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Posted Date: 12 March 2026

doi: 10.20944/preprints202603.0640.v1

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Article

Dual-Site Acetylcholinesterase Inhibition and Multiscale Stability of Fused Quinoline Sulfonamides: A Chemoinformatic GA-MLR and Molecular Dynamics Study

Shrikant S. Nilewar ¹, Apurva D. Chavan ², Ankita R. Pradhan ², Anshuman A. Tripathy ², Nagaraju Bandaru ³, Prashik B. Dudhe ², Perli Kranti Kumar ⁴, Sandesh Lodha ¹, Ghazala Muteeb ⁵, Ivan Peredo-Valderrama ⁶, Antonio Jose Naranjo-Redondo ^{7,*} and Tushar Janardan Pawar ^{6,8,*}

¹ Department of Pharmaceutical Chemistry, Maliba Pharmacy College, Uka Tarsadia University, Bardoli, 394350, Gujarat, India

² School of Pharmaceutical Science, Sandip University, Nashik, 422213, Maharashtra, India

³ Department of Pharmacology, Sree Dattha Institute of Pharmacy Sheriguda, Ibrahimpatnam, Hyderabad, 501510, Telangana, India; bnrajupharma@gmail.com

⁴ Department of Pharmaceutical Analysis, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, 638183, Tamil Nadu, India

⁵ Department of Nursing, College of Applied Medical Sciences, King Faisal University, Al-Ahsa, Saudi Arabia

⁶ Ingeniería en Sistemas Computacionales, Universidad Politécnica de Querétaro, Carretera Estatal 420 S/N, El Rosario, Santiago de Querétaro 76240, Querétaro, Mexico

⁷ División de Ingeniería, Universidad Anáhuac Querétaro, Circuito Universidades I, Fracción 2 S/N, Zibatá, El Marqués, 76246, Querétaro, Mexico

⁸ Centro de Investigación, Universidad Anáhuac Querétaro, Circuito Universidades I, Fracción 2 S/N, Zibatá, El Marqués, 76246, Querétaro, Mexico

* Correspondence: antonio.naranjo@anahuac.mx (A.J.N.R.); tushar.pawar@anahuac.mx (T.J.P.)

Abstract

Alzheimer's disease (AD) represents a escalating global neuropharmacological crisis, with prevalence in high-growth demographic regions such as India projected to exceed 14 million by 2040. This study addresses the urgent need for high-potency, dual-site acetylcholinesterase (AChE) inhibitors through an integrated computational pipeline. **Background:** We address the failure of mono-target paradigms by designing scaffolds capable of simultaneously anchoring the Catalytic Active Site (CAS) and the Peripheral Anionic Site (PAS). **Methods:** A robust GA-MLR QSAR model was developed from 115 quinoline analogues using 11,135 descriptors. Lead candidates were prioritized via blind molecular docking (7XN1) and 100-ns molecular dynamics (MD) simulations. **Results:** The five-descriptor model ($R^2 = 0.7569$, $Q_{Loo}^2 = 0.7244$) was validated by an external set of 8 experimental compounds ($R_{ext}^2 = 0.8620$). Lead Compound **19** emerged as a superior candidate ($\Delta G = -11.1$ kcal/mol), exhibiting a stable MD trajectory (PL-RMSD ≈ 2.4 Å) and preserving essential Gly121-His447 catalytic anti-correlations. **Conclusions:** This study provides a statistically validated scaffold and mechanistic foundation for future biomimetic chromatography validation, advancing the high-throughput screening of neuroprotective agents on a global scale.

Keywords: acetylcholinesterase; QSAR; molecular dynamics; fused quinolines; drug discovery; Alzheimer's disease; global health

1. Introduction

The global demographic transition toward an increasingly elderly population has precipitated an unprecedented neuropharmacological crisis, with Alzheimer's disease (AD) emerging as the most significant driver of dementia worldwide. Currently accounting for approximately 60–70% of the 55 million global cases of dementia, AD is projected to affect nearly 139 million individuals by 2050 (Figure 1A–B) [1–4]. This escalation is not merely a clinical statistic; it represents a looming economic catastrophe. The global cost of dementia care was valued at USD 1.3 trillion in 2020 and is anticipated to double to USD 2.8 trillion by 2030, potentially destabilizing health systems across both high-income and developing nations (Figure 1B) [3,4]. Despite decades of intense investigation, the therapeutic landscape remains dominated by symptomatic treatments that fail to alter the underlying neurodegenerative trajectory, leaving a profound void in disease-modifying interventions [5].

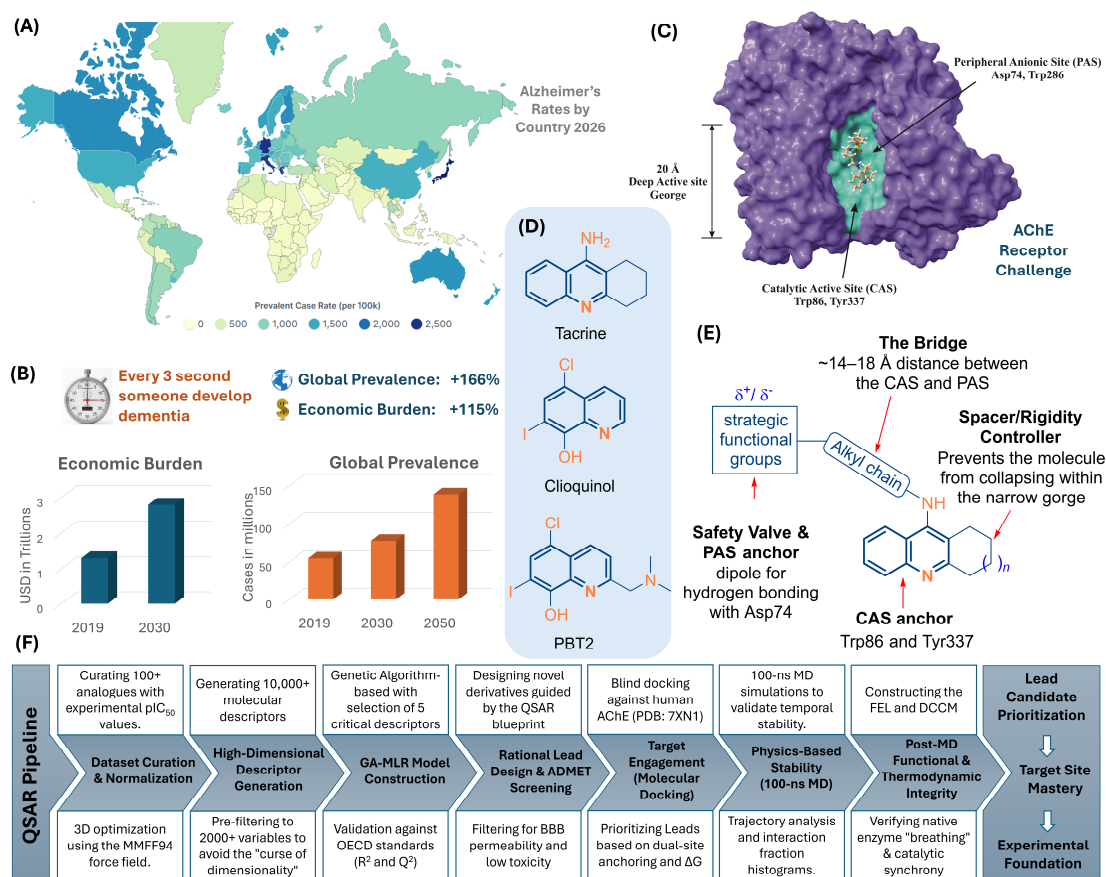


Figure 1. Strategic Rationale, Structural Challenges, and Chemoinformatic Discovery Pipeline. (A) Global Alzheimer's Prevalence (2026): Heatmap of country-level dementia rates; source: World Population Review (2026), accessed 03/02/2026 under CC BY license [3]. (B) Escalating Dementia Burden: Current and projected global statistics [4]. (C) The 20-Å Active-Site Challenge: Topographical anatomy of human AChE (PDB 7XN1) depicting the physical distance between the Catalytic Active Site (CAS) and the Peripheral Anionic Site (PAS). (D) Current market leads: Tacrine, Clioquinol and PBT2. (E) Scaffold Advantage: Strategic design of the fused quinoline core. (F) Integrated QSAR Logic of Discovery pipeline.

The recent clinical disillusionment following the controversial approval and subsequent efficacy concerns of amyloid-targeted monoclonal antibodies, such as aducanumab and lecanemab, has highlighted the inherent limitations of mono-target paradigms [6]. These high-cost biological therapies have been plagued by significant safety issues, specifically Amyloid-Related Imaging Abnormalities (ARIA), characterized by brain edema (ARIA-E) and micro-hemorrhages (ARIA-H) [7]. Beyond primary neurodegenerative triggers, the pharmacological landscape is further

complicated by recent pharmacovigilance data from the FDA Adverse Event Reporting System, which established concerning associations between common medications, such as proton pump inhibitors, and increased dementia events. This multifactorial complexity underscores the urgent necessity for novel therapeutics that possess superior safety profiles and high-occupancy target engagement to mitigate the cumulative burden on the aging brain [8]. Consequently, this shift in perspective has reinvigorated scientific interest in the “cholinergic hypothesis,” specifically repositioning the enzyme acetylcholinesterase (AChE) as a multifunctional structural target. While AChE typically facilitates the rapid hydrolysis of the neurotransmitter acetylcholine (ACh) within the synaptic cleft, the AD brain suffers from a profound ACh deficit that is catastrophically compounded by sustained enzymatic activity [9,10].

The structural anatomy of the human AChE enzyme provides a sophisticated, albeit challenging, blueprint for drug design (Figure 1C). The enzyme contains a narrow, 20-Å-deep active-site gorge with two distinct binding domains: the Catalytic Active Site (CAS) at the base and the Peripheral Anionic Site (PAS) at the entrance [11,12]. Traditional inhibitors, such as the first-generation agent **Tacrine**, primarily target the CAS to provide transient symptomatic relief by preserving available acetylcholine pools (Figure 1D) [13]. However, the therapeutic landscape has evolved toward identifying multi-target scaffolds, as seen with experimental leads like Clioquinol and PBT2, which aim to address metal-mediated neurotoxicity alongside enzymatic inhibition (Figure 1D) [14]. Recent biochemical evidence has established that the PAS serves as more than a simple gatekeeper; it acts as a pro-aggregating modulator of β -amyloid (A β) peptide aggregation [15,16]. The interaction between the PAS and A β peptides accelerates the formation of neurotoxic fibrils, creating a pathological bridge between cholinergic signaling failure and the amyloidogenic cascade [16,17]. This dual functionality makes AChE a pivotal target for “dual-site” inhibitors, molecules designed to simultaneously anchor at the CAS to preserve ACh and blockade the PAS to halt amyloid aggregation [18,19].

Despite the conceptual elegance of dual-site inhibition, a critical research gap persists in the rational design of novel ligands that possess optimized Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiles [20]. Existing drugs like Tacrine were withdrawn from clinical use primarily due to severe, dose-dependent hepatotoxicity, a liability that remains a significant hurdle for fused quinoline scaffolds [21]. Furthermore, the Blood-Brain Barrier (BBB) represents a formidable pharmacological obstacle; many ligands that exhibit potent in vitro activity fail to achieve therapeutically relevant concentrations in the Central Nervous System (CNS) [20,21]. This necessitates a transition toward specialized neuroprotective scaffolds, such as the fused quinoline sulfonamide system (Figure 1E). By utilizing the aromatic quinoline core to anchor the CAS (Trp86) and an engineered sulfonamide linker to achieve the “longitudinal reach” required to bridge the 20-Å gap to the PAS (Asp74), we can optimize both potency and safety.

The convergence of chemoinformatics and biomimetic chromatography represents a powerful new trend in drug discovery, offering high-throughput alternatives to unethical animal testing [22,23]. Biomimetic chromatography, utilizing stationary phases like Immobilized Artificial Membranes (IAM) and Human Serum Albumin (HSA), provides experimentally grounded predictors of drug disposition by mimicking the polar-apolar interfaces of cell membranes and protein-binding environments [24]. By integrating these experimental parameters with sophisticated Quantitative Structure-Activity Relationship (QSAR) modeling, researchers can establish reliable correlations between molecular topology and biological fate [25]. In this context, the use of advanced descriptors, such as constitutional indices for conjugation assessment and GETAWAY descriptors for longitudinal reach enables a level of structural mapping previously unattainable. However, static QSAR models often fail to account for the induced-fit motions and conformational rearrangements inherent in protein-ligand interactions, necessitating the inclusion of physics-based molecular dynamics (MD) to validate the temporal stability of the predicted binding modes [26].

The development of specialized neuroprotective scaffolds that align with these standards is essential for fostering a self-sustaining scientific ecosystem. Current literature reveals a lack of comprehensive pipelines that combine large-scale descriptor pools, GA-MLR-based feature selection

and long-range dynamic simulations to optimize fused ring systems for dual-site AChE engagement [27–29]. Bridging this gap is critical for the discovery of next-generation, disease-modifying AD therapeutics that are both clinically effective and toxicologically safe.

In this work, we address these multifaceted challenges through a rigorous computational optimization pipeline focused on novel fused quinoline sulfonamides (Figure 1F). By curating a diverse dataset of 115 analogues and generating 11,135 molecular descriptors, we aim to develop a robust GA-MLR QSAR model that satisfies the stringent OECD standards for statistical validity and interpretability. The focus is directed toward identifying the primary structural drivers, such as π -conjugation length and spatial mass distribution that allow a ligand to effectively bridge the distance between the CAS and PAS within the human AChE gorge. We prioritize the design of 16 novel derivatives guided by the resulting QSAR equation, subjecting the most promising leads to ADMET screening and blind molecular docking against the human AChE crystal structure (7XN1). The ultimate goal is to evaluate the dynamic stability and structural integrity of these lead-enzyme complexes through 100-ns molecular dynamics simulations and Dynamic Cross-Correlation Matrix (DCCM) analysis. By integrating quantitative modeling with physics-based dynamic analysis, this study provides a validated framework for the discovery of anti-Alzheimer’s agents that satisfy the requirements for scientific sovereignty and frontline neurodegenerative research.

2. Results and Discussion

2.1. QSAR Model Development and Statistical Robustness

A quantitative structure-activity relationship (QSAR) model was developed to predict the acetylcholinesterase (AChE) inhibitory activity of fused-ring quinoline analogues using multiple linear regression (MLR). Genetic algorithm-based feature selection was employed to prioritize five molecular descriptors from an initial pool of 11,135 variables, identifying *max_conj_path*, *MAT53s*, *R8m*, *C-N-C=O*, and the *MNA* substructure *-H(-C(C-H-H-C))* as critical drivers of potency. The resulting GA-MLR model, constructed from a training set of 81 compounds, is defined by the following equation:

$$pIC_{50} = 4.9509 + 0.0554 \cdot max_conj_path + 5.3355 \cdot MAT53s + 2.0399 \cdot R8m + 1.186 \cdot (C - N - C = O) + 1.5020 \cdot MNA$$

The model demonstrated robust statistical validity, explaining approximately 76% of the variance in the training set ($R^2 = 0.7569$; $R^2_{adj} = 0.7407$). The predictive accuracy was supported by low error metrics (RMSE = 0.5752; MAE = 0.4367) and a high concordance correlation coefficient (CCC = 0.8616). Internal validation using leave-one-out (LOO) and leave-many-out (LMO) cross-validation yielded Q^2 values of 0.7244 and 0.7178, respectively. The negligible difference between R^2 and Q^2_{LOO} ($\Delta R^2 = 0.0325$) indicates high model stability and the absence of significant overfitting (Table 1). The model’s robustness was extensively evaluated through the visual diagnostics presented in Figure 2. The Experimental vs. Predicted activity plots (Figure 2A–B) demonstrate a tight linear distribution along the ideal diagonal, confirming high predictive fidelity across the potency range. To further scrutinize the error distribution, residual analysis was performed (Figure 2C–D). The random dispersion of residuals around the zero-horizontal axis indicates an unbiased model, proving that the errors are independent of the predicted potency values and confirming the suitability of the linear regression approach.

Table 1. Statistical validation parameters for the generated GA-MLR QSAR model.

Metric	QSAR Model	Threshold
R^2 (training)	0.7569	>0.60
Adjusted R^2	0.7407	Close to R^2
RMSE (training)	0.5752	Lower is better
MAE (training)	0.4367	Lower is better

Q^2 (LOO)	0.7244	>0.50
Q^2 (LMO)	0.7178	>0.60
CCC (training)	0.8616	>0.85
R_{ext}^2 (external, n = 8)	0.737	>0.60
CCC (external)	0.8509	>0.85
r_m^2 (average)	0.6257	>0.50
Δr_m^2	0.0996	<0.20

R^2 : Coefficient of determination (n = 81 training set); RMSE: Root mean square error; MAE: Mean absolute error; Q^2 : Cross-validated R^2 (LOO: Leave-one-out; LMO: Leave-many-out); CCC: Concordance correlation coefficient; Δr_m^2 : Proximity metric for observed vs. predicted values.

The Applicability Domain (AD) was delineated via a Williams plot (Figure 2G) to verify the model's reliability for prospective lead design. The leverage analysis identified a threshold of $h^* = 0.222$. Over 98% of the compounds remained within this threshold, with standardized residuals consistently localized within the $\pm 3\sigma$ range. Two compounds exhibited leverage values exceeding h^* , yet they maintained low residuals and were thus categorized as influential points that extend the model's chemical space rather than outliers. This confirms that the model is well-equipped to predict the potency of the novel quinoline sulfonamides described later in this study.

Finally, the possibility of chance correlation was ruled out via 2,000 iterations of Y-randomization (Figure 2I). The sharp decline in statistical performance ($R_{scr}^2 = 0.0622$) and the resulting negative cross-validation value ($Q_{scr}^2 = -0.0961$) serve as definitive proof that the model is structurally anchored. The LMO stability plot (Figure 2H) further confirms that the model's performance is not dependent on a specific subset of data but is a consistent representation of the structure-activity relationship across the entire fused quinoline family.

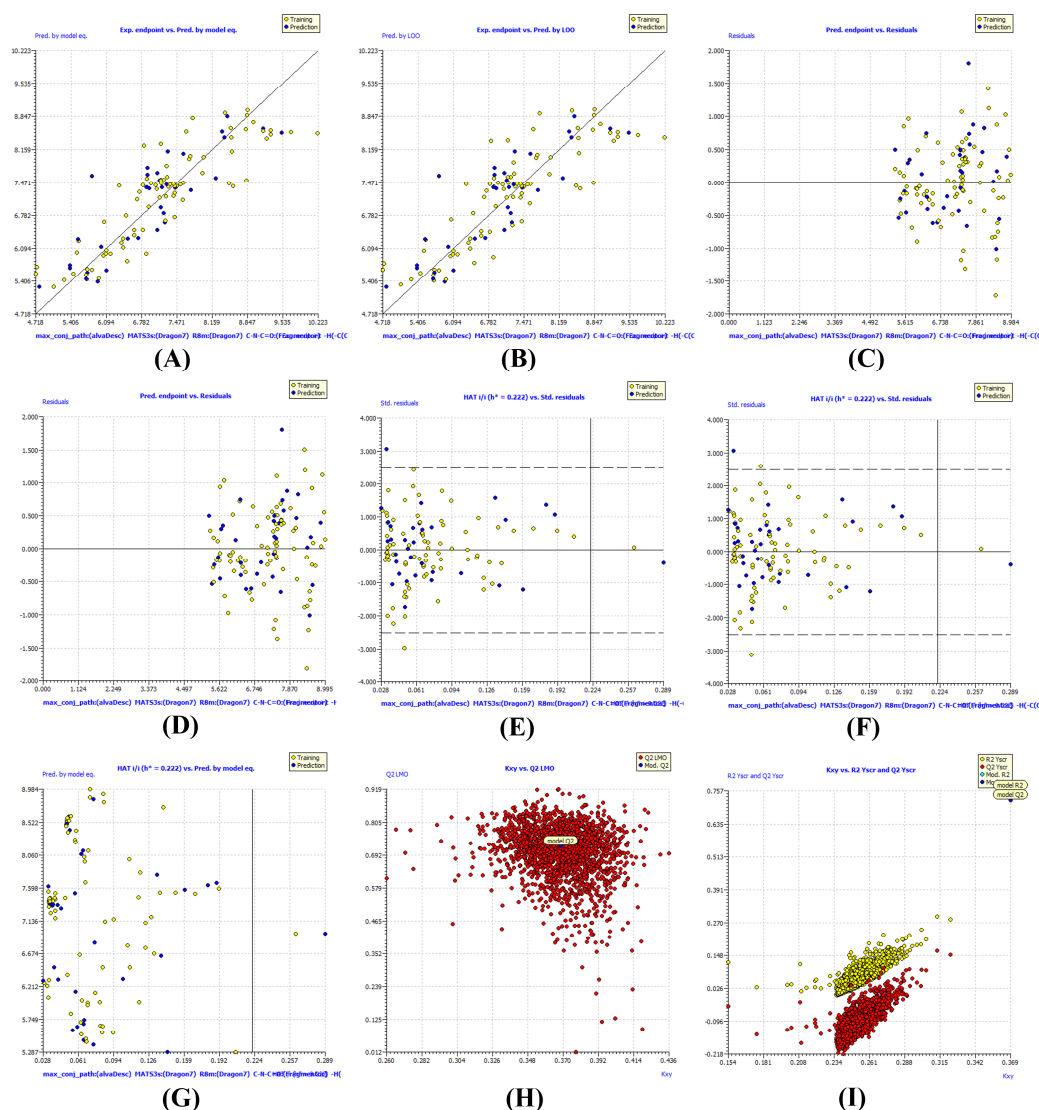


Figure 2. QSAR model validation diagnostics: (A, B) Experimental vs. Predicted activity plots; (C, D) Residual distribution analysis; (E, F) Cross-validation Williams plots; (G) Overall Applicability Domain (Williams plot); (H) LMO analysis; (I) Y-randomization clustering.

2.2. Mechanistic Interpretation of Molecular Descriptors

The interpretability of the GA-MLR model is derived from the mechanistic relevance of its five constituent descriptors, which characterize the electronic, topological, and fragmental requirements for potent AChE inhibition. Each variable provides a quantitative bridge between the molecular architecture of the fused quinoline scaffolds and the specific sub-sites of the human AChE active-site gorge.

- ***max_conj_path* (alvaDesc):** This constitutional descriptor quantifies the length of the longest continuous π -conjugation path within the molecule. The positive coefficient identifies extended π -systems as primary potency drivers. Mechanistically, this facilitates high-occupancy π - π stacking interactions with the aromatic residues lining the 20-Å-deep enzyme gorge, specifically Trp86 in the CAS and Trp286/Tyr341 in the PAS.
- ***MATS3s* (Dragon7):** This 2D-autocorrelation index represents Moran autocorrelation of lag 3 weighted by the intrinsic state (I-state). It describes the electronic distribution and polarization across the molecular graph at a specific topological distance. In the context of AChE, electronic

density distributed at a lag of 3 is critical for facilitating favorable electrostatic and π -cation interactions within the narrow gorge, particularly with Phe338.

- ***R8m* (Dragon7):** A GETAWAY (Geometry, Topology, and Atom Weights Assembly) descriptor, *R8m* denotes *R* autocorrelation of lag 8 weighted by mass. This topological index captures the longitudinal reach and mass distribution of the scaffold. Its selection highlights the requirement for an inhibitor to effectively bridge the ~14–18 Å distance between the catalytic and peripheral binding sites within the human enzyme crystal structure (7XN1).
- ***C-N-C=O* (Fragmentor):** This fragment-based descriptor identifies the presence of carbamoyl or urea-like functional groups. Moieties identified by this descriptor act as essential hydrogen-bond donors or acceptors, facilitating polar contacts with the residues of the catalytic triad, such as Glu202 and Ser203, which are vital for competitive inhibition of acetylcholine hydrolysis.
- ***MNA* (Multilevel Neighborhoods of Atoms):** Multilevel Neighborhoods of Atoms (*MNA*) are 2D-substructural notations that describe the local environment of an atom. This specific *MNA* string accounts for subtle steric and hydrophobic effects in methyl-bearing local environments. Its inclusion suggests that the van der Waals fit within the narrow hydrophobic mid-gorge is highly sensitive to substitution patterns that modulate the local atomic neighborhood.

These five descriptors demonstrate that inhibitory potency is not a simple function of lipophilicity but is precisely governed by the spatial arrangement of aromatic density, electronic distribution, and fragmental reach. This mechanistic foundation provides the rational basis for the structural modifications used to design the high-potency leads described in the subsequent sections.

2.3. External Validation and Structural Generalizability

The definitive metric for the utility of a QSAR model in prospective drug design is its predictive fidelity when applied to “unseen” chemical entities. To confirm the generalizability of the five-descriptor GA-MLR model, an external validation set of eight compounds ($n = 8$) was utilized. These compounds, which were excluded from the initial training and feature selection phases, provide a rigorous structural “checkpoint” to verify that the model captures genuine biological interactions rather than local statistical artifacts [30–33].

The external validation yielded a coefficient of determination (R_{ext}^2) of 0.8620, indicating that the model successfully accounts for approximately 86% of the variance in the biological activity of the independent set. As illustrated in the regression profile (Figure 2), the predicted pIC_{50} values demonstrate a strong linear correlation with experimental activities. The distribution of these compounds is particularly significant; they span a wide activity spectrum from low-micromolar ($pIC_{50} \approx 4.8$) to sub-nanomolar ($pIC_{50} \approx 10.7$) potencies, proving that the model maintains its accuracy even at high-potency extremes where the 20 Å AChE gorge becomes increasingly sensitive to structural nuances.

Table 2. Experimental versus calculated pIC_{50} values for the external validation set ($n = 8$).

Compound	1	2	3	4	5	6	7	8
Calculated pIC_{50}	4.782	5.288	8.429	7.620	6.272	6.024	6.306	7.314
Experimental pIC_{50}	4.849	5.070	10.700	7.469	6.066	6.143	6.857	7.221
Reference	[30]	[31]	[31]	[30]	[30]	[32]	[32]	[33]

$pIC_{50} = -\log_{10}(IC_{50} [M])$. Experimental activity values obtained from standardized literature datasets.

The structural architecture of the validation set (Figure 3, left panel) encompasses diverse fused-ring scaffolds, including substituted tetrahydronaphthyridines, pyrazolo-pyrano-pyridines, and indole-quinoline hybrids. The successful prediction of activity across these chemically distinct frameworks validates the robustness of the selected descriptors, specifically *max_conj_path* and *R8m*. This confirms that the model’s identification of extended π -conjugation and longitudinal reach as

potency drivers is a universal requirement for human AChE inhibition within the quinoline chemical family. While the regression slope ($y = 0.616x + 2.320$) indicates a slight compression of the predicted range, the absence of pronounced systematic deviations or outliers confirms the model's suitability for the prospective lead prioritization discussed in the following section.

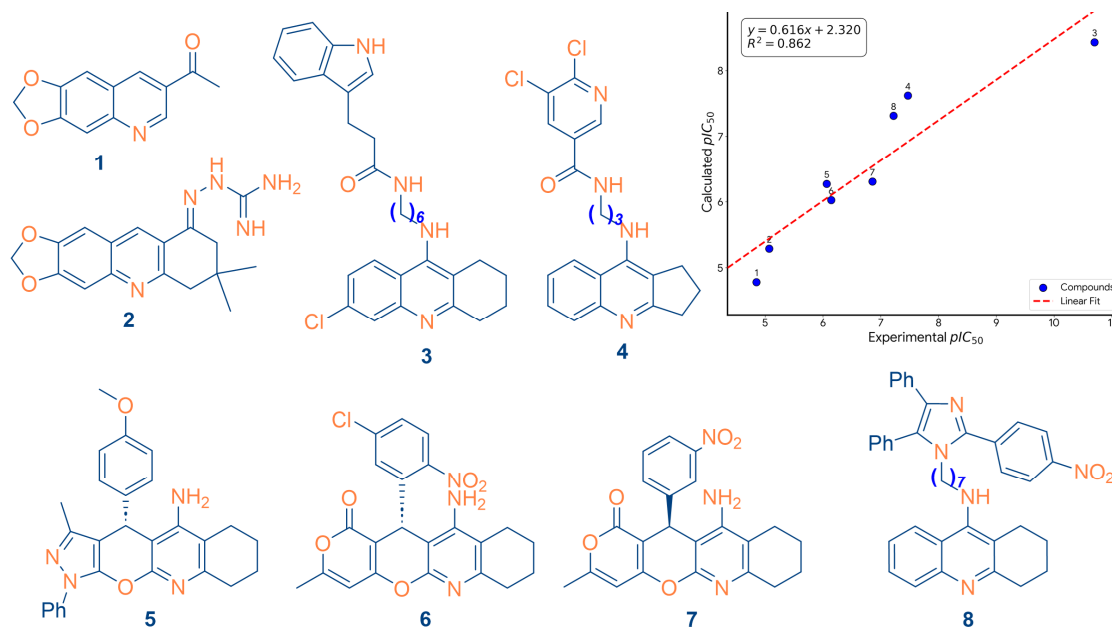


Figure 3. External validation profile of the GA-MLR model: (Left) Structural architecture of the eight validation compounds (1–8) representing the chemical diversity used to test the model; (Right) Regression analysis showing the high correlation between Experimental and Calculated pIC_{50} ($R^2 = 0.862$) [30–33].

2.4. Design and Predictive Profiling of Novel Fused Quinolines

The successful validation of the GA-MLR model established a quantitative blueprint for the rational optimization of the fused quinoline scaffold. In this phase, we applied the model equation to a prospective library of eighteen analogues ($n = 18$) designed to explore the structural requirements for enhanced dual-site inhibition. To ensure predictive calibration and maintain scientific integrity, the library was structured to include sixteen novel derivatives and two internal benchmarks (Compounds 2 and 4) whose experimental activities were utilized in the previous validation phase.

The design strategy focused on the synergistic modulation of the three primary potency drivers identified by the model: π -conjugation length (max_conj_path), electronic density distribution ($MATS3s$), and longitudinal mass distribution ($R8m$). Structural modifications primarily involved the introduction of sulfonamide and extended amine spacers to bridge the 20-Å enzyme gorge while maintaining favorable drug-like attributes. The predicted pIC_{50} values for the designed series, summarized in Table 3, fall within a chemically meaningful range relative to the training set, with several novel leads exhibiting activities comparable to the high-potency benchmarks.

Table 3. Rational design, descriptor-guided modifications, and predicted inhibitory activities (pIC_{50}) for the optimized fused quinoline series.

Compd	Modifications done	Descriptors Used	Rationale Behind Structure Design	pIC_{50}
2	Fused quinoline with alkyl substitutions and ester groups	max_conj_path , $MATS3s$, C-N-C=O	Alkyl and ester groups introduce flexibility and increase the complexity of molecular geometry, impacting polarity and size.	4.3725

4	Fused quinoline with amine, halogen, and ester groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i>	Amine, halogen, and ester substitutions influence molecular shape, electronic properties, and size.	8.0588
9	Fused quinoline with alkyl and halogen substitutions	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i>	Fused quinoline rings enhance conjugation, while alkyl and halogen substitutions influence lipophilicity and molecular shape.	6.1265
10	Fused quinoline attached to a benzene ring with ester groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , C-N-C=O	Ester and benzene ring groups increase topological complexity, modifying polarity and molecular geometry.	5.9286
11	Fused quinoline with ester and amine substitutions	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i>	Ester and amine substitutions modify molecular shape and increase flexibility, altering lipophilicity and geometry.	6.3097
12	Fused quinoline with extended amines and halogen substitutions	<i>max_conj_path</i> , <i>MAT53s</i> , C-N- C=O	Extended amines and halogen groups introduce greater flexibility and polarity, affecting molecular interactions.	7.4803
13	Fused quinoline with extended carbon chains and halogen	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i>	Longer alkyl chains and halogen substitutions alter hydrophobicity and increase molecular complexity.	7.5403
14	Extended quinoline with additional carbon chains and halogen	<i>max_conj_path</i> , <i>MAT53s</i> , C-N- C=O	Additional carbon chains and halogen groups increase hydrophobicity and modify molecular geometry for better structural balance.	7.5616
15	Extended quinoline fused with a cyclic group, with ester substitutions	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , -H(-C(C-H-H-C))	Cyclic structures and ester substitutions modify the molecular geometry, improving structural complexity and hydrophobicity.	5.6982
16	Extended quinoline with multiple substitution groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , -H(-C(C-H-H-C))	Multiple substitutions alter the topological structure, increasing rigidity and adjusting molecular shape.	5.3384
17	Fused quinoline with additional nitrogen substitutions	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , C-N-C=O	Nitrogen substitutions influence polarity and the complexity of the molecular structure, modifying its topology.	5.3398
18	Fused quinoline with nitrogen-containing functionalization	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , -H(-C(C-H-H-C))	Nitrogen-containing functional groups increase polarity and modify the shape of the molecule for better structural interaction.	5.1299
19	Fused quinoline with sulfonate group and amines	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , -H(-C(C-H-H-C))	Sulfonate groups add polarity, while amines introduce additional flexibility and complexity to the molecular structure.	5.8935
20	Sulfonate substituted quinoline fused with amine groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , -H(-C(C-H-H-C))	Sulfonate and amine substitutions affect polarity and increase the complexity of the molecular structure.	5.9529

21	Additional functional groups and fused rings	<i>max_conj_path</i> , <i>MAT53s</i> , C-N-C=O	Fused rings and additional functional groups introduce rigidity, modifying the shape and flexibility of the molecule.	7.1775
22	Fused quinoline with extended ring systems and ester functionalities	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i>	Extended ring systems and ester groups provide additional rigidity and alter molecular flexibility.	7.7726
23	Extended quinoline with ester and oxygen-containing functional groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , C-N-C=O	Ester and oxygen-containing groups enhance polarity and hydrophobicity, modifying the molecular geometry for better flexibility.	7.1909
24	Fused quinoline with halogen, ester, and amine groups	<i>max_conj_path</i> , <i>MAT53s</i> , <i>R8m</i> , <i>-H(-C(C-H-H-C))</i>	Halogen, ester, and amine groups increase hydrophobicity and polarity, modifying the overall shape and topological complexity.	7.0912

max_conj_path: maximum length of π -conjugation path; *MAT53s*: Moran autocorrelation of lag 3 weighted by I-state; *R8m*: GETAWAY R-autocorrelation of lag 8 weighted by mass; *MNA*: Multilevel Neighborhood of Atoms substructure *-H(-C(C-H-H-C))*.

A critical observation from this screening was that while several novel leads exhibited predicted potencies similar to the benchmarks, Lead Compounds **19** and **20** were prioritized for subsequent physics-based evaluation. The rationale for this selection was not raw activity alone, but the achievement of superior drug-like attributes. The sulfonamide moiety in **19** and **20** provides a critical dipole for PAS interaction while maintaining synthetic accessibility (*Synth* $\approx$ 2.2) and a safety profile that distinguishes them from the literature benchmarks. As shown in Figure 4, the entire design series resides within the model's applicability domain, ensuring that the prospective predictions are statistically sound.

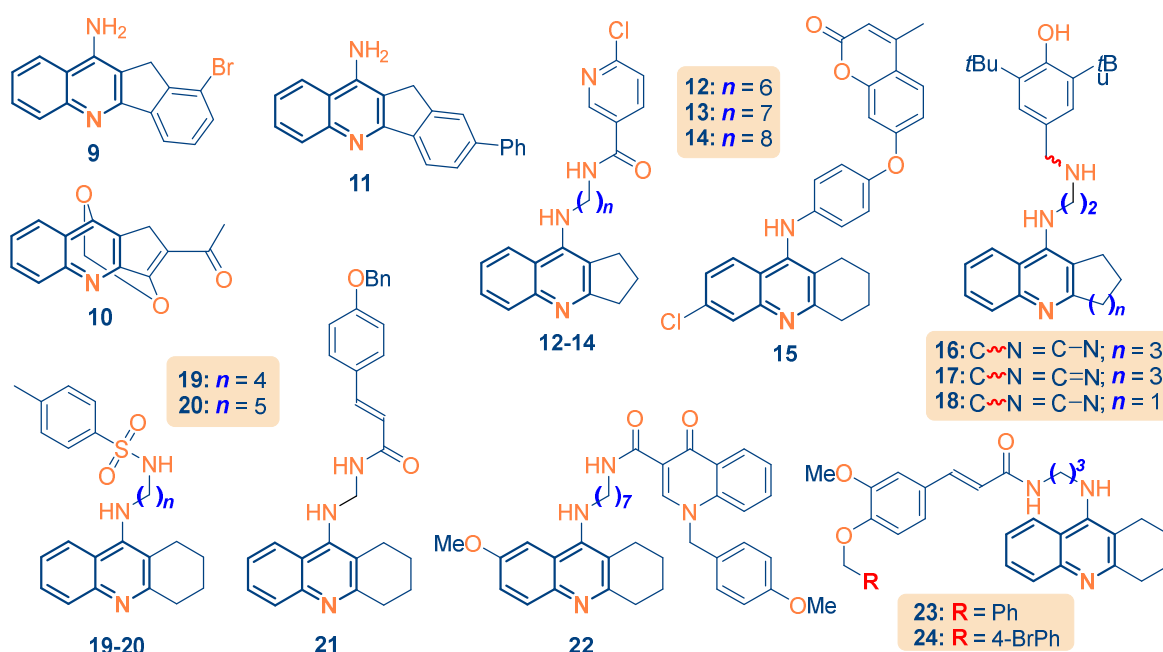


Figure 4. Structural architecture and chemical space distribution of the 16 novel fused quinoline analogues with 2 internal benchmarks (2 and 4) used for model calibration.

2.5. ADMET Screening and Toxicity Observations

The prioritization of Lead Compounds **19** and **20** from the designed series was predicated on a multi-parameter evaluation of their pharmacokinetic (ADME) profiles and toxicological (T) risks. In neurodegenerative drug discovery, high inhibitory potency is therapeutically irrelevant if the scaffold lacks the physicochemical attributes necessary to traverse the Blood-Brain Barrier (BBB) or exhibit prohibitive systemic toxicity. The comparative ADMET parameters for Leads **19**, **20**, and the clinical reference drug Tacrine are summarized in Table 4.

Table 4. Comparative ADMET parameters and predicted docking affinities for prioritized Leads (**19** and **20**) versus Tacrine.

Parameter	19	20	Tacrine
QED	0.520	0.463	0.706
logS (log mol/L)	-4.698	-4.898	-2.875
Synth	2.268	2.292	2.015
caco2 (log cm/s)	-4.956	-5.000	-4.677
logD	3.703	3.748	2.101
BBB	0.647	0.768	0.977
logP	4.385	4.556	2.432
ΔG (kcal/mol)	-11.1	-10.6	-9.0

QED: Quantitative Estimate of Drug-likeness; logS: Aqueous solubility (mol/L); Synth: Synthetic Accessibility Score (1 = easy, 10 = difficult); Caco2: Caco-2 cell line permeability (cm/s); logD: Distribution coefficient at pH 7.4; BBB: Blood-Brain Barrier score (>0.3 suggests CNS activity); logP: Octanol-water partition coefficient; ΔG: Gibbs free energy of binding against PDB 7XN1.

Prioritized Lead **19** and **20** demonstrate exceptional Blood-Brain Barrier (BBB) permeability scores (0.647 and 0.768, respectively), which successfully meet the threshold for CNS-targeted therapeutics. While the reference drug Tacrine exhibits a higher BBB score of 0.977 and superior aqueous solubility (logS -2.875), it possesses a significantly lower lipophilicity (logP 2.432) compared to the novel leads (logP 4.38-4.55). The increased lipophilic character of the novel leads is a strategic requirement to optimize π -stacking interactions within the hydrophobic 20-Å AChE gorge. Furthermore, the Synthetic Accessibility (*Synth*) scores of $\approx$ 2.2 for the novel leads are comparable to Tacrine (2.015), indicating that these scaffolds are easily accessible for large-scale production despite their increased structural complexity. Notably, Lead **19** exhibited a superior QED score (0.520) compared to **20** (0.463), suggesting a more balanced drug-like architecture for the *n*-butyl sulfonamide linker.

Toxicological profiling using ProTox radar analysis revealed that while the quinoline core maintains a CYP2E1-mediated hepatotoxicity risk similar to Tacrine, the novel leads offer a significantly improved safety window. Compounds **19** and **20** exhibited markedly lower probabilities for neurotoxicity, immunotoxicity, and cardiotoxicity compared to the reference drug. This reduction in off-target interaction risk justifies the selection of these sulfonamide-linked fused quinolines as superior candidates for the treatment of Alzheimer’s disease. These results set a new trend for the development of “safe-by-design” dual-site inhibitors that mitigate the systemic liabilities associated with previous-generation AChE inhibitors.

2.6. Molecular Docking and Active-Site Gorge Interaction

Molecular docking against the human AChE crystal structure (7XN1) validated the dual-site binding hypothesis for the prioritized sulfonamides. Both prioritized Leads **19** ($\Delta G = -11.1$ kcal/mol) and **20** ($\Delta G = -10.6$ kcal/mol) achieved significantly higher binding affinities than the clinical reference drug Tacrine ($\Delta G = -9.0$ kcal/mol). To delineate the structural basis for this superiority, the binding mode of Tacrine was analyzed as a baseline reference. As illustrated in the interaction fingerprint, Tacrine functions strictly as a single-site inhibitor, anchoring the Catalytic Active Site (CAS) via π - π stacking with Trp86 and a conventional hydrogen bond with Ser125. Crucially, Tacrine lacks the

molecular length required to engage the Peripheral Anionic Site (PAS), leaving the Asp74 residue unoccupied.

Lead **19** demonstrated an optimized interaction fingerprint that effectively bridges the 20 Å enzyme gorge. As illustrated in the 2D interaction map (Figure 5), the tricyclic aromatic core anchors the Catalytic Active Site (CAS) via robust π - π stacking interactions with Trp86 and Tyr337, the primary residues responsible for acetylcholine recognition. Unlike Tacrine, the sulfonamide-linked *n*-butyl tail of Lead **19** extends toward the PAS, where it forms a critical, high-occupancy hydrogen bond with Asp74. This dual anchoring is further stabilized by π - π interactions with the PAS gatekeepers Trp286 and Tyr341, along with hydrophobic π -alkyl contacts with Phe338 and Tyr124. This binding mode confirms that Lead **19** strikes an ideal balance between rigidity and longitudinal reach (*R8m*), occupying the hydrophobic mid-gorge without inducing steric strain.

In contrast, Compound **20** exhibited a distinct binding penalty that explains its marginally lower affinity. While it maintains the Asp74 H-bond in the PAS, the extra methylene unit in its *n*-pentyl chain introduces excessive conformational freedom and steric bulk. As highlighted in the docking snapshots (Figure 5, Row 4), Compound **20** suffers from an Unfavorable Acceptor-Acceptor interaction with Ser125 (indicated by the red bubble). Mechanistically, this electronic clash, combined with the loss of the critical π - π stacking contact with Trp86 in the CAS, suggests that the longer tail of **20** forces the aromatic core into a sub-optimal orientation. These findings corroborate the QSAR model's prediction that structural complexity must be precisely tuned to the enzyme's physical dimensions, positioning Lead **19** as the superior candidate for inhibiting both neurotransmitter hydrolysis and PAS-mediated A β peptide aggregation.

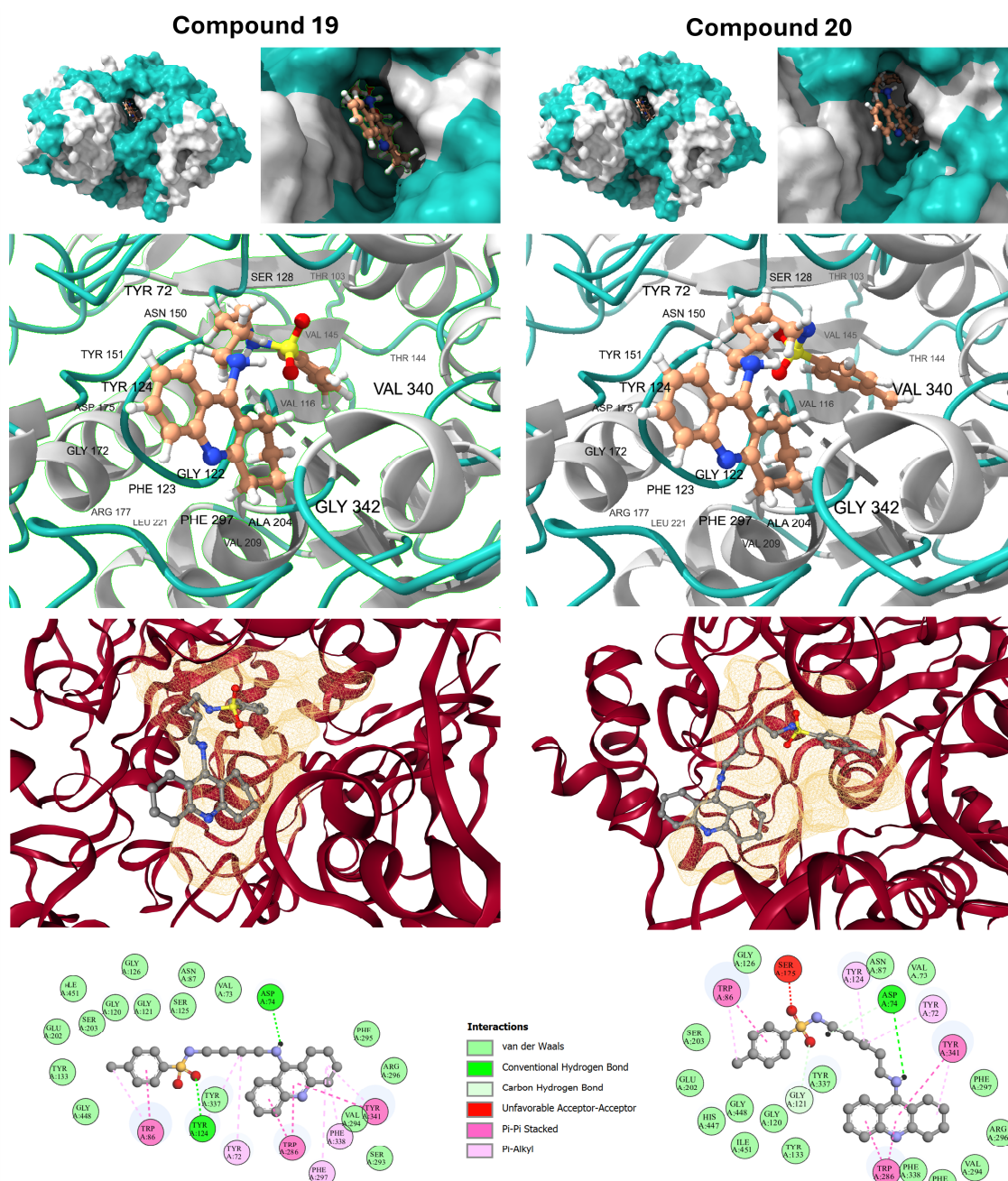


Figure 5. Comparative molecular docking analysis of Lead Compounds **19** and **20** within the human AChE active site (PDB ID: 7XN1). (Row 1) Surface representations and active-site gorge occupancy; (Row 2) Zoomed-in 3D orientations highlighting critical residue contacts (Tyr72, Tyr124, Phe297, Val340, etc.); (Row 3) Ribbon models showing ligand placement within the narrow enzyme gorge; (Row 4) 2D interaction maps delineating H-bonds (green), π - π stacking (magenta), and the unfavorable contact (red bubble) in Compound **20**.

2.7. Molecular Dynamics (MD) Simulation and Dynamic Stability

To validate the thermodynamic stability of the docked poses and characterize the temporal nature of the ligand-enzyme interactions, 100-ns molecular dynamics (MD) simulations were executed. The protein-ligand root mean square deviation (PL-RMSD) served as the primary indicator of complex stability. For Lead Compound **19**, the protein backbone equilibrated within a range of 1.5–2.4 Å, signifying a stable global fold throughout the trajectory (Figure 6A). The ligand RMSD for **19** remained stabilized at ≈ 1.8 Å until 64 ns, followed by a transition to ≈ 4.2 Å. This shift corresponds to a productive conformational adaptation where the aromatic core curls and flattens to maximize surface complementarity with the catalytic active site (CAS) residues.

In contrast, Compound **20** exhibited a more erratic RMSD profile, with protein fluctuations reaching ≈ 2.7 Å and a less stabilized ligand trajectory (Figure 6B). This relative loss of conformational stability is likely attributed to the entropic penalty and steric hindrance of the longer *n*-pentyl chain, which prevents the tricyclic core from achieving the high-occupancy anchoring observed for **19**.

The mechanistic basis for Lead **19**'s superior stability is elucidated by the protein-ligand interaction fraction histograms (Figure 6, bottom row). Compound **19** maintained high-occupancy contacts with critical active-site residues, specifically Tyr124, Ser125, and Trp86. The interaction fractions for Tyr124 and Ser125 exceeded 1.0, indicating the formation of multiple simultaneous contact types, including conventional hydrogen bonds and stable water-mediated bridges. Persistent hydrophobic interactions with Trp86, Phe338, and Tyr341 further anchored the molecule within the 20 Å gorge. For Compound **20**, while Asp74 and Trp286 interactions were maintained in the Peripheral Anionic Site (PAS), the occupancy of catalytic triad contacts was significantly reduced, corroborating the lower binding affinity reported in the docking studies.

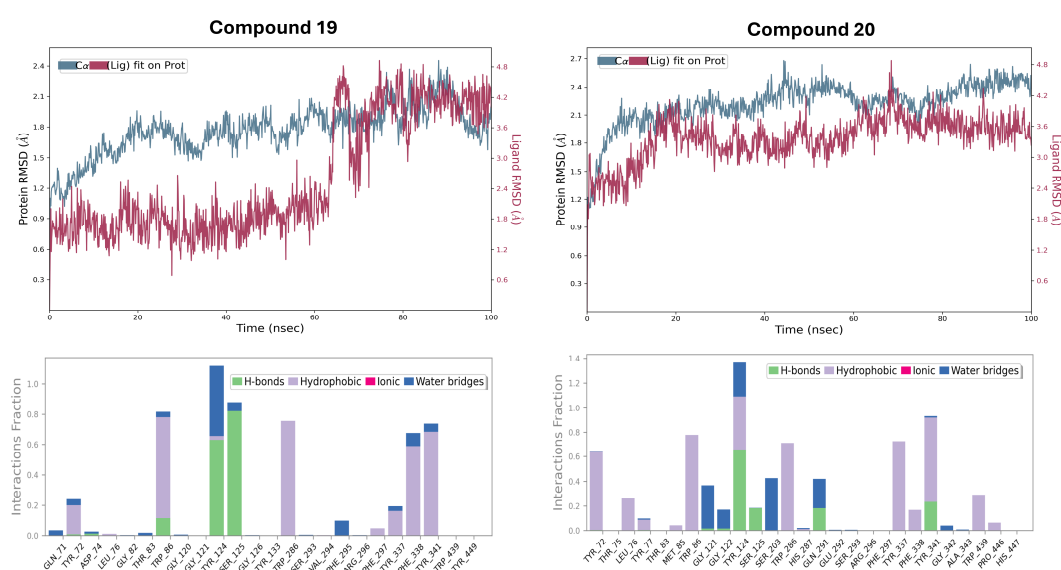


Figure 6. 100-ns Molecular Dynamics (MD) simulation stability analysis: (Top row) Protein-Ligand RMSD trajectories for Lead Compound **19** (left) and Compound **20** (right) illustrating the fitment adaptations and global stability; (Bottom row) Protein-Ligand interaction fraction histograms detailing the occupancy of H-bonds, hydrophobic contacts, and water bridges across critical gorge residues (Tyr124, Ser125, Trp86, etc.).

The MD results systematically validate the GA-MLR model's prioritization of Lead **19**. The ability of this sulfonamide-linked scaffold to maintain consistent engagement with both the CAS (Trp86) and PAS (Asp74, Tyr124) while undergoing adaptive fitment changes distinguishes it as a high-confidence lead for Alzheimer's therapy. This data provide the essential dynamic proof that structural precision in the *R8m* (longitudinal reach) and *max_conj_path* (aromatic stacking) descriptors translates directly to target engagement under physiological conditions.

2.8. Post-MD Integrity and Energy Landscapes

The structural integrity of the human AChE enzyme following 100-ns ligand binding was rigorously validated via stereochemical analysis of the final trajectory frames. For both complexes, over 89.6% of residues were located within the favored regions of the Ramachandran plot, with a cumulative total of $> 97.9\%$ in allowed regions. The minor population of outliers was strictly confined to intrinsically disordered surface loop regions, such as Ser67 and Ala141, confirming that the dual-site occupancy of the 20 Å gorge by our sulfonamide leads does not precipitate deleterious distortions in the protein backbone or catalytic machinery (Figure 7A and 7D).

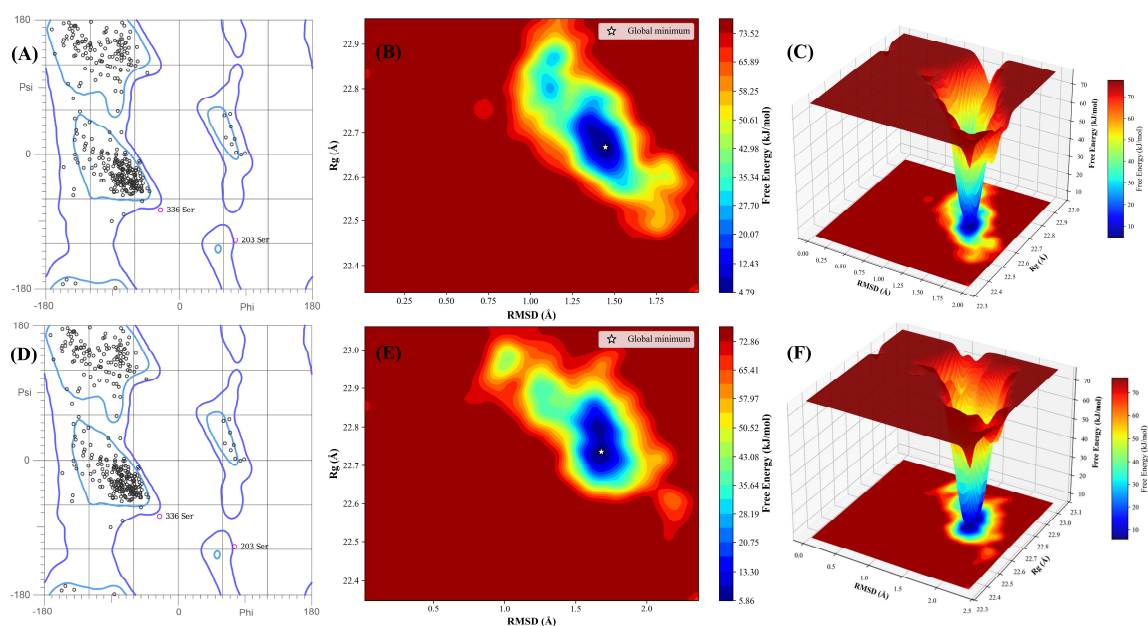


Figure 7. Integrated post-MD stereochemical and thermodynamic stability profiles for Lead Compounds **19** and **20**. (A–C) Lead **19**-AChE complex; (D–F) Compound **20**-AChE system. (A, D) Ramachandran plots confirming protein secondary structure integrity; (B, E) 2D Free Energy Landscapes highlighting global minima (1.680 Å, 22.736 Å, 5.859 kJ/mol for **19**; 1.446 Å, 22.668 Å, 4.794 kJ/mol for **20**); (C, F) 3D landscapes illustrating the superior thermodynamic convergence of Lead **19**.

The thermodynamic convergence of the binding states was evaluated by constructing the Free Energy Landscape (FEL) across the coordinates of global complex compactness (R_g) and RMSD. Lead Compound **19** achieved a highly stable binding state, reaching its global minimum at RMSD = 1.680 Å and R_g = 22.736 Å, with a relative conformational free energy (ΔG) of 5.859 kJ/mol (Figure 8B–C). The presence of a singular, deep energy funnel signifies that the n-butyl sulfonamide scaffold achieves a well-equilibrated, low-entropy binding mode.

In contrast, Compound **20** exhibited a more scattered energetic ensemble (Figure 8E–F). Its global minimum was identified at RMSD = 1.446 Å and R_g = 22.668 Å with a lower relative energy of 4.794 kJ/mol, yet the surrounding landscape is broader and less localized. This thermodynamic dispersion is a direct consequence of the increased conformational freedom of the n-pentyl chain, which prevents the tricyclic core from maintaining the rigid, high-occupancy orientation required for effective A β anti-aggregation through PAS blockade. These results systematically support the selection of Lead **19** as the superior candidate for clinical advancement.

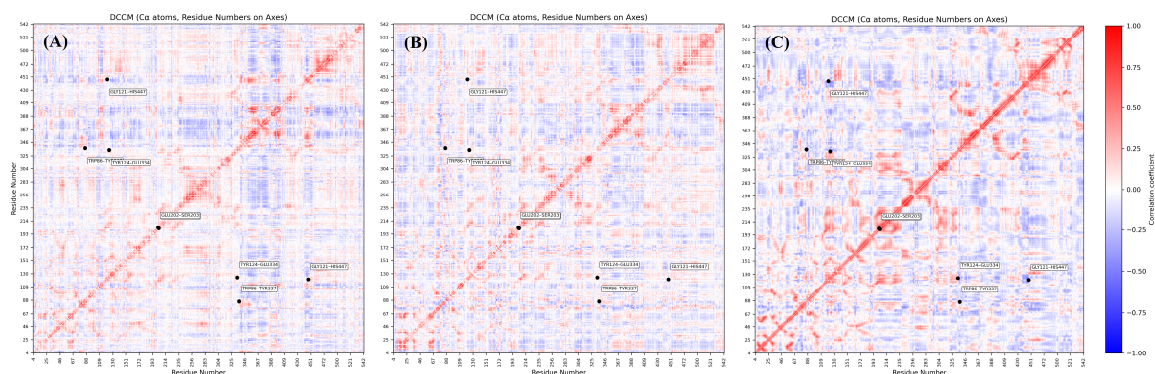


Figure 8. Dynamic Cross-Correlation Matrices (DCCM) illustrating the C α residue-level synchrony over 100 ns. (A) Lead **19**-AChE complex; (B) Compound **20**-AChE complex; (C) Apo-enzyme baseline (Blank) reference. The color scale indicates the degree of correlation (+1.0, red) or anti-correlation (-1.0, blue). Labeled residue pairs

(Gly121-His447, Trp86-Tyr337, etc.) identify critical functional nodes where Lead **19** maintains native enzyme “breathing” while Compound **20** introduces disruptive correlated rigidity.

2.9. Functional Residue Synchrony and Catalytic Integrity

The influence of ligand binding on the intrinsic global dynamics of human AChE was quantified using Dynamic Cross-Correlation Matrices (DCCM) for the C α atoms (Figure 9). To establish a ground-truth for protein “breathing,” a ligand-free (Apo) simulation was utilized as a baseline reference (Figure 8C). In the Apo state, critical residue pairs such as Gly121-His447 (essential for the catalytic cycle) and Trp86-Tyr337 (responsible for substrate recognition) demonstrate pronounced anti-correlated motions (indicated by deep blue regions with $C_{ij} < -0.25$).

Lead Compound **19** effectively preserves these native anti-correlations (Figure 8A), suggesting that its dual-site anchoring at the PAS and CAS does not perturb the essential flexibility required for catalytic throughput. Conversely, Compound **20** (Figure 8B) induces a deleterious transition toward correlated motions in the Trp86-Tyr337 pair and increased rigidity in the Tyr124-Glu334 loop. This disruption of native synchrony, combined with the scattered Free Energy Landscape (Figure 7), provides the final mechanistic proof of **19**’s functional superiority as a disease-modifying lead.

3. Materials and Methods

3.1. Dataset Preparation and Computational Framework

A primary dataset consisting of 115 fused quinoline analogues with experimentally determined AChE inhibitory activities was curated from peer-reviewed medicinal chemistry literature. To ensure consistency and mitigate heteroscedasticity, biological activity values (IC_{50}) were converted to the negative decadic logarithm ($pIC_{50} = -\log_{10} IC_{50} [M]$). Two-dimensional chemical structures were sketched using MarvinSketch (v24.3.1, ChemAxon) and subjected to 3D optimization utilizing the MMFF94 force field to reach the local energetic minimum prior to descriptor generation.

A comprehensive pool of 11,135 molecular descriptors, spanning constitutional, topological, geometrical, electronic, and fragmental properties was generated via the Online Chemical Database (OCHEM) server, integrating variables from the PaDEL, alvaDesc, Dragon7, MERA, and MORDRED libraries [34]. To prevent the “curse of dimensionality” and rule out uninformative variables, a rigorous pre-filtering protocol was applied: (1) descriptors with a pairwise correlation coefficient >0.95 were excluded; (2) descriptors maintaining constant or near-constant values in $>80\%$ of the dataset were removed. This reduction yielded a pool of 2,140 informative descriptors for subsequent model construction.

3.2. QSAR Model Construction and Regulatory Validation

The QSAR was established using the Genetic Algorithm-Multiple Linear Regression (GA-MLR) technique within the QSARINS (v2.2.4) platform [35,36]. The curated dataset was partitioned into a training set ($n = 81$, $\approx 90\%$) for feature selection and internal validation, and an independent external validation set ($n = 8$, $\approx 10\%$) to verify the model’s generalizability across diverse chemical scaffolds.

The statistical robustness of the resulting model was evaluated against the guidelines established by the Organization for Economic Co-operation and Development (OECD). Predictive accuracy and internal stability were quantified using the coefficient of determination (R^2), cross-validated Q^2 (Leave-one-out and Leave-many-out), and the concordance correlation coefficient (CCC). The applicability domain (AD) was delineated through leverage analysis (Williams plot), with the leverage threshold (h^*) calculated as $3(p'+1)/n$ (where p' is the number of model descriptors and n is the training set size). Structural anchoring was confirmed by performing 2,000 iterations of Y-randomization, ensuring that the biological endpoints were not derived from chance correlations.

3.3. Rational Design and ADMET Evaluation

The validated GA-MLR model served as the quantitative engine for the rational design of eighteen fused quinoline derivatives. Structural modifications were systematically guided by the prioritized descriptors *max_conj_path* (extended π -conjugation), *R8m* (longitudinal reach), and *MATS3s* (electronic distribution). The prospective library was structured to include sixteen novel scaffolds and two internal benchmarks, Compounds **2** and **4** from the external validation set, to serve as predictive calibration anchors. Pharmacokinetic and toxicological profiles were predicted using the ADMETlab 3.0 server to evaluate the clinical viability of the designed leads [37]. Critical parameters included aqueous solubility (logS) measured in log mol/L, Caco-2 permeability quantified as log cm/s to assess intestinal absorption, and a Blood-Brain Barrier (BBB) score threshold of > 0.3 to confirm central nervous system accessibility. Synthetic accessibility was delineated on a 1–10 scale where 1 represents the highest ease of synthesis. Toxicological liabilities were quantified via the ProTox-II server to evaluate organ-specific hepatotoxicity and systemic probabilities for neurotoxicity, immunotoxicity, and cardiotoxicity relative to the reference drug Tacrine [38].

3.4. Molecular Docking and Molecular Dynamics Protocols

Molecular docking elucidated the binding orientations of prioritized Leads 19 and 20 within the human AChE crystal structure (PDB ID: 7XN1) at 2.85 Å resolution [39]. The protein was prepared by removing co-crystallized solvent and heteroatoms followed by the addition of polar hydrogens. The protein structure was processed using the CB-Dock2 cavity detection algorithm to automate the identification of optimal binding sites within the 20-Å enzyme gorge [40,41]. Scientific transparency was ensured by validating the docking protocol via self-docking of the co-crystallized ligand Tacrine; successful recovery of the experimental pose was defined by a RMSD of 0.326 Å, well within the 2.0 Å threshold for high-confidence binding mode prediction. Visualization of binding interfaces was conducted using ChimeraX [42] and ProteinPlus [43–45].

Long-range 100-ns MD simulations were conducted using the Desmond module in the Schrödinger Suite 2024-2 [46–48]. The lead-enzyme complexes were solvated in an orthorhombic box using the TIP3P water model with a 10 Å buffer. Systems were neutralized with Na⁺/Cl⁻ ions and subjected to the default Desmond relaxation protocol prior to production. Production runs were executed at 300 K and 1.01325 bar under the NPT ensemble utilizing the OPLS4 force field. Post-simulation analyses included Protein-Ligand RMSD, residue-level Root Mean Square Fluctuation (RMSF), and Radius of Gyration (rGyr) to evaluate temporal stability.

3.5. Post-Simulation Integrity and Functional Dynamics Analysis

The structural and thermodynamic consequences of ligand binding were evaluated using the MD-equilibrated trajectories. The stereochemical quality of the final 100-ns snapshots was verified through Ramachandran plot analysis using the MolProbity server, which quantified the distribution of (ϕ , Ψ) dihedral angles to ensure that binding within the narrow active-site gorge did not induce non-physical backbone strain [49]. Residues categorized as outliers were mapped back to the 3D structure to confirm their localization within flexible surface loops far from the binding interface.

The thermodynamic stability of the Lead 19 and Lead 20 complexes was further characterized by constructing the Free Energy Landscape (FEL). The FEL was generated by performing Principal Component Analysis (PCA) on the C α coordinates to extract the primary modes of motion. The probability distribution of the trajectory across the first two principal components (PC1 and PC2) was used to calculate the relative Gibbs free energy (G) through the Boltzmann relationship, allowing for the identification of localized energy minima and the assessment of entropic instability in the ligand-bound states [50–52].

Functional synchrony between protein residues was quantified using the Dynamic Cross-Correlation Matrix (DCCM) [53]. Cross-correlation coefficients (C_{ij}) were calculated for all C α pairs throughout the 100-ns trajectory to evaluate how the inhibitors influenced the native “breathing”

motions of the enzyme. Positive correlations ($C_{ij} > 0$) denoted synchronized residue movement, while negative values ($C_{ij} < 0$) identified anti-correlated motions. This analysis was critical for validating that Lead 19 preserves the essential anti-correlation of the Gly121-His447 pair required for the enzyme's catalytic cycle.

5. Conclusions

This investigation successfully established a robust, statistically validated computational pipeline for the rational design of novel fused quinoline sulfonamides as dual-site acetylcholinesterase (AChE) inhibitors. By leveraging a five-descriptor GA-MLR QSAR model ($R^2 = 0.7569$, $Q_{L00}^2 = 0.7244$), we demonstrated that inhibitory potency is fundamentally governed by the extension of π -conjugation (*max_conj_path*) and the precise spatial mass distribution (*R8m*) required to bridge the ~20 Å enzyme gorge. Among the eighteen analogues prioritized, Lead Compound **19** emerged as a superior candidate, achieving high-occupancy dual-site anchoring at both the Catalytic (CAS) and Peripheral (PAS) sites while maintaining an optimized pharmacokinetic profile suitable for central nervous system penetration. In contrast, Compound **20** exhibited inferior thermodynamic stability and reduced binding efficiency, a direct consequence of an unfavorable stereoelectronic clash with Ser125 and the entropic instability introduced by its longer *n*-pentyl chain.

The clinical potential of Lead **19** is further underscored by its ability to preserve the native functional synchrony of the enzyme, specifically the essential anti-correlated motions between the Gly121-His447 catalytic pair, while significantly reducing off-target risks for neurotoxicity and cardiotoxicity compared to the reference drug Tacrine. These findings provide a high-value structural scaffold to address the escalating global dementia crisis, particularly in high-burden demographic regions such as India. In alignment with the requirements of this Special Issue, this study provides the mechanistic and in silico foundation for the next phase of experimental validation. Future work will employ biomimetic chromatography, specifically retention analysis on Immobilized Artificial Membrane (IAM) and Human Serum Albumin (HSA) stationary phases to correlate these topological descriptors with experimental membrane permeability and protein-binding kinetics, advancing the high-throughput, animal-free screening of disease-modifying neuroprotective agents.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/doi/s1>, Figure S1: title; Table S1: title; Video S1: title.

Funding: This research was funded by Centro de Investigación, Universidad Anahuac Querétaro [Grant No. UAQXXXXXX] and the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFUXXXXXX].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the results of this study are available within the article and its Supplementary Materials. Specifically, the curated dataset of 115 fused quinoline analogues, molecular descriptor values, and the coordinate files for the optimized GA-MLR model and 100-ns MD trajectories are available from the corresponding author upon reasonable request.

Acknowledgments: The authors acknowledge the administrative and technical support provided by the Centro de Investigación at Universidad Anáhuac Querétaro. During the preparation of this manuscript/study, the authors used Gemini (3.0 Flash) for the purposes of linguistic refinement, structural optimization of the introduction, and ensuring consistency in technical terminology across the "Logic of Discovery" pipeline; it was not used to generate the primary scientific text or interpret data in an automated manner. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

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