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IN SILICO PROFILING OF DIAZA- AND TETRAAZABICYCLONONAN-9-ONES AS NOVEL NICOTINIC ACETYLCHOLINE RECEPTOR LIGANDS

Abstract: Nicotinic acetylcholine receptors (nAChRs) are promising therapeutic targets for treating cognitive disorders and nicotine addiction. This study aimed to identify a novel nAChR ligand from a series of 8 newly designed diaza- and tetraazabicyclononan-9-ones using a comprehensive *in silico* approach. The biological activity spectrum was predicted using the PASS program, revealing a high potential for nAChR antagonism and other neurotropic activities. Pharmacokinetic and drug-likeness properties were assessed with SwissADME, indicating favorable profiles for most compounds. Molecular docking into the orthosteric site of the human α3β4-nAChR (PDB ID: 6PV7) and α4β2-nAChR (PDB ID: 5KXI) was successfully performed for six derivatives. Among them, compound **1** demonstrated a superior calculated JAMDA Score (-2.60), outperforming the native ligand, nicotine (JAMDA Score: -1.99). Analysis of the binding pose revealed that its superior predicted affinity stems from a network of specific hydrogen bonds with key residues (Tyr93, Ser148, Ser150) and an interaction with the backbone of Trp149 in the orthosteric site. Thus, the integrated *in silico* strategy identified compound **1** as a potent putative nAChR ligand, warranting its priority for further experimental evaluation.

Keywords: nicotinic acetylcholine receptors, molecular docking, *in silico* screening, bicyclic nitrogen heterocycles, drug design.

Introduction

Nicotinic acetylcholine receptors (nAChRs), especially the α7 and α4β2 subtypes, are ligand-gated ion channels widely distributed in brain regions essential for cognition, such as the hippocampus and cortex (Guan, 2024). α7 nAChRs exhibit anti-inflammatory and neuroprotective effects through several mechanisms: suppression of NF-κB-dependent cytokine synthesis, activation of Nrf2-mediated antioxidant defense, modulation of JAK2/STAT3 signaling, and enhancement of autophagy. These mechanisms make α7 nAChRs a promising therapeutic target for the treatment of neuroinflammatory and neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, and multiple sclerosis) (Lee & Hung, 2022; Magnussen, 2025).

While several ligands are known, the search for new, selective, and potent modulators with improved safety profiles remains a pressing need in medicinal chemistry (Crestini et al., 2024). The clinical application of nAChR-targeted drugs is limited by low efficacy in the treatment of neurodegenerative diseases and cognitive impairment, as well as safety concerns due to receptor desensitization and peripheral side effects (Recio-Barbero et al., 2021). Non-selectivity of action, pharmacokinetic limitations, and species differences in animal models further complicate the development of successful therapeutic agents (Magnussen, 2025).

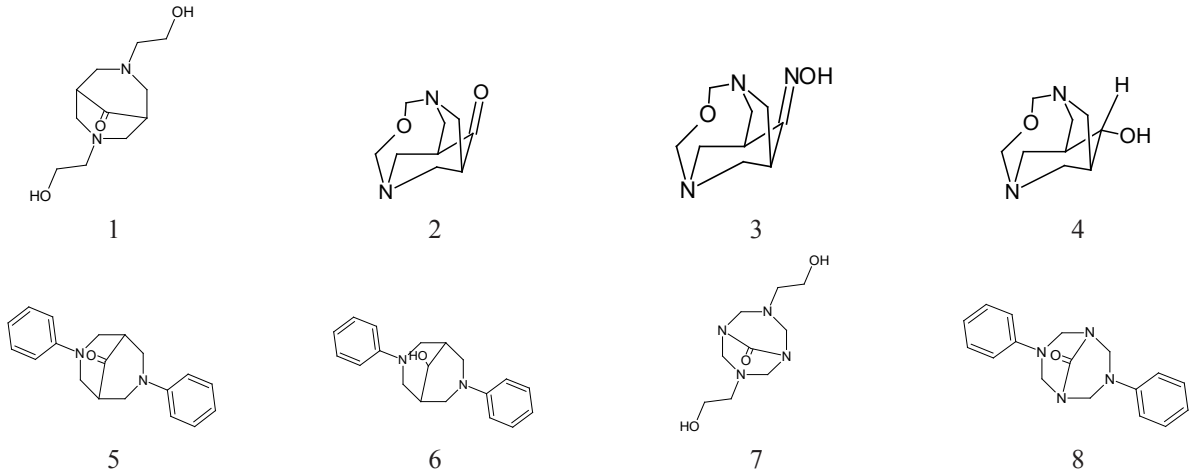
Current neurodegeneration drug research strongly favors heterocyclic scaffolds (e.g., piperidines, indoles, quinolines, pyrazoles) because they can address multiple pathogenic processes in Alzheimer's and Parkinson's disease (Katsoulaki et al., 2025). Among such promising frameworks, di- and tetraazabicyclononan-9-ones are the building blocks for obtaining drugs for prevention and treatment of neurodegenerative diseases (Matthews et al., 2024). The primary objective of this work was to conduct a comprehensive *in silico* profiling of a novel series of diaza- and tetraazabicyclononan-9-ones derivatives

to identify the most promising candidate acting as a nAChR ligand, using a combination of predictive and molecular modeling techniques.

Materials and methods

The series of novel diaza- and tetraazabicyclo[3.3.1]nonan-9-one derivatives (1–8) investigated in this study were designed and synthesized at al-Farabi Kazakh National University. The detailed synthetic protocols and spectroscopic characterization are beyond the scope of this computational work and will be disclosed in a separate paper focused on the synthetic chemistry. For the purposes of this study, the compounds served as the basis for constructing their 3D models for subsequent *in silico* analysis. The structures are shown in the Fig. 1.

Figure 1
New compounds based on diaza-, tetraazabicyclononan-9-ones 1-8



ACD Labs ChemSketch was used to draw chemical structures and generate SMILES notations.

Prediction of biological activity spectra was carried out using the PASS Online (2025), which estimates P_a (Probability "to be Active") and P_i (Probability "to be Inactive") for a wide range of pharmacological effects based on the structural formula of the compound.

The free online platform SwissADME (2025) (from the Swiss Institute of Bioinformatics) was used to determine the drug-likeness, pharmacokinetics and medicinal parameters of the test substances.

Molecular docking was performed for compounds 1–6 using JAMDA (Jupyter-Aided Molecular Docking Application) available through the Proteins*Plus* web service (2025). The JAMDA platform was selected due to its integrated use of machine-learning scoring functions and the gradient-based pose optimization method by Flachsenberg et al. (2021), which ensures a robust and reproducible protocol particularly suited for ranking ligand affinities within congeneric series.

The crystal structure of the nicotinic acetylcholine receptor was obtained from the Protein Data Bank. For molecular docking, the crystal structures of two heteropentameric nicotinic receptor subtypes were used: α3β4-nAChR (PDB ID: 6PV7, resolution 3.34 Å) and α4β2-nAChR (PDB ID: 5KXI, resolution 3.94 Å). 6PV7 was chosen due to its structural similarity to the predicted target α6β3β4α5, the availability of a native ligand (nicotine) for protocol validation, and its high-quality model of a receptor with a β4 subunit. The 5KXI structure was used as a model for subtypes containing a β2 subunit (including the predicted α2β2). This approach allows us to evaluate the interaction of new compounds with two distinct but pharmacologically relevant binding interfaces in the nAChR family, encompassing structural features common to the subtypes predicted as the most likely targets (Gotti et al., 2023) and providing insights into potential subtype selectivity. The docking pocket was identified using the DoGSiteScorer.

Molecular geometry optimization of ligand molecules was performed at the semi-empirical

PM6 level of theory using Gaussian 16 software. The optimized structures were subsequently converted to SDF format for use in docking simulations. Compounds 7 and 8 were excluded from docking analysis due to the inability to generate valid conformations for these sterically complex structures within the automated protocol. This was done to ensure the reliability and reproducibility of the results.

Results and discussion

1. Prediction of biological activity

Prediction of biological activity using the PASS-online program revealed a multi-target pharmacological profile for compounds **1**–**8**. Of interest in the context of the study's objective was the high probability of antagonism to nicotinic acetylcholine receptors (Table 1).

Table 1
Predicted neurotropic activity for compounds **1**–**8** *(Pa > 0.75)*

Compound	nAChR α2β2 Antagonist	nAChR α6β3β4α5 Antagonist	Phobic Disorders Treatment	Anaphylatoxin Receptor Antagonist
1	0.77	0.80	0.89	0.92
2	0.86	0.82	0.84	-
3	0.74	0.68	0.81	-
5	0.90	0.83	0.85	-
6	0.85	0.77	0.78	-
7	-	-	0.80	0.89
8	0.75	-	0.76	-

Compound **2** (P_a=0.86) and especially compound **5** (P_a=0.90) showed the greatest potential as an α2β2 receptor antagonist. Antagonism to the α6β3β4α5 receptor was most pronounced also for compounds **2** (P_a=0.82) and **5** (P_a=0.83). Compounds **1** (P_a=0.89) and **5** (P_a=0.85) showed high potential for the treatment of phobic disorders, and compounds **1** (P_a=0.92) and **7** (P_a=0.89) showed potential as an anaphylatoxin receptor antagonist.

The multi-target profile predicted by PASS, encompassing both specific nAChR antagonism and broader neurotropic activities (e.g., phobic disorders treatment), aligns with the complex pathophysiology of neurodegenerative diseases, which often require polypharmacological approaches (Katsoulaki et al., 2025). The particularly high P_a values for nAChR antagonism in compounds **2** and **5** (P_a > 0.82) strongly justified their selection, along with others from the series, for subsequent molecular docking studies to verify and quantify this predicted activity at a structural level.

2. Evaluation of the pharmacokinetic profile and drug potential

The pharmacokinetic profile and druggability criteria for compounds **1**–**8** were assessed using the SwissADME website. All compounds conform to Lipinski's rule, indicating their fundamental druggability and oral bioavailability.

Analysis of the key descriptors presented in Table 2 and the bioavailability radars (Fig. 2) showed that all compounds fall within the optimal range for parameters such as size, polarity (TPSA), saturation, and molecular framework flexibility.

Table 2
Key Physicochemical Properties of Compounds 1–8

Cmpd	Molecular Formula	MW (g/mol)	TPSA (Å²)	Num. H-Bond Donors	Num. H-Bond Acceptors	Consensus Log P
1	$C_{11}H_{20}N_2O_3$	228.29	64.01	2	5	-0.72
2	$C_9H_{14}N_2O_2$	182.22	32.78	0	4	-0.01
3	$C_9H_{15}N_3O_2$	197.23	48.30	1	5	0.02

Continuation of the table

Cmpd	Molecular Formula	MW (g/mol)	TPSA (Å²)	Num. H-Bond Donors	Num. H-Bond Acceptors	Consensus Log P
4	$C_9H_{16}N_2O_2$	184.24	35.94	1	4	-0.07
5	$C_{19}H_{20}N_2O$	292.37	23.55	0	1	2.63
6	$C_{19}H_{22}N_2O$	294.39	26.71	1	1	2.53
7	$C_9H_{18}N_4O_3$	230.26	70.49	2	5	-1.37
8	$C_{17}H_{18}N_4O$	294.35	30.03	0	1	1.99

Abbreviations: Cmpd – Compound; MW – Molecular Weight; TPSA – Topological Polar Surface Area; Consensus Log P – averaged logarithm of octanol-water partition coefficient.

As shown in Table 2, all compounds exhibit molecular weights below the 500 g/mol threshold, and the number of hydrogen bond donors and acceptors adheres to Lipinski's criteria, supporting their oral drug-likeness. The Topological Polar Surface Area (TPSA) values vary considerably across the series, ranging from 23.55 Å² for compound **5** to 70.49 Å² for compound **7**, which directly correlates with predicted membrane permeability and blood-brain barrier penetration. These physicochemical properties are visually summarized in the bioavailability radar plots (Fig. 2), where the optimal range for each parameter (lipophilicity, size, polarity, saturation, flexibility, and solubility) is represented by the pink area. Compounds whose profiles fall entirely within this area are considered to have a high probability of favorable oral bioavailability.

The BOILED-Egg model (Fig. 3) predicted a high probability of gastrointestinal absorption for compounds 3, 5, 6, and 8. Furthermore, compounds 5, 6, and 8 showed the ability to penetrate the blood-brain barrier, which is particularly relevant for their putative neurotropic action. Importantly, these same compounds do not exhibit structural alerts for the PAINS and Brenk filters, reducing the risk of nonspecific activity or toxicity. In contrast, highly polar compounds 1 and 7 (TPSA > 60 Å², Cons. Log P < -0.7), despite high calculated solubility, are likely to be poorly absorbed when administered orally due to low lipophilicity.

Figure 2
Bioavailability radar of compounds 1–8

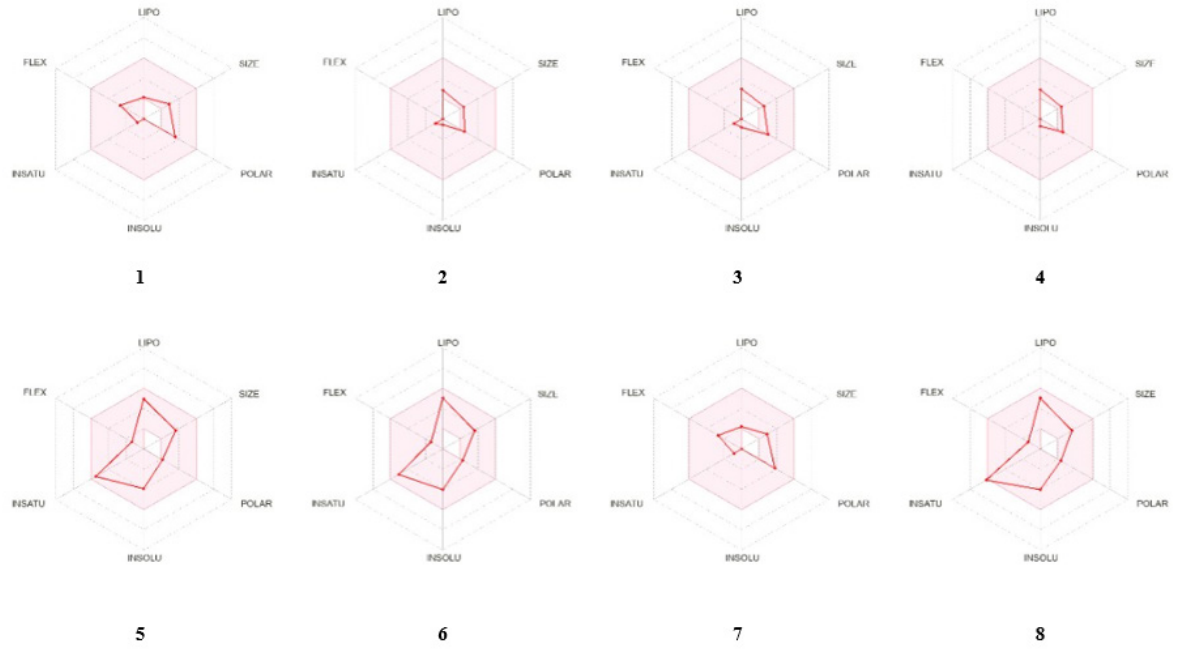
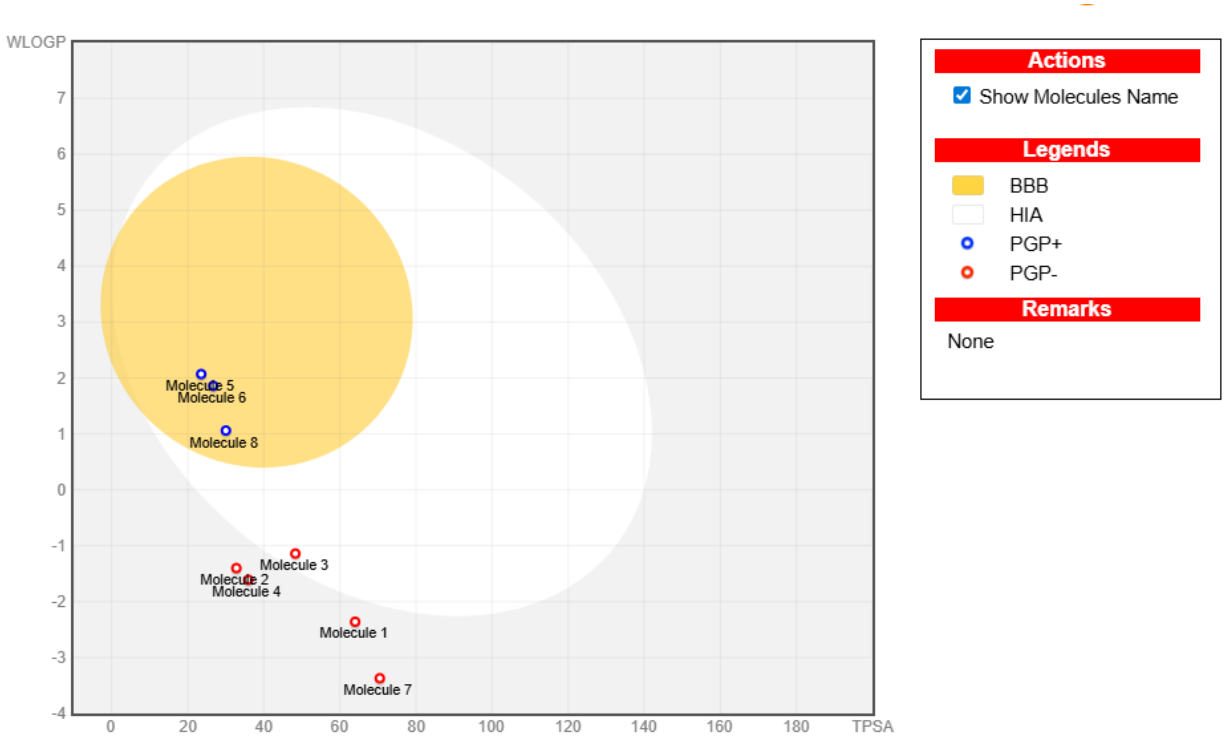


Figure 3
BOILED-Egg model for predicting the absorption of compounds in the gastrointestinal tract (white area) and penetration into the brain (yellow area). BBB – blood-brain barrier; HIA – human intestinal absorption; PGP – P-glycoprotein



These ADME predictions create a context for interpreting the docking results. For instance, compound **1**, which showed the highest calculated binding affinity, falls into the "non-BBB-permeable" and likely low-oral-absorption category according to the BOILED-Egg model, indicating a potential disconnect between excellent target engagement and desirable CNS drug-likeness. This contrast underscores the need for a balanced optimization strategy that considers both potency and pharmacokinetics. Compounds **5**, **6**, and **8**, predicted to have good BBB penetration and gastrointestinal absorption, represent more promising starting points from a drug delivery perspective, provided their binding affinity can be improved through structural modifications informed by the docking poses.

3. Molecular docking into the nAChR orthosteric site

The molecular docking study was conducted to verify the predicted nAChR antagonism and rank the compounds based on their calculated JAMDA Score. Two high-resolution crystal structures of nAChRs were employed. The α3β4 structure (6PV7) was selected as a suitable model for studying interactions with β4-subunit-containing subtypes, including the predicted α6β3β4α5 target from PASS analysis (it is often used to model other subtypes as well, as in the case of the present study) (Noviello et al., 2021). The α4β2 structure (5KXI) was used as a model for subtypes containing the β2 subunit, such as the predicted α2β2 target. The docking protocol was validated by redocking nicotine into both receptor structures. The root-mean-square deviations between the docked and experimental poses were 0.98 Å for 6PV7 and 1.85 Å for 5KXI, both below the 2.0 Å threshold, confirming the reliability of the computational approach. The key interactions of nicotine with the residues of the binding site (hydrogen bond with Tyr96, π-π stacking with Trp154) were correctly reproduced, confirming the suitability of the model for comparative analysis.

The results, expressed as Score, are summarized in Table 3. The JAMDA score is unitless, as it is a machine-learning ranking metric rather than a physical binding energy. Score is based on normalized model features and is used exclusively for comparative analysis of poses and ligands.

Table 3
Docking results (JAMDA Score) for compounds 1–6 and nicotine in the orthosteric sites of nAChR subtypes α3β4 (PDB: 6PV7) and α4β2 (PDB: 5KXI)

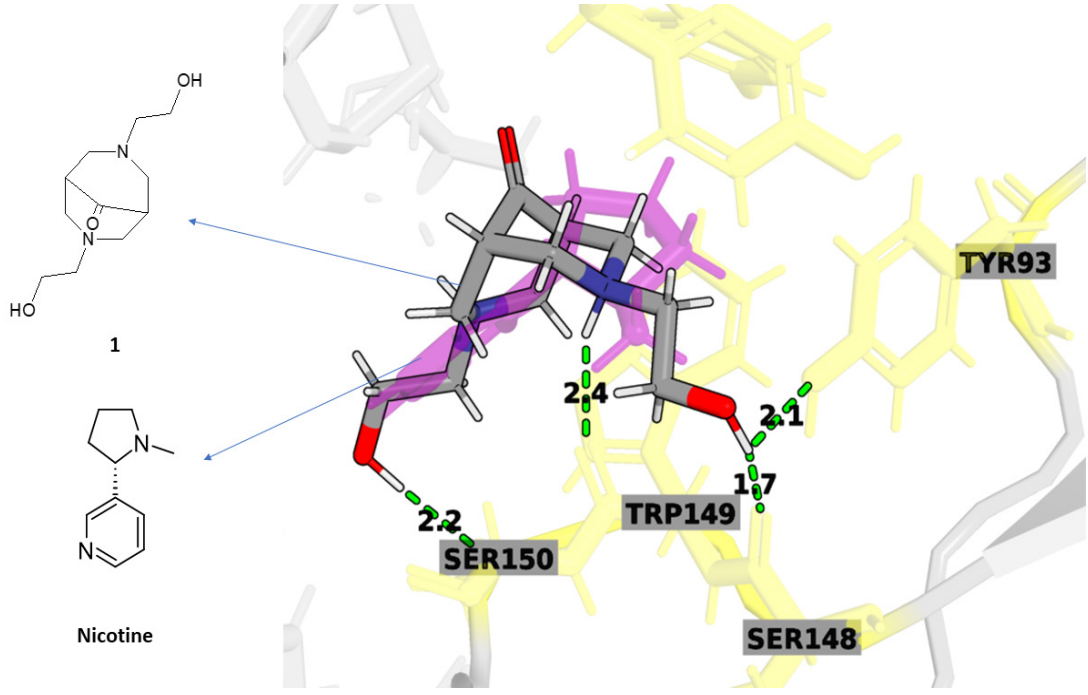
Ligand	JAMDA Score	
	α3β4 (PDB: 6PV7)	α4β2 (PDB: 5KXI)
Nicotine	-1.99	-1.68
1	-2.60	-2.06
2	-1.66	-1.31
3	-1.56	-1.35
4	-1.64	-1.21
5	-1.89	-1.52
6	-1.74	-2.07

The most significant result is compound 1, which demonstrated the best calculated Score in both structural models, significantly outperforming the native ligand nicotine (Table 3). This result is consistent with the PASS prediction data, which predicted compound 1 to have a high probability of antagonist activity against various nAChR subtypes. Compound 6 showed a better result (-2.07) in the α4β2 nAChR model (5KXI) than in the α3β4 model (-1.74), and even outperformed nicotine in this model. This may indicate potential selectivity of this derivative for receptors containing the β2 subunit, which requires further study.

It should be noted that all tested compounds, with the exception of six, demonstrated higher calculated Score for the α3β4 subtype (more negative JAMDA Score values for 6PV7). This trend is consistent with literature data indicating that structural features of the α3β4 nAChR binding site can facilitate stronger interactions with certain classes of ligands (Kanasuwan et al., 2023).

Detailed analysis of the binding pose of compound 1 (Fig. 4) revealed specific interactions with key residues of the orthosteric site. One of the ligand's hydroxyl groups forms a double hydrogen bond: with Tyr93 (2.1 Å) and with Ser148 (1.7 Å), acting as a bridge between these residues (Matera ert.al., 2023); the hydroxyl group of the second substituent forms a hydrogen bond with Ser150 (2.2 Å). In addition, the protonated nitrogen atom of the diazabicyclic fragment forms a fourth hydrogen bond with the carbonyl oxygen of Trp149 (2.4 Å), which provides additional stabilization of the complex.

Figure 4
Molecular model of compound 1 bound to key interacting residues of the orthosteric site of nAChR (PDB: 6PV7). The docking pose of 1 (shown in gray) was presented by structural alignment with the co-crystallized nicotine (shown in purple)



The detailed analysis of the binding pose of compound 1 (Fig. 4) not only explains its superior calculated affinity but also provides valuable insights for future structural optimization. The formation of multiple hydrogen bonds with key serine and tyrosine residues (Ser148, Ser150, Tyr93) and the interaction with the backbone of Trp149 create a stable network specific to the orthosteric site. This pattern is distinct from the simpler interaction profile of nicotine. Comparing the docking scores (Table 3) with the structural features of compounds 1–6 suggests that the presence of specific hydrogen bond donor/acceptor groups in the substituents of the diazabicyclononan-9-one core is crucial for high affinity.

Furthermore, the divergent performance of compounds like 6 in different subtypes (α3β4 vs. α4β2) hints at the potential to modulate selectivity through targeted modifications of the hydrophobic regions of the ligands. These *in silico* derived SAR observations establish a rational basis for designing next-generation analogs with improved potency, selectivity, and pharmacokinetic properties.

Conclusion

The search for new ligands of nicotinic acetylcholine receptors for the treatment of cognitive and neurodegenerative diseases remains highly relevant. We conducted comprehensive *in silico* profiling of novel diaza- and tetraazabicyclononan-9-ones, integrating PASS prediction, SwissADME pharmacokinetic analysis, and molecular docking into the orthosteric sites of nAChR subtypes (α3β4 and α4β2).

The study confirms the initial hypothesis about the high potential of this chemical scaffold for targeting nAChRs. The key finding that fundamentally enriches existing knowledge is the identification of compound 1, a diazabicyclononan-9-one derivative, which demonstrated a superior calculated binding affinity compared to the native ligand, nicotine, particularly in the α3β4 model.

Compound 1 was a leader in terms of affinity for nAChRs, but its inability to penetrate the blood-brain barrier is predicted to limit its potential use in the treatment of CNS diseases. This highlights the need to balance high affinity with optimal pharmacokinetic properties. This result, however, provides a valuable starting point for further medicinal chemical optimization. The structural framework of compound 1 can be modified to maintain high affinity but impart properties that facilitate brain penetration, while its low permeability itself may be useful for the development of peripherally selective agents.

The primary prospective application is the experimental validation of compound 1 *in vitro*, followed by the synthesis of focused analog libraries to either exploit its peripheral selectivity or improve its CNS drug-likeness.

Author contributions

E.M. Yergaliyeva: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. *K.B. Bazhykova*: Supervision, Project administration, Methodology, Validation, Writing – review & editing, Corresponding author. *A.M. Koishybay*: Investigation, Resources, Formal analysis, Writing – review & editing. *M. Dossay*: Investigation, Software, Formal analysis, Visualization, Writing – review & editing. *A. Khozhalepessova*: Software, Formal analysis, Investigation, Writing – review & editing.

Conflict of interest

The authors declare that they have no conflicts of interest.

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