Rational Design of New Phosphodiesterase Inhibitors: An In Silico Approach Oriented Toward Alzheimer's Disease

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Abstract. *Alzheimer's disease (AD) is the leading cause of dementia worldwide and, due to the limited efficacy of current treatments, phosphodiesterase (PDE) has emerged as a promising new therapeutic target. In this context, this study proposed the development of two novel PDE inhibitors using in silico approaches. Docking results revealed high binding affinities and effective enzyme inhibition. Recurring interactions with residues in the PDE active site suggest a stable inhibition mechanism, reinforcing the potential of the designed compounds as promising candidates for AD treatment, while also providing valuable insights for the rational development of new therapeutic agents.*

Resumo. *A doença de Alzheimer (DA) é a principal causa de demência no mundo e, diante da baixa eficácia dos tratamentos atuais, a fosfodiesterase (PDE) tem ganhado destaque como novo alvo terapêutico. Nesse contexto, este estudo propôs o desenvolvimento de dois novos inibidores de PDE por meio de abordagens in silico. Os resultados de docking revelaram altas afinidades de ligação e capacidade de inibição da enzima. Interações recorrentes com resíduos do sítio ativo da PDE sugerem um mecanismo de inibição estável, reforçando o potencial dos compostos planejados como candidatos promissores ao tratamento da DA, além de oferecer insights valiosos para o desenvolvimento racional de novos fármacos.*

1. Introduction

Alzheimer's disease (AD), considered the leading cause of dementia, begins with the abnormal accumulation of the beta-amyloid peptide, which aggregates to form amyloid plaques, accompanied by the formation of neurofibrillary tangles composed of the tau protein. These phenomena result in the progressive degeneration of neurons. In addition, the cholinergic hypothesis suggests that the reduction in acetylcholine levels, a neurotransmitter essential for memory processes, is directly associated with the cognitive decline observed in the disease. The enzyme acetylcholinesterase, responsible for breaking down acetylcholine at synapses, represents one of the main therapeutic targets. However, the currently available drugs for its inhibition do not provide a cure, offering only temporary symptom relief. Therefore, new targets and therapeutic agents are being sought for the treatment of Alzheimer's disease (Dadalto; Cavalcante, 2022; Nour et al., 2022).

Given the limitations of currently available treatments, phosphodiesterases (PDEs), intracellular enzymes that regulate the levels of the cyclic nucleotides cAMP and cGMP, have received increasing attention as potential targets for the treatment of cognitive impairment associated with aging and Alzheimer's disease (AD). This enzyme family plays a fundamental role in various neurological disorders, as it controls the degradation of second messengers involved in cellular signaling (Azargoonjahromi 2023; Quimque et al., 2021; Sheng et al., 2022). Recent research shows that PDE modulation can improve cognitive function, as demonstrated in transgenic models with learning deficits. In addition, studies indicate that selective inhibition of these enzymes may mitigate long-term memory deficits caused by oxidative stress, aging, and the accumulation of amyloid plaques and neurofibrillary tangles (Beato et al., 2021; Cummings 2021; Li, Yu, Tu 2021).

Quantum calculations play a key role in investigating bioactive compounds and rational drug design by providing information on electronic properties, binding sites, and interaction energies (Ahmad et al., 2023; Guan et al., 2025). Low-cost quantum mechanical methods, especially semiempirical approaches like GFN2-xTB, have gained prominence for balancing efficiency and accuracy, delivering performance comparable to hybrid density functional theory functionals (Bannwarth, Ehlert, and Grimme, 2019). Their broad adoption is demonstrated through applications such as predicting bioisosteric substitutions, optimizing cannabinoid derivatives, and estimating stable conformations in enzyme active sites (Losev et al., 2023; Kumaeum and Jaiyong, 2025; Yaakob et al., 2025). Complementarily, molecular docking is a strategic tool for predicting interactions between small molecules and biological macromolecules, enabling virtual screening, stable ligand conformation prediction, and ligand–receptor affinity estimation, key steps in computational drug development (Agu et al., 2023; Asiamah et al., 2023; Danel et al., 2023).

In this context, the phosphodiesterase enzyme has gained prominence as a promising target due to its role in neuronal signaling processes and memory consolidation. Thus, the present study proposes the rational design and *in silico* evaluation of two new inhibitors of this enzyme, using theoretical and computational methods to explore their molecular interaction potential with the target protein, with the aim of contributing to the development of more effective therapeutic strategies to combat Alzheimer's disease.

2. Methodology

The initial stage of this study involved the theoretical-computational design of two novel inhibitors, named INI-P1 and INI-P2 (Figure 1), derived from the reference compounds INI-R1 and INI-R2, which had previously been evaluated in complex with the phosphodiesterase (PDE) enzyme (PDB ID: 5SDU). The hybrid molecules were developed through a rational molecular design approach, focusing on the preservation and combination of bioactive heterocyclic fragments present in the reference structures. The main fragments incorporated were: (1) the substituted pyrazole core containing an amide group and a carbamoyl side chain from Molecule 1; (2) the methylated pyridazine, with potential for π-stacking and electrostatic interactions; (3) the aromatic isoxazole ring from Molecule 2, known for its electronic stability and ability to form

hydrogen bonds; and (4) the phenyl aromatic rings present in both molecules, which contribute to hydrophobic interactions and π-π stacking with aromatic residues in the target protein, as observed in the protein-ligand complex (5SDU).

Subsequently, the structures were subjected to geometric optimization using the semiempirical quantum method GFN2-xTB, implemented in the xTB (Extended Tight-Binding) code developed by Bannwarth, Ehlert, and Grimme (2024). Subsequently, the protonation profiles of the inhibitor species were analyzed as a function of pH using the SwissParam tool (Zoete et al., 2011), aiming to identify the predominant forms at the target pH, particularly the physiological value (7.0). To complement this analysis, the free energy and total charge of the protein at the same target pH were estimated using the PDB2PQR server (Dolinsky et al., 2004), providing additional support for the modeling of the INI-P1 and INI-P2 inhibitors.

Molecular docking studies were conducted using the AutoDock Vina software (Trott and Olson, 2010) for both the reference and designed inhibitors, aiming to characterize intermolecular interactions and estimate binding energies. The grid calculation was performed using the program's default settings, with the center set at x = 91, y = 0, and z = 19. The grid dimensions were defined as 40 × 40 × 40 Å, covering the entire binding site region based on the known ligands (INI-R1 and INI-R2) previously crystallized with the protein and available in the Protein Data Bank.

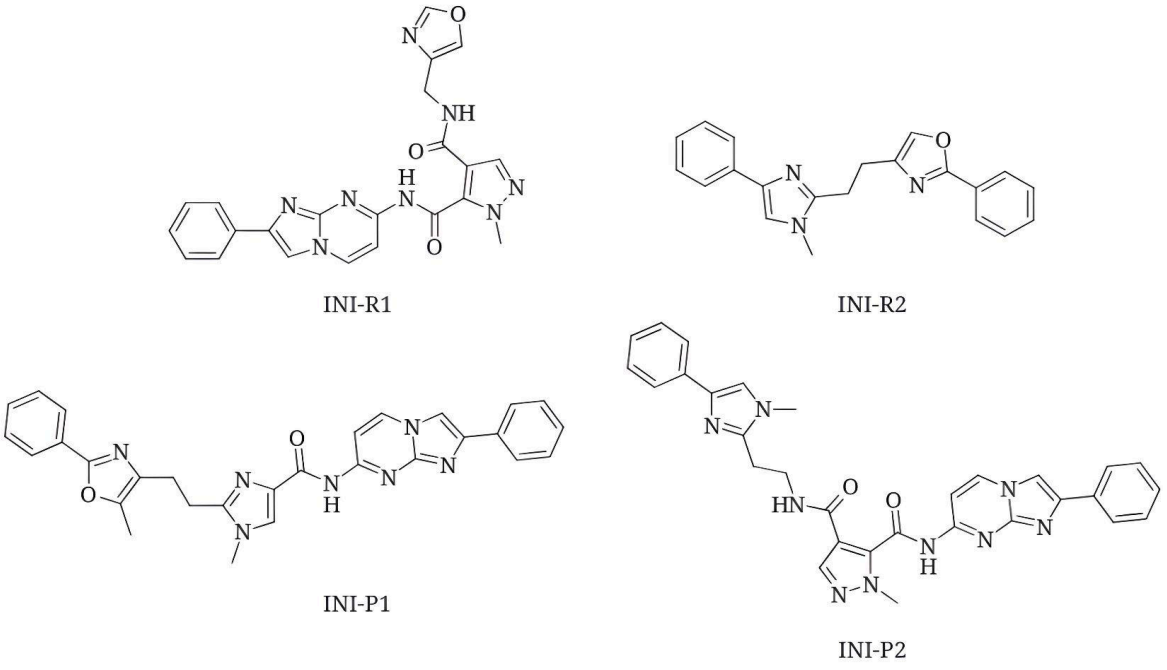

Figure 1 – 2D structures of planned and reference compounds.

Based on the model proposed by Goettig, Chen, and Harris (2024), the inhibition constants (K_i) were calculated using Equation 1, in which $\Delta G°_{(I)}$ represents the standard free binding energy of the inhibitor, R is the universal gas constant, and T=310 K corresponds to human physiological temperature.

$$K_i = exp\frac{\Delta G°_{(I)}}{RT} \qquad Eq.1$$

3. Results and Discussion

The analysis of the microspecies shown in Figure 2, corresponding to the molecules INI-P1 and INI-P2 as a function of pH, revealed the protonation and deprotonation profiles of each compound. For INI-P1, the neutral form became predominant from approximately pH 6 onward, reaching near exclusivity in alkaline environments. The protonated form, represented by microspecies 2, predominated under acidic conditions and progressively decreased as the pH increased, evidencing a clear trend toward deprotonation of INI-P1 as the medium became less acidic. Microspecies 1, 3, and 4 did not exert significant influence within the analyzed pH range. For INI-P2, the neutral form predominated around pH 7, indicating a slightly later transition to the non-ionized state. Protonated form 1 was predominant under acidic pH but its contribution decreased as pH increased, while microspecies 2 predominated over the neutral form only in the pH range of 1 to 2.

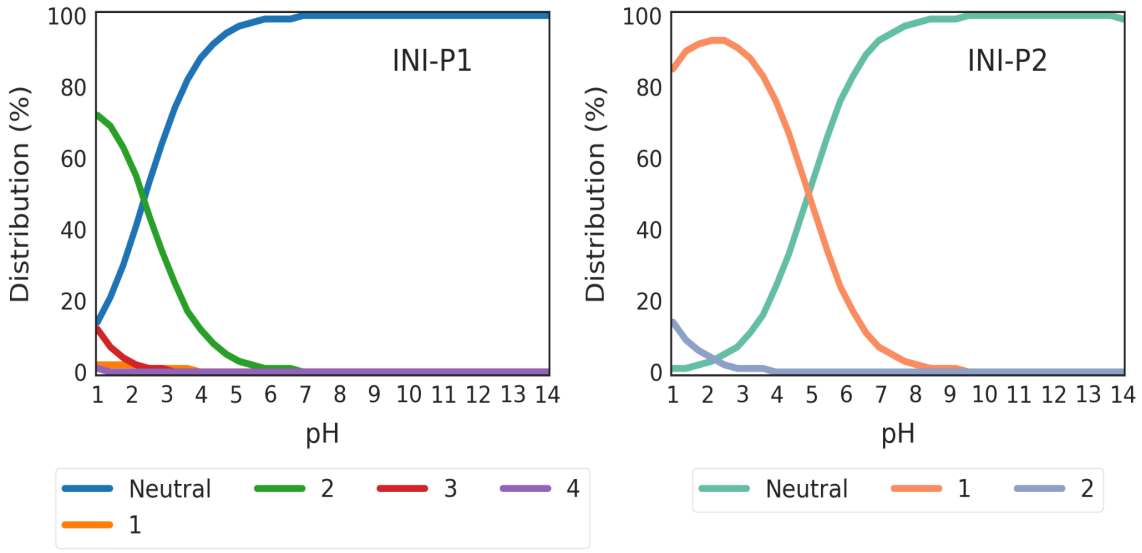

Figure 2 – Distribution of the microspecies of the designed inhibitors as a function of pH.

As illustrated in Figure 3 (A), the analysis of the folding free energy of the PDE (Phosphodiesterase) protein as a function of pH revealed that its maximum conformational stability occurred around pH 7, where the folding free energy reached a minimum value of approximately 2.9 kcal mol^{-1}.

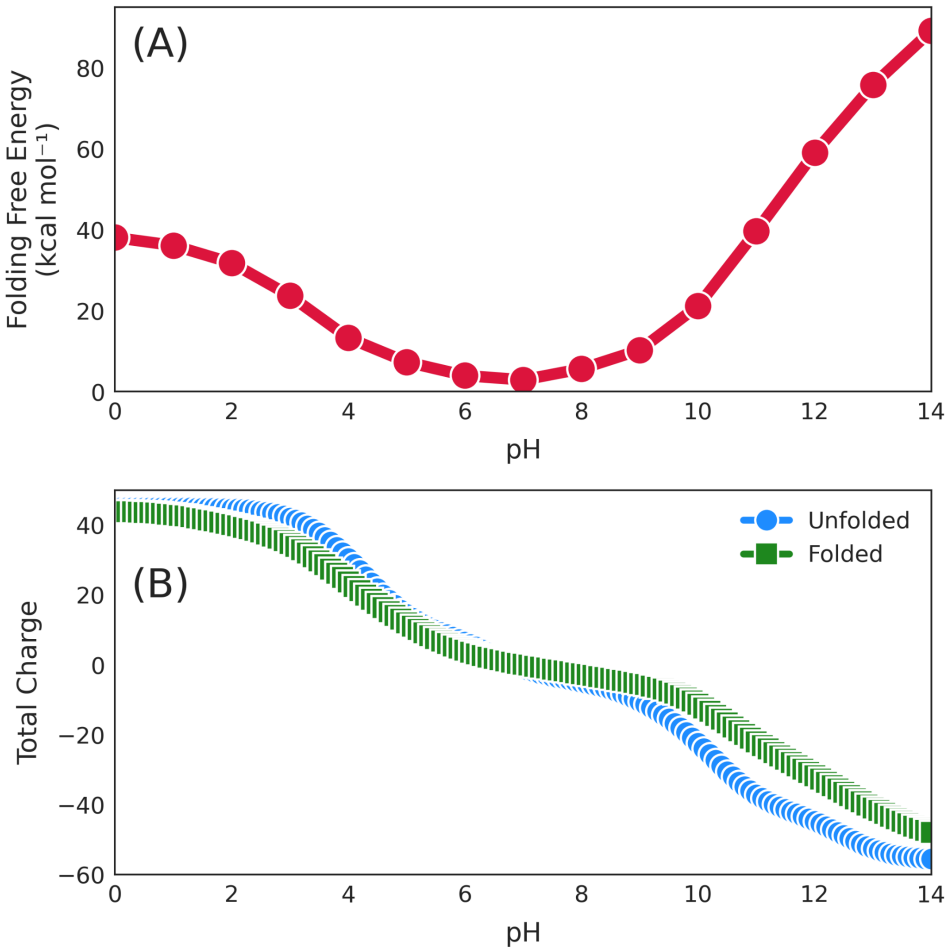

Figure 3 – Free energy and total charge of the protein as a function of pH.

This value indicated that at this pH the protein was in its most stable native form. In contrast, under acidic conditions, between pH 0 and 3, a significant increase in folding free energy was observed, indicating pronounced conformational instability. Similarly, under alkaline conditions, with pH greater than 7, the folding free energy also increased, suggesting that basic environments were unfavorable to maintaining the native structure of the protein. Figure 3 (B) showed the analysis of the protein's total formal charge as a function of pH, indicating that around pH 7 the net charge of the molecule was zero or close to zero, characterizing a state of electrostatic neutrality. This charge balance contributed significantly to the protein's structural stability, as it minimized internal electrostatic repulsions. At extreme pH values, both acidic and basic, the protein acquired significant net electric charges, positive in acidic media and negative in basic media, which could increase intramolecular repulsions and consequently contributed to structural destabilization.

Table 1 presented the structural and energetic parameters of the reference inhibitors INI-R1 and INI-R2, as well as the designed inhibitors INI-P1 and INI-P2. It included the binding energies obtained through molecular docking, the root-mean-square deviations (RMSD) relative to the ligands in the crystallographic structure, and the calculated inhibition constants (K_i), considering the predominant

forms of the inhibitors and the condition of zero total protein charge. The RMSD values were 0.09 Å for INI-R1 and 0.21 Å for INI-R2, both below the widely accepted threshold of 2 Å, which indicated high reliability and accuracy in molecular docking studies (Hosseini et al., 2021).

Table 1 – Energetic, structural, and activity parameters of the designed and reference inhibitors.

	Binding Energy (kcal mol^{-1})	RMSD (Å)	K_i (μM)
INI-R1	-10.50	0.09	1.60×10^{-1}
INI-R2	-10.00	0.21	4.31×10^{-1}
INI-P1	-11.20	----	5.00×10^{-2}
INI-P2	-10.80	----	1.00×10^{-1}

The binding energies calculated for the reference inhibitors INI-R1 (-10.50 kcal mol^{-1}) and INI-R2 (-10.00 kcal mol^{-1}) were consistent with the experimental RMSD values (Root Mean Square Deviation), with INI-R1 standing out for exhibiting the lowest RMSD and the most favorable binding energy between the two. The designed inhibitors INI-P1 and INI-P2 showed even more favorable binding energies, -11.20 and -10.80 kcal mol^{-1} respectively, suggesting potentially more stable interactions with the phosphodiesterase (PDE) active site compared to the reference compounds. The calculated K_i values ($5.0x10^{-2}$ μM for INI-P1 and $1.0x10^{-1}$ μM for INI-P2) indicated higher inhibitory activity for the designed compounds compared to INI-R1 ($1.6x10^{-1}$ μM) and INI-R2 (4.3×10^{-1} μM), establishing the following descending order of inhibitory potency: INI-P1 > INI-P2 > INI-R1 > INI-R2.

Although the designed compounds were theoretical and therefore lacked experimental RMSD data, the results suggested their potential in inhibiting the catalytic activity of PDE. Figure 4 presents the two-dimensional interaction map, along with a legend illustrating the main types of bonds formed between the inhibitors INI-R1 and INI-R2 and the residues located within the active site of the phosphodiesterase (PDE) protein. The INI-R1 inhibitor established an extensive network of interactions with residues from chain A of the target protein. Glutamine (GLN726) played a key role by forming two conventional hydrogen bonds, one with the oxygen atom of the polypeptide backbone and another with the nitrogen atom in the pyrimidine ring of the ligand. Methionine (MET713) contributed through two distinct interactions, one of the π–sulfur type and another of the π–alkyl type, both mediated by the conjugated system formed by the fused Imidazole and Pyrimidine rings. π–π stacking interactions were observed with Proline (PRO712), Glutamate (GLU721), and Phenylalanine (PHE729), involving the aromatic Phenyl and Pyridazine rings. Additionally, Valine (VAL722), Alanine (ALA689), and Isoleucine (ILE692) established π–alkyl interactions resulting from contacts with the Pyridazine, Oxazole, and Phenyl rings present in the ligand structure.

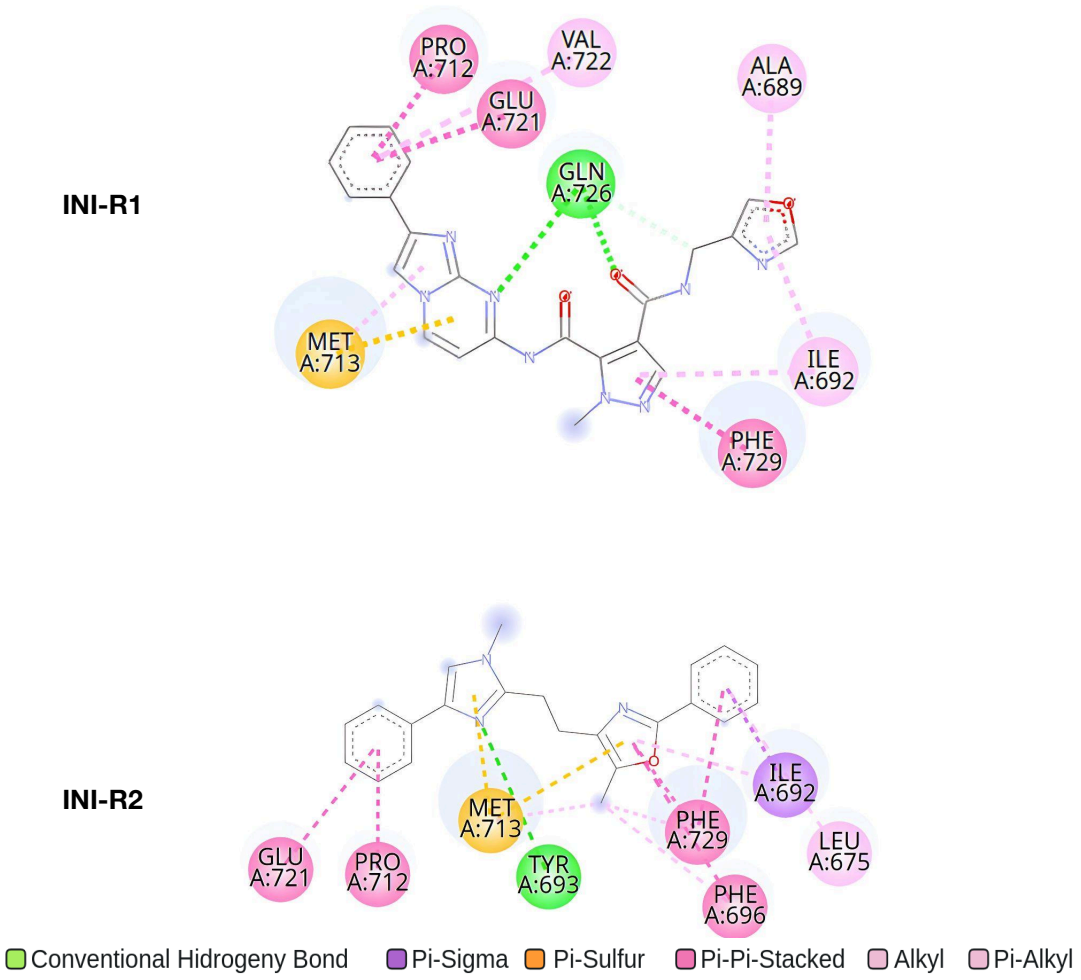

Figure 4 – Two-dimensional interaction map between residues of the PDE protein and the reference inhibitors INI-R1 and INI-R2.

In the INI-R2 inhibitor, a strong hydrogen bond was observed between the NH group of the substituted pyridazine and the residue Tyrosine (TYR693). Methionine (MET713) simultaneously participated in a π–sulfur interaction with the pyridazine ring and the methyl group of the oxazole. Additionally, π–π stacking interactions occurred between the phenyl and oxazole-methyl groups of the inhibitor and the residues Glutamate (GLU721), Proline (PRO712), and Phenylalanine (PHE729 and PHE696). Hydrophobic π–alkyl interactions were present between the Isoleucine (ILE675) residue and the phenyl group, while a π–sigma interaction was formed between the oxazole ring and Isoleucine (ILE692).

Figure 5 presents the two-dimensional interaction map, along with a legend illustrating the main types of bonds formed between the inhibitors INI-P1 and INI-P2 and the residues within the active site of the phosphodiesterase (PDE) protein.

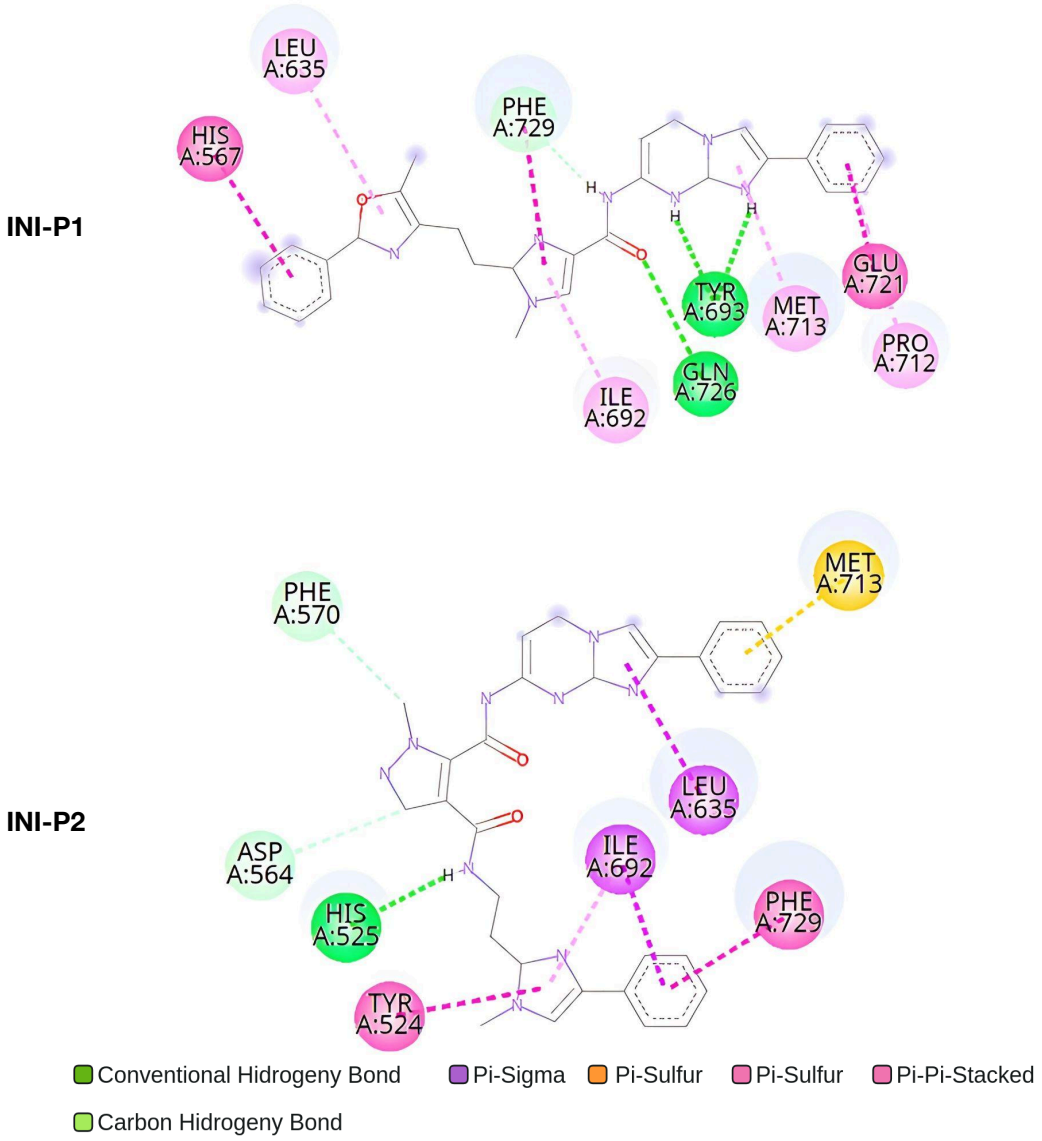

Figure 5 – Two-dimensional interaction map between residues of the PDE protein and the reference inhibitors INI-P1 and INI-P2.

In the INI-P1 inhibitor, conventional hydrogen bonds stood out between the residues Tyrosine (TYR693) and Glutamate (GLU726) and the fused imidazole-pyrimidine rings, as well as with the oxygen atom of the carboline. Additionally, π–alkyl interactions were observed involving the residues Leucine (LEU635), Isoleucine (ILE692), Proline (PRO712), and Methionine (MET713) with the fused rings, oxazole, phenyl, and pyridazine groups of the ligand. π–π stacking interactions also occurred between the residues Glutamate (GLU721) and Histidine (HIS567) and the phenyl ring. The residue Phenylalanine (PHE729) formed both a sigma (C–H) bond and a π–π stacking interaction with the oxazole and amide groups of the ligand. In the case of INI-P2, π–sulfur interactions occurred between the phenyl ring of the ligand and the sulfur atom of Methionine (MET713). π–sigma interactions

involving the residues Leucine (LEU635) and Isoleucine (ILE692) were observed with the fused Imidazole-Pyrimidine, Phenyl, and Pyridazine rings. π–π stacking interactions were detected between Tyrosine (TYR524) and Phenylalanine (PHE729) with the Phenyl-Pyridazine rings, while the residues Phenylalanine (PHE570) and Aspartate (ASP564) participated in sigma (C–H) bonds with the methylated Pyridazine (CH_3). Finally, the residue Histidine (HIS525) established a hydrogen bond with the amide group of the ligand.

Based on the comparative analysis of the molecular interaction maps of the four complexes formed between the ligands and the protein, it can be highlighted that the recurring residues identified in the PDE active site have relevant practical implications for the development of more effective inhibitors. The consistent involvement of MET713, PHE729, and ILE692 suggests that these residues act as key anchoring points, contributing to the stability and molecular recognition of the protein–ligand complex. This finding opens perspectives for structural optimization strategies, in which specific chemical modifications could be introduced in the ligands to strengthen hydrophobic and aromatic interactions with these residues. Furthermore, the participation of PRO712 and GLU721 in three of the four analyzed complexes indicates that targeting these amino acids with additional functional groups could enhance affinity and selectivity. From a therapeutic standpoint, exploiting these critical interactions may lead to the design of more potent and selective inhibitors with a lower risk of adverse effects, contributing to the development of safer and more efficient drug candidates.

4. Conclusions

This study demonstrated, through molecular modeling and semiempirical methods, the potential of two novel phosphodiesterase inhibitors, INI-P1 and INI-P2. The analysis of the protonation profiles, both of the designed ligands and the target protein, indicated that the lowest-energy conformations tend toward a neutral state at physiological pH (7.0), favoring their stability in a biological environment. The designed inhibitors exhibited more favorable binding energies and inhibition constants compared to the reference compounds, with INI-P1 standing out with a binding energy of –11.20 kcal mol^{-1} and an inhibition constant (Ki) of 5.0×10^{-2} μM. The most frequent interactions observed in the protein–ligand complexes involved residues such as Methionine (MET), Isoleucine (ILE), Phenylalanine (PHE), Glutamate (GLU), and Proline (PRO), suggesting that these amino acids play a crucial role in effective anchoring within the phosphodiesterase active site. Thus, the inhibitors designed through in silico approaches demonstrated high potential for interaction with phosphodiesterase, offering promising perspectives for the rational development of new drug candidates with greater affinity, specificity, and efficacy for the treatment of Alzheimer's disease. Therefore, the objectives proposed in this study were fully achieved, as it was possible to evaluate the potential of the designed inhibitors and identify the key residues involved in the interaction with PDE.

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