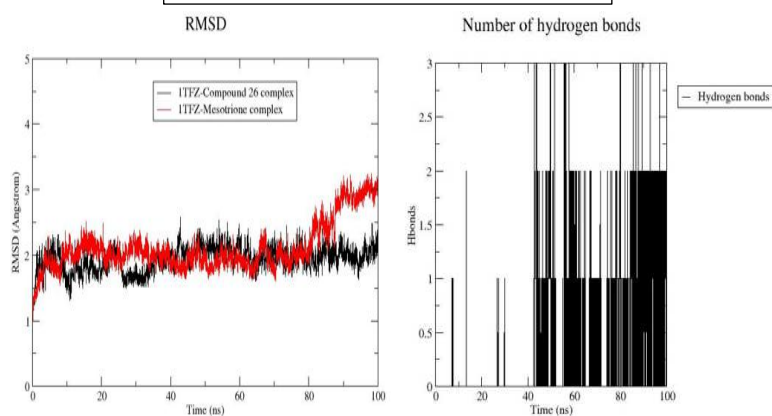
Newly design derivatives

- ✓ 26a
- ✓ 26b
- ✓ 26c
- ✓ 26d
- ✓ 26e

ADMET & Herbicide-likeness

- ✓ High QED score
- ✓ Low toxicity
- ✓ Good pharmacokinetics

Molecular Dynamics Simulation



Stable RMSD & H- bond

1.0 Introduction:

Herbicides show a crucial character in modern agriculture by enabling efficient and large-scale weed control, which is indispensable for supporting crop efficiency and mechanized farming systems. The intensive and long-term reliance on herbicidal chemicals has unintentionally accelerated the emergence of resistant weed species, substantially increasing yield losses and complicating weed control efforts across global agricultural systems (Tukur et al., 2019). In addition to competing aggressively through crops for indispensable resources such as light, nutrients, and moisture, wildflowers frequently function as alternative hosts for plant pathogens, insect vectors, and viral agents. This role often facilitates the spread of diseases, further reducing crop efficiency and threatening food security. Nevertheless, herbicides continue to represent unique of the most practical, affordable, and efficient approaches to weed suppression and remain indispensable within integrated weed management frameworks (Kraehmer et al., 2014). Additionally, subsequently the commercialization of 2,4-dichlorophenoxyacetic acid in 1948, herbicide research and development have undergone substantial transformation. The molecular architecture of this early herbicide provided a conceptual foundation that has guided the evolution of subsequent herbicidal chemistries and continues to influence modern inhibitor development (Peterson et al., 2018). Among the various enzymatic targets investigated, the receptor HPPD has gained prominence owing to its indispensable character in vegetal biochemical pathways.

Moreover, the receptor HPPD shows a fundamental role in the interruption of tyrosine by transforming Para -hydroxyphenylpyruvic acid (HPPA) into homogentisic acid (HGA), a fundamental herald for the manufacture of the two most vital compounds, plastoquinone and tocopherols (Chen et al., 2023). Plastoquinone is mandatory for electron conveyance through photosynthesis and for carotenoid biosynthesis, while tocopherols function as antioxidants that safeguard plant materials from oxidative destruction. When HPPD activity is blocked, these essential processes are disrupted, leading to carotenoid loss, oxidative stress, visible leaf bleaching, and ultimately plant death (Dong et al., 2023). Because of this actual genre of action, HPPD inhibitors require for further than four periods and are appreciated for their broad-spectrum activity, relatively favorable safety profiles, and usefulness in controlling several herbicide-resistant weed species (Nan et al., 2021).

Furthermore, extensive researches of how these inhibitors intermingle with HPPD at the molecular level have delivered appreciated guidance for structure-based herbicide design.

Crystallographic analyses, so that, the mesotrione–AtHPPD complex (PDB ID: 1TFZ), show that strong inhibition largely depends on coordination between the inhibitor's triketone group and the active-site ferrous ion, supported by stabilizing π – π collaborations amid aromatic moiety and residues aligning the binding pocket (Lian et al., 2023). Perceptions from these revisions have permitted the growth of greater than twenty commercial HPPD-targeting herbicides, generally grouped into triketones, pyrazoles, and isoxazoles (diketonitriles) according to their core structures (Cai et al., 2024). Despite their success, most of these compounds share a preserved chemical moiety (2-benzoylethylene-1-ol) motif that is vital for metal coordination in some catalytic site (Chen et al., 2023). Although this structural feature contributes to high potency, its widespread use limits chemical diversity and may increase the likelihood of resistance. While confirmed resistance to HPPD inhibitors is still uncommon and mainly linked to non-target-site mechanisms, continued selection pressure could eventually promote target-site resistance (Owen, 2016). For this reason, there is growing interest in exploring new chemical scaffolds, expanding pharmacophore diversity, and adopting innovative design strategies to sustain the long-term effectiveness of HPPD-directed herbicides (Nishio et al., 2025).

In addition, over years, electronic couple with computer for chemical design has materialized as a influential tool in both pharmaceutical and agrochemical research, offering efficient solutions to the challenges associated with traditional discovery channels. Conventional agrochemical development is often expensive, labor-intensive and time-consuming, with a declining success rate in advancing candidates from early discovery to market approval (Sagiru et al., 2023). To overcome these limitations, computational procedures such as QSAR molding, coupling molecular docking and incredible molecular dynamics (MD) calculations, and ADMET prediction have been broadly adopted to accelerate lead identification and optimization while, reducing development costs and timelines (Abdullahi et al., 2025). The QSAR approaches enable the quantitative extrapolation of biological action centered on molecular descriptors, whereas molecular docking delivers comprehensive discernments into ligand–protein interaction patterns within enzyme active sites (Ibrahim et al., 2023). Complementary ADMET analyses further assess pharmacokinetic behavior, toxicity risks, and overall drug-likeness, supporting informed decision-making during lead optimization (Zakari et al., 2020). Building on our previous work, where 2D-QSAR molding, coupling molecular docking, and pharmacokinetic profiling were successfully applied to assess the herbicidal potential of urea and phenoxy acid derivatives (Tukur et al., 2019), the present

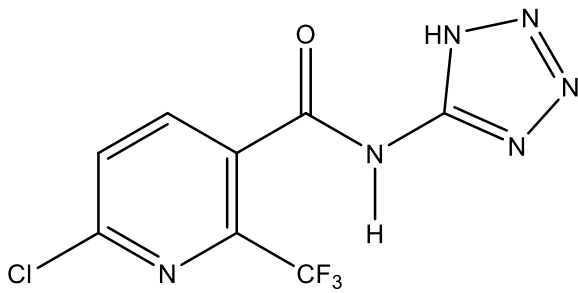
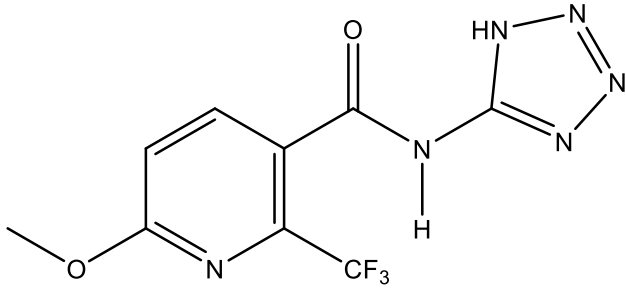
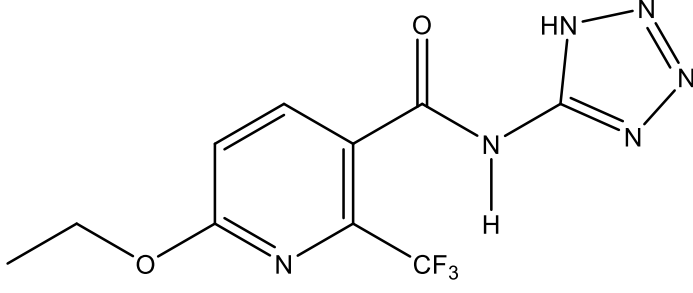
study will integrates advanced *in-silico* strategies to explore and optimize novel HPPD inhibitor scaffolds with improved efficacy and resistance-mitigating potential.

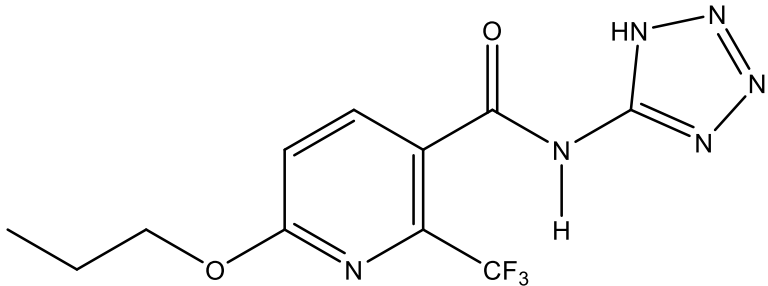
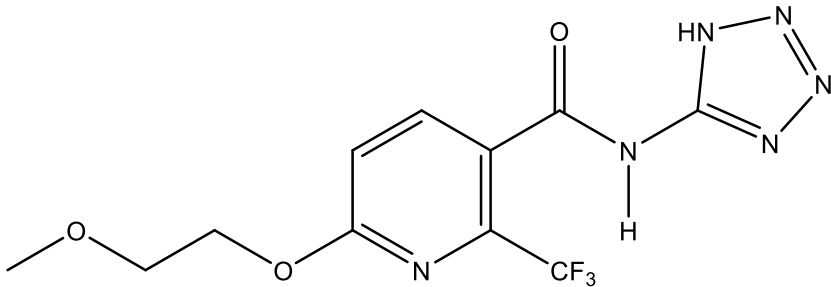
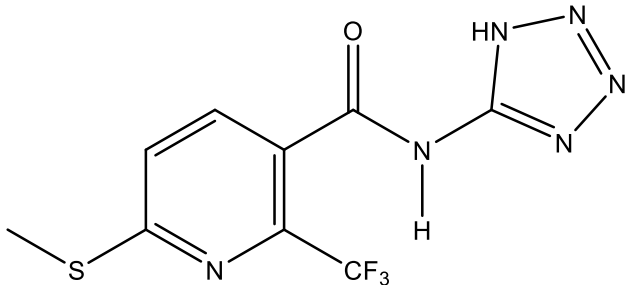
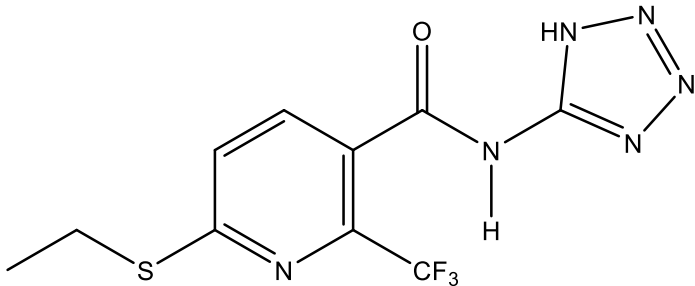
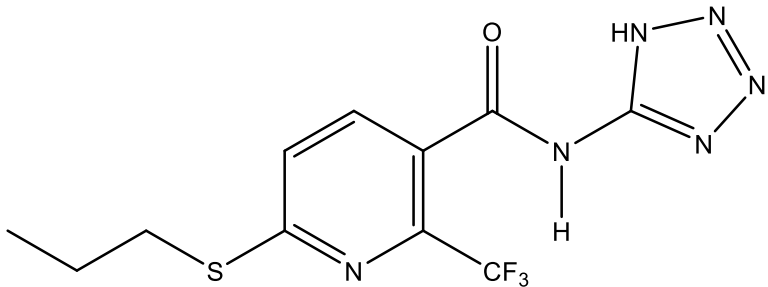
2.0 Materials and Methods

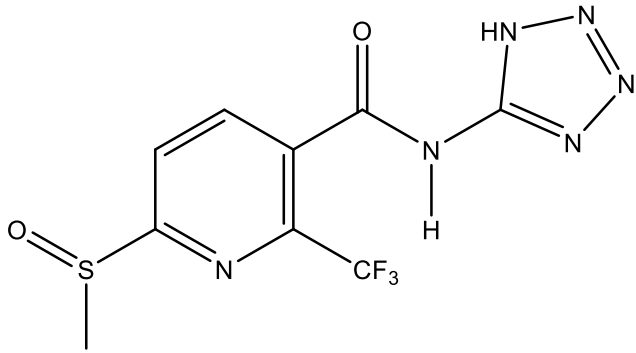
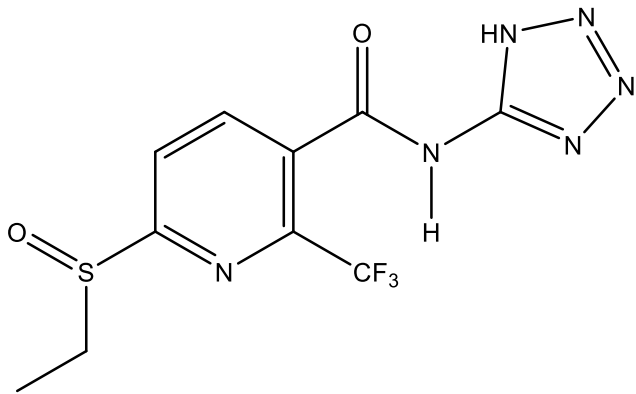
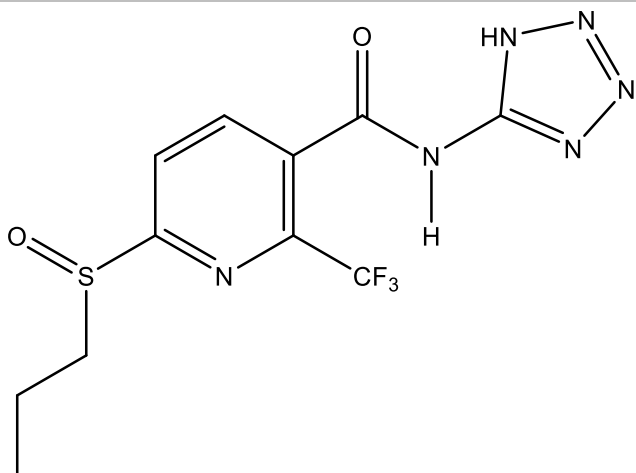
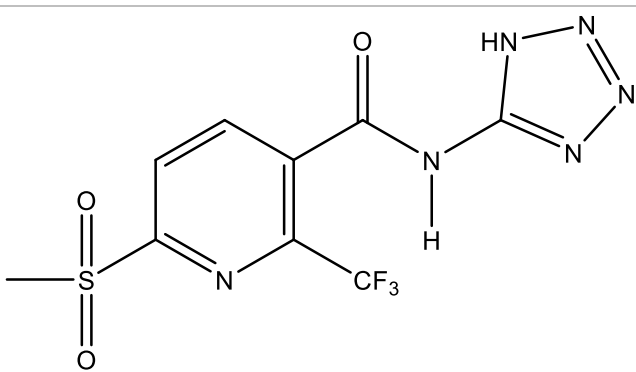
2.1. Assortment of dataset

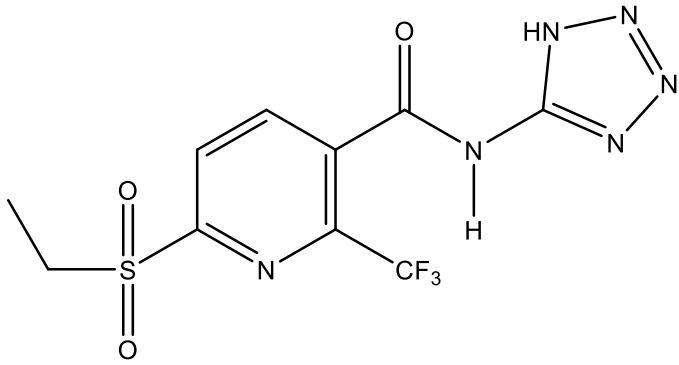
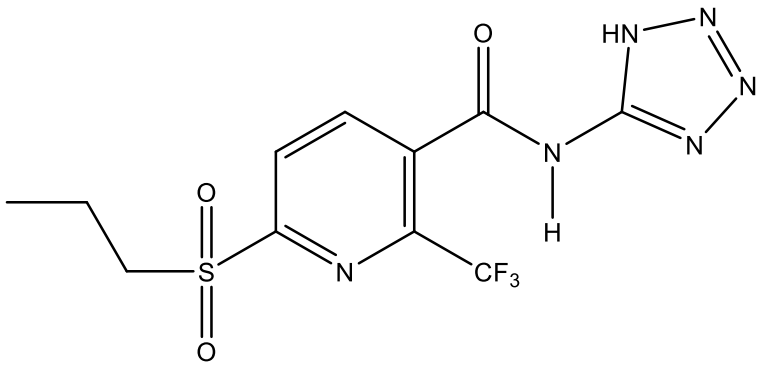
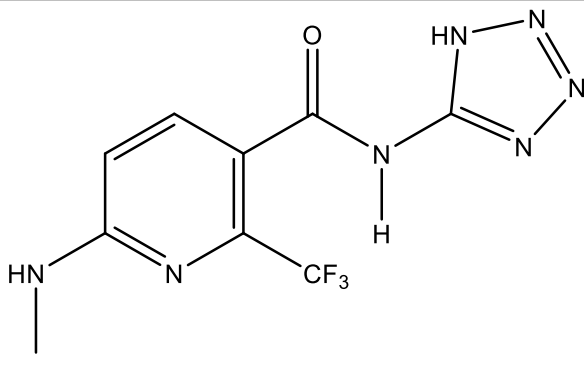
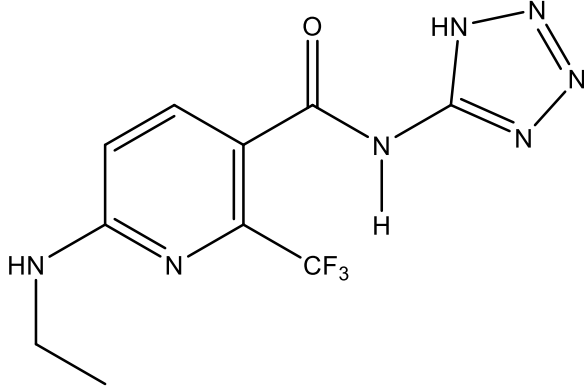
The designated compounds consist of 35 Tetrazolium nicotinamide series recognized as prospective 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-centered herbicides. The inhibitory concentration of both complexes were evaluated by using its half-maximal inhibitory concentration (IC_{50}), expressed in micro molar (μM) (Chen *et al.*, 2023). These IC_{50} values were subsequently transformed to their logarithmic form ($pIC_{50} = -\log IC_{50} \times 10^{-6}$) to enhance the linearity of the dataset as presented using Table 1 below.

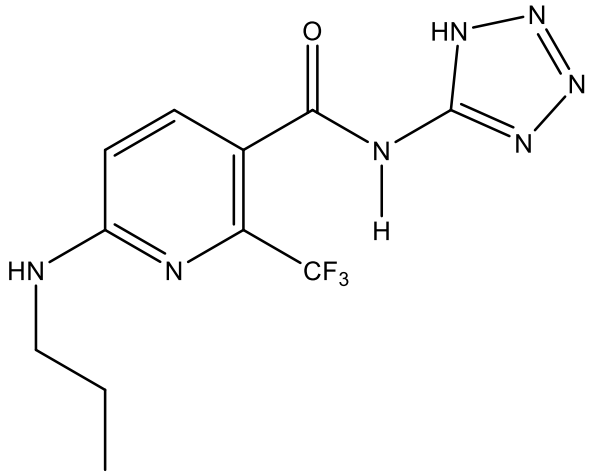
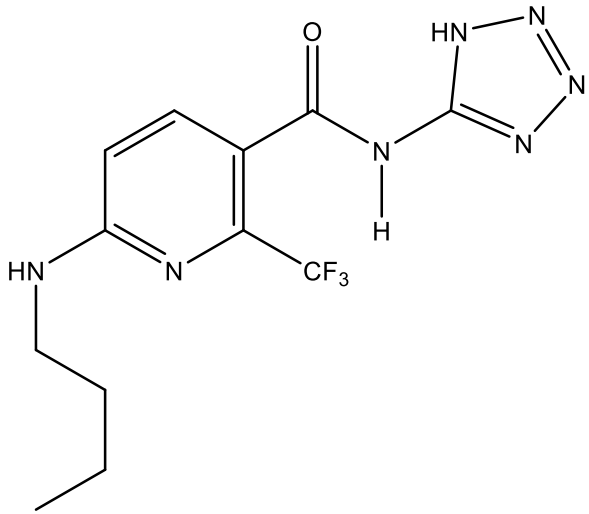
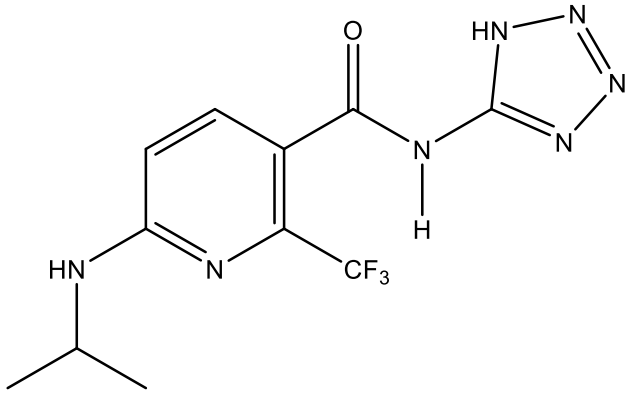
Table 1- 2-Dimensional Arrangements and experimental pIC_{50} values of Tetrazolium nicotinamide derivatives.

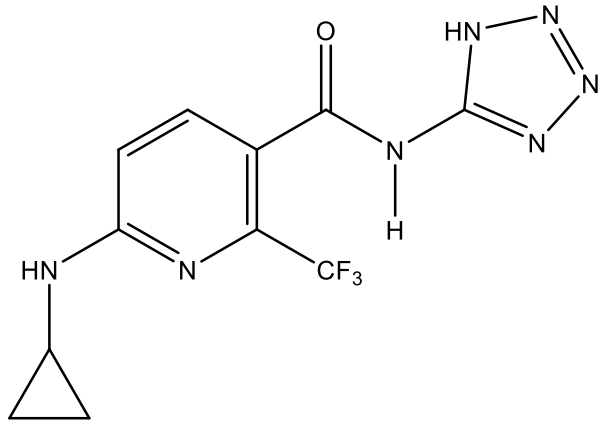
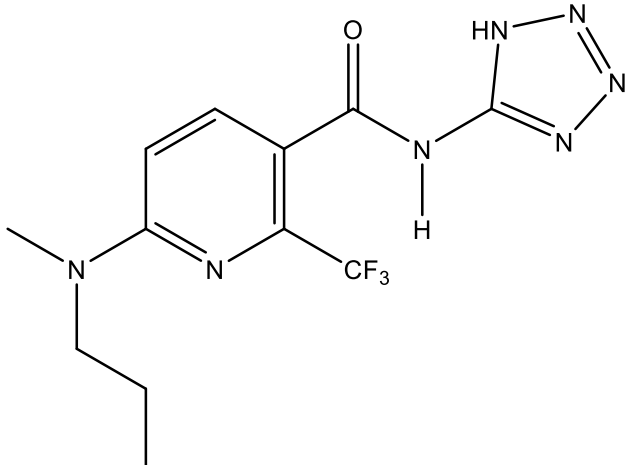
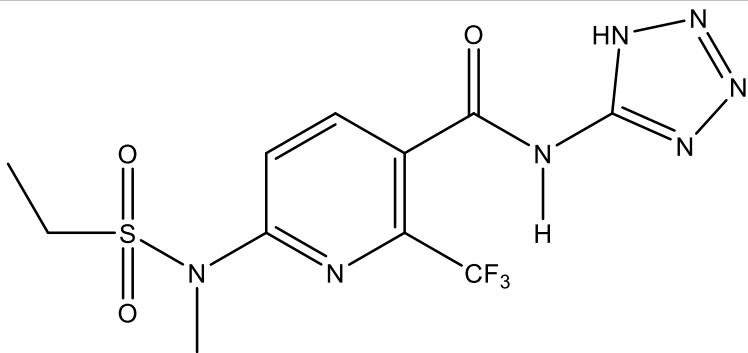
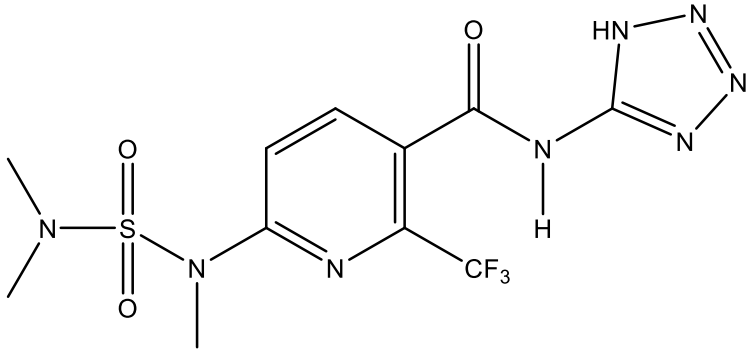
S/no.	2D Structures	pIC_{50}
1		6.730487
2		6.764472
3		6.841638

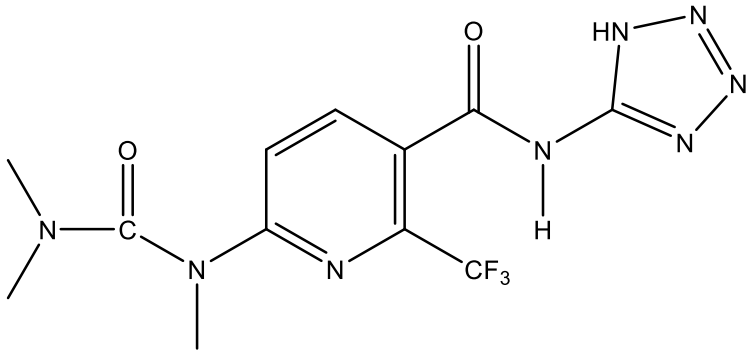
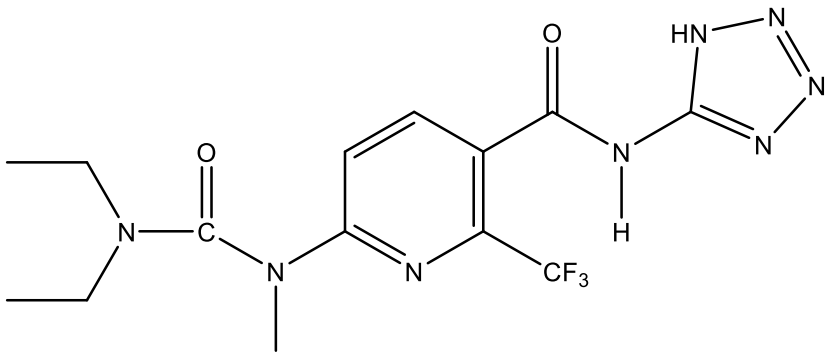
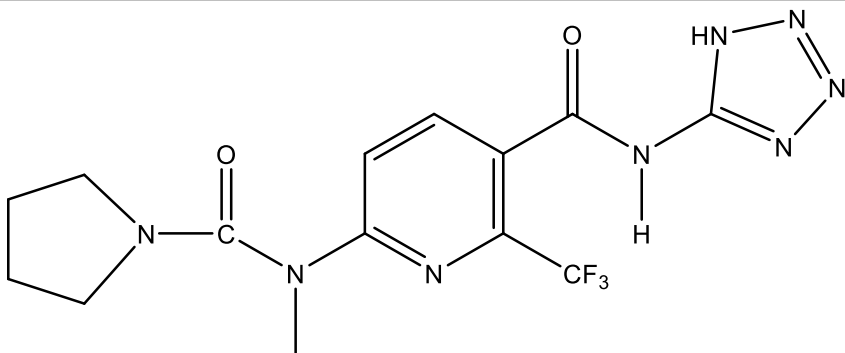
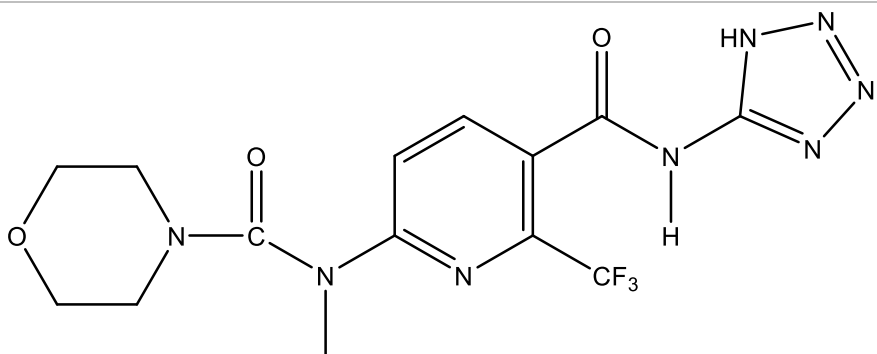
4		6.978811
5		6.614394
6		6.853872
7		7.017729
8		7.075721

9		6.408935
10		6.326058
11		6.372634
12		7.022276

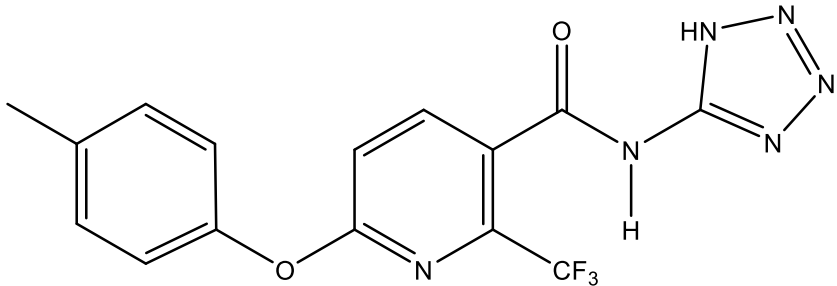
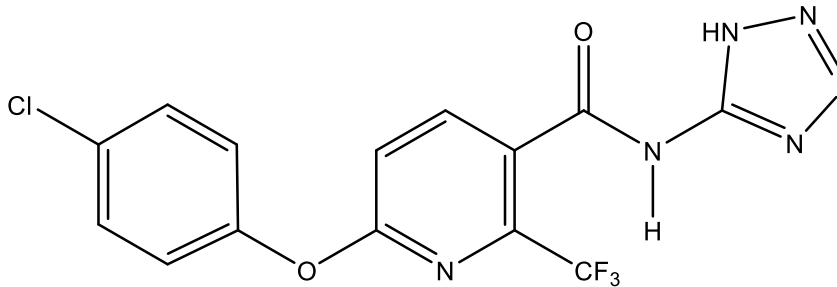
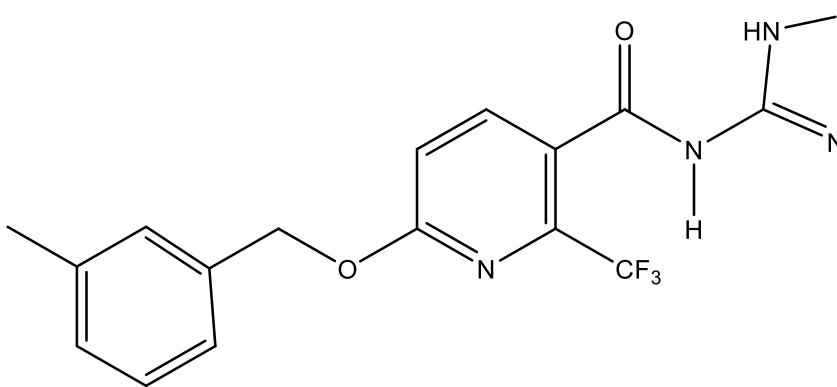
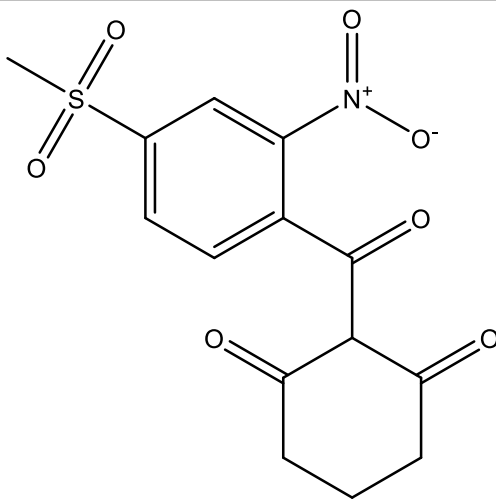
13		7.040959
14		7.055517
15		6.573489
16		6.545155

17		6.505845
18		6.481486
19		6.498941

20		6.492144
21		6.879426
22		6.774691
23		6.89279

24		6.974694
25		7.142668
26		7.013228
27		6.787812

28		7.075721
29		6.844664
30		7.091515
31		7.091515

32		7.102373
33		7.124939
34		7.065502
Mesotione		6.440093

2.2 QSAR Modeling Studies

The 2D molecular configurations of the composites remained cautiously twisted via the ChemDraw 2D- software. These depictions were formerly transformed into 3D orientation models with the incredible Spartan 14 software program, where molecular energy lowering was conceded out by employing the analogy of Molecular Mechanics Force Field (MMFF) method (Zakari et al., 2020). Afterward, the system of Density Functional Theory (DFT) calculations retaining the B3LYP functional with the 6-31G* basis set remained achieved to optimize the geometries and obtain stable conformers with minimum energy. Finally, the software PaDEL calculator was utilized to figure approximately 1,800 molecular descriptor values for all optimized compounds (Sagiru et al., 2023).

2.2.1 Geometry Optimization

Molecular optimization refers to the process of determining molecular symmetry and geometrical pattern or the lowermost energy conformation associated with a molecule (Zakari *et al.*, 2020). The arrangements of the compounds in 2D orientation were primarily sketched using the 2D software sketcher (ChemDraw Ultra version 12.0) and stored in the cdx document file (Ibrahim *et al.*, 2019). The transformed structures remained converted into 3D Cartesian geometrical models and optimized using the Spartan 14.0 software (Abdulfatai *et al.*, 2020). Preliminary optimization was accomplished expending the semi-empirical process to relieve structural strain before conducting the complete DFT modeling. The calculations of DFT were conceded out by expending the Becke three-parameter hybrid functional joined with the Lee–Yang–Parr correlation functional (B3LYP) and the 6-31G* basis set (Malekzadeh & Heshmati, 2013). The completely optimized molecular assemblies remained subsequently saved in the Assembly Data File (SDF) presentation, which is the obligatory input format for the software PaDEL-calculator (Adeniji *et al.*, 2020).

2.2.2 Calculations of Mathematical Descriptors

Molecular descriptor denotes a statistical assessment that represents specific molecular properties derived either through experimental methods or computational algorithms (Ibrahim *et al.*, 2023). The exact descriptors were obtained by Spartan '14 software version 1.1.2, a computational tool designed for quantum chemical calculations (Zakari *et al.*, 2020). Additionally, molecular descriptors of different dimensions (1D, 2D, and 3D) were computed by employing PaDEL- software Descriptor calculator version 2.1.8. In total, 1,875

descriptors were generated comprising 1,444, 1 and 2-dimensional descriptors and 431, 3-dimensional descriptors by using together the PaDEL-calculator and Spartan '14 packages (Zakari *et al.*, 2020).

2.2.3 Normalization of Dataset

The actual descriptors generated by the PaDEL calculator remained standardized using a normalization formula based on their range, defined by the upper and lower values, laterally through the dispersion parameters such as standard deviation and variance. This process ensured that, the descriptors were properly scaled before being converted into a normalized distribution (0, 1, 2, 3, 4... N). Moreover, this normalization approach reduces redundancy and minimizes correlations among several number of descriptors, thereby improving the strength and reliability of the analyses that follow (Panchal *et al.*, 2018).

2.2.4. Pretreatment and Partitioning of Data

The pretreatment and partitioning of data was conceded out purposely to enhance descriptor quality, the complete set of calculated molecular descriptors was first manually screened, followed by further refinement using the Kennard-Stone preprocessing approach (Abdullahi *et al.*, 2025). Descriptors showing very low variability were eliminated with a variance threshold of 0.001, while intensely connected variables were detached using a correlation cut-off of 0.80. After this filtering stage, the optimized set remained distributed into model-building and external authentication sets concluded by the approach of Kennard & Stone sampling method, ensuring a balanced and representative distribution of compounds in both groups (Saporo *et al.*, 2012).

2.2.5. Development of Test Set and Training set.

In mandate to create a consistent model equation, the dataset of 35 derivatives was fragmented into twofold sets (training and testing sets) by the approach of Kennard-Stone algorithm. This segregation was achieved with software developed by the Drug Theoretical Chemo-informatics Laboratory (DTC Lab), where 70% out of 100% of the molecules were allocated to the progress set and the remaining 30% out of 100% were kept for testing (Joseph, 2022). The Kennard & Stone technique is extensively adopted in contemporary QSAR studies because it produces balanced and representative datasets. Consequently, the

model building set was used for model development, while the testing set provided an independent external validation of the resulting model (Xu & Goodacre, 2018).

2.2.6 Assessment of the QSAR Equations

The model equation presentation was evaluated by expending several arithmetical factors, including N (compound numeral) P (descriptor number), R^2 (constant of determination), F-test (Fisher's assessment), Q^2_{cv} (correlation coefficient for cross-validation), and R^2_p (prognostic squared correlation constant for the peripheral testing set). Among these, R^2 and Q^2 are the most critical indicators for determining the dependability and sturdiness of a validated QSAR model (Olasupo et al., 2020; Arthur *et al.*, 2020). A QSAR model is measured as statistically substantial when it satisfies the resulting criteria: $R^2 > 0.6$, $Q^2_{cv} > 0.6$, and $R^2_p > 0.5$. Based on these statistical measures, the model exhibiting the highest Q^2_{cv} and R^2 values was designated as the utmost reliable and extrapolative model (Adawara et al., 2020).

2.2.7. Internal Corroboration of the Designated Model

The core validation (Internal validation) represents the initial phase in assessing the reliability of a model. The outcomes of this authentication process demonstrate the model's degree of stability and predictive consistency (Abdulfatai *et al.*, 2020). However, the constant of determination (R^2) reflects the proportion of the total discrepancy elucidated by the model, serving as a key indicator of its performance. A value of R^2 approaching 1.0 signifies a highly reliable and well-fitted model. Among the various internal validation parameters, R^2 is one of the utmost repeatedly employed and is calculated using Equation 1, as presented below:

$$R^2 = 1 - \frac{\sum(Y_{obs.} - Y_{pred.})^2}{\sum(Y_{obs.} - Y_{training})^2} \quad (1)$$

Where, The terms $Y_{obs.}$, $Y_{pred.}$, and $Y_{training}$ symbolize the practical, predicted, and average inhibition concentrations of the composites within the development dataset, respectively (Umar et al., 2020). The value of R^2 tends to increase with the numeral of descriptors included in the designated model; hence, it ought not to be the sole criterion for evaluating model performance. To account for the sum of descriptors, the value of R^2 is attuned accordingly. The attuned R^2 can be determined using Equation 2 provided below:

$$R_{adj.}^2 = 1 - (1 - R^2) \frac{n - 1}{n - p - 1} = \frac{(n - 1)R^2 - p}{n - p + 1} \quad (2)$$

In this context, n signifies the entire number of designated compounds in the development dataset, while p , and signifies the numeral of descriptors integrated into the equation (Tukur *et al.*, 2019). Additionally, the extrapolative competence of the equation model stood evaluated using the Friedman's Lack of Fitting (LOF), it serves as one of the key parameters for internal validation. The LOF value was computed according to Equation 3 presented below:

$$LOF = \frac{SEE}{\left(1 - \frac{c + dp}{N}\right)^2} \quad (3)$$

Here, SEV refers to the standard error of valuation; it is also recognized as the standard deviation (SD). The variable p , represents the number of autonomous variables included in the model, and the letter d , stands for a user defined leveling factor, c denotes the numeral of expressions in the model and N indicates the total quantity of compounds within the development dataset. (Mahmud *et al.*, 2020) A model is considered robust when it exhibits a lower SEE value. The SEE can be determined using Equation 4, as shown below:

$$SEE = \sqrt{\frac{(Y_{exp.} - Y_{pred.})^2}{N - P - 1}} \quad (4)$$

In this equation, $Y_{exp.}$ and $Y_{pred.}$ correspondingly signify the observed and predicted inhibition concentrations of the molecules within the development dataset ((Ibrahim *et al.*, 2021). Here, N refers to the whole quantity of compounds included in the development set, while P implies the quantity of mathematical descriptors exploited in building the model (Grammatica, 2025). In addition, a key metric used to evaluate the reliability of a model is the essential Leave-One-Out (LOO) cross-validation approach. The cross-validated regression coefficient (Q^2_{cv}) analysis, which reflects the extrapolative accuracy of the model, can be premeditated using Equation 5, as illustrated below:

$$Q^2_{cv} = 1 - \frac{\sum_{i=1}^n (H_{exp.} - W_{pred.})^2}{\sum_{i=1}^n (H_{exp.} - \bar{H})^2} \quad (5)$$

$H_{exp.}$, $W_{pred.}$, and $\bar{H}$ represent the investigational, expected, and average inhibition concentration values of the compounds in the training dataset, respectively (Ibrahim et al., 2020).

2.2.8. External assessment of the selected QSAR Model

The created QSAR regression equation underwent external validation to verify its reliability and predictive strength. The estimation of the external endorsement was built on the R^2 assessment obtained for the ligands in the testing dataset (Mitra et al., 2010). Accordingly, the model's external predictive capability and extrapolation potential were determined using the regression coefficient equation presented as Equation 6 below:

$$R_{test}^2 = 1 - \frac{\sum (M_{pred.test} - M_{exp.test})^2}{\sum (M_{pred.test} - \bar{M}_{training})^2} \quad (6)$$

$M_{pred.test}$, $M_{exp.test}$, represent the forecast and investigational inhibition absorptions of the nominated compounds within the trial dataset, while $\bar{M}_{training}$ denotes the average experimental inhibition absorption of the molecules in the development set (Bahia et al., 2023). Furthermore, the extrapolative performance of the QSAR regression equation was assessed via the compounds excepted from the training procedure. This appraisal was supported out through the root mean of error square (rm^2), considered by Equation 7 as shown below:

$$rm^2 = r_{test}^2 \left(1 - \left| \sqrt{r_{test}^2} - r_{test_o}^2 \right| \right) \quad (7)$$

Where the parameter r_{test}^2 symbolizes the constant of determination for the trial set molecules, although, $r_{test_o}^2$ denotes the squared relationship coefficient derived from the graphical plot of investigational inhibition concentrations against forecast inhibition concentrations of the trial set towards zero intercept (De et al., 2022). The constraints W and W' correspond to the gradients of the regression outlines obtained from plotting the anticipated inhibition concentrations in contradiction of the trial values (Edache et al., 2022). The values of W and W' are determined by the given Equation 8, as articulated below:

$$W = \frac{\sum Y_i \bar{Y}_i}{\sum \bar{Y}_i^2} \quad \text{and} \quad W' = \frac{\sum Y_i \bar{Y}_i}{Y_i^2} \quad (8)$$

Whereas, Y_i and $\bar{Y}_i$ represent the forecast and mean trial inhibition concentrations of the compounds in the training dataset, respectively. Additional statistical relationships between the inhibition concentrations and molecular descriptors were examined using the Y-randomization test, a well-recognized approach for exterior validation of designated QSAR models. Moreover, this procedure, the Y-values (dependent variables) are randomly shuffled, and the novel models are subsequently generated by the similar set of descriptors as in the original, unshuffled model (Onu et al., 2023).

However, a parameter designated as R_p^2 was utilized to evaluate the model's robustness. This parameter adjusts the model's R^2 value by accounting for the variance among the average relationship coefficient squared ($R_{and.}^2$) of the scattered models and the squared relationship constant (R^2) of the original, un-scattered model (Roy et al., 2018). The R_p^2 value was determined using the mathematical expression presented in Equation 9 below:

$$R_p^2 = R^2 \times \sqrt{(R^2 - R_{and.}^2)} \quad (9)$$

The parameter R_p^2 serves to confirm that, the established models remained obtained by chance co-incident. Furthermore, it is generally expected that a reliable and statistically significant model should exhibit and R_p^2 value greater than 0.5, indicating strong predictive validity (Jeong & Choi, 2022).

2.2.9. Y-randomization test

The cR_p^2 parameter is used to attest that the created models existed by random correlation. For a model to be considered reliable and meaningful, the R^2 value should typically exceed 0.5, indicating that the model possesses genuine predictive capability rather than being a product of chance (Li, 2017).

$$cR_p^2 = R[R^2 - (R_r)^2]^2 \quad (10)$$

Here, cR_p^2 represents the constant of fortitude for the Y-scattering test, R denotes the association coefficient obtained from the Y-scattering analysis and R_r indicates the mean R value assign to the randomized models (Yang et al., 2019). In the Y-randomization procedure, Manifold Linear Regression (MLR) equations stayed generated using randomly shuffling the concentration of inhibition values (pIC₅₀) though, keeping the molecular

properties of descriptors constant. The subsequent randomized models typically exhibited significantly lower R^2 and cross-validated Q^2_{cv} values across several trials, confirming that the developed QSAR model maintained genuine predictive performance and reliability (Ibrahim et al., 2020). A total of 10 Y-scattering tests stayed conducted, and the outcomes revealed that roughly four equations created R^2 and Q^2_{cv} values below 0.5. This observation validates that the constructed model was robust, statistically sound, and do not derived by random coincidental (Arthur et al., 2020).

2.2.10 Calculation of the Dominant of Applicability for the Selected Model

The constructed QSAR equation was additionally assessed through the Dominant of Applicability (AD) investigation using the approach of Williams's graph, it represents a graph of consistent residuals against leverage standards (Sagiru et al., 2023). The applicability domain serves to identify potential outliers or influential compounds in mandate to appraise the reliability, uniformity and sturdiness of the developed model equations (Ibrahim et al., 2019). The values of the leverage, which form the foundation of the Williams plot, were computed using Equation 11, as shown below:

$$h_i = X_i(J^T X)^{-k} X_i^T, \quad (i = k, \dots p,) \quad (11)$$

Here, X_i symbolizes the mathematical matrix of the i-th molecule in the development set, X denotes the $n \times k$ mathematical matrix of the development data, and J^T is the diagonal of matrix X castoff in model generation. The space of Leverage acts as a diagnostic apparatus, whereas compounds in either the development or test set with leverage standards ($h_i < f^*$) are regarded as reliably predicted by the model (Abdullahi et al., 2024). A domain demonstrating strong predictive reliability includes compounds (from together development and testing sets) with leverage parameter below the verge ($h_i < f^*$) and standardized residuals within the acceptable range of ± 3 (Etemadi & Khashei, 2024). Conversely, compounds exhibiting ($h_i > f^*$) are considered influential or outliers according to the model's prediction (Ibrahim et al., 2024). For the development dataset, the Williams graph is primarily employed to recognize complexes that play meaningfully to the model's structural framework ($h_i < f^*$) (Ibrahim et al., 2020). The leverage of warning (f^*), which defines the frontier for X-coordinate, can be calculated by the help of Equation 12, as presented below:

$$f^* = \frac{3(b+1)}{n} \quad (12)$$

Wherever, n represents the entire number of molecules included in the training database, and b denotes the quantity of molecular descriptors applied in constructing the model.

2.3. ADMET properties

The website computational platforms, namely SwissADMET and ADMETLab 2.0, were utilized to prognosticate the pharmacokinetic performance and probable noxiousness profiles of the tetrazolium nicotinamide herbicidal candidates (Sultana et al., 2023). Although these tools (SwissADMET and ADMETLab 2.0) were formerly established for estimating herbicides-likeness in pharmaceutical research, they are now broadly embraced in agrochemical discovery because of their effectiveness in identifying compounds that combine strong biological activity with improved environmental safety (Bhandar et al., 2022). The predictive capability of these platforms reduces reliance on extensive laboratory and field-based toxicity assessments, thereby streamlining the early-stage screening and prioritization of promising herbicide leads. In this study, Lipinski's Five Parameters Rule (LFPR) was smeared as an initial screening standard to assess the physicochemical feasibility of the compounds, followed by a more detailed evaluation of key ADMET-related parameters (Milićević et al., 2024).

2.4 Ligand- Molecular Docking Preparation

Molecular docking examination is a computer -based software approach used in structural bioinformatics in order to measure how two interacting molecules fit organized to form a steady complex. In this work, docking analyses remained accomplished for all the 35 synthesized Tetrazolium nicotinamide derivatives. The collaborations amongst these molecules and the functional moeity residues of the enzyme Hydroxyphenylpyruvate dioxygenase (HPPD) enzyme were explored using Molegro Virtual Docker (MVD). The 3D structure of 4-HPPD (PDB ID: 1TFZ; DOI: (<https://doi.org/10.2210/pdb1TFZ/pdb>)) was recovered from the online Data Array of Protein and equipped by eradicating the bound ligand and non-essential crystallographic water molecules (Yang et al., 2024) and residues with structural distortions were also inspected and corrected accordingly. A spherical search area by maintaining a radius of 1.80Å was defined around the enzyme's catalytic pocket to guide the docking simulations. To safeguard the consistency of the docking technique, the native ligand existing in the crystal arrangement was redocked into the same binding compact and the RMSD among the emperical and predicted active-site of Mesotrione was calculated

as a validation step. After protocol validation, all optimized Tetrazolium nicotinamide derivatives were discretely docked into 1TFZ active center. For each ligand, Molegro Virtual Docker (MVD) generated key scoring outputs including the MolDock Score, Rerank Score, and estimated H- bonding energy (Alnemesi, 2016). The docked complexes remained further scrutinized and visualized by the help of discovery studio to describe the specific bio-molecular interactions stabilizing the ligand–enzyme binding.

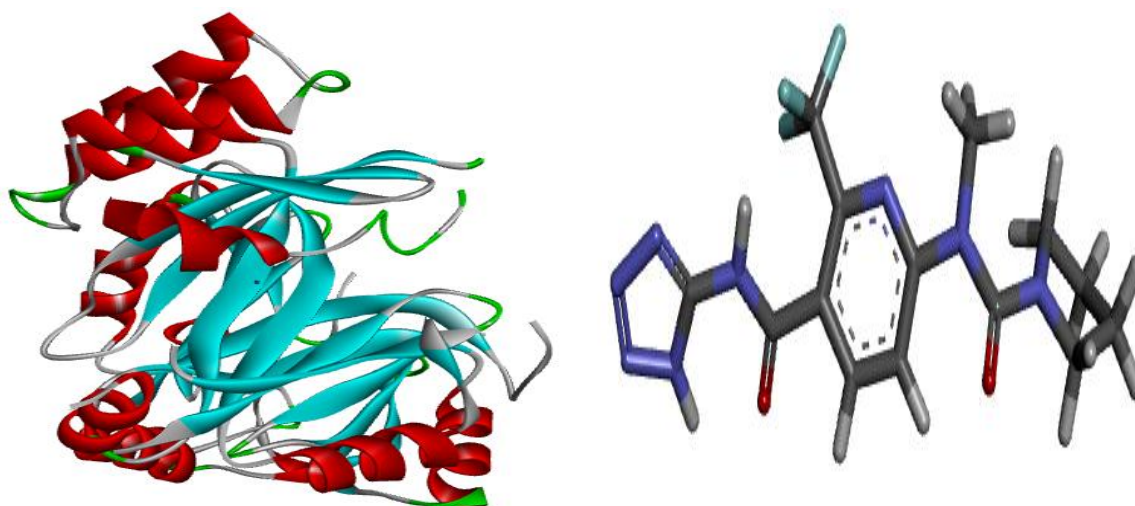


Fig. 1. Prepared crystal diagrams of HPPD protein and the 3D molecular representation of the prepared Ligand (26)

2.6. Preparation of MD simulation

Molecular dynamics (MD) calculations of the protein–ligand systems stood conceded out using software GROMACS 2024 composed with the CHARMM36-jul2021 force field to estimate their structural reliability and dynamic concert (Oduselu et al., 2024). The primed multi- complexes were positioned within a triclinic simulation box, solvent environment using the TIP3P water model, and electrically neutralized via the addition of counter-ions, while periodic borderline and measures were compulsory in all spatial directions. The systems first undertook energy minimization via the sharpest descent method, after which equilibration was achieved consecutively under unbroken volume (NVT) and continuous pressure (NPT) environments at 300 K and 1 bar, regulated by suitable thermostat and barostat algorithms (Ademuwagun et al., 2023). Electrostatic interfaces over elongated spaces remained calculated exhausting the Particle Mesh Ewald (PME) methodology, and bond dimensions comprising H- atoms were alleviated by expending the LINCS constraint algorithm (Tretiakov et al., 2025). Consequently, a 100 ns production simulation was

accomplished employing a 2 femtosecond time increment under meticulous condition of temperature and pressure surroundings. Post-simulation analyses were accompanied using built-in GROMACS utilities to assess essential structural and dynamical indicators considering the root mean square of deviation (RMSD), root mean square of fluctuation (RMSF), the radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen-bond formation, thereby providing a widespread evaluation of system stability and ligand retention inside the binding point over the interval of the simulation (Oduselu et al., 2024).

2.7. Rational design of new inhibitors

The ligand with ID-26, which demonstrated the most viable Gibbs's free energy (-141.002 kcal/mol), was preferred as the lead scaffold for analogue development. This optimal was directed not solitary by its superior Molegro binding score and inhibitory potential but, also by its well-balanced interaction pattern inside the active center of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) (Fu et al., 2020). Detailed inspection of the docking pose revealed a network of stabilizing interfaces, counting H -interactions, hydrophobic contacts, and π - π stacking with significant catalytic deposits, suggesting a robust and specific binding mode. To guide rational optimization, the structural geometry accountable for this high affinity were cautiously surveyed by both 2D interaction diagrams and 3D docking conformations. In particular, the $-\text{CF}_3$ substituent contributed significantly to hydrophobic stabilization and electronic complementarity within the binding pocket, while the tetrazolium ring served as an effective hydrogen-bond donor and acceptor. In addition, the nicotinamide moiety engaged in favorable π - π collaborations through aromatic residues lining the active site, further anchoring the ligand in a productive orientation (Yousef et al., 2022). Moreover, mapping these key interactions provided a valuable insight into the residues and forces responsible for ligand stabilization within the catalytic pocket. Building on these observations, a structure-approach design scheme was engaged to enhance binding affinity while preserving the essential interaction framework of the lead compound.

Furthermore, targeted substitutions were introduced at the CF_3 position, including $-\text{CN}$, $-\text{NO}_2$, $-\text{CF}_2\text{H}$, $-\text{Cl}$, and $-\text{F}$ groups, with the aim of maintaining favorable steric fit and electronic properties within the active site. The structural modifications were deliberately designed to subtly adjust hydrophobic and electrostatic interactions while preserving the binding pose of the parent compound. In particular, targeted substitutions on the tetrazolium and nicotinamide moieties were introduced to enhance hydrophobic contacts and promote additional hydrogen-

bond interactions, without introducing steric strain that could compromise binding (King, 2021). Bioisosteric replacements were also carefully considered to retain the essential electronic and spatial features of key functional groups, thereby strengthening interaction networks and improving the overall stability of the ligand–enzyme complex.

Moreover, all newly designed analogues were initially sketched using ChemDraw and subsequently subjected to geometric optimization in Spartan before docking. Docking was then achieved using Molegro Virtual Docker with constraints that had been formerly validated. To ensure a realistic representation of the catalytic environment, the HPPD active site including the Fe²⁺ cofactor was treated as rigid, while full conformational tractability was permissible for the ligands. Docking scores, together with detailed interaction profiles, remained used to rank the compounds, and only candidates exhibiting binding affinities comparable to or exceeding that of Ligand-26 were advanced for further investigation. Post-docking analyses in Discovery Studio enabled a closer examination of H- interactions, π – π interactions, hydrophobic contacts, and any newly introduced contacts ensuing from the structural modifications. Understandings expanded from these evaluations were subsequently used to guide the iterative refinement of the ligand designs (Huang et al., 2016).

In addition, to further assess their herbicidal potential, the most promising analogues were evaluated using ADMET and herbicide-likeness screening tools, including SwissADME and ADMETLab, with particular attention to solubility, foliar uptake, translocation, and safety toward non-target organisms. Compounds that met key criteria for plant absorption and environmental safety were prioritized for further optimization. However, by combining docking performance, binding-site interaction analysis, and ADMET evaluation, this iterative design-dock-refine approach ensured that the final set of analogues demonstrated binding affinities and plant-compatible pharmacokinetic properties that matched or surpassed those of the original high-affinity scaffold. These results highlight their strong potential as effective 4-HPPD-inhibiting herbicides.

3.0 Results and discussion

3.1. Coupling 2- Dimensional QSAR expending MLR – GFA approach

A combination of Manifold Linear Regression (MLR) and the Genetic Function Approximation (GFA) algorithm was employed to construct a set of five Quantitative Structure Activity Relationship (QSAR) regression equations. Among these equations, equation 1, was identified as primitive highly reliable and statistically sound, as reflected by its strong validation metrics (LOF = 0.01824700; R^2 = 0.95761800; R_{adj}^2 = 0.94349100; Q_{cv}^2 = 0.92339200; R_{pred}^2 = 0.629281). These statistical indices collectively demonstrated that, the model possesses excellent fitting ability, strong internal uniformity and acceptable external extrapolative supremacy. All the validation strictures supporting the robustness, consistency, and predictive métier of the generated QSAR models were obtainable in Table 4. According to widely accepted QSAR modelling criteria, the designated equation fulfills the essential requirements for a dependable predictive model namely; a high constant of fortitude (R^2), strong cross-validated correlation (Q_{cv}^2), low lack-of-fit (LOF), and satisfactory external predictivity (R_{pred}^2) (Lahyaoui et al., 2025). These attributes confirmed that the model is neither overfitted nor structurally unstable.

Model 1

$$pIC_{50} = 1.376443332 * AATS2e - 0.08769636 * AATS5i + 0.000220103 * ATSC3v + 0.000675401 * ATSC7v + 0.351797553 * XLogP + 10.325334944$$

Table 2- Descriptors numerical values, Trial pIC_{50} , Forecast pIC_{50} and Residual values of the Dataset

S/no.	Descriptors					Exp.pIC ₅₀	Pred. pIC ₅₀	Residuals
	AATS2e	AATS5i	ATSC3v	ATSC7v	XLogP			
Training set								
2	9.103278	182.9553	-106.159	-149.419	0.379	6.764472	6.820019	-0.05555
3	8.872879	180.8935	415.2515	-222.319	0.802	6.841638	6.898036	-0.0564
4	8.688532	178.7174	418.9814	-286.624	1.160	6.978811	6.918464	0.060347
5	8.914619	180.6664	68.90833	-346.109	0.189	6.614394	6.599911	0.014483
6	8.890832	179.324	-109.31	-229.001	0.836	6.853872	6.952372	-0.0985
8	8.518929	175.5813	378.0851	-365.734	1.552	7.075721	7.035507	0.040214
9	8.939472	178.5557	-116.911	-227.637	-1.193	6.408935	6.372156	0.036779
10	8.734287	176.9876	200.5634	-300.526	-0.835	6.326058	6.373837	-0.04778
11	8.570283	175.4971	375.8187	-362.063	-0.477	6.372634	6.401763	-0.02913
12	9.071494	177.8605	-124.028	-226.368	-0.385	7.022276	6.898383	0.123893
16	8.686262	180.9947	410.4298	-264.636	0.533	6.545155	6.50802	0.037135
17	8.527142	179.1591	413.6716	-326.148	0.891	6.505845	6.535082	-0.02924

18	8.397399	179.5526	476.0052	-437.071	1.460	6.481486	6.460963	0.020523
21	8.445217	175.1331	127.8713	-450.341	1.454	6.879426	6.826658	0.052768
23	8.876163	172.6005	-889.207	-330.026	-0.166	6.89279	6.929415	-0.03662
27	8.648062	174.868	-74.9399	254.3871	-0.758	6.787812	6.782275	0.005537
28	8.348957	174.1565	971.1178	-12.8815	0.911	7.075721	7.069846	0.005875
29	8.321007	173.4091	1267.942	-466.587	1.031	6.844664	6.898028	-0.05336
32	8.570156	176.3105	-406.762	-169.492	1.667	7.102373	7.042323	0.06005
34	8.476276	173.5301	-752.39	-215.23	1.863	7.065502	7.118924	-0.05342
Test set								
1	9.275653	189.0429	-54.3208	-124.489	0.716	6.730487	6.670224	0.060263
7	8.683322	177.6081	203.7626	-303.121	1.194	7.017729	6.962019	0.055709
13	8.863155	176.421	197.4709	-298.100	-0.027	7.040959	6.886111	0.154848
14	8.694616	175.4258	373.5187	-358.612	0.331	7.055517	6.865226	0.190291
15	8.88454	182.9635	65.3115	-197.867	0.110	6.573489	6.428605	0.144884
19	8.528714	179.354	29.17654	-317.995	0.993	6.498941	6.476918	0.022023
20	8.625268	179.5876	501.1151	-214.510	0.653	6.492144	6.643486	-0.15134
22	8.784938	174.7876	15.25309	-289.969	0.625	6.774691	7.116457	-0.34177
24	8.685661	173.2647	-652.273	-354.491	-0.358	6.974694	6.577035	0.397659
25	8.443701	174.3787	454.581	411.1181	0.488	7.142668	7.204638	-0.06197
26	8.475421	173.68	-121.827	269.2290	0.148	7.013228	6.967252	0.045976
30	8.570156	174.6876	-269.699	-167.296	1.456	7.091515	7.142064	-0.05055
31	8.570156	175.9796	-406.762	-260.536	1.667	7.091515	7.009854	0.081661
33	8.814302	176.916	-167.601	-9.94553	1.641	7.124939	7.47653	-0.35159

Table 2, summarizes the trial and forecast pIC₅₀ values, as well as the corresponding residuals, for the 35 tetrazolium nicotinamide derivatives assessed in this work. The consistently small residuals observed across all compounds indicate a strong alignment between measured and calculated inhibitory activities. This close match establishes that, the selected QSAR equation is both highly accurate and unswerving. The strong correlation further confirms that, the descriptors incorporated during model construction successfully capture the molecular and physicochemical factors that determine herbicidal activity toward the target enzyme. The application of the Genetic Function Estimate (GFE) technique headed to the choice of five key descriptors AATS2e, AATS5i, ATSC3v, ATSC7v, and XLogP as the utmost prominent variables in generating the final linear QSAR equation. These descriptors reflect critical electronic, hydrophobic, and topological characteristics that shape how the ligands interact with the active site. Their presence in the optimal model highlights their importance in governing inhibitory potency within the tetrazolium nicotinamide series. The resulting model built upon these descriptors, shows an excellent capability in foreseeing the pIC₅₀ values of all the compounds included in the dataset, underscoring its robustness and predictive strength (Kumar et al., 2024).

Table 3, provides additional information on each selected descriptor, including its definition and dimensional class. The notable representation of two-dimensional (2D) descriptors in the final equation advocates that these kinds of descriptors effectively encode the structural together with physicochemical features that drive biological activity, even without the need for obvious 3-dimensional molecular information. In summary, the MLR/GFA-based QSAR models developed in this study demonstrates strong statistical performance, high predictive accuracy, and meaningful mechanistic relevance. The descriptors featured in the model offer valuable insight into the structural attributes that enhance herbicidal potency, making the model a useful tool for guiding future optimization and rational design of tetrazolium nicotinamide herbicide candidates.

Table 3- Descriptors types, description and classes.

S/no.	Descriptors	Description	Class
1	AATS2e	Middling Broto-Moreau autocorrelation - lag 2 / weighted by Sanderson electronegativities	2D
2	AATS5i	Ordinary Broto-Moreau autocorrelation - lag 5 / weighted by first ionization potential	2D
3	ATSC3v	Focused Broto-Moreau autocorrelation - lag 3 / weighted by van der Waals volumes	2D
4	ATSC7v	Spotlighted Broto-Moreau autocorrelation - lag 7 / weighted by van der Waals volumes	2D
5	XLogP	XLogP	2D

Table 4, outlines the accepted benchmark values for validation (internal and external) constraints used to gauge the sturdiness and extrapolative dependability of the QSAR equations developed using the Genetic Function Estimation (GFE) approach. These validation criteria deliver a structured outline for formative whether a model demonstrates sufficient stability, predictive strength, and the aptitude to generalize to compounds outside the training set (Cumming et al., 2013). The close agreement between the calculated validation metrics and these recommended thresholds designates that the models constructed in this learning fulfill the essential requirements for a statistically sound QSAR methodology (Tropsha, 2010).

Table 4 - General bottommost commended value for an acceptable 2D -QSAR equation.

S/no.	Validation parameters	Threshold	Developed model	Remark	References
1	Friedman LOF	Low value	0.0182	Passed	Tropsha A. (2010)
2	R-squared	$R^2 > 0.6$	0.958	Passed	Tropsha A. (2010)
3	Adjusted R-squared	$R^2_{adj} > 0.6$	0.943	Passed	Tropsha A. (2010)
4	Cross validated R-squared	$Q^2_{cv} > 0.6$	0.923	Passed	Tropsha A. (2010)
5	Variance between R^2 & Q^2_{cv}	$(R^2 - Q^2_{cv}) < 0.3$	0.035	Passed	Tropsha A. (2010)
6	Precarious SOR F-value (95%)	$F_{test} > 2.09$	2.921	Passed	Tropsha A. (2010)
7	Y-randomization cR^2_p	$cR^2_p > 0.5$	0.819	Passed	Tropsha A. (2010)
8	Association coefficient of the test set R^2_{test}	$R^2_{test} > 0.6$	0.629	Passed	Tropsha A. (2010)
9	Min expt. error for non-significant LOF (95%)	0.050	0.047	Passed	Tropsha A. (2010)

Table 5, provide the Pearson relationship matrix for the five molecular descriptors selected for the final model. The consistently stumpy correlation factors detected among these descriptors suggest minimal overlap in the information they encode (De et al., 2022). This low degree of interdependence is advantageous, as it ensures that, every descriptor adds distinct and complementary evidence to the regression model. Such independence not only reduces the likelihood of multicollinearity but, also enhances both the interpretability and stability of the predictive equation. Overall, the correlation results support the suitability of the chosen descriptors and reinforce the consistency of the resulting QSAR model.

Table 5 - Pearsons relationship matrix for the labeled descriptors employed in the model.

	<i>AATS2e</i>	<i>AATS5i</i>	<i>ATSC3v</i>	<i>ATSC7v</i>	<i>XLogP</i>	VIP	ME
AATS2e	1					6.238916	-3.335360
AATS5i	0.708753	1				5.467342	4.339372
ATSC3v	-0.36004	0.127035	1			2.069743	-0.008450
ATSC7v	0.375607	0.314401	-0.14325	1		1.186758	0.051823
XLogP	-0.28007	0.193753	0.168587	-0.11378	1	1.949564	-0.047390

The Variance Inflation Parameter (VIP) values obtained for the nominated 5 descriptors included in the model were all below the commonly accepted upper limit of 10 and their corresponding Mean Effect (ME) values were all below 5. These results indicate that, the descriptors do not exhibit strong interdependence and can be considered largely orthogonal. Based on the guidelines of Belsley et al. (1980), such values demonstrate that multicollinearity is minimal and that the statistical integrity of the model is well maintained. To further assess descriptor significance, hypothesis testing was achieved to discover the affiliation among the selected molecular descriptors together with the pIC_{50} estimate of the compounds. Moreover, the insignificant hypothesis proposed that, no noteworthy liaison

existed amongst the variables at a 95% confidence smooth ($P < 0.05$). Because all descriptors produced p-values below this threshold, the insignificant hypothesis was forbidden, demonstrating that each descriptor makes a meaningful contribution to explaining the observed variation in biological activity. This encourages their aptness for inclusion in the QSAR model (Travers et al., 2017).

However, to further measure the consistency of the model equation, a Y-scattering test was performed, and the results are abridged in Table 6 below. The consistently stumpy R^2 and Q^2_{cv} values observed across multiple randomization runs suggest that the correlations in the novel equation of model are not the consequence of coincidental (Kaneko, 2019). In addition, the corrected cRp^2 value of 0.8194, which is well above the recommended minimum of 0.5, indicates strong predictive reliability. Overall, these validation outcomes authorize that, the model equation is robust, dependable and proficient of precisely forecasting the inhibitory activities of tetrazolium nicotinamide derivatives.

Table 6 - Y-Scattering Test Parameters.

Model	R	R^2	Q^2
Original	0.978580	0.957618	0.923392
Trial 1	0.501303	0.251305	-0.490160
Trial 2	0.489072	0.239191	-0.283370
Trial 3	0.529147	0.279996	-0.819380
Trial 4	0.460089	0.211682	-0.238460
Trial 5	0.252432	0.063722	-1.468700
Trial 6	0.549170	0.301588	-0.420970
Trial 7	0.335396	0.112490	-0.966370
Trial 8	0.550593	0.303153	-0.542260
Trial 9	0.776433	0.602849	0.152098
Trial 10	0.620761	0.385345	-0.599160
Trial Models Constraints			
Mean r :	0.506440		
Mean r^2 :	0.275132		
Mean Q^2 :	-0.567670		
cRp^2 :	0.819403		

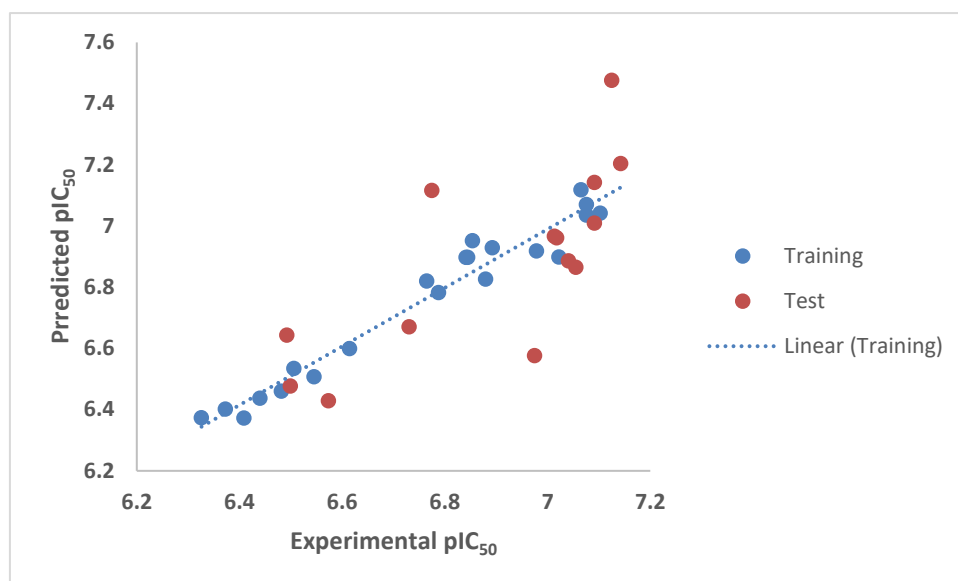


Fig. 2. Graph of experimental pIC_{50} the forecast pIC_{50} standards of training and testing set molecules

Figure 2 above, signifies the scatter graphical plot comparing the experimental pIC_{50} values with the anticipated pIC_{50} standards for together the training and testing sets. The developing set achieved a constant of determination (R^2) value of 0.958, while the test set produced an R^2 value of 0.8403. These results fall within the acceptable range for validated QSAR models and are consistent with the recommended performance criteria for models generated by the Genetic Function Approximation (GFA) approach, as outlined in Table 4. The strong linear association observed in both datasets demonstrates that, the established model holds high extrapolative consistency and steadiness.

Moreover, to scrutinize the domain applicability of the model equation and to perceive any potential outliers or structurally influential compounds, leverage values remained contrived against standardized values of the residuals to produce the Williams plot shown in Figure 3. This diagnostic evaluation determines whether any compounds exceed the standardized residual threshold of ± 3 or surpass the calculated warning leverage value ($f^* = 0.85$). The analysis displayed that virtually all molecules in the model development set and testing sets remained indoors the ± 3 residual range, indicating no outliers were present. In addition, all compounds had leverage values underneath the cautioning limit, suggesting that none unduly influenced the regression model (Subrahmanyam et al., 2024). The uniform placement of compounds within the acceptable leverage zone also reflects the structural similarity of the tetrazolium nicotinamide derivatives, ensuring they all fall comfortably within the model's defined applicability domain. Overall, these conclusions highpoint the sturdiness of the

QSAR model equation and support its consistency in accurately predicting the herbicidal activity of this compound series.

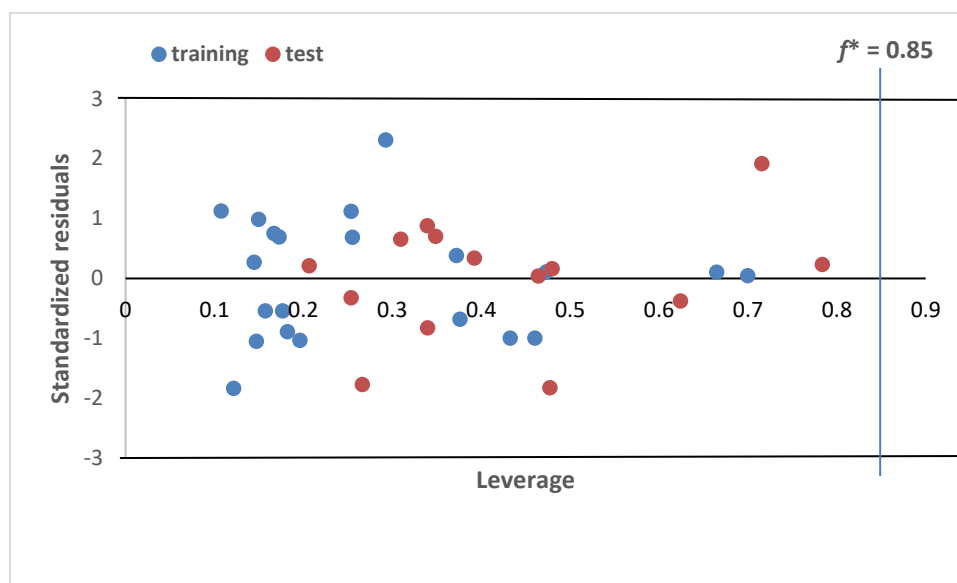


Fig. 3. Williamson's Plot, A graph of standardized residual against Leverage

3.2 Molecular docking results

The molecular docking analysis discovered a stable and energetically favorable alignment of the compound inside the catalytic active center of the objective protein. Visualization of both the three-dimensional interaction surface and the corresponding two-dimensional contact map highlights a complex system of stabilizing forces, including H- bonds, conventional hydrophobic contacts, and additional non-covalent connections, which collectively enhance the binding energy and asset of the ligand - receptor complex. According to the outcomes obtainable in Table 7, four ligands (**25**, **26**, **27** and **28**) exhibited the most favorable binding energies among the 35 series.

Table 7. MolDocking Score, Reranking Score (Kcal/mol) and H- bond energetics of the studies compounds

ID	MolDocking Score (Kcal/mol)	Reranking Score (Kcal/mol)	H- Bond (Kcal/mol)
1	-104.773	-84.7081	-0.96717
2	-114.283	-94.6483	-7.23557
3	-116.946	-93.1755	-6.45056
4	-122.850	-99.3508	0.00000
5	-122.500	-90.7364	-2.10574
6	-113.316	-92.2980	-1.30010

7	-118.639	-97.1280	0.00000
8	-125.493	-101.291	-1.18404
9	-116.145	-95.4187	-0.95492
10	-120.427	-88.8124	-3.40470
11	-126.396	-100.205	-1.80610
12	-120.112	-100.011	0.00000
13	-123.823	-103.009	-0.85565
14	-129.186	-106.295	0.00000
15	-112.825	-92.599	-4.27963
16	-119.304	-95.6163	-1.68522
17	-121.405	-98.8243	0.00000
18	-127.68	-90.988	-8.22759
19	-123.638	-98.1507	-4.99574
20	-125.523	-98.2835	-4.99661
21	-133.105	-106.443	0.00000
22	-126.401	-87.0944	-0.78606
23	-115.385	-101.415	0.00000
24	-131.424	-107.042	-4.69008
25	-139.644	-105.552	-1.40626
26	-141.002	-110.865	-4.60681
27	-136.528	-114.210	-5.15928
28	-140.533	-111.267	-0.72318
29	-139.656	-114.161	0.00000
30	-131.531	-80.2481	-2.74055
31	-128.075	-99.9808	-2.27357
32	-130.190	-99.5362	-2.43462
33	-130.280	-104.764	0.00000
34	-131.696	-99.6692	-2.46635
35	-118.689	-101.442	-4.52561

Table 8, provides the various interaction residues which comprises of the Binding scores, H-Bond and Non - ionic Bond Interaction designed among the Ligands with the utmost Molegro scores and the lively center of the 1TFZ -receptor, out of the 10 receptor- ligands complexes presented in Table 8 below, only four stood designated for conception in 3D and 2D. In addition, ligand 26, in particular, demonstrated the uppermost binding mark of -141.002 kcal/mol, indicating the strongest interaction with the target 1TFZ protein. Owing to its superior binding score and well-positioned docking conformation, ligand 26 was selected as the lead compound and obliged as a reference template for the rational design of additional potent Tetrazolium nicotinamide inhibitors. This lead compound selection provides a strategic basis for subsequent structure-based optimization efforts, facilitating the

development of novel derivatives with enhanced herbicidal activity and improved interactions inside the active center of the target 1TFZ receptor.

Table 8. Binding energies, H-Bond and Hydrophobic Bond Interaction designed among the Ligands with the uppermost Molegro scores and the Active center of the 1TFZ protein target.

S/no.	Ligands ID	Binding scores [Kcal/mol]	PDB Code	H- bond interactions		Hydrophobic bond interactions
				Amino acid Residues	Bond length [Å]	
1	21	-133.105	1TFZ	ASN261	1.59961	HIS287 PHE360 PHE403
2	24	-131.424	1TFZ	ASN261	1.6012	HIS287 PHE360 PHE371 PHE403
3	25	-139.644	1TFZ	PHE398	2.42356 3.08432 2.48382	PHE360 HIS287 PHE403
4	26	-141.002	1TFZ	SER246 ILE260 ASN261	1.96214 2.6722 2.13021	HIS287 PHE403 PHE360 PHE371
5	27	-139.656	1TFZ	SER246 ASN261 GLN286 MET314	2.03369 2.09377 2.43403 2.70204	PHE360 PHE403 LEU244
6	28	-140.533	1TFZ	GLN286 HIS287	2.52344 3.02872	PHE360 HIS287 PHE403
7	29	-139.656	1TFZ	HIS287	2.39714	HIS287 PHE360 PHE371 PHE403
8	30	-131.531	1TFZ	HIS287	2.60099	PHE360 PHE403
9	32	-130.19	1TFZ	SER246 ILE260 ASN261 GLN286	1.99230 2.52409 2.76849 1.83918	PHE403 LEU406 HIS287
10	34	-131.696	1TFZ	SER246 ILE260	2.22964 3.00445	PHE403 HIS287 HIS287

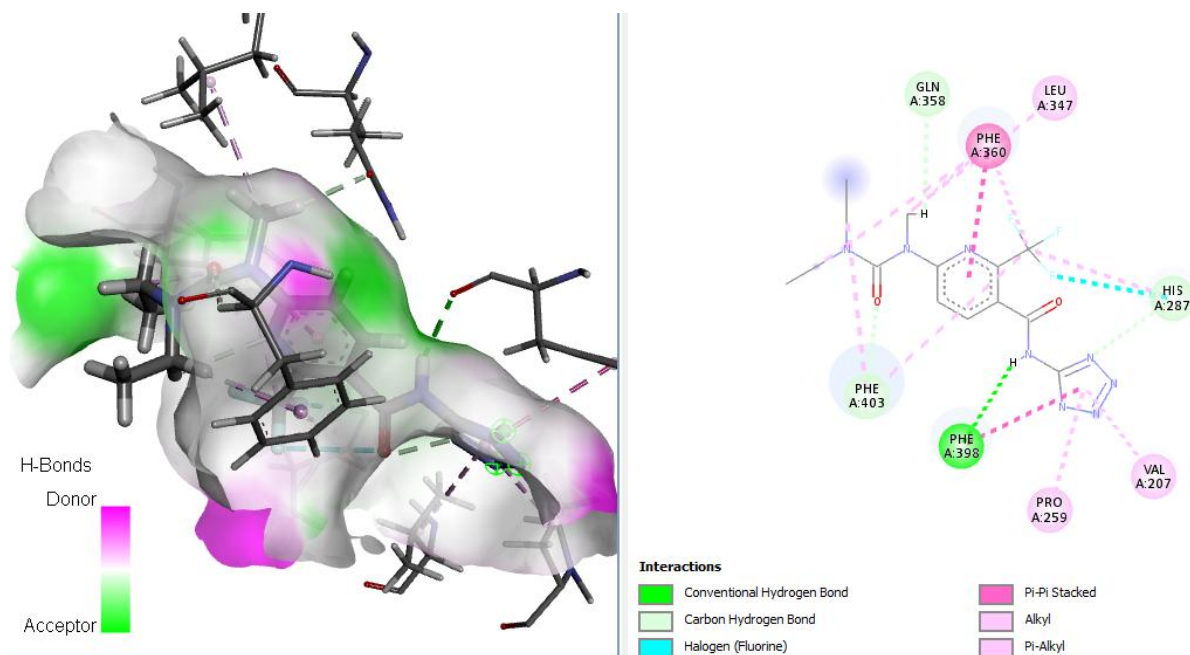


Fig. 4a- 3D and 2D molecular interaction of Ligand -25 with 1TFZ receptor binding site

Figure 4a elucidates the docking pose of Ligand-25, which demonstrated a robust binding score of -139.644 kcal/mol, signifying a stable and energetically promising interaction inside the receptor's target center. The ligand espouses a distinct orientation that is stabilized by a complementary network of non-covalent interactions, collectively contributing to its high binding affinity. A key stabilizing feature is the formation of a classical hydrogen bond with PHE398, which effectively anchors the ligand in the polar region of the binding pocket and helps maintain its optimal positioning. Additional stabilization arises from a halogen bond with HIS287, emphasizing the directional and specificity-driven nature of halogen interactions in enhancing binding complementarity. The ligand also contributes in a pronounced π - π stacking interaction with PHE360, which strengthens its placement within the hydrophobic core of the active site and contributes significantly to overall binding stability through dispersion forces.

Furthermore, a carbon -hydrogen bond with GLN358 provides additional conformational restraint, reducing unnecessary ligand flexibility and supporting a favorable binding geometry. All together, these interactions incorporating hydrogen bonding, halogen bonding, aromatic stacking, and weak C-H contacts form an integrated interaction network that yields a compact and energetically optimized receptor -ligand complex. This well-balanced interaction profile highlights the strong affinity of Ligand-25 and supports its potential as a

auspicious framework for additional optimization in structure-based inhibitor or herbicide design.

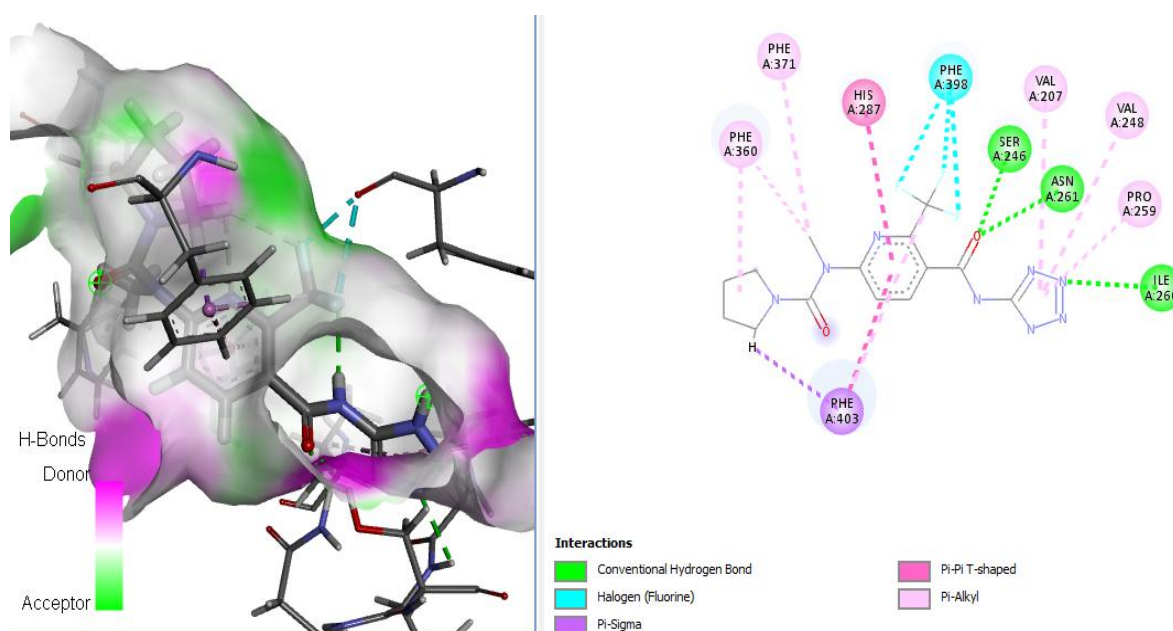


Fig. 4b- 3D and 2D molecular interaction of Ligand -26 with 1TFZ receptor binding site

Figure 4b depicts the docking pose of Ligand-26, which accomplished the most favorable binding score (-141.002 kcal/mol), demonstrating a highly steady and well-organized contact within the receptor's active point. The ligand assumes an evidently demarcated binding orientation that is reinforced by a corresponding combination of polar interactions, aromatic contacts, and halogen-mediated stabilization. Three classical hydrogen bonds formed with SER246, ASN261, and ILE260 which shows a central part in anchoring the ligand inside the polar region of the binding center and contribute considerably to its enthalpic stability. The interactions with SER246 and ASN261 assist in accurately orienting the ligand within the cavity, while the hydrogen bond involving ILE260 likely interceded through the backbone further reinforces proper configuration of the scaffold. In addition, a halogen bond with PHE398 introduces a directional interaction that augments ligand positioning within an aromatic sub-pocket of the active point and the aromatic interactions further strengthen the binding mode. The ligand also participates in a π - π stacking interaction with HIS287, providing favorable dispersion stabilization, while a π -sigma interaction with PHE403 improves hydrophobic complementarity and helps limit conformational flexibility within the binding groove.

Additionally, these hydrogen-bonding, halogen, and aromatic interactions form an integrated and cooperative interaction network that decisively secures Ligand-26 within the receptor. This well-balanced and highly directional interaction profile enlightens the strong binding affinity detected for Ligand-26 and underlines its suitability as a promising lead framework for extra structure-based optimization.

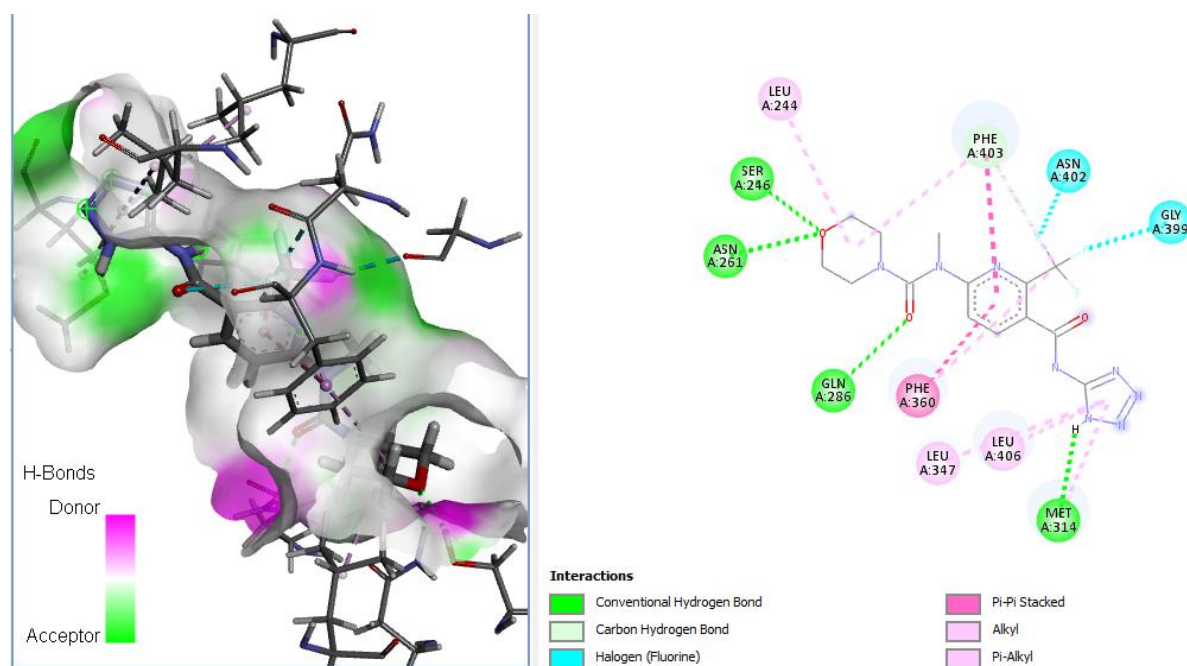


Fig. 4c- 3D and 2D molecular interaction of Ligand-27 with 1TFZ receptor binding site

Figure 4c presents the docking pose of Complex-27, which exhibits a robust binding energy of -139.656 kcal/mol, imitating a stable and well-oriented conformation within the receptor's active site. Ligand-27 is stabilized within the active site by a well-organized network of polar, halogen-mediated, and aromatic interactions that collectively promote a favorable binding geometry. Four H- bonds involving SER246, ASN261, GLN286, and MET314 act as the primary anchoring points, effectively positioning the ligand's polar functionalities within the binding pocket and contributing significantly to enthalpic stabilization. These interactions help lock the ligand into an orientation that is well matched to the local electrostatic environment of the receptor.

However, an extra stabilization arises from halogen contacts with ASN402 and GLY399, where the halogen substituents engage electron-rich regions of the protein, improving spatial complementarity and refining the overall docking pose. The ligand's aromatic core is further secured through a pronounced π - π assembling interaction with PHE360, which reinforces

hydrophobic engagement within the cavity and minimizes conformational freedom. A weaker C–H interaction with PHE403 also contributes by subtly optimizing local packing within the nonpolar region of the active site. In conclusion, these hydrogen-bonding, halogen, aromatic, and C–H interactions form an integrated and cooperative interaction network that stabilizes ligand-27 in a low-energy conformation. This balanced interaction profile not only enhances binding affinity but also reduces conformational entropy, underscoring Complex-27 as an encouraging framework for additional structure-based optimization and analogue development.

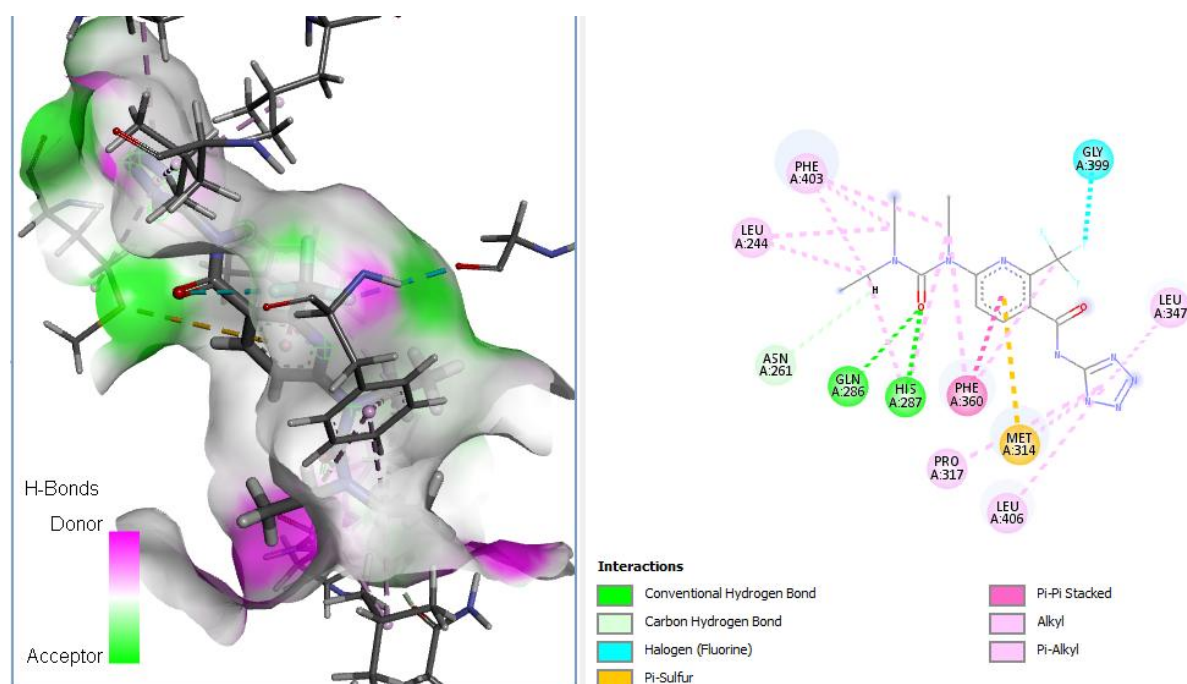


Fig. 4d- 3D and 2D molecular interaction of Ligand-28 with 1TFZ receptor binding site

Figure 4d exemplifies the docking pose of Ligand-28, which achieved a binding score of -140.533 kcal/mol, reflecting a stable and well-defined orientation inside the receptor's active point. The ligand is affixed primarily through two important H-bonds with GLN286 and HIS287, which precisely position its polar groups and provide significant binding stabilization. Another support comes from a halogen interaction with GLY399, enhancing geometric complementarity and confining rotational flexibility around the halogen-substituted moiety. Aromatic interactions also play a crucial role consisting of a strong π – π assembling with PHE360 stabilizes the ligand's delocalized aromatic core within the hydrophobic region of the pocket, while a π -sulfur interaction with MET314 improves packing efficiency by engaging the methionine sulfur atom. The combined contributions of

hydrogen bonding, halogen contacts, π - π stacking, and π -sulfur interactions form a coherent and cooperative interaction network that stabilizes Ligand-28 in a low-energy, favorable binding conformation. This well-balanced interaction profile reflects its strong binding potential and highlights Ligand-28 as a favorable scaffold for structure-guided optimization.

In addition, the comparative docking analyses of Ligands 25, 26, 27, and 28 further designate that all the four ligands form stable and energetically favorable complexes with the receptor. Hydrogen bonds serve as the primary anchoring interactions, ensuring proper ligand orientation within the Polar Regions of the active site. In addition, halogen contacts enhance geometric complementarity and restrict excessive conformational freedom, thereby improving binding specificity. Aromatic interactions most notably π - π assembling coupled with PHE360 and neighboring hydrophobic residues-provide substantial stabilization, while secondary contacts such as C-H bonds, π - σ , and π -sulfur interactions produce a significant protagonist in fine-tuning ligand positioning within the binding pocket. Collectively, these polar and nonpolar interactions promote well-defined, low-energy conformations across all four ligands. Among them, Ligands 26 and 28 exhibit particularly favorable binding contours, distinguishing them as resilient candidates for additional optimization and the development of potent inhibitory or herbicidal agents.

3.4 ADMET Properties of the nominated Tetrazolium nicotinamide derivatives

The ADMET properties summarized in Table 9 offer an herbicide-centric evaluation of the tetrazolium-nicotinamide derivatives, highlighting structural features that affect plant absorption and translocation. All nine derivatives fall within an acceptable molecular weight range and have favorable hydrogen bond donor counts and lipophilicity profiles, indicating that there are no major steric or lipophilic obstacles to movement through plant cuticles and cellular membranes. Nonetheless, several compounds display higher hydrogen bond acceptor counts, relatively elevated topological polar surface areas (TPSA $\approx$ 100 Å²), and acidic pKa values (4.73 - 4.80). These characteristics increase ionization under physiological and foliar pH conditions, raising overall polarity, reducing aqueous solubility for certain analogues (Log S $\leq$ -4), and limiting the fraction of neutral molecules obtainable for inactive diffusion transversely plant tissues. This explains the relatively lower predicted foliar absorption and translocation of these derivatives compared with the commercial HPPD inhibitor mesotrione.

In summary, the physicochemical liabilities particularly the high hydrogen bond acceptor burden, moderately elevated TPSA and strong acidic character likely contribute to reduced absorption and higher predicted plasma approval rates for these compounds. Rational optimization strategies, such as lowering polar surface area, reducing hydrogen bond acceptor density, and fine-tuning pKa to decrease anionic speciation at neutral P^H, are expected to enhance passive permeability. Where structural modifications are constrained by the need to preserve herbicidal activity, complementary formulation approaches may further improve bioavailability. Moreover, these findings provide a clear rationale for targeted chemical refinement and experimental validation, offering guidance for the development of next-generation Tetrazolium nicotinamide herbicide candidates with improved uptake and systemic exposure.

Table 9. Predicted ADMET physicochemical properties of the selected ligands

S/no.	Molecular weight	HBA	HBD	Log P	Log S	TPSA	pka (Acid)	Lipinski rule Violation
	Mesotrione (Template)							
35	339.04	8	0	1.056	-3.135	128.49	5.363	0.00
	Selected ligands with the highest binding scores							
21	329.12	8.0	2.0	2.254	-3.335	99.69	4.456	0.00
24	358.11	10.0	2.0	1.309	-2.757	120.00	4.530	0.00
25	338.889	10.0	2.0	1.924	-2.834	120.00	4.553	0.00
26	384.13	10.0	2.0	1.343	-2.654	120.00	4.518	0.00
27	400.12	11.0	2.0	1.217	-2.601	129.23	4.578	0.00
28	400.16	10.0	2.0	2.050	-2.972	120.00	4.671	0.00
29	412.16	10.0	2.0	1.914	-3.448	120.00	5.246	0.00
30	364.09	8.0	2.0	2.685	-4.377	105.68	4.802	0.00
32	364.09	8.0	2.0	2.524	-4.070	105.68	4.730	0.00
Lipinski's rule	≤500	≤10	≤5	≤5	Low	≤140Å	4 - 6	

The pharmaceutical radar charts for the tetrazolium nicotinamide herbicide selected derivatives (25, 26, 27, and 28) and the commercial herbicide mesotrione (compound 35) were generated using the ADMET-Lab 2.0 platform which provide a clear and intuitive overview of their herbicides-likeness and physicochemical profiles (Figure 5). For the mesotrione template, most physicochemical and ADMET parameters fall comfortably within the optimal range, as indicated by the blue section of the radar chart. The only deviation observed is the absence of hydrogen-bond donors (nHD = 0). Although this represents a

relatively minor shortcoming, it suggests a limited hydrogen-bonding capacity compared with what is commonly observed among highly effective herbicidal agents. In contrast, the selected tetrazolium nicotinamide derivatives exhibit a more balanced and favorable radar profile, with all evaluated constraints outstanding within their corresponding optimal ranges. None of the descriptors exceed recommended thresholds, indicating that the structural modifications presented into the tetrazolium nicotinamide scaffold successfully improved the overall physicochemical balance of the molecules. Notably, derivatives 25, 26, 27, and 28 closely align with molecular topographies characteristically connected with strong herbicidal performance, particularly when benchmarked against mesotrione.

However, all the four derivatives fully comply with Lipinski's rule of five, displaying suitable molecular weight, H-bond donor and acceptor counts, lipophilicity, and molecular flexibility. These characteristics are indicative of favorable absorption properties and efficient translocation within plant tissues. In addition, the compounds satisfy other widely used drug-likeness filters, including the Ghose, Veber, Egan, and Muegge criteria, underscoring their chemical robustness and suggesting a low risk of unfavorable physicochemical liabilities. Further support for their herbicidal potential is provided by the Quantitative Estimate of Herbicide-likeness (QEH), adapted from QED principles. Compounds 25-28 achieved higher QEH scores than mesotrione, reflecting a more optimal balance of molecular size, polarity, lipophilicity, and aromaticity key attributes for effective plant uptake and strong interaction with the HPPD target. Given that QEH values range from 0 (least favorable) to 1 (most favorable), the elevated scores of these derivatives highlight their enhanced suitability for development as next-generation herbicidal agents.

In conclusion, the radar chart analyses, multi-rule herbicide-likeness evaluations, and QEH scoring collectively indicate that compounds 25-28 possess superior physicochemical and ADMET profiles compared with mesotrione. These optimized molecular features make them encouraging lead aspirants for further structural refinement, detailed biological evaluation, and advancement toward next-generation HPPD-inhibiting herbicides.

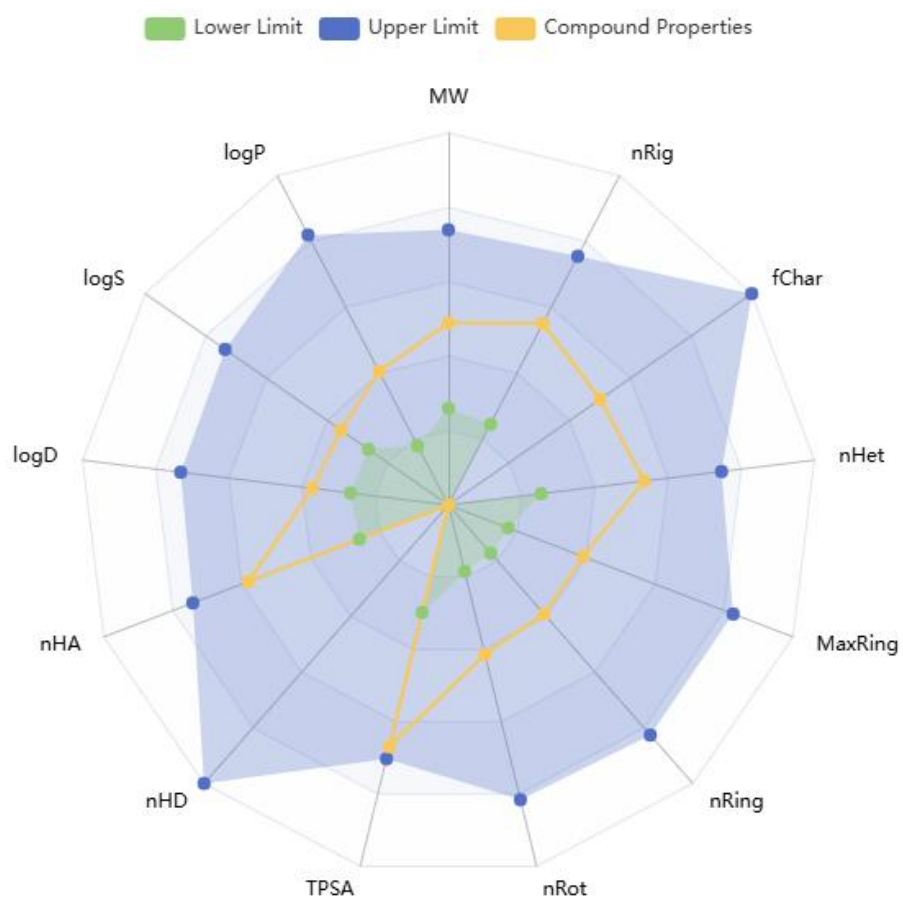
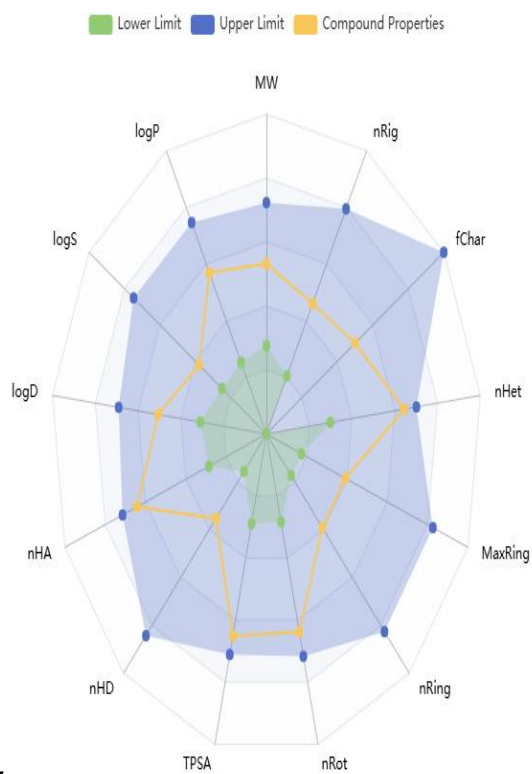
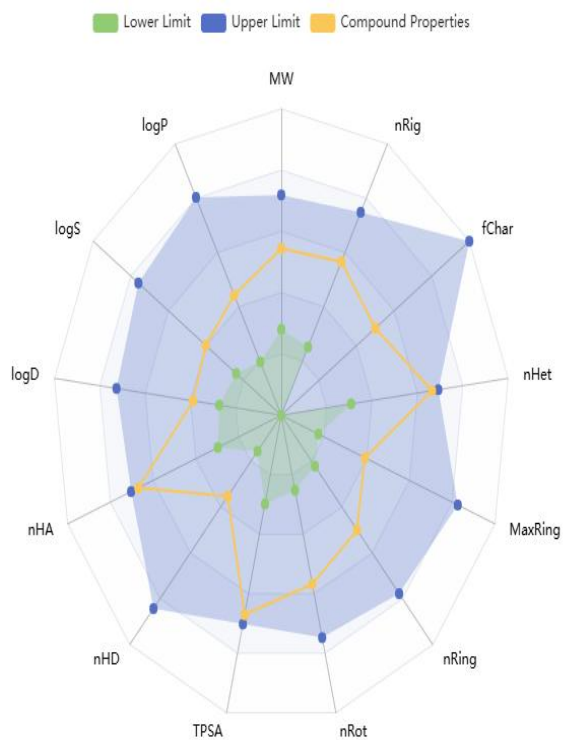


Fig. 5. Pharmacological radar chart of standard Mesotione herbicide (Template)

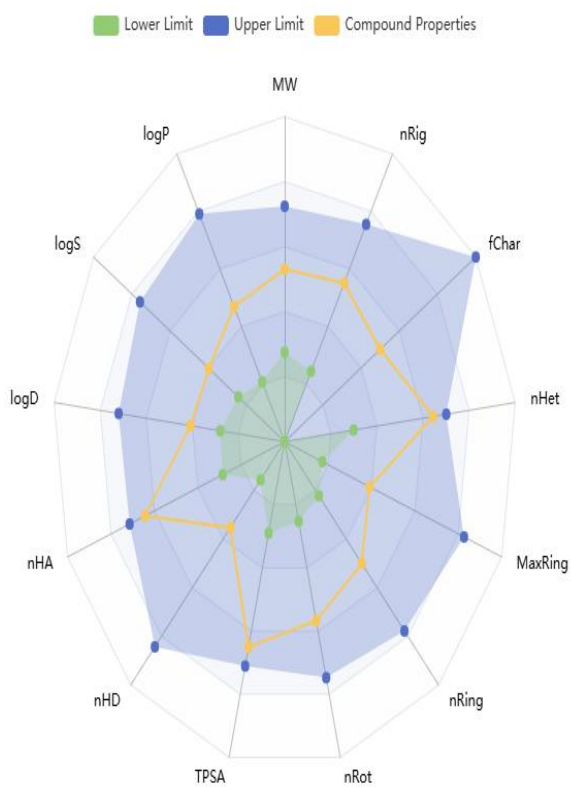
25



27



26



28

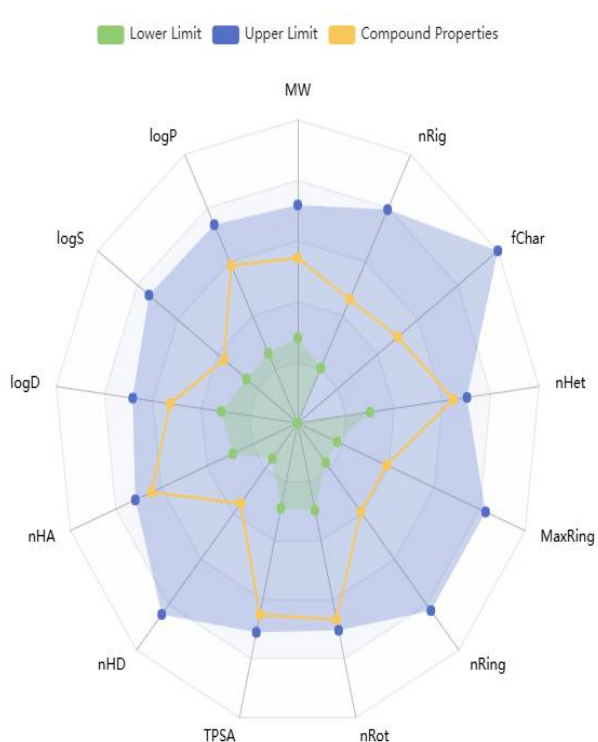


Fig. 6. Pharmacological radar chart of ligand 25, 26, 27 and 28

Table 10. ADMET evaluation parameters of the nominated ligands

	Absorption		Distribution			Metabolism		Excretion		Toxicity		
S/no	Caco-2 Perm.	HIA	PPB	VDss	BBB	CYP		CLplasma	T _{1/2}	AMES	Carcinogenicity	Hematotoxicity
						3A4	2D6					
Template (Ligand -35)												
35	-4.582	---	59.0%	0.539	---	--	---	0.861	2.061	0.472	0.579	0.583
Selected ligands with the highest binding scores												
21	-5.119	---	98.1%	0.662	--	---	---	2.547	1.553	0.267	0.321	0.729
24	-5.299	-	78.0%	1.014	-	---	---	2.582	1.639	0.242	0.247	0.718
25	-5.330	---	95.9%	0.772	+++	---	---	3.646	1.205	0.145	0.138	0.629
26	-5.060	---	90.1%	0.910	-	---	---	2.618	1.659	0.222	0.214	0.611
27	-5.187	---	84.8%	0.853	--	---	---	1.523	2.313	0.245	0.421	0.453
28	-5.345	---	98.1%	0.827	+++	---	---	4.002	1.066	0.137	0.164	0.605
29	-5.385	---	95.5%	0.925	+++	---	---	3.471	1.177	0.127	0.103	0.676
30	-4.985	---	98.5%	0.484	--	---	---	1.793	1.602	0.292	0.319	0.573
32	-5.123	---	97.4%	0.509	---	---	---	1.375	1.741	0.313	0.380	0.558

Hint: prediction likelihood standards are malformed into six probability signs: **0-0.1 (---)**, **0.1-0.3 (--)**, **0.3-0.5 (-)**, **0.5-0.7 (+)**, **0.7-0.9 (++)**, and **0.9-1.0 (+++)**

The ADMET expectations for the template compound (Ligand-35) and the selected high-binding ligands (21, 24, 25, 26, 27, 28, 29, 30, and 32) reveal notable differences in physicochemical properties, pharmacokinetic behavior, and potential toxicity profiles. All the selected compounds displayed Caco-2 permeability values within a low range similar to the template (-4.582), suggesting limited passive intestinal absorption across the series. Among them, ligands 24, 25, 28, and 29 showed the lowest predicted permeability ($\approx$ -5.30 to -5.38), whereas ligand 30 (-4.985) demonstrated permeability closest to the template, indicating comparatively better intestinal uptake. The plasma protein binding (PPB) varied widely across the compounds. The template showed moderate PPB (59.0%), while ligands 21, 25, 28, 29, and 30 exhibited very high PPB values (>95%), implying a substantially reduced fraction of free drug available for activity.

In contrast, ligands 24 (78.0%) and 27 (84.8%) displayed more moderate PPB, closer to the template. Predicted volumes of distribution (VDss) ranged from 0.484 to 1.014 L/kg, indicating moderate tissue distribution for most ligands. Notably, ligand 24 had the highest VDss (1.014 L/kg), suggesting enhanced tissue penetration, while ligands 30 and 32 showed the lowest VDss, implying more confinement to plasma and extracellular compartments. However, no metabolism-related predictions for CYP3A4 and CYP2D6 were available,

limiting conclusions about potential drug - drug interactions and metabolic clearance pathways. Nevertheless, plasma clearance and half-life ($T_{1/2}$) estimates provided additional differentiation. The template exhibited moderate plasma clearance (0.861) and a half-life of 2.061hrs. The following ligands with higher clearance values 21 (2.547), 24 (2.582), 25 (3.646), 28 (4.002), and 29 (3.471) are likely to be eliminated more rapidly, potentially resulting in shorter systemic exposure. Correspondingly, their predicted half-lives were mostly squatter than that of the template, with the exception of ligand 27 ($T_{1/2}$ = 2.313 h), which showed the longest predicted systemic persistence.

In addition, the toxicity predictions were also a critical component of the overall assessment of the candidate compounds. The reference template exhibited moderate predicted risks for AMES mutagenicity (0.472), carcinogenicity (0.579), and hematotoxicity (0.583), indicating potential safety liabilities. In contrast, ligands 25, 28, and 29 showed markedly lower AMES scores (0.137-0.145), suggesting a reduced likelihood of mutagenic effects relative to the template. Ligand 21, however, displayed a comparatively elevated risk of hematotoxicity (0.729), whereas ligands 24 and 29 were allied with considerably minor predicted carcinogenicity values (0.247 and 0.103, respectively), pointing to more favorable safety profiles. When the complete ADMET profiles were evaluated collectively, ligands 24, 26, 27, 30, and 32 emerged as the most well-balanced candidates. Among these, ligand 24 was particularly notable, combining adequate plasma protein binding with the highest predicted volume of distribution, low mutagenicity and carcinogenicity risks, and an acceptable half-life. This combination of properties positions ligand 24 as a strong lead candidate for further development. Ligand 27 also demonstrated a favorable profile, characterized by an extended half-life, moderate plasma protein binding, and reduced predicted carcinogenicity. In addition, ligands 30 and 32 showed encouraging permeability, moderate clearance rates, and relatively low toxicity risks, further supporting their potential as safe and effective candidates for subsequent optimization and development.

Furthermore several ligands exhibit strong binding affinity, those with extremely high PPB (21, 25, 28, and 29) may require structural optimization to enhance free-drug availability. Among all the evaluated compounds, ligand 26 stands out as the most promising candidate, displaying an optimal integration of absorption, distribution, excretion, and toxicity characteristics, and represents a robust lead for extra optimization and experimental validation.

3.5 MD simulations analysis

The structural immovability of the coupling receptor -ligand multiplexes was assessed through the strength Root Mean Square of Deviation (RMSD) analysis over a 100 ns molecular dynamics simulation, as shown in Figure 7. The RMSD is a broadly engaged metric for appraising the convergence and overall conformational stability of biomolecular systems during simulation. Both the 1TFZ -Compound -26 and 1TFZ -Mesotrione complexes demonstrated an early increase in RMSD during the leading ~ 10 ns, reaching values between 1.5 and 2.0 Å. This early rise corresponds to the equilibration phase, during which the complexes adjusted from their initial docked conformations to the solvated environment. Importantly, no abrupt or erratic fluctuations were observed during the simulation, indicating that, both the systems sustained steady conformations and did not encompass significant structural perturbations during equilibration.

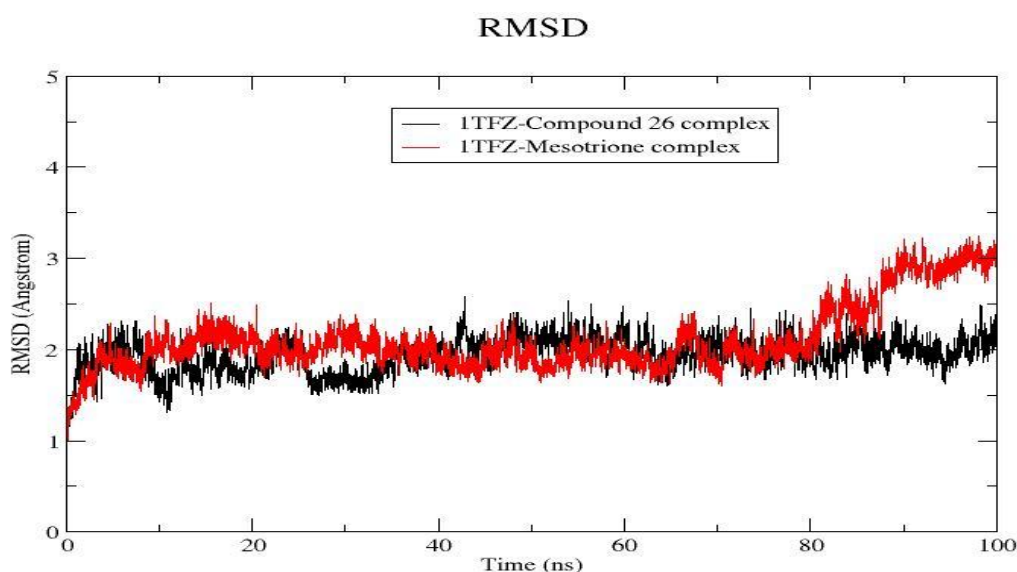


Fig. 7. Root Square of Mean Deviation (RSMD) analysis

However, following equilibration, the 1TFZ -Compound -26 complex endured extremely unwavering during the production phase, with coupling RMSD values largely confined between 1.6 and 2.1 Å from ~ 10 to 100 ns. The narrow fluctuation range and consistent RMSD profile indicated that, the protein backbone was well-maintained and that the molecular ligand retained a steady binding mode during the simulation. In comparison, the 1TFZ -Mesotrione complex showed similar stability during the early and mid-stages of the simulation (~ 10 - 70 ns), with RMSD values fluctuating around 1.8 - 2.2 Å. However, after

~70 ns, the RMSD gradually increased, reaching approximately 3.0Å by the end of the simulation. This rise suggests greater conformational flexibility and potential rearrangements within the complex, indicating a comparatively less steady interaction among Mesotrione and the 1TFZ binding target area (Munei et al., 2023).

Overall, the lower RMSD values and reduced fluctuations observed for the 1TFZ- Compound -26 complex point to the enhanced structural stability relative to Mesotrione. The RMSD values below ~2.5 Å are generally indicative of stable protein ligand systems, further supporting the superior stability of Compound -26. These observations demonstrated that, ligand ID -26 signifies an extra steady complex with 1TFZ over the reference ligand Mesotrione, maintaining conformational integrity throughout the simulation. Such stability suggests stronger and more persistent interactions, which may contribute to its improved binding performance and potential biological activity.

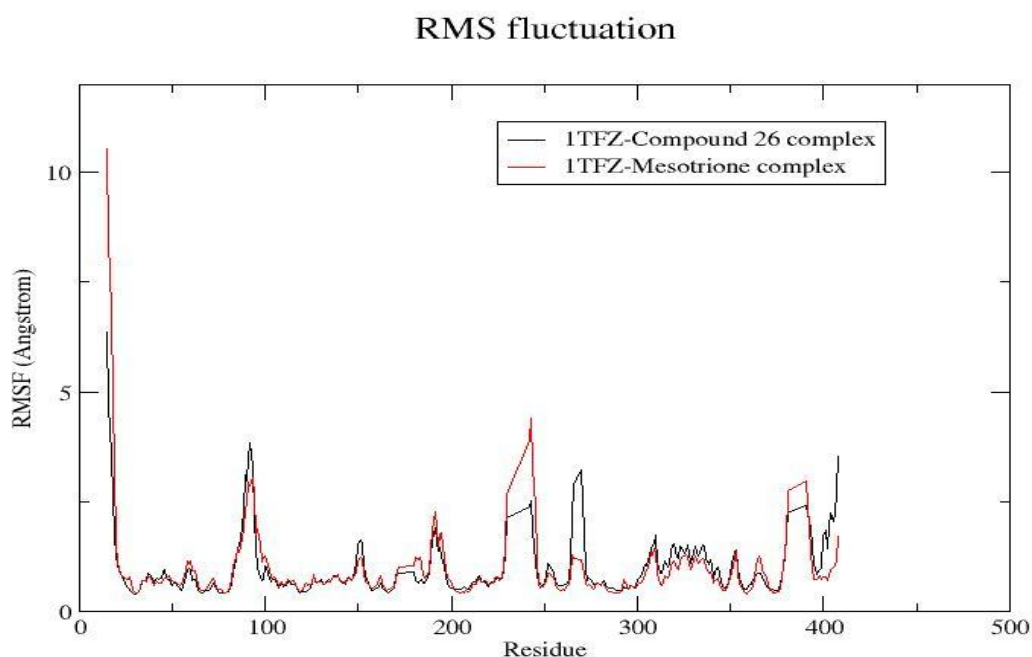


Fig. 8. Root Mean of Square Fluctuation (RMSF) analysis

Figure 8, presents the Root Mean of Square Fluctuation (RMSF) profiles of Ligand-26 and the reference ligand Mesotrione in complex with the 1TFZ receptor during a 100 ns molecular dynamics of simulation. Thus, the RMSF analysis offers residue-wise vision into indigenous conformational dynamics and the impact of ligand binding on protein flexibility. Both complexes displayed comparable overall RMSF profiles, with the majority of residues

fluctuating below $\sim 2.0\text{\AA}$, indicating that, the ligands binding does not source noteworthy global destabilization of the protein structure. However, higher fluctuations were primarily pragmatic in the incurable and loop regions, which are obviously elastic and solvent-exposed. Notably, the N-terminal residues exhibited pronounced flexibility, with RMSF values reaching $\sim 6.0\text{\AA}$ for the 1TFZ -Compound -26 complex and exceeding $10.0\text{\AA}$ for the 1TFZ - Mesotrione complex. This suggests that, while the terminal mobility is expected, mesotrione binding confers comparatively less stabilization than Compound -26. Several distinct RMSF peaks were also observed in loop-rich regions around residues $\sim 80 - 100$, $\sim 180 - 210$, $\sim 230 - 260$, and $\sim 380 - 410$. In these regions, the mesotrione complex consistently displayed higher RMSF values than the Compound -26 complex, indicating increased local flexibility (Lin et al., 2019). Conversely, the reduced fluctuations in the Compound -26 complex suggest enhanced stabilization, likely reflecting stronger protein -ligand interactions or ligand-induced conformational restraint. Importantly, residues near the binding site showed lower RMSF values in the Compound -26 complex, indicating an improved rigidity and preservation of the active-site building. Such stabilization of functionally relevant regions is often concomitant with more obstinate binding and improved inhibitory potential.

In overall, the RMSF analysis demonstrates that, while both ligands maintain the structural integrity of 1TFZ, Compound -26 also induces a more dynamically stable conformation, with reduced residue-level fluctuations compared to Mesotrione. These findings align with the RMSD results and further support the superior steadiness and binding efficiency of Compound -26 within the 1TFZ active site.

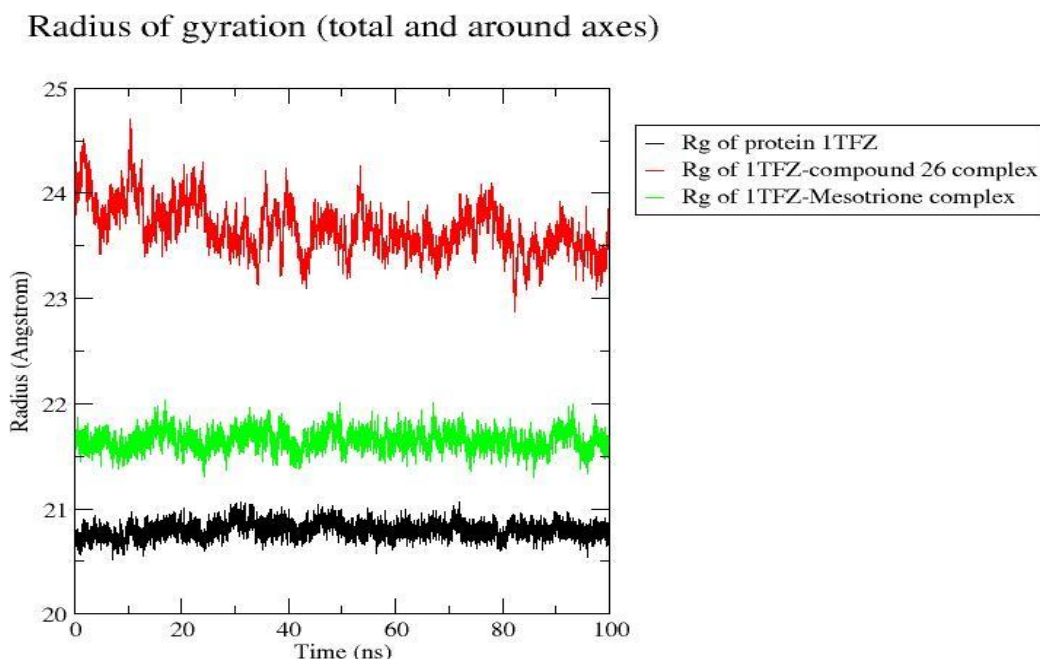


Fig. 9. Radius of gyration (Rg) analysis

The parameter Radius of Gyration (Rg) was examined to evaluate the firmness and conformational steadiness of the 1TFZ target in its apo (absence of cofactor or ligand attached to the receptor) form and in complex with Compound -26 and Mesotrione over a 100 ns molecular dynamics simulation (Figure 9). The Rg provides insight into ligand-induced structural changes and the overall folding behavior of the protein. The apo 1TFZ protein exhibited a stable Rg profile during the simulation, fluctuating narrowly around $\sim 20.7 - 21.0 \text{ \AA}$. This consistency indicates that the protein maintains a compact, well-folded conformation in the absence of ligand binding, with only minor fluctuations arising from intrinsic backbone motions. Upon ligand binding, distinct Rg patterns emerged. The 1TFZ - Compound -26 complex displayed slightly higher Rg values, stabilizing around $\sim 23.4 - 24.0 \text{ \AA}$ after the initial equilibration phase. Despite this modest increase, the Rg stayed unswerving over a period of time with no evidence of progressive expansion or contraction. This suggests that, Compound -26 induces a stable ligand accommodated conformation, characterized by moderate structural expansion while maintaining overall protein compactness and integrity. In contrast, the 1TFZ -mesotrione complex exhibited intermediate Rg values, fluctuating around $\sim 21.5 - 22.0 \text{ \AA}$. The relatively lower Rg compared to the Compound -26 complex indicates more limited conformational rearrangement upon mesotrione binding yet, protein stability was preserved (Wang et al., 2021). The comparative

analysis shows that, ligand binding induces controlled and stable conformational changes in 1TFZ, with Compound 26 promoting a more pronounced but well-equilibrated expansion relative to Mesotrione.

The overall analysis revealed that, the Rg results confirms that, all the systems remain structurally steady through the process of the simulation. The slightly increased nevertheless stable Rg observed for the 1TFZ -Compound -26 complex, unswerving with the RMSD and RMSF outcomes, supports the conclusion that, Compound -26 stabilizes the protein in a dynamically favorable conformation. These discoveries strengthen its purulent as a superior ligand compared to the standard mesotrione.

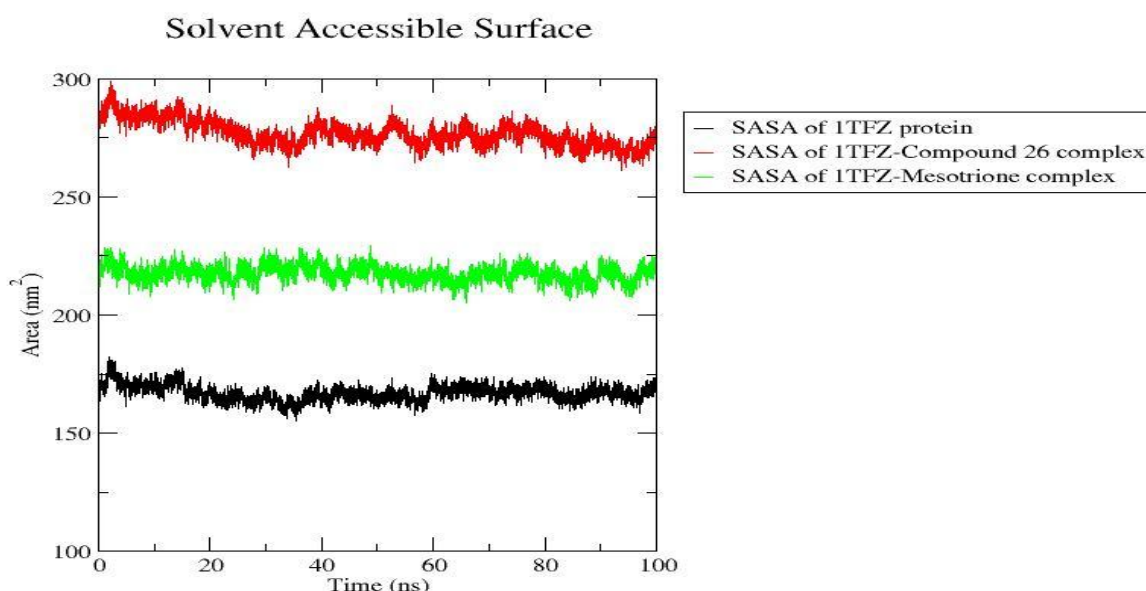


Fig. 10. Accessibility of Solvent Surface Area analysis (SASA)

The parameter Accessibility of Solvent Surface Area analysis (SASA) was analyzed to scrutinize the influence of ligand binding on the conformational constancy and solvent exposure of the 1TFZ receptor throughout the 100 ns molecular dynamics simulations (Fig. 10). The SASA is an important structural parameter that reflects changes in protein compactness and surface accessibility in response to dynamic conformational rearrangements. As shown in Fig.10, all the systems exhibited stable SASA profiles with only moderate fluctuations throughout the simulation, indicating that, the protein maintained structural stability under all conditions. The absence of pronounced drift or abrupt changes suggests that, none of the systems underwent significant unfolding or destabilizing

conformational transitions during the simulation period. However, the apo 1TFZ protein displayed the lowest SASA values, fluctuating around 160 - 170nm², consistent with a relatively compact conformation in the absence of ligand binding. Minor fluctuations observed were attributed to local side-chain and backbone motions inherent to thermal dynamics. Upon ligand binding, SASA increased, reflecting ligand-induced conformational adjustments. The 1TFZ -mesotrione complex exhibited intermediate SASA values, averaging ~210-220nm², indicating moderate structural rearrangements that exposed additional surface regions to the solvent while maintaining overall integrity. In contrast, the 1TFZ -Compound - 26 complex showed the highest SASA values, ranging from 270 - 290 nm², suggesting more pronounced changes in solvent exposure, likely due to induced-fit effects or ligand mediated opening of surface accessible regions.

In addition, the higher SASA observed in the ligand bound complexes, the fluctuations remained stable over the whole simulation, confirming that, these conformational vicissitudes are dependable and do not compromise protein integrity. The distinct SASA profiles further indicate that, different ligands exert unique effects on protein surface properties. Overall, the SASA analysis demonstrated that, ligand binding modulates the solvent exposure and conformational dynamics of the 1TFZ target in a ligand specific manner. The stable behavior across all systems reinforces the reliability of the molecular dynamics simulations and suggests that, the observed differences in solvent accessibility reflect intrinsic binding interactions rather than simulation artifacts.

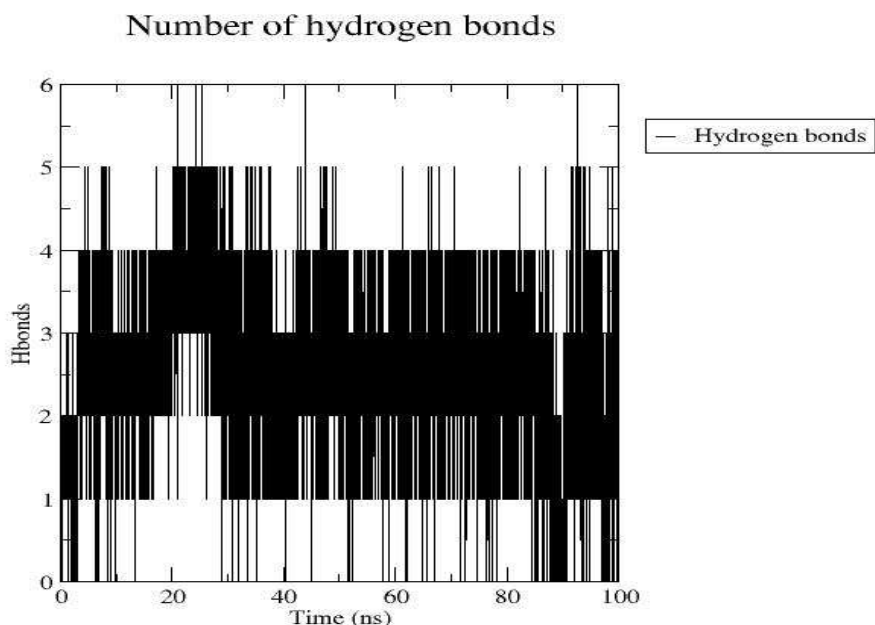


Fig. 11. Number of Hydrogen bond analysis of mesotrione (complex -35)

Figure 11 above, shows how hydrogen bonds evolve over the sequence of the 100 ns molecular dynamics (MD) simulation. These interactions were tracked using standard geometric criteria, and their chronological development delivers appreciative intuition into the dynamic steadiness of the coordination. The quantity of H- bonds fluctuates between zero and approximately six, reflecting the continuous formation and disruption of these interactions on picosecond timescales driven by thermal motion. Importantly, no sustained upward or downward trend is observed in the hydrogen-bonding profile, indicating that the structure remains steady and thriving equilibrated throughout the simulation. For the majority of the trajectory, the system maintains between two and four H- bonds, signifying that these intermediate bonding states are energetically favored. Occasional reductions to zero or one hydrogen bond correspond to brief local rearrangements, while short-lived increases to five or six bonds represent transient yet highly favorable interaction states. These extreme events occur infrequently and do not dominate the overall behavior of the system.

Overall, the hydrogen-bonding pattern remains consistent across the simulation, with no indication of major structural disruptions such as ligand dissociation, protein unfolding, or large-scale conformational rearrangements. This behavior indicates that the complex preserves a stable interaction network while retaining sufficient local flexibility, thereby achieving a balance between structural stability and dynamic adaptability.

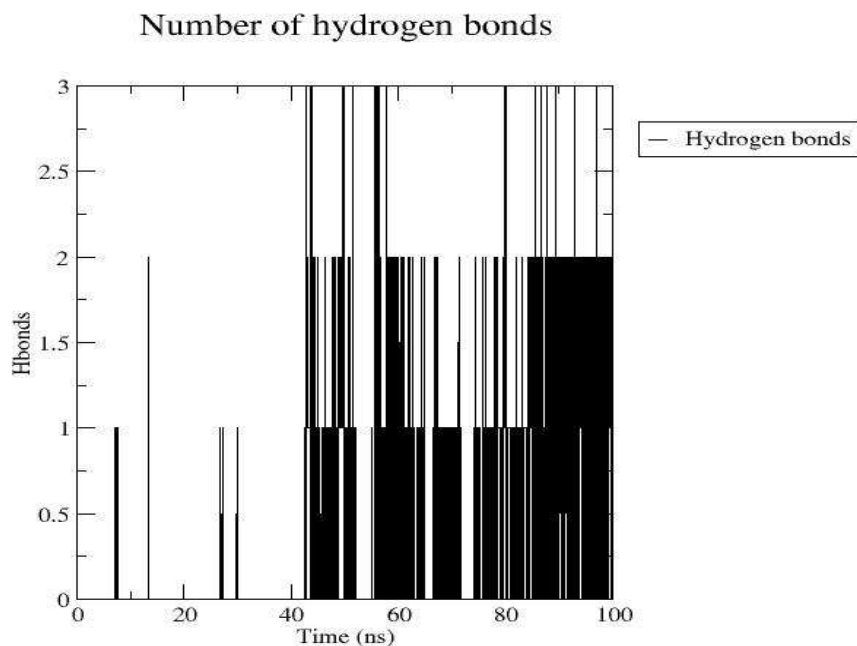


Fig. 12. Number of Hydrogen bond analysis (Complex -26)

Figure 12, illustrates the time evolution of hydrogen bonds formed between Ligand-26, a substituted tetrazolium nicotinamide herbicide and its target receptor over a 100 ns molecular dynamics (MD) simulation. The H-bonds are key non covalent interfaces that govern ligand recognition, binding specificity and complex stability; thus, their persistence and temporal distribution provide valuable insight into the binding behavior of Ligand-26 within the active site. However, through the preliminary phase of the simulation (0 - 30ns), the number of H-bonds remains low, mostly fluctuating between 0 and 1, with only sporadic, short-lived interactions. This early behavior reflects the ligand undergoing conformational adjustments and positional reorientation within the binding pocket. At this stage, Ligand-26 has not yet established a steady H-bonding network with vital amino acid residues, which is typical of the equilibration phase in MD simulations. The transient nature of these early interactions indicates weak or nonspecific contacts as the ligand searches for an energetically favorable binding pose.

Moreover, from approximately 35 - 40 ns onward, a marked increase in hydrogen bond formation is perceived with the number of bonds frequently oscillating between 1 and 2 and occasional peaks reaching 3 hydrogen bonds. This transition signifies the onset of a more stable binding regime, where Ligand-26 becomes better accommodated within the receptor cavity. The tetrazolium ring and nicotinamide moiety likely contribute to these interactions,

acting as hydrogen bond acceptors and donors with polar residues lining the active site. The recurrent formation of multiple hydrogen bonds during this period indicates improved complementarity between the ligand's functional groups and the receptor's hydrogen bonding network. Furthermore, in the later stage of the simulation ($\approx 60 - 100$ ns), H-bond foundation signifying more persistent, with prolonged periods maintaining 1-2 hydrogen bonds and frequent excursions to 2 - 3 bonds. The sustained hydrogen-bonding pattern indicates that the ligand-receptor complex has reached a dynamically stable state. The infrequent occurrence of zero hydrogen bonds, together with the predominance of higher bond counts, suggests that Ligand-26 remains securely anchored within the binding pocket throughout the simulation. These persistent interactions restrict excessive ligand mobility and help preserve key pharmacophoric contacts, conditions that are often linked with improved binding affinity and enhanced inhibitory potential. Overall, the hydrogen-bond analysis reveals a clear progression from initially weak and fluctuating interactions to a well-stabilized complex.

However, during the equilibrated phase, Ligand-26 consistently forms up to three hydrogen bonds, underscoring the importance of its substituted tetrazolium and nicotinamide moieties in mediating strong polar interfaces with the receptor. When evaluated in aggregation with harmonizing molecular dynamics metrics including RMSD, RMSF, SASA, and binding free energy these hydrogen-bonding characteristics further reinforce the candidacy of Ligand-26 as a promising lead for herbicide development, demonstrating both stable binding behavior and sustained molecular interactions over time. Conclusively, the 100 ns MD simulations show that ligand-26 forms a more steady and dynamically favorable complex with the 1TFZ receptor compared to the reference ligand, Mesotrione. The consistently lower RMSD values, reduced residue level fluctuations, stable radius of gyration, and well-maintained SASA profiles indicate that Compound -26 preserves the structural integrity of the protein while promoting effective ligand accommodation. Moreover, the persistent yet flexible hydrogen bonding network reinforces the firmness of the 1TFZ–Compound -26 complex through the simulation. Collectively, these results demonstrate enhanced conformational stability, stronger protein ligand interactions, and improved binding persistence for Compound -26, highlighting its potential as a superior ligand with promising biological activity relative to Mesotrione.

3.6 Combined Ligand and Structure -based analogue design

Table 11 below, presents the molecular descriptor mathematical values and the corresponding forecast pIC_{50} activities of the freshly calculated derivatives (26a, 26b, 26c, 26d, and 26e). Notably, all the five derivatives exhibited predicted pIC_{50} values greater than 7.600, which represents a significant improvement over the template compound (Ligand-26), whose experimentally derived pIC_{50} value was 7.0132 as shown in Table 2. This consistent enhancement in predicted bioactivity across all newly designed entities highlights the beneficial impact of strategic structural modification at the CF_3 substitution site.

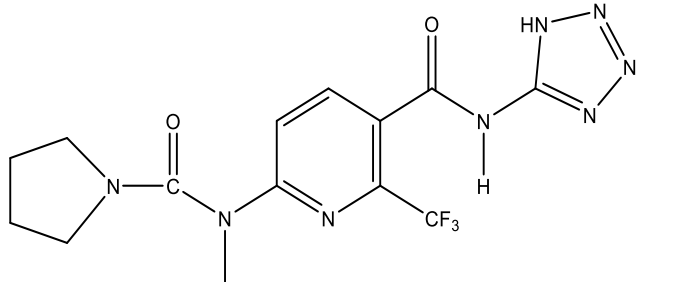
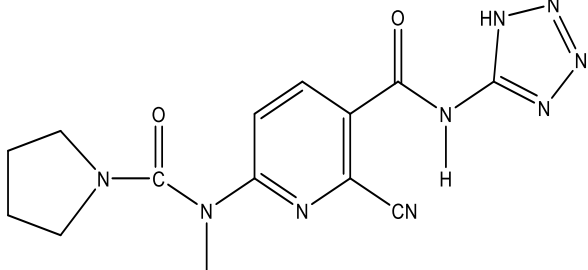
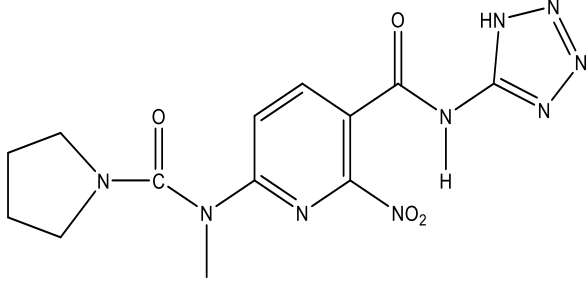
Table 11. Descriptors values, and projected pIC_{50} of the recently calculated compounds

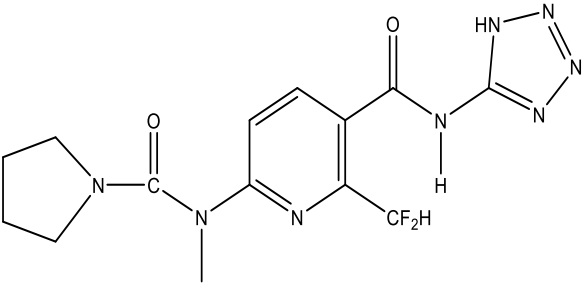
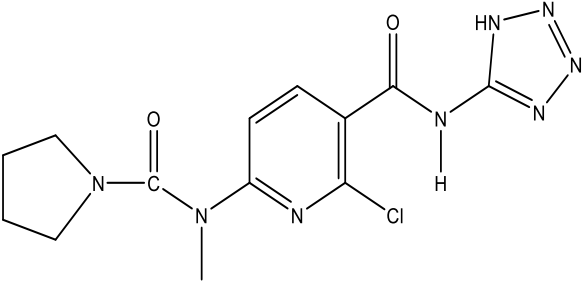
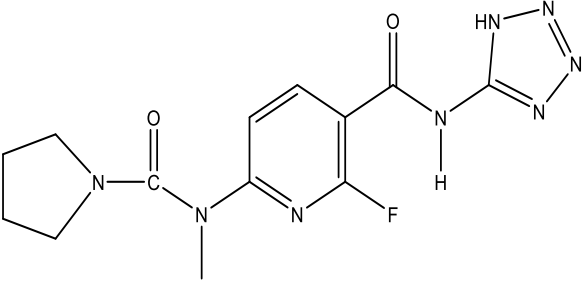
Compound ID	AATS2e	AATS5i	ATSC3v	ATSC7v	XLogP	Pred. activity
26a	8.074768	163.0054	-45.9064	277.3733	0.953	7.657308
26b	8.227649	165.8591	-141.368	205.1411	1.281	7.663080
26c	8.294203	170.3431	-121.945	401.3393	2.200	7.821541
26d	8.294203	170.3431	-121.945	401.3393	2.200	7.821541
26e	8.171236	165.7934	-207.976	205.3814	1.561	7.675195

Furthermore, Table 12 provides the 2D structural representations of the template compound and the five recently calculated analogues, alongside their predicted pIC_{50} values, MolDock scores, and Rerank scores. These computational parameters collectively offer insights into the binding affinity and stability of the ligand receptor interfaces. As evident from the data provided, each of the recently premeditated compounds demonstrates a more favorable MolDock and rerank scores compared to the template thereby, indicating stronger predicted binding interactions with the target protein (PDB ID: 1TFZ) (Fu et al., 2020). The higher (i.e., more negative) docking scores typically correlate with improved ligand receptor complementarity and enhanced binding stability and further supporting the robustness of these design modifications. However, the observed improvements in both predicted activity and docking scores can be directly attributed to the introduction of electron withdrawing substituents ($-CN$, $-NO_2$, $-CF_2H$, $-Cl$, and $-F$) at the CF_3 position of the parent scaffold. These substituents are known to modulate physicochemical properties such as polarity, electronegativity, and electron dispersal, all of which play critical roles in receptor acclaim and H-bonding interactions. Their strategic incorporation likely enhances the overall molecular interaction profile of the compounds inside the active point of the 1TFZ receptor thereby, contributing to improved binding affinity and predicted inhibitory potency.

Moreover, the results from Tables 11 and 12 provide strong computational evidence that, the newly designed derivatives possess superior predicted activity and binding characteristics relative to the template (compound -26). The consistent trend across all evaluated parameters supports the hypothesis that targeted substituent modification at the CF₃ position is an actual approach for optimizing the biological performance of this class of compounds. Lastly these findings justify the further evaluation of these derivatives in subsequent computational, biochemical, or structural studies.

Table 12. 2-Dimensional arrangements of the Template (Ligand -26) and the recently premeditated complexes with their docking marks

S/no.	2D Structures	Predicted pIC ₅₀	MolDocking score	Reranking score
	Template (Ligand -26) 	7.013228	-141.002	-110.865
26a		7.657308	-142.107	-109.394
26b		7.66308	-142.168	-111.374

26c		7.821541	-144.817	-115.221
26d		7.821541	-143.081	-107.912
26e		7.675195	-135.287	-104.149

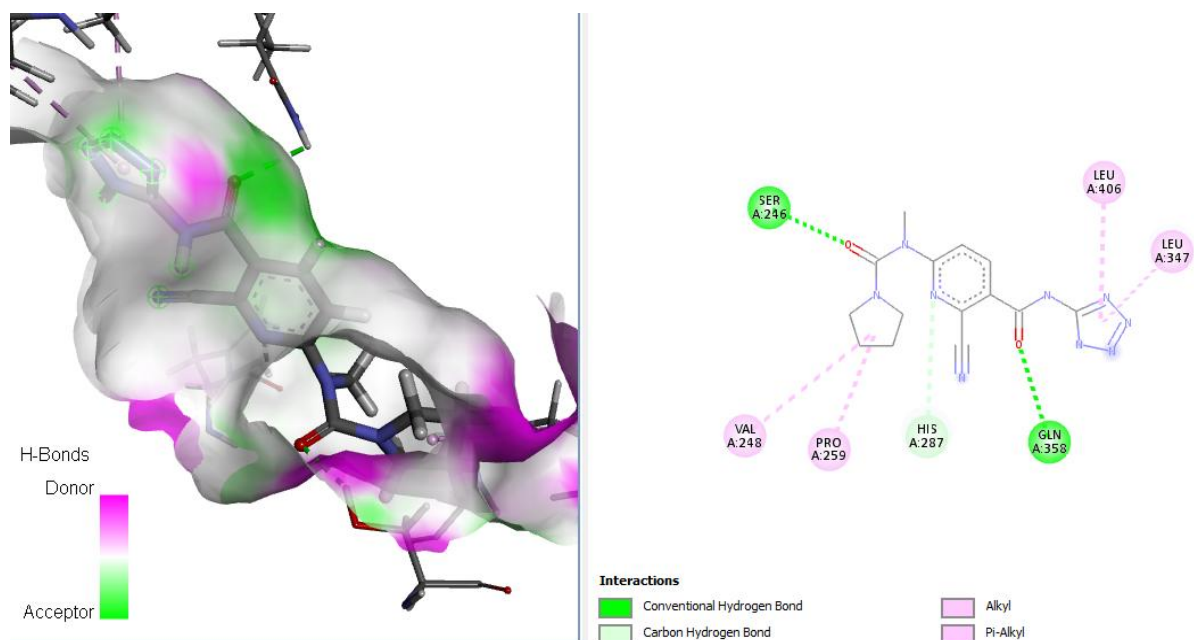


Fig. 12a. 3D and 2D molecular interaction of newly designed Ligand -26a with 1TFZ binding site

Figure 12a illustrates the binding mode of Ligand-26a, which unveils a docking dynamism of -142.107 kcal/mol and adopts a stable, well positioned conformation inside the target's active site. The ligand's stability is supported by a well-coordinated network of interactions. Two hydrogen bonds with SER246 and GLN358 serve as critical polar anchors, carefully orienting the ligand's functional groups inside the binding abridged and donating significantly to its overall steadiness. Hydrophobic interactions further reinforce the binding pose, with alkyl contacts involving VAL248 and PRO259 promoting tight packing within the hydrophobic regions of the active site. Π -Alkyl interactions with LEU347 and LEU406 also help stabilize the aromatic moiety, augmenting van der Waals interfaces and limiting conformational flexibility. Together, these H-bonding and hydrophobic interactions create a cohesive network that keeps Ligand-26a in a favorable low-energy conformation. This detailed interaction map underscores the ligand's promise as a scaffold for future structure-based optimization and rational herbicide design.

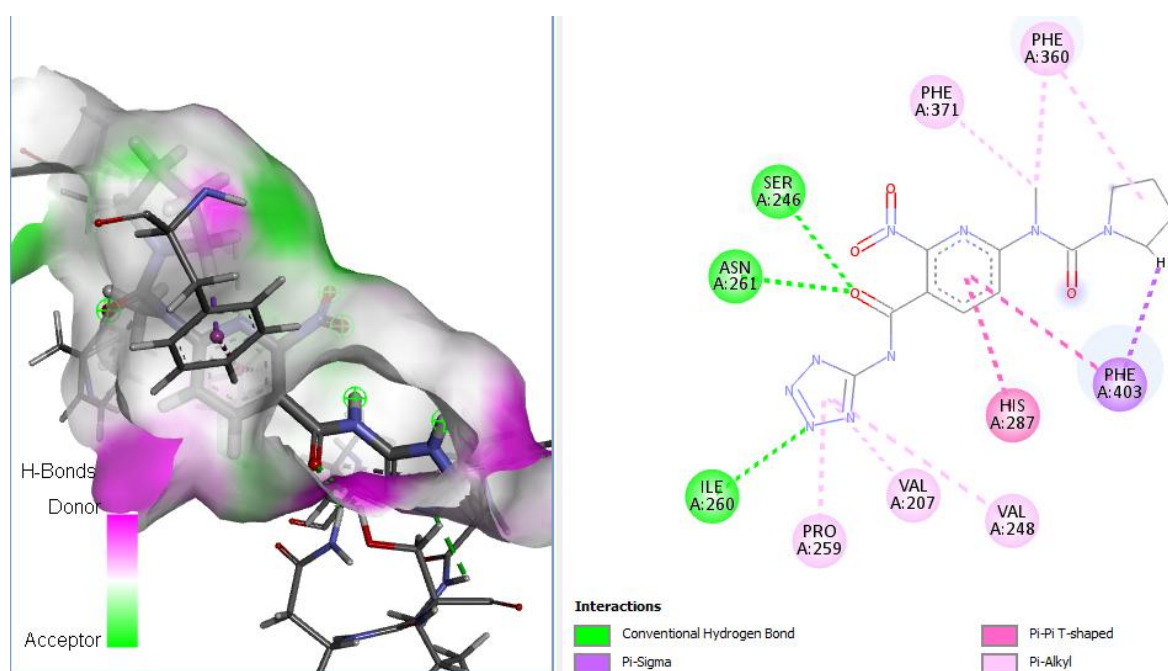


Fig. 12b. 3D and 2D molecular interaction of newly designed Ligand -26b with 1TFZ binding site

Figure 12b depicts the binding mode of complex 26b, which displays a docking scoring value of -142.168 kcal/mol and adopts a stable and well-defined orientation indoors the receptor's active point. The ligand adopts a unwavering binding orientation through a coordinated network of ionic and pi-electron delocalization of aromatic interactions. Three hydrogen bonds involving SER246, ASN261, and ILE260 serve as key anchoring points, appropriately

positioning the ligand's polar functionalities within the binding pocket and contributing substantial enthalpic stabilization.

In addition, beyond these polar contacts, an array of hydrophobic and aromatic interactions further consolidates the docking pose. π -Alkyl interactions with PHE360, PRO259, VAL207, VAL248, and PHE371 promote efficient van der Waals packing, effectively embedding both the aromatic and aliphatic regions of the ligand within the hydrophobic cavity. A π - σ interaction with PHE403 provides additional stabilization by optimizing face-to-edge contacts with the aromatic system, while a π - π T-shaped interaction with HIS287 reinforces the aromatic core through directional stacking and limits conformational flexibility. Collectively, these interactions form a complementary and cooperative network that maintains compound 26b in a low-energy, well-stabilized conformation. This detailed interaction profile underscores the ligand's impending as a robust framework for additional structure-based optimization and rational herbicide design.

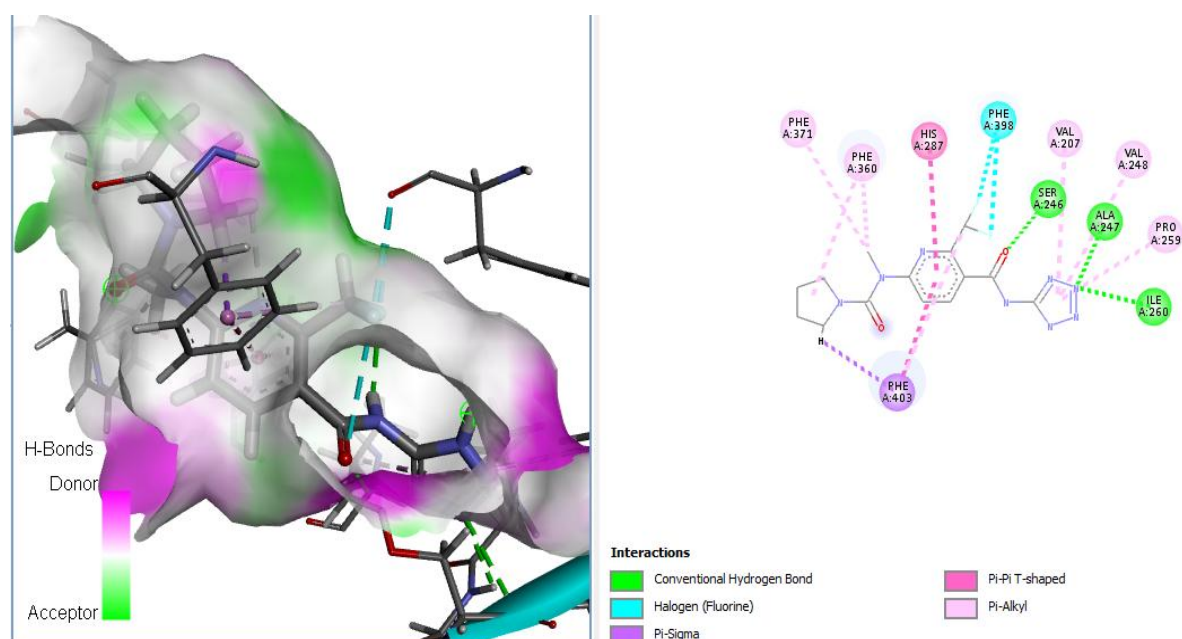


Fig. 12c. 3D and 2D molecular interaction of newly designed Ligand -26c with 1TFZ binding site

Figure 12c illustrates the binding mode of complex 26c, which unveils a docking energy of -144.817 kcal/mol and also adopts a stable, thriving concerned with conformation within the receptor's active point. The complex stability is maintained by a well-coordinated network of polar, hydrophobic, halogen, and aromatic interactions. Three hydrogen bonds involving SER246, ALA247, and ILE260 act as primary anchors, precisely positioning the ligand's

polar groups within the binding pocket and contributing significantly to overall stabilization. However, accompanying these polar contacts, hydrophobic interactions reinforce the binding pose. π -Alkyl contacts with PHE403, PHE360, PHE259, VAL207, VAL248, and PHE371 enhance nonpolar complementarity, securely lodging the ligand within the hydrophobic cavity, restricting conformational flexibility, and strengthening van der Waals interactions. A halogen contact with PHE398 further stabilizes the ligand's halogen substituent by engaging an electron-rich aromatic environment, optimizing its orientation within the pocket. Furthermore, a π - π T-shaped interface with HIS287 stabilizes the aromatic core through directional stacking, further enhancing binding affinity. Lastly, these interactions function synergistically to maintain complex 26c in a low-energy, well-packed conformation. This robust interaction profile highlights the ligand's potential as a strong scaffold for future structure-based optimization and rational herbicide design.

In addition, across the five newly designed ligands (26a–26e), four compounds (26a, 26b, 26c, and 26d) demonstrated improved predicted stability compared with the template ligand 26 (−141.002 kcal/mol), with binding scores of −142.107, −142.168, −144.817, and −143.081 kcal/mol, respectively. However, ligand 26e, demonstrated a lower binding Gibbs's energy value of −135.287 kcal/mol relative to the template, suggesting that the outline of a fluorine atom at C-5 of the pyridine ring in the tetrazolium nicotinamide template reduces binding affinity and overall complex stability. Moreover, complexes 26a and 26b showed modest enhancements in binding affinity, primarily due to additional hydrophobic and aromatic contacts compared to the template's combination of hydrogen bond, halogen and π - π interactions. In contrast, complex 26c demonstrated the most favorable binding energy and the most extensive interaction profile, combining multiple hydrogen bonds, enriched π -alkyl and aromatic contacts and a retained halogen interaction. This results in a deeply stabilized, well packed binding orientation, positioning ligand 26c as the utmost auspicious candidate between the five recently considered candidates, with superior overall stability compared to both the original template and its structural derivatives.

Table 13. Predicted ADMET physicochemical behavior of the newly established compounds

S/no.	Molecular weight	HBA	HBD	Log P	Log S	TPSA	pka (Acid)
Template (Ligand -26)							
26	384.13	10.0	2.0	1.343	-2.654	120.0	4.518
Newly designed analogues of ligand -26							
26a	341.13	11.0	2.0	0.404	-2.260	143.79	4.009
26b	361.12	13.0	2.0	0.417	-2.319	163.14	3.923
26c	366.14	10.0	2.0	0.996	-2.239	120.00	4.394
26d	366.14	10.0	2.0	0.996	-2.239	120.00	4.394
26e	334.13	10.0	2.0	0.860	-2.198	120.00	4.365
Lipinski's rule	≤500	≤10	≤5	≤5	Low	≤140Å	4-6

The physicochemical assessment of the recently designated compounds (26a-26e), summarized in Table 13, provides key perceptions into their herbicides-likeness and acquiescence with Lipinski's rule of five (Ro5) (Chen et al., 2020). Conferring to Ro5, optimal oral bioavailability is typically allied with compounds having a molecular mass/weight (MW) < 500, H-bond acceptors (HBA) ≤ 10, H-bond donors (HBD) ≤ 5, and a partition constant (Log P) < 5. These standards remained used to evaluate the newly developed analogs relative to the template compound (26). However, all the newly designed compounds demonstrated molecular weights below 500, suggesting that, none are likely to encounter permeability or absorption issues due to excessive low size. These values aligns well with the Lipinski's criteria and is comparable to the template compound. Regarding hydrogen bonding, compounds 26c, 26d, and 26e fully comply with the HBA limit (≤10). Compounds 26a and 26b, however, exhibited slightly elevated HBA values of 11.00 and 13.00, respectively. While these deviations exceed the Ro5 cut-off, they do not necessarily preclude oral activity though, they may indicate a potential reduction in membrane permeability or absorption relative to the other analogs. All the designed compounds demonstrated HBD values below the threshold of ≤5, suggesting minimal risk of impaired permeability due to excessive hydrogen bonding (Karami et al., 2022).

In addition, the water fearing lipophilicity of the analogs, articulated as Log P, revealed values below 1.0 for all the newly designed molecules, indicating lower lipophilic character than the template (Log P = 1.343). The reduced lipophilicity may enhance aqueous solubility and reduce nonspecific binding though, it could moderately influence membrane permeability depending on the structural context. Nevertheless, all values remain comfortably within the

acceptable range ($\text{Log } P < 5$), supporting favorable physicochemical behavior. The aqueous solubility ($\text{Log } S$) values ranged from -2.198 to -2.319 across the series, indicating moderate solubility, yet slightly improved relative to the template ($\text{Log } S = -2.654$). The superior solubility detected in the newly designed compounds is anticipated to improve both their absorption and formulation potential. Distinct differences among the analogs are evident when examining their polar surface area of topology (TPSA). Compounds 26c, 26d, and 26e exhibit TPSA values quite below the recommended threshold of $140 \text{ \AA}^2$, suggesting favorable membrane permeability and promising oral bioavailability.

In contrast, compounds 26a and 26b slightly exceed this threshold, with TPSA standards of $143.79 \text{ \AA}^2$ and $163.14 \text{ \AA}^2$, correspondingly. Combined with their higher numbers of hydrogen bond acceptors, this may result in moderately reduced passive membrane permeability and oral absorption relative to the other analogs. The acidic pK_a standards of the new compounds range from 3.923 to 4.394, closely matching that of the reference template ($\text{pK}_a = 4.518$). This resemblance designates that their ionization under physiological conditions is largely maintained, supporting an optimal balance between solubility, absorption, and receptor interactions. Furthermore, most of the premeditated compounds particularly 26c, 26d, and 26e demonstrate a good acquiescence with Lipinski's rule of five and improved solubility equated with the template. While 26a and 26b show minor deviations in TPSA and hydrogen bond acceptor counts, these differences are unlikely to preclude oral activity, though they may slightly affect permeability or absorption. Overall, the physicochemical profiles of these analogs support their drug-like characteristics and competitive performance, positioning them as strong candidates for further ADMET and biological evaluation.

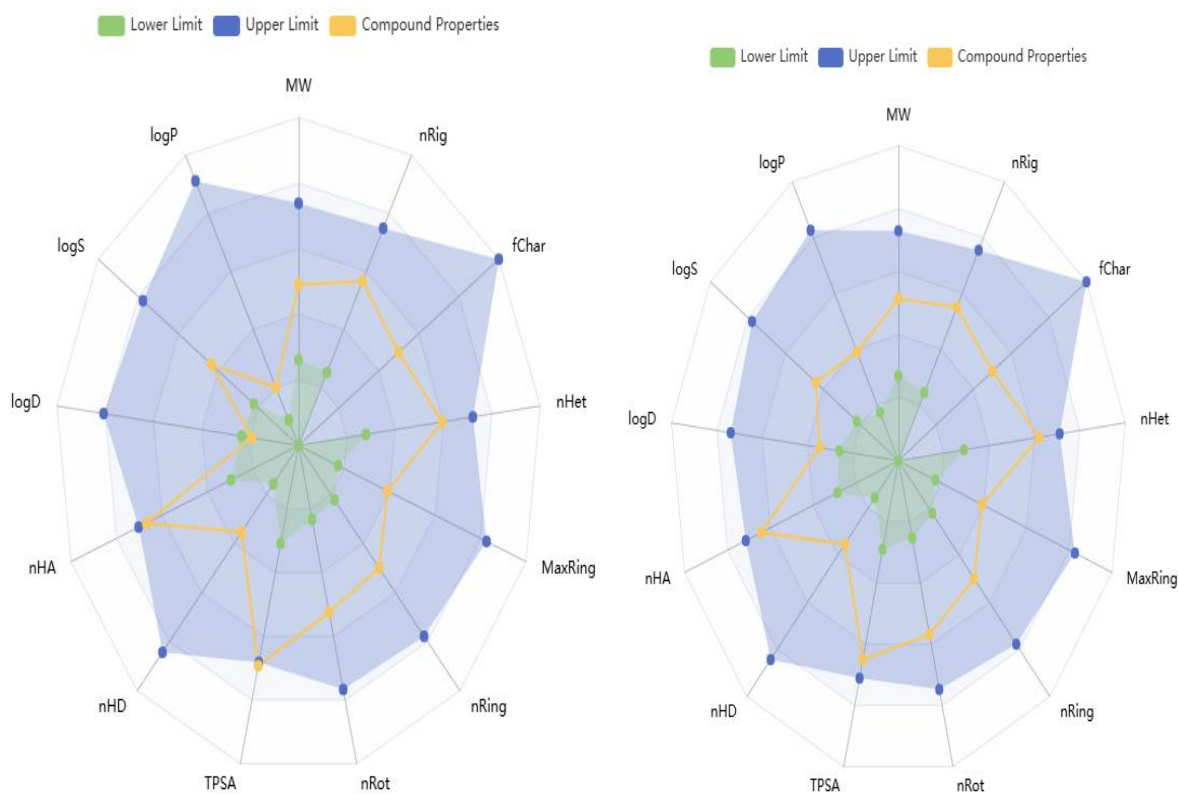


Fig. 13. Pharmacological radar chart of newly designed ligands (26a and 26c)

The pharmaceutical radar charts for the template compound 26, which served as the structural basis for designing five new derivatives (26a–26e), were spawned by the website ADMET-Lab 2.0 platform, as discovered in Figure 6. The radar profile of the template established that, all of its physicochemical and ADMET related parameters fall within the optimal (blue) region, indicating a well-balanced distribution of molecular properties conducive to herbicidal activity. The newly designed derivatives were afterward evaluated using the same profiling approach. Figure 13 above, presents the radar charts for two prioritized derivatives, 26a and 26c, which also show promising placement of nearly all descriptors within the upper limit (blue) region. Considering ligand 26a, only an insignificant increase in topological polar surface area (TPSA) was detected, placing it just above the optimal range. This minor deviance is unlikely to pose a significant limitation, as moderate TPSA increases can sometimes improve solubility or reinforce interactions with the target, contingent on the biological context. Overall, the radar plots of the recently considered derivatives disclose a more stable physicochemical profile compared with the reference template.

Importantly, all the newly synthesized compounds fully mollify the Lipinski's herbicide-likeness criteria, as summarized in Table 13. Their favorable values for key constraints comprising molecular mass, H- bond donors and acceptors, lipophilicity (Log P), coupled with molecular flexibility support efficient absorption and transport within plant systems. Furthermore, the improved radar profiles of compounds 26a and 26c, relative to the template, indicate that the structural modifications introduced during the design process enhanced their overall physicochemical robustness, which could contribute to superior herbicidal performance.

In conclusion, the combined radar chart analysis and Lipinski assessment demonstrate that, the newly designed tetrazolium based derivatives derived from compound -26 possess superior drug-likeness characteristics and more optimal ADMET aligned properties than the parental scaffold. However, these outcomes highpoint compounds 26a and 26c as outstanding lead contenders for additional biological evaluation and potential advance as future next generation herbicidal agents.

Table 14. ADMET evaluation controls of the recently established ligands

S/no	Absorption		Distribution			Metabolism		Excretion		Toxicity		
	Caco-2 Perm.	HIA	PPB	VDss	BBB	CYP		CL _{plasma}	T _{1/2}	AMES	Carcinogenicity	Hemato toxicity
						3A4	2D6					
Template (Ligand -26)												
26	-5.060	---	90.1%	0.910	-	---	---	2.618	1.659	0.222	0.214	0.611
Newly designed analogues of ligand-26												
26a	-5.303	--	81.0%	0.736	---	---	---	2.458	1.259	0.382	0.529	0.441
26b	-5.024	---	78.8%	0.688	--	---	---	2.115	1.327	0.763	0.612	0.783
26c	-5.495	---	85.7%	0.768	---	---	---	2.162	1.584	0.268	0.390	0.611
26d	-5.495	---	85.7%	0.768	---	---	---	2.162	1.584	0.268	0.390	0.611
26e	-5.311	---	77.5%	0.875	--	---	---	2.887	0.983	0.492	0.466	0.639

The comparative ADMET evaluation of the template compound (Ligand-26) and the newly designed analogues (26a, 26b, 26c, 26d and 26e), summarized in Table 14, highlights some key trends in absorption, distribution, excretion, and toxicity profiles. All the newly designed analogues exhibited Caco-2 permeability values within the same low-permeability range as the template, suggesting a comparable passive absorption profile across the series. The predicted plasma protein binding (PPB) values for the analogues ranges between 77.5 -

85.7% and were unswervingly lesser than that of the template (90.1%) therefore, indicating a higher fraction of unbound, pharmacologically active compound in circulation. The predicted volume of distribution (VD) values were mostly dependable athwart all analogues, indicating limited tissue spreading comparable to the parent compound. Plasma clearance and half-life values showed only minor variation within the series, with compounds 26c and 26d exhibiting half-lives closest to that of the template, suggesting a favorable balance between distribution and elimination for these two analogues. The toxicity predictions further distinguished the compounds. Both 26c and 26d displayed low AMES toxicity, consistent with the template, whereas 26b exhibited the highest predicted risks for mutagenicity, carcinogenicity, and hematotoxicity, making it less suitable for further development. When considering all ADMET parameters collectively, the designed analogues show varying degrees of alignment with the reference compound. Compounds 26a and 26e display moderate pharmacokinetic and toxicity profiles, though their predicted carcinogenicity and clearance values merit careful attention.

In contrast, 26c and 26d consistently demonstrate the most favorable overall ADMET profiles, combining good permeability, balanced distribution, moderate clearance, and the lowest predicted toxicity risks within the series. These findings underscore 26c and 26d as the most promising candidates, offering the best overall balance of ADMET properties relative to the template and positioning them as strong leads for additional untried authentication and optimization in this tetrazolium-based herbicidal series.

4. Conclusion

In this education investigation, we employed a comprehensive computational approach to identify and optimize novel tetrazolium-nicotinamide herbicidal candidates targeting the HPPD enzyme (PDB ID: 1TFZ). Our workflow integrated 2D-QSAR modeling, molecular docking, structure-guided chemical modification, agrochemical-focused ADMET evaluation, and molecular dynamics (MD) simulations, providing a systematic framework for interpreting the structural and physicochemical features that govern inhibitory activity. The developed MLR-GFA QSAR model validated a strong statistical reliability, with excellent internal consistency and robust external predictive performance, highlighting its utility in guiding rational lead discovery and optimization. Moreover, molecular docking analyses discovered that, compounds 25-28 form favorable interactions within the HPPD active point, with ligand -26 demonstrating the uppermost predicted binding affinity. This enriched

interaction is sustained by a stable network of hydrogen bonds, halogen contacts, and aromatic interactions, which cooperatively stabilize the ligand within the catalytic pocket. Directed by QSAR descriptors and binding-site insights, beleaguered modifications of the -CF₃ substituent led to the rational design of five new analogues (26a–26e). These derivatives consistently demonstrated greater foretold herbicidal action and enriched docking recital relative to the parent scaffold, emphasizing the favorable role of electron-withdrawing substitutions in refining both receptor compatibility and binding affinity. In addition, the assessment of agrochemical-relevant physicochemical properties and ADMET profiles further indicated that the tetrazolium–nicotinamide derivatives offer improved plant selectivity, favorable absorption characteristics, and reduced environmental and toxicity risks compared with the commercial HPPD inhibitor mesotrione. Progressive tendencies were perceived for foliar uptake, metabolic stability, and minimized off-target effects, providing valued regulation for future lead optimization. Furthermore, the constancy of compound 26 within the HPPD binding center was established over 100 ns molecular dynamic calculations. The complex remained structurally stable throughout, as reflected in steady RMSD values, limited residue mobility, maintained compactness, and stable solvent exposure. Hydrogen-bond analysis revealed a clear transition from initially dynamic interactions to a stable binding phase, during which compound 26 formed adamant H-bonds with key active-site residues, emphasizing the crucial protagonist of the tetrazolium and nicotinamide moieties in anchoring the ligand while restricting excessive conformational flexibility. In conclusion, the integration of QSAR predictions, favorable docking interactions, enhanced agrochemical properties, and dynamic stability locations compound 26 as a auspicious lead for the perfection of next-generation HPPD-inhibiting herbicides. These innovations discovered that apparent substituent modifications within the tetrazolium–nicotinamide scaffold can substantially improve herbicidal performance. Forthcoming work will accentuate on the chemical production of these leads, followed by experimental validation through in vitro enzyme inhibition assays, greenhouse efficacy testing, and crop selectivity studies, advancing compound 26 toward practical agrochemical applications.

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Ethics Approval and Permission to Participate

This study was conducted using computational and theoretical approaches and did not encompass human participants, animals, clinical samples, or personal data. Consequently, ethical approval and informed permission to partake were not required for this research.

Consent to Publish

“Not applicable”

Data Availability Statement

The datasets generated during and /or analyzed during the existing study are accessible from the corresponding author on judicious demand.

Confessional declaration

No prospective conflict of attentiveness was described by the entire author(s).

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Author's Contributions

- 1- S. T. and Z.U. I. - Wrote, edited and compiled the main manuscript text
- 2- G.A.S. and A.U. - Supervised and provide all the software's for the research Project
- 3- M.T.I. - Carried out the QSAR modeling analysis and interpreted the result
- 4- G.O. O. - Conducted the MD Simulation analysis and interpreted the result

5- B.D T. - Prepared all Figures and Tables

6- All authors reviewed the final edited manuscript

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